RESEARCH PEOPLE AND ACTUAL TASKS ON MULTIDISCIPLINARY SCIENCES

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8–10 JUNE, 2011
RESEARCH PEOPLE AND ACTUAL TASKS ON MULTIDISCIPLINARY SCIENCES

Third Conference

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Volume 3


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8–10 JUNE, 2011
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PREFACE

Dear Colleagues!

The International Scientific Conference “Research People and Actual Tasks on Multidisciplinary Sciences” is third International Conference organized in Bulgaria with basic purpose to create the framework for the presentation, debate and publication of the valuable scientific results obtained by both the young members.

United by the idea of Multidisciplinary Sciences, the researchers and faculty will report the results of their research. Thus, the scientists will contribute is to promote exchange of research results, scientific ideas and their practical implementation and development work in the various disciplines.

We hope this meeting will initiate new joint research projects, new friendships. We owe special thanks to all participants, and especially to the supporting organizations.

Chief Editor
Dr. Eng. Atanas Atanasov
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INFLUENCE OF THE CLIFF MICROCLIMATE ON THE POPULATION ECOLOGY OF SOIL PREDATORY MITES (ACARI: MESOSTIGMATA - GAMASINA) FROM ROMANIA

Manu Minodora

Abstract: This ecological study was made in 2010, near to a cliff area from Brebu gorges, from Prahova district, Romania (N: 45° 12' 31,1'”; E: 25° 44' 23,5'”). In order to show the influence of the cliff microclimate on these invertebrates populations, some biotical (type of vegetation) and abiotical factors (type of soil, soil and air temperature, soil humidity and precipitation) were described. 35 species of predatory mites, from 10 genera and 12 families were identified. Species Veigaia nemorensis, Veigaia planicola and Prozercon traegarhi were dominant and constant (which represents 14, 4% from the total number of species), having a wide ecological plasticity and which easily adapt to characteristically cliff environment. The increased number of accessory and accidentally species (54, 3%) showed that the cliff is not a proper habitat for the predatory mites, their migrating from the adjacent forest ecosystems.

The specifically cliff microclimate (the poor vegetation, the sandy soil, the increased air and soil temperature, the decreased soil humidity) had determined a characteristically structure and dynamics of the mite populations.

Keywords: microclimate, mite, population, dynamics.

INTRODUCTION
In trophical soil web, predatory mites have an important role of transformation and transportation of the organic matter, being secondary and third consumers [1], [2]. They are very sensitive to any modifications of the bioedaphical substrate. Cliff habitats are characterized by specifically environmental conditions, vegetation and soil [3], [10]. The modification of the mite populations could be visible on the taxonomical, structural compositions and their dynamics [1], [2], [5], [6], [11], [14]. This study brings new informations about an abundant group of soil fauna, which inhabits near to such atipically ecosystem.

MATERIAL AND METHODS
This ecological study was made in 2010, near to a cliff area from Brebu gorges, from Prahova district, Romania (N: 45° 12' 31,1'”; E: 25° 44' 23,5'”). Altitude was by 537 m. Vegetation was represented by different types of elements: euroasiatic: Rubus saxatilis, Rubus caesius, Hippophae rhamnoides, Populus tremula, Salix caprea, Salvia glutinosa, Campanula sibirica (44,18%); european: Valeriana montana, Crategus monogyna, Berberis vulgaris, Taxus baccata (18,6%); central-european: Centaurea stoebe, Cornus sanguinea, Cytisus nigricans (9,3%); carpathian: Sesleria heuflerana, Thymus pulcherimus, Silene nutans subsp. dubia (11,62%); mediteranous: Cnidium silaifolium, Hedera helix. The highest representation had mesophytes species (42%), followed by xeromesophytes (37%) and mezohygrophyles (9%), and finally xerophytes (7%). More that 20% are pioneer species, as: Cytisus nigricans, Rosa canina and Hippophaê rhamnoides.

Soils are clasified in three classes: clayey till argillaceous on the moderate and strongly inclined peaks, which are serious affected by erosion; brown eumesobasic till pseudogleic, which have a mineral component formed at soil surface, connected to a thin humified organic matter layer; typically alluvial soil.

The abiotic factors were measured: soil and air temperature (T air; T soil), humidity (U%), precipitations (RR).

20 samples/month were collected with MacFadyen corer (5 cm diameter), on 10 cm deep. The soil samples were taken in april, june and october, 2010, around of the cliff.
area. The extraction was performed with a modified Berlese-Tullgren extractor, in ethyl alcohol and the mites samples were clarified in lactic acid. The identification of the mites from the Mesostigmata order was made up to the species level. In total were analysed 120 soil samples, with 35 species and 159 individuals.

After taxonomical identification, the numerical abundance (number of individuals) was the base for the quantification of some structural index as: numerical density (ind./sq.m.); dominance (D%); constance (C%); ecological significance index (W).

RESULTS AND DISCUSSION

Analysing the abiotic factors, was obvious that the most increased soil temperatures were recorded in june and august, and the most decreased in january. The same evolution was obtained for air temperature, with high valued in july - august and low values in january. The humidity had recorded the most increased values in winter month (january and december) and the most decreased values in spring (march and april). The biggest quantity of precipitations falled in june- july, and the lowest in march and january (Fig. 1). The measured abiotical factors had recorded valued specifically for a terrestrial temperate area.

The taxonomical structure of gamasid populations revealed 35 species, belonging to the 20 genera and 12 families: Epicriidae, Parasitidae, Parasitinae, Veigaiidae, Rhodaracaridae, Ascidae, Macrochelidae, Laelapidae, Pachylaelapidae, Pseudolaelapidae, Eviphiidae, Zerconidae. Were recorded 159 individuals, with 15,900 ind./sq.m. Taking account of the numerical densities the most abundat species were: Veigaia planicola, Veigaia nemorensis, Geholaspis manidibularis, Pachylaelaps furcifer, Prozercon carsticus and Prozercon traegardhi (Table 1). These represent 17,14% from the total number of identified species.

The species diversity had recorded decreased values, in comparison with other forest ecosystems (80-100 species), but increased in comparison with meadows, spoil areas, shrubs ecosystems or urban parks (10-30 species) [2], [4], [5], [6], [11], [12], [14]. The same comparison could be made taking account of the numerical densities. These phenornen could be explain due to the soil instability and to the weak content in organic matter (the favourable habitat for these predatory invertebrates and the main trophic source) [10]. In comparison with spoil areas and urban parks, the cliff areas could provide high quantities of nutrients (Mg, Ca, P, K) to the soil, especially on the adjacent areas (as the base of the cliff), creating a favourable habitat for plant developing [15]. The plants
communities from these isolated „spots‖ provides the organic material decomposed by the detritophagous invertebrates (as springtails, nematodes, enchytreids, oribatids), which are the main trophic spectrum for gamasids.

Analysing the dominance index the following species could be classified as eudominant (D>10%): Veigaia planicola, Veigaia nemorensis; dominant (D=5,1-10%): Geholaspis manibularis, Prozercon traegardi; subdominant (D= 2,5-5%): Pergamasus longicornis, Veigaia exigua, Macrocheles sp., Macrocheles matrius, Pachyseius humeralis, Pachylaelaps furcifer, Pseudolaelaps doderoi, Zercon peltadoide, Zercon hungaricus, Zercon foveolatus, Prozercon carsticus. The rest of species (19) are classified as recedent and subrecedent (D= 1,1-2% and respectively D<1,1%).

The constance classes of the investigated species were euconstant (C= 75,1-100%): Veigaia planicola, Veigaia nemorensis; constant (C= 50,1-75%): Leptogamasus obesus; accessory (C= 25,1-50%): Pergamasus longicornis, Pergamasus barbarus, Veigaia exigua, Macrocheles sp., Macrocheles matrius, Geholaspis manibularis, Pachylaelaps furcifer, Pseudolaelaps doderoi, Eviphis ostrinus, Zercon peltadoide, Zercon foveolatus, Prozercon carsticus. The rest number of species (19) were accidently (C= 1-25%).

The ecological significance index (W) showed that in the investigated ecosystem were identified characteristical species Veigaia nemorensis (10<W<20), complementary species Veigaia planicola and Prozercon traegardi (5<W<10), and rest were associated (1<W<5) and accidental (0,1<W<0.1) species (Table 1).

The eudominant- dominant and euconstant-constant species represents 11,4% from the total number of gamasids. Veigaia planicola, Veigaia nemorensis, Geholaspis manibularis, Prozercon traegardi had wide ecological plasticity, easily adapting to characteristically cliff environment. They are ubiquitous predator species, without food preferentiability, capable to migrate in order to find the prey [1], [2], [8], [9], [13].

The increased number of recedent-subrecedent and accessory-accidentally species (54, 3% each) showed that the cliff is not a proper habitat for the predatory mites, their migrating from the adjacent forest ecosystems. This phenomenon is argued through the ecological significance index, which demonstrate that on the cliff aecosystem is not identified any „leader‖ species, only Veigaia nemorensis being described as charachteristical gamasid. It is known as the most coomon species, identified in palearctic area, in all types of ecosystems and habitats.

Table 1: Populational parameters of the identified gamasid mites near to cliff area.

<table>
<thead>
<tr>
<th>Species</th>
<th>No.ind.</th>
<th>Ind./sq.m.</th>
<th>D</th>
<th>C</th>
<th>W</th>
</tr>
</thead>
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<tr>
<td>Epicrius tauricus Bregetova, 1977</td>
<td>1</td>
<td>100</td>
<td>0.63</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td>Leptogamasus obesus Holzmann, 1969</td>
<td>7</td>
<td>700</td>
<td>4.40</td>
<td>70</td>
<td>3.08</td>
</tr>
<tr>
<td>Lysigamasus lapponicus Tragardh, 1910</td>
<td>1</td>
<td>100</td>
<td>0.63</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td>Lysigamasus neoruncatellus Schweizer, 1961</td>
<td>1</td>
<td>100</td>
<td>0.63</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td>Lysigamasus sp.</td>
<td>1</td>
<td>100</td>
<td>0.63</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td>Pergamasus longicornis Berlese, 1906</td>
<td>4</td>
<td>400</td>
<td>2.52</td>
<td>30</td>
<td>0.75</td>
</tr>
<tr>
<td>Pergamasus barbarus Berlese, 1904</td>
<td>3</td>
<td>300</td>
<td>1.89</td>
<td>30</td>
<td>0.57</td>
</tr>
<tr>
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### CONCLUSIONS AND FUTURE WORK

35 species of predatory mites, from 10 genera and 12 families were identified.

Species *Veigaia nemorensis*, *Veigaia planicola* and *Prozercon traegarhi* had the wider ecological plasticity, easily adapting to characteristically cliff environment.

Some populational parameters (dominance, frequency and ecological significance index) that the cliff is not a proper habitat for the predatory mites, their migrating from the adjacent forest ecosystems.

The specifically cliff microclimate (the poor vegetation, the sandy soil, the increased air and soil temperature, the decreased soil humidity) had determined a characteristically structure and dynamics of the mite populations.

The future objectives of this study will be the monitoring of the gamasid populations for at least 3 years and to established some correlations between local abiotic factors and some quantitative parameters of mites.

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KAHRIZES (QANAT’S) ARE THE SOURCES OF ECOLOGICALLY CLEAN AND STABLE WATER

A. Guliyev

Abstract: Kahriz (underground water-supply, qanat’s) systems as a yield of ancient experience and engineering are the most reliable water sources for arid-climate zones. With their unique structure kahrizes are the perfect forms of drainage systems being applied in melioration. They are quite various in terms of nutrition sources and morphometric dimensions, and related with the sciences of contemporary hydrogeology, geomorphology, engineering geology, soil mechanics, soil and etc. At present, in the period of climate change over the world, the restoration of kahrizes and the surveillance over their ecological situation is one of the urgent issues in the Eastern countries which need rather artificial irrigation.

Keywords: kahriz, qanat, well, water systems ecology

INTRODUCTION
Disasters, climate change occurring in the world, as well as reducing the supply of drinking water and the change in the environment encourage us to be vigilant and to use efficiently natural resources.

The Republic of Azerbaijan has always suffered the problem of water. The rivers Kur and Araz being important for the republic in water delivery are exposed to pollution by Armenians and thus the drinking water can only be used from local springs and qanats (drainage gallery). During the summer, small rivers used for irrigation in the lower reaches get dry and therefore in the lower areas their watercourse has no drop of water. There is hope only by groundwater.

The underground water flow has long been used by the population both for water supply and for irrigation in the form of qanat (kariz). The latter is confined to water-bearing formations and includes underground water pipes. The kariz (qanat), as already mentioned is a type of artificial use of subterranean water, the aim of which is a catchment of groundwaters, taking out them to the Earth’s surface by natural flow for irrigation and water supply. They are widespread in the eastern parts of the world where there are shortcomings in drinking and irrigation waters. History of qanats goes to the high antiquity, having not less than about 5-6 thousand years.

Qanats are very harmless system of nature and have no negative effect on the ecosystem. In arid regions they are the most effective sources of water. In recent years, the attention of many scientists and regulatory bodies in the world to qanats shows that they are the most reliable reclamation system for the future.

MATERIAL AND METHODS
Azerbaijan located on the caravan route linking the north, east and west has attracted the attention of historians, geographers, merchants and travellers over the centuries by geographic location, strategic position, surface and underground riches. This region has witnessed many historical events. Already in the second century, the great Greek geographer Claudius Ptolemy in his work "Geography" talked about the history of Azerbaijan (Nakhchivan), the great Italian composer Virgil wrote the song about the beauty of Araz, the Arab scholars Abudzhafar al-Tabari, al-Balazuri, Ibn Hordabek, the French traveller B. Rubruck, the Venetian traveller Marco Polo, the German traveller A. Olearius and historian of Amir Timur Sharafaddin Ali Yazdi, the Turkic traveller E. Chelebi and others gave rich information about Azerbaijan.

Water systems in Azerbaijan, its qanats and monuments on them reflect the historical past of the people, lifestyle, ethnogenesis and are of great importance to the study of ethnography.

In regard to engineering and reclamation, the qanat systems are poorly studied.
There were started to researches of development, working principle, structure, construction and quality, ecologic and economic assessment of qanats in Azerbaijan based on fundamental scientific principle.

There were firstly conducted studies on the geographical distribution of qanats, chemical composition and environmental condition. The study of ancient water systems has the scientific and practical importance.

The field evidence concerning the sound management of qanat water affords an opportunity to restore many of disabled ancient qanats.

On the other hand, studying of ecological state of qanat waters on the basis of sanitary standards provides an opportunity to use them daily round and in irrigation of crops in future.

Analysis of assessment of qanat waters was carried out on the basis of norms of sanitary and hygienic point of view.

In soils where irrigation is carried out by qanat waters there were studied the pedogenenic processes that is of great scientific and practical importance.

This paper was carried out based on field studies, visual observations, and laboratory analysis.

The results of investigation in areas where qanats are distributed make it possible to determine the rise and fall of groundwater level.

RESULTS AND DISCUSSION

According to the data provided by International Centre of kariz (qanats) (qanat) in UNESCO the amount of used groundwater by means of qanats is approximately 8.4 billion m$^3$ of 34255 qanats in Iran within a year, in Afghanistan 1.77 billion m$^3$ from 5984 qanats, in Pakistan 0.510 billion m$^3$ of 841 qanats, in Oman 0.460 billion m$^3$ of 3108 qanats, in Azerbaijan 0.419 billion m$^3$ of 885 qanats. Currently, more than 40 countries use qanats.

Qanat is an artificial drainage, which serves for lowering of level of the groundwater in the sloping plains. Using the system does not lead to waterlogging and salinity. In qanat all harmful salts are dissolved and washing down are removed. In case of irrigation by means of qanat waters, the flora and fauna is developing normally, because the temperature in qanat water conforms to the norm, furthermore it contains nutrient elements.

In subartesian groundwater the temperature is considerably lower and their composition is poor in nutrients. Advantage of qanat system over the subartesian wells is in economic benefits – in qanats water flows by gravity without using extra energy and mechanisms but the subartesian wells cannot do without them.

Using the qanat waters for irrigation has a peculiar effect on soil formation, which is very poorly informed with modern researches in arid zones.

The qanats enabled only in specific circumstances to develop irrigation. Since the lead-in channels of qanats were very short or did not exist, as a consequence of this the seepage losses are reducing.

Nutrition of qanat systemz is carried through various methods commencing from underground tributaries of overlying areas, from the evacuation cone of rivers, infiltration waters of irrigation canals and reservoirs etc.

The ancient qanats had peculiar engineering facilities located just above the water-resistant or aquitard aquiclude, consisting of medium and heavy indigenous clay or clay loam.

The extensive network of qanat systems in areas where qanat waters are used for irrigation canals and irrigation ditches represent peculiar drainage system.

The qanat soils differ significantly from the natural soils and their predecessors. Under influence of qanat water for irrigation and by impact of irrigable crops, the water,
thermal and biological regime of soils is changed that causes modifications in their upper layers. Natural features of the original soil type are preserved only in the upper horizon of soil profile. These soils are called gray soils irrigated by qanats etc.

These are essentially anthropic soils and emerged under the influence of centuries-long qanat irrigation on fine-grained cultural-irrigation alluviations, laid out from qanat waters, by which the irrigation is carried out. The annual layering of qanat irrigation sediments (up to 0.1-0.4 mm/year) resulted in strengthening their capacity to 0.3-0.8 m and more (in some cases up to 1 m), and natural soils were buried under them.

The below specified features are typical for old irrigated qanat soils:

1) Low humus content (0.8-1.5%);
2) Uniform laying-down and homogeneous medium-loamy texture;
3) High saturation (up to 1 m and deeper) of the active microflora;
4) Availability of carbonate and gypsum beds in an area where the influence of sea-lagoon sediments is met in the process of soil formation;

The qanat waters were ecologically clean water sources up to 1950 years. Subsequently, the construction of cities, population movements and the use of reservoirs and subartesian wells made qanats somehow less important. Even in some places they have been completely abandoned.

At present, during the period of climate change all over the world, there was started to the reconstruction of qanats and observations on their environmental conditions. This is one of the important tasks in solving the water problems in Eastern countries, which need artificial irrigation.

CONCLUSIONS AND FUTURE WORK

In modern conditions, causing qanats to be out of service there is set in inadequate drainage outflow, even by immoderate water application, the underground waters are raised, leading to salinization or waterlogging. Either of them reduces sharply the soil fertility. Soils subjected to salinization, which are already in various stages of salt marshes, are particularly common at the periphery of irrigated land where formerly there were operated qanats.

It has been established that the increase of mineralization of qanat waters corresponds to the maximum stand of level of water in reservoirs and the period of intensive irrigation of newly reclaimed land. This phenomenon can be explained by the direct participation of rock salt dissolved with infiltration, irrigation, and seepage waters of reservoirs and channels.

The findings of investigation show that the influence of anthropogenic activities in Azerbaijan, particularly commencing from 1950s, the carried out drainage and irrigation works have led to a change in salinity in water administration of qanats.

The findings of investigation enabled us to prepare the theoretical and experimental methods for restoration of ecological condition of qanats and there were introduced some systems of activities.

The findings of research can be used in research institutions, as well as in other countries (Kazakhstan, Uzbekistan, Turkmenistan, Turkey, Iran, etc.) where there are qanats.

The qanat after being repaired once runs continuously and does not require significant expenditures, and in subartesian wells there are required constant repairs, depreciation costs, and electricity.

Restoration of qanat system will allow also reviving the ancient agriculture in the region (wine growing, melon-growing) based on ancient technologies and production of ecologically clean products. Thus, there is recreated the ancient cultural landscape in its natural form.
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WATER HARVESTING IN RURAL MICRO-WATERSHEDS

C. GÖL, S. EDİŞ, H. YILMAZ, H. ACAR

Abstract: Water harvesting is extra rainfall water’s accumulation in surface or subsurface storage which are seen in rainy period and presented this water for use in the arid period. In semi-arid regions water harvesting is used for specific purposes with the rainwater collecting. In this study water harvesting methods and techniques which are aimed to use rain water more effective and efficient in arid and semi-arid areas are discussed. Water harvesting is the process of intercepting storm water runoff from a surface and putting it to beneficial use. In term of basic looking water harvesting techniques are used in practice for urban and rural ecosystems. We need to recognize that the source of all water on earth is not the river, is not the underground aquifer, is not the lake, well or stream. Rain is the source of all water.

Key words: Water Harvesting, Drought, Watershed

INTRODUCTION

Water crisis will be lived in Turkey as other countries of the world in the future. In these countries problem is, besides the lack of quantity and quality of water resources, they aren’t used with in a suitable way. The solution is efficient and sustainable use of resources. Many methods are being done for water resources development and technical work. One of these methods is water harvesting. Water harvesting is containing important and feasible methods in terms of more efficient usage of water in arid and semi-arid watersheds. In this study applicable water harvesting techniques in arid and semi arid micro watersheds are examined which have not cost huge economic standards.

Different methods of water harvesting as applied on the Earth, classifications and nomenclature of it may vary locally. If these techniques considered as ecosystem-based, different two types of water harvesting can be reported including rural and urban ecosystems. The main purpose of water harvesting is utilizing the highest level of inadequate water supplies.

Water Harvesting in Micro-watershed

Micro catchment water harvesting method is applied for trees (200mm annual precipitation) and for one year plants (300 mm annual precipitation) [1]. The goals of these techniques are collecting storm water in localized basins served by small watersheds. After heavy rainfall, micro catchment should be checked for evidence of overflow. If needed, shallow spillway could be built on the berm to control the outlet of future large flows from the microbasin. Micro catchments can be constructed in a range of sizes to support single or multiple plants with different water needs [2].

Spillways

Spillways could offsetted on micro catchments to create a longer water flow path to encourage more infiltration into the soil. These are appropriate for moderate flows [2]. May need more erosion control measures for overflow areas (Figure 1a).

Microbasin on contour

For second variety micro catchments should be arranged to intercept water running off a ridge. Micro catchments will follow the shape of the contour line around a ridge with the upslope ends of basins at the same elevation (Figure 1b) [2].
Depression holes
In the birch technique for micro catchments, gentle localized depressions could be constructed without constructing associated berms (Figure 2a). They could be made without huge costs.

Negarim microcatchments
They are small diamond-shaped basins, surrounded by low earth bunds. The runoff infiltrates at the lowest point, where the trees are planted (Figure. 2b) [3]. Negarim micro catchments are diamond-shaped basins surrounded by small earth bunds with an infiltration pit in the lowest corner of each. Runoff is collected from within the basin and stored in the infiltration pit. Micro catchments are mainly used for growing trees or bushes. This technique is appropriate for small-scale tree planting in any area which has a moisture deficit. Besides harvesting water for the trees, it simultaneously conserves soil. Negarim micro catchments are neat and precise, and relatively easy to construct [4].

Meskat systems
Meskat systems are suitable for areas with 200-400mm annual rainfall and slopes between 2-15%. The system consists of a catchment area called "meskat", of about 500 m² in size, and a cropping area called "manka" of about 250 m². The entire meskat system is surrounded by a 20 cm high bund, equipped with a spillway to let the runoff flow into the manka plots and surplus water to leave the manka (Figure. 3a). The meskat system is a micro catchment technique which is only used for tree cropping, covering around 300.000 ha in Tunisia, where mainly olive trees are cultivated in the manka plots [3].
Mulch
The goal of this technique is optimizing water harvesting by reducing evaporation from soils. This technique is used in two types, organic and inorganic mulches. Organic mulch has a tendency to float, so make sure basins are deep enough to retain mulch during heavy rainfall events. Inorganic mulches include rock, gravel, cobbles, and decorative rock and other hard materials. So the top layer of inorganic mulch will become hot in summer and might damage heat sensitive plants (Figure 3b).

Organic mulch breaks down with time forming rich soil, and should be replenished as needed. With time, seeds, leaves and twigs dropping from plants in the basins will create a renewable mulch layer (self mulching). Additional mulch can be added if the level of self-mulching is not sufficient. Both inorganic and organic mulch should be periodically maintained to keep soils covered.

Figure 3. a- Meskat system, b- Types of mulches [3].

Hillslope microcatchments
Hillslope micro catchments are micro basins of rectangular shape to supply single trees or bushes with sufficient water. The catchment size is 5-50 m² and the cropping area 1-5 m². This technique can be applied on slopes of 1-50% inclination; normally there are no bunds around the plot. Regular hoeing of the infiltration basin is a prerequisite for the functioning of the system. Hillslope micro catchments can be applied in areas of 200-600 mm annual rainfall (Figure 4) [3].

Figure 4. Hillslope microcatchments
Swales on Contour

Build swales and berms along contour lines within the confines of a gently sloped drainage way. Include overflow spillways to allow excess stormwater to flow downslope (Figure 5a). The advantage of swales is that you can also plant a food forest system on the lower mound side where the water will gradually seep away. A Food Forest will build you a canopy of trees, reintroduce native flora and fauna and generally stabilize fragile eco-systems [7].

Swales off Contour

An off-contour swale is constructed at a slight angle from the contour line. As shown in the illustration, the swales and berms are aligned slightly off parallel with the contour lines. Off-contour swales convey stormwater slowly downslope in a controlled manner to maximize infiltration, support vegetation, control erosion, reduce stormwater flow velocity, and eventually discharge any excess stormwater to a safe location. There are some several variations of off-contour swales.

Where roads and paths slope downhill, install a “water bar” (a very gently sloped berm) across the travel surface to intercept stormwater that might cause erosion (Figure 5a). The water bar should be constructed slightly off-contour to direct water toward an adjacent depression. Extend the water bar to create a hookshaped berm enclosing a basin next to the path. If the watershed area is relatively large, an overflow spillway can be constructed in the hooked portion of the berm to route excess water away from the path. Make sure the water bar is very gently rounded to avoid creating a tripping hazard on pathways.

Endpoints of these arcshaped swales point uphill, and are the low points of the berm. After a swale fills, overflow occurs at one or both end points. Protect end points from erosion using rip rap or other erosion-control approach. Boomerang swales can be installed individually or in a series [2].

CONCLUSIONS

Water must be used most effectively and efficiently in arid and semi arid ecosystems. In these ecosystems, it is not sufficient that both following stream and stored groundwater for managing this resources. Increasing water demand and drought has revealed a new water resources development. At this point water harvesting techniques has gained a importance because of un storage water which is unviable in rainy period. Besides the ecological effects of water harvesting with social and economic effects by reducing the effects of drought on rural areas, water harvesting is drawing an attention. More water,
more and more products mean more revenue. Water harvesting relates many other subjects as rural poverty, migration and socio-economic problems.

Various researches conducted worldwide show that in arid and semi-arid regions more efficient plants have grown with water harvesting techniques. According to the results of this research soil erosion is decreasing, ground water is protected, and also feeding of groundwater is to be the case. Water harvesting, especially in dry areas, at forestry, agriculture, fruit growing, grazing and be beneficial in various sectors such as drinking water needs. Key point is determinated of water production capacity of the ecosystem and appropriate harvesting technique. Water harvesting techniques should be applied in arid regions with public awareness, supporting and training way.

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SPECIFIC CHARACTER OF CREATION THE CHEMICAL ELEMENTS CONTENT OF THE BIOSPHERE

Vyacheslav Korzh

Abstract: It is for the first time that the general regularities for redistribution of average elemental compositions in the biosphere between solid and liquid phases (i.e., within the lithosphere – hydrosphere system) have been found. This process is most active in biogeochemical barriers, i.e. in the localities of "concentrated life" and runs under some nonlinear principle that has not been known earlier.

Key words: environmental protection, biosphere, lithosphere, hydrosphere, elemental composition, evolution, nonlinearity

INTRODUCTION

Global strategy of environmental protection is connected not only with the scale of technogenic influence. The task of revealing “vulnerable points” in complex natural systems becomes one of the most important problems for today. Complex natural systems may be destroyed even in case of slight influence on such points. So, the environmental protection needs the knowledge of regularities and evolution of systems’ vital activity.

The key point of investigation of the specificity of the biosphere elemental composition formation is determination of patterns of redistribution of elemental average concentrations among various phases, like solid - liquid - gaseous (i.e., the lithosphere – the hydrosphere - the atmosphere), which occurs as a result of a global continuous processing of inert matter by living material. Our task here is to investigate this process in the system “lithosphere – hydrosphere” in view of the integrated involvement of living material in it. It is also necessary to take into consideration here that the differences existing between protolithospheric and biospheric elemental compositions can be only found if the regularities in the evolution of the biosphere elemental compositions are determined.

Last years some researches into an evolution of geological processes in the Earth history, an evolution of sea sedimentations and basic sediments [2] and a chemical evolution of the hydrosphere have been reported. This work focused on research of the evolution of elemental composition of the biosphere (namely, the lithosphere and the hydrosphere) is the first attempt to solve the above problem using modern methodological principles [3].

RESULTS AND DISCUSSION

We consider the elemental composition as a comprehensive whole possessing its specific organization level and emergence characteristics, i.e. an irreducibility of the system features to a number of properties of its consistent elements. When passing through the living material, the inert matter changes towards an increase in its order level (i.e., reduction of chaos) and formation of a new structure, combined interrelations of the elements within the system playing a special role in this process. Hence, the used methodology suggests some subordination of the system elements to the general laws of the system evolution.

A.P. Vinogradov has published some data for the contents of chemical elements in the major types of the Earth’s crust rocks and in stony meteorites [4] which underlie our constructions. The graphic presentation of the comparisons of stony meteorites and the lithosphere average elemental compositions as shown in Fig. 1 provides determination of the following groups of elements: \( C_{\text{met}} > C_{\text{lith}} \) (contains only 11 elements), \( C_{\text{met}} \sim C_{\text{lith}} \) (18 elements) and \( C_{\text{met}} < C_{\text{lith}} \) (42 elements).
The correlation coefficient between logarithmically expressed element concentrations for the 71 studied chemical elements in proto planetary material and in the lithosphere is equal to 0.87. The tangent for the regression line inclination is equal to 0.75. This tangent numerical value is a quantitative estimation of the nonlinearity factor for the elemental composition general evolution within the system "proto lithosphere - living material - biosphere".

![Graph showing element concentrations in chondrites and the lithosphere](image)

Fig.1. Ratios for element concentrations in chondrites and in the lithosphere.

The established pattern provides quantitative estimations of the trends in processing of inert matter with living materials on the surface of the Earth. This process leads to a general relative raise of chemical element concentrations in the solid phase according as the element prevalence in the environment drops. Therefore, translation of the chemical elements from more soluble compounds to some less soluble ones is a common & specific reaction of the "living matter" to the deficiency in their concentrations as seen in the habitat.

The new approach to the lithosphere elemental composition research has enabled to explain the regularity discovered by A.P. Vinogradov [4]: the isotopes present in an isotopic mixture in smaller concentrations are in general more actively "collected in the rocks lying closer to the surface of the Earth". The elemental and isotopic compositions within the surface of the lithosphere naturally differ from those in its deeper strata. It was specified by V.I. Vernadsky in his work "On the geochemical balance of the biosphere". As far as the level of scrutiny for the elemental composition of the surface stratum of the lithosphere considerably surpasses our knowledge of its deeper strata, we have every reason to suggest that the lithosphere average elemental composition presented by Vinogradov [4] basically correlates with the biosphere average composition.

When studying mechanisms of the formation of the hydrosphere elemental composition, we take advantage of a cybernetic approach, the same way as with the lithosphere research. Shelving the point regarding the processes occurring in separate parts of the system, we only use the concepts "input" and "output". We regard the ocean as a complex system where transformations of the material that is received through a
geochemical barrier “river - sea” (the input) provide average elemental composition of the ocean (the output) [5, 6].

The diagram for the dependence of average elemental composition of ocean & river waters is presented in Fig. 2. Assuming the quantity of the studied elements is equal to 64, the correlation coefficient is equal to 0.94. The line corresponding to the equation as obtained by the linear regression method makes up angle of 34° with the abscissa axis, \( \tan \alpha = 0.67 \). The fact that all the elements are located on one side of the line (or on the line precisely), forming an angle of tangent equal to 0.70 with the abscissa axis, also deserves attention.

![Fig. 2. Ratios of average concentrations for elements in ocean and river flow.](image)

The line running through Cl, Na, B, Br, Sr, Li, Rb, Cs, Mo, U, W, Tl, Re and Au was found to combine elements that considerably differ in their chemical and physical features through the general pattern of the material transformation & migration on the geochemical barrier “ocean-atmosphere” [5, 6], the characteristic feature of the elements located on this line being the equilibrium of their flows within the “ocean – continent - ocean” system. It is described by the following equation:

\[
C_{A(\text{rivers})} = 10^{-3.4} C_{A(\text{ocean})}^{0.7}
\]

The actual equality of the tangent for the line inclination angle as obtained by the linear regression method and the tangent ratio for the line reflecting the pattern of material transformation & migration in the barrier “ocean – atmosphere” (Fig. 2), indicates the equality of constants for nonlinearity of the average elemental compositions redistribution processes as occurring between the dissolved and solid phases in absolutely different geochemical barriers “ocean - atmosphere” and “river - sea”. The basic generality of geochemical barriers is that they are the places of “the live condensation”. Regression analysis has allowed us to reveal the general tendency of relative increase in microelement concentration indices for the solid phase (and their respective drop in solution) as a result of the inert matter processing by living material both in the lithosphere and in the geochemical barriers like “ocean - atmosphere” and “river - sea”. The general nonlinear character of the dependence of average elemental compositions redistribution processes between the dissolved and solid fractions in the matter that is received through
barsis lithosphere – “living material” – hydrosphere, river – “living material” – sea and ocean – “living material” – atmosphere on their initial concentrations which we have found suggests the following important conclusion. Biogeochemical behaviour of elements existing in micro concentrations in the nature does not generally repeat their behaviour of their chemical analogues that are present in the lithosphere and the hydrosphere in macro concentrations. It is especially significant to consider when solving any environmental problems and also when radioactive isotope behaviour in the biosphere is investigated [7].

CONCLUSIONS

Thus, the general patterns of average elemental compositions redistribution in the biosphere between solid and liquid phases (i.e., lithosphere - hydrosphere) have been determined for the first time. This process is most active in biogeochemical barriers, i.e. in places of “the life condensation” and runs under a nonlinear regularity that has been unknown before.

It is established that this process results in a general relative increase in concentrations of chemical elements in the solid phase in proportion as their prevalence in the environment is reduced. This process running in various natural systems has practically the same parameter of nonlinearity (v) approximately equal to 0.7 (for proto lithosphere – “living material” - sedimentaries system v = 0.75; for river – “living material” – ocean system v = 0.67 and for ocean – “living material” – atmosphere v = 0.7). For the contemporary factual awareness level these estimations of nonlinearity indices are practically negligible. Hence, it is for the first time that the existence of a universal constant of nonlinearity of elemental composition evolution in the biosphere has been proved and its quantitative evaluation has been made.

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SEASONAL VARIATIONS OF PHOSPHATES CONTENTS IN THE GROUNDWATERS OF STARA ZAGORA REGION

Zvezdelina Yaneva, Nedyalka Georgieva*

Abstract: The aim of the present study was qualitative and quantitative evaluation of the seasonal variations of phosphates contents in the groundwaters of Stara Zagora Region during 2010. The latter was provoked as by the deteriorated ecological situation in Stara Zagora Region during the last 8 years, so by the increased phosphorus loading of natural waters worldwide which has become a severe problem since 1960s. The research goal was accomplished by detailed spectrophotometric analyses of $\text{PO}_4^{3-}$ concentrations in real groundwater samples taken from four municipalities (Stara Zagora, Kazanlak, Chirpan, Gurkovo) in the stated region during 2010; comparative estimation of the data obtained from the standpoint of Bulgarian and international water quality standards and statistical interpretation of the results. The statistical significance of the experimental data was also tested. The groundwater monitoring established elevated levels of phosphate pollution in the well waters of Gurkovo City and Cherganovo Village. The measured concentrations surpassed approximately 5 - 10 times Bulgarian groundwater quality standard (0.5 mg/L).

Key words: Phosphates, Groundwaters, Spectrophotometry.

INTRODUCTION

Groundwater is an important water resource whose mode of storage is controlled by hydrology and geologic structure. Its supply is restricted by atmospheric precipitation and the surface water percolation in addition to human activities related to groundwater development and utilization [1].

Over the last several decades, agricultural sources of phosphate (P) have been suggested as a contributing factor to water quality degradation [2]. Although industrial wastes, municipal wastes, and urban runoff contribute to P loading in certain watersheds, P applied directly to cropland through fertilizers and animal wastes can be contaminant sources of P in watersheds with predominately agricultural land uses. Whereas fertilizer P use has increased slightly in the last decade, substantial growth of confined animal feeding operations has occurred [3].

Phosphorus is an important constituent of domestic and agricultural wastewater. The natural capacity of subsurface soil and rock materials to attenuate the mobility of phosphate is limited. Although P is strongly buffered in soils, there is evidence that losses may become significant once a threshold concentration is reached [4]. Thus, phosphate can be a mobile contaminant in groundwater. This mobility is a particular concern where domestic wastewater or agricultural organic-waste systems are located in close proximity to surface water. Groundwater discharge or surface runoff can contribute phosphate loading to the receiving surface water. Unlike nitrate ($\text{NO}_3^-$) which can be converted to N-gases by denitrification, phosphorus removal by soil retention and biotic uptake results in accumulation within the system [5]. Besides, dissolved inorganic phosphorus interacts strongly with sediments and is involved in various precipitation/dissolution reactions, e.g. with calcium carbonate/phosphate and iron/aluminium oxide minerals [6]. Retained P may be released later from vegetation and soils as dissolved P and exported to streams by discharged groundwater [5]. In systems where distinct point-sources can be identified, little is currently known about how river bed-sediments respond to enhanced concentrations in the overlying water and whether such interactions act as a short or long-term sink of phosphorus [6].

In addition, diffuse P sources in the catchments reflect seasonally different agricultural practices. For example, manure is mainly exposed in spring, and plant
coverage is generally low in autumn and winter, thus exposing soils to surface erosion. Overall in rivers, particulate and dissolved P forms are prone to both, intrinsic and extrinsic mechanisms, which cause differences in P concentrations and loads [7]. Phosphate is typically the limiting nutrient present in surface water. Increased inputs of phosphate can increase the growth of algae and other plants and upset the ecological balance of aquatic habitats due to eutrophication [8,9].

The investigations of Neal et al. revealed that focussed remediation is needed to target the situations where aquatic biology is at risk as: the dilution of effluent is particularly low and high phosphorus concentrations occur during the critical spring and summer low flow periods; i.e. when dilution potential is at its lowest and biological activity is at its highest; phosphorus release from contaminated sediments may sometimes be significant for the water column and the contaminated bed sediments themselves may adversely affect the riverbed ecosystem; biological feedback mechanisms adversely affect the ecosystem functioning [10].

The aim of the present study was qualitative and quantitative evaluation of the seasonal variations of phosphates contents in the groundwater s of Stara Zagora Region during 2010. To accomplish the latter phosphate concentrations in real groundwater samples were spectrophotometrically determined; the experimental data was interpreted from the viewpoint of the national and international water quality standards regarding the parameter $PO_4^{3-}$.

**MATERIAL AND METHODS**

As statistically representative samples of the surface and groundwater quality in Stara Zagora Region, Bulgaria, 12 sampling points from four major municipalities, namely: Stara Zagora, Kazanlak, Chirpan and Gurkovo, were chosen for the present study (Table 1).

<table>
<thead>
<tr>
<th>No</th>
<th>Municipality</th>
<th>Sampling Point (s.p.)</th>
<th>Code</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bratya Kunchevi Village - well</td>
<td>302</td>
<td>N 42 29.837° E 25 51.104°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kolyo Ganchevo - well</td>
<td>303</td>
<td>N 42 24.522° E 25 39.030°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulitza Village - well</td>
<td>304</td>
<td>N 42 26.180° E 25 28.775°</td>
</tr>
<tr>
<td>2.</td>
<td>Chirpan</td>
<td>Tekirska River – well (before Chirpan City)</td>
<td>305</td>
<td>N 42 12.686° E 25 19.638°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gita Village - shaft well in a drinking water pumping station</td>
<td>306</td>
<td>N 42 13.816° E 25 24.859°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drinking water drilling (around Malak Yurt Reservoir)</td>
<td>307</td>
<td>N 42 18.057° E 25 18.980°</td>
</tr>
<tr>
<td>3.</td>
<td>Kazanlak</td>
<td>Sheinovo Village - well</td>
<td>308</td>
<td>N 42 68.573° E 25 31.883°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cherganovo Village - well</td>
<td>309</td>
<td>N 42 58.489° E 25 47.041°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Razhena Village - well</td>
<td>310</td>
<td>N 42 53.376° E 25 49.580°</td>
</tr>
<tr>
<td>4.</td>
<td>Gurkovo</td>
<td>Gurkovo City - well</td>
<td>311</td>
<td>N 42 39.699° E 25 47.807°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roadside catchment fountain near Gurkovo City</td>
<td>312</td>
<td>N 42 30.496° E 25 32.164°</td>
</tr>
</tbody>
</table>

The samples were taken and the analyses were conducted during the period December, 2009 - October, 2010. All investigations were performed in triplicate. The concentrations of phosphate ($PO_4^{3-}$; $PO_4^{2-}$-P) were determined on UV/VIS Spectrophotometer DR 5000 (Hach Lange, Germany) using LCK 349 (0.05-1.50 mg/L $PO_4^{3-}$-P; 0.15-4.50 mg/L $PO_4^{3-}$; 0.15-3.5 mg/L $P_2O_5$) cuvette test (Hach Lange, Germany) at $\lambda$ 850 nm, temperature (T) 15-25°C and pH 7-9. The principle of the determination is based on the reaction of phosphate ions with molybdate and antimony ions in an acidic solution and the formation of antimonyl phosphomolybdate complex, which is reduced by
ascorbic acid to phosphomolybdenum blue. The precision and accuracy of the results were regularly checked with the analytical quality assurance system LCA Addista (LCA 704, LCA 700, Hach Lange, Germany).

The statistical significance of the results was tested on the basis of the Standard Deviation (SD, %) values calculated by the Student’s t-test.

RESULTS AND DISCUSSION

The spectrophotometrically determined monthly phosphates concentrations in real groundwater samples taken from 12 sampling points situated in Stara Zagora Region were presented on Fig. 1, Fig. 2. The analyses of the experimental data and the conclusions for the quality of the tested groundwaters were made on the basis of the quality standard - 0.50 mg/L PO$_4^{3-}$ postulated by the Bulgarian regulations on the exploration, use and protection of groundwater and on the quality of water intended for human consumption [11,12]. Obviously, the phosphate concentrations in 64 % of the groundwaters from the three village wells (s.p. 308, s.p. 309, s.p. 310) in Kazanlak Municipality exceeded the regulated standard with approximately 1.2-6 times (Fig. 1).

![Fig. 1. Phosphate concentrations in the groundwaters from Kazanlak Municipality during the period December 2009 - October 2010.](image)

Although Stara Zagora Municipality is the largest and most populated district in the region studied, the measured phosphate concentrations in the groundwater samples taken from that area were definitely lower and even far below the quality standards in s.p. 302, 303 and 304 during the entire monitoring period (Fig. 2). Thus, the waters from the latter three groundwater sources could be regarded as clean. A clearly outlined tendency of a rapid increase of PO$_4^{3-}$ concentrations (from 0.07 to 0.853 mg/L) in the samples from s.p. 301 during the spring and autumn was observed (Fig. 2). The above trend could probably be attributed on the one hand to the greater extend of dilution during the winter and on the other - to the more intensified biological activity during the warmer year seasons. Besides, the highest registered PO$_4^{3-}$ concentrations in the well waters from Novo selo Village (s.p. 301) surpassed with approximately 1.7 times the quality limit.
According to the data obtained, however, the groundwater sources in Gurkovo Municipality (s.p. 311 and 312) outlined as the “hot spots” regarding phosphate pollution as the measured concentrations during the spring and autumn months were more than 10 times greater than the standards cited (Fig. 2). Moreover, the observed situation became even more disturbing, considering their nature, namely a well in Gurkovo City and a catchment fountain near the town. Thus, prompt actions of controlling and restricting the probable sources of phosphates in this area have to be taken.

The measured phosphate concentrations in the groundwater samples from Chirpan Municipality (s.p. 305, 306, 307) were in the range 0.02 - 0.19 mg/L, i.e. far below the guideline value during the entire stated period (Fig. 2).

Consequently, the comparative estimation of the results obtained in the present research proved that the national quality standard regarding the parameter phosphates was exceeded in 50 % of the examined groundwaters.

The calculated SD values, presented in Table 2, proved the statistical significance of the results obtained.

### Table 2. Standard deviation (SD) values regarding PO$_4^{3-}$ concentrations in the groundwaters of Stara Zagora Region.

<table>
<thead>
<tr>
<th>s.p.</th>
<th>301</th>
<th>302</th>
<th>303</th>
<th>304</th>
<th>305</th>
<th>306</th>
<th>307</th>
<th>308</th>
<th>309</th>
<th>310</th>
<th>311</th>
<th>312</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>0.26</td>
<td>0.00</td>
<td>0.02</td>
<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.17</td>
<td>0.67</td>
<td>0.93</td>
<td>1.54</td>
<td>1.70</td>
</tr>
</tbody>
</table>

Impartial conclusions and a complex assessment of the current state of the groundwater quality in Stara Zagora Region during the monitoring period, as well as identification of the primary pollution sources, could be withdrawn after finalization of the integrated water monitoring, which is a part of a significant research subjected to assessment, reduction and prevention of air, water and soil pollution in Stara Zagora Region.

### CONCLUSIONS AND FUTURE WORK

The experimental data obtained in the present research proved that the quality groundwater standard (0.5 mg/L) regarding PO$_4^{3-}$ was surpassed with 1.2 - 10 times in 50 % of the examined groundwaters during December 2009 - October 2010. Thus,
phosphates could be accepted as a priority pollutant of the groundwater resources situated predominantly in Kazanlak and Gurkovo Municipalities, Stara Zagora Region.

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SOIL EROSION FACTORS AND THE SITUATION WITHIN A SMALL CATCHMENT (WESTERN SERBIA)

G. Šekularac and D. Stojiljković

Abstract: Mean annual and total specific erosion-induced sediment yields in the Babović Brook catchment are induced by both natural and anthropogenic factors. The catchment erosion factors evaluated in this study, including relief, geological substrate, soil, climate and vegetative cover, have contributed to the annual erosion intensity of 78.56 m³ km⁻² of soil in the catchment area of the Babović Brook, classified as a small torrential brook and a dry watercourse.

Key words: Factors, Erosion Intensity, Soil, Catchment.

INTRODUCTION

There are different erosion factors that induce changes in both soil and geological substrate, resulting in devastation or complete disappearance of soils. As soil changes can be either slow or fast, erosion can be characterised as either a slow or rapid process.

Erosion-induced soil losses can be predicted by the Universal Soil Loss Equation (USLE) and the Revised Universal Soil Loss Equation (RUSLE).

Above 90% of the total land in the Republic of Serbia suffers from different types and intensities of erosion [3].

The erosion process can have both direct and indirect impacts, inducing permanent soil disappearance. The calculated value of the total annual sediment yield suggests that some 16.0 cm of soil are annually eroded off the 21,000 ha of land in Serbia [6].

An increasing tendency of air temperature and a decreasing tendency of rainfall are quite evident in the region of Čačak [9]. Such climate changes lead to the following: deterioration in soil physical properties, increased soil erodibility, a reduced protective role of vegetation, and hampered natural and manmade revegetation.

All of the above cause intensification of both surface and deep-cutting processes of erosion.

Soil erosion is a global concern. Threats to agriculture, forestry and water management as induced by erosion intensification are becoming an increasing problem; hence the growing need to undertake erosion control and soil improvement activities.

Given the above, the objective of this study is to assess quantitatively soil erosion as induced by a number of different factors as well as to estimate sediment yield in one part of the catchment area of the Kamenica River (part of the Zapadna Morava catchment) i.e. its subcatchment, including its first order right tributary the Babović Brook. The Babović Brook is located near Čačak (43° 53’ N; 20° 21’E), Western Serbia, and belongs to the catchment of the Zapadna Morava River.

MATERIAL AND METHODS

The reconnaissance method is used to analyse and present the catchment configuration elements. This basic method is accompanied by the use of different scale topographic, geological and pedological maps that enable the definition of the traits and impacts of natural agents on the erosion of the catchment concerned.

Meteorological parameters for the catchment area are calculated using the method of interpolation of rainfall data by the rainfall gradient [1], and air temperature calculations for any altitude [2].

The quantitative indicators of soil erosion are calculated using the analytical method [4].
RESULTS AND DISCUSSION

The size, length, circumference and shape of the catchment area are among major catchment elements of importance for the soil erosion process.

The Babovič Brook catchment is 3.41 km² in area (F), 2.50 km in length (L), and 8.25 km in circumference (C).

Major elements of the Babovič Brook catchment, relief and geological substrate characteristics, soil type and soil utilisation method are parameters that determine quantitatively the soil erosion process in the catchment (Tab. 1).

Table 1. Major relief parameters of the Babovič Brook catchment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit of measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The lowest point of the main watercourse and catchment</td>
<td>m</td>
<td>445</td>
</tr>
<tr>
<td>The highest point of the main watercourse</td>
<td>m</td>
<td>720</td>
</tr>
<tr>
<td>The highest point of the catchment (E)</td>
<td>m</td>
<td>778</td>
</tr>
<tr>
<td>Average slope of the main watercourse in the catchment (lₐ)</td>
<td>%</td>
<td>8.7</td>
</tr>
<tr>
<td>Mean altitude of the catchment (Aₘ)</td>
<td>m</td>
<td>641.83</td>
</tr>
<tr>
<td>Mean altitudinal difference of the catchment (D)</td>
<td>m</td>
<td>196.83</td>
</tr>
<tr>
<td>Mean slope of the catchment (Iₘ)</td>
<td>%</td>
<td>19.8</td>
</tr>
<tr>
<td>Coefficient of catchment relief erosion energy (Eᵣ)</td>
<td>m km⁻¹/²</td>
<td>77.44</td>
</tr>
</tbody>
</table>

Table 1 lists major relief parameters of the Babovič Brook catchment, with relief playing a primary role in the occurrence of soil erosion. An increase in relief parameter values results in increasing intensity of soil erosion in the catchment.

The mean altitude (Aₘ) of the Babovič Brook is 641.83 m (Tab. 1). The method used involved making contour lines at every 100 m increase in altitude.

The mean altitudinal difference (D) of the Babovič Brook catchment is 196.83 m, being the result of the difference between mean altitude of the catchment and that of the confluence (Tab. 1).

The mean slope (Iₘ) of the Babovič Brook catchment is defined using the vertical distance between contour lines (h) of 100 m; hence the (Iₘ) value of 19.8% (Tab. 1).

Relief of a region can also be determined by the coefficient of relief erosion energy (Eᵣ), the value thereof for the Babovič Brook catchment being 77.44 m km⁻¹/² (Tab. 1).

Geological substrates as another erosion agent also contribute to the erosion process within the Babovič Brook catchment area, Tab. 2.

Erosion resistance of geological substrates is dependent upon their permeability. The geological substrate found in the Babovič Brook catchment is diabase, accounting for 3.41 km² (100.00% of the total catchment area) and showing poor permeability. The water permeability coefficient of the diabase geological substrate (S₁) is 0.76, suggesting non-resistance of the geological substrate to the erosion process, Tab. 2.

As an erosion agent, soil and its properties contribute to a lesser or larger degree to the erosion process. Due to the effect of pedogenetic factors, the soil type covering the Babovič Brook catchment area is brown skeletoid soil on diabase rock. It is classified as shallow soil. The profile of the brown skeletoid soil on diabase is of the A₉–C type. A strong degree of erodibility is found in the brown skletoid soil on diabase [8].

25
Table 2. Geological substrates, coefficient of their water permeability ($S_1$) and their erosion resistance within the Babović Brook catchment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit of measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{ip}$ – Impermeable rocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Diabase</td>
<td>km²</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.00</td>
</tr>
<tr>
<td>Water permeability coefficient of the geological substrate ($S_1$)</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Erosion resistance of the geological substrate</td>
<td>Non-resistant</td>
<td></td>
</tr>
</tbody>
</table>

The most aggressive climate elements inducing and contributing to soil erosion include rainfall, air temperature, and soil temperature (indirectly, through air temperatures). This region has a temperate continental climate. The mean annual rainfall total ($R$) for the Babović Brook catchment is 786.4 mm, and the mean annual air temperature ($t$) is 8.5°C. The data on the rainfall reaching the catchment surface indicates an important role of rainfall as a climate element on soil erosion in the catchment area observed.

The contribution of another soil erosion agent: vegetation, both autochtonous and anthropogenic, and that of the vegetative cover coefficient ($S_2$) are given in Tab. 3.

Table 3. Plant cadastre and vegetative cover coefficient ($S_2$) of the Babović Brook catchment

<table>
<thead>
<tr>
<th>Design</th>
<th>Parameters</th>
<th>Unit of measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_f$</td>
<td>Forests and coppice of good spacing</td>
<td>km²</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>39.88</td>
</tr>
<tr>
<td></td>
<td>Orchards</td>
<td>km²</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>16.13</td>
</tr>
<tr>
<td>$F_g$</td>
<td>Meadows</td>
<td>km²</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>15.25</td>
</tr>
<tr>
<td></td>
<td>Pastures and devastated forests and coppices</td>
<td>km²</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>11.14</td>
</tr>
<tr>
<td>$\Sigma f_g$</td>
<td></td>
<td>km²</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>42.52</td>
</tr>
<tr>
<td>$F_b$</td>
<td>Arable land</td>
<td>km²</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>17.60</td>
</tr>
<tr>
<td></td>
<td>Infertile soil</td>
<td>km²</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>0.00</td>
</tr>
<tr>
<td>$\Sigma f_b$</td>
<td></td>
<td>km²</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>17.60</td>
</tr>
<tr>
<td>Vegetation cover coefficient ($S_2$)</td>
<td></td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

The total area of land under forests and coppice of good spacing ($F_f$) in the Babović Brook catchment is 1.36 km² (39.88%), some 1.45 km² (42.52%) of land are under grass vegetation ($\Sigma F_g$), and 0.60 km² (17.60%) of land are under bare soil ($\Sigma f_b$), which contributes to the protection of the studied area against the erosion process (vegetative cover coefficient, $S_2 = 0.76$), Tab. 3.
The devastating potential of the watercourse can be determined from the hydrographic and hydrologic traits of the region studied. The traits pertaining to the family of the Babović Brook torrent (F) are as follows: F_c: D; V; Z=0.19, meaning that the Babović Brook is a dry watercourse and a small torrential brook (D) classified as class V of erosion category and having an erosion coefficient (Z) of 0.19 (a very low or slight erosion intensity).

The above traits of the erosion factors in the Babović Brook catchment result in sediment production and soil erosion of particular intensity.

The scale of erosion of the Babović Brook catchment is manifested through the mean annual erosion-induced sediment yield, W/yr of 653.41 m^3 yr\(^{-1}\).

The mean annual volume of the total sediment yield (G/yr) reaching the Babović Brook confluence is 267.90 m^3 yr\(^{-1}\), whereas the specific annual total erosion-induced sediment yield reaching the confluence with the Kamenica River (G/yr_sp \(^{-1}\)) is 78.56 m^3 km\(^{-2}\) yr\(^{-1}\). This finding on the very low erosion intensity is comparable to that on the low-intensity erosion of the Grliška River region (Eastern Serbia) of (G/yr_sp \(^{-1}\)) 209.12 m^3 km\(^{-2}\) yr\(^{-1}\) [7]. The said erosion intensity is manifested at the relief erosion energy coefficient of 83.39, erosion coefficient (Z) of 0.36, mean annual rainfall of 665.40 mm, and at an average annual air temperature of 10.7°C, with about 34% of the total catchment area being under forest soil, and the predominating district cambisol accounting for 34.9% of the total upland area.

The above data show that, in view of the annual sediment yield, about 0.33 ha of soil up to 20 cm depth are eroded off the Babović Brook catchment area i.e. about 0.5 t ha\(^{-1}\) of soil is lost annually. The amount of the eroded soil material can be categorised as class I (0-1 t ha\(^{-1}\) yr\(^{-1}\)) of permissible or tolerable erosion [5].

CONCLUSIONS AND FUTURE WORK

The Babović Brook, a dry watercourse and a small torrential brook, has the following specific traits: class V of erosion category and erosion coefficient (Z) of 0.19, which suggests very low or slight intensity of erosion. These and the other soil erosion agents studied in the catchment area have resulted in the mean annual erosion-induced sediment yield of 653.41 m^3 yr\(^{-1}\), and erosion intensity of 78.56 m^3 km\(^{-2}\) yr\(^{-1}\). The results herewith presented show that the region observed is undergoing erosion. Although the erosion observed in this region is slight and lacking intensity, the key agent in the process is the anthropogenic factor that governs soil utilisation and soil conservation and protection from further erosion-induced degradation and disappearance.

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THE BREEDING BIRD SPECIES FROM THE RESERVOIRS AREA OF THE MIDDLE HYDROGRAPHICAL BASIN OF THE ARGEȘ RIVER (ROMANIA)

M. D. Conete, A. Mestecăneanu, and R. Gava

Abstract: The authors made ornithological observations during 2002 - 2010 in the middle basin of the Argeș River, and observed 122 breeding species that are included in 13 orders, 38 families and 83 genera. 95 of them (77.86%) are certainly breeding species and 27 (22.13%) are probably breeding species. The biogeographic origin, the main habitat occupied, and the diet are taking in account. 21 breeding species are listed in Annex I of the Birds Directive.

Key words: Bird species, breeding, Argeș River, Romania, habitat, protection.

INTRODUCTION

The Argeș River is the most important running water from the Argeș County. A series of artificial lakes was built on its course: Vidraru, Oiești, Cerbureni, Zigoneni, Vâlcele, Budeasa, Bascov, Pitești, Golești.

Besides their important hydroenergetic role, these basins have had a significant impact on the landscape, influencing the composition and also the spatial and temporal dynamics of the bird species in the area [3, 4].

The avifauna is relatively diversified because the birds find here optimal living conditions during the whole year.

The lakes are part of the Argeș River Basins (“Lacurile de acumulare de pe Argeș”, ROSPA0062), a site included in the Important Bird Areas Program and in the Nature 2000 Network.

The Important Bird Areas Program is a worldwide effort to identify the most important areas in order to maintain the wild bird populations and to focus on the conservation activities to protect these areas. The Nature 2000 Network, the European Union’s main instrument for nature conservation, is a network of natural or semi-natural areas where both vulnerable plant and animal species and natural habitats should be protected [8].

Data concerning the Romanian biodiversity are still missing at European level [5].

As far as the avifauna is concerned, in order to fill the gaps in the deficient data, efforts have been made in recent years for the more accurate knowledge of biodiversity [3, 4, 6, 7] and for the adoption of effective measures to protect it [8].

MATERIAL AND METHODS

The Argeș River is 344 km long and the surface of its hydrographical basin measures 12,550 square km. In the Argeș County it is 140 km long. It flows from NW toward SE and drains the most part of the southern versant of the Făgăraș Mountains, the Sub-Carpathian Hills, the eastern side of the Getic Piedmont and most of the Romanian Plain. Thus it meets on its way most of the natural landscapes of Romania.

The observations were made during 2003 – 2010 in the middle hydrographical basin of the Argeș River on the artificial lakes: Vâlcele (408 ha), Budeasa (412 ha), Bascov (162 ha), Pitești (122 ha), Golești (649 ha), (Fig. 1).

The site is placed in the hilly area, which is covered mainly with leaf forests, orchards and agricultural crops. The vegetation of the basins is characteristic to the water zones; it is represented by species of the genera: *Ceratophillum*, *Myriophyllum*, *Carex*, *Juncus*, *Phragmites*, *Typha*, *Salix*, *Alnus*, *Populus*, *Rosa*, *Rubus*, etc. The main habitat is represented by the aquatic habitat (the surface of the water and the permanent flooded
rush-bed). The amphibious habitat is represented by the temporary flooded rush-bed, the edge of the forest and the temporarily flooded pastures. The terrestrial habitat is represented by the arborescent, subarborescent vegetation and the agricultural crops.

The fauna is rich. The vertebrates, besides the bird species, are represented by fish (*Esox lucius*, *Abramis brama*, *Cyprinus carpio*, *Perca fluviatilis*, *Leuciscus cephalus*, *Chondrostoma nasus*), amphibians (*Hyla arborea*, *Bombina variegata*, *Bombina bombina*, *Rana ridibunda*, *Rana esculenta*, *Salamandra salamandra*), reptiles (*Emys orbicularis*, *Natrix natrix*, *Natrix tessellata*, *Lacerta viris*, *Anguis fragilis*) and mammals (*Neomys fodiens*, *Apodemus agrarius*, *Arvicola terrestris*, *Lutra lutra*, *Ondatra zibethica*, etc.).

From the physical and chemical point of view, it is first-class water and the climate is temperate-continental [1].

The study was conducted between 2003 and 2010. We used the itinerary and the fixed point observation methods, too [2]. We walked on the banks of every basin in order to observe the avifauna of the entire water surface and of the neighbourhoods. For identification, we utilized 10 x 50 binoculars, a 10 x 45 terrestrial scope and field guides.

**RESULTS AND DISCUSSION**

During the research we recorded 122 breeding bird species, which belong to 13 orders, 38 families and 83 genera (Table 1). 95 of them (77.86%) are certainly breeding species (CB) and 27 (22.13%) are probably breeding species (PB), (Table 1).

**Table 1.** The list of the breeding birds identified in the studied area.

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<tr>
<th>No.</th>
<th>Species</th>
<th>Breeding</th>
<th>Biogeographic origin</th>
<th>Habitat</th>
<th>Phenology</th>
<th>Diet</th>
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RESEARCH PEOPLE AND ACTUAL TASKS ON MULTIDISCIPLINARY SCIENCES
8 – 10 JUNE 2011, LOZENEC, BULGARIA

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<td>E</td>
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<tr>
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<tr>
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<td>Parus palustris</td>
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<td>E</td>
<td>European species</td>
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<tr>
<td>Parus major</td>
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<tr>
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<td>Remiz pendulinus</td>
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<td>Lanias minor</td>
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<td>In</td>
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<tr>
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<td>OA</td>
<td>European species</td>
</tr>
<tr>
<td>Pica pica</td>
<td>Om</td>
<td>Sedentary species</td>
<td>OA</td>
<td>European species</td>
</tr>
<tr>
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<td>OA</td>
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<td>OA</td>
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<tr>
<td>Corvus corone cornix</td>
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<td>OA</td>
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<td>OA</td>
<td>European species</td>
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<td>OA</td>
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<td>Gr</td>
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<td>OA</td>
<td>European species</td>
</tr>
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<td>OA</td>
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</tr>
<tr>
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<td>European species</td>
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<td>OA</td>
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</tr>
<tr>
<td>Miliaria calandra</td>
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<td>OA</td>
<td>European species</td>
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<tr>
<td>Emberiza schoeniclus</td>
<td>Gr</td>
<td>Granivorous species</td>
<td>OA</td>
<td>European species</td>
</tr>
</tbody>
</table>

Legend:
According to the biogeographic origin (Table 1), the bird breeding species are grouped as follows: 71 species (58.19% - *Podiceps nigricollis*, *Ixobrychus minutus*, *Gallinula chloropus*, *Alcedo atthis*, etc.) - European origin (E), 29 species (23.77% - *Falco tinnunculus*, *Fulica atra*, *Riparia riparia*, etc.) - Transpalearctic origin (Tp), 11 species (9.01% - *Vanellus vanellus*, *Charadrius dubius*, etc.) - Mongolian origin (Mo), 8 species (6.55% - *Merops apiaster*, *Egretta garzetta*, etc.) - Mediterranean origin (M), 2 species (1.63% - *Aythya fuligula*) - Siberian origin (S), and 1 species (0.81% - *Egretta alba*) - Chinese origin (Ch).

Regarding the occupied habitat, 50 of the 122 species, (40.98%) live in the forest and coppice (FC), 31 species (25.41%) live in the wetland (W), 29 species (23.77%) live in the open area (OA) and 12 species live within the built-up areas (BA), (Table 1).

Depending on the diet: 43 species (35.25%) are insectivore species (In), 31 species (25.41%) are zoophagous-poliphagous species (Zoo-p), 30 species (24.59%) are omnivore species (Om), 8 species (6.56%) are carnivore-predator species (CP), 8 species (6.56%) are granivorous species (Gr), and 2 species (1.64%) are ichthyophagous species (Ih), (Table 1).

From the phenological point of view, 66 species (54.09%) are summer visitors and mainly summer visitors in the researched area, 34 species (27.86%) are sedentary or mainly sedentary species, 20 species (16.39%) are partially migratory species and mainly partially migratory species, 1 species (0.81%) is mainly a winter visitor, and 1 species (0.81%) is mainly a passage migrant species (Table 1).

According to the Birds Directive, 21 species (17.21%) of the 122 species breeding in the studied area are listed in Annex I (the species mentioned in Annex I shall be subjected to special conservation measures concerning their habitat in order to ensure their survival and reproduction in their area of distribution) (Table 1).

**CONCLUSIONS AND FUTURE WORK**

We consider that, in spite of the strong anthropogenic impact on the birds in the area, the biodiversity of the breeding species is high.

The number of breeding species in the studied area will continue to increase in correlation with the continuance of the silting process (that involving the enlargement of the rush-bed surface).

In the future, by adopting efficient measures for the protection of birds and their habitats, the list of breeding species presented here will surely increase.

**REFERENCES**


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R. Gava, University of Piteşti, 1 Târgu din Vale, 110040, Piteşti, Argeş, Romania, e-mail: gavaradu@yahoo.com
CREATION OF THE GIS DATABASE FOR SPATIAL ESTIMATION OF IODINE AND SELENIUM CONTRIBUTION TO THYROID DISEASES AMONG RURAL POPULATION OF THE BRYANSK AREA

Elena Korobova, Victor Beryozkin, Alexander Kuvyline, Elena Chesalova, Irina Kurnosova, Valentina Danilova, Nadezhda Korsakova, Lyubov Krigman, Sabzbahor Khushvakhtova

Abstract: The main goal of the study is creation of GIS allowing adequate spatial comparison of the medical, bio- and geochemical data obtained from different georeferenced sources to reveal contribution of iodine and selenium in natural biogeochemical food chains to distribution of the thyroid diseases among the rural population of the Bryansk region subjected to different impact of radioiodine fallout for adequate decision making on the long-term monitoring the Chernobyl consequences and prophylaxis.

The database included: 1) a set of electronic basic maps of the area (parent rocks, soil, vegetation, administrative regions with rural settlements); 2) radionuclide contamination of settlements; 3) medical data on thyroid endemic diseases and cancer with reference to the settlement (data of the Bryansk clinical and diagnostic center, BCDC); 4) original experimental data on iodine content in soils, grasses, milk, potato and drinking water collected in selected private farms of rural settlements with the data on iodine renal excretion obtained by BCDC specialists.

Database for GIS “Bryansk-Iodine-Selenium” was created with the help of ArcGIS 9.3, Geo Graf 2.0 and MS Excel which allowed various spatial estimates and production of a set of output maps characterizing iodine and selenium status of the area and risk assessments.

The main goal of this work is to create GIS database, which include medical, bio- and geochemical data. GIS tools have been used to analyze spatial correlation of thyroid disease's distribution in Bryansk region with geological, geochemical and biological characteristics of this region. The result of this analysis is represented in the final map of medical risk. This GIS may also be used to make long-term monitoring of thyroid disease's distribution after Chernobyl accident.

Key words: biogeochemistry, geochemical ecology, iodine, selenium, GIS database

INTRODUCTION

Iodine belongs to one of the most significant chemical elements in organisms which provide normal functioning of thyroid gland. The latter accumulates about 20-50% of the total iodine entering the organism. In natural conditions thyroid malfunctioning was often related to a deficit of stable iodine in diets leading to serious diseases up to goiter and destruction of cognitive abilities. However stable iodine deficit was easily compensated by systematic consumption of iodine-enriched products.

Development of nuclear weapon and technologies brought to life about twenty of the short-lived radioactive iodine isotopes as fresh fission products. $^{131-135}$I radioactive isotopes form about 20% of the total activity of the young fission products. The total amount of the global $^{131}$I dispersion due to nuclear tests was estimated to equal 700 EBq [7]. Modern estimate of the fraction of $^{131}$I released during the Chernobyl accident approximates 50-60% of the totally produced $2.4-3.2*10^{18}$ Bq [2]. The estimate of the thyroid gland irradiation dose for children aged from 3 to 7 years living in 973 settlements of the Bryansk area contaminated by radionuclides varied from 0,07 to 2 Gy [1].

However the geochemical background of the contaminated area is rather heterogenous and the soil cover is composed of the soil types with a considerably different stable iodine supply and transfer to food chains. In case of deficiency of chemical elements in the environment their bioaccumulation is known to be higher (as a mechanism of adaptation). Recent investigations proved that there was no difference between iodine isotopes in their concentration by thyroid gland [8]. Therefore radioiodine followed the pathway of its stable analogue in the local food chains and could have accumulated to the higher extent in areas with lower iodine status [6]. Selenium is the other chemical element significant for thyroid gland activity. It is required for appropriate thyroid hormone synthesis.
and metabolism. Thyroid gland has the highest Se concentration among all human organs [3-5].

The main goal of the study is creation of GIS allowing adequate spatial comparison of the medical, bio- and geochemical data obtained from different georeferenced sources to reveal contribution of iodine and selenium in natural biogeochemical food chains to distribution of the thyroid diseases among the rural population of the Bryansk region subjected to different impact of radioiodine fallout for adequate decision making on the long-term monitoring the Chernobyl consequences and prophylaxis.

**GIS DATA AND DATA HANDLING**

The data sets included: 1) geochemical data on iodine and selenium content in different soils and their transfer to plants, water and milk; ecogeochemical criteria for iodine and selenium deficiency in soils and plants; 2) a set of basic raster and vector maps of the area (topography, hydrology, rocks, soil, vegetation, administrative regions, population scales: 1:1000 000 and 1:200 000); 3) official data on radionuclide contamination of more than 2000 settlements scattered over the area; 4) medical data on cases of thyroid endemic diseases (cancer and goiter), iodine in urine with reference to the settlement (data of the Bryansk clinical and diagnostic center, BCDC for the period of 1986-2009); 5) data on the number of residents in 1988 and later years; 4) original experimental data on iodine and selenium content in soils, grasses, milk, potato and drinking water collected in the selected private farms of more than 100 rural settlements provided by BCDC with the data on iodine renal excretion (Figure 1).

![Figure 1. A structure of GIS “Bryansk-Iodine-Selenium”](image_url)

Fast expert evaluation of iodine status of the natural and agricultural soil cover around settlements with due regard to the structure of the soil cover is performed according to the equation (1) with the help of the original software.

\[
As = \left( \sum_{i=1}^{n} A_i \cdot P_i \right) / \sum_{i=1}^{n} P_i
\]

where

As - weighted estimate of iodine supply in soils of the area considered;
Ai - iodine concentration in particular soil type;
Pi - quota (percent in the protocol) of the soil type area within the total area considered;
n - number of the soil types within the total area considered.
Radius of 5 km was considered as the most reasonable for the selected soil map of scale (1:1000 000) and the collective farm area used for food production and consumption.

Figure 2. Visualization of the examined settlements

Table 1. Statistical parameters of iodine and selenium variation in components of the food chain collected in Bryansk region

<table>
<thead>
<tr>
<th>Components</th>
<th>Units</th>
<th>Iodine</th>
<th>Selenium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>Soil¹, 0 - 5 cm</td>
<td>mg/kg, dw</td>
<td>95</td>
<td>0.02</td>
</tr>
<tr>
<td>Soil², 5 - 10 cm</td>
<td>mg/kg, dw</td>
<td>68</td>
<td>0.11</td>
</tr>
<tr>
<td>Soil³, 10 - 20 cm</td>
<td>mg/kg, dw</td>
<td>71</td>
<td>0.182</td>
</tr>
<tr>
<td>Agrosoil⁰ – 10 cm</td>
<td>mg/kg, dw</td>
<td>84</td>
<td>0.02</td>
</tr>
<tr>
<td>Agrosoil¹ 10 – 20 cm</td>
<td>mg/kg, dw</td>
<td>67</td>
<td>0.14</td>
</tr>
<tr>
<td>Agrosoil⁰ – 20 cm</td>
<td>mg/kg, dw</td>
<td>98</td>
<td>0.13</td>
</tr>
<tr>
<td>Grasses*</td>
<td>mg/kg, dw</td>
<td>90</td>
<td>0.022</td>
</tr>
<tr>
<td>Water*</td>
<td>µg/l</td>
<td>276</td>
<td>0.6</td>
</tr>
<tr>
<td>Milk*</td>
<td>µg/l</td>
<td>304</td>
<td>0.46</td>
</tr>
<tr>
<td>Potatoe*</td>
<td>µg/kg, fw</td>
<td>289</td>
<td>2.2</td>
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</table>
natural soils used for haying and grazing, three layers of sampling depth, cm; 2) arable soils from private farms, two layers of sampling depth, cm; 3) similar soils, one layer sampling depth, cm; 4) natural pastures; 5) drinking water; 6) produced and consumed in private farms.

Maps have been created with the help of ArcGIS 9.3, Geo Graf 2.0, for table data MS Excel is used.

GIS database will be constantly replenished with new experimental data to select and analyze medical and geochemical information on different levels of data aggregation for regional and local evaluations.

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REFERENCES

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RAIN FACTOR IN AGRO CLIMATIC AREAS OF VOJVODINA PROVINCE

D. Stojiljković, M. Rajić, G. Šekularac

Abstract: We analyzed the precipitation in six climatic regions in Vojvodina - northern part of Serbia. Precipitation and temperature were analyzed for the period from 1966 by 2009. This period was marked by the uniformity of distribution of rainfall a year to two primary and secondary maximum and minimum average monthly amount of rainfall. Rain factor L varies from 49.6 in the northernmost region of agroclimatic to 56.1 in the southernmost region of agroclimatic. Based on the results of Kruskal-Wallisovog test statistically significant difference medial value of monthly precipitation. Not pronounced trend for the period analyzed. Analysis of temperature shows higher variability in the winter months.

Key words: agro-climatic region, rain factor, trend, Vojvodina

INTRODUCTION
In the field of Vojvodina and the southern edge of the Pannonian Basin, may be applied the principle of regional responsibility that aims to further differentiate the specific areas according to their local natural and microclimate conditions, to make it widely available for the rational and optimal use in agriculture.

MATERIAL AND METHODS
We analyzed the time series of precipitation and temperature from 6 meteorological stations (MS) and six agroclimatic area for the period of 1966 of 2009. Calculation of average values in the time distribution of rainfall for the Vojvodina aims to establish a measure of the size and course changes of rainfall during the year for individual meteorological stations, and production agroclimatic zones is the total average amount of rainfall in Vojvodina. Shows the relative fluctuations R by the formula:

\[ R = \frac{(P_{\text{max}}-P_{\text{min}})}{P_{\text{ukup}}} \times 100\% \] ...........................................................................................................(1)

where:
- \( R \) relative fluctuation of rainfall
- \( P_{\text{max}} \) average monthly max precipitation
- \( P_{\text{min}} \) average monthly mini precipitation
- \( P_{\text{ukup}} \) annual sum of precipitation

Based on analysis of temperature in the same meteorological stations (MS) for the same period is determined by Lang's rain factor (L) using the formula:

\[ L = \frac{P}{T_0^\circ C} \] ...........................................................................................................(2)

where:
- \( L \) rain factor of Lang
- \( P \) annual precipitation sum
- \( T_0^\circ C \) average annual temperature

Based on the results of Kruskal-Wallisovog test a statistically highly significantly difference medial values of precipitation. The monthly distributions was explored by means of box-whisker diagram on the base of median (\( M_e \)) upper (\( Q_3 \)) and lower quartiles (\( Q_1 \)) and interquartile range that is a measure of dispersion of the central portion of a distribution (IQR=\( Q_3 - Q_1 \)). This diagram is very useful in establishing skewness of the
distribution and presence of outlier and extreme values. The outlier is defined as the value outside the range of \((Q_1 - 1.5 \cdot I_Q, Q_3 + 1.5 \cdot I_Q)\), and the extreme value is the value outside the range of \((Q_1 - 3 \cdot I_Q, Q_3 + 3 \cdot I_Q)\).

RESULT AND DISCUSSION
The paper presents the average values of the analyzed time series for Vojvodina. Because of the size of the images 1, 2, 3, 4 a statistical analysis of rainfall and temperature for the six meteorological stations in Vojvodina. The average annual precipitation for the area of Vojvodina based on six meteorological stations (MS), is 596.9 mm. The rainiest year was in 1999. years with an average annual sum of precipitation of 847.8 mm and the driest 2000th years with an annual sum of 273.5 mm. The primary annual maximum rainfall in June with an average rainfall of 83 mm. Secondary maximum in this period in November with 48.2 mm \([2, 3]\). The primary minimum is expressed in February with the amount of rainfall of 32.3 mm. Secondary annual minimum was recorded in October of 44.2 mm. The average relative fluctuation of rainfall is \(R = 8.3\%\), the largest in the Central Vojvodina (tab.1. MS Zrenjanin) at least in northern Vojvodina. Monthly average height and annual precipitation sum is greater than the average in the agro-climatic Vojvodina regions of southern Vojvodina (MS Vršac and MS R. Šančevi, tab.1., fig.1.), and lower than average in the agro regions of northern Vojvodina( MS Palić and MS Kikinda, tab.1., fig.2.) .

Table 1. Average months of precipitation (mm) during period 1966-2009.y. in Vojvodina

<table>
<thead>
<tr>
<th>MS</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>SUM P (mm)</th>
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<td>35.1</td>
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<td>54.6</td>
<td>76.2</td>
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<td>55.4</td>
<td>45.</td>
<td>38.</td>
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<td>79</td>
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<td>48</td>
<td>47.8</td>
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<td>39</td>
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<td>85.9</td>
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<td>36.6</td>
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<td>75.7</td>
<td>62.8</td>
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<td>48.7</td>
<td>50.1</td>
<td>663.4</td>
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<td>64.7</td>
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<td>48.2</td>
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<td>46</td>
<td>596.9</td>
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</tbody>
</table>
The average annual temperature in Vojvodina for the analyzed period is 11.2°C. The warmest year was in 2000, with an average annual temperature from 13.0°C. Maximum average annual temperatures for the period varies from 21.7°C in rainiest year, to 24.3°C in driest year. Minimum average monthly temperature in the extreme year 1999. and 2000. year range from 0.56°C to -1.6°C. Average temperatures are seasonal: the maximum is always in July, with air temperature of 21.5°C. Minimum average monthly temperatures in January and amounted to 0.25°C (tab.2.). If we analyze the time series of monthly temperature in the period 1966-2009 for 6 station, and the average temperature calculated as average monthly temperature of individual MS, the linear trend was not statistically significant [1]. If the observed temperature series for individual months in the following charts (fig.3.,4.), one can notice that the January, June, the July and August temperatures exhibited an increasing trend. Using Augmented Dickey-Fuller test statistics
is investigated whether the trend of temperature time series for individual months in the reporting period stochastic or deterministic nature. In all cases, rejecting the null hypothesis of the existence of unit root on the basis of which it follows that all trends deterministic character. Based on the January, June, July and the August temperature in the period 1966-2009, were estimated by a linear trend of temperature models for individual meteorological stations as well as for high temperature \( Y = a + bX \). The coefficient of the trend line is in all cases (except January temperatures in Palić and Vršac) is statistically significant or highly significant.

Table 2. Average months of air temperature during period 1966.-2009.y. (\(^{0}\text{C}\))

<table>
<thead>
<tr>
<th>MS</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>Average years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palić</td>
<td>-0.7</td>
<td>1.5</td>
<td>6</td>
<td>11.3</td>
<td>16.8</td>
<td>20</td>
<td>21.6</td>
<td>21</td>
<td>16.5</td>
<td>11.3</td>
<td>5.3</td>
<td>0.9</td>
<td>11</td>
</tr>
<tr>
<td>Sombor</td>
<td>-0.4</td>
<td>1.8</td>
<td>6.1</td>
<td>11.3</td>
<td>16.8</td>
<td>19.8</td>
<td>21.4</td>
<td>20.8</td>
<td>16.3</td>
<td>11.1</td>
<td>5.4</td>
<td>1.1</td>
<td>11</td>
</tr>
<tr>
<td>R.Šančevi</td>
<td>-0.1</td>
<td>2</td>
<td>6.4</td>
<td>11.5</td>
<td>17</td>
<td>19.8</td>
<td>21.5</td>
<td>21.1</td>
<td>16.7</td>
<td>11.6</td>
<td>5.9</td>
<td>1.5</td>
<td>11.2</td>
</tr>
<tr>
<td>Kikinda</td>
<td>-0.5</td>
<td>1.7</td>
<td>6.2</td>
<td>11.6</td>
<td>17</td>
<td>20</td>
<td>21.7</td>
<td>21.1</td>
<td>16.7</td>
<td>11.5</td>
<td>5.6</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Zrenjanin</td>
<td>-0.3</td>
<td>1.9</td>
<td>6.4</td>
<td>11.6</td>
<td>17.1</td>
<td>20</td>
<td>21.6</td>
<td>21.3</td>
<td>16.9</td>
<td>11.7</td>
<td>5.8</td>
<td>1.2</td>
<td>11.3</td>
</tr>
<tr>
<td>Vršac</td>
<td>0.5</td>
<td>2.6</td>
<td>6.8</td>
<td>12</td>
<td>17.1</td>
<td>19.9</td>
<td>21.6</td>
<td>21.4</td>
<td>17.3</td>
<td>12.3</td>
<td>6.7</td>
<td>2.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Vojvodina</td>
<td>0.25</td>
<td>1.9</td>
<td>6.3</td>
<td>11.5</td>
<td>16.7</td>
<td>19.9</td>
<td>21.5</td>
<td>21</td>
<td>16.6</td>
<td>11.5</td>
<td>5.7</td>
<td>1.3</td>
<td>11.2</td>
</tr>
</tbody>
</table>

Fig. 3. Trends of mean temperature in January calculated from measurements of six meteorological stations in January (1966.-2009.)
Fig. 4. Trends of mean temperature in July calculated from measurements of six meteorological stations in July (1966.-2009.)

Table 3. Lang’s rain factor for years of extreme precipitation and extreme drought

<table>
<thead>
<tr>
<th>MS</th>
<th>Rain P (mm) 1999.y.</th>
<th>Average T (0C) 1999. god.</th>
<th>Rain factor L (P/T) 2000. y.</th>
<th>Average T (0C) 2000. y.</th>
<th>Rain factor L (P/T)</th>
<th>Average L(P/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palić</td>
<td>710.1</td>
<td>11.2</td>
<td>63.4</td>
<td>247.1</td>
<td>12.7</td>
<td>19.4</td>
</tr>
<tr>
<td>Sombor</td>
<td>778.5</td>
<td>11.3</td>
<td>68.8</td>
<td>277.5</td>
<td>12.8</td>
<td>21.6</td>
</tr>
<tr>
<td>R.Šančevi</td>
<td>944.4</td>
<td>11.5</td>
<td>82.1</td>
<td>287.8</td>
<td>13.0</td>
<td>22.1</td>
</tr>
<tr>
<td>Kikinda</td>
<td>831.3</td>
<td>11.5</td>
<td>72.2</td>
<td>223.1</td>
<td>13.0</td>
<td>17.1</td>
</tr>
<tr>
<td>Zrenjanin</td>
<td>884.6</td>
<td>11.6</td>
<td>76.2</td>
<td>278.5</td>
<td>13.2</td>
<td>21.0</td>
</tr>
<tr>
<td>Vršac</td>
<td>938.4</td>
<td>12.2</td>
<td>76.9</td>
<td>327.2</td>
<td>13.4</td>
<td>24.4</td>
</tr>
<tr>
<td>Vojvodina</td>
<td>847.8</td>
<td>11.5</td>
<td>73.2</td>
<td>273.5</td>
<td>13.0</td>
<td>20.9</td>
</tr>
</tbody>
</table>

Lang’s rain factor is $L = 73.2$ (mm/$^0$C) in the rainiest 1999th years, defines more humid type of climate (tab. 3.). Rain factor is the largest in South Backa, the lowest in northern Backa. Rain factor of time for the driest 2000th amounts to $L = 9.20$ (mm/$^0$C) and defines the arid type of climate with the introduction of mandatory irrigation of agricultural areas. Rain factor 2000th was the lowest in the agroclimatic region of northern Banat, and the largest in the agroclimatic region of eastern Banat. Average rain factor Langa is for six agroclimatic regions likely Vojvodina $L = 53.3$ (mm/$^0$C).

**CONCLUSIONS**

In agro-climatic regions for the period of analysis of 1966 by 2009 observed the following:

- in all agro-climatic regions of the primary maximum annual amount of rainfall in June, and the primary minimum is in February
- southern agro-climatic region of Vojvodina are high in this disposition of excess water from rainfall throughout the year. Other agro-climatic regions with different levels of monthly surpluses and deficits of water than the
average; in all agro-climatic regions of northern Vojvodina with severe water shortage throughout the year

- annual and spatial variation of rainfall (R) increases from north to south
- when the rain factor Lang (L) is less than 60, average annual temperatures higher than 11°C, and the average annual amount of rainfall less than 650 mm (dry and warm year). It is characteristic of arid steppe climate and savanna. Mandatory introduction of irrigation in agricultural production
- when the average annual rainfall exceeds 650 mm and the average annual temperature less than 11°C (the rainy and cold) rain factor is greater than 60. It is characteristic of the humid climate of weak forest. Requires the use of land reclamation by the analysis of air and water quality and soil type of biotech culture.

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Abstract: Applying landscape architecture and side ecology there was represented the preliminary design for open pit mining recultivate in the form of multi-purpose anthropogenic water surface. In Vojvodina, these depressions are naturally filled in with ground and atmospheric water, than with water from the canal system DTD or by forming sources with exploitation wells. Even though their surfaces are quite small they contain a mosaic of versatile habitat types and so represent an extraordinary natural replacement of birds’ habitats, small vertebrates and danger invertebrates.

Key words: landscape architecture, open pit mining, recultivate

INTRODUCTION

Mining as well as many other human activities cause temporary or permanent degradation of natural environment. This conflict is specially emphasized in open pit minims where mineral material is exploited. Among the most important conflicts there are: soil occupation and change the purpose of its utilization, water regime change, destruction of habitats of protected species of flora and fauna etc.

MATERIAL AND METHODS

In Vojvodina there is mostly open pit mining that used to be on agricultural land. After many years of exploitation, implementing the established town and regulatory plans, they were positioned nearby urban residential areas or protected parks of nature. Implementation of landscape architecture in abandoned open pit mining recultivate has different importance depending of initial purpose of surface or current ecological value of the location for potential new users.

In open pit mining recultivate generally, there are three solutions that can be considered:

- Recultivate to the level of previous state – that understands fulfilling of depression of open pit mining with barren soil, communal waste, material from newly opened prospects, construction pits etc. and than covering it with humus layer in order to bring the surface to its previous purpose (agriculture, viniculture, orchard);
- Recultivate of depression of the open pit mining in order to establishing ecologically valuable habitats, specially in cases of locations in natural parks of different level of protection or in case of some autochthonous habitat regeneration;
- Recultivate with aim to arranging area for sport and recreation, aqua parks, specially when the open pit mining is nearby settlement;
- Renouncing to spontaneous natural development after exploitation termination. This solution is taken in consideration if the open pit mining edges (slopes) are so shaped that there is possibility of spontaneous or
revitalization with the slightest interventions of a landscape architect. Most often there is necessary the area of the open pit mining to be enclosed, in order to avoid damage that could be caused by people, wild and domestic animals. This solution doesn’t exclude activities according to legal obligations for recultivate.

In order to determine the purpose of depression utilization and procedure how to achieve that, first there are explored basic guidelines, limitations and fundamental natural characteristics of the open pit mining depression. This location purpose depends on natural conditions, size of the surface, existing infrastructure and potential users needs. In the following phase, there are explored elements of recreation program and than follows the solution for recultivate and planning.

RESULTS AND DISCUSSION

Among existing solutions, there is represented the solution of recultivate with aim to arrange recreation area along with ecologically valuable habitats protection. Namely, initial decisions in making the Project for recultivate and planning of the open pit mining refer to: lake constructing, its utilization in recreation purposes and keeping this location as ornithological valuable habitats [1] [2].

The recultivated and arranged area of the open pit mining will be assigned to citizens of Novi Sad and nearby settlements, recreation. Criteria which indicate such decisions are: vicinity of the city, good approach and future lake attractiveness. There would be provided recreation for versatile users:

- Intellectual-healthy type of user who wants to training, taking rest, relaxing, reading, playing games;
- natural-landscape type of user who wants to moving, looking around, playing outside in natural surrounding, playing ball, swimming, fishing, observing and making photos of flora and fauna;
- sport type of user who swims, rows, plays tennis / basketball / volleyball / football etc.

Considering the above named types of users, in the area there should be distinguished and divided two zones – a zone of quiet recreation and a zone of intensive recreation.

The recreation program includes the summer recreation as follows:
- beside water: placement, playing, fishing, preparation and food consumption, birds observing etc.;
- On water: swimming, water polo, rowing, sailing;
- On green free surfaces: settlement, playing, walking, driving bicycle, sports (volleyball, basketball, bowling, tennis) and similar.

Walking is the only activity in the winter part of the recreation program.

Versatile purposes of location are achieved with construction of sport terrains, running tracks, and observation tracks climbing hills, hotels, restaurants, barbeque facilities, view points and small shops for service activities. Along the river or lake bank there are proposed to be constructed plateau / pontoons, universal playgrounds, leveling and banks underpinning, cabins for change, beach, facilities for boats tying etc. The location is advised to have associated roads: paths for approaching to foot paths, cycling paths, running paths as well as a parking place. Drinking water supply as well as technical
water supply is necessary, water, electrical, postal and other infrastructure. Urban street furniture should contain benches, lighting, trashes, information tables / signs. At the end there is given the preliminary y design for green space arranging with seasonal and multi year plants with defined regime of their maintenance.

In the case of multi purpose utilization of bigger anthropogenic accumulations, where the primary purpose would refer to rest and recreation, fishing has an inferior role. But, in smaller anthropogenic depressions, restocking and fishing have the basic role in recultivate and ambient ecology. The preliminary design determines the extent of anthropogenic degradation of terrain [1] [2].

Table 1. Criteria for different types of recreation

<table>
<thead>
<tr>
<th>Recreation type</th>
<th>Convenience criteria</th>
</tr>
</thead>
</table>
| Swimming        | - water body, at least 3 – 5 (ha)  
|                 | - free surfaces bigger than the lake surface  
|                 | - acceptable bacteriological water quality  
|                 | - no permanent phosphates presence  
|                 | - more than a half of water surface water depth more than 3 (m)  
|                 | - acceptable water temperature  
|                 | - constant water level  
|                 | - well formed banks and underwater relief  
|                 | - good relation “meadow – forest – field”  
|                 | - convenient traffic connections  
|                 | - no damages from nearby usage (noise, gases etc.)  
|                 | - no danger for neighbor purpose of the location (noise) |
| Fishing         | - minimal water body surface 2 (ha)  
|                 | - more than a half of water surface water depth more than 2 (m)  
|                 | - without extreme concentrations of salts and pH values  
|                 | - shoal presence (spawning area) |
| Rowing          | - minimal water body surface 20 (ha)  
|                 | - good approach to banks |

Consequently it is not reasonable to apply intensive fish breeding in order to provide big ichthyoproduction. The future presence of fish in the lake should provide both fishing and production in accordance with the lake potential. Organizing and constructing permanent objects with necessary protection measures of water and green surfaces, contribute to the future water ecosystem conservation, stability and quality. At the same moment there are necessary to organize minimum of protection measurements: allowed usage of tools, guard’s service, and prevention of application of prohibited devices or theft of fish. In order to prevent potential eutrofication, excessive usage of baits should be limited. Take into account the quality of water in the ponds[3] [4].
Table 2. The criteria of water quality in ponds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Desirable contents (mg/l)</th>
<th>MAC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water color</td>
<td>30^0</td>
<td>50^0</td>
</tr>
<tr>
<td>Transparency</td>
<td>Transparency</td>
<td>Little muddy water</td>
</tr>
<tr>
<td>O2</td>
<td>4-8</td>
<td>2.5</td>
</tr>
<tr>
<td>Free CO2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>H2S</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>pH</td>
<td>7</td>
<td>6.5</td>
</tr>
<tr>
<td>Alkalinity /mg-ekv</td>
<td>1.8-2</td>
<td>3.5</td>
</tr>
<tr>
<td>Total hardness</td>
<td>5-8</td>
<td>3-5</td>
</tr>
<tr>
<td>N Albuminin</td>
<td>0.5-1.5</td>
<td>2</td>
</tr>
<tr>
<td>Oxidation O2</td>
<td>15-20</td>
<td>40</td>
</tr>
<tr>
<td>NH4 salt</td>
<td>0.5-1</td>
<td>1.5</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Do 0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1-2</td>
<td>0</td>
</tr>
<tr>
<td>Phosphate P2O2</td>
<td>0.2-0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>1-2</td>
<td>4</td>
</tr>
<tr>
<td>Cl</td>
<td>5-10</td>
<td>25</td>
</tr>
<tr>
<td>SO4</td>
<td>10</td>
<td>20-30</td>
</tr>
<tr>
<td>Salinity</td>
<td>1 000</td>
<td>1 500</td>
</tr>
</tbody>
</table>

Plant material is allocated to green areas as a group or in a linear arrangement. Has primarily the role of physical and biological filter. The choice of material depends on many years of natural environmental conditions and degree of land degradation, water and air. Plant material has the following tasks:

- Produktion of oxygen: yew 118 units, pine 164, krupnolisna linden 450, poplar berlinska topola 691 units,
- Retention of particle: poplar *Populus* 34 kg, elm *Ulmus* 28 kg, maple *Acer* 33 kg, mleč *Acer Platanoides* 28 kg, horse chestnut *Castanea* 16 kg..
- Air ionization: greatest impact on the creation of light ions in the air have *Pinus silvestris* – 80%, *Betula nana* – 64%, *Sorbus aucuparia* – 49.
- Protective forest belts
- Barrier thermal
- Barrier sonic
CONCLUSIONS

Primary designs are adapted to space since they do not disturb other neighbor structures, but contribute to the existing ruined state sanation as well as improving the location landscape, they improve microclimate and refine space in general. The estimation of the environment impact is as follows: measures for open pit mining recultivate contribute to the environment protection improvements, do not danger the environment and the existing objects with their contents provide safe functionality and its protection. The rationality of the solution comes out from achieving double benefit – the environment protection as well as space refining, possibility for employing some number of people, possibility for people in neighborhood to offer their goods and services, which altogether lead to decreasing the migration of people from smaller settlements to big cities.

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THE HUMAN IMPACTS AND CONSERVATION ON BRYOPHYTES FROM VÂLSAN VALLEY PROTECTED AREA

Codruţa Mihaela Dobrescu, Liliana Cristina Soare

Abstract: Biodiversity conservation has traditionally relied on the establishment and maintenance of a network of protected areas. For bryophytes in particular, conservation is mainly incidental and occurs through preservation of habitat for other reasons.

The importance of the Vâlsan as a unique area of sculpinperch or Romanian Darter (Romanichthys valsanicola) required its establishment as a National Reservation.

The Moss Flora includes 130 species, belonging to 81 types, from 42 families assigned to divisions Marchantiophyta (Class Hepaticae) and Bryophyta (class Musci). Relationships between bryophyte diversity variables and environmental variables were analyzed and a bigger diversity in the superior tank of Vâlsan River which has a rich precipitation condition and a lower anthropic influence.

A number of factors have influenced and still have an adverse effect on the moss diversity in the Vâlsan River Basin - the destruction of forests, the construction of dams Vidraru, Vâlsan and Dobroneagu, the construction of roads, the growth of the localities. This might also be the reason why some species of our moss flora went extinct or are in danger of extinction.

The preservation and maintenance of existing protected area contributes to the retention of protected area's moss diversity.

Key words: Valsan Valley, human impact, conservation on bryophytes

INTRODUCTION

Biodiversity conservation is a rather new multidisciplinary science that has developed to cope with the crisis facing the global biological diversity [5].

Biodiversity conservation has traditionally relied on the establishment and maintenance of a network of protected areas. For bryophytes in particular, conservation is mainly incidental and occurs through preservation of habitat for other reasons [1].

Threats and mechanisms that are vulnerable bryophytes are becoming increasingly well known, although further research is necessary experiments to better understand the causes of species rarity.

The Vâlsan River Basin it is a Natural Reservation in the Făgăraş Mountain of Argeş County, Romania as a unique area of sculpinperch or Romanian Darter (Romanichthys valsanicola).

Description of the collecting sites is determined by the following geographic coordinates Vâlsan River Basin: N - 45 ° 25'-45 ° 35'; E - 24 ° 40'-24 ° 45'.

MATERIAL AND METHODS

The bryophytes were collected from the Vâlsan River's Basin, of the Făgăraş Mountains, Argeş County and identification was done using relevant bryological literature. Nomenclature was actualized according to Hill & al. [3].

The identification of species was conducted in the laboratory by an Optika stereomicroscope and the microscope Optika B-253 to which a micro-photo camera Canon A630 was attached to take photos.

By processing bryophytes samples I made a list of recorded mosses and I analyzed the Moss Flora under the aspect of anthropic factors influenced and still have an adverse effect on the moss diversity in the Vâlsan River Basin.
RESULTS AND DISCUSSION

In summary, the conclusions reached about mosses, includes 130 species, belonging to 81 types, from 42 families assigned to divisions Marchantiophyta (Class Hepaticae) and Bryophyta (class Musci).

Marchantiophyta division is represented by 16 species belonging to 15 genera belonging to 13 families and Bryophyta division is represented by 114 taxa (seven varieties) belonging to 66 genera, belonging to 29 families.

In the protected area of Vâlsan Valley, the following were identified: three species of mosses are very rare - Dicranella crispa, Hymenostylium recurvirostrum, Plagiothecium neckeroideum; three species as rare - Grimmia trichophylla, Plagiobryum zieri, Hygrohypnum duriusculum; twelve of which are sporadic species - Sphagnum angustifolium, Racomitrium sudeticum, Blindia acuta, Cynodontium polycarpon, Dicranoweisia crispula, Bryum creberrimum, Rhizomnium pseudopunctatum, Eurhynchium angustirete, Campylophyllum halleri, Hypnum bambergeri, Hypnum jutlandicum, Anomodon longifolius in Romania [2].

We can thus say that the environmental analysis of bryophytes species prove the close connection that exists between physical and geographical conditions of their development planning and analysis.

Relationships between bryophyte diversity and environmental variables were analyzed and a bigger diversity in the superior tank of Vâlsan River which has a rich precipitation condition and a lower anthropic influence.

A number of factors have influenced and still have an adverse effect on the moss diversity in the Vâlsan River Basin. The main negative factor is the destruction of forests, which began centuries ago and still continues today. The distribution of a number of forest species is restricted in this way. This might also be the reason why some species of our moss flora went extinct or are in danger of extinction.

The partial or complete systematic drainage of water basins and wetland areas, beginning in the first decades of this century, has also had a negative impact on the diversity of the moss flora. This has resulted in drastic changes in the microclimate of the respective regions and the destruction of the habitats of certain moss species.

Among the most important factors incriminated by the disappearance of "Romanichthys valsanicola" in the Argeș River, Doamnei River and partially in the Valsan River, but also damage vegetation and flora of bryophytes and fanerofite in the area, it refers to hydrotechnical works affecting the riverbed.

The construction of dams Vidraru, Vâlsan and Dobroneagu changed the microclimate and destroys areas that support moss vegetation. The construction of roads, the growth of the localities, are other negative factors influencing moss diversity in the Vâlsan River’s Basin.

Remember the conjugated action of factors whose effects have increased since the inauguration of hydrotechnical works and have intensified logging and construction of access roads, the continuous growth of necessary construction materials provided by forests and the river bed (boulders, rocks, sand) determined profound changes in the structure and dynamics of catchment biocenoses Vâlsan, which resulted in endangering the existence of species.

In past years (2005-2010) climate change represented an important disturbing factor in the moss flora and vegetation of forests in protected area Vâlsan Valley [6], [7].

Significant amounts of rainfall, flash floods and torrents create led to the destruction of entire mountainsides and fallen tree trunks have arrived in the river water (Fig. 1, 2).
Bryophytes are characterized by a profound character morfoanatomic adaptive, functional, biochemical and genetic level can be seen in their evolutionary development of progressive, environmentally driven process, and geographical fitoistoric various environmental conditions in which life develops.

Thus, bryoflora, although affected, has a high capacity of recovery, compared with the fish fauna. At only 1-2 years after these events, the degree saprolignicolous bryophytes fallen trunks occupied (Fig. 3).
Although bryophytes are rarely the most obvious elements in the landscape, they play an important ecological role in terms of water balance, erosion control, nitrogen, or budget, or simply by providing habitat for other organisms [4].

**CONCLUSIONS**

Because of the *Romanichthys valsanicola*, species declared „Nature Monument“ (considered the most endangered species from the European ihtiofauna), the area examined has eco-protective valence, reason for which the ecological reconstruction of the area for the prelevation of the species *Romanichthys valsanicola* is necessary.

The importance of the Vâlsan as a unique area of sculpinperch or Romanian Darter (*Romanichthys valsanicola*) required its establishment as a National Reservation, which involves further ecologic measures: reduction of negative impact of the anthropogenic activities; area monitoring program; respecting the “protected area” status; poaching and disorganized camping.

The preservation and maintenance of existing protected areas contributes to the retention of Vâlsan’s moss flora diversity. At present, the threats faced by local bryophytes are minor, but increasing destruction of forests and global warming may be significant in the long term. Recommendations for the conservation of Vâlsan River bryophytes include setting up protected sites outside of the current conservation areas, reducing destruction of forests, including bryophytes rare in a Red List for Protected Area Vâlsan, training local bryologists, and increasing public awareness.

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MAN-MADE IMPACT ON THE SOIL/PLANTS DISTRIBUTION OF SELENIUM AND MERCURY

Larisa Jovanović, Vadim Ermakov, Valentina Danilova

Abstract: This paper discusses the problem of technogenic influence on the behavior of selenium and mercury in soils and plants. Strengthening technogenesis in a situation of increasing environmental problems in the world are changing the mobility of chemical elements.

Key words: technogenesis, biosphere, soil, plants, selenium, selenate, selenite, mercury, mold, fungi.

INTRODUCTION

Problems of technogenic influence is multifaceted and in some cases, the behavior of vital micro (vitamins, trace elements) becomes the determining for animals and humans. In the technological evolution of the biosphere is important to a comprehensive study of biogeochemical endemies particularly dangerous due to lack or excess of essential chemical elements in the environment and animal and human organisms. Their origin depends on the genetic basis of organisms and features of local biogeochemical cycles of elements. These are determined by processes of weathering and transformation of matter and to anthropogenic factors. This is typical for a number of trace elements including selenium and mercury.

MOBILITY OF SELENIUM AND MERCURY IN SOILS

Mobility of selenium and mercury in soil is determined by its type, parameters, including the level of iron and other metals, the mechanical composition, the presence of clay minerals, the size of silt particles, water regime, exchange properties, redox properties (Eh, pH, acidity) organic matter content, number of soil organisms [2].

The mobility of selenium in soils evaluated by water-soluble fraction, as well as by successive extractions using potassium chloride, hydrochloric acid, potassium chlorate, and others.

In some cases the degree of extraction of selenium compounds by aqueous solutions correlates with the level of selenium content in plants.

In assessing the state of selenium in soils and environment, some information may be obtained from consideration of the diagrams of selenium oxidation. If we consider that the pH of most soils varies from 4.5 to 8.0, and Eh from 300 to 700 mV, it becomes clear that selenate, selenite, elemental selenium and selenides of metals should be stable in the soil. The speed of transformation of selenite to elemental selenium is fast, whereas the corresponding rate of interconversion selenite to selenate is slow. Taking into account environmental factors and microbial transformation, we can assume that almost all the selenium in the soil should ultimately be present in elemental form. However, in real conditions Se in soils dominated by selenite and biselenite ions, while at high pH - selenate ions.

ACCUMULATION of Se and Hg in MUSHROOMS

It is well known that fungi specifically accumulate certain chemical elements (zinc, cadmium, mercury, arsenic, etc.) [3, 8]. Several Macromycetes tend to accumulate radionuclides and some organic compounds of anthropogenic origin and selenium (some Lycoperdaceae, red mushroom, white mushroom) [2, 4, 9]. However, the nature of the accumulation of microelements in Macromycetes is not clear.
Fig. 1: The change of the biological absorption of selenium by plants (selenium concentrations in plants) and various forms of Se in the soils of Eastern Meshchera (Ermakov [4]). The value of concentration and soluble forms (per cent) increased to 10.

Fig. 2. Cycle of selenium in soils

MATERIALS AND METHODS
Fungi were collected in the Moscow region (Eastern Meschera) in a forest dominated by Scots pine in the summer-autumn period. Hg-concentration in fresh and dried mushrooms and the extracts were determined by atomic absorption method [6]. The selenium content was measured spectrofluorimetrically [5] and HPLC. Alkylmercury concentration was determined by gas-liquid chromatography after extraction of mercury species by liquid-liquid distribution and distillation in the presence of copper chloride. Mushrooms were dried under different conditions: at room temperature in air and in an incubator (105°C). Soluble forms of trace elements were extracted from fresh wet biomass after homogenizing 5 g of mushrooms (mostly hats) with 20 ml of solvent (distilled water, 1 M HCl; 0,15 M solution of potassium chloride, ethyl alcohol, acetone, chloroform, hexane). The contents of other metals and arsenic were determined by atomic absorption in flames and by electrothermal options on the devices QUANTUM-2A and KVANT.Z.ETA after decomposition of the material with nitric acid and hydrogen peroxide.
RESULTS AND DISCUSSION

Very interesting fact-specific accumulation of total mercury by certain species of fungi. The existing publications [9, 11] focus on the man-made causes the accumulation of mercury in mushrooms. However, our studies showed that some fungi characterized by the selective absorption of mercury, despite the low concentrations of elements in the environment. First of all, it concerns a large parasol mushroom (Lepiota procera (Fr.ex.Seop.) Quel), individual instances of which accumulate mercury up to 5400 mg/kg wet wt (Table 1).

Table 1: Contents of total mercury in mushrooms and alkylmercury (μg/kg wet wt)

<table>
<thead>
<tr>
<th>Name of the fungus</th>
<th>MMC</th>
<th>EMC</th>
<th>Total Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boletus edulis Fr. Ex Bull (3)</td>
<td>≤ 1,0</td>
<td>≤1,0</td>
<td>156-380</td>
</tr>
<tr>
<td>Krombholzia aurantica (Bull) Glib. (3)</td>
<td>≤1,0</td>
<td>≤1,0</td>
<td>60-85</td>
</tr>
<tr>
<td>Krombholzia scarab (Bull) Karst. (3)</td>
<td>≤1,0</td>
<td>≤1,0</td>
<td>26-34</td>
</tr>
<tr>
<td>Lepiota procera (Fr.ex.Seop.) Quel (5)</td>
<td>≤1,0</td>
<td>≤1,0</td>
<td>302-5400</td>
</tr>
<tr>
<td>Clitocibe rivulosa (Fr.ex.Pers) Quel. (2)</td>
<td>4,0-4,8</td>
<td>20,0-22,5</td>
<td>78-101</td>
</tr>
<tr>
<td>Amanita muscaria (L.ex.Bolt) (5)</td>
<td>11,4-16,8</td>
<td>2,4-8,6</td>
<td>75-94</td>
</tr>
<tr>
<td>Marasmius oreades Fr.ex.Bolt (5)</td>
<td>≤1,0</td>
<td>≤1,0-1,6</td>
<td>202-450</td>
</tr>
<tr>
<td>Marasmius scorodonius Fr. (3)</td>
<td>1,2-1,8</td>
<td>1,4-1,6</td>
<td>540-720</td>
</tr>
<tr>
<td>Phodopaxillus nudus (Fr.ex.Bull) (5)</td>
<td>9,0-36,8</td>
<td>5,2-5,8</td>
<td>126-495</td>
</tr>
<tr>
<td>Russula delica Fr. (3)</td>
<td>1,4-1,8</td>
<td>2,3-9,6</td>
<td>20-36</td>
</tr>
<tr>
<td>Lactarius lignyotus Fr.ex.Scop. (2)</td>
<td>≤1,0</td>
<td>2,1-4,8</td>
<td>160-181</td>
</tr>
<tr>
<td>Entoloma rhodopolium (Fr.) Quel.</td>
<td>≤1,0</td>
<td>≤1,0</td>
<td>78</td>
</tr>
<tr>
<td>Lycoperdom perlatum Pers. (5)</td>
<td>≤1,0</td>
<td>≤1,0</td>
<td>317-1500</td>
</tr>
<tr>
<td>Lactiporus sulphures (Bull.ex.Fr.) Bond et Sing (2)</td>
<td>≤1,0-1,3</td>
<td>10,1-21,4</td>
<td>86-92</td>
</tr>
<tr>
<td>Champignon - Agaricus sp (10)</td>
<td>≤ 1,0</td>
<td>≤ 1,0</td>
<td>88-3500</td>
</tr>
</tbody>
</table>

Note: number of test samples indicated in parentheses, MMC - methilmercurychlorid, EMC - ethilmercurychlorid.

To the fungus Hub mercury should also include mushrooms. For example, in the region on the sandy loam soil with a mercury content 20 mg/kg of mushroom is found, the level of mercury which is 258 mg/kg. On calcareous chernozem Moldova with total mercury concentration in the A-horizon 37 mg/kg, the content of the element in champignons reached 1860 mg/kg wet wt. Near Sparrow Hills (Moscow) with the content of mercury in the soil 38 mg/kg of metal concentrations in fresh mushrooms varied from 88 to 3500 mg/kg. In this case, the young mushrooms contain far less mercury (88-135 mg/kg) than older (1700-3500 mg/kg).

For comparison, note that in the lamellar mushrooms collected in the mine Chauvay mercury concentration is 1850 mg/kg, while herbaceous plants contain metal 1780-22460 mg/kg.

Due to the fact that moderate doses of selenium compounds have a detoxifying effect on mercury, we have analyzed the ratio of trace elements in fresh mushrooms collected in Moscow and Moscow region. The data set is 50 to include a list of the most common fungi identified in the Table. 1.

It turned out that between the content of mercury and selenium in Macromycetes planned hyperbolic dependence - the more selenium in mushrooms, the less mercury (Fig. 3). This trend needs to check on more material. Nevertheless, marked by the fact of some interest from the toxicological point of view.

Alkylmercury is found in fungi (Table 1). Several species of mushrooms contain both MMC and EMC. Of particular interest are mushrooms Ryadovka purple, accumulating methylmercury in the amount 29,0-36,8 mg/kg of raw material, as well as a red mushroom with a concentration MMC 11,4-16,8 mg/kg. Moreover, the same types of fungi, selected
from different places, invariably contain high amounts of mercury and methylmercury. Other fungi (govorushko red, novator, ramar racemose, trutovik sulfur-yellow, and limatsella brilliant) accumulate ethylmercury.

![Graph](image.png)

*Fig. 3. Relationship between concentrations of mercury and selenium in Macromycetes (n = 50, data on crude biomass).*

The coefficient of biological concentration alkilmercury in these organisms is 20-40. The mushrooms did not contain phenyl and metoksyethylmercury.

When extracting mercury from mushroom various solvents was found that in aqueous extracts, as in organic solvents becomes less than 1% of the metal. Weak acid (01 M HCl) and 1 M NaOH extracted mercury 12-20% of its total content. In the process of drying of mushrooms at 105°C up to 50-60% of the mercury evaporates.

In fungi Hub mercury, such as mushroom umbrella big Lepiota procera (Fr.ex.Seop.) Sulfur content was increased to 0.25%, as in the white mushrooms (Boletus edulis Fr. Ex Bull). Perhaps this is due to increased synthesis of methionine.

With regard to selenium, this dependence is set to Macromycetes [1]. Lepiota procera contain mercury, which is 38% extracted 0.15 M solution of KCl. The maximum amount of mercury was accumulated in the fraction of proteins with a molecular weight of 20-30 thousand daltons. These proteins contain mercury 30 ng/mg protein, and sulfur - 3%.

**CONCLUSION**

1. In conclusion, it should be noted the important role of knowledge on soil biogeochemistry in the modern era due to the increasing selenium deficiency associated with both the common features of the global cycle of selenium, as well as man-made factors: the extensive land use, fertilizer use, erosion etc. [7 , 8].

2. Selenium tends to migrate to the borders of continents in a soluble form or adsorbed on mechanically colloidal particles and deposited with thin sediments and
organic matter in lakes, rivers and seas. He removed from the water before they reach the sea, and the content in sea water is very low.

2. In the process of weathering selenium enriched subtly weathered products, and colloidal particles rich in organic matter, often enriched with iron, aluminum and manganese. Sediments that contain little colloidal material and clay minerals (quartz and sand-rich feldspar, sand dunes or sea shore sand), deficient in selenium and alkali and lime-rich soil may enhance its migration.

3. Toxic concentrations of selenium, in contrast to mercury - an extremely rare phenomenon, and industrial pollution, waste and agriculture (waste smelting plants, thermal plants - ash, fertilizers, etc.) increase the selenium content in soil, but toxic levels are impossible because most soils potentially deficient in selenium, and Se quickly removed from the solution to the solid phase.

4. Heavy metal concentrations in mushrooms are declining in the following order: Fe, Zn> Cu, Mn> Mo, Pb> Cd> Co> Ni. However, mercury does not fit into this series. Some fungi accumulate two or more trace elements together (red mushroom (Amanita muscaria Fr. Ex L.), the battery as Se, and Zn and Cd).

5. Within the same ecological and trophic groups of fungi, there are significant differences in metal content. The closest relationship observed between the composition of elements and the type of substrate on which the mycelium develops, and the regime of humidity and temperature environments. For mercury plays a significant role age of fungi bodies.

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POLICY AND PRACTICE OF SUSTAINABLE MANAGEMENT OF FOREST RESOURCES IN SERbia: EXPERIENCE AND CHALLENGES

Vesela Radović, Larisa Jovanović

Abstract: Due to increased pressures and demands that are placed in front of forest ecosystems and resources needed to make efforts to prevent the adoption and implementation of the adverse decisions of other sectors (finance, economy, transport and others) that lead to forest degradation. Such activities are realized by using the mechanism of evaluation of environmental impact and improving inter-sectoral cooperation in resolving potential conflicts among stakeholders. The essence of the theory of sustainable multiple forestry is an equal priority of all forest functions. The principle of profit maximization is slowly changing into the principle of harmony and coordination in the use of forest resources.

Key words: sustainable development, natural resources management, forest resources, sustainable forest management

INTRODUCTION

Since the notion of sustainable development was popularized by the World Commission on Environment and Development Report (a.k.a. the Brundtland Report) in the late 1980s, both developed and developing countries have embraced, at least in theory, sustainable management of their natural resources. However, the practice of sustainable management of natural resources presents enormous challenges especially for developing countries because of excessive pressure to combat poverty, and weak programmatic and institutional capacity that makes it difficult to implement even well intended policies. The Republic of Serbia faces economic, social and political problems partly contributed by its civil war and ethnic conflicts in the 1990s. Its economic misfortune is further exacerbated by the current global economic recession. Nevertheless, there are reports of improvements in Serbia’s governance as an emerging stable democracy, and the country is striving to attain full membership in the European Union (EU). It is believed that EU membership would further enable Serbia conform to standards of good governance and stewardship of natural resources.

In 2008, Serbia adopted a national Sustainable development strategy with the objective of balancing economic development with social and environmental development, emphasizing the imperative of rational use of natural resources. A major segment of this strategy is to achieve sustainable management of forest through a sound protected area regime (15). Forests represent a significant environmental, economic and social potential Republic of Serbia (14).

National Biodiversity Strategy and Action Plan of the RS is pending adoption to take place in the first quarter of 2011 for period 2011-2018. Law on forests specifies that the forest resource of general interest that must be used in a sustainable manner so as to preserve and increase their value and usefulness, ensure durability and protection and is constantly improving growth and yield (16).

FOREST RESOURCES AND NATURAL PROTECTED AREAS IN SERBIA

Forests and woodland cover 28% of Serbia, 40% is arable land and 21% of land used as permanent pastures. Total forest area in Serbia is 2,360,400 ha and forest cover 26.7%, slightly lower than the average forest cover in Europe. Area state forests that are managed by public enterprises is 1,375,553 ha, which was 51.4% of all forests and forest land in Serbia.

The structure of the forest stands and tree species most common are pure stands of 64.3% of the area (59% deciduous and coniferous 4.7%), followed by mixed stands of sawmills with a share of 30.5% and conifer forests with about 6%. The total growing stock...
of broadleaved account for 90.7%. Of all the most common tree species are beech and oak. Beech forests are represented with 27.6% of the total area of forests, oak forests with 24.6%, other 6.0% hardwood, poplar 1.9%, other 0.6% softwood and mixed stands of broadleaved 30%, conifers with 6.0%, and mixed deciduous and coniferous forests 3.3%.

Forest seed origin account for 39.6%, 34.6% coppice, forest culture from 14.7%, 5.6% scrub and scrub 5.5%, which means that coppice and degraded forests cover an area of 45.7%.

Volume of timber estimated to be about 235 million m$^3$ and the total current increment is more than 6 million m$^3$.

The concept of sustainable forest management is fully implemented in forest management in protected areas (17). Support for this concept is achieved by defining a clear and balanced determination of priority functions of forests with respect to the economic possibilities of the state and needs of the population in the capital, rural areas.

Serbia has so far in various ways put under some form of protection around 543,000 hectares (6.1%) of the territory of which 95 national parks (Fruska Gora, Kopaonik, Tara, Shar mountains, and Iron Gate - Iron Gate), 15 nature parks, 50 severe and 21 special nature reserves, 284 natural monuments, 16 areas of outstanding features, 37 areas of cultural or historical significance, and 642 extremely rare. Natural goods on the territory of the Republik of Serbia (1.123) and 98 reservations of nature are included into National Forest Inventory of the Republik of Serbia (20).

State forests which represent half of all forests in Serbia. Public forest are managed by Srbijašume Vojvodinašume (93%), National parks (5.8%), JP-Borjak Vrnjacka spa (0.6%), Faculty of Forestry, University of Belgrade (0.4%) and various agricultural and water management organizations (1.3%).

Professional and technical jobs in private forests made JP for Forest Management and National parks. The basic unit of management in private forests has parcels.

The average plot size is 0.3 ha, which greatly complicates management.

Total growing stock of Serbia is 235 million m$^3$, a volume increment is estimated at over 6 million m$^3$. The average level of cuts in Serbia is approximately 2.5 million m$^3$ and level of afforestation in 2003. year was 3661 ha. Measures of care in state forests conducted annually at over 30 000 ha, and in private for about 15 000 ha.

Recorded damage to forests in 2003. year were related to: illegal felling of 16,720 m$^3$, other damages caused by man 2914 m$^3$, damage from insects, 10,384 m$^3$, of 8812 m$^3$ of natural disasters, plant diseases from 5690 m$^3$ and 37,520 m$^3$ of fire i.e. about 1400 ha.

According to the National Forest Inventory of the Republic of Serbia, finishing 2006 (published 2009), the forest includes all inventory units larger than 0.5 ha, covered with forest trees whose crowns cover more than 10% of the area, where trees must be able to reach the minimum 5m height at age of maturity for harvest (20). Of the total area of the territory of the Republic of Serbia 29.1% is under forest and other forest land, which belong to an international definition of scrub and scrub, including 4.9% of the territory, which is the total amount of 34.0% or 36.3% in relation to the productive land area of Serbia. Compared to the postwar forest area increased by about 1,000,000 ha. The total area of forests in the Republic of Serbia is 2,252,400 ha.(20). Since it is a state-owned 1.194 million hectares or 53.0%, a privately owned 1,058,400 hectares or 47.0%. Forest cover, compared to the global aspect, close to the world that is 30%.

**MANAGEMENT OF FOREST RESOURCES IN RS**

In the management of forests there exist public, private and nonprofit sectors:
- The public sector has a key role in preserving this natural resource.
- The private sector, regardless of what takes care of the restoration and protection, and works on innovations in the field of forestry in terms of technology, is focused on increasing productivity, profitability and new products.
- Non-profit sector consists of NGOs for the protection of forests.

Movements to protect forests in the world are focused on the problems of deforestation and lack of professional management in less developed countries and countries in transition. Modern management of forest resources requires education experts in the area of new forest technologies. Genetic studies, investigations of fast-growing trees and development of new methods for production of quality wood are necessary (8).

Management of forest resources, which is a complex complex ecosystem, must be oriented to achieve sustainable and rational continued alignment of natural and technology factors of production (1).

By various management practices the good qualities of forest communities can be enhanced, while the bad traits suppressed or restricted. In the management process harmful and useless trees are gradually removed, suppressed or weakened, while all economically and ecologically good sides are improved (10).

The different methods of cultivation and exploitation of forests are created to manage forests. Nurturing of selected trees and tree species, forming the crown and trunk so that they produce the best examples of wood for the settlement of certain economic needs.

The theory of sustainable forestry encourage development of new techniques of cutting timber and require to plant more trees during afforestation than they were cutted. It also requires a longer period between harvesting and forest regeneration.

Growing and exploitation of forest are compatible processes, because most works related to the forest are connected with cutting of trees. These processes must take place to maintain a better balance of the complex environmental factors and the prevention of forest as much to protect forest from the bad influence of abiotic, biotic and technogenic factors (4).

Management of forests resources is a permanent process of work and investment funds. The economic effect can be expected only after 40-50 years, and ecological effects after 3-4 years, so the state is obliged to allocate funds for expanded reproduction and to initiate the projects of afforestation.
Environmental education is an aspect of humanity's debt to nature. Our understanding of the usefulness of forests for mankind must be expanded to take into account many recently observed uses of forests (2).

Science must achieve unity in the assessment of consequences of technological development, to increase forecast accuracy and focus to stop side effects. It is necessary to incorporate principles and rules of environmental protection not only into national development strategy and investment in the economy, but also into awareness of the population (3, 5).

In the field of environmental protection Ministry of Agriculture, Forestry and Water Management (MAFWM) performs duties related to forestry policy. Forest Management, an administrative authority within the Ministry of Agriculture, Forestry and Water Management, performs specialized tasks related to: forestry policy, forest conservation, improvement and use of forests and wildlife, implementation of forest and wildlife protection measures, control of the seed and seedlings in forestry and other tasks specified by law.

Forestry Directorate as an administrative body within the Ministry, performs the tasks of state administration and professional activities related to: forestry policy; forest preservation; forestry policy; forest preservation; promotion and utilization of forests and wildlife; enforcement of measures to protect forests and wildlife; carries out other activities in this area.

The Directorate comprises the following units:
1. Division for Strategic Planning and Sustainable Development in Forestry and Hunting;
2. Section for Forestry Improvement

Provincial Secretariat for Agriculture, Water Management and Forestry has the jurisdiction over water management on the level of Autonomous Province Vojvodina. Provincial Secretariat of Agriculture, Water Management and Forestry is responsible for creating multi-annual and annual work plans of those services that are located on the territory of the Autonomous Province of Vojvodina, monitoring of these services, as well as their evaluation. Number of services that cover this territory is 12 (84 extension agents in total). The operation of these services is financed by the Provincial Secretariat of Agriculture, Water Management and Forestry.

Up to now the following steps to protect forest resources and rational management of them are taken:
- introduction of tax and investment policies in most countries,
- initiation of basic projects to protect and conserve forests worldwide,
- ongoing monitoring of the environment,
- education and training of professional staff and residents, as well as
- introduction of environmental standards.

CONCLUSION

The traditional attitude of people towards forests was reflected in the uncontrolled spending of wood, which led to the replacement of forests to agricultural land. Humans have recently been under the delusion that nature has the power of limitless regeneration and that it can absorb and apply all the negative consequences they caused. Natural resources are capable of reproduction and regeneration, but only to a certain extent, which, unfortunately, humans long gone. In the 20th century, we found that the true value of forests is not only the material that can be sold in international markets. Multiple benefit functions of forests received more importance with the development of environmental awareness (6).
Multiple interests included the increase in growing stock by afforestation of abandoned agricultural lands, construction of necessary road network and small and medium-sized plants for timber with easily changeable programs in rural areas are also sufficient grounds for establishing a lasting and stable, profitable system of financing of such programs, but also in the areas of tourism, recreation in the nature complemented with software that would be the valuation of natural, historical, cultural and other values of the area (13). Objective in Forestry Development Strategy is to create sustainable and economically efficient wood industry sector to be competitive on world markets and thereby contribute to the advancement of the forestry sector, environmental protection and competitiveness of the economy. In this goal is to create opportunities for starting the process of certification of wood products. Agricultural Development Strategy of Serbia (2005), contains elements of forestry policy as an inseparable part of rural development. For the Strategy is important preparation and adoption of the National Programme of Environmental Protection of RS, ie, local environmental action plans (LEAP).

Republika of Serbia is finalising the Programme for the Development of Forestry in Serbia 2011-2020. It is expected that the Programme will be adopted in 2011.

At the end of this paper we need to summarize multiple benefit and general social functions of forests:

- Protection of land from erosion, torrents and floods, wind erosion and prevention of drying wetlands;
- Impact on water regime and water composition (water treatment);
- Impact on soil fertility and agricultural production;
- Protection and improvement of the environment;
- Impact on the climate - control of air temperature, the impact on increasing atmospheric precipitation;
- Creating of oxygen (absorption of carbon dioxide);
- Slowing growth of microorganisms (fungi and bacteria);
- Herbs (tropical plants from which one receives many food products and drugs);
- Creation of favorable conditions for the rest, recovery and recreational tourism;
- The development of hunting tourism, etc.

Bearing in mind the importance of forests and the functions they perform, raising and reforestation (increase forest cover) is not only an obligation of ecology and forestry as a science which concerns the forests, but the state should have an active role in resolving the acute problems of deforestation. Due to the great importance of benefit functions of forests for the society, special attention and care to enhance forest ecosystem must be devoted (9, 11, 12).

Forestry sector in Serbia, considering the generally accepted principle of balancing economic, environmental, social and cultural functions of forests, can provide a significant contribution to the sustainable development of the Republic of Serbia.

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THE STUDY AND MAPPING OF BIODIVERSITY AROUND LAKE CUEJDEL NATURAL RESERVE

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Abstract: Cuejdel lake is the youngest natural barrage lake in Romania. Its birth determined the emergence of new environmental conditions resulting in a high biological diversity. A multidisciplinary study was necessary to investigate the biodiversity of Cuejdel Lake and its surroundings. This research aims to achieve a biodiversity database for Cuejdel Lake Nature Reserve together with a distribution map for each natural habitat and species of community interest and an extensive analysis of spatial scale distribution of habitats. We are also following the state of conservation of natural habitats and species of conservation interest within the area. So far a number of 161 plant species were recorded; the investigations on the animal biodiversity reveal 8 invertebrates species (Odonata and Lepidoptera Order) and 27 bird species.

Key words: Cuejdel Lake, natural reserve, biodiversity, landslides.

INTRODUCTION

Environmental factors directly contribute to how an ecosystem is born, organized and developed. Among them, landslides are an example of geomorphic disturbances that can produce important losses of biodiversity. Being seen only as a destabilizing factor, ecological role brought by landslides is often overlooked, but changing the initial conditions, they turn back the clock of succession, providing new places for different organism to colonize them [7]. Natural disturbances to plant communities are simultaneously a source of mortality for some individuals and a source of establishment sites for others [4]. In our case, a newly formed lake gives us the opportunity to investigate how new physical conditions could create such a well shaped biodiversity around Cuejdel Lake. Recently, through elaboration of laws on the natural protected areas regime, preservation of natural habitats, wild flora and fauna started in 2001 and continuing in 2004 through a government decision on creation of the protected natural area regime for new areas, the lake is a protected area of national interest - Cuejdel Lake Nature Reserve.

For all these reasons the objective of this present study is to show a descriptive account of biodiversity (vegetation, invertebrates and birds) around a newly formed natural landslide lake and to identify species or natural habitats of community importance, in order to create a management plan.

MATERIAL AND METHODS

Study site

Cuejdel lake is located in the southern part of Stânişoarei Mountains, a range of the eastern Carpathians, at an elevation of 665 m, with the nearby crest about 980 m (N 47° 2'25.25"; E 26°12'50.84"). The study area is about 21 km northern of the city Piatra Neamţ, the small villages of Cracăul Negru is located 5 km north. The lake covers an area of 12.2 ha, an average depth of 7.44 m and by its volume (907,000 m²) is the largest lake in the country in its category.

The area has temperate-continental climate, the annual average temperature is between 7-9.2°C and an annual rainfall 641 mm. The dominant soil is brown podzolic [11].

The geological substrate consist of Cretaceous deposits represented by layers of Sărata consisting of black clayey schist, siliciferous limestones, silicities, siderite marl-limestone and sandstone. The lake was formed due a landslide that began in 1978 which completely blocked the Cuiejdi river, a tributary of Bistriţa river. Nowadays, the current phase can not be considered completely stabilized, the phenomenon of landslide being able to activate again [8].
**Methods**

In order to quantitatively sample the plant communities, using the principles and methods developed by the European Central School Braun-Blanquet, we randomly selected sample areas (stations) along the lake and the adjacent zones. Fixed points were established where all species present were noted, evaluating the quantitative participation of the species. GPS coordinates, altitude and size of the sample were noted. The size of the sample was determined by the type of vegetation, thus surveys of 25-100 m² were conducted for the marsh and meadow vegetation and 400 m² for the forest. All plant species have been identified; nomenclature follows Ciocârlan [2].

For identifying birds community composition we used itinerary line transects in all types of habitats: forest, pasture and around the lake shore. We recorded all the bird species we saw or heard between the transect and 50 meters left and right. We estimated the size of the population according to the number of birds observed and habitat availability.

The preliminary research in the area did not target the invertebrate fauna around the lake, but a very brief inventory was made, based on photographic data, recorded along transects for avian fauna. The species identification and distribution were made using the keys from Askew [1], Cîrdei and Bulimar [3], Dijkstra [5], Niculescu [9], [10], and Tolman [16].

**RESULTS AND DISCUSSION**

Before the landslide occurred, the investigated area was entirely covered by forest. Nowadays, numerous tree stumps can be seen in the middle of the lake, giving the scenery an eerie look reminding of the old forest that existed here before the formation of the lake. But the event that sculpted the landscape 33 years ago generated favorable places for the installation of new habitats.

Preliminary results of the study reveal a well defined plant biodiversity, although the lake ecosystem is very young. 161 species were identified, some taxa present in the national Red List [12]: *Hepatica transsilvanica* (Ranunculaceae) – endemic for Eastern and Southern Carpathians; *Ranunculus carpaticus* (Ranunculaceae) – rare and endemic species for Carpathians; *Epipactis helleborine* (Orchidaceae) – rare.

The area adjacent to the lake is occupied by large beech forests (Dacian beech forest) in combination with other tree species. Tree layer consists largely of *Fagus sylvatica* ssp. *sylvatica* and *Abies alba* with specimens of *Acer pseudoplatanus*, *Carpinus betulus*, *Ulmus glabra*, *Fraxinus excelsior*. The canopy attains heights of up to 25 m for fir or for old beech individuals. Depending on local characteristics the tree layer can be formed only by fir. Shrub layer is represented by *Lonicera xylosteum* or *Corylus avellana*. Floristic composition of the herbaceous layer consist of *Paris quadrifolia*, *Pulmonaria rubra*, *Geranium robertianum*, *Glechoma hederacea*, *Asperula odorata*, *Oxalis acetosella*, *Mercurialis perennis*, *Brachypodium sylvaticum*, *Campanula abietina*, *Lamium maculatum*, *Sanicula europaea*, *Salvia glutinosa* and others; and in wet area of: *Chrysosplenium alternifolium*, *Stellatia aquatica*, *Caltha palustris*, *Petasites albus*, *Dryopteris filix-mas*. Species such as *Atropa belladonna* are installed almost dominantly on the right bank of the lake, looking upstream, fast occupying deforested areas, which represents a normal stage of succession to beech forest [6].

Along the lake a series of aquatic plant communities were identified. *Thypha angustifolia/Thypha latifolia* communities grow near the banks, where water depth is less than 50 cm. The predominant species are: *Thypha angustifolia*, *Thypha latifolia* with *Alisma plantago-aquatica*, *Sparganium erectum*, *Mentha aquatica*, *Juncus effusus*. 
Eleocharis palustris communities is a transition association from marsh vegetation to the mesophilic, preferring marshy places, where water level is not very high (less then 10 cm) [15]. Floristic composition includes species like: Mentha aquatica, Thypha angustifolia, Agrostis stolonifera, Lytrum salicaria and others.

Schoenoplectus lacustris communities are present only downstream occupying a relatively small area but with many individuals. The characteristic specie grows along with Phragmies australis, Rumex hydrolapathum, Eupathorium canabinum, Mentha aquatica, Lycopus europaeus, Solanum dulcamara etc.

Three odonate species (Order Odonata) and five butterfly species (Order Lepidoptera) (Table 1) were identified as follows:

Table 1. List of invertebrates species from Cuejdel and their protective status

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>IUCN Red List</th>
<th>Habitat Directive</th>
<th>National red list</th>
<th>Distribution in Romania</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Platycnemis pennipes</em> (Pallas, 1771)</td>
<td></td>
<td></td>
<td></td>
<td>In Romania it is widespread [1],[3],[5].</td>
</tr>
<tr>
<td>2</td>
<td><em>Aeshna cyanea</em> (Müller, 1764)</td>
<td></td>
<td></td>
<td></td>
<td>In Romania is distributed within the Carpathian arc [1],[3],[5].</td>
</tr>
<tr>
<td>3</td>
<td><em>Sympetrum sanguineum</em> (Müller, 1764)</td>
<td></td>
<td></td>
<td>Present on the entire territory of Romania with the exception of the steppic region [1],[3],[5].</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Pieris rapae</em> (Linnaeus, 1758)</td>
<td></td>
<td></td>
<td>In Romania it is a common species, being present with 2 subspecies [9],[16].</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Pieris napi</em> (Linnaeus, 1758)</td>
<td></td>
<td></td>
<td></td>
<td>In Romania it is a widespread species [9],[16].</td>
</tr>
<tr>
<td>6</td>
<td><em>Lycaena dispar</em> (Haworth, 1802)</td>
<td>NT</td>
<td>Annex II</td>
<td>VU</td>
<td>Widespread in Romania with the largest populations in Danube Delta, Transylvania and Banat regions [13],[16].</td>
</tr>
<tr>
<td>7</td>
<td><em>Polygonia c-album</em> (Linnaeus, 1758)</td>
<td></td>
<td></td>
<td></td>
<td>Common species in the entire territory of Romania [10],[16].</td>
</tr>
<tr>
<td>8</td>
<td><em>Argynnis paphia</em> (Linnaeus, 1758)</td>
<td></td>
<td></td>
<td></td>
<td>In Romania, one of the most common species in summer months [10],[16].</td>
</tr>
</tbody>
</table>

Lycaena dispar (Haworth, 1802) is the only specie that has a special protective status: near threatened (NT) species on IUCN red list and listed in Annex II Habitat Directive. The species has vulnerable (VU) status on national red list [14].

The lake and forest from Cuejdel is a good habitat for forest birds. We identified for this pilot study in September 2010 a number of 28 species, most of them being sedentary birds. Occasionally some migratory birds were observed. The time of the survey was at the end of the migratory period, and most of them already started their migration.

The most abundant species we recorded and map was the Common Chaffinch *Fringilla coelebs* and the European Robin *Erithacus rubecula*.

By their conservation status, only one species is protected under the Birds Directive and 8 of them are species of national concern according to OUG 57/2007 (Table 2)
Table 2. List of bird species from Cuejdel and their protective status

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>OUG 57/2007</th>
<th>Birds Directive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buteo buteo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Carduelis carduelis</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Certhia familiaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Coccothraustes coccothraustes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Corvus corax</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Delichon urbica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Dendrocopos major</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Dendrocopos syriacus</td>
<td>Annex 3</td>
<td>Annex I</td>
</tr>
<tr>
<td>9</td>
<td>Erithacus rubecula</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Fringilla coelebs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Garrulus glandarius</td>
<td>Annex 5C</td>
<td>Annex II/2</td>
</tr>
<tr>
<td>12</td>
<td>Loxia curvirostra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Motacilla alba</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Motacilla cinerea</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Nucifraga caryocatactes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Parus ater</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Parus caeruleus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Parus major</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Parus palustris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Phoenicus ochrurus</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Phylloscopus trochilus</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Pyrrhula pyrrhula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Regulus regulus</td>
<td>Annex 4B</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Saxicola rubetra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sitta europaea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Troglydites troglodytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Turdus merula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Turdus philomelos</td>
<td>Annex 5c</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS AND FUTURE WORK

Preliminary observations on the biodiversity of Cuejdel Lake was finalised with the identification of an important number of plants, invertebrates, birds species and habitats. This represented the first step of our study, to be followed by the completion of the species list for all groups, measuring the size and state of protected species population on site. Further studies are necessary in order to establish a database on biodiversity of the Lake Cuejdel natural reserve, a distribution map for each natural habitat and wild species of community interest and presenting the state of conservation of natural habitats and wild species of conservation interest within the area.

REFERENCES


ABOUT THE AUTHORS
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Abstract: The studies revealed that the abundance and distribution of the plant species on the surface of the cliffs (Brebului Gorges) is influenced by geological and geo-morphological characteristics of the cliffs. Most of the plant species are clonal developing specific growth strategies on cliffs, being difficult to establish the exact number of individuals. The micro-climatic factors acting on the Brebu cliffs are highly variable. Due to limited soil availability, micro-climatic extremes and water stress the plant species growing on these cliffs support physiological constrains and their stability is precarious due to the habitat instability.

Key words: Romania, Cliffs, Vegetation, Multidisciplinary research.

INTRODUCTION

Cliffs are usually considered lifeless and hostile, thus cliff researches are seldom performed around the world [13]. Cliff represent: primary habitats for many plant species; unique environment being able to support a specialized group of plants, many endemic or rare species; refuge for native flora and fauna [7]; low potential productivity habitat as core habitat for the wide variety of other species particularly sensitive to disturbance [8]; habitat for plant species not specifically adapted to cliffs [7] [13]. The plant communities growing on cliffs might be different from the surrounding areas or might be species from the neighbouring areas of the cliffs [13] [16].

The factors influencing the cliffs are: rock type and strength, climate and the processes of physical and chemical weathering (freeze-thaw activity). Chemical weathering of the rock is directly controlled by precipitation amount and chemistry, rock temperature and geochemistry. The rates of rock fall and rock particle size have a strong influence over the organisms that occur on the cliffs [12]. A very important factor is vegetation structure growing on rocks, their roots penetrating the rocks. It is not yet known the functional significance of the deep root morphology and architecture [5] [6] [18] and also their functional significance and their contribution to the whole-ecosystem processes [2]. The hydrological balance and the carbon and nutrient cycling of the whole ecosystem are greatly influenced by the depth at which the plants are able to grow roots [2] [14].

Due to their verticality and physical structure, cliffs represent one of the harshest and heterogeneous habitats for plants; the micro-sites favourable for vegetation development are quite rare. The way that the plant species adapt at the cliffs conditions are less understood yet. Nowadays is known that in the conditions of reducing available soil resources, the growth, survival and fecundity of plant species decrease but increase the competition underground [3]. On the cliffs walls there are usually fissures and crevasses where might be accumulated: soil, organic matter, water, thus forming a substrate suitable for plant growth. Once the fissures are interconnected they form a wide expanded network favourable for rhizomatous plant growth and spread [5] [11] [14]. Clonal plants successfully survive in this extreme habitat forming large vegetative individuals (genets) [15].

Most of the studies developed in areas with rocky substrate from Romania are reduced to general description of the flora and vegetation, aspects of distribution and conservation of some saxicolous plant species. On Doftana Valley have been performed studies regarding the diversity and primary productivity of hill beech forests [17] and geomorphology of the basin [1].
MATERIAL AND METHODS

Brebului Gorges (massif vertical cliffs cut in Inferior Miocene conglomerate) are located in the hilly area of Southern Romanian Subcarpathians on Doftana Valley (N: 45°12’31,1’’; E: 25°44’23,5’’ at 535 m altitude. Rock structure has a small and medium resistance to erosion and is characterized by the dominance of conglomerate intercalate with compact sandstone sandy and clay rocks, quartzite, micaschists, amphibolites, gneisses. Rock fall happens often because of the weakness of the conglomerate that forms the escarpment.

The main objective of the study (2008-2010) is to quantitatively sample the plant communities associated with different cliff expositions, structures and micro-climate. Depending on slope climbing accessibility (using ladder) have been established transects on cliff faces with 50-85 degree slopes, southern and northern expositions (including more humid N-W exposition vertical joint). On transects have been established 4 fix 1 m² monitoring plots, one at every 1.5 m up to transect height. Vertical cliffs are difficult to sample and requires special attention for safety. Plants records were performed monthly from May to August. Aerial shoots were recorded, together with coverage (%) of lichen and bryophytes. Establishing the exact number of individuals is difficult for clonal plants.

For microclimate evaluation, several EL-USB-2 Humidity/Temp USB data logger from Lascar Electronics were used. The loggers (setup to record the readings once 30 minutes) were installed at northern and southern cliff walls (at approximately 4m high) and in a vertical joint, between two conglomerate pinnacles (on the N-W slope). Data were downloaded once every 2-4 months to avoid loss (some loggers were not found again, due to rock fall, and/or local people or amateur climbers). Thus, a period of 21 decades (10 days interval) from 20.06.2008 to 20.01.2009 was entirely recorded for all three sites. For mezzo-scale characterization of the climatic factors are used data provided by regional Câmpina Meteorological Agency.

The relative numerical abundance of the plant species was calculated and similarity matrix of the transects based on Jaccard’s similarity coefficient. The species nomenclature followed [4].

\[ S_j = \frac{a}{(a+b+c)} \] where \( S_j \) – Jaccard’s similarity coefficient; \( a \) – number of species in sample A and B (cumulative); \( b \) - number of species only in sample A (not also in B); \( c \) - number of species only in sample B (not also in A).

RESULTS AND DISCUSSION

The slope and the micro-climate of the cliff surfaces from Brebu Gorges vary greatly. The average temperature variation from southern and northern slopes follows the average temperature variation on regional level (Figure 1), but the hourly temperature variation is high. The average temperature in the era of vertical joint (N-W slope) is lower than the average temperature of the two main slopes. Northern and southern expositions determine changes in species diversity and numerical abundance (Table 1). On the northern comparative with southern slope, the woody species dominance, size and projective cover is higher, *Hippophäe rhamnoïdes* being dominant. Saxicolous species present on Northern slope (transects N1-N10) are typical for boreal, subalpine and even alpine regions (*i.e.* Carpathians endemics *Sesleria heuflerana, Saxifraga corymbosa, Thymus pulcherrimus*). On southern slope (transects S1-S6) there are species typical for dry grasslands (*Melica ciliata*) and forests (*Brachypodium sylvaticum*). Similarity matrix (Table 2) highlights the great differences between the transects situated even on slopes with the same exposition. Landslides usually happen on slopes with 45-50 degree with a structured soil cover (Transect S4-S6) and even on steeper slopes, re-shaping the cliff surface and contributing
to the vegetation dynamic. On the top of the cliff were installed diverse woody species having small shrubs shape, remarkable being the rare and relict species *Taxus baccata*.

![Graph](image)

**Legend:** I – decade 1-10; II-decade 11-20; III-decade 21-30 (31)

**Figure 1:** Comparison between Brebu Gorges (three sites) and Campina Meteo Station of the average temperature’s variation in the time interval 20.06.2008-20.01.2009 (10 days period).

**Table 1:** Relative numerical abundance (%) of the plant species from different cliff slopes

<table>
<thead>
<tr>
<th>Species</th>
<th>Northern slope</th>
<th>Southern slope</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td><em>Asperula rumelica</em></td>
<td>0.58</td>
<td>0.69</td>
</tr>
<tr>
<td><em>Asplenium ruta-muraria</em></td>
<td>0.18</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Cnidium silaifolium</em></td>
<td>0.3</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Cytisus nigricans</em></td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td><em>Galium mollugo</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Hedera helix</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Hippophaë rhamnoides</em></td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td><em>Rubus caesius</em></td>
<td>0.18</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Salvia glutinosa</em></td>
<td>0.51</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Saxifraga corymbosa</em></td>
<td>0.69</td>
<td>0.92</td>
</tr>
<tr>
<td><em>Sesleria heuflerana</em></td>
<td>2.41</td>
<td>2.38</td>
</tr>
<tr>
<td><em>Stachys officinalis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Thymbus pulcherrimus</em></td>
<td>2.38</td>
<td>6.13</td>
</tr>
<tr>
<td><em>Valeriana montana</em></td>
<td>0.46</td>
<td>0.72</td>
</tr>
</tbody>
</table>

**Only on northern Slope:** species relative abundance < 0.08%: *Alnus incana, Asplenium trichomanes, Berberis vulgaris, Campanula sibirica, Chamecytisus hirsutus, Inula ensifolia, Mycelis muralis, Rubus saxatilis, Salix caprea, Solidago virgaurea, Sorbus aucuparia, Taxus baccata, Thuja occidentalis.*

**Only on southern slope:** species relative abundance < 0.2%: *Brachypodium sylvaticum, Convolvulus arvensis, Crataegus monogyna, Epipactis helleborine, Euphorbia cyparissias, Festuca gigantea, Fraxinus excelsior, Hypericum perforatum, Lathyrus niger, Melica ciliata, Pimpinella saxifraga, Populus tremula, Reseda lutea, Rosa canina, Salvia verticillata, Silene nutans subsp. dubia, Teucrium chamedrys, Verbascum sp.*

**Common to both slopes:** species relative abundance < 0.03%: *Campanula rapunculoides, Centaurea biebersteinii, Clematis vitalba, Cornus sanguinea, Erysimum odoratum, Galeopsis tetrahit, Sedum maximum, Taraxacum officinale*
Table 2: Transects’ similarity matrix based on Jaccard’s similarity coefficient.

<table>
<thead>
<tr>
<th></th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>N6</th>
<th>N7</th>
<th>N8</th>
<th>N9</th>
<th>N10</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>1.00</td>
<td>0.53</td>
<td>0.53</td>
<td>0.78</td>
<td>0.41</td>
<td>0.46</td>
<td>0.44</td>
<td>0.46</td>
<td>0.21</td>
<td>0.50</td>
<td>0.41</td>
<td>0.06</td>
<td>0.43</td>
<td>0.19</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>N2</td>
<td>0.53</td>
<td>1.00</td>
<td>0.42</td>
<td>0.19</td>
<td>0.32</td>
<td>0.50</td>
<td>0.41</td>
<td>0.45</td>
<td>0.29</td>
<td>0.36</td>
<td>0.37</td>
<td>0.06</td>
<td>0.40</td>
<td>0.26</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>N3</td>
<td>0.53</td>
<td>0.42</td>
<td>1.00</td>
<td>0.17</td>
<td>0.31</td>
<td>0.36</td>
<td>0.31</td>
<td>0.34</td>
<td>0.15</td>
<td>0.38</td>
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Some roots of *Hippophae rhamnoides* species, dominant on northern slope, can reach more than 3 m length (from up cliff to down reaching the soil from the cliff base). The aboveground biomass is reduced, the medium height of the shoots reaching 50-60 cm. *Thymus pulcherrimus* present on both slopes grow in small or big clumps distributed almost on the entire surface of the cliff.

**CONCLUSIONS AND FUTURE WORK**

The abundance and distribution of the plant species on the surface of the cliffs is influenced by geological and geo-morphological characteristics of the cliffs. All these factors act synergistic with heterogeneity of cliff surface, the degree of solid rock disintegration and climatic factors. Cliffs support plant communities amenable to physiological constrains due to limited soil availability, micro-climatic extremes and water stress, and provide unique environment for plant species adapted to these conditions. Future work will include: experiments regarding architectural plasticity and growth responses [9] [11] [12] of different plant species growing naturally on cliffs; studies using techniques from other disciplines [5] [8] [10], including molecular techniques [15], studies on plant relationship with micro-organisms, invertebrates, vertebrates (including human beings climbers) and environmental factors.

The driving forces (physical, chemical, biogenic) of rock degradation act simultaneously in different proportion and rate. The complexity of the process asks for multidisciplinary and multi-analytical approach including: geology, petrology, mineralogy, geochemistry and material sciences [12].

For all the challenges raised by cliff studies it is necessary a complementary and multidisciplinary research team.

**REFERENCES**


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AGRO-SYSTEM INFLUENCED DIVERSITY AND ITS VALUE IN HUNGARY

Szilvia Kovács

Abstract: Profit chasing in agriculture resulted in efforts to maximize profit, maximise croplands, and maximise subsidies. Have been doing this, the approach caused environmental degradation from the aspects of landscape and biodiversity. Diversity is an obvious and widely appreciated factor of the environment. The hypothesis is that we must face some degradation but it is still not irreversible, and there are methods or tools to be used parallel in order to set the value of diversity. The objective of this paper is to present the results of using a tool based on preference to set the value of diversity, which is not only one and has got its disadvantages to be applied. The research work focused on the influence of crop management in a region in Hungary which was known by the researcher and the respondents too. An effective intervention would last eight years and cost around 20 million euros at regional level, based on relevant literature. From this angle, the value of diversity expressed in monetary unit is in accordance with the respondents’ opinion about necessary sums meant to be spent on the issue. That is the current value of diversity by the respondents under the circumstances they live and sense the issues at the moment.

Key words: diversity, landscape, value, agro-system, crop management

INTRODUCTION

Agro-environmental programs in Europe focus on lighting the burden that agriculture represent on the natural environment. It is clear, however, that affects of agriculture are more severe in the areas where agriculture is one of the main sources of socio-economic system. This is also true for Hungary, where intensive agriculture feature most areas of the country. It is highly true, furthermore, for crop production that uses 58% of productive land in Hungary.

Applied technologies are very intensive which means a developed way of crop production and sometimes negative affects on the area itself at the same time. If crop production is in the focus, then those effects are external effects. External effects of technology coming from operations are far not stressed upon. A well known case is the wetlands’ case which are in danger all over the world (Kerekes at al., 1994; Kerekes and Szlávik,1999). Presently it occurs often that value of biodiversity has to be expressed in monetary units, but it also demands efforts because of value factors independent of use. There are tools developed and used, but they differ in applicability. Evaluation of such tools by Pearce at al. (1999) is available in order to embed them in practice. At the same time evaluation of agri-products has not embedded properly in practice, but some reports budgeting 20-30 million euros for a program of amendment using such a product and related to the theme are available (Felföldi, 2008a; 2008b).

To express external effect in monetary term, the beginning step is to be aware of the influence-response relations. Influences might appear in parallel or can build up one another, similarly to the generated responses of environment. To improve actual situation, it is suggested that a belt of plants around units of cropland should be applied to contribute to biodiversity and landscape improvement, assist game management, and enhance public satisfaction. To be as precise as possible, it is suggested that we take into account only clear responses (Felföldi, 2008a).

The hypothesis is that we must face some degradation but it is still not irreversible, and there are methods or tools to be used parallel in order to set the value of diversity. The objective of this paper is to present the results of using a tool based on preference to set the value of diversity.
MATERIAL AND METHODS

The research work focused on the influence of crop management in a region in Hungary which was known by the researcher and the respondents too. Driving factors of the research fit to the results of paper by Felföldi (2008a), who revealed influence-response relations by using an agri-product to improve environmental factors under the pressure of crop production. Values of diversity as results of the research work stemmed from using different aspects such as replacement, preference, and productivity. This paper shows the results of the work done using a tool based on preference only. A survey was made targeted at a generation of 20-24 years of age to reveal their preference to landscape and diversity. This generation is a 643000 head population, of which 100000 heads live in the related region. Out of this population it was 25000 heads that met the preliminary of the survey. The basic characteristic of the issues determined the group of respondents, since such respondents were needed who could realise the importance and influence of the issues, and were capable of thinking about a scale fitting to practical aspects. One of the practical aspects was that the time frame of the issues. Since the survey was on the expression of the value of landscape and diversity in money terms, therefore, there were needed respondents who would be able to earn the money they were willing to pay for landscape and diversity. People with BSc and MSc diploma were meant to meet the criteria before. Thus, respondents were searched for among students at high educational institutions. The sample provided us with 514 and 511 valid respondents related to issues of landscape and biodiversity, and relative cover, respectively.

RESULTS AND DISCUSSION

Value of landscape

To set the value of landscape, two questions were used in the survey. The first question was addressed to the issue that how much monthly sum per person the respondents consider to be paid for improving landscape degradation caused by intensive crop production. The second one was addressed to the issue that how much monthly sum the respondents themselves had been able to afford for this purpose.

Related to first question per capita figures a year are shown by figure 1.

![Figure 1. Sums to be spent on landscape a year per capita by frequency](image-url)
Considering the sum such a respondent lives on a month, which is 250-300 euros per month, the money they would spend on landscape is reasonable. This money is around 4 euros a month, which is the sum that two pockets of cigarettes charged for. The mean is 47 euros per year and the mode is 44 euros, which means that they could agree with spending a sum of 44 euros on landscape improvement.

![Figure 2. Affordable sums on landscape a year per capita by frequency](image)

Presently, however, they could afford some less money such as 32 euros in average (figure 2.). But the mode was only 22 euros, which means that they consider 22 euros as reasonable sum for such a purpose. Considering the sum such a respondent lives on a year, which is 3000-3600 euros per year, the money they could afford is acceptable.

**Value of biodiversity**

To set the value of biodiversity, other two questions were used in the survey. The third question in the survey was about biodiversity addressed to the issue that how much monthly sum per person the respondents consider to be paid for improving diversity degradation caused by intensive crop production. The fourth question in the survey also was about biodiversity, which was addressed to the issue that how much monthly sum the respondents themselves could presently afford for this purpose.

Related to the third and fourth questions, per capita figures a year are shown by figure 3. and 4., respectively.

Taking into account the same respondents, living on 3000-3600 euros per year, the money they would spend on biodiversity is also reasonable. The mean is 44 euros per year and the mode is 44 euros also, which means that they could agree with spending a sum of 44 euros on biodiversity improvement. Presently, however, they could afford some less money such as 31 euros in average (figure 4.). But the mode was only 22 euros, which means that they consider 22 euros as reasonable sum for such a purpose.
Diversity value at regional level

The targeted generation was of 20-24 years of age to reveal their preference to landscape and biodiversity. Out of this generation living in Hungary some 100000 heads live in the related region. Out of this population it was 25000 heads that met the preliminary of the survey, being potential money makers and tax payers. Figures related to them are shown in Table 1.

Table 1. Yearly value figures for the region

<table>
<thead>
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<th>Statistics for represented population (thousand EUR/year in total)</th>
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<td>landscape value</td>
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This represented population could budget 2,2 million euros for amendment of diversity from the angle of crop management, which might be the value of landscape and biodiversity calculated on a year base. Using a dynamic aspect, an effective intervention would last eight years and eight times yearly sum is 18,1 million euros. Nowadays they can afford only 1,5 million euros a year, which is 12,3 million euros in 8 years. That is the current value of diversity by the respondents under the circumstances they live and sense the issues at the moment.

CONCLUSIONS AND FUTURE WORK

This paper uses diversity as a two component feature of natural environment, since issue under examination here stems from intensive crop production effecting both landscape and biodiversity. The way of intervention taken into consideration will also affect both component such as landscape and biodiversity. During the survey, diversity was divided into those components to make it more understandable to and easier to be expressed in money terms by the respondents. The need for diversity is rather dynamic than static, thus expressing the value on year base allow us to calculate but the need to finance programs addressed to it rises year after year. In this paper setting the value of diversity is based on the sums set by the respondents as necessary money meant to be spent on the issue. Timeframe of such a program, taking into consideration current practice, might be 4-5 years, but the longer the better. A program of eight year can have significant influence. A program of amendment affecting both landscape and biodiversity that lasts eight years would cost 20-30 million euros at regional level, based on relevant literature. From this angle, the value of diversity expressed in monetary unit is in accordance with the respondents’ opinion about necessary sums meant to be spent on the issue.

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INVESTIGATION OF PHYSICAL PROCESSES TAKING PLACE ON THE OPEN CHANNEL’S AIR-WATER INTERFACE

A. Vaideliene, V. Vaidelys

**Abstract:** Steadily growing global pollution of atmosphere and open water reservoirs challenge researchers to search new techniques for mediums purification. One of the methods of water purification is stimulation of self-purification processes. The latest are highly dependent on the processes taking place in the air-water interface. The paper deals with the mathematical description of the processes acting in the air-water interface under conditions of turbulence. Mathematical simulation was based on the classical equations of diffusion.

**Key words:** air, water, aeration, diffusion, absorption, adsorption.

**INTRODUCTION**

The phenomena and processes taking place in the open water reservoirs interface between air and water are the investigation objects of researchers working in the large range of activity, including chemical, mechanical and environmental engineering. From the point of view of environmental engineering the processes of aeration is especially important, because the anthropological activity with its toxic pollution highly reduces natural amount of oxygen dissolved in the water. Toxic pollution continuously growing due too sour rains, chemical agents, streaming from cities and towns sewerage, and due too agriculture activity can be noticeable reduced through water self-purification process. Potential possibilities of this process i.e. on what value water can to resist pollution itself can be evaluated only under thorough investigation of air and water mass transfer, diffusion, heat exchange, passive impurities sedimentation processes and their correlation. These processes can be described with motion, heat transfer and passive impurities balance equations that are common for gas and fluids flows [1]. Some references physical processes in the air-water interface exclude as a most important [2-7]. We agree such a point and there examine the processes taking place in the air-water interface. All these phenomena are closely related with diffusion. For example, aeration and reaeration as well as evaporation and adsorption are based on diffusion phenomenon [8-10]. All these processes are solitary instances of diffusion dependent on media characteristics and boundary conditions.

Except of common nature features, typical for air and water mediums there are very specific features important only for nature flows. Particularly specific conditions are on air-water interface. Firs of all they are determined by water surface surging, roughness of water surface, increased surface friction, turbulence flows of kinetic energy in the water mass. The spectrum of natural turbulent flows is very large. It is produced by interferences of atmosphere that also act surface of open water.

Thus in the air-water interface the natural as well as artificial physical processes are continuously in the progress. Water quality of the natural water reservoirs (lakes and dams) and flows (rivers) most influences the gas transfer phenomena: aeration and reaeration. Therefore it is important them properly examine and describe mathematically. Though this problem is purely physical, developed mathematical dependences can serve as a good tool for the environment engineering.
MATERIAL AND METHODS

The goal of this paper was to describe mathematically diffusion process taking place in the open water reservoirs air-water interface under turbulence conditions.

Low solubility gases (such as a $O_2$, CO, CO$_2$, NO) transfer across air-water interface depends on resistance in the liquid side. This process is determined with molecules interaction and turbulence transfer. The sources of natural turbulence can be divided in to three main types (figure 1): surface shear turbulence caused by wind, turbulence caused by bottom shear in the rivers and similar flows and convectional turbulence caused by surface cooling (for example in artificial channels).

One of the most important factors acting in the turbulence transfers between air and water is wind. The wind higher as 3 – 8 m/s produces turbulence caused by shear [11]. River bottom caused turbulence (figure 1C) acts below air-water interface and stimulate diffusion in to surface. Thus bottom turbulence as well as surface turbulence influences diffusion process. Describing these processes mathematically with diffusion equations we evaluate gas transfer between liquid and air, and evaporation from water’s surface.

Oxygen transfer through air-water boundary

The rate of oxygen transfer through air-water interface can be written as follow [12].

$$\frac{\partial C_{O_2}}{\partial t} = V \cdot k_{O_2} \cdot M_{O_2} \left( C_{O_2, \text{gas}} - C_{O_2, \text{w}} \right),$$  \hspace{1cm} (1)

where $V$ – volume fraction of the water phase, $k_{O_2}$ – coefficient of oxygen volumetric mass transfer, $M_{O_2}$ – oxygen molar mass, $C_{O_2, \text{gas}}$ – oxygen molar concentration at gas – water interface, $C_{O_2, \text{w}}$ – oxygen molar concentration in bulk water. Rubisov and Papangelakis gave the mathematical expression for $k_{O_2}$ and $C_{O_2, \text{gas}}$:

$$k_{O_2} = 0.5667 \cdot e^{-\frac{500}{T_w \text{ surface}}}$$

where $T_w \text{ surface}$ – water surface temperature.
where \( P_{O_2} \) – partial pressure of oxygen, \( k_H \) – Henry constant:

\[
k_H = \frac{10^8}{\rho_w} e^{15.98 + \frac{1.58 \times 10^4}{T_{gas}} - \frac{4.33 \times 10^6}{T_{gas}^2} - \frac{3.50 \times 10^8}{T_{gas}^3}}
\]  

(3)

Where \( \rho_w \) – water density

Evaporation from water surface can be described with the equation as follow [13]:

\[
\frac{\partial C_{w,\text{evap}}}{\partial t} = \frac{2E}{2 - E} \left[ \frac{M_w}{2\pi RT_{gas}} \left( 1 - e^{-\frac{4\sigma M_w}{\rho_w RT_{gas}}} \right) \right] P_{sat} V_{gas} V_w,
\]

(4)

where \( E \) – evaporation coefficient, \( M_w \)– water molar mass, \( R \) – universal gas constant, \( T_{gas} \)– gas surface temperature, \( \sigma \) – surface tensor, \( V_{gas} \) – volume fraction of gas mixture.

Because both present – evaporation and gas circulation between air and water, we propose gas transfer through air-water interface describe as a gas and water diffusion:

\[
\begin{align*}
\frac{\partial C_w}{\partial t} &= \chi_{12} C_w - \chi_{21} C_{air} \\
\frac{\partial C_{air}}{\partial t} &= -\chi_{12} C_{air} + \chi_{21} C_w
\end{align*}
\]

(5)

Were \( C_w \) is gas concentrations of water phase, \( C_{air} \) is water concentration of air phase, \( t \) is time. \( \chi_{12} \) and \( \chi_{21} \) are diffusion rates of water phase gas and water, respectively, which are expressed as \( \chi_{12} = \alpha_{12} i_{H_2O} \) \( \chi_{21} = \alpha_{21} i_{air} \), where \( \alpha_{12} \) and \( \alpha_{21} \) are sticking coefficients of water gas and water from air phase to the surface, respectively, which range from 0 to 1 and \( i_{H_2O} \) and \( i_{air} \) are the relative fluxes of gas from water and from air to the surface, respectively.

Solution of differential equations (5), gives the following dependences:

\[
\begin{align*}
C_w &= \frac{\chi_{12}}{\chi_{12} + \chi_{21}} C_w(0) - \frac{\chi_{12} e^{-[\chi_{12} + \chi_{21}] t} - 1}{\chi_{12} + \chi_{21}} C_{air}(0) \\
C_{air} &= -\frac{\chi_{21}}{\chi_{12} + \chi_{21}} C_w(0) + \frac{\chi_{12} + \chi_{21}}{\chi_{12} + \chi_{21}} e^{-[\chi_{12} + \chi_{21}] t} C_{air}(0)
\end{align*}
\]

(6)

Where \( C_w(0) \) and \( C_{air}(0) \) – initial conditions of water and gas concentrations.

RESULTS AND DISCUSSION

Air and water diffusion process we simulated with equations (5) and solution of this equation with equation (6). For simulation we used experimental data published by H.Chanson, T. Brattberg [14], F.Murzyn, H.Chanson [15] and L.Toombes, H.Chanson [16]. These authors obtained experimental data in artificial channel made special for their experiment.
Fig. 2 illustrates the solution equations calculated for different initial concentrations [17] (Fig. 2). Range of initial water bubbles relative concentration we assumed as from 0.01 to 0.3 and air initial concentration from 0.7 to 0.99 (Fig.2).

![Graph](image)

**Fig. 2.** Air and water adsorption curves, with $\alpha_{12} = 0.8$, and $\alpha_{21} = 0.2$

As can be seen from figure 2, with water and air sticking to the surface probabilistic coefficients respectively $\alpha_{12} = 0.8$ and $\alpha_{21} = 0.2$, air and water concentration dependence on time changes according exponential law. Asymptotes values of all curves are equal to probabilistic sticking coefficients. The shape of curves depends on initial concentration of air and water.

**CONCLUSIONS**

1. A hydrodynamic and kinetic model of diffusion at air-water interfaces has been developed. These processes can be described with mathematical equations.
2. Character of proposed simulation equations solutions of air and water diffusion processes depends on initial conditions.
3. Solutions of proposed mathematical simulation equations of air and water diffusion processes show that in thin air and water layers these processes run according exponential law.

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THE NUMERIC DOMINATION OF LUMBRICIDAE POPULATIONS FROM TWO WOODLAND ECOSYSTEMS OF CÂNDEȘTI PIEDMONT (ARGEȘ COUNTY - ROMÂNIA)

Gheorghița Brînzea

Abstract: The purpose of the study was to determine the dominating lumbricidae from two different woodland ecosystems, as follows: a hardwood ecosystem, a spruce ecosystem and a meadow, all in the Cândesti Piedmont (county Arges-Romania). The worms have been sampled during two periods of time: March-October 2007 and March-October 2008. In order to determine the numeric domination of lumbricidae species from these examined sites, the following parameters have been taken into consideration: the aggregation level (λ), the dispersion level (i), the expansion level (E), the domination (D) and the full receptivity (R). It has been proven that the numeric domination cannot be known before knowing the aggregation, dispersion and expansion level of the lumbricidae species. Each species has its own role inside the ecosystem, fact which requires for the numeric domination to be determined for each single species, which, along with other criteria, draws attention on important aspects at biocenotic level.

Key words: Lumbricides, deciduous, spruce.

INTRODUCTION

The present study describes the lumbricid fauna of two forest ecosystems (deciduous, spruce) considered different in terms of vegetation, types of soil that influence the presence or absence of certain species of lumbricids in the soils of these ecosystems. Given that the area is geographically located in the region of high hills reveals a wide variety of flora, and the differences in height and average temperatures between the northern and southern territory, and the variety of landforms, exhibition and inclination of the slopes, lead to the formation of specific topoclimates, with their characteristic soils and differentiated vegetation [1], [2], [3].

For these reasons, the ecosystems under study may have a different specific composition of lumbricid populations, and indices of aggregation, dispersion, expansion and dominance can vary from species to species, illustrating the function of each species, with higher or smaller role within that ecosystem.

MATERIAL AND METHODS

The samples were taken from two different ecosystems (deciduous and spruce) from March to October 2007 and March-October 2008, in the village of Dobresti, Arges County (Piedmont Cândesti) with an area of 50/50m² each. There have been collected 10 sample units for each area (25/25cm) on levels from 10 to 10 cm depth to a depth of 40cm. Earthworms were extracted from each soil level and put in 9000 alcohols in labeled containers provided with tightly fitting lid. The containers were taken to the laboratory. Earthworms were identified according to the species level under the stereo microscope, using the Identification Device [4].

To highlight the numerical dominance of lumbricid species in the ecosystems studied, we used the methodology set forth by Debouche (1962), which involves a series of ecological indicators. These ecological indices are: aggregation index (λ), dispersion index (i), expansion index (E) numerical dominance index (D) and total responsiveness (R) of the species in the three ecosystems [5].

The aggregation index is dependent on the number of existing units and individuals' density in these units. It increases while decreasing the number of units and individuals density within the unit increases. When the whole population is united in one group, the index reaches the maximum value and increases only if the group increases.

The calculation formula for this index is:
where: $\lambda = \text{aggregation index}$, $s = \text{standard deviation}$, $m = \text{the average number of organisms in the sample}$. The aggregation index value may alter depending on the habitat conditions or ecological tolerance of species. Aggregation of lumbricid species is closely related to their dispersion, calculated using the formula:

$$i = \sqrt[3]{\frac{s^3}{\sum x}}$$

where: $\lambda = \text{aggregation index}$, $\Sigma x = \text{maximum possible aggregation}$.

The dispersion coefficient is a value whose ecological significance is highlighted only in association with the expansion degree of the species. The expansion index is obtained by multiplying the dispersion coefficient with density, resulting in dispersion of the whole population at a time. The formula for calculating the expansion index is:

$$E = m\left(1 - \frac{\lambda}{\sqrt{\Sigma x}}\right)$$

where $m = \text{average number of bodies}$, $1 - \frac{\lambda}{\sqrt{\Sigma x}} = \text{dispersion index}$.

The expansion index value is directly proportional to the size of a population. Dominance, is a parameter that expresses one or more species influence on the structure and function of biocenosis. It expresses the percentage ratio between a species number of individuals and the total number of individuals in a sample and is calculated using the formula:

$$D = \frac{n_A}{N} \times 100$$

where: $n_A = \text{species number of individuals}$, $N = \text{total number of individuals}$.

Numerical dominance is an expression of responsiveness to the habitat of certain species. In this respect, we calculated the overall responsiveness ($R$) representing the sum of all numerical dominants of species forming biocenosis for which we used the formula:

$$R = \sum D$$

RESULTS AND DISCUSSION

In the deciduous forest ecosystem, the aggregation index of lumbricid species during March-October 2007 had values between 1.19-3.09 (figure 1). The highest aggregation value was reached by *Octolasion lacteum* species in August (3.09), but the species reached high levels of this index in almost all the period analyzed. The aggregation index value was as follows: 2.75 for *Aporrectodea rosea rosea* species, in March 2007, 2.19 for *Allolobophora Leoni*, in September, 2.19 for *Dendrobaena alpina* in April and 2.57 for *Dendrobaena octaedra* in March. The remaining species inhabiting deciduous forest ecosystem had their aggregation index values ranging from 1.19 to 1.78.

From March to October 2008, (figure 2) *Octolasion lacteum* species had high values of this index and reached the highest value (3.07) in August as in the previous period. *Aporrectodea rosea rosea* did not exceed values higher than 1.97 throughout the period; higher values were recorded in 2008 for *Allolobophora caliginosa caliginosa* species in April (2.04), *Dendrobaena byblica* in August (2.52) and *Lumbricus rubellus* in October (2.82). The highest values of the coefficient of dispersion were observed for *Lacteum Octolasion* species in 2007 and 2008, but the species *Aporrectodea rosea rosea* had higher almost constant values of this index in 2008 (0.56 - 0.80), throughout the period.
The expansion index of lumbricids in the two periods analyzed (2007-2008), varies from month to month depending on the density achieved by each species, but higher values of this index were observed for Octolasion lacteum, Aporrectodea rosea rosea species in the two years. Other low density species achieve small expansion in this ecosystem: Allolobophora leoni (April 2007, 0.10), Allolobophora dacica (October 2007, 0.04), Dendrobaena alpina (September 2007, 0.06), Dendrodrilus rubidus rubidus (April 2007 - 0.09).

In 2008, as expected, lumbricids expansion in the deciduous forest ecosystem had higher values due to their higher numerical density recorded in this period. The dominant deciduous forest species in 2007 and 2008 were Aporrectodea rosea rosea and Octolasion lacteum which had high levels of numerical dominance index. Species that reached levels higher than 10% were observed only in certain months: Allolobophora caliginosa caliginosa (25% in October 2007, 50%, 11.36% - March 2008), Dendrobaena byblica (11.11% - April 2007 ) Allolobophora dacica (18.86% - May 2008), but these species cannot be dominant in this ecosystem during the two years. Most species had numerical dominance index values between 2.5 and 9.09% (subdominant species) and 1.21% - recedent species).

In the spruce forest ecosystem in the period March-October 2007, the aggregation index of Aporrectodea rosea rosea and Octolasion lacteum was high throughout the study period, ranging from 1.52 to 3.39 in Aporrectodea rosea rosea species and from 1.11 to 3.49 in Octolasion lacteum. Higher values of this index were met with Allolobophora caliginosa caliginosa (2.20) in October and Dendrobaena byblica species (2.19) in May, (figure 4).

From March to October 2008, aggregation of lumbricid species was much higher compared with the period March - October 2007, (figure 5). There was an aggregation increase in Dendrobaena byblica throughout the study period of 2008 in the spruce forest ecosystem, with values ranging from 1.26 to 3.09; Dendrobaena octaedra species with values between 1.26-2 , 27 and Lumbricus rubellus species (1.26 to 2.19). Unlike the previous year (2007), Octolasion lacteum species had lower values of aggregation index in 2008 which did not exceed 1.89 throughout the study period. Only in Aporrectodea rosea rosea species, aggregation was observed throughout the study period, but values of this species did not exceed 2.90, compared with 2007.
The dispersion coefficient values in spruce forest ecosystem did not differ greatly from species to species and month to month in the two study years, but the expansion index showed high values for *Aporrectodea rosea rosea* and *Octolasion lactum* species. If the expansion index of *Aporrectodea rosea rosea* had values ranging from 0.08 to 1.07 in 2007, the highest value (1.07) being reached in September, the values increased the following year ranging from 0.68 to 4.10, the highest value being reached in July, while in August and September, the index values were 3.23 and 3.21 respectively.

The analysis of numerical dominance index of lumbricids in the spruce forest (2007), revealed *Octolasion lactum* and *Aporrectodea rosea rosea* as eudominant species. We also found that *Aporrectodea rosea rosea* species had low values of numerical dominance index (2, 5 and 4.65) in April and May, which made it a subdominant species. *Allolobophora dacica*, *Allolobophora caliginosa caliginosa* and *Dendrobaena byblica* species, reached values that place them as eudominant species in certain months of 2008. *Dacica Allolobophora* species was eudominant in September, *Allolobophora caliginosa caliginosa* in October and *Dendrobaena Byblica* was eudominant in April and October and dominant in May. The remaining species were recedent and subdominant.

From March to October 2008, the number of eudominant species in the spruce forest ecosystem increased compared with the previous period. Therefore, in addition to *Aporrectodea rosea rosea* and *Octolasion lactum* species which are eudominant throughout the period analyzed in 2008, there appears *Dendrobaena byblica* species with dominance index values which placed it as eudominant in May, June, July and October and dominant in March, August and September. Of the nine species identified in the spruce forest ecosystem in 2008, only two species were subdominant *Dendrodrilus rubidus rubidus* and *Octodrilus lissaensis*; the remaining were eudominant and dominant.
Analyzing the overall responsiveness in the two periods analyzed (2007-2008), we noticed that it was nearly 100% in all months of the period analyzed, with the exception of March 2008 when responsiveness was only 21.85%. In terms of overall responsiveness to lumbricid species of deciduous forest ecosystem, the index values ranged between 90.31% - 100% in 2007 and 86.34% - 99.98% in 2008 (figure 6).

CONCLUSIONS AND FUTURE WORK

The analysis of aggregation, dispersion, expansion, dominance and responsiveness indices in the biotopes studied during 2007 and 2008, revealed that Aporrectodea rosea rosea and Octolasion lacteum were the key species (eudominant) with higher values of these indices in the two periods analyzed; they fulfilled the main functions in relations established between the various biocenoses. Stronger aggregation of species existing in the biotopes in certain months, showed the existence of a smaller number of points with the best food and physiological conditions. We also concluded that a high density population achieved a higher degree of expansion, which gave the species a determining role within the biocenotic complex.

The dominance index values of Allolobophora caliginosa, Allolobophora dacica, Dendrobaena byblica, Dendrobaena octaedra, Lumbricus rubellus, Lumbricus terrestris, with some position variations in the biotopes, form the top side of the general picture of numerical dominance, followed by other species, each with its higher or lower value, which gives them a precise position in the ecosystem. In terms of overall responsiveness to lumbricid species, the biotopes show higher values of this index, slightly but not significantly lower in the spruce forest biotope.

REFERENCES


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THE REQUIREMENTS OF DIETARY GUIDELINES AND THEIR IMPACT ON CONSUMERS

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Abstract: The food style is polarized between food consumers who maintain a sustainable health (healthy eating behavior) and satisfaction and pleasure in the moment of food consumption (appetite behavior), even if all consumers are aware of the relationship between proper diet and health. This paper make a comparative analysis of dietary guidelines from several countries (UK, USA, Canada, Chile, Greece and Romania) made by nutrition professionals as a reference diet for a healthy diet in the population of those countries. We will try to identify whether these dietary guidelines are compatible in terms of the two aspects mentioned for the consumers. In these pilot study, based on student perceptions specialized in public nutrition, we found significant differences in terms of attractiveness of the dietary guidelines as follows: attractive (Greece, Chile), unattractive (Canada, UK).

Key words: dietary guidelines, healthy diet, healthy eating behavior, appetite behavior.

INTRODUCTION

The alimentary style represents an ensemble of conceptions and behaviors that characterizes the satisfaction ways of food needs of the organism for its normal function. The factors that affect the alimentary style are [1]:

- the human organism by its genetic, physiological, psychological, age, sex, weight, health characteristics, etc;
- life style, especially the work, financial resources that are available, the way free time is used (sedentary or physical activities);
- culture and civilization level, religion, traditions, determine what aliments are consumed and how are they consumed (religious holidays determine the consume of specific aliments);
- ways used to satisfy food needs: dishes, tableware, glasses, napkins, tablecloths, equipment to create a nice ambient – furniture, floral ornaments, music, etc;
- the sources of acquiring the food distribution in populous zones (alimentary stores, supermarkets), the semi-prepared food existence and food already cooked in this stores, the quality of products and services in public alimentary units (restaurants, fast-foods, patisserie, sweets shop).

The appetite behavior (emotionally concerning food) is influenced by the pleasures and satisfactions obtained by direct sensorial taste at the foods consumption (smell, taste, flavor, color, consistence) [2]. The factors that determine the attraction and preference of the consumers regarding foods are [3]:

- psychological factors: the needs motivation, needs and desires, cognitive perception, studying, attitudes;
- cultural factors: culture as social ambience, ethnical or regional culture segments;
- social factors: membership and reference groups, social, environmental and professional categories.

From the factors mentioned earlier, the psychological factors has a major incidence on the consumer behavior, being the most hard predictable for those who analyze the consumer’s behavior.
In a study made by Rozin [4] regarding the population’s attitude on foods, study made on subjects groups adults and young from 4 countries (SUA, France, Japan and Belgium), had in plan the following aspects:

- bond between diet and health;
- attitude regarding the aliment (satisfaction, pleasure, repulsion);
- the consumption degree of foods that are considered healthy (products with low content in salt, sugars and fats);
- tendency of the association food with nutritional and energetic value;
- healthy diet appreciation;
- importance of the aliment, as positive force in life.

Rozin’s research showed the following conclusions:

- women from all groups and men from SUA group are oriented on a healthy diet;
- men from Belgium and France are preferentially oriented on foods that give satisfaction and pleasure;
- men from Japan group have a middle attitude having as preference a diet that give them health and pleasure at the same time.

At the question: “If they consider their selves healthy consumers?”, paradoxically, with the exception of american subjects, all other consider that they are healthy consumers.

MATERIAL AND METHODS

First, we make a comparative analysis of dietary guidelines from several countries (UK, USA, Canada, Chile, Greece and Romania) made by nutrition professionals as a reference diet for a healthy diet in the population of those countries. The food style is polarized between food consumers who maintain a sustainable health (healthy eating behavior) and satisfaction and pleasure in the moment of food consumption (appetite behavior), even if all consumers are aware of the relationship between proper diet and health. Secondly, we tried to find students perception on the attractiveness of dietary guidelines from the 6 countries. We mention that the study is make from 40 students, in the third year of study, specialized in Public Nutrition at the University of Craiova and „Aurel Vlaicu” University, which have participated at the Nutrition courses. After students have analyzed the diet of each guide, they were questioned about the attractiveness of the dietary guidelines for six countries and asked to rank them: rank 1 for the most attractive food pyramid, rank 6 for the less attractive food pyramid.
RESULTS AND DISCUSSION

To come in help of consumers that are oriented on a healthy diet, in the majority of countries in this world were given documents called “dietary guidelines” from the side of nutritional and food safety experts. The essential message of this diet guide is that a healthy diet must contain different foods, to be well equilibrated and in the same time moderate [5]. These diet guides are addressing to population under colored graphics of different construction (pyramids, trays, scales, rainbow, etc.) which must contain visual messages and compressed information but they have to be enough to understand [6].

In the below figures are presented different guides of diet specific to culinary habits, but also the healthy alimentation desiderate from many countries:

In the Great Britain, The Eatwell Plate (Figure 2) is based on the five commonly accepted food groups. These are: Fruit and vegetables; Bread, rice, potatoes, and pasta; Meat, fish, eggs, beans, and other non-dairy sources of protein; Milk and dairy foods; Foods and drinks high in fat and/or sugar. The Food Standards Agency from Great Britain recommends foods from the two largest groups (fruit and vegetables, and bread, rice, potatoes, and pasta) should be eaten most often; foods in the next two largest groups (meat, fish and alternatives, and milk and dairy) should be eaten in moderation; and foods from the smallest group (those containing fat and/or sugar) should be eaten least often.

Figure 2. British diet guide represented by The Eatwell Plate, 2010, http://www.diettrific.com/2007/04/16/balanced-nutrition-the-facts-revealed/

Figure 3. Alimentary diet guide in SUA, Dietary Guidelines for Americans, 2010, http://www.mypyramid.gov/pyramid/

SUA’s diet actual guide (Figure 3) is represented by a pyramid with six aspects (2010):

Grains group (orange, first fascicle) made from wheat, rice, oats, cornmeal, barley or another cereal grain, bread, pasta, oatmeal, grits is a grain product.

Vegetables group (green, second fascicle), Fruits group (red, third fascicle) and Milk group (blue, fascicle five) are the most representative. All fluid milk products and many foods made from milk are considered part of this food group. Foods made from milk that retain their calcium content are part of the group. Most dairy group choices should be fat-free or low-fat.

Oils group (yellow, fascicle four) is very slow represented. Oils are fats that are liquid at room temperature, like the vegetable oils used in cooking. Oils come from many different plants and from fish. Most oils are high in monounsaturated or polyunsaturated fats, and low in saturated fats. Solid fats are fats that are solid at room temperature, like butter and shortening. Protein foods group (violet, fascicle six) is the same very slow represented. All foods made from meat, poultry, fish, dry beans or peas, eggs, nuts, and seeds are considered part of the protein foods group. Most meat and poultry choices
should be lean or low-fat. Fats, oils occupy the least level of the pyramid, being recommended in a sober consumption.

Canada’s diet guide (Figure 4) is represented by a four level rainbow. The biggest share of the rainbow is represented by cereals, being followed in order by vegetables and fruits, milk products and finally meat and derivates. Canada’s diet guide is similar in essential points with SUA’s diet guide, this similarity can be placed in on the culinary similarity traditions in the two states.

Chile’s diet guide (South America, Figure 5) is represented by a five level pyramid. At the pyramid basis are cereals, potatoes and fresh vegetables. Fruits are on the second level together with preserved vegetables. The third level of the pyramid is divided by milk products, meat and eggs. At the four level of the pyramid are oils, fats and raw seeds (pumpkin, sun flower). On the last level is sugars and sugar products, being recommended a moderate consumption of these products. Chile’s diet guide is similar with SUA’s diet guide, but in Chile, fats and sugars are on different levels of the pyramid (sugar is considered to be more harmful comparing with oils and fats).

Interesting is the Greece population’s diet (Figure 6), on the basis which are the following principles: variety, moderation, proportionality, equality between ingested energy and spent energy in human being. From the recommended foods to be consumed a lot are vegetables and fish. From the other presented diet guides, the Greek’s diet guide does not forbidden to much the eggs consumption, poultry, potatoes, sweets and reject just red meat. The Mediterranean Diet is grounded on the principles of enjoyment and pleasure. Foods, drinks and meals are best eaten with others, when possible, and savored.

In Romania the Food Pyramid (Figure 7) is structured at 6 levels. We should mention a few special features: water consumption and weight control as the base of the pyramid (First level and Second level) and limiting consumption of pasta, sweets, red meat indicates at Level six: Red meat, butter (little and often) and White rice, white bread, potatoes, pasta, sweets (little and often). Third level indicates: Whole grains and vegetable oils: olive, sunflower, corn, etc. Level four indicates: Fruits and vegetables (2-3 times a day), beans, peas, beans, chickpeas, etc. and Fruits (1-3 times per day): walnuts, hazelnuts, almonds, etc. Level five indicates: Dairy products and calcium (1-2 times per day) and White meat and fish, eggs (0-2 times per day).
Unlike other dietary guidelines last two offers clear proportions on eating certain foods. It is difficult to determine for a consumer which products can cover dietary recommendations, because most often we use food products derived from basic foods.

All analyzed diet guides are in harmony with the conceptions and alimentary behavior of populations from the origin countries and they have the task to guide the population towards functional aliments consumption, that protect the health on long term. Whatever the culinary habits, all diet guides put cereals on the first consumption level, being followed by fruits, vegetables, lactates, meat and finally sugars and fats.

Statistical processing was done with SPSS13. We recode the data: rank 1 was given to ranks interval [1-3] and that means the most attractive diet and rank 2 was given to ranks interval [4-6] and that means the least attractive diet. In terms of attractiveness for consumers (Romanian students), significant differences were obtained in the following diet guide (Chi-square): Greece (90%) and Chile (80%) have the most attractive diet. Regarding the lack of attractiveness were significant differences obtained from these dietary guidelines: Canada (87.5%) and Great Britain (77.5%).

Table 1. Comparison of the 6 country food guides. Romanian student’s perceptions of attractiveness for the 6 dietary guidelines.

<table>
<thead>
<tr>
<th>Pyramid Dietary Guidelines</th>
<th>Most often consumed</th>
<th>Moderation consumed</th>
<th>Least often consumed</th>
<th>Attractiveness The most</th>
<th>The least</th>
<th>Chi-square Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Britain</td>
<td>Fruit, Vegetables, Bread and cereals</td>
<td>Milk and Meat Fish</td>
<td>Fat Sugar</td>
<td>22.5%</td>
<td>77.5%</td>
<td>12.10 Significant p&lt;0.01</td>
</tr>
<tr>
<td>SUA</td>
<td>Grains group, Vegetables group, Fruit group, Milk group</td>
<td>Protein foods, group: meat, poultry, fish, dry beans or peas, eggs</td>
<td>Oils group</td>
<td>65%</td>
<td>35%</td>
<td>3.6 Insignificant p&gt;0.05</td>
</tr>
<tr>
<td>Canada</td>
<td>Cereals</td>
<td>Vegetables, Fruits, Milk products</td>
<td>Meat and derivates</td>
<td>12.5%</td>
<td>87.5%</td>
<td>22.5 Significant p&lt;0.01</td>
</tr>
<tr>
<td>Chile</td>
<td>Cereals, Potatoes, Fresh vegetables</td>
<td>Milk products, meat, eggs and oils, fats and raw</td>
<td>Sugars and sugar products</td>
<td>80%</td>
<td>20%</td>
<td>14.4 Significant p&lt;0.01</td>
</tr>
</tbody>
</table>
Dietary guidelines are recommendations for healthy eating behavior, but they sometimes come into strong conflict with consumer’s food habits and preferences. In this case, habits / food preferences of Romanian students seem to be in accordance with the proposals of dietary guideline from Greece and Chile.

CONCLUSIONS AND FUTURE WORK

In context with the other diet guides, on the basis of analyzed diet guides and in the vision of the students take in the study, we can conclude that the Greek’s diet guide is the one that suits better with satisfaction of desires expressed by consumers (the right aliment is a pleasure source and also a health source). It seems that there is a preference for less restrictive diets and covering a wide variety of products consumed frequently. Diet of Chile for example is restrictive regarding sweets, but extremely flexible for other common products. Unlike the all diet guides, Greek’s diet guide is the one that has fewer restrictions, offering satisfaction and pleasure to the consumer.

This is a pilot study, but it would be very interesting to identify why a particular diet is preferred/ rejected and also a study to include the perceptions of students from different countries on these dietary guidelines.

REFERENCES

INFLUENCE OF MEDIA ON EDUCATION IN THE GLOBAL ENVIRONMENT

A. Langović-Miličević, T. Cvetkovski, Z. Langović

Abstract: This paper focuses on the influence of media on education in global environment. In the era of information, there are all the time more proponents of the idea that there is crisis of education. Development of new technologies, and media as well caused great changes in education. When we wonder whether media educate or not, we can agree with the view that the contents educate, but not media. Development of new technologies, and media as well caused great changes in education. Learning due to media begins to look like self-activity, individualism, communication and community. Teachers' role is changing and they become mentors and active partners in conversation with students about the contents in media which helped students gain knowledge.

The main problem in the study of mass communication is the change occurring in the area at an incredible rate, and most of the results obtained from researches become obsolete too quickly. The fact is that mass media influence the direction of human development. Additionally, although we are not always able to find the cause and consequence of each individual case, there is a cause - effect relationship between human behavior and exposure to media.

Key words: media, global environment, culture, education.

INTRODUCTION

The form "Media Education" began to be used in the sixties in the international circles that are engaged in the research of the problem of education, especially in the circles associated with UNESCO. During this period, experts' forecasts were focused on the apparent explosion of mass communications, especially television. Without any particular order the following topics were considered, among which we mention only the most common - the power of the new magical device for literacy of the broad masses, which were not included by the existing educational structures and qualified personnel, teachers reluctance to accept television as a legitimate access to the bringer of knowledge, the necessity of critical assessment of the dangers of media manipulation. In all these cases, it seemed necessary to acquire knowledge that would enable an impartial consideration of these issues. But out of a variety of meanings the dominant one sticks out and it is still present on the international level. Sometimes the media are distributors of information, sometimes they are just resources that are shaping our attitudes and opinions, which convince us, and sometimes even the means of socialization. Research in the framework of formal education is still limited. Thanks to the media we spontaneously learn about art, travel, history, science. Our informal learning takes place before, during and after our training. Most of us have learnt about animals, plants and various open spaces just from TV shows, books, magazines, and children learn from the Internet. That way the media keeps us emotionally and intellectually alive and active.

EFFECT OF MEDIA ON EDUCATION AND MEDIA EDUCATION

By "media education" we mean acquiring the ability for critical reading of the media, whatever the type of media. The aim is to reduce the distance from the media by understanding their functioning and learning about their content. Educational media obviously have a different function. And their definition implies specific problems. Where does the sense of urgency required media education come from?
1. high consumption and a plethora of media to which we reach
2. ideological significance of the media, especially commercials
3. emergence of information management in enterprises
4. increasing penetration of media in democratic processes
5. growing importance of visual and information communication in all fields
6. expectations of youth to be educated so that they can understand their good sides
7. national and international growth of the privatization of information technology [21]

When education is organized this way, without the hassle we understand that it becomes a battle for the adoption of some democratic ideas.

In an article three of the British researchers noted as follows: "Like any new enterprise, and media education in Britain was marked by excessive demands on the objectives to be achieved. It is often claimed that it can lead to profound political changes, both in students and in school programs. The militant fervor is not bad in itself, but not teachers, responsible for media, all the more convinced that such a completely ill-founded claims, and that the teaching and study of the media put a lot more problems than they would their counsel would like to believe."

Conceptual phrase "media education", which includes access to the study of some of the general media environment of modern times, is most often associated with media literacy (in the sense of elementary education) and education for the media (the training of certain professional profiles).

"Media Education" can be truly successful only if we take into account the relationship between two key factors specific (given) the media and the way they understand the specificity of the learners.

Media education cannot be achieved without parallel processes of education concerning the promotion of universal cultural values, which together with the true democratic process, helping not only the survival of culture and core values of the civil world, but also the foundations of humanistic values. News media education should not only mean the process of learning about the media, which includes direct training for the use of new communication means, but it should also include all other processes of mediation communications, which affect not only the culture of mass media, but also the kind of socialization, which contributes to survival and development of world culture, observed not only in the context of media, but also on humanistic grounds built systems thinking, learning and assessment.

It should be noted that the one who makes use of mass media without their understanding will be used by their masters. Underdevelopment of an adequate education is not random and it completely fits in the visible problem - the general crisis of liberal education throughout the world. Modern states are more happy with a responsible individual functionally adjusted to the economic mechanisms rather than an independent and critical citizen. In contemporary society, media systems and educational systems are located and they interact. This means that they influence each other and to two-way exchange of values and consequences of actions.

Defining the term education often one takes into account education. Today it is accepted that the school cannot just educate, without upbringing. There are lots of parents who feel that they are only able to commit to the education of their children, and that it can never be acquired at school. For Freud education is actually a "failure" because it tends to incompatible goals. So it is necessary to keep from mixing - the school as an institution, the source of strength and weakness, and teachers. They are brokers. Basically, they should attempt to satisfy the conflicting wishes of parents and schools, and to consider the wishes of children. First of all they are employees of the institution that is their employer.

Dominant position of democratic societies focuses on child development and the blooming of its quality. There is no little doubt that the traditional concept that education is looked on as the introduction of children into accepting certain norms of social class, is still present. It also explains the persistence of some apparent contradictions.
Educated population is much less a subject to the influence of the media. It obtains its information primarily from the Internet because it has a much larger and more diverse selection of information, than on TV, where information primarily suits the owners, in order to achieve higher profits. However, excessive consumption of media (especially internet) now, can lead to distancing from each other and from ourselves. You need to learn how to rationally use them and gain only the best of them. Mostly working class spend their free time watching TV, and therefore, they start to believe in all of its contents and it becomes an easy target of manipulation. Intellectual class people would rather go to the theater, exhibitions, read quality books and thus they are less subject to media influence. Educated population does not take all the information for granted, but they know which ones to adopt and which to avoid in a wide arc.

SOCIALIZATION, FORMAL AND INFORMAL EDUCATION

When we ask whether the media are educated or not, we agree with the view that certain contents educate, but not the media in general. Development of new technologies, and the media, brought great changes in education. Learning thanks to them begins to look like self-activity, individualism, communication and fellowship. The teacher's role is changing and they become mentors and active partners in conversation with the student about the contents from which he gained knowledge through the media.

All knowledge is mediated by the media today. Great strides have been made in the implementation of media education in terms of the use of modern technology. Having access to the media in the educational process, without clearly marked ways to use media in the process, brings more problems than benefits, because the teacher is left to himself. The most important part in this process is educating teachers to use information and communication strategy, or it comes to education in the media literacy.

The experts, who see the danger of new technologies for learning, explain that schools are at risk, because they are not oriented to the new technology, which actually means you are going too slowly to meet the future. The attitude that other media, especially printed, are very important for education has more and more supporters. Schools should show how to actually operate with the new media, transferring knowledge through them.

In the process of learning each individual, using and mixing different media is very individual. However, some general trends are visible in all functions of education. This is all the more affecting work habits, health, energy, home environment, heredity, individual activities, families, schools, teachers, and of course the media material used for these purposes. Some of the reasons why the broadcast media do not use computers in schools are the following: teachers are traditionally focused on printed materials. Only recently teachers have started to use electronic material for teaching. In the society there is a general view that electronic media are used more for entertainment than for education, most of the available material has low quality that broadcasters demand from it, decisions that introduce electronic media generally are made at the administrative level, and it creates even more resistance from teachers, especially if they have not undergone proper training, electronic media are very expensive, unless you are not used in training a large number of students, content of electronic materials is a major problem. [5]

Developing media literacy in children is different from country to country. The term media now mostly includes television, radio, print, internet, and in most cases it is forgotten that books are also mass media, that supports the media. So it's not good to deprive media from modern information technologies, and it is also bad to reduce the knowledge about the media to information technology only. Switzerland is an example of
how to ignore their own media content in favor of modern media classrooms. Here, then, we talk about computer education, and not the education for the media. Here, special educational TV series, treatment and dramatization of literature and various other facilities are used to learning in schools and at home.

It is not easy to keep up with technological innovations, but we cannot lose pace with them. New technology enhances the quality of learning, giving them access to various sources of knowledge. They help to look at ideas and concepts differently and to use them more efficiently. If the technology is developing quickly and thoughtfully, and if it is deliberately used in education, we will have experts who will be able to recognize the right information, and will know the right way to deal with the overarching influence of the media. The right choice will be when the media in education are equally represented in both quantitative and qualitative way.

**CONCLUSIONS AND FUTURE WORK**

The media are now an indispensable component of human life. They surround us on all sides and we cannot live without them. Their importance is multiple and complex. Thanks to them, we come to the important information that we need daily. Information is power, and changes are happening at the speed of light. Information Technology has an impact on us, it defines what we buy, how to dress, what we think and for whom we vote. They have enabled the creation of a global culture, linking people and reducing centralization, with the introduction of new channels and technologies now available to a wide audience. The media are also used for entertainment. The man is just put out in the world in which images, one by one make up everyday life and paint it with the new colors.

To be able to use the mass media in education, it is necessary above all to understand its functioning, set the media messages in different contexts and perspectives, as well as the critical consumption of media. Experts in this area say the six reasons why the media are essential in the education process:

1. Incorporation of new technologies in management processes
2. The huge consumption of media and their plethora of
3. Ideological significance of the media, especially advertising
4. The growing penetration of media in the development of the democratic process
5. Expectation of students to understand the times we live in
6. The growth of the role of information and visual communication in all areas of life and work

Information has become commodity, products and their role is greatly changing [5]. Education through the media has faced many problems trying to break into the programs of elementary and secondary schools. These problems are poor motivation and lack of appropriate organization of state institutions to the teaching programs include some form of education through the media.

In countries where education has been developed for the media, the biggest problem are the teachers who have an inadequate organization of the system. We can talk about the quality of media education only when the teachers motivate students about the media, from the media and with the media. Dynamic changes in the media area mean short-term topical content and high costs of publishing, because of their continuous adjustment of the same. Successful achievement of the objectives of education for the media need the support of the highest educational - educational institutions, and aim is a consumer who will not be a subject to any form of media manipulation, who knows how to select only high quality information and to responsibly and properly communicate to the media and in all other processes.
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STUDY REGARDING THE MICROBIOLOGICAL QUALITY OF CONFECTIONERY PRODUCTS

C. C. Vişan

Abstract: Consumers are taking unprecedented interest in the way food is produced, processed and marketed, and are increasingly calling for producers to take greater responsibility for food safety and consumer protection.

In order to determine the efficiency of the HACCP system I monitored two confectionery units during June 2007 and August 2009, in the city area of Focşani, (Vrancea county, Romania), the technological flow in the critical points that assure the prevention of contamination and elaboration of good quality products.

Key words: microbiological quality, confectionery products, consumer protection, HACCP system.

INTRODUCTION
Confidence in the safety and integrity of the food supply is an important requirement for consumers. Food borne disease outbreaks, involving agents such as *Escherichia coli*, *Salmonella*, *Bacillus cereus* and chemical contaminants, highlight problems of food safety and increase public anxiety that modern farming systems, food processing and marketing do not provide adequate safeguards for public health.

Factors which contribute to potential hazards in confectionery include improper agricultural practices; poor hygiene at all stages of the food chain; lack of preventive controls in confectionery processing and preparation operations; misuse of chemicals; contaminated raw materials, ingredients and water; inadequate or improper storage, etc. [1]

Due to the chemical composition rich in nutrients and high humidity, the cakes are favorable environments for the development of microorganisms. Therefore, respecting the steps of technological process, specific to each group of cakes and respecting the working parameters (time, temperature, relative air humidity) will ensure the attainment of healthy products which do not endanger consumers’ health.

MATERIAL AND METHODS
The methods used for determining the microbiological analysis products (presence of *Escherichia coli*, *Bacillus cereus*, *Salmonella* species, coagulase-positive *Staphylococcus*) are official methods, commonly used in Food microbiology laboratory.

The data obtained were statistically processed for objective assessment of food safety in the area monitored. Findings and recommendations on the quality of such food have been developed.

RESULTS AND DISCUSSION
Microbiological charges of cakes filled with cream on the total number of mezophyl aerobic germs (TNMAG) have values within the limits of admissibility, which shows that:

- the conditions of hygiene in production areas and storage areas have been followed;
- good quality raw and auxiliary materials were used;
- the technological process was successfully complied;
- during working hours, the staff met the mandatory health and hygiene rules.
Results of microbiological investigations performed in unit A and unit B show that the presence of *Escherichia coli*, *Salmonella*, coagulase-positive *Staphylococcus*, *Bacillus cereus*, *Listeria* are absent for all creamy cakes investigated.

Assortment of cakes *Chocolate Cake*, *Éclair with vanilla cream* and *Savarin* are prepared both in drive A and in drive B. Medium TNMAG values presented in Table 1, were graphically represented (Fig. 1.)

In the graphical representation we estimated the value TNMAG with a 95% (amount varies by ± 5%).

### Table 1. TNMAG values - cakes prepared in unit A and unit B

<table>
<thead>
<tr>
<th>No.</th>
<th>Cakes with cream</th>
<th>TNMAG/g medium value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unit A</td>
<td>Unit B</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Chocolate Cakes</em></td>
<td>11260.00</td>
<td>11916.67</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Éclair</em></td>
<td>4566.67</td>
<td>6675.00</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>Savarin</em></td>
<td>16200.00</td>
<td>16833.33</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing TNMAG medium values of cakes](image)

Fig. 1. *TNMAG medium values of cakes prepared in unit A and unit B*

By analyzing the graphic we can observe higher NTGAM values for all the cakes prepared in unit B compared with the same assortment of cakes prepared in unit A.

TNMAG medium values are almost equal for *Chocolate cakes* and *Savarin*, the average charge being 5.83% and 3.90% higher in the cakes of unit B compared with those of unit A.

TNMAG higher differences were recorded for *Éclair with vanilla cream*, the average value being 46.16% higher in the cakes of unit B, compared with those of unit A.

The causes for a larger charge of TNMAG may be determined by:
- improper cleaning of equipment and premises;
- hygiene non-compliance by the staff of unit B;
- the usage of raw materials and auxiliary nonetheless qualitatively;
- failure of technological flow compliance;
- improper storage conditions of temperature and relative air humidity of raw materials for finished products or half-baked products.
Points to check:
- the formality of the staff establishment to the rules of personal hygiene, working hygiene, periodic monitoring of health;
- rotation of raw and auxiliary materials stocks to avoid exceeding the validity, ensuring traceability of raw materials and changing suppliers in case of repeated failure to provide good quality products;
- compliance sequencing and working parameters during the course of technological process;
- cleaning storage areas for raw materials and finished goods storage;
- respecting the space and microclimate parameters: temperature, relative humidity of air and respecting the microclimate parameters: temperature, air relative humidity;
- reviewing the sanitation plan and accountability of personnel for performing cleaning and sanitation of the controlling process.

CONCLUSIONS

Needless to say, food is essential for our lives and safety should come first. Food hygiene is a classic issue in the public health programme, and today it is still a globally significant issue.

Responsibility for food safety is shared by everyone whose actions involve food, from production to consumption, including growers, processors, regulators, distributors, retailers and consumers.

The determined microbiological quality proves the HACCP conditions have been improved and the measures are compliant in both units, showing a big concern for client safety and high quality products.

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THE ETHNOLINGUISTIC ANALYSIS OF THE IDIOM FORMATION IN LANGUAGES OF DIFFERENT FAMILIES

L. Ramazanova

Abstract: It is assumed that people speaking diverse languages think and apprehend the world in different ways. Idioms, being widely current among native speakers of a language, can reconstruct the past of a nation thus disclose its character, traditions and mentality.

The research is an attempt to summarize the reasons for the presence of convergences and divergences in phrase formations in languages of various families or different branches of a language family. On the factual material, the categories and the peculiarities of the idiom formation are determined. The phrases of Azerbaijani (Turkic), Lezghian (Caucasian), English (Germanic), French (Romanic) and Russian (Slavic) languages are the basis of the work. The material is taken from dictionaries and literary works of different types and genres.

Key words: idioms, divergences, derivation, language family

INTRODUCTION

History of a nation is a unique mirror for their progeny, looking at which helps to evaluate what was done yesterday and how to avoid mistakes in the future. When history definitely influences the way different ethnic groups perceive the world, the language can reflect their culture, traditions.

The object of this work is to generalize the reasons for the presence of convergences and divergences in phrase formations in languages of various families or different branches of a language family. The phrases of Azerbaijani, Lezghian, English, French and Russian languages are the basis of the work. Before going over to the factual research of stable word-complexes, it is necessary to give a preliminary description of the languages abovementioned:

1) Azerbaijani - the language of the people, belonging to the Turkic branch of the Altaic family. Azerbaijani is member of the Oghuz branch of the Turkic languages and is closely related to Turkish, Qashqai and Turkmen. Azerbaijani is the official language of Azerbaijan Republic. It is spoken in southwestern Asia, primarily in Azerbaijan and northwestern Iran, and many other countries by immigrants from Azerbaijan.

2) Lezghian - the language belonging to the Lezgic languages (the Lezgic languages are one the seven branches of the Northeast Caucasian language family). It is spoken by the Lezgians, who live in southern Dagestan and northern Azerbaijan. Lezghian is a literary language and an official language of Dagestan. It is classified as "vulnerable" (the total number of speakers is about 783,720) by UNESCO's Atlas of the World's Languages in Danger. Lezghian is spoken in Russia, mainly Southern Dagestan, and in mainly the Qusar, Quba, Qebele and Khachmaz (Kaçmaz) provinces of northeastern Azerbaijan, in Kazakhstan, Kyrgyzstan, Turkey, Turkmenistan, Ukraine, and Uzbekistan by immigrants from Azerbaijan and Daghestan. There is also small population in Balikesir and Yalova in Turkey.

3) English is an Indo-European language belonging to the West Germanic branch. It is the official language of Britain, the US, most parts of the Commonwealth, and certain other countries. It is the native language of over 280 million people and is acquired as a second language by many more. English is an international language.

4) French is an Indo-European language belonging to the Romance group. It is the official language of France: also an official language of Switzerland, Belgium, Canada, and certain other countries. It is the native language of approximately 70 million people; also used for diplomacy.

5) Russian is an Indo-European language belonging to the East Slavonic branch. It is the official language of Russia. Russian is widely spoken by over 130 million people in the
territory of the former Soviet Union, and many other countries by immigrants from the former Soviet Union.

MATERIAL AND METHODS

All the five languages abovementioned belong to the people of different culture and traditions whose history was somehow intertwined at different periods of their life. Some linguistic interactions can be observed below in the given work. The research was conducted with the use of historical, comparative, contrastive, and descriptive methods of linguistics.

As it is generally known in modern linguistics, the reasons for the phrase formation can be divided into 3 groups: 1) the phrase formation is caused by communicative needs; 2) idioms are the result of an emotive function of a language; 3) a stable word-complex is an effect of satisfactions of nominative needs. However, it should be noted that the idiom formation is, first of all, caused by communicable needs. It derives from the ontology of a language – the result of the realization of communicative needs. Figurativeness and nomination are the means of communication; figurativeness is inherent in nomination when a lexical unit is formed [1].

As the overwhelming majority of idioms appear on the national or ethnic base, their sources are diverse and related to different epochs. Images of idioms are taken from different spheres of material, cultural, and socio-economic life of people, where their history, way of life, culture, spirit, and way of thinking are reflected [4]. So, idioms, as a synthesis of spiritual values of people, reconstruct their past, disclose their character, and familiarize with their customs.

The richness and diversity of the sources of a phrase derivation allows dividing stable word-complexes into two big etymological categories: phrases of nonterminological and terminological derivations. Phrases of nonterminological derivation can be generally classified as:

a) Phrases relating to some historical facts or events. Such stable word-complexes reflect the history of a nation and remind progenies about the existence of significant facts that formed country. Compare: azer. Nikolaydan qalmış (remained from Nicholas) – smth that is not too old yet disused. This expression appeared after the Russian Revolution in 1917. Nicholas II (1868 - 1918), son of Alexander III, reigned 1894 - 1917. Forced to abdicate after the Russian Revolution in 1917, he was shot along with his family a year later; lezg. хан Батый рекъер кутумир (don’t lay the way like Batu khan) – don’t walk along the bad new roads/places. This phrase appeared in the 1230s, the period of the Mongol overlordship. Then Batu khan attacked brutally the settlements of the lezghians; engl. to send to Coventry - to punish someone for disloyalty to his companions or workmates by refusing to speak to him’. This phrase finds its origin in the Civil War between King Charles I and Parliament in 1642-1646 when Royalist prisoners captured at Birmingham were sent to Coventry, a Parliamentary stronghold, where some of them were beheaded; hence the association of ‘sending to Coventry’ with the punishment of disloyalty, which later took the form of not speaking to the offender.; fr. bâtir (faire) des châteaux en Espagne (to build castles in Spain) – to build castles in the air. This expression is related to the prince of France Henry of Burgundy (1057-1114), who went to Spain together with his knights to fight against moors for the king of Castile - Alphonso VI. For such a help, Henry of Burgundy could marry a royal daughter and get lands in Spain where he started to build castles. His success aroused envy among most of his countrymen who also desired ‘to build castles in Spain’, which was very prestigious. russ. вот тебе, бабушка, и Юрьев день! (here is, Granny, St. George’s day!) - here’s a fine how d’ye do!. On St. George’s day, serfs had the right to pass from one landlord to
another. The expression is related to the abolition of this right by Boris Godunov, Russian tsar, in 1580-90.

b) Phrases derived from historical quotations. These stable word-complexes are based on phrases said by famous persons. Compare: azer. Bir kere qalxan bayraq bir daha enmez (the flag hoisted once can never be lowered) – the sense of independence once felt by a person can never be ousted from him. This phrase was said by Mammademin Rasulzade, the first president of the Independent Republic of Azerbaijan, during his statement to the nation in 1918; lezg. Лукларин дуя Аллагьдиз къыдыч (the pray of slaves will not be heard by Allah) – don’t surrender to fate but develop yourself and try to change your life. These words were said by Yaraşqy M., a spiritual enlightener, to his listeners, during one of his lessons in madrasah; engl. A pessimist sees the difficulty in every opportunity; an optimist sees the opportunity in every difficulty. - Winston Churchill; fr. Après nous le déluge – after us the deluge. This expression was uttered by Louis XV (1710-1774), who was very selfish. However, there is an idea that it might have been said by Madame de Pompadour while consoling the king who was sad due to the defeat in the battle. russ. Иду на вы!/- I come against ye!. This historical phrase used as declaration of war was first uttered by a famous general of early Rus, the Grand Prince Svyatoslav I Igorevich of early Rus (955—972).

c) Phrases taken from a literary source. Most of such phrases derived from literature of different genres and epochs. Knowing the situation or context helps to understand the expression better, thus the mood and mentality of the author and the spirit and life standard of the nation he belongs to. Compare: azer. Gəztlü o adamdə ki, özünü görə bilsin (that man is sighted who can see himself). This expression belongs to Bakikhanov A.A., the outstanding scientist, poet, and writer of Azerbaijan, who created an image/character of ‘a little man/little people’ in literature; lezg. акунар алай къелеч капур (the sword of a special form from the fortress) – smb. or smth. seemingly dangerous/threatening but actually non-effective. It came from the Lezghin epos about Sharvily, the national hero, who had a sword of two edges which could cut anything into three parts. Sharvily hardly ever used his sword. He killed the enemies of the nation with his fists but his sword was driven into the ground in front of the fortress he protected to keep strangers in fear; engl. To be or not to be, that’s the question is the opening line of a soliloquy from William Shakespeare’s play Hamlet (written about 1600), act three, scene one. It is one of the most famous quotations in world literature and the best-known of this particular play; fr. Allons, saute, marquis! (Come on, jump, marquis!) - dance with joy! This expression is taken from the comedy ‘The Gamester’ by Jean- François Regnard, which was first performed in 1696. This phrase was repeated several times by the self-satisfied marquis when he was admiring himself and his own imaginary virtues. russ. Твърди ли я дрожащая или право имею…? (Am I a despicable creature or do I have a right to…?) These words were said by Raskolnikov, the central character of the novel ‘Crime and Punishment’ by Fyodor Mikhaylovich Dostoyevsky.

d) Phrases relating to the names of parts of body. This category of stable word-complexes forms the majority of the phraseological stock of all the five languages. Moreover, most somatic phrases coincide in the languages and may seem international. Compare: azer. başdan ayağacan (from head to leg); lezg. къывелэй къеачел (from head to leg); engl. from top to toe; fr. de pied en cap (from foot to head); russ. с головы до ног (from head to legs).

e) Phrases relating to the nature: natural phenomena, flora, abiocoen, etc. Compare: azer. buzу gün erider, insani xecalat (a person will be melted away (destroyed) by problems like ice by the sun); lezg. са къулач тухуналди рук къутляъ жедч (the forest will not get thinner if you take away a cart of wood); engl.; it never rains but it pours – small
problems are generally followed by big problems; fr. selon le vent (depending on the wind) – depending on the situation/circumstances; russ. гром не грянет, мужик не перекрестится (if there is no clap of thunder, a man will never cross) - they won't lock the barn door till after the horse is stolen.

The list of stable word-complexes of nonterminological derivation can be described in such groups as phrases relating to legends, anecdotes, animal world, religion, family life, etc. Meanwhile, during the research it was noticed that all the five languages are rich in phrases relating to parts of body, the nature, religion, family life. However, in Azerbaijani and Lezghian, the number of phrases relating to historical facts, historical quotations, or literary sources is limited.

Phrases of terminological derivation are based on free word combinations that, in many cases, are used in their direct meaning even today. They can be generally classified as:

a) Phrases relating to soldiery and military science. Compare: azer. gılınç açmayan yeri pul açar (what cannot be slain by a sword can be taken by money); lezg. капур акъатна – иви аваыъна! (the sword is taken out – blood will be shed) – so said so done; engl. a flash in the pan [2] – fiasco, a thing or person whose sudden but brief success is not repeated or repeatable. The origin of this phrase comes from priming of a firearm and the flash arising from an explosion of gunpowder within the lock; fr. brûler sa denière cartouche (to shoot the last bullet) – to use your final chance/last resort; russ. встретить в штыки (to meet with bayonets) — give a hostile reception.

b) Phrases relating to hunting. Compare: azer. ovçuya bir göz de kifayetdir (for a hunter even one eye is enough) – a professional can do without many things; lezg. лишан такъуна яда (one can fire without aiming) – one is very professional and does not need to prepare for; engl. the bait hides the hook – danger/problems may be hidden behind the temptations proposed; fr. romper les chiens (to call back the dogs) – to stop the conversation started ineptly; russ. потерять нюх (to lose flair) — to depart from the rules of etiquette, to misbehave.

c) Phrases relating to jurisprudence and legal proceedings. The stable word-complexes of this group reflect the judicial customs, morals, and manners of the past. Compare: azer. yazan Hadji Mansur, pozan Hadji Mansur (Hadji Mansur writes, Hadji Mansur erases) – the positive or negative outcome of a case depends on the same person and one should deal with him; lezg. кавхадин гъакъ-нигъакъ (the rightness and wrongness according to the judge) - double standards; engl. circumstances alter cases – what may be good in some cases may be bad in the others; fr. faire amende honorable (to do an honorable fine)– to apologize in public, to admit errors; russ. на нет и суда нет (for 'No' there is no case) — you can't do the impossible.

d) Phrases of marine derivation. Compare: azer. дənizçi denizde olmalıdır (a seaman should be in the sea) – everybody should first do their own job; lezg. гимичи гимдə къисизба (the captain is ousted from his own ship) – to encroach on legitimate rights, to reject smb’s authority; engl. to take down a peg. If it is said that someone should be brought/taken down a peg, it means that they should be made to realize that they are not so important or wonderful as they think they are; fr. jeter/mettre/attacher le grappin sur qn (to throw/put/fix a hook at smb.) – to bring smb. under control, to override; russ. отдать концы (cast off (all ropes)) — to die, to cut one's cable

The description of phrases of terminological derivation can be continued in such groups as idioms relating to different handicrafts, games, sport, medicine, etc. Azerbaijani and Lezghian are poor in phrases derived from such sources as sport and medicine.

Historically gradually in every language, appear the word-complexes commonly used in general literary language compared to which the other theoretically possible word-complexes can be regarded as their variants [3]. Therein lies one of the causes of
RESULTS AND DISCUSSION

Some reasons for the presence of divergences in phrase formations can be comprised into the following categories:

1. **Lexical divergences**;
   a) Different words participating in the process of a phrase formation. Compare: azer. baş-başa (head to head); lezg. чин-чина (face to face); engl. face to face; fr. bec à bec (nose to nose); russ. с глазу на глаз (eye to eye).

2. **Divergences in semantic capacity of lexical units.** Compare: azer. асаблердүү ойнамаq (play on the nerves); lezg. нервийра эяле эвун (prod the nerves); engl. get on (fray) smb.’s nerves; fr. porter sur les nerfs (fall onto the nerves); russ. действовать на нервы кому-либо (act on smb.‘s nerves).

3. **Divergences in synonymic relations and synonymic potentials.** Compare: azer. боzжandын тутмаq (yapıståmaq) (take (stick to) by the throat); lezg. къамалай къун (take by the throat); engl. catch (grab, take) by the throat; fr. prendre qn. à la gorge (take smb. by the throat); russ. взять (хватить, держать) за горло (за плотку, за язык) (take (catch, hold, grab) by the throat (gills)).

4. **Divergences caused by peculiarities of a nomination.** Compare: azer. ürəýинин генiшлиyinden (due to the breadth of the heart); lezg. рускя ацаяшлый (due to the fullness of the heart with a sense); engl. in the fullness of one’s heart; fr. d’abondance de cœur (due to the abundance of the heart); russ. от щедрости душь (from the generosity of the soul).

2. **Grammatical divergences (complete or partial)**
   Compare: azer. burunu бойұмәк (nose to grow); lezg. нер цава къун (nose in sky keep); engl. turn up one’s nose; fr. lever le nez (turn up the nose); russ. задирать нос (lift up nose).

3. **Semantic divergences.**
   Compare: azer. dal гевирмәк – 1) to leave; 2) to resent; 3) to refuse; 4) to turn aside; lezg. дапу элкъүрөө – 1) to ignore; 2) to hate; engl. turn up one’s back – 1) reject; 2) to sit/to stand back to.; fr. tourner le dos à qn, qch – 1) to ignore; 2) to reject; 3) to stampede; russ. поворачивать спину – 1) to show indifference, disregard.

4. **Stylistic divergences.**
   Compare: azer. qoq qıpируемa (during the wink of an eye); lezg. вулпайдаи (sooner than an eye can wink); engl. in the twinkling of an eye; fr. en un clin d’œil (in the wink of an eye); russ. в молниеого ока (in the twinkling of an eye).

CONCLUSIONS AND FUTURE WORK

It can be concluded that all the five languages possess a rich phraseological stock perfectly describing morals and manners of the nations. So, Azerbaijani and Lezghian
cannot boast a large number of phrases relating to medicine, sport, historical facts and quotations, or literary sources. It can be explained by the fact that these languages are not investigated enough and most written sources are lost in the mists of time due to multiple attacks from the side of stronger enemies. However, these languages are rich in phases relating to religion, everyday life, parts of body, nature, etc. Meanwhile, English, French, and, Russian demonstrate the richness of their phraseological stock in all the categories. It should also be noted that today the English language is the main source of the formation of international idioms.

As is obvious, in spite of lexical, grammatical, semantic, and stylistic divergences, international idioms give the general purport of an expression. However, there are a number of stable word-complexes where not only ideas but also means of their expression coincide. Such parallel stable word-complexes derived from a common source can hardly be attributed to adoptions of one language from another. Most likely what is at issue is that such stable word-complexes formed independently of each other but in analogous social and historical conditions by the use of identical vocabulary. Compare: azer. başını itirmek; lezg. кьил квадарун; engl. lose one’s head; fr. perdre la tête; russ. потерять голову.

Most explicable reason for the presence of convergences and divergences in phrase formations in languages of various families or different branches of a language family can be phraseological adoptions, which are the result of a mutual influence and enrichment of different languages. Phraseological adoptions, as a rule, derive from one source which is sometimes impossible to determine. However, most of them are based on some historical facts, antique mythology, religion, literature, movies, etc. This type of a phrase formation due to its capacity requires an individual research.

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MARKETING AUDITING OF THE MACRO FACTORS IMPACT OVER THE ROMANIAN AGRO FOOD INDUSTRY

GABRIELA OFELIA CHIRLA, SABINA FUNAR, RAMONA TIPA

Abstract: The macro factors as part of the marketing environment exercise an influence of a certain degree over the economic area. The nature of the impact (positive or negative) and its intensity depend on the capacity of analysis and prevention of each industry or enterprise. Identifying the main changes in the macro environment is part of the marketing audit process.

The agro food industry is very important for each country’s economy, being the most directly attached to the consumer’s needs of food supplies and health. Therefore, the aim of this article is to audit the macro factors and their influence over the agro food industry from Romania in order to identify the main opportunities and threats. The key-task of a marketing audit process is to offer solutions for the enterprises to exploit the opportunities and also to avoid the threats.

Results of the study using PEST analysis indicates that economical and political factors have the higher degree of influence providing many threats for the enterprises in the agro food industry.

Key words: Marketing Auditing, Marketing Environment, Marketing Audit, Opportunities, Threats, PEST Analysis, Macro Factors, Agro Food Industry.

INTRODUCTION

The marketing audit process is an examination of marketing environment, objectives, strategies and activities of a company or industry with a view to identify the major threats and opportunities and recommending a plan of action in order to improve its performance [2].

The first step of a full marketing audit is analyzing the macro-environment which consists in six factors and forces that could affect the activity of a company but over which the company has very little control. The detail of the examination depends on the involvement of the enterprise or the involvement required by the industry [3].

The aim of a marketing audit is to identify the trends for each of the macro-factors and their implications for the company in order to avoid threats and exploit the opportunities. The agro food industry is closely attached to the consumers needs for food supplies and health so a strong marketing orientation is necessary [1]. Its’ efficiency can be evaluated with the aid of the marketing audit methodology. The agro food industry from Romania is being represented by 6456 of enterprises from a total of 772 259 of enterprises at the end of year 2007 according to the statistic offered by Borg Design, a complex database of Romanian enterprises.

Because of the major role of the agro food industry both for the consumers but for the national economy also, the aim of this article is to perform the first step from a marketing audit process- the environmental audit- for the Romanian agro food industry to identify the influence of the macro factors for the sector and highlight the main opportunities that could be exploited and the threats that must be avoided. The research was performed with the help of some variables chosen for each of the four macro factors of the marketing environment according to their potential impact for the agro food industry.

MATERIAL AND METHODS

The sub domains of the agro food industry considered in the research are: processing and conservation of meat and meat products, fish and fish products, conservation of vegetables and fruits, fabrication of oils and grease, fabrication of dairy products, fabrication of milling products, and fabrication of beverages.

The method used is the PEST analysis which is a specific tool for the marketing audit process. The name is an acronym from the main factors which are taken into
consideration when implying this “valuable framework for identifying opportunities and threats in the macro environment” [2]. The macro factors are: political-legal factors, economical factors, socio-cultural factors and technological factors. The PEST tool is used in this case to analyze the position of an industry (agro food industry) within the marketing environment and it is based upon a grid with four quadrants [4] which interacts with one another. This marketing audit tool does not offer a clear framework because of the unlimited variables of each dimension therefore the need of prioritizing the variables according to their impact for the industry studied [5]. Consequently the variables chosen because of their relation with the Romanian agro food industry from each of the four dimensions of the PEST analysis are:

- for political-legal factors: tax policy, legislation;
- for economical factors: populations’ incomes and expenditures, the categories of consumption, importation and exportation, internal commerce, gross domestic product (GDP), interest rate, inflation rate, unemployment rate;
- for socio-cultural factors: lifestyle trends, demographical trends, consumer attitudes and opinions, the image of the brand, population consumption models, trends, religious factors;
- for technological factors: technology trends.

Those variables were analyzed using statistical data from the Romanian National Institute of Statistics, the official site of the Romania National Bank, The Fiscal Code but also from the consumer tracking studies, with the scope of examining their relation and influence over the agro food industry.

RESULTS AND DISCUSSION

The political-legal factor from Romania has a great influence for the agro food industry throughout the measures adopted. The notable aspect for this macro factor consists in the fact that Romania is a member of the European Union since 2007 which offered a huge opportunity by facilitating the commercial trade. The positive aspect is that Romania has access to the European market and the negative aspect is the huge competition for the local agro food production and the foreign one. The law for increasing the value added tax (VAT) from 19% to 24% had a huge impact for the agro food prices and a negative impact for the consumption. Taxing the meal tickets had a negative impact for the employs, many enterprises eliminated these facility for them and the consumption was reduced especially at the food products.

The first variable from the economic factor refers to the average incomes and expenditures of the Romanian urban population which is situated around 200 euros per person [7]. Another aspect refers to the small difference between incomes and expenditures indicates that the population buys only the products strictly necessary for their daily use. In the third trimester of 2010 the difference between incomes and expenditures reached the level of 23 euros. The impact for the industry consists in the limited power of consumption of the population and is also related with the prices which have to be kept at an accessible level. As referring to the categories of consumption analyzing the data from 2008 to 2010 it revealed that approximately 40% of the monthly consumption is generated for agro food products followed by the household expenses which indicates a positive impact for the industry because there is a strong demand for agro food products [9]. The evolution of the expenses for the agro food products indicates a descending trend from 2008 to 2010. Also it can be concluded that the expenses for these products have a boom period in the second trimester of each year and a depression period in the third trimester, so it indicates a seasonality in the consumption which the
enterprises from the industry have to take into consideration when programming their production.

A statistical analysis of the variable regarding the categories of products exported and imported revealed that the agro food category has the lowest share in the total of exported and imported products, the average from 2007 to 2010 indicates a level of 7% \[10\]. The statistical analysis of the internal commerce reveals an ascending trend regarding the agro food category of products purchased from 9528 million lei in 2000 to 54574 million lei in 2008. The increasing trend indicates that the consumer buys more agro food products from a year to another instead of producing himself inside the household \[8\].

A brief analysis of the contribution to the GDP of each sector of the national economy from 2002 to 2008 indicates that the agriculture sector is the most deficient because negative values were registered in 2005 and 2007 \[7\]. For the agro food sector this aspect represents a threat because of the raw materials supply and the need to import them if the national agriculture does not support the internal demand.

The importance of the variable “interesting rate” for the agro food industry consists in the access to credits for investments in new technologies or raw materials. The analysis of statistical data from 2006 to 2010 shows that the level of the interest rate reached the highest level in the second semester of 2008 and first semester of 2009, with an average of 10.25%. From June 2009 the level started to decrease reaching the point of 6.25% in May 2010 when it remained stable \[11\]. In the year 2010 it reached the lowest level from the last four years which means that the National Bank of Romania tries to stimulate the credit, which can be considered an opportunity for investments.

The inflation rate indicates that the actual level is 7.60% but the objectives for this year and next year of the National Bank is 3% and 2% for 2013. The reducing level of inflation is an opportunity for the agro food industry because it encourages the consumption and maintains the prices at an accessible level \[12\].

The unemployment rate at the end of January 2011 was at the level of 6.74% and it decreased with 0.13% comparing with December 2010. The average rate of unemployment from the European Union is 9.5% so Romania is situated below this level. The impact for the agro food industry reflects in the limited number of people working inside the factories but is also a positive impact because of the consumption which is increasing when the unemployment rate is at lower level.

The analyze of the socio-cultural macro-factor is based on some consumer tracking studies which indicated that Romanian people are more preoccupied by a healthy alimentation than in the past years. So a study \[6\] revealed that the products associated with healthy food are fruits and vegetables and dairy products which, for the agro food enterprises represents a positive information and also an opportunity for increasing the production and varying the offer. The general opinion of the Romanian people regarding a complete meal is that it must contain meat. Pork meat is preferred by male population while chicken meat, by women and children. A cultural aspect is related with the idea of home cooked meals which are the women task. The same study indicates that young people (high schools pupils and students) are attracted by fast food products. The agro food enterprises must find a proper solution for adapting their offer according to this social aspects because of their potential strong impact.

The same study reveals that the consumption preferences for breakfast are changed. While margarine was preferred in the past as a usual morning food, the pate and the yoghurts are now in top of the preferences. For the juice consumption it has been observed that the noncarbonated ones are mostly preferred but not the sugar free juice. People associate the sweet taste with the natural taste which is quite the opposite. Another important fact from the study refers to the increasing preference for the retailers
brands from 3% to 7%. The phenomenon is explained by the low prices for this products. So a negative aspect for the agro food industry and a potential threat are the retailer brands, the low prices being the reason for this competition.

The demographical trends analyzed show that the natural increase for Romania has been negative since 1992 [7], which represents a threat not only for the agro food industry but for all sectors of the economy, because of the reduction of young population which represents a buying force and also a working resource.

The variable “consumption models” was approached with the help of a study applied by Mednet [13], which revealed that 76% of the people interviewed reduced their consumption in 2010, others bought cheaper products in smaller quantities or the same products they used before, but in smaller quantities. A possible threat for the agro food industry is represented by the new alimentary convictions like the vegan and the vegetarianism. The religious variable is very important for Romanian consumption because of the high percent of orthodox people (85%), and the four religious posts over the year that summarize almost 100 days when meat and dairy products are prohibited. To avoid the reducing level of the consumption, the agro food industry must adapt the offer by producing soy products, fruit and vegetables cans.

The technological factor refers to the widespread of the Internet and the possibilities for the consumers of a better information about the agro food products purchased. The technological trend vary for each of the agro food enterprise according to its domain of activity.

CONCLUSIONS AND FUTURE WORK

It can be concluded that the variables for each of the four dimensions of the PEST analysis have an impact for the agro food industry either positive or a negative one. The method implied offered a lot of freedom to the auditor when choosing the variables. The political factor can have a strong impact for the industry because of the rapid changes occurred within it, changes that can occur from one month to the other and that is why it must be analyzed frequently. The opportunity identified using the variables mentioned before refers to the easiest access to the European market. The threats identified consist in the increasing value of VAT which reduce the power of consumption and the taxing over meal tickets that affects the consumption.

The opportunities identified in the economical dimension are: the highest share of the agro food products in the monthly consumption, the boom period in the second trimester of each year, the increasing trend for the agro food products in the internal consumption, the lower share in the export categories. The lower level registered for the interest rate in also an opportunity for investments into new technology. The objectives of the national organisms of reducing the inflation rate is a future opportunity because of the stimulation of consumption. The unemployment rate at a low level is an opportunity because of the higher mass of consumers. The threats identified refer to: the low level of incomes and the high level of expenses, the huge differences in agro food consumption during the year, the seasonality of the consumption, the deficiency in the agricultural sector that affects the raw material supply, the low unemployment rate which can affect the job demand for the agro food sector.

The opportunities identified analyzing the socio-cultural factor are: the Romanian beliefs regarding the consumption of meat products which are needed daily, the association of dairy products with the concept of health, the preference for sugar juice noncarbonated, the increasing number of people which prefer pate products and yoghurts for breakfast. The threats identified refer to: the preference of young population for fast food products, the natural increase which for the past 19 years has been negative, the
retailers’ products which are cheaper, the changes occurred in the consumer behavior because of the economic crisis (they buy in smaller quantities or they reduce their consumption). The alimentary trends like vegan and vegetarianism are a threat for the meat production enterprises but can represent a possible target and opportunity for the ones oriented to the conservation of fruits and vegetables.

The religious variable must be taken into consideration because of the religious post period when meat and dairy products are prohibit but an opportunity arise for the enterprises specialized in soy products.

The technological factor have to be analyzed for each specific enterprise according with the domain of activity because of the specific technologies for each of them. The common threat is the easier access to information and the fact that consumers are much better informed related to the food consumption and products.

As a marketing audit tool, the PEST analysis offers a framework but it is not a punctual analysis. It has to be repeated periodically for giving the possibility for the enterprises to anticipate the changes occurred in the marketing environment.

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RURAL DEVELOPMENT BASED ON RENEWABLE ENERGY SOURCES – CASE STUDY FROM EASTERN CROATIA

Gorana Heffer, Davor Kralik, Goran Heffer

Abstract: This paper presents a case study of Municipality in Eastern Croatia that its development based on the exploitation of renewable energy sources for activities in agriculture, energetic and tourism, which should significantly improve the lives of the local population and the wider community. In the paper is presented the main potential of renewable energy sources and plans for their use. The main potential of renewable energy is the source of geothermal water. By its quality and capacity is among the best in Croatia, which offers the possibility of developing different forms of agricultural production, energy and tourism. It was especially presented projects of greenhouses and fish farming.

Key words: Eastern Croatia, rural development, renewable energy sources

INTRODUCTION
Sustainable community development in rural areas is one of the basic priorities of the EU, and also in Croatia. Therefore, the development of such environments strongly encourage numerous incentives and partner programs, as well as funds to finance local development activities and projects at the regional level (counties). It should be noted that 40% of funds from the EU budget is intended for rural development [6].

Practice in the EU confirms that the local communities, where investing in the development of “green industry” and renewable energy sources, recorded rapid growth. This area is particularly important for local communities in Croatia, where the preserved natural environment is a valuable capital. With active protection of environment and use of renewable energy sources can be open the new prospects and strengthen competitiveness. This allows long-term sustainable community development, creating new jobs and higher living standards.

Especially important role in creating and managing of local development in such situation receive the local governments. They have the task to recognize development opportunities and chances, establish and define the real development vision, strategic goals and priorities of local development, as well as initiate and launch strategic development activities, programs and projects for the practical realization of the aims of local development.

Croatia is predominantly rural country. Over 90% of inland areas are the rural areas where live about 50% of Croatian population. Over 75% of rural areas are lagging behind in development. Especially it relates to Eastern Croatia. Development of these areas is mainly based on agriculture and processing of agricultural products. But, there are also development opportunities based on energy potentials, especially renewable energy sources.

In Croatia is a long time known the availability of geothermal energy from deep boreholes, which can be useful for the economic application. There have been individual attempts to starting with commercial projects based on geothermal energy, but from them, except building the spas, give up already in the initial stage.

Most geothermal resources are located exactly in the Croatian rural areas, and therefore their importance for the development of these areas is very large.

MATERIAL AND METHODS
This paper deals with the applicability of geothermal resources as a basis for the development of Croatian rural areas. Geothermal resources and their locations are explained, as well as the possibility of their applications for economic purposes.
It was presented a case study of exploitation of geothermal resources in the Eastern Croatia – in the Municipality of Babina Greda, where was analyzed the concrete project.

**Geothermal resources in Croatia**

Possibility of applying some geothermal source is determined by the geothermal gradient $G$ ($^\circ$C/m). This is an increasing temperature of water per unit depth of the source [4].

Two sedimentary basins cover almost the whole area of Croatian – the Pannonian basin and the Dinarides [2]. Between them are great differences in geothermal gradients, which are established in exploration activities with the aim of finding oil and gas. However, geothermal gradients in both basins are significantly higher than the average gradients of other geothermal resources in Europe. The average value of geothermal gradient in Europe is $0,03 \ ^\circ$C/m, while the Croatian basins have gradients of $0,018 \ ^\circ$C/m – the Dinarides and $0,049 \ ^\circ$C/m – Pannonian Basin (Figure 1).

![Figure 1. Geothermal gradients ($^\circ$C/m) of sedimentary basins in Croatia, [2]](image1)

Geothermal potentials in Croatia can be divided into three groups, as shown in figure 2.

![Figure 2. Geothermal Resources in Croatia, [2]](image2)
The shown groups of geothermal resources are:
- middle temperature reservoirs of steam and hot water \((T = 100\text{ – }200 \, ^\circ\text{C})\),
- low temperature hot water reservoirs \((T = 65\text{ – }100 \, ^\circ\text{C})\),
- Geothermal warm water springs \(< 65 \, ^\circ\text{C}\).

**The applicability of geothermal energy in Croatia**

Applicability of geothermal energy is mainly limited to the area around the production boreholes because of transportation costs of thermal energy. Therefore, for implementation of programs of exploitation geothermal energy are interesting just those locations where it can engage as many economic activities in a relatively small area.

Geothermal energy from middle temperature reservoirs can be exploited for space heating and for different technological processes, as well as for electricity production by binary process. Energy of low temperature reservoir and source of warm water can be used in different technological and economic purposes with lower levels of warming.

Croatia has a long tradition of exploiting geothermal energy from natural resources for medical and recreation purposes. Such sources are the basis of the economic success of many spas in Croatia (Varazdin, Daruvar and Stubica spa, Lipik, Topusko, etc.).

Besides the spas, geothermal water was used for the heating of water and space (heat exchangers), in the growing of fish and plants, as well as sanitary water, as drinking water (water supply system) or as table and mineral water (bottling).

**RESULTS AND DISCUSSION**

**Exploitation of geothermal resources in Babina Greda – Case Study**

Municipality of Babina Greda is located in the west of Vukovar-Srijem County, which is the easternmost county in Croatia (Figure 3).

Babina Greda is one of the largest Slavonian village and also one of the largest Croatian Municipalities. It covers the area of 8,009 ha, of which are 1,151 ha forests, and the rest are mainly treated agricultural areas.

Relief is flat with an altitude of about 83-85 m, characterized by a past flooded valley of the river Sava, and today rugged by numerous canals and small rivers water stream Beravica.

Climate in this area is a moderate continental with very cold winter. Average minimum temperature is \(-0,5 \, ^\circ\text{C}\) (in January), and the average maximum temperature is 21,5 °C (in July). Average annual temperature is 11,5 °C.

Average number of days for space heating is 203 days/year (room temperature 20-22 °C), and for greenhouses 158 days/year (room temperature 15-18 °C).

Municipality of Babina Greda is in the area of special state concern (war-affected area). Big problem of the Municipality is the displacement of people (especially young), caused by a lack of employment opportunities. Municipality is classified in the category of undeveloped since the economy, employment, incomes and budget are at very low level. About 90% of families in the municipality are engaged in agriculture, which is insufficiently developed and in heavy condition.

A deep borehole for geothermal water Babina Greda (BaG-1) was built in year 1984 and 1985. Locality of the borehole is located about three kilometers northwest from the
town center. Geothermal gradient of source is very high at 0.0621 °C/m. This positive geothermal gradient was obtained during the maximum output, based on the measured temperatures of 120.0 °C at 1.750 m depth. Calculations showed that the borehole can produce 2,400.00 m³/day hot water with a temperature of 100 °C and with a dynamic pressure of 6 bar.

The program of economic use of geothermal energy at the location Babina Greda occurs in the tourist and economic zone that includes an area of approx. 45 ha (Figure 4).

![Figure 4. Geothermal touristic and economic zone Babina Greda. [8]](image)

In the tourist and economic zone Babina Greda is planned production of electricity, as well as complementary use of geothermal energy in greenhouse farming for vegetables and flowers, fish farming and in other manufacturing and service sectors that need heat and cooling. It was also planned build of tourist Center and the district heating of Babina Greda.

- For the purpose of growing vegetables in the first stage is planned to build a 2.5 ha of greenhouses, and the following second stage is 2.5 ha. The designed heating system ensures the air temperature of +15 °C indoors at the outside temperature of -18 °C. In the heating season air temperature during the night is held at 15-17 °C, and during the day at 18-24 °C, depending on the intensity of sunlight. For a total area of 5 ha is required power of about 5 MW, which will be fully ensure from geothermal energy.
- For the purpose of growing flowers in the first stage is planned to build a 2.5 ha of greenhouses, and in the following second stage, as well as with vegetables, build a 2.5 ha. Average temperatures of heating and required specific thermal power are the same as in greenhouses for growing vegetables. Geothermal energy will fully ensure energy of about 5 MW.
- For fish farming will be used water from the river Beravica. The total daily amount of water with a temperature of 20-25 °C, which is constantly changing in the pools, is 810 m³/day. Maximum required power for heating of pool water is approx. 770 kW, which will be fully ensure from the warm water distribution system.

Development of Babina Greda based on the exploitation of geothermal resources

Through implementation of the planned program are created a real basis for the development of Municipality Babina Greda and improve of living conditions of local residents.
The program ensures employment for about 200 people, and therefore creating conditions to existence for about 200 families. Improving economic conditions will be indirectly affected on the return and stay of people in rural areas, as well on the introduction of new technologies by using renewable energy sources. The program also introduces new forms of agricultural production in the Vukovar-Srijem county, which will positively affect on the overall development of agriculture in Eastern Croatia.

The program satisfies a number of conditions, among which are the most important: profitability, reduce unemployment, suitability for local conditions, wider social benefit and environmental acceptability. Direct benefit will be also for the Vukovar-Srijem County.

CONCLUSIONS AND FUTURE WORK

The program of economic applications of geothermal energy at the location Babina Greda offers real possibilities for positive result because of:

- affirmative social climate and establishing a new system for evaluating of environmental protection and renewable energy sources, including higher quality to their economic evaluation, thus they become economically reasonable,
- designing the appropriate technological and business model of application of geothermal energy with a group of complementary projects, which offer the high level of application of total available energy and attractive product program.

This program is original technological and economical solution, which should promote as national energy and economic project, so to provide the adequate social support in the implementation. The program supports important national development priorities: renewable energy sources, food production, employment and reduced need for imports.

Successful implementation of the presented application of geothermal energy can serve as the model for economic activation of about 65 similar existing sources (boreholes) with the possibility of production of geothermal water on the Croatian territory.

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A THEORETICAL APPROACH OF THE EUROPEAN FLOWER MARKET INTERACTION

CARMEN SAMOILĂ, SABINA FUNAR
Abstract: The main objective of the flower market prospecting is to determine the consumption level of cut flowers, mainly referring to species, varieties, colours and quality mostly preferred by consumers and also the interaction between the European flower markets. The largest amounts of flowers traded inside the European Union are: carnations, roses, orchids, chrysanthemums and gladioli. Those species are cultivated in Romania also. The flower trade is based both on the local production and the imports. In Romania the export level is very low compared with the import level.

Of all the European countries, the Romanian flower market mainly interacts with Holland, Italy and Hungary. The seasonality of demand is influenced by the time of year, for example: special occasions, religious holydays, academic events, and also by the national offer affected by climate.

The interaction between the European flower markets requires a permanent adaptation to the internal and external influences of the markets. In order to obtain a financial growth on the flower market it is necessary that each country adapt their production to the international demand.

Key words: Market Flower, Interaction, Local Production, Seasonality of Demand, Import, Export, International Demand.

INTRODUCTION
Because of the important role of flowers in peoples’ daily life, the interest for their cultivation and commercialization is intensified in almost every country of the European Union. The flower trade has a significant evolution in correlation with the market share. The highest quantities of flowers traded inside the European Union are the roses because of their varieties of species, colours and perfumes. Roses are traded mostly as cut flowers.

The economic recession from the last three years had a great impact over the Romanian imports and their volume that is superior comparing to the Romanian exports and national production. The high levels of flower production from the European countries influence the price of acquisition that is reduced comparing with the ones from the national production.

The main scope of this article is a theoretical approach of the Romanian imports and exports and also from the other European countries in order to highlight their interactions. The internal flower sector is also analyzed and its' potential development which is mainly affected by the seasonality, spring being the most profitable season with the highest level of sales.

Another aspect approached refers to the requirement of adopting suitable legislation to prohibit the use of chemical substances for flower treatment of the international producers and encouraging the ecological production.

MATERIAL AND METHODS
The main method used in this article is the literature review and the analyze of statistical data from the Romanian National Institute of Statistics, the National Agency for Consumer protection, the Agricultural Fond for Rural Development and the Ministry of Agriculture and Rural Development.

RESULTS AND DISCUSSIONS
Romania is considered a flower market of high dimensions by the big European distribution chains that justifies their presence throw the enormous quantities imported and commercialized. The permanent research of the consumer behaviour of flowers is the main condition in order to increase the market share, constant and durable communication being the key to success [1].

In Romania the quantities of cut flowers imported in the first eleven months of 2010 reached 6.516 tones with a total amount of 20.4 millions of Euro. Comparing with the
quantities from 2009 the volume exceeded with 1.138 tones. In 2009 the level of imports was 17.5 millions of Euro [8].

The largest amounts of cut flowers imported by Romania are: carnations, roses, orchids, chrysanthemums and gladioli because of their longer period of life (being treated with chemical substances to increase their resistance) and also for their small price of acquisition (being purchased in high quantities)[6].

The principal countries from where Romania imported the cut flowers during January 2010 – November 2010 are Holland (3.620 tones in amount of 14.39 millions Euro), Hungary (229.6 tones in amount of 1.534 millions Euro) and Turkey (2.210 tones in amount of 2.48 millions Euro). Other countries like Italy, Spain and Denmark registered lower quantities of cut flowers imported by Romania [2].

Regarding the exports, it has been observed an ascending trend during January 2010 –November 2010 comparing with the export from the same period from 2009. still the Romanian level of export is situated below its’ natural potential. In 2009 the quantities of cut flowers exported reached the level of 5.9 tones in amount of 916.914 Euro. In 2010 the exports reached the level of 398 tones in amount of 927.607 Euro [5].

The quantities of cut flowers exported in 2010 there are 165 times smaller than the imports. The national trend is represented by the increasing surfaces of cultivated flowers and the increasing level of production because of the European funds allocated for rural development like the measures offered by The Agricultural Fond for Rural Development: 112 (for installing the young farmers) and 121 (the modernization of agricultural exploitations) the one condition is the compliance with the business plan [4].

The destination countries for the Romanian exports are: Hungary (196 tones in amount of 18.392 Euro), Holland (81.2 tone in amount of 315.502 Euros), Bulgaria (51.3 tones in amount of 199.543 Euros) while for other destinations goes a total of 69.5 tones [5].

The main advantages of the imports refer to the higher quantities of flowers purchased at low prices. This is the reason why the national production is not very profitable unless the production increases and the prices will be reduced.

There is an ecological alternative of production and commercialization of flowers, but they imply huge production costs. The main consequence is the decreasing level of the demand for the Romanian consumers, leading to a balance between the demand and the offer [7].

In Romania the current trends are to increase the surfaces cultivated with flowers with compliance to the European standards and the Regulation (CE) no. 889/2008 regarding the ecological production.

According to the statistics, the flower merchants are confronted to the highest level of demand of 60% during the springtime because of the special occasions, religious and laic holydays while the lowest level (25%) is reached during the winter season.

**CONCLUSIONS AND FUTURE WORK**

It can be concluded that is very important to permanently analyze the flower market interaction, the flower production and also the international trade regarding them.

The interest for the increasing level of flower production and the flower trade is strongly influenced by the consumers’ increasing demand. The constant analyze of the products and services offered by the merchants is necessary.

The main conclusion is that Holland has the leading role in the international flower trade.
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DEVELOPMENT AND ANALYSIS OF NEW SEMISOLID PREPARATIONS WITH HEALING ACTION BASED ON PLANT EXTRACTS

C. E. Gird, T. D. Balaci, L. E. Dutu, V. Hirjau, A. A. Stanescu and A. C. Fita

Abstract: The objective of this study was to use lyophyllization as a laboratory method for obtaining extracts from plant materials (Calendulae flores, Sambuci flores, Alchemillae herba, Millefolii flores and Liquiritiae radix), to standardize the active ingredients content of each extract and then to combine this extracts based on their analyzed constitution in different portions to obtain various topic preparations.

Key words: Plant materials, Topic preparations.

INTRODUCTION
There are a lot of nutritive supplements, but most of them are not standardized. This paper presents the methodology for to obtain dry standardized extracts, which will be further include in a topic pharmaceutical preparation.

Taking into account the data from scientific literature, we select some herbal drugs which have anti-inflammatory, astringent and antibacterial activities: Calendulae flores, Sambuci flores, Alchemillae herba, Millefolii flores, Liquiritiae radix.

Calendulae flores is well-known in phytotherapy. It has wound-healing effect (due to flavones, carotenoids and saponins; oleanolic acid increase the synthesis of soluble-cholagen), anti-inflammatory activity, anti-edematous activity (due to flavones and saponins) and immunostimulating effect (due to calendulosides). Sambuci flores is diaphoretic and diuretic, due to flavones, saponins and mineral salts (potassium). Alchemillae herba has astringent, wound-healing effect, hemostatic and antibacterial activity, due to tannins, which precipitate bacterial proteins. Millefolii flores has anti-inflammatory and hemostatic activity, due to volatile oil, flavones, triterpens, tannins and vitamine K. Liquiritiae radix has anti-inflammatory activity cortizonic-type (due to glycyrrhizic acid) and antispasmodic activity (due to flavones and coumarins) [4, 6, 10].

MATERIAL AND METHODS
Materials: Calendulae flores, Sambuci flores, Alchemillae herba, Millefolii flores, Liquiritiae radix, which were bought from Romanian pharmacies; hydroalcholic extractive solutions, obtained from previous raw materials; dry selective extracts, which were obtained at Faculty of Pharmacy (Bucharest), Departament of Pharmacognosy Phytochemistry Phytotherapy.

Ointment bases with hydrophyllic polymers (polyacrylic acid, cellulose derivatives, polyvynilic alcohol) were prepared, with concentrations mentioned in the scientific literature (table 1). The ointment bases were selected according to the some known principles from the pharmaceutical technology like: stability, concordant plastic and pseudoplastic flowing, slow release of drug, optimum viscosity and penetrability. The preparation methods vary according to the nature of the macromolecule [3, 5, 9].
Table 1. Hydrophillic ointment bases

<table>
<thead>
<tr>
<th>Components</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyacrilic acid (Carbopol 940)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methylcellulose (Methocel MC)</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.65</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Preservative solution to</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Dry vegetal extracts were incorporated in these ointment bases, in concentration of 2.5% (table 2).

Table 2. Hydrogels with vegetal extracts

<table>
<thead>
<tr>
<th>Components</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sambucci flores extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Liquiritiae radix extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Calendula flores extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Alchemilla herba extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Millefolii flores extract</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ointment base I to</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ointment base II to</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ointment base III to</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Ointment base IV to</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

For the identity checking and determination of quality for raw vegetal materials it was applied the pharmacognostic analysis.

The identity was appreciated through a macroscopic exam, a microscopic exam (powder preparations clarified with a 800g/L chloral hydrate solution) and a qualitative chemical analysis. For the qualitative analysis the raw materials were successively extracted with polar solvents (alcohol, purified water). Half of the above alcoholic and aqueous solutions were hydrolyzed. Specific reactions were carried out in initial and hydrolyzed solutions, with the purpose to identify the active principles.

In order to evaluate the quality, the content of flavones was determined, using a spectrophotometric method, which is based on the reaction with aluminium chloride. The standard calibration curve was obtained using rutin (Merck). For the spectrophotometric assay a UV-VIS Cecil Series 2000 spectrophotometer was used.

The hydroalcoholic extractive solution was concentrate in a rotary evaporator (Rotavapor R-215, Buchi) and then was evaporated to dryness in a lyophilizer (Christ Alpha 1-2, B. Braun Biotech International).

Were determined the organoleptic properties, the pH (by potentiometry) and some rheologic properties of the products, such as spreadability (by the extensiometric method Ojeda – Arbussa) and the viscosity (using a Rheotec RC1 apparatus) [10, 11].

The determinations were made after preparation and after certain intervals in time (three months, six months).
RESULTS AND DISCUSSION

The morphologic characteristics of our raw materials correspond to those mentioned in scientific literature for these herbal drugs, e.g.: Calendulae flores: ligulate florets consists of orange-yellow ligule, with a three toothed apex; Sambuci flores: small flowers, with a white to yellowish-white corola; Alchemillae herba: cylindrical pubescent stems, petiolate or sessile leaves, with a pair of large stipules at the base, and 5 – 9 lobes, small yellowish-green flowers, arranged in flat-topped clusters; Millefolii flores: white to yellowish-white capitula, arranged in a corymb; Liquiritiae radix: yellow cylindrical fragments of roots, with a sweet taste.

The following anatomic characteristics were observed in powdered preparations: Calendulae flores: spherical pollen grains with a spiny exine, biseriate covering-trichomes, secretory trichomes with biseriate multicellular stalk and a large, biseriate multicellular head, bulbous papillae, endothecium; Sambuci flores: spherical pollen grains with very finely pitted exine, endothecium, idioblasts containing numerous sandy crystals of calcium oxalate; Alchemillae herba: fragments of palisade parenchyma and spongy parenchyma, numerous acuminate covering-trichomes, with thick lignified walls, somewhat enlarged and pitted at the base; Millefolii flores: spherical pollen grains with a spiny exine, endothecium, uniseriate covering-trichomes with four small cells at the base and a long, often tortuous terminal cell; Liquiritiae radix: fragments of xylem, yellow thick-walled fibres, often accompanied by prisms of calcium oxalate.

Only active principles which are soluble in polar solvents were investigated, with the aim to justify the association of hydroalcoholic extracts in a pharmaceutical preparation. All raw materials contain flavones, saponins and tannins.

After that, hydroalcoholic extractive solutions was obtained, using each of these raw materials and alcohol 20%, 50% and 70%, respectively, as solvents. The content of flavones was analysed in these solutions. The results are enclosed in table 3.

### Table 3. Quantitative analysis of raw material - the content of flavones

<table>
<thead>
<tr>
<th>Herbal drug</th>
<th>Flavones (g%, expressed as rutin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcohol 20%</td>
</tr>
<tr>
<td>Calendulae flores</td>
<td>0.090-0.101</td>
</tr>
<tr>
<td>Sambuci flores</td>
<td>0.082-0.100</td>
</tr>
<tr>
<td>Alchemillae herba</td>
<td>0.120-0.138</td>
</tr>
<tr>
<td>Millefolii flores</td>
<td>0.099-0.112</td>
</tr>
<tr>
<td>Liquiritiae radix</td>
<td>0.105-0.182</td>
</tr>
</tbody>
</table>

The results suggest that alcohol 70% is the solvent which extract the highest amount of flavones, so in further study we used this solvent for to obtain selective extracts.

Because the yarrow volatile oil has important therapeutic properties, but it lost when the raw material is heat under reflux, a extractive solution was obtained, by infusation Millefolii flores with purified water, for 30 minutes. The content of flavones was determined for this solution. 0.115 – 0.120 g% flavones, expressed as rutin was obtained.

Dry extracts were prepared by heating under reflux with ethanol 70% (for Calendulae flores, Sambuci flores, Alchemillae herba, Liquiritiae radix), or by infusation with water (for Millefolii flores), following by concentrating the solutions in a rotary evaporator and then evaporating to dryness in a lyophilizer. The dry extracts are hygroscopic, yellowish-brown (Calendulae extract, Sambuci extract, Millefolii extract), yellow (Liquiritiae extract) or yellowish-green (Alchemillae extract).
The reactions for flavones, saponins and tannins were positive in all five extracts, so we considered that our extraction method is correct, any degradative products formed.

The content of flavones was determined for these extracts. The results are enclosed in table 4.

Table 4. Quantitative analysis of dry extracts - the content of flavones

<table>
<thead>
<tr>
<th>Extract</th>
<th>Flavones (g%, expressed as rutin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calendulae extract</td>
<td>0.133 - 0.368</td>
</tr>
<tr>
<td>Sambuci extract</td>
<td>0.218 – 0.929</td>
</tr>
<tr>
<td>Alchemillae extract</td>
<td>0.309 – 1.391</td>
</tr>
<tr>
<td>Millefolii extract</td>
<td>0.251 – 1.035</td>
</tr>
<tr>
<td>Liquiritiae extract</td>
<td>0.108 – 0.406</td>
</tr>
</tbody>
</table>

The rheograms of the gels show that each gel manifests a pseudoplastic flow, typical for such systems, and some a tixotropic flow: the gel bases with methylcellulose had pseudoplastic behaviour and gels based on Carbopol are significant thixotropic [9].

By incorporating the vegetal extracts in the methylcellulose and polyvinyl alcohol gels, their rheologic properties are slightly modified, compared to the Carbopol gels (figures 1,2).

During 6 months of different storage conditions, the formulations were stable and did not demonstrate changes of physical and chemical properties, such as aspect, pH and rheologic profile.
CONCLUSIONS AND FUTURE WORK

The analysis of rheological behavior represents an essential factor in the evaluation of quality, stability and bioavailability of topical preparations.

Based on the results, it can be concluded that all semisolid systems with the formulas proposed in the study have an optimal rheological behavior and an adequate consistency. These characteristics allow an easy application on the skin, a good stability and, due to the active principles in the incorporated vegetal extracts, have the potential of being used for healing, anti-inflammatory and astringent activity.

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COMPARATIVE PHARMACOGNOSTIC STUDY ABOUT WHITE MULBERRY LEAVES (*MORUS ALBA* L.) AND BLACK MULBERRY LEAVES (*MORUS NIGRA* L.)

L.E. Dutu, C.E Gird, T.D. Balaci, A.C. Fita and M.A. Mitu

**Abstract:** This study proposes a comparative botanical and a phytochemical examination of the leaves harvested from white and black mulberry. Pharmacognostical tests suggest that both leaves could be used in therapy. After that, a selective extract and then a pharmaceutical preparation (tablets) were obtained and analysed. Preliminary research results regarding the formulation and the preparation of laboratory-scale tablets, can be the basis for obtaining a new type of tablets with hypoglycemic activity.

**Key words:** Morus alba, Morus nigra, selective extract, tablets, diabetes.

**INTRODUCTION**

The *Morus* genus consists of about 15 woody species. The most frequently cited in the scientific literature are *Morus alba* (fig. 1) and *Morus nigra* (fig. 2).

![Fig. 1. Morus alba L.](image)

![Fig. 2. Morus nigra L.](image)

Mulberry leaves, particularly those of white mulberry, are important as the sole food source of the silkworm (*Bombyx mori*), the pupa / cocoons of which is used to make silk. *Mori folium* is used internally in therapy for treatment of diarrhea and diabetes (adjunct). [3,5,9].

This study proposes a comparative botanical and a phytochemical examination of the leaves harvested from white and black mulberry, in view to predict if therapeutic activities differences exist. After that, according with these preliminary results, a selective extract and a pharmaceutical preparation (tablets) were obtained. choose plants as an alternative to a full air conditioning solution.

**MATERIAL AND METHODS**

The raw materials are two samples of leaves, harvested from Vrancea district (Romania), at their full maturity, in 2009. The leaves were naturally dried in the shadow, and conserved in laboratory conditions.
1. Pharmacognostic characterization of raw materials

The identity of the two samples was verified through a macroscopic exam. After that, a microscopic examination on the powder drug was carried out. Chloral hydrate solution 800 g/L as reagent and a Zeiss Imager D1 microscope (ob. 10x and 40x) were used.

Qualitative and quantitative phytochemical studies were carried out, too. For the qualitative analysis the raw material was successively extracted with different solvents (ethyl ether, alcohol, purified water). Half of the above alcoholic and aqueous solutions were hydrolyzed. Specific reactions were carried out in initial and hydrolyzed solutions, with the purpose to identify the active principles. In order to evaluate the quality the contents of some active principles were determined. Spectrophotometric methods were used for to evaluate the content of flavones (reaction with aluminium chloride, etalone curve of rutin, detection at 427 nm) and polyphenolcarboxylic acids (formation of oxymes in the presence of sodium nitrite / hydrochloric acid and sodium hydroxide, etalone curve of caffeic acid, detection at 510 nm). For the spectrophotometric assay a UV-VIS Cecil Series 2000 spectrophotometer was used. Preliminary, parameter „loss on drying” was carried out [1,2,6,7].

2. Obtaining and chemical characterization of selective extract

For to obtain a selective extract, the vegetal drug was heat under a reflux condenser, using purified water as solvent, the extractive solution was concentrate in a rotary evaporator (Rotavapor R-215, Buchi) and then was evaporated to dryness in a lyophilizer (Christ Alpha 1-2, B. Braun Biotech International). The dry extract was characterized, by the following parameters: loss on drying, the content of flavones and the content of polyphenolcarboxylic acids. Foregoing methods were applied.

3. Formulation, preparation and quality control of mulberry extract tablets:

Materials: standardized mulberry leaf extract; microcrystalline cellulose (Avicel PH 102); sodium starch glycolate (Primogel); polyvinylpyrrolidone K 30; colloidale silicon dioxide colloidale anhydrous (Aerosil); talc; magnesium stearate.

Equipment used for preparation and quality control tests of the tablets: Mettler Toledo electronic balance; Erweka cubic mixer; Wet Granulator Type WG - 30 PharmaG; Tablet Compression machine Triowin TP 1400 Tablet Press; Disintegration Tester Sotax DT 3; Hardness tester Vanderkamp VK 200; Vankel friability tester.

We begin the study by making several experimental combinations between the plant extract and excipients in different proportions. The next step was to granulate all the obtained mixes using a binder solution of polyvinylpirrolidone. Then we dried the granules, and after adding the lubricant auxiliary substances we have studied for this new mixtures some physical characteristics such as the flow rate, poured and tapped bulk density and compresibility (Carr’s compressibility index and Hausner ratio).

Based on the tests results we established qualitative and quantitative composition of the tablets, as presented in Table I.

The technological process of tablets making involves several steps: weighing of the raw materials, grinding and sifting of plant extract and excipients, homogenisation of the initial mix, wet granulation, drying and uniformizing of the granules, preparation of the compression material by adding lubricant excipients to the dried granules, compression with an excentric tablet machine equipped with a 12 mm die and punch.

After compression stage we could move on to the quality control tests of the tablets that includes: appearance, mass uniformity, disintegration time, hardness and friability. [4,6,7,8].

<table>
<thead>
<tr>
<th><strong>Tabelul I . Tablets formulation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>132</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

1. Black-mulberry*leaves are simple, often lobed, serrated on the margin, slightly pubescent especially on the abaxial surface and on the veins. White-mulberry*leaves are quite small.

The aspects of our leaf samples correspond to those are mentioned in scientific literature for the leaves of Morus nigra and Morus alba, so we considered that the identity of the two raw materials was verified [5,9]

The following anatomic characteristics were observed in both leaf*microscopic preparations: epidermal cells with anomocytic stomata, spirally and annularly thickened vessels, long covering trichomes with striated cuticle, and numerous idioblasts.

Sterols, carotenoids, saponins, catechic tannins, mucilages and reducing compounds, flavones and polyphenolcarboxilic acids were identified in both leaves'samples, by specific chemical reactions.

The results for quantitative analysis are enclosed in table II.

<table>
<thead>
<tr>
<th>Table II: Mulberry leaves - quantitative analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Loss on drying (%)</td>
</tr>
<tr>
<td>Flavones (mg %, expressed as rutin)</td>
</tr>
<tr>
<td>Polyphenolcarboxilic acids (mg %, expressed as caffeic acid)</td>
</tr>
</tbody>
</table>

All these qualitative and quantitative results suggest that both leaves (from Morus alba and Morus nigra) could be used in therapy.

2. The selective extract is a pale-brown, odourless powder.

The results for quantitative analysis are enclosed in table III.
Table III: Mulberry extract - quantitative analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying (%)</td>
<td>EP general method (105 °C)</td>
<td>4.85</td>
</tr>
<tr>
<td>Flavones (mg %, expressed as rutin)</td>
<td>spectrophotometric method</td>
<td>535.361</td>
</tr>
<tr>
<td>Polyphenolcarboxilic acids (mg %, expressed as caffeic acid)</td>
<td>spectrophotometric method</td>
<td>507.223</td>
</tr>
</tbody>
</table>

3. In the formulation stage, because we had to satisfy both the demand of having high dose of extract / tablet and the need to maintain an acceptable size of the final product, we chose only the excipients that can exercise their technological and biopharmaceutical role at low concentrations.

Well-known for his good capacity of water absorption, followed by swelling and pronounced increase in volume, the sodium starch glycolate (Primogel) was used for an optimal dissolution effect. Because of his concentration per tablet the efficacy of the disintegration is not affected by the presence of hydrophobic lubricants or by the increase of the compressing strength.

Based on the results of the physical characteristics of the different compression mixtures it was selected the optimal formula which showed higher values for the poured and tapped bulk density as well as higher compressibility. The Carr’s index values (31.3) indicates a favorable compression behavior and the small values of Haussner ratio (1.4) designates good flow properties of the mixture.

The characteristics of the experimental formulation allowed a good application of the standard technological process for obtaining laboratory-scale tablets, as evidenced by the results obtained from measurements performed in the quality control (Table IV).

The controled aspects have been within the limits allowed by regulations.

Tabelul IV . Quality control of the tablets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect</td>
<td>uncoated, coated, flat, edges intact and smooth</td>
</tr>
<tr>
<td>Thickness</td>
<td>3.8 mm</td>
</tr>
<tr>
<td>Diameter</td>
<td>12 mm</td>
</tr>
<tr>
<td>Colour</td>
<td>yellow (cream)</td>
</tr>
<tr>
<td>Average mass</td>
<td>0.650 g</td>
</tr>
<tr>
<td>Desintegration</td>
<td>7 minute</td>
</tr>
<tr>
<td>Hardness</td>
<td>17 Kp</td>
</tr>
<tr>
<td>Friability</td>
<td>0.92 %</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND FUTURE WORK

There are not different morphologic and anatomic characteristics between Morii albae folium and Mori nigrae folium.

The qualitative and quantitative results suggest that both leaves (from Morus alba and Morus nigra) could be used in therapy.

The dry selective extract (obtained by using purified water as solvent) contains 535.361 mg% flavones (expressed as rutin) and 507.223 mg% polyphenolcarboxilic acids (expressed as caffeic acid).
Preliminary research results regarding the formulation and the preparation of laboratory-scale tablets, combined with the results of preclinical and clinical experiments, can be the basis for obtaining a new type of tablets with hypoglycemic activity.

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THEORETICAL CONSIDERATIONS REGARDING PSYCHOSOMATIC SYMPTOMS IN PSYCHOANALYSIS
Abstract: The psychoanalytic concept of drive is placed at the limit between psychic and somatic. It is the psychic representative of the somatic stimuli, stimuli that impose a certain process to the psychic apparatus. On the one hand, a certain Freudian model of the psychic apparatus (as it appears in the Interpretation of dreams) presents it as having two «windows»: perception and motricity. On the other hand, deficits in the psychic process – especially regarding the capacity of mental representation – can lead to somatisation, which is the manifestation of the psyche on a somatic ground. The paper proposes outlining a comparison between the psychosomatic phenomena and other «exit» directions of the excitation from the psychic apparatus: on the one hand, the dream as hallucination of the perception, on the other hand, the act as a motricity’s manifestation towards the exterior.

Key words: psychosomatic, drive, dream, acting, representation.

DREAM AND ACT AS EXPRESSION OF DRIVE

S. Freud speaks about the „limit” in 1915, when he defines the concept of drive: “[...] the drive appears to us as a concept on the frontier between the mental and the somatic, as the psychical representative of the stimuli originating from within the organism and reaching the mind, as a measure of the demand made upon the mind for work in consequence of its connection with the body” (Freud, 1915, p. 64).

Besides the meaning „limit of concept” (Green, 1997, p. 48), we are also dealing with the idea of frontier between psychic and somatic looking from inside the psychic apparatus towards its exterior (the drive is already a psychic representative).

In The Interpretation of Dreams, the psychic apparatus seems to have two “windows”: perception and motricity; the excitation – thus the drive –“move” around or towards one another.

Given the points of view that arise from the first Freudian topic, combined with the idea of „drive movement” particular to the second topic, I would like to put forward the theme in the title taking into account two aspects as terms of reference.

The first is the dream, a regressive process unto the perceptive pole, with the hallucination of perception: the representation becomes sensoriality and, says Freud, “the conceptual content is not thought, but is transformed into visual images, to which we give credence, and which we believe that we experience” (Freud, 1900, p. 490-498, my emphasis).

For him the dream represented the „royal” way towards the Unconscious. And Melanie Klein used it a lot, in order to illustrate the mechanism of projective identification, a mechanism that was later considered an utmost under an interpersonal light and which led to a dual functioning model in cure. The dream has an extremely useful ambiguity for the study of the Unconscious: it is lived by the subject as an external reality, on the other hand, at awakening and when recalled no doubt rests on his subjective nature of internal reality (on the author and the dream’s inner source), although felt as “foreign”.

The other extremity taken into account for the psychic apparatus, that of motricity, is the frontier towards act. The act was described as an essential element for the matter of borderline states, unlike neuroses that „deal” better with representations.

Serving as illustrations, two „cases” come to my mind. One is The Kreutzer Sonata by Tolstoi, where Pozdnišev’s murder act, although a real, external act, is perceived in an unconscious way as though being dreamt, internal, reversible; only by looking at his dead wife, in the coffin, three days after the murder does he have the revelation of the irreversible objective time, that vectorilly passes by, from life to death:
“Then only, when I saw her dead face, did I understand all that I had done. I understood that it was I, I, who had killed her [...] I was the cause [...] and there was no way, never, nowhere, of repairing this thing” (Tolstoi, p. 103, my emphasize). The character also seems to experience the revelation of his clearly distinguished Ego. Moreover, he tries to equate "never" with "nowhere" and "no way" in a similar failed attempt to avoid the traumatic revelation of his limits and of the external reality limits.

The second “case” is one related to the reference point „act“ namely the case patented as illustrating borderline pathology, of M. Little. In accounting her analysis with Winnicott, she relates a fragment that took place in one of the first sessions. Overwhelmed by the fact that she will never be able to communicate with the analyst, she started to gander the cabinet and then thought: “I contemplated throwing myself out of the window, but felt that he would stop me?]. Then I thought of throwing out all his books, but finally I attacked and smashed a large vase filled with white lilac, and trampled on it. In a flash he was gone from the room [...] Next day an exact replica had replaced the vase and the lilac […] Neither of us ever referred to it again, which seems odd to me now” (Little, 1985, p. 122).

I noticed that, out of all the scene, the only version that became act was the one not thought consciously. The dream-act analogy would be that, as „images seem to think themselves“ in the dream (Bion, Lacan), in such borderline states „the act seems to think” itself, without a subject to think.

THE PSYCHOSOMATIC EXPRESSION OF DRIVE

From a clinical point of view, patients associated with psychosomatics mainly present a somatic symptom – functional or tissual – and a psychic symptom.

Although he was never particularly preoccupied with this matter, Freud thought of the psyche and body as representing a unit. However he clearly established the boundaries of his field of exploitation, as being strictly the psychological field, while the organic field couldn’t or wouldn’t be exploited.

S. Ferenczi argued that the illness could be a defence measure against unconscious psychic processes. He even assumed that a psychotic pathology, for instance melancholy, could be cured following an organic illness. M. Klein imagined the source of the organic illness as staging and dramatizing some archaic phantasms. Moreover, the illness would also be a defensive method against psychosis.

A concept that turned in a true paradigm, especially in France, appeared during the 60’s. The main idea was that of somatic disorders due to a deficiency in mentalization, related to a psychic dysfunction of an economic type. The representatives of the Paris Psychosomatic School are P. Marty, M. De M’Uzan, C. David, M. Fain. The fundamental notion is that of „deficiency“ or „psychic deficit“, some sort of negative of the neurotic functioning, distorting negative, that has no neurotic mental defences capable of adjusting the psychic energetics. We are not dealing with a meaning of malady, but with a disinvestment or counter-investment of an economic nature.

“Operative thinking” is described as specific to this so called „linear“ functioning, because it lacks affect, it focuses on factual, on describing, on concrete instances and is poor in associations that send to a phantasmatic or oniric. The relationship with the other is described as „white“, without affective involvement, and the intellectualization degree of the communication has a stereotype nature, with a weak symbolist capacity. The effort on a pre-conscious level seems quasi absent. The subject seems to maintain a distance from all forms of psychic conflicts.

The concept of essential depression or „depression without an object“ is associated to the operative thinking, which is however difficult to diagnose, as it lacks the depressive
affect. This thing proves that the psychic economy involved not only reaches thinking, but also the affective sphere. Such a “disaffection”, that McDougall called “pulverisation of affects”, was seen as a true “survival technique” of the subject confronted with an intolerable perspective of sufferance. Reaching the affective sphere determined Marty to modify the “operative thinking” term in “operative life”.

Marty describes a true ierarhization of psychic functions, starting from the most sophisticated mental functions and reaching the somatic functions. The operative pathology conditions determine a dis-ierarhization: from a mental level, the operative defence mechanisms and the essential depression lead to somatisation through psychic disarray.

More recently, M. Aisenstein shows that the emergence of a somatic illness is the result of numerous factors, among which we may find the psychic factor. She mentions the following fact as proof of the economic (energetic) aspect’s intervention in psychosomatics: severe mental disorders, like melancholy, temporarily disappear in the course of an intercurrent organic illness (2010, p. 1367). Starting from the Freudian definition of drive as « demands of the psychic effort » the same author formulates a further step: drive as demand for representation.

Therefore, the psychic connection with soma implies from the first the necessity to represent what belongs to it, by taking the form of corporal the body unlike the soma is already « dwelt » by the psychic, meaning it already has a representation. In other words, Aisenstein’s theory implies a body (capable of being imagined) that interposes between psychic and soma. Thus, the representation effort would start with an effort through which the body appears where the soma was. (2010, p. 1369).

In 1920, Freud modified the first theory regarding the psychic apparatus (unconscious, pre-conscious, conscious) elaborating his second theory in which psychic instances are Id (unorganized drive container of life and destructive drives) Ego and Superego. If the first theory satisfyingly explained the neurotic functioning (with the hysteria paradigm), the second theory represented an adaptation to the clinic’s non-neurotic pathologies. Today, we can say that the non-neurotic functioning includes not only the borderline pathology, but also the addictions and psychosomatic manifestations.

Though, nowadays, psychoanalysis asserts that the neurotic functioning is the closest to healthy psychic functioning, or one of its conditions. In the psychoanalytic treatment an indicator of progress is the psychic transformation in the sense of « neurotisation » or « hysterisation ». Hence, the first Freudian model not only cannot be abandoned in the psychoanalytic theory, but it (re)becomes an actual alternative for the research of any pathologic functioning, including the psychosomatic one. In the first model the accent is laid on the unconscious representation, while, in the second model, the economic, quantitative aspect of the drive’s movement force and act gain importance. This is also a model applicable to the study of affect as the affect is also defined as drive movement.

Therefore, psychosomatics might be dealing with a psychic organization in which the drive force becomes more important than the meaning (Aisenstein, 2010, p. 1373). The meaning implies a representation capacity which also implies an integration of the subject’s passive position, opposed to the « acting » tendency. The representation is an inner transformation, the act tends to transform the external reality. Thus, the psychoanalytic cure of patients with borderline and somatic organizations might have the purpose of passing from a process directed towards act to a process capable of producing representations.

For P. Marty soma is a field of dis-psyching the drive. In other words, somatisation is a « relapse » of the drive movement from the psychic in its somatic source, a regression of the drive towards its origin.
If neurosis (with its paradigm of hysteria) is based on the hallucinating functioning illustrated by the dream effort, the borderline and psychosomatic organizations originate from a trauma and destructivity matter.

On the other hand, L. Grinberg stated that the act is a dream that cannot be accomplished. Through the language’s transforming function, in the psychoanalytic cure, the «acting» tendency shall become a dream or the capacity to dream.

B. Rosenberg emphasized the role of the waiting function in the metabolization and mentalization capacity, a waiting that implies the mobilisation of a primary erogenous masochism, needed for tolerating tension or pain. This waiting makes possible the psychic effort, thus the representative effort.

But wasn’t the dream called the «the guardian of sleep», meaning an element that makes the somatic «waiting» possible until at the right time the awakening can occur? The dream puts the soma in waiting, delays the awakening act. The dream requires passivity, and the psychosomatic organization is based on an early traumatism that compromised the already passive position. (Savvopoulos, 2010, p. 1404).

In psychosomatic patients, the deficiency of the primary masochism leads to running from the inner psychic conflictuality, leaving disorganization a free pass. That is why the «somatic solution» was qualified as being an «economic emergency solution» (Potamianou, 2010, p. 1361).

CONCLUSIONS

Psychic movements (and the drive, as psychic representative of somatic stimuli is already psychic) are headed, as expression, towards three registers: soma, behaviour and psychism. (Potamianou, 2010, p. 1360). I shall resume the reference points laid down at the beginning of the paper, that is dream and act.

The dream is about representation, that regresses from thinking to sensoriality and hallucination of perception. The movement is limited to the psychic space, to the internal reality, to the reversible subjective time. Somatisation shows the drive’s impossibility to «remain» in the representative and conflictual psychic space. The psychic tension is evacuated at the psychism border, but outside it, in soma, his territory of origin. However, the inner tension is not evacuated through the act, in the external reality, of the irreversible time. This means that the «soma» forms, looking from inside the psychic space, a space located between the internal reality and the external reality of the behaviour.

The Skin-ego also expressed the ego’s activity of «spacing» drives, as a way of giving them form (Savvopoulos, 2010, p. 1394), a condition of representation. Thus, somatisation could be seen, following Grinberg’s idea, as a drive movement that couldn’t be «dreamt» nor transformed in act, as a consequence of her impossibility to pass through the body. F. Pasche (1999) defined the body as a representation projected by self at the Ego’s frontier, as a psychic protection analogue to skin which guards the physical body.

Thus, the comparative discussion regarding the somatisation process in relation with the dream and act reveals another difference, that between the body and soma. Freud considered that they were synonyms, it was just one body with psychic and somatic valances even in the case of actual neurosis. Marty thought that while the body is invested with libido thus pertaining to the psychic territory, being capable of elaboration, either representing for the subject himself or his object), soma lays on the ground of the de-psyching of drive. (Savvopoulos, 2010, p. 1397).

A question arises, are we dealing with an evacuation in the de-psyching movement? (Bion). If yes, the following hypothesis emerges. Trying to evacuate the psychic tension towards the exterior, in the form of an act, doesn’t the subject have a certain type of
confusion between the body image and soma? Can we anticipate this kind of confusion at the two protagonists taken as landmarks at the beginning of the paper, the Kreutzer Sonata (that mistook the act as dream) and M. Little (that maybe mistook the act as thought). The body lies within the psychic space, the soma outside of it. Then, the confusion they bring along would constitute a particular means of the generic pathological non-differentiation between the internal and the external reality.

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FORMULATION AND EVALUATION OF SOME MULTIVITAMIN TABLETS

Anca Nicoară, Teodora Balaci, Andreea Stănescu, Mircea Hîrjău, Oana Karampelas, Emma Budura

Abstract: We have aimed our research towards obtaining a flavoured vitamin combination in the form of chewable tablets and effervescent tablets. These two types of tablets, due to their way of administration and their improved taste, are an attractive alternative, especially to children of young ages, who have difficulty in swallowing. The added advantage of effervescent tablets is that the active ingredients are already in solution at the time it is consumed. Thus, the absorption is faster and more complete than with conventional tablet.

Key words: effervescent tablets, chewable tablets, direct compression.

Introduction

Vitamins are essential micronutrients required for healthy development, growth and maintenance of life. They have a variety of functions in the body, regulating the metabolic process, converting carbohydrates and fat into energy and forming tissue and bone. Vitamins, generally, cannot be synthesized by humans and, therefore, must be supplied in the diet. Because food contain only small quantities of the vital micronutrients, it is difficult to meet the requirements for vitamin intake through a normal diet. Furthermore, children, the elderly, pregnant women belong to a risk group for lack of vitamins. Thus, to avoid deficiency diseases, supplementation with vitamin products can be of particular importance.

Effervescent tablets are uncoated tablets that generally contain acid substances and carbonates or bicarbonates which react rapidly in the presence of water by releasing carbon dioxide. They are intended to be dissolved or dispersed in water before use. Many studies have demonstrated that effervescent tablets enhance absorption of a number of active ingredients, because the carbon dioxide created by the effervescent reaction can induce enhanced active-ingredient permeability, due to an alteration of the paracellular pathway. It is theorized that the carbon dioxide alters (widens) the intercellular space between cells, which leads to greater absorption of active ingredients (both hydrophobic and hydrophilic).

Chewable tablets represent a particular form of oral dosage, not intended to be swallowed intact, but to be chewed in the mouth by the patients, which are, most of the times, children or people who either refuse to swallow an intact tablet or may have difficulty doing so.

Materials and methods

We have developed, laboratory scale manufactured and tested several formulations of effervescent tablets (formula I) and chewable tablets (formula II) containing water soluble vitamins (thiamine mononitrate, riboflavin, pyridoxine hydrochloride, nicotinamide, calcium D-pantothenate, ascorbic acid, cyanocobalamin) and fat-soluble vitamins (vitamin A, E) in the form of dry powders (fat-soluble vitamins combined with a protective colloid, sugars, emulsifiers, antioxidants). Fat-soluble vitamins as dry powders can be dispersed in cold water, making them suitable to use in preparations which need to be dissolved or dispersed in water prior to use (effervescent and chewable tablets).

The components of the studied tablets are shown in table 1.

Table no. 1. Effervescent and chewable tablets formulations
### Development of the formulas

The purpose was to provide a simple method of producing stable effervescent tablet having a short dissolving time, using the most efficient types and quantities of excipients.

As a source of carbon dioxide were used tartaric acid and sodium bicarbonate, which are very common in effervescent formulas and produce a clear solutions after tablet disintegration.

Ludipress is an all-purpose direct compression excipient, based on Lactose monohydrate, Kollidon 30 and Kollidon CL, which functions as a filler, binder and disintegrant. Having a low hygroscopicity, improves the stability of the active ingredients and of the tablets themselves.

Polyethylene glycol 6000 was chosen as lubricant due to the fact that is water soluble and not hygroscopic (qualities important in formulating effervescent tablets).

Fructose was introduced in formulation due to its multiple function: tablet diluent, sweetening agent, flavor enhancer, dissolution enhancer. Fructose is sweeter than the sugar alcohols mannitol and sorbitol, which are commonly used as tableting excipients and tablets of satisfactory hardness and friability can only be produced by direct compression.
Sorbitol and sucrose are common chewable tablet diluents, which permit smooth disintegration at a satisfactory rate, in the same time having a pleasant taste and feel in the mouth. Kollidon® VA 64 is a vinylpyrrolidone-vinyl acetate copolymer in the form of a free flowing powder, water soluble, with a faint characteristic odor and practically no taste. We have elected this modern binder due to its high plasticity (it produces mixtures that is less susceptible to capping and tablets that are less brittle), being best suited for tablets of low hardness and medium friability (in ex. chewable tablets) and for actives of low compressibility (in ex. vitamins).

Aerosil 200® (colloidal silicon dioxide) was chosen as glidant and flow improver of powders due to its small particle size and large specific surface. Furthermore, being odorless and tasteless makes it suitable for chewable tablets.

Flavoring agents and sweeteners, other then sugars, had to be added in formulations to mask the unpleasant taste of vitamins. Artificial sweeteners have the advantage of reducing the bulk volume, considering the quantity of sugars (sucrose, sorbitol, fructose) required to produce the same degree of sweetness.

For each formulation, all the components were mixed, passed through a 0.8 mm sieve, mixed again and pressed with high compression force in a single-punch press fitted with 20-mm (formula 1) or 12mm (formula 2) flat punches. All the manufacturing steps took place in an air-conditioned room (relative humidity <25% and temperature <25°C).

Direct compression method is most suitable for tablets containing vitamins (which are highly unstable active ingredients, can be degraded in the presence of oxygen, light, temperatures above 25°C, humidity, oxidizing or reducing substances) because its a fast procedure, requiring few production operations (mixing and tableting) so that the stability of the active ingredients is higher, due to low exposure to degrading factors during production.

After preparation, the effervescent tablets were packed in plastic tubes with fitted caps containing a desiccant. Desiccants are used to absorb any free moisture in the tablet or in the air, thus preventing the effervescent reaction from starting prematurely. This packaging system was then verified performing the leak test.

The tablets were analyzed after preparation and after 3 and 6 months, the following determinations being performed:

- organoleptic characteristics;
- weight uniformity;
- mechanical resistance;
- friability;
- disintegration.

All the determinations were made under controlled conditions: air-conditioned room (relative humidity <25% and temperature <25°C).

The equipment used for these determinations was:

- for the determination of the mechanical resistance: Erweka type TBT apparatus and the method described in the Eur. Ph. 6th edition;
- for the determination of the friability: Vankel friabilator and the method described in the Eur. Ph. 6th edition;
- for the determination of the disintegration time: Sotax DT3 Disintegration tester and method A (formula 2) and method B (formula 1) described in the Romanian Pharmacopoeia, X th edition.
- for the weight uniformity: Mettler-Toledo balance;
RESULTS AND DISCUSSIONS

Table no. 2 Experimental results of the quality control performed on effervescent tablets (formula 1)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Initial</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
</tr>
<tr>
<td>Diameter</td>
<td>20 mm</td>
<td>20 mm</td>
<td>20 mm</td>
</tr>
<tr>
<td>Thickness</td>
<td>4 mm</td>
<td>4 mm</td>
<td>4 mm</td>
</tr>
<tr>
<td>Theoretical weight</td>
<td>2150 mg</td>
<td>2150 mg</td>
<td>2150 mg</td>
</tr>
<tr>
<td>Average weight actual</td>
<td>2148 mg</td>
<td>2150 mg</td>
<td>2152 mg</td>
</tr>
<tr>
<td>Friability</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Hardness</td>
<td>95 N</td>
<td>93 N</td>
<td>92 N</td>
</tr>
<tr>
<td>Disintegration time</td>
<td>1 min</td>
<td>50 sec</td>
<td>50 sec</td>
</tr>
</tbody>
</table>

Table no. 3 Experimental results of the quality control performed on chewable tablets (formula 2)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Initial</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
<td>Slightly yellow, round, biplanar tablets, characteristic odor</td>
</tr>
<tr>
<td>Diameter</td>
<td>12 mm</td>
<td>12 mm</td>
<td>12 mm</td>
</tr>
<tr>
<td>Thickness</td>
<td>3.7 mm</td>
<td>3.6 mm</td>
<td>3.7 mm</td>
</tr>
<tr>
<td>Theoretical weight,</td>
<td>580 mg</td>
<td>580 mg</td>
<td>580 mg</td>
</tr>
<tr>
<td>Average weight actual</td>
<td>575 mg</td>
<td>577 mg</td>
<td>578 mg</td>
</tr>
<tr>
<td>Friability</td>
<td>1.4%</td>
<td>1.4%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Hardness</td>
<td>60 N</td>
<td>62 N</td>
<td>62 N</td>
</tr>
<tr>
<td>Disintegration time</td>
<td>6 min</td>
<td>5 min</td>
<td>5 min</td>
</tr>
</tbody>
</table>

The tablets were evaluated for appearance, thickness, diameter, weight variation, hardness, friability, dissolution time. The appearance of tablets remained unchanged for the whole period of testing. The tablets obtained were of uniform weight, with acceptable variation (±1%). Friability of the effervescent tablets below 1% along with the hardness found to be 92-95N was an indication of good mechanical resistance of the tablets. A friability above 1% for the chewable tablets and a lower hardness (60-62N) are general acceptable because these tablets have to be easily chewed in the mouth. A disintegration time of 1 minute for the effervescent tablets is within the acceptable limits of Romanian Pharmacopoeia for this type of tablets. A higher disintegration time (6 min) for the chewable tablets is normal to be obtained because these tablets do not contain disintegrants, the disintegration is facilitated by chewing.
CONCLUSIONS AND FUTURE WORK

We have prepared several formulations in order to appreciate how the composition may influence the quality and stability of the tablets and then we have analysed each of these products performing following determinations: organoleptic characteristics, weight uniformity, mechanical resistance, friability, disintegration. All this quality tests were repeated with good results after 3 and 6 months.

The characteristics of the tablets studied suffered minor variations (within acceptable limits) during storage under controlled conditions (relative humidity <25% and temperature <25°C) and it can be concluded that the preliminary researches on effervescent and chewable tablets formulations are satisfactory and may lead to further optimization.

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STUDIES TOWARDS OBTAINING A NEW PHOTOPROTECTIVE DERMOCOSMETIC PRODUCT

Andreea Stănescu, Teodora Balaci, Eleonora Mircia, Anca Nicoară, Mirela Mitu, Marcela Untaru

Abstract: The present study focuses on obtaining a new dermo-cosmetic photoprotective emulsion with good physical and chemical stability as well as suitable organoleptic and rheological properties, in order to ensure the innocuity and pleasant administrating features of the product.

We have used an organic filtering photoprotective substance obtained through a condensation process between the para-aminosalicylic acid and a substituted acetanilide derivate which showed no toxicity on the skin, inorganic screen photo protective substances, vegetal extracts, collagen, antioxidants, natural products having a slight photoprotective effect as well as hydrating and emollient properties, preservatives which prevent the development of microorganisms, natural perfumed composition.

The results of this study may lead to the possibility of using these photoprotective products after clinical researches are made.

Key words: Photoprotective dermo-cosmetic product

INTRODUCTION

Prolonged exposure to UV-B (290-320nm) and UV-A (320-400nm) radiations increases the risk of developing carcinoma and accelerates skin aging. Filtering photoprotective substances containing organic compounds that absorb the UV-B radiations and also screen substances that absorb the UV-A radiations are commonly used as a solar protection.

Photoprotective products are considered borderline between cosmetics and pharmaceuticals due to physiological implications they have on skin’s health considering the environmental changes (radiations, global warming, and the diminished ozone layer) and due to the high demands regarding the balance between the efficacy and the safety of the consumer.

If the early 1990’s the main purpose was to obtain products with higher photoprotective rates (in some countries the SPF reached 100), over time, taking more into consideration the consumer’s safety made it necessary to impose a limit regarding the maximum SPF value. This is why it was admitted lately a limited concentration of filter and screen substances in the formulation of dermo-cosmetic products.

The new trends in research and development of photoprotective products are towards decreasing, down to the elimination if it’s possible, of the negative influences this tipe of preparation may have on the skin (high allergenic potential, risk of tissue accumulation, hyperpigmentation) throughout the accomplishment of complex formulations. These formulations have the role of obtaining a targeted action of the pharmaceutical form and to incorporate natural bioactive substances witch can increase the efficiency of the synthetic photoprotective compounds.

The studies we have carried out in this paper consist in the following: selection of the excipients and active ingredients (organic and inorganic filter and/or screen substances, natural products, antioxidants) and the use of a proper technological process for obtaining a dermo-cosmetic product having a good physical and chemical stability, as well as suitable organoleptic and rheological properties in order to ensure the innocuity and pleasant administrating features.
MATERIAL AND METHODS

We have used the following ingredients:

- one organic filtering photoprotective substance: an organic substance obtained through a condensation process between the para-amino salicylic acid and a substituted acetanilide derivate which showed no toxicity on the skin;

- two inorganic screen photo protective substances, insoluble in water and oil: titanium dioxide consisting in particles (having a mean size of 15 nm and a specific surface of 80 m²/g) coated with alumina and silicon (titanium dioxide M170) for improving the stability of the emulsion. We have added in composition zinc oxide which is usually associated with titanium dioxide, who acts on the entire UVA and UVB spectrum;

- vegetal extracts having a well determined content in active principles: carrot and marigold extracts. Based on the content in bioactive principles and on the therapeutic effects mentioned by the traditional and modern medicine, we have selected two vegetal products having a well defined content in active principles. The extraction of bioactive principles from selected plants was made by cold maceration method to avoid altering the thermosensitive principles;

- one proteic substance: collagen used for the skin elasticity improvement and also known for having an intense skin hydration, firmness and revitalizing effect. Collagen is given much attention today by biologists, chemists, physicists, doctors and pharmacists due to his special qualities in the treatment of burns, in cosmetics, in various activities of restoration and regeneration of old leather. Also it was also proven that combined with the vitamins it’s increasing the protective activity of the products with 5-30%, depending on the type of vitamin used;

- natural antioxidants: vitamin A and E; synthetic phenolic antioxidants (butylated hydroxy anisole-BHA);

- natural products having a slight photoprotective effect, inferior to synthetic organic substances, and a hydrating and emollient effect;

- preservatives which prevent the development of microorganisms during the storage period;

- natural perfumed composition which ensures the desired organoleptic characteristics of the final product. Qualitative and quantitative composition is shown in table 1.

The physical and chemical methods used to characterize the raw materials and the final products are as following:

- to find out the amount of inorganic substances (with screening role) the samples were incinerated for two hours at 800°C;

- the quantity of organic substance (with the UV filtering role) was determined by an UV-VIS spectrophotometric method;

- vitamins identification was done according to the European Pharmacopoeia VI-th edition;

- the photoprotective ability was tested by applying a standard amount of emulsion on a synthetic skin device (0,2g/cm²);

- to test the microbial stability were applied the European Pharmacopoeia VI-th edition stipulations;

- to estimate the compatibility with the human skin a standard quantity of sample (0,2g/cm²) was laid under an occlusive patch. The test was made on a 25 cm² surface, on healthy volunteers. After 30 minutes the color intensity of the produced erythema was determined;

- the hydrating effect was evaluated through instrumental methods, after applying a sample on a test skin area located on the forearm;
RESULTS AND DISCUSSION

The preliminary tests have allowed us to establish the necessary quantities of substance for obtaining a homogenous product, pleasant after application which can provide a uniform film and assure optimal photoprotective characteristics.

The physical and chemical characteristics of the final product are shown in table 2.

In order to create an uniform film on the skin surface the forms for dermal application must present the rheological behavior of a liquid. This means that during the contact with the skin the two phases of the emulsion must separate and this separation leads to the decrease of the viscosity down to values that characterize the aqueous solutions. Spreading the emulsion on the skin makes it possible to evaporate the aqueous phase and to obtain a uniform film of photo protective substances. The tixotropic structure of the emulsion ensures an optimal distribution of the photo protective film on the skin’s surface. The viscosity returns to high values in a short time after the friction stops (figures 1, 2).

Table 1. Qualitative and quantitative composition for the photoprotective emulsion

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>The organic compound obtained through a condensation process between the para-aminosalicylic acid and 2,6-dichloro-acetanilide</td>
<td>1g</td>
</tr>
<tr>
<td>Titanium dioxide (M170)</td>
<td>1g</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>1g</td>
</tr>
<tr>
<td>Carrot extract</td>
<td>1g</td>
</tr>
<tr>
<td>Marigold extract</td>
<td>1g</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>300.000 UI</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Shea Butter</td>
<td>5</td>
</tr>
<tr>
<td>Olive oil</td>
<td>35g</td>
</tr>
<tr>
<td>Lanolin</td>
<td>7g</td>
</tr>
<tr>
<td>Wax</td>
<td>5g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1g</td>
</tr>
<tr>
<td>Whale spermaceti wax</td>
<td>3g</td>
</tr>
<tr>
<td>Cetylic alcohol</td>
<td>3g</td>
</tr>
<tr>
<td>Solution of sodium hydrogenocarbonate 2%</td>
<td>10g</td>
</tr>
<tr>
<td>Methyl p-hydroxybenzoate</td>
<td>0.05g</td>
</tr>
<tr>
<td>Butylated hydroxyanisole</td>
<td>0.05g</td>
</tr>
<tr>
<td>Volatile oil-based fragrance</td>
<td>q.s.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>60g</td>
</tr>
</tbody>
</table>
Table 2. The physical and chemical characteristics of the product obtained

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>homogenous</td>
</tr>
<tr>
<td>Colour</td>
<td>White or slightly yellow</td>
</tr>
<tr>
<td>Odor</td>
<td>Pleasant parfumated</td>
</tr>
<tr>
<td>pH</td>
<td>6</td>
</tr>
<tr>
<td>Stability at constant temperature</td>
<td>Stable, with no segregation of phases</td>
</tr>
<tr>
<td>Volatile substances content</td>
<td>58 %</td>
</tr>
<tr>
<td>Oily phase content</td>
<td>18 %</td>
</tr>
<tr>
<td>Photoprotective organic substances content</td>
<td>1%</td>
</tr>
<tr>
<td>Photoprotective value</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 1. Viscosity variation depending on the speed rate

Figure 2. Variation of the shear stress depending on the speed rate;
Microbiological characteristics: The total number of aerobic germs and fungi was determined by the decimal dilution method or the multiple tube test. For identifying pathogenic species we have used specific tests.

The evaluation of photodegradation was carried out through the variation of photoprotective factor of the studied product kept 60 minutes under UV radiation at 65 °C. The tests revealed the preservation or even a slight increase in the SPF.

The use of screen substances in the formulation of photoprotective products results in undesirable sensory characteristics: visible white film on the skin surface, unpleasant sensation, dry or oily skin after application. Therefore we considered necessary to evaluate the cosmetic characteristics after application of the obtained product.

The volunteers who applied the product on the skin appreciated the sensory characteristics such as: easiness of use, pleasant texture, easy absorption without oily traces, appearance, odor and pleasant color.

The results of hydration effect tests are presented in figures 3, 4.

Figure 3. Determination of hydration of the skin before applying the product

Figure 4. Determination of skin hydration after 30 minutes after application

The values of the erythema are relatively low, which indicates that the product is compatible with the skin and has no sensitizing potential.

Having a complex composition of ingredients with varying degrees of unsaturation, with the major organic structure, the product is subjected to destructive processes at the impact of UV radiation and atmospheric oxygen. As a result this organic substances undergo structural changes, embodied in the initial color change, division of chain, forming oxidizing action groups and oxygen free radicals that continue the destructive process. Because of these features, the product was subjected to accelerated tests of thermal and photo destruction. As a result of these tests, the product’s characteristics have not changed significantly.
CONCLUSIONS AND FUTURE WORK

The aim of the study was to obtain a dermo-cosmetic product containing a new organic substance that has been proven to have photoprotective action. The first phase was to sort out the natural products that contain bioactive compounds and to find the best associations with photo protective (filtering and screen) substances. After the formulation stage, we studied the physical, chemical and biological features of the final product, and then its cosmetic efficiency.

REFERENCES

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THE USE OF DIRECT COMPRESSION ON ACTIVE INGREDIENTS FORMULATED IN LARGE DOSES

Balaci Teodora, Lupuliasa Dumitru, Mircia Eleonora, Budura Emma, Şaramet Gabriel, Hîrjău Mircea, Nicoară Anca

Abstract: The present study is based on practical aspects, and its aim was to optimise the manufacturing technological processes for obtaining tablets with Acetylsalicylic acid and Ibuprofen and to extend the direct compression procedure to active substances with a high therapeutic dosage. On such materials, the following parameters were studied: particle shape and size, flowing properties, volumetric characteristics, humidity content, behaviour during compression. The materials were compressed using an eccentric machine and a rotating machine, and subsequently, the physico-mechanical properties of the resulting tablets were evaluated. The behaviour during compression is different for each material.

Key words: Directly compressible forms, compression properties.

INTRODUCTION

Currently, there is a growing tendency to replace the older compression methods, involving wet or dry granulation of mixtures of drugs and excipients, with simplified and more efficient procedures such as direct compression. Modification of the structure of some active substances difficult to compress, with high therapeutic doses, were done, in order to optimise the flowing and compacting properties. Although designed for direct compression, many of these products raise problems during production and require fine tuning of the technological process and establishing all process parameters.

In the present study, several pharmacotechnical properties, such as the behaviour during compression, were determined for two materials with a granular structure containing Acetylsalicylic acid and Ibuprofen, in order to establish the optimal parameters for the manufacture of tablets containing doses of 500 mg of drugs.

MATERIAL AND METHODS

- Aspirin DC 90 Granules - Shanghai Hygeia Chem. Industry Co., Ltd., China;
- Ibuprofen DC 85 Granules - BASF, Germany.

The following parameters were studied on these materials: particle shape, by means of microscopy, particle size, by employing the sieving and sorting method, flow time and speed, by timing the duration in which a certain mass of material flows through a standardized orifice, angle of repos by the fixed cone method, bulk and tap densities, tapping behaviour, Haussner balance, Carr index, humidity content.

The behaviour of the materials, when various compression forces are applied, was studied on an eccentric type Korsch EK-O machine with tensiometric devices adapted to the upper punch cylinder and on a highspeed rotating Kilian S 250 machine.

For each DC material several batches of tablets with a dosage of 500 mg of drug were obtained.

The pressure was gradually increased, starting from values that do not ensure optimal properties for the tablets (too low mechanical resistance, friability values bigger than 3%), continuing with pressure values used in the industry, and up to values for which the physical parameters of the tablets exceed the acceptable values (the disintegration duration is more than 15 minutes).

The pressure range was 40-220 MPa for the eccentric machine and 10-20 KN for the rotating Kilian machine.
Pressure-height, pressure-disintegration, pressure-mechanical resistance, pressure-friability diagrams were traced.

RESULTS AND DISCUSSION

Both studied granular materials have an almost spherical shape, and the drug is entrapped in the granular structure, with no crystals (figure 1). The granulometric analysis shows that the sizes most of the particles are within the range of 150-600 μm for Aspirin DC and 250-700 μm for Ibuprofen DC. The values for the flow duration and angle of repose indicate that the two materials have optimal flow properties. Using the bulk and tap density values, the Haussner ratio and Carr index were calculated, and the results show a good compression behaviour (table 1).

![Aspirin DC](image1.png)  ![Ibuprofen DC](image2.png)

Figure 1. Particle shape of the DC products

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aspirin DC</th>
<th>Ibuprofen DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle shape</td>
<td>spherical</td>
<td>spherical</td>
</tr>
<tr>
<td>Particle size (μm)</td>
<td>150 – 600</td>
<td>250 – 700</td>
</tr>
<tr>
<td>Flow time (sec)</td>
<td>8.34</td>
<td>7.12</td>
</tr>
<tr>
<td>Angle of repos (°)</td>
<td>34.50</td>
<td>29.35</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>0.69</td>
<td>0.70</td>
</tr>
<tr>
<td>Tap density (g/cm³)</td>
<td>0.78</td>
<td>0.84</td>
</tr>
<tr>
<td>Haussner balance</td>
<td>1.13</td>
<td>1.19</td>
</tr>
<tr>
<td>Compressibility %</td>
<td>11.53</td>
<td>15.96</td>
</tr>
<tr>
<td>Humidity content %</td>
<td>0.52</td>
<td>1.80</td>
</tr>
</tbody>
</table>

For the tablets resulting after compression with Korsch excentric type machine, the pressure-height, pressure-disintegration, pressure-mechanical resistance and pressure-friability diagrams were traced.

By analyzing the diagrams for the two DC product studied, (figure 2,3,4,5) the following conclusions can be drawn: Aspirin DC does not have an optimal compressibility with 40-220 MPa. The tablets resulting under a compression pressure of 40-130 MPa have a low mechanical resistance and friability values above 1%. Optimal compression of this material occurs at a pressure of 150-220 Mpa, when all the studied properties have acceptable values.
The Ibuprofen DC has shown an optimal behaviour in the compression pressure range of 80-130 MPa, with the friability of less than 1%, and the values for the mechanical resistance which ensure the integrity of the tablets during usual manipulation. The duration of the disintegration is the critical parameter, being relatively long for all series and exceeding the imposed limits, when the applied pressure is above 150 MPa.

When using the high speed rotating Killian machine, the optimal compression pressures are 16-18 KN for Ibuprofen DC and 20 KN for Aspirin DC.

Figure 2. The variation of the tablet height with the pressure applied

Figure 3. The variation of the tablet disintegration time with the pressure applied
CONCLUSIONS AND FUTURE WORK

The studied substances are specially designed for direct compression, being previously processed in granular forms, with optimal flow and compression properties. It can be observed that, although the materials contain different active substances, the values obtained for some parameters, such as particle shape, flow time, volumetric properties are similar.

The behaviour during compression is different for each material. The optimisation of the technological processes is necessary in order to obtain high quality tablets.
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STUDIES ON STABILITY OF SOME MOUTHWASHES PREPARED WITH ESSENTIAL OILS

Emma Budura, Dumitru Lupuleasa, Ancuța Fița, Oana Karampelas, Mirela Mitu, Mircea Hîrjău

Abstract: In the studied formulas of the mouthwashes, we have associated active ingredients (peppermint essential oil, menthol, eucalyptus essential oil and thyme essential oil), an anti-inflammatory agent (ά-bisabolol), sodium fluoride for protection against tooth decay, two preservatives (benzoic and salicylic acids), sweetener (sodium saccharin), citric acid / sodium citrate buffer, solubilizing and stabilizing agents, Cremophor RH 40 and Lutrol F 127, and a hydroalcoholic vehicle. After preparing the mouthwashes, we have performed the quality control of the resulting solutions, studying also their stability after three months.

Key words: mouthwashes, peppermint essential oil, eucalyptus essential oil, thyme essential oil

INTRODUCTION

Research studies have shown that almost half of the heart attack cases in the world are based on anterosclerosis and pericarditis developed because of bacteria from untreated oral infections. Dentists recommend the use of mouthwashes, both for patients with dental problems and for those without, in order to ensure the daily hygiene. These have different roles: antiseptic, bactericidal, fungicide, and they can be used both for preventing and treating infections on the oral cavity, and also for their odorant role, being aromatic, they ensure fresh breathing.

Based on these facts, the aim of this paper was to prepare some mouthwashes based on different essential oils, using a modern technology.

MATERIALS

The materials used for their manufacture are produced by BASF / Germany. In the formulas, we have associated active ingredients (peppermint essential oil, menthol, eucalyptus essential oil and thyme essential oil), an anti-inflammatory agent (ά-bisabolol), sodium fluoride for protection against tooth decay, two preservatives (benzoic and salicylic acids), sweetener (sodium saccharin), citric acid / sodium citrate buffer, solubilizing and stabilizing agents, Cremophor RH 40 and Lutrol F 127, and a hydroalcoholic vehicle.

The components of the studied mouthwashes are shown in table 1.

Table 1 - The formulation of the mouthwashes

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.1 formula</td>
<td>No.2 Formula</td>
</tr>
<tr>
<td>Peppermint essential oil</td>
<td>2.00</td>
</tr>
<tr>
<td>Eucalyptus essential oil</td>
<td>0.09</td>
</tr>
<tr>
<td>Thyme essential oil</td>
<td>0.06</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.04</td>
</tr>
<tr>
<td>á-Bisabolol</td>
<td>-</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>-</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>0.06</td>
</tr>
</tbody>
</table>
The peppermint essential oil is obtained by water vapor drive of the leaves or of the fresh flowering tops, harvested from Mentha x pieperita species, Lamiaceae family, with a content of at least 50% total alcohols expressed in menthol, and at least 4% of esters expressed in mentil acetate. It is a clear, light yellow liquid, with characteristic smell, and a burning taste, with a lower density than water, and practically insoluble in water.

The peppermint essential oil contains alcohols, of which menthol is a major constituent, 30-50%, and is accompanied by neomenthol, izomenthol, neoizomenthol (figure 1), piperytol, piperytenol, izopiperytenol, ketones, 20-30% (mentona, izomentona, neomentona, neoizomentona, piperytona, piperytenona, izopiperytona), acetylated esters of menthyl, neomenthyl, and neoizomenthyl, butyrate and izovaleryanate of menthyl, oxides (cyneol, menthofuran, piperytonoxid), monoterpenic hydrocarbons (ά- pinen, β- pinen, limonen), sesquiterpenic hydrocarbons, coumarins, sulfur compounds, alkylic alcohols.

It has many therapeutic effect, but it was chosen in the studied formula for its anti-infective action, and as a flavoring.

The Eucalyptus essential oil is obtained by water vapor drive of the fresh leaves harvested from the old branches of Eucalyptus globus Labill. Species, and of other Eucalyptus species, Myrtaceae family, with a content of at least 70% 1,8- cineol (eucalyptol). It is a clear, light yellow liquid, with characteristic flavored smell, of cineol, and a burning taste at the beginning, then refreshing, and practically insoluble in water.
The major constituents are 1,8-cineol, cytonelal, piperytona, cryptona, methyl cinamate, but also monoterpenes, sesquiterpenes, monoterpenic alcohols, sesquiterpenic alcohols, phenols. It, also has a lot of effects, but we chose it for its anti-infective and anti-inflammatory actions, and as a flavoring.

The thyme essential oil is obtained by water vapor drive of the floering aerial parts, harvested from the Thymus vulgaris L. Species, Lamiaceae family. It looks like a volatile liquid, light yellow when is fresh, then becoming red, with characteristic flavored smell, of thymol, and a flavoured taste, practically insoluble in water, used for its excelent anti-infective effect.

Its predominant constituents are geraniol, linalol, p-cimen, monoterpenic alcohols, thymol, and carvacol, accompanied, in small quantities by in cantitati mai mici, monoterpenic hydrocarbons, and terpinoidic phenols. [1]

The menthol - (1R, 3R, 4S) – 3 – p – mentanol (figure 1) is obtained from the peppermint essential oil (natural menthol) or by synthesis (synthetic menthol). It is shown as acicular brittle crystals, without colour, with a strong smell of mentha and a burning taste at the begining, then becoming refreshing. At the room temperature it volatilizes. It is very easily soluble in alcohol, chloroform, and ether, easily soluble in oils, including the essential ones, but very sparingly soluble in water. It is preserved at cool place, away from light. It has antiseptic effect, and it is in the same time flavouring agent for the studied mouthwashes.

The α-(-)–Bisabolol, known also as levomenol, is a monocyclic sesquiterpenic alcohol, which looks like a colourless viscous oil, with a pleasant floral smell, and is the major compound from the essential oil of Chamomilla recutita and Myoporum grassifolium. It is practically insoluble in water and glycerine, and easily soluble in alcohol. It is used as flavouring agent, but especially for its anti-inflammatory, healing, and anti-infective effects.

The sodium fluoride is a white powder, odorless, with a weak salty taste, 1:2.5 soluble in water, poorly soluble in alcohol, and is kept away from light. It is irritant over the skin, but it is frequently used to prevent tooth decay.

The salicylic acid is a crystalline white powder, odorless, with a mellow characteristc taste, becoming sour, easily soluble in alcohol and in boiling water, 1:100 soluble in glycerine and 1:500 in water. Heated with caution sublimates, and by sudden heating decomposes presenting phenol smell. It is used in the present formulas as a preservative agent.

The sodium saccharin is a white odorless powder, very sweeey, soluble in water, used as a sweetener.

The sodium citrate forms together with the citric acid the buffer of the solution, bringing its pH close to neutrality, in order to be well tolerated by the oral mucosa. They are both white odorless powders, easily soluble in water. [2]

Cremophor RH 40 is Polyoxyl 40 Hydrogenated Castor Oil (PhEur/USP-NF) produced by ethoxylation of hydrogenated castor oil. Cremophor RH 40 is a nonionic solubilizer used to solubilize vitamins, poorly water soluble substances, and essential oils in water or mixtures of water and alcohol. It acts as surface-active solubilizer in water and forms the structures of micelles. The micelle that envelops the active substance is so small that it is invisible, giving the aspect of a solution.

Lutrol F 127 also named Poloxamer 407 (DAC, USP-NF) is a block polymer consisting of 73 % of polyethylene glycol and 27% polypropylene glycol with an average
molecular weight of 12000. The main applications for Lutrol F 127 is of solubilizer and stabilizer in bucal and topical solutions, gel former, and stabilizing agent in external suspensions. Together with Cremophor RH 40 acts like a solubilizer by a mechanism based on hydrophilisation. In 2-5% amounts it does not modifies the viscosity of solutions. [3]

Glycerol is a polyhydric alcohol, obtained by fat saponification, is a polar and hydrophilic solvent, miscible in any proportion with water and alcohol. Its solubilising power increases by heating, when its viscosity decreases. It looks like a thick soppy liquid. It is used as a cosolvent.

METHODS

INCREASING THE SOLUBILITY BY HYDROTROPHY

The compounds with strong hydrophylic polar groups in their molecule increase the solubility of poorly soluble substances by the effect named as hydrothropy. This is based on the activation of hydrogen bridges and on the decrease of interfacial tension. The hydrogen bridges influences the water structure (they are breaking it) and this influences the solubilization of some substances. In the studied formulas, as hydrothrops substances, acts Lutrol F 127 and glycerol.

MICELLAR SOLUBILIZATION

The micellar solubilization represents the bringing in solution of some poorly or insoluble substances with the help of the surfactants. By micellar solubilization is understood an increased of the solubility by active links at the surface, which are capable to transfer poorly or insoluble substances in water clear solutions, without modifying the chemical structure of the actives. As solubilizing agents are used bipolar tensioactive substances.

As mediators of dissolution we used for the presented solutions the Cremophor RH 40. Its ability of solubilization is based on forming molecular aggregates or micelles. Micelles are formed in the solution over a concentration named critical micellar concentration. The micelles are formed spontaneously, and the micellar systems are thermodinamical stable. The substances which reach in the center of the micelles are protected by the degradation reactions.

The alcohols, like glycerol, are increasing the ability of solubilization of the tensioactives incorporating over the hydrogen bridges in the palisadic layer of the micelles and enhancing their strength, this being the reason for which we choosed it in the studied formulas. The phenomena is known under the name of cosolubilization. [4]

THE MANUFACTURE OF THE MOUTHWASHES

For the first formula:
The mixture prepared from menthol, peppermint, Eucalyptus, and thyme essential oils, with Cremophor RH 40 is heated at 60°C. Separatly, it is prepared a solution by dissolving salicylic acid, benzoic acid, sodium saccharin, citric acid, sodium citrate, glycerine and Lutrol F 127 in the properly quantities of ethanol and water. The solution is brought at the same temperature, of 60°C, and is added slowly to the well stirred mixture previously obtained.

For the second formula:
The mixture prepared from menthol, Eucalyptus essential oil, á-bisabolol, and Cremophor RH 40 is heated at 60°C. A solution from sodium fluoride, sodium saccharin, buffer of citric acid/sodium citrate, and Lutrol F 127 dissolved in the vehicle composed by glycerol, alcohol and water, is made. The solution is brought at the same temperature, of 60°C, and is added slowly to the well stirred mixture previously obtained.
RESULTS AND DISCUSSION

Quality control of the resulting solutions was performed, the organoleptic properties, pH (by potentiometric method) and viscosity were determined, immediately after preparation, and also their stability after three months.

The resulting solutions are clear, slightly yellow, with a characteristic menthol and eucalyptol smell, and a refreshing mint taste. These characteristics remained unchanged during preservation for 90 days.

The pH of the solutions was within the limits provided by the Romanian Pharmacopoeia Xth edition, with values between 6.4 and 6.7, values which are compatible with oral mucosa. In conclusion the preparation is not irritant during administration and the pH created by the buffer ensures an optimal stability for the dissolved active ingredients.

The viscosity represents the property of the fluids to oppose resistance at the slip of two neighboring layers during their flow. In the international system, the dynamic viscosity unit is pascal-second. 1cP=1mPa.s. It is measured with the rheometer, when the size of the torque forces needful to oppose resistance to the rotation of a cone inside the solution, is determined.

The viscosity was determined using the Rheometer RC 1 (RheoTec GmbH, Meßtechnik GmbH, Radenburg, Germany), a rotational rheometer type cone / plate with an electronic unit which allows the control of the rotation speed and the recording of the determined values. The viscosity of the prepared solutions was of 8.0 mPa.s for the first formulation, and 7.5 mPa.s for the second one at 22°C, these values remained unchanged for three months. [5,6]

CONCLUSIONS

The oral cavity, the first segment of the alimentary canal, is exposed to a multiple mechanic, chemical and toxic exogenous irritations, this allowing the easy development of microbial and fungical infections. This is the reason for what it is recommended the use of mouthwashes with an odorant, refreshing and antiseptic role.

In the present study, there were formulated and prepared two external solutions with mouthwash role. At their preparation we used, in order to bring in solution the poorly water soluble substances (menthol and essential oils), the micellar solubilization and hydrothropy techniques, using modern agents (Cremophor RH 40 and Lutrol F 127). After obtaining them, the quality control of the solutions was performed, following them during a tree months period.

The solutions have adequate characteristics, within the limits provided by rules into force and a satisfactory stability, thus being suitable for pharmaceutical use.

To improve the appearance of the solutions and also to make the easier to recognize, being only for external use, coloring agents can be added. Also, in the case that is difficult for the patient their use due to the too strong taste or smell, they can be diluted before the use with a different quantity of water, the solutions maintaining their effects and qualities.

REFERENCES


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MANUFACTURING PROCESS AND CHARACTERIZATION OF SOME L/H EMULSIONS WITH ESSENTIAL OIL OF *Melaleuca alternifolia* (*Tea Tree Oil*)

Mirela Mitu, Emma Budura, Victoria Hîrjău, Teodora Balaci, Andreea Stănescu, Anca Nicoară

**Abstract**: In the present study we formulated and studied three L/H emulsions with 5% tea tree essential oil. The obtained emulsions were characterized by determining the physico-chemical parameters, the appearance, stability, pH, colour, density, viscosity, and also antimicrobial efficiency was tested.

**Key words**: tea tree essential oil, L/H emulsions, *Melaleuca alternifolia*

**INTRODUCTION**

L/H emulsions used as liquid pharmaceutical forms for topical application in dermatology and cosmetics present a lot of advantages compared with ointments and creams. They present a faster and higher penetration through skin, thus providing to the active ingredients a better bioavailability than the semisolid forms, due to the intervention of both hydrophilic and lipophilic phases and of the emulsifier. Also, they have a good facility of administration, the miss of some allergic phenomena, and they are washable.

All these advantages of the external emulsions, L/H type, as a pharmaceutical form with skin application of actives with topical effect, determined us to study in the present research the formulation, preparation, quality control and antimicrobial action of some emulsions with tea tree essential oil obtained through leaves distillation of the *Melaleuca alternifolia* plant. This essential oil presents a complex action: antimicrobial, antifungal, antiviral, antiacne and anti-inflammatory. It was used by aborigines in Australia in order to treat different skin diseases being one of the most effective natural antiseptics and antifungics known.

It is recommended to use the essential oil in proportion of 3-10%, without being irritating, in ointments, creams, gels and L/H emulsions. [1,2,3]

In the present study we have formulated, prepared, and characterized some L/H emulsions with tea tree essential oil obtained through leaves distillation of the *Melaleuca alternifolia* plant. Though it is known for its good antiseptic properties from 1920, this essential oil is still very popular, being a safe and effective natural antimicrobial.

**MATERIALS**

Tea tree essential oil is a volatile liquid, clear, colourless to light yellow, with characteristic smell, and with all the properties mentioned in table no. 1.

Table no. 1. Physical properties of the essential oil from *Melaleuca alternifolia* (*Tea tree oil*) (NSW Agriculture, Analytical Services, Environmental Laboratory, Wollongbar, Australia)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, without visible water</td>
</tr>
<tr>
<td>Colour</td>
<td>Colourless to light yellow</td>
</tr>
<tr>
<td>Smell</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Relative density (20°C)</td>
<td>0.885–0.906</td>
</tr>
<tr>
<td>Refractive index (20°C)</td>
<td>1.475–1.482</td>
</tr>
<tr>
<td>Optical rotation (20°C)</td>
<td>+5° – +15°</td>
</tr>
<tr>
<td>Miscibility in 85% (v/v) ethanol (20°C)</td>
<td>Less than 2 volumes</td>
</tr>
</tbody>
</table>
By chemical point of view, tea trea essential oil is a complex formed from 100 different compounds, mainly monoterpenes and their alcohols, as described in table no. 2. [4,5,6,7]

Table no. 2. Chemical composition of the essential oil from Melaleuca alternifolia (Tea tree oil) (NSW Agriculture, Analytical Services, Environmental Laboratory Wollongbar, Australia)

<table>
<thead>
<tr>
<th>Compound</th>
<th>%</th>
<th>Limits %</th>
</tr>
</thead>
<tbody>
<tr>
<td>α–tuien</td>
<td>0,8</td>
<td></td>
</tr>
<tr>
<td>α–pinen</td>
<td>2,5</td>
<td>1–6</td>
</tr>
<tr>
<td>cadinen</td>
<td>0,2</td>
<td>tr–3,5</td>
</tr>
<tr>
<td>β–pinen</td>
<td>0,7</td>
<td></td>
</tr>
<tr>
<td>mircen</td>
<td>0,8</td>
<td></td>
</tr>
<tr>
<td>α–terpinen</td>
<td>9,0</td>
<td>5–13</td>
</tr>
<tr>
<td>limonen</td>
<td>0,9</td>
<td>0,5–4</td>
</tr>
<tr>
<td>1,8–cineol</td>
<td>3,4</td>
<td>0–15</td>
</tr>
<tr>
<td>γ–terpinen</td>
<td>19,4</td>
<td>10–28</td>
</tr>
<tr>
<td>p–cimen</td>
<td>2,0</td>
<td>0,5–12</td>
</tr>
<tr>
<td>terpinen–4–ol</td>
<td>37,4</td>
<td>30–40</td>
</tr>
<tr>
<td>α–terpineol</td>
<td>2,5</td>
<td>1,5–8</td>
</tr>
<tr>
<td>terpinolen</td>
<td>3,5</td>
<td>1,5–5</td>
</tr>
<tr>
<td>aromadendren</td>
<td>1,4</td>
<td>tr–7</td>
</tr>
<tr>
<td>δ–cadinen</td>
<td>1,1</td>
<td>tr–8</td>
</tr>
<tr>
<td>globulol</td>
<td>0,3</td>
<td>tr–3</td>
</tr>
<tr>
<td>viridiflorol</td>
<td>0,2</td>
<td>tr–1,5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>86,1</td>
<td></td>
</tr>
</tbody>
</table>

In the formulas, we have associated active ingredient (tea trea essential oil 5%), as a lipophile faze, the liquid paraffin which is chemical inert, emollient, and has a good stability in time, and as emulsifiers we chose both L/H and H/L types associated in different proportions, in order to give a better stability to the final emulsions, isopropyl myristate, glycerol monostearate, sodium cetylstearyl sulphate, span 40, tween 80, stearine, triethanolalanine, the vehicle being the water. [8]

The components of the studied L/H emulsions are shown in table no. 3.
Table no. 3. The formulation of the L/H emulsions with 5 % essential oil from *Melaleuca alternifolia* (Tea tree oil)

<table>
<thead>
<tr>
<th>Ingredients (g)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Tea tree essential oil</td>
<td>5</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>13</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>2</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>Glycerol monostearate</td>
<td>3</td>
</tr>
<tr>
<td>Span 40</td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>8</td>
</tr>
<tr>
<td>Sodium cetylstearylsulphate</td>
<td>3</td>
</tr>
<tr>
<td>Stearine</td>
<td>4,4</td>
</tr>
<tr>
<td>Triethanolanine</td>
<td>3</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

**METHODS**

The basic emulsions were prepared by heating the two phases: hidrophile and lipophile at 70°C, the emulsifier being dissolved in the phase in which it is soluble. The components were mixed and emulsified continuously stirring at 900 rpm. The obtained preparations were preserved in brown recipients and stored at the room temperature. They were controlled, by following: dispersibility, and stability.

Quality control of the resulting emulsions was performed as follows: the organoleptic properties (aspect, colour, smell, and homogeneity), pH and specific electric conductivity (Ohm$^{-1}$ · cm$^{-1}$), at 19,2°C, with INOLAB level 1 pH-meter, density (g/cm$^3$) with picnometer, at 19°C, dynamic viscosity (η, cP), at 19°C, with Höpler viscozymeter, and antimicrobial efficiency were determined, immediately after preparation, and also after three months. [9,10,11]

**RESULTS AND DISCUSSION**

*The dispersibility* was determined by diluting the emulsions with water 1 : 10 and examined with the magnifier (4,5×), when all the prepared emulsions remained stable.

*The stability* was followed in graded cylinders by observing, at different periods of time, the instability phenomena: cremation, coalescence, phase separation, and we noticed that all the prepared emulsions remained stable.

*The aspect:* all the emulsions are white milky, stable, homogeneous, and have a characteristic smell.

*The pH* varies between 5.15 – 8.09 limits, except the second formula which has an easy alkalin pH (8.09), the first one has a neutral pH (6.8), and the third emulsion has an easy acid pH (5.15), the pH of the skin being 4-6.5.

*The specific electric conductivity* has small values, 1,78 · 10$^{-4}$ Ohm$^{-1}$ · cm$^{-1}$ for the first emulsion, 4,03 · 10$^{-4}$ Ohm$^{-1}$ · cm$^{-1}$ for the second one, and 1,29 · 10$^{-4}$ Ohm$^{-1}$ · cm$^{-1}$ for the third formula.

*The density* is subunit for all the emulsions, 0,9728 g/cm$^3$ for the first, 0,9483 g/cm$^3$ for the second, and 0,9588 g/cm$^3$ for the third emulsion.

*The dynamic viscosity* varies between 5,3488 – 12,7122 m · Pa · S, 12,7122 m · Pa · S for the first formula, 10,7757 m · Pa · S for the second one, and 4,7590 m · Pa · S for
the third one, being lower for the last two formulations due to the addition of the alcohol as an absorption promoter.

The antimicrobial efficiency was determined by using the agar gel diffusion method, for the bacteria using MULLER-HINTON culture medium, and for Candida, SABOURAUD medium.

The testing products were stored in metallic cylinders, of stainless steel (Ø = 8mm) in volumes of 0.4 ml.

In comparison with the emulsions, the antimicrobial efficiency of tea tree essential oil, and of its 5% solution in alcohol, respectively propylenglycol, were determined, using as control positive standards ampicillin and nystatin.

The inhibition area diameters were registered after 24 h of incubation at 37°C for bacteria, and after 40 h at 30°C for the fungus.

The variations of the inhibition area of the tested emulsions are presented in table no. 4.

### Table no. 4. The antimicrobial efficiency of the emulsions with 5 % essential oil from *Melaleuca alternifolia* (Tea tree oil) and of the standards

<table>
<thead>
<tr>
<th>Formula</th>
<th>Volume in cylinders</th>
<th>The inhibition area diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.4 ml</td>
<td>8 18 8 0 15 20 20 12</td>
</tr>
<tr>
<td>II</td>
<td>0.4 ml</td>
<td>0 8 6 0 15 22 23 27</td>
</tr>
<tr>
<td>III</td>
<td>0.4 ml</td>
<td>0 15 6 0 15 18 20 14</td>
</tr>
<tr>
<td>Tea tree essential oil</td>
<td>20 μl</td>
<td>25 22 32 13 35 50 40 22</td>
</tr>
<tr>
<td>5% Tea tree essential oil in alcohol</td>
<td>0.4 ml</td>
<td>24 35 34 22 34 30 28 36</td>
</tr>
<tr>
<td>5% Tea tree essential oil in propylenglycol</td>
<td>0.4 ml</td>
<td>21 32 20 28 34 34 30 35</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 μg/disc</td>
<td>24 40 8 28 29 – – –</td>
</tr>
<tr>
<td>Nystatin</td>
<td>10 μg/disc</td>
<td>– – – – – 25 – 25</td>
</tr>
</tbody>
</table>


It can be observed that the first formula presents a better antimicrobial efficiency for all the tested micro-organisms, in comparison with the second and third formulas, which are not active on the Staphilococcus aureus.

All three prepared 5% tea tree essential oil emulsions present a better antifungal than antimicrobial efficiency.

### CONCLUSIONS

In the present study, they are presented the results of the researches made on some L/H emulsions containing 5% tea tree essential oil.

There were formulated and prepared a number of three L/H emulsions. The emulsions were characterized by determining the physico-chemical parameters: aspect, stability, pH, colour, density, electric conductivity, viscosity, and also antimicrobial efficiency was tested.

It was noticed that in the first formula the isopropyl myristate contributed, along with tea tree essential oil at a better antimicrobial efficiency on the bacteria, than the second and third formulas, which are containing ethanol as an absorption promoter. Instead, the last two emulsions had a higher antifungal efficiency, than the first one.
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THE STUDY OF THE FORMULATION OF ORODISPERSIBLE PARACETAMOL TABLETS

M. Hîrjău, V. Hîrjău, D. Lupuliasa, E. Budura, O. Karampelas, A. C. Fiţa

Abstract: The aim of this study was to formulate and obtain orodispensible paracetamol tablets. For the formulation of the tablets, we have considered several excipients, correlated with the tableting method applied, in order to reach an optimal balance between a fast dispersion and an adequate tablet hardness. We have studied four tablet formulations. The tablets were obtained by direct compression, as well as by wet granulation, and they were analysed using compendial methods. The quality control has revealed that we have achieved a satisfactory balance between a fast dispersion (in about 60 seconds) and an adequate hardness for the formulas containing a superdisintegrant.

Key words: Orodispersible tablets, Paracetamol.

INTRODUCTION

According to compendial definitions, orodispensible tablets are uncoated tablets which rapidly disperse in the saliva in the mouth (in less than 3 minutes after administration), before being swallowed [1, 2].

A modern variant of the orodispensible tablets is represented by Fast Dissolving/Dispersing Oral Tablets. Their distinctive characteristic is their capacity to rapidly dissolve or disperse in saliva (in 15-30 seconds) once placed on the tongue, before being swallowed. These tablets are suitable for patients with swallowing difficulties, such as elderly patients and children, HIV patients or patients following a radiotherapy [3, 4, 5, 6, 7].

The aim of this study was to obtain paracetamol tablets as an alternative to conventional oral dosage forms containing this drug (tablets and capsules), for pediatric use.

Paracetamol (acetaminophen) is a p-aminophenol derivative with moderate analgesic and antipyretic effect. Its indications are minor to moderate pain (nerve or muscle pain, headaches, dismenorrhea), fever of various etiology (microbial or viral infections). It is considered an antipyretic of first election for young children, in viral infections and in rheumatic pains [8].

As for other active substances (analgesic, anti-inflammatories, migraine medication, antidepressants, neuroleptics, antispastic and antiparkinsonian medication), a rapid onset of the therapeutic effect is desired in the case of paracetamol.

MATERIAL AND METHODS

The following substances of pharmaceutical grade were used in the formulation of the paracetamol tablets: Paracetamol (Skyopen Medipharma Co. Ltd., China), DC lactose (Tablettose, Meglle Pharma, Germany), lactose monohydrate (Mastermind International Ltd., China), starch (Roquette, Italy), microcrystalline cellulose (Vivapur 102, S&D Chemicals Ltd.), polyvinylpyrrolidone K 30 (BASF AG, Germany), sodium starch glycolate (Explotab – Ed. Mendell Co.), sodium carboxymethylcellulose (Crosscarmellose sodium – Vcarefarma, India), magnesia stearate (Union Derivan SA / Arnaud Romania), colloidal silica dioxide (Aerosil 200, Degussa/Astron Chemicals), banana flavor, aspartame.

Both direct compression and compression after wet granulation were used for the production of the tablets.

The pharmaco-technical evaluation of the tablets was conducted using methods described in the Romanian Pharmacopoeia for the following parameters: uniformity of mass, hardness, friability, disintegration.
1. **The formulation of the paracetamol orodispersible tablets**

The objectives of the formulation of the tablets were the selection of the excipients and of the manufacturing technology, in order to result in rapidly dissolving tablets with a suitable hardness, the masking of the unpleasant (bitter) taste of the active substance (given that by dispersing the tablet in saliva, the taste buds come in direct contact with the tablet components for a few seconds).

Four formulations of paracetamol tablets were taken into study.

<table>
<thead>
<tr>
<th>Tablet components</th>
<th>Role in formulation</th>
<th>Formula 1</th>
<th>Formula 2</th>
<th>Formula 3</th>
<th>Formula 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>Active ingredient</td>
<td>0,1250</td>
<td>0,1250</td>
<td>0,1250</td>
<td>0,1250</td>
</tr>
<tr>
<td>Lactose DC</td>
<td>Directly compressible filler</td>
<td>0,1063</td>
<td>0,1063</td>
<td>0,1063</td>
<td>-</td>
</tr>
<tr>
<td>α-Lactose monohydrate</td>
<td>Filler</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0,1500</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Filler / Binder</td>
<td>0,1063</td>
<td>0,1063</td>
<td>0,1063</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>Filler / disintegration agent</td>
<td></td>
<td></td>
<td></td>
<td>0,0326</td>
</tr>
<tr>
<td>Sodium starch glicolate</td>
<td>SuperDisintegrant agent</td>
<td>0,0586</td>
<td>-</td>
<td>0,0293</td>
<td>0,0293</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>SuperDisintegrant agent</td>
<td>-</td>
<td>0,0586</td>
<td>0,0293</td>
<td>0,0293</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone K 30 (Ethanol solution 5%)</td>
<td>Binder</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0,0300</td>
</tr>
<tr>
<td>Magnesia stearate</td>
<td>Lubricant / antialderent</td>
<td>0,0012</td>
<td>0,0012</td>
<td>0,0012</td>
<td>0,0012</td>
</tr>
<tr>
<td>Aerosil</td>
<td>Glidant</td>
<td>0,0020</td>
<td>0,0020</td>
<td>0,0020</td>
<td>0,0020</td>
</tr>
<tr>
<td>Saccharin</td>
<td>Sweetener</td>
<td>0,0003</td>
<td>0,0003</td>
<td>0,0003</td>
<td>0,0003</td>
</tr>
<tr>
<td>Banana flavor</td>
<td>Flavor</td>
<td>0,0003</td>
<td>0,0003</td>
<td>0,0003</td>
<td>0,0003</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>0,4000</td>
<td>0,4000</td>
<td>0,4000</td>
<td>0,4000</td>
</tr>
</tbody>
</table>

2. **The production of paracetamol orodispersible tablets**

The direct compression applied for the formulas 1 – 3 comprises of the following stages: weighing the components, dry mixing, lubricating the mix and compression.

The stages of the compression following (after) wet granulation method applied for formula 4, were: weighing the ingredients, spraying the binder solution on the dry components, blending of the mix, kneaving of the wet mass, sieving of the wet mass, drying of the granules, tableting (Triowin machine with a set of 12 cm punches and die).

3. **Pharmaco-technical evaluation of the paracetamol orodispersible tablets**

The following parameters were evaluated using compendial methods (Romanian Pharmacopoeia, Xth ed.): characteristics (appearance, color, taste, odor), tablet thickness and hardness (VanKel VK 200 Tablet Hardness Tester), friability (VanKel Friability Tester), mass uniformity (Sartorius pharmaceutical scale) and disintegration of the tablets.

**RESULTS AND DISCUSSIONS**

*Organoleptic characteristics*
For all four formulas, we have obtained disc-shaped tablets, white, plane or convex, spotless, with no cracks and with intact margins, sweet, odorless. All parameters meet the before-mentioned requirements.

**Tablet thickness and diameter**

The tablet thickness and diameter show small variations, indicating that the material that was to be compressed has good flow properties and that the compression stage was properly conducted.

![Average Thickness and Diameter (mm)](image)

*Figure 1. Average thickness and diameter*

**Mass uniformity**

The results of this test are shown in the figure below.

![Average mass (g)](image)

*Figure 2. Average tablet mass*

The results show that the tablets meet the requirements of the Romanian Pharmacopoeia (average mass ± 5%).

**Tablet hardness and friability**
Figure 3. Average Tablet hardness and friability for the four formulas

The results show the correlation between the tablet hardness and the friability. The tablet hardness is sufficient to ensure their integrity during handling and a friability within accepted limits (less than 1%).

**Disintegration test**

All formulations have disintegrated rapidly, in less than 60 seconds. The fastest disintegration (40 seconds) could be observed for formula 3, prepared by direct compression and including two superdisintegrating agents.

Figure 4. Disintegration time

**CONCLUSIONS**

Four orodispersible tablet formulations containing paracetamol were prepared. All formulas have shown appropriate characteristics, an acceptable hardness and, most of all, a rapid disintegration. An optimal balance between tablet hardness and disintegration time was obtained for the tablets prepared by direct compression for which the formula contained two superdisintegrants.

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SPECIFIC METHODS OF INTERVENTION TO ADDRESS SPEECH AND LANGUAGE DELAYS IN THE CASE OF CHILDREN WITH DOWN SYNDROME

I. Iacob, C. Muşuroi, and A. Spătaru

Abstract: The present paper presents some specific methods of intervention addressing children with Down Syndrome’s speech and language delays. These methods consist of using structured teaching, visual materials for each new concept, symbolic and role-play games, daily speech therapy activities, and building opportunities for the child to succeed. These methods have been assessed during a two-year intensive intervention program started with an eight-year-old boy with Down Syndrome, recently continued with other children with Down Syndrome.

Key words: Down Syndrome, speech and language delays, intervention program

INTRODUCTION

Children with Down Syndrome have significant delays in the area of speech and language skills. This particularity of the syndrome has considerable impact on the development in other important areas such as reasoning, remembering, not to mention the resulting difficulties in their ability to communicate and to develop an adequate social behavior. Therefore, the aim of the present research was to develop a program adapted to the specific difficulties and needs of the children with Down Syndrome in order to diminish the psychological impact of the language impairment on these children and their families. The intervention program was elaborated during a two-year research and it has been recently introduced in the therapy of other children with Down Syndrome of different ages.

Each child with Down Syndrome has its own development pace, different degrees of anxiety and lack of motivation. Consequently, in order to have the best results with a child, the therapy team should keep the structure of the program while being flexible and adjusting to the child’s reactions.

MATERIAL AND METHODS

We started from the assumption that if Down Syndrome has its own characteristics, the intervention program should be adjusted to address them. The first subject of the intervention program was an eight-year-old child who was almost completely non-verbal and any therapy started in different centers was unsuccessful. Analyzing the child’s behavior, two main causes of this situation have been identified. Firstly, the child had a high anxiety that prevented him to be opened to the information coming from an environment perceived as insecure. He had panic attacks with severe somatic responses. The second cause identified was that the therapies were general, following the same pattern as for any other types of disabilities. For example, the language and speech therapy were based on auditory learning, on practicing sounds and words with no visual support. This method is completely inefficient when working with a child with Down Syndrome whose short-term auditory memory is impaired and who may have difficulties in hearing. Approximately 75% of the children with Down Syndrome have either unilateral or bilateral hearing impairment caused by commonly found middle ear problems and possible central auditory processing difficulties. Taking into account that these children have decreased hearing ability it is obviously inefficient to use teaching methods addressing their hearing.
In order to design an efficient program, these two causes had to be eliminated. The child needed a safe learning environment to be sure that his attention would not be disturbed by his anxiety reactions. Therefore, the first year, the team worked only at the child’s home, in a room having the specific destination of a classroom. The first things he was taught to express in gestures and words were the refusal and his needs. Thus, the child was given the possibility to get control over what was happening around him. We discovered that his anxiety started to decrease as he became more able to reject or ask for things. Today, the child has no more panic attacks and he can control his anxiety almost in all new situations. We concluded that there is a strong relation between the child’s ability to talk and his anxiety. The drawbacks of the hearing-based learning program were eliminated by introducing much and diverse visual support. The words were written down and illustrated by realistic images. The child started to learn words and concepts at a very fast pace and to use them appropriately.

Language was firstly targeted. We noticed that the child did not feel comfortable in the speech sessions. The mimo-gymnastics, the sounds articulation, and practicing words were very frustrating and usually made the child aggressive or it raised his anxiety. Any source of stress was initially avoided in order to build child’s motivation to participate to the lesson and learn. The vocabulary was introduced on categories, using flashcards and permanently checking the child’s proper understanding by matching exercises.

The program had two important features: it was structured and intensive. It was an everyday program, except for the weekend, starting with three hours a day and getting to five hours daily. This condition ensures the rapid acquisition pace by building learning skills, exercising the attention, the memory, the reasoning. The intervention program became part of the child’s daily routine and he developed habits related to the program that made him feel safe.

The characteristic of a structured program is essential for the child’s success. Children with Down Syndrome usually lack learning motivation because they have poor coping strategies with new learning tasks. They may lack or have reduced problem solving skills; therefore they prefer to use their abilities to avoid a task than to solve it. We discovered that in time the problem solving skills can be improved by exercising some successful patterns. These patterns have been trained by introducing the child in a structured program. Each new task was introduced progressively, by breaking the solving process in stages. The child was assisted through all the stages and he was encouraged to firstly complete by himself the last one. After he had been able to do the last stage independently, he was trained to complete the previous one. This way the child learns to think sequentially, he understands the cause-effect relation and he is given the opportunity to be successful which raises his motivation in doing the task independently.

During this intensive, structured program the basic skills needed for efficient learning were trained in the beginning. The child was encouraged and trained to maintain eye contact, to imitate the educator, to sit at the table and to keep his attention on a task for gradually longer periods of time. Initially, the boy had poor eye contact and he rarely would imitate the interlocutor. At present these two abilities are excellent and facilitate an easier, faster learning pace.

In order to register his objective progress, the subject’s IQ was assessed at a score of 38 by using Portrage Checklist, in March 2009. Portrage Checklist is a very useful instrument as it helps to keep a record of the child’s achievements in different areas – cognitive, social behavior, self-help, motor and language areas. It also works as a guide, indicating the specific acquisition a child must have at a certain age. The checklist lists 140 behaviors which represent guidelines for developing skills relevant for these covered behaviors. In two years, following the guidelines, the child scored at his last evaluation,
January 2011, an IQ of 56. Other parallel evaluation based on a non-verbal test recorded an IQ score of 60.

During the therapy not only the language was aimed. The child used to perform daily brain training exercises addressing his memory, reasoning processes, attention, reaction speed, visual intelligence. The used materials were puzzles, association games, memory games, building blocks. We started with very simple patterns – a four pieces puzzle or a memory game involving two pairs - and achieved the level of 24 pieces puzzles or 20 pairs of card for the memory game. The tutor working directly with the child always ensured the child’s success, thus he started enjoying these games.

The fastest progress was registered in the cognitive area. In this area, the eight-year-old child was at an age of 2 years and a half. In January 2011, he was assessed at a cognitive age of 5 years and 11 months. In two years his cognitive progress was approximately of three years and a half which reflects the rapid pace of the learning process. The important aspect of this result is that his language – especially the receptive one – started developing constantly, permitting the information to be understand and processed by the child. He was encouraged to use images, gestures and sounds/syllables to approximate words in order to answer the questions when tested. As we underlined before, the language was our first target because it supports the reasoning, the memory, and the understanding processes. The first year we pay little attention to the speech area because verbalization was very frustrating for the child and during these sessions he used to become aggressive, to develop self-stimulation behavior and to become defensive.

After the child had become more confident in his language skills, we started the speech therapy. It was an experimental and difficult decision as we accepted the fact that even if the child had a rather diverse receptive language no one outside his close family could understand him and his parents often had difficulties in correctly understanding his requests. Still the assessment of his learning behavior had indicated that we could lose the child motivation if we put him in a stressful learning situation. Children with Down Syndrome experiment high stress when directly addressed with a difficult task. They will display different reactions to avoid fulfilling what they are asked to. The risk is high to accentuate their retard by making them block themselves in a safe refusal. Research claims that children with Down Syndrome are stubborn and that they may have difficult behavior. Their obstinacy may be a reaction to a stressful stimulus. They become defensive by refusing any collaboration in a situation they perceive as stressful. This reaction cannot be educated by explanations or punishments. The solution is to turn the situation in something unthreatening, familiar and easy to cope with. Therefore the speech training was split in three short sessions (10 minutes/session) placed between two other very pleasant activities. The child had a board with his activities written down. He was warned that a 10 minutes speech session was coming followed by a longer period of his favorite activity as a prize. It depended on his performance how much he could play his favorite games. The anticipation is very important as it decrease anxiety and frustration. The child was in control – he had the timetable, and the duration of the activity was represented as a row of 10 squares. After each minute he could put a sticker in one square. Therefore, the verbalization part of the program became more tolerable. The speech training was also based on visual materials in order to help the child to distinguish pair sounds as t-d, p-b or to become aware of the presence of the final sounds. One sound was exercised until it was mastered in the initial, median and final position.

The results are significant and they indicate the efficiency of our concept of a therapy adjusted to the specificity of Down Syndrome and to the child’s particular needs.

At the end of the first year of therapy, the child was introduced in a constant program of symbolic games and story reading. The symbolic game is a pleasant way for the child to
experiment difficult or stressful situations in a safe environment. Playing at the doctor helps the child to process the information about what happens in a doctor’s office without feeling threatened. The symbolic game is an essential instrument to let the child get familiar with unpleasant situations and to train adequate behaviors. As he gets involved in the game he becomes more relaxed and he accepts and learns the pattern from the real life. Therefore, when visiting the doctor, the child became more cooperative and calm.

RESULTS AND DISCUSSION

Our research has developed an intensive, practical programme tailored to the specific impairments of a child with Down Syndrome and to the subject’s personal needs. The results of the programme were significant, measured in an average IQ increase of 20 units, according to Portrage Checklist and The Comprehensive Test of Nonverbal Intelligence. We also recorded a significant increase of the subject’s learning motivation and a dramatic decrease of his anxiety level. The child is open to new educational tasks, and he manifests his curiosity and interest in new things. He explores new objects and uses words to describe them, usually in one or two words structures, rarely longer. He has a positive attitude towards learning and using language. His frustration regarding verbalization has disappeared.

We conclude that developing language by using images, written words, sounds and gestures in the case of children with Down Syndrome with a significant language and speech impairment is very efficient for developing their mental abilities. There are variations in the rates of the speech production ability among children with Down Syndrome. Our subject had a very severe delay in the area of speech and language, not because he could not understand language, but because he had difficulties in producing sounds and words. In this case it was extremely useful to develop his receptive language without pressing the child to correctly articulate words. After having built up a rich and diverse vocabulary (approximately up to 600 words) we started improving his articulation, by constantly prompting the child to say the word as close to the model as possible. Meanwhile we had developed his sound, syllables and words awareness by offering constant written support and by playing rhythm or sound and intonation discrimination games. The therapy regarding the motor control of mouth, tongue and vocal tract was included since the beginning of the therapy. However, in order to ensure the child’s success in the activities included in the intervention programme, during the first year we slightly and rarely asked the child to produce sounds and words correctly.

We recommend that children with Down Syndrome with speech production difficulties should be integrated in a therapy following this sequence of stages: first developing vocabulary and then insisting on the accuracy of the speech production in order to avoid frustrating the child and delaying his progress in the language area.

CONCLUSIONS AND FUTURE WORK

Children with Down Syndrome must be introduced in an intervention programme addressing their specific learning profile and typical delays. Besides the Down Syndrome specificity, the child’s own particularities must be taken into account. We observed that if the child feels secure and the therapists build an affectionate relationship obtaining the child’s trust this contributes essentially to the success of the therapy. The programme designed during this research is based on diverse visual material, on the concept of structured learning, on daily intensive training, on symbolic games and social stories. It principally aimed the development of the language as a support for the child’s mental evolution. The success of the programme is certified by the child’s significant IQ score increase, by his stronger learning motivation and diminished anxiety level. The programme
has been introduced in the therapy of other two children with Down Syndrome and the progress obtained so far is steady and faster than before the therapy.

We believe that parents should be trained to understand their children specific learning profile in order to be able to contribute to their faster development in all areas. The therapists also should consider the emotional and behavioral particularities of children with Down Syndrome to design an efficient intervention programme. A national data base containing definite programs, activities, learning materials such as flashcards, videos illustrating how to work with the child, according to the age and to the approached impairment is a necessity in the countries where families having children with Down Syndrome lack professional support and guidance.

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EXPERIENCE OF APPLYING NEW INFORMATION TECHNOLOGIES ON BUSINESS SYSTEMS - EXPERIENCE IN SERBIA

Z. Langovic, B. Pazun, A. Langovic-Milicevic

Abstract: The use of new IT, such as cloud computing, web2.0, SQL Server, RAID, Oracle database, blade servers, enables the creation of business benefits of a system. In other words, due to these new technologies, business systems and the whole economy become more efficient.

In some cases, the given technology does not achieve desired results; however, failed project incorporates the valuable information that could be applied in the future.

Efficiency of IT implementation is reflected in optimization of operating costs, capital investment and reduction of unnecessary costs.

Cloud computing as a new technology that recently has found application in business systems, supports well business agility, that is, speed and flexibility of implementation of business changes.

Because of the flexibility to use the shared resources that it offers, and the fact that this concept in most cases requires less time and effort to provide additional resources for existing applications or new resources for new applications, it is more efficient than the on-site model.

Besides the advantages that given technologies have, it is necessary to consider their shortcomings and to compare them to more cost-effective implementation of business systems.

The aim of this paper is to show how new information technologies affect business system.

Key words: Business System, Cloud computing, Information technology, project.

INTRODUCTION

Today we have witnessed substantial changes in the field of IT. There is a phenomenon that the computing power of computers is growing and at the same time components price falls. Information systems whose structure has modern information technology allows easier managing of business systems that is, information systems could be used more than ever.

Efficiency of IT implementation is reflected in optimization of operating costs, capital investment and reduction of unnecessary costs.

Technology that defines an essential change in this area is the Cloud computing. Data technology is a platform that allows users more efficiently, easier and, of course, cheaper use of IT resources.

The infrastructure of this technology consists of the following layers or units i.e. services: Infrastructure as a Services, Platform as a Services, Software as a Services, DaaS (Desktop as a Service). Cloud computing infrastructure components are top quality products that guarantee high availability, security and economy: VMware vSphere platform for Cloud technology, HP Blade servers, NetApp storage systems, Cisco networking products, Microsoft OS, Linux, SQL Server and others.

CHARACTERISTICS OF THE CLOUD

When we talk about the architecture of the Cloud, we can define the following types: Private cloud, Community cloud, Public cloud and Hybrid Cloud (composition of two or more models).

The main characteristic of the Cloud platform is resources management ability in accordance with the current needs of an application owned by a particular business system. One of the great challenges that all companies face is to take time and complexity of installing new servers and applications in line with growing IT demands. Business systems using Cloud technology are able to automate and to simplify these processes. For example, new applications can be run in several hours, whereas in the past it took several weeks. This enables company to meet in very short time demands of external environment...
and internal requirements of a business system, in other words, the company is able to respond quickly to the market requests. The question is which organization is ideal for using Cloud technology, and the answer is the following: all organizations that have a need to simplify the IT infrastructure, scalability, high availability and want to pay only for those resources they use. Practically, the company rents IT resources from the Cloud provider in the scope corresponding to their current requirements. What could define the company’s success, which applies the Cloud concept, is productivity and efficiency, whose intensity, or, values are large scale. This platform is the most represented in two forms of Private Cloud, whose infrastructure belongs to one company, and therefore its safety is at a higher level. Another form of this platform is Public cloud where more than one business systems share the resources of a given system, where the safety is at a lower level compared to the previous form.

The main economic advantages of the cloud centers could be highlighted. Main advantages of economies of scale that brings scalability are:

1. Savings on the side of the bidder: thanks to extent, cloud centers have lower costs per unit of the server than a simple collection of independent cloud systems.
2. Savings on the demand side: thanks to the aggregation cloud centers can support multiple clients simultaneously which directly contributes to the greater utilization of the server.
3. Other savings that are brought by growing number of clients: hosting a larger number of clients reduces administrative overhead costs and reduces the server cost per client which directly affects the reducing cloud costs per client.

The first strong factor in favor of the Cloud is client’s possibility to use resources uniformly. In that way total costs decrease, therefore, administrative costs decrease.

Another strong factor in favor of the Cloud is the elasticity of cost. Client pays resource only when it uses.

In the IT field there is the concept of virtualization. The question set up here is the difference between the concept of Cloud and virtualization. To a given question it could be answered with sufficient accuracy that Cloud technology means evolution of virtualization concept or, it may be roughly defined as the initial stage in the development of this technology.

### APPLYING NEW INFORMATION TECHNOLOGIES ON BUSINESS SYSTEMS - EXPERIENCE IN SERBIA

The most represented utilization of the Cloud technology is the form of software as a service (SaaS). When it comes to the Balkans and therefore Serbia, systems are at an early stage of development. The market imposes a need for these systems; therefore companies more easily decide to use such technologies.

Here we induce examples of the Cloud application, and his “older brother” – virtualization, in Serbian companies:

1. **Disaster recovery solution in Tarkett EE based on VMware SRM technology**
   Tarkett Eastern Europe (EE) is a leading company in design and manufacturing of floor coverings in Eastern Europe. It has been established in 2002 as a joint venture of domestic Sintelon and Tarkett Sommer. Today Tarkett EE is an integral part of Tarkett with about 1,500 employees. With production and marketing companies in Serbia, Russia and Ukraine and significant presence in all markets of Eastern European region, Tarkett EE business is high degree dependent on efficient and prompt information flow and IT infrastructure that provides. Therefore, the Tarkett EE IT organization based at the facility in Backa Palanka, Serbia, traditionally has been recognized as one of the most
progressive local IT environments - among other achievements, there have been
distinguished two parts: first, Storage Area Network technology introduction among the
first in the country, and the second, immediately after, the SAP based information system
introduction.

The following business requirements and its own tradition of innovation and
introduction of advanced technology, Tarkett EE IT management has decided to embark
into the project creating of Disaster recovery solutions.

In response to this request, the expert team of the company Coming - Computer
Engineering has made the project Disaster Recovery (DR) solutions and start off its
implementation. Since the Tarkett EE production environment has been heavily virtualized
using VMware VI3 virtual infrastructure, it has been estimated that the optimum DR
solution should be based on VMware Site Recovery Manager (SRM) technology. This
technology allows a high degree of automatization in the process of services migration and
virtual machines from the primary to the backup location (failover) and the service return to
the primary site (failback). Since the construction of the magnificent location for backup
data center has been rejected as noneconomic, in cooperation with Telekom Serbia it has
been designed a solution that provides backup location existence in the Telekom data
center in Belgrade. Location in Belgrade is associated with the primary data center in
Backa Palanka (remoted over 100km link speed of 100 Mb / s).

Due to the large number of services and the need to store large amounts of data, it
has been decided that the part of project should be also consolidation of storage systems,
therefore in both locations has been introduced one HP StorageWorks XP 20000 storage
system from HP's high-end line of storage systems. For data replication between sites it
has been used HP Continuous Access technology. Existing HP Proliant blade and Integrity
server environment at the primary location has been expanded with the new one HP
Proliant BL495c servers at the backup location.

2. Virtualization of the productive environment in Wiener Städtische insurance by
principle stretched Data Center with VMware technology

Wiener Städtische insurance company a.d.o. Belgrade has operated at the Serbian
market since February 2003. The company has been founded as a "greenfield" investment
of Wiener Städtische Allgemeine Versicherung (in 2006 the Group name has been
changed into Vienna Insurance Group). Vienna Insurance Group, which we are part of, is
Austria's leading insurance company in Central and Eastern Europe, with a business long
tradition over 180 years. Due to combination of economic strength, expertise and market
knowledge Wiener brought confirmed quality and safety which provided them a leadership
position in life insurance, and the total product portfolio is among the leading insurance
companies in the domestic market. Its mission from the beginning was creating and
developing insurance culture and services provision whose quality meets contemporary
international business standards. Therefore, the company constantly develops products
that are consistent with the European security concept and these products adapts to a
modern needs and conditions of the domestic market. In due to make good functioned
environment, it is necessary that the IT sector of the company provides a high service level
and ensures the functioning of all branches across the whole of Serbia. Considering the
nature of the firm, it is necessary to provide a high level of data protection, even in case of
serious damage.

According to the customer requirements and current capabilities, the expert team of
Coming - Computer Engineering company has responded with data center design and
solution. The implementation of a virtual data center in prolix performance has been
launched, thanks to VMware Virtual Infrastructure 3 and two EMC storage systems which
are connected by using Cisco networking equipment. Existing HP DL 360/380 servers are
reinforced with additional processors, memory cards and HBA cards for optical SAN infrastructure. Two groups were formed with two ESX hosts. This improved hardware has enabled the implementation of complex and highly demanding services, with full redundancy, since the data from storage replicate periodically and asynchronously. These four host are being controlled and constantly monitored by one VCMS server, and VCB server with Vizioncore vRanger software takes over from storage complete virtual machines with data in the form of compressed archive. These archives are temporarily stored on HP MSA70 disk enclosure. From here using Symantec BackupExec software they overreach to tapes using device MSL 4042 (robotic library tape). As Wiener has two system rooms in different parts of the building, data center is stretched to these two locations.

Services (either brand new or an existing virtualized) are transferred to the new infrastructure, which as the final result gave 100% virtualized production pipeline. Extended data center provides a significant dose of security, because each of data center segments could take over the entire production pipeline operations in case of damage in the other part of the building. On the virtual infrastructure have been built: production services which are core of company business, such as domain controllers, DMZ services (mail server) performed on the Exchange 2007 technology with over 500 mailboxes and productivity database under SQL2005 64-bit system. In this way, the company has received an additional level of security, given that these essential services were on physical servers without a cluster, and now they are at the Zware cluster and they are highly available. So, the entire infrastructure that currently enables 900 + employees has been carried out using 6 servers, of which 4 ESX hosts, two storage systems and Cisco communications equipment. There successfully functions 25 + virtual servers, including AD, Exchange, SQL Server, IIS and other services. Calculation has showed that this method of service implementation is five times cheaper for maintenance (the power cost, cooling cost, cost of required storage space), not to mention the flexibility and scalability of the same (in terms of easiness and expansion speed of the necessary resources when demands coming from the user interface increase).

3. Company EUnet

So far, the only option for the Cloud Hosting were public Cloud platforms offered by Amazon and the similar to it, hosted in the U.S. or EU. From now on, companies in Serbia and the region can use the Cloud service based on a AppLogic platform and hosting in London and Belgrade.

EUnet offers the first complete Cloud Computing Platform in SEE based on AppLogic™. This is the first turnkey commercial Cloud computing product, designed from the ground up to provide a complete Private Cloud infrastructure solution. AppLogic is a turn-key cloud computing platform for scalable applications and web services. AppLogic runs distributed transactional and streaming applications on arrays of commodity hardware.

CONCLUSIONS AND FUTURE WORK

The use of new IT, such as cloud computing, enables the creation of business benefits of a system. In other words, due to these new technologies, business systems and the whole economy become more efficient.

Efficiency of IT implementation is reflected in optimization of operating costs, capital investment and reduction of unnecessary costs.
Cloud computing as a new technology that recently has found application in business systems, supports well business agility, that is, speed and flexibility of implementation of business changes.

Today we witness the application of cloud that somehow combines a modern IT technology in a new concept, computing power, expressed in appropriate units MIPS increases and cost per MIPS in rapid decline.

Because of the flexibility to use the shared resources that it offers, and the fact that this concept in most cases requires less time and effort to provide additional resources for existing applications or new resources for new applications, it is more efficient than the on-site model.

Besides the advantages that given technologies have, it is necessary to consider their shortcomings and to compare them to more cost-effective implementation of business systems.

We can say that today the new IT technologies combined in the form of Cloud concept represent the future of modern IS in modern business systems.

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ASPECTS OF THE WORKPLACE STRESS
AT TIMIS COUNTY INSTITUTION OF PREFECT

C. Mușuroi, E. Băcală

Abstract: The object of the research is represented by the assessment of the organizational stress faced by the employees of a large-scale public administration institution and the analysis of the stress level depending on the gender and seniority of the subjects. The stress test applied comprised 32 questions describing the way in which the relation between the individual and the environment he or she works in reflects itself upon their personality. The study demonstrates that, under the same working environment conditions, the employee’s stress level differs depending on gender and seniority within the institution, due to the statistically significant differences concerning the ability to communicate with superiors and workmates, being aware of and observing one's own physical and professional limits, assessing and planning activities based on time intervals, establishing priorities, self-control.

Knowing the causes of stress as well as the influence exerted by the employee’s gender and seniority allows the elaboration of strategies meant to defeat stress at the workplace by means of approaching the individual within the three environments of his or her existence, the family, the organisation and their own person.

Key words: professional stress, the effects of stress, risk factors, professional satisfaction

INTRODUCTION

Within the European Union work stress represents a health issue related to one’s professional activity, coming second, as a pathological manifestation, behind spinal disorders. Identified with 28% of EU employees [5], work stress may be caused by psychological factors within the work environment such as activity design, work management and institutional management.

Explaining disfunctionalities and finding solutions regarding stress at the workplace may be achieved only by understanding individual mechanisms of triggering, maintaining and diminishing this phenomenon.

Stress must be regarded as an imbalance perceived subjectively, between the demands which the body is subjected to and its response capacity, at the workplace and outside it. The impact with stressors is followed by the assessment of the danger degree, as well as of the individual response capacity, relying on personal experience, resources and time available in order to react. However, we are dealing with an evolutionary process, a permanent transaction between the environment and the individual, which responds, leaves its mark upon the environment with by means of this response and expects a result which would reflect the suitability of the response to the demands of the outer environment. We are talking, therefore, about a continuous negotiation which, in order to be satisfactory for the individual, has to maintain unaltered the somatic and psychic equilibrium of the human organism.

Each workplace is characterised by stress factors to which, under conditions of optimal physical and psychic functionality, the individual responds without repercussions concerning his or her health state. Moreover, moderate psycho-neuro-endocrinal stimulation maintains the equilibrium, the physical and psychic tone, the health state and induces a positive adaptation to the environment.

Stress factors of high intensity resulting from overloading, intense and prolonged over-stimulation create a conflict between individuals and the environment, which exceeds their ability to provide a response, their adaptation physical and psychic resources, with the occurrence of behavioural disorders and even the setting in of stress somato-psychic disease phenomena.

Stress related to professional activity occurs when the demands of the work environment exceed the employee’s ability to cope with them or keep them under control.
The current professional activity subjects the individuals bearing multiple role statuses and acting as members of various organisations (with different structures) to multiple socio-professional pressures, which affect, either consciously or unconsciously, their personal adaptation resources [8].

MATERIAL AND METHODS

The present research was performed at the Institution of Prefect – Timiş County in Romania.

The Institution of Prefect – Timiş County, founded in 1990, is organised and operates as a public institution, with judicial personality, with its own patrimony and budget, meant to fulfil the prefect’s prerogatives.

The staff within the prefecture, 45 persons, comprises clerks, special status clerks and contract staff. The assignment, respectively the circumscription, as well as modification, suspension and cessation of work relations and, respectively, of work relations pertaining to the prefecture staff, are performed by means of the prefect’s order, under the conditions stipulated by the law.

The main goal of this research was that of assessing professional stress manifested in the present institution and identifying the relation between professional stress and the gender, respectively the seniority of the person in question, within this context. The study group comprised employees of the Institution of Prefect – Timiş County, with a seniority exceeding twenty years of activity, in Romania.

The work sampling was achieved based on the voluntarism criterion and, out of the 45 subjects, clerks, special status clerks and contract staff, 38 employees of the institution responded to our initiative. The subjects belong to both genders, with ages between 23 and 50, the average age being 36.

The tool employed was the structured interview, which made it possible to collect demographic data such as age, gender, seniority, finalised level of education, marital status, etc. The professional stress perceived was measured by means of the 32 items of the Tim Hindle’s survey [10]. The respondents circled the number best fitting the 32 statements (1 – never, 2 – occasionally, 3 – often, 4 – always), the next step having stress operationalized through the item answers. From a procedural point of view, the pen and paper variant was used, the subjects being ensured both verbally and in writing (during the instructions stage) that there were no good or bad answers and each and everyone’s answers would be confidential.

RESULTS AND DISCUSSION

The data obtained following the implementation of the survey and answers to the structured interview were processed with the help of the SPSS 13.0 software for Windows.

The verification of the hypothesis was achieved using the Mann-Whitney nonparametric test, the survey items being used as variables (on an ordinal scale). The results obtained pursuant to the implementation of the survey are displayed in the table below.

Table 1. Average values of the Tim Hindle’s survey scores for the employees of the Institution of Prefect – Timiş County, as per seniority at the workplace

<table>
<thead>
<tr>
<th>Item</th>
<th>Seniority</th>
<th>N</th>
<th>Mean Rank</th>
<th>Z (Mann-Whitney)</th>
<th>p (Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i1</td>
<td>less</td>
<td>28</td>
<td>17.43</td>
<td>-2.492</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>over</td>
<td>10</td>
<td>25.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i18</td>
<td>less</td>
<td>28</td>
<td>17.00</td>
<td>-2.565</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>over</td>
<td>10</td>
<td>26.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i19</td>
<td>less</td>
<td>28</td>
<td>17.07</td>
<td>-3.166</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>over</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The aspects of professional stress were operationalized by means of statistically significant answers to the following items:

- Item i1, “I blame myself if things take a bad turn at work.”
- Item i18, “I ignore my own professional and physical limits”.
- Item i19, “I give up my hobby and interests because work takes all my time.”
- Item i28, “I must attend to all important matters on a daily basis.”
- Item i32, “I become anxious if the slightest delays occur.”

Item i1 refers to the feeling of self-guilt which the employees working for more than 10 years for the Institution of Prefect – Timiş County have when things take an unsatisfactory path at the workplace. This feeling may be destructive for the individual should it persist for an extended period of time.

Item i18 reveals the fact the employees with a higher seniority overexercise themselves both physically as well as intellectually and emotionally, even in case they should feel this overexercise exceeds the body’s ability to respond to external aggression, thus risking to become ill following the efforts they subject themselves to. As one could infer from the quantitative analysis, item i19 is the most noticeable one, with a high level of significance, which means that the employees of the Institution of Prefect – Timiş County sometimes give up even the pleasure of hobbies or personal interests for the benefit of work. The fact that they no longer have the necessary time for their own person may be due to a large volume of work or a highly rigorous work procedure. In addition to that, activity design and work management play a very important role.

As in the case of item i18, where one can observe that employees even take their own faculties or means to the extreme in order to handle the demands of the job, the workload and meeting deadlines, item i28 displays the same tendency of sacrifice which is, in fact, useless or, more than that, has unfavourable outcomes. Thus, the persons hired more than 10 years ago are concerned about the daily fulfilment of all tasks, even if this seems unachievable. This overexercise the individuals subject themselves to carries psychic distress, which may have repercussions both upon the individual and upon the institution.

Item i32 suggests that in the case of the persons with a seniority exceeding 10 years within the Institution of Prefect – Timiş County there is a permanent race against the clock in solving the tasks, and any delay occurring from various reasons causes the state of panic to set in.

Table 2. Average values of the Tim Hindle’s survey scores for the employees of the Institution of Prefect – Timiş County as per the gender variable

<table>
<thead>
<tr>
<th>Item</th>
<th>Gender</th>
<th>N</th>
<th>Mean Rank</th>
<th>Z(Mann-Whitney)</th>
<th>p(Sig.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i1</td>
<td>femal</td>
<td>20</td>
<td>22.00</td>
<td>-1.895</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>18</td>
<td>16.72</td>
<td>-2.004</td>
<td>0.045</td>
</tr>
<tr>
<td>i18</td>
<td>femal</td>
<td>20</td>
<td>22.60</td>
<td>-1.895</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>18</td>
<td>16.06</td>
<td>-2.004</td>
<td>0.045</td>
</tr>
<tr>
<td>i19</td>
<td>femal</td>
<td>20</td>
<td>22.90</td>
<td>-2.792</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>18</td>
<td>15.72</td>
<td>-2.792</td>
<td>0.005</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As one can infer from table 2, aspects of professional stress have a prevalent frequency in the case of female employees hired at the Institution of Prefect – Timiş County, and are less frequent in the case of male employees. The answers were statistically significant for the items presented.

As one may notice, a significant and relevant item is i1 since, out of the answers provided by both genders, we can see that male subjects do not display just as intensely as female subjects the tendency of assuming self-guilt if things take a negative turn at the workplace. Male subjects display a better self-control and more balanced reactions to stress than female subjects do.

Item i18 reveals the fact that male subjects display a better management of their own resources and a tendency of limiting excesses regarding the level of response to stressors, which makes them respond with more easiness to professional tasks in comparison with female subjects. Female subjects have the tendency to exceed that “final point” that marks signing out of the physiological limits of response and adaptation, which have, in fact, individualized values.

Male subjects achieve a better time management than female employees, they do not consider that work steals all their time and do not give up their hobbies in favour of it, as demonstrated by item i19. All these indicate that male subjects enjoy a better balance between private life and professional life in comparison with female subjects.

CONCLUSIONS AND FUTURE WORK

The purpose of the present research was that of assessing the level of the stress state in the case of the employees within the Institution of Prefect – Timiş County, from the point of view of its relation with the individual’s gender and seniority at the workplace. The study demonstrated that the stress level increases in parallel with accumulating an increasing number of years at the same workplace, the data being statistically significant in favour of the employed personnel with seniority in excess of ten years within the Institution of Prefect – Timiş County.

The employees of the Institution of Prefect – Timiş County occasionally sacrifice their spare time in favour of work, ignore their own limits and take the blame upon themselves if work tasks are not adequately fulfilled, which leads to the increase of the stress degree, respectively to a higher degree of psychological stress.

We admit that each and every job has a potential of stress-prone factors and the position of clerk, special status clerk or contract personnel involves intense psychological stress, taking into account the fact that they do not offer a high degree of decisional control.

Male subjects employed within the Institution of Prefect – Timiş County manifest a superior ability in the fields of time management, problem solving and emotional equilibrium. The degree of psychic stress at an institutional level is increased, and yet male subjects cope much better with stressors in comparison with the female personnel.

Consequently, the results of this study may be the starting point for drafting guidances designed to reduce stress at the workplace:

1. Measures taken to prevent the accumulation of significant seniority at the workplace. We are making no reference to the transfer to another institution. Changes may take into account the job, a reduced number of workmates, the position, the work tasks. Any change may be a beneficial one as long as demands do not exceed the individual’s response capacity.

2. Correlating tasks at the workplace with the individual’s physical and psychic potential, in relation with his or her gender. Discrimination is out of the question.
here, the point being the identification of ways to increase the capacity of response and adaptation to the work task, both for men and for women, in equal measure.

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Abstract: Computing technologies has emerged as an innermost part of our daily life, continually challenging researchers to provide more accurate explanations of psychological phenomena surrounding them. Flow refers to a special state of enjoyment, satisfaction, playfulness, engagement, and absorption that is frequently experienced by users in online environments. This paper aims to provide an analysis of the main psychological components of flow experience, and the relationships among them, as well as of their potential to inform the design of user interfaces with new technologies.

Key words: Flow, User Experience, Human-Computer Interaction

INTRODUCTION

Flow is a positive, highly enjoyable state of consciousness that occurs when a person’s perceived skills match the perceived challenges that he or she is undertaking. When the goals are clear, the skills are up to the challenge, and feedback is immediate, people become involved in that activity. Sometimes, this involved is so powerful that one loses his or her sense of self and time distorts. The experience becomes autotelic or intrinsically rewarding; the person performs it for its own sake [2]. It should be clear that the flow experience has little to do with the well-known figure of speech, “go with the flow”, which means to abandon oneself to a situation that feels good, natural and spontaneous. It is an experience that rather requires skills, focused attention and perseverance.

But how can we explain the enjoyment, satisfaction, playfulness, engagement, absorption and other related states of involvement experienced by many users in interaction with the new technologies? Computer environments seems to be natural inducers of flow, and it may explain the large adoption of this technology. Flow is "a sense humans have developed in order to recognize patterns of action and transmitting over time" [2, p.34], that makes people tend to repeat activities they enjoy, to return to the same websites over and over again, or to reject products or technologies that fails in engaging users in an authentic way.

Developing a better understanding of the flow experiences of Web users is important for many reasons. First, there is evidence to suggest that the behavior of users toward new information technologies is shaped by their holistic experiences with the technology, and not just the usability or instrumentality of the technology. Second, our current understanding of user concerns, derived from the old world of computer based activities seems to be less adequate for dealing with the special design challenges presented by new emerging technologies, like the Web 2.0 and social media, where users are actively designing their virtual environments and generating new content. Thus, a more coherent understanding of what enjoyment actually is and how it can be addressed by new products and processes. Following these idea, this paper will review research on the nature of flow and related concepts, and look at possible applications.

FLOW AS AN OPTIMAL EXPERIENCE

Many people experience flow while engaging in more mundane activities such as gardening, driving, talking with friends or browsing the web. Despite the obvious differences between these kind of activities, people consistently describe flow experiences in rather similar terms [2, 3]:
- a sense of potential control;
- a merging of action and awareness;
One of the most distinctive features of the flow experience is the effective merging of action and awareness. As Csikszentmihalyi [2] describes it, sometimes "people become so involved in what they are doing that the activity becomes spontaneous, almost automatic; they stop being aware of themselves as separate from the actions they are performing" (p. 53). This merging of action and awareness is made possible by the focusing of attention. In everyday life, human minds is often diverted from the main goal and activities by doubts and questions that constantly compete for attentional resources. In flow, however, the activity at hand can occupy a person's attention to the point of excluding all other irrelevant thoughts, and the usual dualism between action and awareness disappears [3].

Another commonly reported dimension of the flow experience is a distorted sense of time, with the subjective feeling of time going very fast. Not only Internet users are experiencing this phenomenon: in some exceptional cases, people in flow find themselves very sensitive to the passage of time. In most of these situations, keeping track of time is one of the skills needed to perform well in a specific activity.

When an activity exhibits many of the characteristics described in the preceding sections, the experience becomes autotelic, or worth doing for its own sake. According to Csikszentmihalyi [2], an autotelic experience is "a self-contained activity, one that is done not with the expectation of some future benefit, but simply because the doing itself is the reward" (p. 67). Because autotelic experiences are so satisfying, they create a strong desire to repeat the activity that produced the experience. Once it starts, what keeps it going is the activity itself. Activities such as art, music and sport often lead to autotelic experiences. People do them, not to receive some extrinsic reward, but simply because they enjoy the experience. In contrast, many of the activities we perform every day are exotelic.

In addition to the six characteristics mentioned above, [2] also identifies a set of three conditions necessary for an activity to provide a flow experience:

- clear activity goals and distinct steps to reach the goals: the person knows exactly what needs to be done next.
- balance between the challenge of the activity and the skill of the individual, that ensures that an activity is experienced as both interesting and manageable.
- clear and immediate feedback: a person in flow should know at each step how well he or she is doing in meeting the goals of the activity.

Altogether, these requirements create a condition in which the six experiential characteristics discussed earlier can occur. Csikszentmihalyi [3] suggests that many activities, including religious rituals, games, sports, and artistic performances are designed to promote the experience of flow through ordered rules and clarity of goals. Moreover, flow theory claims that any activity can provide a flow experience if it is structured to meet these basic requirements.

In one of the first studies of flow in computer-based activities, [1] found that web users frequently reported feelings of inspired involvement, loss of self-consciousness, excitement and fascination, enjoyment, and timelessness. In a paralleling work, Hoffman and Novak extended Csikszentmihalyi’s work to consumer navigation on the web, and defined flow online [6] as the psychological state occurring during network navigation which is: (a) characterized by a seamless sequence of responses facilitated by machine interactivity; (b) intrinsically enjoyable; (c) accompanied by a loss of self-consciousness; and (d) self-reinforcing.
People who experience flow tend to be more playful, exploratory and willing to try new things. Furthermore, users return to products or web sites that facilitate flow. Hence, Hoffman and Novack states that, from a design perspective, flow has a number of positive consequences [6, 7, 8], including:

- increased user learning,
- exploratory behavior and positive behavior,
- positive subjective experience
- perceived sense of control over their interaction.

All theses results suggest that designers and online marketers should be aware of these implications and offer enough “flow opportunities” for their intended users.

**CREATING FLOW “OPPORTUNITIES” IN VIRTUAL ENVIRONMENTS**

As discussed above, flow occurs under a limited set of circumstances.

Empirical data shows that prerequisite of flow are rather similar in online and offline contexts, and is determined by [7]: (a) high levels of skills and control, (b) high level of challenge and arousal, and (c) focused attention; especially in web environments flow is enhanced by (d) interactivity and telepresence.

Users can experience flow only when their computer-based activities proceed smoothly, with fast response, immediate and unambiguous feedback, clear navigation and minimal distractions. When experiencing flow, users feel their skills matching available challenges. In testing various commercial websites and web applications (chat rooms, newsgroups, etc.), [6, 7] found that interaction speed had the most important effect on the amount of time spent online and on frequency of visits for web applications. However, for repeat visits, the most important factors seems to be the followings: the perceived level of control by matching skill-challenge balance, important content, and fast response time.

**Interactive speed and unambiguous feedback.** Speed is a significant factor in all models of user satisfaction, contributing significantly to ease of contact and variety. For both online and offline content, pages should load quickly and minimize the variability of delay; slow response should be also avoided after pages are loaded. Most flow experiences are reported to occur within activities that are goal-directed and bounded by rules [3]. Flow activities allow a person to focus on clear goals and to receive meaningful feedback. Hence, fast, unambiguous feedback should be provided for user input in online environments. Information elements like: links, navigation widgets, display performance variables (server load, cache state, page/file sizes, download progress bars) help users to evaluate the progress made towards a stated goals. Sometimes, as in the case of computer mediated conversation, the goals and rules governing an activity are negotiated on the spot. As Csikszentmihalyi explains, “unless a person learns to set goals and to recognize and gauge feedback in such activities, she will not enjoy them” [3, p. 55].

**Skills and challenges.** The flow experience is something that requires skills, focused attention and perseverance [2]. In order to make a web site compelling enough for users to return to, it should offer a perceived level of control by matching challenges to user skills. An adaptable/adjustable interface can give users control over their environment’s complexity that is appropriate to their skill level. The flow experience is often described as involving a sense of control, or more precisely, an absence of any concerns about failure. However, if the challenges of an activity are too high relative to one’s skills, one experiences anxiety or fear of failure. On the contrary, if challenges are too low, one experiences boredom. If challenges and skills are both low, one experiences apathy and the overall quality of the subjective experience is the lowest. If challenges and skills are both high, the likelihood of experiencing flow is maximized and the overall quality of the subjective experience is the highest [3].
Designing for fun and utility. Confirming their previous work, [8] describes two types of flow: experiential (associated with recreational surfing) and goal-directed (associated with research, shopping, etc.). While less-experienced users tend to approach the web in a hedonic, playful way, associated with recreational surfing, more experienced users tend to view the web in a utilitarian way, or a means to accomplish specific task-oriented activities, such as research, work, and shopping. This finding suggest that the design of computer environments should accommodate both type of activities.

Offering a rich yet responsive experience, plus tools to help users accomplish their goals quickly and easily is a prerequisite of flow in online environments. Closely related to the role of flow in human-computer interactions, is the playfulness construct. There is ample anecdotal and empirical evidence that people find an element of playfulness in their experiences with computers. Because computers follow logical rules and produce very immediate outcomes, they create work environments that resemble many recreational games, and they foster exploratory learning and what-if fantasizing.

Further more, Webster et al [9, p. 412] defined the state of computer playfulness as „an aspect of users' subjective experiences during computer interactions that is characterized by perceptions of pleasure and involvement‖, and proposed that the flow construct is comprised of four interrelated dimensions: control, attention focus, curiosity and intrinsic interest. They further hypothesized that flow would be significantly correlated with perceptions of a program's flexibility and modifiability, experimentation and expectations of future computer use.

Simple and transparent design. Humans have a limited attention capacity. Based on humans ability to recognize about seven chunks of information per limit of time, Csikszentmihalyi estimated that human users can processes about 126 bits per second. This limited bandwidth is one reason why designers are advised to minimize distractions on the web. Simplicity of design, with uncluttered layout and minimal features reduce the attention load. Making navigation and performance transparent is possible by using signposts such as site maps, breadcrumb trails, and “you are here” landmarks help visitors find their way so they can easily form a mental model of the site or work environment. In an interview for the Wired magazine (no. 9/1996), Csikszentmihalyi claims that “the key attribute is that it should be very user-friendly and transparent at first, but one should immediately be able to find complexity in it, so as to find quickly the right level of opportunities for “action” that match one’s skills. These “challenges” include the visual aspects as well as the content.”

CONCLUSIONS

In the early history of computers and internet, users were required to follow relatively inflexible path and existing navigational structures. In the new type of web 2.0 environment, users are taking more and more in the control their navigational experiences and have much more freedom to do things there are interested in doing [8]. Underlying the idea of facilitating flow experiences online, user experience is very important in online environment, and designers need to focus on the nature of what is important in this context. One such characteristics is that online user behavior is grounded in both goal-directed and non-directed motivations [6]. This distinction may explain some internal changes noticed in the structure of the flow construct developed by many authors. What it was noticed was the following: while skills and importance increased with web experience, attention, challenge, telepresence, flow and exploratory behavior were decreasing. In other words, as users use the web longer and longer, they use it for skill-based, goal-directed purposes, and less as recreational tool.
Csikszentmihalyi [8] describes the flow experience as “one of complete involvement of the actor with his activity” (p. 36), and he has identified a number of elements that are indicators of its occurrence and intensity. These indicators include: the perception that personal skills and the challenges provided by an activity are imbalance, centering of attention, loss of self-consciousness, unambiguous feedback to a person’s actions, feelings of control over actions and environment, and momentary loss of anxiety and constraint, and enjoyment or pleasure.

Despite its obvious relevance to computer-mediated environments, flow has been constantly proved to be an elusive construct to define. The difficulties that researchers have encountered in their studies of the flow experiences of computer users suggest that the topic is in need of further conceptual development. A better understanding of the concepts pertaining to flow in the Web environment could help to resolve uncertainties and contribute to more precise and complete explanations about its nature and subsequent implications.

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ENGLISH LOANWORDS AS AN INTERNATIONAL LANGUAGE
PHENOMENON

Anica Perković, Georgeta Rata, Alka Turalija, Jasna Vujčić

Abstract: No doubt that English today is the most widely used language in the international communication. Most languages are daily subjected to a powerful English influence. Why? Several factors have brought about this situation. Namely, modern technology such as film, television (satellite and cable) Internet, cell phones put people directly within the reach of the wider world, culture and linguistic assimilation.

The paper aims to provide examples and areas English words are borrowed from. We shall also try to answer the question how to cope with invasion of English loanwords.

Key- words: English words, international, language, phenomenon

INTRODUCTION

Traditionally a national language was used for national education and culture. England, France, German, Italy, Russia and Spain were countries with well established culture and language which extended their lexical range to cover new language needs in the period before the 19th century.

English was in Europe introduced in schools at the end of 17th century as a foreign language aiming to help in acquiring knowledge on other culture and communication beyond national boundaries.

Croats, as many other countries, used to borrow English words to name new objects and concepts, thus, to fill lexical gaps (bajt, čip, kompjutor, CD, akumulator, parkirati,). Many of the analysed such English borrowings (derived from Latin or Greek and were already integrated in the English language- internationalisms) entered our national languages as anglicisms. Once the English word is borrowed the adaptation process starts. The words are adapted on phonological, morphological and semantic levels.

However, English words enter Croatian lexicon regardless the fact that there are already Croatian equivalents for them (e.g. kompjutor – računalo; struktura – sastav; tenzija – napetost; vizija – videnje; kompatibilan – skladan; kompaktn – zbijen).

English language has a vital role in EU serving as an official language in an internal communication within the European institutions. Countries, applicants for EU, such as Croatia and others use English in various forms of communication. Being as European lingua franca English is thus, feared to be means of new joint identity and mechanism resulting in “erosion of regional languages” [1].

Today we live in the era of television, film, cell phone, computer which enable us to reach the wider world, culture and linguistic assimilation. It is also a period when multiculturality makes us live in a pluri-lingual world. Developing business and the aforesaid technology resulted in the English language dominance since it prevails in all the above mentioned. Croatian linguist Filipović [2] said that word borrowing occurs if there is a requirement for naming new objects, phenomenon or ideas in the receiving language since it doesn't have an adequate equivalent. However, English borrowings (anglicisms) are used regardless the fact that there is a required term in a receiving language. Especially young people are affected by anglicisms. English used in other national languages also has a stylistic function. Namely many firms get English names (Avenue Mall, City Centre 1, Autosound, Eurocomputer systems, Eurotrade, Zapad Commerce, 3D Design Studio, Agrigenetics, Agristar etc.) and commercials are either all in English or partially (smiley ne košta ništa (Tele 2), I'm lovin it – (Mc Donald’s), Coca-Cola – Zero % šešera). This is due
to the global language prestige. All areas of life (fashion, sports, music etc.) have been influenced by the English borrowings.

**Pseudo-borrowings** have recently appeared as a new phenomenon. Namely, they do not exist as a model in the English language and are freely designed (kuler, darker, kaubojke, koledžice etc.).

How do puritans respond to such imperialism in written and spoken national languages?

They protest requiring national languages to keep “pure” without new forms. They have also taken some steps related to the above mentioned. For example the Japanese want to dump poorly understood foreign words; Iranians ban usage of foreign words; Koreans argue against “blatant foreignisms” on two premises: they are “foreign words”, and any of them are difficult to understand; the British think foreign words are seldom de rigueur. Some others have sought not merely to replace foreign words but to provide a way of producing needed new words. The German writer, linguist, educator and publisher Joachim Heinrich CAMPE developed approx. 11, 500 translations for foreign words, of which about 300 survived. The Icelander Vikor (cited by Michael T Corgan) suggested three systematic strategies for developing new words and meanings for Icelandic:

1) to give new meanings to existing words
2) to derive words from concepts already existing and
3) to make compound words of existing Icelandic terms to fashion required new words

Romeans purists tried to replace the French cravate (from <Croate, because Croatian soldiers used to wear such an accessory) meaning “tie” by Romanian degatlegau (literally “necktie”) – which did not work.

Examples of Turkish words (jok, sirče, vala, vriča etc.) used in Slavonia (Eastern Croatia) can be found in works of Croatian writers (R. Aleksiš, J. Vončina) who considered them unnecessary. Some English words (airplane) have been tried to replace by the Croatian (zrakomlat) ones but not for long.

Writing about the process of linguistic borrowing and adaptation of English foreign words to the rules of Croatian Branimir Belaj and Goran Tanackoviš Faletar (2007) suggest a new model for the analysis of linguistic borrowing. It says that English words, prior entering the Croatian language, should pass a “ladder” whose height could be jumped over only by words meeting three “norm principles” of:

- functional justifiability
- maximum possible of substitution
- complete adaptation at all levels of the language description.

Our paper aims to answer the questions:

- Can we really fight the massive invasion of English words in almost every area of life? If yes, how?
- Will English borrowings jeopardise national languages?

**MATERIAL AND METHODS**

The paper aims to study English loanwords specific to various aspects such as lexical, syntactic, pragmatic etc. Examples were selected from the spoken language, magazines, TV commercials, survey from the Faculty of Philosophy in Osijek (taboo English words).
RESULTS AND DISCUSSION

Lexical aspects of the English borrowings in Croatian language can be seen on the examples followed:

Examples selected from the expert books (agriculture) have been entered into the English word stock either from Latin or Greek: agriculture - agrikultura, poljoprivreda; economy - ekonomija; ecology - ekologija; embryo - embryo; esterification - esterifikacija; fertilization - fertilizacija; photosynthesis - fotosinteza; galactose - galaktoza; hydrometer - hidrometar; cholesterol - kolesterol; park - park etc.

Electronics: accumulator - akumulator; converter - converter, pretvarač; detector-detector etc.

Computer science: nouns (bug - bug; file - fajl; font-font; modem-modem; scanner-skener; server - server

verb: download-downlodati; e-mail-milati, mejlati; forward-forwardirati, surfati (po internetu),

Everyday life: nouns (drink, life, mesidž, meil, keš, freak /frik/, filing, party, frend /frendica/, luzer, song, baskit, bajk, fighter/fajter/

verbs: sprintati, biti in/out/cool, skulirati se, muvati, ići na coffee,

modal verb must is used as a noun in Croatian language. Mala crna haljina je apsolutni must ovoga ljeta.

adjective: kul, ful, best, fancy

adverbs: tu mač, baj d vej (by the way)

prepositions: in and out are used in Croatian as adjectives (everyday conversation)

Posao koji se na Manhattanu odavno smatra “in”je -šetač pasa. [6]

Hijerarhija je definitivno “out”, a suradnja postaje pravi brend.

phrases: Ovo je shortcut. Shit happens! Ovo je must have ove sezone.

English abbreviations: CV, DJ, DVD, IQ

English borrowed lexems are adapted at morphological level by adding suffix for female gender e.g. trendseterica, frendica, head-hunterica

Syntactic aspects of the English influence in Croatian language can be seen in the examples followed:

word order: Ovo je najbolji film koji sam gledala ikad (ever) instead of (…koji sam do sada gledala…)

passive instead of active: Rečeno mu je instead of Rekli su mu (He was told)

redundant use of possessive pronouns: He is drinking his coffee. Pije svoju jutarnju kavu.

My mother told me a story. Moja majka mi je ispričala priču. [5]

constructions as “sulfonil-, acil-” are foreign to Croatian language. We should write complete names like “hidroksikiseline and aminokiseline or use description.

The study conducted at the Faculty of Philosophy in Osijek [7] was based on recordings and transcripts of speech data collected during a classroom discussion in three separate groups of the third year students of English. There were 33 examinees, 24 males and 9 females. The aim of the study was to show how students misuse the pragmatic aspect of taboo language.

Taboo language words used were as follows:

Jesus! Idiot! Shut up! Blood! Sht! God! Screw you! Don’t give a shit! Bloody hell! Crap! Drop dad!
Students were asked to tell the sources they used English taboo language from. The answers were as follows: **cinema, TV, friends, street, lecture room, media (internet, newspapers, journals) books**.

As for the reasons why they use taboo language students provide answers as follows:
- it is useful for expressing strong emotions (anger, pain or frustration)
- for fun
- to draw attention

It is also interesting that students prefer using English taboo language words than their mother tongue since for them English taboo words don't have the same emotional power as words expressed in their mother tongue.

Students are constantly exposed to English taboo words from media in all possible contexts and are not taught on the adequate use of these words. Namely, native English speaking people are rather willing to use less offensive expressions instead of the English taboo words.

**CONCLUSIONS AND FUTURE WORK**

In spite of being powerful in many European countries the English language doesn’t jeopardise other languages. However, it is a part of multi-language structure offering each language to have its place and function.

Today it is inevitable for languages to be mixed due to the globalization processes, fashion, and imitation. When English lexemes enter a certain language they are subjected to adaptation process of the recipient language at morphological, lexical and semantic level.

It is obvious that the old language system that was developing for thousands of years is getting disappeared giving way to restructuring of the world hierarchy. Graddol [3] said that English language dominance will finally be replaced by oligarchy of several big languages whereby English will probably keep its special position in multi-language societies as the only one which will be used in all language combinations worldwide.

Vesna Muhvič Dimovski [4] concluded that neither extreme purism nor excessive openness is good. However, a language should be stable on one hand and sufficiently elastic on the other one able to follow communication needs of its interlocutors.

However, further study on this phenomenon should be conducted aiming to see forthcoming changes and steps that should be taken in terms of the abovementioned.

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MODERN TECHNICAL RESOURCES IN FOREIGN LANGUAGE TEACHING

R. Stan

Abstract: This paper is the result of some experiments which set forth the idea that foreign language teachers must make a concerted effort to educate their students in the utilization of communications technology resources. The paper points to Internet technologies as effective resources that enhance instruction. Emerging technologies make what we learn today nearly obsolete by next week, but a good instructor should be reasonably conversant with any of the technologies used in the classroom. It is essential that we make informed decisions about how the Internet can be successfully integrated into the language classroom. The Internet's educational potential is immense, in spite of its limitations and obstacles.

Key words: Technology, Communication, Educational potential

INTRODUCTION
Technology has pervaded many aspects of education and business over the past decades. The Internet has emerged as a prominent new technology, used in all aspects of the educational, business, and economic sectors of our world. Internet technologies have been embraced by many foreign language teachers as effective resources that enhance instruction. However, many more have not done so for a variety of reasons: lack of knowledge, lack of access, and inadequate professional role models. All these contribute greatly to the underuse of communications technology in the foreign language classroom at both secondary and tertiary levels. The consequence is many foreign language teachers marching through their language curriculum seldom seeing technology implemented in the classroom. They therefore are little inclined to include communications technology as part of their own teaching. To break this cycle, foreign language methods instructors must make a concerted effort to educate their students in the utilization of communications technology resources.

MATERIAL AND METHODS
A myriad of communications technologies are available for the foreign language teacher, who should just look for information. Many articles and books exist that explain all of these technologies and concepts in great detail. After acquiring some basic knowledge about several electronic communications technologies the foreign language teacher can apply that knowledge in practical situations encountered in the classroom.

Electronic mail, also known as e-mail, is probably the most common method of using the Internet. You must have access to the Internet to use e-mail. Many teachers can access the Internet through their home institution.

Once you have access, you also need software so that your computer can talk to other computers; this involves sending commands, receiving and sending mail, and any other general communication functions computers do. You need to install the software, set the parameters, and begin sending and receiving mail.

How does e-mail work? You can send and receive original messages, include parts of messages in other messages, reply to messages, forward messages, and save your messages to a file. These functions are all done by commands, which differ from system to system. It is, therefore, very important to understand how your system works.
RESULTS AND DISCUSSION

TASK:a) E-mail is all over the world and is used by people in all languages. Foreign language teachers will want to take advantage of this by putting students in communication with native speakers of the respective target languages. The students can be asked to send mail to each other or to send mail to the teacher from their own e-mail account. b) The teacher may ask the student to click on a mail link. A mail dialogue box will appear with the beginnings of a mail message addressed to the teacher. The student has to fill in the other lines and the CC: to themselves if they wish.

The students can opt to join or subscribe to an electronic discussion group, also called a list. Thousands of these groups exist on the Internet; they join together for a common interest (professional, leisure, research). A list with a broad appeal may have hundreds or even thousands of members, while a list that is very specific in nature and confined in terms of topic definition might only attract a small subscribership. Membership is generally free and one need not actively participate by posting regularly in most cases. Users who read but do not post ("lurkers") are usually welcome on lists as well. Later in the lessons, the students will have an opportunity to subscribe to a discussion list. There are two addresses associated with most lists: the mechanics address and the discussion address. Your discussion contributions are directed to the address whereby your comments are posted and joined by those of other members.

TASK:a) The students will join an electronic discussion list and follow one particular thread for several days. In essence, they will be "lurkers". They may participate if they wish, but it is always wise to observe for a short while before entering the discussion. The list the teacher has in mind is a Foreign Language Teaching System. It is a rather large and active list and it is dedicated to the improvement of foreign language instruction and learning at all institutional and instructional levels. b) The students are asked to subscribe to the following address:

WTCD@WTCD.EDU

and put nothing in the subject line. In the body of the message, write:

SUBSCRIBE WTCD FIRSTNAME and LASTNAME.

The teacher asks the students to send this message, without anything else in the message field. They will shortly receive a confirmation message that will ask them to respond, thus making a complete circuit between their computer account and the WTCD account. c) Once this connection is established, the student will receive a Welcome message. This message is very important. It will give all sorts of useful information about how to send and receive WTCD messages, appropriate postings, and other valuable data.

Participation in usenet groups is a way to join Internet discussions, which functions like bulletin boards, where students post their messages and respond to others. They differ from discussion lists in that they do not join but rather access the postings via a usenet reader, if the service provider gives this option. Also, the postings are "on the bulletin board" and do not come to anyone's mail account or computer. In this way, Usenet groups take up much less space on the computer or in the Internet account. A thread of discussion can be followed, and the Usenet reader can keep track of the messages in that thread that the student has or has not read. In some ways, Usenet groups are easier for beginners to manage. They require much less commitment and are perhaps not taken nearly as seriously as membership in a discussion list. These groups provide a means for students to make contact with peers who are native speakers of the target language and then engage in conversations with them via e-mail.

TASK:a) The students access the postings via a newsreader, which keeps track of the groups which the students follow and, within those groups, of the threads and messages
the students have read. The first step is to determine if the service provider gives the Usenet option. To ascertain this, the students must check with the service provider or the systems manager. b) Once the students have their newsreader installed they are asked to spend some time looking around for some newsgroups they might want to "join" or participate in by posting messages.

Gopher is an Internet application that allows browsing many different kinds of resources by looking at menus or listings of information available. Gopher was really pretty cool in its heyday. The menus in the Gopher system allowed to see what information was there; the Gopher client on the system then brought the information required to the computer screen. The Gopher servers of the world were all interconnected and were compared to a large library full of resources. Gopher is no more used as all these resources have been ported to WWW formats; however one day someone may be on "Jeopardy" and a question about Gopher might arise just in case.

Telnet allows two different computers to communicate with each other. Telnet allows the user to log on to an account on a remote computer. This is useful for people who are traveling or are going to be away from their own computer and their local account but need to have access to the latter. Someone might be in another town or country and need some information that is stored on a file in the account at the home institution. Telnet allows getting into the home institution files that are on the remote server, and retrieve the information.

World Wide Web (WWW) pages have the ability to present multiple media, text, images, sounds, and moving pictures simultaneously. This puts it in a different class compared to other applications. The user can interact with web pages to perform functions, such as sending and retrieving information. Web pages can be sent for, received, and viewed via Web browsers. Several different browsers exist (Netscape, Internet Explorer), and some are available free to educators. Many of these can be downloaded from the Internet. A request can be sent via browser, for a particular page. Each page has its own address, in the form of a Uniform Reference Locator (URL). The address can include the name of the computer where the file is located and the directory. Web pages are transmitted to the WWW by Hypertext Transfer Protocol (HTTP). This protocol lets the computer know that a file is formatted in Hypertext Markup Language (HTML). The HTTP and the HTML designations should look familiar if the student has ever seen a WWW page with its address. All URLs begin with http:// and many end with html tags. URLs frequently have many unusual letters or character combinations; that is why much care is needed when entering them. The page that appears in the browser contains information with links to other locations, either in the same document or in others at other places. These links are usually indicated by underlining and/or by being a different color than the rest of the text. A click on a link means submitting a URL for the browser to retrieve. A new page with the new location will appear to replace the old one, but one can always retrace steps by clicking on the "back" button or icon in the tool bar at the top of the browser. TASK: a) Students can access current information from countries around the world. They can obtain cultural, geographical, economic, social, political information from the countries in which the target language is spoken. They can read web versions of daily newspapers and same-day news reports from various sources. Such experience can allow learners to participate in the culture of the target language, which in turn can enable them to further learn how cultural background influences one's view of the world. b) The Internet also serves as a medium for experiencing and presenting creative works. While students can peruse the information on the Net, they can also use it as a platform for their own work such as essays, poetry, or stories. Numerous public schools, for example, are making use of the World Wide Web for publishing student work which can be accessed by other web
users. Students therefore become not only consumers of content, but in fact generate the content.

Internet Relay Chat (IRC) is a popular tool that permits synchronous conversation among users around the world. It creates channels that users can log on to and then chat away. The conversations are seen—because the participants have to type rather than talk—simultaneously by anyone who is on that channel and can be responded to by anyone as well. Any user can issue a command to see what channels are active at any given moment. Channel names generally reflect the topic of conversation. It is also possible to create a private channel for a closed classroom discussion. Software for IRC is available through the Internet as either shareware or freeware.

**TASK:** a) To participate in IRC, students need client software that is compatible with their system. There are several IRC software programs available on the Internet. The students’ first assignment is to download the client software, necessary to participate in an IRC session. Once they have obtained the IRC software, they should follow the instructions to install it on their computer. b) Now the students are ready to try out IRC. In the documentation that came with IRC client software, they will find information on how to participate in IRC and what commands are basic to IRC operations. The students should take some time to familiarize themselves with this information; it might be wise to print out the commands and directions for their first foray into the IRC world. Then they should launch their IRC client, pick a nickname, find a friendly server, and look at a list of the channels available. c) The students should find channels on foreign servers where people are conversing in their particular target language. d) They should spend about 15 minutes on a target language channel of IRC to get a sense of how people “talk” using this application. e) They will have a complete record of the IRC conversation, which they can print out and turn in as completion of the assignment.

**CUSeeMe** is an application that enables videoconferencing. The software was developed by Cornell University and White Pine as an endeavor to create affordable and workable desktop videoconferencing. A video transmission device, such as a camera, is needed.

**TASK:** a) The students should see and talk to someone at the same time. They can send and receive both audio and video messages, or one and not the other. They can do this between them and one other person or between them and a whole conference room full of people—or a whole classroom of foreign language speakers. b) The students will be asked to download the CUSeeMe software and install it on their computers. c) The students go to a public site and try out CUSeeMe for awhile.

**MUDs, multi-user domains**, as well as **MUDs/object-oriented (MOOs)**, are text-mediated virtual environments that allow users to connect to the same place at the same time and interact with each other. They “talk” by typing and “listen” by reading; in this way, it is much like IRC. But MOOs differ in that they create a virtual environment - a cafe, a library, a house - and the users manipulate cyber-objects (using words) that assist in their conversations, self-definitions, and in creating the virtual environment itself. Many MOOs exist where target language speakers and learners can interact regularly.

**TASK:** The students should get involved into both private or public conversations; they can include objects being exchanged, personal notes passed, and the like.

**CONCLUSIONS AND FUTURE WORK**

We must admit that in spite of its limitations and obstacles, the Internet’s educational potential is immense. It is essential that we make informed decisions about how the Internet can be successfully integrated into the language classroom.
Undoubtedly, we are in the center of a "monumental technological paradigm shift, one which will eventually change the way that all instructors teach and the way students learn" (Jensen, 1993). While technology should not take over the language classroom, it must be embraced in order to allow educators to do those things which they are unable to do themselves.

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OXIDANT/ANTIOXIDANT STATUS IN INFECTED WITH TRICHINELLA SPIRALIS MICE AFTER ZINC SUPPLEMENTATION

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Abstract: The oxidative-antioxidant and zinc status of mice infected with Trichinella spiralis and supplemented/unsupplemented with 2Gly.ZnCl₂.2H₂O was studied. The markers were malondialdehyde content, glutathione peroxidase and Cu,Zn-superoxide dismutase activity. Zinc levels were determined in liver and plasma. The parasite burden in mice was studied. Trichinellosis caused increased oxidative stress and zinc imbalance. Zn supplementation restored Zn status, and improved antioxidant system in the infected mice. It reduced muscle larvae. The results encourage the use of 2Gly.ZnCl₂.2H₂O combined with antihelminthic.

Keywords: Trichinella spiralis, oxidant/antioxidant status, mice

INTRODUCTION
Zinc is a structural constituent of many enzymes, and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins. It is an important antioxidant element [13].

Trichinellosis is one of the major parasitic diseases in some European countries. The tissue damages in the infected hosts are most probably mediated by reactive oxygen species [3].

We studied the effect of 2Gly.ZnCl₂.2H₂O on the response of mice to a primary infection with the parasitic nematode Trichinella spiralis. Malondialdehyde (MDA) levels, glutathione peroxidase (GPx), CuZn-superoxide dismutase (CuZn-SOD) activities, zinc plasma and liver levels were the parameters of oxidative/antioxidant status that have been investigated in our study.

MATERIAL AND METHODS
Male BALB/c mice, 9-10 weeks, body weight about 25 g were allocated into three groups, each comprising eight mice: group 1, control (healthy and non-supplemented mice); group 2, experimentally infected with T. spiralis and non-supplemented, and group 3, experimentally infected with T. spiralis and supplemented with 2Gly.ZnCl₂.2H₂O (Zn-Gly) compound. The mortality and body weights of the mice were recorded in each group. In parallel to groups 1, 2 and 3, separate groups 1p, 2p, and 3p each of five rats were used for the determination of biochemical parameters and parasite counts as well as body weights and mortality. Mice from groups 2 and 2p, 3 and 3p were inoculated per os with 100 T. spiralis larvae. Treatment with Zn-Gly of groups 3 and 3p initiated 2 weeks before the infection and continued 3 weeks after the infection. Daily doses of 0.5 mg Zn/ 1 ml H₂O in the form of Zn-Gly was administrated to each mouse from these groups.

2Gly.ZnCl₂.2H₂O was synthesized from the aqueous solution of ZnCl₂ using the method of isothermal decrease of the supersaturation [1].

T. spiralis larvae (code ISSO3) were obtained from the International Trichinella Reference Centre, Rome, Italy. The number of muscle larvae in the experimental mice was determined by recording the larvae isolated from the diaphragm. This organ alone was used because it is the usual predilection site of the Trichinellae according to literature data [11]. Biochemical studies. CuZn-superoxide dismutase (CuZn-SOD, E.C. 1.15.1.1) activity in washed erythrocytes was determined by epinephrine method according to Misra and Fridovich
Glutathione peroxidase (GPx, E.C. 1.11.19) activity in full blood samples was measured spectrophotometrically according to Beutler [2]. Plasma and liver zinc content was evaluated by Atomic Absorption Spectrophotometry using Varian Techtran, Model AA 220. Lipid peroxidation was determined by the estimation of the concentration of serum malondialdehyde (MDA) as malondialdehyde-thiobarbituric adductor [12]. Statistical evaluation of data was done following Student’s t-test. A difference was considered significant at p<0.05.

RESULTS AND DISCUSSION

MDA serum levels are presented in Fig. 1. MDA level in control mice remained stable during the experiment. In the infected mice MDA level increased up to week 8 compared to the controls. MDA concentration in the group 3 was lower compared to that in group 2 and higher to that in the group 1. MDA level was reduced in the infected mice after zinc supplementation.

The activity of antioxidant enzyme CuZn-SOD is presented in Fig. 2. SOD activity in the controls remained almost unchanged during the experiment. SOD activity in the infected mice was changed compared to the control. It was increased at the 8 week p.i. SOD activity in the infected and supplemented mice was elevated in comparison to that in the group 1 and 2.

The activity of the blood antioxidant enzyme GPx is presented in Fig. 3. It was constant in the control mice throughout the experiment. GPx activity was significantly increased in the infected mice from week 4 up to the end of the experiment. No statistically significant differences between mice in group 2 and 3 were established.

The plasma and liver Zn contents are presented in Figs. 4. The Zn levels in the control group were quite stable during the experiment. The infected mice exhibited significantly lower Zn content in plasma and higher Zn content in liver in comparison with infected mice. In the infected and supplemented mice plasma and liver Zn level was increased in comparison to that in the infected mice without any side effects.
The resulting average intensity of the worm burden at week 8 pi was 290 muscle larvae per gram tissue in mice without Zn supplementation and 200 muscle larvae per gram tissue in mice with Zn supplementation. Therefore the larvae reduction after Zn application was 31%.

The values of the body weights of the animals were slightly reduced in the infected animals. At the end of the experiment body weights in the mice infected and supplemented were increased in comparison to these in the infected animals but they were lower in comparison to the controls.

The number of survived animals up to the end of the experiment was not changed.

The mortality of control animals was a normal state. Zn supplementation did not affect the mortality.

The present study was performed to find out the modifications induced by Zn supplementation on the antioxidant/oxidative status in infected with *T. spiralis* mice.

The alterations in the MDA, enzymes CuZn-SOD and GPx, and plasma and liver zinc in *T. spiralis* mice clearly show a development of antioxidant imbalance in the infected mice. Lipid peroxidation is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics [8]. It has been suggested that the parasite infection may induce oxidative stress by producing hydroxyl radicals, nitric oxide and hydrogen peroxide [13]. Increased MDA content implies that the infected organism may not have a sufficient amount of antioxidant enzymes to cope with the increased oxidative stress. The results showed significant reduction in serum MDA content in the infected mice after Zn supplementation. Supplemental Zn may suppress the lipid peroxidation by affecting many different cellular functions. Dietary Zn supplementation has been demonstrated to inhibit the pathway of free radicals generation by hepatic cytochrome P450 enzyme system [4].

The activity of the main antioxidant enzymes SOD and GPx. was monitored. Their activity was significantly changed in the infected untreated mice, compared with the controls. The alteration of the enzyme activity may be attributed to parasitic infection and increased oxidative stress. Therefore, increased oxidative stress in parasitic infection was probably exacerbated by the altered availability of antioxidant enzymes. A prolonged Zn treatment almost normalized the Zn balance leading to increased CuZn-SOD activity in
infected animals. Zinc is a component of CuZn-SOD and plays an important role in the antioxidant system. Our results are in good accordance with those of Sidhu et al [14] who established protective effects of Zn on oxidative stress.

To evaluate the status of zinc during *T. spiralis* infection, we assessed Zn levels in plasma and in the liver. It is observed Zn redistribution between them in the infected mice – depressed Zn plasma and elevated Zn liver concentration. Zn homeostatic control mechanisms are involved in the protection against the parasite. The decline in plasma Zn content seen in the infected mice is probably due to hepatic sequestration of the metal as happened with a variety of stressors. This may be because of the inability of the infected animals to synthesize the hepatic protein metallothionein, which sequesters the metal [13].

Several studies have shown the trace element imbalance developed due the parasitic infections. The mineral imbalance influenced host immune and antioxidant responses to the parasite [15].

The protective effects of zinc on activities of antioxidant enzymes in animals infected with parasites has been obtained with parasitic nematode infections – *Strongyloides ratti* [6], *Nippostrongylus brasiliensis* [5], *Fasciola hepatica* [9]. These observations lead to the assumption that when the parasite burden increases, the antioxidant status of the host may be overwhelmed by the free radical-induced oxidative damage. Zinc application reduces the above damages.

The reduction of muscle larvae was established at week 8 in infected and Zn supplied mice. The improved antioxidant system as well as immune response in mice after Zn supplementation may be the possible reason for the significant reduction in the number of muscle larvae. Our finding is in a goof agreement with the results of Fenwick et al [6, 7]. They show that zinc deficiency impairs the expulsion of *T. spiralis* in rat. According them Zn deficiency in the host impaired either the migration of larvae or their subsequent encystment in muscle, or both.

**CONCLUSIONS AND FUTURE WORK**

Administration of 2Gly.ZnCl₂.2H₂O compound has been shown to be useful for the improving of antioxidant and Zn status the infected with *T. spiralis* mice. In such a context, the use of the highly bioavailable 2Gly.ZnCl₂.2H₂O helps to increase the security of an adequate Zn status in the animals. 2Gly.ZnCl₂.2H₂O is a promising source of Zn for food fortification or dietary supplements in order to prevent Zn deficiency and improve the antioxidant defense systems during trichinellosis. The results encourage the use of 2Gly.ZnCl₂.2H₂O combined with antihelminthic. Future studies are needed to study the combined effect of 2Gly.ZnCl₂.2H₂O with antihelminthics on various helminthoses.

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IN VIVO BEHAVIOR OF CALCIUM PHOSPHATE BONE SUBSTITUTES IN A RABBIT MODEL

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Abstract. The study compares the in vivo behavior of three calcium phosphates (mono-phase Zn-β tricalcium phosphate, Mg—β tricalcium phosphate and a bi-phase mixture of hydroxyapatite and β-tricalcium phosphate) in a paste form and implanted in rabbits. Radiological, histological, and biochemical studies were done. The phase composition and ion (Zn and Mg) modification do not change their behavior - they have a similar degradation degree and equivalent promote bone neoformation to autologous bone. The pastes have good biocompatibility and are promising materials as bone substitutes.

Key words: bone implants, in vivo behavior, rabbit

INTRODUCTION

Bone defects occur in a wide range of clinical situations, namely loss of bone substances due to congenital, neoplastic, or traumatic reasons. It is widely recognized that calcium phosphate ceramics, cements and injectable paste are suitable bone substitutes in orthopedic, reconstructive and maxillofacial surgery because structurally they are close to the hard bone tissues and have good biocompatibility and extensive bone conductivity [2]. Recently, calcium phosphate (CP) cements and injectable paste attracted much attention as a bone substitute which would overcome practical problems associated with the ceramics [1]. Hydroxyapatite (HAP) and tricalcium phosphate (TCP) are currently used as bone graft substitutes or coatings on metallic prostheses because of their excellent biocompatibility and osteoconductivity [7]. The practically insoluble mono-phased bio-ceramics of dense HAP do not actively participate in the process of bone remodeling, however at a contact with body fluids they cooperate for the formation of a surface layer from bone-like apatite. The mono-phased α - TCP and β -TCP have a problem of high reactivity and they degrade rapidly in vitro and in vivo [6]. Synthesis of metal ion-modified CPs has drawn much scientific interest since metal ion substitution has been shown to improve the mechanical properties and bioactivity of implants [1, 6]. Zn - modified TCP ceramics was found to possess lower solubility than pure ones and hence reduce the resorption rate [10]. Bi-phased mixtures of HAP and β TCP ceramics were developed in order to improve the biological behaviour of the mono-phased materials [8].

This study compares the in vivo behavior of three calcium phosphates with different chemical and phase composition, microstructure and solubility, using autologous bone as a control, in a rabbit model. The implanted materials were mono-phased Zn - β tricalcium phosphate, Mg - β tricalcium phosphate and a bi-phase mixture of hydroxyapatite and β-tricalcium phosphate. Bone radiological, blood biochemical and clinical studies were done after the implantation.

2. MATERIAL AND METHODS

2.1. Chemical synthesis

2.1.1. Materials – Simulated body fluids. The popular conventional simulated body fluid (SBFc) [5] was used as a base to assure electrolyte medium for the materials preparation. Modified calcium free conventional simulated body fluid (SBFc-Cam) was used as a solvent for K₂HPO₄ and modified phosphorous free conventional simulated body fluid (SBFc-Pm) was used as a solvent for CaCl₂, MgCl₂ and ZnCl₂ respectively, thus
preliminary precipitation was avoid [9]. The all simulated body fluids used in the experiments were prepared by successive mixing of preliminary prepared solutions of KCl, NaCl, MgCl$_2$.6H$_2$O, CaCl$_2$.2H$_2$O, ZnCl$_2$.2H$_2$O, NaHCO$_3$, Na$_2$SO$_4$, and K$_2$HPO$_4$ salts in a distilled water, the (Ca+Zn)/P and Ca/P ratio was 1.67. The pH of the solutions was adjusted to 8.2 - 8.4 using 0.1M HCl or 0.05M Tris (hydroxymethyl) aminomethane.

2.1.2. Precursors precipitation. CPs precursors were biomimetically synthesised by application of two methods – quick mixing and continuous co-precipitation. Zn-modified CP precursors was synthesised by the method of quick mixing of the all 3 modified simulated body fluids (P solution, Ca solution and Zn solution) at a room temperature and under intense stirring, keeping pH 10 by an 1M NH$_4$OH. The precipitates were filtered, immediately washed with water and with acetone (solid-to-liquid ratio of 1:1) and freeze-dried for a week. The SBF-modified as well as Mg-modified CP precursors were synthesized by application of the method of continuous co-precipitation. Two reagents (P solution and Ca solution or Ca and Mg solution) with a (Ca$^{2+}$+Mg$^{2+}$)/P ratio of 1.67 were added to precipitate in a glycine buffer with a rate of 3 ml/min at room temperature, keeping pH 8 by 1M KOH. The precipitates were matured in the mother liquid for 24 h at room temperature and then the thick suspensions were subjected to gelling with Xanthan Gum, lyophilized at -56°C, washed (solid-to-water-ratio 1:100), and secondary lyophiliz.

2.1.3. Calcination. The freeze-dry precursors were heated at 600°C and atmospheric pressure in high-temperature furnace, type VP 04/17 of LAC Ltd Company. The working regime was heating with rate 10°C/min till the desire temperature and keeping it for 2 hours.

2.2. Characterization.

2.2.1. Chemical analysis. The sum of Ca$^{2+}$ and Mg$^{2+}$ ions in the solid samples was determined complexometrically with EDTA at pH 10. The concentrations of Zn$^{2+}$, Mg$^{2+}$, K$^+$ and Na$^+$ ions were analyzed by a THERMO M5 AAS; and the concentrations of P-PO$_4^{3-}$ and Cl$^-$ ions, spectrophotometrically by a NOVA 60 equipment, using Merck and Spectroquant® test kits.

2.2.2. X-ray diffraction - The polymorphous phase transformations of the amorphous and high temperature treated calcium phosphates were determined by a Bruker D8 advance XRD, operating at 40 kV and 40 mA with CuK$_\alpha$ radiation and SolX detector within the 2θ range 10-90° 2 θ, step 0.04° 2 θ and counting time 1s/step.

3.1. In vivo experiments on calcium phosphate paste

3.1.1. Experimental animal model and implantation procedure. A total of 24 New Zealand white male rabbits were used as an animal model in the study. The method, consisting in the creation of a 4 mm diameter cavity bone defects in the distal metaphysis of the rabbit femur, was described by Katthagen and Mittelmeier [4]. The rabbits were divided into 4 groups of 6 rabbits, according to the material used for filling the defects: control group - with autologous bone implantation; gr. 1$^{st}$ – with Zn - β tricalcium phosphate implantation, gr. 2$^{nd}$ - with a bi-phase mixture of hydroxypatite and β tricalcium phosphate implantation and gr. 3$^{rd}$ – with Mg - β tricalcium phosphate. Powders of the compounds were used in the paste form after mixing with a hyaluronic acid.

3.1.2. Studies on rabbits post implantation. Clinical observations, comprising body weight gain, body temperature, heart and respiratory rates) were performed during the operation period. The blood biochemical studies were done prior operation (prior-op), at the end of 1$^{st}$ week and 14$^{th}$ week. The serum bone markers calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), alkaline phosphatase (AP) and bone – alkaline phosphatase (BAP), were measured using Human diagnostic kits by the Screen master 588 LiHD 111. After the 14 weeks, the rabbits were euthanized and the femurs retrieved for the radiological evaluation.
RESULTS AND DISCUSSION

Biomimetic synthesis of ion-modified mono-phased and bi-phased calcium phosphates. Three metastable XRD amorphous calcium deficient phosphate precursors – SBF- modified CP (with a Ca\(^{2+}/P\) ratio of 1.27), zinc-modified (with a (Ca\(^{2+}+\text{Zn}^{2+})/P\) ratio of 1.35), and magnesium-modified (with (Mg\(^{2+}+\text{Ca}^{2+})/P\) = 1.38), were biomimetically precipitated in modified simulated body fluids. SBF, as an electrolyte medium, plays a crucial role in the precipitation processes and influences the composition of the precipitated precursors. Part of these calcium vacancies could be occupied by free Na\(^+\), K\(^+\), Zn\(^{2+}\), and Mg\(^{2+}\) ions from the solution, thus forming Posner`s clusters with a common formula Ca\(_w\)Mg\(_x\)Na\(_y\)K\(_z\)(PO\(_4\))\(_v\)(CO\(_3\))\(_6\)-(w+x+y+z<9). Fine powders of mono-phased Zn-β-TCP (with a Zn\(^{2+}/\text{(Ca}^{2+}+\text{Zn}^{2+}+\text{Mg}^{2+})\) ratio of 0.01) and Mg-β-TCP (with Mg\(^{2+}/\text{(Ca}^{2+}+\text{Mg}^{2+})\) ratio of 0.10) as well as of bi-phase SBF-modified HAP and β-TCP calcium phosphates (with a Mg\(^{2+}/\text{(Ca}^{2+}+\text{Mg}^{2+})\) ratio of 0.005) were obtained after 600°C sintering of the precursors. Their mineral composition was closest to those in the hard tissues enamel, dentin and bone mineral [1] (Table 1).

<table>
<thead>
<tr>
<th>Composition</th>
<th>Ca</th>
<th>P</th>
<th>Zn</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>(Ca+Zn)/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn - β TCP</td>
<td>8.74</td>
<td>6.6</td>
<td>0.1</td>
<td>0.06</td>
<td>0.25</td>
<td>0.7</td>
<td>0.06</td>
<td>1.35</td>
</tr>
<tr>
<td>Mg - β TCP</td>
<td>7.76</td>
<td>6.34</td>
<td>-</td>
<td>0.98</td>
<td>0.06</td>
<td>0.02</td>
<td>&lt;0.05</td>
<td>1.38</td>
</tr>
<tr>
<td>β-TCP + HAP</td>
<td>6.29</td>
<td>4.97</td>
<td>-</td>
<td>0.04</td>
<td>0.05</td>
<td>0.02</td>
<td>&lt;0.05</td>
<td>1.27</td>
</tr>
<tr>
<td>Enamel, Dentin, Cementum, Bone (Dorozhkin, 2009)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>5.4</td>
<td>0.02</td>
<td>0.22</td>
<td>2.10</td>
<td>0.03</td>
<td>0.1</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>9.1</td>
<td>5.7</td>
<td>0.29</td>
<td>0.39</td>
<td>4.9</td>
<td>-</td>
<td>1.73</td>
<td>-</td>
</tr>
</tbody>
</table>

Clinical studies. No complications were observed throughout the physical examination of rabbits. Body weights of growing rabbits increased steadily in all groups during the observed period 14 weeks. There were no significant differences between any of groups.

Radiological studies. The radiological survey did not show any complications in treated bone. The radiographic evaluation confirmed that all materials implanted were biocompatible. After 14 weeks, the images suggested some remodeling of the calcium phosphates, although the materials were still present. There was not a remarkable growth of the new bone both in bones with implants as well as in authologous bone at 14 week.

Serum biochemical studies. To evaluate the bone metabolism we have used Ca, P, Mg, Zn, AP and BAP. The mean serum Ca, Mg, Zn and P levels varied non-significantly between different groups prior to operation. After the implantation period of 1 week they increased in all groups in comparison to the results of the beginning. The levels of Ca and P were higher in all animals but the increase was better expressed in gr. 2 and 3 (Fig. 1).
The increased blood levels of Ca and P levels by the end of the 14 week may be attributed to increased mineral turnover in the skeleton. Mg level was significantly higher in group 3 and Zn level was non-significantly higher in group 1 compared to those in the rest groups at the 14 week. The levels of Ca, P, Zn and Mg were in the physiological values. Mg is one of the most important bivalent ions, which is an important factor in the qualitative changes in the bone matrix that determines bone fragility. Mg depletion adversely affects all stages of skeletal metabolism, causing low bone growth, decreases osteoblastic and osteoclastic activities [9]. It has been reported that Mg can be incorporated into TCP structure by substitution of Ca [3]. Zn is also an essential trace element with stimulatory effects on bone formation. In vivo studies on Zn doped calcium phosphates on rabbit femora showed over 50% more newly formed bone over Zn-free calcium phosphate composition [10]. It has been reported that Zn can be incorporated into HAP by substitution of Ca [10]. However, the release of Zn from Zn-doped HAP is very slow due to low solubility of HAP. Zn-doped TCP has also been studied in past several years [10]. Zn-doped TCP exhibits stimulatory effects on osteoblast bioactivity and bone formation. In all groups AP and BAP values fluctuated with no marked change prior-op. The activity of AP and BAP was decreased by the end of the 1st week compared to those prior-op. AP and BAP were non-significantly changed in the animals of all groups at the end of the experiment. They varied non-significantly among the groups (Fig. 2). AP is considered to be a marker of bone formation. Increased osteoblastic activity has been shown to increase the AP activity. In the research setting [3].

CONCLUSION AND FUTURE WORK
It was found that the phase composition and ion (zinc and magnesium) modification of the calcium phosphates do not change their in vivo behavior. They have a similar degradation degree and equivalent promote bone neoformation to autologous bone. They do not have a negative influence on biological response of organism. The studied
pastes have good biocompatibility and are promising materials as bone substitutes. Further studies might be done to optimize the properties of the materials.

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HEAVY METALS ACCUMULATION IN HARES INFECTED WITH ENDOPARASITES

M. Gabrashanska, V. Nanev, V. Ermakov, S. Tyutikov, and M. Panayotova-Pencheva

Abstract: Our study was to assess the accumulation of heavy metals in hares (Lepus europaeus Pallas, 1778) infected with endoparasites. The effect of cestodes Mosgoviaia pectinata or nematodes Trichostrongylus retortaeformis on Cd, Pb, Co, Hg, Zn and Cu concentrations was established. Hosts infected with cestodes had lower Cd and Pb levels than hosts infected with nematodes. Levels of Co, Zn and Cu were similar in the hosts infected with cestodes or nematodes. The concentration of Cd, Pb and Cu was higher in kidneys than in livers. The content of heavy metals in hosts was decreasing with increasing of cestode burden.

Key words: heavy metals, hares, cestodes, nematodes

INTRODUCTION

Metals are natural constituents of the components of all ecosystems, and under natural conditions they are cycled within and between the geochemical spheres. Human activities have altered natural concentrations of many substances in the environment and added numerous new chemicals. In general, there was a relationship between metal concentrations among several species. Wild animals are naturally exposed to basal levels of heavy metals in their natural habitats. They are the direct bioindicators for heavy metal pollution in the ecosystems [3]. Among possible biomonitoring species, hares (Lepus europaeus Pallas, 1778) have been widely used to assess environmental contamination [4, 8]. They are widely distributed and are the most abundant herbivorous animals in Bulgaria. Annual captures in Bulgaria range about 386 950 hares in 2008 year. Because the hares are frequently infected with endoparasites it is necessary to follow their possible influence on heavy metal levels in the tissues of hares, which are used in the monitoring of environment pollution. There is need for sentinel organisms reflecting small-scale changes in heavy metal pollution of different habitats and the role of terrestrial mammalian parasites is an important field of research aimed at the potential use of parasitic models as bioindicators.

In recent years there have been increasing information showing how parasitism and pollution can interact with each other in aquatic organisms [5, 6]. Some data have shown that metal composition in the small mammals was influenced by the parasitic infection [1, 2, 3].

Our study was to assess the accumulation of heavy metals in hares (Lepus europaeus Pallas, 1778) infected with endohelminths. The effects of the most abundant intestinal helminth species (Mosgoviaia pectinata, Cestoda or Trichostrongylus retortaeformis, Nematoda) on heavy metal concentrations (Zn, Cu, Co, Pb, Hg and Cd) in tissues of hares under industrial polluted field conditions were established.

MATERIAL AND METHODS

Field researches were conducted in the industrial emissions affected area of the town Pernik. A total of 52 shot male hares, aged about one year, were sampled in 2008 year. The animals were conditioned to 0°C before they were opened for removal of liver, kidneys, muscle (m. semimembranosus of the hind leg) and digestive tract. The digestive tracts were analyzed for intestinal cestodes and nematodes. Parasitological negative hares and hares with mixed infections (cestodes and nematodes together) were excluded from this study. Liver, muscle, and kidneys were deep-frozen until posterior processing for analysis. Heavy metal contents (Cd, Pb, Co, Hg, Zn, Cu) were determined in the samples taken from liver, kidney and muscle. The analysis of Zn, Cu, Co, Cd and Pb was done...
using flame atomic absorption spectrometry using a Perkin-Elmer (Polo Alto, CA) 2380 Instrument. The method was cold vapor flameless atomic absorption spectrometry (CV-AAS)(Sanso, HG-3000) for Hg determination.

Statistical analysis was performed according to Statview 4.5 software package. For all tests, a significance level of $p<0.05$ was applied.

**RESULTS AND DISCUSSION**

The most abundant intestinal helminth species in hares were *Mosgovayia pectinata*, Cestoda and *Trichostrongylus retortaeformis*, Nematoda. Nineteen hares were infected with *M. pectinata*, eleven hares – with *T. retortaeformis* and seventeen – with both *M. pectinata* and *T. retortaeformis* or with other helminth species. Five hares were without endohelminths. The prevalence of *M. pectinata* was significantly higher in the hares ($p<0.01$). The mean intensity of *M. pectinata* was $4+/1$ and this of *T. retortaeformis* – $121+/14$. The metal concentrations were determined in the hares infected with *Trichostrongylus retortaeformis* (Nematoda) and in the hares infected with *Mosgovayia pectinata* (Cestoda) in comparison.

Element concentrations presented in different tissues of hares infected with cestodes or nematodes are shown in Fig. 1, 2, 3 and 4.

![Fig 1. Heavy metals in tissues of hares infected with *M. pectinata* (Cestoda)](image1)

![Fig 2. Heavy metals in tissues of hares infected with *T. retortaeformis* (Nematoda)](image2)
The levels of Cu, Zn and Co were similar in hares infected with cestodes or with nematodes (Fig. 1, 2 and 3). Cu and Zn were the lowest in the muscle and the highest – in the kidney of the hares. Co concentration was the highest in the liver in comparison to the muscle and kidney.

The levels of Cd, Hg and Pb were the highest in the kidney of the host and the lowest – in the muscle. There were significant differences in the levels of Cd and Pb. The level of Cd was lower in the kidney and muscle than this in the liver. It was higher in the kidney and muscle from hares infected with T. retortaeformis compared to that in hares infected with Mosgovayia pectinata. Pb concentration in the kidney and liver in hares infected with cestode were significantly lower than these levels found in the hares infected with nematode. Hg was not found in the muscle. Its level was similar in the hares infected with T. retortaeformis or Mosgovayia pectinata. The content of heavy metals in hosts was decreasing with increasing of cestode burden.

The impact of the helminth burden on bioaccumulation of heavy metals in the hares was well demonstrated in our study. Comparison of heavy metals content in the organs of hares infected with cestodes or nematodes, respectively, showed higher concentration of Cd and Pb, in general, in hares infected with nematodes. A statistically significant difference was found for these metals concentrations mainly in the kidneys and livers as the target organs of metal pollution. Variation in the metal concentrations among tissues in the infected hares indicates differences in the degree of accumulation. Cu and Zn are
involved in a series of metabolic processes and are therefore found in fairly large amounts in all three tissues. Our data confirmed the finding that the kidney is an organ in which metals are present in the highest concentration for terrestrial mammals (7, 8).

The parasite species-response models to particular heavy metals are presented in the hares.

The metal levels in the hares infected with parasites have reflected the environmental contamination with heavy metals. The hares used in this study were captured in industrial emission affects area of the town Pernik.

We found out that hosts infected with cestodes had lower Pb and Cd level in the hosts than those infected with nematodes. The mechanism whereby cestode-infected animals accumulate less metals than these infected with nematodes is unknown. It may be due to their metabolic rates. It is possible that the host organism performs a barrier function to the access of some elements to the parasites when there is increased concentration of them in the environment [7]. Our results are corresponded with these obtained by Jankovska et al [1]. The author has been studied the levels of Cd, Cr, Cu, Mn, Ni, Pb and Zn in the liver and kidney of the field voles (Microtus agrestis) and field mice (Apodemus flavicollis) infected with cestodes or nematodes in comparison. The host infected with cestode infection (Paranoplocephala sp) had lower contents of metals in their livers and kidneys compared to the hosts with the nematode infection (Mastophorus muris).

The influence of the anthropogenic and migration processes in hares, makes this host-parasite system a possible bioindicator for heavy metals pollution. In our case not only the influence of the environment on the metal migration in the environment-host-parasite chain can be seen but and also the relative stability in the metal status (concerning biogenic elements Zn, Cu and Co) of the host-parasite system.

CONCLUSIONS AND FUTURE WORK

It is important to use organisms that are known to be infected, especially in studies examining the question of whether exposure to certain chemicals affects the physiological homeostasis of a test organism. Most free-living animals used for biomarker studies are believed to be infected with a variety of parasite. Therefore, results from pollution test can be falsified if infected test organisms are involved. Similarly, this is probably the most interesting area for ecotoxicological research with parasites because it questions the previous use of biomarkers as indicators of pollution if parasites were not considered as "stressors" having a specific and often severe effect on the physiology of the host [2]. Our data give grounds to re-evaluate heavy metal pollution in the environment using the system host-endohelminths.
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JUNIPERUS COMMUNIS AND JUNIPERUS VIRGINIANA TETRAHYDROFURAN EXTRACTS

C.M. Pleșa, N.G.Hădărugă, A. Ardelean, D.I.Hădărugă, A.G.Branic and A.X.Lupea

Abstract: The extracts of various juniper parts were obtained by refluxing method. The compositions of berries, needles and branches from two species of Juniperus (Juniperus communis and J. virginiana) were comparatively analyzed by GC-MS and Kovats indeces. All the analyzed species contained 15 main compounds, the most common compounds being α-pinene, β-phellandrene and limonene.

Key words: Juniperus communis, Juniperus virginiana, tetrahydrofuran, refluxing extraction.

INTRODUCTION

J. virginiana L. (Family Cupressaceae) commonly called eastern red cedar is a widely distributed species in the USA and parts of Canada [1]. Some Juniperus species are present in the Romanian flora: J. virginiana and J. communis L. [2].

The overall objective of the programme was to point out possible differences between Juniperus communis and Juniperus virginiana three parts – branches, needles and berries – collected from five regions. Essential oils of these two species have been intensively investigated [1, 3-8], but solvent extracts were not sufficiently investigated [9].

MATERIAL AND METHODS

The species investigated belongs to genus Juniperus (Juniperus communis L. and Juniperus virginiana L.). Needles, ripe berries and branches were harvested in October 2009 from several wild plants, at an altitude of 700m, from three Romanian’s regions, and an altitude of 1000m, in Austria and Syria. After picking, the plant material was air-dried at room temperature and stored in paper bags at ambient temperature, protected from direct light, until further analysis.

The solvent, tetrahydrofuran from Chimopar, București and Na₂SO₄ from Fluka, Switzerland.

Refluxing with solvent

The plant material was dried and stored at room temperature. Dried berries, needles and branches (2g in each case) were chopped in small pieces, treated with 15mL of solvent, and refluxed for 30 min.

GC-MS analysis

A Hewlett Packard 6890 Series gas chromatograph was used for analysis of the extracts. The GC conditions used were: programmed heating from 50°C to 250°C at 6°C / min. Helium was the GC carrier gas. The GC was fitted with a mass spectrometer, MS, model Hewlett Packard 5973.

The relative percentage concentration of the volatile compounds of two species of juniper was computed from the GC peak areas. The identification of the main compounds was performed by using our previous Kovats indices data obtained for standard compounds [10, 11] and/or by matching the experimental mass spectra with those from the NIST/EPA/NIH Mass Spectral Library 2.0.
RESULTS AND DISCUSSION

We examined needles, branches and berries of J. communis and J. virginiana to point out possible interesting differences in the chemical composition between different parties of the shrub. A total of 100 different compounds were identified and significant differences were observed between juniper species and regions. Of the large number of compounds found in all extracts only the most important ones (15) were selected and is reported in Tables 1-3.

Table 1. RETENTION TIME, $t_R$, KOVATS INDECES, $I_K$, AND CHROMATOGRAPHIC AREA PERCENTAGES OF COMPOUNDS IDENTIFIED IN REFLUXING EXTRACTS OF BRANCHES OF J. COMMUNIS AND J. VIRGINIANA (% OF TOTAL AREA)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$\alpha$-Pinene</td>
<td>5.71</td>
<td>936</td>
<td>9.36</td>
<td>8.22</td>
<td>12.69</td>
<td>12.12</td>
<td>7.65</td>
</tr>
<tr>
<td>2.</td>
<td>$\beta$-Pinene</td>
<td>7.055</td>
<td>986</td>
<td>0.85</td>
<td>0.75</td>
<td>0.89</td>
<td>0.77</td>
<td>0.25</td>
</tr>
<tr>
<td>3.</td>
<td>Limonene</td>
<td>8.118</td>
<td>1023</td>
<td>1.37</td>
<td>9.54</td>
<td>0.41</td>
<td>0.57</td>
<td>0.33</td>
</tr>
<tr>
<td>4.</td>
<td>$\beta$-Phellandrene</td>
<td>8.336</td>
<td>1030</td>
<td>0.26</td>
<td>0.63</td>
<td>0.15</td>
<td>7.05</td>
<td>1.07</td>
</tr>
<tr>
<td>5.</td>
<td>3-Carene</td>
<td>9.023</td>
<td>1052</td>
<td>0.15</td>
<td>0.16</td>
<td>0.19</td>
<td>1.11</td>
<td>1.89</td>
</tr>
<tr>
<td>6.</td>
<td>Terpinolene</td>
<td>9.759</td>
<td>1075</td>
<td>0.39</td>
<td></td>
<td>0.28</td>
<td>0.26</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Copaene</td>
<td>16.027</td>
<td>1264</td>
<td>-</td>
<td>0.89</td>
<td>0.47</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>8.</td>
<td>$\beta$-Elemene</td>
<td>16.497</td>
<td>1278</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
<td>1.33</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Caryophyllen</td>
<td>17.402</td>
<td>1306</td>
<td>0.1</td>
<td>0.27</td>
<td>0.24</td>
<td>1.98</td>
<td>0.46</td>
</tr>
<tr>
<td>10.</td>
<td>$\tau$-Elemene</td>
<td>17.531</td>
<td>1310</td>
<td>0.2</td>
<td></td>
<td>-</td>
<td>-</td>
<td>0.94</td>
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<tr>
<td>11.</td>
<td>$\alpha$-Humulene</td>
<td>18.307</td>
<td>1334</td>
<td>0.03</td>
<td>0.24</td>
<td>0.11</td>
<td>0.2</td>
<td>0.4</td>
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<tr>
<td>12.</td>
<td>$\beta$-Cubebene</td>
<td>18.918</td>
<td>1353</td>
<td>0.42</td>
<td>1.5</td>
<td>0.39</td>
<td>-</td>
<td>0.87</td>
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<tr>
<td>13.</td>
<td>Germacrene D</td>
<td>18.979</td>
<td>1355</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.96</td>
<td>1.43</td>
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<tr>
<td>14.</td>
<td>$\tau$-Cadinene</td>
<td>19.676</td>
<td>1376</td>
<td>-</td>
<td>1.14</td>
<td>0.18</td>
<td>-</td>
<td>0.35</td>
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<tr>
<td>15.</td>
<td>$\beta$-Cadinene</td>
<td>19.775</td>
<td>1380</td>
<td>-</td>
<td>0.54</td>
<td>-</td>
<td>-</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Total, %</td>
<td></td>
<td></td>
<td>13.13</td>
<td>23.88</td>
<td>16</td>
<td>25.06</td>
<td>18.11</td>
</tr>
</tbody>
</table>

Table 2. RETENTION TIME, $t_R$, KOVATS INDECES, $I_K$, AND CHROMATOGRAPHIC AREA PERCENTAGES OF COMPOUNDS IDENTIFIED IN REFLUXING EXTRACTS OF NEEDLES OF J. COMMUNIS AND J. VIRGINIANA (% OF TOTAL AREA)

<table>
<thead>
<tr>
<th>No.</th>
<th>Components</th>
<th>$t_R$</th>
<th>$I_K$</th>
<th>J. c. SYR</th>
<th>J. c. SYR</th>
<th>J. c. R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$\alpha$-Pinene</td>
<td>5.71</td>
<td>936</td>
<td>10.4</td>
<td>6.51</td>
<td>1.51</td>
</tr>
<tr>
<td>2.</td>
<td>$\beta$-Pinene</td>
<td>7.055</td>
<td>986</td>
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<tr>
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<td>1278</td>
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<td>-</td>
<td>0.22</td>
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<tr>
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<td>-</td>
<td>0.11</td>
<td>0.2</td>
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<td>0.38</td>
<td>0.12</td>
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<tr>
<td>12.</td>
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<td>-</td>
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<tr>
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<td>36.66</td>
<td>24</td>
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</table>
Table 3. RETENTION TIME, $t_R$, KOVATS INDECES, $I_K$, AND CHROMATOGRAPHIC AREA PERCENTAGES OF COMPOUNDS IDENTIFIED IN REFLUXING EXTRACTS OF BERRIES OF J. COMMUNIS AND J. VIRGINIANA (% OF TOTAL AREA)

<table>
<thead>
<tr>
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<tr>
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<td>9.25</td>
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<td>Limonene</td>
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<td>1023</td>
<td>4.74</td>
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<tr>
<td>4.</td>
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</tr>
<tr>
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<td>1052</td>
<td>0.71</td>
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<td>Copaene</td>
<td>16.027</td>
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<td>β-Elemene</td>
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<td>1278</td>
<td>2.22</td>
</tr>
<tr>
<td>9.</td>
<td>Caryophyllene</td>
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<tr>
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<td>β-Cubebene</td>
<td>18.918</td>
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<td>Germacrene D</td>
<td>18.979</td>
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<td>14.</td>
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<tr>
<td>15.</td>
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<tr>
<td></td>
<td>Total, %</td>
<td></td>
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<td>59.57</td>
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</table>

It is obviously that were variations in the percentage of total area tetrahydrofuran extracts compounds between the locations and juniper parts. The branches from Syria and Roumania were found to be richer in α-pinene than from the other regions, while limonene were the richest on Austria’s branches. (Figure 1).

Figure 1. The main compounds from Juniperus communis (JC) and J. virginiana (JV) branches extracts obtained by refluxing extraction (R1, R3-Romanian’s regions, AUS-Austria, SYR-Syria)

Some compounds as α-pinene, β-phellandrene, β-pinene, caryophyllen, germacrene, α-humulene, β-cadinene and copaene were present in higher amounts in J. communis needles (Syria) extract than in Roumanian or Syrian J. virginiana needles extracts (Figure 2).
Figure 2. The main compounds from *Juniperus communis* (JC) and *J. virginiana* (JV) needles extracts obtained by refluxing extraction (R3-Romanian region, SYR-Syria)

As it can be seen from Figure 3, the main compounds from berries tetrahydrofuran extract was β-phellandrene in *J. virginiana* Roumanian extract, but from *J. communis* (Roumanian extract) was identified α-pinene, followed by β-pinene and β-phellandrene.

Figure 3. The main compounds from *Juniperus communis* (JC) and *J. virginiana* (JV) berries extracts obtained by refluxing extraction (R2, R3-Romanian’s regions)

**CONCLUSIONS AND FUTURE WORK**

In this study, it has been tried to compare qualitative characteristics of juniper tetrahydrofuran extracts. It was found that α-pinene, β-phellandrene and limonene were the most common compounds for all juniper extracts. In the future we want to try other solvent refluxing extraction from this two juniper species.

**Acknowledgements**

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ASPECTS CONCERNING THE MULTIPLICATION AND IN VITRO CONSERVATION OF THE FERN POLYPODIUM VULGARE L.

E. Vișoiu, L.C. Soare and C.M. Dobrescu

Abstract: The in vitro multiplication of ferns currently has numerous applications, which allow the regeneration of the species bearing interest for ornamental, medicinal, or environmental purposes, or of endangered species. The biological material obtained (gametophyte or sporophyte) can be conserved in vitro, thus laying the bases for germplasm banks. In species Polypodium vulgare, the vegetable material used for initiating the in vitro culture was collected in the Valea Vâlsanului protected area, as the species holds medicinal properties. The explants, made up of sporangia (green sori) were initiated on a 1/2 MS, hormone free, agarized culture medium. Out of each inoculum cultivated on the medium a colony of gametophytes (prothallia) was obtained. Gametophytes with archegonia were obtained after 40 days from the initiation of the culture. In spite of the excellent multiplication of the gametophyte, the sporophyte was obtained a year after the initiation of the culture. The lack of the antheridia on the prothallia points to the sporophyte being formed through apogamy.

Key words: in vitro culture, Polypodium vulgare, gametophyte, sporophyte.

INTRODUCTION

The in vitro multiplication of ferns currently has numerous applications, which allow the regeneration of the species bearing interest for ornamental, medicinal, or environmental purposes, or of endangered species. There is a large production of ferns in the world by micropropagation, the fern Nephrolepis being considered as one of the largest among in vitro propagated plants [8]. Polypodium vulgare is a saxicolous species, frequently encountered in România [1]. It is mentioned as a medicinal species in Flora R.P.R. (The flora of Romania), vol. I [4]. Relatively recent researches show that the plant has ways of achieving the biosynthesis of the ecysterois: the latter were identified in the rhizome, the roots, the leaves, and, in much larger amounts, in the gametophyte cultivated in vitro [9]. In vertebrates, the pharmacological action of the ecysterois manifests itself at the level of proteic metabolism, of carbohydrates and lipids, of the nervous system, the heart and the circulatory system, the liver, the kidneys, etc. [5]. In keeping with the above elements, the purpose of the present research is to multiply and conserve in vitro the gametophyte and the sporophyte of that species.

MATERIAL AND METHODS

The vegetable material needed to realize the multiplication and in vitro conservation of the species Polypodium vulgare was collected in the protected area Valea Vâlsanului (the valley of the Vâlsan River). The leaves with sporangia were fragmented and hydrated in water for 30 minutes. They were sterilized with calcium hypochlorite 6% for 5 minute, and then washed three times with sterile distilled water [3]. The sporangia were sampled off the leaves by means of a lanceolate needle, and were inoculated on the hormone free medium 0,5xMS (1952) [7], distributed in Petri dishes. The cultures dishes were maintained in the growing room at a temperature of 25±1°C, with a photoperiod of 16 hours of light, 8 hours of darkness.

In order to establish the experimental protocol susceptible to secure the long-term maintenance of the gametophyte, cultures were initiated with explants of the gametophyte type on the following culture media (6 month into the initiation of the in vitro culture):

1. Water + 20g/l saccharose/sucrose+6g/l agar; 2. M&S (1962) modified (½ macroelements) +110g/l saccharose + 6g/l agar; 3. M&S (1962) + 20g/l saccharose + 6g/l agar + 1mg/l BAP +0,5mg/l AIA; 4. M&S (1962)+ 20g/l saccharose + 6g/l agar + 1mg/l agar + 0,5mg/l AIA.
BAP + 0,5mg/l IBA; 5. M&S (1962) + 20g/l saccharose + 6g/l agar + 1mg/l BAP + 0,5mg/l ANA; 6. M&S (1962) + 10g/l saccharose + 10g/l mannitol + 6g/l agar + 1mg/l BAP + 0,5mg/l AIA; 7. M&S (1962) + 10g/l saccharose + 5g/l mannitol + 6g/l agar + 1mg/l BAP + 0,5mg/l AIA; 8. M&S (1962) + 20g/l mannitol + 6g/l agar + 1mg/l BAP + 0,5mg/l AIA; 9. M&S (1962) + 10g/l mannitol + 6g/l agar + 1mg/l BAP + 0,5mg/l AIA.

The vegetable material obtained in vitro was examined macroscopic and microscopic. The photos were taken under an Optika B250 microscope, with a Canon Power Shot A630 camera, and the Optika SZR stereomicroscope.

RESULTS AND DISCUSSION

The differentiation of the gametophyte begins 7 days after the culture is started, when the inoculum becoming green can be observed macroscopically. The process of differentiation undergoes the stages characteristic of leptosporangiates, namely: prothallic filament, prothallic, and prothallic cordate blade [2]. When 6 weeks passed after the culture was initiated, prothallic blades, and young prothallic cordate blades can be seen under the microscope (Fig. 1). Out of each explant cultivated in vitro a colony of prothallia is obtained.

The prothallia differentiate secretory one-cell and two-cell trichomes, both on the edges of the wings, and at the surface. Both at the surface of the gametophyte, and on its edges, prothallic trichomo-rhizoidogeneous cells were observed, which differentiated both a trichome and a rhizoid (Fig. 2). Such cells were also described for Cyrtomium falcatum and Dryopteris dilatata [9]. The emergence of the trichomes is preceded by an uneven division of a prothallic cell (Fig. 3); the resulting smaller cell is the mother-cell of the trichome. The trichomes produce wax, which plays a crucial part in protecting the gametophyte from dehydration.

Very much as in other species whose gametophyte was multiplied in vitro, the process of vegetative reproduction of the gametophyte [3] was noticed in Polypodium vulgare, through the forming of ramifications, each of which is capable of producing a new prothallium (Fig. 4). That process has a special significance because a larger amount of vegetable material is obtained, and the gametophytes thus formed have an aposporic nature. The excellent in vitro multiplication of the gametophyte can be exploited to obtain active principles. Thus, in the gametophyte of Polypodium vulgare cultivated in vitro there occurs the ecdysteroid biosynthesis, and the amount of substance obtained from that type of tissue is bigger than that obtained from sporophytic tissues (rhizome, roots, leaves) [9].

The gametophyte produces, in large numbers, only archegonia (Fig. 5), while the antheridia failed to be noticed in any one of the development stages. The archegonia were only identified on the prothallia 40 day after the in vitro culture was started. The missing antheridia on the gametophyte makes impossible the sexual formation of a zygote, although the archegonia are functional. The formation of sporophytes from somatic cells, i.e., without the intervention of sexual organs is named apogamy. The term “obligate apogamy” describes a cycle in which a sporophyte is regularly produced in this manner. Approximately 10% of ferns and unknown proportion of other Monilophytes have life cycles of this type [6].

The apogamous formation of the sporophyte was noticed one year after the in vitro culture was started (Fig. 6, 7). Thus, out of the prothallic tissue an spogamous embryo is organized, located towards the apical, cordate region.
Fig. 1 *Polypodium vulgare*: filaments and prothallic blades. (x160, orig.); Fig. 2 trichomo-rhizoidal cell (x 1600, orig.).

Fig. 3 Initial cell of trichome (x 1600, orig.). Fig. 4 *Polypodium vulgare*: gametophyte branching (x 400, orig.);

Fig. 5 *Polypodium vulgare*: arhegonia in apical region of gametophyte (x 160, orig.);
Fig. 6 First leaf of apogamous sporophyte (x10, orig.).
The first leaf that is differentiated from the apogamous embryo has a lamina described as ± circular, whole, and crossed by a dichotomically ramified vein. The following leaves differentiated have a differently incised lamina (Fig. 8). The leaves are protected by trichomes similar to those on the gametophyte, and also by pluricellular trichomes and paleae. The plantlets obtained were individualized from the colonies of prothallia and acclimatized.

As far as the conservation of the gametophyte on the culture media proposed, the following observations were made: a) the development of the gametophyte occurs on the medium variants V2, V3, V4, V5, V6, V7; b) variants V1 and V2 allow to obtain the sporophyte, one year after the in vitro culture was started; c) the transfer of the gametophyte from the V1 V3, V4, V5, V6, V7, V8, V9 onto V2 conduces to the formation of the sporophyte in cases V1, V3, V4, V6, V7, V8, V9.

CONCLUSIONS AND FUTURE WORK

Obtaining and multiplying the gametophyte from the sporangia (green sori) occurred on simple media with no supplement of hormones, of the type M&S (1962), in which the amount of macroelements was reduced to one half. To continue the process of multiplication and to maintain the gametophyte over a long period of time, adding hormones (auxins and cytokinines) is necessary, in concentrations of 0.5-1mg/l, in the initial culture medium. Adding mannitol to the culture medium (5-10g/l) as a retarding agent, in combination with 10g/l saccharose, led to diminishing the multiplication processes of the gametophyte. The formation of the sporophyte took place on simple media, with no supplement hormones, in the presence of saccharose as an energy support; when the gametophyte phase was prolonged on more complex media, the formation of the sporophyte only occurs after the transfer on simpler media. Since on the prothallia archegonia are differentiated, the sporophyte is differentiated through apogamy. Our further researches concerning the in vitro multiplication and conservation of the gametophyte and sporophyte of that species will be oriented towards obtaining the sporophyte over a shorter period of time, and realizing, characterizing and testing a number extracts of a therapeutical nature.
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CULTURE CONDITIONS OF R2A MEDIUM FOR THE ENUMERATION OF BACTERIA IN SPRING WATER AND BOTTLED WATER

Slavica Ćirić and Slobodan Grašić

Abstract: The aim of this study was to determine the optimal cultivation conditions of low-nutrient R2A medium in obtaining the count of aerobic mesophilic bacteria in spring and bottled waters. Significantly higher number of bacteria was recorded at lower temperatures after prolonged incubation. Optimal method for microbiological monitoring of these waters include inoculation on R2A medium by spread plate technique and incubation at 20 ºC for 7 days.

Key words: Spring water, Bottled water, R2A medium, Culture conditions.

INTRODUCTION

The total count of heterotrophic bacteria, as an important microbiological parameter of the quality of drinking water, and of other water types is defined as the total count of aerobic mesophilic bacteria. It represents the count of viable aerobic and facultatively anaerobic bacteria. Only a small fraction (less than 1%) of waterborne bacteria are thought to belong to the group of culturable heterotrophic bacteria.

In Serbia, the method of determining the count of these bacteria is legally regulated and involves the inoculation of samples on Nutrient Agar (made by Serbian Institute "Torlak", Belgrade) and incubation at 37 ºC for 48 hours [13]. Official, authorized laboratories are obligated to perform this procedure, while unauthorized laboratories have used Plate Count Agar (PCA) for a long time, which is less nutrient and more acceptable in the investigation of cleaner waters, such as drinking water. However, this medium, too, is high-nutrient for the cultivation of oligotrophic microflora.

Using newer detection methods, it is possible to significantly increase the proportion of heterotrophic bacteria that can be cultured from drinking water [6]. The use of media with low nutrient levels (e. g., R2A), which are better suited to the needs of water microflora, allows on increase in the proportion of waterborne microorganisms that can be determined by the cultivation method [12].

R2A medium was developed to maximize bacterial recoveries; it yielded higher counts when incubated for 5-7 days at 20 ºC or 28 ºC [9, 5, 8, 2, 10, 11, 3] and permitted the examination of larger sample volumes by membrane filtration methods [12].

The aim of this work was to define the most optimal cultivation conditions of R2A medium in determining the count of aerobic mesophilic bacteria in spring water and bottled water.

MATERIAL AND METHODS

Jastrebac is the highest mountain in central Serbia, close to the town of Kruševac (Fig. 1). Amongst all Balkan mountains Jastrebac Mt. features the dense forests and abundance of waters and springs. In order to examine the most optimal method for the bacteriological investigation of clean waters, ten major springs of Jastrebac Mt. was sampled. It is considered that the water of these springs potentially could be bottled and used as drinking water. A total of 120 samples were taken. Samples were inoculated on two media: standard - PCA medium and low nutritive R2A medium, in triplicate for each of the applied inoculation technique: pour plate, spread plate and membrane filtration. One such set of inoculated samples was incubated at 37 ºC for 48 hours and 7 days; the second set was incubated at 20 ºC for 48 hours, 7 and 30 days, while the third set was incubated at 8 ºC during the same period.
Along with spring waters, the examination of the most commonly consumed bottled water in this region was conducted, in the same way. The characteristics of this bottled water source are very similar to the sources of Jastrebac. A total of 30 samples of bottled water were taken, a month after filling.

By the statistical method of variance analysis [4], the significance of differences obtained between the applied media, inoculation techniques and incubation conditions was tested.

RESULTS AND DISCUSSION

Count of aerobic mesophilic bacteria in ten springs, obtained using different cultivation methods, is shown in Table 1. The values ranged from 3 CFU/ml on PCA medium, using the pour plate inoculation technique and incubation at 37 °C for 48 h, to 805 CFU/ml on R2A medium, using the spread plate technique with incubation at 20 °C for 30 days.

Table 1. Results of the CFUs in springwaters

<table>
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<th>37 °C</th>
<th>Cultivation conditions</th>
<th>20 °C</th>
<th>8 °C</th>
<th>30 d</th>
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<tr>
<td>PCA-sp</td>
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<tr>
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<tr>
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<td>R2A-mf</td>
<td>23</td>
<td>49</td>
<td>80</td>
<td>345</td>
<td>662</td>
</tr>
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</table>

- Colony Forming Units; d - days; pp - pour plate; sp - spread plate; mf - membrane filtration

In the bottled water, minimum count of bacteria was recorded under the same conditions of cultivation as for springwaters (308 CFU/ml), while the maximum number was recorded after 7 days using the same method as above (90 650 CFU/ml) (Table 2). Results of bacterial count in bottled water obtained after 30 days are not shown because they were "too numerous to count". In all samples, with all the techniques of inoculation and all incubation conditions, higher count of bacteria was detected on R2A medium as compared to PCA. It means that the R2A in which, among other things, enters ten times less peptone, five times less yeast extract and half of glucose compared to the PCA, provides better conditions for the growth of bacteria present in very clear waters. In the
springwaters, the number of bacteria on R2A was higher than on the high-nutritive medium 1.4 to 5.9 times, depending on the conditions of cultivation. In bottled water which, on average, contained 79 times more bacteria than springwaters, the differences between media were more expressive (from 1.1 to 16.4 times more bacteria was recorded on R2A). It means that the superiority of R2A medium was more expressive in more bacteriologically loaded samples.

Table 2. Results of the CFUs in bottled water

<table>
<thead>
<tr>
<th>Medium and inoculation methods</th>
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<tbody>
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<td></td>
<td>48 h</td>
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<tr>
<td>PCA-mf</td>
<td>441</td>
</tr>
<tr>
<td>R2A-pp</td>
<td>4425</td>
</tr>
<tr>
<td>R2A-sp</td>
<td>9254</td>
</tr>
<tr>
<td>R2A-mf</td>
<td>5121</td>
</tr>
</tbody>
</table>

Forming Units; d - days; pp - pour plate; sp - spread plate; mf - membrane filtration

Statistical analysis also showed a significant advantage of R2A medium. Significantly (p<0.05) and greatly significant (p<0.01) higher number of bacteria on R2A was recorded in all samples of water, at all temperatures, with all lengths of incubation and all inoculation techniques. An exception was the cultivation at lower temperatures (8 °C and 20 °C) after 48 hours, when there were no significant differences between the media. This was because the bacterial growth is slower at lower temperatures, so it takes longer to form colonies [1, 3].

Since the clear superiority of R2A is demonstrated, those values were used for determination of the most optimal incubation conditions and inoculation techniques to be combined with this medium. Regarding the technique of inoculation, the highest number of bacteria was recorded using the spread plate method, and the lowest using pour plate method. The differences between these two techniques were generally statistically significant, regardless of incubation conditions. More bacteria was obtained by membrane filtration than by the pour plate, but these differences were not statistically significant. This was also noted in the examination of tap water and purified water [8, 7]. By spread plate technique, the highest count of bacteria was recorded at 20 °C after 7 and 30 days (Tables 1 and 2; Fig. 2).

Figure 2. Results of the CFUs in spring and bottled waters on R2A medium using spread plate inoculation technique
Statistical analysis showed clear advantage of incubation at 20 °C (Table 3). The incubation period of 30 days was not suitable for testing these waters. In fact, after 30 days it was obtained the useless information for bottled water ("too numerous to count"), while the results for spring water were not significantly different from those after 7 days (Table 4). In addition, the incubation period of 30 days is a long time waiting for the results. This is unacceptable for water that is directly used for drinking, since, in case of adverse findings, a large number of people have already been exposed to health risks.

Differences in the number of bacteria between 48 hours and 7 days were statistically significant, except at 37 °C. Temperature of 37 °C does not match the natural microflora, since it is far from the temperature conditions prevailing in their nature environments.

<table>
<thead>
<tr>
<th>Water source</th>
<th>Incubation periods</th>
<th>Compared incubation temperatures</th>
<th>Statistical of in bacterial count applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 vs. 20°C</td>
<td>8 vs. 37°C</td>
<td>20 vs. 37°C</td>
</tr>
<tr>
<td>Springs</td>
<td>48 h</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>7 d</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>30 d</td>
<td>NS</td>
<td>-</td>
</tr>
<tr>
<td>Bottled water</td>
<td>48 h</td>
<td>GS</td>
<td>GS</td>
</tr>
<tr>
<td></td>
<td>7 d</td>
<td>GS</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 4. Statistical significance of difference in bacterial count between different periods of incubation on R2A using spread plate technique

<table>
<thead>
<tr>
<th>Water source</th>
<th>Compared incubation periods</th>
<th>37 °C</th>
<th>20 °C</th>
<th>8 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Springs</td>
<td>48 h vs. 7 d</td>
<td>NS</td>
<td>GS</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>48 h vs. 30 d</td>
<td>-</td>
<td>GS</td>
<td>GS</td>
</tr>
<tr>
<td></td>
<td>7 d vs. 30 d</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Bottled water</td>
<td>48 h vs. 7 d</td>
<td>NS</td>
<td>GS</td>
<td>GS</td>
</tr>
</tbody>
</table>

NS - Not significant; S - Significant (p<0.05); GS - Greatly significant (p<0.01)
CONCLUSIONS AND FUTURE WORK

The optimum method for bacteriological testing of bottled and spring waters involves the inoculation of samples on R2A medium and incubation at 20 °C for 7 days. R2A medium, with low concentration of nutrients is appropriate for microflora inhabiting oligotrophic environment. Temperature of 20 °C is close to the temperature at which these organisms live in nature, while the incubation period of 7 days is the most optimal for obtaining real results.

It was noted that, in addition to quantitative, and qualitative differences exist between the standard and R2A medium, mostly as a greater number of pigmented forms on R2A. Further studies will relate to the isolation and identification of these species, bearing in mind that pigmented bacteria mainly belong to opportunistic pathogens.

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STUDY ON CHEMICAL CHARACTERISTICS OF THE TAILINGS DUMPS FROM MOLDOVA NOUA FROM ROMANIA

Lavinia Micu, Al. Moisuc, D. Petanec, M. Lazarovici, Otilia Cotuna, Isidora Radulov

Abstract: In this paper we present the results of chemical analyses concerning chromium, cadmium, and nickel content of the sterile, at different depths, i.e. 0 cm (surface), 10 cm - 120 cm. The necessity of these analyses resulted from the fact that most fodder plants (graminaceae, legumes) have most of their roots (including the tap root) down to 20 cm in the soil. Therefore, we were interested mainly in the heavy metal content of the sterile up to this depth. But there are also plants whose roots can reach a depth of over 20 cm (for instance, couch grass) but they are not important as feed. In exchange, they can contribute to the stabilisation of the sterile and to the restoration of the area.

Key words: sterile, heavy metals, chemical analyses

INTRODUCTION

In Romania, float ponds have expanded and started to be centres of deterioration of the environment and ecological unbalances, with a strong impact on the environment. At Moldova Noua (Caraş-Severin County), processing subterranean and surface ore resulted in a total amount of 5.4 t of sterile annually. The material deposited in the ponds has varied physical, chemical, and mineralogical features depending on the initial features of the processed ore and on the ore processing technology (4, 5). Sterile deposits at Moldova Noua are the largest in the country; they have been established as “field” ponds and are the most disfavoured from the point of view of wind frequency and speed of all the areas with sterile deposits (2).

Chromium soil pollution is rarely an issue for environmentalists since it is phyto-toxic only as oxidised anion (hexavalent chromium), a form that can occur only in certain conditions of pH and potential redox that cannot last long in the soil (1).

MATERIAL AND METHODS

The material we have analysed chemically was sampled from the Tăuşani pond. We first designed a profile in the pond and then sampled.

In the Plant Protection Laboratory of the Banat University of Agricultural Science and Veterinary Medicine in Timişoara (Timiş County, Romania), raw samples were weighed and then we obtained laboratory samples through the quarter method. They were wrapped in paper bags and labelled and then sent to the Agrochemistry Laboratory where they were analyse.

To determine the heavy metal content of mining tailings, samples were extracted with a mixture of hydrochloric acid and nitric acid, maintained for 16 hours at room temperature, followed by boiling under reflux for 2 hours. The extract was then clarified and brought to volume with nitric acid. The content of microelements in the extract was determined by atomic absorption spectrometry method. [3].

RESULTS AND DISCUSSION

To see if the sterile at Moldova Noua (in the waste dumps) is a soil capable of developing and of maintaining vegetation, though inferior from a qualitative point of view, but not toxic for animals, it should meet the standards stipulated by the Expert Committee of the Health Office in Berlin (Germany). This means that heavy metal content in the sterile should not be above tolerable concentrations of different elements in the soil taking into account the limits admitted for plants (table 1). Results presented in Table 1 allow us to
say that the test based on limit differences showed zinc 80 cm deep in the sterile had a very significant concentration compared to the tolerable zinc concentration of the soil, while 100 cm deep in the sterile zinc concentration compared to the control (tolerable concentration in the soil) was significant. There were lower zinc concentrations compared to the control 40 cm deep in the sterile (distinctly significant).

On surface sterile (0 cm) and 10 cm -100 cm deep in the sterile, zinc concentrations did not reach minimum level (300 ppm) of statistic significance.

As for the rest of heavy metals determined in the sterile (Cr, Cd, and Ni), they has lower concentrations than tolerable soil concentrations (very significant).

Analysing Figures 1-4 we can easily see that 40 cm deep in the sterile heavy metals under study (Zn, Cr, Cd, and Ni) can only be found in small amounts compared to the other strata.

As a result, we can say that between 20 and 40 cm, sterile is made up of coarse sand that allowed a stronger levigation of heavy metals and that lowered heavy metal content deeper than 40 cm compared to the sterile stratum (0-20 cm).

Figures 1-4 show that the stratum 60-80 cm is made up of fine sand that retain larger amounts of heavy metals levigated from the sterile surface (except for cadmium, in smaller amounts 80 cm deep in the sterile, i.e. 56.97% compared to the cadmium amount 60 cm deep in the sterile). The stratum 80-100 is made up of coarse sand that allows an easy levigation of heavy metals towards the stratum 100-120 cm, made up of finer sand and that retains a larger amount of heavy metals.

As we can see, the sterile at Moldova Nouă in the Tausani pond is made up of alternating strata of fine sand – coarse sand – very fine sand – coarse sand etc.
Table 1

Summary of experimental results and determining the significance of differences compared to the tolerable concentration of these metals in soil

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>V1 (0 cm)</td>
<td>230.33</td>
<td>- 69.67</td>
<td></td>
<td>202.6</td>
<td>- 93.01</td>
<td>0.15</td>
<td>12.46</td>
<td>- 2.85</td>
<td>0.00</td>
<td>349.16</td>
<td>- 93.81</td>
<td>0.93</td>
</tr>
<tr>
<td>V2 (10 cm)</td>
<td>270.25</td>
<td>- 29.75</td>
<td></td>
<td>202.6</td>
<td>- 95.4</td>
<td>0.4</td>
<td>5.85</td>
<td>- 2.6</td>
<td>0.00</td>
<td>349.16</td>
<td>- 94.14</td>
<td>5.31</td>
</tr>
<tr>
<td>V3 (20 cm)</td>
<td>369.33</td>
<td>69.33</td>
<td></td>
<td>202.6</td>
<td>- 94.75</td>
<td>0.46</td>
<td>13.43</td>
<td>- 3.0</td>
<td>0.00</td>
<td>349.16</td>
<td>- 95.14</td>
<td>12.04</td>
</tr>
<tr>
<td>V4 (40 cm)</td>
<td>37.4</td>
<td>-262.6</td>
<td>0.00</td>
<td>202.6</td>
<td>- 95.14</td>
<td>0.0</td>
<td>12.04</td>
<td>- 3.0</td>
<td>0.00</td>
<td>349.16</td>
<td>- 94.75</td>
<td>12.04</td>
</tr>
<tr>
<td>V5 (60 cm)</td>
<td>349.16</td>
<td>49.16</td>
<td></td>
<td>202.6</td>
<td>- 93.51</td>
<td>0.93</td>
<td>14.11</td>
<td>- 2.07</td>
<td>0.00</td>
<td>349.16</td>
<td>- 94.75</td>
<td>12.04</td>
</tr>
<tr>
<td>V6 (80 cm)</td>
<td>643.66</td>
<td>***</td>
<td></td>
<td>202.6</td>
<td>- 93.75</td>
<td>0.53</td>
<td>14.25</td>
<td>- 2.47</td>
<td>0.00</td>
<td>349.16</td>
<td>- 95.14</td>
<td>12.04</td>
</tr>
<tr>
<td>V7 (100 cm)</td>
<td>561.16</td>
<td>261.16</td>
<td></td>
<td>202.6</td>
<td>- 94.04</td>
<td>0.4</td>
<td>13.4</td>
<td>- 2.6</td>
<td>0.00</td>
<td>349.16</td>
<td>- 95.14</td>
<td>12.04</td>
</tr>
<tr>
<td>V8 (120 cm)</td>
<td>361.83</td>
<td>61.83</td>
<td></td>
<td>202.6</td>
<td>- 93.93</td>
<td>0.7</td>
<td>11.39</td>
<td>- 2.3</td>
<td>0.00</td>
<td>349.16</td>
<td>- 95.14</td>
<td>12.04</td>
</tr>
<tr>
<td>olerable soil</td>
<td>300</td>
<td>Witness</td>
<td>- 100</td>
<td>Witness</td>
<td>3</td>
<td>50</td>
<td>M Witness</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>DL 5% = 177.42</td>
<td>DL 5% = 31.36</td>
<td>DL 5% = 0.89</td>
<td>DL 5% = 12.87</td>
<td>DL 5% = 24.41</td>
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<tr>
<td>DL 1% = 244.38</td>
<td>DL 1% = 43.19</td>
<td>DL 1% = 1.23</td>
<td>DL 1% = 17.73</td>
<td>DL 1% = 24.41</td>
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</tr>
<tr>
<td>DL 0.1% = 338.44</td>
<td>DL 0.1% = 59.47</td>
<td>DL 0.1% = 1.69</td>
<td>DL 0.1% = 17.73</td>
<td>DL 0.1% = 24.41</td>
<td></td>
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</tr>
</tbody>
</table>

Conclusions and future work

As a result of statistical processing of the data we obtained, we can draw the following conclusions: the largest amount of zinc is 80 cm deep in the sterile (643.66 ppm), and the smallest amount is 40 cm deep in the sterile (37.4 ppm);

- chromium can be found in large amounts both on sterile surface (0 cm), and 120 cm deep in the sterile (6.97 ppm); it can be found in small amounts 10 cm deep in the sterile (4.6 ppm);

- as for cadmium content, there was a higher value 60 cm deep in the sterile (0.93 ppm), while 40 cm deep in the sterile there was no cadmium at all; therefore, cadmium completely levigated through the sand stratum 40-60 cm deep in the sterile;

- nickel was found in large amounts 60 and 8 cm deep in the sterile (14.11 ppm and 14.25 ppm), while the smallest amounts were 10 cm deep in the sterile (5.85 ppm).

Therefore, the levigation of the four heavy metals (zinc, chromium, cadmium, and nickel) under study differs in the seven substrata (10-120 cm) of the sterile. Thus, 120 cm deep in the sterile, the depth reached by most of the roots, cadmium has the lowest value (0.46 ppm), and zinc has the largest amount (369.33 ppm).

Of the four chemical elements, zinc had a very significant concentration compared to the concentration tolerated for the soil. Excessive zinc in the soil results in changes of its physical and physico-chemical features and reduces biological activity. Rather large amounts of zinc identified in the sterile are, among others, the cause of the lack of vegetation of the waste dumps at Moldova Nouă. Zinc in plants becomes toxic at concentrations higher than 400 ppm because it hinders the absorption of other elements.
In general, sterile samples from the Tăuşani pond at Moldova Nouă (Caraş-Severin County) had high concentrations of zinc and nickel.

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A COMPARATIVE STUDY OF THE CALLUS EXTRACTS FROM TWO VITIS VINIFERA L. VARIETIES REGARDING THE ANTHOCYANIN CONTENT UNDER THE INFLUENCE OF BIOTIC AND ABIOTIC ELICITORS

R. Mihai and A. Brezeanu

Abstract: Anthocyanin biosynthesis has been extensively studied based on its large biotechnological applicability. Plant cell culture proved to be a feasible experimental system for anthocyanin biosynthesis using V. vinifera cultivars. Elicitation with biotic or abiotic stimuli provides an opportunity to enhance production of these compounds over levels found in intact plants and to identify possibilities for improving the biosynthesis using tissue culture. In this context, we analyze the possibilities to stimulate anthocyanin biosynthesis using elicitors in a long-term callus culture initiated from Vitis vinifera L. cvs. Isabelle pericarp and in a primary callus culture of Vitis vinifera L. cvs. Negru Vartos, started from leaf fragments. The results of the experiments revealed that the highest concentration of anthocyanins in the callus extracts of Vitis vinifera cvs. Isabelle could be achieved using a two-stage culture system. This involves the presence of 2mM mannitol for the proliferative and 40 μM jasmonic acid for the biosynthetic stages. The use of abscisic acid (20 μM) in the second stage proved to be the most efficient regarding anthocyanin biosynthesis in Vitis vinifera cvs. Negru Vartos. The original data obtained by us permitted to design new systems for the modulation of callus biosynthetic potential regarding anthocyanins.

Key words: Anthocyanin, Vitis vinifera L., Elicitors, Two-stage culture system.

INTRODUCTION

Increased market demand, due to the large biotechnological applicability, has fuelled the development of techniques for anthocyanin production. Plant cell culture has been suggested as a feasible technology for the production of these compounds which triggered considerable efforts for anthocyanin biosynthesis improvement. Among the productivity enhancement techniques developed, application in the plant cell tissue cultures of physical, chemical and biological elicitors has been shown to be the most efficient strategy to enhance the anthocyanin production. The elicitors employed include culture filtrates and cell extracts of fungal and bacterial origin, phycocyanin, UV light, fluoridine, methyl jasmonate or jasmonic acid, ethylene, β-glucan, chitosan, ibuprofen, riboflavin and inorganic ions such as Ca, Mn, Zn, Co, Fe. Many researchers have reported anthocyanin production in a vast number of plant cell and tissue cultures. The well studied plant systems are Vitis vinifera, Fragaria sp., Daucus carota, Perilla frutescens and Aralia cordata [1]. Knowing that potentially the most valuable component of grape pomance are the anthocyanin pigments, we investigate the influence of some biotic (fungi extracts) and abiotic elicitors regarding the anthocyanin accumulation in a callus cell line of two Vitis vinifera L. cultivars. The study was achieved by carrying out a quantitative analysis of anthocyanin biosynthesis using the pH differential method.

MATERIAL AND METHODS

Plant Material and Growth Conditions

The first source of inoculum consists in a stock culture of a long-term callus from the Institute of Biology collection, previously initiated from immature pericarp of grape berries (V. vinifera L. cvs. Isabelle) [2].

The cell line was maintained through periodic subcultivations (once a month) on a basal Gamborg-B5 (1968) medium variant, supplemented with 0.1 mg/l NAA (α-naphtalen acetic acid), 0.2 mg/l kinetine, 2 g/l casein hydrolysate, 30g/l sucrose, 8g/l agar (Difco), which has been referred as control variant (Var IX).

For the second source of inocula was used a primary callus cell line generated from leaves of *Vitis vinifera* L. cv. Negru Vartos.

Based on our previously research on the production of the anthocyanins [3], we established a two-stage experimental system, testing the influence of different concentration and elicitors on these callus cultures.

In case of the *long-term* callus of *Vitis vinifera* cvs. Isabelle nine variants of the experimental medium were analyzed (Table 1) for 37 days and each treatment was repeated 4 times. For the first stage of the experiment the *long-term* callus was grown 30 days on the medium variants supplemented with 2mM mannitol (Man), 10-20µM salycilic acid (SA), or 20 µM jasmonic acid (JA) to provide the callus proliferation. Further subcultivation of the callus for 7 days during the second stage of the experiment ensured the biosynthetic capacity of the callus. The elicitors for this experiment, with the exception of the fungal ones, were solved in 1 ml methanol (MeOH).

In parallel was tested another two-stage system for the primary callus induced from leaf explants of *Vitis vinifera* cvs Negru Vartos using for the proliferative stage only the elicitor SA in a concentration of 10µM. Var IX represented the control, the cell line being growth with the lack of elicitors.

The elicitor preparation from fungi extracts of *Botrytis cinerea* and *Fusarium oxysporum* used for this experiment consisted in sonicated pellets.

<table>
<thead>
<tr>
<th><em>Vitis vinifera</em> sp.</th>
<th>Experimenta l medium variants</th>
<th>First Stage</th>
<th>Second Stage</th>
<th>First Stage</th>
<th>Second Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Manitol (mM)</strong></td>
<td><strong>Salycilic acid (µM)</strong></td>
<td><strong>Jasmonic acid (µM)</strong></td>
<td><strong>Etephon (mM)</strong></td>
<td><strong>Extract Fusarium oxysporum (ml)</strong></td>
</tr>
<tr>
<td><em>V. vinifera</em> L. cvs. Isabelle</td>
<td>Var I</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
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<tr>
<td></td>
<td>Var II</td>
<td>2</td>
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<td></td>
<td>Var III</td>
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<td>Var IV</td>
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<td>Var V</td>
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<td>10</td>
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<tr>
<td></td>
<td>Var VI</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>0.1</td>
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<td></td>
<td>Var VII</td>
<td>-</td>
<td>10</td>
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<td></td>
<td>Var VIII</td>
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<tr>
<td></td>
<td>Var IX</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td><em>V. Vinifera</em> L. cvs. Negru Vartos</td>
<td>Var X</td>
<td>-</td>
<td>10</td>
<td>-</td>
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<tr>
<td></td>
<td>Var XI</td>
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<td>Var XII</td>
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<td></td>
<td>Var XIII</td>
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<td>10</td>
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<td>Var XIV</td>
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</tr>
</tbody>
</table>

**Table 1.** Types and concentrations of elicitors used in the two-stage culture system.

**Sample preparation and extraction of anthocyanins**

Four samples each one of 3 g callus from the all medium variants were grounded using mortar and pestle. The disintegrated samples were extracted in refrigerator for 24 h with 3 ml MeOH solution containing 0.1% HCl, at 4 °C in a dark environment. The extract was centrifuged (4000 rpm, 15 min) and the clear supernatant was collected and used for further biochemical analysis.

**Morphometric parameters**
Rate of growth was represented as the fresh weight of callus grown for 37 days on different medium variants starting from the idea that all treatments were initiated from callus samples with fresh weight of 1g. The water content was calculated as difference between the fresh and the dry weight expressed as percentage from fresh weight.

**Measurement of total monomeric anthocyanins using the pH differential method**

Monomeric anthocyanins reversibly change color with a change in pH (the colored oxonium form exists at pH1.0 and the colorless hemiketal form predominates at pH 4.5. The difference in absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyaniding-3-glucoside basis. Polymerized anthocyanin pigments and non-enzymatic browning pigments do not exhibit reversible behavior with pH, and thus are excluded from the absorbance calculation.

\[
\text{Anthocyanin pigment (cyaniding-3-glucoside equivalents, mg/L) = } \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\varepsilon \times 1}
\]

Where \( A = (A_{520nm} - A_{700nm}) \) pH 1.0- \( (A_{520nm} - A_{700nm}) \) pH 4.5; where MW (molecular weight) = 449.2g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established; \( 1 = \) pathlength in cm; \( \varepsilon = 26900 \) molar extinction coefficient, in L X mol \(^{-1}\) X cm \(^{-1}\), for cyd-3-glu; and \( 10^3 = \) factor for conversion from g to mg.

**Indices for pigment degradation, polymeric color and browning**

Indices for anthocyanin degradation can be derived from a few absorbance readings of a sample that has been treated with 0.68M sodium bisulfite solution. Anthocyanin pigments will combine with bisulfite to form a colorless sulfonic acid adduct. Polymerized colored anthocyanin-tannin complexes are resistant to bleaching, whereas the bleaching reaction of monomeric anthocyanins will rapidly go to completion. The difference in absorbance at 420 nm, 520 nm and 700 nm of the bisulfite-treated sample serves as an index for browning. The ratio between monomeric and polymerized anthocyanin complexes can be used to determine the degradation index.

\[
\text{Index degradation} = \frac{(A_{420} - A_{700}) + (A_{520} - A_{700}) \times \text{DF in water}}{(A_{420} - A_{700}) + (A_{520} - A_{700}) \times \text{DF in bisulfite}} \times 100
\]

**RESULTS AND DISCUSSION**

The investigation of the elicitors influence regarding the callus growth revealed that in case of *V. vinifera* cvs. Isabelle all the elicitors used (Man, SA, JA) slightly decreased growth of the callus cells.
Probably the initial callus proliferation was promoted by elicitors, but a further subcultivation on media supplemented with biotic elicitors, ethephon and JA induced an inhibition of the growth. The V. vinifera cvs. Negru Vartos callus after the exposure period in the two stage culture system revealed almost the same aspect of the growth as in the control conditions (Var XIV), with a slightly increase (Var XII). (Figure 1)

In this experiment the influence of the elicitors on the callus growth could not be correlated with the effect of the same elicitor combination on the anthocyanin accumulation. With the exception of the fungal elicitor prepared from Botrytis cinerea, all the elicitors used induced an increased yield of anthocyanins in the calli used.

The callus cells of V. vinifera cvs. Isabelle recorded the maximum yield of anthocyanins in the culture two-stage system containing SA (10 µM) and JA (40 µM). (Figure 2)

![Figure 2. Values for total monomeric anthocyanin content of the calli under the influence of the two-stage system culture. Positive responses of cell cultures to SA elicitation are possibly associated with the fact that SA is one of the key endogenous signals involved in activation of numerous plant defense responses, such as the induction of the expression of pathogenesis-related genes (PR genes) and biosynthesis of secondary metabolites [4]. The stimulatory effect of JA was supported by previously reported positive effect on anthocyanin accumulation in hypocotyls of soybean seedlings, in shoots of wild-type of Arabidopsis, in detached corollas of Petunia, in cell culture of Vaccinium pahalae, in suspension cultures of Vitis vinifera [5].

Production of anthocyanins was enhanced in both kinds of calli (long-term and primary) from Vitis vinifera cultivars (Isabelle and Negru Vartos) by adding in the medium culture the combination of the elicitors SA (10 µM) and abscisic acid (10 µM). A possible explanation for the positive effect of abscisic acid is due to the fact that this elicitor can enhance the mRNA accumulation of VvmybA1, a putative regulatory gene of anthocyanin biosynthesis of grape [6].

The anthocyanin biosynthesis in the calli of V. vinifera cvs. Isabelle is also induced by the two-stage culture system containing JA (20 µM) and ethephon (0.1 mM). The ethephon represent the ethylene precursor. Even though ethylene itself induces production of few plant secondary metabolites, it most often interacts with the JA signaling pathway, leading to secondary metabolites production, in this case affecting positively the accumulation of anthocyanins.

The high monomeric anthocyanin content in the calli of both V. vinifera varieties grown in the presence of elicitors SA and abscisic acid is correlated with the smallest polymerized anthocyanin production (Figure 3). This triggers the idea that the two-stage
system culture containing SA (10μM) in combination with abscisic acid (10μM) is the optimum growth system for the V.vinifera calli regarding the anthocyanin enhancement.

![Anthocyanin Content](image)

**Figure 3.** The browning anthocyanin content of the calli under the influence of medium variants.

**CONCLUSIONS AND FUTURE WORK**

The study regarding the anthocyanin biosynthesis of the V. vinifera long-term callus (cvs. Isabelle) and the primary callus (cvs. Negru Vartos) demonstrate that the application of the two-stage culture system containing as elicitors SA (10μM) and ABA (10μM) represents the optimum condition for increasing the anthocyanin production.

The data presented in this paper also revealed that the reactivity of the culture towards elicitors tested differs both with the cultivars physiological and peculiarities of culture. Our experiment demonstrate that the primary callus of V.vinifera cvs. Negru Vartos had a more positively reactivity than the long-term callus from cvs. Isabelle under the influence of the best elicitor system used for anthocyanin biosynthesis.

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CORRELATION OF MERCURY AND METHYLMERCURY IN SURFACE WATERS OF ARID AND HUMID REGIONS, AND THE ROLE OF HUMIC ACIDS IN MERCURY MIGRATION

Valentina Danilova, Sabzbakh Khushvakhtova, Vadim Ermakov

Abstract: Mercury high content levels in waters of arid landscapes (e.g., in Kyrgyzstan) do not activate metal methylation process. The features of the interaction of mercury and humic compounds like fulvic (FA) and humic (HA) acids are discussed. The FA are found to promote mercury dispersion in the biosphere due to formation of fulvic complexes and abiogenic methylation of mercury resulting in the toxic organomercuric compounds. The HA manifest high sorption capacity levels in relation to mercury ions and serve as sorbents. At a competitive interaction in the system FA-Hg-HA, $K_d$ for mercury drops sharply with an increase in FA concentration and pH system.

Key words: Humic Acid, Fulvic Acid, Mercury, Methylmercury, Water

INTRODUCTION

Mercury is one of the most toxic trace element and included in the list of priority pollutants. Possessing some specific physical & chemical features, mercury is a good migrant in the environment, depending on the current conditions accumulating and redistributing in the components of water and surface ecosystems, gas and water environments [2, 7]. Among the wide range of various interactions running in natural environments, the following processes are outlined as principal for the solution phase: a) hydrolysis of mercury (II) ions and their complex formation to FA [9], and b) abiogenic and biogenic methylation of mercury [3-6].

MATERIALS AND METHODS

Mercury interaction with FA was studied by a method of solubility in microvariant. The molecular-mass distribution of mercury (II) fulvic complexes was studied by filtration through a sephadex G-25 column. The experiments for mercury sorption on HA and the interactions in a three-component system were conducted in cylindrical teflon vessels with screw-on lids. The mercury content levels in the solutions were determined using a method of cold vapor with atomic-fluorescent registration of the analytical signal, and also AES-ICP method, FA concentrations were determined using a spectrophotometric method from solution absorbancy indices at $\lambda=420$ nm. The methylmercury concentrations were determined using gas-liquid chromatography with an electronic capture detector following an extraction concentrating with benzol (toluene) from acid solutions in the cold [1, 8].

RESULTS AND DISCUSSION

In the course of the surveillance of various areas with different mercury levels in the environment (the Northern Dvina, Moscow suburbs, Kuban, and South Kyrgyzstan) some data array has been obtained on mercury and alkyl mercury in various natural objects including natural surface waters. Methylmercury was found to exist in water ecosystems and water organisms only. Neither methyl- (MMC) nor ethylmercurchloride was found in the soils, soil microorganisms biomass or plants with the method sensitivity level of 0.5 to 1.0 ng/g [2].

The water analyses revealed the following:
- methylmercury concentrations in surface waters vary ranging from $< 2$ to 80 ng/l, making 4 to 50% of their total content at a solution (filtrate) phase;
- at a sharp increase in total mercury concentrations in natural waters, methylmercury levels rise slowly while methylated mercury percentage drops (see Fig. 1);
- a tendency of a positive correlation between methylmercury content levels in the waters and the bottom deposits (i.e., soil or silt) is noted;
along with the higher coefficients of mercury biological absorption by organisms in arid regions as compared to the humid ones, a tendency of still more intensive mercury alkylation in a water environment is observed. However, in the conditions of a hot climate methylmercury can migrate from reservoirs to the atmosphere and decompose being affected by solar radiation.

Among the aspects of abiogenic methylation, reactions with implication of humic substances are of substantial significance.

The humic substances (HS) are complex mixtures of high molecular organic compounds of natural origin formed through plant and/or animal organic residues decomposition. They represent a macrocomponent of the organic matter of both the soil and the water ecosystems. HS are subdivided into three constituents, namely humin, which is an inextractable residue, humic acids (HA) and fulvic acids (FA).

To estimate the role of fulvic acids in organomercuric compounds formation, the influence of time period of mercury (II) exposure to FA solution(s) upon mercury alkylation process has been investigated on a model system (see Fig. 2). As follows from the figure, the methylation process, having reached a certain level, is replaced or hampered by a reverse process of dealkylation.

We chose elemental mercury and mercuric sulfide as the original mercury (II) compounds in the fulvic acid - Me model system.

Fig. 1. Dependence of methylmercury concentrations in waters on total mercury content indices (for the Ferghana and the Arkhangelsk biospheric subregions).

Nevertheless, it should be noted that the interaction of mercury (II) with FA in natural environments results not only in appearing some rather toxic organomercuric compounds formed through abiogenic methylation, but also in drastic growth of mercury migratory capacity as seen both in waters and soils due to formation of stable & soluble mercury (II) fulvic compounds.

Fig. 2. Dependence of methylmercuric chloride (MMC) concentrations on time (pH=6.4).
Elemental mercury is a major form of mercury existence in the atmosphere; when reaching the earth surface together with atmospheric precipitates, it interacts with soil and/or water organic substances.

Also, investigations into model systems using mercuric sulfide as the element basic mineral form as seen in the Earth’s crust were of some interest for understanding of mercury migration mechanism in the biosphere.

The dependence of elemental mercury and mercuric sulfide solubility on fulvic acid concentrations was investigated at various pH values of 5.0, 6.0 and 7.0.

Together with the growth of FA concentrations in the systems under investigation, mercury concentrations also increased notably (Fig. 3), which is due to the complex formation processes. The data for the solubility method allow identification of the mercury fulvic complexes composition and stability indices. The Hg:FA correlation in the complex compounds was graphically established as a slope ratio of the lines. The obtained $\tan \alpha$ values point to the prevalence of fulvic complexes with Hg:FA composition =1:1 in equilibrium solution for FA associate with the average molecular weight $M_w=2200$ characteristic for pH=5.0.

The results of the calculations for conditional constants of the mercury (II) fulvic complexes stability as obtained in the course of analyzing the Hg$^0$-FA and HgS-FA systems are summarized in Table 1.

We determined that highly durable & soluble complex compounds are formed in the fulvic systems containing elemental mercury and mercuric sulfide. The values of conditional stability constants for mercury (II) fulvic complexes linearly depend on pH, which is conditioned by some FA specific features, namely by their tendency to make reversible associations and a possibility of structural rearrangements within FA molecules due to pH rates growth.

The molecular-mass distribution of the mercury (II) fulvic complexes was

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**Table 1. Conditional stability constants for mercury fulvic complexes of the composition Hg: FA =1:1.**

<table>
<thead>
<tr>
<th>The system studied</th>
<th>pH</th>
<th>Mean $\beta_{11}$</th>
<th>$\lg \beta_{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg$^0$ – FA</td>
<td>5.0</td>
<td>3.60×10$^4$</td>
<td>7.56</td>
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<tr>
<td></td>
<td>6.0</td>
<td>1.03×10$^{10}$</td>
<td>10.01</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2.32×10$^{11}$</td>
<td>11.37</td>
</tr>
<tr>
<td>HgS – FA</td>
<td>5.0</td>
<td>7.52×10$^{2}$</td>
<td>7.88</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2.99×10$^{12}$</td>
<td>12.48</td>
</tr>
</tbody>
</table>
studied using a gel filtration method. The experimental data suggest that more than 70% of mercury is eluted in fractions with the output volumes characteristic of substances with molecular weights ranging from 600 to 5000 Da.

Thus, in the model system Hg-FA the high molecular mercury complexes with associated FA forms drastically prevail in the solutions, which promotes metal migration within environmental objects.

Being somewhat higher molecular and slightly soluble, the humic acids in natural objects behave as complex-forming sorbents, promoting metal concentration in soils, water suspensions and bottom deposits. Using the Hg-humic acid model system, characteristics of ash-free HA preparations as extracted from turf, marine sediments and sapropel, as well as the mechanism of their interactions with mercury ions, were studied. The HA sorption capacity indices with relation to mercury ions are high enough reaching 300 to 340 mg/g for HA extracted from turf and sapropel, and 175 mg/g for the HA found in marine sediments (see Fig.4). From the sorption isotherms, the conditional constants of mercury affinity to HA sorption centers were calculated using a method of quantitative physical & chemical analysis, their logarithm values ranging within 4.1 to 4.95.

Thus, HA serve as an effective natural geochemical barrier concentrating mercury.

The actual mercury mobility in the biosphere is controlled by the competition of metal-FA versus HA complex formation processes (Fig. 5). With the growth of FA concentrations, mercury distribution ($K_d$) in the FA-Hg(II)-HA model system drops noticeably. Table 2 demonstrates the data for $K_d$ values dependence on pH system.
At the competitive interactions in the systems mercury fulvate-HA and Hg(II)-FA-HA (see Table 1), mercury distribution coefficient drops by one or two orders: a) with the increase in FA concentrations (at a constant pH value), b) with the pH growth from 2 to 6 (at a constant FA concentration).

Consequently, with the growth of pH values and water color indices due to the competitive mercury-FA or –HA complex forming, mercury dispersion over natural landscapes pronouncedly grows up, while at low pH levels and weak water color the mercury sorption on HA dominates, i.e. there is a process of mercury accumulation in environmental objects.

CONCLUSION AND FUTURE WORK
There are the peculiarities of interaction of mercury with humus substances – fulvo-(FA) and humic acids (HA). It was shown that FA promotes to mercury dispersion in the biosphere in result of formation of fulvic complex and abiogenic methylation of mercury with producing toxic Hg-organic compounds. HA, in contrast, has higher sorption volume as to mercury ions and implements role of sorbents. $K_d$ of mercury in system FA-Hg-HA is fall significantly at competition interaction with increasing concentration of FA and pH of system. Thus, the humic acid are regulators of mercury migration in the biosphere. Nevertheless, there is a need to evaluate the competitive interaction of different metals with the active points of humic acid in the presence of mercury.

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NOWADAY DEVELOPMENT OF BIOGEOCHEMISTRY AS AN INTEGRATED SYSTEM OF KNOWLEDGE

Vadim Ermakov, Margarita Gabrashanska, Larisa Jovanovich

Abstract: The analysis of the formation of biogeochemistry as an integrated system of knowledge regarding the biogenic migration of chemical elements and some functions of the biosphere is given. Biogeochemical endemics having wide circulation worldwide, like fluorosis, endemic goiter and some human and/or animal pathologies associated with the selenium deficiency in the environment and the food chain are summarized. The contemporary development of biogeochemistry is closely linked to the need for making assessments of the environment and the effect it has upon the quality of agricultural products and human health. There is a requirement to develop and implement national and international programs for the correction of microelementoses in plants, animals and/or humans.

Key words: Biogeochemistry, Living matter, Man-made evolution, Microelementose

INTRODUCTION

It turned out that the biosphere that was evolving for billions of years has been subjected to man-made impacts, their extent being aggravated at present. The technogenesis is a transformation of the Earth resulting from human economic activities. Quantitatively, this process can be characterized by a correlation of the magnitudes of natural, agricultural and residential areas, the population size, the extent of the natural resources consumption, and the decrease in biodiversity.

Its determinants are the problems arising with the nutrition and the energy, raw materials, food quality, and the habitat of living organisms in general. Nowadays we clearly differentiate the concepts defined as “the biosphere” and “the technosphere” or the biosphere seized by technogenesis, the contemporary period representing the stage of the biosphere adaptation [6, 8].

In the realities of the XX century and the beginning of the XXI century the effect of natural resources scarcity (i.e., depletion) principle manifested. The mankind has dramatically altered the planetary balance of resources as the anthropogenic impacts qualitatively change the properties of its habitat. Hence, the resources that seemed inexhaustible may become significantly limited. In addition, we strongly feel the principle of globalization with respect to migrations of various substances (e.g., radionuclides), while economic problems require both the replenishment of biospheric resources and the compensation of the damage caused to the environment and human health. The above problems require an integrated systemic approach to the assessment of the biosphere functioning, and the interactions of the biosphere and the technosphere. One of the first attempts of such integrated approach were made by V.I. Vernadsky [3].

BIOGEOCHEMISTRY

When developing the environmental trends as observed in the views of European naturalists of the 18th century, V.I. Vernadsky working in the 30-ies of the last century developed a scientific biogeochemical concept of the biosphere as a geological shell of the Earth, the structure and the energy of which having been generated by the activities of living matter in the course of the planet’s geological history. His ideas on the geochemical significance of living matter, the formation of the environment suitable for life, and the unity of the life and the geochemical environment have led to the creation of a new section in natural sciences, namely, biogeochemistry, which studies life in the aspect of atomic migrations and energy transformation. These ideas of the researcher, along with the concepts of technogenesis of the biosphere and of the noosphere are the foundation of the up-to-date biospheric investigations [2,3].
Currently, biogeochemistry is a system of knowledge, i.e., a system science dealing with the elemental composition of living matter and its role in the migration, transformation and concentration of chemical elements and their compounds in the biosphere, and with geochemical processes involving organisms, their interactions with the geochemical environment, and geochemical functions of the biosphere.

This is a priority research field, its relevance is due to the technological evolution of the planet and searching for appropriate ways of interaction between the mankind and the nature. The knowledge of biogeochemistry is required in medicine and agriculture, it determines the strategy of forming noospheric technologies.

The basic trends in biogeochemical researches were determined by V.I. Vernadsky very extensively, ranging from the geological (or geochemical) role of living matter to the biological role of chemical elements and their compounds. Environmental aspects of biogeochemistry have been implemented within a concept of biogeochemical provinces [4] and geochemical ecology [12]. Systematic study of organism elemental composition specificities brought V.I. Vernadsky and other researchers of Biogeochemical Laboratory he had set up to the conclusion that evaluation of biological significance of chemical elements, and the activities of A.P. Vinogradov [4] and V. V. Kovalsky [12] aimed at investigation of biogeochemical provinces, which subsequently influenced the formation of ecology, medical geography and geomedicine in general, were of special significance here.

Intensive development of biogeochemistry was a result of differentiation of the science branch and the emergence of new trends like carbon and organic matter biogeochemistry, trace element biogeochemistry, isotope biogeochemistry, biogeochemical methods for mineral deposit prospecting, paleobiogeochemistry, radiobiogeochemistry, soil & plant biogeochemistry, and ocean biogeochemistry (Fig. 1).

It must be stressed that when discussing biochemical problems V.I. Vernadsky repeatedly emphasized that it is a living organism that is the object of biogeochemistry. Nevertheless, biogeochemistry abroad developed towards the study of organic matter and organism remains, resulting in the formation of new directions in biogeochemistry. In the contemporary conditions of biospheric technogenesis the role of biogeochemistry has been increasing drastically.

The key notions in the biogeochemistry are living matter as a collection of living organisms and biogenic migration of chemical elements, i.e. atomic migration in the
biosphere due to organism vital activity. The above terms were first proposed by V. I. Vernadsky in 1930 [3]. The biogenic migration reflects the unceasing interactions of organisms (i.e., living matter) and the lithosphere, and is one of the driving forces of planetary geochemical processes. The concept of geochemical (or biogeochemical) energy is also closely associated with the biogenic migration. In his report “Problems of biogeochemistry” V.I. Vernadsky stated: “the free energy, manifested by the living matter in the biosphere, reducible to the work related to atomic migration, and manifesting itself in the migration of living matter, was termed by me as a biogeochemical energy in 1925” [3].

The researcher has emphasized that the biogeochemical energy is manifested in various ways, e.g., in omnipresent prevalence of the life, i.e. the rate of a certain organism population over the planet (or the equator).

Having a faculty of a comprehensive perception of both exogenous and endogenous phenomena, V.I. Vernadsky was one of the first to appreciate the paramount significance of the transformation of light energy as conducted by plants. He calculated the amount of the energy that the earth’s biosphere receives from the heat rays of the Sun through the green plant chlorophyll. The index turned out to be equal to 1.7x10^{14} calories. The up-to-date calculations show that V.I. Vernadsky has even slightly understated the actual index of the cosmic energy being absorbed by the biosphere. For example, terrestrial green vegetation absorbs annually as much us 1.6x10^{17} kilocalories of energy, and given the green algae/weeds of the ocean this amount should be raised to at least to 10^{19} [5]. To estimate the biogenic migration of chemical elements and their compounds, their system-to-system transitions coefficients (Kb), the capture coefficients (m) and flow (F) are used [10]. In the process of biogenic migration some vital chemical elements like carbon, oxygen, nitrogen, sulfur, phosphorus, calcium, magnesium, iron, manganese, etc. are primarily involved. Due to photosynthesis and continuous interrelated biogeochemical cycles, a relative homeostasis of the biosphere (i.e., its organization) is provided. Biogenic migration of the substance intensifies with time, but the process is not unlimited. Under the conditions of the biosphere technogenesis, the masses of biogenic matter are being reduced under the influence of humans, while the masses of man-made matter are continually growing, and the man-made component of the biogeochemical cycles must not exceed the natural flows of matter here. The biochemical criteria for chemical element migration and the data on isotope biogeochemistry are used for expert assessments of ecological state for such areas [5].

Biogeochemistry as an integrated science uses the knowledge and methods of other science branches, primarily analytical chemistry (spectroscopy, chromatography, electrochemistry, and/or radiochemistry). For elemental analysis of substances, ICP-mass spectrometry, NAA, AAS, X-ray fluorescence analysis, and some others are quite effective. There is a close relation of biogeochemistry with biological sciences (like biochemistry, physiology, genetics, ecology, and soil science), and Earth sciences (e.g., geology, geochemistry, and geography), and oceanography. This provides solving some complex problems within the framework of biogeochemistry to assess biogenic migration of chemical elements & foreign substances, and evolution processes, clarifying of the organism adaptation processes, regulation of macro-and micronutrients, territorial zoning, diagnostics, microelementoses prevention, and many others.

Biogeocenology and landscape geochemistry are the branches closest to biogeochemistry. Biogeocenology has a number of signs identical to those for general ecology. Interaction of organisms with geochemical environment and dependence of their chemical constitution on medium composition, give an environmental focus to biogeochemistry. This is especially true for biogeochemical cycles being considered in the ranks of general ecology. Nevertheless, it is impossible to identify biogeochemistry with
ecology since they differ both in the subject and the objects of study, as well as in the general methodology and processes to be observed. The research areas close to biogeochemistry are landscape geochemistry, environmental geochemistry, geochemistry of the biosphere and biogeocenology, which together with biogeochemistry are a foundation of the doctrine on the biosphere. However, at the heart of biogeocenology some environmental systemic principles prevail, while landscape geochemistry is based on evaluation of landscape, i.e. evaluation of chemical element migration within the soil-plant system, taking into account the fine differentiation of landscape [5].

The problems of the evolution of living matter elemental composition, the genesis of biolytes and deposits, isotope fractionation, the processes and mechanisms for matter exchange in the system units like atmosphere-lithosphere (the ocean), biogeochemical zoning of biospheric taxons, and a number of others are solved through proper biogeochemistry.

ACHIEVEMENTS AND PROBLEMS OF BIOGEOCHEMISTRY

The achievements in biogeochemistry that should be noted are as follows: introduction of a systematic approach to the study of local and/or global cycles of chemical elements, formation and development of a new branch of biogeochemistry & ecology like geochemical ecology, development of biogeochemical methods for mineral resources prospecting, development of new biogeochemical processes to extract rare elements, zoning and environmental assessment of areas through using biogeochemical criteria, development and improvement of technologies for plant, animal and/or human microelementoses correction, and some others [1].

Nevertheless, we do not know much about metabolism balance in the biosphere-cosmos system. Living matter of the biosphere requires some “inventory”. The biorhythms of macro- and micronutrients are not taken into account. The atmospheric component of the cycles, especially the processes of plant respiration (i.e., evapotranspiration) and of aerosol transportation from the ocean onto the continents, as well as the link of sedimentation to erosion and technogenesis are poorly investigated.

Biogeochemistry has entered deeply into the mysteries of the life genesis, but is still very far from its explanation. Today the researchers are basically modelling abiotic processes preceding prokaryotes emergence. Apparently, biogeochemistry will be more closely tied to genetics in the near future, and it will influence the works on determination of the life origin.

BIOGEOCHEMICAL ENDEMICS AND MICROELEMENTOSES

Determination of the nature of biogeochemical endemics as extreme manifestations of geochemical factors of the organism habitat is one of important tasks of biogeochemistry. We know some human biogeochemical endemics like endemic goiter, chondrodystrophies, Keshan disease, or gout. Beside goiter and fluorosis, animal endemics include white muscle disease, enzootic ataxia, nutritional anemia, and boracic enteritis. Some plant biogeochemical endemics like endemic lodging of cereals, chloroses and necroses of leaves, etc. are also known. In every case, their genesis is due to some imbalance in macro-and micronutrient metabolism or uptake (see Table 1).

The basic functions of chemical elements seem to be the following ones: plastic (structural), catalytic, conformational and detoxifying. Formation of a pool of chemical elements and their metabolism is largely controlled genetically. The endogenous physiological & biochemical role of some trace elements is well known, it is a catalysis of biologically active substances including vitamins, enzymes, hormones, involvement in respiration, nerve conduction, light perception, and neutralization of xenobiotics, excessive peroxides & free radicals, etc. [9].

Formation of a pool of macro-and micronutrients depends on many factors, the major of them being the concentration levels of chemical elements, forms of the compounds, and
intake specificities. The parameters of intake, accumulation and removal of some chemical elements in animal organism are studied insufficiently as yet.

Typically, the etiological role of the chemical elements in the pathogenesis of biogeochemical endemics is rather complicated. To understand the organismic reactions to geochemical environment factors, the main points of chemical element implications into biochemical processes, as well as their groups involving the whole organism in the response to a certain element deficiency or excess, should be determined, i.e., it is necessary to establish the causal dependences and explain the significance of every stage of the organism integral response. A biocenotic approach to the investigation of animal/human microelementoses, e.g., at parasitic, bacterial or viral pathologies is of interest as well. In this case, the requirement for essential trace elements in the host organism usually increases [11].

Some hypo-and hypermicroelementhoses in animals and their diagnostics [10]

<table>
<thead>
<tr>
<th>Trace element</th>
<th>Excess</th>
<th>Diagnosis</th>
<th>Deficiency</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>Acute selenosis, Alkaly disease</td>
<td>The high level of Se in the environment and animal tissues</td>
<td>White muscle disease, exudative diathesis, liver degeneration</td>
<td>Level of selenium in the environment, feed, blood, organs. Decrease in activity of glutathione-peroxidase</td>
</tr>
<tr>
<td>J</td>
<td>Goytorogenic effects (hyper-function of thyroid gland)</td>
<td>Iodine levels in feed and blood</td>
<td>Various forms are goiter (hypo-function of thyroid gland)</td>
<td>Iodine levels in the blood, the content of iodine-containing hormones</td>
</tr>
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<td>B</td>
<td>Boric enteritis</td>
<td>The level of B in the environment and the body. Increasing of protease activity</td>
<td>Dysplasia of bone</td>
<td>The level of boron in the feed the organs and tissues</td>
</tr>
<tr>
<td>F</td>
<td>Fluorosis</td>
<td>Dental health, a change of the skeleton, the level of F in feed</td>
<td>Dental caries (?)</td>
<td>Problematic</td>
</tr>
<tr>
<td>Cu</td>
<td>Gipokuproz, copper jaundice</td>
<td>Hematology, serum bilirubin in the blood content of Cu in the blood and feeds</td>
<td>Enzootic ataxia</td>
<td>Cu levels in forage and blood, activity of oxidase, superoxide-dismutase, ceruloplasmin, etc.</td>
</tr>
<tr>
<td>Mo</td>
<td>Molibdenozis, chronic Mo toxicosis</td>
<td>The level of Mo and copper in feed, tissues and organs, the activity of xanthine-oxidase</td>
<td>The animals were not described in detail</td>
<td>Problematic. Possible to determine Mo in blood and activity of xanthine-oxidase</td>
</tr>
</tbody>
</table>

In case of geochemical endemics, apart from specific signs of metabolism disorders, there are generalized welfare disorders. The latter ones for livestock include a drop in productivity, impaired reproductive ability, a decrease in immunobiological characteristics of the organism. Currently, along with monohyper- and hypomicroelementhoses in animals, a problem of complex elementhoses caused by an extensive type of agriculture has emerged, mainly referring to highly productive animals.
Special attention should be paid to the processes of interaction of biologically active macro-and micronutrients. Competitive and synergistic relationships among macro-and micronutrients are observed at the stage of intake, the phase of penetration through membranes, both at molecular and genetic levels. Such interactions are well known between calcium and zinc, calcium and cadmium, calcium and strontium, calcium and phosphorus, copper and molybdenum, between sulphate levels and copper, selenium and iodine, etc.

Effective prevention of the endemics provides for improvement of the parameters and criteria for their diagnosis and correction (e.g., application of special food supplements, microfertilizers, introduction of new technologies of regulation of chemical element intake in plant/animal organisms, predictive assessment of endemic areas using modern information technologies).

CONCLUSION AND FUTURE WORK
The contemporary development of biogeochemistry is closely linked to the requirement for evaluation of environmental conditions and their influence upon agricultural products quality and human health. Such assessments should be based on the results of both fundamental and/or applied biogeochemical studies of biospheric taxons. Meanwhile, in the recent decades, this research trend has not received adequate support. Elimination of this gap at the up-to-date level requires the organization of appropriate structures with an optimal state funding.

In any case, development and implementation of special intergovernmental programs for correction of the microelementhoses (like fluorosis, endemic goiter, selenium deficiency-based pathologies, and others) is under demand. Development of regional criteria and parameters of both macro- and micronutrient biogenic migrations as a fundamental basis for environmental and biogeochemical forecasting of the state of areas, an also upgrade of training in the field of biogeochemistry and environmental geochemistry are equally important.

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BIOLOGICAL INDICATION OF SELENIUM ECOLOGICAL STATUS

Vladimir Safonov, Sergey Tjutikov, Vadim Ermakov, Valentina Danilova, Sabsbakhor Khushvahtova, Elena Krechetova, Alexander Degtyarev, Yuriy Kovalsky

Abstract: Selenium was detected spectrofluorometrically and/or using AAS in livestock hair cover and blood samples. Se-deficiency manifestations were not found on farms of the Moscow and the Voronezh regions, while the other regions (i.e., the Zabaikalye Territory, North Caucasus and the Kirov region) are characterized by diverse degrees of selenium deficiency which is manifested in the form of white muscle disease among younger animals and/or endemic goiter, selenium content levels in blood correlating with the microelement concentrations in tail bunch hair samples of cows.

Key words: Animals, Blood, Dairy Farms, Hair, Selenium

INTRODUCTION

Selenium is an essential trace element with unique biochemical functions and a wide spectrum of biological effects of Se compounds. Selenium plays an important antioxidant role in the biosphere. Se-deficiency may cause cardiovascular diseases and weakening of the immune system. In zones of Se-deficiency some diseases are widespread, like white muscle disease of livestock and also Keshan disease, and an endemic Urov Kashin-Beck disease [1,2]. The purpose of this article - to reveal the relationship between content of selenium in the hair cover and the blood of animals in assessing the status of this trace element.

MATERIALS AND METHODS

The field biogeochemical investigations were carried out on farms of the Moscow, the Kirov and the Voronezh regions, the Zabaikalye Territory, Kabardino-Balkaria and North Ossetia. The microelement status on commercial dairy farms had been evaluated earlier in the period of 2005 to 2009 [4]. The farms of the Moscow and the Voronezh regions were determined as non-deficient for selenium, while the other grounds (namely, the Zabaikalye Territory, North Caucasus and the Kirov region) were characterized by variable degrees of the microelement natural deficiency with cases of both sporadic and mass scale manifestations of white muscle disease being observed in livestock.

Whole blood and cow hair tail samples were taken routinely from 346 milking cows of 3 to 8-year age on various farms located in the above regions, and 1% heparin solution was used as a coagulant. Hair cover samples were taken from the switch of the tail and cleaned through washing in distilled water as per the existing method [5].

Selenium indices were determined spectrofluorometrically after the biological material decomposition with a mixture of perchloric and nitric acids followed by selenium chemical reduction to Se (IV) HCL and diazoselenol production in the reaction between selenite and 2,3-diaminonaphthalene. The standard deviation for selenium additives in 10 tests was 97.3+6.3%. It should be noted that the selenium spectrofluorometrical detection in the whole blood became possible not until excessive iron was removed using a strong-acid cation-exchange resin column and adding some complexing agents (e.g., acetic acid or sodium fluoride) to the solution [3]. As standard samples, hair coat samples CRM NCS DS 73347 and CRM 397 were used (the determined selenium content 0.60±0.03 mg/kg, the certified index 0.61±0.02 mg/kg).

Besides, some attempts of selenium quantitative evaluation in blood through atomic-absorption spectroscopy were made using a device “QUANTUM-Z.ETA (“KORTEK”) following the material hydrolysis with nitric acid and hydrogen peroxide. Nevertheless, the results obtained there were reproducible only at Se levels exceeding 120 to 150 μg/l [3].
RESULTS AND DISCUSSION

The data for selenium concentrations in blood of the milking cattle from Black Soil zone are represented in Fig. 1. The microelement average content level was some 148 ± 3 μg/l (n= 115) ranging within 102 to 224 μg/l, i.e. the variation range reached 2 units. The selenium levels in blood showed an extremely irregular distribution pattern, while the general distribution pattern corresponds with the normal prevalence. The highest frequency of selenium concentrations ranging within 131 to 140 μg/l is noticeable. The following maximal level falls on interval 161 to 170 μg/l. The selenium level in blood seems to be a complex function depending on animal age, physiological state and some other factors.

![Fig. 1. Alteration of selenium concentrations in cattle blood for the Voronezh region.](image1)


Alteration in selenium concentrations in the blood of milking cows from the Kirov region farms (Nonblack Soil zone) is represented in Fig. 2.

![Fig. 2. Alteration of selenium concentrations in blood for cattle from the Kirov regions.](image2)

The selenium content intervals (μg/l) are as follows: 1 – 30-40, 2 – 41-50, 3 – 51-60, 4 –
As is easy to see that in this case the microelement concentration distribution pattern is either complicated. The most abundant selenium concentrations range within 71 to 110 μg/l (the first peak). The second peak falls on interval 131 to 140 μg/l. The average selenium rate in cattle whole blood samples from the Kirov region was 96 ± 4 μg/l fluctuating from 33 μg/l (minimal level) to 181 μg/l (maximal level), i.e., the deviation range reached 6 units. The obtained indices approach to selenium content levels in other regions of the Nonblack Soil Zone (e.g., for North Ossetia it is 75 to 117 μg/l and for the Moscow region 80 to 125 μg/l).

What stands out here is the selenium content indices in the whole blood of cows from the Kirov region farms are comparatively low. On average, they are 1.54 times as lower than the selenium concentrations in cattle blood for the Voronezh region, the difference being reliable and rather substantial (P<0.001). The differences are apparently due to some geochemical factors. The Kirov region is known as a Se-lacking area as regards to feeds. Consequently, here we can see a distinct link between the selenium content levels in livestock blood and the biogeochemical specificities of biospheric taxons. Nevertheless, for making an elaborate analysis of this problem the selenium concentrations are to be arranged with regard to animal age, productivity, physiological features, some feeding factors and the current biogeochemical situation as a whole.

The data on the comparison of selenium content levels in cattle blood and hair coat are summarized in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Sampling place</th>
<th>Se in Blood, μg/l</th>
<th>Se in hair, μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \bar{x} \pm s )</td>
<td>Min</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Voronezh district</td>
<td>148 ± 3</td>
<td>102</td>
</tr>
<tr>
<td>“Moskovetsky”, Moscow district</td>
<td>119 ± 15</td>
<td>89</td>
</tr>
<tr>
<td>“Nemchinovka”, Moscow district</td>
<td>127 ± 12</td>
<td>80</td>
</tr>
<tr>
<td>“Lenin state farm”, Moscow district</td>
<td>132 ± 5</td>
<td>91</td>
</tr>
<tr>
<td>Vladimir district</td>
<td>101 ± 16</td>
<td>86</td>
</tr>
<tr>
<td>Kirov district</td>
<td>96 ± 4</td>
<td>33</td>
</tr>
<tr>
<td>“Becklemishevo”, Trans-Baikal Region</td>
<td>102 ± 10</td>
<td>82</td>
</tr>
<tr>
<td>“Trubachevskoe”, Trans-Baikal Region</td>
<td>98 ± 17</td>
<td>65</td>
</tr>
<tr>
<td>Nerchinsky Zavod, Trans-</td>
<td>79 ± 23</td>
<td>62</td>
</tr>
</tbody>
</table>
The sampling grounds are arranged descendingly for Se levels in blood as follows: the Voronezh region, “Lenin state farm”, “Nemchinovka”, “Moskvoretksy”, settlement Unal (North Ossetia), Tirniauz (Kabardino-Balkaria), Becklemishevo, the Vladimir region, the Kirov region, “Trubachevskoye” and the Nерchinsky Zavod (Trans-Baikal territory). For example, while the cow blood selenium levels from the Voronezh region farms sometimes reached 224 μg/l, the cow blood samples from the Nерchinsky Zavod and the Kirov region showed the selenium levels of 30 to 60 μg/l. On a farm “Nemchinovka” the selenium levels often reached 120 to 130 μg/l. The similar picture is observed for selenium distribution in animal hair coat samples.

It should be noted that selenium levels both in blood and hair depend on animal (especially calves) treatment with sodium selenite or intake of feeds containing selenium compound additives. That hampers selenium status interpretation. Nevertheless, for selenium concentration analysis in hair cover a positive correlation is determined (r =+ 0.83 at P ≤ 0.01), depending on selenium levels in feeds and/or pasture plants. In this case the average concentrations in hay and pasture plants were considered for the Nерchinsky Zavod, Dogye, Trubachevo, Unal and Tirniauz, where the above index varied from 20 to 160 μg/l, and average selenium concentration in animal hair cover ranged within 261 to 463 μg/kg.

Not any effect of livestock age peculiarities upon selenium content level in hair cover was determined. The influence of hair colour on selenium levels for livestock hair cover was not observed either. The obtained data correlate with a selenium status selective evaluation carried out by other researchers in the Chita region (the Zabaikalye Territory) and the Belgorod region [4].

**CONCLUSION AND FUTURE WORK**

Thus, some geographical specificities of selenium accumulation in animal blood and/or hair cover have been determined, and the selenium concentration distribution patterns for every region were abnormal and varied. There are animal groups having both low and comparatively higher levels of the element. Nevertheless, selenium concentration levels both in animal blood and hair coat positively correlate and comply with biogeochemical criteria used at ecological state evaluation of various regions [4].

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EVOLUTION OF THE DEGRADATION PROCESS OF ANIMAL FAT DURING FREEZING AND DEFINE A MATHEMATICAL MODEL FOR THIS PHENOMENON

I. Manea, L. Manea

Abstract: The purpose of this study was to follow the degradation process of lipids in the meat during freezing storage. To analyze the fat degradation process was dosed the value of the peroxide index and the 2-thiobarbituric acid (TBA) in the fatty tissue of the pork, under different freezing.

The results have been statistically processed. In this study there have been tested five types of simple regression models (the linear, the logistic, the potential, the exponential and the multinomial models) which all use the trend line. As an independent variable (X) has been considered the analyzed evolution time and \((Y)\), as a dependent variable, has been considered the oxidation product taken as the value of the peroxide index and the thiobarbituric acid.

The mathematical regression allows a dynamical data processing so it has correctly modeled the interdependence of different bio-chemical parameters for a long period of time. Consequently, it has finally lead to the creation of some models which are very close to reality and on the basis of which we can make predictions. Have demonstrated that the lipid oxidation is a biochemical process which can be described by an power mathematic trend model for de evolution peroxide value.

Key words: Thiobarbituric acid, animal fat, regression models

INTRODUCTION

Freezing is an essential method of meat preservation which is based on the criobiosis principle. Freezing has many advantages for the preservation of meat and facilitates its marketing but there is some destruction of muscle fiber due to the formation of ice crystals. [2]

There are also colour modifications caused by the oxidation of the muscle pigment-myoglobin and flavour and smell modifications of fats are determined by the hydrolisis phenomena caused by tisulare enzymes and the spontaneous oxidation. The most important cause of the flavour degradation is the lipid oxidation which affects especially the polyunsaturated fatty acids. [6,9]. Oxidation of fats is preceded by an induction period during which the changes are relatively slight. The duration of the induction period is of practical importance since it determines the storage life of the fat-containing product. Once the phase of rapid oxidation has set in, characteristic rancid odor and flavour quickly appear [12]

The lipid spontaneous oxidation is produced at the same time as the first phase of the hydroperoxides formation, which are degraded into malonaldehyde (MA) and several other reactive compounds. Some of the most common methods for assessing stability or rancidity of a fat or fatty food are thiobarbituric acid assay and peroxide value [3,7]

To analyze the fat degradation process we quantizes the value of the peroxide index and the 2-thiobarbituric acid (TBA). [4,5]

In order to establish a mathematical model able to define the evolution of the lipid degradation process during the freezing of the meat, there have been tested five types of simple regression models. The mathematical analysis of the analytical data has been realized using Microsoft Excel Xp – Trend lines. All the mathematical lines use trend lines. The evolution of the processes has been analysed using the mathematical simulation. [10, 11]

The process of lipid oxidation is described by a mathematical model dependent on time. The trend lines are the most efficient if the value of R squared is 1 or close to 1.
MATERIAL AND METHODS

This study has analyzed the pork and the beef in their semi-carcasses. Once the animals have been slaughtered the semi-carcasses have been stored for twenty-four hours at the refrigeration temperature. After this, they have been frozen at different temperatures: -25 degrees C and -10 degrees C. In the next study we did the following notes:

- **P1** - pork meat preservation at -10 degrees C;
- **P2** - pork meat preservation at -25 degrees C;
- **V1** - beef meat preservation at -10 degrees C;
- **V2** - beef meat preservation at -25 degrees C.

The samples have been analyzed for twelve months.

The degree of fat oxidation of the fatty tissue from the meat was analysed by:

- the peroxide value (PV) using the AOCS method (AOCS 1998). In this method the peroxides (R-OO-H) are reacted with potassium iodide (KI) in the presence of acetic acid, and the liberated iodine is measured after addition of Na₂S₂O₃. The AOCS peroxide value is based on the amount of Na₂S₂O₃ used to change the colour of the liberated iodine (I₂) in the aqueous phase from purple to colourless iodide (I⁻), which was converted into peroxide value. [1]
- the thiobarbituric acid (TBA) assay using the method after Pensel 1990. It is based on the reaction of malonaldehyd (MA) and TBA, which generate a TBA-MA complex with an absorbance maximum at 530nm. It is measured spectrophotometricaly and the values are expressed as [mg MA/g].

The mathematical analysis of the analytical data has been realized using Microsoft Excel X – Trend lines. All the mathematical lines use trend lines. The evolution of the processes has been analysed using the mathematical simulation [MEXP] [11]

RESULTS AND DISCUSSION

The peroxide value measures the accumulation of the peroxides formed by the oxidation of polyunsaturated fatty acids which resulted from the simple triglycerides hydrolysis. The higher the oxidation is the higher the accumulation of the peroxides.

The results obtained after the PA dosage are revealed in Figure 1 for pork and for beef.

As it results from figures 1 with the ninth months the peroxide values for the pork and for beef as well raise considerably if the freezing temperature is of -10 degrees C.

The meat stored at -20deg.C, accumulates a smaller quantity of peroxides and at the end of the period it even records a peroxide diminution.

The light diminution of the peroxide value after twelve months of storage at the temperature of -20 deg.C can be caused by the meat concentration which because of the water losses through the process of evaporation has diminished its weight.

As far as the TBA method is concerned, we notice essential modifications of the malonaldehyde (MA) for the pork and the beef as well. The TBA method is based in the first place on the determination of the TBA-MA coloured complex concentration which is formed through the reaction between the malonaldehyde and the 2- thiobarbituric acid.

As far as the 2- thiobarbituric acid is concerned the obtained values for the pork and beef stored at the two temperatures mentioned are presented in the Figure 2.

The process of lipid oxidation which takes place during the freezing can be described understanding of the production mechanisms.
Figure 1- The evolution of the lipid oxidation process, expressed by the peroxide value

A mathematical model allows a better configuration of the experiments, a better understanding of the results and it can force to develop an intuitive thinking.

Figure 2- The evolution of the lipid oxidation process, expressed by the thiobarbituric acid assay

All the mathematical models use trend line. They are used to represent graphically the data trends and to analyze prediction issues referred to as regression analyses. The trend lines are the most efficient if the value of R squared is 1 or close to 1.

A linear trend line is a straight line which approximates the best simple linear data sets. The data are linear if the model, in its data points, looks like a line. The linear model figures out the approximation of the smallest square roots for a line represented by the following equation:

\[ y = mx + B \]

where, \( m \) represents the inclination and \( B \) the intersectation. The relations obtained by the modeling and the specific values of the determination coefficient are: \( y \)- the process of lipid oxidation rendered by the peroxide value, respectively TBA-MA; \( x \)- the time of storage at the freezing temperature.
As the linear model hasn’t rendered in the best way the evolution of the analysed phenomenum, there has been attempted a polynomial model and power model.

In the analyzed study the best modeling results have been obtained using a power trend model, for de peroxide value, where R squared has the biggest value (Figure 3).

Calculates the least squares fit through points by using the following equation:

\[ y = cx^b \]

where \( c \) and \( b \) are constants

The process of lipid oxidation is described by a mathematical model dependent on the time as it is represented in Figure 4 for the TBA-MA.

![Figure 3 - Exponential evolution model of the lipid oxidation process expressed as PA value, in the pork and beef meat](image)

The polynomial trend model is a curved line which is used to illustrate the data fluctuation. Calculates the least squares fit through points by using the following equation:

\[ y = b + c_1x + c_2x^2 + c_3x^3 + \ldots + c_6x^6 \]

where \( b \) and \( c_1, \ldots, c_6 \) are constants.

![Figure 4 - Exponential evolution model of the lipid oxidation process expressed as thiobarbituric acid assay, in the pork and beef meat](image)
The polynomial trend model hasn’t rendered in the best way the evolution of the lipid oxidation for peroxide value. The curved line of the model, its equation and the determination coefficient are present in Figure 3 and Figure 4. In all the analyzed cases the calculated coefficient $R^2$ (R squared) has raised – above 0.91. The freezing temperature offers clear conditions for an polynomial and power evolution.

CONCLUSIONS AND FUTURE WORK

Fat peroxide or hydroperoxide are the primary active products of lipid auto-oxidation. The evolution of the lipid oxidation processes which is well rendered by the polynomial trend model, for de TBA, where the calculated determination coefficients have values close to 1 (0.9323-0.976). The lipid oxidation is a biochemical process which can be described by an power mathematic trend model for de evolution peroxide value $R^2 = 0.9255-0.9778$. Continue to study a possibility to prevent fat rancidity.

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CHARACTERIZATION BY BIOCHEMICAL MARKERS OF THE INTERPOPULATIONAL VARIABILITY OF FERNS SPECIES WITH BIOTECHNOLOGICAL PROPERTIES

Cristian Banciu, Florentina Aldea, Florenta Helepciuc, Liliana Cristina Soare

Abstract: Ferns are an important group of vascular plants characterized by a great variety of shapes, living in all areas of the world that have aroused the interest of many research teams, thanks to their special ability to adapt and their biotechnological and ornamental properties.

In this context, studies aiming conservation of ferns from various regions of Romania have attracted particular interest, one of them being the protected area of Valsan Valley. For these reasons, our research has focused on species Asplenium trichomanes and Asplenium scolopendrium. For this purpose, in a first stage we considered very useful their characterization using biochemical markers.

Comparative studies were performed on 10 samples from two populations of each species. By analyzing the isoenzymatic spectra of main enzymes that are sensitive to changes of the environmental conditions (peroxydases, esterases, alkaline phosphatases and the total protein content) it was made a comparative estimation of in situ plant variability. We could observe the existence of differences in the isoenzymatic spectra at intra- and interpopulational level.

Key words: Asplenium trichomanes, Asplenium scolopendrium, isoenzimatic spectra.

INTRODUCTION

Pteridophyta is a group of plants that have existed for more than 300 million years and is characterized by a great variety of shapes, being found all over the world. In time, ferns attracted the interest of many research teams, due to their age and their special ability to adapt and not the least to their importance as ornamental plants and as important source of pharmacologically active principles. A brief global analysis led to the conclusion that about 20 000 plant species are endangered, rare or under extinction [3]. For these reasons, finding effective methods of long-term conservation of germplasm is a major objective [4].

Studies concerning conservation of ferns from different regions of the country have received particular interest. Such a protected area is Valsan Valley region, rich in flora and fauna, being populated particularly by different species of ferns. Delimited by the region near the banks of the river Valsan, the natural protected area is mixed with rare species of plants and animals.

In order to evaluate the variability using biochemical markers, there are available electrophoretic and spectrometric techniques. Generally, the biochemical markers analyses show sensible modifications due to genetic alterations, although sometimes secondary metabolites synthesis is determined by the physiological status of cells. Isoenzymes are used in plant breeding studies and population genetics, as biochemical markers for plant species identification and characterization. Protein or enzyme electrophoresis analyses are widely used for new varieties identification.

We have selected few representative fern species from Valsan Valley of ornamental importance, in order to develop further studies.

In our study, individuals from the natural habitat belonging to Asplenium trichomanes and Asplenium scolopendrium species have been characterized using biochemical analyses. Using the isoenzimatic analysis through isoforms electrophoresys of the most sensible enzymes to the enviromental conditions, comparative estimations of the plant variability in the natural habitat have been done. Apparently the presence of isoenzymatic modifications has been determined by the heterozygosity between the individuals [1].
This kind of experiments presents an important degree of novelty, not being cited in national literature.

**MATERIAL AND METHODS**

Biochemical analyses were based on the use of enzymatic markers with the purpose to highlight any changes in the spectra of the main types of isoenzymes sensitive to variations in environmental conditions.

Extraction of total proteins from cytosol was performed by grinding the tissue in 0.1 M phosphate buffer, pH 7 at 4°C. The supernatant obtained after centrifugation at 15,000 rpm for 10 minutes, was used for electrophoretic analysis.

Analyses were based on the principle of electrophoretic migration of samples in electrical field at 20mA for 2 hours in a batch system. The samples were run in an 8% concentration polyacrylamide gel (PAA), a 4% polyacrylamide stacking gel and a 0.05M Tris-Gly buffer, pH 8.3. Samples were loaded in the wells of stacking gel and subjected to electrophoretic migration process at a voltage of 10 mA through the stacking gel for 30 minutes and then at a voltage of 20 mA through the separating gel for 90 minutes, at 4°C. As a marker highlighting the front of migration, bromphenol blue was used. It was used an Biometra electrophoresis apparatus. After samples migration, the gels were specifically stained for each enzyme.

The expression of peroxidase activity (POX) was marked out by incubating the gel in 0.5m acetate buffer, pH 5 containing 0.08% benzidine and several drops of H$_2$O$_2$.

The activity of alkaline phosphatase (AKP) was revealed in the presence of the following solutions:
- α and β-naphtylphosphate, 0.05 M
- 0.1% Fast Blue BB
- 0.25 M MgCl$_2$ and 0.5 M MnCl$_2$ – few drops;

Reaction medium was represented by:
- tris-citrat buffer 0.01 M with pH 8.3.

Bands of esterases (EST) were developed in a solution of 0.1 M phosphate buffer with pH of 6.5, containing 0.2% α-and β naphtylacetate as the substrates and 0.05% Fast Blue RR as the dye.

Also the spectra of total proteins extracted from each sample was showed by gel staining with Coomassie Brilliant Blue solution. The gels were photographed and interpreted by comparison of the similar bands of the samples belonging to the same population and also from the two different populations.

**RESULTS AND DISCUSSION**

Samples from *in situ* populations of the species studied represent a small fraction of the diversity in habitat. Especially in areas uninfluenced by human activities such as forests, biodiversity of species is the result of natural evolutionary processes, like genetic recombination and accumulation of mutations due to DNA replication errors. These variations are dependent on genetic recombination and mutagenic rates in connection with species’ genome size.

Evaluation of genetic diversity analysis on pteridofites species was achieved by the use of izoenzimatic spectra of several enzymes. In the present experiment we tested peroxydase, esterases, alkaline phosphatases and total protein spectra.

In this study we compared 10 samples from each of the two populations analyzed belonging to the species *Asplenium trichomanes* and *Asplenium scolopendrium*. 
In case of the species *Asplenium trichomanes* the isoenzymatic spectra have revealed that the greatest degree of polymorphism is present in esterases. Peroxydase generally revealed the existence of single isoforms, except for a single sample from population 2. This additional isoform may be a result of the adaptation to local stress conditions, possibly different light, or soil structure [2] (Fig. 4). Esterases isoforms showed no significant differences between the two population samples, except for a single individual that expressed an additional band (Fig. 1). In alkaline phosphatase, samples of the two populations are relatively homogeneous (Fig. 2). The only change is the presence of an additional isoform, in one of the individuals. Total protein spectra do not present qualitative differences but only quantitative, expressed as the intensity of the electrophoretic bands (Fig. 3).

The analyses on the *Asplenium scolopendrium* specis individuals present similar characteristics. Thus, the esterases of the first population samples reveals a higher polymorphism degree (5 isoforms) comparing with the second population that presents less different isoforms (3 extra bands). We can remark band intensity differences between the individuals of the same population (Fig. 7). Both populations present three weak revealed isoforms of alkaline phosphatase (Fig. 6). The total protein spectra on this species (*Asplenium scolopendrium*) do not presents qualitative intra and interpopulational differences (Fig. 5).
Fig. 3 Isoenzimatic total protein spectra on first population (left) and second population (right) of *A. trichomanes* species

Fig. 4 Isoenzimatic peroxidase spectra on first population (left) and second population (right) of *A. trichomanes* species

Fig. 5 Isoenzimatic total protein spectra on first population (left) and second population (right) of *Asplenium scolopendrium* species

Fig. 6 Isoenzimatic alkalin phosphatase spectra on first population (left) and second population (right) of *Asplenium scolopendrium* species
CONCLUSIONS AND FUTURE WORK

Analysis of electrophoretic peroxidase spectra on *Asplenium trichomanes* and *Asplenium scolopendrium* species showed no differences except the occurrence of one band from a single individual of the species *Asplenium trichomanes*. In the case of esterases isoenzymatic spectra of the two species have revealed the existence of intra- and interpopulational variability, especially for species *A. scolopendrium*. Alkaline phosphatase showed a very low degree of variability with both species. The only exception is the species *A. trichomanes* which shows an additional isoform in a single individual. Total protein shows similar spectra on both populations of each species. Future studies concerns *in vitro* multiplication of ferns for medium and long term conservation.

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STUDIES OF CHL A AND CHL B LOADED LIPOSOMES USING ABSORPTION SPECTROSCOPY AND FLUORESCENCE TECHNIQUES

Alina Ortan, Cristina Parvu, Mihaela Ghica, Mirela Mitu

Abstract: Planar lipid bilayers represent a well defined and useful model for biological membranes, but they are not such a good model in case of photosynthesizing membranes, because it is difficult to incorporate proper quantity of chlorophylls in order to obtain adequate results. This problem was overcome by using liposomes - bilayer, vesicular, colloidal, biodegradable structures, formed when phospholipid molecules are dispersed in water. However, in this case it is difficult to prepare unilamellar lipid vesicles, well-defined shape and size, and to select the lipid type with optimal interactions with chlorophyll molecules, in order to ensure the incorporation and stabilization of chlorophyll in the bilayers of the liposomes.

The objectives of this work were the comparative study of chlorophyll a (chl a) and chlorophyll b (chl b) loaded liposomes by absorption spectroscopy and screening of the influence of pH on the liposomal suspensions. We also tried to highlight the possibility of studying the energy transfer between chl b and chl a entrapped in liposomes using fluorescence techniques.

Studies have shown that sensitivity of the formulations is mainly determined by chlorophylls and chl b loaded liposomes present higher stability. We also found that energy transfer can be qualitative indicated only for approximately equal quantities of chl a and chl b entrapped in the same liposomal suspension.

Key words: liposome, chlorophylls, stability

INTRODUCTION

Over the past twenty years, liposomes changed their status from laboratory research topic into a powerful industrial tool, the distance between their ideal desired characteristics and technical feasibility continuously decreasing.

Liposomes can be defined in a simplistic way as lipidic vesicles with an aqueous content. They were brought to the attention of the scientific world in 1965 by AD Bangham, being proposed as a model for cell membranes [1]. Many researchers, such as Dervichiani, Luzzati, and Chapman have studied these systems from different points of view, clarifying certain properties of membranes [2, 3].

Planar lipid bilayers are a well-defined and very useful model for biological membranes, but for the study of photosynthesizing membrane is difficult to incorporate into these systems sufficient chlorophyll in order to get good spectra. This problem does not exist when using liposomes, which can incorporate a much larger amount of chlorophyll. In this case, however, it is difficult to prepare unilamellar vesicles, well defined shape and size, and to select the lipid type with optimal interactions with chlorophyll molecules, in order to ensure the incorporation and stabilization of chlorophyll in the bilayers of the liposomes [4].

Liposomes containing chlorophylls are very useful systems as models for photosynthesizing membranes, for studying transport phenomena through lipidic membranes (using chlorophyll as a marker, due to its absorptions and emission spectral properties) and also for studying the photoelectric and photovoltaic phenomena [5].

The objectives of this work were the comparative study of chlorophyll a (chl a) and chlorophyll b (chl b) loaded liposomes by absorption spectroscopy and screening of the influence of pH on the liposomal suspensions. We also tried to highlight the possibility of studying the energy transfer between chl b and chl a entrapped in liposomes using fluorescence techniques.
MATERIAL AND METHODS

Obtaining chlorophyll a and chlorophyll b. Chlorophyll a and chlorophyll b were obtained using Strain and Sweck method, which is based on the chromatography of a mixture of photopigments (extracted from 200 g nettle leaves) on a powdered sugar with 4% starch column. As solvents we used petroleum ether 2,5-2% n-propane. Chlorophylls were kept in n-pentane.

The VIS absorption spectra were performed using a Perkin-Elmer Lambda 2S spectrophotometer, and fluorescence spectra were plotted with an Amico-Bowman spectrofluorimeter. The purity of the chlorophylls were spectrophotometrically estimated, and the results showed that the used pigments were of high purity (>99%).

Preparation of liposomes. Liposomes were prepared using ovallecinth phospholipids - EPC (MERCK), with average molecular weight GM = 800 g/mol, zero net charge at pH = 7 and transition temperature Tc = -10°C.

Multilamellar vesicles (MLV) were prepared accordingly to the thin film hydration method. Lipid solutions were obtained by dissolving precise amounts of phosphatidylcholine and chlorophylls in chloroform [6]. We prepared liposomes with chlorophyll a (chl a), chlorophyll b (chl b) and chlorophyll a+b (chl (a+b)), the molar ratio for all the lots being:

\[ \frac{q}{c_c} = \frac{1}{10} \]

Where: cc – chlorophyll concentration;
     cl – lipid concentration.

5.0 mL from each solution was introduced in a 100 mL round-bottomed flask. The solvent was evaporated in a Heidolph Laborota 4000 rotaevaporatory, at 20°C. The obtained dry lipid film was hydrated with 5 mL phosphate buffer KH2PO4 – Na2HPO4 (0.5 M). The mechanical stirring of the lipids was performed with the rotaevaporatory equipment at 25°C and by manual stirring in the water bath, for 2h, at the same temperature. This suspension was allowed to hydrate for 2 h in order to anneal any structural defects. Unilamellar vesicles (SUV) were obtained by sonication of the MLV liposomes, in a bath-type sonicator (Sono Swiss SW 6L) for 30 min. (6x5min.). The sonication temperature was above Tc of the lipids (a temperature under Tc determines structural defects in the bilayers of the liposomes, which conducts to the fusion of vesicles).

The emission spectra were plotted using an Amico-Bowmann spectrofluorimeter, and the absorption analysis were performed using a Perkin Elmer Lambda 2 S spectrophotometer, at λ – 660 nm in case of chl a and 642 nm in case of chl b.

Comparative study of chl a and chl b loaded liposomes were performed by absorption spectroscopy, on the preparation day and at 4 days after preparation.

The influence of pH on the liposomes suspensions was determined by calculating the relative fluorescence intensity at 1 day after preparation for liposomes with chl a. The pHs of the phosphate buffer used in these experiments were: 4.8; 5.8; 6.5; 7.2; 7.6 and 8. The liposomes suspensions were excited at λ = 430 nm (maximum of absorption spectrum from Soret band, for chl a).

For highlighting the energy transfer between chl a and chl b in liposomes, we prepared vesicles with chl a, chl b and chl (a+b), the last containing an approximately equal quantity of chl b and chl a. The molar ratio for all the liposomes was 1/10.

RESULTS AND DISCUSSION

Comparative study of chl a and chl b loaded liposomes. The liposomes containing chl a and chl b were prepared accordingly to the method presented in previously chapter. The spectroscopy analysis was performed for both types of liposomes immediately after
preparation and at 4 days after preparations. We registered the maximum extinction ($E_{\text{max}}$) and the corresponding wavelength. The obtained data are presented in table 1.

Data presented in the table show that in case of chl a loaded liposomes, after 4 days, a strong photo bleaching appeared due to the sensitivity of chlorophyll determined by light and oxygen from the air. The photo-oxidation of chl a conducts to a spectral change in Soret band, in the sense of reversal ratios between the two extinctions [7]. We used the ratio between extinction at 437 nm and 419 nm in order to estimate the photo-oxidation of chl a entrapped in these liposomes: $\frac{E_{437}}{E_{419}} = 0.84 < 1$, which confirms the photo – bleaching of chl a. On the other hand, in case of chl b loaded liposomes the spectrum does not indicate such a modification, which could probably mean that liposomes containing chl b remained in a stable state, aggregated in a trimer form, just before sonication.

**Influence of pH on the liposomal suspensions.** In order to monitor the influence of pH we prepared several lots of liposomes with chl a, lipidic films being suspended in phosphate buffer with following pH: 4.8; 5.8; 6.5; 7.2; 7.6 and 8. The experimental liposomes were excited at $\lambda = 430$ nm, maximum of the absorption spectrum from Soret band for chl a. and the relative fluorescence intensity was calculated. The obtained data are presented in table 2.

By analyzing the data presented in the table it can be observed that for all pHs, relative fluorescence intensity increases after 24h. The high percentage of the increased
fluorescence intensity (16% - 28.8%) shows that the evaporation of the solvent could not be the cause of that, but fluorescence extinction by concentration immediately after preparation [8]. After 24h, because of the mobility of the lipids, this effect is removed (the chlorophyll molecules are dispersed on the surface of the lipid bilayer).

Data presented in table 2 also show that at basic pHs (neutral – basic) there is a shift to smaller wavelengths, which can be explained by the tendency of chlorophyll molecules to deeper penetrate into bilayer.

For next experiments we prepared several lots of liposomes with chl a (Lchla), chl b (Lchlb), chl (a+b) (equal quantities) (Lchl a+b) and we also mixed liposomes with chl a with liposomes with chl b in equal quantities. The data obtained from processing the emission spectra are presented in table 3.

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>$\lambda_{\text{max excitation}}$ (nm)</th>
<th>$\lambda_{\text{max emission}}$ (nm)</th>
<th>$I_{\text{rel}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lchla</td>
<td>415</td>
<td>676</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>676</td>
<td>57</td>
</tr>
<tr>
<td>Lchlb</td>
<td>415</td>
<td>663</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>667</td>
<td>118.5</td>
</tr>
<tr>
<td>Lchl (a+b)</td>
<td>415</td>
<td>676</td>
<td>103.5</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>676</td>
<td>85.5</td>
</tr>
<tr>
<td>Lchla+ Lchlb mixed</td>
<td>415</td>
<td>676</td>
<td>112.5</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>662</td>
<td>82.5</td>
</tr>
<tr>
<td>Lchla+ Lchlb 1h after mixing</td>
<td>415</td>
<td>676</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>438</td>
<td>663</td>
<td>72</td>
</tr>
</tbody>
</table>

It can be observed that, in case of the suspension obtained by mixing equal quantities of liposomes with chl a and liposomes with chl b, there are two emission peaks, corresponding to chl a, respectively chl b in liposomes. This shows that there was no energy transfer, probably because of the large distances between chlorophyll molecules [9].

By exciting the liposomes with chl (a+b) at Soret band absorption peaks of chl a and chl b in liposomes, a single peak appears, at $\lambda = 676$ nm, corresponding to the maximum of chl a in liposomes[9]. This, together with an increase in relative fluorescence intensity of chl a and a decrease in relative fluorescence intensity of chlb conduct to the conclusion that there was an energy transfer between chl b and chl a.

**CONCLUSIONS AND FUTURE WORK**

Experiments conducted for comparison of liposomes loading chl a and chl b show that liposomes containing chl b are more stable than the ones containing chl a (in case of chl a loaded liposomes a strong photo-oxidation takes place at 4 days after preparation).

Studies of the effect of pH on the stability of liposomes showed a change in emission spectra at 1 day after preparation (a shifting toward smaller wavelengths and an increase of relative fluorescence intensity appeared in case of neutral – basic pHs.

The experiments performed for the qualitative demonstration of the energy transfer between chl b and chl a entrapped in liposomes showed that this might be possible when using approximately equal quantities of chl a and chlb and incorporating the chlorophylls in the same suspension.
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RESEARCHES CONCERNING MICROBIOTA IN THE FERN RHIZOSPHERE FROM VÂLSAN VALLEY PROTECTED AREA

L.C. Soare, I. Deliu, C.M. Dobrescu

Abstract: Soil is an important ecosystem with various microorganisms; they play a role in soil formation, fertility, stabilization and evolution. Soil microorganisms have very diverse biochemical characteristics; they take part in all chemical processes in soil. Many factors influence the activities of bacteria, microfungi, algae and protozoans which constitute the soil microbiota. In this paper are presented the quantitative and qualitative data about the fern rhizosphere microbiota (for four species of fern from Valsan Valley protected area), especially about eubacteria, cyanobacteria and algae. The soil samples were obtained from different spots, from rhizosphere of four species of fern and were further processed in laboratory by directly inoculation in sterile media of soil dilution. The samples presented bacteria ($8938.3 \times 10^6 - 27652.72 \times 10^6$ CFU/ml) and an important taxonomic diversity of cyanobacteria and soil algae.

Key words: microbiota, cyanobacteria, soil algae

INTRODUCTION

Soil is an important ecosystem with various microorganisms. Soil microorganisms have very diverse biochemical characteristics; they take part in all chemical processes in soil. Many factors influence the activities of bacteria, microfungi, algae and protozoans which constitute the soil microbiota. Edaphic algae are an important autotrophic component of the biota in all terrestrial habitats. They play a significant role in soil genesis, stabilization of substrata and the formation of organic matter in natural and technogenic soils [6, 9, 10, 13]. The bacteria are most numerous and most active microorganism from soil, and Gram negative bacteria are more frequent than Gram positive bacteria [11]. The microorganisms influence plants through changes of soil structure and through decomposition of different toxic substances, accumulation in the soil which could lead to plants death [14]. The aim of this study was to analyze the bacteria and algae from some fern rhizosphere.

MATERIAL AND METHODS

The soil samples were obtained from different spots, from rhizosphere of four species of fern: Asplenium scolopendrium L. (3 sites), A. trichomanes (2 sites), Cystopteris fragilis, Selaginella helvetica. The soil samples were collected from Valea Valsanului area. The sampling was conducted in compliance with all rules of conserving sterility. The samples were collected and kept until the research in labelled sterile packs made of hard paper [2]. To identify algae the indirect method was used, by spontaneous culture [4] and the following determiners: Hindák et al. (1975) [5], Ionescu & Peterfi 1979, 1981 [7, 8], Nogy-Toth & Barna, 1998 [12]. Arranging the algae in systematic classification units was done according to Cavalier-Smith [1].

The soil samples were processed in laboratory by making a suspension in sterile saline solution for viable bacterial microbiota. Decimal dilutions of suspensions ($10^{-6}, 10^{-7}, 10^{-8}$) were inoculated by incorporation in nutrient agar in three repetitions each. The bacterial colonies were obtained after five days at 22°C. The colonies were counted using the Funke Gerber colony counter and the number of colony - forming units per ml (CFU/ml) was calculated, taking into account the average values of all the three repetition and the factor of dilution. For those species which had more than one site, we calculated the average of values.
RESULTS AND DISCUSSION

Were identified 18 taxa in Asplenium scolopendrium soil samples; these belong to phyla: Cyanobacteria, Ochrophyta (subphylum Diatomeae) and Chrysophyta (Table 1). The soil samples quantitative analysis of cyanobacteria and edaphic algae denote prevailing of taxa from Bacillariophyta phylum (67%), followed by Cyanobacteria phylum (28%) and Chrysophyta phylum (5%) - Figure 1. The largest number of specimen/soil gram was registered for Synechococcus (39100 specimen/soil gram in 060 sit sample and 10760 specimen/soil gram in 074 sit sample), followed by Cylindrospermum sp. 5720 specimen/soil gram and Pinularia with 5140 specimen/soil gram. The entire number of specimen/soil gram was varied between 7400 and 50360.

In Asplenium trichomanes soil samples were identified 11 genus/species from Cyanobacteria, Ochrophyta and Chlorophyta phyla (Table 1). The bacillariophyta were prevailing with 55 percentages, followed by cyanobacteria with 36% and green algae with 9% (Figure 1). Conforming with quantitative registered data, the largest number of specimen/soil gram was determined for Pinularia sp. (12200 specimen/soil gram in 060 sit sample and 3420 specimen/soil gram in 071 sit sample), followed by Navicula nivalis with 4800 specimen/soil gram. The entire number of specimen/soil gram was varied between 8820 and 20110.

<table>
<thead>
<tr>
<th>Identified cyanobacteria and algae</th>
<th>Pteridophyta</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>As</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Cyanobacteria</td>
<td></td>
</tr>
<tr>
<td>Cylindrospermum Kützing</td>
<td>5720</td>
</tr>
<tr>
<td>Nostoc commune Vaucher</td>
<td>60</td>
</tr>
<tr>
<td>Oscillatoria Vaucher ex</td>
<td></td>
</tr>
<tr>
<td>Gomont</td>
<td></td>
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<tr>
<td>Phormidium Kützing ex Gomont</td>
<td></td>
</tr>
<tr>
<td>Synechococcus sp.</td>
<td></td>
</tr>
<tr>
<td>Ochrophyta</td>
<td></td>
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<tr>
<td>Amphora sp.</td>
<td></td>
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<tr>
<td>Asterionella formosa Hassall</td>
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<tr>
<td>Cyclotella sp.</td>
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<tr>
<td>Diatoma vulgaris Bory</td>
<td></td>
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<tr>
<td>Fragilaria capucina</td>
<td></td>
</tr>
<tr>
<td>Hantzschia amphioxys (Ehrenberg) Grunow</td>
<td>200</td>
</tr>
<tr>
<td>Navicula nivalis Ehrenberg</td>
<td></td>
</tr>
<tr>
<td>Navicula sp.</td>
<td></td>
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<tr>
<td>Navicula sp.1</td>
<td></td>
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<td>Navicula sp.2</td>
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<tr>
<td>Navicula sp.3</td>
<td></td>
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<tr>
<td>Nitzschia amphibia Grunow</td>
<td></td>
</tr>
<tr>
<td>Pinnularia viridis (Nitzsch) Ehrenb.</td>
<td>2840</td>
</tr>
<tr>
<td>Synedra sp.1</td>
<td></td>
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<tr>
<td>Synedra sp.2</td>
<td></td>
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<tr>
<td>Chlorophyta</td>
<td></td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td></td>
</tr>
<tr>
<td>Chrysophyta</td>
<td></td>
</tr>
<tr>
<td>Tribonema sp.</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria and algae (whole number)</td>
<td>50360</td>
</tr>
</tbody>
</table>
Were identified 12 taxa from *Cyanobacteria* and *Ochrophyta* phyla in *Cystopteris fragilis* rhizosphere (Table 1). Like previous samples, was observed the prevailing of bacillariophyta (67%), followed by cyanobacteria with 33% (Figure 1). The quantitative analysis indicate the largest number of specimen/soil gram was registered for *Navicula* sp.1 (46260 specimen/soil gram), followed by *Pinularia* sp. (14920 specimen/soil gram) and *Nitzschia* sp. (13280 specimen/soil gram). The whole number of specimen/soil gram was varied between 60 and 46260.

Among the identified taxa for *Selaginella helvetica* rhizosphere soil sample was noticed, like previous samples, the prevailing of diatoms, with 73%, followed by cyanobacteria with 27% (Figure 1). The quantitative analysis indicate that the whole number of specimen/soil gram for this soil sample was approximately 12000. The largest number of specimen was registered for *Pinularia* sp. (6780 specimen/soil gram).

![Figure 1](attachment:Figure1.png)

**Figure 1** The share of cyanobacteria and algae phyla in studied samples.

The largest number of genera and species, 15 genera and 18 species was determined in *Asplenium scolopendrium* rhizosphere (Figure 2).

![Figure 2](attachment:Figure2.png)

**Figure 2** Taxonomic diversity (genera/species) of identified cyanobacteria and algae.

The *Hantzschia amphioxys* and *Pinnularia viridis* species were identified in all samples. *Hantzschia amphioxys* can be considered cosmopolitan, being found in different soil types [3].

From a quantitative point of view, the bacterial microbiota was properly represented. The results of soil samples analysis are presented in Figure 3.
Was observed that the determined values are approximately between $900 \times 10^6$ CFU/ml and $27000 \times 10^6$ CFU/ml; these values are normal for a microbiota from abundant soil. The lowest values was registered for *Asplenium scolopendrium* rhizosphere soil sample, and the largest values was determined for bacterial microbiota from *Selaginella helvetica* rhizosphere.

![Graph](image)

**Figure 3** The quantitative determination for bacterial microbiota from soil samples

**CONCLUSIONS AND FUTURE WORK**

The largest number of genera and species of cyanobacteria and algae was determined in *Asplenium scolopendrium* rhizosphere. The *Hantzschia amphioxys* and *Pinnularia viridis* species were identified in all samples. In *Cystopteris fragilis* rhizosphere was determined the largest number of specimen/soil gram. The determined values were not homogenous for bacterial microbiota, but without important differences between the soil samples, depending on fern species rhizosphere. In the future work we will analyze the rhizosphere of other species of ferns.

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ACKNOWLEDGEMENTS

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COMPOSITION AND DISTRIBUTION OF CHEMICAL ELEMENTS IN AEROSOLS FROM NORTHERN REGIONS OF THE CASPIAN SEA

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Abstract: Characteristic features of the composition and distribution of chemical elements in aerosols over northern Caspian were considered. They can be used for a more reliable prediction of possible negative consequences of atmospheric pollution related to the beginning of the large-scale exploitation of oil and gas deposits in the shelf zone Caspian Sea. It was shown that the contents of aerosols, their grain-size composition, and major element composition change under the influence of different factors (transboundary transport of terrigenous dust by air masses, variations in the intensity of turbulent and convective mixing in the near-surface atmosphere, variations in air humidity). The contents of chemical elements in the samples were determined by the neutron activation analysis and mass spectrometry.

Key words: elemental composition, aerosols, neutron activation analysis and mass spectrometry, environmental protection, transboundary transport.

INTRODUCTION
Development of oil and gas deposits on the Caspian sea shelf extends. Industrial emissions of polluting matter and first of all toxic elements can be increased. They can be transferred in aerosol form together with air masses on large distances and worsen quality of atmospheric air. It was important to estimate a role of regional sources in formation of chemical composition of aerosols and possibility of environment pollution in connection with their transfer. Among the main sources reflecting regional composition of aerosols - mainly sea salt; aluminosilicate minerals, carbonate rocks, and elements – indicators are Na, Br; Al, Fe, Sc, Ti, K and Ca, Mg, Ba, Sr, accordingly [1].

Earlier similar problems we solved for oceanic aerosols of northern hemisphere [2]. In this work as the first stage of the decision problems mentioned above we have tried to estimate influence of natural processes and substance sources on distribution in aerosols of the mentioned elements reflecting: presence of sea salt, silicate minerals and carbonate material.

SAMPLING AND ANALYSIS
Sampling of atmospheric aerosols was carried out during 3 expeditions to Northern Caspian sea: in August, 2000 (Chistaya Banca Island); in November, 2000 (~10 km from Lagan); in May-June, 2001 (repetition of supervision in Lagan region, fig. 1).

Samples were selected on nuclear filters (small fraction, particles size <2 microns), metal plates (large fraction, size 2-10 μm) and Petrjanov filters. During the sampling we considered also weather conditions (wind speed and direction (see fig. 3), temperature and humidity of air, atmospheric pressure, etc.). The content of elements in aerosols were measured by neutron-activation analysis [3,4] and mass-spectrometry method (NAA, fig. 2 and MS).
Fractionation factors was used for identification of substance sources in the aerosols

\[ (K_{fr}) = \frac{C_i}{C_r} \text{ aerosol sample} / \frac{C_i}{C_r} \text{ matter source}, \]

where \( C_i \) and \( C_r \) are the contents of element \( i \) and the reference element \( r \), respectively, in the aerosols and sources (in ppm).

**RESULTS AND THEIR DISCUSSION**

1. Factors Controlling Seasonal Variability in the Major-Elements of Aerosols

From the data, given, for example, in tabl.1, it is possible to make a conclusion about the seasonal changeability of aerosols composition. However, the variability trends of reference elements in the basic assumed components of aerosols composition are not identical. Let us examine briefly each of them.

**Aluminosilicate minerals.** For Fe, K, Ti it is characteristic (3-10) - multiple increase in concentration in the interval from spring till autumn while for Sc and Al the return tendency - reduction of their maintenance in 1,5 – 3 times in the autumn in comparison with spring is observed.

Observable seasonal fractionation (table. 1) specified lithophile elements in aerosols can be explained due to the following factors:
Table 1. Seasonal variations in the concentration of indicator elements in the aerosols of the near-surface atmospheric layer over the Northern Caspian (ng/m³ of air)

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>1) 2**) 3***)</td>
<td>1  2  3</td>
<td>1  2  3</td>
</tr>
<tr>
<td></td>
<td>Al</td>
<td>Fe</td>
<td>K</td>
</tr>
<tr>
<td>Al</td>
<td>284</td>
<td>546</td>
<td>85</td>
</tr>
<tr>
<td>Fe</td>
<td>546</td>
<td>5049</td>
<td>658</td>
</tr>
<tr>
<td>K</td>
<td>85</td>
<td>1940</td>
<td>658</td>
</tr>
<tr>
<td>Sc</td>
<td>0.45</td>
<td>2.16</td>
<td>0.86</td>
</tr>
<tr>
<td>Ti</td>
<td>15</td>
<td>284</td>
<td>546</td>
</tr>
<tr>
<td></td>
<td>Aluminosilicate minerals</td>
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</tr>
<tr>
<td>Ca</td>
<td>682</td>
<td>10779</td>
<td>3901</td>
</tr>
<tr>
<td>Mg</td>
<td>168</td>
<td>2126</td>
<td>673</td>
</tr>
<tr>
<td>Sr</td>
<td>2.7</td>
<td>116.0</td>
<td>36.5</td>
</tr>
<tr>
<td>Ba</td>
<td>9.2</td>
<td>40.7</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>Carbonate minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>384</td>
<td>3121</td>
<td>1491</td>
</tr>
<tr>
<td>Br</td>
<td>1.2</td>
<td>10.2</td>
<td>4.4</td>
</tr>
</tbody>
</table>

1*, 2**, 3***) - are the minimum, maximum and average concentrations of chemical elements, respectively

1) change in granulometric composition of aerosols under the influence of ascending and descending air streams and 2) regional variations of the basic chemical composition of initial terrigenous material, caused by change in the directivity of air masses - from the North Western (in spring), to southeast (in autumn) and vortex (in summer).

From data of table 1 also follows high (10 times) difference between the minimum and maximum content of the elements in ground air of studied areas that is possible to explain by influence of consecutive passage of the air masses generated in the Central Asian deserts and in the south of the European part of Russia.

The above mentioned tendencies of seasonal changeability for Fe, K, Ti content from one side, and for Sc, Al content - with another, can be assumed, they are caused, first of all, by different chemical composition of the terrigenous matter, supplied from the Central-Asian deserts (autumn) and the south of Russia and Ukraine (spring). It is obvious that the local sources of terrigenous material play auxiliary role in the forming of the seasonal changeability of the distribution of lithophile elements in the aerosols.

Carbonate minerals. A decrease in the concentration of all alkali earth elements (Ca, Mg, Ba, Sr) in air was observed in autumn compared with the other seasons (table 1). This could be due to the dissolution of submicrometer-sized carbonate particles with increasing air humidity. The maximum concentrations of these elements were documented in summer during the intense development of phytoplankton and macrophytes around Chistaya Banka Island, when biogenic carbonate minerals are precipitated both in water and on the leaves of aquatic plants. Thus, biogeochemical processes in the water of the Northern Caspian significantly affect the contribution of alkali earth elements into the chemical composition of aerosols during the summer period.

Sea salt. The content of sodium, which is the main component of sea salt, shoved an almost tenfold increase in the near-surface layer in autumn compared with spring. Since the content of Na in aerosols over the Northern Caspian in autumn is higher by factor of
than that over the ocean [1], it is reasonable to suppose that the salt particles were transported from the surface of the dried Aral Sea. This suggestion is based on the fact that the sandy soils of the Lagan’ district of Kalmykia show a Na content $2.38 \times 10^{-2}$ g/g. The content of Na in seawater is 0.46 mol/l or 0.29 g/g of salt formed by its evaporation in air. This implies that, at equal masses of salt and terrigenous material input into the lower atmosphere, the content of Na in the silicate component of aerosols will be an order of magnitude lower than in the salt component. Bromine forms volatile compounds and shows the lowest content in the near-surface layer in summer time (probably, owing to high temperature) whereas its content increase in spring and autumn by a factor 30 and 135, respectively.

It is interesting to notice also that the content of chemical elements in atmosphere over Northern Caspian increases (table 2) with height and the maximum of their concentration is on horizon of 1200 m.

It is connect with the process of movement of air masses from Central Asian deserts to the Northern Caspian, the grain-size and chemical compositions of aerosols change. It is origins under influence of the following factors: a) dry sedimentation of transported material, the intensity of which depends on the rate of the gravitational settling of particles and the coefficient of the turbulent diffusion of the air flow and b) washing out of aerosols in a cloud owing to the condensation of water vapor on their surface. The efficiency of this process varies depending on the composition and size of particles from 90-100 % for sea salt and large particles to 50-60% for terrigenous particles from 0.02 to 0.2 μm in radius. In during of washing out of sea salt and large particles from the air column beneath the cloud base.

Table 2. Increase in the concentration of aerosols and their enrichment factor in chemical elements at altitude of 700 and 1200 m above the Northern Caspian relative to the near-surface air layer (Spring2001)

| Element | Concentration, ng/m$^3$ | | | | | | Enrichment factor relative to surface air |
|---|---|---|---|---|---|---|---|---|
| | 150 m | 1200 m | 150 m | 1200 m | 150 m | 1200 m | 150 m | 1200 m |
| Al | 1485 | 2586 | 7518 | 12411 | 0.96 | 1.9 | 1.9 | 9.1 |
| Fe | 3150 | 6142 | 5602 | 8004 | 1.4 | 1.9 | 2.5 | 3.6 |
| Ti | 616 | 916 | 1018 | 1457 | 10.2 | 15.3 | 17.0 | 24.3 |
| K | 2245 | 4307 | 4550 | 7511 | 3.4 | 6.5 | 6.9 | 11.4 |
| Sc | 1.3 | 2.7 | 2.6 | 3.6 | 1.5 | 3.2 | 3.0 | 4.2 |
| Ca | 18091 | 34792 | 40434 | 47986 | 4.6 | 8.9 | 10.4 | 12.3 |
| Mg | 1485 | 3958 | 2586 | 12411 | 2.2 | 5.9 | 3.8 | 18.4 |
| Sr | 43.1 | 78.7 | 88.7 | 77.1 | 1.2 | 2.2 | 2.4 | 2.1 |
| Ba | 64 | 168 | 160 | 224 | 2.5 | 6.5 | 6.2 | 8.6 |


The combined influence mentioned above processes (and also dust storms) results in relative accumulation of terrigenous particles with radii from 0.05 to 0.1 μm at an elevation of 1200 m in comparison with horizon of 150 m, which was responsible for the enrichment of air by chemical element from Al to Ba (table 2).

In general, it can be concluded that the seasonal variability in the major-element salt composition of aerosols in the near-surface air of the Northern Caspian is controlled mainly by the flux of atmosphere during the transboundary movement of air masses. Biochemical processes in the underlying land and ware area of the Northern Caspian play an important role in the distribution of alkali earth elements in aerosols (fig. 2).
2. Factors Controlling Variability in the Major-Elements of Aerosols (by cyclonic and anticyclonic types of air circulation)

Except a seasonal course of changes of general movement of air masses additional (short-period from 1 till 9 days) variations of the meteorological situations were also observed caused by passage of cyclones (an ascending stream), anticyclones (a descending stream) and streams of air weights along a spreading surface. Duration, capacity and a parity of these streams strongly influence on composition of aerosols.

In atmosphere during this period there is a transformation granulometric and a chemical composition of aerosols. It has been confirmed in the course of studying of aerosols composition in a cyclone and especially anticyclones in August, 2000 (12.08 and 21.08) on an island Pure Bank by comparison of reference elements distribution in two granulometric fractions of aerosols with the size <2 μm s and 2–10 μm (fig. 4).

So, it has been established that on August, 12th, 2000 on an outcome anticyclonic period the content of major of investigated chemical elements in ground air prevailed in fine dispersed (<2 μm) aerosols fractions, and on August, 21st, 2000 concentration of lithophylic elements (Sc, Fe) in air has been caused by coarse dispersed aerosols which have arrived from the Central Asian desert.

As to behavior of K, it usually is in structure of clay minerals of submicronic size and consequently it is more extended in fine dispersed fractions of soils and in aerosols. The case of the noticeable presence of potassium in aerosols fraction with the size 2-10 μm (Fig. 4, f) can be explained by both the condensation and coarsening of clay minerals under the effect of the atmospheric moisture and by entering of soil particles in the period of cyclone (on August 21, 2000.)
Distribution of Ba in granulometric fractions, apparently, influences both an atmospheric moisture, and the content of SO$_4^{2-}$ ions in it which form aerosols with diameter more than 2 μm, and BaSO$_4$ compound is badly solvable. These processes, possibly, also influence in basic Ba distribution (fig. 4, e). Redistribution of Sr between granulometric fractions of aerosols has similar mechanism.

The revealed examples of interrelation between the basic chemical composition and granulometric fractions at passage of cyclones and anticyclones to region testifies about presence of trends of short-period variability of quality of ground air during several days.

3. Diurnal variations in the parameters of geophysical processes and the major-element compositional of aerosols.

It has been noticed that more favorable conditions for aerosols sedimentation are at night time, and in the afternoon there is their carrying out from a spreading surface because of the influence of turbulent and convective processes in atmosphere.

These and supervision set allow to ascertain that the basic chemical composition of terrigenous material after its mobilization in atmosphere undergoes considerable changes depending on a season of year, heights of transboundary transfer, type of atmospheric circulation, and also a daily course of dynamic and physical and chemical processes in a ground layer of air. All it influences on fractionation the basic chemical composition of aerosols over Northern Caspian sea concerning their likelihood sources, namely – soils and sedimentary rocks.
4. Fractionation of the basic chemical composition of aerosols over Northern Caspian sea.

As it was marked, sources of terrigenous matter arriving in aerosols of Northern Caspian sea under the influence of transboundary transfer of air masses can be a Near-Caspian lowland (summer), the south of Russia and Ukraine (spring), Kazakhstan and the Central Asian deserts (autumn). The spreading surface of Near-Caspian region also can make essential impact on the basic chemical composition of aerosols under the influence of mobilization of soil particles because of cyclonic and convective streams of air.

We used fractionation factor \( (K_{fr}) \) as an indicator of influence of possible sources of matter on chemical composition of aerosols.

As possible sources of chemical elements receipt in atmosphere by us were considered: a) sedimentary rocks (average composition of earth crust and its fine dispersed clay-silt component are accepted according to [5,6]); soils of the Astrakhan reserve located in a flood plain of Volga river chemical composition of which was generated in the processes of geochemical transformation of a solid river drain [6]. This composition reflected, thus, specific properties of terrigenous material of all water modular area of Volga (including the south of Russia) and including submicronic (\(<1 \mu m\)) and large (\(>10 \mu m\)) particles of Kalmykia soils [1].

Data is resulted in table 3. It follows from them that the average content of Ca in sedimentary rocks in comparison with that in their clay-silt component is 3 times higher, and Ca and Sr (in two granulometric fractions of Kalmykia soil) – 1.6 - 1.7 times higher. For all other chemical elements the opposite trend is observed: growth of their concentration in 1.5 – 4 times in submicronic particles.

The average content of the basic chemical elements in soils of a flood plain of the Volga river is (within errors) at level of their contents in studied granulometric fractions of Kalmykia soils. It is obvious that such distribution of chemical elements in flood plain soils is caused granulometric content of soils generated as a result of mixture of river sand and clay silt. The exception makes Ca, which content is in 2 – 4 times above, than in Kalmykia soils. The most likelihood reason of such enrichment is presence of carbonate particles of bowls detritus in the flood plain of Volga river.

**Table 3. Average content of elements in the major chemical composition of the possible sources of terrigenous material for the aerosols of the Northern Caspian**

<table>
<thead>
<tr>
<th>Element</th>
<th>Sedimentary rocks, % [5, 6]</th>
<th>Soils of the Caspian region, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Composition of the Earth's crust</td>
<td>Clay-silt rock</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>4.0</td>
<td>4.97</td>
</tr>
<tr>
<td>Fe</td>
<td>3.46</td>
<td>5.0</td>
</tr>
<tr>
<td>Ti</td>
<td>0.3</td>
<td>0.39</td>
</tr>
<tr>
<td>Ca</td>
<td>3.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Mg</td>
<td>1.33</td>
<td>1.33</td>
</tr>
<tr>
<td>Sr</td>
<td>0.025</td>
<td>-</td>
</tr>
<tr>
<td>Ba</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Na</td>
<td>0.58</td>
<td>0.48</td>
</tr>
<tr>
<td>Br</td>
<td>0.0006</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>1.26</td>
<td>1.54</td>
</tr>
<tr>
<td>Sc</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4. Fractionation factors of chemical elements in the aerosols of the Northern Caspian relative to sedimentary rocks for various seasons

<table>
<thead>
<tr>
<th>Element</th>
<th>Spring 2001</th>
<th>Summer 2000</th>
<th>Autumn 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.19</td>
<td>0.19</td>
<td>7.1</td>
</tr>
<tr>
<td>K</td>
<td>1.53</td>
<td>1.56</td>
<td>22.8</td>
</tr>
<tr>
<td>Sc</td>
<td>0.053</td>
<td>0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Ti</td>
<td>0.59</td>
<td>15.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Ca</td>
<td>3.82</td>
<td>87.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Mg</td>
<td>15.1</td>
<td>-</td>
<td>3.6</td>
</tr>
<tr>
<td>Sr</td>
<td>4.5</td>
<td>1.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Ba</td>
<td>1.6</td>
<td>12.2</td>
<td>17.0</td>
</tr>
<tr>
<td>Na</td>
<td>7.6</td>
<td>14.2</td>
<td>242.0</td>
</tr>
<tr>
<td>Br</td>
<td>21.5</td>
<td>17.1</td>
<td>-</td>
</tr>
</tbody>
</table>

1* – Average composition of the Earth’s, 2* – Clay-silt rock.

Table 5. Fractionation factors of chemical elements in the aerosols of the Northern Caspian relative to the soil cover of the Caspian region for various seasons

<table>
<thead>
<tr>
<th>Element</th>
<th>Spring 2001</th>
<th>Summer 2000</th>
<th>Autumn 2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.27</td>
<td>0.23</td>
<td>10.2</td>
</tr>
<tr>
<td>Ti</td>
<td>0.44</td>
<td>1.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Ca</td>
<td>57.3</td>
<td>252.7</td>
<td>135.0</td>
</tr>
<tr>
<td>Mg</td>
<td>1.6</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Sr</td>
<td>1.8</td>
<td>28.0</td>
<td>26.6</td>
</tr>
<tr>
<td>K</td>
<td>1.8</td>
<td>0.81</td>
<td>26.6</td>
</tr>
</tbody>
</table>

1* and 2* - Kalmykia, soil particles <1 μm and > 10 μm in diameter, respectively, 3** - Average chemical composition of soils from Astrakhan nature reserve in the floodplain of Volga. Notes: Concentration of Al in the air was taken to be 1000 ng/m³.

The obtained data was used then for calculation of value (K_{fr}) chemical elements in aerosols concerning a possible source of terrigenic matter. Was accepted that if the source of terrigenous material plays a dominating role in formation of the basic chemical composition of aerosols it was supposed that K_{fr} value will fluctuate within 1.0 ± 0.5.

As appears from data in tables 4 and 5, during autumn time enrichment (within an order) of aerosols by lithophyle elements concerning sedimentary rocks and soils of Near-Caspian region (that confirms earlier assumption of dust from the Central Asian deserts receipt in atmosphere of Northern Caspian sea, together with the South Caspian cyclones) is observed.

The similar tendency of seasonal variability took place for Na and Br, testifying to receipt of sea salt from a high part of Aral sea.

In summertime aerosols are depleted by Br (as it was marked, apparently, as a result of Br evaporation from their surface at high temperatures), as K_{fr} concerning sedimentary rocks decreases for two order in comparison with other seasons.

For group alkali-earth elements other law of seasonal variability of fractionation process in aerosols is fixed: in the summer - enrichment of aerosols by Ca (concerning sedimentary rocks and Kalmykia soils), Sr (rather fine dispersed fractions of Kalmykia soils) and Ba (concerning sedimentary rocks). There are reasons to believe that it is caused by influence of biogeochemical processes on alkali-earth elements migration in system water mass - atmosphere.
Presence of a trend of seasonal variability for processes fractionation lithophyle and alkaline elements in aerosols testifies to a dominating role of transboundary transfer of terrigenous matter in formation of chemical composition of aerosols over Northern Caspian sea.

Thus, it is possible to conclude that by working out of forecasting models of quality of air environment in the bottom layers of atmosphere in investigated region of Northern Caspian sea it is necessary to consider the revealed trends of natural time variability for the basic chemical composition of aerosols and, thus, to predict accumulation, transfer and deducing of toxic components from region.

CONCLUSIONS
1. The composition of aerosols over the Northern Caspian shows seasons variability. The concentrations of Fe, K and Ti, which are transported with aluminosilicates, show an increase by a factor of 3-10 during the period from spring to autumn, whereas the content of aluminosilicate Al and Sc decrease simultaneously by a factor of 1.5-3.0/ The same tendency (a tenfold increase in concentration in autumn compared with spring) was observed for Na, the main component of sea salt. The observed fluctuations are related to the transboundary transport of material by air masses between southern Russia and Central Asian deserts. The alkali earth elements are associated with carbonates, and their contents in air decrease in autumn/ The maximum concentration of these elements in summer can be attributed to intense biochemical processes in the water region of the Northern Caspian/

2. The seasonal variations in the chemical composition of solid aerosols over the Northern Caspian are accompanied by short-period changes in the composition of aerosols under the influence of cyclones and anticyclones/ They affect the grain size composition of aerosols, which results in variations in the chemical composition with a periodicity of 2-9 days/ In the experiments above Chistay Banka Island in august 2000, the high concentrations of Ba, Fe, and Sc were related to coarse aerosols fractions, and that of K, to finely dispersed aerosols.

3. The concentrations of all of the elements studied in the air column over the Northern Caspian increase from the underlying surface to an altitude of 1200 m.

4. Based on the normalization of element contents to the reference concentrations in the possible sources of materials, was established that, in the spring of 2001, the main sources of Fe, Ti, Sr, and K in aerosols were the soils of Kalmykia and that main sources of Mg and K were the soils of the Caspian nature reserve. In the summer of 2000, Fe was supplied into the aerosols of the Northern Caspian from Kalmykia.

REFERENCES
INFLUENCE OF PECTIN CONCENTRATION AND STORAGE TIME ON THE COLOR STABILITY AND ANTIOXIDANT PROPERTIES OF RASPBERRY JAM

M.A. Poiana, D. Moigradean, D. Dogaru, I. Gergen, C. Mateescu and T. Trasca

Abstract. In this study have been evaluated the impact of different pectin concentrations and storage time on the antioxidant activity, color quality (expressed by color intensity and percent of polymeric color) and on the some bioactive compounds (total polyphenols, monomeric anthocyanins, vitamin C) of raspberry jam. Increasing the pectin dose induces retention of greater amounts of bioactive compounds in raspberry jam. Also, jams color intensity was more pronounced and the polymeric pigment formation occurred in a lesser extent. During storage at room temperature of raspberry jams both the content of investigated compounds and color quality recorded some depreciations whose magnitude increases with storage time. 

Key words: FRAP, Polyphenols, Vitamin C, Polymeric color, Anthocyanins, Low-sugar raspberry jam

INTRODUCTION
Raspberries have received much attention due to their positive role in human health and disease prevention [3]. Multiple technologies have been employed to process fresh raspberries into various value-added products for long-term preservation. Processing methods varying in the number of processing steps, heating temperature, duration, pectin concentration, sugar content, storage time can markedly affect the anthocyanin content, colour stability and antioxidant capacity of obtained jams [3, 7]. The quality of the colour may influence consumer’s acceptance. It is therefore essential that jam is prepared and stored at a temperature which will maximise colour stability. Anthocyanins have a critical role in the colour quality of processed raspberry. They are a good source of natural antioxidants, but they are quite unstable during processing and storage. Temperature, pH, oxygen and water activity are considered to be important factors influencing its stability. During processing and storage, degradation and polymerisation usually lead to its discolouration [3, 8]. Pectin is primarily used in food industry as a gelling agent for jams, jellies, and other foods. To obtain low-sugar jams are recommended low methoxyl (LM) pectin with degree of esterification DE<50% [8, 9]. LM pectin gelation is considered as the formation of a continuous network of ionic cross linkages via calcium bridges. These develop between the carboxyl groups belonging to two different chains in close proximity [5]. Because the information is limited regarding the influence of pectin concentration and storage time on the nutritional quality and color of raspberry jam, the purpose of this study is to assess the impact of these factors on the antioxidant capacity, total phenolics, vitamin C, monomeric anthocyanins and color quality of raspberry jam. Color and bioactive compounds were monitored during three months of storage at 20°C. Practical application of this work is that the obtained data will be very useful to optimize the receipt of raspberry jam and storage conditions in order to improve the quality of these products.

MATERIALS AND METHODS
Samples. Raspberries fruits (harvested in 2009 at the fully ripe stage from western Romania) were washed, drained and frozen using a home type freezer. The fruits were stored in freezer at -18°C in closed plastic boxes for less than 1 month prior to processing. Prior to jam preparation, fruits were semi-thawed. For jams preparation followed materials were used: fruits (1000 g), sucrose (470 g), low methoxyl pectins LM 40 (3, 5, 7 and 10 g) and citric acid (4 g). Fruits, larger part of sucrose and citric acid were mixed and cooked at 80°C. Pectin was mixed with part of sucrose and added at the final stage of the jam cooking. When the cooked mass reached 45°Brix the jams were filled into hot glass jars,
capped and pasteurized at 80°C for 10 min. They were allowed to cool at room temperature and stored in the dark until analysis. 

**Jams were analyzed** postprocessing after 1 day, and after 1 and 3 months of storage at room temperature (20°C).

**Sample analysis.** For total anthocyanins, total polyphenols, vitamin C and antioxidant capacity evaluation, three replicates of jam extracts were prepared according to Kalt et al. (1999) [6]. In the case of anthocyanins and antioxidant activity evaluation for extraction was used 95% (v/v) ethanol acidified with HCl (0.1%, v/v). For phenolics and vitamin C extraction was used 95% (v/v) ethanol.

**Total phenolics content** was measured spectrophotometrically using the Folin-Ciocalteu reagent [11]. Quantification of the data was calculated based on the calibration curve generated using gallic acid as the standard and the results was expressed as mM of gallic acid equivalents (GAE) per 100 g dry substances (ds) of jam.

**Total monomeric anthocyanins content** of fruits extracts were determined by the pH-differential method in accord with Giusti and Wrolstad (2001) [4]. The content of total anthocyanins was expressed as mg of cyanidin-3-glucoside per 100 g ds of jam.

**Color analysis.** Color density, polymeric color and percent of polymeric color of these samples were determined using the bisulfite bleaching method [4].

**Vitamin C (VC)** was measured by titration with a 2,6-dichlorophenolindophenol sodium salt solution and chloroform was used for intensely colored extracts [1].

**Total antioxidant capacity** was evaluated by FRAP (ferric reducing antioxidant power) assay. The FRAP method consists in the reduction of Fe³⁺ ions to Fe²⁺, which forms a blue-coloured complex with 2,4,6-tripiridyle-s-triazine (TPTZ). This reduction was monitored by absorption change measuring at 595 nm. The intensity of the colour depends on the antioxidant concentration [2]. Results were expressed as mmol of Fe²⁺ equivalents per 100 g ds of jam.

**RESULTS AND DISCUSSIONS**

Results of total phenolics content, vitamin C, monomeric anthocianyns content and FRAP values, comparatively presented for jams after 1 day of processing and after 1 and 3 months of storage, are presented in the Table 1. By analysis of results showed in Table 1 we can see that with increasing of pectin dose, the bioactive compounds are retained by a greater extent in raspberry jam.

<table>
<thead>
<tr>
<th>Raspberry jam</th>
<th>Anthocyanins (mg/100g ds)</th>
<th>FRAP values (mM Fe²⁺/100g ds)</th>
<th>Total phenolics (mM GAE/100g ds)</th>
<th>Vitamin C (mg/100g ds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>after 1 day of processing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10‰ LM 40</td>
<td>14.34</td>
<td>31.78</td>
<td>14.5</td>
<td>80.98</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>13.59</td>
<td>29.42</td>
<td>14.07</td>
<td>75.97</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>11.93</td>
<td>26.75</td>
<td>12.42</td>
<td>72.44</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>10.71</td>
<td>25.74</td>
<td>11.03</td>
<td>68.15</td>
</tr>
<tr>
<td>after 1 month of storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10‰ LM 40</td>
<td>12.31</td>
<td>28.09</td>
<td>12.93</td>
<td>77.29</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>11.27</td>
<td>25.71</td>
<td>11.84</td>
<td>70.87</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>9.64</td>
<td>23.01</td>
<td>10.24</td>
<td>65.97</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>8.39</td>
<td>21.78</td>
<td>8.7</td>
<td>61.19</td>
</tr>
<tr>
<td>after 3 months of storage</td>
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<tr>
<td>10‰ LM 40</td>
<td>10.52</td>
<td>24.59</td>
<td>10.65</td>
<td>72.22</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>9.61</td>
<td>21.16</td>
<td>9.57</td>
<td>66.19</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>7.92</td>
<td>18.82</td>
<td>7.82</td>
<td>60.31</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>6.89</td>
<td>17.26</td>
<td>6.57</td>
<td>53.57</td>
</tr>
</tbody>
</table>
It was noticed that increasing the dose of pectin from 3 to 10‰, monomeric anthocyanins content increased by 33.89%, total polyphenols content with 31.46%, vitamin C content with 18.82% and antioxidant capacity with 23.46%. Anthocyanins have a crucial role in the color quality of raspberry jams. LM pectins probably interact with anthocyanins more easily because they have fewer methoxyl groups [9]. The jam colour stabilization effect may be based on electrostatic interactions between the anthocyanin flavilium cation and the dissociated carboxylic groups of the pectin, in a similar manner as calcium ions are bound in pectin. Due to this association, anthocyanins may be prevented from water attack, which leads in turn to colour stabilisation [5]. With increasing of pectin concentration has been more effective anthocyanins stabilization due to formation of molecular associations between them and pectin. Due to these associations, anthocyanins may be prevented from water attack, which leads in turn to colour stabilisation [8, 9].

In the Figure 1 are given the losses incurred in the total monomeric anthocyanins content (A), vitamin C content (B), FRAP values (C) and total phenolics content (D) during jam storage.

![Figure 1. The losses of monomeric anthocyanins content (A), vitamin C content (B), FRAP values (C) and total phenolics content (D) during jam storage](image)

The effect of pectin concentration and storage time on raspberry jam color was quantified by measuring the color density, polymeric color and percent of polymeric color. Changes in the color parameters are shown in the Table 2. The percentage of polymeric color is a measure of the degree of anthocyanins polymerization [10, 12]. In terms of color quality alteration, it is important to mention that by increasing of pectin dose in the jam recipe, has registered a more intense color and the polymeric pigment formation occurred in a lesser extent. After a month of storage, the color intensity decreases with 2,6-6.8% of the value recorded after 1 day of processing, reaching 4,5-10% after three months of storage.
storage at room temperature. The color density showed a similar trend over time for all jam samples obtained with different pectin concentrations.

Table 2

<table>
<thead>
<tr>
<th>Raspberry jam</th>
<th>color intensity</th>
<th>polymeric color</th>
<th>percent of polymeric color (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>after 1 day of processing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10‰ LM 40</td>
<td>6.75</td>
<td>0.49</td>
<td>7.26</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>6.604</td>
<td>0.64</td>
<td>9.69</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>6.461</td>
<td>0.94</td>
<td>14.55</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>6.233</td>
<td>1.12</td>
<td>17.97</td>
</tr>
<tr>
<td><strong>after 1 month of storage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10‰ LM 40</td>
<td>6.573</td>
<td>0.57</td>
<td>8.67</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>6.351</td>
<td>0.78</td>
<td>12.09</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>6.123</td>
<td>1.05</td>
<td>16.50</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>5.807</td>
<td>1.23</td>
<td>20.03</td>
</tr>
<tr>
<td><strong>after 3 months of storage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10‰ LM 40</td>
<td>6.446</td>
<td>0.71</td>
<td>11.01</td>
</tr>
<tr>
<td>7‰ LM 40</td>
<td>6.195</td>
<td>0.92</td>
<td>14.85</td>
</tr>
<tr>
<td>5‰ LM 40</td>
<td>5.917</td>
<td>1.39</td>
<td>23.49</td>
</tr>
<tr>
<td>3‰ LM 40</td>
<td>5.606</td>
<td>1.67</td>
<td>29.79</td>
</tr>
</tbody>
</table>

It is remarkable that the rate of color loss is much slower than the rate of anthocyanin degradation. Similar to other thermally processed products [12, 13], anthocyanin losses during storage were accompanied by increases in percent of polymeric color because the anthocyanins were extensively polymerized during storage at 20°C. The progressive increase in polymeric color, as a measure of color degradation, was observed during the storage period of investigated jams: after 1 months the polymeric color of raspberry jam were situated in the range 8-20%, and after 3 months has reached 11-30%.

**CONCLUSIONS AND FUTURE WORK**

Color stability and antioxidant properties of raspberry jam are significantly influenced by the dose of pectin used in the formulation and storage period. By increasing the pectin concentration has been increase of monomeric anthocyanins content, total phenolics, vitamin C and FRAP values. Also, raspberry jams color intensity was more pronounced and the polymeric pigment formation occurred in a lesser extent. Jams obtained with pectin doses between 7-10‰ retained higher levels of anthocyanins, total phenolics, vitamin C and FRAP value and had lower polymeric color values than jams obtained with 3-5‰ pectin concentrations. During storage at 20°C of raspberry jams both the content of bioactive compounds and color quality recorded some depreciations whose magnitude increases with storage time; the antioxidant activity decreased due to decrease of total polyphenols and anthocyanins content. By increasing the storage period at room temperature it was observed the progressive increase in polymeric color, as a measure of color degradation in parallel with color intensity decreasing. Optimization of pectin dose in jam recipe and storage time would help to conserve the bioactive compounds and color quality.
Acknowledgments. This study was supported by S.C. Etco Europe Trade Company from Sebis, Arad country, research project no. 637/21.01.2009, Theme: Studies regarding the impact of technological treatments on the antioxidant characteristics of products obtained from berries, Project Manager: Ph.D Assoc. Prof. Mariana-Atena Poiana

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EFFECT OF CHAMPION 50WP FUNGICIDE ON HAEMATOLOGICAL AND BIOCHEMICAL PROFILES OF MARSH FROG (PELOPHYLAX RIDIBUNDUS)

Alina Păunescu and Cristina Ponepal

Abstract: Champion 50WP is a foliar fungicide with protective action which comprises copper hydroxide who governs the toxicity of the product. Pelophylax ridibundus is one of the most common amphibians and comprise a large proportion of their total number in the Romania. Adult male and female frogs Pelophylax ridibundus were exposed to 0.125x10^{-3}mg Champion 50WP/g body weight administrated by intraperitoneal injection, 1 injection at 2 days in a scheme of 3 weeks. The animals were kept at 4-6°C, respectively at 22-24°C in tap water tank. Total red blood cells (RBCs), total white blood cells (WBCs), glycaemia value, plasma cholesterol and triglycerides value, hepatosomatic index value were studied as compared to control animals. We observe increased the number of RBCs and decreased plasma cholesterol and triglyceride, decreased the number of WBCs, glycaemia and hepatosomatic index value. These changes were more powerful at 22-24°C than 4-6°C.

Key words: Champion 50WP, frog, red blood cell, white blood cell, glycaemia value, cholesterol and triglycerides value, hepatosomatic index.

INTRODUCTION
Champion 50 WP is a contact fungicide based on copper, used in preventing and combating mildew and bacterial burns in vines, fruit trees, vegetables, potatoes. It contains 50% Cu as Cu (OH)_{2}. Fungicides based on copper compounds are produced to prevent mold growth (anticriptogamic action).

Copper is an essential nutrient for plant and animal life, being involved in many biological processes [9]. Over 30 enzymes are known to use copper as a cofactor [4], among which peroxidase, cytochrome oxidase and superoxide-dismutase. Lack of copper in the body causes severe symptoms. On the other hand, excess of copper is toxic; it represents a direct or indirect source of pollution [1]. It penetrates the aquatic ecosystems, either through excessive use of pesticides based on copper or industry and affects aquatic organisms. Unlike organic pollutants, this compound does not undergo changes in aquatic organisms and bioaccumulates in the target organs such as liver, kidney, muscle, intestine, spleen, causing alterations of these structures.

The toxic action of copper is shown by blocking biochemical reactions acting on -SH group protein or by eliminating trace elements which are active centers of enzymes. These changes induce reduction of cell metabolism, stimulation of lipid peroxidation, inhibition of oxidative phosphorylation, disruption of calcium homeostasis and changes in cell membrane structure and permeability [3].

To illustrate Champion 50WP fungicide toxic effect in Pelophylax ridibundus, we used a concentration of 0.25x10^{-3}mg Cu(OH)_{2}/g body weight, respectively Champion 50WP 0.125x10^{-3}mg/g body weight, administrated by intraperitoneal injection, one injection every two days in a 3-week schedule.

MATERIAL AND METHODS
The animals used in this study were adult of Pelophylax ridibundus, of both sexes, captured in spring (April-May) from the surrounding areas of the city Pitești (South Romania). The animals were kept in laboratory condition in aquaterrarios filled with tap water for five days to test their health and accommodate them for the experiment. The water was changed daily to avoid the accumulation of toxic substances.
After adaptation in the lab, the frogs were separated in lots, which were used separately for the following experiments: two lots of control individuals, containing animals kept in laboratory at 4-6°C, respectively at 22-24°C with no treatment, in running water which was changed everyday, (1) one lot containing animals which were subjected to treatment with Champion 50WP in a dose of 0.125x10^{-3}/g of body weight and kept at 4-6°C, (2) a second lot containing animals which were subjected to treatment with Champion 50WP in a dose of 0.125x10^{-3}/g of body weight and kept at 22-24°C in a thermostatic chamber. Ten animals were used for each lot. The toxic was administered by intraperitoneal shots, one shot every two days, in a scheme of 3 weeks. The administered dosage of toxic was not lethal as none of the subjects died through the experiment.

After three weeks at treatment blood specimens were withdrawn from the frogs by cardiac puncture after chloroform anesthesia. The values of operational factors under discussion were determined by using standard automated method. The hepatosomatic index was calculated using formula:

\[ HSI = \frac{\text{liverweight}}{\text{bodyweight}} \times 100 \]

Hematological, biochemical and HIS results were expressed as means ± standard deviation (SD). Statistical analysis was performed as control lot versus treated lot using the Student’s t-test. The chosen level of significance is p<0.05.

RESULTS AND DISCUSSION

Studying the changes of haematological, biochemical and hepatosomatic indices can provide information on the immune system, growth rates, efficiency of food assimilation, carbohydrate metabolism stage using Champion 50WP fungicide treatment.

The action of Champion 50WP fungicide on the liver occurs primarily by decreasing its weight, which is evidenced by the decrease of hepatosomatic index value for the two groups studied (figure 1).

![Figure 1. The influence of Champion 50WP fungicide upon hepatosomatic index on marsh frog (b* p<0.05 vs control, a p<0.05 vs treated lot)](image)

In animals treated with Champion 50WP fungicide and kept at a temperature of 4-6°C, the index value decreased by 10.29% compared to the control value, while in animals kept at a temperature of 22-24°C and treated with the same concentration of fungicide, the index value decreased by 17.76%. The analysis of these changes shows a more pronounced decrease in liver weight in animals kept at a higher temperature.

Similar changes in the hepatosomatic index value of Pelophylax ridibundus were also found by Papadimitriou and Loumbourdis [6] when using treatment with copper.
Modifying the parameter values investigated is consistent with liver histological changes recorded by Păunescu et al. [7] in *Pelophylax ridibundus* when using Champion 50WP fungicide. The authors have reported the presence of large quantities of Pearl's reagent material in Kupffer cells, dilatation of blood vessels, peri-hepatocyte, peri-centrilobular, peri-sinusoidal and periportal fibrosis, an expansion of Disse spaces, presence of leukocyte infiltrates, vacuolated hepatocytes with small pyknotic nuclei, and necrotic areas in the parenchyma. Histological changes were more powerful at animals that were kept at 22-24°C than 4-6°C.

The hematological picture shows anemia associated with Champion 50WP administration in both groups of animals. Thus, there is a decreased number of red cells by 18.41% compared to control value for animals kept at a temperature of 4-6°C, and 14.89% compared to control value for animals kept at 22-24°C (figure 2). These determinations are consistent with histological observations in liver, where there were observed many iron deposits in the form of hemosiderin, as a result of intensification of hemolysis. The decrease of red blood cells causes the increase of white blood cells in response to inflammatory action triggered by Champion 50WP fungicide action in *Pelophylax ridibundus* (figure 3).

The analysis of this figure shows an increase by 11.08% in the number of white blood cells compared to control value in animals treated with Champion 50WP and kept at a temperature of 4-6°C, while for animals treated with the same concentration of toxic and kept a temperature of 22-24°C, the increase is 16.08% compared to the control value.

![Figure 2. The influence of Champion 50WP fungicide upon number of erythrocytes on marsh frog](image)

![Figure 3. The influence of Champion 50WP fungicide upon number of leukocytes on marsh frog](image)

Hematological indices (number of erythrocytes and leukocytes) have significantly modified values compared to control values in animals treated with the same concentration of fungicide and kept at two thermic levels. They can provide rapid information on long-term effects of environmental pollution. Therefore, hematological measurements can be indicators of rapid assessment of aquatic pollution. Biochemical diagnoses provided additional data that have completed the body damage picture induced by this toxic action.

Champion 50WP fungicide active substance, copper is known to influence the body carbohydrate metabolism. This nutrient accumulation in the liver probably stimulates liver glicogenogenesis and results in reducing the amount of circulating blood glucose. Thus, both studied groups show a decrease of blood glucose by 18.18% compared to control group for animals kept at 4-6°C and 30.43% for animals kept at 22-24°C (figure 4).
Copper also works by changing the quantity of plasma cholesterol (figure 5) and triglycerides (figure 6). Plasma cholesterol records a decreased value by 13.63% compared to control group for animals treated with Champion 50WP fungicide and kept at a temperature of 4-6°C, and 13.57% compared to control for animals kept at a temperature of 22-24°C.

The index decrease is explained by Paik et al. [5] by the accumulation of copper in the body which results in inhibiting the enzyme HMG-CoA reductase activity (3-hydroxyl-3-methyl-glutaryl-Co-A) involved in the biosynthesis of cholesterol.

In terms of triglyceride level, there was a decrease of their value in both groups studied by 10.16% compared to control group for animals kept at 4-6°C, and 10% for animals kept at 22-24°C. Bakalli et al. [2] showed that the excess of copper in the body triggers a smaller number of triglycerides.

Similar changes in biochemical indices (blood glucose, cholesterol and triglycerides level) were reported by Rahman et al. [8] in broiler chickens that received more copper.
Summary of changes in the values of physiological parameters under the action of Champion 50WP fungicide is presented in Table 1.

Table 1. Percentage changes of haematological, biochemical and hepatosomatic indices in Pelophylax ridibundus under the action of Champion 50WP fungicide in concentration of 0.25x10^{-3}mg Cu(OH)_{2}/g body weight.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>4-6°C</th>
<th>22-24°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatosomatic index (IHS)</td>
<td>3 weeks</td>
<td>-10.29</td>
<td>-17.76</td>
</tr>
<tr>
<td>Number of erythrocyes</td>
<td>3 weeks</td>
<td>-18.41</td>
<td>-14.89</td>
</tr>
<tr>
<td>Number of leukocytes</td>
<td>3 weeks</td>
<td>+11.08</td>
<td>+16.08</td>
</tr>
<tr>
<td>Glycaemia</td>
<td>3 weeks</td>
<td>+18.18</td>
<td>+30.43</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3 weeks</td>
<td>-13.63</td>
<td>-13.57</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>3 weeks</td>
<td>-10,16</td>
<td>-10</td>
</tr>
</tbody>
</table>

The analysis of this table reveals a stronger toxic effect of Champion 50WP fungicide for animals kept at a temperature of 22-24°C.

CONCLUSIONS AND FUTURE WORK

From the present study it is obviously that Champion 50WP fungicide in a dose of 0.25x10^{-3}mg Cu(OH)_{2}/g body weight cause hematological and biochemical alteration in marsh frog at both thermic level who may lead to increase mortality rate and decline the population rate.

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CONTENT OF AVAILABLE IRON AND MANGANESE OF PSEUDOGLEY IN MID RIVER PART OF WESTERN MORAVA BASIN

N. Gudžić, M. Aksiš, S. Gudžić, N. Deletić, V. Djurić, and S. Stojković

Abstract: Numerous chemical factors limit normal growth of plants on acid soils. In pseudogley soils high concentrations of soluble and available to plants forms of Fe and Mn can potentially be a risk factor for successful production of grown crops. This investigation has been aimed to establish content of available forms of Fe and Mn (by extraction with 0.1M HCl) in mid river part of pseudogley area in Western Morava river basin.

Results of the study offer possibility for defining dynamics of available forms of Fe and Mn in pseudogley profile, as well as for determining precise locations where concentrations of the investigated elements exceed MAC values.

Key words: Pseudogley, Iron, Manganese, MAC.

INTRODUCTION

Soil pH value primarily is a consequence of natural factors, but it is also affected by direct and indirect influence of man. Destructive effects of acid rainfall (Brady, 1990) and application of physiologically acid fertilizers (Khonje et al., 1989; Bolan, 1991) are especially prominent factors leading to soil pH value reduction, i.e. soil acidification, that are of human origin.

In acid soils, regardless to the origin of soil acidity (natural or anthropogenous), a series of specific chemical factors and their interaction can limit plant growth, so agricultural production efficiency too. More precisely, in the conditions of soil acidity, crops suffer harmful effect of high concentrations of H⁺, Al³⁻ and some organic acids. A serious problem appearing at high aluminum concentrations is inhibition of root growth. It was observed that aluminum has a special effect on forming incompletely developed and shallow root system with a limited capability of mineral nutrients uptake, so it simultaneously increases risk of drought induced stress (Marcshner, 1991). Other problems appearing in acid soils are related to insufficient amount of some elements (Ca, Mg), and low solubility of some (P, B, especially Mo) or increased solubility of the other elements (Fe, Mn, Cu), especially of the heavy ones (Pb, Cd, Cr, etc).

From the plant nutrition point of view, very interesting cases are those when high concentrations of necessary elements call into question their confirmed essentiality for plants, and make them dangerous. Such case can be seen when available forms of Fe and Mn are observed, and these elements’ content rises by increased soil acidity. In soils with pH<6.5 those two elements’ mobility is increased, and so their availability for grown crops (Kabata-Pendias, 2004), which is particularly obvious at pH values below 5.5 (Jakovljević et al., 1997; Milovac et al., 1997). In the conditions of increased mobility and content of toxic forms of Mn and Fe ions plants suffer so called “stress conditions” (Foy, 1988). It should be pointed out to toxicity of increased manganese concentration, which makes it being one of the main obstacles for plant growth (Marcshner, 1991; Kochian et al., 2004), and some examples of damage in grain crops confirm the mentioned (Radanović and Predić, 1997).

High concentrations of Fe and Mn also are indirectly harmful to plants, where the main problem is difficult nutrition by phosphorus. More precisely, in very acid soils, precipitation of phosphate ions occurs because of high concentrations of Al, Fe and Mn. The result is forming of aluminum phosphates (e.g. variscite, AlPO₄·2H₂O) and iron phosphates (e.g. strengite, FePO₄·2H₂O) (Jomo et al., 2007), which causes a deficiency of soluble phosphorus forms and lack of normal nutrition of crops by this nutrient.
The high observed acidity of pseudogley soils in Western Morava valley (Ţivković and Dugalić, 2001; Bošković-Rakočević and Bokan, 2005) limits their fertility and suitability for a successful plant production. An important factor for limited fertility of pseudogley in this area could be the content of available trace elements, especially of Fe and Mn.

MATERIAL AND METHODS

In order to determine content of available Fe and Mn forms in pseudogley of mid river part of Western Morava basin the following two localities were chosen: Gračac (Vrnjačka Banja) and Globoder (Trstenik). In both localities soil was sampled down to 60 cm of depth, from the all horizons (Ah, Eg, Ebtg). Depth of the horizons mainly was uniform. Surface Aoh horizon was down to 15 cm of depth in average, Eg horizon from 15-35 cm, and below it was Ebtg horizon which was sampled down to 60 cm.

Soil samples were analyzed for basic soil properties and content of available Fe and Mn. Among basic soil properties the following ones were observed: pH of soil suspension in H2O and 1M KCl (potentiometry), humus (Kotzman), and available P2O5 and K2O (Egner-Riehm).

Content of available Fe and Mn was determined after extraction by 0.1M HCl. The analyzed elements were extracted by shaking 10 g of soil sample for one hour with 100 cm3 of 0.1M HCl. Concentration of the analyzed elements in filtrate was carried out by atomic absorption spectrophotometry.

The obtained data regarding available Fe and Mn content were processed by analysis of variance, and significance between horizons and localities was tested by LSD test.

RESULTS AND DISCUSSION

Characteristic properties of pseudogley located in mid river part of Western Morava basin were its low pH value and available phosphorus content (tab. 1). Comparing acidity (active and substitutional) of different horizons one can see that it was stronger in Eg horizon than in Aoh horizon, while it mostly was a little bit weaker in Ebtg horizon. Particular problem was very low content of available phosphorus, which constantly decreased by depth. In deeper horizons we only found trace content of phosphorus which was just 1.43 mg/100 g (Gračac) or 1.84 mg/100 g (Globoder). Acid soil reaction and low content of available phosphorus in pseudogley of Kraljevo valley, which is a part of Western Morava basin, was reported by Dugalić et al. (2002; 2004), as well as by Bošković-Rakočević and Bokan (2005). Content of humus and available potassium in most cases was within levels of moderate supplies. Humus content decreased by depth, while content of available potassium was constant.

Table 1. Basic chemical properties of pseudogley.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Horizon</th>
<th>pH</th>
<th>Humus (%)</th>
<th>mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H2O</td>
<td>KCl</td>
<td>P2O5</td>
</tr>
<tr>
<td>Gračac</td>
<td>Aoh</td>
<td>5.48</td>
<td>4.35</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>Eg</td>
<td>5.33</td>
<td>4.18</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Ebtg</td>
<td>5.32</td>
<td>4.45</td>
<td>0.21</td>
</tr>
<tr>
<td>Globoder</td>
<td>Aoh</td>
<td>5.78</td>
<td>4.43</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>Eg</td>
<td>5.51</td>
<td>4.51</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Ebtg</td>
<td>5.69</td>
<td>4.68</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Iron and manganese are necessary and irreplaceable elements in plant nutrition, by the moment when their concentration reaches levels potentially dangerous to plants. Available iron content was very high at both locations and in the all analyzed samples (tab. 2). However, concentration of physiologically active Fe did not exceed 85 mg/kg, which was much lower than maximum allowable concentration (MAC) for this element.

Reducing conditions are suitable to Fe solubility increase, and these are characteristic for pseudogley, especially during wet periods and in deeper layers. This could be the reason for the observed higher Fe content in both localities, especially in Eg horizon. At the location Graćac in this horizon the average Fe content of 41.74 mg/kg was observed, which was high-significantly greater comparing with the horizons Aoh (28.99 mg/kg) and Ebtg (30.22 mg/kg). In the location Globoder highly significant difference was established only between the horizons Eg (39.06 mg/kg) and Aoh (24.31 mg/kg).

Table 2. Content of available Fe (mg/kg of soil).

<table>
<thead>
<tr>
<th>Locality (B)</th>
<th>Horizon (A)</th>
<th>Average (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aoh</td>
<td>Eg</td>
</tr>
<tr>
<td>Graćac</td>
<td>28.99</td>
<td>41.74</td>
</tr>
<tr>
<td>Globoder</td>
<td>24.31</td>
<td>39.06</td>
</tr>
<tr>
<td>Average (A)</td>
<td>26.65</td>
<td>40.40</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>7.68</td>
<td>6.28</td>
</tr>
<tr>
<td>0.01</td>
<td>10.37</td>
<td>8.48</td>
</tr>
</tbody>
</table>

There was not any significant difference between the investigated locations, and one could point out to a pretty uniform content of available iron in wider area. That is confirmed by the results of Boskovic-Rakocevic and Bokan (2005) for pseudogley in upper part of Western Morava basin (around Kraljevo), while in the same area Dugalić et al. (2006) found a higher content of this element.

Average values of extracted manganese point to its content being above levels of optimal supplies (tab. 3). Especially it was the case in Globoder, where in Aoh horizon were noticed values of manganese content over MDC level, with the maximum of 307 mg/kg. The highest average Mn content was observed in surface Aoh horizon (203.71 mg/kg) and constantly decreased by depth, reaching in Ebtg horizon average value of 116.21 mg/kg. Differences in available manganese content between Aoh and deeper Eg and Ebtg horizons were highly significant.

Table 3. Content of available Mn (mg/kg of soil).

<table>
<thead>
<tr>
<th>Locality (B)</th>
<th>Horizon (A)</th>
<th>Average (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aoh</td>
<td>Eg</td>
</tr>
<tr>
<td>Graćac</td>
<td>118.85</td>
<td>100.57</td>
</tr>
<tr>
<td>Globoder</td>
<td>203.71</td>
<td>140.07</td>
</tr>
<tr>
<td>Average (A)</td>
<td>161.28</td>
<td>120.32</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>20.68</td>
<td>16.88</td>
</tr>
<tr>
<td>0.01</td>
<td>27.92</td>
<td>22.79</td>
</tr>
</tbody>
</table>
Globoder and Gračac were highly significant. Differences between horizons were observed, with the tendency of decrease by depth. A highly significant difference was observed between the horizons Aoh and Ebtg, while between Aoh and Eg, as well as between Eg and Ebtg, there was not any significant difference in available Mn content.

Similar results concerning content of physiologically active Mn forms in pseudogley of Kraljevo valley were reported by Dugalić et al. (2006), and especially by Boskovic-Rakocevic and Bokan (2005).

CONCLUSIONS AND FUTURE WORK

Results of the investigation of available iron and manganese content of pseudogley in mid river part of Western Morava basin point to a very high content of these two elements.

The highest content of available iron was found in Eg horizon, but the highest observed values were far below the maximum allowable concentration (MAC) for this element.

The obtained results showed a uniform concentration and dynamics of available iron in this part of Western Morava basin.

Results of available manganese concentration lead us to worriedness, especially at locations where cases of Mn content above maximum allowable concentration or its borderline values were observed.

Cases with alarming high content of manganese were observed in the downstream part of the analyzed area that is in the location Globoder and that problem ought to be properly treated in further investigation.

Future research ought to be directed to finding proper agrotechnical solutions in order to decrease potentially danger concentrations of toxic elements, especially manganese, or to introducing and spreading such genotypes of grown crops capable to suffer high concentrations of these two elements.

REFERENCES


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Abstract: The consequences of the researches of the light reflecting features of soil depending on its different characteristics and length of electromagnetic waves are conducted in the offered work. In particular, from the compotition of humus, carbonate, humidity, density on the phone of relatively insignificant changes of the different forms of iron, also with the calculation of the changes of soluble salt on dense residue.

Keywords: chestnut soils, meadow-serozem soils spectrograms, electromagnetic waves

INTRODUCTION

In relation with the obvious theory by Maltus about disproportion between increase of the quantity of the population and its food provision, a problem of the soil guard become more important and complicated every day, by acquiring a strategical importance. Thousand million of hectare of the fertile land go out of agricultural rotation every year. In particular, for erosion, salinization, pollution of the different industrial wasters and also other natural process exposing to degradation of soil.

In order to reveal this unfavorable process and warn their further development in good time it is necessary to use of more active and reliable methods of the control for a state of the natural environment. To this method belongs distance exploring of the soil surface (Andronikov, 1979; Kondratev, 1986; Kronberg, 1988). We should note that under distance investigations it is necessary to have dealings with the coefficient of the light reflect of the surface layer of the soil at first., Under study of light reflect of the soil, under natural conditions side by side with a quality of the soil and its environment it is necessary to take into account the Sun pose in the moment of conducting of the distance measures. The laboratorial methods are deprived of these difficulties and allow to study different types of the soil under equal conditions of the experiment, in particular light, density, humidity, length of the wave and etc. (Karmanov 1974, Orlov and others, 2001; Mikhaylova, Orlov, 1986; Azizov and oth. 1990; Babayev and oth. 1987). On the basis of study of spectral curve reflects of the different horizons of the soil on profile it is possible to compose transmission function of the relation between upper and low horizons allowing extrapolation in the deepest horizons under distance measures.

Three groups of the substances play a great role in formation of the state of being coloured of the soil. This is a compotation of the organic substances, carbonate, soluble forms of Ferric oxide. Decomposition of the organic substances in the soil promotes creation of the dark and grey tones; carbonate calcium, silicon, combinations, caoline and some other minerals are reason for formation of white tones; ferric combinations take part in formation of red, orange-coloured. If the formation of the colour of upper horizon happens at the expense of decomposition of the organic combinations, then the coloured tones of the transitional horizons form at the expense of ferric-oxide and silicon, the maternal rock mainly influences on formation of the coloured tones of the low horizons.

The object and methods of the researches

The object of the research was selected in the soil of the step of Shirvan, distinguishing by a diversity of the ancient and temporary deposits of the different historical period, including different conglomerations, sand, grain of sand, silt, limestone, loam, gravel and etc. At present an application of proluvial- alluvial deposits is continued by the rivers of the Great Caucasus. Irrigative agriculture has been conducted, process of salinization, erosion and pollution of the soil has widely been developed here, since the beginning of time. On the whole, the Shirvan steppe presents itself non-homogeneous accumulative plain from
genetic point of view. From geomorphological point of view the object divides into three zones: foothill proluvial-alluvial, foothill deluvial-proluvial plain and mezorelief distinguishing by a diversity of alluvial deposits (Salayev, 1994). Subsoil water is formed at the expense of the water from mountain rivers, irrigations, spring floods, and etc. (Mamedov, 1989). On the whole the depth of the subsoil waters changes in the wide limits, a degree of the salinization which increases depending on a removal sources in particular within the limits of the approach the low-land part of the steppe. The most characteristic are hydrocarbonate, sulphate-hydrocarbonatic, chloride-sulphatic and sulphate-chloridic types of salinization here. A climate belong to the dry subtropic. The evaporation forms a quantity of the order more than 1000 mm, increases a quantity of atmospheric precipitations 2-3 times. The natural vegetations is presented by a typical semidesert wormwood-ephemer formation (Prilipko, 1970).

The chemical analyses of the soils (composition of humus, carbonate, salt and etc.) are mainly carried out by classical methods, adopted in the countries of UNS (AU-union, 1973; Oreshkina, 1988). For measurement of the reflective features of the soil the spectrophotomete-SF-18 allowing to investigate coefficients of the soil reflection is used in the visible field of the electromagnetic waves.

The soil samples are ready for an analysis, situated in the special cuvette entering the complete of spectrophohometre SF-18. The principle of the influence is that monochromatic small bunch of the light is shared (by Roshon’s prism) into two plane polarized small bunches. One of which is diaphragmed, the second passes through Volloston’s prism, sharing again into two small bunches, polarizing in two mutual perpendicular planes. The further small bunches are singly covered again by revolving modulators. The light intensity in every small bunch. Changes trapezoid form. The beginning of the opening of one small bunch corresponds to the beginning of the closing of the other. The light, passing through a soil sample and reference substance, occurs in the interirating ball and light up photo-element. Appearing electrical signal after intensification turns Roshon’s prism and leads to drum motion, fixing optical density of the research sample.

**The discussion of the research results**

The chestnut, meadow-serozem, serozem-meadow, meadow-marshy soils and also solonchak in limits of the Shirvan steppe including the Kur-Araz lowland were selected for experimental researches.

The chestnut soils are mainly situated in the foothill parts of the Shirvan steppe. On the basis of the laboratorial investigations (picture 1.) it is established that a quantity of humus in the upper horizon of the soil (0-36 sm) is found at average in limits of 4,65 %.

A quantity of carbonate-calcium on the profile of soil changes in a large limit of 3.9-24.3%. In distribution of carbonates is observed its leaching from upper horizons to low ones.

The capacity of absorption of this soil changes in limits of 26,7-31,6 mg-ekv. in 100 g of the dry soil. In sum of the absorbed bases the most quantity fall per share of ions of calcium (67.8-72.8 %), then follows magnesium (21,1-27,4 %) and sodium (3,5-6,2). The dense residue of salt on the whole profile changes in an intervale of 0,120-0,194 %. Granulometric competition of this soil is mainly mean clayey (Jafarov, 2009).
The packet of spectrogram chestnut soils (section 6). The main influencing on light reflection of the soil factors are presented to the right.

<table>
<thead>
<tr>
<th>Indices, %</th>
<th>Genetic horizons, sm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-19</td>
</tr>
<tr>
<td>Humus</td>
<td>2.1</td>
</tr>
<tr>
<td>CaCO3</td>
<td>1.0</td>
</tr>
<tr>
<td>Dense residue</td>
<td>8.937</td>
</tr>
<tr>
<td>Silt</td>
<td>32.20</td>
</tr>
<tr>
<td>Clay</td>
<td>60.80</td>
</tr>
<tr>
<td>Humidity</td>
<td>4.6</td>
</tr>
<tr>
<td>Fe</td>
<td>5.00</td>
</tr>
</tbody>
</table>

The spectrograms on profile of the chestnut soils in limits of the Shirvan steppe, going into the Kur-Araz lowland are presented on picture 1. It is obvious from the picture that the spectrum of the reflection of every horizon distinguish between themselves. In order to explain the purposes of these differences a table influencing on factors on motion of the spectral curve soil horizons is presented to the right. Under increase of the, scale of the picture, the differences are clearly visible and that’s why it is possible to find corresponding explanation of these differences for the concrete horizon of the investigative soil. So, for example from picture 1. it is obvious that spectral curve horizon of 129-165 sm is situated on the same upper picture. Such location of this curve is explained by the given tables arranging to the right of table. From the table data it is, obvious that the most quantity of carbonate calcium, under minimum quantity of humus, fall into that horizon. It is possible to explain an arrangement of the spectral curve genetic horizons of 100-129 and 63-100 sm.

The coefficients of the light reflection of meadow-serozem soils, past stage of the deep steppization and the most characteristic for the innestigative steppe are presented on pic.2

<table>
<thead>
<tr>
<th>Indices, %</th>
<th>Genetic horizons, sm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-37</td>
</tr>
<tr>
<td>Humus</td>
<td>1.9</td>
</tr>
<tr>
<td>CaCO3</td>
<td>22.0</td>
</tr>
<tr>
<td>Dense residue</td>
<td>0.46</td>
</tr>
<tr>
<td>Silt</td>
<td>31.68</td>
</tr>
<tr>
<td>Clay</td>
<td>53.6</td>
</tr>
<tr>
<td>Humidity</td>
<td>3.2</td>
</tr>
<tr>
<td>Fe</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The composition of humus on upper horizons of this soil arranges in limits of 2.02-2.55%. Further decrease from the depth and on horizon of 88-136 sm a quantity reaches 0.95-1.10 %. The composition of carbonates from the depth of horizons increases from 6.8-9.7 % (0-27 sm) to 12.2-13.8 % (88-136 sm). The capacity of absorption changes in limits of 21.9-25.6 mg-ekv in 100 sm of dry soil. Per share of calcium falls 57.1—65.0 %, magnesium 26.4-36.2 %, sodium 7.4-9.1 %. The dense residue of salts on the upper
horizon forms a quantity of the order 0.260 %. Granulometric composition of this soil is mainly loamy.

On the occasion of serozem-meadow soils the distribution of the curve reflection of the soil is in the narrowest small bunch, that is evidence of the relative homogeneity of this soil on profile.

The spectral curve samples of serozem-meadow soils, taken from section 9, are distinguished from curve meadow-seroszem soils by a character. Firstly the width of the curve small bunch increases visibly by enlarging of the length of the wave, secondly the spectral coefficients change in the widest range, that also coordinates with the composition of the genetic horizons.

On the whole the composition of humus is in limits of 2.25-2.62 % on the upper horizon (0-18 sm). On the horizon of 19-165 sm its size becomes 0.15-0.16 %. The composition of carbonates on profile changes in limits of 12.6-18.9 %, the most quantity falls per share of the horizon 18-37 sm. The capacity of the absorbing bases changes in limits of 25.7-29.3 mg-ekv for 100 g of the dry land. From them per share of calcium falls 47.6-52.7 %, magnesium-32.9-35.2 %, sodium-12.5-18.2. the dense residue of salt on the upper horizon changes in limits of 0.203-0.225 % from the depth it sharply increases and reaches the quantity 1.662 %. On granulometric composition this soil belongs to average and heavy clayey, a size of the physical clay in this soil reaches the size of 93.60 %.

The capacity of absorption changes in a small intervals of 32.1-32.9 mk-ekv for 100 g dry land. From a general sum per share of calcium falls 49.1-49.5 %, magnesium 41.2-42.3 %, sodium 8.5-9.5 %. The soil is salinized weakly and middling, the dense residue of salt changes in limits of 0.383-0.790 %. On granulometric composition this soil is clayey, the composition of physical clay changes in limits of 77.3-79.8 %, lightens down on profile a little.

The meadow marshy soils spread in the limited quantity, mainly, in falls, in the places where subsoil water is close to soil surface and close to the river of Kur. This soil is distinguished by a high composition of organic substances and moisture. Here mainly hydrophilous vegetation spread (Garayzade, 2009). A quantity forms the size of the order 5.2%, on the upper horizon, from the depth decreasing on the level of one metre it reches 1.32 %. The composition of carbonates is found in limits of 5.8-12.9 %.

**Packet of the spectrogram of serozem-meadow soil (sec.9).**

<table>
<thead>
<tr>
<th>Indices, %</th>
<th>Genetic horizons, sm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-35</td>
</tr>
<tr>
<td>Humus</td>
<td>2.1</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>11.8</td>
</tr>
<tr>
<td>Dense residue</td>
<td>0.145</td>
</tr>
<tr>
<td>Silt</td>
<td>19.80</td>
</tr>
<tr>
<td>Clay</td>
<td>51.20</td>
</tr>
<tr>
<td>Humidity</td>
<td>5.7</td>
</tr>
<tr>
<td>Fe</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Packet of the spectrogram of meadow-marshy soil (sec. 2). To the right of affecting factors.

<table>
<thead>
<tr>
<th>Indices, %</th>
<th>Genetic horizons, см</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-21</td>
</tr>
<tr>
<td>Humus</td>
<td>2.30</td>
</tr>
<tr>
<td>CaCO3</td>
<td>13.2</td>
</tr>
<tr>
<td>Dense residue</td>
<td>0.157</td>
</tr>
<tr>
<td>Silt</td>
<td>5.68</td>
</tr>
<tr>
<td>Clay</td>
<td>59.92</td>
</tr>
<tr>
<td>Humidity</td>
<td>4.4</td>
</tr>
<tr>
<td>Fe</td>
<td>5.30</td>
</tr>
</tbody>
</table>

The spectral characters of meadow-marshy soils are presented on picture 4. As is obvious, the profile of this soil is visibly differential. It can be observed on the picture.

The spectrograms of solonchak which has the most complicated picture 5. It can be connected with the distribution of the influencing on reflective peculiarity of the soil components.

Solonchaks in the Shirvan steppe belong to chloride-sodium type of salinity. On the upper horizon of this soil a quantity of humus changes about 2.1 %. A quantity of carbonates of this soil changes in limits of 1.1-10.6 % on profile. The capacity of the absorbing bases for 100 g of the dry soil forms a quantity of the order 16.2-21.3 % mg-ekv. Per share of calcium falls 38.1-49.85 %, magnesium 30.5-39.8 %, sodium 19.0-20.5 %. The dense residue of water extract on the upper horizon of solonchak forms 6.-579-8.937%. A quantity of physical clay in this soil changes in limits of 35.36-90.80 %.

Packet of the spectrogram of solonchak (sec.13). to the right the main influencing on light reflection of soil factors are presented.

Thus on the basis of spectrophotometric analysis of the different types of the soil in limits of the Shirvan steppe, the influence of the component composition of this soil is established on character and form of the curve light reflecting coefficient. The results are recommended for the decision of the reverse problems, in particular for the determination on spectral curve effecting on reflection of the soil factor, and also for usage under determination of the soil colour.
CONCLUSION

In this work the spectral curve reflections on a profile of soils are investigated, transfer functions of communication between data of the top and bottom horizons are made, that is allowing at remote measurements to extrapolate the distantly obtained soils properties for the horizon data on deeper horizons. The ancient and modern sediments of the various historical period including various conglomerates, sands, sandstones were objects research, or lime-stones, loams etc. The irrigated agriculture here is from time immemorial conducted, the processes of salinization, erosion and pollution are widely developed. Here are carbonate, sulfate-hydro-carbonate, chloride-hydro-carbonate and sulfate-carbonate types of salinization. The received spectrograms on a profile of the investigated soils have shown distinction not only depending on type of soils, but also within their profile. All distinctions have been compared from structures and a condition of soils and have received corresponding explanations.

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