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Biologia

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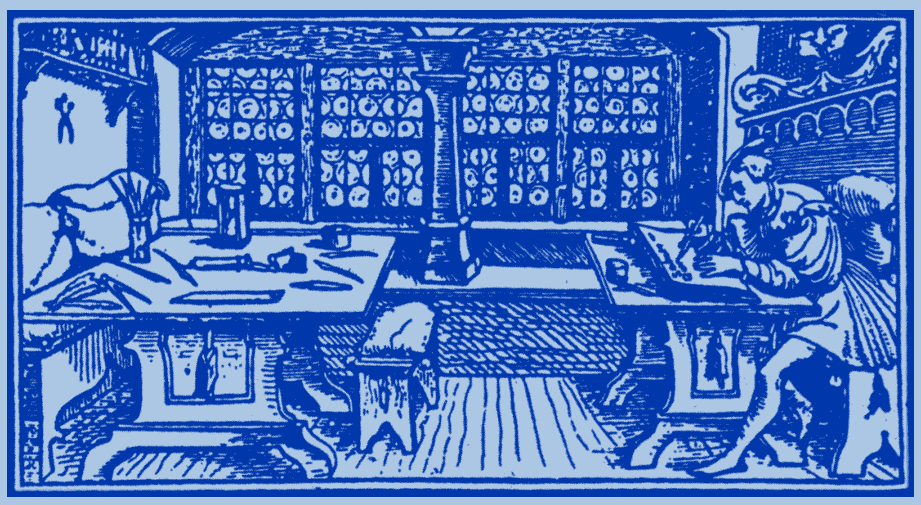

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 1, 2006 (p. 3 – p. 6)

SPHINGONAEPIOPSIS GORGONIADES (HÜBNER, [1819]) (LEPIDOPTERA: SPHINGIDAE), AT ITS FIRST CERTAIN RECORD IN ROMANIA

SERGIU MIHUŢ[1] and VLAD DINCĂ[2]

Summary. The species *Sphingonaepiopsis gorgoniades* (Hübner, [1819]) is recorded as a certitude for the Romanian entomofauna. The biology, ecology and distribution of this species are discussed.

KEYWORDS: *Sphingonaepiopsis gorgoniades*, Romania, Dobrogea, distribution.

Introduction. During the period 1-2 May 2005, the authors collected lepidopterological material in southern Dobrogea (Dumbrăveni and Esechioi forests) (Fig. 1). Among several local species little known from Romania such as *Cucullia fraterna* Butler, 1878, *Paradrymonia vittata bulgarica* de Freina, 1983, *Dasycorsa modesta* (Staudinger, 1879), we identified one specimen belonging to the species *Sphingonaepiopsis gorgoniades* (Hübner, [1819]).

Material: 1♂, 1. V. 2005, Dumbrăveni Forest (southern Dobrogea), leg. S. Mihuţ.

By the shape, colour and wing pattern, *Sphingonaepiopsis gorgoniades* cannot be confounded with any other hawk moth species present in Romania (Fig. 2). Due to its wing span (25-32 mm, our specimen 28 mm), this is the smallest Sphingidae species from Romania.

Type locality: Southern Volga, Russia. Several subspecies and forms of *S. gorgoniades* have been described (eg. ssp. *chloroptera*, ssp. *pfeifferi*), but the taxonomic status of these subspecies is not very clear; the early stages of most of the described subspecies and forms seem to be identical (http://tpittaway.tripod.com/sphinx/s_gor.htm).

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Fig. 1. The collecting site of *Sphingonaepiopsis gorgoniades* Hb.

Taking into consideration the habitus of the collected specimen and the collecting locality, it belongs to the nominal form, *Sphingonaepiopsis gorgoniades gorgoniades* (Hübner, [1819]) (http://tpittaway.tripod.com/sphinx/s_gor.htm).

Fig. 2. *Sphingonaepiopsis gorgoniades* (Hübner, [1819]), Dumbrăveni Forest (Southern Dobrogea), 1. V. 2005

Biology and Ecology. It is a crepuscular species but it may also be active at dawn (de Freina and Witt, 1987), generally occurring in open scrub or steppe areas, in small and local colonies (http://tpittaway.tripod.com/sphinx/s_gor.htm).

We collected this species using a light trap (250 W mercury vapor bulb) placed in a sylvo-steppe type habitat with *Quercus pubescens* occurring on a limestone substratum (Fig. 3).

Fig. 3. Calcareous slopes with *Quercus pubescens* - habitat of *Sphingonaepiopsis gorgoniades* at Dumbrăveni Forest, 1. V. 2005

S. gorgoniades is a two brooded species, on wing between 1/2V-VI and 1/2VII-VIII according to altitude and regional climatic conditions (de Freina & Witt, 1987, http://tpittaway.tripod.com/sphinx/s_gor.htm). The specimen collected by us belongs to the first brood; it was on wing particularly early as the imago is known to emerge starting second half of May (see above). The larvae feed mainly on *Galium* (especially *Galium verum*), but were also reported on other species of Rubiaceae (http://tpittaway.tripod.com/sphinx/s_gor.htm).

Distribution. *S. gorgoniades* has a poorly known distribution. It has been reported from eastern Bulgaria (Beshkov, 1990), Croatia, Macedonia, central Greece, southern Ukraine and Crimea, southern Russia and southern Urals, Kazakhstan, the Caucasus, southern Turkmenistan and Turkey to the Tian Shan and Afghanistan (http://tpittaway.tripod.com/sphinx/s_gor.htm, de Freina and Witt, 1987).

There are also several old as well as more recent unconfirmed reports that the species may occur in Hungary; however, these may refer to the Croatian population, as Croatia was formally part of the Austro-Hungarian Empire (http://tpittaway.tripod.com/sphinx/s_gor.htm). This subspecies may also occur in Mongolia and northern China (http://tpittaway.tripod.com/sphinx/s_gor.htm).

According to Karsholt and Razowski (1996) *S. gorgoniades* is present in Romania, probably based on the species' known distribution in Eastern Europe. König (2003) also mentions the species from Romania, but he doesn't mention any collecting locality. However, due to the lack of relevant records, this species has been excluded from the Romanian Lepidoptera Catalogue (Rákosy *et al*., 2003). Yet, the presence of this species in south-eastern Romania was to be expected as it has been quite recently recorded from north-eastern Bulgaria (Beshkov, 1990), more precisely near Balcic (northern Black Sea coast), not very far from the place where we found it. Taking into consideration the ecological preferences of the species, it is possible that it is also present in several other places in southern Romania.

Acknowledgements. The authors would like to thank Dr. Roger Vila for his useful comments and suggestions regarding the present paper.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 1, 2006 (p. 7 – p. 10)

NEW DATA CONCERNING THE PRESENCE OF THE SPECIES *BOLORIA AQUILONARIS* (STICHEL, 1908) IN THE ROMANIAN ENTOMOFAUNA (LEPIDOPTERA, NYMPHALIDAE)

SERGIU MIHUŢ[1] and VLAD DINCĂ[2]

Summary. New data about the occurrence of *Boloria aquilonaris* (Stichel, 1908) in Romania are given. The authors provide information on a new site where the species is present: Ic Ponor area (Western Carpathians). Certain data on the distribution and the biology of this species are also given.

KEYWORDS: bogs, *Boloria aquilonaris*, protection, Romania.

Introduction. *Boloria aquilonaris* (Stichel, 1908) is a species widely distributed in the northern regions of Europe, while in Central Europe its areal is limited to the mountainous, subalpine and alpine bogs occurring in the Alps, Jura, Vosgi, Ardennes, and Carpathians. Towards East, the species' areal extends along the arctic Russia, southern Ural, reaching Western Siberia and the Altai (Tolman and Lewington, 2002). Therefore, *Boloria aquilonaris* is distributed almost all over the Palaearctic, missing from its southernmost regions, with discontinuous (isolated) populations unevenly distributed – this pattern favourising the allopatric speciation and the existence of several infraspecific taxa.

In Romania (Fig. 1), the species was until now known only from the bogs of Poiana Stampei (Vatra Dornei) where a local colony survives – considered to be the only doubtless population.

In the Ludovic Beregszászy collection there are two specimens determined as *B. aquilonaris* collected from the Retezat Mountains (Berhina) on 14. VII. 1977 (leg. Beregszászy) (Stănescu, 1995). Because the species was never again recorded in this region, we questioned the presence of the species in this area.

A very old record (before 1900) is known from Transylvania (Rákosy *et al*., 2003).

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Being aware of the ecological preferences of this species and knowing its phenology, the authors made field trips visiting certain bogs from the Western Carpathians in order to see if *Boloria aquilonaris* is present at these locations.

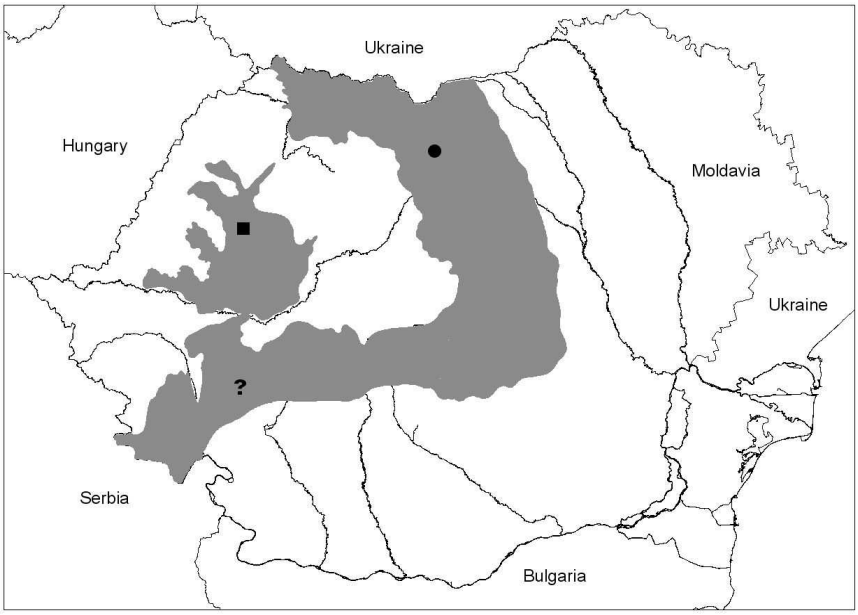

Fig. 1. Recordings of *Boloria aquilonaris* in Romania during the last century
■ = Ic Ponor (Western Carpathians), ● = Poiana Stampei (Vatra Dornei),? = Berhina (Retezat Mountains).

Materials and methods. During the field work made in the Western Carpathians, the authors identified a new population belonging to this species; this population is restricted to one of the oligotrophic bogs from Ic Ponor area (Fig. 2).

Identified material: 4 specimens (3 ♂♂, 1 ♀), Western Carpathians (Ic Ponor), 18. VII. 2001, leg. & coll. V. Dincă & S. Mihuț.

Besides the four collected specimens, we also observed four specimens flying in the same perimeter. We could find the adults only around the bog, being therefore extremely local.

Fig. 2. Overview of the habitat of *Boloria aquilonaris* from the Western Carpathians (July 2001).

Results and discussion. The population of *Boloria aquilonaris* present at Ic Ponor is situated close to the southern limit of its range in Europe and also represents the second certainly known location for this species in our country. The maintenance of the species in the southern regions of its range is conditioned by the presence of the oligotrophic bogs were the larval food plant grows (*Vaccinium oxycoccos* and *V. microcarpum*). Nevertheless, it seems that *B. aquilonaris* uses other food plants as well (eg. *Polygonum bistorta*), being polyphagous mainly on herbaceous plants (Kostrowicki, 1969). As there are no published studies about the biology of any Romanian population of *B. aquilonaris* and as we didn't have the opportunity to make such a study, we cannot tell for sure if the larvae of the population from Ic Ponor feed on *Vaccinium* (which is reported to be the most common larval food plant) or on other herbaceous plants.

It has been noticed that the larvae prefer to consume young flowers or leaves. We also observed that in unfavourable conditions the larvae may hibernate twice (a phenomenon usually present at high latitudes or at high altitudes). On bad weather, the larvae find shelter among *Sphagnum* (Lepidopterologen-Arbeitsgruppe, 1987), which is also abundant in the bogs from Ic Ponor area.

B. aquilonaris is threatened in several regions of Europe due to the degradation or disappearance of bogs (exploitation, drainage, eutrophization due to fertilizer infiltration, etc.).

Despite these facts, the species is not considered to be threatened at European level, being present in 20 countries with a distribution class of 5-15% and stable populations (van Swaay and Warren, 1999).

Conclusions. In our country, the population from Poiana Stampei is strongly menaced by the destruction of the marshlands; under these circumstances, the extinction of this population is quite probable in the near future. Other authors also consider that *B. aquilonaris* is a critically endangered species in Romania (Rákosy, 2002).

Unfortunately, there is absolutely no data regarding the biology of the Romanian populations belonging to this species.

We believe that the population from Ic Ponor, although it is very local and apparently not very abundant, has real chances of survival because the bogs present in the region are still not exposed to a high human pressure. As long as these habitats remain unaltered, the species is not at risk although certain monitoring actions to survey the evolution of this population would be extremely useful in order to prevent its loss or decline in case of human disturbance.

We also consider that further researches made in favourable habitats are likely to point out the presence of this species at other locations than those very few known at the present moment.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 1, 2006 (p. 11 – p. 22)

STATUS OF THE AVIFAUNA IN THE SIGHIŞOARA AREA IN 1948 AND PRESENT: A COMPARATIVE APPROACH

COSMIN IOAN MOGA[1]

Summary. This paper presents the actual situation of the avifauna of the town named Sighişoara and its surroundings compared with the one in 1948. The species' richness, phenological situation and repartition of species in different habitat types from the two periods of time chosen are compared and contrasted. The status of the avifauna in 1948 was evaluated from the manuscript of Fostrat (1948). The data from Fostrat (1948) were compared with data gathered by more recent studies, carried out in 2003, 2004 and 2005, all phenological phases being covered. The bird species' composition was affected by the long term changes in the habitats from this area. A total number of 161 species has been recorded for both intervals under study. The forest clearings and the high anthropic impact on the forest habitats are documented by the disappearance of some species of large prey birds (*Hieraaetus pennatus*, *Circaetus gallicus* and *Milvus migrans*). As a consequence of the river regularization species relying on the vegetation that characterises the large flooded areas (*Nycticorax nycticorax*, *Circus aeruginosus*, *Rallus aquaticus*, *Locustella luscinioides*, *Acrocephalus schoenobaenus* and *Emberiza schoeniclus*) have not been recorded anymore, while new species, depending on the recently-formed permanent ponds (*Tachybaptus ruficollis*, and *Ixobrycus minutus*) have occurred.

KEYWORDS: avifauna, long term changes, Sighisoara.

Introduction. Globally, the decrease of biodiversity as well as the increase of the number of endangered species pleads for the enhancement of ecological studies and more focus on conservation strategies for some threatened species, at risk of extinction. Among the essential objectives in the view of nature conservation are the identification of species, species' communities and habitats at risk as a result of human activity (Hannon *et al.* 2004).

The replacement of traditional agricultural methods with intensive farming led to the degradation of numerous natural habitats, with negative impact on the avifaunal species (Böhning and Bauer 1996, Kleijn and Sutherland 2003).

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At the landscape scale, the coexistence of a mosaic of habitats contributes to the increase in biodiversity (Tews et al, 2004), being favourable especially for double territory species such as prey birds. (Newton, 2002). In time, the disappearance of some habitats, as well as the establishment of new habitats in the landscape, determine changes in the regional bird species' composition and in the populations' quota.

This study compares the richness of bird species recorded in 1948 in the Sighişoara area to the current situation, with focus on breeding birds. The comparison concerns the phenology of the bird species and their distribution according to the habitat types.

Study area. The area under study is located at about 15 km from the Sighişoara town (46°13, 165'; 24°47, 583'), the main habitat types being represented by forests, open habitats and wet areas (Fig. 1).

At present, the forest habitats cover the hill slopes with altitudes between 457m and 687m. The forests are heterogeneous, both as age and as tree species' composition. The open habitats are mainly located along the valleys and on the lowermost third part of the slope of the hills, and consist of grass lands and pastures with bushes, trees and hedgerows, in various amounts. The wet areas are represented by permanent and temporary ponds as well as by the occasionally flooded areas along the rivers. The Şaeşului and the Bendorfului Valleys represent the main watercourses in the studied area. The permanent ponds were formed due to the works carried out for changing the course of the Târnava Mare River, and represent its fossil branches. Due to the same reason, the occasionally flooded area corresponding to this river is currently very limited. Along the smaller valleys and the Târnava Mare River, long corridors of alluvial forest developed.

In comparison, in 1948 the area covered by forests was larger, and the extended meadows of the Târnava Mare River – which was not regularised in that time - showed larger flooded areas with temporary ponds and wide canals of common reed (*Phragmites australis*). However, the permanent ponds were not present in the study area.

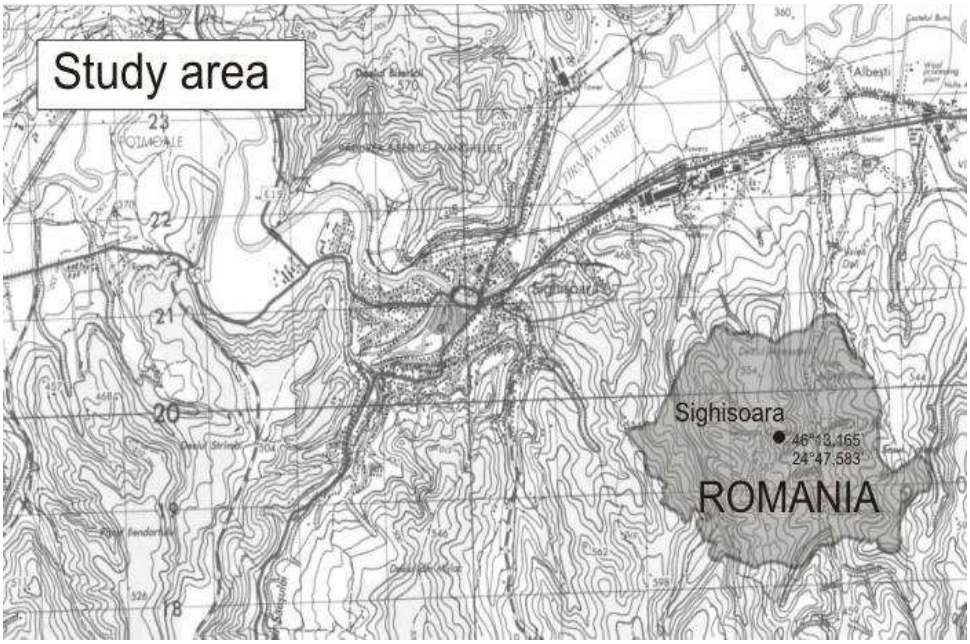

Fig. 1. Study area.

Methods. The current data are the results of field campaigns performed in the years 2003, 2004 and 2005, when all the phenological stages have been accomplished. The line transect method was used in the case of the open habitats, while the point counts method in the case of the forest habitats (Bibby et al, 2000). The bird species have been identified by direct observation with the binocular 7-29x50 and based on the features of the males' song.

The status of the avifauna in 1948 was evaluated from the manuscript of Fostrat (1948). The paper of Salmen (1980) was used for checking the phenological assignment of the species.

Results and discussions. A total number of 161 species has been recorded for the two study intervals (Table 1).

Table 1.

Comparative situation of the avifauna in the Sighisoara Area

Species	Avifauna in 1948	Actual avifauna	Habitat
Gavia arctica	P	-	Aq
Podiceps cristatus	-	P	Aq
Podiceps nigricolis	-	P	Aq
Tachybaptus ruficollis	-	Br	Aq
Ixobrychus minutus	-	Br	ReedAq
Ardeola ralloides	P	-	Aq
Nycticorax nycticorax	Br	-	Aq
Ardea cinerea	Br	Br	Aq
Ardea purpurea	P	-	Aq
Ciconia ciconia	Br	Br	Op
Ciconia nigra	Br	Br	Op
Anas platyrhynchos	Br	Br	Aq
Anas penelope	P	P	Aq
Anas querquedula	P	P	Aq
Aythya ferina	-	P	Aq
Aythya nyroca	P	-	Aq
Aquila pomarina	Br	Br	Fo
Hieraaetus pennatus	Br	-	Fo
Circaetus gallicus	Br	-	Fo
Buteo buteo	Br	Br	Fo
Buteo lagopus	Wi	Wi	Fo
Pernis apivorus	Br	Br	Fo
Accipiter gentilis	Br	Br	Fo
Accipiter nisus	Br	Br	Fo
Milvus migrans	Br	-	Fo
Circus aeruginosus	Br	-	ReedAq
Falco peregrinus	P	P	Op
Falco subbuteo	Br	Br	Op
Falco columbarius	-	Wi	Op
Falco vespertinus	P	-	Op
Falco tinnunculus	Br	Br	Op
Bonasa bonasia	Wi	-	Fo

Species	Avifauna in 1948	Actual avifauna	Habitat
Perdix perdix	Br	Br	Op
Phasianus colchicus	-	Br	Op
Coturnix coturnix	Br	Br	Op
Rallus aquaticus	Br	-	ReedAq
Porzana porzana	Br	Br	Wet
Crex crex	Br	Br	Op
Gallinula chloropus	Br	Br	Aq
Fulica atra	-	Br	Aq
Vanellus vanellus	-	P	Wet
Charadrius dubius	Br	Br	Wet
Scolopax rusticola	Br	-	Fo
Actitis hypoleucos	Br	Br	Wet
Larus ridibundus	-	P	Aq
Sterna hirundo	-	P	Aq
Columba oenas	Br	Br	Fo
Columba palumbus	Br	Br	Fo
Streptopelia turtur	Br	Br	Op
Streptopelia decaocto	-	Br	Op
Cuculus canorus	Br	Br	Fo
Otus scops	-	Br	Op
Athene noctua	-	Br	Op
Bubo bubo	Br	Br	Fo
Strix uralensis	Br	Br	Fo
Asio otus	Br	Br	Fo
Asio flammeus	Wi	-	Op
Strix aluco	Br	Br	Fo
Tyto alba gutata	P, Occ	-	Op
Caprimulgus europaeus	-	Br	Fo
Apus apus	Br	Br	Op
Alcedo atthis	Br	Br	Aq
Merops apiaster	-	Br	Op
Coracias garrulus	P	-	Op
Upupa epops	Br	Br	Op
Picus viridis	Br	Br	Op
Picus canus	Br	Br	Fo
Dendrocopos major	Br	Br	Fo
Dendrocopos syriacus	-	Br	Op
Dendrocopos medius	Br	Br	Fo
Dendrocopos minor	Br	Br	Fo

Species	Avifauna in 1948	Actual avifauna	Habitat
Dendrocopos leucotos	-	Br	Fo
Dryocopus martius	-	Br	Fo
Jinx torquilla	Br	Br	Op
Galerida cristata	Br	Br	Op
Alauda arvensis	Br	Br	Op
Lullula arborea	Br	Br	Op
Riparia riparia	Br	Br	Op
Ptyonoprogne rupestris	Occ	-	Op
Hirundo rustica	-	Br	Op
Delicon urbica	-	Br	Op
Anthus trivialis	Br	Br	Op
Anthus pratensis	Br	Br	Op
Anthus spinoletta	P	-	Op
Anthus petrosus	Occ	-	Op
Motacilla flava	Br	Br	Op
Motacilla cinerea	P	-	Op
Motacilla alba	Br	Br	Op
Lanius collurio	Br	Br	Op
Lanius minor	-	Br	Op
Lanius excubitor	-	Br	Op
Oriolus oriolus	Br	Br	Fo
Sturnus vulgaris	Br	Br	Op
Bombycilla garrulus	Wi	-	Fo
Garrulus glandarius	Br	Br	Fo
Pica pica	-	Br	Op
Corvus monedula	Br	Br	Op
Corvus frugilegus	Br	Br	Op
Corvus corone cornix	Br	Br	Op
Corvus corax	Br	Br	Fo
Troglodytes troglodytes	Br	Br	Fo
Cinclus cinclus	Wi	-	Aq
Prunella modularis	Br	Br	Fo
Locustella luscinioides	Br	-	ReedAq
Locustella fluviatilis	Br	Br	Wet
Locustella naevia	-	Br	Wet
Acrocephalus schoenobaenus	Br	-	ReedAq
Acrocephalus palustris	Br	Br	Wet
Acrocephalus arundinaceus	Br	Br	ReedAq
Sylvia nisoria	Br	Br	Op

Species	Avifauna in 1948	Actual avifauna	Habitat
Sylvia hortensis	P	-	Op
Sylvia borin	Br	Br	Fo
Sylvia atricapilla	Br	Br	Op
Sylvia communis	Br	Br	Op
Sylvia curruca	Br	Br	Op
Phylloscopus trochilus	P	-	Fo
Phylloscopus collybita	Br	Br	Fo
Phylloscopus sibilatrix	Br	Br	Fo
Regulus regulus	Wi	Wi	Fo
Regulus ignicapillus	Wi	-	Fo
Ficedula albicollis	Br	Br	Fo
Ficedula parva	Br	Br	Fo
Muscicapa striata	P	-	Fo
Oenanthe oenanthe	Br	-	Op
Saxicola rubetra	-	Br	Op
Saxicola torquata	-	Br	Op
Phoenicurus phoenicurus	Br	Br	Fo
Phoenicurus ochruros	Br	Br	Op
Erithacus rubecula	Br	Br	Fo
Luscinia megarhynchos	Br	Br	Wet
Luscinia luscinia	Br	Br	Wet
Turdus merula	Br	Br	Fo
Turdus philomelos	Br	Br	Fo
Turdus viscivorus	Br	Br	Op
Turdus pilaris	Br	Br	Op
Parus palustris	Br	Br	Fo
Parus caeruleus	Br	Br	Fo
Parus ater	Br	Br	Fo
Parus major	Br	Br	Fo
Aegithalos caudatus caudatus	Br	Br	Fo
Aegithalos caudatus europaeus	-	Br	Fo
Sitta europaea	Br	Br	Fo
Certhia familiaris	Br	Br	Fo
Passer domesticus	Br	Br	Op
Passer montanus	Br	Br	Op
Fringilla coelebs	Br	Br	Fo
Fringilla montifringilla	Wi	-	Fo
Pyrrhula pyrrhula	Wi	Wi	Fo
Coccothraustes coccothraustes	Br	Br	Fo

Species	Avifauna in 1948	Actual avifauna	Habitat
Serinus serinus	Br	Br	Op
Carduelis chloris	Br	Br	Op
Carduelis spinus	Wi	-	Fo
Carduelis carduelis	Br	Br	Op
Carduelis cannabina	Br	Br	Op
Carduelis flammea	Wi	-	Op
Loxia curvirostra	Wi	Wi	Fo
Miliaria calandra	Br	-	Op
Emberiza rustica	Wi	-	Fo
Emberiza schoeniclus	Br	-	ReedAq
Emberiza citrinella	Br	Br	Op
Plectrophenax nivalis	Wi	-	Op

Legend: Br- breeding species, Wi- winter visitors, P- passage species, Occ- occasionally species, Op- open land, Fo- forest, Aq- aquatic, ReedAq- paludal vegetation (*Phragmites australis* and *Typha latifolia*) around the permanent and temporary ponds, Wet-marshy area, with its characteristic vegetation.

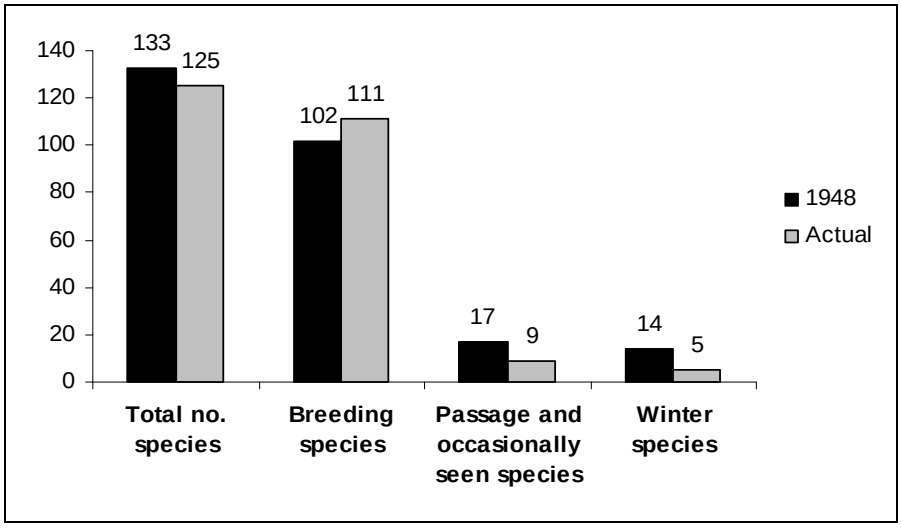

Fig. 2. Total number of bird species and their phenology in 1948, as compared to the present status.

In 1948, a total number of 133 species has been recorded, of which 102 breeding species, 17 passage and occasionally seen species, and 14 winter species (Fig. 2). As a result of the current study, a total number of 125 bird species was identified, of which 111 breeding species, 9 passage species, and 5 winter species (Fig. 2).

Concerning the breeding species, 88 of them have been recorded both in 1948 and at the present, 11 species were recorded as breeding only in 1948, while 21 species have been registered as breeding only in the present study. The difference between the total number of breeding species in the present and that back in 1948 is of 9 species. The species *Streptopelia decaocto* has extended its European distribution between 1925-1957, from Bulgaria to Denmark (G. de Lattin 1967, cited by Bănărescu 1970). This species has not been recorded as breeding species in 1948, probably due to its later occurrence in the colder and more humid valleys of the studied area, which are not the most suitable environments for this thermophile species. *Hirundo rustica* and *Delicon urbica* were not mentioned among the breeding species in 1948; probably the author did not take them into consideration due to their synanthrope nature. Also the species *Pica pica* was not considered, probably as a consequence of its omnipresence. It is worth mentioning that three large prey birds: *Hieraaetus pennatus, Circaetus gallicus* and *Milvus migrans* were recorded among the breeding species only in 1948. Thus, *Hieraaetus pennatus* was recorded for the first time as breeding species in the neighbourhoods of Sighişoara on the 25th of May, 1900, in an identified nest (Leonhard cited by Salmen, 1982). The same author had found on the 10th of May, 1901, a nest with fresh leaves but lacking eggs, while on the 7th of July, he noticed the presence of one offspring in the same nest. On the 29th of June, 1902, Leonhard identified another nest containing two offsprings. Weber and Mitruly (2001) have remarked this species as a breeding one in the neighbourhoods of Mediaş town (35 km west from Sighişoara) by recording 3 pairs identified around the Richiş and Jidvei villages. It is possible that the species also breeds in the valleys around Sighişoara, taking into account the proximity of the two areas. In 1903, Leonhard (cited by Salmen, 1982) found an egg-containing nest of *Circaetus gallicus* in the neighbourhoods of Sighişoara. In Transylvania, this species is rarely considered as a breeding one (Salmen, 1982). *Milvus migrans* has been recorded as breeding species in the Sighişoara area, on the 1st of May, 1905, based on a nest containing 3 eggs identified in the Greinmerich forest (Leonhard cited by Salmen, 1982). The nest was located 50m away from a nest occupied by *Milvus milvus*, a species that was considered a breeding one neither by Fostrat in 1948, nor by us during the present study. It is worthy to mention the species *Scolopax rusticola*, which was also recorded as breeding species in the study area only in 1948. In 1903, Leonhard (cited by Salmen, 1982) recorded this species as a breeding one in the vicinity of Sighişoara. On the 3rd of May, 1933, Höhr (cited by Salmen, 1982) has identified a nest containing 4 eggs on the Breite Plateau, located about 5 km away from

Sighişoara. *Scolopax rusticola* was also remarked as breeding species in the neighbourhoods of Mediaş (Weber, 1973), currently been very rarely recorded in the Târnava Mare Valley area.

Regarding the distribution of the bird species in the various habitat types, in 1948 most of the species (43) belonged to the forest habitat, followed by the open habitats (42 species) and the wet area (7 species), the other habitat types containing small numbers of species (Fig. 3). Most of the current avifaunal species (56) belong to the open habitats, followed by the forest habitat (41 species) and the wet area (8species) (Fig. 3).

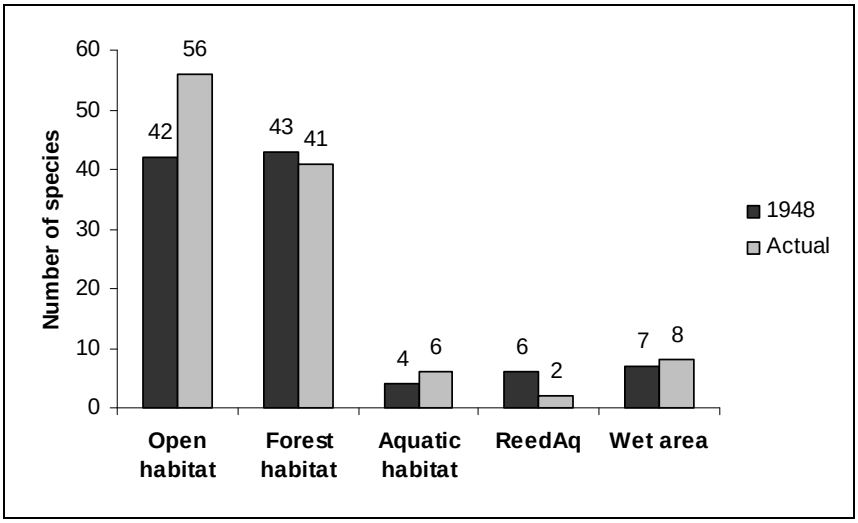

Fig. 3. Distribution of bird species according to their habitats in 1948, as compared to the present status.

Even if there is a small difference between the number of breeding species recorded in the forest habitat in 1948 as compared to the present (2 species), the larger number of species currently living in the open habitats as well as the disappearance from the study area of three species of large prey birds (see above) point out the anthropogenic impact on the forest habitats and the extension of the open lands.

The record only in 1948 of the breeding species *Nycticorax nycticorax, Circus aeruginosus, Rallus aquaticus, Locustella luscinioides, Acrocephalus schoenobaenus* and *Emberiza schoeniclus,* relying on swampy/marshy areas with extended reed development plead for the former existence of large flooded areas along the Târnava Mare River that was not submitted to regularisation works in those times. In the present, due to the change of the river's watercourse and to the dams built along its banks, permanent ponds 2.2 - 4 hectares in surface, surrounded by paludal vegetation (e. g. the Şercheş and Vânători ponds) have formed. As a result, in

the present study the breeding species *Tachybaptus ruficollis* and *Ixobrychus minutus*, depending on this habitat type have been recorded, species that were not mentioned in the evaluation from 1948.

Even if there were changes within some habitat types from one interval to the other, the coexistence of a mosaic of habitats on a relatively restricted area both in 1948 and at the present, contributes to the high diversity of species. The preservation of the habitat heterogeneity is needed as a prerequisite for the conservation of the avifaunal diversity in the region.

Conclusions. A total number of 161 species has been recorded for the two intervals under study. In 1948, a number of 133 species has been recorded, of which 102 breeding, 17 passage and accidentally seen, and 14 winter visitors. Under the present study, 125 species have been registered, of which 111 breeding, 9 passage, and 5 winter visitors.

In 1948, most of the species (43) belonged to the forest habitat, followed by the open habitats (42 species), the wet area (7 species), the temporary and permanent ponds with rich paludal vegetation (6 species), and the permanent ponds (4 species). At the moment, the open lands host the largest number of bird species (56), followed by the forest habitat (41 species), the wet area (8 species), the temporary and permanent ponds with rich paludal vegetation (2 species), and the permanent ponds (6 species). Now, the extension of the open lands on account of the forests is followed by the increase in the number of species belonging to the open habitats (56). The forest clearings and the high anthropgenic impact on the forest habitats are documented by the disappearance of some species of large prey birds (*Hieraaetus pennatus, Circaetus gallicus* and *Milvus migrans*).

The change of the watercourse of the Târnava Mare River and the dams build along it led to the disappearance of the large flooded areas with their typical vegetation, and to the formation of some permanent ponds, practically representing former river branches. As a consequence, in the present study the species relying on the vegetation characterising the large flooded areas (*Nycticorax nycticorax, Circus aeruginosus, Rallus aquaticus, Locustella luscinioides, Acrocephalus schoenobaenus* and *Emberiza schoeniclus*) have not been recorded anymore, while new species, depending on the recently-formed permanent ponds (*Tachybaptus ruficollis*, and *Ixobrycus minutus*) have occurred.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, I, 2006 (p. 23 – p. 29)

EFFECTS OF COPPER AND ZINC ON STRAWBERRY*(FRAGARIA ANANASSA D.)* FRUIT QUALITY

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Summary. Long term experiments were design to evaluate tolerance and metabolic changes when copper and zinc were applied in aqueous solution to leaves of strawberry plants in order to establish the usefulness of this taxa to be cultivated in polluted areas. Appropriated plant selection and evaluation is crucial for a successful soil use in contaminated areas. Field-based investigations are strongly needed to clarify whether the fruit of such contaminated plants can be used. Fruit harvested in 2001, 2002 and 2003 were characterized by lower carbohydrate and ascorbic acid content compared to control

KEYWORDS: carbohydrate synthesis, heavy metals, L-ascorbic acid strawberry, polluted areas.

Introduction. Contamination of soil, air and water presents a topical problem for human health. Heavy metals polluted areas are sources of contamination of ground and surface water. Heavy metal contaminated sites often support characteristic plant species that thrive in these polluted soils. Some of these taxa are able to accumulate or tolerate unusually high concentrations of metals.

Because some plants possess a range of potential mechanisms that may be involved in the detoxification of heavy metals, they manage to survive under metal stresses (Zha, *et al.*, 2004). High tolerance to heavy metal toxicity could rely either on reduced uptake or increased plant internal sequestration, which is manifested by an interaction between a genotype and its environment. The growing application of molecular genetic technologies has led to increased understanding of mechanisms of heavy metal tolerance/accumulation in plants (Yang *et al.*, 2005).

To avoid metal toxicity, organisms have evolved mechanisms including efflux of metal ions from cells and sequestration into internal cellular compartments. Members of the ubiquitous cation diffusion facilitator (CDF) family are known to play an important role in these processes (Zha, *et al.*, 2004). The plant CDF family member metal tolerance protein 1 (MTP1) from the Ni/Zn hyperaccumulator *Thlaspi goesingense* (*TgMTP1)* may suppress the Zn sensitivity of the plant. Expression of

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TgMTP1 leads to lowering of Zn accumulation and an increase in Zn efflux from the cells (Kinu *et.al.*2004).

Metal immobilization away from metabolically active sites within the cell represents the last step in both the homeostasis of metals and the detoxification of metal in excess. The vacuolar storage of Zn was confirmed, but no Zn accumulation at all was observed in *Arabidopsis halleri* protoplasts after Zn exposure. Specific metal tolerances were also found at the cellular level in the hyperaccumulating plants, highlighting that specific adaptations to metal ions exist in the cells as well as in the whole plants (Marques *et al.* 2004).

Plant physiological processes such as seed germination (Li *et al., 2005*), plant growth and chlorophyll synthesis (Lehman *et al*., 2004*)* and mitochondrial respiration (Chang *et al.,* 2005) seem to be inhibited by heavy metals such as copper and zinc.

The ecological hazards related to the heavy metals are not dependent on their total contents in the soil but rather on their form of bonding and therefore their bioavailability (Burzynski *et al.,* 2004).

The present study is focused on the ability of strawberry plants to tolerate different concentration of Zn and Cu, two cations which are often associated in polluted areas. These heavy metal represent the greatest potential source of contamination in the mine tailings areas. The aim of our research was to identify the condition which may associate an important fruit production with an effective absorption and translocation of heavy metals.

The quality of fruit is given by their carbohydrate and L-ascorbic acid (vitamin C) content. Although plants are the main source of vitamin C in the human diet, there is still a limited understanding of how plants synthesise L-ascorbic acid (AsA) and what regulates its concentration in different plant tissues. In particular, the enormous variability in the vitamin C content of storage organs from different plants remains unexplained. Possible sources of AsA in plant storage organs include *in situ* synthesis and long-distance transport of AsA synthesised in other tissues via the phloem (Hancock *et al.,* 2003).

In plants, L-ascorbic acid (AsA) is essential for photosynthetic activity via the detoxification of superoxide and hydrogen peroxide in chloroplasts in the absence of catalase (Noctor and Foyer, 1998).. AsA is also crucially involved in the regeneration of α-tocopherol and zeaxanthin and the pH-mediated modulation of PS II activity In addition to its general antioxidant functions, AsA has been implicated in cell division, cell wall metabolism, cell expansion, and plant-pathogen interactions (Smirnoff and Wheeler, 2000).

Appropriated plant selection and evaluation is crucial for a successful soil use in contaminated areas. Field-based investigations are strongly needed to clarify whether the fruit of such contaminated plants can be used.

Materials and methods. Long term experiments were design to evaluate tolerance and changes in carbohydrate accumulation and ascorbic acid level when Zn and Cu were applied to strawberry plants in order to establish of usefulness of this taxa to be cultivated in polluted areas.

The field experiment was located in Viile Satu Mare at the Institute of Horticultural Research and Production at 12 km distance from the Turt mine tailing, (nonferrous metals). The strawberry field was initiated in year 2000 using Redgauntlet cultivar. Redgauntlet is a Scottish variety with large, handsome, fruit fair in flavor, easy to pick, which is least subject to *Botrytis*. It is liked for the fresh fruit market, being considered too large for best processing.

Plants were grown in a mixture of sand 7, 6% (medium grade 2 to 0.2 mm), 48, 0% fine grade(0, 2 to, 02 mm), 20, 7% ultrafine grade <0, 002 mm, clay < 0, 01mm, and 1, 37% humus at a pH of 6, 3, with P_{AL} 34, 5 ppm, K_{AL} 148, 0 ppm, interchangeable Ca 4, 79 (ml/100g), interchangeable Mg 0, 69 (ml/100g). Plants were watered daily and fed weekly with a complex nutritive solution containing 1:1:1 NPK. The culture was treated with Elbatan as herbicide, and with 1%, $CuSO_4$, 0, 5-1% Topsin, 1% Systhane and 0, 05 – 0, 08%.Demitan as insecticides.

Ten different treatments have been used with replicates per each, as follows: V_1 – control (H_2O), V_2 – $CuSO_4$ c = 5.10^{-4}%, V_3 – $CuSO_4$ c = 5.10^{-3}%, V_4 – $CuSO_4$ c = 5.10^{-2}%, V_5 – $ZnSO_4$ c = 5.10^{-4}%, V_6 – $ZnSO_4$ c = 5.10^{-3}%, V_7 – $ZnSO_4$ c = 5.10^{-2}%. Besides, 3 mixed treatments have been tested consisting of V_8 – $CuSO_4$ + $ZnSO_4$ c = 5.10^{-4}%, V_9 – $CuSO_4$ + $ZnSO_4$ c = 5.10^{-3}%, and V_{10} – $CuSO_4$ + $ZnSO_4$ c = 5.10^{-2}%. The metals were applied monthly in aqueous solution to leaves.

Morphological measurements were performed daily, Total carbohydrate content (according modified Bertrand method, 1935) and L-ascorbic acid were measured consecutively three years (2001, 2002, 2003) after the ripening of the fruit. The procedure for the determination of ascorbic acid was based in its reducing reaction on mono (1, 10-Phenanthroline)-iron (III) and following the spectrophotometric determination of tris (1, 10-Phenan-throline)-iron (II). The absorption spectra measured against reagent blank shows its maximum absorption at 510 nm (Schilt, 1969). The results were statistical analyzed.

Results and discussion. The content of total carbohydrate of strawberry fruits differ according to the applied treatment. For most of the variants the copper and zinc treatment induce an inhibition of the carbohydrate synthesis, the recorded values being lower than the control, as presented in Table 1.

Increasing the applied copper concentration, the total amount of fruit carbohydrate decreased. Compared to control, the decrease is between 14, 82% for variant V_2 ($CuSO_4$ c=5.10^{-4}%), and 56, 8% for the variant V_4 ($CuSO_4$ c=5.10^{-2}%).

All the samples treated with zinc solutions had registered lower carbohydrate concentration than the control. The difference compared to the copper treatment consists of increase of carbohydrate concentrations with the increased zinc concentration applied.

Table 1.
Influence of copper and zinc on total carbohydrate content (%) of strawberry fruit

Variants	Total carbohydrate content g%				% of control	r compared to control	t compared to control	t Significance
	Annual avarages			General avarage				
	2001	2002	2003					
V_1 martor	8, 1	6, 4	7, 3	7, 266	100	-	-	-
V_2 $CuSO_4$ c=5.10^{-4}%	6, 9	6, 3	6, 4	6, 533	89, 908	0, 921	-2, 234	0
V_3 $CuSO_4$ c=5.10^{-3}%	4, 2	6, 2	5, 6	5, 333	73, 394	-0, 966	-1, 799	0
V_4 $CuSO_4$ c=5.10^{-3}%	3, 5	5, 9	4, 6	4, 633	63, 70	-1	-2, 280	0
V_5 $ZnSO_4$ c=5.10^{-4}%	3, 5	5, 4	3, 9	4, 266	58, 715	-0, 959	-2, 835	*
V_6 $ZnSO_4$ c=5.10^{-3}%	3, 9	6	4, 2	4, 7	64, 678	-0, 937	-2, 274	0
V_7 $ZnSO_4$ c=5.10^{-2}%	6	6, 3	6	6, 1	83, 944	-0, 882	-2, 007	0
V_8 $CuSO_4$+$ZnSO_4$ c=5.10^{-4}%	4, 2	5, 8	4, 6	4, 866	66, 972	-0, 970	-2, 489	0
V_9 $CuSO_4$+$ZnSO_4$ c=5.10^{-3}%	3, 9	5, 6	4, 1	4, 533	62, 385	-0, 928	-2, 709	0
V_{10} $CuSO_4$+$ZnSO_4$ c=5.10^{-2}%	3, 6	5, 2	3, 9	4, 233	58, 256	-0, 952	-3, 127	*

t significance at degrees of freedom$_e$ = 4
0 – no significance
* – significant differences
r - correlation coefficient

The mixed treatments (V_8-V_{10}) induced the highest inhibition of the carbohydrate synthesis in fruit, directly correlated with the metal concentration applied.

As can be seen in table 1, there are differences among the results obtained during the three years of the experiments, probably due to environmental conditions. In 2003 we had registered values with small differences compared to control, that year being warmer (with and average of 1.2°C) and wetter than the previous years. Annual variation in soluble solids and phytochemical composition was also observed by other researchers among nine strawberry genotypes, which was likely attributable to variations in solar radiation and air temperature (Del Pozo *et al*., 2006)

Table 2 presents the influence of copper and zinc on ascorbic acid content of the strawberry fruit. As can be seen the content of ascorbic acid differ according to the applied treatment. For all the variants, the heavy metal treatments induce a lower level of the ascorbic acid than the control. Comparing the effects of the two microelements applied, it may be seen that zinc has a stronger influence than the copper on this parameter. Increasing the applied copper concentration, the total amount of ascorbic acid increased. Compared to control, the increase is from 38, 73% for variant V_2 ($CuSO_4$ c=5.10^{-4}%), to 98, 73% for the variant V_4 ($CuSO_4$ c=5.10^{-2}%). The same, increasing the zinc concentration the level of ascorbic acid increase from 36, 82 at variant V_5 ($ZnSO_4$ c=5.10^{-4}%) to 51, 11% at variant V_7 ($ZnSO_4$ c=5.10^{-2}%).

L-Ascorbic acid (vitamin C) in fruits and vegetables is an essential component of human nutrition. Surprisingly, only limited information is available

about the pathway(s) leading to its biosynthesis in plants. It was reported the isolation and characterization of GalUR, a gene from strawberry that encodes an NADPH-dependent D-galacturonate reductase. There are evidences that the biosynthesis of L-ascorbic acid in strawberry fruit occurs through D-galacturonic acid, a principal component of cell wall pectins. Expression of GalUR is correlated with changing ascorbic acid content in strawberry fruit during ripening and with variations in ascorbic acid content in fruit of different species of the genus Fragaria.

Table 2.

Influence of copper and zinc on ascorbic acid content (mg/100g) of strawberry fruit

Variants	Ascorbic acid content (mg/100 g)				% of control	r compared to control	t compared to control	t significance
	Annual avarages			General avarage				
	2001	2002	2003					
V_1control	110	100	105	105	100	-	-	-
V_2 $CuSO_4$ c=5.10^{-4}%	53	32	37	40, 6666	38, 7301	0, 957	-17, 545	***
V_3 $CuSO_4$ c=5.10^{-3}%	65	38	47	50	47, 619	0, 982	-10, 718	***
V_4 $CuSO_4$ c=5.10^{-2}%	108	98	105	103, 6667	98, 7301	0, 974	-2, 00	0
V_5 $ZnSO_4$ c=5.10^{-4}%	48	31	37	38, 6666	36, 8254	0, 986	-30, 347	***
V_6 $ZnSO_4$ c=5.10^{-3}%	59	39	47	48, 3333	46, 0317	0, 993	-19, 126	***
V_7 $ZnSO_4$ c=5.10^{-2}%	68	43	50	53, 6666	51, 1111	0, 969	-10, 917	***
V_8 $CuSO_4$+$ZnSO_4$ c=5.10^{-4}%	104	96	105	101, 6667	96, 8254	0, 811	-1, 89	0
V_9 $CuSO_4$+$ZnSO_4$ c=5.10^{-3}%	89	67	75	77	73, 3333	0, 988	-7, 766	**
V_{10} $CuSO_4$+$ZnSO_4$ c=5.10^{-2}%	76	58	65	66, 3333	63, 1746	0, 992	-16, 086	***

t significance at degrees of $freedom_e$ = 4
0 – no significance
* – significant differences
** – distinct significant differences
*** very significant differences
r - correlation coefficient

Reduced pectin solubilization in cell walls of transgenic strawberry fruit with decreased expression of an endogenous pectate lyase gene resulted in lower ascorbic acid content. (Agius *et al.,* 2003). Our results may be explained by the interaction of heavy metals with cell wall molecules leading to lower ascorbic acid content.

Conclusions. 1. Different concentrations of copper and zinc applied monthly in aqueous solution to leaves, induced an inhibition of the carbohydrate synthesis, the recorded values being lower than the control.

2. Increasing the copper concentration, the total amount of fruit carbohydrate decreased.. The difference compared to the copper treatment consists of the increase of carbohydrate concentrations when zinc concentration increase.

3. For all the variants, the heavy metal treatments induce a lower level of the ascorbic acid than the control. Comparing the effects of the two microelements applied, it may be seen that zinc has a stronger influence than the copper on this parameter.

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THE PHOTOCHEMICAL ACTIVITY RELATED TO THE XANTHOPHYLL CYCLE DURING PHOTOINHIBITION AND TO THE RECONVERSION PERIOD IN THE GREEN ALGA *Mougeotia sp.*, STRAIN AICB 560

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Summary. The effect of various light intensities on PS II photochemistry and xanthophyll cycle and also their recovery in the dark are presented. The green alga *Mougeotia sp*., strain AICB 560 displayed a slower growth. Under 1500µmol.m^{-2}.s^{-1} light the fluorescence has been enhanced the first 60 minutes, especially F_V, and then has decreased according to the exposure time. The quantic efficiency and PS II quantic yield were equal to the control. Chlorophyll a has decreased, chlorophyll b has enhanced and the high content of anteraxanthin, lutein and carotens was highlighted. The presence of zeaxanthin proves the operation of the xanthophyll cycle. It becomes functional the lutein → lutein 5.6-epoxide interconvertibilily with photoprotective role. The 3000 µmol.m^{-2}.s^{-1} light caused the increase of F_0, the decrease of F_M, F_V and F_V/F_M, certifying the installation of photoinhibition. The level of zeaxanthin and anteraxanthin has grown, and this certifies the increasing of the excess excitation energy dissipation. The photochemical activity has dramatically decreased by exposure to 4500 µmol.m^{-2}.s^{-1}. The xanthophylls of the xanthophyll cycle were maintained at high values, as well as the protein content. The recovery of the photochemical activity was more obvious in those variants treated with the first two lights, and it has a bimodal graphic shape. The level of violaxanthin increased by the epoxidation of zeaxanthin, whose concentration has decreased, as well as the anteraxanthin concentration. It was proved the PS II damage irreversibility in dark

KEYWORDS: chlorophyll fluorescence; carotenoid; growth curve; HPLC analysis; recovery period.

Introduction. The organisms that develop oxygenic photosynthesis convert the light energy into chemical energy. The light is absorbed into thylacoidal membranes of the

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algae and higher plants by means of the light-harvesting antenna complexes that bind the chlorophyll (LHCs) in connection to the reaction center of PS I and PS II.

When the photosynthetic apparatus absorbs the excess light over the necessary of the photosynthesis process, the PS II becomes the subject of irreversible photooxydative damage. These selective damages affect the PS II reaction center proteins, this leading to the potential decrease of photosynthesis level and to diminution of plants growth and productivity (Powles and Critcheley, 1980; Powles, 1984).

Under excess light violaxanthin is quickly converted (de-epoxidation) through anteraxanthin, to zeaxanthin, while the opposite reaction (epoxidation) is renewed under low light. This reversible sequence of the two independent reactions is named *xanthophyll cycle* (Demming-Adams and Adams, 1996). The carotenoids that participate to this cycle are present exclusively in the thylacoidal membrane which suffers concentration changes due to the light. According to Demming-Adams (1990) the xanthophyll cycle has an important role in the protection of the photosynthetic apparatus against the damages of the photoinhibition, through the involvement of zeaxanthin in the dissipation of the excess energy as heat (non-photochemical energy). Alternatively, in the zeaxanthin epoxidation reactions (reactions that take place only in dark), the reactive oxygen radicals such as singlet oxygen are consumed (Siefermann-Harms, 1987; Schindler and Lichtenthaler 1996; Schubert *et al*., 1994; Young, 1991).

In this article there are represented the connections between the photochemical activity of PS II and the xanthophyll cycle activity during the various light intensities induced photoinhibition and the reconstruction way of these activities in the absence of light.

Materials and methods. *Strains and growth conditions.* The green alga *Mougeotia sp*. Agardh, strain AICB 560, derives from the I.C.B. Cluj-Napoca Collection of algae cultures (Dragoş *et. al*., 1997). Strain AICB 560 was grown in Bold (BBM) nourishing solution, under air agitating, continuous illumination with 560 $\mu mol.m^{-2}.s^{-1}$, at a 26^{o}C temperature. The cultivation period was of 21 days. The development of the algal suspension was estimated by drawing the growth curve on the basis of $\Delta \mathbf{log_2}$ of the optical density (Stein, 1973).

The luminous treatment. The 1500, 3000 and 4500 $\mu mol.m^{-2}.s^{-}1$ PAR (photosynthetically active radiation) light intensities were applied for 120 minutes, at room temperature (illumination period). As light source there have been used 500W halogen bulbs. The suspension was magnetically stirred during the whole period of light exposure in order to homogenize the cell, and they were covered with a water filter to avoid the heating. The development of the photochemical activity during the recovery period (post-illumination) was also investigated.

Pigments and proteins analysis. Pigments analysis was performed in the end of each luminous treatment and recovery period. The alga suspension was grinded and extracted with acetone in presence of $CaCO_3$. The chlorophylls (chlorophyll a and chlorophyll b) and carotenoids contents were spectrophotometrically estimated on the basis of absorption specific coefficients (Arnon, 1949; Lichtenthaler and Wellburn, 1983). The proteins were detected by Lowry *et al*. (1951) method, by hydrolyzing the algal suspension on water bath for one hour with 0, 1 N NaOH, and then performing the reaction with Folin reagent.

Carotenoids analysis by HPLC. The algal suspension was concentrated by filtration, and the pellet was saponified with a 30% KOH in ethanol solution (1:1 ratio with the algal suspension), at room temperature, for 10 hours. The carotenoids were extracted with ethylic ether by repeated washing and then they evaporated. The resulted residue was suspended in 5 ml ethyl acetate, and it was used for (HPLC). The analysis was performed on two systems:

- System 1 – for carotenoids identification: built by Altex 110 A pumps (Kontron AG), Altex mixing chamber, Altex automatic gradient controller, Waters 990 photodiode matrix detector and Waters 990 soft for data gathering and processing.

- System 2 – for quantitative analyses: the Aglient 1100 system includes degasor, the quaternary Aglient G1311A pumps system and the Rheodyne injector equipped with 20µl loop, EC 250/4.6 Nucleosil 120-5 C18 column, an UV/VIS Agilent G1314A detector and a Chemstation Aglient software. The used gradient: 10 min: 10% B / 90% A; 20 min: 70% B / 30% A (A represents the acetonitrile:water mixture, which is 9:1; B is ethyl acetate). The chromatograms represent the major carotenoids according to the retention time. The separation analysis was performed at 450 nm wavelength, at 35^{o}C temperature and 144 bar pressure.

Chlorophyll fluorescence analysis. The fluorescence of chlorophyll has been measured with a PAM-210 fluorometer, according to Schreiber *et. al*. (1986). Fluorescence parameters and quenching analysis were performed applying the saturation pulse method. The quantic yield of photochemical energy conversion were detected by = $\Delta F/F_M$, while the ratio F_V/F_M ($F_V/F_M = F_M\text{-}F_0/F_M$) expresses the photochemical quantic production of the closed PS II reaction centers. The fluorescence parameters of the algal suspensions grown in normal conditions were used as control sample.

Results and discussions. *1. The growth process.* The growth curve of *Mougeotia* cultures in 560 µmol.m^{-2}.s^{-1} light is represented in Fig 1. The cultures displayed a slower growth, especially between the days 9 and 13, with an exponential growth rate (R) = 0.175, a medium growth rate (R_m) = 0.069 and a

generation period (g) = 5.7. The HPLC chromatogram including major carotenoids in the algal suspensions considered to be control proved the lutein and carotenes dominance. On the other hand, there was detected a low concentration of violaxanthin and a significantly growth of anteraxanthin concentration. The lack of zeaxanthin is due to the medium light intensity in the growth period, which is not in excess, and consequently, the xanthophyll cycle is not working. The chlorophyll a concentration was also very high because of the medium intensity of the growth light (Table 1).

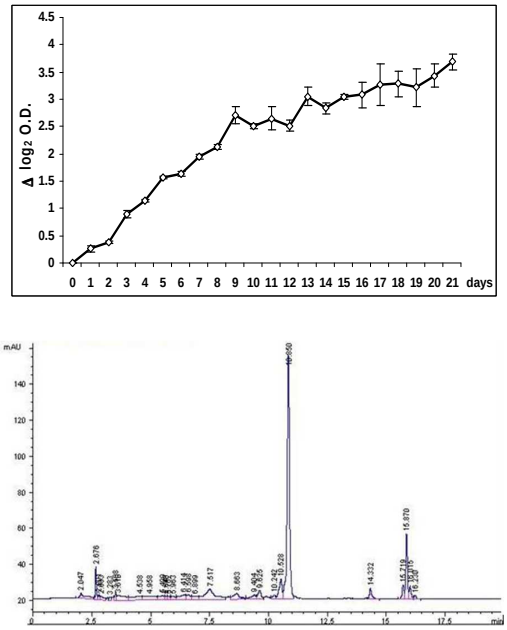

Fig. 1. Exponential growth curve and carotenoids chromatogram in algal suspensions grown in normal conditions and thought to be controls.

2. PS II phocotchemical activity during illumination period. Under 1500 $\mu mol.m^{-2}.s^{-1}$ light the chlorophyll fluorescence increased during the first 60 minutes and then gradually decreased in time (Fig. 2.). The values were superior to the control. The PS II quantic efficiency and also the photosynthetic performance of the entire electrons carrier chain (yield) were about equal to the control values. The increase of the variable fluorescence certifies the increment of oxidized Q_A (the plastoquinone volume increases), contributing to increment of the harvesting rate of the excitation energy by the antenna and to high-maintained energy harvesting efficiency by the reaction centers.

Concentration of chlorophyll a decreased, while concentration of chlorophyll b increased under light action (Table 1). Between all major carotenoids

detected there was observed a high content of anteraxanthin, lutein and carotenes. It also was noted the presence of zeaxanthin, as a proof for the xanthophyll cycle operation under tested light intensity. Xanthophylls interconvertibility process in the sense of violaxanthin (di-epoxide) → anteraxanthin (mono-epoxide) → zeaxanthin (without epoxide) by addition or removal of the epoxide group, was obviously intensified with the production in high concentration of the intermediary product. With this reaction, the lutein → lutein 5.6-epoxide interconvertibility reaction becomes functional. The xanthophylls which derive from α-caroten and also lutein are LHC_S structural constituents, and contribute to the dissipation of the light energy excess (Niyogy *et al.*, 1997). The content of cell proteins obviously increased (Table 1). Xanthophylls are related to the chlorophyll of the membranous integrated protein complexes – LHC which absorb and transfer the excitation energy to the photosynthetic reaction centers to be used in electrons' transport, this reactions having the role of converting light energy to chemical energy to be used for fixing CO_2 and producing carbohydrates.

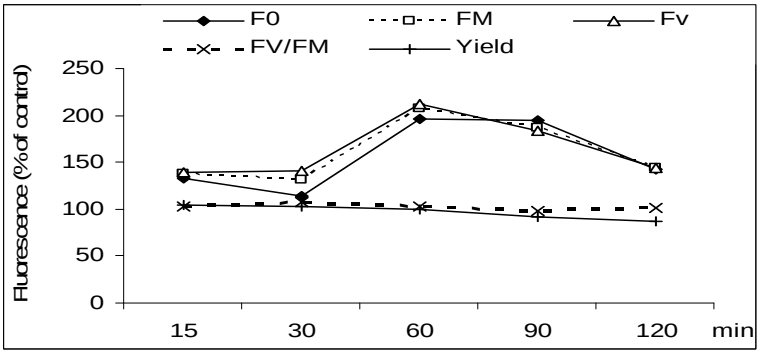

Fig. 2. Development of the chlorophyll fluorescence under 1500 µmol.m^{-2}.s^{-1}.
F_0 = minimal fluorescence; F_M = maximal fluorescence; F_V = variable fluorescence; Yield = quantic production.

Lutein, which is a zeaxanthin structural isomer, differing from it only by one double bundle, has one de-epoxidated cycle group (analogous to anteraxanthin), and may have a role in energy dissipation. The xanthophyll cycle which implies lutein 5.6-epoxide seemed to take place in the parasite plant *Cuscuta* which lacks neoxanthin. The violaxanthin de-epoxidase operates on 5.6-epoxide which is de-epoxided to lutein that has a photoprotective function by loosing epoxide (Bungard *et al.*, 1999).

The illumination with 3000 µmol.m^{-2}.s^{-1} lead to the increase of the chlorophyll florescence during the first 60 minutes (Fig. 3). The long-term exposure, over 60 minutes, caused the increment of F_0, decrease of F_M, F_V and FV/FM ratio (PS II photochemical efficiency) and also of quantic yield at electrons

linear carrier. Generally, the fluorescence values were over the control values. The decrease of variable fluorescence certifies the decrease of oxidized Q_A (the plastoquinone volume decreases), helping to the decrease of PS II excitation energy catching rate, and the FV/F_M, ratio distinguishes a low quantic yield of the closed reaction centers of PS II. The development of the chlorophyll fluorescence parameters after 60 minutes exposure period, certifies the inducement of photoinhibition in the photosynthetic apparatus. By the decrease of the FV/F_M ratio it is proved the inducement of the PS II photosystem photoinhibition, and implicitly the photosynthetic capacity decrease.

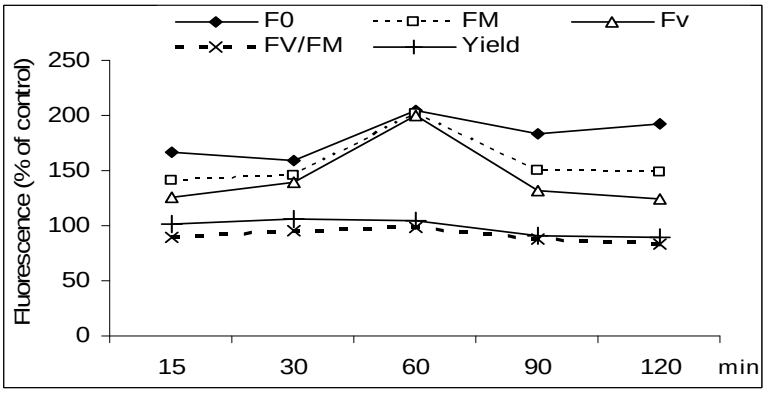

Fig. 3. Development of chlorophyll fluorescence under 3000 $\mu mol.m^{-2}.s^{-1}$ light intensity.

The major plant carotenoids are lutein, β-carotene, vilaxanthin and neoxanthin. β-carotene is related to PS II reaction center, while xanthophylls are related to the LHC_S antenna proteins. The increase of lutein content leads to the decrease of violaxanthin concentration and increment of total carotenoids content (Pogson and Rissler, 2000). The decrease of violaxanthin content is related to plastochinone oxidation state (Pfündel and Bilger, 1994).

After exposing the algal suspensions to 3000 $\mu mol.m^{-2}.s^{-1}$ light intensity there was observed a significant increase of carotenoids content (Table 1). The concentration of xanthophylls implied in the xanthophyll cycle, such as zeaxanthin and anteraxanthin increased, this certifying the increasing of the excess energy dissipation at the level of photosystem's antenna. The high β-carotene content explains the excess energy dissipation at the level of PS II reaction center. The cell protein content decreased below control values (Table 1).

Table 1.

Quantity of chlorophylls and carotenoids, and the retention time (minutes) in *Mougeotia* suspensions under various light intensities.

Parameters	Control		1500 µmol.m^{-2}.s^{-1}		3000 µnmol.m^{-2}.s^{-1}		4500 µmol.m^{-2}.s^{-1}	
	con	*rt*	*con*	*rt*	*con*	*rt*	*con*	*rt*
chlorophyll *a* mol.10^{-5}	15.22	-	9.43	-	7.33	-	7.95	-
chlorophyll *b* mol/mol chl. *a*	0.37	-	0.39	-	0.36	-	0.39	-
neoxanthin*	2.13	2.05	8.83	1.82	12.61	1.85	4.65	1.85
violaxanthin	0.61	3.45	1.96	3.45	4.42	3.45	0.58	3.48
anteraxanthin	3.12	10.53	80.59	10.91	102.38	10.64	35.85	10.71
lutein	32.1	10.85	92.69	11.06	76.61	10.87	34.40	10.91
zeaxanthin	-	-	5.70	11.36	8.66	11.15	3.07	11.19
lutein 5, 6-epoxid	1.23	14.33	1.01	14.55	1.30	14.30	-	-
α-carotene	1.36	15.7	8.23	15.87	7.76	15.68	0.65	15.69
β-carotene	6.46	15.87	38.96	16.02	39.53	15.83	1.95	15.84
9Z-β- carotene	1.36	16.01	7.13	16.16	7.76	15.98	0.65	15.98
15 Z-β-carotene	0.34	16.23	2.74	16.38	3.53	16.19	-	-
a / *b*	2.64	-	2.51	-	2.71	-	2.51	-
a + *b* / *c*	4.47	-	0.88	-	0.81	-	2.65	-
Proteine (g.g^{-1} s.u.)	4.84	-	5.18	-	4.41	-	5.69	-

Legend: * = carotenoids are expressed in mmol/mol chlorophyll *a*
a = chlorophyll *a*; *b* = chlorophyll *b*; *c* = carotenoid; *con* = content; *rt* = retention time

The photosynthetic activity dramatically decreased after exposing the algal suspensions to 4500 µmol.m^{-2}.s^{-1} light, and fluorescence parameters were reduced in value comparatively to the control (Fig. 4). The minimal fluorescence was value situated over the maximal fluorescence which was maintained at a low value, distinguishing the low quantity of closed Q_A acceptors.

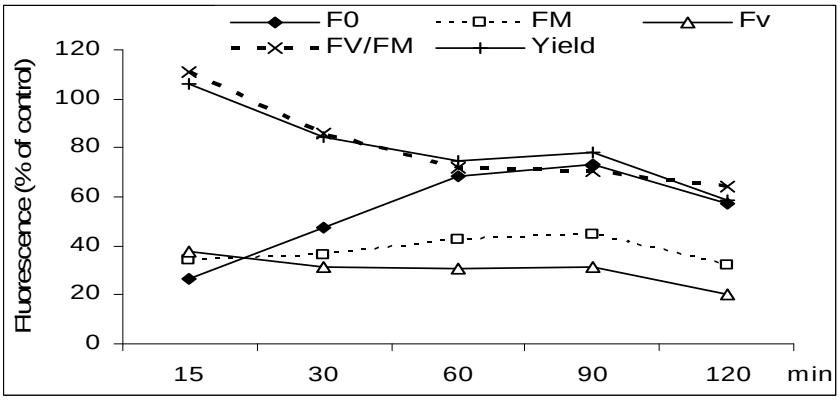

Fig. 4. Development of chlorophyll fluorescence under 4500 µmol.m^{-2}.s^{-1} light intensity.

The low values of minimal fluorescence explain the oxidative state of Q_A acceptor, and the life period it short (Laisk *et al*., 1997). The photochemical

efficiency of the reaction centers and the electrons linear transport quantic yield were significantly reduced during light exposure, which certifies the installation of the stable photoinhibition or photoinactivation state at the level of the photosynthetic apparatus, leading to the cell photosynthetic capacity decrement. In these conditions, there are two processes that take place in order to adjust the photoinactivation: a – the changes in photochemical activity regarding the capacity of charges stable separation and b – the constant rate of non-photochemical activity (down regulation) which contributes to photoprotection by reducing photoinhibition targets (Oxborough and Baker, 2000).

The chlorolphylls content of the photosynthetic apparatus increased due to the increment of the chlorophyll b concentration, which lead to the change in components ratios (Table 1). Major carotenoids concentration has significantly decreased except lutein and anteraxanthin. The photoinhibition that was produced by excess light affected very much the carotenes. The xanthophylls of the xanthophyll cycle were maintained at high values, and the lack in 5.6-epoxide certifies photodamages at the level of photosystems' antennae after photoinhibition, contributing to the photochemical activity decrease. The content of cell proteins has significantly increased (Table 1).

3. PS II photochemical activity during the recovery period. The chlorophyll fluorescence is an important indicator at the level of photosynthesis. Chlorophyll is the antenna's major pigment driving the absorbed energy toward the reaction center where the photochemical conversion of the excitation energy takes place.

During the recovery period, when the light is lacking, after exposing the algal suspensions to 1500 $\mu mol.m^{-2}.s^{-1}$, the fluorescence parameters displayed values upper than the control values, with two peaks at 30 and 90 recovery minutes (Fig. 5). The fluorescence bimodal aspect certifies the energized state whish is still maintained at the level of thylacoidal membranes. The thylacoids photosynthetic performance in the recovery period expressed through the indicators F_V/F_M and $\Delta F/F'_M$ was slowly reduced comparatively to the control.

The decrease of chlorophylls content causes the decrease of light-harvesting polypeptides (Maxwell *et al.*, 1995). The carotenoids concentration has significantly enhanced during the recovery period. Between the xanthophylls of the xanthophyll cycle, it was distinguished the increase of violaxanthin due to the drastic decrease of anteraxanthin (Table 2). The lutein content increased through the disappearance of lutein 5.6-epoxide element. The neoxanthin content has significantly increased, but the carotenes were maintained at same values. To observe that in this period the xanthophyll and lutein cycle operates very efficient in order to produce violaxanthin by zeaxanthin epoxidation, and to produce lutein by consuming the quantity of lutein 5.6-epoxide. The increase of violaxanthin content is related to the reducing state of plastoquinone. The low pH leads to the increase of plastoquinone reduction and thus the violaxanthin quantity increases. The content of cell proteins has significantly decreased, being situated below the control values (Table 2).

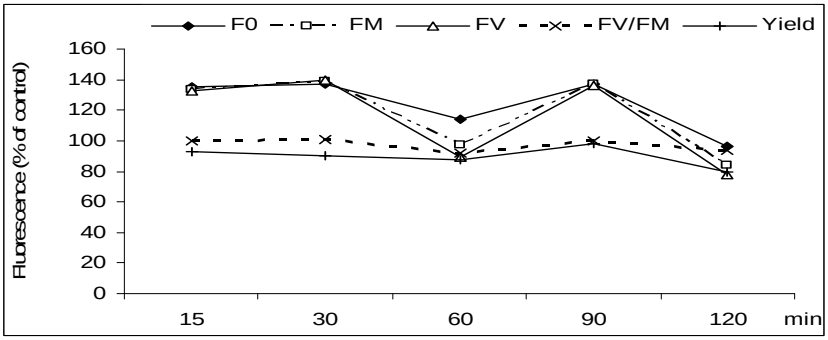

Fig. 5. Development of chlorophyll fluorescence in the recovery period after exposing the algal suspensions to 1500 µmol.m^{-2}.s^{-1} light.

The chlorophyll a content was significantly reduced, leading to the changing of the ratios between the photosynthetic apparatus components (Table 2).

Table 2.

Quantity of chlorophylls and carotenoids and the retention time (minutes) in *Mougeotia* suspensions during the recovery period.

Parametrii	Control		1500 µmol.m^{-2}.s^{-1}		3000 µmol.m^{-2}.s^{-1}		4500 µmol.m^{-2}.s^{-1}	
	con	*rt*	*con*	*rt*	*con*	*rt*	*con*	*rt*
chlorophyll *a* mol.10^{-5}	15.22		7.33		6.03		8.25	
chlorophyll *b* mol/ mol chl. *a*	0.37		0.39		0.38		0.38	
neoxanthin*	2.13	2.05	29.642	2.22	9.201	2.06	0.56	2.14
violaxanthin	0.61	3.45	59.285	2.81	5.366	3.42	5.60	3.49
anteraxanthin	3.12	10.53	11.663	10.42	81.128	10.32	2.88	10.27
lutein	32.08	10.85	143.23	10.81	53.448	10.61	21.90	10.59
zeaxantin	-	-	6.662	11.09	4.050	10.89	1.18	10.87
lutein 5, 6-epoxid	1.25	14.33	-	-	0.787	14.09	1.73	14.1
α-carotene	1.36	15.72	7.059	15.67	6.007	15.48	1.25	15.52
β-carotene	6.46	15.87	35.295	15.82	31.750	15.63	6.27	15.67
9Z-β- caroten	1.36	16.02	7.059	15.96	6.007	15.77	1.25	15.81
15 Z-β-caroten	0.339	16.23	-	-	2.574	15.98	0.63	16.02
a / b	2.64		2.53		2.61		2.57	
a + *b* / *c*	4.47		0.88		1.07		5.05	
Proteins (g.g^{-1} s.u.)	4.84		3.83		4.15		5.35	

Legend: * = carotenoids are expressed in mmol/mol chlorophyll *a*
a = chlorophyll *a*; *b* = chlorophyll *b*; *c* = carotenoid; *con* = content; *rt* = retention time

During dark-recovery (dark-adaptation) after exposure to 3000 µmol.m^{-2}.s^{-1}, the fluorescence parameters enhanced in the first 30 minutes, attending a rapid decrease and then an increase after a physiological rebalance of 90 minutes (Fig. 6). The minimal and maximal fluorescence distinguished the existence in equal concentrations of the Q_A reaction centers in oxidized and reduced state, and even so the photochemical efficiency of the closed reaction centers and the photosynthetic

performance were maintained below the control values. Removal of the photoinhibition effects was significant in the first 30 minutes of recovery.

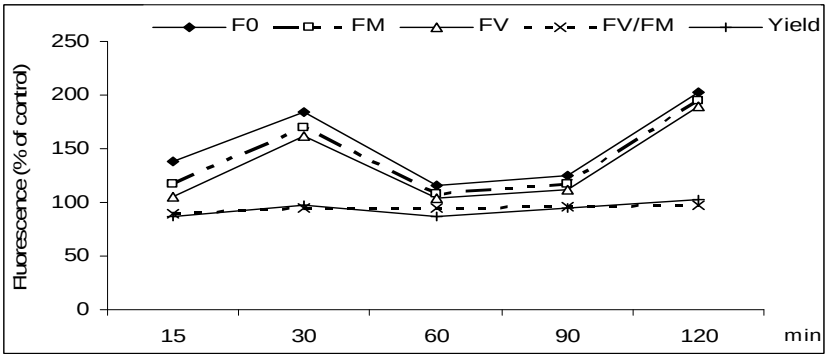

Fig. 6. Development of chlorophyll fluorescence during the recovery period after exposing the algal suspensions to 3000 $\mu mol.m^{-2}.s^{-1}$ light.

The assimilator pigments content of the algal suspensions during the recovery period after being exposed to 3000 $\mu mol.m^{-2}.s^{-1}$ light has significantly decreased. The chlorophylls quantities have decreased leading to changes of the ratio between the photosynthetic apparatus' elements, this having consequences on the photosynthetic capacity and performance (Table 2). Carotenoids concentration was reduced in thylacoids. Lutein and neoxanthin quantities have also decreased, while the decrease of the carotenes was insignificant. On the other hand, the violaxanthin quantity enhanced through zeaxanthin epoxidation whose concentration decreased as well as anteraxanthin. The violaxanthin and lutein cycle operates in the photosynthetic balances recovery. Meanwhile, the cell proteins concentration decreased (Table 2).

During dark adaptation after irradiating the algal suspensions with 4500 $\mu mol.m^{-2}.s^{-1}$ light, the development of chlorophyll fluorescence parameters is presented in fig. 7. The increase of the chlorophyll fluorescence parameters and of photosynthetic performance in 90 minutes of dark adaptation was recorded, and after that the photosynthetic activity was reduced because of the absence of light necessary for the photochemical processes. The minimal fluorescence finally reached the control values. Holding the variable fluorescence at low values because of the maximal fluorescence that remained low, emphasizes the low photochemical efficiency of the closed reaction centers that dominate the membranes' structure, while it is distinguished the photodestruction irreversibility at the level of PS II that which is hardly removed in dark.

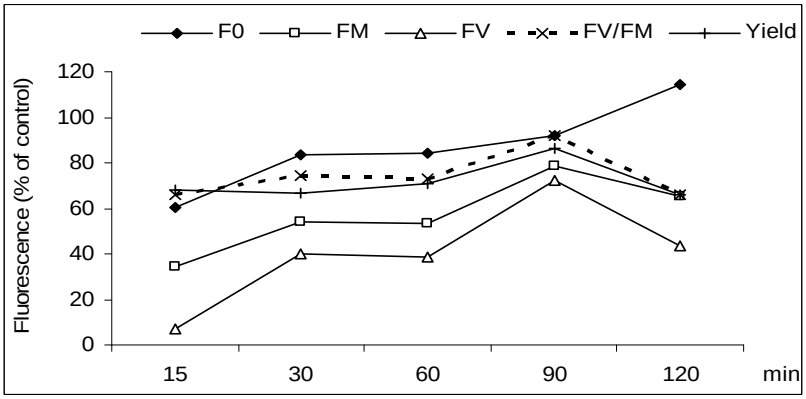

Fig. 7. Chlorophyll fluorescence development in the recovery period after exposing the algal suspensions at 4500 $\mu mol.m^{-2}.s^{-1}$ light.

In the end of the recovery period at the 4500 $\mu mol.m^{-2}.s^{-1}$ light-treated variant there was observed a substantially increase of chlorophyll a, this leading to ratios changes between the pigment compounds (Table 2). Major carotenoids were also significantly reduced. There was observed a relative recovery of the carotenes compounds that were strongly inhibited by excessive light. The xanthophylls cycle that took place produced a high quantity of violaxanthin due to the zeaxanthin epoxidation and through decrease of anteraxanthin content. It also started to operate the lutein cycle by decrease of lutein content and enhancement of lutein 5.6-epoxide concentration. The accumulated high protein quantity during illumination maintained at high values during dark adaptation of the photosynthetic structures, indirectly distinguishing the photodestructions that take place at the level of the photosystems antennae (Table 2).

Conclusions. PS II photochemical activity during the illumination period under various light intensities rapidly reacts to the stability state assessed by photoinhibition. The high light intensities proportionally modify the chlorophyll fluorescence parameters.

Thus, at lower experimented light intensities, the increase of variable fluorescence certifies the increase of plastoquinone volume, contributing to the increment of the energy harvesting rate by the reaction centers. The PS II open reaction centers proportion was reduced, this meaning that a big part of the excitation energy was non-photochemically dissipated. Among carotenoids there were the high anteraxanthin, lutein and carotenoids content and also the presence of zeaxanthin that were emphasized, as a proof for the xanthophyll cycle. It also becomes functional the interconvertibility reaction lutein → lutein 5.6–epoxide.

The long excessive light exposure lead to the increase of F_0, the decrease of F_M, F_V, photochemical efficiency and quantic yield, resulting in the decrease of the

cell photosynthetic capacity. Also the concentration of the xanthophylls involved in the xanthophyll cycle such as zeaxanthin and anteraxanthin increased, this certifying the intensification of the excess excitation energy dissipation at the level of photosystem's antenna. The protein content was generally maintained at high values.

The PS II photochemical activity in the dark adaptation period remained tributary to those effects that were induced by various experimented light intensities. At the variants that were exposed at low or moderated-intensity of light, the recovery of the photochemical activity was significant comparatively to the control. To notice that during this period the xanthophyll and lutein cycle operates at high efficiency in order to produce violaxanthin by epoxdating zeaxanthin, respectively producing lutein by decreasing the lutein 5.6-epoxide content. The increase of violaxanthin content is related to the reduction state of plastoquinone. The cell proteins content has decreased.

At the strong-light exposed variant, the recovery of the photochemical activity was difficult and slow, distinguishing the irreversibility of the photodestruction at the level of PS II, these being difficultly removed in dark. The xanthophyll cycle produced a high quantity of violaxanthin by epoxidating the zeaxanthin and by decreasing the anteraxanthin content. The high proteins quantity accumulated during illumination was also maintained at high values during dark recovery.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 1, 2006 (p. 45 – p. 55)

EXCITATION PRESSURE AND ENERGY NONPHOTOCHEMICAL DISSIPATION UNDER EXCESS LIGHT IN *Mugeotia sp.*, GREEN ALGA STRAIN AICB 560

VICTOR BERCEA[1], BOGDAN DRUGĂ[1], CĂTĂLINA VASILESCU[1] and NICOLAE DRAGOŞ[2]

Summary. Excitation pressure effects on PS II under light excess and nonphotochemical dissipation of excitation energy (q_N) concomitantly with violaxanthin de-epoxidation in green alga *Mugeotia sp*. are presented. Light intensities of 1500, 3000 and 4500 $\mu mol.m^{-2}.s^{-1}$ were applied for 120 minutes in the alga exponential growth phase. The excitation pressure on PS II reaction centers enhanced under 1500 $\mu mol.m^{-2}.s^{-1}$, increasing the number of closed (reduced) Q^-_A while the photochemical reactions decreased and q_N. At high light, the excitation pressure decreased, being correlated to q_p increment, which denotes a Q^-_A reoxidation, possible due to stromatic reactants that operate on plastoquinone pool through the chlororespiration. The q_N enhanced meaning that the reaction centers were gradually closed with the exposure period. Violaxantin de-epoxidation that was observed by absorption changes at 505 nm enhanced with the exposure period leading to zeaxanthin enhancement, being correlated with q_N. De-epoxidation was correlated with the tylacoidal membrane conformational changes displayed by the absorption changes at 535 nm and 700 nm. During the post-illumination period, q_p has decreased with the reduction of the q_N in the absence of light.

KEYWORDS: chlorophyll fluorescence; PS II and PS I reaction centers; primary acceptor; redox state; violaxanthin de-epoxidation.

Introduction. Under photooxidative conditions, light absorption performed through chlorophyll is the main cause of damages. The processes that absorb and stock light energy with great efficiency are located into thylacoids. PS II is susceptible to photooxidative damages, and the recovery of processes is difficult because of the photoinbibition. Thylacoids contain specific constituents and some processes related to a photoprotective role. The light-harvesting system

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develops a great structural flexibility between two different states: one for light-harvesting, and another one for excess excitation energy dissipation as heat.

The energy dissipation requires the existence of LHC membrane proteins and particular-type carotenoids which causes the connection between the two states. Under excess light there appears the mechanism for photoprotective reduction of light –harvesting efficiency (Kobližek *et al*., 2001).

Xanthophylls are related to chlorophyll – as structural entities – into the LHC complexes which absorb and transfer the excitation energy that is necessary for the photosynthetic transport of the electrons to the reaction centers, these reactions converting the light energy into chemical energy. The xanthophylls cause the de-excitation of the singlet chlorophyll (^{1}Chl) - accumulated in the LHC complexes under excess light and were measured as NPQ. NPQ enhancement is correlated with zeaxanthin and anteraxanthin synthesis starting from violaxanthin. Singlet zeaxanthin and anteraxanthin can directly accept the excitation energy from chlorophyll. Excited chlorophylls are returned to the basic state through nonradiative heat dissipation (Niyogi *et al*., 1997).

Zeaxanthin and anteraxanthin are synergistically acting with the trans-thylacoidal ΔpH gradient in dissipation of the excitation energy excess, but the precise photoprotection mechanism is still unknown (Thayer and Björkman, 1990; Horton *et al*., 1991; Gilmore and Yamamato, 1991; Königer *et al*, 1995).

In this article there are presented the effects of excitation pressure on PS II photochemical activity under excess light and the nonphotochemical dissipation of the excitation energy correlated with violaxanthin de-epoxidation.

Material and methods. *Strains and growth conditions.* The green alga *Mougeotia sp.* Agardh, strain AICB 560, derives from the Collection of Algae Cultures of I.C.B. Cluj-Napoca (AICB) (Dragoş *et al*., 1997). Stain AICB 560 was grown in nutritive Bold (BBM) solution, under continuous air stirring and continuous illumination with 560 $\mu mol.m^{-2}.s^{-1}$ at 26^{o}C.

Light treatment. The 1500, 3000 and 4500 $\mu mol.m^{-2}.s^{-}1$ PAR (photosynthetically active radiation) light intensities were applied for 120 minutes, at room temperature (illumination period). As light source there have been used 500W halogen bulbs. The suspension was magnetically stirred during the whole period of light exposure in order to homogenize the cell, and they were covered with a water filter to avoid the heating. The development of the photochemical activity during the recovery period (post-illumination) was also investigated.

Chlorophyll fluorescence analysis. Chlorophyll fluorescence was measured with PAM-210 fluorometer like Schreiber *et al*. (1986). Fluorescence parameters and quenching analysis were performed by saturation pulse method. *In vivo* PS II excitation pressure was estimated by $1-q_p$. Algal suspensions growth in normal conditions were used as controls.

Violoxanthin de-epoxidation, respectively light-depending zeaxanthin-forming were analyzed *in vivo* by measuring the absorption changes at 505 nm (ΔA_{505}) (Bilger *et*

al., 1989); Q_A quinone acceptor state at 320 nm (Q^-_A - Q_A); state of PS I reaction center at 700 nm (Melis, 1989).

Results and discussions. The indicator function of chlorophyll fluorescence is proved by the fluorescence emission that is complementary to other alternative de-excitation ways, which are the photochemistry and heat dissipation. Regarding the fluorescence emission there are two competitive processes of de-excitation, that are fundamentally different:

- photochemical energy conversion in PS II reaction centers,
- the energy non-photochemical losses in antenna and in the reaction centers.

Due to the both mechanisms the maximal fluorescence is decreased. Fluorescence decrement implies the photochemical rate intensifying and the enhancement of the excess excitation energy dissipation rate.

The excitation pressure in PS II reaction center enhanced, with a bimodal aspect on the graphic, under 1500 $\mu mol.m^{-2}.s^{-1}$, this meaning the enhancement of the proportion of reduced Q^-_A whose lifetime is longer and the decrement of photochemical reactions and of open reaction centers (Fig.1). The excess light causes PS II reaction centers closure because of the excitation pressure enhancement (Maxwell *et al*., 1995). In the presence of Q^-_A the lifetime of the excitation is determined by other competitor processes for decrease of excitation energy such are the thermal dissipative ones (Laisk and Oja, 2000).

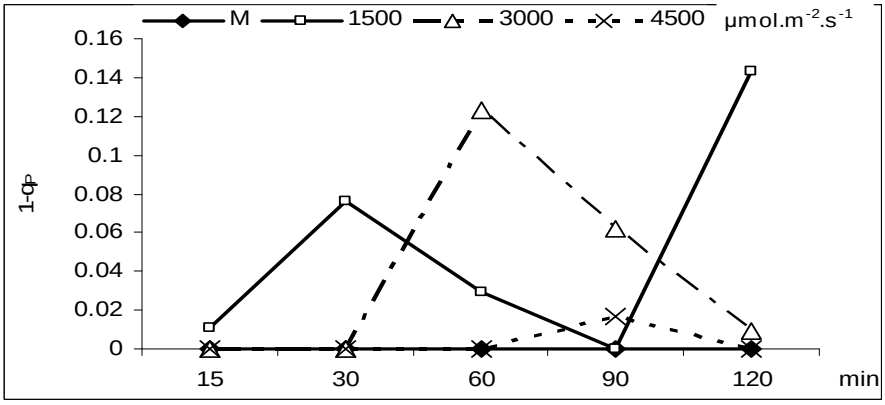

Fig. 1. Evolution of excitation pressure in PS II at *Mougeotia* strains.

At high light used in this experiment, the excitation pressure was decreased emphasizing the decrease of the photosynthetic electrons linear transport activity. This decrement was correlated with q_p quencher which means an intensification of the Q^-_A oxidation reaction, possibly due to the stromatic reactants that act on the plastoquinone pool by chlororespiration.

Under 1500 $\mu mol.m^{-2}.s^{-1}$ light the PS II open reaction centers was reduced proportionally to the light-exposure period which means that a part of excitation

energy was nonphotochemically dissipated (Fig. 2). This decline proves that the photochemical conversion of quanta was saturated. Under 3000 µmol.m^{-2}.s^{-1} light, the Q_A open reaction centers excelled control values, this meaning that the excitation energy was efficiently converted during the photochemical reactions leading to the stimulation of the photosynthetic electrons transport. Under 4500 µmol.m^{-2}.s^{-1} light, the concentration of Q_A open reaction centers maintained within control values, certifying the balance between the photochemical and non-photochemical reactions in PS II.

The changes that occurred in the absorption at 320 nm emphasize the high quantity of primary acceptor in oxidized state (Q_A), this state being correlated with q_p evolution (Fig. 3). This oxidized state makes an exception from the rule in some moments of excess light exposure, such is between 60-90 minutes at 1500 µmol.m^{-2}.s^{-1} light intensity.

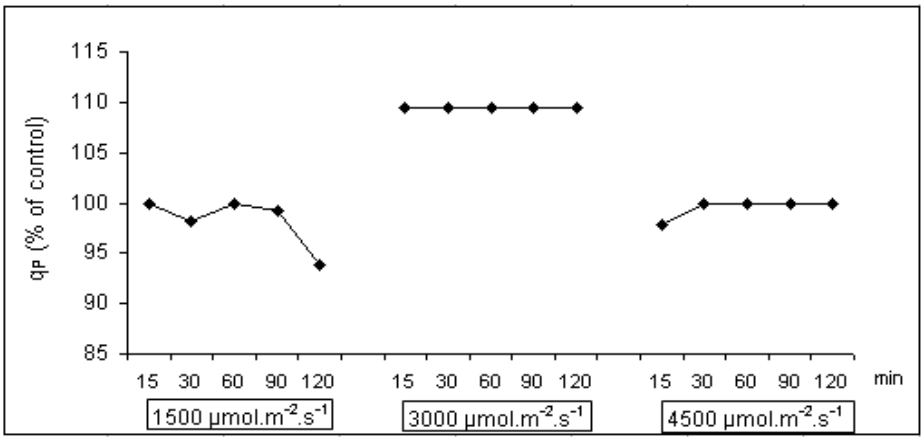

Fig. 2. Evolution of photochemical quencher during illumination for 120 minutes.

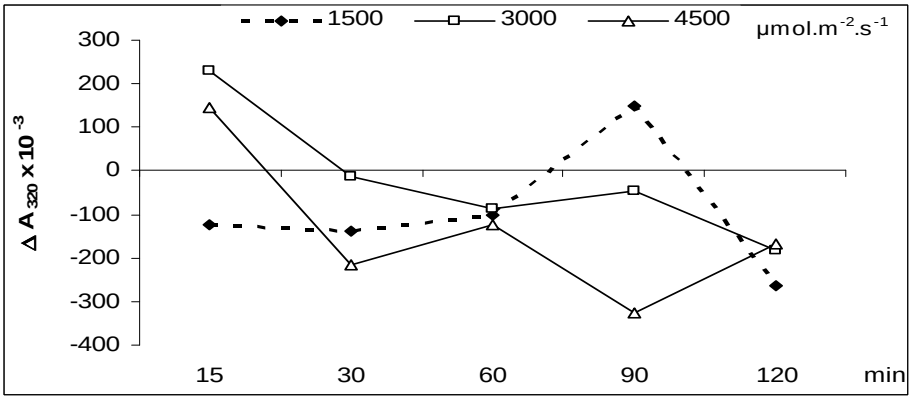

Fig. 3. Absorption changes occurred at 320 nm regarding the redox state of the Q_A primary acceptor.

The photoinhibition state is related to the reaction center and antenna alteration and leads to the decrease of PS II photochemical production. By decreasing the variable fluorescence the non-photochemical dissipation of the excitation energy was intensified (q_N). Thus, the dissipation was much more intense under 1500 µmol.m^{-2}.s^{-1} and 4500 µmol.m^{-2}.s^{-1} light and lower under 3000 µmol.m^{-2}.s^{-1} (Fig. 4.). This means that the reaction centers were gradually closed with the exposure time, leading to the decrease of the photosynthesis performances by decreasing the non-cyclic electrons transport. In this case, the energization state of the thylacoidal membranes is raised as well as the rate of closing the PS II reaction centers in light (Kobližek *et al*., 2001).

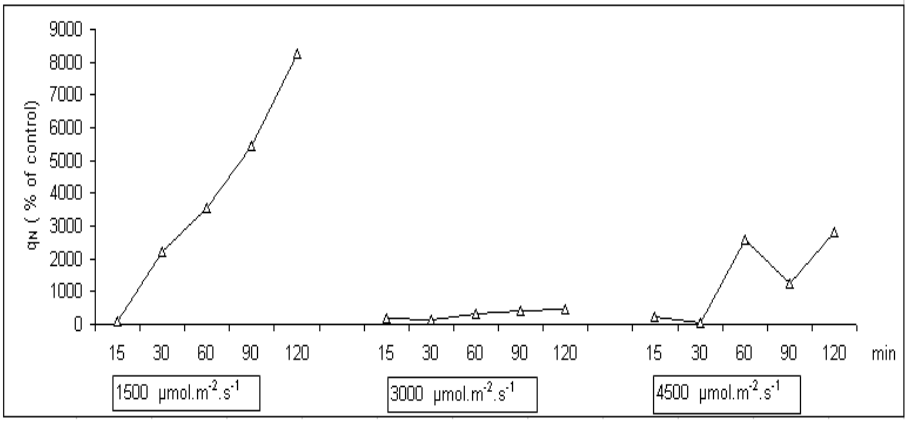

Fig. 4. Evolution of nonphotochemical quencher during illumination for 120 minutes.

The protection of photosynthetic apparatus against damages takes place through the dissipation of the excess excitation energy which can be measured with q_N induced by the pH of the thylacoidal lumen as a result of electrons transport (Ruban *et al*., 1993). When the photochemical decrease is insufficient, the excitation excess must be non-photochemically decreased (q_N), this being a physiological protective mechanism called"down regulation", which maintains constant the light-independent excitation lifetime and reduces the effects of inactivation (Lazár, 1999, Netto *et al*., 2002). Between the components of the excitation energy dissipation mechanism, q_E is the reversible reduction of the photochemical efficiency factor providing protection against photoinhibition.

The dissipation of the excess excitation energy is produced directly in the chlorophyll-carotenoid antennary complexes for PS I and PS II. These processes need a high proteins concentration in the thylacoidal membrane. The molecular mechanism of this process is based on: simple and direct excess excitation energy transfer from chlorophyll to

zeaxanthin, activated by the pH gradient; -protonation-dependent chlorophyll de-excitation is indirect and non-stoichiometrically modulated by zeaxanthin (Demming-Adams, 1990).

The violaxanthin de-epoxidase that was examined through absorption changes at 505 nm emphasizes its intensification with light exposure time leading to zeaxanthin enhancement. Enzyme activity, namely the enhancement of zeaxanthin, correlates with the dissipation of the excitation energy (q_N) (Fig. 5). The absorption changes at 505 nm are related to the xantophyll cycle activity and it is due to the absorption differences between violaxanthin and zeaxanthin (Niyogi *et al*., 1997). The de-epoxidase activity is controlled by the pH of the thylacoidal lumen coupled with the membranal protons lead by the ATP synthesis (Pfündel et al., 1994). There is a close connection between the q_E component of the excitation dissipation mechanism and the violaxanthin de-epoxidation to zeaxanthin, respectively the absorption change at 535 nm (Ruban *et al*., 1993). Xanthophylls are located exclusively in the pigment-protein light harvesting complexes (LHC), emphasizing the implication of the PS II antenna in the excitation dissipation. In fact q_E derives from the structural changes regarding the pigments coupling at LHC II, that is synergistically influenced through LHC II polypeptides protonation and violaxanthin re-replacement by zeaxanthin.

The conformational changes that occur in the thylacoidal membrane are shown in Fig. 6. There is a similarity in the evolution of the absorption changes at 505 nm and 535 nm. These changes are due to the membrane energization by H^+ absorption, respectively LHCs protonation in the presence of zeaxanthin leading to the structural features alteration (Niyogi *et al*., 1997; Müller *et al*., , 2001). The pH decrement leads to the cytochrome b/f reduction inhibition and to plastoquinone reduction enhancement. The result is the enhancement of violaxanthin content by decreasing the plastoquinone oxidation rate in the cytochrome b/f (Pfündel *et al*., 1994). The absorption changes at 535 nm depend by the balance between the activity of the in-dark ATP-consuming reactions and light that produces membranes energization (Bilger *et al*., 1989).

The activity of PS I reaction center distinguishes the oxidative state dominancy, that correlates with the intensification of the electrons carrier chain quantic yield (Fig. 7). Within 60 and 90 minutes there occurred a decrement of the oxidative state, this being correlated with the decrease of fluorescence, and of quantic yield in the photosynthetic chain (Bercea *et al*., 2006). The LHC II aggregating state is shown by absorption changes at 700 nm.

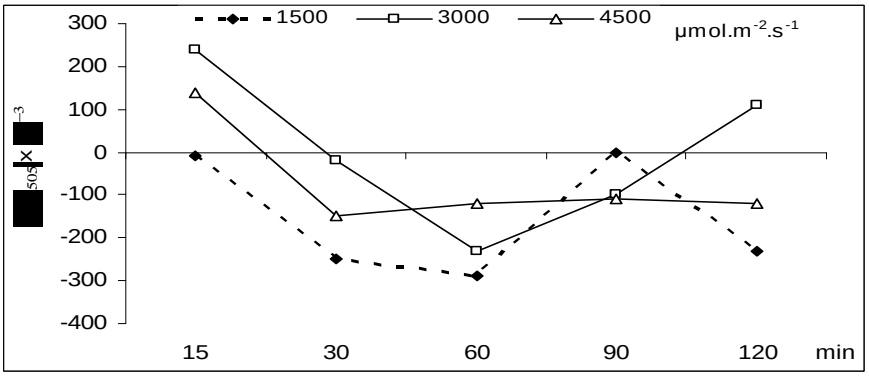

Fig. 5. Absorption changes produced to the 505 nm regarding the epoxidase activity.

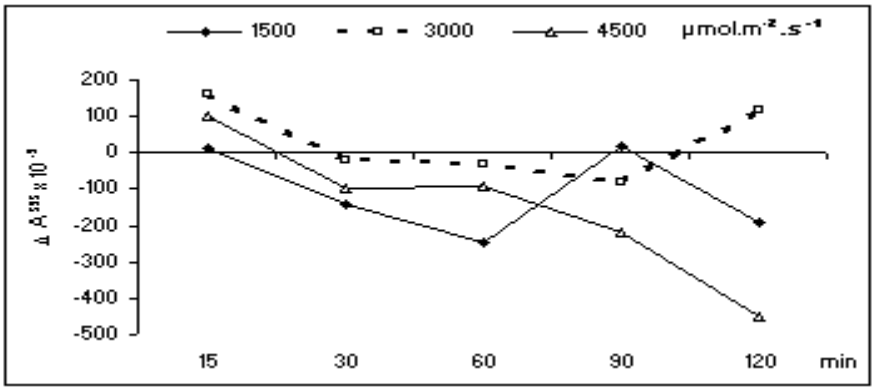

Fig. 6. Absorption changes at 535 nm regardind the thylacoidal membranes redox state.

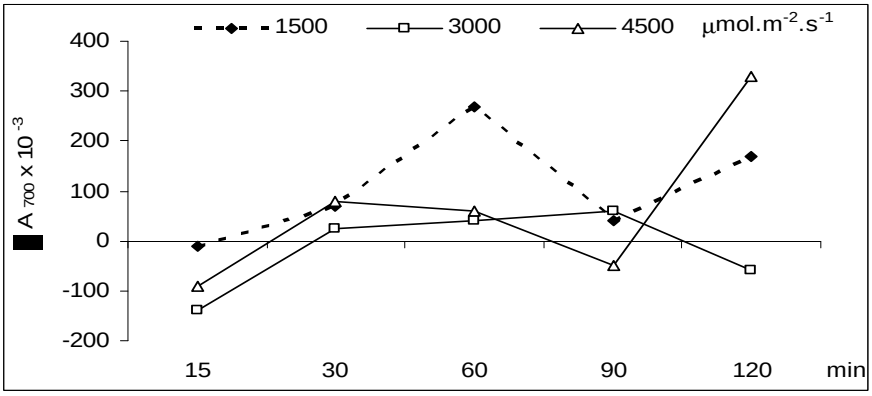

Fig. 7. Absorption changes at 700 nm regarding the P_{700} reaction center activity.

During the post-illumination period (darkness period), q_P has decreased after illumination with 1500 µmol.m^{-2}.s^{-1}, excepting the peak from 90 minutes; by keeping the algae in dark for recovery; it also maintained over control values in the variant exposed at 3000 µmol.m^{-2}.s^{-1} with a minimum at 60 minutes and remained constant to the variant exposed at 4500 µmol.m^{-2}.s^{-1} (Fig. 8). This evolution displays a liniar correlation with the quantic yield. Where q_p was decreased, the proportion of open Q_A was significantly reduced and thus the photochemical conversion efficiency of the quanta was reduced.

In the absence of light, the q_N coefficient that shows the non-photochemical dissipation of the excess excitation energy has significantly decreased in those variants treated with 1500 and 3000µmol.m^{-2}.s^{-1} light (Fig. 9). The slow relaxation of the energy dissipation distinguishes the fact that the q_I component acts, correlated with PS II photoinactivation. In the variant treated with 4500 µmol.m^{-2}.s^{-1} light, the non-photochemical dissipation of the excitation energy in the dark maintained at high values, displaying a maximum at 30 minutes, this certifying that at the level of PS II took place some photodestructions that can be very heavily removal through dark relaxation.

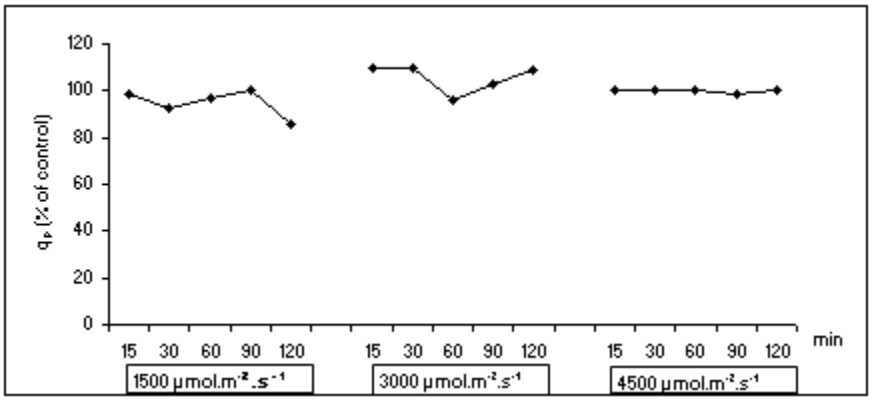

Fig. 8. Evolution of photochemical quencher during the recovery period of 120 minutes.

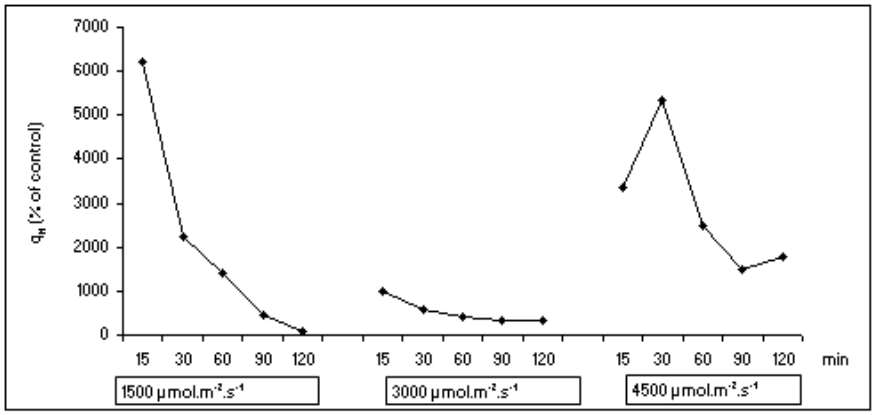

Fig. 9. Evolution of nonphotochemical quencher during dark recovery period of 120 minutes.

Conclusions. The competitive processes of de-excitation which refer to photochemical energy conversion from PS II reaction centers and to non-photochemical losses of excitation energy in antenna and the reaction centers contribute to fluorescence decrement under excess light.

The excitation pressure on PS II reaction centers enhanced under 1500 $\mu mol.m^{-2}.s^{-1}$ light, keeping a high proportion of reduced Q^-_A, whose lifetime is protracted while the number of photochemical reactions and open reaction centers decreases, this certifying that a part of the excitation energy was non-photochemically dissipated.

Under high light, the excitation pressure decreased distinguishing the decrement of the photosynthetic electrons linear transport activity. This decrease was correlated with the q_p enhancement, this proving the re-oxidation of Q^-_A centers, maybe due to the stromatic reactants that act on the plastoquinone pool through chlororespiration.

By decreasing the variable fluorescence, the non-photochemical dissipation of the excitation energy (q_N) enhanced, meaning that the reaction centers were gradually closed with exposure time leading to the photosynthetic performances decrement through electrons non-cyclic transport decrease.

The violaxanthin de-epoxidation activity observed through absorption changes at 505 nm intensified with the exposure time leading to the enhancement of zeaxanthin content and being correlated with excitation energy dissipation (q_N). The de-epoxidation was related to the thylacoidal membrane conformational changes and the LHC II aggregation state displayed by the absorption changes at 535 and 700 nm.

During the post-illumination period the photochemical quencher q_p decreased in the dark, and the open Q_A proportion decreased, thus the photochemical conversion efficiency of light quanta also decreased, being related to the quantic yield. In dark, the non-photochemical dissipation of the excess excitation energy was also reduced.

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STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LI, 1, 2006 (p. 57 – p. 70)

DIVERSE HEPATIC ULTRASTRUCTURAL MORPHOLOGY INDUCED BY CHRONIC ETHANOL FEEDING OF RATS

CORNELIU TARBA[1] and ADRIAN FLOREA[2]

Summary. Male Wistar rats were maintained in our animal facility for 14-18 weeks on a normal diet, with free access to water. One group of the rats served as control (C), while in another group (A) each rat was supplemented individually with 1.5 ml of 48% ethanol/100 g body weight daily. At the end of the period, the rats were sacrificed and most of their liver used for preparation of mitochondria, on which several functional assays were performed *in vitro*, as previously described (Tarba and Suărăşan, 2003; 2004). A small piece of the original hepatic tissue as well as mitochondrial sediment from the incubation vessel were always prepared for electron microscopy. The electron micrographs of the hepatic tissue from control animals confirm the state of good health of our rats. Thus, the nuclei and the rest of the organelles have a normal appearance. Mitochondria observed *in situ* are dominated by the so-called orthodox configuration, while *in vitro*, the great majority of them have a condensed conformation and intact membranes. For most rats, chronic ethanol consumption has grave consequences on the hepatic and mitochondrial ultrastructure, as demonstrated by the images obtained from the A group. Thus, the cell nuclei have little chromatin and large nucleoli, with intense activity, the smooth endoplasmic reticulum is highly dilated and vesiculated, a clear peroxysome proliferation is present in cells with a reduced amount of lipids, while lipid droplets are present in cells where peroxysomes are rare. The mitochondria *in situ* have an irregular (polymorphous) shape and an electron transparent matrix, apparently lacking the cristae, while most of the isolated mitochondria present a condensed and ultracondensed or falciform aspect, with a rarefied central zone and a matrix condensed at the periphery. In the presence of calcium the mitochondria are swollen or even disintegrated. The ultrastructural morphology observed supports our previous functional results.

KEYWORDS: chronic ethanol feeding, rat hepatic ultrastructure.

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Introduction. Relatively recent data have shown that chronic ethanol consumption or its acute administration is able to initiate a process of hepatic apoptosis, followed very often by necrosis and leading eventually to specific hepatic structural and functional alterations known under the name of alcoholic liver disease (ALD) (Nanji, 1998; Hill *et al*., 1998; Deaciuc *et al*., 1999; 2001; Tsukamoto and Lu, 2001; Tsukamoto *et al*., 2001; Higuchi *et al*., 2001; Adachi and Ishii, 2002; Ishii *et al*., 2003). Although the mechanism by which alcohol induces the disease is not entirely known, chronic ethanol exposure has been associated with mitochondrial oxidative stress and partial decrease in ATP synthesis (Halestrup *et al*., 1997; Kurose *et al*., 1997; Cunningham and Van Horn, 2003; Wu and Cederbaum, 2003). On the other hand, the same type of factors are implicated in membrane permeabilisation and release of the so-called apoptogenic factors, residing in the mitochondrial intermembrane space, *i.e.*, factors and events that are leading to apoptosis (Kroemer *et al*., 1995; 1997;1998; Petit *et al*., 1996; Marchetti *et al*., 1996; Scarlet and Murphy, 1997; Susin *et al.*, 1997; Hirsh *et al*., 1998; Bernardi *et al*. 1999; Reed, 2000; Bernardi *et al.* 2001; Tarba, 2001). In general, the apoptotic sequence following the liberation of the apoptogenic factors from mitochondria is well known and understood, while the way in which the release occurs is still a matter of debate. Most researchers agree that the central event in this release is a drastic change in mitochondrial membrane permeability associated with the formation of a so-called permeability transition pore (PTP), considered as an irreversible phenomenon (Kroemer *et al*., 1995; 1997; 1998; Bernardi *et al*., 1999; Crompton, 1999; Petronilli et al., 2001). The exact sequence of events leading to PTP formation, however, continues to be disputed. One point of view holds that the opening of the pore is accompanied by membrane potential collapse, uptake of electrolytes and water, matrix swelling and rupture of the mitochondrial outer membrane. As a consequence, several factors present in the intermembrane space, among which cytochrome c (cyt c), are liberated into the cytosol. There is, however, an alternative point of view, which holds that cyt c and probably other proapoptotic factors can be released without a permeability transition and outer membrane breaking, due to certain pores created into the outer membrane by several proapoptotic agents, such as Bax and Bid (Eskes *et al*., 1998; Martinou *et al*., 1999; von Absen *et al*., 2000; Kim *et al*., 2000; Gogvadze *et al*., 2001). Even if the first alternative is accepted, the exact relationship between membrane potential collapse and swelling (*i.e.*, which is the cause and which is the effect) is not completely elucidated.

The present paper belongs to a series of studies regarding the sequence of events involved in the liberation of the apoptogenic factors from the intermembrane space of the rat hepatic mitochondria. For this purpose, we selected chronic ethanol feeding as a natural model of producing hepatic apoptosis. In combination with the use of suspending media of different ionic contents and the addition of different concentrations of calcium, also known to induce oxidative cell injury and apoptosis (Thor *et al*., 1984; Grijalba *et al.*, 1999; Ermak and Davies, 2002; Orrenius *et al*., 2003), we used a DA spectrophotometer to monitor, in parallel, membrane potential,

calcium release and matrix swelling. Liver slices and aliquots of both supernatant and mitochondrial sediment taken at specific moments of incubation were prepared or saved for later analysis, including electron microscopy (EM). The present article describes and discusses the EM (ultrastructural) results of our study, while most of the functional studies have already been reported (Tarba and Suărăşan, 2003; 2004) A preliminary report of the present results has also been published (Tarba and Florea, 2005).

Materials and methods. Male white Wistar rats were maintained in our animal facility for 14-18 weeks, starting from an average weight of 120 g/individual, while the evolution of their weight was assessed periodically. The rats were kept on a normal diet (a premix containing all the ingredients of the Larsen diet), with free access to water. One group served as control while in another group each rat was supplemented daily with 1.5 ml of 48% ethanol/100 g body weight, administered in the morning, on a little piece of bread, before getting access to the food. At the end of the period, the rats were fasted for 24 hrs and sacrificed by decapitation after a slight anaesthesia. Small pieces of liver were taken in some cases and prepared for electron microscopy, according to standard techniques, while the rest of the liver was used for the preparation of mitochondria, essentially as previously described (Johnson and Lardy, 1967; Tarba, 1983), in a medium containing 200 mM mannitol, 70 mM sucrose, 5 mM Hepes-KOH (pH 7.37) and 0.5 mM Na-EDTA. The washing and preserving medium lacked the chelating agent (EDTA). The suspending media that are of interest for the present work are designated as MKS (110 mM mannitol, 65 mM KCl, 40 mM sucrose, 1.5 mM $MgCl_2$, 1 mM KP_i, 5 mM Hepes) and KSW (100 mM KCl, 50 mM sucrose, 5 mM KP_i, 10 mM Hepes), also known as the swelling medium. In both cases the pH was 7.35. Mitochondria were suspended in one of the above-mentioned media and NADH-dependent respiration was abolished by the addition of 8 μM rotenone before the introduction of succinate (2.5 mM) as a respiratory substrate. In combination with the two suspending media of different ionic contents and the addition of different concentrations of calcium, membrane potential and calcium fluxes were each monitored in parallel with the swelling by the use of appropriate probes and a diode array (DA) spectrophotometer, as previously described (Tarba and Suărăşan, 2003; 2004).

Liver slices and aliquots of both supernantant and mitochondrial sediment taken at specific moments of incubation were prepared or saved for later analysis, including electron microscopy. For EM, the material was processed as described previously (Tarba and Crăciun, 1990) and the ultrastructural images were obtained using a JEOL JEM 1010 transmission electron microscope.

Results and discussion. *The control rats*. Hepatic ultrastructure. Our EM images obtained from the liver of the control rats are representative for the normal state of the liver, but also show appreciable interindividual variations, which, for

simplicity, can be grouped into two distinct types. In both cases, the nuclei have a normal aspect, with large and active nucleoli, but with a slightly dilated perinuclear space (Fig.1 and 2). Biliary canaliculi are normal and the microvilli are present in a great number in the Disse space. However, in one case (case 1), illustrated by Fig.1, the smooth endoplasmic reticulum is slightly dilated, the lipids are present in a small quantity while glycogen is almost absent. The mitochondria *in situ* have an orthodox configuration (conformation), with a normal aspect, both as form and electron density. In the second case (case 2), as illustrated in Fig.2, lipids seem to be absent from the cytoplasm while glycogen is present in large quantities, as black granules. The mitochondria look similar to the first case, although the matrix electron density seems to be higher. Also, in a few situations (not illustrated here), the nuclei have a slightly irregular contour. It can be concluded that these are interindividual variations which are circumscribed within the limits of the normal. We mentioned these two types of variation, however, because they seem to be recognizable (*i.e.*, partially preserved) even in the ethanol-fed rats, as we shall see later.

Ultrastructure of isolated *(in vitro)* mitochondria. The mitochondria obtained from the liver of the control animals, suspended in the presence of succinate and in the absence of exogenous calcium, have generally a condensed configuration, with a normal electron density of the matrix and intact membranes, as illustrated in Fig. 3 and 4. The presence of either orthodox or ultracondensed forms is rare. Even at a rather high concentration of calcium (50 μM), some preparations of the control mitochondria succeed in accumulating this calcium and preserving their functionality (at least for a while), as our previously reported spectrophotometric measurements have demonstrated (Tarba and Suărăşan, 2003; 2004). This situation seems to be also confirmed by the ultrastructural images obtained in the present work, such as those illustrated in Fig.5 (representing the case-2 mitochondria), where the condensed aspect is generally preserved, although a few organelles have a swelling tendency. Even in the presence of very high calcium concentrations (200 μM; case-1 mitochondria suspended in MKS), when there is an evident swelling process taking place, one can observe some condensed organelles (Fig.6). In fact, even in the swelling medium (KSW), one can still observe the traces of mitochondrial cristae and the presence of some intact membranes for case-2 mitochondria (not presented here).

All these observations are in agreement with our previous functional results which indicate that the so-called massive calcium efflux and the membrane potential collapse in mitochondria of normal (control) rats occur at relatively high concentrations of calcium and only associated with an appreciable advance of the mitochondrial swelling process (Tarba and Suărăşan, 2003; 2004).

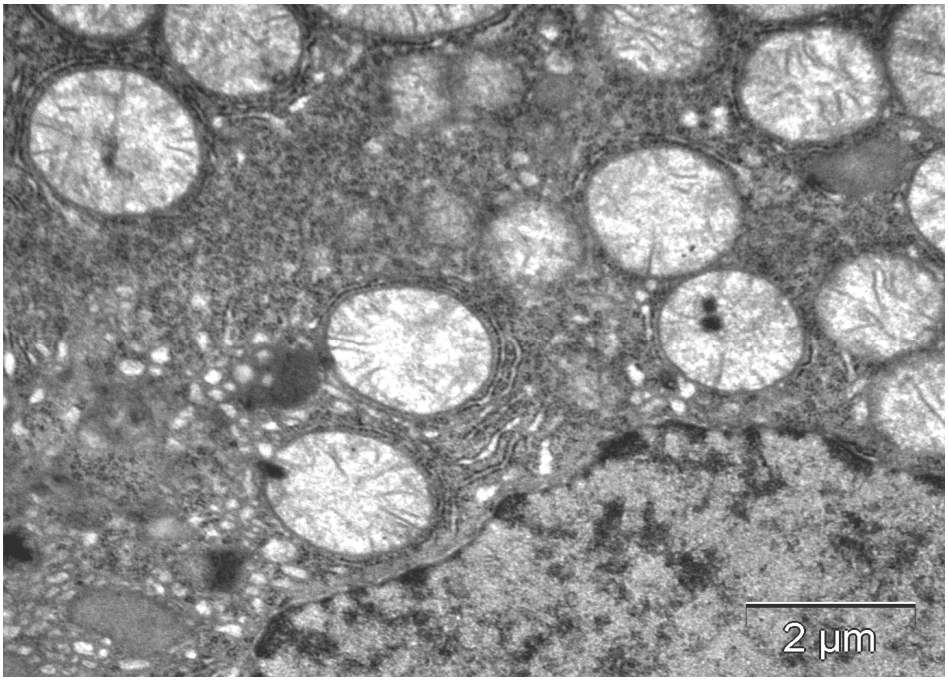

Fig. 1. Ultrastructural aspects of case-1 control liver.

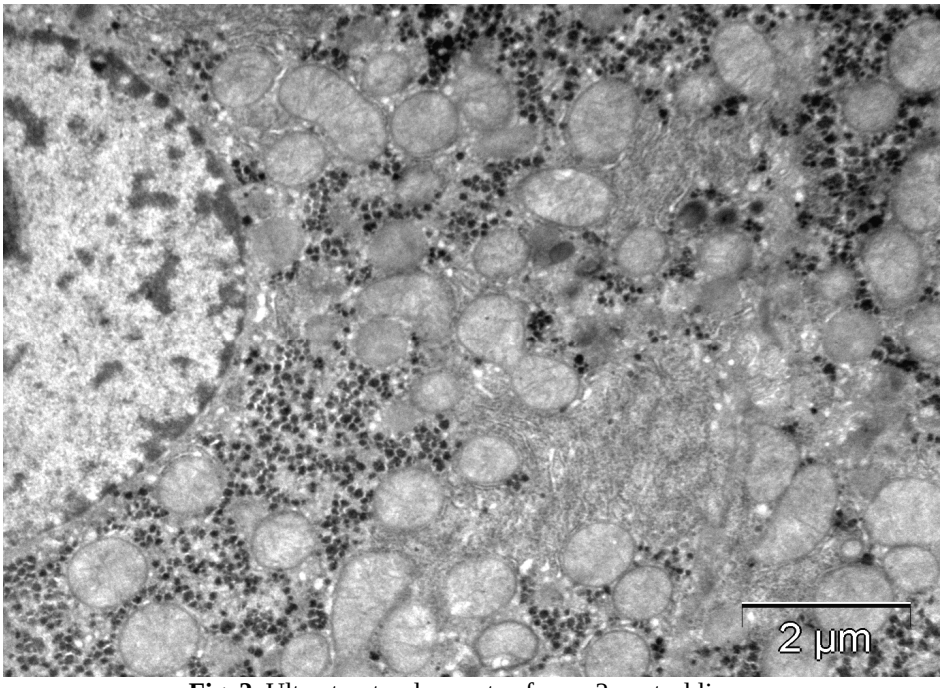

Fig. 2. Ultrastructural aspects of case-2 control liver.

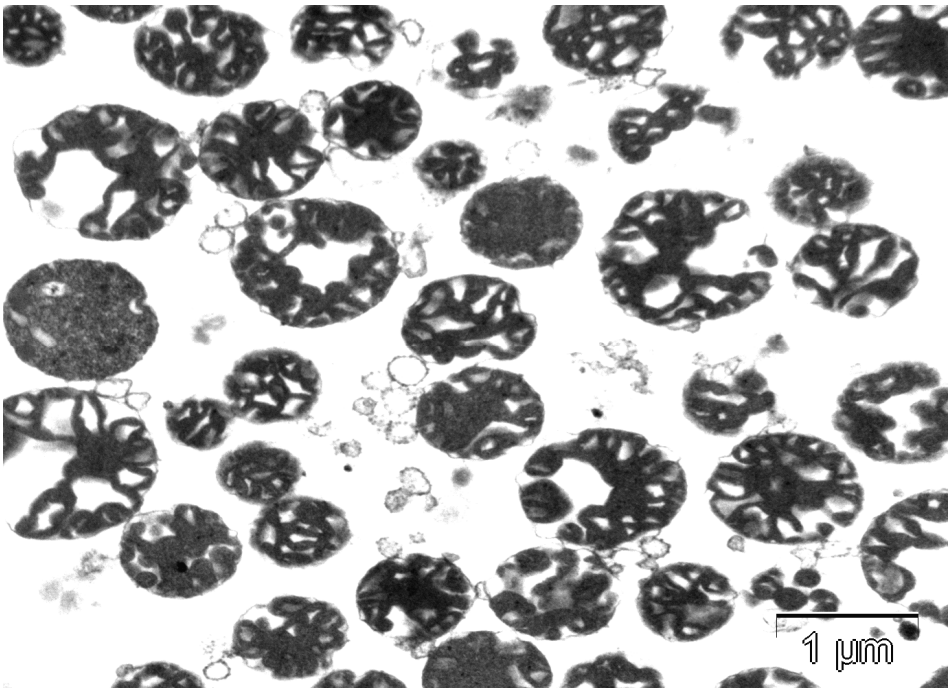

Fig. 3. Ultrastructural aspects of case-1 mitochondria *in vitro.*

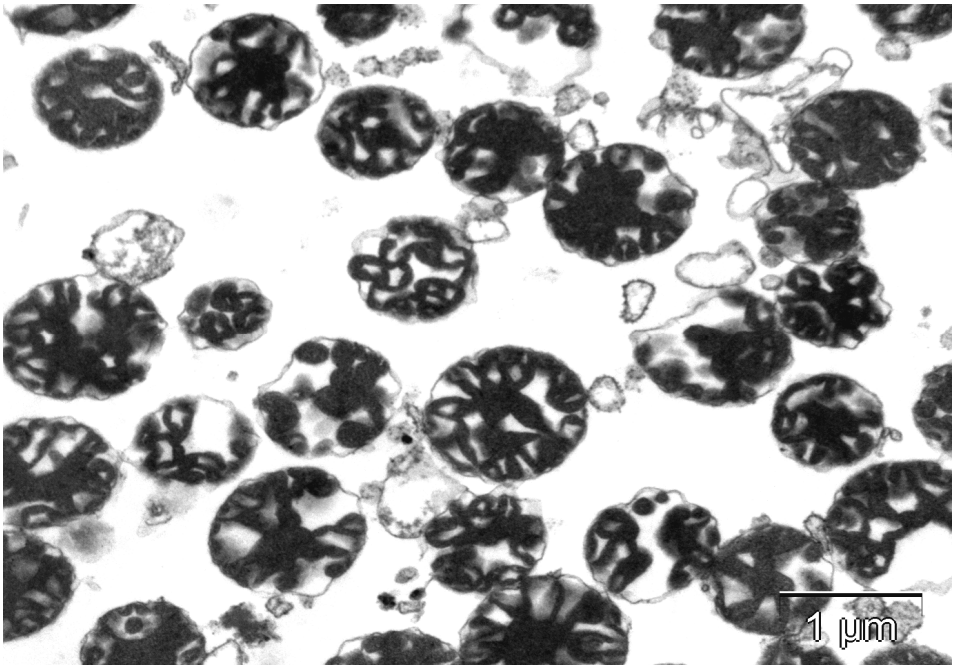

Fig. 4. Ultrastructural aspects of case-2 mitochondria *in vitro.*

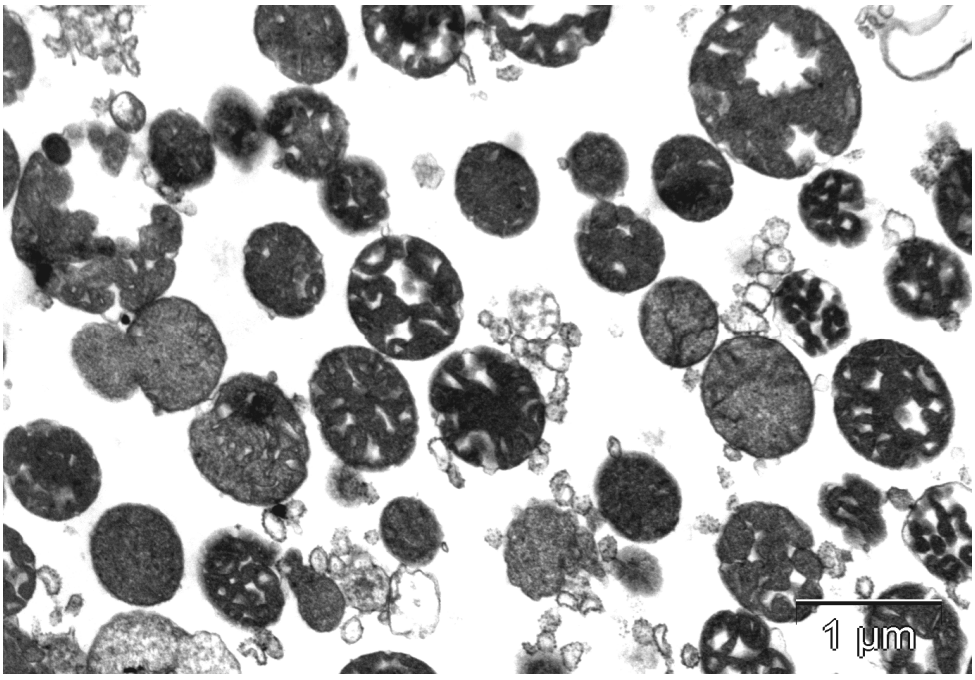

Fig. 5. Control mitochondria suspended in MKS in the presence of 50 µM calcium.

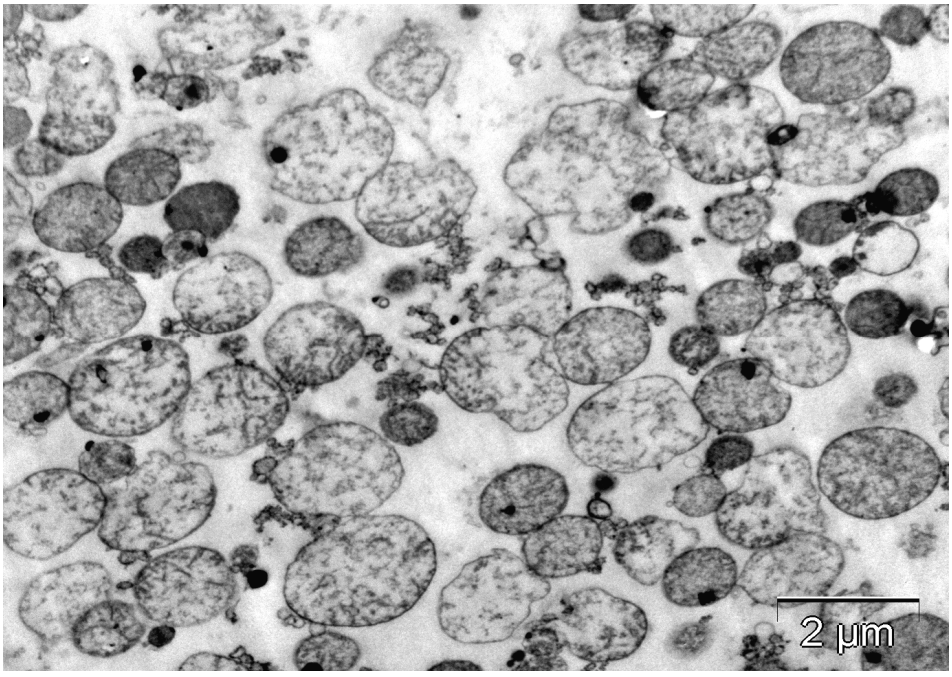

Fig. 6. Control mitochondria suspended in MKS in the presence of 200 µM calcium.

Chronically ethanol-fed rats. Hepatic ultrastructure. All the hepatic cells and structures are visibly affected by the chronic ethanol consumption, although certain interindividual differences are present here, too. Thus, in most liver preparations from this group, the nuclei have little heterochromatin and large nucleoli, with intense activity. The smooth endoplasmic reticulum is highly dilated and fragmented (vesiculated) whereas the lipids and glycogen are lacking from the cytoplasm. In turn, one can observe a strong peroxysomal proliferation. Mitochondria are enlarged and deformed (polymorphous mitochondria), with an electron transparent matrix and lack of cristae, as can be seen from an overall image of a hepatocyte presented in Fig.7.

In other rats of this group (a minority of about 20-25%), the situation is better, most of the nuclei having a normal aspect, with a slight dilation of the perinuclear space, similar to the control. However, even here one can often observe nuclei with an irregular contour and chromatin marginally agglomerated, which are morphological signs of apoptosis. The endoplasmic reticulum is also dilated, but only partially fragmented. Unlike in the first case, peroxysomes are rare and lipids are well represented, sometimes as large droplets. The biliary canaliculi and the microvilli in the Disse space appear normal. The *in situ* mitochondria, although having a normal distribution of electron density, display polymorphous tendencies, as illustrated in Fig.8, which presents a detail image with mitochondria, endoplasmic reticulum and a large lipid droplet.

Ultrastructure of isolated (*in vitro)* mitochondria. For most of the animals in this group (6 out of 8 rats), as found by us before (Tarba and Suărăşan, 2003; 2004), the mitochondria had a typical functional behaviour, characteristic (in our opinion) for the chronic ethanol consumption (low resistance to calcium loads, easy swelling and rapid collapse of the membrane potential). The situation is paralleled by the ultrastructural aspects revealed by the present work. Thus, the majority of the mitochondria tested *in vitro* in the presence of succinate (with no other metabolic challenge) have a condensed or ultracondensed conformation, most of them being either falciform or with the matrix circularly condensed and an electron transparent central zone. The outer membrane is visible in some cases (due to its detachment) but in many others it seems to be broken. A few organelles, that have an apparently orthodox shape, are more likely on the course of disintegration. Some others, still, are completely collapsed, having lost most of their content. Such details are illustrated in Fig.9. All these alterations are indicative of the disastrous effect of alcohol. They correlate well with both *in situ* hepatic ultrastructure and with our functional results mentioned above (for more details, see Tarba and Suărăşan, 2003; 2004). The results mentioned so far are additionally confirmed by the poor resistance of mitochondria to calcium loads, as can be seen in Fig.10, where the majority of the organelles are swollen and even destroyed (lysed) in the presence of 200 μM calcium. The situation is almost the same even at 50 μM calcium (not shown here).

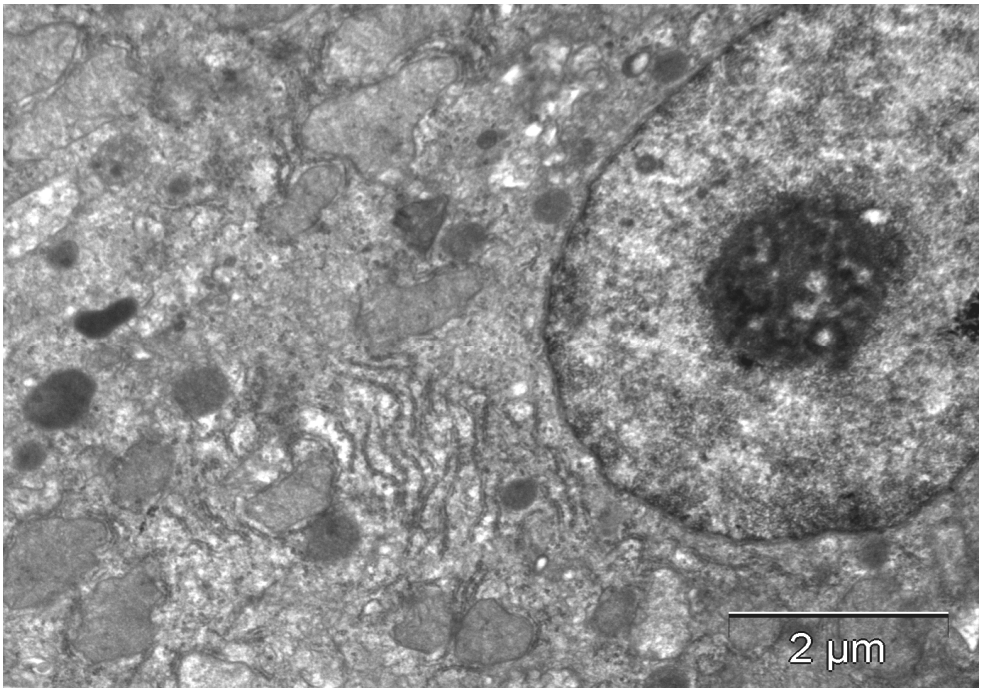

Fig. 7. Overall image of a hepatocyte from an ethanol-fed rat (typical case).

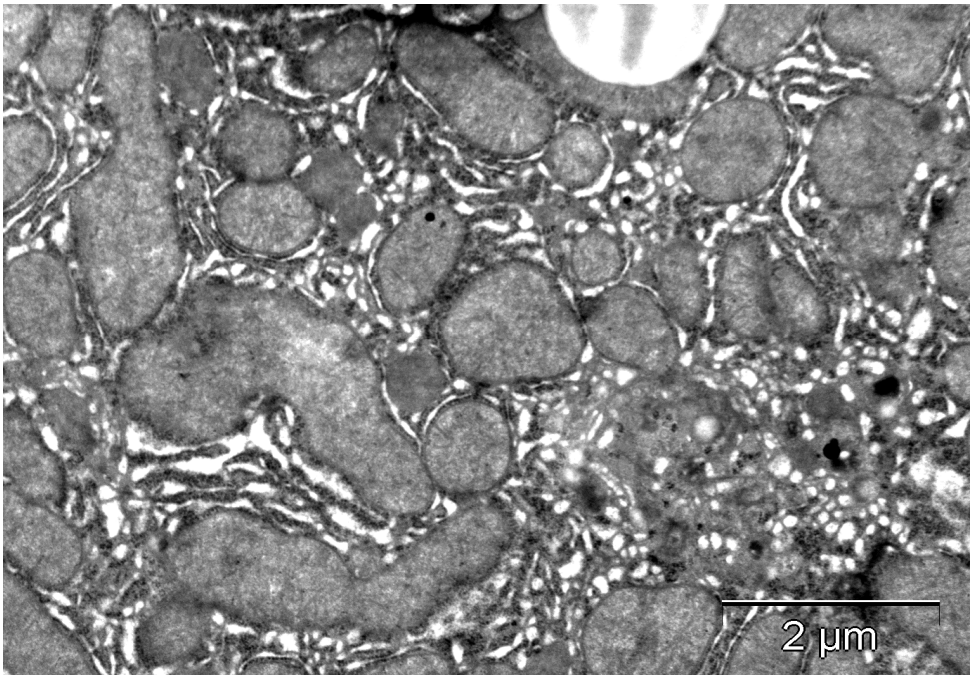

Fig. 8. Ultrastructural detail of a hepatocyte from an ethanol-fed rat (atypical case).

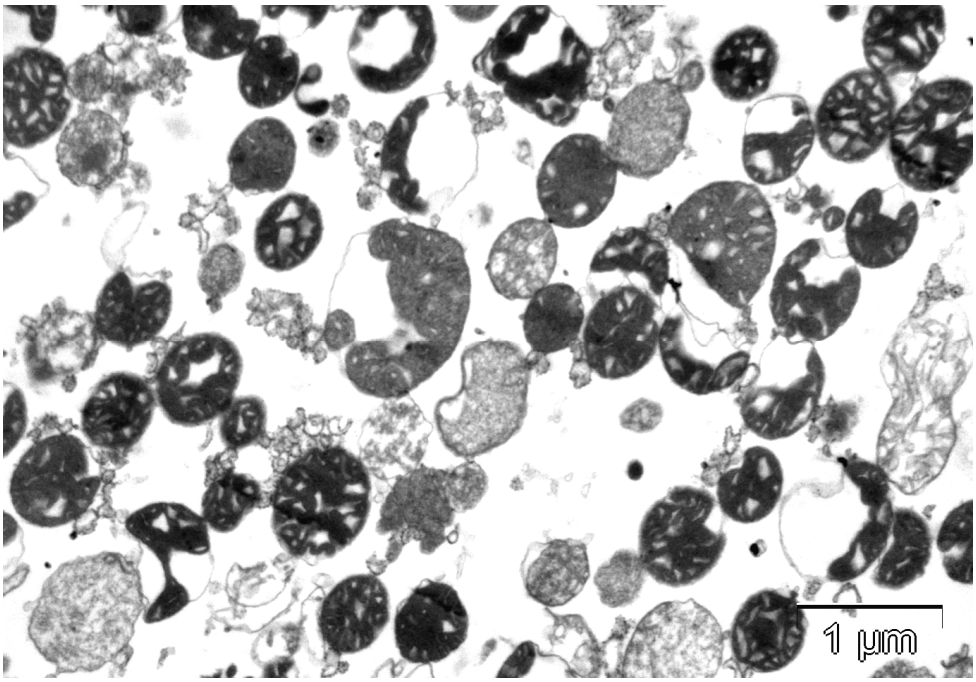

Fig. 9. *In-vitro* mitochondria from ethanol-fed rats (typical case).

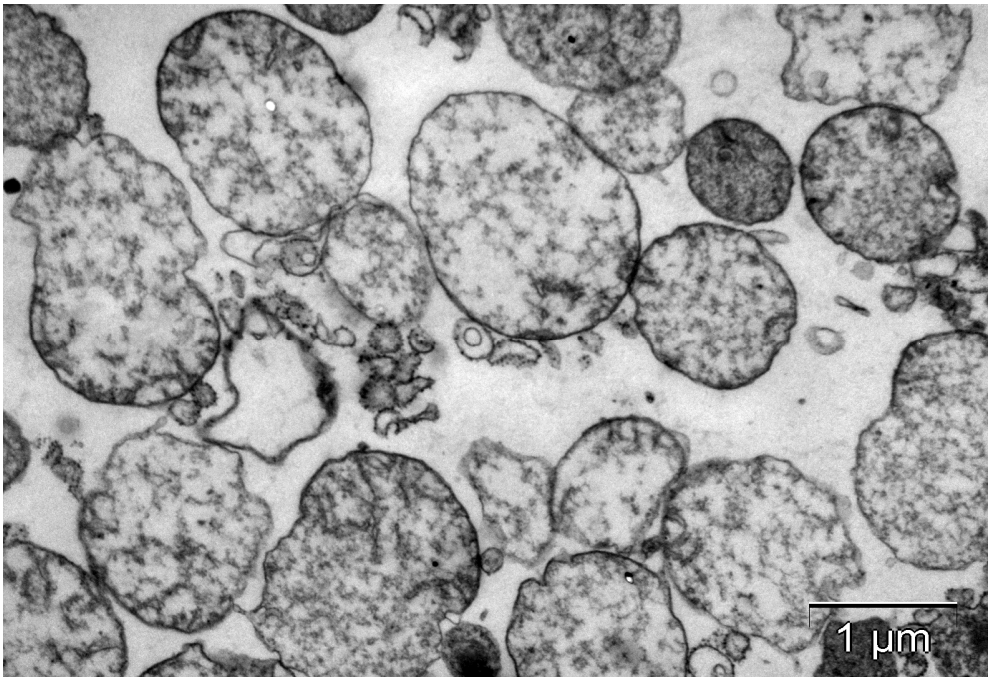

Fig. 10. *In-vitro* mitochondria from ethanol-fed rats in the presence of 200 µM calcium.

As for the better appearance and behaviour of a minority of hepatic and mitochondrial preparations (2 out of 8 rats), although we have very clear confirmatory results, both functionally and ultrastructurally, we do not want to insist because of the small number of samples, but it appears that there is a proportion of at least 20% of our rat population which seems to be resistant to a long-term moderate-to-high alcohol consumption. This statement has to be considered in the light of the fact that a 4-month period in the life of a rat is equivalent to around 10 years of a human life and that, if one takes into account the specific metabolic rate, which is almost 5 times higher in rats, the amount of ethanol received by a rat would be equivalent to about 250 ml of vodka taken daily by a 75-kg adult person.

While it is sure that some of these effects and differences are due to an interindividual variation in the induction capacity of the enzymes implicated in alcohol metabolism, it is not clear yet whether they are solely a consequence of this variation or that some other factors are also involved. For example, some of the effects could be due to differential sensitivity to, or to a different degree of penetration of the bacterial endotoxin, which is known to get through the intestinal wall of the chronic alcoholics and to produce variable effects (Deaciuc *et al*., 1999; Wheeler, 2003). Therefore, our next work in this series will be devoted to problems related to the acute endotoxin treatment of both normal and ethanol-fed rats.

Conclusions. From the results obtained in the present study, which lend further support to our previous functional observations, it is clear that the hepatic effect of a long-term ethanol consumption in rats is in most cases devastating. At the same time, at least 20% of the animals seem to be resistant to this rather tough treatment.

It is very important that the ultrastructural images obtained in this work not only confirm our previous functional results, including the existence of an alcohol-resistant subpopulation, but also suggest possible physiological mechanisms through which some of the differences might be achieved or explained. While for the typical, alcohol sensitive case, the ultrastructural images suggest a structural-functional alteration of the necrotic type (swelling of the organelles, especially of the endoplasmic reticulum and mitochondria, dilation of the Disse space, destruction of the microvilli and biliary canaliculi), for the alcohol resistant case, our images are more compatible with a limited apoptotic process. Thus, the mitochondria found *in situ* have a normal electron density whereas their swelling *in vitro* requires greater metabolic challenges. At the same time, the microvilli and the biliary canaliculi appear in good shape while chromatin is agglomerated marginally in many nuclei, a morphological feature usually associated with apoptosis.

It is not clear whether these results are an exclusive consequence of the variation in the induction capacity for alcohol-metabolising enzymes or that other genetic and/or physiological mechanisms are also involved.

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ANALYSIS OF THE ORIENTATION OF CYANOBACTERIA IN BAHAMIAN STROMATOLITE MATS USING A DIGITAL IMAGE ANALYSIS AND GIS-BASED APPROACH

ALEXANDRU I. PETRIŞOR[1] and ALAN W. DECHO[2]

Summary. Oldest known macrofossils on Earth, marine stromatolites are known to result from microbially mediated processes, and are still forming in remote environments. The microbial mats of stromatolites are fueled by cyanobacterial autotrophy. Several studies documented that cyanobacteria are able to move within these environments. Previous observations of stromatolites documented a preferential orientation of cyanobacteria, but there was no mathematical model available to test it. This study quantitatively analyzed images obtained with a scanning confocal laser microscope using a combination of image analysis and Geographical Information Systems techniques in conjunction with a mathematical model to test whether there is a preferential orientation of cyanobacteria. Analyses indicate that the vertical orientation is significantly predominant in Type 1 mats ($p=0.0010$) and below the top of mats ($p=0.0342$). Even though there are limitations of this method due to the effects of microscale topology, mounting of samples for microscopic analyses, image classification aspects, and mathematical modeling, the approach used to address the research questions presented in this paper is the only quantitative one. Future studies should focus on examining the influence of several known factors, such as light conditions, on the orientation of cyanobacteria.

KEYWORDS: stromatolites, cyanobacteria, phototaxis, GIS, digital image analysis.

Introduction. Marine stromatolites represent dynamic biogeochemical systems with a long geological history. As the oldest known macrofossils on Earth (Grotzinger and Knoll, 1999), marine stromatolites are now known to result from microbially mediated processes, and are still forming in remote regions of shallow, open-water marine environments (Reid *et al.*, 2000). Stromatolites are ideal systems for studying microbial distributions, and for suggesting a possible mechanism for how precipitation of horizontal micritic laminae occurs in these mats(Reid *et al.*, 2000). Lamination is likely a result of the development, change,

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and reorganization of microbial communities in response to extrinsic and/or intrinsic stressors. The microbial mats of stromatolites are fueled by cyanobacteria autotrophy (Pinckney and Reid, 1997). The surface mat communities repeatedly cycle through three distinct stages (termed Type 1, 2 and 3) that can be categorized by characteristic changes in precipitation products (Reid *et al.*, 2000).

Along with analyses of the role of cyanobacteria in the microbial mats, several studies documented that cyanobacteria are able to move within these environments. The cell wall of some motile species presents pores through which mucilage could be secreted. This secretion constitutes a propulsive force that moves the trichome (Hoiczyk and Baumeister, 1998). Nevertheless, *Phormidium uncinatum*, a highly motile species, does not present pores, although it secretes polysaccharide (Häder, 1987). Regardless of the propulsive mechanism, other researchers investigated the factors that could explain the motility, finding three different reactions: phototaxis, photokinesis, and photophobic responses(Häder, 1987). Phototaxis is a motion oriented to the direction of light, photokinesis is a motion with a speed proportional to the intensity of light, and the photophobic response is a reverse in the direction of the movement as a result of a change in the intensity of light (Stall, 2000).

Other studies focused on the periodicity of cyanobacterial movements. A daily cycle has been documented for *Oscillatoria sp.* and *Spirulina subsalsa* (Garcia-Pichel *et al.*, 1994). Potential causes are high concentrations of oxygen in the light, which could determine photo-oxidative reactions (Eloff *et al.*, 1976), and ultraviolet radiation (Bebout and Garcia-Pichel, 1995). A hot spring cyanobacterium, *Oscillatoria terebriformis*, moves down in the sediment until it reaches the sulfide layer, which inhibits its motility, trapping eventually the entire population (Stall and Moezelaar, 1997).

Since the movement of cyanobacteria has been documented, it could be inferred that these organisms could change their position as a result of different environmental conditions. Previous observations of stromatolites documented a preferential orientation of cyanobacteria, but there was no mathematical model available to test it. Our previous studies have developed an approach to quantitatively analyze images obtained with a scanning confocal laser microscope (SCLM) using a combination of image analysis and Geographical Information Systems (GIS) techniques (Petrisor and Decho, 2004). This study utilizes the approach in conjunction with a mathematical model to test whether there is a preferential orientation of cyanobacteria.

Materials and Methods. *Collection and mounting of samples.* Stromatolites, from which samples were collected, came from a subtidal marine environment at Highborne Cay in the Exuma Chain of islands in the Bahamas, 760 49' W; 240 43' N (Kawaguchi and Decho, 2000). This site is under current investigation through the Research Initiative on Bahamian Stromatolites (RIBS)

project (http://www.home.duq.edu/~stolz/RIBS/index.html). Freshly collected intact stromatolites were sectioned using sterile razor blades. Immediately after collection, the sections of stromatolite were preserved in 3% buffered formaldehyde in seawater. Sections were initially trimmed using a sterile razor, then placed in BEEM embedding molds. Nanoplast® resin (Ted Pella Co., Redding, CA, USA) and catalyst were thoroughly mixed on site and then added to moulds containing the stromatolites sample. The moulds were placed in a temperature-controlled heat block at 25°C for 48-60 h to allow slow penetration and complete mixing of the Nanoplast® resin with the hydrated sample. After penetration of the medium, the temperature is raised to 40°C for 48 h to dry and then 60°C for 48 h to harden the medium into blocks. The resulting blocks were thick sectioned, mounted on glass microscope slides using Epon 812 and then observed using CSLM (488/ 680 nm; excitation/ emission).

Microscopy. Images were obtained using a Nikon Eclipse TE 300 compound microscope (Nikon, Tokyo, Japan) equipped with an MRC 1024MP single and multiphoton system (BioRad Laboratories, Hercules, CA.). Image resolution was 512 x 512 pixels. For CSLM imaging, three internal detectors were used, each with a 6-position emission filter wheel and a variable confocal aperture. Sample slides were viewed using Nikon Plan Apo 20x, 40x, 60x, or 100x objectives. The 60x and 100x were used with immersion oil produced from synthetic hydrocarbons and advanced polymers by Stephens Scientific (catalog # M4004), having a refractive index of 1.515. The final output was represented by colored composite images exported in a Tagged Image File Format (TIFF). A sample image is displayed in Fig. 1.

Images were sampled within each mat type from two regions: the top of the mat, and a region situated below at approximately 4.4 µ below. In all cases, a special attention was paid to the condition that the top of the image should parallel the top of the precipitate.

Image classification. Images were classified using a specific ArcView GIS/ ArcGIS extension called "Feature Analyst" (Visual Learning Systems, Inc., 2002). To increase the accuracy of the classification process, "supervised classification" was used (Fig. 2). Supervised classification requires the user to select a sample of representative areas defining each feature within an image (F i g. 2 A). This sample is used by the program to classify the entire image (i.e., "image classification") or identify all pixels corresponding to a feature (i.e., "feature extraction"). Our analyses used only "feature extraction" to identify cyanobacteria (Fig. 2 B). The program has a specific application that allows the computer to draw in the next step vector lines for each linear feature identified within an image (Fig. 2 C). All lines delineating cyanobacteria were saved in ArcView GIS shapefiles corresponding to each imagine.

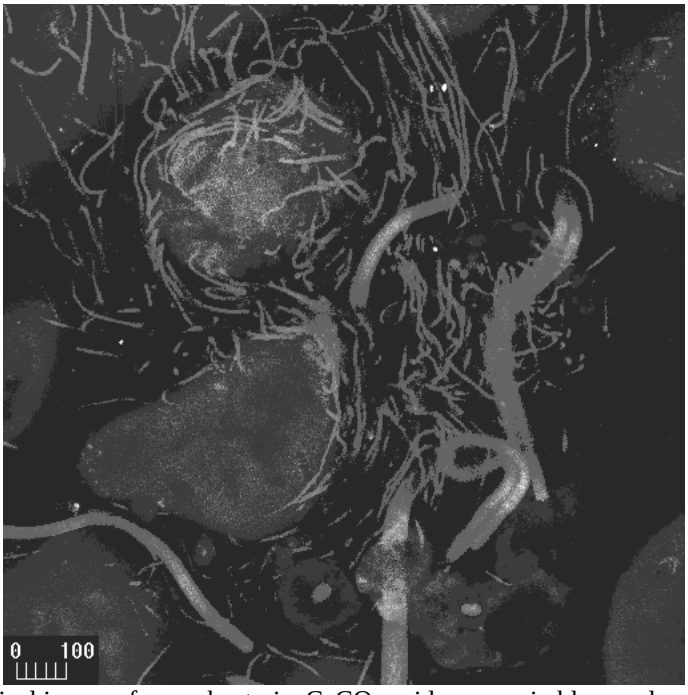

Fig. 1. Typical image of cyanobacteria. $CaCO_3$ ooids appear in blue, and cyanobacteria appear in red.

GIS modeling. Most of the GIS software allows for "modeling". Modeling is a procedure that allows the user to describe all the analytical procedures applied to an image in a flowchart format. The advantage of modeling is that the same model could be used for analyzing more features following the same steps. Therefore, the entire process is automated. The model showed in Fig. 3 is presented in GIS terms; however, the most important steps are the actual calculations of angles and lengths. Angles were computed using a specific ArcGIS extension, CalcAngle 1.0, developed for this project by a GIS programmer. Angles were measured between the orientation of each segment and the "North" vector (oriented vertically, pointing to the top of each image). Segments were classified as "vertical", if the angle formed with the "North" vector was between 0^0-45^0, 135^0-225^0, or 315^0-360^0, and "horizontal" otherwise. Lengths of each segment were computed using an ArcView GIS extension called "Animal Movement" (more details at *http://www.absc.usgs.gov/glba/gistools/animal_mvmt.htm*). The model produced two numbers for each image, representing the total length of "vertical" segments and the total length of all segments (in pixels; one pixel was 2.86 μ).

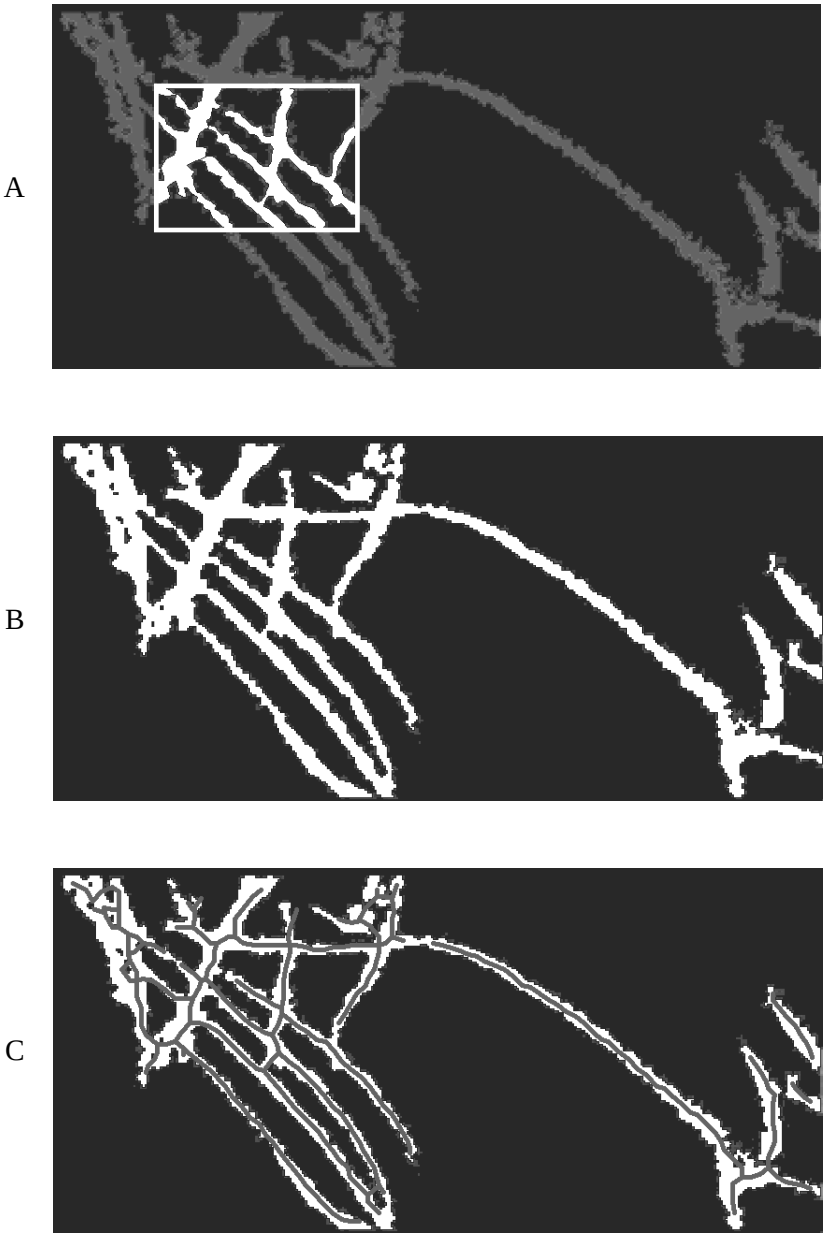

Fig. 2. Details of image classification: (A) selection of training pixels from within representative areas; (B) automated recognition of all similar pixels within the entire image; (C) identification of lines corresponding to cyanobacteria.

Statistical analyses. Data were analyzed using SAS (Cody and Smith, 1997). Logistic regression analysis was used to examine differences in the proportion represented by "vertical" segments of all cyanobacterial segments, and *t-tests* were used to detect whether the proportion of "vertical" segments differs from what is expected to be if the orientation is random (i.e., 50%). Analysis of Variance (ANOVA) was used to look at differences between the regions with respect to the total length of all cyanobacterial segments, a measure of the density of cyanobacteria.

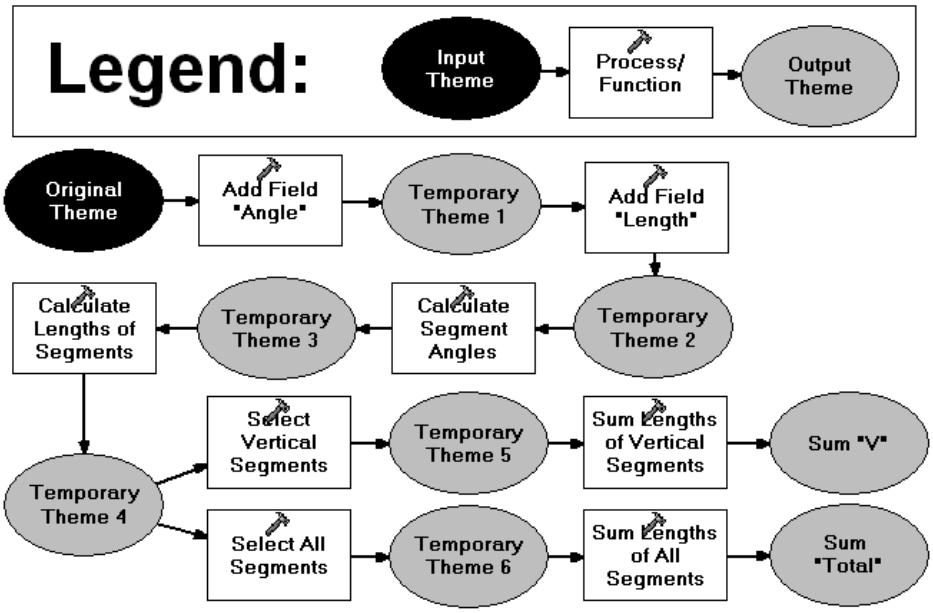

Fig. 3. GIS model used to determine the orientation of cyanobacterial segments.

Results and Discussion. Summary statistics for all data are displayed in Table 1, and the results of statistical analyses are displayed in Table 2.

Table 1.

Summary statistics for all data

Type	Region	Total length of cyanobacterial segments	Average percentage of "vertical" segments
1	Top	110686	0.6204
1	Below	98774	0.6288
1	All regions	209460	0.6264
2	Top	86700	0.5309
2	Below	120117	0.6020
2	All regions	206817	0.5665
All types	Top	197386	0.5757
All types	Below	218861	0.6154

Table 2.

Results of statistical analyses

Research question	Statistical method	Result
Are proportions of "vertical" segments different from 0.5 in the four groups?	*t-test*	All proportions are significantly greater than 0.5 ($p<0.0001$).
Are there any differences in the proportion of "vertical" segments?	Logistic regression analysis	Significant differences detected ($p<0.0001$).
Are there differences in the proportion of "vertical" segments between Type 1 and Type 2 mats?	Logistic regression analysis	Proportion of "vertical" segments significantly greater in Type 1 mats ($p=0.0010$).
Are there differences in the proportion of "vertical" segments between the "top" region and regions below it?	Logistic regression analysis	Proportion of "vertical" segments significantly greater below the top ($p=0.0342$).
Are any specific combinations of regions and mat types (e.g., "top of Type 1 mats") significantly different from the others?	Logistic regression analysis	No significant interactions between the mat type and region ($p=0.0510$).
Are there any differences in the total length of segments, a measure of the total number of cyanobacterial cells?	ANOVA	No significant differences detected ($p=0.1101$).

Limitations of the methodology. The limitations are a result of the effects of microscale topology on the classification process. First, the top of the precipitate presented irregularities, and the parallelism between the top of the image and the top of the precipitate, even though controlled as well as possible, could be subjected to a variation with an angle of approximately 30^0 in one direction or the other one. Furthermore, the control of the orientation of images was easier to achieve at the top, where the actual top of the precipitate could be seen, and far away hardly achieved below, where references where hardly identifiable.

Microscope stage limitations. The microscope stage limitations affected the second set of samples, incubated before fixation under light/ dark conditions. The limitations are related to the mounting of samples for microscopic analyses: instead of being mounted in an aqueous environment, these samples were mounted into oil. Due to the high refractive index of this oil, the z-section was considerably reduced. Therefore, the amount of observable cyanobacteria was reduced. This reduction can be seen when looking at the total length of cyanobacterial segments. Nevertheless, comparability was not affected with respect to both total length of all segments in all images, and the comparison of the orientation of cyanobacteria at the top of the precipitate vs. regions situated below.

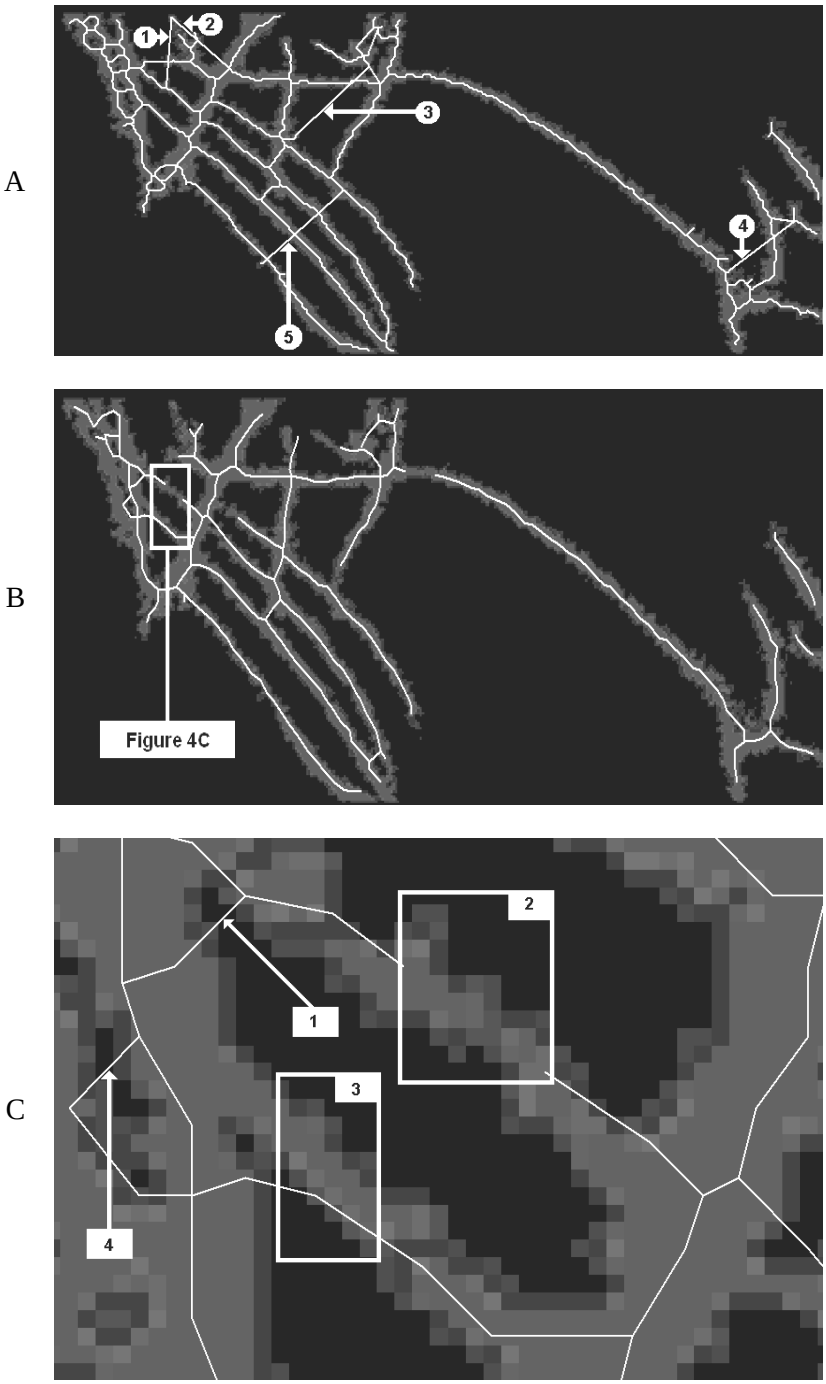

Fig. 4. Image classification limitations: (A) poor classification due to choosing the wrong parameters; (B) good classification; (C) detail presenting some errors within the results of a good classification.

Image classification limitations. The image classification is the methodological approach that presents the uppermost influence on the final results apart from the microscopy imaging stage. Supervised classification was preferred because it allows the user to carefully chose the most suitable parameters for the process. Nevertheless, the selection process must be based on both general experience and analyses of representative images for calibration purposes, and also must rely on selecting as many representative pixels as possible, even though this process is laborious and time-consuming. F i g. 4 displays an example of the transformation of polygonal areas consisting of all pixels identified as belonging to cyanobacteria into lines. Selection of inappropriate parameters results into "poor" results (F i g. 4 A, where some of the errors have been indicated using numbers); even a "good" fit of lines (F i g. 4 B) does not appear to be error-free when zoomed in (F i g. 4 C).

Definition of "vertical" and "horizontal" segments. Let us examine the effect of actual topography on two definitions of the angle (F i g. 5). The top line shows the effect of a change of 22.5^0 in the actual topography when two classes are defined: "vertical" (1), when the angle formed between a given segment and the "North" vector is between 0^0-45^0, 135^0-225^0, or 315^0-360^0, and "horizontal" (2) for any other angles. Let us imagine that the actual topography is rotated 22.5^0. The effect of this rotation is indicated in the top right figure; reported to the vertical class, FP areas represent the "false positive" (i.e., "horizontal" segments misclassified as "vertical"), and FN areas represent the "false negative" (i.e., "vertical" segments misclassified as "horizontal"). Obviously, the complementary applies to "horizontal" segments, since there are only two classes. In summary, a percentage of 37.5% for both vertical and horizontal segments is classified correctly. For each class, this percentage represents 75% of the width of the class.

The bottom line indicates the effect of operating with a more precise definition of the angle, which leads to the creation of new classes. In this case, "vertical" segments (1) form angles between 0.0^0-22.5^0, 157.5^0-202.5^0, or 337.5^0-360.0^0, horizontal segments (2) form angles between 67.5^0-112.5^0 or 247.5^0-292.5^0, and "other" segments (3) form angles within the remaining ranges. Let us imagine that the actual topography is rotated 22.50. The effect of this rotation is indicated in the bottom right figure; reported to the vertical class, FP areas represent the "false positive" (i.e., "horizontal" segments misclassified as "vertical"), and FN areas represent the "false negative" (i.e., "vertical" segments misclassified as "horizontal"). Reported to the horizontal class, FP' areas represent the "false positive" (i.e., "vertical" segments misclassified as "horizontal"), and FN' areas represent the "false negative" (i.e., "horizontal" segments misclassified as "vertical"). Complementary definitions pertaining to FP, FN, FP' and FN' areas apply to the "others" class. In summary, a percentage of 12.5% for both vertical and horizontal segments is classified correctly. For each class, this percentage represents 50% of the width of the class.

This example illustrates that more precise definitions of "horizontal" and "vertical" segments are more likely to be affected by the actual topography of the image. This is the reason for preferring the first definition, as presented in F i g. 5.

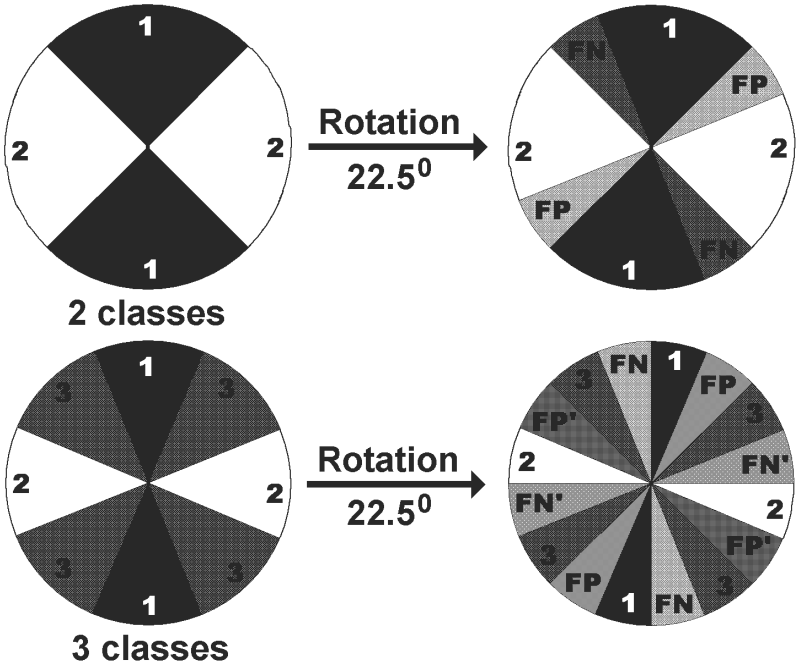

Fig. 5. Diagram showing the effect of actual topography given different definitions of the orientation.

Conclusions. 1. Vertical orientation is significantly predominant in Type 1 mats and below the top of mats.

2. Even though there are limitations of this method due to the effects of microscale topology, mounting of samples for microscopic analyses, image classification aspects, and mathematical modeling, the approach used to address the research questions presented in this paper is the only quantitative one.

3. Future studies should focus on examining the influence of several known factors, such as light conditions, on the orientation of cyanobacteria.

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THE EFFECT OF GREEN-MANURE ON ENZYMATIC ACTIVITIES IN A BROWN LUVIC SOIL

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Summary. Actual and potential dehydrogenase, catalase and nonenzymatic catalytic and phosphatase (measured in unbuffered, acetate buffer and borax buffer reaction mixtures) activities were determined in the 0–10–, 10–20– and 20–30–cm layers of a brown luvic soil submitted to a complex fertilization (green-manure) experiment. It was found that each activity decreased with increasing sampling depth. The fertilization with green-manure led to a significant increase in each of the seven enzymatic and nonenzymatic activities determined. The enzymatic indicators of soil quality calculated from the values of enzymatic activities depending on the kind of fertilizers, showed the order: lupinus + rape + oat > lupinus > rape + lupinus > vetch + oat + ryegrass > lupinus + oat + vetch > unfertilized plot. This order means that by determination of enzymatic activities valuable information can be obtained regarding fertility status of soils.

KEYWORDS: catalase, dehydrogenase, green-manure, phosphatase.

Introduction. Soil enzymes are the biological catalysts of innumerable reactions in soils. Although some enzymes (e.g. dehydrogenase) are only found in viable cells most soil enzymes can also exist as exoenzymes secreted by microorganisms or as enzymes originating from microbial debris and plant residue that are stabilised in complexes of clay minerals and humic colloides. Since it is difficult to extract enzymes from soils, enzymes are studied indirectly by measuring the activity via assays (Samuel *et al.*, 2000, 2005). Nonetheless, studying soil enzyme activities provides insight into biochemical processes in soils and is sensitive as a biological index (Bőhm *et al.*, 1991; Haluszczak *et al.*, 1991).

The effect of green-manure on soil enzymatic activities were studied in many countries, including Romania (Chiriță *et al.*, 1980; Ștefanic, 1991; Ștefanic *et al.*, 1983, 1984). In order to obtain new data on the soil enzymological effects of soil management

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practices we have determined some enzymatic activities in a brown luvic soil submitted to a complex fertilization experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

The first data regarding the influence of green-manure on this soil were published by (Domuța *et al.*, 2004, 2005). They studied the effect of green-manure associated with mineral fertilization on the physical and chemical properties of a brown luvic soil and found that the mixture of the green-manure resulted in higher physical and chemical indicators. They published no paper on the soil enzymological effect of green-manure.

Our results are in good agreement with the literature data reviewed by (Dick *et al.*, 1994; Kanazawa, 1986; Ştefan and Radu, 2005; Tang, 1987) and constitute novelties for the enzymological characterization of a brown luvic soil submitted to complex management practices.

Material and methods. The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5, medium humus (23.2%) and P (22 ppm) contents, but it s rich in K (83 ppm).

The experimental field was divided into plots for comparative study of green-manure fertilization at rates of 47.8 t/ha lupinus, 29.9 t/ha vetch + oat + ryegrass, 39.7 t/ha lupinus + oat, 23.9 t/ha lupinus + rape + oat, 20 t/ha rape, 19.1 t/ha rape + lupinus.

The green-manure was maintained on the soil surface 7 days and after that the land was ploughed. The plots were installed in three repetitions.

In July 2005, soil was sampled from the 0–10–, 10–20– and 20–30–cm depths of the plots under maize crop. The soil samples were allowed to air-dry, then ground and passed through a 2- mm sieve and, finally, used for enzymological analyses.

We have determined six enzymatic activities (actual and potential dehydrogenase, catalase and phosphatase measured in unbuffered, acetate buffer and borax buffer reaction mixtures) and one nonenzymatic catalytic activity (H_2O_2 splitting in autoclaved samples).

Actual and potential dehydrogenase activities were determined according to the methods described in (Drăgan-Bularda, M. 2000b). The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2, 3, 5-triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose. All reaction mixtures were incubated at 37ºC for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm.

The reaction mixtures for catalase activity consisted of 3.0 soil, 2.0 ml H_2O_2 3% and 10 ml buffer solution. The buffer solution was prepared as recommended by Drăgan- Bularda, M. (2000a).

For determination of phosphatase activities, disodium phenylphosphate served as enzyme substrate (Drăgan-Bularda, M. 2000c; Öhlinger, R. 1996).

Three activities were measured: phosphatase activity in unbuffered reaction mixtures, acid phosphatase activity in reaction mixtures to which acetate buffer

(pH 5.0) was added and alkaline phosphatase activity in reaction mixtures treated with borax buffer (pH 9.4). The reaction mixtures consisted of 2.5 g sol, 2 ml toluene (antiseptic), 10 ml distilled water or buffer solution and 10 ml 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the controls. All reaction mixtures were incubated at 37ºC for 2 hours. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2, 6-dibromoquinone-4-chloroimide.

Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced from 2, 3, 5-triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 hours, whereas catalase and nonenzymatic catalytic activities are recorded as mg of H_2O_2 decomposed by 1g of soil in 1 hour. Phosphatase activities are expressed in mg phenol/g soil/2 hours.

The activity values were submitted to statistical evaluation by the two-way *t*-test (Sachs, L., 1968).

Results and discussion. Results of the enzymological analyses are presented in Table 1, and those of the statistical evaluation are summarized in Table 2.

Variation of soil enzymatic activities in dependence of sampling depth. It is evident from Table 1 that each enzymatic activity and nonenzymatic catalytic activity decreased with sampling depth in all plots under maize crop. In addition, Table 2 shows that the mean values of each activities also decreased with increasing soil depth.

Comparison of the three phosphatase activities measured. At the same soil depths (0–10–, 10–20– and 20–30–cm) in all plots under maize crop, the activities decreased in the order: phosphatase activity measured in unbuffered reaction mixtures > acid phosphatase activity > alkaline phosphatase activity (Table 1). This decreasing order is also valid for the mean values of the three activities (Table 2).

Enzymatic indicators of soil quality. Significant ($p < 0.05$ to $p < 0.001$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the type of activity and the nature of green-manure. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the seven plots:

actual dehydrogenase activity: lupinus + rape + oat > rape + lupinus > lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilized plot;

potential dehydrogenase activity: lupinus + rape + oat > lupinus > rape + lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilized plot;

catalase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus + oat > lupinus > rape > rape + lupinus > unfertilized plot;

phosphatase activity measured in unbuffered reaction mixtures: vetch + oat + ryegrass > lupinus + oat > lupinus + rape + oat > lupinus > rape > rape + lupinus > unfertilized plot;

acid phosphatase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus > lupinus + oat > rape + lupinus > rape > unfertilized plot;
alkaline phosphatase activity: vetch + oat + ryegrass > lupinus + rape + oat > lupinus + oat > lupinus > rape > rape + lupinus > unfertilized plot.

Table 1.

The effects of soil management practices on enzymatic and nonenzymatic catalytic activities in a brown luvic soil under maize crop

Soil enzymatic activity*	Soil depth (cm)	Type of green – manure**						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
ADA	0-10	9.01	6.95	7.31	11.82	6.10	11.56	5.52
	10-20	7.31	4.59	5.61	10.20	4.70	8.50	4.52
	20-30	5.10	2.72	3.91	5.76	3.40	5.10	2.72
PDA	0-10	22.78	16.66	14.28	24.28	11.22	16.32	10.60
	10-20	15.30	10.20	11.22	16.66	9.50	12.24	9.41
	20-30	8.33	8.16	10.37	15.30	8.67	9.86	7.88
CA	0-10	1.98	2.07	1.96	2.44	1.79	1.09	0.89
	10-20	1.79	1.95	1.85	2.23	1.33	1.07	0.83
	20-30	1.60	1.95	1.67	2.03	0.95	0.92	0.71
CAn	0-10	0.51	0.56	0.54	0.55	0.56	0.51	0.51
	10-20	0.52	0.54	0.51	0.46	0.50	0.49	0.48
	20-30	0.41	0.54	0.44	0.36	0.45	0.44	0.45
UPA	0-10	2.87	2.97	2.94	2.96	2.83	2.80	2.77
	10-20	2.84	2.96	2.92	2.91	2.79	2.76	2.61
	20-30	2.81	2.93	2.90	2.87	2.67	2.60	2.55
AcPA	0-10	2.85	2.94	2.81	2.96	2.81	2.79	2.69
	10-20	2.81	2.87	2.75	2.89	2.69	2.75	2.38
	20-30	2.74	2.81	2.69	2.85	2.20	2.32	2.30
AlkPA	0-10	1.72	1.97	1.90	1.94	1.85	1.71	1.67
	10-20	1.53	1.93	1.67	1.84	1.38	1.35	1.31
	20-30	1.40	1.83	1.51	1.76	1.34	1.31	1.29

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
CA – Catalase activity
CAn – Nonenzymatic catalytic activity
UPA – Phosphatase activity measured in unbuffered reaction mixtures
Ac PA – Acid phosphatase activity
Alk PA – Alkaline phosphatase activity

V_1 - Lupinus
V_2 – Vetch + oat + ryegrass
V_3 – Lupinus + oat
V_4 – Lupinus + rape + oat
V_5 - Rape
V_6 – Rape + lupinus
V_7 – Unfertilized plot

It is clear from these orders that seven plots presented either a maximum or a minimum value of the six soil enzymatic activities. Consequently, these orders don't make it possible to establish such an enzymatic hierarchy of the plots which takes into account each activity for each plot. For establishing such a hierarchy, we have applied the method suggested in (Kiss *et al.*, 1991).

Table 2.

Significance of the differences between enzymatic and nonenzymatic catalytic activities in a brown luvic soil submitted to a fertilization experiment

Fertilization experiment	Soil enzymatic activity*	Soil depth (cm)	Mean activity values in fertilization experiment			Significance of the differences
			a	b	a-b	
1	2	3	4	5	6	7
Lupinus (a) versus vetch + oat + ryegrass (b)	ADA	0-30	7.14	4.75	2.39	0.01 > p > 0.001
	PDA		15.47	11.67	3.80	0.50 > p > 0.10
	CA		1.79	1.99	-0.20	0.50 > p > 0.10
	CAn		0.48	0.54	-0.06	0.50 > p > 0.10
	UPA		2.84	2.95	-0.11	0.01 > p > 0.001
	Ac PA		2.80	2.87	-0.07	0.02 > p > 0.01
	Alk PA		1.55	1.91	-0.36	0.02 > p > 0.001
Lupinus (a) versus lupinus + oat (b)	ADA	0-30	7.14	5.61	1.53	0.02 > p > 0.01
	PDA		15.47	11.95	3.52	0.50 > p > 0.10
	CA		1.79	1.82	-0.03	0.50 > p > 0.10
	CAn		0.48	0.49	-0.01	0.50 > p > 0.10
	UPA		2.84	2.92	-0.08	0.01 > p > 0.001
	Ac PA		2.80	2.75	0.05	0.01 > p > 0.001
	Alk PA		1.55	1.69	-0.14	0.02 > p > 0.001
Lupinus (a) versus lupinus + rape + oat (b)	ADA	0-30	7.14	9.26	-2.12	0.50 > p > 0.10
	PDA		15.47	18.74	-3.27	0.50 > p > 0.10
	CA		1.79	2.23	-0.44	0.01 > p > 0.0001
	CAn		0.48	0.45	0.03	p > 0.50
	UPA		2.84	2.91	-0.07	0.02 > p > 0.01
	Ac PA		2.80	2.90	-0.1	0.01 > p > 0.001
	Alk PA		1.55	1.91	-0.36	0.02 > p > 0.01
Lupinus (a) versus rape (b)	ADA	0-30	7.14	4.73	2.41	0.05 > p > 0.02
	PDA		15.47	9.79	4.25	0.50 > p > 0.10
	CA		1.79	1.35	0.44	0.10 > p > 0.05
	CAn		0.48	0.50	-0.02	0.50 > p > 0.10
	UPA		2.84	2.76	0.08	0.50 > p > 0.10
	Ac PA		2.80	2.56	0.24	0.50 > p > 0.10
	Alk PA		1.55	1.52	0.03	p > 0.50
Lupinus (a) versus rape + lupinus (b)	ADA	0-30	7.14	8.38	-1.24	0.50 > p > 0.10
	PDA		15.47	12.80	2.67	0.50 > p > 0.10
	CA		1.79	1.02	0.77	0.01 > p > 0.001
	CAn		0.48	0.48	0.00	-
	UPA		2.84	2.72	0.12	0.10 > p > 0.05
	Ac PA		2.80	2.62	0.18	0.10 > p > 0.05
	Alk PA		1.55	1.45	0.10	0.50 > p > 0.02
Lupinus (a) versus unfertilized plot (b)	ADA	0-30	7.14	4.25	2.89	0.02 > p > 0.001
	PDA		15.47	9.29	6.18	0.50 > p > 0.10
	CA		1.79	0.81	0.98	0.01 > p > 0.001
	CAn		0.48	0.48	0.00	-
	UPA		2.84	2.64	0.20	0.05 > p > 0.02
	Ac PA		2.80	2.45	0.35	0.10 > p > 0.05
	Alk PA		1.55	1.42	0.13	0.50 > p > 0.10

Table 2 *(continued)*

1	2	3	4	5	6	7
Vetch + oat + ryegrass (a) versus lupinus + oat (b)	ADA	0-30	4.75	5.61	-0.86	0.10 > p > 0.005
	PDA		11.67	11.95	-0.28	p > 0.50
	CA		1.99	1.82	0.17	0.10 > p > 0.005
	CAn		0.54	0.49	0.05	0.50 > p > 0.010
	UPA		2.95	2.92	0.03	0.02 > p > 0.01
	Ac PA		2.87	2.75	0.12	0.01 > p > 0.001
	Alk PA		1.91	1.69	0.22	0.50 > p > 0.10
Vetch + oat + ryegrass (a) versus lupinus + rape + oat (b)	ADA	0-30	4.75	9.26	-4.51	0.05 > p > 0.02
	PDA		11.67	18.74	-7.07	0.01 > p > 0.001
	CA		1.99	2.23	-0.24	0.50 > p > 0.10
	CAn		0.54	0.45	0.09	0.50 > p > 0.10
	UPA		2.95	2.91	0.04	0.10 > p > 0.05
	Ac PA		2.87	2.90	-0.03	0.10 > p > 0.05
	Alk PA		1.91	1.84	0.07	0.10 > p > 0.05
Vetch + oat + ryegrass (a) versus rape (b)	ADA	0-30	4.75	4.73	0.02	p > 0.50
	PDA		11.67	9.79	1.88	0.02 > p > 0.01
	CA		1.99	1.35	0.64	p > 0.50
	CAn		0.54	0.50	0.04	0.50 > p > 0.10
	UPA		2.95	2.76	0.19	0.05 > p > 0.02
	Ac PA		2.87	2.56	1.24	0.50 > p > 0.10
	Alk PA		1.91	1.52	0.39	0.10 > p > 0.05
Vetch + oat + ryegrass (a) versus rape + lupinus (b)	ADA	0-30	4.75	8.38	-3.63	0.05 > p > 0.02
	PDA		11.67	12.80	-1.13	0.50 > p > 0.10
	CA		1.99	1.02	0.97	0.01 > p > 0.001
	CAn		0.54	0.48	0.06	0.10 > p > 0.05
	UPA		2.95	2.72	0.23	0.05 > p > 0.02
	Ac PA		2.87	2.62	0.25	0.50 > p > 0.10
	Alk PA		1.91	1.45	0.46	0.05 > p > 0.002
Vetch + oat + ryegrass (a) versus unfertilized plot (b)	ADA	0-30	4.75	4.25	0.50	0.10 > p > 0.05
	PDA		11.67	9.29	2.38	0.50 > p > 0.10
	CA		1.99	0.81	1.18	0.10 > p > 0.05
	CAn		0.54	0.48	0.06	0.05 > p > 0.02
	UPA		2.95	2.64	0.31	0.05 > p > 0.02
	Ac PA		2.87	2.45	0.42	0.05 > p > 0.02
	Alk PA		1.91	1.42	0.49	0.05 > p > 0.02
Lupinus + oat (a) versus lupinus + rape + oat (b)	ADA	0-30	5.61	9.26	-3.65	0.10 > p > 0.05
	PDA		11.95	18.74	-6.79	0.10 > p > 0.05
	CA		1.82	2.23	-0.41	0.01 > p > 0.001
	CAn		0.49	0.45	0.04	p > 0.50
	UPA		2.92	2.91	0.01	0.50 > p > 0.10
	Ac PA		2.75	2.90	-0.15	0.01 > p > 0.001
	Alk PA		1.69	1.84	-0.15	0.50 > p > 0.10

Table 2 *(continued)*

1	2	3	4	5	6	7
Lupinus + oat (a) versus rape (b)	ADA	0-30	5.61	4.73	0.88	0.05 > p > 0.002
	PDA		11.95	9.79	2.16	0.05 > p > 0.002
	CA		1.82	1.35	0.47	0.10 > p > 0.05
	CAn		0.49	0.50	0.01	p > 0.50
	UPA		2.92	2.76	0.16	0.10 > p > 0.05
	Ac PA		2.75	2.56	0.19	0.50 > p > 0.010
	Alk PA		1.69	1.52	0.17	0.50 > p > 0.010
Lupinus + oat (a) versus rape + lupinus (b)	ADA	0-30	5.61	8.38	-2.77	0.10 > p > 0.05
	PDA		11.95	12.80	-0.85	0.50 > p > 0.010
	CA		1.82	1.02	0.80	0.50 > p > 0.010
	CAn		0.49	0.48	0.01	0.05 > p > 0.002
	UPA		2.92	2.72	0.20	0.05 > p > 0.002
	Ac PA		2.75	2.62	0.13	0.50 > p > 0.10
	Alk PA		1.69	1.45	0.24	0.05 > p > 0.002
Lupinus + oat (a) versus unfertilized plot (b)	ADA	0-30	5.61	4.25	1.36	0.05 > p > 0.002
	PDA		11.95	9.29	2.66	0.05 > p > 0.002
	CA		1.82	0.81	1.01	0.05 > p > 0.002
	CAn		0.49	0.48	0.01	0.50 > p > 0.10
	UPA		2.92	2.64	0.28	0.05 > p > 0.002
	Ac PA		2.75	2.45	0.30	0.50 > p > 0.10
	Alk PA		1.69	1.42	0.27	0.05 > p > 0.002
Lupinus + rape+ oat (a) versus rape (b)	ADA	0-30	9.26	4.73	4.53	0.10 > p > 0.05
	PDA		18.74	9.79	8.95	0.001 > p > 0.001
	CA		2.23	1.35	0.88	0.02 > p > 0.01
	CAn		0.45	0.50	-0.05	0.50 > p > 0.10
	UPA		2.91	2.76	0.15	0.05 > p > 0.002
	Ac PA		2.90	2.56	0.34	0.50 > p > 0.10
	Alk PA		1.84	1.52	0.32	0.10 > p > 0.05
Lupinus + rape + oat (a) versus rape + lupinus (b)	ADA	0-30	9.26	8.38	0.88	0.50 > p > 0.10
	PDA		18.74	12.80	5.94	0.05 > p > 0.02
	CA		2.23	1.02	1.21	0.01 > p > 0.001
	CAn		0.45	0.48	-0.03	p > 0.50
	UPA		2.91	2.72	0.19	0.01 > p > 0.001
	Ac PA		2.90	2.62	0.28	0.50 > p > 0.10
	Alk PA		1.84	1.45	0.39	0.05 > p > 0.002
Lupinus+ rape + oat (a) versus unfertilized plot (b)	ADA	0-30	9.26	4.25	5.01	0.05 > p > 0.02
	PDA		18.74	9.29	9.45	0.05 > p > 0.02
	CA		2.23	0.81	1.42	0.01 > p > 0.001
	CAn		0.45	0.48	-0.03	p > 0.50
	UPA		2.91	2.64	0.27	0.50 > p > 0.10
	Ac PA		2.90	2.45	0.45	0.05 > p > 0.02
	Alk PA		1.84	1.42	0.42	0.05 > p > 0.02

Table 2 *(continued)*

1	2	3	4	5	6	7
Rape (a) versus rape + lupinus (b)	ADA	0-30	4.73	8.38	-3.65	0.10 > p > 0.05
	PDA		9.79	12.80	-3.01	0.50 > p > 0.10
	CA		1.35	1.02	0.33	0.50 > p > 0.10
	CAn		0.50	0.48	0.02	0.50 > p > 0.10
	UPA		2.76	2.72	0.04	0.10 > p > 0.05
	Ac PA		2.56	2.62	-0.06	0.50 > p > 0.10
	Alk PA		1.52	1.45	0.07	0.50 > p > 0.10
Rape (a) versus unfertilized plot (b)	ADA	0-30	4.73	4.25	0.48	0.10 > p > 0.05
	PDA		9.79	9.29	0.50	0.50 > p > 0.10
	CA		1.35	0.81	0.54	0.50 > p > 0.10
	CAn		0.50	0.48	0.02	0.50 > p > 0.10
	UPA		2.76	2.64	0.12	0.10 > p > 0.05
	Ac PA		2.56	2.45	0.11	0.50 > p > 0.10
	Alk PA		1.52	1.42	0.10	0.50 > p > 0.10
Rape + lupinus (a) versus unfertilized plot (b)	ADA	0-30	8.38	4.25	4.13	0.10 > p > 0.05
	PDA		12.80	9.29	3.51	0.10 > p > 0.05
	CA		1.02	0.81	0.21	0.01 > p > 0.001
	CAn		0.48	0.48	0.00	-
	UPA		2.72	2.64	0.08	0.50 > p > 0.10
	Ac PA		2.62	2.45	0.17	0.50 > p > 0.10
	Alk PA		1.45	1.42	0.03	0.05 > p > 0.02

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
CA – Catalase activity
CAn – Nonenzymatic catalytic activity
UPA – Phosphatase activity measured in unbuffered reaction mixtures
AcPA – Acid phosphate activity
Alk PA – Alkaline phosphatase activity

Briefly, by taking the maximum mean value of each activity as 100%, we have calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator which is considered as an index of the biological quality of the soil in a given plot. The higher the enzymatic indicator of the soil, the higher position of the plots is in the hierarchy. Table 3 shows that the first positions are occupied by those plots in which enzymatic activities were the highest. The soil under the unfertilized maize plot occupying the last position can be considered as the last enzyme-active soil.

Table 3.

Enzymatic indicators of soil quality

Position	Plot	Enzymatic indicator of soil quality
1	Lupinus + rape + oat	594.93
2	Lupinus	441.49
3	Rape + lupinus	421.69
4	Vetch + oat + ryegrass	421.36
5	Lupinus + oat	425.86
6	Rape	370.66
7	Unfertilized plot	347.29

The conclusions of the present research are:

1. The soil enzymatic activities decreased with increasing sampling depth.

2. The soil phosphatase activities decreased in the order: phosphatase activity measured in unbuffered reaction mixtures > acid phosphatase activity > alkaline phosphatase activity.

3. The enzymatic indicators of soil quality calculated from the values of enzymatic activities determined in the plots under maize crop showed the order: lupinus + rape + oat > lupinus > rape + lupinus > vetch + oat + ryegrass > lupinus + oat > rape > unfertilized plot.

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THE EFFECT OF IRRIGATION ON THE ENZYMATIC ACTIVITIES IN A BROWN LUVIC SOIL

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Summary. We have determined five enzymatic activities (actual and potential dehydrogenase, catalase, acid and alkaline phosphatase) and one nonenzymatic catalytic activity (H_2O_2 splitting in autoclaved samples) in the 0–10–, 10–20– and 20–30–cm layers of a brown luvic soil submitted to a complex irrigation and crop rotation (2– and 3–crop rotations) experiment. Each activity in both non-irrigated and irrigated soil under wheat and maize crops was significantly higher in the intermediate layer than in the upper, respectively deeper layers. Non-irrigation – in comparison with irrigation – resulted in significantly higher soil phosphatase activities in the 0–10–, 10–20– and 20–30–cm layers, whereas dehydrogenase and catalase activities were significantly higher in irrigated soil. The soil under wheat or maize was more enzyme-active in the 3– than in the 2–crop rotation and in the monoculture. In the monoculture and in the 2–crop rotation, higher enzymatic activities were registered under wheat than under maize. In the 3–crop rotation, higher enzymatic activities were recorded maize under wheat. The enzymatic indicators of soil quality decreased depending on the nature of crops and kind of irrigation in the following order: maize (3–crop rotation) > wheat (3–crop rotation) > wheat (2–crop rotation) > maize (2–crop rotation) > wheat (monoculture) > maize (monoculture).

KEYWORDS: catalase, crop rotation, dehydrogenase, irrigation, phosphatase.

Introduction. Enzymes are a special group of proteins which catalyze biochemical processes in soil. As such, they may be used as indicators of microbiological and biological activities of soil (Ionescu-Sişeşti and Ştefanic, 1984).

Soil enzymes include a wide spectrum of oxidoreductases, transferases, hydrolases and lyases. Soil enzymes are mainly of bacterial and fungal origin. Only a small fraction is excreted by plants and/or animals. Outside the producing cell,

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the enzymes function in the soil solution or are immobilized on cell fragments, inorganic and organic soil components. Soil enzymes play a role in the degradation of litter and artificial substances (Freney *et al.*, 1985).

Any management practice (Angers *et. al* 1993; Dick, 1992; Dick *et al.*, 1994; Dormaar and Sommerfeldt, 1996; Lovell *et al.* 1995; Kannan and Oblisami, 1990; Pulford and Tabatabai, 1988; Zelles *et al*., 1987) that effects the biological populations of soil could be expected to result in some change in soil enzyme levels. This would apply especially to intracellular enzymes, and to extracellular enzymes either in soil solution or attached to living cells.

Following our investigations, during which in October 1997 (Samuel *et al.*, 2005a; Samuel *et al.*, 2005b) we determined the effects of tillage, crop rotation and fertilisation on soil enzymatic activities, now we report the effects of irrigation and crop rotation on soil enzymatic activities.

We have determined five enzymatic activities (actual and potential dehydrogenase, catalase, acid and alkaline phosphatase) and one nonenzymatic catalytic activity in a brown luvic soil submitted to a complex irrigation and crop rotation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

It is well know that the dehydrogenase and catalase activities are considered as indicators of the global and respiratory activity of soil, whereas phosphatase activities are related to the P cycling in soil.

The first enzymological data on the soil regarding the effects of irrigation on the enzyme activities in this soil, were obtained by Ştefanic and his collaborators (Ştefanic, 1991; Ştefanic and Picu, 1989; Ştefanic *et al.*, 1984), but he published no paper on this investigation.

Material and methods. The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5, medium humus (2.32 %) and P (22 ppm) contents, but it is rich in K (83 ppm).

The experimental field was divided into plots and subplots for comparative study of irrigation and non-irrigation and rotations of 2– and 3–crops. The crops of the 2– and 3–crops rotations are specified in Table 1.

Table 1.

Crops of the two rotations

Year 2005	Monoculture	Rotation of 2 crops	Rotation of 3 crops
	Plots	Plots	Plots
	Wheat Maize	Wheat Maize	Wheat Maize Soybean

Each plot consisted of two subplots representing the irrigation and non-irrigation variants. The plots (and subplots) were installed in three repetitions.

In October 2005, soil was sampled from the 0–10–, 10–20– and 20–30–cm depths of the subplots under wheat and maize crops. The soil samples were allowed to air-dry, then ground and passed through a 2–mm sieve and, finally, used for enzymological analyses.

We have determined five enzymatic activities (actual and potential dehydrogenase, catalase, acid and alkaline phosphatase) and one nonenzymatic catalytic activity (H_2O_2 splitting in autoclaved samples).

Actual and potential dehydrogenase activities were determined according to the methods described in (Drăgan-Bularda, 1983). The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2, 3, 5- triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose. All reaction mixtures were incubated at 37º C for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. The reaction mixtures for catalase activities consisted of 3.0 g soil and 2 ml H_2O_2 3% and 10 ml buffer solution. The buffer solution was prepared as recommended by (Drăgan-Bularda, 1983).

Disodium phenylphosphate served as enzyme substrate (Drăgan-Bularda, 1983; Őhlinger, 1996). Two activities were measured: acid phosphatase activity in reaction mixtures to which acetate buffer (pH 5.0) was added and alkaline phosphatase activity in reaction mixtures treated with borax buffer (pH 9.4).

The reaction mixtures consisted of 2.5 g soil, 2 ml toluene (antiseptic), 10 ml buffer solution and 10 ml 0.5 % substrate solution. Reaction mixtures without soil or without substrate were the controls. All reaction mixtures were incubated at 37º C for 2 hours. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2, 6-dibromoquinone-4-chloroimide.

Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced from 2, 3, 5-triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 hours.

Catalase and nonenzymatic catalytic activities are recorded as mg of H_2O_2 decomposed by 1 g of soil in 1 hour. Phosphatase activities are expressed in mg phenol / g soil / 2 hours.

The activity values were submitted to statistical evaluation by the two *t*-test (Sachs, 1968).

Results and discussion. Results of the enzymological analyses are presented in Table 2, and those of the statistical evaluation are summarized in Tables 3 and 4.

Variation of soil activities in dependence of sampling depth. It is clear from Table 3 that enzymatic activities were significantly higher (at least at $p < 0.05$) in the intermediate layer of the non-irrigated soil, excepting actual dehydrogenase activity which was unsignificantly higher ($p > 0.05$). In the deeper layer of the non-irrigated soil, enzymatic activities were significantly higher (at least at $p < 0.05$) than in the upper layer, excepting actual dehydrogenase activity which was unsignificantly ($p > 0.05$). In the irrigated soil,

enzymatic activities were significantly higher in the intermediate than in the upper and deeper layers, excepting actual dehydrogenase activity which was unsignificantly higher.

Table 2.

The effects of soil management practices on enzymatic and nonenzymatic catalytic activities in a brown luvic soil

Soil enzymatic activity*	Soil depth (cm)	Monoculture**				Rotation of 2–crops				Rotation of 3–crops			
		Wheat		Maize		Wheat		Maize		Wheat		Maize	
		N	I	N	I	N	I	N	I	N	I	N	I
ADA	0-10	7.84	8.40	7.28	8.14	8.96	11.08	7.84	8.84	9.00	11.26	8.00	8.96
	10-20	8.54	11.4	8.68	9.52	9.03	12.40	9.12	11.20	10.08	12.80	11.20	11.40
	20-30	8.22	11.20	8.68	8.24	9.00	11.20	8.94	9.80	9.96	11.52	11.72	9.96
PDA	0-10	23.52	31.60	24.08	29.60	25.64	32.40	29.80	31.20	29.28	35.20	30.36	32.96
	10-20	33.60	34.00	29.04	35.12	35.48	37.40	33.44	36.09	38.28	38.96	37.04	38.68
	20-30	28.00	33.88	25.16	31.08	35.20	36.88	32.79	32.17	38.04	37.88	36.43	37.07
CA	0-10	4.47	4.60	4.23	4.74	4.60	4.77	4.34	4.80	4.67	4.90	4.50	5.33
	10-20	5.27	5.87	4.93	5.70	5.50	5.87	5.20	5.83	5.77	5.97	5.26	5.87
	20-30	4.50	5.67	4.37	5.60	4.64	5.87	5.11	5.73	4.66	5.93	5.22	5.74
CAn	0-10	2.60	2.87	2.34	2.87	2.77	3.02	2.46	2.97	2.90	3.06	2.54	2.99
	10-20	2.97	3.17	2.57	2.97	3.00	3.19	2.77	3.02	3.12	3.21	2.91	3.10
	20-30	2.77	2.97	2.40	2.87	2.87	3.07	2.70	3.00	2.94	3.17	2.81	3.07
AcPA	0-10	0.159	0.120	0.163	0.129	0.167	0.135	0.176	0.144	0.173	0.143	0.189	0.179
	10-20	0.179	0.140	0.180	0.155	0.191	0.168	0.187	0.185	0.190	0.161	0.195	0.188
	20-30	0.165	0.125	0.174	0.144	0.189	0.141	0.180	0.173	0.189	0.152	0.190	0.185
AlkPA	0-10	0.061	0.050	0.065	0.053	0.073	0.067	0.071	0.062	0.076	0.070	0.083	0.068
	10-20	0.084	0.079	0.082	0.071	0.089	0.082	0.083	0.077	0.091	0.085	0.095	0.080
	20-30	0.067	0.055	0.071	0.066	0.078	0.070	0.077	0.070	0.083	0.075	0.087	0.076

* ADA – Actual dehydrogenase activity
CAn – Nonenzymatic catalytic activity
** N – Non-irrigation
PDA – Potential dehydrogenase activity
Ac PA – Acid phosphatase activity
I - Irrigation
CA – Catalase activity
Alk PA – Alkaline phosphatase activity

The effect of irrigation on the enzymatic activities in soil. Actual and potential dehydrogenase and catalase activities were significantly higher (at least at $p < 0.05$) in the three soil layers analysed of the non-irrigated soil, excepting potential dehydrogenase activity in the deeper layer and catalase activity in the intermediate layer which were unsignificantly higher ($p > 0.05$). Nonenzymatic catalytic activity was significantly higher (at least at $p < 0.01$) in the three soil layers of the non-irrigated soil. These findings are valid under each crop.

The effect of crop rotations on the enzymatic activities in soil. For evaluation of this effect, the results obtained in the three soil layers analysed in the two subplots of each plot were considered together.

Table 3.

Significance of the differences between enzymatic and nonenzymatic catalytic activities in a brown luvic soil submitted to irrigation

Soil depth	Soil enzymatic activity*	Mean activity values			Significance of the differences
		a	b	a-b	
1	2	3	4	5	6
Non-irrigated soil Upper layer (a) versus intermediate layer (b)	ADA	8.15	9.44	-1.29	0.05 > p > 0.02
	PDA	27.11	34.48	-7.37	0.002 > p > 0.001
	CA	4.47	5.32	-0.85	0.0001 > p
	CAn	1.74	2.89	-1.15	0.001 > p > 0.0001
	Ac PA	0.171	0.187	-0.016	0.01 > p > 0.002
	Alk PA	0.071	0.087	-0.016	0.001 > p
Upper layer (a) versus deeper layer (b)	ADA	8.15	9.42	-1.27	0.10 > p > 0.05
	PDA	27.11	32.60	-5.49	0.02 > p > 0.01
	CA	4.47	4.75	-0.28	0.50 > p > 0.10
	CAn	1.74	2.75	-1.01	0.02 > p > 0.01
	Ac PA	0.171	0.181	-0.01	0.05 > p > 0.02
	Alk PA	0.071	0.077	-0.006	0.01 > p > 0.002
Intermediate layer (a) versus deeper layer (b)	ADA	9.44	9.42	0.02	0.10 > p > 0.05
	PDA	34.48	32.60	1.88	0.50 > p > 0.10
	CA	5.32	4.75	0.57	0.05 > p > 0.02
	CAn	2.89	2.75	0.14	0.001 > p > 0.0001
	Ac PA	0.187	0.181	0.006	0.05 > p > 0.02
	Alk PA	0.087	0.077	0.01	0.002 > p > 0.001
Irrigated soil Upper layer (a) versus intermediate layer (b)	ADA	9.45	11.45	-2.00	0.001 > p > 0.0001
	PDA	32.16	36.71	-4.55	0.001 > p > 0.0001
	CA	4.86	5.85	-0.99	0.001 > p > 0.0001
	CAn	2.96	3.11	-0.15	0.01 > p > 0.002
	Ac PA	0.141	0.166	-0.025	0.01 > p > 0.0.02
	Alk PA	0.061	0.079	-0.018	0.01 > p > 0.002
Upper layer (a) versus deeper layer (b)	ADA	9.45	10.32	-0.87	0.10 > p > 0.05
	PDA	32.16	34.83	-2.67	0.01 > p > 0.002
	CA	4.86	5.75	-0.89	0.001 > p > 0.0001
	CAn	2.96	3.03	-0.07	0.05 > p > 0.02
	Ac PA	0.141	0.153	-0.012	0.05 > p > 0.02
	Alk PA	0.061	0.057	0.004	0.01 > p > 0.002
Intermediate layer (a) versus deeper layer (b)	ADA	11.45	10.32	1.13	0.002 > p > 0.001
	PDA	36.71	34.83	1.88	0.05 > p > 0.02
	CA	5.85	5.75	0.10	0.05 > p > 0.02
	CAn	3.11	3.03	0.08	0.05 > p > 0.02
	Ac PA	0.166	0.153	0.013	0.05 > p > 0.02
	Alk PA	0.079	0.057	0.022	0.02 > p > 0.01

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
CA – Catalase activity
CAn – Nonenzymatic catalytic activity
AcPA – Acid phosphatase activity
Alk PA – Alkaline phosphatase activity

Table 4.

Significance of the differences between enzymatic and nonenzymatic catalytic activities in a brown luvic soil submitted to different management practices

Management practices	Soil enzymatic activity*	Soil depth	Mean activity values in management practices			Significance of the differences
1	2	3	4	5	6	7
Irrigation (a) versus non-irrigation (b)	ADA	0-10	8.15	9.45	-1.30	0.01 > p > 0.002
		10-20	9.44	11.45	-2.01	0.02 > p > 0.01
		20-30	9.42	10.32	-0.90	0.01 > p > 0.002
	PDA	0-10	27.11	32.16	-5.05	0.01 > p > 0.002
		10-20	34.48	36.16	-2.23	0.05 > p > 0.02
		20-30	32.60	34.83	-2.23	0.10 > p > 0.05
	CA	0-10	4.47	4.86	-0.39	0.02 > p > 0.01
		10-20	5.32	5.85	-0.53	0.10 > p > 0.05
		20-30	4.75	5.75	-1.00	0.001 > p > 0.0001
	CAn	0-10	1.74	2.96	-1.22	0.002 > p > 0.001
		10-20	2.89	3.11	-0.22	0.01 > p > 0.002
		20-30	2.75	3.03	-0.28	0.001 > p > 0.0001
	Ac PA	0-10	0.171	0.141	0.030	0.001 > p > 0.0001
		10-20	0.187	0.166	0.021	0.02 > p > 0.01
		20-30	0.181	0.153	0.028	0.02 > p > 0.01
	Alk PA	0-10	0.071	0.061	0.010	0.001 > p > 0.0001
		10-20	0.087	0.079	0.008	0.01 > p > 0.002
		20-30	0.077	0.057	0.020	0.001 > p > 0.0001
The same crop in the two rotations						
Wheat in monoculture (a) versus in 2–crop rotation (b)	ADA	0-40	9.27	10.28	-1.01	0.05 > p > 0.02
	PDA		30.77	33.83	-3.06	0.05 > p > 0.02
	CA		5.06	5.21	-0.15	0.01 > p > 0.002
	CAn		2.89	2.99	-0.10	0.02 > p > 0.01
	Ac PA		0.148	0.165	-0.017	0.01 > p > 0.002
	Alk PA		0.066	0.076	-0.01	0.01 > p > 0.002
Wheat in monoculture (a) versus in 3–crop rotation (b)	ADA	0-40	9.27	10.77	-1.50	0.01 > p > 0.002
	PDA		30.77	36.27	-5.50	0.05 > p > 0.02
	CA		5.06	5.32	-0.26	0.50 > p > 0.10
	CAn		2.89	3.07	-0.18	0.01 > p > 0.002
	Ac PA		0.148	0.168	-0.02	0.001 > p > 0.0001
	Alk PA		0.066	0.080	-0.014	0.002 > p > 0.001
Wheat in 2–crop rotation (b) versus in 3–crop rotation (b)	ADA	0-40	10.28	10.77	-0.49	0.05 > p > 0.02
	PDA		33.83	36.27	-2.44	0.002 > p > 0.001
	CA		5.21	5.32	-0.11	0.05 > p > 0.02
	CAn		2.99	3.07	-0.08	0.01 > p > 0.002
	Ac PA		0.165	0.168	-0.003	0.05 > p > 0.02
	Alk PA		0.076	0.080	-0.004	0.02 > p > 0.01
Maize in monoculture (a) versus maize in 2–crop rotation (b)	ADA	0-40	8.42	9.29	-0.87	0.02 > p > 0.01
	PDA		29.01	32.58	-3.57	0.05 > p > 0.02
	CA		4.93	5.17	-0.24	0.10 > p > 0.05
	CAn		2.67	2.82	-0.15	0.01 > p > 0.002
	Ac PA		0.157	0.174	-0.017	0.01 > p > 0.002
	Alk PA		0.068	0.073	-0.005	0.01 > p > 0.002

Table 4 *(continued)*

1	2	3	4	5	6	7
Maize in	ADA	0-40	8.42	10.21	-1.79	0.01 > p > 0.002
monoculture	PDA		29.01	35.42	-6.41	0.01 > p > 0.002
(a) versus	CA		4.93	5.32	-0.39	0.02 > p > 0.01
maize in 3–	CAn		2.67	2.90	-0.23	0.01 > p > 0.002
crop rotation	Ac PA		0.157	0.187	-0.03	0.002 > p > 0.001
(b)	Alk PA		0.068	0.081	-0.013	0.002 > p > 0.001
Maize in 2–	ADA	0-40	9.29	10.21	-0.92	0.50 > p > 0.10
crop rotation	PDA		32.58	35.42	-2.84	0.05 > p > 0.02
(a) versus	CA		5.17	5.32	-0.15	0.50 > p > 0.10
maize in 3–	CAn		2.82	2.90	-0.08	0.002 > p > 0.001
crop rotation	Ac PA		0.174	0.187	-0.013	0.05 > p > 0.02
(b)	Alk PA		0.073	0.081	-0.008	0.01 > p > 0.002
Different crops in the same rotation						
Monoculture	ADA	0-40	9.26	8.42	0.84	0.10 > p > 0.05
Wheat (a)	PDA		30.76	29.01	1.75	0.02 > p > 0.01
versus maize	CA		5.06	4.93	0.13	0.50 > p > 0.10
(b)	CAn		2.89	2.67	0.22	0.05 > p > 0.02
	Ac PA		0.148	0.157	-0.009	0.01 > p > 0.002
	Alk PA		0.066	0.068	-0.002	0.02 > p > 0.01
2–crop	ADA	0-40	10.28	9.29	0.99	0.05 > p > 0.02
rotation	PDA		33.83	32.58	1.25	0.01 > p > 0.002
Wheat (a)	CA		5.21	5.17	0.04	0.05 > p > 0.02
versus maize	CAn		2.99	2.82	0.17	0.01 > p > 0.002
(b)	Ac PA		0.165	0.174	-0.009	0.05 > p > 0.02
	Alk PA		0.076	0.073	0.003	0.05 > p > 0.02
3–crop	ADA	0-40	10.77	10.21	0.56	0.001 > p > 0.0001
rotation	PDA		36.27	35.21	0.85	0.01 > p > 0.002
Wheat (a)	CA		5.31	5.32	-0.01	0.01 > p > 0.002
versus maize	CAn		3.07	2.90	0.17	0.01 > p > 0.002
(b)	Ac PA		0.168	0.187	-0.019	0.05 > p > 0.02
	Alk PA		0.080	0.082	-0.002	0.02 > p > 0.01

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
CA – Catalase activity
CAn – Nonenzymatic catalytic activity
AcPA – Acid phosphate activity
Alk PA – Alkaline phosphatase activity

The soil enzymological effect of the same crop in the two rotations. As wheat and maize were crops in monoculture and both rotations, it was possible to compare the soil enzymological effect of the monoculture and of the 2– and 3–crop rotations. The soil under both plants was more enzyme-active in the 3–crop rotation. In the soil under wheat, the enzymatic activities were significantly higher in the 3–crop rotation, excepting catalase activity which was unsignificantly higher in the 3–crop rotation than in the monoculture. In the soil under maize, the differences between the rotations was significant (at least at $p < 0.05$) in the 3–crop rotation than in the monoculture. Nonenzymatic catalytic activity was significantly higher (at least at $p < 0.01$) in the 3– than in the 2–crop rotation and monoculture under each crops.

The soil enzymological effect of different crops in the same rotation.

The monoculture. Potential dehydrogenase and nonenzymatic catalytic activities measured in the wheat soil exceeded significantly ($p < 0.02$ and $p < 0.05$, respectively) the corresponding activities recorded in the maize soil, whereas actual dehydrogenase and catalase activities were unsignificantly higher ($p > 0.05$ and $p > 0.10$, respectively) in the wheat soil. Acid and alkaline phosphatase activities were higher under maize.

The 2–crop rotation. Each enzymatic activity and nonenzymatic catalytic activity measured in the wheat soil exceeded significantly (at least at $p < 0.05$) the corresponding activity recorded in the maize soil, excepting acid phosphatase activity which was higher under maize.

The 3–crop rotation. Significant ($p < 0.05$ to $p < 0.001$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and the nature of crop. Dehydrogenase activities and nonenzymatic catalytic activity were significantly higher (at least at $p < 0.01$) in the wheat soil, while catalase and phosphatase activities were higher in the maize soil.

Enzymatic indicators of soil quality. For establishing a hierarchy of the plots admitting equal importance for the enzymatic activities, we have used the method, referred to in (Kiss *et al.*, 1990), to calculate the enzymatic indicators of soil quality.

The results obtained (Table 5) show that in the hierarchy of the six plots, the first positions are occupied by the crops of the 3–crop rotation, while the last positions are occupied by the crops of the monoculture.

Table 5.

Enzymatic indicators of soil quality

Position	Plot	Enzymatic indicator of soil quality
1	Maize (3–crop rotation)	492.41
2	Wheat (3–crop rotation)	487.41
3	Wheat (2–crop rotation)	468.47
4	Maize (2–crop rotation)	455.98
5	Wheat (monoculture)	425.86
6	Maize (monoculture)	418.17

The conclusions for the present research are:

1. The soil enzymatic activities under wheat and maize crops in both non-irrigated and irrigated soil were higher in the intermediate layer than in the upper, respectively deeper layers.
2. Non-irrigation – in comparison with irrigation – resulted in higher phosphatase activities, whereas dehydrogenase and catalase activities were higher in irrigated soil.
3. The 3–crop rotation – as compared to the 2–crop rotation and monoculture – led to higher enzymatic activities in the soil layers under maize or wheat.

4. In the monoculture and in the 2–crop rotation, higher enzymatic activities were registered under wheat than under maize.
5. In the 3–crop rotation, higher enzymatic activities were recorded maize under wheat.
6. The enzymatic indicators of soil quality calculated from the values of enzymatic activities determined showed the order: maize (3–crop rotation) > wheat (3–crop rotation) > wheat (2–crop rotation) > maize (2–crop rotation) > wheat (monoculture) > maize (monoculture).

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CORRELATIONS BETWEEN ENZYMATIC ACTIVITIES AND CHEMICAL INDICATORS IN A BROWN LUVIC SOIL

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Summary. Actual and potential dehydrogenase and acid phosphatase activities and nitrate, ammonium and mobile phosphorus contents were determined in the 0–10–, 10–20– and 20–30–cm layers of a brown luvic soil submitted to a complex tillage and crop rotation experiment. Each activity and each chemical content in both non-tilled and conventionally tilled soil under crops of both rotations decreased with increasing sampling depth. It was found that no-till – in comparison with conventional tillage – resulted in significantly higher soil enzymatic activities and nitrate, ammonium and mobile phosphorus contents in the 0–10– and 10–20–cm layers and in significantly lower activities and chemical contents in the deeper layers. The soil under wheat or maize was more enzyme-active in the 6– than in the 2–crop rotation. In the 2–crop rotation, higher enzymatic activities were registered under maize and under wheat, excepting acid phosphatase activity; nitrate, ammonium and mobile phosphorus contents were significantly higher under maize. In the 6–crop rotation, higher enzymatic activities were registered under wheat, excepting actual dehydrogenase activity; ammonium and mobile phosphorus contents were higher under wheat. There were positive correlations between enzymatic activities and chemical indicators under each drop of both rotations.

KEYWORDS: ammonium, dehydrogenase, nitrate, phosphatase, phosphorus.

Introduction. Soil microorganisms contribute to better plant development. They colonize mainly the organic matter at the microsites (Kandeler and Murer, 1993).

Clay minerals also serve as carriers of organisms, enzymes (Lovell *et al.*, 1995), metabolic products, and growth and inhibitory substances. The air and water filled pore spaces in the soil are an essential requirement for the life of most organisms. The microbial

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counts rapidly decrease with soil depth due to the deterioration of the substrate (Dick, 1992).

The metabolic activity of soil microorganisms is essential for organic matter turnover. The mobilization and immobilization of inorganic nutrients and trace elements are also mainly a result of microbial activities (Dormaar and Lindwall, 1989). Metabolic activities are determined by the species composition, which in turn is influenced by the available litter, the soil type and other environmental conditions (Doran, 1980a, 1980b).

It is well known that the dehydrogenase activity of a soil is thus the results of the activity of different dehydrogenases, which are an important component of the enzyme system of all microorganisms (enzymes of respiratory metabolism, citrate cycle, and nitrogen metabolism). Dehydrogenase activity is thus an indicator of biological redox-systems, and can be taken as a measure for the intensity of microbial metabolism in soil (Dick *et al.*, 1994; Dick, 1984).

Phosphatases are inducible enzymes that are produced predominantly under conditions of low phosphorus availability (Canarutto *et al.*, 1995; Clarholm and Rosengren-Brinck, 1995). Phosphomonoesterase enzymes play an important role in P cycling in soil and, consequently, in P nutrition of plants, as these hydrolytic enzymes eliberate plant-available, mineral o-phosphate from organic P compounds, namely from P monoesters.

The effects of tillage (Groffman, 1985), crop rotation (Angers *et al.*, 1993) on soil enzymatic activities were studied in many countries, including our country (Ştefanic *et al*., 1983, 1984).

In continuation of our investigations (Samuel and Kiss, 1999; Samuel *et al*., 2000), we studied some enzymatic activities and their correlations with chemical properties in a brown luvic soil submitted to a complex tillage and crop rotation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

Material and methods. The ploughed layer of the studied soil is of a mellow loam texture and it has a pH value of 5.5.

The experimental field was divided into plots and subplots for comparative study of no-till and conventional tillage and rotations of 2– and 6–crops. The crops of the 2– and 6–crops rotations were wheat and maize.

Each plot consisted of two subplots representing the no-till and conventional tillage variants. The plots (and subplots) were installed in three repetitions.

In October 2005, soil was sampled from the 0–10–, 10–20–, 20–30– and 30–40–cm depths of the subplots. The soil samples were allowed to air-dry, then ground and passed through a 2–mm sieve and, finally, used for enzymological analyses.

Actual and potential dehydrogenase activities were determined according to the methods describe in (Drăgan-Bularda, 2000). The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2, 3, 5- triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose. All reaction mixtures were incubated at 37º C

for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. Disodium phenylphosphate served as enzyme substrate. Acid phosphatase activity was measured in reaction mixtures to which acetate buffer (pH 5.0). The buffer solution was prepared as recommended by (Öhlinger, 1996a). The reaction mixtures consisted of 2.5 g soil, 2 ml toluene (antiseptic), 10 ml acetate buffer and 10 ml 0.5 % substrate solution. Reaction mixtures without soil or without substrate solution were the controls. All reaction mixtures were incubated at 37º C for 2 hours. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2, 6-dibromoquinone-4-chloroimide.

Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced from 2, 3, 5-triphenyltetrazolium chloride (TTC) by 10 g of soil in 24 hours, whereas phosphatase activities are expressed in mg phenol / g soil / 2 hours.

Chemical indicators were determined according to the methods described in (Kandeler, 1996a, 1996b; Öhlinger, 1996b).

The activity values were submitted to statistical evaluation by the two *t*-test (Sachs, 1968) and the correlations between the enzymatic activities and chemical indicators were determined according to the methods described in (Snedecor, 1968).

Results and discussion. Results of the enzymological analyses are presented in Tables 1 and 2, and those of the statistical evaluation are summarised in Tables 3 and 4. Fig. 1, 2 and 3 show correlations between enzymatic activities and chemical indicators.

Variation of the enzymatic activities and chemical properties in dependence of sampling depth. It is evident from Table 1 that each enzymatic activity decreased with sampling depth in both subplots under each crop of both rotations. In addition, Table 3 shows that the mean values of each of the three activities in both non-tilled and conventionally tilled subplots also decreased with increasing soil depth.

The chemical indicators also, decreased with increasing sampling depth.

The effect of tillage practices on the enzymatic activities and chemical properties in soil. Each of the three enzymatic activity determined was higher in the upper (0–10– and 10–20–cm) layers of the no-tilled subplots. But only actual dehydrogenase activity and acid phosphatase activity in the 0–10–cm layer were significantly higher (at least at $p < 0.05$ and $p < 0.02$, respectively).

Potential dehydrogenase activity was unsignificantly higher (at least at $p < 0.10$) in the upper layers, whereas acid phosphatase activity was unsignificantly higher ($p > 0.05$) in the 10–20–cm layer. The reverse was true (at least at $p < 0.05$) in the deeper (20–30– and 30–40–cm) layers. These findings are also valid for chemical indicators.

Table 1.

The effects of soil management practices on enzymatic activities in a brown luvic soil

Soil enzymatic activity*	Soil depth (cm)	Rotation of 2 crops**				Rotation of 6 crops			
		Wheat		Maize		Wheat		Maize	
		N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
ADA (mg TPF/10g soil/24 hours)	0-10	6.48	6.16	6.64	6.52	8.92	8.68	8.84	8.60
	10-20	5.58	5.49	6.38	6.24	8.54	8.23	8.48	8.24
	20-30	4.36	5.04	5.06	5.38	6.60	6.72	7.07	7.62
	30-40	3.52	4.13	4.24	4.98	4.66	5.38	5.26	6.11
PDA (mg TPF/10g soil/24 hours)	0-10	20.15	19.63	21.18	20.87	24.83	22.97	23.23	21.75
	10-20	19.31	19.15	21.09	20.95	23, 56	22.18	22.05	20.63
	20-30	17.86	18.74	18.42	19.81	20.21	20.75	19.18	20.15
	30-40	15.07	16.22	15.05	15.83	16.33	17.56	16.73	16.84
AcPA (mg TPF/10g soil/24 hours)	0-10	0.310	0.252	0.294	0.206	0.344	0.298	0.352	0.312
	10-20	0.198	0.188	0.190	0.180	0.245	0.209	0.213	0.200
	20-30	0.131	0.177	0.141	0.173	0.149	0.195	0.142	0.209
	30-40	0.072	0.140	0.097	0.110	0.112	0.156	0.104	0.123

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
Ac PA – Acid phosphatase activity

**N.t. – No-till
C.t. – Conventional tillage

Table 2.

The effects of soil management practices on chemical properties in a brown luvic soil

Chemical indicators	Soil depth (cm)	Rotation of 2 crops*				Rotation of 6 crops			
		Wheat		Maize		Wheat		Maize	
		N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
$N\text{-}NO_3$ (mg N / kg soil)	0-10	0.42	0.41	0.46	0.43	0.57	0.51	0.59	0.54
	10-20	0.40	0.38	0.44	0.41	0.56	0.50	0.57	0.51
	20-30	0.31	0.34	0.31	0.37	0.38	0.46	0.34	0.42
	30-40	0.23	0.24	0.21	0.28	0.28	0.30	0.25	0.28
$N\text{-}NH_4$ (mg N / kg soil)	0-10	1.33	1.28	1.37	1.32	1.39	1.36	1.42	1.39
	10-20	1.27	1.20	1.35	1.31	1.35	1.31	1.41	1.37
	20-30	0.66	0.74	0.61	0.69	0.82	0.85	0.80	0.86
	30-40	0.38	0.40	0.40	0.43	0.41	0.45	0.42	0.44
P_2O_5 (mg P_2O_5 / kg soil)	0-10	12.5	12.1	12.4	12.2	14.2	13.6	14.7	14.0
	10-20	11.7	11.6	12.0	11.8	12.7	12.0	13.6	13.3
	20-30	9.8	10.7	10.5	11.1	10.5	12.1	12.3	12.9
	30-40	7.9	8.2	8.8	9.1	9.2	10.2	9.4	11.2

* N.t. – No-till
C.t. – Conventional tillage

Table 3.

Significance of the differences between enzymatic activities in a brown luvic soil submitted to different management practices

Management practices	Soil enzymatic activity*	Soil depth	Mean activity values in management practices			Significance of the differences
			a	b	a-b	
No-till (a) versus conventional tillage (b)	ADA	0-10	7.72	7.49	0.23	0.02 > p > 0.01
		10-20	7.25	7.05	0.20	0.05 > p > 0.02
		20-30	5.77	6.19	-0.42	0.05 > p > 0.02
		30-40	4.42	5.15	-0.73	0.001 > p > 0.0001
	PDA	0-10	22.35	21.30	1.05	0.10 > p > 0.05
		10-20	21.50	20.73	0.77	0.50 > p > 0.10
		20-30	18.92	19.86	-0.94	0.02 > p > 0.01
		30-40	15.80	16.61	-0.81	0.05 > p > 0.02
	Ac PA	0-10	0.325	0.267	0.058	0.02 > p > 0.01
		10-20	0.212	0.194	0.018	0.10 > p > 0.05
		20-30	0.141	0.188	-0.047	0.002 > p > 0.001
		30-40	0.096	0.132	-0.036	0.50 > p > 0.10
The same crop in the two rotations						
Wheat in 2– crop rotation (b) versus wheat in 6– crop rotation (b)	ADA	0-40	5.10	7.22	-2.12	0.002 > p > 0.001
	PDA		18.27	21.04	-2.77	0.01 > p > 0.002
	Ac PA		0.184	0.214	-0.03	0.002 > p > 0.001
Maize in 2– crop rotation (b) versus maize in 6– crop rotation (b)	ADA	0-40	5.68	7.53	-1.85	0.002 > p > 0.001
	PDA		19.15	20.07	-0.92	0.02 > p > 0.01
	Ac PA		0.174	0.207	-0.033	0.05 > p > 0.02
Different crops in the same rotation						
2– crop rotation Wheat (a) versus maize (b)	ADA	0-40	5.10	5.68	-0.58	0.01 > p > 0.002
	PDA		18.27	19.15	-0.88	0.10 > p > 0.05
	Ac PA		0.184	0.174	0.01	0.50 > p > 0.10
6– crop rotation Wheat (a) versus maize (b)	ADA	0-40	7.22	7.53	-0.31	0.0001 > p
	PDA		21.04	20.07	0.97	0.01 > p > 0.002
	Ac PA		0.214	0.207	0.007	0.50 > p > 0.10

* ADA – Actual dehydrogenase activity
PDA – Potential dehydrogenase activity
AcPA – Acid phosphatase activity

The effect of crop rotations on the enzymatic activities and chemical properties in soil. For evaluation of this effect, the results obtained in the four soil layers analysed in the two subplots of each plot were considered together.

Table 4.

Significance of the differences between chemical indicators practices in a brown luvic soil submitted to different management practices

Management practices	Chemical indicators	Soil depth	Mean activity values in management practices			Significance of the differences
			a	b	a-b	
No-till (a) versus conventional tillage (b)	$N\text{-}NO_3$	0-10	0.51	0.47	0.04	0.05 > p > 0.02
		10-20	0.49	0.45	0.04	0.02 > p > 0.01
		20-30	0.34	0.40	-0.06	0.02 > p > 0.01
		30-40	0.24	0.28	-0.04	0.10 > p > 0.05
	$N\text{-}NH_4$	0-10	1.38	1.34	0.04	0.01 > p > 0.002
		10-20	1.35	1.30	0.05	0.002 > p > 0.001
		20-30	0.72	0.79	-0.07	0.02 > p > 0.01
		30-40	0.40	0.43	-0.03	0.02 > p > 0.01
	P_2O_5	0-10	13.45	12.97	0.48	0.02 > p > 0.01
		10-20	12.50	12.20	0.30	0.10 > p > 0.05
		20-30	10.78	11.70	-0.92	0.05 > p > 0.02
		30-40	8.82	9.68	-0.86	0.10 > p > 0.05
The same crop in the two rotations						
Wheat in 2–crop rotation (b) versus wheat in 6–crop rotation (b)	$N\text{-}NO_3$	0-40	0.34	0.45	-0.11	0.10 > p > 0.05
	$N\text{-}NH_4$		0.91	0.99	-0.08	0.001 > p > 0.0001
	P_2O_5		10.56	11.81	-1.25	0.001 > p > 0.0001
Maize in 2–crop rotation (b) versus maize in 6–crop rotation (b)	$N\text{-}NO_3$	0-40	0.36	0.44	-0.08	0.01 > p > 0.002
	$N\text{-}NH_4$		0.94	1.01	-0.07	0.02 > p > 0.01
	P_2O_5		10.99	12.68	-1.69	0.0001 > p
Different crops in the same rotation						
2–crop rotation Wheat (a) versus maize (b)	$N\text{-}NO_3$	0-40	0.34	0.36	-0.02	0.10 > p > 0.05
	$N\text{-}NH_4$		0.91	0.94	-0.03	0.50 > p > 0.10
	P_2O_5		10.56	10.99	-0.43	0.02 > p > 0.01
6–crop rotation Wheat (a) versus maize (b)	$N\text{-}NO_3$	0-40	0.45	0.44	0.01	0.05 > p > 0.10
	$N\text{-}NH_4$		0.99	1.01	-0.02	0.10 > p > 0.05
	P_2O_5		11.81	12.68	-0.87	0.01 > p > 0.002

The soil enzymological and chemical effects of the same crop in the two rotations. As wheat and maize were crops in both rotations, it was possible to compare

their effect on soil enzymatic activities. The soil under both crops was more enzyme-active in the 6– than in the 2–crop rotation. In the soil under each crops, the difference between the two rotations was significant (at least at $p < 0.05$) in the case of each enzymatic activity. These findings are valid for chemical indicators, excepting nitrate content which was unsgnificantly higher ($p > 0.05$) in the 6– than in the 2–crop rotation under wheat.

The soil enzymological and chemical effects of different crops in the same rotation.

The 2–crop rotation. Actual dehydrogenase activity was significantly higher ($p < 0.01$), while potential dehydrogenase and acid phosphatase activities were unsignificantly higher ($p > 0.05$ and $p > 0.10$, respectively) in the maize than in the soil under wheat.

In the case of chemical indicators, only phosphorus content was significantly higher ($p < 0.02$), while nitrate and ammonium contents were unsignificantly higher in the maize than in the soil under wheat.

The 6–crop rotation. Actual dehydrogenase activity was higher in the maize soil, while potential dehydrogenase and acid phosphatase activities were higher in the wheat soil.

Regarding chemical indicators, ammonium and phosphorus contents were higher in the soil under wheat, whereas nitrate content was higher in the soil under maize.

Correlations between enzymatic activities and chemical indicators. We have determined the correlations between actual dehydrogenase activity and nitrate content (Fig. 1), potential dehydrogenase activity and ammonium content (Fig. 2), acid phosphatase activity and phosphorus content (Fig. 3), under each crop of both rotations. It was found that each of the three enzymatic activity was positively correlated ($r = 0.229$ to $r = 0.992$) with the chemical indicators.

Based on the results presented above, we can conclude that:

1. The soil enzymatic activities and the chemical indicators decreased with increasing sampling depth.
2. No-till – in comparison with conventional tillage - resulted in higher enzymatic activities in the 0–10– and 10–20–cm layers and in lower activities in the 20–30– and 30–40–cm soil layers under each crop of both rotations. These findings are valid for chemical indicators.
3. The 6–crop rotation – as compared to the 2–crop rotation – led, in general to higher enzymatic activities and nitrate, ammonium and phosphorus contents in the soil layers under maize or wheat.
4. Each of the three enzymatic activity was positively correlated with the chemical indicators.

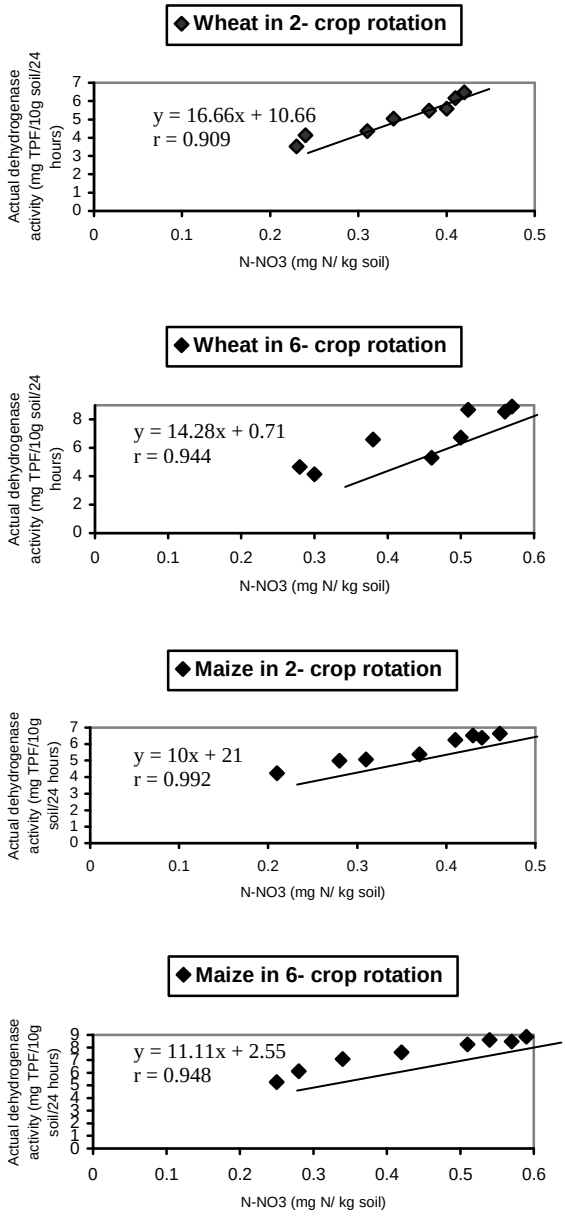

Fig. 1. Correlation between actual dehydrogenase activity and nitrate content.

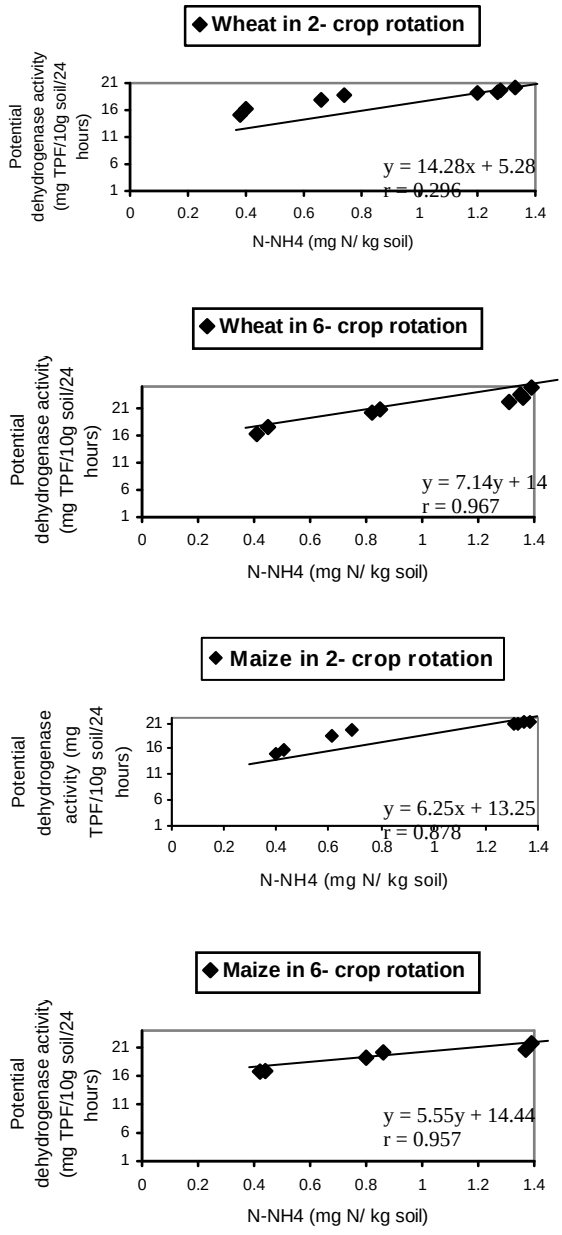

Fig. 2. Correlation between potential dehydrogenase activity and ammonium content.

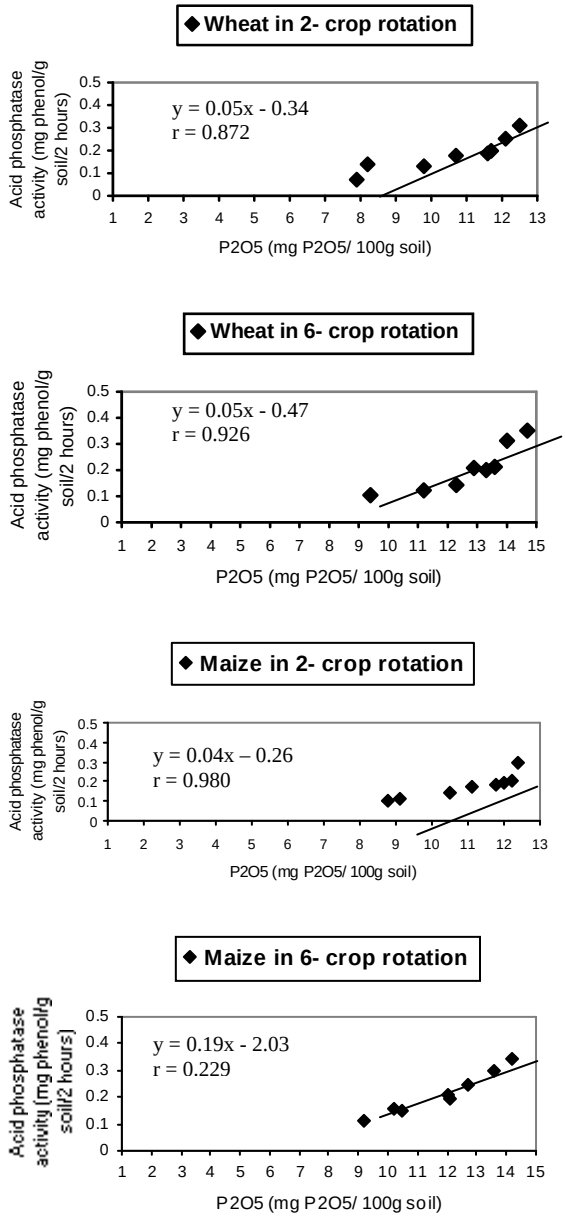

Fig. 3. Correlation between acid phosphatase activity and mobile phosphorus content.

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CONF DR. *FRANCISC PÈTERFI*
(7 MAI 1917- 13 AUGUST 2005)

Născut la 7 mai 1917 în Cluj, ca fiu al renumitului briolog şi botanist Márton Péterfi, frate mai mic al algologului academician István Péterfi. Tânărul Francisc urmează şcoala primară şi gimnaziul la fostul Gimnaziu al Piariştilor din Cluj în perioada 1924-1937, dobândind o temeinică educaţie orientată spre cultura clasică (latină, greacă, franceză) dar şi spre ştiinţele naturii. Renumiţi dascăli ai vremii i-au îndrumat paşii spre tainele fizicii, iar apoi spre cele ale biologiei. Companioni în anii tinereţii i-au fost cele 18 volume din seria monografică „Brehm", precum şi renumite determinatoare pentru insecte (Cserey, Spuller, Abafi, s.a.). Impreună cu fratele său mai mare au efectuat nenumărate excursii de colectare şi observare a insectelor în împrejurimile Clujului. Bine cunoscute i-au fost încă din copilărie Fânaţele Clujului, Valea Gîrbăului, Vârful Peana, Pădurile Hoia şi Făget. Pasiunea şi interesul stârnit în anii copilăriei de natură şi în special de nevertebrate, nu-l vor părăsi toată viaţa.

Urmează cursurile Facultăţii de Ştiinţe Naturale şi Geografie, fiind un mare admirator al prelegerilor şi operei profesorilor Emil Pop şi Aristide Grădinescu. Facultatea este absolvită cu o interesantă lucrare de diplomă dedicată „Morfologiei, anatomiei, biologiei şi taxonomiei lepidopterelor din familia Sphingidae", iar pentru partea de geografie cu lucrarea"Morfologia Munţilor Gilău". Pasiunea pentru fluturi pe care au avut-o cei doi fraţi István şi Francisc persistă şi se concretizează la primul într-o valoroasă colecţie, iar la cel de-al doilea într-o lucrare de doctorat cu titlul"Macrolepidopterele din Transilvania – caracterele sexuale secundare cu referiri speciale asupra solzilor alari"

Din 1941 este numit preparator la catedra de Zoologie unde în 1946 devine asistent, apoi în 1949 conferenţiar. In paralel, in perioada 1948-1952 predă şi cursul de entomologie agricolă, în limba maghiară la Institutul Agronomic din Cluj. La Facultatea Bolyai, devenită mai târziu Babeş-Bolyai, a predat, cu foarte mici întreruperi, zoologia nevertebratelor precum şi zoogeografie, apicultură, creşterea viermilor de mătase, ecologie animală, colectarea şi prepararea animalelor, ş.a. în limba maghiară şi română. A organizat şi supravegheat practica studenţilor şi a coordonat zeci de lucrări de licenţă. In perioada 1949-1959 este şeful Catedrei de Zoologie, între 1953-1954 prodecan, 1954-1958 decan. Intre anii 1961-1970 este iniţiatorul şi coordonatorul Societăţii de Ştiinţe Biologice, filiala Cluj, fiind şi membru în comisia superioară pentru acordarea titlului de doctor în ştiinţe biologice.

Pe latura ştiinţifică, prof. dr. Francisc Péterfi a avut interes şi preocupări susţinute pentru Lepidoptere (printre altele semnificaţia solzilor de pe aripi), Tardigrade, din care a semnalat 9 specii noi pentru fauna ţării, dintre care *Echiniscus viridissimus* Péterfi, este o specie nouă pentru ştiinţă. Cele mai multe lucrări le-a dedicat dipterelor şi dintre acestea Chloropidelor (muştele cerealelor), studiindu-le biologia, ecologia, taxonomia şi semnificaţia lor economică. A semnalat 63 de specii noi de Chloropide pentru fauna

României, dintre care 5 sunt noi pentru ştiinţă (*Elachiptera stratifrons, Meromyza hybrida, M. lucida, M. obtusa şi M. puriseta*). Devenit o somitate europeană pentru dipterele Chloropide, Francisc Péterfi a întreţinut o foarte bogată corespondenţă cu cei mai reprezentativi specialişti ai grupului din Europa, Asia şi America. Lucrările sale şi speciile nou descrise sunt citate şi menţionate în toate marile monografii dedicate Cloropidelor şi Tardigradelor. Ca zoolog, a participat la toate marile proiecte derulate pe teritoriul României, coordonate fie de Academia Română, fie de Academia de Ştiinţe Agricole. Prin traducerea lucrării lui H. Fabrie „Cartea coleopterologului"(1958) a impulsionat cercetarea acestui grup în România.

Dintre lucrările de sinteză realizate menţionăm: 1958 - Determinator pentru insectele dăunătoare agriculturii. Ed. Agro-Silvică, Bucureşi, 1-428; 1954, 1957 - Zoologie part. I (limba maghiară) – curs litografiat; 1962 – Zoologia nevertebratelor (limba maghiară), Ed. Didactică şi Pedagogică,. Bucureşti, 1-461.

Prof. dr. Francisc Péterfi s-a dedicat carierei didactice, căreia i-a afectat cel mai mult timp. Imbinând harul didactic cu asiduitatea muncii ştiinţifice, a reuşit să formeze şi iniţieze numeroşi studenţi, dintre care unii continuă şi azi, cu succes activitatea didactică şi de cercetare. Modest şi tăcut s-a pensionat şi apoi a plecat dintre noi fără valuri, pe nesimţite, precum fâlfâitul unui fluture de noapte. A lăsat însă în urmă rodul muncii şi gândirii sale, concretizate în diverse forme. Cele 6 specii noi pentru ştiinţă descrise de F. Péterfi vor aminti pe veci de cel care a trudit cu pasiune şi dârzenie multe decenii întru cunoaşterea celor care nu cuvântă. Colegii şi studenţii, toţi cei care l-au cunoscut, deplâng cu pioşenie pierderea unui maestru, coleg, prieten sau pur şi simplu a unui a unui om drept şi onest. Va dăinui în amintirea celor care l-au cunoscut, iar sufletul se va înălţa la locul cuvenit, însoţit de miraculoasele zburătoare pe cere le-a îndrăgit şi studiat atîta vreme.

László RÁKOSY

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IN MEMORY OF ERNST MAYR – A PUBLICISTIC EPILOGUE TO A LIFE DEDICATED TO SCIENCE

Recently, in the year 2004, Humanitas publishing house, released under the name of the prestigious book series „Science Masters", the volume „**De la bacterii la om – Evoluția lumii vii (What Evolution Is?)**" by Ernst Mayr. A good translation by Elena Maria Badea has completed an exceptional apparition in the scientific literature available in Romanian. As Professor of Zoology at Harvard University, Ernst Mayr was no less than 97 years old when he published this book in 2001.

„What Evolution Is?" is a science book reviewing in a highly accessible manner the „Synthetic Theory of Evolution" or the „Neo-Darwinism" conclusions and ideas aquired during 74 (!) years of life dedicated to science, particularly to the field of evolution theories and science philosphy.

The book has a foreword by Jared M. Diamond (Professor of Physiology at University of California, Los Angeles) and a preface by Ernst Mayr. The text is divided into four chapters: „What evolution is ?", „How we explain the evolutionary changes and the adaptability", „Origin and evolution of diversity: cladogenesis" and „Evolution of man". Each chapter brings up-to-date observational and experimental facts, strongly supporting the synthetic theory of evolution. The target readers are the large mass of people interested in the theories of evolution, and, nonetheless, the students, researchers and teachers being willing to have clear, concise, actual and interesting review on the evolution aspects.

Due to his claimed sighting of red-crested pochards in Germany, a species that had not been seen in Europe for 77 years,.Ernst Mayr started his career with an introduction to Erwin Stresemann. After a tough interrogation, Stresemann accepted and published the sighting as authentic. Mayr was invited to work as a volunteer at the Berlin Museum while studying medicine. He subsequently took great interest in ornithology and earned a doctorate in the same field. He was introduced, during a congress in Budapest, to Lord Walter Rothschild, a rich banker and naturalist, who had a comprehensive private bird collection. Mayr was sent by him to New Guinea, collected several thousands bird skins (he named 26 new bird species during his lifetime) and, in the same time, naming 38 new orchid species. In 1931 he moved to the American Museum of Natural History, where he played the important role of stock brokering and acquiring the Rothschild collection of bird skins. As a traditionally trained biologist with little mathematical experience, Mayr was often highly critical of early mathematical approaches to evolution such as those of J. B. S. Haldane, famously calling in 1959 such approaches "bean bag genetics". He continued to reject the view that evolution is the mere change of gene frequencies in populations, maintaining that other factors such as reproductive isolations had to be taken into account. In a similar fashion, Mayr was also quite critical of molecular evolutionary studies such as those of Carl Woese. In many of his writings, Mayr rejected reductionism in evolutionary biology, arguing that evolutionary pressures act on the whole organism, not on single genes, and that genes can have different effects depending on the other genes present. He advocated a study of the whole genome rather than of isolated genes only.

Current molecular studies in evolution and speciation indicate that although allopatric speciation seems to be the norm in groups (possibly those with greater mobility) such as the birds, there are numerous cases of sympatric speciation in many invertebrates (especially in the insects). Mayr joined the faculty of Harvard University in 1953 and retired in 1975 as *emeritus* professor of zoology, showered with honors. Following his retirement, he went on publishing more than 200 articles, in a variety of journals. Even as a centenarian, he continued to write books. He received awards including the National Medal of Science, the Balzan Prize and the International Prize.

Ernst Mayr died in february 2005 at 101 years old, leaving behind a strong print in the modern biological thought, imposing the neodarwinian trend in the evolutionary theories, „the most important concept in biology", as he claimed in the opening of the „What Evolution Is ?" book.

Horia BANCIU