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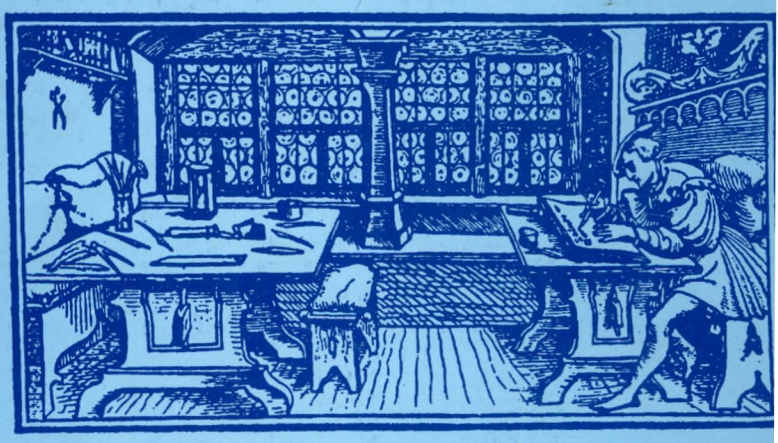

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ENZYMOLOGY OF SOILS AFFECTED BY INDUSTRIAL EMISSIONS

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SUMMARY. — Three directions were delineated in the studies dealing with enzymology of soils affected by industrial emissions: I. studies of the soil enzymological effects of the components of industrial emissions through experiments modelled in the laboratory or in *in situ* artificial microcosms; II. studies of the soil enzymological effects of industrial emissions originating from a point source (an industrial plant) and III. studies of the soil enzymological effects of industrial emissions originating from multiple sources (many industrial plants manufacturing different products, but situated in the same, industrial area).

In the present paper, studies in the first direction are not dealt with as excellent reviews on these studies are already available [3, 10, 31, 32, 35, 37, 56, 59, 69, 106, 107, 116]. Only the studies in the second and third directions and studies on some related topics, including enzymology of urban and roadside soils are reviewed.

Introduction. Many industrial plants emit gaseous, liquid and solid, particulate pollutants to the atmosphere. The air pollutants are then dispersed over smaller or larger areas and deposited in wet or dry form on the landscape affecting soils and waters, in which they can accumulate. Most of the pollutants, *e.g.* heavy metals may be very harmful to natural and man-made ecosystems, to health of humans, animals and plants, may exert detrimental effects on soil life.

Increasing environmental pollution encountered in so many areas all over the world has become a subject of extensive concern and has led to a vast literature in the field of soil enzymology, too.

The effects of heavy metals on the enzyme activities in soils were first studied in the '40s and '50s by the founders of soil enzymology (J. P. Conrad, Ed. Hofmann, V. F. Kuprevich) (see *e.g.* [54, 101]). These first studies did not aim at enzymological indication of soil pollution; their purpose was to gather additional data proving that the reactions attributed to soil enzymes are really catalysed by enzymes, as they are inhibited or inactivated by heavy metals.

The soil enzymological effects of industrial emissions as environmental pollutants are studied under laboratory and natural conditions.

In laboratory experiments, the effects of some components of industrial emissions on enzyme activities in soils are estimated. These components (heavy metals, mineral acids, fluoride, phenol etc.) are added in form of pure compounds to soil samples and the changes induced by the additions in soil enzyme activities are determined. Thus, the emissions from nonferrous metallurgical plants are *modelled* by using

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Cu, Zn, Pb, Cd etc. salts with which soil samples are treated. The acid rains are *simulated* by treating soil samples with mineral acids (H_2SO_4, HNO_3 or HCl) or SO_2. The *in situ*, artificial microcosm studies are, conceptually, similar to the laboratory ones.

In the studies carried out under natural conditions, enzyme activities in soil samples collected in the vicinity of the pollution source and in those taken at different distances from this source are compared, *i.e.* soil enzyme activities are determined along a pollution gradient.

The pollutants originate from a point source (an industrial plant) or from multiple sources (many industrial plants manufacturing different products, but situated in the same, industrial area).

Consequently, three directions can be delineated in the studies dealing with enzymology of soils affected by industrial emissions:

I. studies of the soil enzymological effects of the components of industrial emissions through experiments modelled in the laboratory or in *in situ* artificial microcosms;

II. studies of the soil enzymological effects of industrial emissions originating from a point source (an industrial plant) and

III. studies of the soil enzymological effects of industrial emissions originating from multiple sources (many industrial plants manufacturing different products, but situated in the same, industrial area).

The studies in the first direction as laboratory or artificial microcosm studies contribute to the understanding of the soil enzymological effects of pollutants from industrial emissions, but their results can not be extrapolated as perfectly valid for the natural conditions.

The studies in the second and third directions, evidencing the soil enzymological effects of industrial emissions from point or multiple sources, make it possible to establish the responsability of industries in environmental pollution and to evaluate the efficiency of the decontamination technologies applied.

I. Studies of the soil enzymological effects of the components of industrial emissions through experiments modelled in the laboratory or in i n s i t u artificial microcosms

Such studies are numerous, being described in hundreds of papers. Many synthesis papers were also published. They review the studies dealing with the soil biological and biochemical (including soil enzymological) effects of heavy metals [3, 10, 32, 37, 56, 107, 116], simulated acid rain [31, 32, 37, 106], SO_2 [59], pollutant inorganic and organic chemicals [69]. Due to the existence of these excellent reviews, the studies in the first direction will not be dealt with in our present paper.

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Within the review of studies in the second direction, 11 sections could be delineated according to the type of industries emitting pollutants to the atmosphere (nonferrous metallurgical plants, ironworks, ore enrichment works, coking plants, refractory brickworks, pulp and paper mills, synthetic fibre factories, oil production plants, other chemical factories, oil- or coal-fired power plants and atomic energy power plants). In additional sections, two activities causing point source pollution (military waste disposal and rocket destruction operations) are also considered. Within each section, the reviewed studies are grouped by countries, the order of which was established based on the year in which the first paper on the topic of section had appeared. This criterion of grouping by countries is also applied in reviewing studies in the third direction and in the addenda dealing with enzymology of urban and roadside soils.

II. Studies of the soil enzymological effects of industrial emissions originating from a point source (an industrial plant)

1. Nonferrous metallurgical plants. *Sweden.* The first soil enzymological studies in areas, where the unique, point source of pollution is a nonferrous metallurgical plant, were performed in Sweden. Rühling and Tyler [96] have studied the area around Fliseryd. This village in south-eastern Sweden has only one industry processing metal oxides for production of batteries. The village is surrounded by pine *(Pinus sylvestris)* and spruce *(Picea abies)* forests. In April 1972, the F_1 needle mor layer (usually 3—5 cm deep) was sampled in 100 sites situated along a transect extending 12.5 km from the industrial plant. The elements emitted in large amounts from the plant are lead, nickel and cadmium. The Pb concentrations in mor samples from sites situated within 0.5 km from the plant were, on average, 26 times higher than in samples from sites situated more than 7 km away. The corresponding factors for Ni and Cd were 54 and 22, respectively. Small gradients were found in vanadium (factor $\sim$ 7), copper ($\sim$ 2), iron ($\sim$ 1.4) and chromium ($\sim$ 1.3).

The mor samples were also analysed for determining their dehydrogenase activity and respiration (CO_2 evolution) rate. Highly significant ($p < 0.001$) negative correlations were found between dehydrogenase activity and concentration of Pb, Ni, Cd and V, respectively or the sum of Pb+Ni+Cd concentrations (Fig. 1).

Tyler [109—117] has performed detailed chemical, microbiological and biochemical (including enzymological) studies in the Gusum area, too. Ebregt and Boldewijn [19] and Nordgren *et al.* [75] also carried out studies in this area. In Gusum, a small town in south-eastern Östergötland, a brass foundry has been operating since 1661. In 1966, the old foundry in central Gusum was replaced by a modern one outside the town, at 1.5 km from the

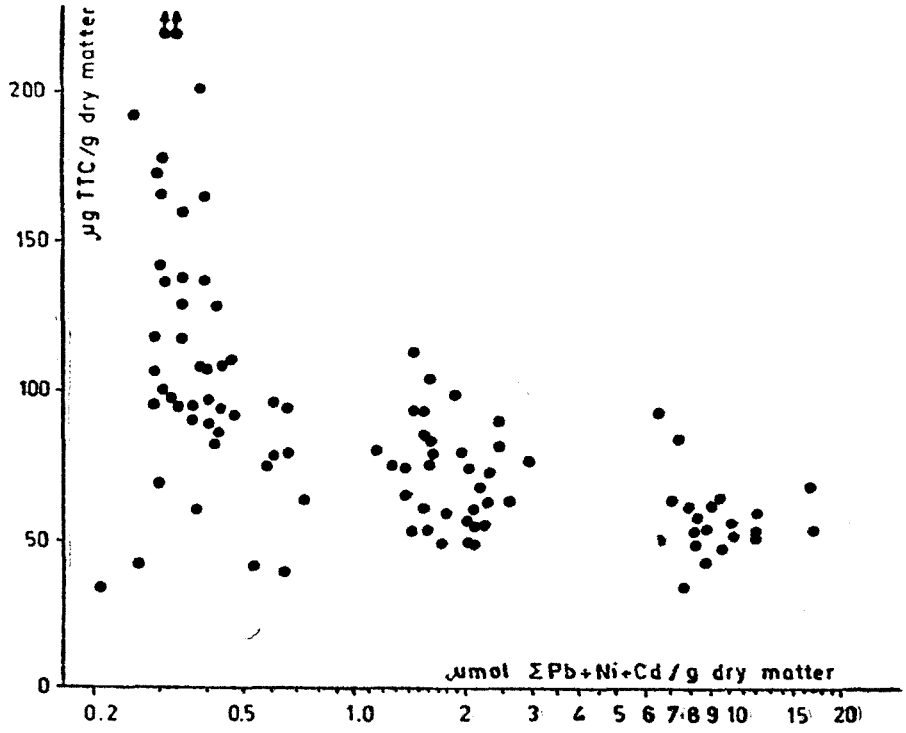


Fig. 1. *Dehydrogenase activity in the Fliseryd needle mor, related to heavy metal concentration* [96]

TTC — 2,3,5-Triphenyltetrazolium chloride reduced at 18°C in 48 hrs.

old foundry. The new foundry, having no smoke-cleaning equipment, remained the unique, point source of heavy metal pollution. About 98% of total emissions of heavy metals as alkaline oxides are copper and zinc, and there are only small emissions of lead and cadmium. The vegetation in the Gusum area, mainly coniferous woodland dominated by spruce *(Picea abies)*, developed on podzolic soils. Close to the foundry the vegetation was severely injured, most spruces being dead or dying.

The organic topsoil (the F_1 layer of spruce needle mor) was sampled in many sites.

In July 1973, Tyler [110] collected mor samples from 40 sites, distributed in all directions within a radius of 3 km from the foundry. Distinct metal concentration gradients were recorded in the samples. Thus, the samples taken close to the foundry (at 0.2—0.3 km from the main outlet) were exceedingly high in Cu and Zn (11,000—17,000 ppm and 16,000—22,000 ppm dry weight, respectively) compared with those taken about 3 km away (40—140 ppm and 200—600 ppm, respectively).

Urease and acid phosphatase activities, like respiration (CO_2 evolution) rate, correlated negatively and highly significantly with the Cu+Zn concentration of the mor samples. Within the recorded concentration range of Cu+Zn (240—39,000 ppm), with increasing Cu+Zn concentration the urease activity was reduced by a factor of 10*, acid phosphatase activity and respiration rate by a factor of 5. At the same time, no negative effect of high concentrations of Cu and Zn on the β-glucosidase activity has been established.

The spruce mor samples taken in March-April 1974 from 80 sites, distributed in a stratified random way in all directions within 7 km of the foundry, were used for determination of nitrogen mineralisation rate (increase of $NH_4^+ + NO_3^- + NO_2^-$ content) during aerobic storage of mor samples in undisturbed stratification in a humid chamber at 22°C and constant water content corresponding to 47—50% of the water-holding capacity, for 10 weeks. It was found (Tyler [111]) that heavy metal pollution, especially Cu is a limiting factor for N mineralisation rate, and even moderate amounts of Cu and Zn, exceeding only three times the background levels registered at 7—9 km from Gusum (Cu: 12—20 ppm; Zn: 100—200 ppm), are sufficient to reduce the available N to plants.

Fourty spruce mor samples collected in July 1973 and 150 samples taken in March—April 1974 were used by Tyler [114] for determination of the rates of decomposition (dry weight loss) and phosphorus mineralisation (increase in acetic acid-soluble *o*-phosphate content) during 5-month aerobic storage of the samples in a humid chamber at 22°C, keeping their water content at 72—75% of the water-holding capacity. There were highly significant negative correlations between the Cu or Zn or Cu+Zn concentration of samples and the decomposition rate, as well as between the Cu+Zn concentration and the P mineralisation rate. In 5 samples containing > 20,000 ppm Cu+Zn, no organic carbon decomposition could be recorded. In stored samples, very close inverse relationships were observed between heavy metal concentration and acid phosphatase activity and high positive correlation coefficients were obtained between phosphatase activity, decomposition and P mineralisation rates.

It was evidenced by statistical analyses that Cu is more responsible for reducing phosphatase activity than Zn at about equal concentrations. Thus, even in the range of 30—200 ppm Cu there is an inverse relationship ($p < 0.05$) between Cu concentration and phosphatase activity of samples. The relationship is indicated even in the range of 15—80 ppm Cu. The Cu concentration in sites beyond the influence of pollution is 12—20 ppm. In other words, the negative effect of Cu on phosphatase

* Urease activity was also determined in 150 mor samples collected in March-April 1974 (Tyler [112]). In these samples, the concentration range comprised only the low and middle parts of the metal gradient (corresponding only to 15—2,000 ppm Cu). Within this concentration range, the highly significant correlation between urease activity and heavy metals decreased in the order: Cd (−0.633) > Zn (−0.571) > Cu (−0.556). In other words, when the most metal-polluted mor samples are excluded, urease activity is reduced to the largest extent not by the most abundant Cu and Zn, but by the less abundant Cd.

activity is measurable already at a low degree of pollution. Other important findings: a higher pH of the mor sample may, to some extent, counteract the activity decrease; edaphically poor sites are more sensitive to heavy metal pollution as indicated by stronger decrease in phosphatase activity.

Tyler's report to the National Swedish Environment Protection Board published in Swedish in December 1974 and in English in February 1975 [112] is a synthesis of the studies we have referred to above. "The phosphatase map" presented by Tyler in this report (see Fig. 2) shows that a certain reduction of acid phosphatase activity is noticeable as far as 3—5 km from the old foundry, that is within an area of about 5,000 ha. In the vicinity of foundry, as in the residential area, phosphatase activity is very much reduced.

Tyler's communication at the International Conference on Heavy Metals in the Environment, held in Toronto in 1975, is also a review of the investigations performed mainly in the Gusum area [113]. In the communication, mention was also made of a reactivation experiment described in detail in a 1977 report (Tyler [115]).

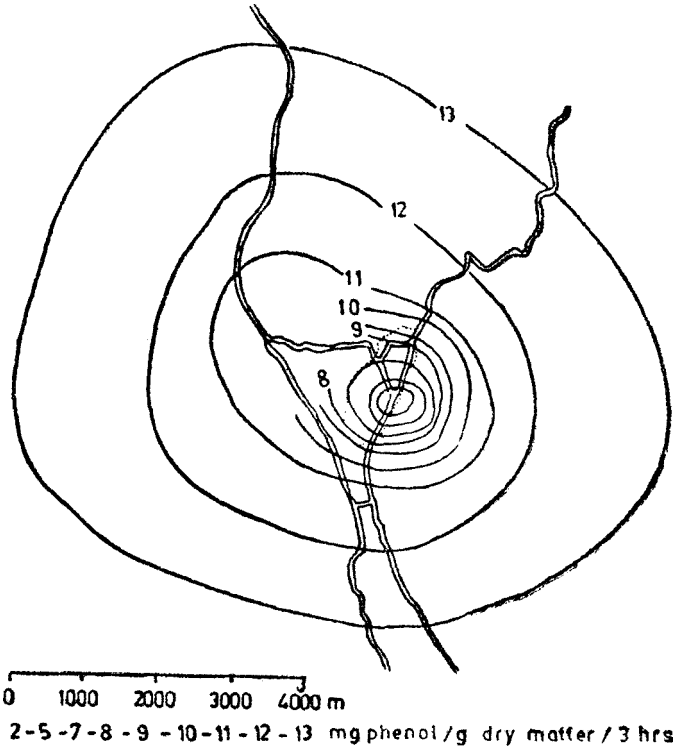


Fig. 2. *"The phosphatase map" of the spruce needle mor samples in the Gusum area* [112]

A metal-chelating agent, namely Na-EDTA (0.05 M) was used to reactivate acid phosphatase and urease in 10 + 10 spruce needle mor samples collected in the surroundings of the old and new brass foundries, respectively, at Gusum in the autumn of 1975. Ten needle mor samples from locally unpolluted areas in south-eastern Östergötland and, thus, containing only background levels of Cu and Zn served as controls.

Reaction mixtures were prepared, with and without Na-EDTA addition, for determination of acid phosphatase activity (at pH 5.0) and urease activity (at pH 6.7), and incubated at 22°C for 3 hrs. The difference between activity values measured in the precence and absence of Na-EDTA was calculated and expressed in percentage, too, for each mor sample.

The 30 mor samples were divided into 4 groups according to their Cu+Zn concentration: < 0.3, 3.4—11.9, 16.2—22.3 and 28.5—45.8‰ of the mor dry weight. The mean percent differences in enzyme activities measured in reaction mixtures with and without Na-EDTA addition were the following in the 4 mor groups: + <1, +17, +55 and + 36% (acid phosphatase activity), and —8, +26, +37 and +5% (urease activity). In other words, Na-EDTA had no effect on phosphatase activity and slightly inhibited urease activity in the unpolluted mor samples, but brought about a partial reactivation of both enzymes in the polluted mor samples. The reactivating effect of Na-EDTA was most pronounced in the mor samples containing 16.2—23.3‰ Cu+Zn.

Ebregt and Boldewijn [19] have determined amylase activity and rates of basal respiration (no organic substance added) and starch-induced respiration in spruce needle mor samples from 50 sites selected in a stratified random way to secure an approximately logarithmic distribution with regard to distance from the foundry. Amylase activity significantly decreased with the sum of Cu+Zn+Pb+Cd concentration increasing from 200 to 50,000 ppm. Both basal and starch-induced respirations behaved like amylase activity.

In a 1983 paper, Nordgren *et al.* [75] emphasise that the organic topsoil (spruce needle mor) close to the new foundry replacing — as already mentioned — the old one in 1966, reached in 15 years the same levels of heavy metals, about 20,000 µg of Cu and 20,000 µg of Zn/g oven-dry soil as those around the old foundry. By studying the microfungi in topsoil samples collected in October 1979 and May 1980 these authors have established that soil moisture and organic matter contents had little influence on the microfungal community compared with the heavy metal pollution, along both the whole gradient and its most polluted part, whereas at the less polluted places soil moisture and organic matter accounted for more of the observed changes than did the Cu and Zn concentrations.

Tyler and Westman [118] and Nordgren *et al.* [76] performed soil studies in the area of the primary smelter in Rönnskär (Skellefteå region, northern Sweden), the strongest single point source of heavy metals in this country. The smelter has been emitting a series of heavy metals and other pollutants for 50 years, the dominant metals

being Zn, Pb, Cu and As, whereas Cd, Hg, V, Ni, Cr, Se are present in smaller amounts. The emissions also contain SO_2 in large amounts.

The vegetation of the area is dominated by spruce forests, frequently mixed with pine and deciduous trees.

In October 1978, Tyler and Westman [118] have collected spruce needle mor samples from 68 sites located at 10-km distances from each other along 11 transects long of up to 80 km from the smelter. In such a way, the sites along the transects are situated circularly around the smelter.

Concentrations of 5 metals (Zn, Pb, Cu, Cd and As), pH_{KC}, phosphatase and urease activities, and respiration rate were determined in each sample. Then, the mean values of these variables in samples taken at sites located in 10-km intervals (0—10, 10—20 ..... 60—70 and 70—80 km) from the smelter were calculated. We quote only the results obtained in samples from the distances of 0—10, 20—30 and 70—80 km: metals (μg/g dry mor), *i.e.* Zn 352, 71 and 51; Pb 290, 78 and 39; Cu 115, 35 and 22*, Cd 9.60, 1.56 and 0.84; As 107, 53 and 32*; pH_{KCl} 3.30, 3.30 and 3.49; phosphatase activity (mg phenol/g organic dry matter of mor/2 hrs) 4.69, 6.68 and 9.32; urease activity (μg NH_4—N/g organic dry matter/2 hrs) 113, 146 and 206; respiration rate (μg CO_2—C/g organic dry matter/24 hrs) 65, 94 and 138.

It is evident from these data that the metal concentrations decreased, pH increased with 0.2 units, enzyme activities and respiration rate considerably increased with increasing distance from the smelter.

The metal concentrations positively correlated with each other and negatively with phosphatase and urease activities and respiration rate. The correlation between these three indices and metal concentration was most significant in the case of Cd and Cu. The correlation of pH_{KCl} was positive and significant with phosphatase activity and respiration rate and positive but insignificant with urease activity.

As Tyler and Westman [118] point out, elevated levels of several heavy metals in the topsoil of forested areas are measurable at great distances (at least 70—80 km) from the smelter, and the pollution has a measurable effect on enzyme activities and respiration rate in coniferous forest soils over a territory of at least 2,500—4,000 km^2.

These investigations were continued by Nordgren *et al.* [76]. In September 1981, they collected mor samples at 12 sites from a northern transect (2.4—40 km north of the smelter) and at 9 sites from a southern transect (3—55 km south of the smelter). Several chemical, microbiological and biochemical indices were determined in the samples. The different heavy metals were 5 to 75 times higher at the site closest to the smelter compared with background levels. Despite emission of sulphur, no decrease in pH was recorded. Due to the pollutants, of which arsenic was found to be the representative one, urease activity

* The Cu and As concentrations in mors even at 70—80 km from the Rönnskär smelter are higher than the normal values (Cu 8—12 ppm; As < 10 ppm) registered in mors in the middle part of Norrland (northern Sweden) [118].

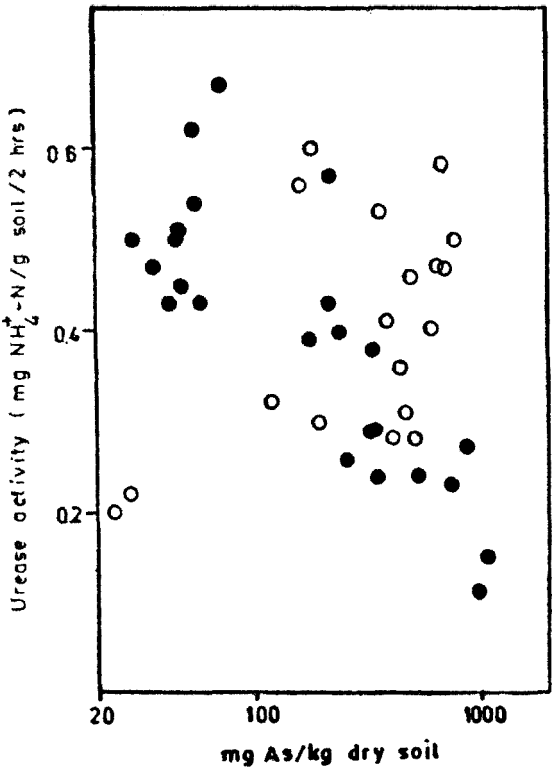


Fig. 3. *The effect of metal pollutants, represented by arsenic, on soil urease activity* [76].
The full and empty circles show data from the northern and southern transect, respectively.

decreased by about a factor of 4. Fig. 3 shows that the lowest urease activity values were registered at the highest As concentrations on the northern transect and at the lowest As concentrations on the southern transect. At the same time, phosphatase activity did not decrease significantly with pollution level. Respiration rate, like urease activity, decreased by about a factor of 4, whereas the pollution-induced decrease in numbers of bacteria producing acid from or hydrolysing various substrates was usually 8—11-fold. Mycelial lengths, like phosphatase activity, were unaffected by the pollutants.

One can state that some results obtained by Nordgren *et al.* [76] are in good agreement with and some other results differ from the results registered by Tyler and Westman [118].

USA. Pancholy *et al.* [86] have studied the soil factors preventing revegetation of a denuded area near an abandoned zinc smelter. First, they present a brief history of the studied area. A zinc roaster

and smelter were operated at Henryetta in Okmulgee county, Oklahoma from 1916 to 1953, and the smelter was operated alone from 1953 to 1968. The area adjacent to the roaster and smelter was originally covered with post oak-blackjack oak (*Quercus stellata—Q. marilandica*) forest, but an area approximately 400 ha in extent was completely denuded downwind from the plant. P a n c h o l y *et al.* selected a test plot of several hectares in the bare area and a control plot in a post oak-blackjack oak forest at about one mile from the test plot. Eight soil samples were taken in the test plot and 8 in the control plot from the 0—15—cm depth in December 1972 and again in March 1973.

Soil dehydrogenase and urease activities were significantly lower ($p < 0.01$) in the bare test plot than in the control plot, the mean values being 0.51 and 7.37 μl H/10 g soil/24 hrs, and 0.57 and 4.69 mg NH_3/g soil/24 hrs, respectively. Mean numbers of bacteria, actinomycetes and microfungi and those of *Nitrosomonas* and *Nitrobacter* were also considerably lower in the bare than in the control soil. *Azotobacter* was lacking in both soils at both sampling periods. The weak biological potential of the bare soil is related to the high concentrations of Cd and Zn combined with the secondary effects of low pH.

Poland. A seminary paper, presented by K o b u s [55] in 1975, contains some data on soil investigations conducted in the area of the zinc smelter in Miasteczko Slaskie (Katowice voivodship). For analyses soil samples were taken along a transect, at 0, 0.5, 1.3 and 2.1 km from the smelter during 1972—1975. In this period, Zn concentration in soil increased from 80 to 1250 ppm at 0 km and to a lesser extent around the smelter, thus to 1/6 of these values at 0.5 km. Soil dehydrogenase and amylase activities did not exhibit considerable changes along the transect, but invertase activity increased with increasing distance from the smelter. In total numbers of microorganisms, the annual variations were broader than the changes along the transect. Exceptionally, the number of *Azotobacter* cells was highest at 0 km.

In the area of this smelter, B a d u r a *et al.* [4] also performed soil chemical, microbiological and enzymological investigations. Soil samples were collected from three pine *(Pinus sylvestris)*—dominated forest stands located at 1.4, 2.6 and 5.5—8 km from the smelter. For determination of Zn content the soils were analysed seasonally in 1979; total numbers of microorganisms were determined monthly in the period of January 1979-September 1980; the ammonium-oxidising capacity and cellulase activity were determined monthly in the November 1979-May 1980 period.

The total Zn content (extractable with 8N HNO_3) showed considerable differences in the soils of the three forest stands, the highest content being always found in the soil of the stand closest to the smelter. In change, the water-extractable Zn content was of the same level in the three soils.

Total numbers of bacteria, actinomycetes and microfungi varied to a larger extent depending on season than on the distance from the smelter. Similar results were obtained in the estimation of ammonium-oxi-

dising capacity and cellulase activity. All these findings were explained by uniformity of the mobile, biologically available Zn content in soils situated along a pollution gradient determined mainly by the total Zn content emitted from the smelter, which means that in these soils a large part of pollutants were immobilised and, thus, became biologically unavailable.

Greszta *et al.* [40] performed field experiments by using dusts, collected from electrofilters operating in zinc and copper smelting works. The dusts, in which the metals are present as oxides, were several times mixed with the 0—30—cm (mellow sandy or slightly clayey sandy) soil layer of experimental plots installed in an only slightly polluted forest nursery at Klaj, in the south-eastern part of the Niepolomice Forest. Three types of dusts, namely a Pb-Zn dust (containing 6.30% Pb, 39.42% Zn), a Pb-Cu dust (7.41% Pb, 16.50% Cu) and a Cd-Pb-Zn dust (2.44% Cd, 15.29% Pb, 55.70% Zn), and a Pb-Cu sludge — wet dust extract from a copper smelter (8.68% Pb, 0.57% Cu, 3.75% Zn) were used in the following percent proportions to the soil weight: 50, 25, 10 and 1%. Plots to which no dust or sludge was added served as controls. Onto the plots, 2-year-old forest tree seedlings were planted. The plots were set up in the autumn of 1974. For enzymological and microbiological analyses soil samples were taken from the 3—5—cm depth, in June, August and October 1975.

As expected, the soil enzyme activities studied (urease, invertase and catalase) decreased with increasing amounts of dust or sludge additions. The only exception was urease activity in the Cd-Pb-Zn dust-treated soil: urease activity decreased in the soil treated with this dust in 1 and 10% proportions, but, when the soil contained 25 and 50% dust, this activity increased and even exceeded the activity value registered in the control soil. The urease activity-decreasing effect of the other heavy metal materials showed the order: Pb-Cu sludge $\approx$ Pb-Cu dust $<$ Pb-Zn dust.

The reducing effect of the Cd-Pb-Zn dust on invertase activity was a little weaker than that of the other heavy metal materials.

The order in which these materials reduced catalase activity was: Cd-Pb-Zn dust $<$ Pb-Zn dust $\approx$ Pb-Cu sludge $<$ Pb-Cu dust.

The finding that the Cd-Pb-Zn dust acted less inhibitorily on enzyme activities contrasts other findings: this dust had the most detrimental effect on soil microorganisms (excepting the ammonifying bacteria, whose number, like urease activity, increased at the highest Cd-Pb-Zn dust addition) and on the growth of tree seedlings.

In August 1980, a new experiment was initiated in the Niepolomice Forest, at 25 km north-east of Cracow [38, 39]. The plots of 240 m^2 each were installed in a mixed pine-oak forest, dominated by about 40-year-old pines *(Pinus sylvestris)* and were treated with dusts from electrofilters of 6 industrial plants: zinc, cadmium and aluminium smelters, ironworks, electric power plant and cement plant. The main components and their amounts in dusts were the following: Zn dust 22.06% ZnO, 43.74% SiO_2; cadmium dust 3.02% CdO, 4.07% PbO, 21.83%

Al_2O_3, 45.34% SiO_2; aluminium dust 21.42% Al_2O_3, 43.18% SiO_2; ironworks dust 40.94% Fe_2O_3, 12.44% CaO, 20.92% SiO_2; power plant dust 10.75% CaO, 68.21% SiO_2; cement plant dust 47.04% CaO, 28.42% Al_2O_3, 16.26% SiO_2. Rate of dust application was: 0, 100, 500, 1000, 2000 and 5000 t/km^2/year during 1$^1/_2$ years. The first quarter of the annual dose was applied on 20—25 August 1980 and the other quarters were administered at 3-month intervals.

For enzymological and microbiological analyses Z w o l i ń s k i *et al.* [136] took soil samples from the 0-5-cm layer (A_1 horizon) of the control (untreated) plot and of those treated with dusts from the cadmium and aluminium smelters, ironworks and power plant. The samplings were carried out twice prior to the dust treatment (in May and June 1980) and 4—5 times annually in the 1980—1983 period.

The enzyme activities measured are specified in Table 1 together with the mean of the activity values recorded in the 1980—1983 period. Besides the mean values (x), the standard deviations (s), correlation coefficients (r) between the dust rate and enzyme activity, significance of correlations and number of analyses (n) are also given in Table 1.

One can see from this table that the effects of the 4 dusts on the 6 enzyme activities studied varied with the rate and source of dusts and with kind of enzymes.

At the largest rate applied, all dusts reduced activity of each enzyme. The correlation coefficients between the rates of Cd and Al dusts and the enzyme activities were always negative and significant. Asparaginase, phosphatase and dehydrogenase activities were the most sensitive to the Cd dust, and again phosphatase and dehydrogenase activities manifested the highest sensitivity to the Al dust. The correlations of ironworks dust rates were insignificant with all enzyme activities, whereas the correlations of power plant dust rates were significantly negative with phosphatase activity and significantly positive with dehydrogenase activity.

The mean values for the 1980—1983 period also showed that the Cd dust reduced significantly the total numbers of soil bacteria and actinomycetes and insignificantly the number of microfungi. The Al dust exerted significant effect only on the number of actinomycetes, the effect being positive. The dust from the ironworks influenced negatively and significantly only the number of microfungi. None of the microbial groups was significantly affected by the power plant dust.

Intensity of ammonification was not significantly influenced by any of the 4 dusts, while nitrification intensity significantly decreased under the influence of Cd dust. Intensity of cellulose decomposition was significantly reduced by the Cd, Al and ironworks dusts, and respiration (CO_2 evolution) was significantly decreased by the Cd and Al dusts.

It is evident from these results that the effects of Cd dust were very detrimental to the soil life.

In continuation of the studies reviewed above, S t a r z e c k a [104] has determined protease, C_x cellulase, acid and alkaline phosphatase activities in the surface soil layer (more precisely, in the 5-6-cm thick

Soil enzyme activities in plots not treated and treated with industrial dusts [136]

Source of dusts	Rate of dusts (t/km²/year)	Asparaginase[1] x+s	r	Urease[1] x+s	r	Phosphatase[1] x+s	r	β-Glucosidase[1] x+s	r	Invertase[1] x+s	r	Dehydrogenase[1] x+s	r
Control	0	2.37±0.20		6.18±0.75		5.64±0.11		3.1±0.3		2.3±0.4		1.37±0.33	
Cadmium smelter	500	2.46±0.16		5.71±0.84		5.73±0.24		3.0±0.5		2.2±0.4		1.14±0.19	
	1000	2.26±0.31	−0.699	4.31±0.96	−0.537	4.97±0.36	−0.868	2.7±0.6	−0.569	1.8±0.4	−0.540	0.76±0.25	−0.723
	2000	2.01±0.12	p<0.001	3.76±0.42	p=0.02	4.63±0.11	p<0.001	2.4±0.4	p=0.01	1.5±0.3	p=0.02	0.59±0.16	p<0.001
	5000	1.75±0.17	n=20	2.88±0.71	n=20	4.18±0.20	n=20	1.9±0.4	n=20	1.2±0.1	n=20	0.46±0.21	n=20
Aluminium smelter	500	2.41±0.10		4.31±0.43		5.13±0.30		2.7±0.1		2.2±0.3		1.34±0.33	
	1000	2.27±0.21	−0.495	3.55±0.38	−0.472	4.66±0.28	−0.902	2.2±0.1	−0.405	1.9±0.2	−0.558	0.95±0.18	−0.675
	2000	2.03±0.11	p=0.02	2.77±0.49	p=0.05	4.13±0.17	p<0.001	1.9±0.2	p=0.05	1.7±0.1	p=0.01	0.96±0.39	p=0.001
	5000	1.69±0.31	n=20	2.51±0.31	n=20	3.54±0.10	n=20	1.8±0.3	n=20	1.4±0.2	n=20	0.43±0.22	n=20
Ironworks	500	2.54±0.17		5.71±0.25		5.77±0.25		3.2±0.5		2.4±0.6		1.53±0.37	
	1000	2.58±0.29	−0.352	6.28±0.29	−0.358	6.06±0.85	−0.327	3.2±0.6	−0.295	2.5±0.5	−0.380	1.85±0.64	0.336
	2000	2.41±0.23	p>0.05	6.65±0.76	p>0.05	5.72±0.38	p>0.05	3.4±0.6	p>0.05	2.3±0.4	p>0.05	1.71±0.35	p>0.05
	5000	2.16±0.25	n=20	4.08±0.92	n=20	5.10±0.61	n=20	2.8±0.6	n=20	2.0±0.4	n=20	1.30±0.41	n=20
Power plant	500	2.18±0.07		5.15±0.43		5.66±0.49		2.8±0.3		2.1±0.4		1.40±0.17	
	1000	2.45±0.16	0.209	6.08±0.34	−0.361	5.88±0.34	−0.460	3.3±0.7	−0.272	2.6±0.4	0.465	1.54±0.31	0.562
	2000	2.57±0.17	p>0.05	6.12±0.89	p>0.05	5.63±0.58	p=0.05	3.7±0.7	p>0.05	2.8±0.6	p>0.05	1.74±0.17	p=0.02
	5000	1.80±0.15	n=16	3.80±0.57	n=20	4.39±0.24	n=20	2.1±0.3	n=16	1.8±0.3	n=16	1.17±0.10	n=16

[1] Expression of activities: asparaginase and urease in mg NH_3/10 g soil: phosphatase in mg P_2O_4/100 g soil; β-glucosidase and invertase in ml 0.1 N $Na_2S_2O_3$/10 g soil; dehydrogenase in mg triphenylformazan/10 g soil.

fermentation-humus layer — AoFH) of three types of experimental plots (A, B and C) installed in the southern part of the Niepolomice Forest. Plots A (of 240-m^2 each) were installed in 1981 when they were treated with Cd dust at a rate of 0, 100, 500, 1000 and 2000 t/km^2/year. In 1986—1987, half of each plot A was fertilised (720 kg NPK/ha) and limed (3000 kg magnesium-dolomite lime/ha); the fertilised and limed halves of plots A were designated plots B. In the spring of 1987, one of the 240-m^2 control plots (not treated with Cd dust in 1981) was divided into 1-m^2 "mini" plots (plots C), to which then Cd dust was added at rates proportional to those used in 1981 for plots A. All plots were sampled for soil enzymological and microbiological analyses in the period of June-October 1987. Thus, plots A and B served for assessing the long-term effects and plots C for estimating the short-term effects of Cd dust.

The analytical data have indicated that protease activity was nearly the same in all plots. This means that the Cd dust exhibited neither long- nor short-term effects on this activity which remained at an approximately constant level also in the fertilised and limed plots.

Contrarily, C_X cellulase activity sharply decreased with increasing Cd dust rate in plots A. The decrease was not so sharp in plots B treated with the two highest rates of Cd dust, which may be attributed to the fertilisers and lime. In plots C, the activity showed an increasing trend with the amount of Cd dust.

Activity of acid phosphatase was much higher than that of the alkaline phosphatase. In plots A, both phosphatase activities tended to decrease with the rate of Cd dust addition. In plots B, fertilisation and liming weakened the decreasing effect of the largest amount of Cd dust on both phosphatase activities. In plots C, the Cd dust-induced decrease of acid phosphatase activity was sharper than that of the alkaline phosphatase activity.

Due to the long-term effects of Cd dust in plots A, a considerable reduction also occurred in the total numbers of soil microorganisms (heterotrophic bacteria, actinomycetes and microfungi) and in the intensity of soil respiration (CO_2 evolution), but the short-term effects of Cd dust in plots C were partly different: the total number of actinomycetes slightly decreased, whereas the total numbers of heterotrophic bacteria and microfungi and intensity of respiration increased.

Fertilisation and liming of plots B, as compared with plots A, resulted in increases in both microbial numbers and respiration intensity.

In conclusion one can state that the soil enzymological and microbiological effects of Cd dust are persistent as the enzymatic and microbial indices measured in 1987 showed more negative effects of Cd dust in plots A treated with Cd dust in 1981 than in plots C to which the same amounts of Cd dust were added in 1987. Some indices were even higher in the Cd dust-treated plots C than in the untreated, control plot C.

Romania. Effects of the emissions from the aluminium works in Slatina (Olt county) on soil dehydrogenase activity and some microbiological indices were dealt with by E l i a d e *et al.* [22] and I o n e s c u

et al. [46]. The main pollutants in these emissions are fluoride, SO_2 and dust. Soil was sampled at 50, 500 and 700—1200 m from the works. Soil dehydrogenase activity, total numbers of bacteria, actinomycetes, microfungi, endosporogenic bacteria, N mineralisation and nitrification capacities were lowest in the vicinity of the pollution source.

S o r e a n u [103] carried out soil chemical and enzymological studies in mixed, deciduous-coniferous forest stands heavily polluted by emissions from the lead smelter in Baia Mare (Maramureş county). Four places were selected for soil sampling; they are located at 0.1, 1, 2 and 10 km from the source of pollution. The place at 10 km is the unpolluted control.

On the polluted area, there were experimental plots installed. They were limed (5 t/ha) and fertilised with N (50 kg ha) or P (70 kg ha) or with both (50 kg N and 70 kg P ha) by using ammonium nitrate or/and superphosphate. Unlimed and nonfertilised plots served for comparison.

The soils sampled in June and September 1976 and 1977 were analysed to determine their Pb content and dehydrogenase, invertase, urease and phosphatase activities. As expected, the Pb content was much higher in the polluted than in the unpolluted area. Thus, the 0-4-cm soil layer at 0.1 km from the smelter contained 8,000 ppm Pb which was about 30 times higher than the Pb content in the 0-4-cm layer at 10 km. Of the 4 enzyme activities determined, dehydrogenase and urease activities were found to clearly differentiate the unpolluted soils from the polluted ones, and urease activity was also found to be a good indicator of the beneficial effects of liming and fertilisation on Pb-polluted soils in forest stands.

During 1985, R ă u ţ ă *et al.* [93] conducted complex pedological investigations on an area of about 36 km^2 around the Zn-Pb smelter in Copşa Mică (Sibiu county). Nine profiles were described and 42 litter and soil samples were taken for analyses. In the most polluted zone, concentrations of heavy metals in samples of the litter and the 0-3-cm soil layer were the following: Zn > 1800 and 370 ppm; Pb > 1300 and 200 ppm; Cu > 100 and 25 ppm; Cd > 50 and 10 ppm, respectively. No dehydrogenase activity could be detected in these heavily polluted samples. In both litter and soil, numbers of bacteria decreased, whereas those of cellulolytic microfungi increased with increasing concentrations of heavy metals.

Canada. F r e e d m a n and H u t c h i n s o n [33, 34] have reported on extensive studies of the pollutants and their effects on soils and vegetation in the area of a large Ni-Cu smelter at Sudbury, Ontario. In this area mining and smelting activity began about 1885. Initially, ore was roasted using open roastbeds which, by 1928, was forbidden, and all further roasting was carried out at three smelter facilities located near Sudbury (at Copper Cliff, Coniston and Falconbridge). Since 1972, all smelting activity is centred either at Copper Cliff, where pollutants are vented through a 380-m "superstack" (the world's tallest smokestack) or at Falconbridge with a 93-m stack. The Coniston smelter

was closed in 1972, with all production transferred to Copper Cliff. The stacks emit huge amounts of SO_2 and fine particulates, mostly iron oxides, although Cu and Ni emissions are also significant.

Along a 60-km transect originating at the Copper Cliff smelter, the aerial deposition of smelter-derived pollutants was estimated, foliar analyses as well as soil chemical, microbiological, enzymological and faunal studies were carried out in the 1975—1977 period.

It was found, for example, that the foliage of the 17 plant species studied had consistently higher concentrations of Cu and Ni at sites closer to the smelter, relative to sites further away. Differences of 10- to 50-fold occurred between the 60-km site and those at 3.0 or 1.6 km for both foliar Cu and Ni. Although the within-site variation was high, Cu and Ni and (to a lesser extent) sulphate were also markedly elevated in surface soil horizons close to the smelter. Differences of up to 60-, 30- and 11-fold for Cu, Ni and sulphate, respectively, occurred between sites at 76.5 km from the smelter and those at < 5 km.

Table 2 comprises data on total Cu and Ni concentrations, respiration rate and acid phosphatase activity in duff-humus soil samples collected from sites at various distances from the Copper Cliff smelter, on 2 June 1977. Duff is defined as the material consisting of recognizable leaf fragments, and humus as the amorphous organic matter. Mean of the values recorded for 10 replicate collections per site and standard deviation are presented.

It is evident from this table that both respiration rate and acid phosphatase activity were lowest in the mineral soil at the denuded site and lower in the duff-humus soils at the contaminated than at the control forest sites. Correlation coefficients calculated using the data of the table showed a stronger inverse relationship of both respiration rate and acid phosphatase activity with total copper than with total nickel concentration. Respiration rate and phosphatase activity also significantly correlated.

Chemical, microbiological and enzymological studies in the area of another Canadian smelter were performed by Dumontet *et al.* [18]. This, Cu-Zn smelter is located on the north-east side of the city of Rouyn-Noranda (south-western Québec). Soil sampling was carried out in May 1986 along two transects oriented in the direction of prevailing winds; one transect is in north-north-east (NNE) and the other in east-south-east (ESE) direction, from 1 to 27.5 and 2 to 42.5 km away from the smelter. Nine sites (4 on the NNE and 5 on the ESE transect) were selected. The samples were taken from the surface litter and the 0-15- and 15-30-cm mineral soil layers. Concentrations of Cu, Zn, Ni, Cd, Pb and total heavy metals (TM) were determined in the litter and both soil layers, whereas respiration (CO_2 evolution) rate and acid phosphatase activity were measured only in the 0-15-cm layer. and expressed in mg CO_2—C/kg dry soil/day and μmol *p*-nitrophenol/g dry soil/hr, respectively.

At each site, concentration of TM decreased in the order: litter $>$ 0-15-cm layer $>$ 15-30-cm layer. The maximum amount of TM (29.5

Table 2

Total copper and nickel concentrations, respiration rate and acid phosphatase activity in duff-humus soil samples collected in sites at different distances from the Copper Cliff smelter [34]

Nature of site	Distance of site from the smelter (km)	Total ppm dry weight		Respiration rate[1]		Acid phosphatase activity[2]	
		Copper	Nickel	per fresh weight	per dry weight	per fresh weight	per dry weight
Denuded (devegetated) hilltop	3.0	210±110	220±200	0.06±0.02	0.07±0.02	8.5±10.0	9.2±10.8
Contaminated forest site	3.0	2400±700	1400±400	0.18±0.09	0.26±0.14	41.6±16.2	63.9±19.3
Contaminated forest site	3.7	2600±800	1900±1400	0.31±0.16	0.58±0.38	46.2±19.3	78.5±44.7
Contaminated forest site	5.2	1400±400	1200±400	0.38±0.17	0.72±0.40	73.9±58.5	144.8±114.0
Control forest site	34.3	470±170	590±190	0.59±0.19	0.98±0.38	191.7±47.7	316.5±82.4
Control forest site	47.8	450±100	460±130	0.51±0.15	0.94±0.32	105.5±49.3	205.6±114.7
Control forest site	50.2	230±70	270±100	0.39±0.15	0.62±0.28	180.2±75.5	248.7±122.4

[1] Expressed in μl CO_2/min/g fresh or dry weight.
[2] Expressed in μg *p*-nitrophenol/20 min/g fresh or dry weight.

meq 100 g dry matter or 13,000 ppm) was recorded in the litter of the site at 1 km away from the smelter on the NNE transect, whereas the less polluted litter (0.38 meq TM/100 g dry matter) was collected at 27.5 km from the smelter on the ESE transect. However, little correlation was found between heavy metal concentrations in the litter and the distance from the smelter. At the same time, in the 0-15-cm soil layer the log-concentration of individual heavy metals and TM decreased significantly ($p < 0.05$ or 0.01) with the log-distance from the smelter; the only exception was Ni as its decrease was not significant.

Table 3

Correlations between heavy metal concentrations, respiration rate and acid phosphatase activity in the 0—15—cm soil layer [18]

	Zn	Cd	Pb	Total metals	Respiration rate	Acid phosphatase activity
Cu	0.449	0.856**	0.929**	0.938**	−0.790*	−0.223
Zn		0.519	0.726*	0.725*	0.076	−0.524
Cd			0.895*	0.874**	−0.856**	−0.114
Pb				0.993**	−0.690	0.041
Total metals					−0.647	0.041
Respiration rate						0.388

Significance: * $p<0.05$; ** $p<0.01$.

Table 3 shows that the correlations among heavy metal concentrations were always positive and, usually, significant. Respiration rate was negatively affected by the heavy metals with the exception of Zn, but only the effects of Cu and Cd were significant. Acid phosphatase activity was negatively, but insignificantly influenced by Cu, Zn and Cd concentrations and was not affected at all by Pb and total metals. Neither was significant the positive correlation between respiration rate and acid phosphatase activity. This activity correlated significantly ($p < 0.05$) only with the content of both soil C and N. One can deduce from these findings that in the area of the Rouyn-Noranda smelter soil respiration rate indicated more sensitively the heavy metal pollution than did acid phosphatase activity.

Russia. Skvortsova *et al.* [102] have determined asparaginase activity in brown forest soil samples collected at 0.6, 2.6 and 3.4 km from a lead smelter located in the Primorye area. The samples at the three distances contained 300, 295 and 100 mg Pb, 215, 175 and 95 mg Zn, 17, 0.9 and 0.5 mg Cd/kg soil. Asparaginase activity was 5—6 times

lower at 0.6 km than at 2.6 or 3.4 km from the smelter. Microbial numbers showed a trend similar to that of asparaginase activity.

In the 1976—1993 period, Evdokimova and her associates [23—27] have performed extensive investigations in the area of the "Severonikel" Ni-Cu smelter complex, located in the northern taiga, near the town of Monchegorsk (Kola Peninsula). The area is dominated by adverse climatic conditions. The emissions contain mainly Cu, Ni and Co (in form of sulphates, chlorides, sulphides, oxides) and SO_2. Due to their huge amounts, these pollutants affect large territories, within which four zones were selected for the investigations: the epicentre, the impact zone ("technogenic desert"), the buffer zone and the background zone.

In each zone, not only the virgin podzolic forest soils (already affected by the smelter emissions for many decades) were investigated, but experimental plots were also installed.

In one of the experiments, the surface (0-30-cm) layer of the soil plots installed in the four zones mentioned above, namely at 0.2—1 km (epicentre) and at 5, 15 and 60 km from the smelter, was removed and replaced with cultivated mineral soil, at the end of May 1976. After 10 years, more precisely on 3 June 1986, 1-m^2 30-cm deep microplots taken from the plots were reintroduced to a "pure", unpolluted area located at 60 km from the smelter. The 0-10-cm layer of the microplots was sampled on the day of reintroduction and, thereafter, periodically, for determination of the Cu, Ni and Co contents, invertase and urease activities, nitrification capacity and other chemical and microbiological parameters. Table 4 comprises some of the results obtained during the first 15 months following reintroduction of microplots to the unpolluted area.

It is evident from this table that during 10 years high amounts of Cu, Ni and Co accumulated in the soil of epicentre and the accumulation decreased with increasing distance from the smelter. After reintroduction of soils to the unpolluted area, the heavy metal contents in the soil from the epicentre decreased considerably in 3.5 months, but during the next ~12 months, decrease of heavy metal contents was much smaller*. These findings are explained by the migration of mobile forms of heavy metals from the surface to the deeper soil layers.

In the soils from the 5-, 15- and 60-km distance, very little changes occurred in their heavy metal contents during the whole 15-month period.

Table 4 also shows that in the soils exposed to the effects of smelter emissions in the epicentre, invertase activity was very low and urease activity and nitrification capacity were not demonstrable. In the epicentre soil reintroduced to the unpolluted area, invertase activity increased during the 15-month period, urease activity became detectable after 3.5 months, whereas a weak nitrification capacity was de-

* The decrease remained extremely low in the 1988—1992 period, too [26]. It was deduced from other studies ([25, 26] that complete decontamination from Cu and Ni would take place in 42—700 and 21—190 years, respectively.

Table 4

Dynamics of the heavy metal contents, enzyme activities and nitrification capacity in the 0—10—cm layer of 30—cm deep microplots of initially unpolluted podzolic soils after their exposure to the effects of emissions at different distances from the smelter for 10 years, followed by their reintroduction to an unpolluted area [24]

Sampling date of the reintroduced soil	Distance at which the initially unpolluted soil was exposed to the effects of smelter emissions for 10 years			
	Epicentre	5 km	15 km	60 km
	Copper content (mg/kg soil)			
3.VI.1986	6232	86	70	49
23.IX.1986	3347	62	51	42
4.VI.1987	3603	84	57	34
3.IX.1987	3176	81	52	35
	Nickel content (mg/kg soil)			
3.VI.1986	2106	126	97	62
23.IX.1986	1004	137	83	53
4.VI.1987	1201	147	79	47
3.IX.1987	1003	139	95	43
	Cobalt content (mg/kg soil)			
3.VI.1986	84	18	18	9
23.IX.1986	50	13	13	13
4.VI.1987	62	17	18	13
3.IX.1987	46	15	16	17
	Invertase activity (mg glucose/g soil)			
3.VI.1986	0.6	12.0	9.6	10.3
23.IX.1986	0.9	11.3	10.5	14.4
4.VI.1987	0.8	17.4	13.1	12.5
3.IX.1987	1.2	10.2	9.8	8.5
	Urease activity (mg NH_4^+ /2 g soil)			
3.VI.1986	0	2.9	2.5	2.2
23.IX.1986	0.2	2.2	2.2	1.8
4.VI.1987	0.3	2.3	3.2	2.3
3.IX.1987	0.2	3.3	2.8	1.9
	Nitrification capacity (mg NO_3—N/kg soil)			
3.VI.1986	0	35	41	31
23.IX.1986	0	40	53	46
4.VI.1987	0	50	55	56
3.IX.1987	0.5	40	40	40

monstrable after 15 months. The soils from the 5-, 15- and 60-km distance manifested both enzyme activities and nitrification capacity which remained nearly at the same level after reintroduction of these soils to the unpolluted area.

In the 1988—1993 period, invertase and urease activities and nitrification capacity showed a trend to further increase in the soil reintroduced from the epicentre and exhibited only small changes in the soils reintroduced from the 5-, 15- and 60-km distance [26].

Other soil microbiological indices such as numbers of bacteria and actinomycetes, bacterial and fungal biomass, species diversity of endosporogenic bacteria, microfungi and algae, respiration (CO_2 evolution), decomposition of cellulose and plant residues, production of free amino acids, N_2 fixation (acetylene reduction), like enzyme activities and nitrification capacity, were negatively affected by the smelter emissions [23—27].

It has also been proved that farmyard manuring of soils affected by smelter emissions led to some diminution of the heavy metal contents in the surface layer, which was attributed to formation of soluble heavy metal compounds with organic components of the farmyard manure and to migration of these compounds from the surface to the deeper soil layers [23, 27].

Kazakhstan. Skvortsova *et al.* [102] have established that in close vicinity of a lead smelter (Chimkent district, southern Kazakhstan) asparaginase activity in the polluted common serozem (containing 1000 mg Pb, 400 mg Zn, 50 mg Cu, 10 mg Cd/kg soil) suffered a 3.25-fold diminution compared with the unpolluted soil. Numbers of microorganisms were also lower in polluted than unpolluted soil.

The area of a metallurgical plant located in northern Kazakhstan was also studied from soil enzymological and microbiological viewpoints (Aristovskaya and Chugunova [1]). Samples were taken from chernozemic soils under wheat, along a 30-km transect. Soil urease activity was twice and respiration (CO_2 evolution) was 1.5 times as high at 30 km from the plant as in its vicinity. Intermediary values were registered at distances of 2.5, 5 and 10 km from the plant.

Switzerland. Wallis (Rhonetal), where Polomski [90] has performed soil studies, is a meteorologically nearly closed inner alpine valley which during many decades had been affected by floride-containing emissions originating from an aluminium smelter. The floride emission has been controlled efficiently only since 1981. In 1983, Polomski has sampled the 0-6-cm layer of soils from 8 spruce forest and 10 grassland sites located at different distances from the smelter. All samples were analysed chemically, but only the forest soil samples were submitted to enzymological analyses. The coefficients of correlations between the analysed parameters were calculated.

The content of water-soluble fluoride decreased with increasing distance from the smelter and correlated significantly and negatively with pH and $CaCO_3$ content and positively with ammonium acetate-extractable Al content. Phosphatase and dehydrogenase activities increased and β-glucosidase activity decreased with the distance from the smelter; more precisely, there were negative significant correlations between fluoride content and phosphatase and dehydrogenase activities ($r = -0.90$ and $r = -0.83$, respectively), whereas the fluoride-β-glucosidase correlation was positive, but less significant ($r = +0.61$).

Austria. Pohla *et al.* [89] and Kandeler *et al.* [51] described multidisciplinary investigations in the area of a copper smelter in Brix-

legg (located on the Inntal lowland, Federal Region of Tirol). Along a pollution gradient, four grassland sites (each of about 1000 m²) were studied. They are situated at 300 m (site A, most polluted), 1125 m (site B), 2425 m (site C) and 5900 m (site D, unpolluted) from the smelter. Samples were taken from the 0-5- and 5-10-cm soil depths in September 1990 and May 1991. The results obtained at the two sampling periods were similar.

Concentration of each heavy metal determined (Zn, Cu, Pb, Ni, Cd) decreased with increasing distance from the smelter. At site A, the 5-10-cm layer contained more heavy metals (excepting Cd) than the 0-5-cm one, whereas at the other sites both layers contained nearly the same amounts of heavy metals. In the two layers of the four sites the mean heavy metal concentrations (mg/kg dry soil) were the following: Zn 3138, 856, 228 and 96.7; Cu 2746, 664, 131 and 45.7; Pb 1530, 225, 50.8 and 36.8; Ni 72.4, 48.4, 23.8 and 35.3; Cd 15.9, 3.8, 1.2 and 0.4, respectively.

As shown in Fig. 4, dehydrogenase activity was higher in the 0-5-cm than in the 5-10-cm layer at each site and increased with decreasing heavy metal pollution (from site A to site D). The increase was insignificant in the 0-5-cm layer and significant ($p < 0.01$) in the 5-10-cm one.

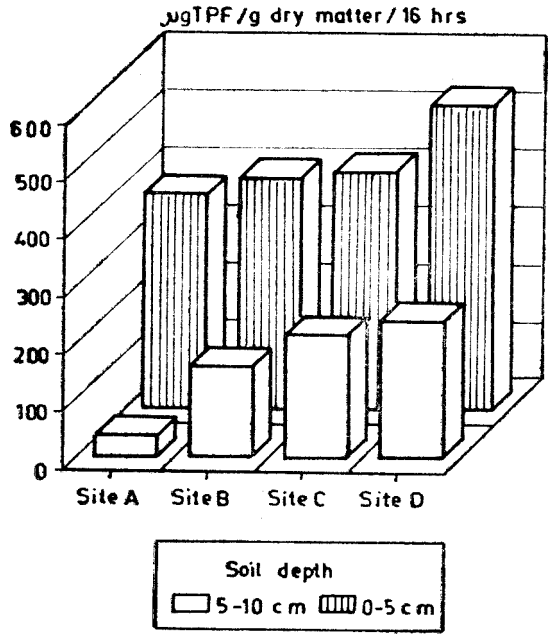


Fig. 4. *Influence of heavy metal pollution on soil dehydrogenase activity at four grassland sites* [51].
TPF — Triphenylformazan.

At site A, as compared with site D, other enzyme activities (protease, alkaline phosphatase and arylsulphatase) as well as basal and substrate (glucose)-induced respirations and nitrification were also significantly ($p < 0.001$) inhibited. At the same time, other enzyme activities (cellulase, xylanase and β-glucosidase) were significantly higher, whereas urease activity and N mineralisation were insignificantly higher at site A than at site D.

It was also found that organic fertilisation of site A led to diminution or even to elimination of the inhibitory effects of heavy metals in the 0-5-cm layer. Heavy metal pollution exerted negative effects on the soil fauna, too.

The conclusion was drawn that the microbial parameters and enzyme activities measured in the 5-10-cm soil layer are suitable for bioindication of heavy metal pollution.

Finkernagel and Schinner [30] have compared a forest site in the Brixlegg area with a less polluted forest site near another locality in Tirol, Terfens. Enzyme activities (dehydrogenase, urease, phosphatase and xylanase) and respiration (CO_2 evolution) rate were found to be significantly lower in the polluted than in the less polluted forest soil.

On both sites, four 4-m^2 experimental plots were installed for comparing the effects of liming and acid treatment on the enzyme activities and respiration in the two soils. The plots received the following treatments: 1 — control (no treatment); 2 — 1 kg lime; 3 — 20 l sulphuric acid solution of pH 3.2; 4 — 1 kg lime + 20 l sulphuric acid solution of pH 3.2. During the 7-month experimental period, lime was applied only once (at the beginning of the experiment), whereas the acid treatment was carried out at the beginning and still 3 times at regular intervals.

Liming resulted in increased pH, dehydrogenase activity, respiration rate, in decreased phosphatase activity and in little changes in urease activity in both soils. The very low xylanase activity in the polluted soil remained at the same low level after liming, too. In contrast, liming of the less polluted soil led to decreased xylanase activity during the first two months, then the activity tended to increase.

The acid treatment brought about minor changes in pH, dehydrogenase and urease activities in both soils. Respiration was stimulated in both soils. Phosphatase and xylanase activities remained unchanged in the polluted soil, whereas in the less polluted soil phosphatase activity reached, after an initial decrease, the level of the control, and xylanase activity increased after the first and third acid treatments, then decreased to the level of the control.

Liming plus acid treatment, as compared with liming alone, induced a loss in activities.

2. **Ironworks.** *Sweden.* Rühling and Tyler [97] have carried out enzymological studies in the areas of two ironworks (at Avesta and

Trollhätten), both emitting chromium as quantitatively most important pollutant element. The Averta emissions also contain large amounts of Mo and Ni and small amounts of V, Cu, Co, Pb, Mn, Cd and Zn. At Trollhätten, Cr and also Ca are major, whereas other metals (Mo, Ni, V, Cu, Mn) are minor components of the emissions.

Both areas are dominated by spruce forests. Needle mor was sampled at 60 sites in the Avesta area in October 1977 and at 88 sites in the Trollhätten area in April 1978. The sampling sites were selected in such a way that all parts of the metal gradient were well represented.

Soil phosphatase activity and respiration (CO_2 evolution) rate were determined in both areas and, in addition, urease activity and N mineralisation rate were also estimated in the Trollhätten area.

In the Avesta mor samples degree of pollution (expressed as Cr concentration) significantly and negatively correlated with both phosphatase activity and respiration rate. The correlations were strongest at higher pH levels.

In the Trollhätten mor samples degree of pollution significantly and negatively correlated with phosphatase activity only at pH levels > 4.2 and with urease activity only at pH levels > 3.5. At the same time, no relationship was discernible between degree of pollution and respiration or N mineralisation rate. But these two microbial indices showed close correlation with pH, the correlation being positive with respiration rate and negative with N mineralisation rate.

At comparable degrees of pollution, the enzymatic and microbial processes were affected to a larger extent in the Avesta than in the Trollhätten mors.

Stepwise regression analysis indicated that the Mo component of the emissions from both metallurgical plants had a greater negative effect on the enzymatic and microbial processes than the Cr component.

Poland. The studies of Zwoliński *et al.* [136] on the soil enzymological and microbiological effects of ironworks dust have already been mentioned on page 14.

3. **Ore enrichment works.** *Sweden.* Rühling [95] has performed soil chemical, microbiological and enzymological studies in the area of the lead mine in Laisvall. Underground mining of lead ore (PbS, galena) began here in 1943. Direct contribution of mining operations to pollution of air with fine particles of PbS and also of other heavy metal (Zn, Cu, Cr, Ni, Ag) minerals was small. More important source of air pollution was the ore enrichment works as the emissions resulted mostly from the drying process.

During the 36th week of 1979, 80 mor samples were taken in the surrounding coniferous forests at different (up to 10-km) distances from Laisvall. Pb content in the samples varied between 11 and 10,900 ppm (this very high value was registered in a sample taken from the close vicinity of the mine). Around the mine, on an about 2-km^2 territory, the Pb content was higher than 1000 ppm. At 3 and 5 km from the

mine, the Pb content was still higher than 500 and 100 ppm, respectively. Contents of the other heavy metals were also highest in the vicinity of mine.

The Pb and other heavy metal contents very significantly ($p < 0.001$) correlated with each other. Acid phosphatase and urease activities and respiration rate also correlated ($p < 0.001$) with each other, but not with the Pb content. The lack of correlation suggests that the lead despite of its high content in the polluted mor is, biologically, nearly completely unavailable which should be evenly valid for the Pb in form of PbS and the Pb strongly bound to organic matter.

Correlation of Cr content was negative and significant ($p < 0.01$) with phosphatase activity and respiration rate, and negative, but insignificant with urease activity. At the same time, each of these three indices gave significant ($p < 0.001$) positive correlation with soil pH and Ca content.

Russia. The ore enrichment works at Kostomuksha (northern Karelia) emits to the atmosphere heavy metals and sulphur causing pollution of the surrounding boreal forests. The most polluted area around the works (impact zone) has a radius of 6—8 km. The heavy metal and sulphur pollutions affect, to a lesser extent, broader areas up to 10 and 50—70 km from the works, respectively (Zagural'skaya [124] and Zagural'skaya and Zyabchenko [125]). These investigators have described soil microbiological and enzymological studies carried out during the first 9 years since the works has been operating. At three forest sites on podzolic soils located at 0.5 and 5 km from the works (impact zone) and at 23 km (considered as background zone), samples were collected from the litter layer (medium thickness: 4—5 cm) and from the superior, 5-12-cm thick layer of the mineral soil.

Results of these long-term investigations have shown that only the litter microorganisms and enzymes were evidently affected by the emissions and these effects appeared rather stimulatory even in the 9th year of the works. Thus, mean values and variation ranges of the numbers of ammonifying, mineral nitrogen-assimilating, oligonitrophilic, oligotrophic and cellulolytic bacteria, as well as those of the total numbers of actinomycetes and microfungi were higher in the litter of the impact zone than in the background zone. Intensity of cellulose decomposition, ammonification and production of free amino acids were also more pronounced in the impact than in the background zone. Catalase activity behaved similarly, but protease and urease activities remained at the same level in the two zones.

The conclusion drawn from these findings was that the heavy metals from the emissions, during the first 9 years of the works, played the role of microelements (trace elements) in the nutrition of litter microorganisms. How long will this stimulatory effect of emissions on the life of litter microorganisms last? For elucidating this problem further investigations are needed.

4. Coking plants. The emissions from coking plants contain a large variety of organic and inorganic substances (*e.g.*, phenol, thiocyanates, SO_2, H_2S, NH_3, pyridine) bound or unbound to soot [11—15, 52, 58, 122].

Ukraine. The enzymological study of soils affected by emissions from coking plants was initiated by D o l g o v a [11]. First, she studied phenol oxidase and peroxidase activities in such soils; later, other soil enzyme activities were also dealt with. The enzyme activity measurements were accompanied by microbiological analyses. For remediation of such soils, biotechnologies were tested and evaluated; for evaluation, enzyme methods were also applied [11—15, 58]. All these studies will be summarised below.

A polluted tree plantation growing at 100—150 m from a coking plant was compared with a similar plantation on an unpolluted area. The dominant tree species were small-leaf lime *(Tilia cordata)*, horse chestnut *(Aesculus hippocastanum)* and common lilac *(Syringa vulgaris)*. In both areas, the soil is of the same type (chernozem). During the vegetation period (April-November), samples were taken from the rhizospheric and nonrhizospheric soil of each tree species for determination of phenol concentration, phenol oxidase and peroxidase activities and number of phenol-oxidising microorganisms (capable of growing on mineral media to which phenol was added as sole carbon and energy source). All determinations gave higher values in the polluted than in the unpolluted soil under each tree species. The pollutant phenol enhanced the growth of phenol-oxidising microorganisms and induced the synthesis of phenol oxidase. As expected, most enzyme and microbial values were higher in rhizospheric than nonrhizospheric soil.

Concerning the other soil enzyme activities studied, it was found that ascorbate oxidase activity increased, but other, both oxidoreductase (dehydrogenase, catalase, nitrate reductase, nitrite reductase) and hydrolase (invertase, urease, protease) activities decreased in the polluted soil as compared with the unpolluted one. For exemplification, Table 5 presents soil dehydrogenase activity values measured in the polluted and unpolluted soils during the vegetation period.

Table

Dynamics of soil dehydrogenase activity in polluted and unpolluted plantations, during the vegetation period [12]

Tree species	Sampling area	Dehydrogenase activity (mg TPF/ 100 g soil)			
		April	June	August	November
Small-leaf lime	Polluted	0.182	0.060	0.230	0.310
(Tilia cordata)	Unpolluted	0.220	0.155	0.783	0.760
Horse chestnut	Polluted	0.243	0.087	0.035	0.105
(Aesculus hippocastanum)	Unpolluted	0.428	0.108	0.760	0.240
Common lilac	Polluted	0.120	0.137	0.093	0.150
(Syringa vulgaris)	Unpolluted	0.253	0.255	0.835	0.800

Significant negative correlations were registered between soil dehydrogenase activity and soil concentrations of both phenols and thiocyanates.

For remediation of the polluted soil, two biopreparations (cultures of the bacteria *Pseudobacterium lacticum* strain 392 and *Pseudomonas liquefaciens* strain 399) were tested. Experimnetal plots were set up in the vicinity of the coking plant. Farmyard manure (7 t/ha) and mineral fertilisers (90 kg N, 60 kg P and 90 kg K/ha) were added to the plots, then they were sown with bluegrass *(Poa pratensis)* seeds previously treated with strain 392 or 399 (at a rate of 2 g biopreparation for 20 g seeds). Seeds not submitted to the bacterial treatment served for comparison. Phenol oxidase activity, number of phenol-oxidising microorganisms and phenol-degrading capacity of soil were determined during the vegetation period. Each of these three indices increased in the bacterial treatments as compared with the untreated control. In addition, the bacterial preparations exhibited an increasing effect on soil catalase and invertase activities, too. Strain 399 was always more efficient than strain 392.

In another remediation experiment, the plots were fertilised with complex NPK (500 kg/ha), sown with bluegrass or orchard grass *(Dactylis glomerata)* and wetted with water or Na humate solution (at a rate of 0.01% Na humate on NPK weight basis). The control plots received no NPK and no humate. During the vegetation period, phenol oxidase and peroxidase activities in the soil of plots showed the following order: NPK + humate > NPK > control. Number of phenol-oxidising microorganisms and phenol-degrading capacity were also highest in the humate-treated plots.

In both remediation experiments, a more vigorous growth of plants in the bacterial and humate treatments could also be observed; the improvement in growth was of 20—30%.

Great Britain. Killham and Wainwright [52] performed investigations in the area of a coking plant at Chapeltown, South Yorks, England. Soil (brown earth podzol) was taken from beneath the canopies of sycamore *(Acer pseudoplatanus)* trees growing 500 m downwind of the coking plant. Polluted sycamore leaves are covered with a thin layer of black, atmospheric pollution deposit (APD) largely composed of soot. Such leaves were randomly picked from trees; APD was collected from their surface by dry brushing, then dry-sieved to remove leaf debris and aphids, and used in a soil incubation experiment. Unsterilised (native) and sterilised (autoclaved) 200-g samples of the soil taken from beneath the canopies of sycamore trees were amended with sterilised APD (1% weight/weight) and the sterilised samples were, in addition, inoculated with *Fusarium solani* spores. Unsterilised samples left unamended were the controls. All samples (containing 30% moisture) were incubated under aerobic conditions, at 25°C for 30 days, after which they were submitted to enzyme and other analyses.

Table 6 shows that in the unsterilised, APD-amended soil, as compared with the control soil, arylsulphatase activity markedly increased

Table 6

Arylsulphatase activity in unamended and APD-amended soil samples after their aerobic incubation at 25°C for 30 days [52]

Soil treatment	Arylsulphatase activity (μg *p*-nitrophenol/g soil/h)
Unsterilized, unamended	83.53
Unsterilized, amended with APD	142.51
Sterilized, amended with APD and inoculated with *F. solani* spores	94.60
Sterilized, amended with APD	0

during incubation. The increase was lower in the sterilised, APD-amended and inoculated soil. The activity increase was accompanied by increases in the concentrations of LiCl-extractable sulphur oxyanions: thiosulphate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$) and, particularly, sulphate (SO_4^{2-}). Based on these findings, it has been assumed that APD contains both elemental and reduced forms of sulphur and organic sulphur (ester-sulphate) and, during the incubation of soil, microbially and enzymatically mediated oxidation and mineralisation processes were taking place. Due to these processes, the APD reaching the topsoil below sycamore canopies can give rise to S-containing ions more available to microorganisms and plants. In other words, the oxidation and mineralisation processes may result in decontamination of APD-polluted soils.

Enzymological aspects of these investigations were referred to in another paper, too [122].

5. Refractory brickworks. *Great Britain.* The emissions from refractory brickworks, like those from coking plants, contain reduced forms of sulphur. This explains the interest of Wainwright [119, 121] in studying the effects of brickworks emissions on the activity of one of those soil enzymes that participate in the biological cycle of S. The studied enzyme, rhodanese catalyses the conversion of $S_2O_3^{2-} + CN^-$ to $SO_3^{2-} + SCN^-$, an early reaction in the microbial oxidation of $S_2O_3^{2-}$ to SO_4^{2-} in soils.

Polluted and unpolluted soils were sampled at Loxley, Sheffield, England. The polluted samples were collected from a wooded area situated 500 m downwind of a refractory brickworks. Leaves of the predominant tree species (sycamore) growing in this area showed signs of SO_2-induced damage and were generally covered with a thin layer of soot. The unpolluted samples were obtained from a similar wooded area 2500 m upwind of the brickworks. Here no signs of SO_2-induced leaf injury were seen. The vegetation and soil type on both polluted and unpolluted areas were similar. Soil was sampled below and at a distance of 5 m from the canopy of sycamore.

Table 7

Rhodanese activity in soil profiles in an area polluted by emissions from a refractory brickworks as compared with an unpolluted area [119]

Soil depth	Rhodanese activity (nmoles SCN^-/g soil/hr)			
	Polluted area		Unpolluted area	
(cm)	Below canopy	Away canopy	Below canopy	Away canopy
0—2	135.3±13.5	133.7±2.6	137.0±0	178.7±10
2—4	308.7±5.3	250.2±39	171.0±33	193.0±12
4—6	336.7±8.4	271.2±0	135.0±28	174.2±7.7
6—8	189.1±12	289.7±3.7	135.0±19	165.0±10
8—10	167.5±18	403.0±26	130.0±10	154.0±4.5

Sampling depths and the numerical results (and standard deviations) obtained in determination of rhodanese activity are presented in Table 7. This table shows that the polluted soil samples consistently had the highest rhodanese activities. In the top 6 cm, the greatest activities were found in soil samples taken from below the canopy of polluted sycamore. Below 6 cm, however, the highest activities were recorded in soil samples collected away from the polluted tree. In the unpolluted area, the highest activities were always found in soil samples taken away from the canopy. The pollution-induced high soil rhodanese activity suggests that such soils become capable of rapid oxidation of $S_2O_3^{2-}$, an important step in the decontamination process.

Wainwright [120] has also performed an experiment in which columns (height: 10 cm; diameter: 6.5 cm) were removed from the top of soil beneath the canopy of sycamore at an unpolluted site, then transported to a polluted site and buried beneath the canopy of a similar sized sycamore. The original litter was replaced and the introduced soil columns were left exposed to the effects of brickworks emissions for one year. Thereafter, activity of a series of soil enzymes participating in the C, N, P and S cycles were determined, columns prepared in the same way but left at the unpolluted site serving as controls (Table 8).

Table 8

Effects of exposure to refractory brickworks emissions on soil enzyme activities [120]

Enzyme activities[1]	Exposed soil columns	Control soil columns
Dehydrogenase	4.43±1.7	4.46±1.1
Carboxymethylcellulase	631±95	539±46
Urease	7.12±1.3	7.14±2.3
Phosphatase	114.9±0.3	113.9±2.1
Arylsulphatase	5.68±0.3	5.62±0.5
Rhodanese	637±13.7	601±15.9

[1] Expression of enzyme activities: dehydrogenase in μM H/g soil/24 hrs; carboxymethylcellulase in μg glucose/g soil/24 hrs; urease in μg NH_4^+—N/g soil/hr; phosphatase and arylsulphatase in μg *p*-nitrophenol/g soil/hr; rhodanese in nmoles SCN^-/g soil/hr.

It is evident from this table that the one-year (*i.e.* a relatively short-term) exposure did not affect soil enzyme activities. Neither occurred significant changes in microbial numbers, rates of respiration, nitrification and solubilisation of insoluble phosphate. The rate of ammonification was, however, higher in the exposed than in the control soil columns.

6. Pulp and paper mills. *Russia.* The soil enzymological and microbiological effects of emissions, containing methyl mercaptan (CH_3SH), carbon disulphide (CS_2), hydrogen sulphide (H_2S), phenol and other pollutants, from a pulp and paper mill operating near the southern shore of the Baikal Lake (South Siberia) were studied by Barykova [5]. Experimental plots were installed in forests on brown earths, along the lake shore at 0.1, 0.3, 1, 5 and 12 km from the mill. Plots at 25 km were the controls.

Soil gelatinolytic and cellulolytic activities were highest in the plots at 0.1 or 0.3 km from the mill, but ureolytic (urease) activity did not exhibit marked changes depending on the distance from the mill.

7. Synthetic fibre factories. *Belorussia.* Soil enzymological effects of the emissions from the synthetic fibre factory "Khimvolokno" at Mogilev were the subject of several studies (Prokopenko *et al.* [92]; Ivleva et al. [47, 48]; Efremov [20]; Lovchii *et al.* [66]). This factory produces the polyester fibre poly(ethylene-terephthalate). The emissions contain methanol, *p*-xylene, ethylene glycol, dimethyl terephthalate etc., among which methanol has the highest concentration.

During the vegetation period of 1981, Prokopenko *et al.* [92] have determined dehydrogenase activity and some microbiological parameters in soil samples taken at 0.3, 1, 3, 5, 10 and 15 km from the factory. Along this transect, dehydrogenase activity in the 0-5-cm soil layer was lowest at 1 km and tended to increase with increasing distance from the factory; phase I nitrification capacity was lowest at the 3-km and highest at the 15-km distance; total number of bacteria increased, numbers of sporogenic bacteria and actinomycetes did not change considerably and that of microfungi decreased with increasing distance from the factory.

Activities of other soil enzymes were studied in both croplands [48] and spruce forests [20, 47, 66] on podzolic soils around the factory. Along a transect, the sampling sites were established at 1, 5, 10, 15 and 35 km from the factory. The site at 35 km was the unpolluted control. Sampling depths were 0—5 and 5—20 cm in croplands and 0—30 cm (litter + mineral soil) in forests.

It has been found that the pollutants (methanol etc.) affected more consistently the 0-5-cm part of the arable layer and the forest litter than the deeper soil layers. In the croplands, soil protease activity was significantly lower, whereas urease activity was higher at 1—5 km than at 35 km. Due to pollution, the forest litters exhibited lower protease,

urease, catalase, polyphenol oxidase and higher invertase and phosphatase activities.

In a microplot experiment, 25-cm thick, 1-m^2 surface soil layers were taken from the unpolluted site (at 35 km from the factory) and introduced to polluted cropland sites in place of the original polluted layers. The introduced soil layers were kept uncropped to be exposed to the effects of emissions for one year. During this (short) period, evident changes in enzyme activities did not occur [48].

8. Oil production plants. *Sweden.* The Kvarntorp area, studied by Nohrstedt [73], is situated in the central part of South Sweden. From 1942 to 1966 this area was heavily exploited for production of oil fuels from the local alun shale containing 7% S. During these years, huge amounts (somewhere between 6.10^5 and $1.2.10^6$ t) of S, mainly in the form of SO_2, were released into the atmospere, damaging the surrounding forests.

Nohrstedt's studies started in 1982, the year before in the Kvarntorp area a central plant for destruction of hazardous chemical residual products from the industry went into operation; the studies were planned to be repeated at regular intervals in the future. The 1982 studies aimed at determining biological activities in the soil of 10 plots located in the surrounding forests. The plots were sized 300 m^2 and were covered by closed deciduous stands. The forest floor (0—3 cm) was sampled in June and August. Four plots, closest (within 2 km) to the previous oil production plant (nearby plots) were compared with the other plots located at greater distances (distant plots). In the period of oil production, the air pollution-induced reduction of tree growth was 30% or more in the nearby plots and about 10% or less in the distant plots. In the zone of nearby plots, the damage was so severe that it resulted in a total harvesting of coniferous trees, and the birch became the dominant tree species.

Analyses of the forest floor samples have shown that organic C content, C/N ratio and total heavy metal content (sum of Cd+Cr+Cu++Pb+Zn) were significantly higher in the nearby than in the distant plots, whereas pH and dehydrogenase activity (nmol TTC/g C/hr) gave significantly lower values in the nearby plots (3.59 and 87.7, respectively) than in the distant plots (4.35 and 546.1, respectively). Dehydrogenase activity significantly ($p < 0.01$) decreased with decreasing distance from the previous oil production plant. At the same time, the two groups of plots did not differ significantly as regards respiration (CO_2 evolution), N mineralisation (net production of inorganic N) and phosphatase activity.

The finding that, in the forest floor samples from the Kvarntorp area, dehydrogenase activity strongly and positively correlated with pH has been confirmed in another study (Nohrstedt [74]), in which the 0-3-cm deep floor samples taken from 20 coniferous and deciduous forest stands in Central Sweden were analysed. As dehydrogenase activity also correlated with stemwood productivity, it was suggested that

this enzymatic activity may be used as a biological index for soil fertility and, consequently, the decreased activity may serve as an indicator of soil acidification.

9. Other chemical factories. *Russia.* Filippova *et al.* [29] have determined soil dehydrogenase, catalase, invertase, amylase, urease, acid, neutral and alkaline phosphatase activities in close vicinity of a factory manufacturing urea, formaldehyde and cyclohexane and at 4 and 30 km from the factory. Soil was sampled from the rhizosphere of herbaceous plants in May, July and September 1972 and 1973. The results obtained have clearly shown that the seasonal variations in enzyme activities were, in many cases, much higher than the differences between the activity values recorded in the unpolluted soils and in the polluted ones. Therefore, an accurate evaluation of the soil enzymological effects of pollutants was not possible.

Around a factory manufacturing phosphate fertilizers from Karatau phosphorite and emitting mainly phosphorus anhydride, fluoride and heavy metals to the atmosphere, soil studies were carried out by Kuz'mina *et al.* [61]. Along 4 transects (northern, north-western, southern and south-western), soil (serozem) was sampled from the 0-5-cm depth at 0.5, 3, 5, 7 and 10 km from the factory. Organic matter content showed a nearly two-fold decrease along the northern and north-western transects and also an about two-fold increase along the other two transects. Dehydrogenase and phosphatase activities expressed on soil weight basis behaved similarly; dehydrogenase activity when reported to organic matter content remained highest and phosphatase activity when reported to mobile P content became lowest at 0.5—3 km from the factory. It has been concluded that the pollutants create anaerobic conditions stimulating dehydrogenase activity and enrich the soil in mobile P inhibiting phosphatase activity.

Aseeva *et al.* [2] have compared enzyme activities in cropped podzolic soils at 0.4, 10 and 40 km from a factory producing mineral fertilisers. All activities studied were lowest in the vicinity of the factory during the whole vegetation period (May-August). Dehydrogenase and urease activities were more sensitive to pollution than were catalase and invertase activities. The sensitivity also depended on the nature of crop plants, being much greater in soils under barley and oats than under timothy. Microbial biomass, N_2-fixing and denitrifying capacities also decreased in the polluted soils.

Austria. A chemical factory at Mürztal, Upper Styria had polluted the surrounding spruce forest with SO_2 during 30 years when Härtel and Cerny's [42] investigations began in this area. Based on SO_2 content in air and signs of spruce injury, unpolluted, slightly and heavily polluted sites were selected for soil sampling. The sites had the same aspect and soil type and similar vegetation.

Fig. 5 shows that each of the four enzyme activities measured was negatively affected by emissions of the factory. This effect was per-

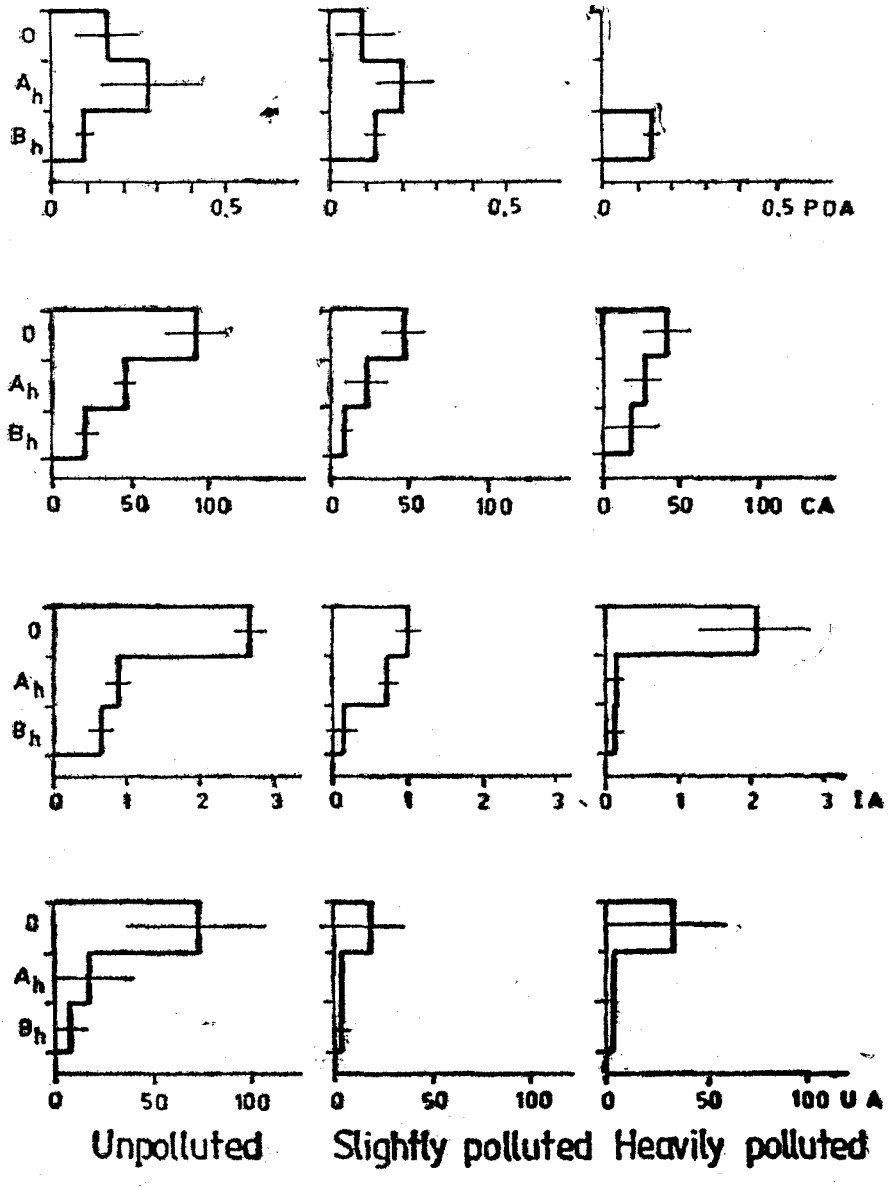


Fig. 5. *Enzyme activities in soil profiles at unpolluted, slightly and heavily polluted sites in the Mürztal area* [42].

PDA — Potential dehydrogenase activity. CA — Catalase activity.
IA — Invertase activity. UA — Urease activity.
The horizontal lines indicate standard deviations.

ticularly evident on dehydrogenase and catalase activities in the higher horizons and on invertase and urease activities in the lower horizons. Soil respiration (CO_2 evolution) and cellulolytic capacity were also negatively affected. Another observation should also be emphasised: when the spruce forest broke down due to the emissions, a deciduous bushwood grew up spontaneously, enabling the soil to acquire activity levels approaching those of the unpolluted site, even under continuous influence of emissions.

Poland. As Żurawska [135] specifies, for revegetation of the areas denuded by emissions (SO_3, SO_2, HF, HCl)* from the inorganic chemical factory "Polchem" at Torun, experimental plots (each of 5×20 m) on the poor soils (loose and slightly loamy sands) of these areas were installed in 1976, at 500-m distance from the factory. The experiment comprised many variants: control (not treated); minerally, NPK-fertilised (150 kg N, 150 kg P_2O_5 and 150 kg K_2O/ha); NPK + sewage sludge (100 and 200 t/ha) + ash (10 t/ha). The sludge originated from a municipal-industrial and an industrial effluent treatment plant and the ash from a brown coal power plant. The fertilisers, sludge and ash were mixed into the top 30-cm soil layer. Half of each plot (*i.e.* 50 m²) was sown with a mixture of grasses and legumes, and the other half was planted with trees and bushes.

In 1979, Żurawska has analysed enzymologically and microbiologically the soils from plots sown with the grass-legume mixture. Samples were taken from the 5-10-cm soil depth on day 15 of each month in the May-November period. Each treatment led to increased dehydrogenase activity as compared with the untreated control. The highest increase was recorded in a complexly treated plot, namely in that treated with NPK + 100 t/ha sludge from industrial effluents + 10 t ha ash. The activity values significantly correlated with numbers of heterotrophic microorganisms. These results fully coincided with those concerning development of herbaceous vegetation in the plots.

Ukraine. The nitrogen oxides, ammonia and other N-containing pollutants emitted to the atmosphere from a nitrogen fertiliser factory strongly affected soil enzyme activity, too, as established in long-term investigations conducted by Dolgova and Pavlyukova [16, 17, 87, 88]. Soil N contents (total, easily hydrolysable, nitrate, nitrite and ammonium N) were higher and much higher in the vicinity of the factory than at slightly polluted and unpolluted (control) sites, respectively. The reverse was true for soil dehydrogenase, catalase, protease and invertase activities. But urease, nitrate reductase and nitrite reductase activities were higher in the heavily than in the slightly polluted and unpolluted soils. These increased activities should play a role in decontamination of soils.

The soddy-podzolic soils in the area affected by emissions (containing SO_3, N oxides, CO, chlorinated hydrocarbons and particles of

* They pollute the environment along a distance of 5.5, 3.5, 1.2 and 1 km from the factory, respectively.

tetraethyl Pb, Zn, Cd, Cu, Al, Mg and Hg) from the chemical factory "Klorvinil" located in the Carpathian region of Ukraine were studied, from enzymological and microbiological viewpoints, by Stefurak [105]. During the vegetation periods of 1984—1986, soil samples were collected from the 0-10-, 10-20- and 20-30-cm depths of experimental plots installed many years before on the territory of the factory as well as at 2 km downwind and at 25 km upwind of the factory. The plots at 25 km were the controls.

In the strongly polluted soils, catalase activity could not be detected, polyphenol oxidase and peroxidase activities markedly decreased at each soil depth, N_2-fixing and cellulolytic capacities behaved like oxidase activities. Total numbers of bacteria and actinomycetes, numbers of *Azotobacter* cells and N_2-fixing clostridia increased, but total number of microfungi decreased with distance from the factory.

10. Oil- or coal-fired power plants. *Sweden.* Tyler's [115] analyses have shown that the emission dust collected from the oil-fired power plant at Karlshamn in the autumn of 1976 contained on average 16% metals, namely 3.8% V, 2.6% Ni, 2.4% Fe, 4.8% Na, 1.3% Ca and several other metals in smaller amounts. One-g samples (on air-dry basis) of a spruce needle mor with low metal contents were treated with 1—500 mg emission dust and immediately submitted to determination of acid phosphatase activity in reaction mixtures not buffered (initial pH 5.75) or buffered (with acetate) to pHs 5.0 and 3.6, and incubated at 22.8°C for 3 hrs. Other 1-g mor samples were treated with 1—100 mg dust and preincubated at 3°C for 6 days before phosphatase activity determination in unbuffered reaction mixtures.

The emission dust strongly inhibited phosphatase activity in mor samples. Thus, at the lowest rate (1 mg dust/g mor), the inhibition was ~20% in unbuffered reaction mixtures (not preincubated and preincubated) and ~5% in reaction mixtures buffered to pH 5.0; at this dust rate, phosphatase activity was not affected in reaction mixtures buffered to pH 3.6. At the dust rate of 100 mg/g mor, the inhibition was ~70% in reaction mixtures unbuffered or buffered to pH 5.0 and ~60% in those buffered to pH 3.6. At the highest rate (500 mg dust/g mor), the inhibition reached ~90% in all reaction mixtures.

Russia. Nikitina *et al.* [72] and Naprasnikova [70] have described soil enzymological and microbiological investigations in area of the network of power plants constructed in 1967 in the Nazarovo Basin which belongs to the Kansk-Achinsk Fuel-Energetic Complex (Siberia). For electricity generation, these power plants use the brown coal stripmined in this basin. The emissions, consisting mainly of calcium, silicon, iron and aluminium oxides, affect a large area (> 100 km^2), increase the Ca carbonate and hydrocarbonate contents of soils even up to 9% and pH up to 8.2. Sampling places were selected on gray forest soils at 5, 40 and 100 km from the power plants.

In soils sampled in the affected area in 1977, urease activity exhibited, on average, a 50% increase. In contrast, neutral and acid phos-

phatase activities decreased in the affected soils, the averaged decrease being of 31 and 78%, respectively. The changes in enzyme activities were attributed to pH changes. Cellulolytic and gelatinolytic capacities of soils were not significantly affected by the emissions.

Poland. Zwoliński and co-workers' [136] studies related to the soil enzymological and microbiological effects of power plant dust have already been referred to on page 14.

11. Atomic energy power plants. *Russia.* Effects of the Chernobyl disaster (1986) on soil enzyme activities were studied by Egorova [21]. In July 1993, she took 23 samples from soddy-podzolic soils in the Zlynka and Vygonichi districts (Bryansk region) for determination of ^{137}Cs, Zn and Cd concentrations and invertase, dehydrogenase and catalase activities. The Zlynka soils showed radioactive pollution, their γ-activity varying between 10^{-7} and 10^{-6} Ci/kg soil, whereas the unpolluted, Vygonichi soils exhibited a much lower γ-activity (10^{-8} Ci/kg soil). Significant positive correlation was found between the concentrations of ^{137}Cs and Zn. The soil samples were grouped along the ^{137}Cs and Zn gradient.

Activity of each of the three enzymes studied increased in soil samples with increasing ^{137}Cs and Zn concentrations up to a maximum value, then two of the activities (invertase and dehydrogenase) decreased in soil samples with higher ^{137}Cs and Zn concentrations. Thus, each activity was highest in samples containing around 60 mg Zn/kg soil, but in samples, in which the Zn concentration was around 110 mg/kg soil, invertase and dehydrogenase activities represented only 23 and 30% of their maximum values, respectively, whereas catalase activity maintained its maximum value.

In 6 soil samples, in which the Cd concentration ranged from 0.7 to 3.8 mg/kg soil, the relationship between enzyme activities and Cd concentration was also studied. Maximum invertase and dehydrogenase activities were measured at 1.7—2.4 and at 2.4 mg Cd/kg soil, respectively, while catalase activity continuously decreased with increasing Cd concentration. Each activity was lowest at 3.8 mg Cd/kg soil.

Addenda

1. Military waste disposal operations. *USA.* Kuperman and Carreiro [60] have conducted soil chemical, enzymological and microbiological investigations on an area in the U.S. Army's Proving Ground at Aberdeen, Maryland. This area is the Toxic Burning Pits (TBP) area, an open field of aproximately 3.6 ha. Between the late '40s and '80s, the pits were used to dispose of chemical agents, bulk chemical wastes, high explosives, nerve, incapacitating and blister agents, and chlorinated solvents. Methods applied for disposal included open burning and open detonation. The pits were maintained by pushing burned

soil and ash toward an adjacent area referred to as the "pushout" area (PA).

In October-November 1994, soils were sampled from the 0-10-cm depth on the PA, near the TBP and in the local background site (LBS), approximately 30 m from the TBP. A reference site (RS), with similar soil characteristics, was selected in Gunpowder Falls State Park, 7 km west of TBP.

The three sites are grasslands which showed significant differences among the mean above-ground vegetation biomasses: 22.6, 155.9 and 676.1 g/m^2 in PA, LBS and RS, respectively.

Concentration of each of the 7 metals analysed (As, Cd, Cr, Cu, Ni, Pb, Zn) and expressed in either mg or mmol/kg dry weight soil was significantly higher in PA than in LBS and, except for As, significantly higher in LBS than in RS. Total mean metal concentrations (mmol/kg dry weight soil) were 39.50 in PA, 7.69 in LBS and 1.75 in RS, which means a 22.5-fold significant increase in PA and a 4.4-fold significant increase in LBS as compared with RS. The order of abundance of the metals (mmol/kg dry weight soil) was Zn > Cu > Pb > Cr > Ni > As > Cd in both PA and LBS and Zn > Cr > Ni > Cu > Pb > As > Cd in RS.

As shown in Table 9, soil enzyme activities in the three sites decreased in the order: RS)) LBS > PA, the differences, excepting alka-

Table 9

Enzyme activities in soils of three grassland sites near or in Aberdeen Proving Ground, Maryland, USA [60]

Enzyme activities	Reference site	Local background site	Pushout area
Endocellulase[1]	88.7 a	16.3 b	9.2 b
	1912 a	769 ab	361 b
β-Glucosidase[2]	0.629 a	0.089 b	0.012 c
	13.567 a	4.191 b	0.445 c
N-Acetylglucosaminidase[2]	0.268 a	0.065 b	0.016 c
	5.785 a	3.044 b	0.623 c
Acid phosphatase[2]	1.782 a	0.402 b	0.081 c
	38.814 a	19.098 b	3.011 c
Alkaline phosphatase[2]	0.296 a	0.118 b	0.145 b
	6.439 a	5.914 a	5.901 a

[1] Enzyme activity is expressed as viscosimetric units per g dry weight soil per hr (first row) and per g ash-free dry weight soil per hr (second row).
[2] Enzyme activity is expressed as mmoles of converted substrate per g dry weight soil per hr (first row) and per g ash-free dry weight soil per hr (second row).
Numbers followed by the same letter in a row are not significantly different at p = 0.05.

line phosphatase activity, being significant among the sites. Significant negative correlations were found between enzyme activities (excepting again alkaline phosphatase activity) and total metal concentrations.

Microbial indices such as fluorescein diacetate (FDA)-stained (active) bacterial biomass, fungal biomass and fungal length, total fungal bio-

mass and length, substrate (glucose)-induced respiration had the significantly lowest values in the soil of PA. The enzyme activities (excepting endocellulase and alkaline phosphatase activities) significantly correlated with FDA-stained bacterial biomass, and all enzyme activities significantly correlated with total fungal length and substrate-induced respiration.

In conclusion, the results obtained indicate that the military waste disposal operations had detrimental effects on soil enzyme activities and microorganisms, for which the high heavy metal concentrations appear primarily responsible.

2. **Rocket destruction operations.** *Russia.* Gaponyuk and Klyueva [36] have carried out a soil enzymological study in the Volgo-Akhtubinsk flood plain, some areas of which are used for destruction of rockets by open detonation. It was calculated that perchlorate (ClO_4^-), the main component of burning rockets, is deposited on the soil of detonation site in an amount of 3 mg/kg soil after each detonation. Will the detonation-generated perchlorate and, generally, will rocket destruction by detonation affect dehydrogenase activity considered as a global indicator of microbial life in soils? To answer this question, soil samples were taken for determining dehydrogenase activity before and after detonation of rockets on 6 sites.

Site-dependent variation of dehydrogenase activity (μl H_2/g soil/24 hrs) ranged from 15.0 to 80.1 before detonation and from 22.3 to 83.6 after detonation. Detonation resulted in 3—35% reductions in dehydrogenase activity on three sites and in 3—49% activity increases on the other three. The conclusion could be drawn that the detonation-generated perchlorate and, generally, destruction of rockets by detonation did not exhibit significant effect (at least instantaneous or short-term effects) on soil dehydrogenase activity. Neither were the soil chemical characteristics affected.

III. Studies of the soil enzymological effects of industrial emissions originating from multiple sources (many industrial plants manufacturing different products, but situated in the same, industrial area)

Sweden. Rühling and Tyler [96] were the first to perform soil enzymological studies in an area affected by industrial emissions from multiple, more precisely from two pollution sources. In the town of Finspång (central Sweden) there are two large industries, an alloy factory with a copper smelter and, in the close vicinity, a turbine industry. The surrounding woodland is dominated by spruce *(Picea abies)* growing on soils of podzolic types. For sampling (on 7—9 October 1971) 49 spruce sites were selected along a heavy metal concentration gradient up to a distance of 13 km from the main pollution source. At each site, three fractions of predominantly spruce needle litter were collected: fraction *a* consists of the uppermost part of the litter layer,

fractions *b* and *c* correspond to the F_1 and F_2 layers of the mor, respectively.

The Cu concentrations of needle litter and needle mor from sites situated within 0.5 km from the pollution source were, on average, about 100 times higher than from sites situated more than 10 km away. The corresponding factors for other heavy metals were about 25 (for Zn), 30 (Cd), 10 (Ni and V), 4 (Pb), 2.5 (Fe and Co) and 2 (Cr). Opposite gradients with concentration increases with the distance from the pollution source were measured for Ca, Mg, K and Mn.

Dehydrogenase activity significantly decreased with heavy metal concentration (increased with the distance from the pollution source) in fractions *b* and *c*. For example, as an average in faction *b*, about 45 μg TTC were reduced in samples with > 10 μmol Zn+Cu+Cd+Ni per g dry matter and about 85 μg TTC in samples with < 10 μmol/g. The corresponding values in fraction *c* were about 40 and 70 μg TTC, respectively. Respiration (CO_2 evolution) behaved like dehydrogenase activity. The lack of correlations in fraction *a* was attributed to low water content in most samples of this fraction.

Poland. Olszowski [82—85] described 7-year fertilisation experiments in an about 40-year-old pine *(Pinus sylvestris)* forest stand strongly damaged by emissions from the Upper-Silesian Industrial District. The soils of the forest belong to the medium-podzolised soil group made up by loose sands. The experiments comprised two stages. In the first stage (1971—1976), experimental plots were installed in 8 variants: I. control (not treated); II. fertilised with NPK (100 kg N, 120 kg P_2O_5 and 100 kg K_2O/ha); III. treated with bentonite (10 t/ha to improve the loose sandy texture); IV. treated with bentonite (30 t/ha); V. limed with caustic CaO (2 t/ha); VI. NPK + bentonite (10 t/ha); VII. NPK + + bentonite (30 t/ha); VIII. NPK + lime (2 t CaO/ha). Bentonite and lime were applied in the autumn of 1971 and NPK in the spring of 1972.

During 1972—1974, — besides botanical and plant morphological studies, plant and soil chemical analyses —, soil enzymological and microbiological analyses were also carried out. Dehydrogenase, catalase, invertase, β-glucosidase, urease and asparaginase activities as well as total numbers of bacteria, actinomycetes and microfungi, and numbers of ammonifying, nitrifying, denitrifying, N_2-fixing and cellulolytic microorganisms were determined in soils sampled from the 0-5-cm depth (A_1 horizon) 8—9 times in each year.

At the beginning of the second stage (1976—1978), namely in the spring of 1976, NPK fertilisation was repeated (N, P_2O_5 and K_2O each at a rate of 150 kg/ha), then the same studies and analyses were carried out as in the first stage. In addition, soil phosphatase activity was also determined.

The results have shown that during the first year (1972), soil enzyme activities and microbial numbers gave similar values in all variants, but increases occurred in 1973 and further increases in the next years in the treated variants. The increases were always highest

in variant VIII (NPK-fertilised and CaO-limed). The enhanced soil enzymatic and microbial potential was accompanied by a 14.7% yearly forest stand volume increment and by improvement in the morphological properties of pines.

Russia. Marusina and Vazhenin [67] and Krasnova [57] have studied the soddy-podzolic soils strongly affected by emissions from large metallurgical, chemical and power plants in the area of Cherepovets (Vologda region). Soil samples were collected from experimental plots installed at 0.5, 2, 5, 7 and 10 km from the metallurgical plant in the direction of prevailing winds. The plot at 10 km served as control. Sampling depths were: 0—1, 1—5, 5—10 and 10—20 cm. The 0-1- and 1-5-cm soil layers contained 1.5—4 times more Fe, Mn, Zn, Cr, Pb in the plots closest to the plant as compared with the control plot. Considerable changes in enzyme activities occurred only in these two layers. According to Marusina and Vazhenin [67], invertase and urease activities were significantly lower ($p = 0.05$) in the polluted plots than in the control one. But the numerical data published by Krasnova [57] show that invertase, urease and dehydrogenase activities were highest at 5 km, and catalase and sulphoxide oxidase activities at 2 km from the plant. The level of enzyme activities in plot at 7 km and control plot was similar.

The area of Meshchera, in which Belitsyna and co-workers [7, 8] performed soil studies, is located in the north of the Moscow region. The soils in this area (humic gley and soddy-podzolic soils) are affected by emissions from factories manufacturing chemicals and pharmaceutical products or preparing ceramics. The main polluting elements are Zn, Pb and Cd. Soil dehydrogenase and invertase activities were found to decrease with increasing level of heavy metal pollution. For example, in a humic gley soil sample containing 45 mg Zn, 43 mg Pb and 3.4 mg Cd/kg soil, dehydrogenase and invertase activities were relatively high (15.5 mg TPF/10 g soil/24 hrs and 22.3 mg glucose/g soil/24 hrs, respectively). The activities were only 1.1 and 5.6, respectively, in another humic gley soil sample in which the Zn, Pb and Cd concentrations were 308, 76 and 3 mg/kg soil, respectively. Both activities disappeared in a third sample containing 407 mg Zn, 71 mg Pb and 16.7 mg Cd/kg soil, but dehydrogenase activity, as compared with invertase activity, was, in general, more sensitive to heavy metals.

Germany. Hüttermann *et al.* [44] have determined β-glucosidase, aminopeptidase, acid phosphatase and phosphodiesterase activities and nitrification rate in brown earth sampled from different depths within soil profiles, at Haard, Nordrhein-Westfalen (North Rhine-Westphalia). Haard is a small hilly forest area located north of the Ruhr region; it was heavily affected by industrial emissions from the Ruhr region for several decades. Beech trees fail here to regenerate naturally; they die off within a few years. Only red oak and pine forests survive.

It was found that the enzyme activities and nitrification rate within soil profiles in both red oak and pine forests gave measurable

values in the top 15-cm layer and were practically not detectable in the mineral soil below 15 cm of depth. This finding was interpreted as evidence proving that "Life has retired here almost completely from the mineral soil". This interpretation was supported by the observation that the depth-dependent decline of enzyme activities and nitrification rate within the 10-30-cm layer of similar soil profiles in an unpolluted forest (Göttinger Wald) was not so steep.

Czech Republic. SO_2 pollution of forest stands in the Ore Mountains (north-western Bohemia) led to withering of the spruce, which — according to Lettl's [62—65] investigations performed during 1982—1985 — was not accompanied by disappearance of microbial life in the soil. Development of grassy vegetation and natural replacement of spruce with stands of more resistant, deciduous trees, namely mountain ash or birch have a clearly positive effect on the soil microflora and enzyme activities.

For the investigations four areas were selected in the vicinity of Nová Ves. The areas located close to each other are covered by a residual original spruce stand (I), a withered spruce stand (II), a sparse mountain ash stand (III) and a birch stand (IV). Area I has no ground vegetation, whereas on the other areas an abundant ground vegetation dominated by *Calamagrostis villosa* has developed.

Samples were taken from the fermentation, humus and mineral A horizons for chemical, microbiological and enzymological analyses.

Numbers of aerobic and ammonifying bacteria in the fermentation and humus horizons, but not in the A horizon, were evidently lower in the residual spruce stand without ground vegetation than in the other stands. Number of microfungi, rates of respiration, ammonification, nitrification and elemental sulphur oxidation gave similar values in the corresponding horizons of all stands. Of the enzyme activities determined in the fermentation and humus horizons, only rhodanese activity in the fermentation horizon was markedly lower in the two spruce stands than in the other two, while invertase, α-amylase, urease and asparaginase activities in both horizons showed only minor differences among the stands.

Rejšek [94] has determined ectomycorrhizal acid phosphatase activity in mature spruce *(Picea abies)* pure stands affected by repeated short periods of heavy air pollution, in four localities at the south-eastern outskirts of the Ostravsko-Karvinský Coalfield (Moravian-Silesian Beskids). The pollution degree was different at the four localities selected: Řečice is the least polluted, Salajka is medium-polluted, and Smrčiny and Albinovo námešti are heavily polluted. For enzyme analysis, ectomycorrhizal roots were sampled from each locality in June 1989. The analytical results showed that acid phosphatase activity of ectomycorrhizal spruce roots significantly decreased with increasing degree of soil pollution.

Great Britain. Press *et al.* [91], who measured arylsulphatase activity in blanket peats from 29 sites in northern England and Wales,

point out that blanket peats, on these territories, differ markedly in their proximity to major sources of atmospheric pollution. The southern Pennines have extensive blanket peatlands, some of which are close to large industrial towns, and which have been subjected to high rates of acidic deposition for two centuries (for example, the peatland at Holme Moss). Other blanket bogs are more remote from urban and industrial areas and, thus, are relatively unpolluted (for example, the peatland in the Berwyn Mountains and that in Migneint, both in North Wales).

At each of the 29 sites studied, surface (0—2 cm) samples were collected and at two sites (Holme Moss and Berwyn Mountains) deeper samples from the 0-30-cm layer were also taken, between February and May 1983.

Arylsulphatase activity in the 0-2-cm layer was low in sites close to industrial towns, the lowest values being recorded in the Holme Moss peat. Sites remote from industrial areas had activities an order of magnitude higher.

At Holme Moss, arylsulphatase activity was evenly low at each depth within the 0-30-cm layer. But the activity, high in the 0-2-cm layer, decreased rapidly with depth in the Berwyn Mountain site, and at 30 cm the activity was less than 20% of that measured at the surface.

The effect of present-day pollution on arylsulphatase activity was also studied. Surface peat was collected from the relatively unpolluted Migneint site in February 1983. Seed trays were filled with this peat to a depth of 2 cm, then placed ("transplanted") on the bog surface in the Berwyn Mountain and Holme Moss sites. The transplanted peat had only minimal contact with the underlying peat.

Arylsulphatase activity, determined in the transplanted peat over 12 weeks (Fig. 6), showed little changes in the relatively unpolluted Berwyn Mountain site, but in the Holme Moss site, exposed to pollution, the activity declined rapidly and in 12 weeks the transplanted peat lost 50% of its initial activity. In other words, arylsulphatase activity was a sensitive indicator even of a short-term (12-week) present-day pollution.

USA. Arylsulphatase activity in peat exposed to pollution (acid precipitation) was studied in the USA, too, by Jarvis *et al.* [49]. Their study site was Big Run Bog, a 15-ha *Sphagnum*-dominated wetland in the Appalachian Mountains of West Virginia. In May 1984, 30-cm deep cores of *Sphagnum*-derived peat were collected, then cut into 6 sections (0—3 cm, 5—10 cm etc.). Arylsulphatase activity and total S and ester-sulphate S concentrations were determined in each section.

Mean arylsulphatase activity was lowest in surface peat (0—5 cm) and generally increased with depth to a maximum value at 25—30 cm. Total S and ester-sulphate S concentrations were lower at 0—5 and 25—30 cm than at intermediate depths. But, near peat surface, SO_4^{2-} availability was higher due to the high loading from both incident acid precipitation and minerotrophic runoff from the surrounding forested watershed.

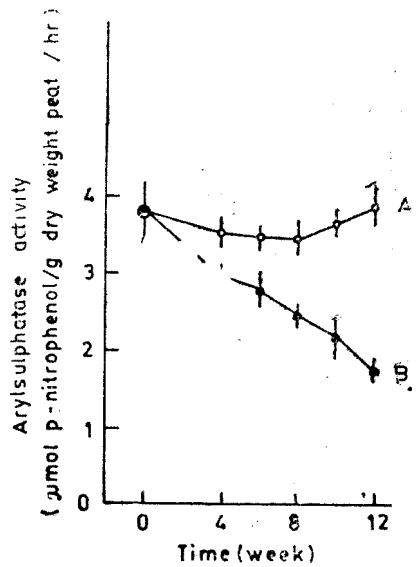


Fig. 6. *Arylsulphatase activity in an initially unpolluted peat from Migneint after its transplantation to a relatively unpolluted peatland site (Berwyn Mountains) (A) and to the Holme Moss site, exposed to pollution (B)* [91].

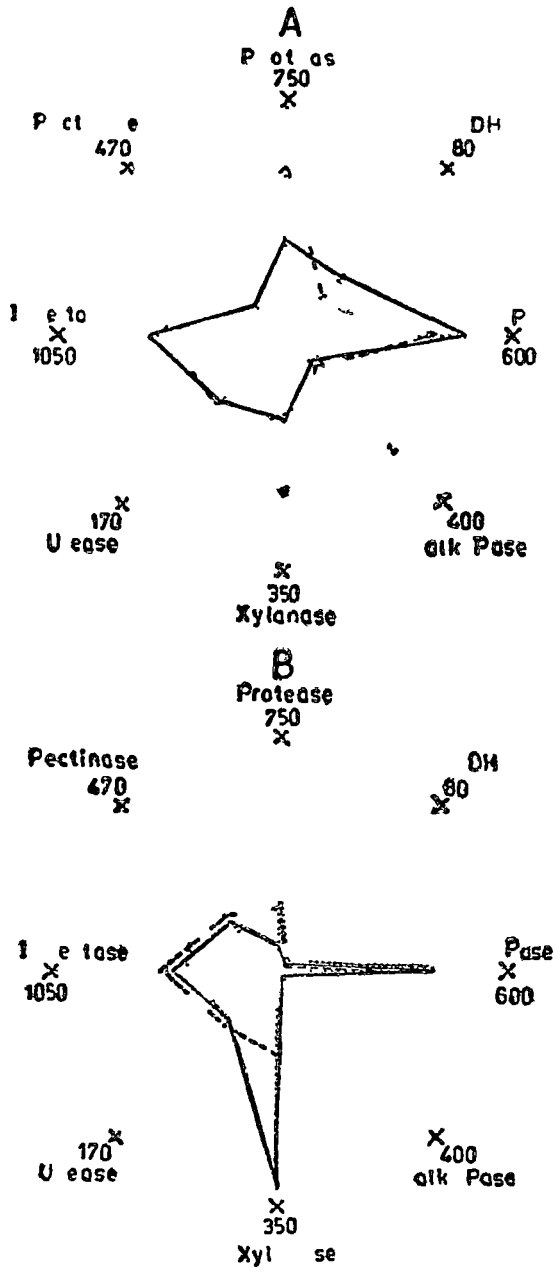
Vertical bars represent the standard errors.

The low arylsulphatase activity in the surface peat may be the result of the high SO_4^{2-} availability: the free sulphate ions inhibited, by feedback mechanism, the microbial synthesis of arylsulphatase. In deeper peat layers, such an inhibition does not occur because of the lower SO_4^{2-} availability which would explain the depth-dependent increase of arylsulphatase activity.

Austria. Kinzel and co-workers [6, 53, 126] have conducted investigations in the Wienerwald (Vienna Woods), namely in 11 beech forest sites, in the west and north-west of Vienna, to assess the effects of pollutants (acid precipitation, heavy, metals, especially Pb and Cd, soot) on soil properties.

For soil sampling, the stemflow (Stammabfluß) method has been applied: soil samples are taken from a zone between the stems or far from the stems and from a zone at the stem (trunk) base which is more polluted owing to the rainwater flowing down on the surface of stems and containing a great part of pollutants collected by the canopy. After removal of litter, the uppermost, 0—5 cm of the Ah horizon is sampled downhill and uphill, right and left of the stem (trunk) at different distances from it.

Besides a series of chemical and microbiological parameters, many enzyme activities were also determined in soil samples collected in the 1986—1988 period.



Γ ς 7 *St pl t f 8 ɔ t t l f tl b h f t t E lb g (th w t f V*) [53]

A — S l b t t m B — S l t th t m b

F ll l — Ap l 1987 D tt d l — J ly 1987 D h d l — S pt mber 1987

DH — D hydr g P — Ph ph t t l pH lk P — Alk l pl ph t E ym t t xp es d μg t p d t TPF (d hy dr g) ph l (b th ph ph t es) gl (yl) NH —N ( ) gl (er t) g l t d (p t) ty (p t)/g dry ght l/h

Fig. 7 presents a star plot of the mean values of 8 enzyme activities in the soil of the strongly polluted beech forest site at Exelberg. One can see from the star plot that dehydrogenase and alkaline phosphatase activities were measurable in the less polluted zone between the stems, but were lacking in the intensely polluted zone near the stems. At the same time, phosphatase activity measured at the natural pH of soil (*i.e.* without addition of buffer solution to the reaction mixtures) was less affected by the pollutants in stemflow. The enzymes participating in the N cycle (protease, urease) were more sensitive to pollutants than were those taking part in the C cycle (invertase, xylanase, pectinase).

Microbial biomass was more abundant, respiration and ammonification were slightly stimulated, whereas N_2 fixation and nitrification were markedly inhibited in the soil of the stemflow zone as compared with the soil of the intermediate (between-stem) zone.

Another finding was that the differences in enzyme activities between sites were greater than the seasonal differences at the same site.

At the Schöneben beech forest site in the Böhmerwald remote from industrial emission sources, the stemflow did not bring about any evident changes in soil properties, including phosphatase (at soil pH), dehydrogenase, urease, protease and β-glucosidase activities as well as microbial biomass and respiration.

Using the stemflow method, Zehner *et al.* [127] have studied chemically and enzymologically a beech forest site in the Wienerwald. Around 6 beech trees, soil was sampled along a 1-m line in the direction of stemflow and also from the opposite direction. Sampling depth was 0—5 cm.

Dimethylsulphoxide reductase, dehydrogenase and arylsulphatase activities in the soil of stemflow zone of each tree were strongly depressed in comparison with the activities measured in the opposite zone. The depressions were of up to 55, 80 and 87%, respectively, and significantly correlated with soil pH decrease.

For estimating the effects of airborne pollutants on soil microbial and enzymatic activities in the Federal Region of Salzburg, Tscherko *et al.* [108] have selected four long-term observation sites, namely a "pure air" site, a site far from and two sites near to emission sources. In soil samples taken from the 0-5- and 5-10-cm depths in May and September 1995, besides a lot of chemical parameters, the following 7 microbial and enzymatic activities were determined: substrate-induced respiration, N mineralisation, potential denitrification, xylanase, urease, phosphatase and arylsulphatase activities.

The numerical values of activities were grouped into three categories comprising low, medium and high activities, respectively. Then, the percent distribution of the three categories for each site was calculated (Table 10).

Table 10 shows that the microbial and enzymatic activities in the soil of the four sites decreased in the order: I < II < III < IV, *i.e.* in concordance with their degree of pollution.

Table 10

Distribution of low, medium and high soil microbial and enzymatic activities at four long-term observation sites in the Federal Region of Salzburg, Austria [108]

Sites	Low	Medium	High
	activities (%)		
I. St. Koloman, ,,pure air" site, grassland	11.9	40.5	47.6
II. Saalfelden-Ramseiden, site far from emission sources, grassland	19.1	52.4	28.5
III. Hallein-Gamp, site near emission sources, grassland	40.5	38.1	21.4
IV. Salzburg Freisaal, site near emission sources, arable land	78.6	7.1	14.2

The differences in activities among the three grassland sites were marked in the 0-5-cm soil layer, whereas the activities and differences among sites decreased in the 5-10-cm layer.

Based on the well-documented effect of acid precipitation to decrease soil pH and mobilise toxic Al ions, Illmer and Schinner [45] have selected forest sites in the Federal Region of Tirol for studying the relationship between Al concentration and some microbial and enzymatic parameters. The acid soils in the selected sites contained different amounts of Al (between 5 and 85 μmol/g dry matter), but their chemical, pedological, phytosociological, climatic and topographic characteristics were similar.

Significant negative correlations ($p < 0.001$) were found between Al concentration and microbial biomass, N mineralisation and protease activity. These three microbial and enzymatic parameters significantly correlated also with each other.

Finland. In 1987, Ohtonen and co-workers [77—81] have initiated complex soil biological investigations on 20 sites (of about 100 m² each), all representing mature pine *(Pinus sylvestris)* forest stands belonging to the middle boreal vegetation zone, around the industrialised city of Oulu located near the coast of the Bothnia Bay in northern Finland. A pulp mill and a chemical factory as well as local heating plant and motor vehicles emit to the atmosphere large amounts of SO_2, NO_x, CO, H_2S, dust and smaller amounts of heavy metals, polluting the area for the last 30—40 years. For example, the annual emissions of SO_2, NO_x, H_2S and Pb are 6275, 7010, 507 and 20 t, respectively.

The 20 sites selected lie on sulphur and nitrogen concentration gradients from 1.3 to 2.8 g S/kg and from 7.9 to 16.3 g N/kg in mor humus. Ten of the sites are considered to belong to the less polluted zone (5—40 km from the emission sources) and 10 sites to the more polluted zone (1—5 km from the emission sources).

Dehydrogenase and cellulase activities were determined from the total mor humus layer, the thickness of which was from 2.3 to 8.9 cm and increased towards the emission sources. Both activities were significantly higher at the less polluted than at the more polluted sites. For example, dehydrogenase activity determined in mor humus sampled from the 10 + 10 sites many times during 1987 and 1988 gave the

following mean values and standard errors (expressed in μmol TPF g organic matter/24 hrs): 2.08 ± 0.06 (less polluted sites) and 1.68 ± 0.05 (more polluted sites).

Other soil biological variables, namely basal respiration rate, microbial biomass, amount of mycorrhizal pine roots, diversity and sporophore production of mycorrhizal fungi, numbers of enchytraeids and nematodes have also been found to decrease towards the polluted end of the S and N concentration gradients. But length of the FDA-stained (living) fungal hyphae was not significantly affected by pollution degree.

Dehydrogenase activity significantly and positively correlated with pH ($CaCl_2$) and number of nematodes in mor humus. Cellulase activity significantly correlated with basal respiration and decreased simultaneously with the increase in the thickness of the humus layer which indicates that decomposition of organic matter has been retarded as a result of pollution.

Addenda

1. Enzymology of urban soils. In urban areas, constant pressure from human activity is a feature of the soils. Emissions from industrial and heating plants, exhausts from motor vehicles, presence of manufactured materials, housing constructions, trampling by humans etc. all are disturbing factors for urban soils, too (Harris [41]).

Poland. Żukowska-Wieszczek [133, 134] and Zimny and co-workers [128—132] have described investigations, in which enzyme activities, microbial and chemical parameters were analysed in soils of Warsaw during 1974—1990.

Of the enzyme activities, dehydrogenase was always determined. In many studies, urease activity was also assayed. Invertase activity was also determined in the first studies, but this activity — contrarily to dehydrogenase and urease activities — proved to be insensitive to assess differences among urban soils. Therefore, in further studies invertase activity was not determined. The 5-10-cm soil layer was analysed in all studies, and the 10-20- and 20-40-cm layers were submitted to enzyme analysis only in some studies.

Different urban soils were compared enzymologically.

a) Streetside lawns and park lawns. The streetside lawns are lawns in open areas, unsheltered by trees and/or bushes, near blocks of flats, whereas park lawns are far from highways, sheltered by trees and/or bushes, far from buildings.

Table 11 comprises average and range of enzyme activities measured monthly in the April-November 1976 period in soils of 3 streetside lawns and 5 park lawns. It is evident from this table that both dehydrogenase and urease activities were much lower in the unsheltered streetside lawn soils than in the sheltered park lawn soils at each sampling depth. It should be added that the differences were persistent not only in the average values but in the monthly values, too.

Table 11

Average and range of dehydrogenase and urease activities in soils of three streetside lawns and five park lawns at three depths [134]

Soils	Depth (cm)					
	5—10		10—20		20—40	
	Average	Range	Average	Range	Average	Range
Dehydrogenase activity	(μl H_2/10 g dry weight soil)					
Streetside lawn soils	6.8	0.2—29	3.4	0.2—16	1.8	0.0—13
Park lawn soils	49.6	7.5—93	24.7	7.5—52	12.4	1.0—31
Urease activity	(mg NH_3—N/10 g dry weight soil)					
Streetside lawn soils	0.54	0.001—1.69	0.69	0.01—1.72	0.49	0.01—1.68
Park lawn soils	2.93	2.13—3.43	2.72	2.20—3.36	2.67	1.99—3.46

b) Trees along streets and park trees. Nine sites were selected for this study. In April, July and November 1978—1979, soils were sampled from the 5-10-cm depth at 20-30-cm distance from the trunk of trees *(Acer platanoides, A. pseudoplatanus* and *A. negundo)* growing along streets and in parks. Average dehydrogenase activity was lowest in soils near trees growing inside pavements, along busy streets, with a free non-grassy surface area of about 1 m^2. A little higher activity was registered in soils near trees growing inside pavements, along busy streets, but with a free grassy surface area of about 1 m^2. As expected, the activity was highest in soils near trees growing in parks.

c) Allotment gardens. Nine gardens, located in three housing estates, were studied. The cultivated plants were tomatoes, roses and apple trees. The 5-10-cm soil layer was sampled in April, July and November 1978—1979. The garden soils presented the following increasing order of the average dehydrogenase activity: apple trees < roses < tomatoes.

Comparison of dehydrogenase activities in all urban soils studied made it possible to establish the following order: streetside soils < park soils < garden soils, and within each soil group the soils under trees were less dehydrogenase-active* than those under other plants, including weeds.

Total number of microorganisms, cellulose decomposition and respiration (CO_2 evolution) rates were also higher in less polluted than in more polluted urban soils.

Inorganic fertilisation of streetside and park lawn soils at a rate of 100 kg N, 60 kg P and 80 kg K/ha/year during 1976—1979 led to increased dehydrogenase and urease activities and cellulose decomposition rate in the first year, but later the activity-increasing effect of NPK remained significant only in the more polluted streetside lawn soils.

* The soils of other tree species growing along streets *(Betula verrucosa, Fraxinus excelsior, Populus nigra, Salix alba, Tilia cordata, Quercus robur)* were also found to be less dehydrogenase- and urease-active.

Significant negative correlations were found between soil dehydrogenase and urease activities and heavy metal, particularly Zn, Cu and Pb contents.

Germany. Studying the urban soils in Trier and Bonn-Bad Godesberg, Weritz and Schröder [123] have selected, for chemical, enzymological and microbiological analyses, 70 places, including park lawns, streetside green stripes and other green spaces as well as laylands, grounds covered with rubbles or used for depositing scrap-iron. A meadow and a sand ground were the controls. Soils were sampled from the 0-15-cm depth in the autumn of 1987. Catalase, xylanase and urease activities and microbial biomass were higher in the green areas than in the other grounds. Variation of these indices, like that of C content, in urban soils of the same use was characterised by a broad amplitude, which was most marked in soils covered with rubbles. Significant correlations were registered between the three enzyme activities and the microbial biomass, as well as between xylanase and urease, and between catalase and urease. But the indices did not significantly correlate with C content of soils. It was supposed that the lack of the correlation could be related to pollution of urban soils with harmful substances.

Studying soils in the city of Dorsten, located at the northern rim of the Ruhr industrial area, Keplin and Broll [51a] have found that soil dehydrogenase activity and biomass of earthworms decreased from ancient garden land to grassland and arable land.

Russia. Semenov *et al.* [100] have determined cellulase activity in two degraded soddy-podzolic soils in Moscow. One soil is under forest (birch-pine) in a suburb, the other in a park (birch-mountain ash). The park soil was found to be a little more cellulase-active which might be related to periodical manuring of the park soil with a peat-soil mixture.

Finland. In the Oulu area, the sites closest to the city are located on urban soils (see pages 48—49).

2. Enzymology of roadside soils. *USA.* Neal and Herbein [71] studied the effect of vehicle disturbance on sulphatase activity in arctic tundra soils at a wet and a drier site located in upland tussock tundra east of Slope Mountain, 175 km south of Prudhoe Bay, Alaska. Tracked vehicles had partially removed the vegetative mat and compacted the tundra surface approximately 9 years prior to the study. The soils are permanently underlain with permafrost. At both sites, samples were collected to a depth of 10 cm from under tussocks in vehicle track and adjacent undisturbed tundra in the autumns of 1979 and 1980. At the wet site, sulphatase activity in both years was significantly less in disturbed (vehicle track) than in the adjacent undisturbed soil. At the drier site, sulphatase activity of the soil sampled in 1979 was not significantly different between disturbed and undisturbed tundra, but in 1980 (in which the soil moisture levels were higher throughout the

growing season) the disturbed soil was significantly less sulphatase-active than the undisturbed soil.

In another study, in which the disturbed (vehicle track) and adjacent undisturbed arctic tundra soils sampled during the second week of August were compared, Herbein and Neal [43] found that at the wet site, approaching water saturation, vehicle disturbance caused a significant decrease in acid phosphomonoesterase and phosphodiesterase activities. In contrast, the activities were not significantly affected by vehicle disturbance at the drier, well drained site.

In Alaska, the effect of road dust on soil enzyme activities was also studied, more precisely Moorhead *et al.* [68] have studied the effect of dust raised from the Dalton Highway on soil endocellulase, exocellulase and acid phosphatase activities. The Dalton Highway is a gravel road stretching 577 km from Fairbanks to Prudhoe Bay. Dust raised from this road is deposited on a total area of about 1154 km². A 500-m transect was established perpendicular to the highway in tussock tundra near the Toolik Lake Long-Term Ecological Research site, on which the vegetation is dominated by vascular plants (55%) and mosses (35%) and among the vascular plants *Eriophorum vaginatum* accounts for the greatest cover. Soil samples were collected between *E. vaginatum* tussocks (0-3-cm depth) at 5-, 7.5- 10-, 50- and 500-m distances from the road, along the perpendicular transect.

It results from the analytical data summarised in Table 12 that the enzyme activities increased with distance from the road, increasing with organic C content and decreasing with dust loading. Sensitivity of activities to dust showed the order: endocellulase > exocellulase > acid phosphatase. Thus, endocellulase, exocellulase and acid phosphatase activities within 5 m of the road were reduced by 88, 74 and 45%, respectively, of the activity levels at 500 m. It was also found that the relationships among activity levels, soil organic C content and dust loading were remarkably similar among enzymes.

Table 12

Soil characteristics along a dust depositional gradient in a tussock tundra, Alaska [68]

Distance from road (m)	Organic C content (% dry weight)	Dust loading (g/m²/day)	Enzyme activities[1]		
			Endocellulase	Exocellulase	Acid phosphatase
5	14.01	0.750	30	90	2900
7.5	15.12	0.660	40	120	3100
10	15.78	0.580	120	205	4100
50	34.55	0.075	175	275	4250
500	39.11	0.010	250	350	5250

[1] Expressed as units of viscosity changes (endocellulase), μg glucose (exocellulase) and μmoles *p*-nitrophenol (acid phosphatase), respectively/g ash-free dry weight soil/hr.

Fenn *et al.* [28] have devoted a study to urease activity in two arid, silty clay loam soils (from Harkey and Saneli), each represented by a cultivated, cotton *(Gossypium hirsutum)* field and an adjacent roadbed site (40 years without cultivation). Urease activity was found to be much lower and more variable in the roadbed soils than in the cultivated ones.

Russia. Samoilova [98] and Samoilova and Gorbatyuk [99], studying the soil biological effects of motor vehicle exhausts along a highway with a very high traffic density (50,000 vehicles/24 hrs), took soil samples in an adjacent field cultivated with oats, at 15 and 250 m from the road. Amylase activity was 1.6 times, acid and alkaline phosphatase activities were 1.4 and 2 times higher at 250 m than at 15 m from the road. Invertase activity tended to increase with increasing distance, whereas urease activities at 15 and 250 m did not differ significantly. Total numbers of bacteria and actinomycetes increased, but that of microfungi did not change significantly with increasing distance from the road.

India. Joshi *et al.* [50] have determined enzyme activities in extracts of leaf litters of alder *(Alnus nepalensis)* and pine *(Pinus kesiya)* growing in a subtropical forest stand polluted due to its location at 15-m distance from the Shillong-Jowai Highway (with a traffic density of 8,000—9,000 vehicles/24 hrs) in the East Khasi Hills district of Meghalaya, North-East India. Another, relatively unpolluted forest stand about 500 m away from the road served for comparison. The two forest stands were similar with regard to soil, vegetational composition, tree height, extent of canopy closure, depth of forest floor and microenvironmental conditions. Alder and pine litters were sampled monthly during 1990 and 1991 and submitted to chemical, enzymological and microbiological analyses.

Cellulase, amylase and invertase activities in extracts of both litters from both forests exhibited monthly varations, and each activity was higher in the alder than in the pine litter. The activities, like numbers of bacteria and microfungi, were lower in the polluted than in the unpolluted forest stand. The correlations between cellulase and amylase activities and bacterial and fungal numbers were, in general, more significant in polluted than unpolluted litter. At the same time, invertase activity never correlated significantly with either bacterial or fungal numbers, but always gave significant correlations with total N and total sugar contents of litters.

Bulgaria. Deribeeva [9] determined enzyme activities in the 0-20-, 20-40- and 40-60-cm depths of a leached smolnitsa soil located on the side of the E-80 Highway, in the Slivnitsa district near Sofia. Sampling places had distances of 10, 50, 100 and 150 m from the road, in an uncultivated and a cultivated land. Invertase, urease and protease activities in the uncultivated smolnitsa increased with the increase in distance from the road. The same was true for invertase, protease and catalase activities in the cultivated smolnitsa. These trends were most

evident in the 0-20-cm soil layer. Surprisingly, there was no correlation between decrease in enzyme activities and amounts of Pb, Zn and Cd in the studied smolnitsa soil.

In another study, Deribeeva and Belichki [9a] have established a transect originating at the Sofia-Kulata Highway. Along the transect on a leached cinnamonic forest soil under different crop plants, samples were taken at 10, 50, 100 and 150 m from the road. Sampling depth was 0—20 cm. Of the enzyme activities assayed, invertase was found to be a sensitive indicator of pollution. This activity increased, urease activity decreased, protease and catalase activities did not change considerably with increasing distance from the road. Numbers of bacteria, oligonitrophilic microorganisms and *Azotobacter* cells, like invertase activity, increased, while numbers of actinomycetes, microfungi and cellulolytic microorganisms remained at a similar level along the transect.

Conclusions. The studies performed in many countries (in alphabetical order: Austria, Belorussia, Bulgaria, Canada, Czech Republic, Finland, Germany, Great Britain, India, Kazakhstan, Poland, Romania, Russia, Sweden, Switzerland, Ukraine, United States of America) have proved that enzyme activities are, in most situations, sensitive indicators of *a)* soil pollution caused by industrial emissions (and motor vehicle exhausts) and *b)* efficiency of the decontamination technologies applied. In addition, there are accumulated soil enzymes capable of participating even directly in decontamination of some polluted soils. But soil enzymes, like soil microorganisms, are not infallible. This is why prevention of pollution should remain the best way for environmental protection.

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POLYDESMUS HAMATUS VERHOEFF 1897 ŞI CELE TREI SUBSPECII ALE SALE

TRAIAN CEUCA* şi DELIA CRIŞAN*

SUMMARY. — Polydesmus hamatus Verhoeff 1897 and Its Three Subspecies. The present paper is a reconsideration of Verhoeff's concept regarding the three subspecies of *Polydesmus hamatus (Polydesmus hamatus hamatus* Verhoeff 1897, *Polydesmus hamatus burzenlandicus* Verhoeff 1925 and *Polydesmus hamatus furculatus* Verhoeff 1925).

Pentru că cele trei subspecii, care sunt menţionate în cele ce urmează, au fost dscrise din România şi pe care le-am la dispoziţie din diferite staţiuni, consider necesare câteva lămuriri.

Sigur că Verhoeff nu a cunoscut (în 1897) existenţa „speciei" *Polydesmus hamatus* Brandt 1841 [3], altfel nu ar fi dat încă o dată acelaşi nume unui alt (adevărat) polidesmid!

Este meritul celor doi colegi Golovatch şi Hoffman [3] care au arătat că statutul zoologic al lui *Polydesmus hamatus* Brandt 1841 a fost complet neclar „chiar din descrierea originală", inclusiv afirmaţia referitoare la proveniența acelui exemplar ca „patria ignota". Mai departe, aceşti doi colegi arată că diplopodul *Polydesmus hamatus* Brandt pare să aparţină genului malgaş *Dalodesmus* Cook 1896.

Cele două homonime apărute mai târziu: *Polydesmus hamatus* Verhoeff 1897 [9] din Carpaţi şi *Polydesmus hamatus* Loksa 1960 din China de Sud [6] trebuie discutate pe rând. *Polydesmus hamatus* Verhoeff 1897, specie clar descrisă de autor şi devenită mai apoi subspecie nominată, poate rămâne formă bună, pentru că este un polidesmid tipic şi pentru că numele specific de *hamatus* este foarte adecvat, tibiotarsul gonopodial având aspectul de cârlig-coasă (Fig. 1), îndoit în formă de semicerc cu marginile simple „netivite".

Cele trei forme sunt de altfel menţionate şi de către Attems [1].

Polydesmus hamatus hamatus Verhoeff 1897 este deci subspecia (tipică), bine delimitată, cunoscută de la Păltiniş, Cheile Cibinului, Tuşnad, Plăieşi şi Munţii Călimani.

Polydesmus hamatus burzenlandicus Verhoeff 1925 se deosebeşte de subspecia tipică prin aspectul tibiotarsului gonopodial îndoit aproape brusc şi este „tivit" pe toată lungimea lui [10], delimitându-se astfel clar de precedenta (Fig. 2). Se cunoaşte din Bucegi, Ciucaş şi Valea Lomaş (Munţii Călimani).

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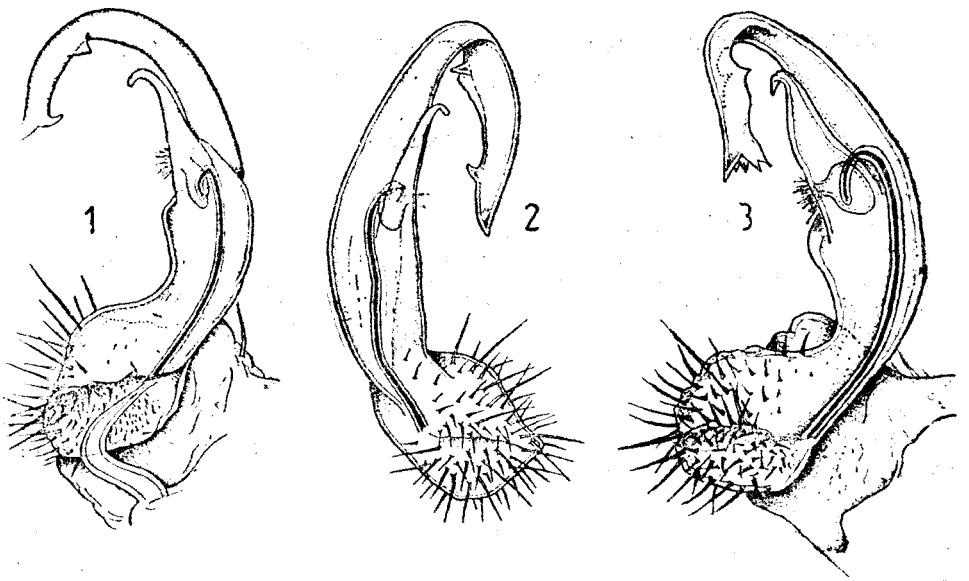

Fig. 1–3. Polydesmus hamatus *Verhoeff 1897*.
1 — Gonopodul drept, profil intern al unui exemplar ♂ de *Polydesmus hamatus hamatus* Verhoeff 1897 de la Cheile Cibinului. 2 — Gonopodul stâng, profil intern al unui exemplar ♂ de *Polydesmus hamatus burzenlandicus* Verhoeff 1925 de pe Valea Lomaş (Munţii Călimani). 3 — Gonopodul drept, profil intern al unui exemplar ♂ de *Polydesmus hamatus furculatus* Verhoeff 1925 de pe Valea Pleşa (Maramureş).

Polydesmus hamatus furculatus Verhoeff 1925 se caracterizează prin tibiotarsul lat după îndoire. Între dintele subapical şi vârful tibiotarsului există 2—3, rareori 4 dinţişori evidenţi (Fig. 3). Se cunoaşte de la noi din Valea Vinului (Munţii Rodnei), Valea Pleşa (Maramureş), Munţii Suhard şi staţiunea Zgureni.

In 1984, Tabacaru descrie, după un singur exemplar ♂, specia *Polydesmus costobocensis* din pasul Rotunda (Munţii Rodnei) [8], făcându-se numeroase referiri şi comentarii la alte specii ale subgenului *Polydesmus*, de altfel inutile, pentru că în mod evident gonopodele acestei forme sunt de tip *hamatus*, aspect care se observă de la prima vedere, încadrându-se ca o subspecie alături de *Polydesmus hamatus hamatus* şi *Polydesmus hamatus burzenlandicus*. De altfel, încă din 1961 Stojalowska [7] citează această subspecie din Polonia. Pe lângă figura dată de Verhoeff*, Stojalowska prezintă alături şi una după material propriu ([7], Fig. 127, p. 112), din care, fără nici un dubiu, reiese clar aspectul identic cu al lui *Polydesmus costobocensis* ([8], Fig. 1, p. 149). Personal am colectat această subspecie de pe Valea Pleşa

* Acea muchie de pe prelungirea femorală a gonopodelor, probabil că nu este decît o cută accidentală, care nu poate avea o valoare taxonomică, fiind poate doar un aspect singular.

(afluent al râului Vaser din Maramureș) în 1961 și am inclus-o în teza de doctorat ([2], Fig. 111, p. 180). În plus, mai am la dispoziție 36 ♂♂ și 8 ♀♀, colectate din stațiunea Zgureni și Munții Suhard. Probabil că Tabacaru nu a avut la dispoziție lucrarea „Krocionogi *(Diplopoda)*" a lui Stojalowska editată în 1961 [7], altfel nu se poate explica această scăpare.

Discuții. *Polydesmus hamatus* Verhoeff 1897 poate rămâne cu acest nume, cu toate că numele specific este atribuit și deci rămas asupra lui *Dalodesmus* (dovedit a nu fi fost un *Polydesmus).* Consider că nu există nici un motiv ca două specii aparținând la două genuri diferite de diplopode (*Dalodesmus* și *Polydesmus*) [4] să aibă același nume specific de *hamatus.* Astfel de situații mai pot fi întâlnite în zoologie, de exemplu în volumul *Pseudoscorpionidea* (Beier, 1963) există *Chthonius leruhti* (Fig. 40, p. 47) și *Neobisium leruhti* (Fig. 160, p. 155), sau din grupul păsărilor: *Coracias garrulus* și *Bombycilla garrulus.* Nu văd motivul pentru care autorii mai sus menționați arată că numai *Polydesmus burzenlandicus* este valid ca nume înlocuitor pentru *Polydesmus hamatus* Verhoeff 1897, știut fiind faptul că *Polydesmus burzenlandicus* nu a fost niciodată descris de autor ca specie de sine stătătoare, ci direct ca o subspecie a lui *Polydesmus hamatus* Verhoeff 1897, deci *Polydesmus hamatus burzenlandicus.* Le mulțumesc foarte mult celor doi autori mai sus menționați pentru apreciere și atribuirea numelui meu „subspeciei" *Polydesmus burzenlandicus ceucai,* dar îmi pare rău că nu văd de loc justificată înlocuirea lui *Polydesmus hamatus* Verhoeff 1897 cu *Polydesmus burzenlandicus ceucai!*

Bineînțeles că odată cu descrierea altor două subspecii, *Polydesmus hamatus* devine subspecia nominată *Polydesmus hamatus hamatus.*

„Specia" *Polydesmus hamatus* Loksa 1960, descrisă dintr-o peșteră din sudul Chinei [6], așa cum spun cei doi autori Golovatch și Hoffman [3], nu este nici *Polydesmus* și nici *hamatus.* După apariția lucrării lui Loksa în 1960 [6], am rămas foarte surprins văzând că numește această specie *Polydesmus hamatus,* după ce cu câțiva ani mai înainte (1954) menționează pe *Polydesmus hamatus* Verhoeff 1897, într-o lucrare în care dă și figura gonopodelor [5]. Consider necesar să menționez aici că atunci, imediat după apariția lucrării lui Loksa în 1960 [6] i-am scris, arătându-i (foarte colegial) eroarea făcută. Nu mi-a răspuns nimic; am crezut că a remediat-o; nefiind interesat, nu am mai urmărit modificarea necesară.

Este meritoriu faptul că Golovatch și Hoffman [3] au încadrat „specia" lui Loksa [6] într-un gen și o specie aparte — *Epanerchodus sinensis,* „mai mult pe baza dovezilor geografice decât anatomice", nefiind descrisă și ilustrată în suficientă măsură.

Acestea fiind spuse, consider că *Polydesmus hamatus burzenlandicus* Verhoeff 1925 poate rămâne pe mai departe o subspecie clară, menționând din nou că această formă nu a fost niciodată descrisă ca specia aparte.

În ceea ce privește *Polydesmus hamatus furculatus* Verhoeff 1925, cred că am arătat în suficientă măsură stabilitatea acestei forme, menționată de Stojalowska și din Polonia [7].

Am redactat această lucrare pentru a arăta locul acestor trei subspecii din România, relevând, în același timp, valorosul aport al celor doi distinși colegi Golovatch și Hoffman în lămurirea celor două forme de *Polydesmus hamatus* Brandt și *Polydesmus hamatus* Loksa, acestea nefiind polidesmide.

Părerea mea referitoare la paternitatea lui Verhoeff asupra lui *Polydesmus hamatus* ca un adevărat polidesmid, cu subspeciile menționate, vrea să fie o restabilire a punctului de vedere verhoeffian.

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STUDIA UNIV. BABEŞ-BOLYAI, BIOLOGIA, XLII, 1—2, 1997

DIPLOPODE *(GLOMERIDA-GLOMERIDAE)* DIN ROMÂNIA — ZONA DORNELOR, MOLDOVA ŞI NORDUL OLTENIEI. Nota a III-a

DELIA CRIŞAN* şi TRAIAN CEUCA**

SUMMARY. — **Diplopods (Glomerida-Glomeridae) from Romania — the Dorna Area, Moldavia and the North of Oltenia.** *Note III.* Two new species of the genus *Glomeris*, *Gl. mauriesi* and *Gl. olarii* are described. Designs of their tergites and of other two species, *Glomeris pachytelopoda* Ceuca 1989 and *Glomeris prominens* Attems 1903 are also presented.

Genul *Glomeris* cuprinde un grup omogen de specii răspândite în aproape toată Europa [5], dar care este reprezentat printr-o mare variabilitate a desenului de pe tergite [2] şi din contră o slabă diferenţiere a telopodelor (gonopodelor) [3]. Cu toate acestea (deşi deocamdată avem puţine exemplare la dispoziţie), vom arăta, în cele ce urmează, la noile specii descrise, atât aspectul desenului spatelui (tergitelor), cât şi conformaţia telopodelor.

În această a treia notă asupra diplopodelor din zona Dornelor, ne vom referi nu numai la două specii noi, ci vom da şi o completare cu aspectul inedit al desenului spatelui la *Glomeris pachytelepoda*. Ceuca 1989, la care există, aşa cum am arătat în lucrarea respectivă [1], un dicromism sexual, precum şi la *Glomeris prominens* Attems 1903. Sigur că cercetări ulterioare, pe mai mulţi indivizi, mai cu seamă la primele trei specii vor arăta eventualul grad de variabilitate. Menţionez că silueta (conturul) spatelui speciilor este după Schubart [4].

*Glomeris mauriesi*** n. sp.

Lg. = 14 mm; lt. = 4 mm. ♀ este la fel de mare, deşi se ştie că în cadrul acestui gen, de regulă, femelele pot fi mult mai mari. Referitor la culoarea corpului (Fig. 1), la prima vedere s-ar părea că există o mare asemănare cu unele forme ale lui *Glomeris hexasticha* [4]. Există, pe fondul brun întunecat, aproape negru, câte trei şiruri de pete oblice albegălbui, separate medial de o bandă neagră, încadrată, de o parte şi de alta, din câte o succesiune de pete albe, aproximativ triunghiulare, orientate cu vârfurile anterior, pe fiecare tergit. Scutul preanal are două pete deschise separate, pe linia mediană, de culoarea de fond întunecată, în formă de T răsturnat. La ♂, acest scut are marginea terminală evident excavată.

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** Cu omagiu distinsului coleg J.P. Mauriès de la C.I.M. din Paris.

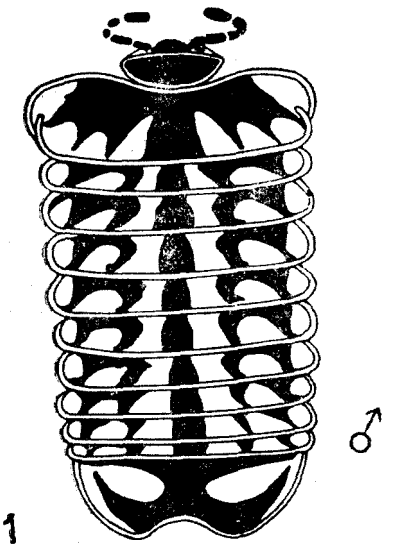

Fig. 1. *Glomeris mauriesi* n. sp.
Aspectul spatelui la ♂.

La cap, atât antenele, ocelii, cât și organul lui Tömösváry au formele și dimensiunile obișnuite. Scutul cervical are cele două șanțuri paralele obișnuite. Bisintergitul sau scutul toracic se pare că nu are striurile transversale obișnuite.

La ♂ perechea a 17-a de picioare (Fig. 2) este, ca de regulă, mai redusă, având coxele înalte, aproximativ până la nivelul prefemurelor. Perechea a 18-a de picioare (Fig. 3) are sincoxitul cu o excavație în formă de unghi de aproximativ 90°, aspect nemaiîntâlnit până acum, telopoditele fiind și aici mai reduse. Perechea a 19-a de picioare sau telopodele (Fig 4) sunt neobișnuit de îngroșate (bondoace), depășind, pare-se, pe cele ale speciei *Gl. pachytelopoda* Ceuca 1989, care la timpul acela aveau un aspect inedit [1, (Fig. 1)]. Iată că această specie a depășit-o nu numai prin grosimea articolelor telopodelor, ci și prin aspectul de ansamblu, care pare că ar fi încadrat într-un pătrat. Lobul sincoxitului (Fig. 6) este relativ înalt, încadrat de cele două prelungiri ale sale care-l depășesc lateral, dar ale căror capete terminale cu greu pot fi observate, din cauza prelungirilor digitiforme ale prefemurelor care le acoperă parțial; terminal au vârfurile simple, nebifurcate.

Bineînțeles, ca de obicei, aceste prelungiri laterale ale lobului sincoxitului au, pe laturile lor mediale, mici țepi. Prefemurele au un aspect globulos cu prelungirile digitiforme mult alungite. Femurele deosebit de dezvoltate par mai înalte (lungi) decât late; prelungirile lor digitiforme, ceva mai scurte decât ale prefemurelor, sunt destul de greu de observat, fiind acoperite, aproape total pe toată lungimea lor, de cele ale prefe-

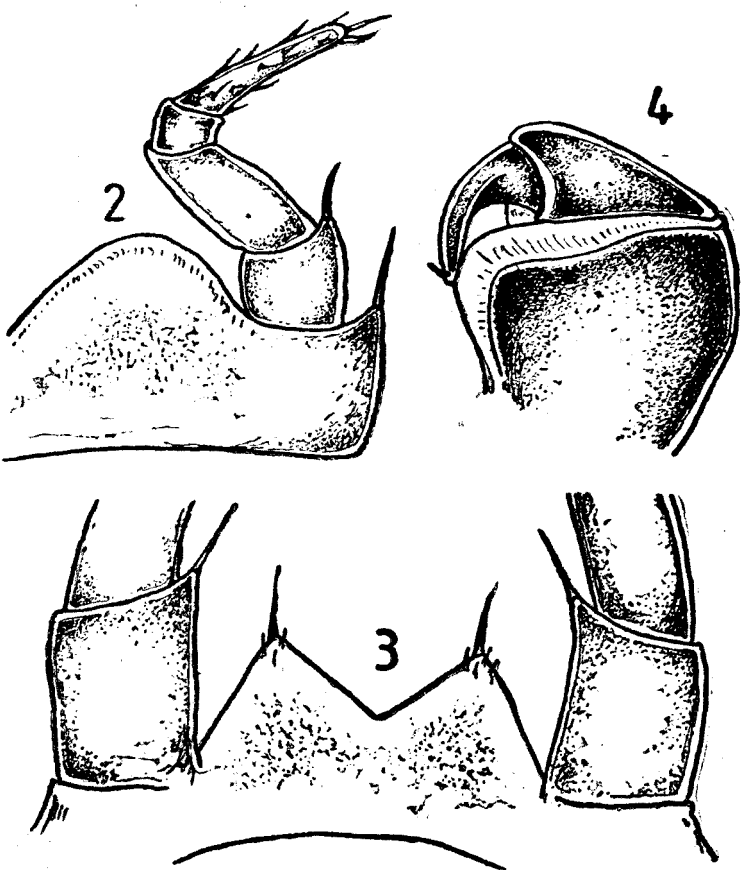

Fig. 2—4. *Glomeris mauriesi* n. sp. 2. Piciorul stâng al perechii a 17-a de picioare. 3. Sinocoxitul perechii a 18-a de picioare. 4. Tibiotarsul perechii a 19-a de picioare, văzut posterior.

murelor. Ambele prelungiri digitiforme au terminal câte o setă lungă. Pe părțile posterioare ale femurelor există câte o prelungire lată (Fig. 4). Tibiile groase și ele, văzute anterior, par mai lungi decât late (având o poziție orizontală), sunt lipsite de părțile lor posterioare, de micile prelungiri obișnuite ale acestora (ca și la *Gl. pachytelopoda).* Tarsele abia vizibile anterior sunt relativ lungi și subțiri, îndoite mult posterior (Fig. 4).

Proveniența: au fost colectate 1 ♂ + 1 ♀ în septembrie 1995 de la Gura Haiti, jud. Suceava de L. Olaru.

Pentru a pune în evidență, mai clar, diferențele, dăm mai jos o paralelă între cele două specii.

Glomeris pachytelopoda Ceuca 1989

— Spatele este diferit colorat la ♂ şi la ♀ (Fig. 11—12).

— Telopodele au articolele mai degajate cu prelungirile digitiforme ale prefemurelor şi femurelor, distanţate; cele ale femurelor sunt mici şi învăluite de acestea [1].

— Femurele acestora sunt uşor mai late decât lungi.

— Tarsele sunt mai scurte şi mai groase [1].

— Lobul sincoxitului este rotund (emisferic), iar prelungirile laterale sunt evident mai subţiri, înclinate medial şi cu „peri" atât pe laturile interne, cât şi pe cele externe.

— Scobitura sincoxitului perechii a 18-a de picioare este în formă de ogivă.

Provenienţa: nordul Olteniei.

Glomeris mauriesi n.sp.

— Spatele este la fel colorat la ♂ şi la ♀ (Fig. 1).

— Telopodele au articolele mult înghesuite, având în ansamblu o formă de pătrat cu prelungirile digitiforme ale prefemurelor suprapuse peste cele ale femurelor (Fig. 1).

— Femurele acestora sunt evident mai lungi decât late.

— Tarsele sunt mai svelte şi uşor alungite (Fig. 4).

— Lobul sincoxitului este uşor conic, iar prelungirile laterale ale acestora sunt paralele, cu „peri" doar pe laturile mediale (Fig. 6).

— Scobitura perechii de picioare a 18-a are sincoxitul în unghi drept (de 90°) (Fig. 3).

Provenienţa: nordul Moldovei.

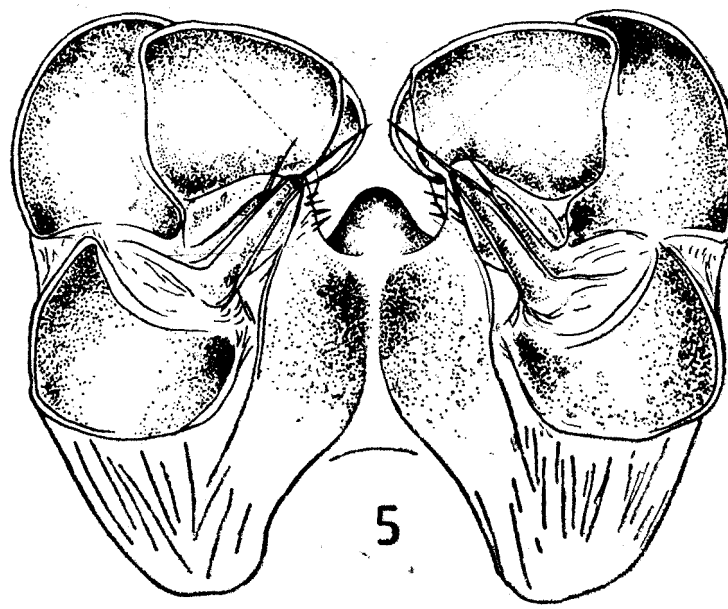

Fig. 5. *Glomeris mauriesi* n. sp. Perechea a 19-a de picioare (telopodele).

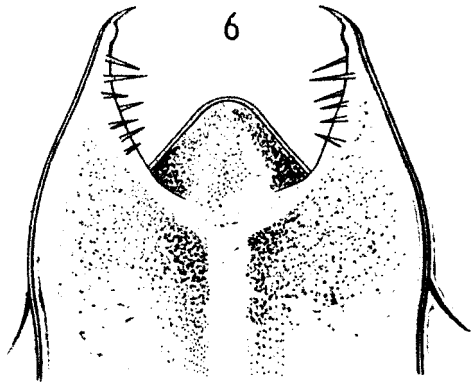

Fig. 6. *Glomeris mauriesi* n.sp. Sincoxitul perechii a 19-a de picioare.

Glomeris olarii n.sp.

Lg. = 12 mm; lt. = 4 mm atât la ♂ cât și la ♀. În ceea ce privește culoarea corpului, acesta este întrucâtva asemănătoare cu cea a lui *Gl. prominens* Attems 1903; aici petele paramediane deschise sunt triunghiulare și oblic dispuse pe tergite fiind uneori mai late sau mai înguste (aspect oarecum asemănător cu cel al lui *Gl. helvetica* [4]). Pe scutul preanal există doar două pete deschise, aproximativ rotunde. La ♂ marginea posterioară a acestuia este evident ușor scobită (Fig. 7). Ocelii, organul lui Tömösváry și antenele au aspectele obișnuite. Scutul cervical are cele două striuri paralele ușor bombate posterior. Scutul toracic sau bisintergitul este prevăzut cu trei striuri transversale, dintre care numai primul este continuu, celelalte două sunt întrerupte la mijloc.

La ♂ perechea a 17-a de picioare (Fig. 8) are coxele bine dezvoltate, depășind în înălțime prefemurele lor; telopoditele lor, ca de obicei, for-

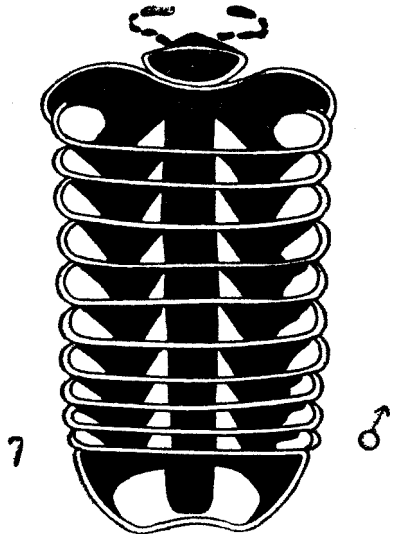

Fig. 7. *Glomeris olarii* n. sp. Aspectul spatelui la ♂.

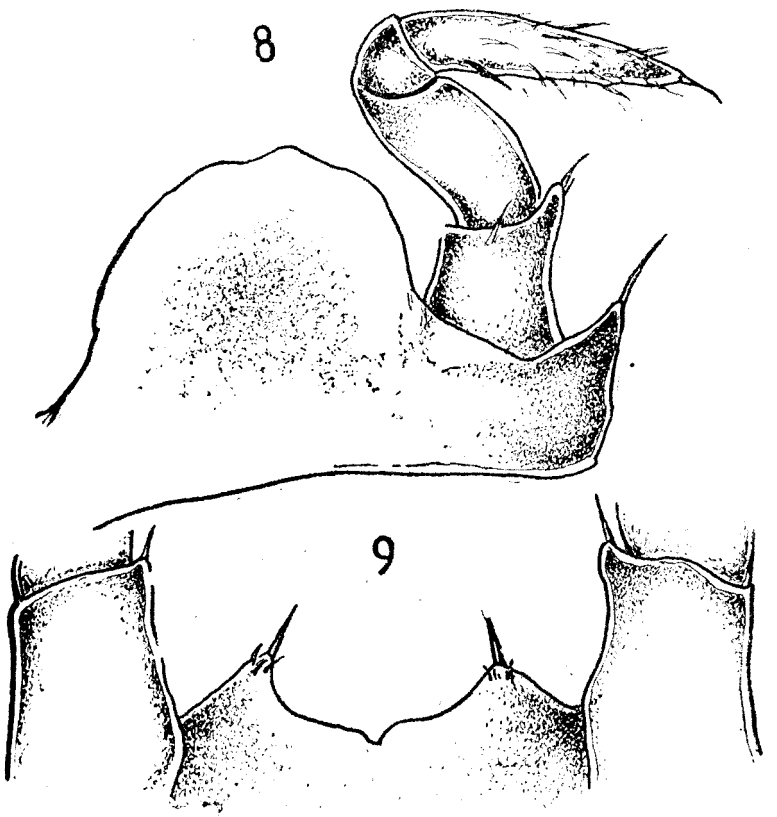

Fig. 8—9. *Glomeris olarii* n. sp. 8. Piciorul stâng al perechii a 17-a de picioare. 9. Sincoxitul perechii a 18-a de picioare.

mate doar din patru articole, sunt mai reduse. Perechea a 18-a de picioare (Fig. 9), și ea mai redusă, are despicătura sincoxitului largă. Perechea a 19-a de picioare sau telopodele (Fig. 10) au articolele ușor mai îngroșate ca de obicei. Lobul sincoxitului aici caracteristic este puțin înalt, fiind evident înclinat posterior. Acest aspect iese și mai clar în relief datorită muchiei orizontale de la baza acestui lob, de pe ale cărui margini se desprind cele două prelungiri laterale, subțiri și înclinate medial; ele au vârfurile evident bifurcate și, ca de obicei, cu mici „peri" pe laturile lor interne. Prefemurele au prelungirile digitiforme lungi și ușor curbate bazal. Femurele, mai groase decât prefemurele, se continuă pe fețele lor posterioare cu câte o prelungire mediană lată, deasupra cărora, pe fețele anterioare, sunt situate prelungirile digitiforme ale acesteia, relativ lungi și ele, prevăzute, ca de obicei, cu câte o setă lungă. Tibiile au prezente, tot pe fețele lor posterioare, prelungirile obișnu-

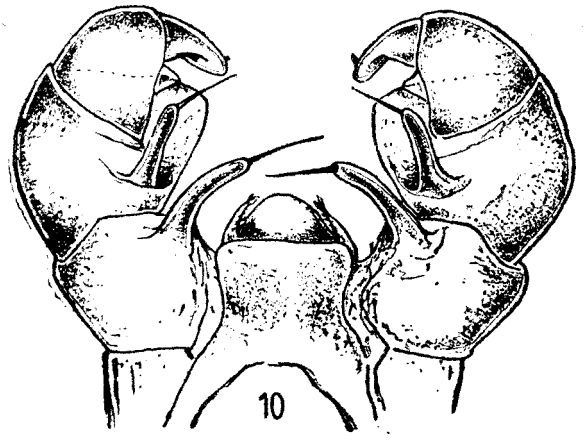

Fig. 10. *Glomeris olarii* n. sp. Perechea a 19-a de picioare (telopodele).

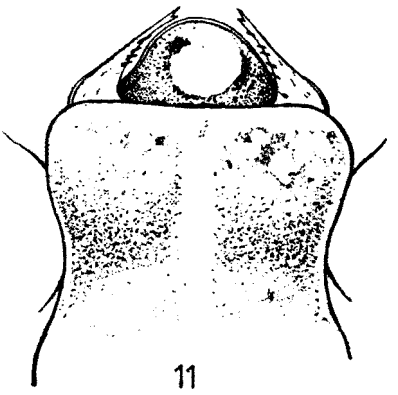

Fig. 11. *Glomeris olarii* n. sp. Sincoxitul perechii a 19-a de picioare.

ite, relativ mici dar evidente; ca de obicei, tarsele sunt subțiri și ușor curbate posterior, în vârf cu scurta setă obișnuită.

Proveniența: au fost colectați 2 ♂♂ + 1 ♀ de pe Muntele Suhard în 27. VIII. 1994 și 1 ♂ de la Crucea în 18. VIII. 1994, leg. L. Olaru.

Prin neobișnuitul aspect al lobului sincoxitului perechii a 19-a de picioare, înclinat oblic posterior, al muchiei de la baza lui, precum și al prelungirilor lui laterale, dispuse convergent, această nouă specie se de-

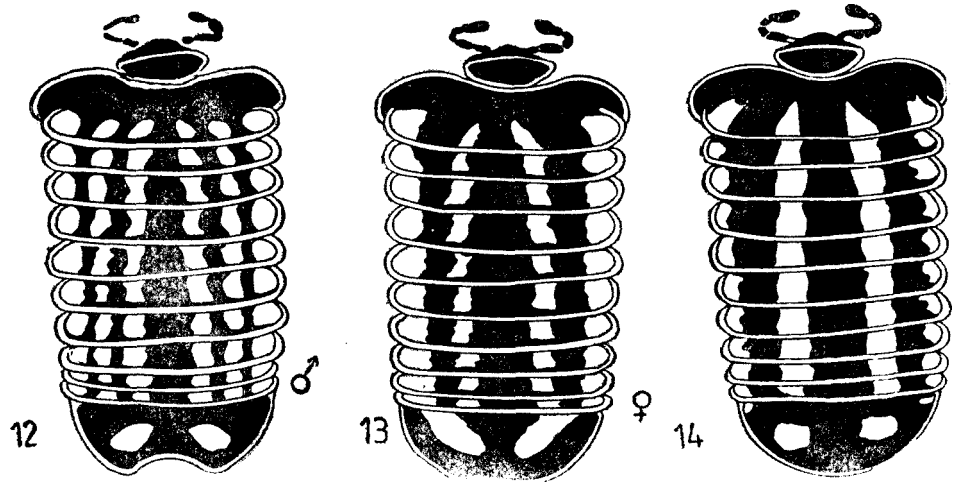

Fig. 12—13. *Glomeris pachytelopoda* Ceuca 1989. 12. Aspectul spatelui la ♂. 13. Aspectul spatelui la ♀.

Fig. 14. *Glomeris prominens* Attems 1903. Aspectul spatelui.

osebeşte clar de formele de la noi; prin şirurile de pete deschise de pe tergite, ar putea fi confundat, la prima vedere, cu *Gl. prominens* Attems 1903.

* * *

Pentru că în lucrarea [1] nu am avut condiţia necesară de a reda şi aspectul spatelui (tergitelor) la specia *Glomeris pachytelopoda* Ceuca 1989, care prezintă şi un dicromism sexual, am socotit să includem în această notă acest aspect. Iată în Fig. 12 şi 13 am redat desenele de pe tergitele celor două sexe. La ♂ , există atât pe bisintergit, cât şi pe fiecare tergit câte 3+3 pete deschise, pe când la ♀ există doar câte 2+2 şiruri de pete. În plus, se poate observa că scutul preanal la ♂ este mai adânc scobit, decât la ♀ . Deşi Jermy [2] dă doar câteva jumătăţi de bisintergite şi tergite plus desenul scutului preanal la *Gl. prominens*, am considerat că nu ar fi de prisos de a da (Fig. 14) aspectul întregului spate al acestei specii.

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ILYOCYPRIS DECIPIENS MASI 1906 *(CRUSTACEA, OSTRACODA)*, ESPÈCE NOUVELLE POUR LA FAUNE DE LA ROUMANIE

VALENTIN POPA*

SUMMARY. — Ilyocypris decipiens Masi 1906 (Crustacea, Ostracoda), **a New Species for the Fauna of Romania.** It was collected in the southern part of the country in a swamp. The features of this species are: the shape of the valves that show pronounced denivelation for the dorsal margin, the general aspect of the valve surface with numerous prominences, the length of the A2 setae that extend beyond the extremity of the last joint.

La sous-famille *Ilyocyprinae* fait partie de la grande famille *Cyprididae*, qui renferme la plupart des Ostracodes d'eau douce. Ses représentants sont encadrés dans un genre unique — *Ilyocypris*. Certains auteurs y ajoutent d'autres genres, tels que *Orygoilyocypris, Rhinocypris* et *Damonella*, mais d'autres sont d'avis que ceux-ci ne sont que des sous-genres d'*Ilyocypris*.

Selon Hartmann [5], la sous-famille comprend 20 espèces actuelles et 43 espèces fossiles. Bronstein [3] décrit 10 espèces répandues sur le territoire de l'ex Union Soviétique. Des données importantes sur la taxonomie et la distribution géographique des *Ilyocyprinae* dans l'Europe de l'Est ont été rapportées par Petkovski [6, 7]. Les références concernant la présence en Roumanie des *Ilyocyprinae* sont peu nombreuses, jusqu'à présent n'étant citées que trois espèces, à savoir: *Ilyocypris gibba* Ramdohr 1808, *I. bradyi* Sars 1890 et *I. getica* Masi 1906 [1, 2, 4, 8—10].

Des études faunistiques que nous avons effectuées dans une mare temporaire des environs du chantier naval de Giurgiu nous ont permis d'identifier *Ilyocypris decipiens*, espèce auparavant inconnue dans la faune du pays.

MATÉRIEL. Le matériel biologique a été collecté en raclant l'épibenthos à l'aide d'un filet benthonique et conservé ensuite en alcool à 70%. La détermination a été faite sur deux exemplaires femelles, desquels on a prélevé les antennes 1 et 2, les palpes mandibulaires, les maxilles, les appendices thoraciques et la fourche. Ces organes ont été inclus en glycérine et examinés au microscope, les dessins étant effectués à la chambre claire. La description qui suit se rapporte aux caractères morphologiques utilisés couramment pour la différentiation des espèces d'*Ilyocypris*.

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DESCRIPTION D'ILYOCYPRIS DECIPIENS.

Valves. Vues en profil, elles ont la forme rectangulaire qui caractérise toutes les espèces du genre. Etablies sur les deux exemplaires, leurs dimensions sont les suivantes:

Valve droite: L = 0,600 mm, H moyenne = 0,305 mm, H postérieure = 0,330 mm, H antérieure = 0,330 mm (Fig. 1a);

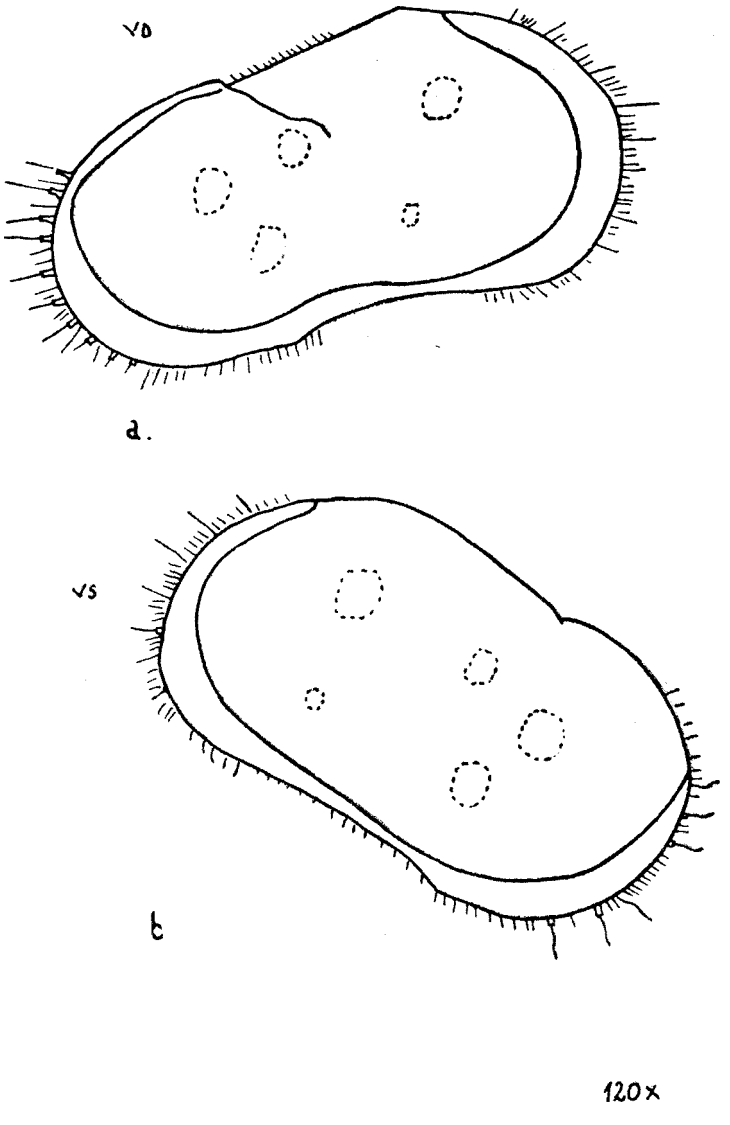

Fig. 1. Valve droite (a) et valve gauche (b); vues latérales.

Valve gauche: L = 0,605 mm, H moyenne = 0,315 mm, H postérieure = 0,330 mm, H antérieure = 0,340 mm (Fig. 1b).

En conformité avec la description originale (Masi 1906 citée par Petkovski [6]), on constate que la valve gauche est légèrement plus longue que la valve droite. Chez les deux valves, la hauteur mesurée sur la ligne médiane est égale à la moitié de la longueur, tandis que la hauteur maximum est atteinte dans la partie antérieure. Sur la surface des valves il y a des proéminences ressemblant à des bosses, bien plus nombreuses que chez *Ilyocypris gibba*.

Antenne 1 (antennule). Elle se compose de 7 articles, de plus en plus rétrécis vers l'extrémité apicale. L'article basal porte trois soies, dont

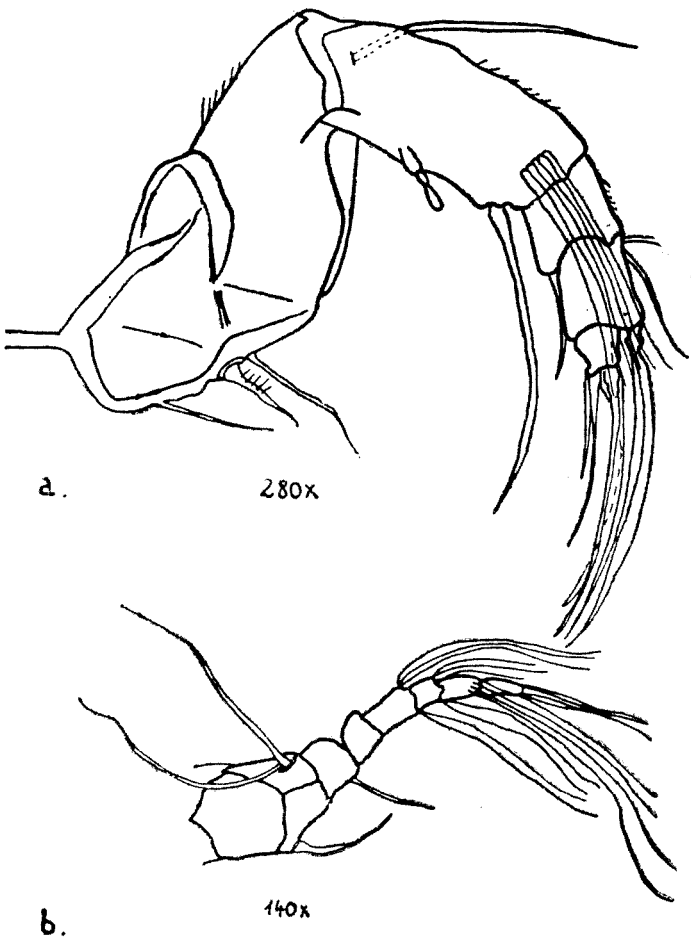

Fig. 2. Antenne 2, face latérale droite (a) ; antenne 1, face postérieure (b).

les deux situées sur le bord externe sont plus iongues que celle placée sur le bord interne; leurs points d'insertion sont très rapprochés. L'article 2 a une seule soie. L'article 3 est dépourvu de soies. Les articles 4—7 présentent sur les deux bords (externe et interne) des soies natatoires de longeurs différents. Le sommet de l'antenne est pourvu de deux épines rigides en forme de griffe (Fig. 2b).

Antenne 2 Elle est formée de 5 articles ayant les dimensions suivantes: 1 = 0,013 mm, 2, 3, 4 = 0,014 mm, 5 = 0,010 mm. A sa base se trouvent trois soies, dont celle médiane, caractéristique pour le genre *Ilyocypris*, est plus épaisse et couverte des poils très fins. L'article basal présente sur son bord externe des petites soies fines. L'article 2 a sur le bord interne une soie sensitive constituée de trois pièces, ainsi qu'une longue soie, qui dépasse la moitié de la longueur des griffes terminales. Sur le bord externe il y a une soie qui arrive jusqu'au niveau de l'article suivant. De même que sur l'article 3, ce bord porte des poils très fins. Dans la partie apicale de l'article 2 se trouve six soies natatoires, dont la pointe atteint la base des griffes terminales. L'article 3 pourvu de trois soies, deux sur le bord externe et une sur le bord interne. A l'extrémité apicale de l'article 4 il y'a trois griffes, et à celle de l'article 5, deux griffes inégales et une soie fine (Fig. 2a).

Palpe mandibulaire. Il est formé de 4 articles. L'article basal présente une petite plaque respiratoire orientée obliquement vers le haut. Sur le bord interne des articles 1 et 2 se trouvent des nombreuses soies poilues. Sur le bord externe de l'article 2 il y a deux soies épaisses et poilues. L'article 3 porte trois soies sur le bord externe et une soie épaisse sur le bord interne. L'article terminal a des nombreuses griffes et soies, dont la fonction est de filtrer le limon (Fig. 3b).

Maxille. D'aspect pyriforme, elle est pourvue d'une petite plaque respiratoire, à la base de laquelle s'insèrent trois soies orientées vers le palpe maxillaire. La base de la maxille est constituée de trois lobes (processus) maxillaires, pourvus de dents aigues qui forment un véritable filtre. Dans la partie proximale du lobe interne se trouvent deux soies, une longue et poilue, l'autre courte et mince (Fig. 3a).

Palpe maxillaire. Il est biarticulé. L'article basal est pyramidal, celui terminal est hémisphérique et présente plusieurs dents rigides et longues. A la base du premier article il y a deux soies (Fig. 3a).

Patte 2. Elle se compose de 5 articles. Le rapport entre la longueur de la patte et celle de la griffe terminale est de 1,286. Dans leur partie proximale, les articles 3 et 4 portent chacun une soie dilatée vers sa base. Le dernier article présente des soies fines et courtes (Fig. 4b).

Patte 3. Orientée obliquement vers le haut, elle est formée de 4 articles. L'article 2 porte une soie sur le bord externe de la partie dis-

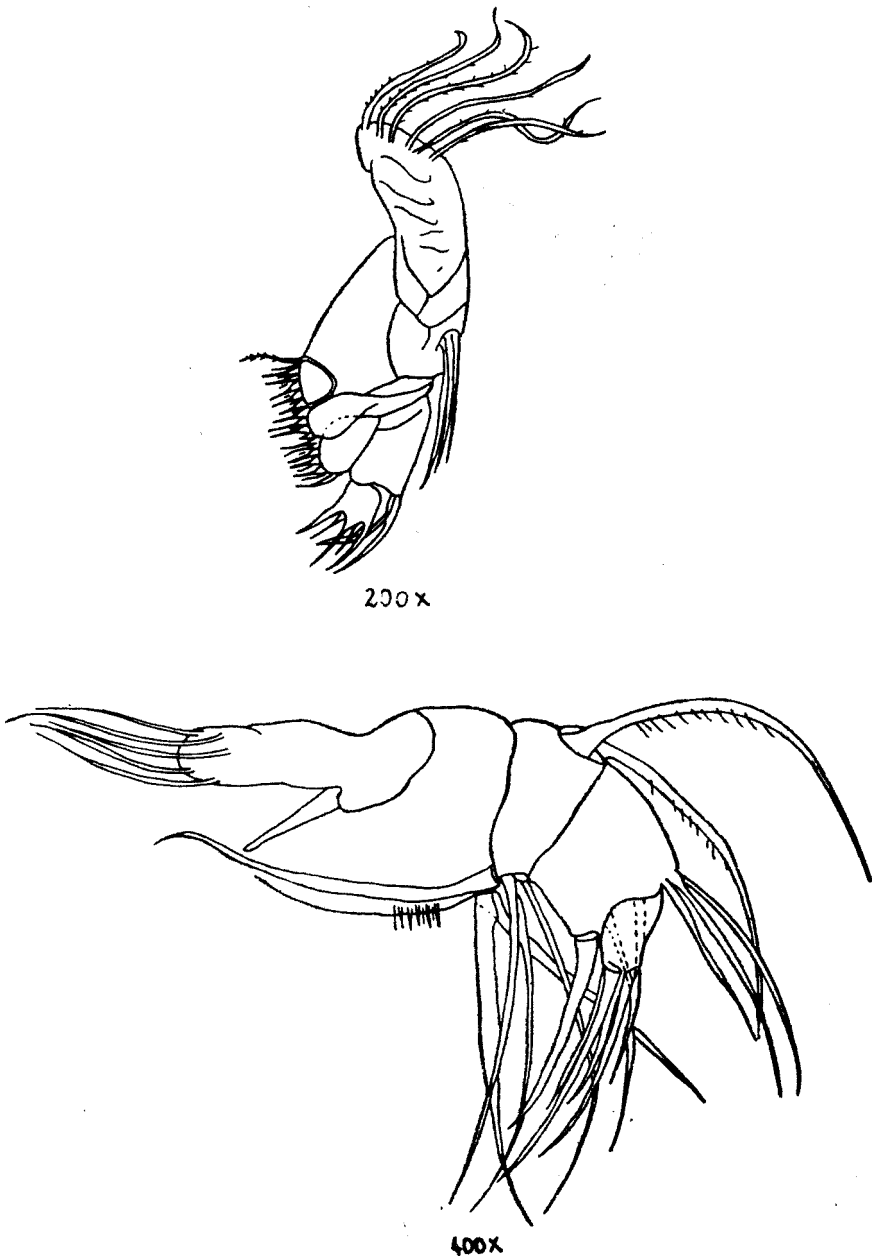

Fig. 3. Maxille, face antérieure (a) ; palpe mandibulaire, face latérale gauche (b).

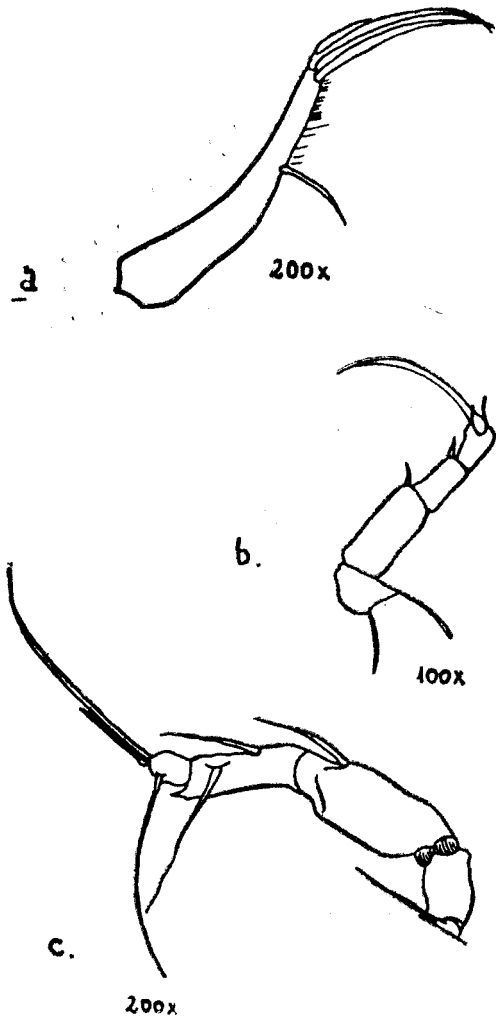

Fig. 4. Fourche, face latérale droite (a); patte 2, face latérale droite (b); patte 3, face latérale droite (c).

tale. L'article terminal est pourvu de trois soies de longeur différente (Fig. 4c).

Fourche. Elle est visiblement courbée. Les griffes terminales ont à peu près la même longueur. Entre celles-ci et la soie latérale il y a des p ils fins (Fig. 4a).

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STUDII OSTEOMETRICE ASUPRA SCHELETULUI CENTURII SCAPULARE ŞI A MEMBRELOR LA ŞORECARUL COMUN *(BUTEO BUTEO* L., CL. *AVES)*

ERIKA GÁL*, EUGEN KESSLER* şi ŞTEFAN KOHL**

SUMMARY. — Osteometrical Studies of the Pectoral Girdle and Forelimb Skeleton of Buzzards *(Buteo buteo L., Cl. Aves).* In this paper the authors present the results of their morphological and biometrical studies of the partial and entire skeletons of 204 buzzards. The osteological material belongs to the bird collection of the Secondary School No. 2. in Reghin (Mureş county, Romania). Parts of the collection relevant to other bird species will be described in other papers.

Şorecarul comun *(Buteo buteo* L.) este o specie de pasăre răpitoare de zi foarte frecventă în Europa, deci şi în ţara noastră. În cadrul prezentei lucrări vom trata caracteristicile osteologice şi biometrice ale celor 204 de schelete parţiale şi integrale existente în colecţia Liceului nr. 2 din Reghin (jud. Mureş).

Caracteristicile osteologice ale speciei au fost studiate de mai mulţi autori, dintre care citãm lucrările lui Otto [4] şi Schmidt-Bürger [5], dar studiile lor se bazează pe examinarea unui număr mult mai mic de exemplare (67). Comparativ vom folosi şi aceste date — alături de observaţiile şi măsurătorile efectuate de către noi asupra exemplarelor din colecţiile de la Oradea (Muzeul „Ţării Crişurilor"), Budapesta (Muzeul de Istorie Naturală) şi Cluj-Napoca (Catedra de Zoologie a Facultăţii de Biologie şi Geologie, Universitatea „Babeş-Bolyai"). Având în vedere faptul că autorii sus amintiţi nu au efectuat în toate cazurile măsurători identice cu cele ale noastre, am luat în considerare doar datele comparabile.

Scopul final al studiilor noastre osteologice şi biometrice asupra exemplarelor din colecţia de la Reghin este nu numai valorificarea lor ştiinţifică, ci şi obţinerea unor puncte de reper cât mai sigure în vederea determinării resturilor fosile şi subfosile de şorecar din materiale paleontologice şi arheozoologice atât din punct de vedere morfologic, cât şi biometric. Din motivele arătate mai sus, am făcut un număr cât mai mare de măsurători la fiecare tip de os (Fig. 1—7). Întrucât colecţia de la Reghin s-a format prin păstrarea resturilor scheletice rămase neutilizate în urma naturalizărilor efectuate, marea majoritate a pieselor aparţin scheletului axial, centurii scapulare, plus humerusul şi femurul. Dintre aceştia, doar coracoidul, omoplatul, humerusul şi femurul prezintă interes pentru noi din punct de vedere paleornitologic. Celălalte tipuri de

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piese scheletice (ulna, radius şi tibiotars) au fost tratate mai sumar având în vedere numărul redus de piese existente în colecţie, dar cu menţionarea caracterelor morfologice evident specifice şi ale dimensiunilor măsurate. Numărul mare de oase în cazul celor patru tipuri de piese sus menţionate ne-a obligat, ca alături de dimensiunile limite (minimă şi maximă) să calculăm şi media aritmetică ($\bar{x}$) abaterea standard (S) şi coeficientul de variaţie (S%), cu menţiunea că aceştia nu au nici o însemnătate din punct de vedere paleornitologic.

În cadrul tabelelor cu dimesniunile pieselor am menţionat separat apartenenţa lor la masculi sau femele, având în vedere dimorfismul sexual evident şi la această specie. De asemenea, am menţionat separat datele provenite de la exemplare juvenile, cu menţiunea că ele figurează aşa în Catalogul întocmit de către Ş. Kohl [3], dar din punct de vedere osteologic parvin de la exemplare capabile de zbor şi imposibil de diferenţiat de exemplare mature, având toate caracterele morfologice bine conturate şi dimensiuni caracteristici speciei nominate.

Terminologia anatomică folosită de noi este cea din tratatul lui Baumel şi colaboratori [1], iar caracterele menţionate în cadrul diagnozelor sunt prezentate alături de modelele de măsurare ale oaselor (Fig. 1—7).

Menţionăm că autorii au publicat deja datele biometrice şi diagnozele privind părţile scheletice ale altor două specii de *Accipitridae* din această colecţie: *Accipiter gentilis* [2] şi *Accipiter nisus* (sub tipar).

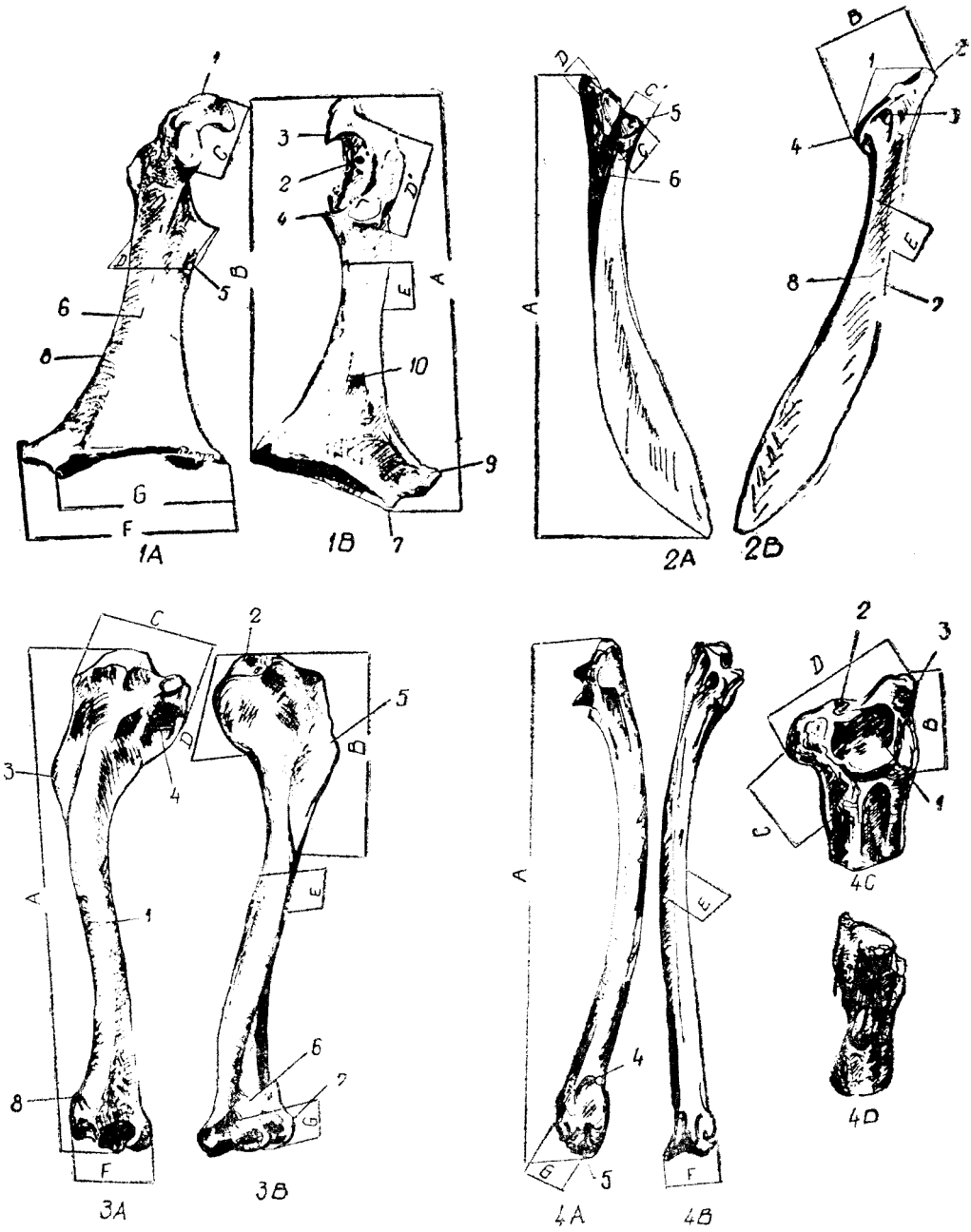
DIAGNOSIS

Coracoid (Fig. 1A, 1B)

Coracoidul în sine apare de factură robustă. *Acrocoracoidul* (1) este rotunjit, mai puţin ascuţit. *Foramen pneumaticum* (2) este slab reprezentat, cu orificiu redus ca mărime, înconjurat cu bare osoase. *Tuberculum dorsale* (3) este ascuţit şi încovoiat, formând un arc cu *processus scapularis* (4). *Processus scapularis* este ascuţit şi sub el se află, aproape de margine, sub formă de fantă *foramen supracoracoideum* (5). *Linea medialis* (6) este puţin proeminentă şi se termină într-un *apex lateralis* (7) şters. *Margo lateralis* (8) se subţiază spre *processus lateralis* (9) formând o creastă. *Tuberositas sternocoracoidalis* (10) apare ca o proeminenţă aplatizată, însă bine conturată. *Foramen ventrale scapulae* absent.

Scapula (Fig. 2A, 2B)

La *caput scapulae* (1), sub *apex acromialis* (2) se află un *cavitas articularis* (3) bine dezvoltat, de formă triunghiulară. *Apex coracoidalis* (4) este proeminent şi rotunjit. *Facies articularis humeralis* (5) este oval şi relativ aplatizat în raport cu caput scapulae. *Collum scapulae* (6) este străbătut oblic de o liine care leagă *margo medialis* (7) de *facies articularis humeralis*. *Corpus scapulae* (8) are forma de lamă turtită, curbată lateral.



Planşa 1. Fig. 1A şi 1B. *Coracoid* — Vedere cranială şi caudală. Fig. 2A şi 2B. *Scapula* — Vedere dorsală şi ventrală. Fig. 3A şi 3B. *Humerus* — Vedere medială şi laterală. Fig. 4A, 4B, 4C şi 4D. *Cubitus* — Vedere ventrală şi dorsală, *epifiza proximală* — Vedere ventrală şi *epifiza distală* — Vedere laterală.

Humerus (Fig. 3A, 3B)

Corpus humeri (1) este suplu și cu o curbură mai puțin evidentă. *Caput humeri* (2) este relativ ascuțit. *Tuberculum ventrale* (3) se ridică oblic deasupra *fossa pneumatica* (4). *Crista pectoralis* (5) este mai îngustă, dar mai alungită și de formă triunghiulară. *Impressio m. brachialis* (6) este bine conturat. Epifiza distală este mai îngustă. Pe partea mediodorsală a *epicondylus dorsalis* (7) se află o fosă ovoidă puțin adâncă. *Epicondylus dorsalis* prezintă o proeminență evidentă. Marginea mediană a *epicondylus ventralis* (8) este puțin evidentă.

Cubitus (Fig. 4A, 4B)

Facies articularis ventralis (1), *facies articularis dorsalis* (2) și *facies ligamenti* (3) sunt mai adânci decât la genurile *Accipiter* și *Circus*, și asemănători cu genul *Milvus*. Capătul proximal al crestei *condylus externus* (4) este mai rotunjit decât la alte specii și genuri. Epifiza distală văzut lateral este mai îngustă decât la genul *Accipiter*.

Radius (Fig. 5A, 5B)

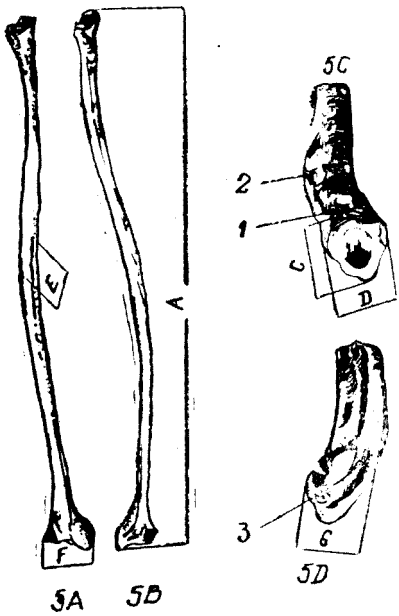
Facies articularis ulnaris (1) este estompat și mai redus decât la *Accipiter* sau *Milvus*. Este redus și *tuberculum bicipitale* (2). La capătul distal *tuberculum mediale* (3) este mic și puțin proeminent.

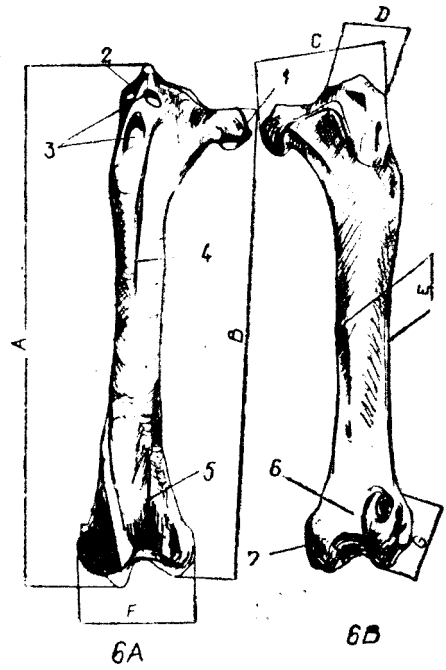
Femur (Fig. 6A, 6B)

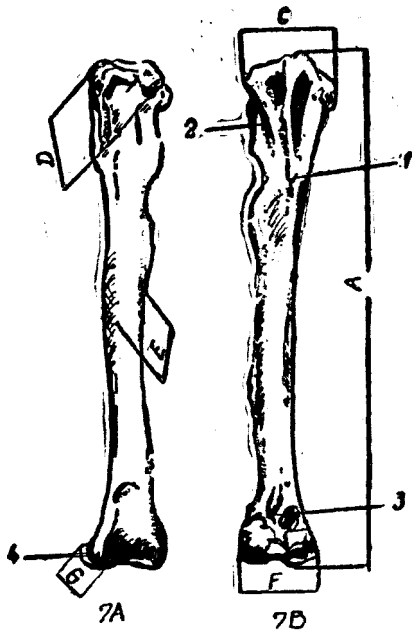
Fovea lig. capitis (1) este o fosă rotundă și adâncă. *Crista trochanteris* (2) este bine dezvoltată. *Foramen pneumaticum* (3) este dublu, orificiul de sus fiind mai mic și rotund, iar cel din jos este oval și mai mare. *Linea trochanterica cranialis* (4) este evidentă, dreaptă și se întinde până în mijlocul diafizei. *Sulcus patellaris* (5) este larg și de formă semiovală. *Fossa poplitea* (6) este mărginită cranial de o linie orientată oblic dinspre *condylus medialis* (7).

Tibiotars (Fig. 7A, 7B)

Tuberozitatea (1) la baza *crista tibiae* (2) este puțin evidentă, ștearsă comparativ cu genurile *Circus* și *Accipiter*. *Crista tibiae* văzut lateral prezintă un aspect triunghiular. *Pons supratendineus* (3) este drept, asemănător cu genul *Milvus*, cu deosebire la *Circus* și *Accipiter*, unde este curbat. *Condylus distalis medialis* (4) văzut medial este îngust, cu deosebire de celelalte genuri.







Planşa 2. Fig. 5A, 5B, 5C şi 5D. *Radius* — Vedere ventrală şi dorsală, *epifiza proximală* — Vedere caudală şi *epifiza distală* — Vedere medială. Fig. 6A şi 6B. *Femur* — Vedere dorsală şi caudală. Fig. 7A şi 7B. *Tibiotars* — Vedere plantară şi dorsală.

Tabel 1

Dimensiunile coracoidului la Buteo buteo (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-max.	$\bar{x}$	S	S%
A	R.	adult	masc.	92	39,0—47,9	43,51	0,83	1,91	fem.	104	43,0—48,1	45,59	0,81	1,78
		juv.		4	41,7—44,4	43,3	0,58	1,34		2	43,0—43,1			
		total		96	39,0—47,9	43,51	0,35	0,80		106	43,0—48,1	45,55	0,10	0,12
	Bp.	adult								1	45,2			
	Cj.	,,								1	43,4			
	M.	,,		22	40,2—47,2	43,30	1,81	4,18		26	41,0—46,6	44,47	1,35	3,04
	Or.	,,								1	43,6			
B	R.	adult	masc.	92	36,0—42,0	38,97	0,28	0,72	fem.	104	38,6—45,2	41,63	0,39	0,94
		juv.		4	38,0—40,6	39,1	0,58	1,41		2	38,1—38,7			
		total		96	36,0—42,0	38,97	0,37	0,96		106	38,1—45,2	41,56	0,82	1,97
	Bp.	adult								1	39,8			
	Cj.	,,								1	39,6			
	M.	,,		22	36,9—42,8	39,59	1,48	3,74		26	38,1—42,1	40,60	1,18	2,91
	Or.	,,								1	40,0			
C	R.	adult	masc.	76	10,4—13,5	11,83	0,58	4,90	fem.	84	11,6—14,6	13,03	0,19	1,46
		juv.		4	11,3—13,1	12,16	0,40	3,29		2	12,1—12,8			
		total		80	10,4—13,5	11,85	0,58	4,89		86	11,6—14,6	13,02	0,36	2,76
	Bp.	adult								1	12,8			
	Cj.	,,								1	13,1			
	Or.	,,								1	12,1			
D	R.	adult	masc.	92	7,7—10,6	9,41	0,15	1,59	fem.	104	9,1—11,3	10,12	0,31	3,06
		juv.		4	9,2—10,3	9,8	0,58	5,92		2	9,7—10,0			
		total		96	7,7—10,6	9,43	0,23	2,44		106	9,1—11,3	10,11	0,26	2,57
	Cj.	adult								1	8,8			
D'	R.	adult	masc.	92	9,6—13,4	11,48	0,28	2,48	fem.	101	10,6—13,9	12,19	0,42	3,45
		juv.		4	10,4—11,8	11,02	0,58	5,26		2	11,1—11,7			
		total		96	9,6—13,4	11,47	0,20	1,74		103	10,6—13,9	12,19	0,24	1,97
	Cj.	adult								1	11,7			
E	R.	adult	masc.	92	4,0— 6,9	5,94	0,22	3,65	fem.	104	5,2— 7,5	6,35	0,57	8,98
		juv.		4	5,6— 6,4	6,0	0,58	9,67		2	5,4— 6,5			
		total		96	4,0— 6,9	5,94	0,12	2,06		106	5,2— 7,5	6,36	0,09	1,42
	Bp.	adult								1	6,3			
	Cj.	,,								1	6,4			
	Or.	,,								1	6,1			
F	R.	adult.	masc.	92	17,3—21,6	19,82	0,36	1,82	fem.	103	19,4—22,6	20,96	0,36	1,72
		juv.		4	18,5—20,1	19,40	0,58	2,99		2	19,6—19,8			
		total		96	17,3—21,6	19,80	0,41	2,06		105	19,4—22,6	20,94	0,17	0,81
	Bp.	adult								1	20,0			
	Cj.	,,								1	20,9			
	M.	,,		26	18,0—21,1	19,72	0,77	3,90		28	18,2—21,8	20,37	0,99	4,86
	Or.	,,								1	20,8			
G	R.	adult	masc.	92	14,5—17,7	16,11	0,36	2,23	fem.	103	15,3—19,1	18,13	0,30	1,67
		juv.		4	14,8—16,7	15,90	0,58	3,65		2	15,5—16,5			
		total		96	14,5—17,7	16,11	0,36	2,23		105	15,3—19,1	18,08	0,37	2,05
	Bp.	adult								1	17,0			
	Cj.	,,								1	17,2			
	M.	,,		26	14,2—17,4	15,78	0,93	5,89		28	14,3—17,5	16,21	0,88	5,43
	Or.	,,								1	16,8			

Tabel 2

Dimensiunile scapulei la B u t e o b u t e o (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	94	47,9—61,0	56,60	0,58	1,02	fem.	102	55,1—63,8	58,22	0,28	0,49
		juv.		4	56,8—58,2	57,28	0,58	1,01		1	55,4			
		total		98	47,9—61,0	56,62	0,76	1,34		103	55,1—63,8	58,19	0,68	1,18
	Cj.	adult								1	58,6			
	M.	,,		27	52,9—60,5	56,91	2,03	3,59		25	54,2—60,8	57,22	1,77	3,09
	Or.	,,								1	58,8			
B	R.	adult	masc.	94	11,0—13,8	12,37	0,34	2,75	fem.	102	11,6—14,8	13,29	0,18	1,35
		juv.		4	11,8—12,8	12,21	0,58	4,75		1	12,2			
		total		98	11,0—13,8	12,37	0,15	1,21		103	11,6—14,8	13,28	0,14	1,05
	Cj.	adult								1	13,8			
	M.	,,		28	11,9—14,1	12,96	0,64	4,94		26	11,9—14,3	13,26	0,57	4,30
	Or.	,,								1	13,7			
C	R.	adult	masc.	94	6,9—9,7	8,20	0,23	2,80	fem.	102	7,2—10,1	8,52	0,26	3,05
		juv.		4	8,0—8,4	8,18	0,23	2,81		1	7,8			
		total		98	6,9—9,7	8,20	0,26	3,17		103	7,2—10,1	8,52	0,23	2,70
	Cj.	adult								1	8,1			
	Or.	,,								1	7,3			
C'	R.	adult	masc.	94	3,0—5,3	4,33	0,11	2,52	fem.	102	3,2—5,4	4,59	0,12	2,61
		juv.		4	4,1—4,8	4,40	0,24	5,51		1	4,0			
		total		98	3,0—5,3	4,33	0,20	4,53		103	3,2—5,4	4,58	0,18	3,93
	Cj.	adult								1	4,5			
D	R.	adult	masc.	92	5,2—7,8	6,34	0,24	3,79	fem.	102	5,5—7,5	6,66	0,05	0,75
		juv.		4	5,9—6,6	6,28	0,20	3,18		1	4,8			
		total		96	5,2—7,8	6,33	0,20	3,16		103	5,5—7,5	6,64	0,19	2,86
	Cj.	adult								1	6,3			
E	R.	adult	masc.	94	3,2—6,1	4,98	0,08	1,61	fem.	102	4,4—6,2	5,29	0,20	3,78
		juv.		4	4,8—5,0	4,95	0,18	3,67		1	5,4			
		total		98	3,2—6,1	4,98	0,13	2,61		103	4,4—6,2	5,29	0,16	3,02
	Cj.	adult								1	5,4			
	Or.	,,								1	5,0			

Tabel 3

Dimensiunile humerusului la Buteo buteo (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	5	99,0—108,7	103,80	0,50	0,48	fem.	5	101,7—112,4	107,85	0,50	0,46
	Bp.	,,								1	109,2			
	Cj.	,,								1	104,8			
	M.	,,		20	97,3—109,7	103,42	3,24	3,13		22	99,5—116,6	106,84	3,76	3,52
	Or.	,,								1	104,7			
B	R.	adult	masc.	5	35,8—40,7	38,40	0,50	1,30	fem.	5	38,8— 43,3	41,77	0,50	1,20
	Cj.	,,								1	41,1			
	Or.	,,								1	38,7			
C	R.	adult	masc.	5	20,3—21,6	20,78	0,50	1,74	fem.	5	21,1— 22,7	21,79	0,50	2,29
	Bp.	,,								1	21,2			
	Cj.	,,								1	21,5			
	M.	,,		23	18,8— 21,6	19,92	0,77	3,87		25	18,3— 21,8	20,46	0,73	3,57
	Or.	,,								1	20,5			
D	R.	adult	masc.	5	20,4— 24,5	22,30	0,33	1,48	fem.	5	20,9— 25,4	23,27	0,50	2,15
	Cj.	,,								1	24,2			
	Or.	,,								1	23,2			
E	R.	adult	masc.	5	7,3— 8,3	7,87	0,5	6,35	fem.	5	7,5— 8,5	7,87	0,50	6,35
	Bp.	,,								1	7,5			
	Cj.	,,								1	7,5			
	M.	,,		22	6,7— 8,4	7,33	0,45	6,14		25	6,8— 8,2	7,56	0,32	4,23
	Or.	,,								1	7,3			
F	R.	adult	masc.	5	17,1— 17,9	17,50	0,5	2,86	fem.	5	17,9— 18,7	18,40	0,50	2,72
	Bp.	,,								1	17,8			
	Cj.	,,								1	17,4			
	M.	,,		21	16,3— 18,4	17,32	0,68	3,93		24	16,9— 18,8	17,83	0,66	3,70
	Or.	,,								1	17,1			
G	R.	adult	masc.	4	9,4— 10,0	9,70	0,58	5,98	fem.	5	9,6— 10,7	10,03	0,50	4,99
	Bp.	,,								1	9,5			
	Cj.	,,								1	9,2			
	Or.	,,								1	9,4			

Tabel 4

Dimensiunile cubitusului la B u t e o b u t e o (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	1	124,6				fem.					
	Bp.	,,								1	125,0			
	Cj.	,,								1	124,3			
	M.	,,		19	112,9-128,3	121,07	4,05	3,35		22	118,3—128,3	123,26	3,23	2,62
	Or.	,,								1	124,0			
B	R.	adult	masc.	1	10,6				fem.					
	Bp.	,,								1	10,5			
	Cj.	,,								1	10,7			
	M.	,,		19	8,5— 9,5	8,92	0,30	3,36		23	8,6— 9,9	9,24	0,30	3,25
	Or.	,,								1	10,4			
C	R.	adult	masc.	1	11,9				fem.					
	Bp.	,,								1	12,0			
	Cj.	,,								1	12,6			
	Or.	,,								1	11,8			
D	R.	adult	masc.	1	13,3				fem.					
	Cj.	,,								1	13,2			
	M.	,,		19	12,6—14,8	13,32	0,62	4,65		23	11,2—14,3	13,73	0,69	5,02
E	R.	adult	masc.	1	6,4				fem.					
	Bp.	,,								1	6,0			
	Cj.	,,								1	6,3			
	M.	,,		16	5,3— 6,3	5,61	0,26	4,63		18	5,1— 6,3	5,78	0,32	5,54
	Or.	,,								1	5,7			
	R.	adult	masc.	1	10,3				fem.					
	Bp.	,,								1	11,0			
	Cj.	,,								1	9,6			
	Or.	,,								1	8,8			
G	R.	adult	masc.	1	9,6				fem.					
	Bp.	,,								1	9,0			
	Or.	,,								1	8,9			

Tabel 5

Dimensiunile radiusului la Buteo buteo (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	1	ap. 118,0				fem.					
	Bp.	,,		1	118,0									
	Cj.	,,								1	117,4			
	M.	,,		19	106,—120,9	114,94	4,07	3,54		23	111,2—122,4	116,87	3,43	2,93
	Or.	,,								1	117,5			
C	R.	adult	masc.	1	6,3				fem.					
	Bp.	,,		1	6,3									
	Cj.	,,								1	6,0			
	M.	,,		19	4,9—6,7	6,13	0,39	6,36		24	5,8—6,7	6,35	0,22	3,46
	Or.	,,								1	6,1			
D	R.	adult	masc.	1	4,5				fem.					
	Bp.	,,		1	4,2									
	Cj.	,,								1	4,3			
	M.	,,		19	3,9—4,6	4,26	0,24	5,63		24	4,0—4,8	4,35	0,22	5,13
	Or.	,,								1	4,5			
E	R.	adult	masc.	1	3,4				fem.					
	Bp.	,,		1	3,3									
	Cj.	,,								1	3,3			
	Or.	,,								1	3,4			
F	R.	adult	masc.	1	8,5				fem.					
	Bp.	,,		1	8,0									
	Cj.	,,								1	8,3			
	M.	,,		19	7,4—8,9	8,09	0,44	5,44		24	7,1—8,9	8,26	0,44	5,34
	Or.	,,								1	8,0			
G	R.	adult	masc.	1	4,8				fem.					
	Cj.	,,								1	4,3			

Tabel 6

Dimensiunile femurului la B u t e o b u t e o (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	95	67,0—79,0	74,48	0,44	0,59	fem.	98	71,2—84,2	77,76	0,50	0,64
		juv.		5	73,4—78,8	75,92	0,87	1,15		1	74,4			
		total		100	67,0—79,0	74,55	0,65	0,87		99	71,2—84,2	77,73	0,40	0,51
	Bp.	adult								1	77,3			
	Cj.	,,								1	74,1			
	M.	,,		44	71,0—78,2	74,48	2,17	2,91		45	72,8—80,8	76,97	1,93	2,51
	Or.	,,								1	74,0			
B	R.	adult	masc.	95	64,9—76,5	72,03	0,64	0,89	fem.	98	67,0—79,3	74,87	0,84	1,08
		juv.		5	70,2—76,3	73,15	0,61	0,83		1	71,5			
		total		100	64,9—76,5	72,09	0,45	0,62		99	67,0—79,3	74,84	0,30	0,40
	Bp.	adult								1	74,4			
	Cj.	,,								1	71,1			
	M.	,,		44	68,3—75,3	71,51	2,25	3,15		45	70,5—77,7	73,89	1,82	2,46
	Or.	,,								1	71,0			
C	R.	adult	masc.	95	13,0—15,9	14,00	0,21	1,56	fem.	98	13,5—16,9	15,22	0,38	2,50
		juv.		5	13,5—14,0	13,88	0,37	2,67		1	13,4			
		total		100	13,0—15,9	13,99	0,25	1,79		99	13,4—16,9	15,20	0,27	1,78
	Bp.	adult								1	15,0			
	Cj.	,,								1	14,5			
	M.	,,		44	12,8—15,6	13,68	0,63	4,61		45	13,8—15,6	14,56	0,41	2,82
	Or.	,,								1	16,2			
D	R.	adult	masc.	95	8,4—10,7	9,70	0,19	1,96	fem.	98	9,3—13,7	10,47	0,22	2,10
		juv.		5	8,7—10,1	9,76	0,50	5,12		1	9,4			
		total		100	8,4—10,7	9,70	0,31	3,16		99	9,3—13,7	10,46	0,23	2,20
	Bp.	adult								1	10,0			
	Cj.	,,								1	9,7			
	M.	,,		44	7,3— 8,7	8,09	0,33	4,08		45	8,0— 9,0	8,60	0,30	3,49
	Or.	,,								1	11,3			
E	R.	adult	masc.	95	6,1— 7,6	6,82	0,21	3,08	fem.	98	6,2— 8,6	7,24	0,13	1,80
		juv.		5	6,4— 7,7	6,89	0,26	3,77		1	6,7			
		total		100	6,1— 7,7	6,82	0,11	1,61		99	6,2— 8,6	7,24	0,26	3,59
	Bp.	adult								1	7,0			
	Cj.	,,								1	7,2			
	M.	,,		44	6,0— 7,4	6,50	0,47	7,23		45	6,0— 7,4	6,72	0,35	5,21
	Or.	,,								1	7,4			
F	R.	adult	masc.	95	13,7—16,0	14,85	0,33	2,22	fem.	98	14,3—16,7	15,84	0,19	1,20
		juv.		5	14,5—15,3	14,87	0,39	2,62		1	13,6			
		total		100	13,7—16,0	14,85	0,36	2,42		99	13,6—16,7	15,82	0,18	1,14
	Bp.	adult								1	16,0			
	Cj.	,,								1	15,3			
	M.	,,		44	14,1—16,0	14,84	0,57	3,84		45	14,8—17,0	15,75	0,51	3,24
	Or.	,,								1	15,5			
G	R.	adult	masc.	95	10,2—12,7	11,69	0,21	1,80	fem.	98	10,8—13,6	12,40	0,21	1,69
		juv.		5	10,9—11,9	11,62	0,34	2,93		1	10,8			
		total		100	10,2—12,7	11,68	0,34	2,91		99	10,8—13,6	12,38	0,27	2,18
	Bp.	adult								1	12,0			
	Cj.	,,								1	11,0			
	M.	,,		44	10,3—12,2	10,88	0,59	5,42		45	10,6—12,6	11,48	0,49	4,27
	Or.	,,								1	11,2			

Tabel 7

Dimensiunile tibiotarsului la Buteo buteo (L.)

Dim.	Prov.	Vârsta	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%	Sex	Nr.	Min.-Max.	$\bar{x}$	S	S%
A	R.	adult	masc.	1	103,6				fem.					
	Bp.	,,								1	105,0			
	Cj.	,,								1	102,9			
	M.	,,		31	97,7—106,9	102,39	2,51	2,45		23	100,3—108,3	103,90	2,06	1,98
	Or.	,,								1	102,8			
C	R.	adult	masc.	2	11,7—11,9				fem.					
	Bp.	,,								1	14,0			
	Cj.	,,								1	11,6			
	Or.	,,								1	13,4			
D	R.	adult	masc.	2	13,1—14,4				fem.					
	Bp.	,,								1	12,0			
	Cj.	,,								1	14,4			
	Or.	,,								1	11,4			
E	R.	adult	masc.	2	6,9— 7,2				fem.					
	Bp.	,,								1	6,4			
	Cj.	,,								1	6,1			
	M.	,,		31	5,0— 6,3	5,69	0,30	5,27		23	5,6— 6,7	6,03	0,35	5,80
	Or.	,,								1	7,0			
F	R.	adult	masc.	1	12,4				fem.					
	Bp.	,,								1	12,7			
	Cj.	,,								1	11,6			
	M.	,,		31	11,0—13,2	11,85	0,56	4,73		23	12,2—13,1	12,63	0,28	2,22
	Or.	,,								1	11,6			
G	R.	adult	masc.	1	7,7				fem.					
	Bp.	,,								1	8,7			
	Cj.	,,								1	7,9			
	M.	,,		31	7,1— 8,5	7,71	0,33	4,28		23	8,0— 8,6	8,23	0,14	1,70
	Or.	,,								1	7,2			

Notă explicativă pentru prescurtările folosite în tabele

A — Lungimea maximă a osului. *B* — Lungimea minimă a osului. *C* — Lățimea epifizei proximale. *C'* — Altă lățime a epifizei proximale (Fig. 2A). *D* — Grosimea epifizei proximale. *D'* — Altă grosime a epifizei proximale (Fig. 1B). *E* — Lățimea diafizei. *F* — Lățimea epifizei distale. *G* — Grosimea epifizei distale.

R. — Colecția Liceului Nr. 2. din Reghin. *Bp.* — Colecția Muzeului de Istorie Naturală din Budapesta; măsurătorile au fost efectuate de E. Kessler. *Cj.* — Colecția Catedrei de Zoologie a Universității „Babeș-Bolyai" din Cluj-Napoca. *M.* — Colecția Facultății de Medicină veterinară din München; date din lucrările lui Otto [4] și Schmidt-Bürger [5]. *Or.* — Colecția Muzeului „Țării Crișurilor" din Oradea; măsurătorile au fost efectuate de E. Kessler.

Dim. — Dimensiunea piesei. *Prov.* — Proveniența piesei. *Nr.* — Numărul exemplarelor măsurate. *Min.* — Limita minimă a parametrului măsurat. *Max.* — Limita maximă a parametrului măsurat. $\bar{x}$ — Media aritmetică a parametrului măsurat. S — Abatere standard. S% — Coeficient de variație. *masc.* — Exemplare de sex masculin. *fem.* — exemplare de sex feminin.

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PRELIMINARY STUDIES CONCERNING THE FAUNA OF SMALL MAMMALS IN SOME MOUNTAIN ZONES OF THE SOMEŞUL MIC BASIN, APUSENI MOUNTAINS, ROMANIA

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SUMMARY. — The paper presents the results of our studies in two zones of the Someşul Mic basin, Apuseni Mountains. In both stations, Beliş and Răcătău, 10 species of micromammals have been identified: 4 insectivore species *(Sorex araneus, S. alpinus, Crocidura leucodon* and *Neomys fodiens)* and 6 rodent species *(Apodemus sylvaticus, A. flavicollis, Mus musculus, Microtus arvalis, M. agrestis* and *Clethrionomys glareolus).* The faunistic spectrum is rather different in the two zones: *Crocidura leucodon* and *Neomys fodiens (Insectivora)* are absent in our captures in Beliş and *Sorex araneus, S alpinus (Insectivora), Microtus arvalis* and *M. agrestis (Rodentia)* are missing in our captures in Răcătău. The insectivore populations are more numerous in Răcătău than in Beliş, which suggests that in the Răcătău zone there exist better ecological conditions for insectivores, especially for *Neomys fodiens.* In both zones the adult individuals are prevailing and the general sex ratio has slight oscillations around the 1 : 1 value.

In Romania we have some studies on the micromammal fauna of the mountain zones [2, 4, 10, 11, 15, 17—19], but we have only few data about the small mammals in the mountain zones of north-western Romania [16, 21]. By studying the mountain fauna, we can observe the geographical variability of the species and establish the limits of their ecological valences. For this purpose we have carried out researches in two localities of the Someşul Mic basin situated in the Apuseni Mountains.

Materials and methods. The studies have been carried out in the surroundings of the locality Beliş, situated near the Fântânele accumulation lake, in the Someşul Cald river bed, and in the surroundings of the locality Măguri-Răcătău, situated on the Someşul Rece bank. In Beliş, at the altitude of 1100 m, two forest ecosystems have been selected for studies. Their spontaneous vegetation consists of *Oxalo-Picetum abietis* and *Luzulo sylvaticae-Picetum abietis* plant communities. In the Măguri-Răcătău zone which is situated at the altitude of 1219 m, some forest ecosystems have been selected, too. Their vegetation consists of *Oxalo-Picetum abietis, Vaccinio-Picetum abietis* and *Chrysanthemo-rotundifolio-Piceo-Fagetum sylvaticae* plant communities.

The small mammals have been caught with baited live traps, 70 in each locality, during 10 days in each station (16—25 June and 6—15 September 1995). The traps were placed linearly, at a distance of 8 m from one another. All the captured animals have been measured, weighed and studied in the laboratory, where their species, sex and age were determined. By statistical methods we have calculated the relative abundance, the biodiversity of the species, using the Simpson index, and the ecological affinity of species communities (the similarity between the two zones), using the Jaccard index.

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Results and discussion. In the two localities we made about 1400 traps/10 nights, and 283 individuals of small mammals, belonging to 10 species of two orders, have been caught (Table 1). In the mountains the

Table 1

Numbers of micromammals caught in the Beliș and Răcătău zones

Species	Beliș		Răcătău	
	No. ind.	%	No. ind.	%
Ord. Insectivora	19	11.45	24	20,51
1. Sorex alpinus	6	2.41	—	—
2. Sorex araneus	13	9.04	—	—
3. Crocidura leucodon	—	—	7	5.98
4. Neomys fodiens	—	—	17	14.53
Oed. Rodentia	147	88,55	93	79,49
1. Apodemus sylvaticus	32	19,28	51	43,59
2. A. flavicollis	21	12.65	17	14.53
3. Mus musculus	11	6.63	3	2.56
4. Microtus arvalis	12	7.23	—	—
5. M. agrestis	47	28.31	—	—
6. Clethrionomys glareolus	24	14.46	22	18.80
Total micromammals	166	100	117	100

average temperature is lower and the rains are more frequent in summer and autumn than in other zones. Consequently, the abundance and the specific diversity are lower in the mountains than in other ecosystems. In Beliș we caught two species belonging to insectivores, *Sorex araneus* and *S. alpinus*, and in Răcătău two species of insectivores: *Crocidura leucodon* and *Neomys fodiens*. The number of insectivore species is very low comparing with other ecosystems, situated at a lower altitude, but the number of individuals is rather high, comparable with other studied ecosystems [1, 3, 8, 9, 11—14].

In both localities the rodents' number is higher than that of the insectivores (Table 1), which is due to their higher density and to the larger limits of their ecological valences in comparison with the insectivores. In the Răcătău zone the number of insectivores is higher than in Beliș (Table 1). It is possible that this difference is due to the different ecological conditions and to the different period of catching (at the beginning of the reproductive period in Beliș, and at the end of the reproductive period in Răcătău).

The number of rodent species is greater in the Beliș zone (6 species) than in Răcătău (4 species) Individuals of *Microtus* species are missing in our captures from the Răcătău zone (Table 1). It is possible that they had no satisfactory ecological conditions for reproduction that year. Contrarily to this situation, in Beliș the *Microtus* species are

rather abundant, *M. agrestis* being the dominant species, which constitutes about 30% of all the animals of the zone, with a dominance index of 0.49.

The *Apodemus* species *(A. sylvaticus* and *A. flavicollis)* occur in both studied zones (Table 1). In Răcătău *A. sylvaticus* is the dominant species with a dominance index of 0.62 and constitutes almost half of all the micromammals caught in this locality. In Beliş this species is on the second place, after *Microtus agrestis. Clethrionomys glareolus* is rather abundant in both stations, being on the second place in Răcătău and on the third place in Beliş (Table 1). The abundance of this species is mentioned in several studies on mountain fauna [4—6, 10, 20]. The house mouse *(Mus musculus)* has been caught in the traps placed near the houses only. Their density is much lower than in the low altitude zones.

Dispersion of the species within the studied perimeters of the zones depends on the ecological requirements of the animals. The species with preferences for damp biotopes, such as *Neomys fodiens,* have been caught in the traps placed on the river bank only. As to the house mouse, we have already mentioned that it has been caught in the traps placed near the houses. We caught *Crocidura leucodon* in the forest glades with rather high degree of dryness, far from water.

The biodiversity index of the Beliş zone is equal to 0.18 and that of the Răcătău zone is 0.27 which means that species diversity in Beliş is greater than in Răcătău. The similarity between the two studied zones is 44.4% which means that less than half of the species are common for both zones.

The sex ratio and the age groups in each locality have been established. In both zones the sex ratio for all species is close to 1 : 1 value (1.1 : 1 in Beliş and 1.4 : 1 in Răcătău with the prevailing of males). In most species from Beliş the males are more numerous, except for *Mus*

Table 2

Sex ratio of micromammals caught in the Beliş and Răcătău zones

Species	Beliş				Răcătău			
	♂♂		♀♀		♂♂		♀♀	
	No. ind.	%	No. ind.	%	No. ind.	%	No. ind.	%
Ord. Insectivora	9	53.3	10	46.7	15	62.5	9	37.5
1. Sorex alpinus	1	16.67	5	83,33	—	—	—	—
2. Sorex araneus	8	53.3	5	46.7	—	—	—	—
3. Crocidura leucodon	—	—	—	—	7	100	—	—
4. Neomys fodiens	—	—	—	—	8	47.06	9	52.94
Ord. Rodentia	78	53.06	69	46.94	53	56.99	40	43.01
1. Apodemus sylvaticus	22	68.75	10	31.25	27	52.94	24	47.06
2. A. flavicollis	14	66.67	7	33.33	13	76.47	4	23.53
3. Mus musculus	4	36.36	7	63.64	—	—	3	100
4. Microtus arvalis	4	33.33	8	66.67	—	—	—	—
5. M. agrestis	25	53.19	22	46.81	—	—	—	—
6. Clethrionomys glareolus	9	37.5	15	62.5	13	59.09	9	40.91
Total micromammals	87	53.09	79	46.91	68	58.12	49	41.88

musculus, *Microtus arvalis* and *Clethrionomys glareolus*, where the females are prevailing (Table 2). In Răcătău the males are also more numerous in the majority of species, except for *Neomys fodiens* and *Mus musculus*, where the females are prevailing (Table 2).

Concerning the age groups, in both localities the adult individuals are prevailing. Juvenile individuals were found in the Beliș zone only, and in the *Microtus* species only (Table 3) which is due to their earlier

Table 3

Age groups in micromammals caught in the Beliș and Răcătău zones

Species	Beliș						Răcătău			
	Adults		Subadults		Juveniles		Adults		Subadults	
	No. ind.	%	No. ind.	%	No. ind.	%	No. ind.	%	No. ind.	%
Ord. Insectivora	19	100	—	—	—	—	17	70.83	7	29.17
1. Sorex alpinus	6	100	—	—	—	—	—	—	—	—
2. Sorex araneus	16	100	—	—	—	—	—	—	—	—
3. Crocidura leucodon	—	—	—	—	—	—	—	—	7	100
4. Neomys fodiens	—	—	—	—	—	—	17	100	—	—
Ord. Rodentia	101	68.71	32	21.77	14	9.52	63	67.74	30	32.26
1. Apodemus sylvaticus	26	81.25	6	18.75	—	—	42	82.35	9	17.65
2. A. flavicollis	15	71.43	6	28.57	—	—	9	60	8	40
3. Mus musculus	11	100	—	—	—	—	3	100	—	—
4. Microtus arvalis	4	33.33	4	33.33	4	33.33	—	—	—	—
5. M. agrestis	29	61.7	8	17.02	10	21.28	—	—	—	—
6. Clethrionomys glareolus	16	66.67	8	33.33	—	—	9	40.9	13	59.1
Total micromammals	120	71.6	32	19.75	14	8.65	80	68.38	37	31.62

start of reproduction (end of May — beginning of June). The number of juveniles is rather low, less than 9% of all the animals (Table 3).

All the captured individuals of *Sorex araneus* and *S. alpinus* were adult, because their reproductive period begins later (middle of June). Juvenile individuals had not been born yet and the subadults from the previous year had already attained the adult's size. Contrarily to this fact, all the shrews *Crocidura leucodon* from Răcătău were subadults, because of the end of reproductive period (September), when the juvenile shrews attained the subadult's size. All individuals of water shrew were caught near the Someșul Rece river only and they all were adults. It is possible that subadults of *Neomys fodiens* are more suspicious or they are negatively affected by the aggressive behaviour of the adults which were very territorial [7].

The only species in which the number of subadults exceeded that of the adults was *Clethrionomys glareolus* in Răcătău. In other species the adult individuals were prevailing and constituted more than 60% of the animals (Table 3).

Conclusions. In the studied biocenoses of the Apuseni Mountain ecosystems, in the Someşul Mic basin, 10 species of small mammals (rodents and insectivores) have been identified. In the Beliş zone two species of insectivores and 6 species of rodents and in the Răcătău zone 2 species of insectivores and 4 species of rodents have been found. Abundance and specific diversity of the micromammal species were lower in the mountains than in the zones at low altitude. The faunistic spectrum is rather different in the studied zones: in Beliş *Crocidura leucodon* and *Neomys fodiens (Insectivora)* and in Răcătău *Sorex araneus, S. alpinus (Insectivora)* and *Microtus arvalis* and *M. agrestis (Rodentia)* were absent. The dominant species was *Microtus agrestis* in Beliş and *Apodemus sylvaticus* in Răcătău. Sex ratio in both zones had slight oscillations around the 1 : 1 value, but in most species the males were more numerous. The adults were prevailing in both studied localities.

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PRELIMINARY DATA ON THE MICROMAMMAL FAUNA IN THE SOMEŞUL MIC BASIN (ROMANIA) ACCORDING TO *ASIO OTUS OTUS L.* PELLETS

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SUMMARY. — The pellets have been collected in two localities in the Someşul Mic basin: Cluj-Napoca and Cojocna. From the total of 2131 pellets, 1403 came from Cluj-Napoca and 728 from Cojocna; 5599 bones of birds and micromammals have been separated: 3776 from Cluj-Napoca and 1823 from Cojocna. The birds constitute 9.01% in Cluj-Napoca pellets and 0.93% in Cojocna pellets. 16 species of micromammals have been identified: 16 from Cluj-Napoca and 9 from Cojocna. The insectivores are missing in Cojocna pellets and in Cluj-Napoca pellets constitute 6.47%. *Microtus arvalis*, which constitute 64.08% in Cluj-Napoca pellets and 78.29% in Cojocna pellets, is the dominant species in both localites. Other species of rodents and insectivores have a very low percentage.

In the last years, many studies concerning the diet of avian predators, especially that of the long-eared owl *(Asio otus)* were performed. Most of these researches were carried out in the central and southern parts of our country [3, 5, 8, 9, 14, 15, 17—19]. There are only three papers dealing with the diet of long-eared owl from the north-western zone of Romania [2, 4, 6]. But the trophic spectrum of this bird of prey may offer important data on the micromammal fauna from certain regions [1]. Therefore, we are presenting below the results of our researches on the *Asio otus* pellets, collected in two localities in the Someşul Mic basin.

Materials and methods. The *Asio otus* pellets were collected in the city of Cluj-Napoca, namely in the Botanical Garden during the winter months of 1994—1995, when there lived a colony of 15—20 individuals of *Asio otus*. The colony had favourable trophic conditions due to an abundant and diverse vegetation in the Botanical Garden and. to numerous hunting areas in the surroundings of the city, dominated by different plant associations. Cojocna, situated east of Cluj-Napoca, at about 30 km from the Someşul Mic river, was the second locality of our studies. This zone is characterized by a hilly relief, with salty swamps and lakes and a lot of cultivated lands. The pellets were collected during the winter months of 1995—1996. The collected material was studied in laboratory and the species of micromammals and birds have been identified.

Results and discussions. Out of the total of 2131 collected pellets, 1403 came from the Botanical Garden (Cluj-Napoca) and 728 from Cojocna. After the preparation of pellets, 5599 bones of birds and mammals

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have been separated: 3776 bones from Cluj-Napoca and 1823 from Cojocna. The number of individuals per pellet was varying between 1 and 7, the average being 2.08 bones per pellet in Cluj-Napoca and 2.4 bones per pellet in Cojocna. After the quantitative and qualitative analyses, we have established that in Cluj-Napoca the micromammals constitute a high percentage (90.99%) in the diet of the owls, while the birds *(Passeriformes)* constitute only 9.01% (Table 1). In Cojocna the bird

Table 1

Composition of *Asio otus* prey as identified from pellets

Diet components	Cluj-Napoca No. ind.	Cluj-Napoca %	Cojocna No. ind.	Cojocna %
Passeriformes	382	9.01	17	0.93
Insectivora	274	6.47	—	—
Rodentia	3582	84.52	1806	99.07

percentage is very low (0.95%), the insectivores are missing, while the rodents constitute 99.07% (Table 1).

The specific composition of micromammals is rather different in the two zones (Table 2). In Cluj-Napoca the number of determined species is much higher, because here there are more varied biotopes than in the

Table 2

Micromammal species identified in *Asio otus* pellets

Species	Cluj-Napoca No.	Cluj-Napoca %	Cojocna No.	Cojocna %
Ord. Insectivora	274	7.11	—	—
1. Sorex araneus	87	2.26	—	—
2. S. minutus	15	0.39	—	—
3. Crocidura leucodon	118	3.06	—	—
4. C. suaveolens	54	1.49	—	—
Ord. Rodentia	3582	92.89	1806	100
1. Microtus arvalis	2471	64.08	1414	78.29
2. M. agrestis	153	3.97	234	12.96
3. Pitimys subterraneus	120	3.11	9	0.5
4. Clethrionomys glareolus	7	0.18	13	0.72
5. Arvicola terrestris	3	0.08	—	—
6. Apodemus sylvaticus	433	11.23	86	4.76
7. A. flavicollis	129	3.35	15	0.83
8. A. agrarius	73	1.89	1	0.06
9. Mus musculus	150	3.89	30	1.66
10. Micromys minutus	29	0.75	4	0.22
11. Rattus norvegicus	5	0.13	—	—
12. Muscardinus avellanarius	9	0.23	—	—
Total micromammals	3856	100	1806	100

Cojocna zone. We have identified 16 micromammal species in the Cluj-Napoca pellets and only 9 species in Cojocna. In both zones *Microtus arvalis* in the most numerous species in the diet of *Asio otus* and it represents more than half of the eaten micromammals (64.08% in Cluj-Napoca and 78.29% in Cojocna). This fact points out that, during 1994—1995, this species had favourable conditions for development, which contributed to its excessive reproduction in both zones. *Apodemus sylvaticus* is situated on the second place in Cluj-Napoca with 11.23% and *Microtus agrestis* occupies the second place in Cojocna with 12.96%. The other identified species have accumulated less than 5%.

It is known that the excessive numerical growth of the individuals from the dominant species is reflected immediately in the specific composition of the food, by an increase in the frequency of this species in pellets. The low density of the dominant species in nature leads to the growth of the specific diversity of the owl's diet [6], which results from our findings as well (Table 2). In Cluj-Napoca, where the percentage of the dominant species is lower than in Cojocna, the specific diversity is greater.

Such species as *Sorex minutus (Insectivora), Clethrionomys glareolus, Arvicola terrestris, Mus musculus, Rattus norvegicus* and *Muscardinus avellanarius (Rodentia)* in Cluj-Napoca and *Pitimys subterraneus, Clethrionomys glareolus, Apodemus flavicollis, A.agrarius* and *Micromys minutus (Rodentia)* in Cojocna represent less than 1% in the long-eared owl's diet. Therefore, these species have a secondary importance in the owl's diet and are hunted only occasionally. This fact is due to the lower density of these species in comparison with the dominant species. As to the rat *(Rattus norvegicus)*, it is very frequent in the localities. But it is not easy at all for the long-eared owl to capture such a big and aggressive prey, that's why the owls don't prefer rats [4]. In many researchers' studies the rat did not accumulate more than 1% in owl's pellets [4—6, 9, 14, 17].

The *Microtus* species are the preferred prey of many *Strigiformes* predators [11, 12]. The proportion of *Microtus* voles in the diet of these birds of prey varies in close accordance with their density in the field [13]. The preference of long-eared owl for the *Microtus* voles, especially for *Microtus arvalis* is mentioned in many studies [2, 4, 7]. Even in the years, when the number of voles was low, the long-eared owl changed its diet only to a slight extent [7]. The preference of owls for the common voles is conditioned by the accessibility of the latter, by their great tendency to conggregate in groups and to live in microhabitats with low vegetation, which are very frequent in Cojocna, as well as by their high prolificity. Predation is responsible for the reduction of the number of voles during autumn and winter (non-reproductive period). Also, the predation may reduce the prey population to lower levels and increase the interval between successive peaks [16].

The indirect action of one species of prey upon another can be seen in the case of insectivores, especially of shrews, which comprise an alternative prey type for the long-eared owl. Thus, the owls shift to

shrews when the number of voles declines, but the abundance of shrews apparently does not affect this shift in diet [13]. In our results the shrews' proportion was 6.47% of all the identified animals and 7.11% of all identified micromammals in Cluj-Napoca pellets. We did not identify any shrews in Cojocna pellets, which can be explained by the great number of *Microtus* voles: the proportion of both *Microtus* species constitutes more than 90% in pellets (Table 2), Therefore, the occurrence of shrews in the owl's diet is inversely related to the percentage of *Microtus* voles in the diet. Many researches have obtained similar results, where the proportion of shrews was varying from 0 to less than 10% (Table 3). From the 4 identified shrew species (Table 2) the lowest percentage was accumulated by the pigmy shrew *(Sorex minutus)* — 0.39%, because of its tiny size and, consequently, because of its low energetic value. A rather high proportion was recorded for *Sorex araneus* (2.26%) and *Crocidura leucodon* (3.06%), which have greater body size and, consequently, higher energetic value.

The birds, like the shrews, are hunted by the owls under the conditions of a low density of rodents. In our pellets the birds *(Passeriformes)* constitute 9.01% of all the animals in the Cluj-Napoca zone and only 0,93% of all the animals in the Cojocna zone. The low percentage of birds in the long-eared owl's diet in Cojocna is determined by the

Table 3

Proportion of insectivores and birds in the *Asio otus* diet in Romania

Insectivora (%)	Aves (%)	References
5.8	15.4	Barbu and Popescu, 1965 [5]
0—2.7	0.1—1.8	Barbu, 1966 [2]
3.2	5.8	Schnapp, 1968 [17]
0.13	1.08	Homei and Popescu, 1969 [9]
1.43	12.38	Cătuneanu et al., 1970 [6]
0—4.9	1.4—6.5	Barbu and Barbu, 1972 [3]
0.3	9.6	Barbu and Korodi Gal, 1972 [4]
0.92	2.28	Murariu et al., 1982 [15]
0.15	11.67	Murariu et al., 1991 [14]

same fact that explains the absence of the shrews as stated above. Thus, the proportion of the birds, like that of the shrews, decreases when rodent population density increases. Therefore, the numerical variations of the birds in the owl's diet results from the rodent fluctuations, which is mentioned by other researches, too [5, 9]. We can also see (Table 3) that the birds' density is not dependent on the shrews' density, being dependent only on the rodent density [10].

Conclusions. The micromammals, which constitute 90.89% in Cluj-Napoca pellets and 99.07% in the Cojocna ones, are the main food for *Asio otus* in both localities. The shrews are present in Cluj-Napoca pel-

lets in a proportion of only 6.47% and are absent in Cojocna pellets. The dominant species in pellets is *Microtus arvalis* with 64.08% in Cluj-Napoca and with 78.29% in Cojocna. The second place is occupied by *Apodemus sylvaticus* in Cluj-Napoca with 11.23% and *Microtus agrestis* in Cojocna with 12.96%. The other species have a very low percentage. The specific diversity is greater in Cluj-Napoca, because of lower density of the dominant species compared with Cojocna. The great proportion of rodents in *Asio otus* diet shows the considerable importance of this species, which must be protected, due to its utility for us and for the natural biocenoses.

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INFLUENŢA ORIGINII EXPLANTULUI ASUPRA EMBRIOGENEZEI ÎN CULTURI CELULARE DE *DAUCUS CAROTA* L.

CONSTANTIN DELIU*, CORNELIA MUNTEANU-DELIU şi ANCA BUTIUC***

SUMMARY. — Influence of the Origin of Explant on the Embryogenesis in Cell Cultures of Daucus carota L. Several cell lines, exhibiting distinctive characteristics, were induced from *Daucus carota* explants obtained from hypocotyls, petioles and roots. The cell lines induced from hypocotyls showed a lowered growth index but exhibited the highest embryogenetic potential, as compared with the ones obtained from petiole and root. The number of embryos that reached an advanced developmental state (the torpedo stage) was also higher in the case of this line, and the formation of somatic embryos required the shortest period of time.

Morcovul a fost prima specie la care s-a demonstrat embriogeneza somatică [10]. Cu toate acestea, culturile celulare de morcov au rămas şi astăzi un sistem model pentru studiul proceselor de embriogeneză somatică la dicotiledonate. Culturile celulare de morcov care posedă potenţialul de a produce embrioni somatici sunt invariabil caracterizate prin prezenţa de agregate alcătuite din mai multe celule mici, cu citoplasmă densă, ce înconjoară una sau mai multe celule cu vacuolă mare, alcătuind aşa numitele mase proembriogene (MPE). Din una sau mai multe celule aflate la suprafaţa acestor MPE se vor dezvolta, în anumite condiţii, embrionii somatici.

În această lucrare am urmărit atât obţinerea culturilor celulare de *Daucus carota* L. cv. Nantes din diferite explante, cât şi influenţa naturii explantului asupra capacităţii lor embriogene. De asemenea, am studiat şi proporţia în care se formează embrionii somatici aflaţi în diferite stadii de dezvoltare, în funcţie de organul din care au fost induse suspensiile celulare respective.

Material şi metode. *Materialul vegetal.* Pentru obţinerea culturilor celulare de *Daucus carota L.* cv. Nantes au fost utilizate: a) explante din calusurile induse din rădăcină tuberizată; b) explante din hipocotil; c) explante din peţiol. Explantele de peţiol şi hipoctil au fost prelevate din plantule de 18 zile, germinate steril. Ele au fost inoculate în vase conice cu mediu lichid şi menţinute pe un agitator rotativ orizontal (98 rpm), la un regim de lumină/întuneric de 16/8 ore, la 25°C.

Mediul nutritiv folosit pentru inducerea suspensiilor celulare din cele trei tipuri de explante a fost alcătuit după reţeta dată de Murashige şi Skoog (MS) [8] cu 2,26 μ M 2,4-D. Pe parcursul experimentelor au mai fost utilizate şi alte tipuri de medii cu compoziţii diferite: Monnier (M) [7] şi Gamborg şi colab. (B5) [4]. Subcultivările s-au efectuat la un interval de 14 zile. Raportul

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dintre inocul: mediu proaspăt a fost de 1:4. Selecția de linii celulare s-a realizat prin metoda clonării de mici agregate celulare.

Creșterea s-a determinat prin recoltarea (la diferite intervale de timp) biomasei celulare (4—5 vase) și cântărirea ei atât sub formă proaspătă, cât și uscată timp de 24 ore la 60°C). Indicele de creștere (I.c.) s-a calculat după formula $Gt_2 - Gt_1/T_2 - T_1 \times 100$, Gt_2 = greutatea biomasei celulare proaspete sau uscate la timpul când a fost recoltată, iar Gt_1 = biomasa celulară la inoculare.

Determinarea potențialului embriogenic al culturilor. În acest scop, din suspensiile celulare aflate în ziua 14-a de cultivare s-au selectat agregate de cca 55 μ m în diametru, prin trecerea lor prin site cu ochiuri de diferite mărimi. Ele au fost spălate cu medii fără auxine și inoculate apoi tot în medii fără hormoni, astfel ca densitatea finală a agregatelor să fie de 900/ml. Potențialul embriogenic al culturilor s-a exprimat prin rata formării embrionilor (RFE), respectiv raportul dintre numărul de embrioni formați și numărul total de agregate celulare inoculate per ml. Determinarea a fost realizată prin numărarea embrionilor (400—500 embrioni aflați în toate stadiile de dezvoltare) cu ajutorul unei lupe binoculare, într-o cutie confecționată din plăci de sticlă de microscop, la care pe placa bazală s-au trasat pătrate cu latura de 1 cm.

Rezultate și discuții. *Obținerea culturilor de suspensii celulare.* Se cunoaște că pentru inducerea embriogenezei somatice un factor important îl constituie natura hormonilor adăugați la mediul de cultură, adaos necesar și pentru inițierea și dezvoltarea culturilor celulare. Dintre aceștia, 2,4-D se pare că este reglatorul de creștere cu o influență deosebită asupra formării embrionilor somatici în culturile celulare de morcov. Din acest motiv, mediul nutritiv MS folosit de noi pentru cultivarea celulelor de *Daucus* a cuprins în alcătuirea sa și 2,4-D într-o concentrație de 2,26 μM. Dintre cele trei tipuri de explante utilizate, calusul indus din rădăcina tuberizată de morcov a dat naștere foarte rapid unei suspensii celulare. Astfel, după doar trei pasări succesive pe același tip de mediu, s-au format culturi celulare veritabile, alcătuite din celule libere și din agregate celulare de diferite mărimi, cele mai mari atingând un diametru de 2,5 mm.

În general, prin trecerea explantelor de hipocotil sau de pețiol direct în mediu lichid nu s-a obținut rapid o cultură celulară. Totuși, timpul de inducere a suspensiilor celulare este mult mai scurt în comparație cu cel parcurs de cultura în care se interpune și faza de calus.

Pentru a găsi un mediu cât mai adecvat creșterii culturilor celulare, am testat câteva medii cu o compoziție diferită. respectiv, MS, M și B5, la care s-a adăugat ca reglator de creștere 2,4-D într-o concentrație de 2,26 μM. Aceste medii se deosebesc între ele în special prin cantitatea și proporția macroelementelor ce intră în compoziția lor. Cel mai bogat mediu din acest punct de vedere este mediul MS. Cu toate acestea, așa cum se observă și din Tabelul 1, efectul cel mai benefic asupra dezvoltării culturilor celulare l-a avut mediul M, indiferent de originea explantului din care au fost induse suspensiile. Diferențe marcante există însă și între variante în funcție de natura explantului, diferențe ce sunt exprimate atât prin cantitatea de biomasă celulară acumulată pe parcursul ciclului lor de creștere, cât și prin indicele de creștere. Astfel, dintre toate variantele, culturile celulare induse din rădăcina tuberizată etalează cel mai ridicat indice de creștere.

Tabel 1

Efectul compoziției mediului asupra creșterii suspensiilor celulare de morcov în funcție de originea explantului

Originea explantului	Mediul de cultură	Biomasa celulară (g/l)		Indice de creștere*
		Proaspătă	Uscată	
Rădăcină tuberizată	MS	425,1	15,29	92,1
	M	534,6	19,27	120,5
	B5	347,3	12,51	72,2
Pețiol	MS	247,4	11,24	63,1
	M	297,8	13,61	80,4
	B5	188,2	8,60	44,3
Hipocotil	MS	120,5	6,73	31,0
	M	146,8	8,21	41,5
	B5	100,1	5,58	22,7

Durata cultivării: 14 zile. Medii utilizate: MS — Murashige-Skoog [8]. M — Monnier [7]. B5 — Gamborg și colab. [4].

* Indicele de creștere s-a calculat în funcție de biomasa uscată.

Rezultatele obținute ne-au determinat ca în continuare să utilizăm, ca mediu nutritiv de bază pentru toate culturile celulare, mediul Monnier [7]. Acest mediu este, după autor, mult mai puțin toxic pentru celule, iar datorită cantității mari de Ca^{2+} ce intră în alcătuirea lui a avut un efect stimulator și asupra embriogenezei somatice în culturile celulare de morcov [1].

Pentru obținerea de linii celulare am utilizat metoda de selecție prin clonarea de mici agregate celulare. În acest mod am reușit să izolăm mai multe linii care își au originea în cele trei tipuri de explante. Ele au fost urmărite atât din punct de vedere al creșterii, cât și al capacității lor embriogene.

Suspensiile celulare, respectiv liniile 1, 2 și 4, inițiate din calus de rădăcină, etalează o creștere intensă, astfel că, după 14 zile de cultivare, biomasa celulară (proaspătă sau uscată) s-a mărit de peste 10 ori (linia 1) (Tabelul 2). În schimb, suspensiile celulare induse direct din hipocotil (liniile 10, 13 și 20) sau din pețiol (liniile 5, 6 și 8) se dezvoltă mai slab. Dacă ne referim doar la vârsta explantelor din care au fost induse suspensiile, fenomenul este destul de interesant, deoarece era de așteptat ca țesuturile tinere să dea naștere la culturi cu o creștere mai intensă. Totuși, chiar după 4 luni de la obținerea acestor suspensii, culturile induse din hipocotil și pețiol continuă să arate același indice mic de creștere. Deci, probabil că în acest caz și originea explantelor joacă un rol important.

Inducerea embriogenezei somatice în culturile celulare de morcov. Primele experimente efectuate în scopul inducerii embriogenezei soma-

Tabel 2

Creşterea unor linii celulare de morcov, induse din diferite organe şi potenţialul lor embriogenic*

Originea explantului	Linia celulară	Biomasa celulară		Potenţial embriogenic [RFE (%)]**
		Proaspătă (g/l)	Uscată (g/l)	
Rădăcină tuberizată	1	600,32	20,62	5,1
	2	542,1	19,71	9,6
	4	460,0	17,48	7,5
Peţiol	5	260,2	13,01	14,0
	6	355,0	14,96	21,7
	8	276,1	12,79	17,5
Hipocotil	10	148,2	8,01	30,4
	13	167,2	9,36	33,8
	20	125,0	7,25	29,2

* Mediul de cultură utilizat pentru creşterea suspensiilor celulare a fost mediul M cu 2,26 μM 2,4–D.

** RFE — Rata formării embrionilor somatici. Mediul de cultură folosit pentru inducerea embriogenezei somatice este lipsit de hormoni. Mărimea agregatelor celulare selectate pentru inducerea formării embrionilor somatici a fost de 55 μm la o densitate de 900 agregate/ml, iar durata cultivării de 21 zile.

tice au fost realizate cu toate liniile celulare obţinute. În acest scop, agregatele celulare, ce aparţin liniilor respective, au fost inoculate în mediul M fără hormoni, astfel ca densitatea lor finală să fie de cca 900 agregate/ml. Această operaţie este necesară, deoarece se ştie că pentru a induce formarea de embrioni somatici de morcov este nevoie ca celulele să fie plasate într-un mediu lipsit de auxine şi la o densitate cât mai redusă [9].

Se consideră că celulele şi masele celulare embriogene se formează sub influenţa unei auxine, în special a 2,4-D şi că ele sunt de fapt embrioni în stadiul incipient, opriţi din dezvoltarea lor chiar de auxina prezentă în mediul de cultură [3]. Odată cu înlăturarea 2,4-D, celulele embriogene îşi vor continua dezvoltarea, transformându-se treptat în embrioni somatici, primul stadiu fiind cel globular.

Analizele efectuate în ziua a 21-a de cultivare relevă că în toate liniile celulare se întâlnesc embrioni somatici aflaţi în cele trei stadii de dezvoltare, respectiv globular, cordiform şi de torpilă. Totuşi, numărul total de embrioni somatici este extrem de mic în special la suspensiile liniilor 1, 2 şi 4, rata formării lor fiind cuprinsă între 5 şi 10%. Deşi din literatura de specialitate reiese clar că la morcov orice organ poate da naştere la embioni somatici [2], până în prezent nu am găsit nici o comunicare cu privire la o posibilă legătură între natura sau originea explantului şi intensitatea formării embrionilor somatici. Cu toate acestea, se observă că există diferenţe semnificative între ratele de formare

ale embrionilor (RFE), la liniile formate din cele trei organe (Tabel 2). Astfel, toate liniile inițiate din rădăcina tuberizată etalează un nivel al RFE mult mai redus în comparație cu cele induse din hipocotil sau din pețiol. Dintre toate liniile testate cel mai ridicat potențial embriogenic îl prezintă linia 13 (inițiată din hipocotil).

În continuare, am urmărit câteva linii pentru a observa dacă ele își conservă sau nu capacitatea embriogenică. Din analizele efectuate am remarcat faptul că ele își păstrează, și după un număr mare de subcultivări, caracterul de linii cu un potențial embriogenic slab (linia 2 inițiată din rădăcină), mijlociu (linia 6 inițiată din pețiol) sau bun (linia 13 inițiată din hipocotil).

Pentru a determina modul de evoluție a embrionilor somatici pe parcursul unei perioade de cultivare în funcție de linia celulară, agregatele celulare ale liniilor 2, 6 și 13 au fost transferate în mediul M, fără hormoni (Fig. 1). Se remarcă, astfel, că embrionii somatici (forma globulară) apar deja în ziua a 4-a de cultivare în cazul liniei 13, în ziua

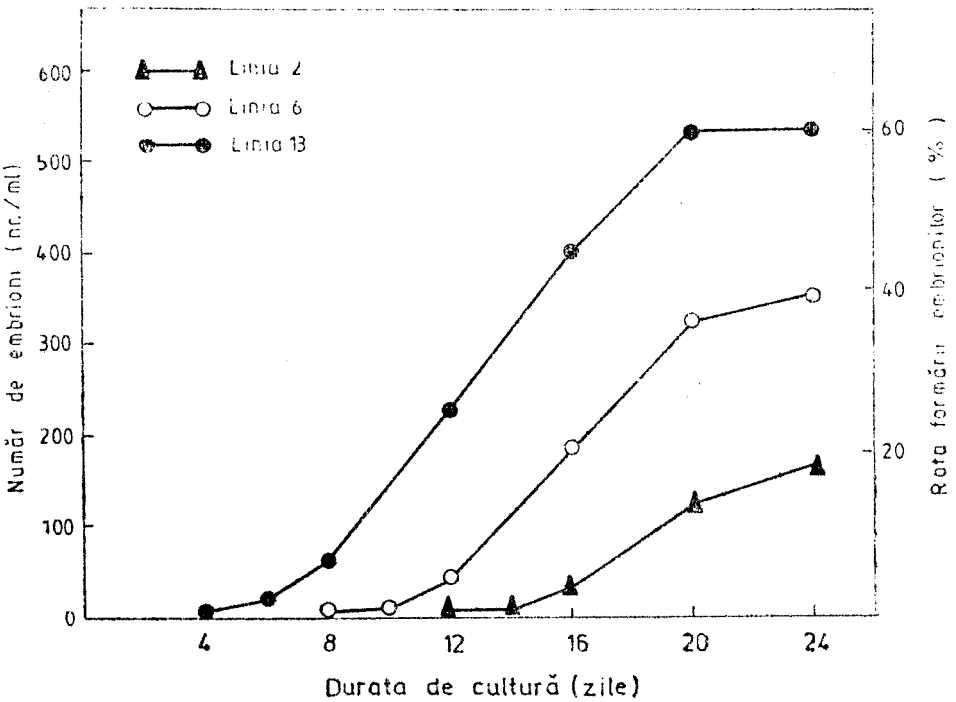

Fig. 1. *Formarea embrionilor somatici din agregatele celulare ale liniilor 2, 6 și 13, în mediul M, lipsit de hormoni.*

Agregatele celulare cu un diametru de 55 µm au fost obținute după procedeul descris în text și au fost inoculate la o densitate de 900 agregate/ml. Rata formării embrionilor s-a calculat după numărul total de embrioni aflați în diferite stadii de dezvoltare.

a 8-a la linia 6 şi de abia în ziua 12-a la linia 2. De asemenea, valoarea RFE este diferită în funcţie de linie, fiind foarte mică la linia 2. Numărul cel mai mare de embrioni se formează din agregatele celulare ale liniei 13 şi atinge maximum în ziua a 20-a de cultivare. La liniile 2 şi 6, embrionii ajung la un număr maxim abia în ziua a 24-a de cultivare. Se pare deci că originea explantului din care au fost induse liniile respective are o influenţă majoră atât asupra numărului de embrioni care se formează, cât şi asupra momentului în care apar primele forme de embrioni somatici (stadiul globular), liniile formate din rădăcină fiind cele mai recalcitrante în acest sens. Fenomenul se repetă şi în cazul raportului în care se află cele trei stadii de dezvoltare ale embrionilor, respectiv globular, cordiform şi de torpilă (Fig. 2). Astfel, după 24 de zile de la inoculare se întâlnesc toate stadiile de dezvoltare, la toate cele trei linii luate în studiu, indiferent dacă agregatele au fost transferate în mediul MS sau M. Dar, aşa cum se observă şi din Fig. 2, între cele trei linii există diferenţe care constau în primul rând în proporţia embrionilor aflaţi în stadiul globular. Astfel, linia 2, cultivată fie

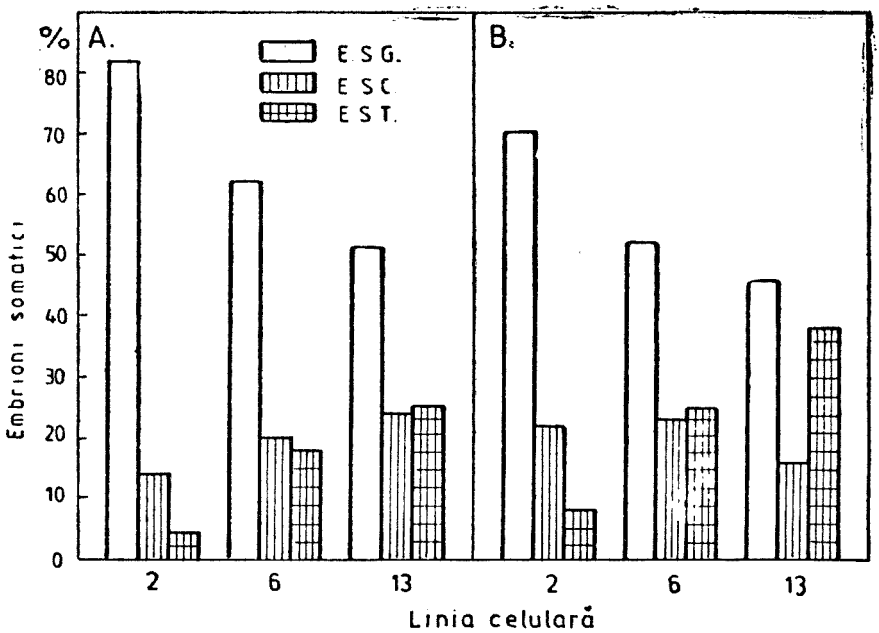

Fig. 2. *Proporţia în care se formează embrionii somatici din agregatele a trei linii celulare cu origine diferită, cultivate în mediul MS şi M, fără hormoni.*

Densitatea agregatelor este de 900 agregate/ml.

A — Mediul MS. B — Mediul M. E.S.G. — Embrioni somatici în stadiu globular. E.S.G. — Embrioni somatici în stadiu cordiform. E.S.T. — Embrioni somatici în stadiul de torpilă.

Determinările au fost efectuate în ziua a 24-a de cultivare.

în mediul MS, fie în M, etalează acelaşi procent extrem de ridicat de embrioni sub formă globulară şi unul foarte mic de embrioni cordiformi şi de torpilă. Acest fenomen se poate produce şi datorită unui proces de inhibiţie a dezvoltării embrionilor la nivelul stadiului globular. Faţă de linia 2, la celelalte linii şi în special la linia 13, numărul de embrioni aflaţi în stadiul cordiform şi în cel de torpilă este mult mai mare. Proporţia în care se găsesc embrionii liniei 13 în stadiul de torpilă este de 38% faţă de linia 2 în care aceştia se găsesc la un nivel ce nu trece de 10%. Se pare că la anumite linii aşa cum este linia 2, linie derivată din rădăcina tuberizată, este redus nu numai potenţialul lor embriogenic, dar există şi un proces general de inhibiţie a dezvoltării embrionilor.

Deoarece linia 13 s-a dovedit a avea, dintre toate liniile celulare folosite, potenţialul embriogenic cel mai ridicat, majoritatea experimentelor efectuate ulterior au fost realizate cu această linie. Urmărind cinetica dezvoltării embrionilor somatici în mediul M, se observă (Fig. 3) că forma globulară apare deja în ziua a 4-a de cultivare, iar numărul acestor embrioni creşte urmând o curbă ascendentă până în a 20-a zi, când numărul lor începe să scadă. În ziua a 8-a apar primii embrioni

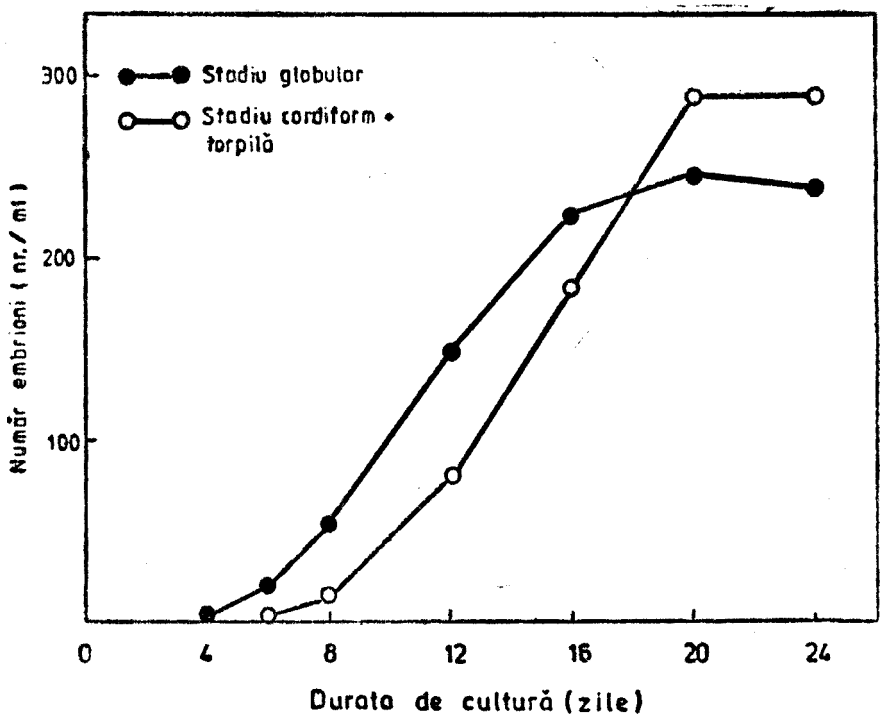

Fig. 3. *Cinetica formării embrionilor somatici din agregatele celulare ale liniei 13 după trasferul lor în mediul M, fără hormoni.*

cordiformi și de torpilă, al căror număr descrie de asemenea o curbă ascendentă asemănătoare și paralelă cu a celor globulari, până în ziua a 20-a, când se pare că dezvoltarea lor intră într-un stadiu staționar. În această perioadă, numărul lor îl întrece pe cel al embrionilor globulari. Cu toate acestea, numărul de embrioni globulari rămâne încă mare, ceea ce ar putea fi explicat, așa cum arătam mai sus, fie printr-o blocare a dezvoltării embrionilor la nivel globular, sau din cauza că agregatele rămase în mediu se transformă în embrioni globulari într-o proporție mai mare, decât cea a embrionilor cordiformi. De asemenea, este posibil ca o creștere a biomasei de embrioni de torpilă să conducă la o sporire a consumului de nutrienți în competiție cu cea a populațiilor de embrioni globulari [5].

Este foarte greu de a da o explicație acestor diferențe care există între liniile celulare, în funcție de organul din care au fost induse, deoarece există încă destule necunoscute legate de procesul de diferențiere și de dobândire a potențialului embriogenic de către celule. Totuși, o posibilă explicație ar fi aceea legată de sensibilitatea celulară la auxină. Explantul primar poate răspunde la auxina conținută în mediul de cultură prin generarea unor celule capabile de diviziune și proliferare sau poate genera celule care se divid și dobândesc capacitate embriogenetică sau organogenetică.

Există deja un exemplu clasic de celule care răspund diferit la tratamentul hormonal: celulele de tutun, indiferent de originea țesutului, au proprietăți organogenetice, pe când celulele de morcov, independent de originea lor, sunt embriogene. Este greu de precizat dacă e vorba de o selecție, sau se poate considera un răspuns diferențial la tratamentul hormonal. În urma investigării sensibilității țesuturilor de coleoptil, hipocotil și rădăcină la acțiunea 2,4-D s-a constatat că preparatul de membrane realizat din hipocotil etalează capacitatea cea mai mare de a lega 2,4-D, fiind țesutul cel mai puternic modulat [6].

Acest proces de modulare a răspunsului diferențial al celulelor la auxine are semnificație biologică în procesele de embriogeneză și organogeneză. Izolarea, din liniile celulare embriogenice de morcov, de linii care proliferează normal, dar nu au capacitate modulatoare și nici capacitate de diferențiere, indică faptul că răspunsul generat de hormon, ce duce la diviziunea celulară, este independent de răspunsul care duce la achiziționarea totipotenței și generarea PEM în prezența auxinei. Prin urmare, aceste răspunsuri diferite sunt posibile, datorită a două clase de proteine de legare a auxinei, una responsabilă de diviziunea celulară și alta capabilă de inducerea atât a diviziunii celulare, cât și a regenerării [6].

Concluzii. 1. Culturile celulare de morcov induse din trei tipuri de explante cu origine diferită, respectiv rădăcină tuberizată, pețiol și hipocotil, au caracteristici specifice, așa cum sunt creșterea și potențialul embriogenic, care le diferențiază una de alta.

2. În scopul găsirii unui mediu de cultură optim pentru creșterea suspensiilor celulare s-au studiat mai multe medii cu o compoziție di-

ferită. Dintre ele cel mai bun mediu s-a dovedit a fi mediul M cu 2,4-D într-o concentrație de 2,26 μM.

3. Originea tipului de explant și/sau juvenilitatea țesuturilor constituente a avut o influență majoră asupra potențialului embriogenic. Astfel, liniile celulare formate din hipocotil prezintă cea mai ridicată rată de formare a embrionilor somatici, procentul cel mai mare de embrioni sub formă de torpilă și un timp de apariție a embrionilor mult mai scurt în comparație cu liniile induse din pețiol și rădăcină.

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ACUMULAREA DE PROTEINE ŞI AMIDON ÎN EMBRIONII SOMATICI DE MORCOV *(DAUCUS CAROTA* L.)

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SUMMARY. — Protein and Starch Accumulation in Carrot (Daucus carota **L.) Somatic Embryos.** In this set of experiments we investigated the influence of cytokinins, sucrose and some amino acids on the formation of somatic embryos originating from the hypocotyl of *Daucus carota,* as well as on the synthesis of proteins and starch. Introduction of the cytokinins zeatin and 2-isopentenyladenine into the culture medium led to an increased embryogenic potential, to enhancement of protein accumulation and to inhibition of precocious germination of the somatic embryos. Supplementation of the culture medium with sucrose stimulated starch synthesis in the embryos that were in the torpedo stage. The amino acids influenced in a different way the somatic embryos. Glutamine led to a higher ratio of germination, to an increased biomass quantity of embryos and to a higher protein content, arginine caused a decreased rate of embryo formation, while proline led to an enchanced embryogenic potential.

Embriogeneza somatică deţine un potenţial ridicat pentru o regenerare eficientă, pe scară mare, comercială, a plantelor elită şi transgenice. Unul din avantajele embriogenezei somatice faţă de alte procedee de regenerare, aşa cum este de exemplu propagarea de lăstari, este numărul mare de plantule ce pot fi obţinute cu uşurinţă chiar în bioreactoare de laborator. Embrionii somatici pot fi deshidrataţi sau incluşi în diferite matrice pentru producerea de seminţe artificiale, în scopul facilitării manipulării lor ulterioare, a conservării şi în final a semănării.

Depozitarea rezervelor de stocare în embrionii somatici este foarte importantă pe timpul germinării şi al transformării lor în plante. Cu toate că anumite aspecte ale dezvoltării embrionilor somatici şi a celor zigotici, aşa cum ar fi cel morfologic, biochimic sau al toleranţei la deshidratare şi al germinării, sunt asemănătoare în multe privinţe [3], există şi diferenţe. Astfel, embrionii somatici, spre deosebire de seminţe, conţin cantităţi relativ mici de proteine şi de amidon de rezervă datorită faptului că ei sunt lipsiţi de endosperm sau de cotiledoane pe deplin dezvoltate [7]. Zaharoza este sursa majoră de carbon necesară pentru sinteza amidonului în seminţe, dar şi sursa cel mai des utilizată pentru dezvoltarea embrionilor somatici [4], astfel că o suplimentare cu zaharoză a mediului de cultură poate să conducă la mărirea cantităţii de amidon în embrionii somatici. De asemenea, adaosul de compuşi cu azot în mediul de cultură al embrionilor somatici modifică acumularea

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rezervelor de proteine. În acest sens, suplimentarea cu glutamină sau cu alte forme de azot redus a mărit sinteza proteinelor de stocare la embrionii somatici de lucernă [6]. Obiectivul studiului de față a fost acela de a determina efectul unor citochinine, al unor aminoacizi și al zaharozei asupra formării embrionilor somatici de morcov, precum și asupra acumulării de amidon și proteine.

Material și metode. Procedeul de obținere a culturilor celulare de morcov a fost descris într-o lucrare anterioară [2]. Explante de hipocotil, prelevate din plantule de 18 zile, au fost inoculate în vase conice cu mediu Monnier (M) [10] lichid, ce conținea 2,4-D într-o concentrație de 2,26 μM. Vasele au fost menținute pe un agitator rotativ orizontal (98 rpm), la un regim de lumină/întuneric de 16/8 ore și la 25°C. După inducerea și selecția liniilor celulare, suspensiile au fost cultivate în același mediu și în aceleași condiții ca cele descrise mai sus. Pentru inducerea embriogenezei somatice s-au utilizat suspensiile liniei celulare 13. Agregatele celulare considerate a fi embriogene, având o dimensiune de 55 μm în diametru, s-au obținut prin trecerea suspensiilor celulare prin site cu ochiuri de diferite mărimi. Ele au fost transferate apoi tot în mediul M, fie lipsit de hormoni, fie cu adaos de citochinine — zeatină (Z) ori 2-izopenteniladenină (2iP). Pentru anumite experiențe, în care în mediul de cultură s-a introdus glutamina sau zaharoza în anumite concentrații, au fost utilizați doar embrioni în stadiul de torpilă. Ei au fost selectați după morfologia lor, înainte de a germina. Astfel, s-au selectat embrioni cu cotiledoane rudimentare, dar vizibile.

Embrionii s-au considerat a fi germinați, dacă radicula era alungită. Pentru determinarea ratei de formare a embrionilor (RFE) s-au numărat 400—500 embrioni aflați în toate stadiile de dezvoltare. Embrionii somatici au fost analizați și din punctul de vedere al capacității lor de biosinteză a unor metaboliți primari importanți pentru dezvoltarea ulterioară, cum sunt proteinele și amidonul. În acest scop s-au recoltat, separat, cu ajutorul unor site, embrioni aflați în cele trei stadii de dezvoltare, respectiv globular, cordiform și de torpilă. Analizele au fost realizate din biomasa proaspătă a unui număr de 700 de embrioni.

Amidonul s-a dozat prin determinarea fotocolorimetrică a glucozei [12, 15] rezultată din hidroliza lui cu HCl. Proteinele totale au fost dozate spectrofotometric conform metodei lui Lowry și colab. [8].

Rezultate și discuții. Embrionii somatici depozitează proteinele și amidonul de stocare pe timpul maturării lor, dar acest proces se produce în absența cotiledoanelor pe deplin dezvoltate ori a endospermului, care se întâlnesc doar în semințe. Slaba vigurozitate a embrionilor somatici a fost de cele mai multe ori atribuită nivelului redus atins de rezervele de stocare din embrioni [1].

Primul experiment efectuat de noi a constat în transferul agregatelor celulare ale liniei 13 în mediul Monnier [10], lipsit de auxine, mediu la care s-au adăugat, înainte de autoclavare, două citochinine într-o concentrație foarte scăzută (0,5 μM). Așa cum se observă și din Tabelul 1, adaosul de citochinine a stimulat sinteza de proteine, ceea ce a condus la sporirea acumulării lor în țesuturile embrionilor de morcov, indiferent de stadiul în care se află aceștia. Totuși, există diferențe marcante care privesc mai ales embrionii în stadiul de torpilă. Astfel, sub influența citochininelor, conținutul în proteine totale a crescut de la 4,4 μg/embrion la 8,1 μg/embrion. Dintre cele două citochinine testate, zeatina și 2-izopenteniladenina, efectul cel mai favorabil în acest sens îl are 2iP, deoarece procentul de proteine sintetizate de embrionii formați și

crescuți în mediu cu 2ip este de 1,35 ori mai mare decât al embrionilor din mediul cu zeatină. Dacă ne referim și la celelalte stadii de dezvoltare ale embrionilor de morcov, se remarcă faptul că, la aceștia, acumularea proteinelor parcurge un drum ascendent în funcție de etapa de dezvoltare la care a ajuns embrionul respectiv. Astfel, cantitatea de proteine crește cu fiecare stadiu parcurs, fiind maximă în stadiul de torpilă.

Asupra amidonului, introducerea de citochinine a avut ca efect inhibiția biosintezei lui. Efectul este mai pregnant în cazul în care în mediu se găsește 2iP. În această variantă, cantitatea de amidon scade în embrionul sub formă de torpilă de la 7,5 µg/embrion la 3,2 µg/embrion. Și aici, ca și la proteine, se observă o acumulare treptată a metabolitului în funcție de etapa de dezvoltare pe care o parcurge embrionul, cantitatea cea mai mare de amidon fiind întâlnită în stadiul de torpilă.

Creșterea în greutate proaspătă a embrionilor, aflați în diferite stadii de dezvoltare, nu a fost afectată de adaosul de citochinine la mediul utilizat pentru inducerea embriogenezei somatice. În schimb, rata de formare a embrionilor s-a mărit în mod semnificativ (cu 50%) în mediile la care s-a adăugat 2-izopenteniladenină sau zeatină.

Un efect interesant l-au avut cele două citochinine și asupra germinării embrionilor somatici. În general, după mai multe zile de cultivare în același mediu standard, fără hormoni, se remarcă apariția unui fenomen de germinație precoce. Totuși, în mediile în care există o citochinină, procentul de germinație scade drastic, în special în mediul cu zeatină (Tabelul 1). După rezultatele obținute de noi și după datele din

Tabel 1

Influența unor citochinine (05 µM) asupra formării embrionilor somatici din agregatele liniei celulare 13 cultivate în mediul M o n n i e r [10] fără auxine, precum și asupra sintezei de proteine și amidon

Varianta de mediu	Embrioni (stadii)	Substanță proaspătă (mg/ES)	Proteine totale (µg/ES)	Amidon (µg/ES)	RFE (%)	EG (%)
—	Sămânță	—	33,9	115,1	—	—
M	G	0,080	2,5	3,0	39,8	0,8
	C	0,148	3,5	5,1		
	T	0,235	4,4	7,5		
Z	G	0,085	2,4	3,2	68,2	0,0
	C	0,160	4,2	3,9		
	T	0,240	6,0	4,5		
2iP	G	0,079	2,3	2,7	71,6	0,5
	C	0,159	5,2	2,9		
	T	0,242	8,1	3,2		

Determinările au fost efectuate la 24 de zile de la inoculare. RFE — Rata formării embrionilor somatici; densitatea agregatelor = 900 agregate/ml. EG — Embrioni germinați. M — Martor. Z — Mediu cu zeatină. 2iP — Mediu cu 2-izopenteniladenină. G — Globular. C — Cordiform. T — Torpilă.

literatura de specialitate este foarte greu de dat o explicaţie, deoarece efecte similare au fost constatate doar în urma utilizării acidului abscisic sau a unor concentraţii mari de zaharoză [5].

Proporţia în care se găsesc diferitele forme de embrioni a fost de asemenea afectată de prezenţa în mediu a celor două citochinine. Astfel, chiar dacă numărul de embrioni globulari nu scade semnificativ sub acţiunea 2iP sau a Z, creşte în schimb procentul de embrioni în stadiul de torpilă (Fig. 1). Acest efect este mult mai pregnant în cazul zeatinei.

Se ştie că zeatina este utilizată pentru inducerea embriogenezei somatice la morcov şi poate avea un efect benefic asupra acestui proces [13]. De asemenea, se consideră că citochininele sunt hormoni vegetali ce stimulează diviziunea celulară şi sinteza de proteine [11]. Deci, probabil că cele două citochinine naturale, zeatina şi 2iP utilizate de noi, au fost implicate în acest mod, atât în stimularea formării embrionilor somatici, cât şi a acumulării de proteine de către aceştia. Cu toate acestea, până în prezent, nu am întâlnit nici o comunicare care

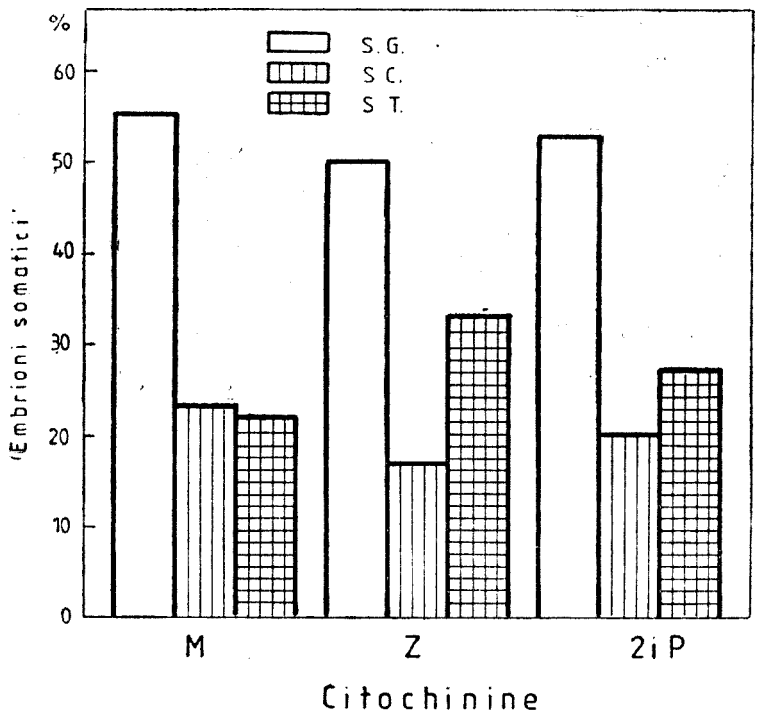

Fig. 1. *Influenţa unor citochinine* (0,5 μM) *asupra proporţiei în care se formează embrionii somatici din agregatele liniei 13, cultivată în mediul M, fără auxine.*

M — Martor. Z — Mediu cu zeatină. 2iP — Mediu cu 2-izopenteniladenină. S.G. — Stadiu globular. S.C. — Stadiu cordiform. S.T. — Stadiu de torpilă.

să ateste că asemenea suplimentări pot să afecteze şi acumularea de amidon.

Una dintre metodele utilizate în mod curent pentru sporirea numărului de embrioni somatici, precum şi a cantităţii de proteine şi amidon acumulate de către aceştia, este suplimentarea mediului de cultură cu aminoacizi sau zaharoză. Primele experimente realizate de noi în acest sens au constat în mărirea concentraţiei de zaharoză a mediului M, lipsit de hormoni, de la 3% până la 5%. Zaharoza a fost adăugată în mediu înainte de inocularea agregatelor celulare ale liniei 13. În urma analizelor efectuate am constatat că după 24 de zile nu a crescut numărul de embrioni, ci doar biomasa lor.

În a doua serie experimentală am utilizat aminoacizii: L-asparagină, L-glutamină şi L-prolină într-o concentraţie de 10 mM. Ei au fost introduşi, ca şi zaharoza, tot de la început în mediul de cultură M al aceleiaşi linii celulare.

Determinările efectuate în ziua a 24-a de cultivare scot în evidenţă că suplimentarea mediilor cu asparagină şi glutamină a condus la mărirea cantităţii de agregate celulare în dauna numărului de embrioni, rata formării lor scăzând cu peste 50% faţă de martor (Fig. 2). În

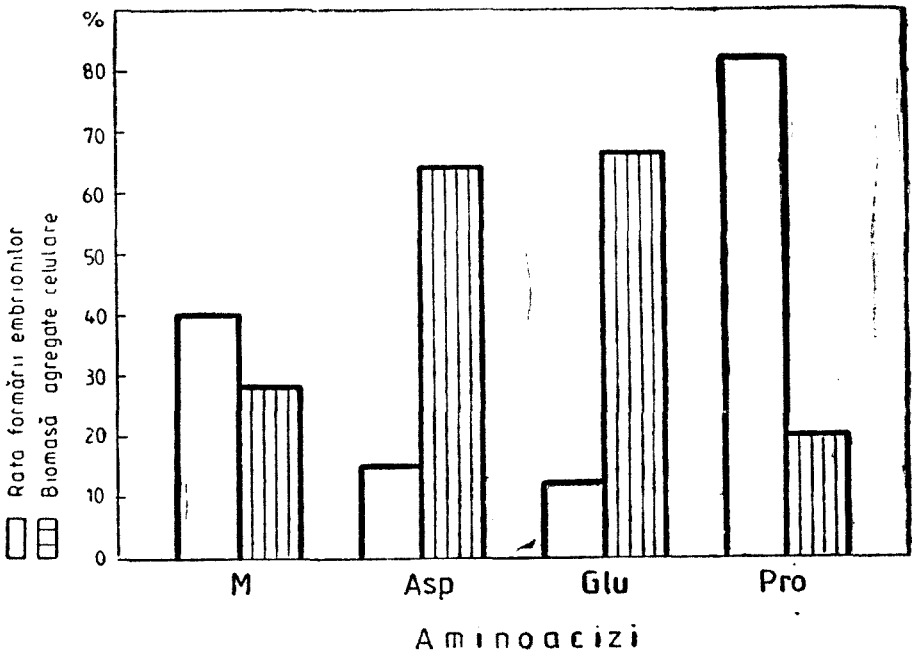

Fig. 2. *Efectul unor aminoacizi* (10 mM) *asupra formării embrionilor somatici totali şi a creşterii biomasei de agregate celulare.*

A fost utilizată linia celulară 13. Aminoacizii au fost introduşi înainte de autoclavare în mediul de cultură M, lipsit de fitohormoni, iar determinările s-au efectuat după 24 zile de cultivare.

M — Martor. Asp — Asparagină. Glu — Glutamină. Pro — Prolină.

schimb, în mediile cu prolină RFE creşte foarte mult ajungând la peste 80%, iar biomasa agregatelor celulare scade. Totuşi, modificarea cantităţii de proteine totale acumulate în embrioni nu se observă în nici una din variante.

Din rezultatele obţinute reiese clar că, dintre aminoacizii testaţi, doar prolina a avut un efect benefic asupra măririi ratei de formare a embrionilor somatici de morcov. În acest sens există date care confirmă stimularea embriogenezei somatice de către prolină şi în alte culturi celulare cum sunt cele de *Agrostis* [14] sau de *Medicago* [1].

O explicaţie a acestui fenomen este că probabil prolina se leagă de metabolismului purinic. Informaţii anterioare asupra formării embrionilor somatici din explante de peţiol de *Medicago sativa* atestă că, la 72—120 de ore după tratamentul cu auxine al acestor explante, se produce o diviziune celulară periclinală, urmată de o serie de diviziuni ce conduc în final la formarea embrionilor globulari [16]. O condiţie necesară pentru diviziunea celulară este replicarea ADN şi de aceea se presupune că pe timpul celor 72 de ore înainte de apariţia embrionilor somatici, se produce sinteza de ADN care necesită la rândul ei sinteza de purine. De aceea, mărirea sau modificarea metabolismului purinic poate să fie un eveniment biochimic anterior al acestui proces. Având în vedere că prolina, în anumite celule tumorale animale, stimulează metabolismul prolinic legat de cel purinic, este posibil ca acest efect să-l aibă şi asupra celulelor vegetale [9]. Rezultatele obţinute de noi sunt în concordanţă cu această ipoteză, deoarece dintre cei trei aminoacizi cu care s-a lucrat, doar prolina a avut ca efect intensificarea procesului de embriogeneză somatică, deci de transformare a maselor proembriogene în embrioni. Asparagina şi glutamina, introduse de la început în mediul de cultură, se pare că au influenţat cu precădere formarea de agregate neembriogene în defavoarea celulelor embriogene.

Al treilea aspect al experimentelor efectuate de noi în acest context a constat în testarea efectului zaharozei în concentraţii de 0,09, 0,12 şi 0,15 M, precum şi al glutaminei în concentraţii de 1, 5 şi 15 mM asupra creşterii biomasei embrionilor somatici în stadiul de torpilă şi asu-

Tabel 2

Efectul zaharozei şi al glutaminei asupra creşterii embrionilor somatici şi a sintezei de proteine şi amidon

Embrioni în stadiu de torpilă	Zaharoză (M)			Glutamină (mM)*		
	0,09	0,12	0,15	1	5	15
Substanţă proaspătă (mg/ES)	0,242	0,287	0,331	0,363	0,387	0,412
Proteine totale (µg/ES)	4,5	4,4	4,6	7,3	8,5	10,7
Amidon (µg/ES)	7,2	10,1	12,6	6,8	6,5	6,1
Germinaţie (%)	76,2	78,8	73,3	83,5	88,1	96,6

În ziua a 14-a de cultivare embrionii în stadiul de torpilă au fost transferaţi la o densitate de 900 embrioni/ml, în medii fără hormoni ce conţineau cei doi compuşi, zaharoza şi glutamina. Determinările s-au efectuat după alte 10 zile de cultivare. Experienţele au fost realizate cu linia celulară 13. Greutatea iniţială a unui embrion a fost de 220 µg.
* Glutamina a fost introdusă în mediul M cu 3% zaharoză.

pra conținutului lor în proteine și amidon (Tabelul 2), precum și asupra capacității lor de germinație.

Având în vedere rezultatele anterioare, au fost selectați doar embrionii aflați în stadiul de torpilă. Embrionii triați trebuiau să prezinte anumite caracteristici, adică să fie bine dezvoltați, cu cotiledoane rudimentare și cu o axă embrionară evidentă. Ei au fost transferați în continuare tot în medii M, fără hormoni, ce conțineau fie zaharoză, fie glutamină în concentrațiile amintite. După 10 zile de la inocularea în aceste medii, embrionii sub formă de torpilă au fost recoltați și analizați din punct de vedere biochimic.

Din datele obținute se remarcă faptul că odată cu mărirea concentrației de zaharoză se mărește și cantitatea de amidon acumulată de embrioni, precum și biomasa proaspătă, dar sinteza de proteine totale rămâne la același nivel întâlnit la martor. Fenomenul este explicabil, deoarece, în mod normal, zaharoza este sursa majoră de carbon pentru sinteza amidonului în țesuturile de rezervă din semințe și este și sursa de carbon pentru embrionii somatici [7].

Introducerea glutaminei în mediu de cultură conduce la intensificarea acumulărilor de proteine totale, proporțional cu creșterea concentrației ei, astfel că la 15 mM cantitatea de proteine este de două ori mai mare decât a martorului. De asemenea, se constată și mărirea greutății proaspete a unui embrion. Acumularea amidonului a fost mai puțin afectată, valorile lui fiind foarte apropiate de cele ale martorului. Desigur, cantitatea de proteine și amidon acumulată de către acești embrioni este mult mai mică decât cea întâlnită la sămânța de morcov (Tabel 1), însă nu trebuie pierdut din vedere faptul că embrionii somatici sunt lipsiți de endosperm, respectiv de cotiledoane bine dezvoltate. Dintre cei doi compuși testați, glutamina în concentrația de 15 mM produce în paralel și o mărire a capacității de germinare a embrionilor, proporția lor crescând de la 76% (embrionii crescuți în mediu cu zaharoză 30 g/l) la peste 96%. De fapt, între puterea de germinație a embrionilor și cantitatea de proteine pe care o acumulează aceștia se constată o corelație pozitivă, amidonul neavând nici un rol în acest proces. O explicație posibilă a acestui fenomen este aceea că endospermul semințelor de *Apiaceae*, familie din care face parte și morcovul, sau cotiledoanele de *Leguminosae* conțin ca substanțe de rezervă proteinele, astfel de semințe fiind de tip aleuronic. Deci, este posibil ca din acest motiv embrionii care etalează un conținut crescut de proteine să germineze cu o frecvență mult mai mare și probabil că și procentul de transformare al lor în plăntuțe este mai ridicat. Cantitatea de amidon nu este afectată de glutamină, ea fiind foarte apropiată de cea întâlnită la martor.

Rezultatele obținute de noi sunt în concordanță cu cele realizate și cu alte plante așa cum este lucerna [7] și reflectă faptul că sporirea cantității unor metaboliți primari acumulați de embrionii somatici poate avea un efect benefic asupra dezvoltării lor ulterioare.

Concluzii. 1. Adăugarea de citochinine în concentraţii foarte mici la mediul fără auxine a condus: la creşterea ratei de formare a embrionilor şi a numărului de embrioni maturi; la sporirea cantităţii de proteine acumulată în special de embrionii sub formă de torpilă şi la inhibiţia germinării precoce a embrionilor somatici.

2. Adaosul de aminoacizi (glutamină, asparagină şi prolină) în mediul de cultură, înainte de inducerea embriogenezei somatice, a avut efecte diferite în funcţie de aminoacizii utilizaţi: scăderea ratei de formare a embrionilor şi creşterea biomasei de agregate celulare (glutamină şi asparagină) şi mărirea potenţialului embriogenic (prolină).

3. Suplimentarea mediului de cultură al embrionilor în stadiu de torpilă cu zaharoză a avut ca efect creşterea în greutate a embrionilor şi mărirea cantităţii de amidon acumulate de aceştia, fără să afecteze sinteza de proteine. Adăugarea de glutamină a condus, în schimb, atât la creşterea biomasei embrionilor, cât şi a cantităţii de proteine sintetizate de ei.

4. Între cantitatea de proteine pe care o acumulează embrionii în stadiul de torpilă şi procentul de germinare al lor există o corelaţie pozitivă.

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THE EFFECT OF SINGLE AND COMBINED UV-RADIATION AND LEAD TREATMENT ON THE CYTOPLASMIC STREAMING RATE WITHIN WHEAT *(TRITICUM AESTIVUM* L.) ROOT HAIRS

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SUMMARY — The effects of UV-irradiation (254 nm) applied in doses of 0.5, 1.0, 1.5, 2.5 and 3.5 kJ/m² on the cytoplasmic motility within wheat root hairs emphasize at beginning a strong and UV-dose-dendent inhibition, followed by a partial recovery process. Depending on the applied lead concentration (100, 200, 2,000 and 10,000 ppm), the cytoplasmic streaming rate is decreased in the first 15 min after treatment. After this time an approximate steady state of the lead action on the cytoplasmic flow is induced. In the combined treatments the inhibitory effects of UV-radiation are predominant and even more pronounced in the presence of lead.

Root hairs and other tubular plant cells (algae, pollen tubes, staminal hairs) are characterized by vigorous cytoplasmic and organelle movements [2, 7, 16, 28]. It is well known that root hairs extend root surfaces [14], but they also have many distinctive physiological and structural characteristics as specialized respiratory cells [3], which make them useful models in plant cell research [3, 14, 15].

Studies on the cytoplasmic streaming within root hairs have shown a very high sensitivity of this phenomenon when the entire root segment was experimentally exposed to UV-radiation or a UV-microirradiation to root hairs was applied [5, 11, 22, 23, 25]. Generally, all types of UV-radiation (UV-A, UV-B and UV-C) damage living organisms to a greater or lesser extent [8—10, 18—21]. The primary alterations are located especially at the level of DNA [4], but more knowledge about the mechanism and tissue specificity of DNA-repair in plants is required [4, 18]. On the other hand, the literature contains frequent reports which emphasize the toxic effects of lead and other polluting heavy metals on plants [1, 13, 27, 29]. Moreover, the studies reported to date have been mainly conducted to analyse the physiological effects induced by only one stress. In fact, little is known about the physiological interaction mechanisms on plant cells in response to the combined action of two or many abiotic stressors.

The aim of the present paper, which continues our previous investigations [22, 23], is to test whether there is an interaction between UV-irradiation and the application of lead as an additional stress on intracellular movements. The cytoplasmic streaming velocity within wheat root hairs was used as a quantitative parameter.

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Material and methods. **Plant material.** Seeds of winter wheat (*Triticum aestivum* L., cv. Arieşan) were germinated in Petri-dishes on filter paper wetted with a mixture of tap and distilled water (1 : 1), at 21—22°C and in darkness. The time needed for germination was 2—3 days. The experiments were carried out on cutted root segments and a root hair length of about 750—1,000 µm [17] was chosen. Before starting the measurements of the cytoplasmic flow, the root segment was kept in the bright field of the microscope to remove the preparative shock.

Cytoplasmic streaming. The velocity of the cytoplasmic streaming within root hairs was measured under a light microscope (Zeiss Nf with phase-contrast optics, ob. 40x, oc. 16x), by recording the time necessary for a cytoplasmic particle to traverse a given distance. The experimental time was established at 2 hours; every 15 minutes the solutions under the coverglass were renewed and 50 measurements of streaming rate were obtained using the stopwatch method as described in our previous studies [11, 12, 22—25[. The bathing medium for streaming rate control was a 1 : 1 mixture of tap and distilled water. All measurements were made on the same root hair and a mean streaming rate for each 15 minute interval was calculated.

UV light source and lead treatment. UV light was obtained from an artificial source of radiation (Universal UV-Lampe, "Camag", Germany) with a main emission peak at 254 nm. Quartz microscope slides and coverglases were used; the UV-irradiation in doses of 0.5, 1.0, 1.5, 2.5 and 3.5 kJ/m^2 on root segment always was applied 30 min after starting the experiments. The effect of lead solutions in form of nitrate salt in concentrations of 100, 200, 2,000 and 10,000 ppm Pb on cytoplasmic streaming rate was investigated by replacing the control solution under the coverglass with lead nitrate solutions through percolation procedure [7]. For every lead concentration, the experiments were repeated 3—5 times. In the combined treatment, a lead nitrate solution of 200 ppm Pb was used, its effect on streaming velocity being investigated by administration before and after UV-irradiation or only after UV-irradiation. For this type of treatment only one dose of UV-light (1.5 kJ/m^2) was used.

Results and discussion. The effect of lead. The effects of various lead concentrations (100, 200, 2,000 and 10,000 ppm) on the cytoplasmic streaming rate are given in Fig. 1. The measurements made every 15 min during a total experimental period of two hours show that the streaming velocity decreases in a concentration-dependent manner. The effect is immediately manifest in the first 15 min after lead application, particularly at high lead concentration (probably, as a biochemical shock). As it could be seen in Fig. 1, lead applied in concentrations of 100 and 200 ppm causes only a slight decrease in the cytoplasmic streaming rate, the induced inhibition exceeding no more than 5%. With increasing lead concentrations to 2,000 and 10,000 ppm, the streaming velocity is immediately decreased up to 20% in the first 15 min from the start of treatment. After this initial phase of lead inhibitory action, an approximate steady state of its action on cytoplasmic streaming rate could be estimated (Fig. 1), although the lead solution was renewed every 15 minutes over 2 hours. At the highest lead concentration used, side effects on cell content, like aggregation of the protoplasm, were also seen. On short distance and for short time, this mass of cytoplasm could be put in motion.

The present and previous results [23] suggest that the cells of wheat root hairs tolerate large amounts of lead for short time of incubation, knowing that cytoplasmic motility is directly connected with the normal

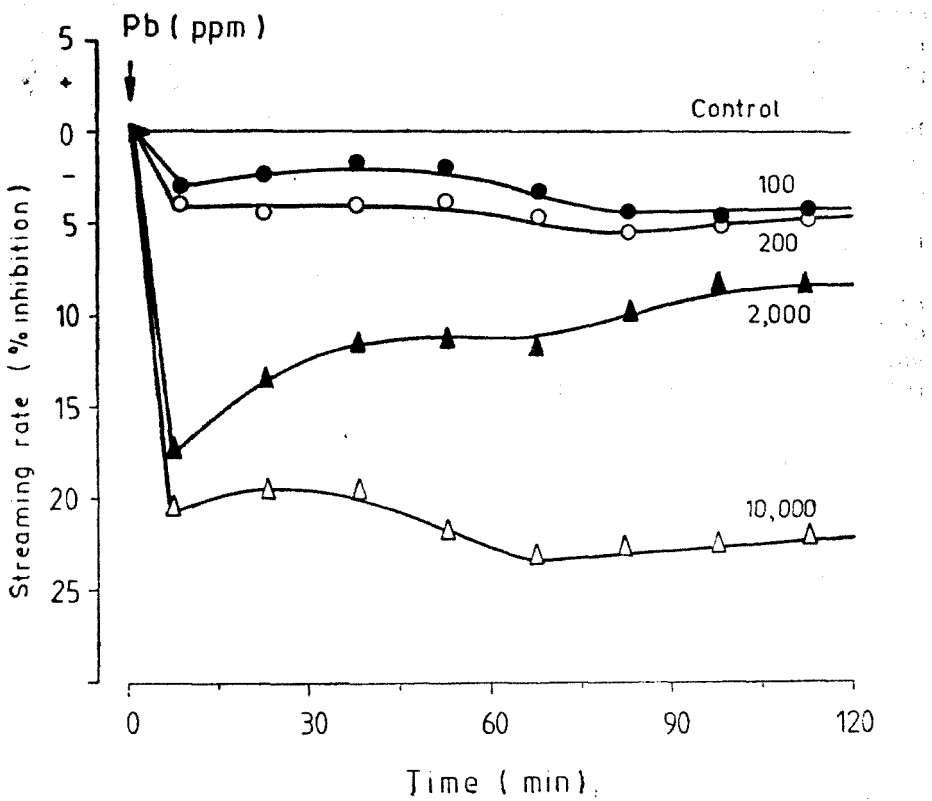

Fig. 1. *The effects of lead on the cytoplasmic streaming rate within wheat* (Triticum aestivum L.) *root hairs.*

biochemical activity of the cell [2, 3, 7, 12, 16, 28]. On the other hand, our results can be related to recent researches conducted on onion root as a model system, where it was found that the process of lead uptake is very intense during the first 15 min of incubation, and for the first 2—6 hours the amount of lead in cell walls increases gradually without its penetration into the protoplast [27]. This kind of lead uptake during the first hours may be an explanation for an approximate steady state of its action on cytoplasmic streaming rate. If the time of incubation is longer, high toxic effects of lead on plant cell appear [13, 27, 29].

The effect of UV-irradiation. The effects of different UV-doses (0.5, 1.0, 1.5, 2.5 and 3.5 kJ/m^2) on the velocity of the cytoplasmic streaming are given in Fig. 2. In agreement with our previous studies and those of other authors [6, 7, 11, 22, 23, 25], the present results show that the cytoplasmic streaming rate is immediately and drastically reduced after UV-irradiation in a dose-dependent manner. This initial and UV-dose-dependent inhibition is followed by a slow re-

covery process of the cytoplasmic movement. As shown in Fig. 2, the effects caused by UV-irradiation in doses of 0.5, 1.0, 1.5 and 2.5 kJ/m² emphasize a similar fashion, in both the inhibition and recovery action. The recovery process of cytoplasmic flow appearing after UV-irradiation can be noticed up to 2.5 kJ/m². Increasing the UV-flux to 3.5 kJ/m² only a slow oscillation in the streaming rate can be recorded at 45—60 min from the start of UV-irradiation (Fig. 2). Changes in the morphology of the moving cytoplasm, like aggregation, have been observed at high UV-doses.

The effect of combined treatment. Fig. 3 summarizes the results of measurements in combined treatment. In this type of treatment, if lead acts after UV-irradiation (Fig. 3, UV + Pb), the values recorded for streaming velocity are very closed to those registered after UV-irradiation alone, without significant modifications. A grea-

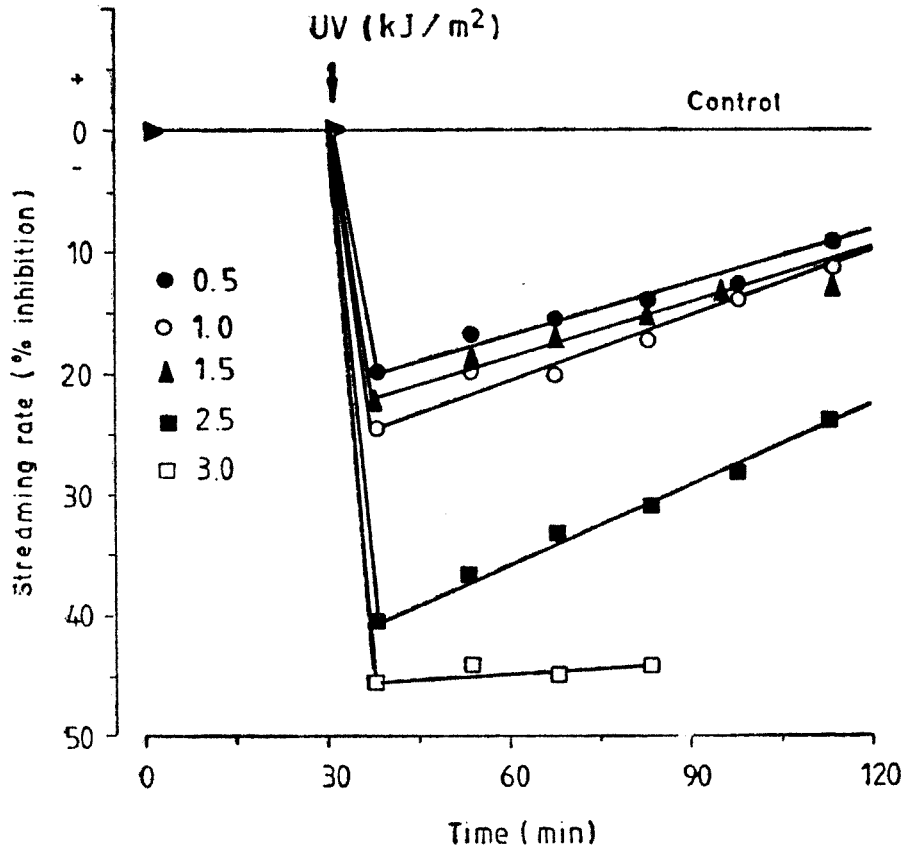

Fig. 2. *The effects induced by various UV-doses on the cytoplasmic streaming rate within wheat* (Triticum aestivum *L.*) *root hairs.*

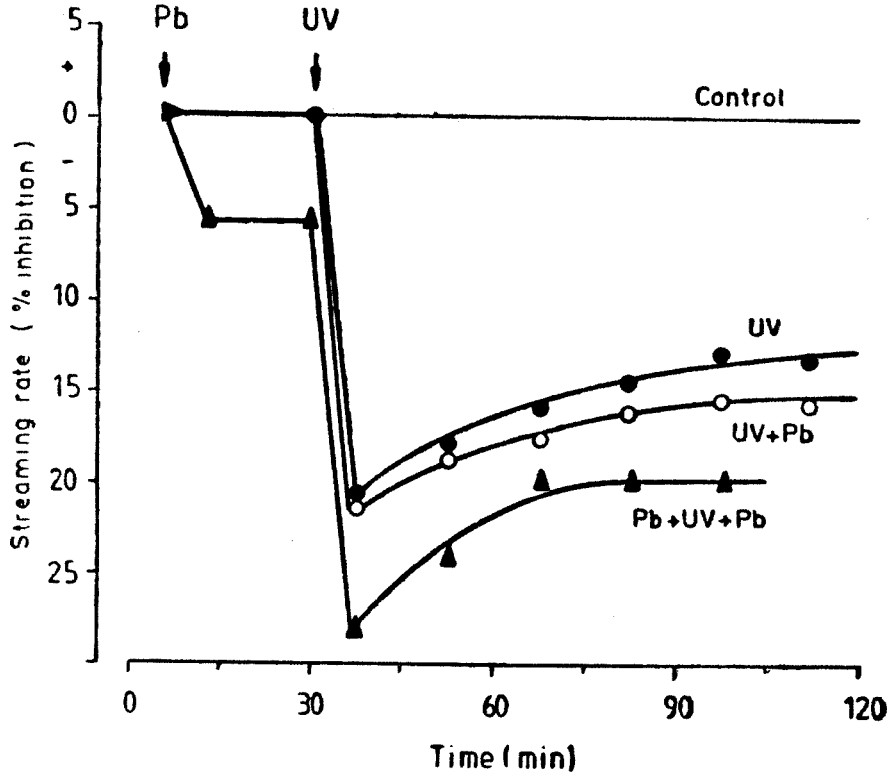

Fig. 3. *The effects of combined treatments with lead and UV on the cytoplasmic streaming rat within wheat* (Triticum aestivum *L.*) *root hairs.* UV — Single UV-irradiation. UV + Pb — Lead applied after UV—irradiation. Pb + UV + Pb — Lead applied before and afte UV—irradiation.

ter inhibitory action is obtained when lead treatment was made before and after UV-irradiation (Fig. 3, Pb + UV + Pb), but the patter is similar to that obtained after single UV-irradiation. During the second treatment, the cytoplasmic movement is stopped after 90 minutes from the start.

The mechanism of UV-action on cytoplasmic motion is not very clear, but the experiments have shown that the cytoplasmic streaming within plant cell is very sensitive to whole or partial cell irradiations with UV-light [5, 7, 11, 22, 23]. While wheat root hair control shows a very active cytoplasmic streaming (or cyclosis) with a streaming velocity between 7.50—8.50 μm/s, due to a natural variability, which was analyzed in tomato root hairs [2], after UV-irradiation this dynamic property of cytoplasm is transitorily lost depending on the applied doses (Fig. 2).

Today, it is well known that the cellular action of UV radiations takes place by interactions with many functionally important molecules, such as nucleic acids and proteins [4, 6, 18]. Concerning the mechanism of the cytoplasmic movement, the present knowledge indicates that cytoskeletal proteins organized in a contractile actomyosin complex are involved [16, 26, 28]. Our results indicating the transitory loss of dynamic properties of the cytoplasm after UV-irradiation may be correlated with the UV-action on proteins, especially the damage of the actin molecules [6]. Further investigation and consideration merit to be done.

It can be **concluded** from these results that UV special effects on cytoplasmic streaming within wheat root hairs can be aggravated by lead.

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ACŢIUNEA IONILOR DE MAGNEZIU ŞI A VITAMINEI B_6 ASUPRA MORFOLOGIEI TESTICULARE LA ŞOBOLANII ALBI JUVENILI TRATAŢI CU L-GLUTAMAT MONOSODIC

CONSTANTIN PUICĂ*

SUMMARY. — The Action of Magnesium Ions and B_6 Vitamin upon the Testicular Morphology of White Juvenile Rats Treated with Monosodium L-Glutamate. Juvenile male Wistar rats were treated with monosodium L-glutamate in a daily dose of 20 mg/kg body weight for 30 days, and with mosodium L-glutamate in the same dose for 30 days plus magnesium sulphate in a dose of 30 mg/kg and B_6 vitamin in a dose of 15 mg/kg body weight for 10 days. The monosodium L-glutamate treatment resulted in a diminution of the testis volume and in disorganisation of the seminal epithelium with signs of cytolysis and dystrophy. Because genital gland is a hormone-dependent organ, it may be assumed that monosodium L-glutamate has an indirect action, manifested through the activation of hypothalamo-pituitary-gonadal axis.

The combined administration of magnesium ions and B_6 vitamin brings about a tendency to normalising the testis structure.

Patologia structurii testiculare îmbracă aspecte variate, de la distrofia epiteliilor seminifere, a celulelor Sertoli şi Leydig, până la neeroza acestora, provocate de diverşi agenţi chimici şi fizici. Interesând toată structura testiculară, cu răsunet direct sau indirect asupra celulelor sexuale, sunt inflamaţiile necrotice provocate de unele substanţe chimice sau medicamentoase, dintre substanţele chimice cu acţiune indirectă asupra structurii şi funcţiei testiculare putând fi enumerat şi L-glutamatul de sodiu (GLU).

GLU reprezintă excitoneurotransmiţătorul major al vertebratelor [2, 5, 11, 13], care, în anumite condiţii (administrare în exces), determină o serie de aspecte negative (distrugeri neuronale ireversibile) la nivelul sistemului nervos central (SNC), la mamifere [1, 4, 7—12, 17]. Cu toate acestea, GLU este şi actualmente larg utilizat în variate forme în industria farmaceutică, precum şi pentru a adăuga savoare în anumite concentrate destinate industriei alimentare şi zootehniei.

Studiile întreprinse îndeosebi în ultimul deceniu au confirmat neurotoxicitatea GLU administrat atât parenteral cât şi *per os*, cele mai sensibile regiuni fiind retina şi ariile circumventriculare hipotalamice, unde bariera hematoencefalică este deficitară, aceste structuri hipotalamice fiind cuprinse în aşa numita „arie hipofizotropă" (AH) [2, 4, 9—11, 13].

Postulatul este următorul: orice acumulare a glutamatului de sodiu în SNC, peste un anumit nivel, produce distrugeri ireversibile ale celulelor nervoase.

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Neuronii AH, grație proceselor metabolice citoplasmatice, sintetizează hormoni peptidici care datorită funcției de scurgere axoplasmică (axoplasmic flow) ajung la terminațiile nervoase din zona eminenței mediane, de unde sunt puși în circulație sub formă de factori de eliberare (releasing factors — RF) sau factori de inhibare (inhibiting factors — IF) ai hormonilor din adeno- și neurohipofiză, constituind baza materială a multiplelor mecanisme de feed-beck, prin intermediul cărora se realizează în ultimă instanță reglarea neuroendocrină. Acești factori acționează atât direct, pe receptori specifici, cât și indirect, prin intermediul unor glande endocrine care le reglează activitatea.

Din cauză că nucleul arcuat hipotalamic, centrul regulator neuroendocrin, este una din zonele AH cele mai afectate, animalele tratate cu GLU în perioada juvenilă manifestă multiple dezechilibre neuroendocrine, însoțite de un habitus anormal în faza de adult, caracterizat prin capacitate redusă de reproducție, hipoplazia adenohipofizei și a gonadelor, însoțite de o scădere a nivelului sanguin al hormonilor luteinizanți și al prolactinei în ser [2, 6, 9—13, 15, 17].

Studii recente [3, 6, 14] au demonstrat *in vitro* că acțiunea unor excitotoxine, în care este inclus și GLU, asupra unor neuroni retinali și din hipocamp depinde de prezența Na^+ și a Cl^- în mediul de incubație. Alte studii susțin că distrugerea neuronală este indusă de un influx crescut al ionilor de calciu la nivel neuronal [2, 3, 5, 6, 11], de unde rezultă că ar exista cel puțin două mecanisme prin care excitotoxinele ar induce moartea celulară, unul dependent de ionii de calciu și unul independent de aceștia [2, 5].

Utilizarea în diverse domenii (terapeutica umană, industria alimentară și zootehnie) a GLU a pus problema atenuării sau chiar a contracarării efectelor secundare — excitotoxice, care sunt însoțite de tot apanajul manifestărilor incluse în „sindromul monosodium glutamat" (MSG-syndrome).

Unii autori au ajuns la concluzia că ionii de magneziu, ca blocanți ai canalelor calcice de la nivelul receptorilor specifici neuronali pentru GLU, ar putea fi utilizați pentru contracararea efectelor secundare-excitotoxice ale GLU. Astfel, utilizarea ionilor de magneziu ca agenți fiziologici ce străbat ușor bariera hematoencefalică (BHE) ar putea determina blocarea canalelor calcice cuplate cu receptorii N-metil-D-aspartat (NMDA) pentru GLU [2, 5, 8, 11, 13, 16].

Experimentul descris de Wolf și colab. [16] este în consens cu alte studii privind efectul neuroprotector al ionilor de magneziu [2, 5, 8, 16]. Boylan și Spallholz [1] au demonstrat *in vivo* că fosfatul vitaminei B_6 formează cu ionii de magneziu un complex capabil de a facilita transportul și acumularea Mg^{2+} în celule.

În lucrarea de față vom urmări efectul complexului $MgSO_4$-vitamina B_6 în vederea atenuării efectelor induse de tratamentul cu GLU la nivel testicular.

Material şi metodă. Experimentul s-a efectuat pe şobolani albi Wistar masculi juvenili, care au fost crescuţi în condiţii zooigienice corespunzătoare. Animalele au fost grupate în 3 loturi experimentale:

— *lotul M*, martor, în număr de 8 animale, având greutatea de 80 g;

— *lotul G*, tratat cu GLU în doză de 20 mg/kg greutate corporală, în număr tot de animale, având greutatea de 80 g;

— *lotul GMB*, tratat cu GLU în aceeaşi doză, căruia i s-a administrat concomitent $MgSO_4$ în doză de 30 mg/kg greutate corporală şi vitamina B_6 în doză de 15 mg/kg greutate corporală, în număr tot de 8 animale, având greutatea de 80 g.

Tratamentul cu GLU s-a efectuat prin gavaj intragastric, zilnic, timp de 30 de zile, iar administrarea complexului $MgSO_4$-vitamina B_6, prin injectare intraabdominală, a durat 10 zile, începând cu ziua 20 de tratament cu GLU.

La sfârşitul perioadei de tratament, animalele au fost sacrificate prin dizlocare cervicală, recoltându-se gonadele care au fost fixate în lichidul Bouin timp de 48 de ore, fiind ulterior prelucrate în vederea includerii la parafină. Secţiunile având grosimea de 5 μ au fost colorate cu hematoxilină-eozină şi studiate la microscopul fotonic.

Rezultate. *Lotul M* prezintă aspectul normal, caracteristic al structurii testiculare, celulele liniei seminale (spermatogonii, spermatocite şi spermatide), precum şi celulele Leydig şi Sertoli fiind bine reprezentate. În lumenul tubilor seminiferi se remarcă prezenţa spermatozoizilor (Fig. 1).

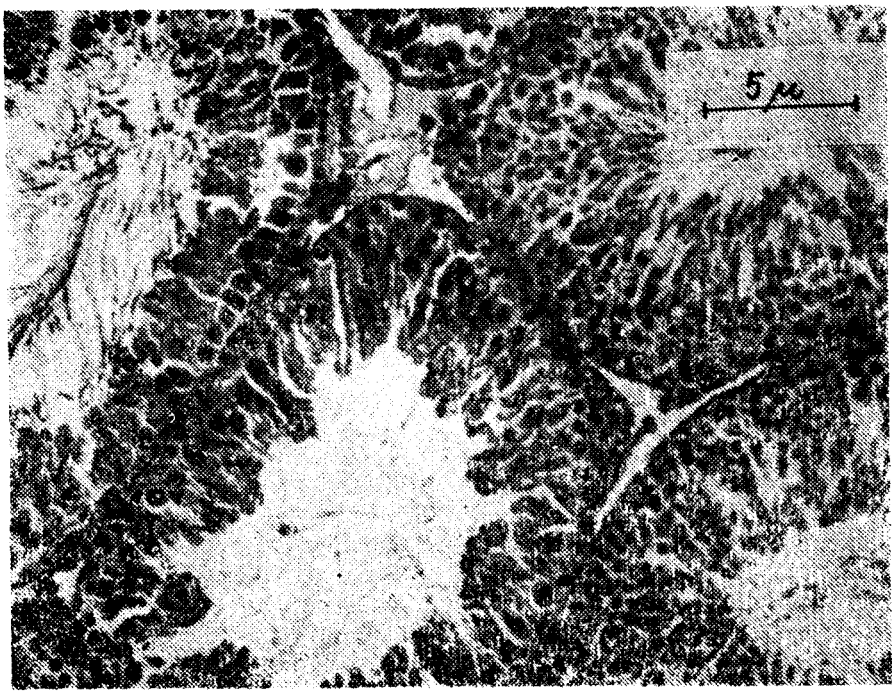

Fig 1. *Aspectul structurii testiculare la lotul M.*

Lotul G la examinarea macroscopică a relevat o reducere în volum a testicolelor, comparativ cu lotul M, respectiv o scădere în greutate cu 16,8%. La nivelul structurii testiculare s-au observat zone ce prezintă modificări morfopatologice caracterizate prin fenomene de distrofie și citoliză a epiteliilor germinative, reducerea marcată a volumului spermatogoniilor și al spermatocitelor, precum și a numărului spermatozoizilor în lumenul tubilor seminiferi. Toate aceste modificări ilustrează alterarea funcției spermatogenetice la animalele acestui lot experimental (Fig. 2).

Lotul GMB. Administrarea concomitentă a ionilor de magneziu și a vitaminei B_6 determină o tendință de revenire spre aspectul normal al structurii testiculare, fenomenele de citoliză și distrofie de la nivelul celulelor liniei seminale fiind mai reduse ca intensitate, în comparație cu cele semnalate la lotul G (Fig. 3). Și la acest lot experimental se constată o reducere, cu 10,7%, a valorii medii a greutății testiculare.

Discuții. Tratarea animalelor cu GLU induce afectarea structurilor periventriculare hipotalamice, respectiv a nucleului arcuat hipotalamic (centrul regulator neuroendocrin), unde se eliberează printre alți hormoni și gonadoliberinele ce controlează secreția hormonilor gonadotropi adenohipofizari foliculostimulator și luteinizant (FSH și LH) [2, 6, 9, 11,

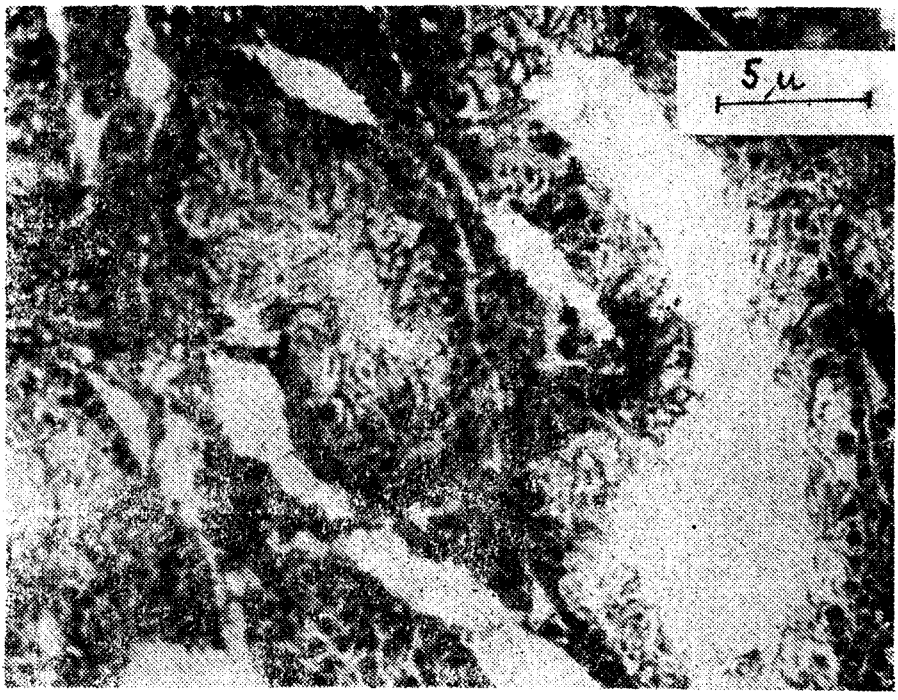

Fig. 2. *Aspectul structurii testiculare la lotul G.*

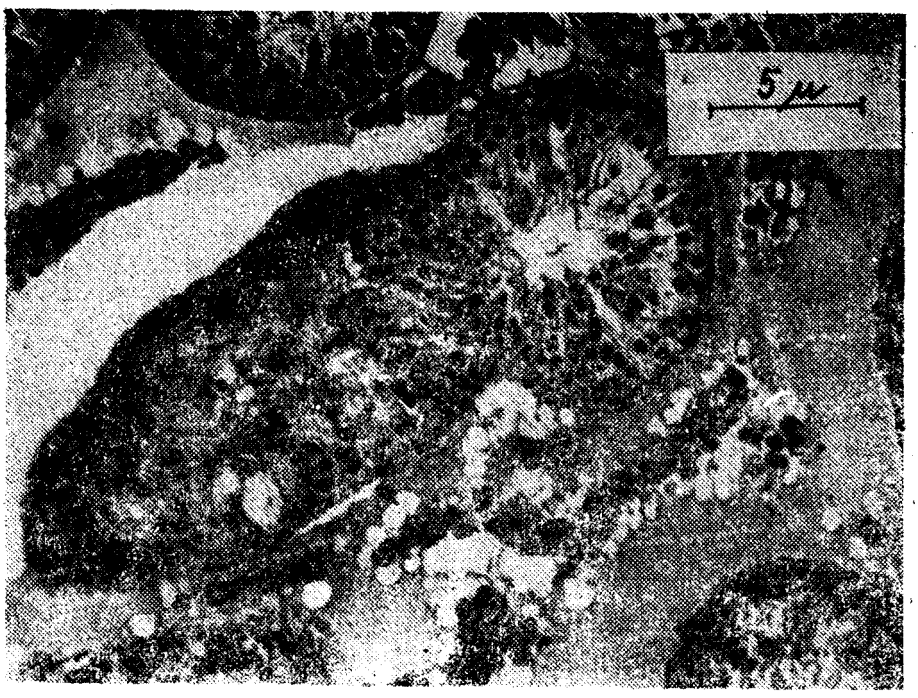

Fig. 3. *Aspectul structurii testiculare la lotul GMB.*

12, 17]. Afectarea nucleului arcuat hipotalamic induce şi hipoplazia adenohipofizei, respectiv a celulelor secretoare de hormoni gonadotropi, toate aceste modificări având drept consecinţă afectarea etajului inferior al axei — structura şi funcţia gonadică. Aspectele degenerative remarcate la nivelul structurii testiculare sugerează o acţiune indirectă, prin intermediul axei hipotalamo-hipofizare, a GLU.

Cum s-a prezentat mai sus, unul din mecanismele prin care excitotoxinele, din care face parte şi GLU, induc moartea celulară, îl reprezintă influxul ionilor de calciu prin membranele neuronale depolarizate, ori, actualmente este bine cunoscut faptul că utilizarea ionilor de magneziu ca antagonişti ai ionilor de calciu, asociaţi cu vitamina B_6, determină blocarea canalelor calcice cuplate cu receptorii N-metil-D-aspartat (NMDA) pentru GLU [1, 7, 9, 10, 13, 16].

Administrarea concomitentă a complexului $MgSO_4$-vitamina B_6 (vitamina B_6 facilitează transportul şi acumularea magneziului în celule [1, 7, 16]) determină o tendinţă de revenire spre aspectul normal al structurii testiculare, zonele cuprinzând afectarea celulelor liniei seminale, precum şi a celulelor Leydig şi Sertoli, fiind mai reduse, sugerând un efect protector indirect, prin intermediul axei neuroendocrine hipotalamo-hipofizare, al acestui complex activ, ca urmare a acţiunii GLU la nivel neuronal.

Concluzii. 1. Tratamentul cu GLU în doză de 20 mg/kg la șobolani masculi juvenili induce afectarea întregii structuri testiculare, respectiv a celulelor liniei seminale.

2. În cazul administrării concomitente a GLU în aceeași doză de tratament și a complexului $MgSO_4$-vitamina B_6, aspectele morfopatologice ale testicolului sunt mai puțin intense, fiind mai apropiate de cele ale lotului martor, sugerând un efect protector al acestui complex asupra structurii testiculare.

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GENETIC ASPECTS OF ICHTHYOSIS

DINA COPREAN*, NICOLAE COMAN and CRISTINA HAŞ***

SUMMARY. — Ichthyoses are caused either by the mutation of an autosomal dominant gene (ichthyosis vulgaris) or of an autosomal recessive gene (lamellar ichthyosis) or of a recessive gene with X-linked transmission (X-recessive ichthyosis). In the forms of ichthyosis determined by the mutation of autosomal genes, both sexes are affected. In the X-recessive ichthyosis, only males are affected, females being carriers of the STS mutant gene. Formation of scales can be the consequence of an enzymatic deficit which disturbs the equilibrium of their production-elimination process. All forms of ichthyosis produce cosmetic handicap. The family and population incidence of these diseases can be reduced by carrier detection and genetic counselling.

Ichthyoses represent a group of genetically and clinically heterogeneous skin diseases, having as a common feature the presence of visible scales on a large part or all over the skin surface. The scale formation can be caused by retention of cells in the stratum corneum through lack of desquamation, epidermal proliferation, acantho-keratosis, enzymatic deficit.

There is an anomaly in the production-elimination equilibrium of scales, which can affect the keratocytes or the intracellular cement [1, 5, 7—11].

The various forms of ichthyosis differ by mode of transmission, clinical picture, histopathologic aspects and associated systemic anomalies.

We attempted to study the genetic aspects of these disorders producing cosmetic handicap because researches on ichthyosis are few and sporadic in Romania.

Material and methods. For the genetic study of the various forms of ichthyosis, we investigated a number of patients admitted to the Clinic of Dermatology of Cluj-Napoca with the diagnosis of ichthyosis, during the period 1990—1996.

The genetic investigations included family enquiry, genetic records and pedigree reconstruction.

Results. The majority of the investigated patients (20 cases) showed symptomatology characteristic of the most frequent form of ichthyosis: ichthyosis vulgaris. Figs. 1 and 2 show the suggestive pedigrees of some families affected by ichthyosis vulgaris.

The pedigrees of two families were investigated and reconstructed, the diagnosis of ichthyosis being established (Figs. 3 and 4).

Patients with lamellar ichthyosis were also investigated (2 cases) and pedigrees reconstructed (Figs. 5 and 6).

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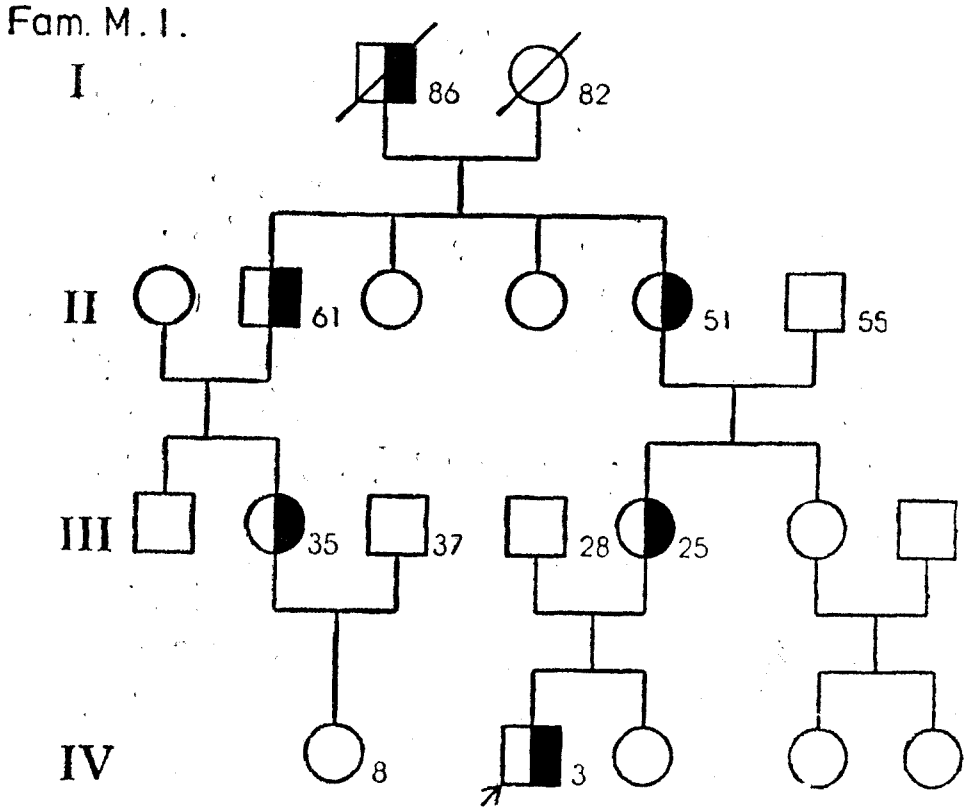

Fig. 1. *Pedigree of family M.I.*

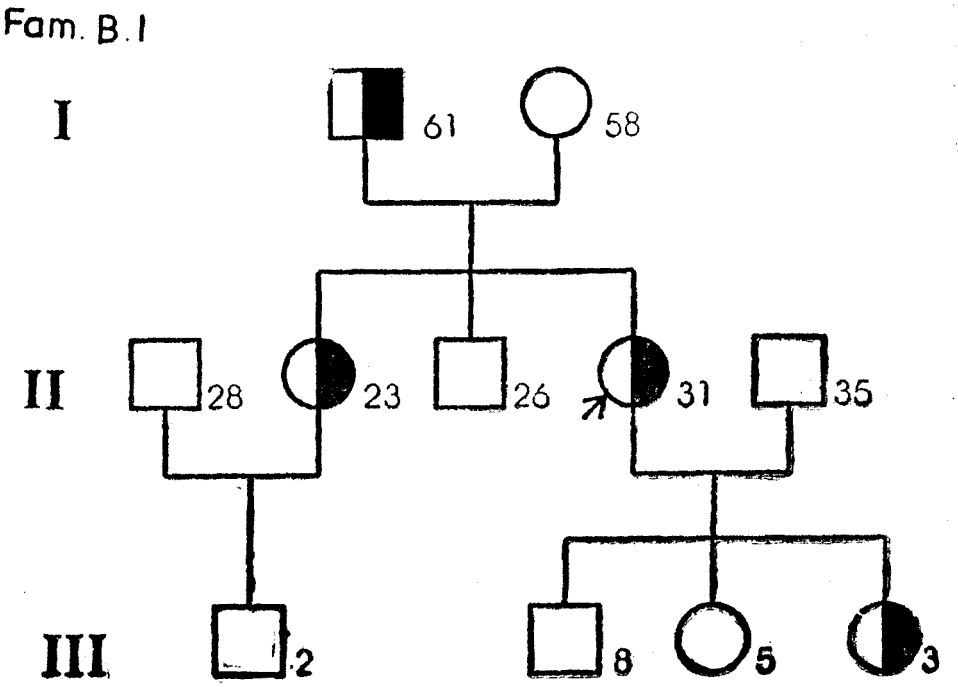

Fig. 2. *Pedigree of family B.I.*

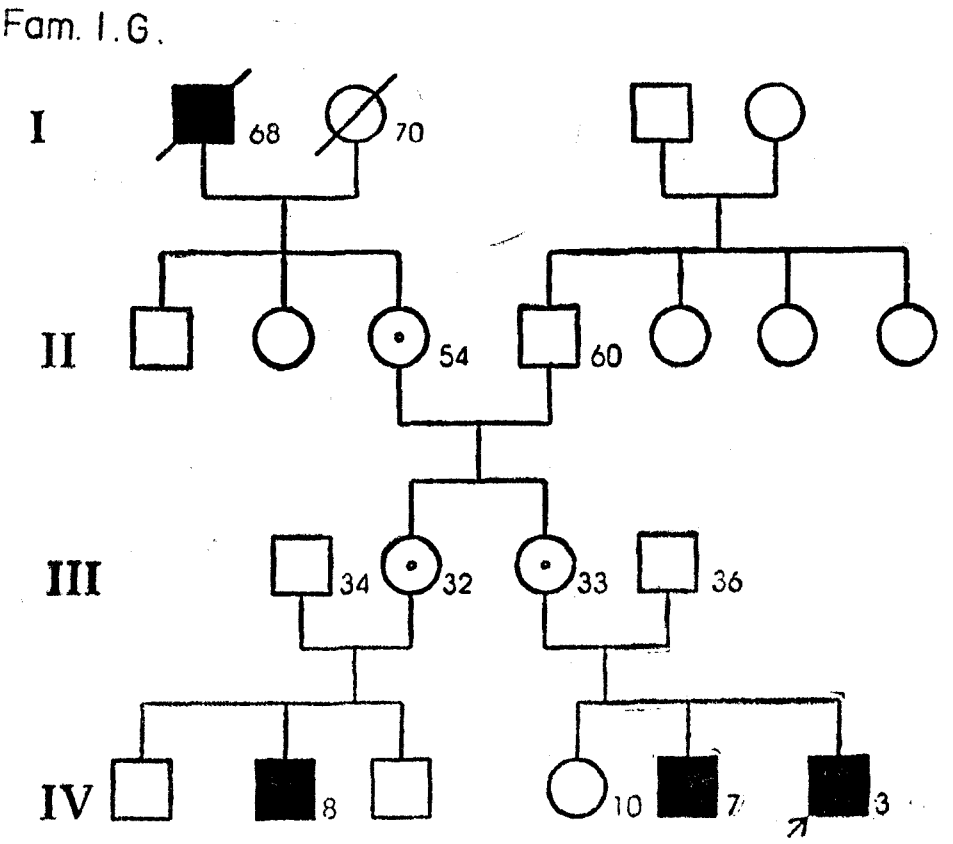

Fig. 3. *Pedigree of family I.G.*

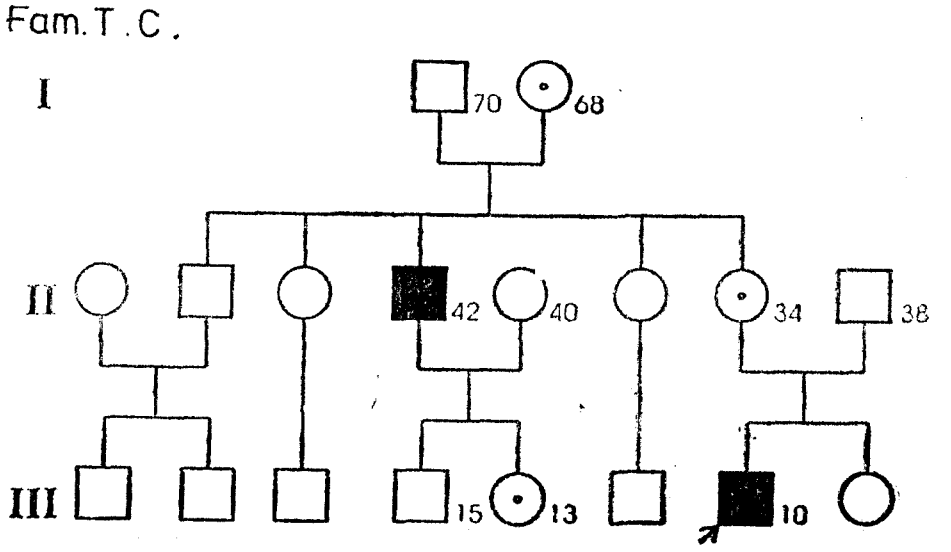

Fig. 4. *Pedigree of family T.C.*

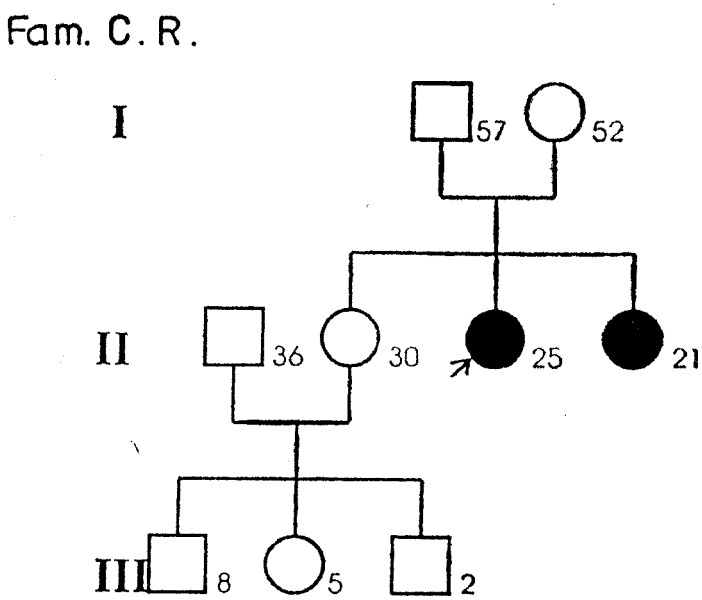

Fig. 5. *Pedigree of family C.R.*

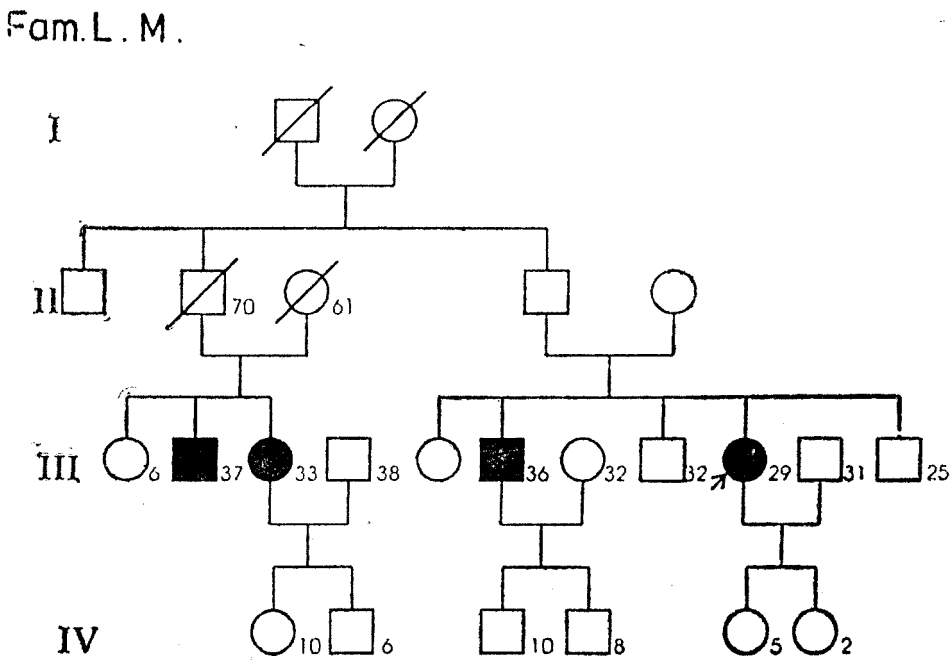

Fig. 6. *Pedigree of family L.M.*

Discussions. Ichthyosis vulgaris is the most common form of ichthyosis and it has a benign evolution. It affects both sexes, and the onset is usually situated during the first year of life (after the first three months). The evolution of the disease is progessive, with abnormally dry skin and symptoms of atopic dermatitis in over 50% of the cases [1—4, 10, 11].

The scales are fine and white, grey or brown. Ichthyosis vulgaris has an autosomal dominant mode of inheritance. The deleted gene has full penetrance and variable expression. In the general population, the incidence of ichthyosis vulgaris is of 1:250 [2, 3].

The study of the pedigrees in the investigated families clearly shows the autosomal dominant mode of transmission of ichthyosis vulgaris. In all families, both sexes are affected, and the deleted gene is transmitted from generation to generation, being expressed in the phenotype (Figs. 1 and 2).

The severity of the clinical picture of patients with ichthyosis vulgaris varies from abnormally dry skin with white scales to symptoms of atopic dermatitis, grey scales and pilar hyperkeratosis. These aspects confirm the variable expression of the deficient gene of ichthyosis vulgaris.

Ichthyosis with recessive X-linked transmission (X-recessive ichthyosis) is caused by the mutation of a recessive gene, the STS gene, whose locus is situated on the short arm of the X chromosome, in the pseudoautosomal region. The STS gene locus is close to the gene locus for Xg blood group [2, 10].

Mutations in the STS gene cause steroid sulphatase and arylsulphatase C deficit. This enzymatic deficit leads to an increase in the cholesterol sulphate concentration in the stratum corneum. Excessive cholesterol sulphate produces changes in the intracellular cement and causes a delayed separation of scales.

In X-linked ichthyosis there is also a reduced mobility of low density proteins.

X-linked ichthyosis has an early onset, being situated in 83% of the cases during the first three months of life, and in 17% of the cases, at birth (the congenital form) [1, 4]. The symptomatology is characterized by brown scales, highly adherent to the limbs, trunk and neck.

Incidence of the X-recessive ichthyosis in the population is 1 in 6,000 boys, without ethnic or racial associations.

The carrier females for STS mutant gene are asymptomatic, but some of them show placental sulphatase deficit.

Males affected by X-recessive ichthyosis transmit the deficient gene to their daughters, who will be carriers. In the case of a female carrier for STS deleted gene, the risk of her daughters being carriers is 50% (1:2).

The study of the investigated pedigrees (Figs. 3 and 4) clearly shows the X-linked recessive mode of inheritance of this type of ichthyosis. In

these families, the boys are affected, and the girls are carriers of the abnormal STS gene.

Lamellar ichthyosis or congenital ichthyosiform erythroderma is a severe dermatologic anomaly characterized by lamellar, dark-coloured scales on the whole skin surface and, frequently, ectropion [2, 4, 7, 8].

The onset of lamellar ichthyosis is at birth, the newborn having a particular appearance, as if being enveloped in a collodium membrane. This membrane disappears soon and erythroderma and generalized ichthyosis occur.

Lamellar ichthyosis is transmitted in an autosomal recessive mode. The gene of lamellar ichthyosis is located on the long arm of chromosome 14, at 14q11, and encodes the synthesis of enzyme transglutaminase 1 [6—8, 11]. The incidence of this form of ichthyosis is 1 in 100,000 births, both sexes being affected.

The analysis of the investigated pedigrees shows the autosomal recessive mode of transmission of lamellar ichthyosis (Figs. 5 and 6). In all families, the probands have clinically healthy parents, but they affected collaterals and brothers. The descent of the affected persons is healthy. The presence of healthy ancestry and descent confirms the autosomal recessive mode of inheritance of this disease.

Treatment in all forms of ichthyosis consists of general treatment with retinoids and daily applications of emollient creams containing lactic acid, urea and glycolic acid. These creams relieve the suffering of the patients.

A decrease in the family and population incidence of these disorders causing cosmetic handicap can be achieved by carrier detection and genetic counselling.

In the future, as the genetic and molecular defect of these diseases, especially of the severe ones: X-recessive ichthyosis and lamellar ichthyosis will be known, they will be treated by gene therapy.

Conclusions. 1. Ichthyoses are a group of genetically heterogeneous skin diseases, caused by mutations either of autosomal dominant and recessive genes, or of recessive genes with X-linked transmission.

2. In inchthyosis vulgaris, the risk of recurrence for a heterozygote is 1:2 (50%) of the descendants of both sexes. In lamellar ichthyosis the risk of recurrence for a carrier is 1:4 (25%) of the descendants of both sexes. The risk of recurrence for a female carrier of (X-recessive) STS gene is 1:2 (50%) of male descendants.

3. Ichthyosis generates cosmetic handicap.

4. Therapy in ichthyosis consists of general treatment with retinoids, and local treatment with keratolytic and emollient drugs.

5. A decrease in the frequency of these disorders can be achieved by carrier detection and genetic counselling.

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CONJUGATIVE DNA TRANSFER IN THE GRAM-NEGATIVE PHOTOSYNTHETIC BACTERIUM *THIOCAPSA ROSEOPERSICINA*

ELENA MANOLACHE*, LÁSZLÓ DANKÓ, GÁBOR RAKHELYI** and KORNÉL L. KOVÁCS****

SUMMARY. — *Thiocapsa roseopersicina* BBS, a Gram-negative purple sulphur photosynthetic bacterium was used as recipient for DNA conjugative transfer. The plasmids transferred by conjugation originated from two *Escherichia coli* strains (*E. coli* C600 carrying the Amp^r, Te^r, Km^r RP4 plasmid and *E. coli 294* harbouring the RP4—Gm^r plasmid which is Amp^r, Gm^r). The conjugant *T. roseopersicina* grew on plates containing Km or Gm as selective antibiotic, respectively. A conjugation frequency between 2.6×10^{-2} — 1.4×10^{-1} for Km-resistant conjugants and between 2.5×10^{-4} — 1.1×10^{-3} for Gm-resistant conjugants was found. The conjugating system succeeded also when using a spontaneous rifampicin-resistant mutant *T. roseopersicina* BBS strain as recipient, the conjugation frequencies were 5.6×10^{-2} for Rif^r/Km^r conjugants and 1.3×10^{-2} for Rif^r/Gm^r products, respectively. In the case of using *T. roseopersicina* BBS protoplasts, a very low frequency of conjugation was observed. During the plasmid analysis the existence of a megaplasmid in *T. roseopersicina* BBS wild strain was detected.

The genetic analysis and manipulation of bacteria and the molecular cloning of prokaryotic genes have been greatly facilitated by the use of antibiotic resistance-carrying transposons [6]. Transposons are specific DNA segments with the ability to move as a unit in a more or less random fashion from one genetic locus to another [1].

In order to use a transposon as a mutagenic agent, a replicon is needed that both carries the transposon and can be introduced into the organism to be mutagenized. Futhermore, a means for selecting against stabilization of the transposon carrier replicon in the recipient strain is required. If these conditions are met, transposition events can be isolated simply by selecting for transposon-mediated antibiotic resistance [6].

Since the genetic analysis of bacteria other than *E. coli* has attracted increasing interest, methods have been developed that make transposon technology applicable to other bacterial species. Drug resistance plasmids of the IncP-group, which are known to be transferable to a wide range of Gram-negative hosts [3, 4] were modified to render them unstable in the recipient strain. They could then be used as vehicles for the introduction of transposons into non-*E. coli* hosts [2, 13].

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We have used the transfer functions of the broad host range plasmid RP4 to mobilize plasmids from *E. coli* into another Gram-negative bacterium, *T. roseopersicina*. In the literature, there is no known gene transfer vehicle for *T. roseopersicina*.

Materials and methods. *Bacterial strains.* Experiments were carried out with *Thiocapsa roseopersicina* strain BBS, a gift from Prof. E. N. Kondrat'eva (Department of Microbiology, Moscow State University, Russia) and with a spontaneous Rif^r *T. roseopersicina* BBS strain, obtained in our laboratory, as recipient. Either the *E. coli* C600 strain or the *E. coli* 294 strain was used as donor. The donor strains were obtained from Prof. H. Trüper (University of Bonn, Germany).

Media and growth conditions. The *T. roseopersicina* strains were cultivated under standard photosynthetic conditions [7, 10] in 100-ml bottles in mineral NS medium. NH_4Cl was supplied as nitrogen source to repress nitrogenase synthesis [8, 10]. The composition of the NS medium (to 1000 ml) was: KH_2PO_4, 1 g; Na_2S, 0.5 g; $NaCl$, 20 g; $MgCl_2$, 1 g; KCl, 1 g; $NaHCO_3$ 2 g; $Na_2S_2O_3$, 2 g; Na acetate, 2 g; NH_4Cl, 1 g; solution of microelements, 1 ml; Fe-EDTA solution (concentration 3360.5 mg/l), 1 ml; B_{12} vitamin, 2—3 mg. The composition of the solution of microelements for 1000 ml: Selecton B_2, (diNa-EDTA), 2975 mg; H_3BO_3, 300 mg; $CaCl_2 \times 6H_2O$, 200 mg; $ZnSO_4 \times 7\ H_2O$, 100 mg; $MnCl_2 \times 4H_2O$, 30 mg; $Na_2MoO_4 \times 2H_2O$, 30 mg; $NiCl_2 \times 6H_2O$, 20 mg; $CuCl_2 \times 2H_2O$, 10 mg. Rifampicin was added to the NS medium of the *T. roseopersicina* Rif^r strain at a concentration of 25 μg/ml. *E. coli* donor strains were cultivated in 2YT medium [12] with the appropriate antibiotic (25 μg/ml Km for *E. coli* C600 and 5 μg/ml Gm for *E. coli* 294) at 37°C for 3 hours.

Bacterial conjugation protocol. — Conditions. The *E. coli* donor strain has to be grown until the OD_{600} was 0.7—0.8 (4—5 hours in LB medium or 2—3 hours in 2TY medium) at 37°C. The donor must be in the exponential growth phase. The recipient bacterium must be in the stationary phase (the *T. roseopersicina* culture should not be older than one week). Cultures having $OD_{900} = 2.7 — 2.8$ are routinely used.

Technique. First day. Three sterile Eppendorf tubes have to be prepared (one each for the donor, for the donor + recipient and for the recipient). 500 μl of cell suspension is pipetted into the tubes (the donor + recipient tube contains 1 ml suspension). Cells are sedimented by centrifugation at 13.000 rpm for 5 minutes. The supernatants are carefully discarded but one drop is left in each tube and the cells are resuspended in it. Cell suspensions are pipetted onto sterile nitrocellulose membranes (0.45 μm) placed on top of Petri dishes (one for each tube) containing a medium that allows the growth of both the donor and the recipient. In our case, a medium containing LB and NS media is used. The plates are put into an anaerobic jar under an atmosphere of $H_2 + CO_2$ and incubated in the light for 20 hours.

Second day. The nitrocellulose membranes are removed from plates and put into sterile Eppendorf tubes containing 1 ml of LB and NS medium. Cells are washed off the filter by gentle pipetting. Dilutions between 10^{-1} to 10^{-6} are prepared from all three tubes. 50 μl and 100 μl suspensions from all dilutions are spread on plates containing an adequate medium (LB + Km or Gm for the donor, NS + Km or Gm for conjugant *T. roseopersicina*, and normal NS medium for recipient controls). The antibiotic concentrations in the plates are 25 μg/ml for Km and 5 μg/ml for Gm, respectively. The plates for conjugate and recipient are solidified with Phytagel (0.5%). They are placed into an anaerobic jar and incubated under standard photosynthetic conditions for 1—2 weeks. The *E. coli* has been incubated at 37°C for one day.

Protoplast preparation. Protoplasts were prepared by lysozyme treatment in 0.5 M sucrose. Cells from 1 ml culture were sedimented by centrifugation and resuspended in 1 ml 0.5 M sucrose. The bacterial wall was digested by lysozyme treatment (50 μg/ml) at 30°C for 15—30 minutes followed by centrifugation (10,000 × g for 10 minutes) [9].

Direct plasmid analysis. Lysis of whole cells in agarose gels and subsequent electrophoresis to detect plasmids were done by using a modified method described by Eckhardt [5]. Cells from one, two or three colonies were suspended in 25 μl lysis buffer (8% sucrose, 2% Ficoll-400, 50 mM EDTA, 450 mM Tris-HCl, pH 8, containing 0.5 μg/ml RNase and 4 mg/ml lysozyme). The mixture was pipetted into the wells of a 1.0% agarose gel (0.5% TBE, 0.5% SDS) and incubated at room temperature for 30—60 minutes, followed by electrophoresis at 35 V for 16 hours. Conventional agarose electrophoresis and plasmid isolation were performed by standard techniques [11].

Estimation of conjugation frequency. 10^{-4}—fold dilution 50 μl cell number = X, 10^{-4} dilution 100 μl cell number = Y. Average (A) = (X + (Y/2))/2. Conjugant cell number = A × 20 × 10^4/ml. Nonconjugant *Thiocapsa* cell number was calculated as above, with appropriate modifications. The *conjugation frequency* = conjugant cell number/nonconjugant cell number.

Chemicals. Bacto-agar and Yeast extract were from Difco Laboratories (USA), Ficoll-400 was from Pharmacia Fine Chemicals (Sweden), Phytagel was from Sigma Chemical Co. (USA), Agarose was from Koch Light Ltd. (England), Sodium dodecylsulphate was from Serva (USA). Lysozyme (EC 3.2.1.17) and other chemicals were from Reanal (Hungary).

Results. Four new types of *Thiocapsa roseopersicina* were produced: Km-, Gm-, Rif/Km- and Rif/Gm- resistant, using the conjugative DNA transfer method and the transfer functions of the broad host range plasmid RP4 to mobilize plasmids from *E. coli* into *T. roseopersicina.*

The conjugation frequency between 2.6 × 10^{-2} and 1.4 × 10^{-1} was calculated for Km^r conjugant *T. roseopersicina,* and between 2.5 × 10^{-4} and 1.1 × 10^{-3} for Gm^r conjugant *T. roseopersicina.* The conjugation frequency obtained in the case of using *T. roseopersicina* rifampicin-resistant mutant as recipient was 5.6 × 10^{-2} for Rif/Km^r and 1.3 × 10^{-2} for Rif/Gm^r, respectively. While working with the Rif^r strain a limitation of growth of the *E. coli* donor strains was observed. This may be due to the inhibitory effect of any residual rifampicin in the conjugation system. Therefore, in an ongoing experiment in this study, residual rifampicin will be carefully removed by suspending the cells into normal *Thiocapsa* medium before conjugation.

Conjugation experiments were also done with *T. roseopersicina* protoplasts based on the note that this bacterium contains a slime capsule which may interfere with the conjugative gene transfer. A very low frequency of conjugation was obtained (practically only the undiluted suspension grew, and for this reason we could not calculate the conjugation frequency). This result does not necessarily show a deficiency of the plasmid transfer itself; it indicates rather a very pronounced sensitivity of protoplasts. The fragile protoplasts tended to be ruined during the manipulation.

After SDS agarose electrophoresis plasmid analysis, a megaplasmid (~ 60 kb) has been seen both in the conjugant *T. roseopersicina* and also in the nonconjugant wild colonies (Fig. 1). After the plasmid purification, the agarose gel electrophoresis showed similar results, the plasmidial DNA bands of the conjugant strains being a bit thicker, suggesting a possible superposition of the both, native and transferred plasmids (Fig. 2).

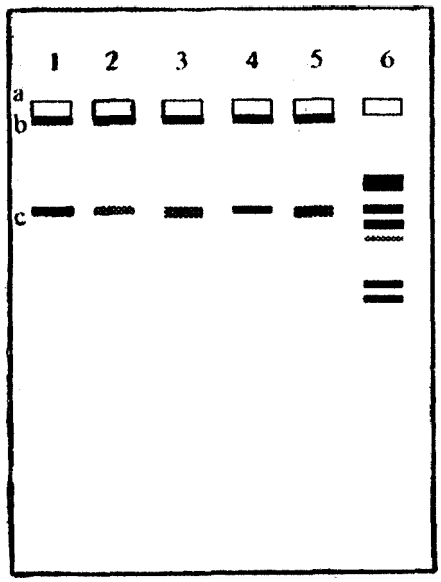

Fig. 1. *Results of the direct plasmid analysis by SDS–agarose gel electrophoresis.* a — Loading places. *b* — Chromosomal DNA. c — Plasmidial DNA. 1 — *E. coli* C600 (donor). 2 — *T. roseopersicina* BBS (nonconjugant). 3 — *T. roseopersicina* Km^r (conjugant). 4 — *T. roseopersicina* Gm^r (conjugant). 5 — *E. coli* 294 (donor). 6 — λ marker.

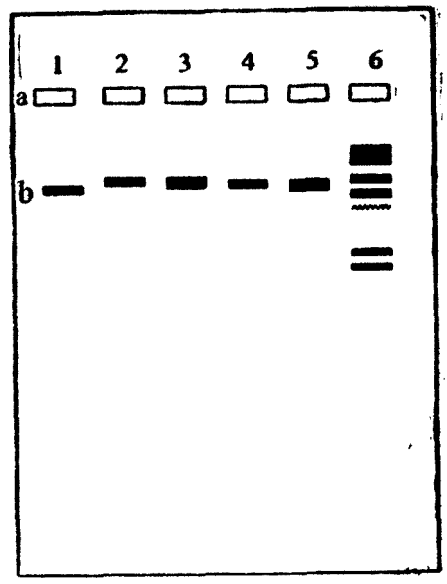

Fig. 2. *Results of the plasmid DNA purification by agarose gel electrophoresis.* a — Loading places. b — Plasmidial DNA. 1 — *T. roseopersicina* BBS (nonconjugant). 2 — *E. coli* C600 (donor). 3 — *T. roseopersicina* Km^r (conjugant). 4 — *E. coli* 294 (donor). 5 — *T. roseopersicina* Gm^r (conjugant). 6 — λ marker

Discussion. We have succeeded in obtaining antibiotic resistant strains of the Gram-negative purple sulphur bacterium *T. roseopersicina* using a DNA conjugative transfer of RP4 and RP4-Gm plasmids from *E. coli* C600 and *E. coli 294*, respectively. The only previous similar attempt for gene transfer in this family of photosynthetic bacteria has been done at the University of Bonn, Germany, recently. In the German experiment, the purple sulphur phototrophic bacterium *Chromatium vinosum* was used as recipient (Drs. H. Trüper and C. Dahl, unpublished data).

It is known that both *Thiocapsa* and *Chromatium* belong to the same family *(Chromatiaceae)* [12]. The two bacterium species are very similar in their physiology, metabolism, distribution and orientation of the hydrogenase, etc. [9, 12]. Our preliminary results also indicate the existence of a plasmid in *T. roseopersicina* which does not exist in *C. vinosum* (Drs. H. Trüper and C. Dahl, unpublished data). The existence

of this megaplasmid in *T. roseopersicina* remains to be established with further methods (such as plasmid digestion with restriction enzymes) as well as its role, if any, in the hydrogenase expression.

Conclusions. 1. Four new strains of *Thiocapsa roseopersicina* were produced: Km-, Gm-, Rif/Km- and Rif/Gm-resistant, using conjugative DNA transfer method.

2. The best conjugation frequency ranged between 1.1×10^{-3} and 1.4×10^{-1} for Gm^r and Km^r conjugant *T. roseopersicina* and between 1.3×10^{-2} and 5.6×10^{-2} for Rif Gm^r and Rif/Km^r conjugant *T. roseopersicina*, respectively.

3. During the direct analysis of plasmids and after their purification, the preliminary results showed the presence of a megaplasmid in both conjugant and nonconjugant *T. roseopersicina* strains.

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FERMENTAREA EXTRACTULUI MUSTULUI DE BERE DE CĂTRE UNELE TULPINI DE DROJDII INDUSTRIALE

LETIŢIA OPREAN*

SUMMARY. — Fermentation of Wort Extract by Some Industrial Yeast Strains. Eleven industrial yeast strains belonging to the genus *Saccharomyces* were studied. Six of them are brewer's yeast strains (*S. uvarum* A, B, 66, T_{18}, $Tanc_{11}$ and 15), two are baker's yeast strains (*S. cerevisiae* CA and CP), one is a spirit yeast strain (*S. cerevisiae* CF) and two are wine yeasts (*S. oviformis* and *S. ellipsoideus*). During the fermentation (9 days at 20°C), the apparent wort extract and the apparent fermentation degree were determined daily and at the end of the fermentation period the real fermentation degree was also assessed for each yeast strain, by using standard methods

The results have shown that most of the yeast strains studied decreased substantially the apparent wort extract and exhibited a high apparent fermentation degree even during the first day of fermentation. At the end of the fermentation period, the lowest apparent wort extract values and the highest apparent and real fermentation degrees were registered with *S. cerevisiae* CF, *S. uvarum* A and *S. ellipsoideus*.

Capacitatea de fermentare a extractului mustului de bere este o însuşire importantă a drojdiilor utilizate în diferite ramuri industriale. Această însuşire este evidenţiată prin determinarea extractului aparent şi a gradului aparent şi real de fermentare a mustului de bere, care în prealabil a fost inoculat cu drojdia studiată şi supus incubării [1—5, 7].

În lucrarea de faţă descriem cercetări comparative privind dinamica extractului aparent şi a gradului aparent de fermentare, precum şi gradul real final de fermentare, în prezenţa unor tulpini de drojdii industriale aparţinătoare genului *Saccharomyces*.

Materiale şi metode. Am studiat 11 tulpini de drojdii industriale, dintre care 6 sunt tulpini de drojdii de bere provenite de la fabricile de bere din ţară, iar 5 tulpini de drojdii (două de panificaţie, una de spirt şi două de vin) au fost obţinute sub formă de culturi pure în cadrul Laboratorului de microbiologie al Catedrei de tehnologia produselor alimentare, Universitatea Lucian Blaga, Sibiu. Cele 6 tulpini de drojdii de bere (*S.uvarum*) sunt notate prin A, B, 66, T_{18}, $Tanc_{11}$ şi 15. Cele două tulpini de drojdii de panificaţie aparţin speciei *S.cerevisiae* şi sunt notate prin CA şi CP. Drojdia de spirt aparţine tot speciei *S.cerevisiae;* notarea tulpinii: CF. Cele două tulpini de drojdii de vin sunt tulpini de *S.oviformis*, respectiv *S.ellipsoideus*.

Pentru cultivarea tulpinilor de drojdii a servit, drept mediu nutritiv, mustul de bere industrial preparat din malţ, sterilizat în prealabil, nehameiat şi cu un extract real de 13,31%. Mustul de bere a fost disrtibuit în cantităţi de 200 ml în baloane cu fund plat de 250 ml, sterilizate şi închise cu ventile de fermentaţie cu

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acid sulfuric concentrat. Pentru inocularea mustului de bere, din culturile stoc s-au preparat culturi tot pe mustul de bere sterilizat. Fermentația mustului de bere a fost condusă la temperatura camerei (20°C) timp de 9 zile (192 ore).

În cursul fermentației am determinat zilnic extractul aparent și gradul aparent de fermentare a mustului de bere, iar la sfârșitul fermentației am determinat și gradul real de fermentare, pentru fiecare tulpină de drojdie studiată. Am utilizat metodele de analiză curente, în conformitate cu STAS-ul în vigoare [6].

Pentru determinarea extractului aparent al mustului de bere se folosește o probă decarbonată reprezentată de filtratul obținut în urma centrifugării mustului în fermentație, în cantitate de 100 g. Pentru determinarea extractului inițial (primitiv) se folosește o probă de 100 g, reprezentată de filtratul obținut în urma centrifugării mustului inițial, nefermentat, deci lipsit de alcool. Se determină cifra de apă a picnometrului, după care picnometrul este folosit pentru determinarea densității relative a filtratului obținut în urma centrifugării mustului de bere supus, respectiv nesupus fermentației. În final, se stabilesc extractul inițial (primitiv) și extractul aparent al mustului de bere, exprimate în % masice (g extract/100 g must), cu ajutorul unor tabele [3, 7].

Gradul aparent și gradul real de fermentare, exprimate tot în %, se stabilesc cu ajutorul unor formule [3, 7].

Rezultate. Rezultatele obținute în studierea dinamicii extractului aparent al mustului de bere sunt trecute în Tabelul 1. Se poate vedea din acest tabel că deja după 24 ore de fermentație are loc o scădere

Tabel 1

Dinamica extractului aparent al mustului de bere sub acțiunea celor 11 tulpini de drojdii industriale studiate

Tulpini de drojdii (Saccharomyces)	Extract real inițial (% masice)	Extract aparent (% masice)							
		Durata de fermentație (ore)							
		24	48	72	96	120	144	168	192
S. uvarum A (bere)	13,31	4,52	3,42	1,96	1,84	1,80	1,52	1,31	1,11
S. uvarum B (bere)	13,31	6,31	3,69	3,24	3,09	2,93	2,88	2,52	2,24
S. uvarum 66 (bere)	13,31	7,27	3,24	3,17	3,04	2,64	2,26	2,11	1,96
S. uvarum T_{18} (bere)	31,31	5,53	4,04	2,47	2,39	1,76	1,76	1,71	1,66
S. uvarum $Tanc_{11}$ (bere)	13,31	6,77	3,99	2,78	2,47	2,39	2,29	1,83	1,56
S. uvarum 15 (bere)	13,31	5,12	4,47	4,19	2,99	2,38	1,84	1,71	1,66
S. cerevisiae CA (panificație)	13,31	5,63	4,39	4,09	3,27	2,65	2,16	1,96	1,69
S. cerevisiae CP (panificație)	13,31	5,86	3,64	2,88	2,14	2,06	1,99	1,84	1,66
S. cerevisiae CF (spirt)	13,31	4,81	3,44	2,73	2,65	1,99	1,54	1,31	1,06
S. oviformis (vin)	13,31	4,99	3,32	2,96	2,78	2,44	2,26	2,14	1,36
S. ellipsoideus (vin)	13,31	3,97	3,91	2,89	2,54	1,81	1,64	1,32	1,22

substanțială a extractului aparent al mustului sub acțiunea tulpinilor de drojdii studiate, cu mici variații de la o tulpină la alta. Cea mai scăzută valoare a extractului aparent se remarcă la drojdia de vin *S.ellipsosoideus*, fiind egală numai cu 54,6% în raport cu drojdia de bere *S.uvarum* 66, ce prezintă cea mai ridicată valoare a extractului aparent. *S.ellipsoideus* este urmat de drojdia de bere *S.uvarum* A și de

drojdia de spirt *S.cerevisiae* CF, sub acțiunea cărora, în ordinea enumerată, mustul prezintă valori ale extractului aparent egale cu 62,2 și 66,2% în raport cu *S.uvarum* 66.

La sfârșitul fermentației, sub acțiunea drojdiei de spirt *S.cerevisiae* CF, mustul prezintă cel mai scăzut extract aparent care este egal cu numai 47,3% în raport cu *S.uvarum* B, drojdia cu cea mai ridicată valoare a extractului aparent. *S.cerevisiae* CF este urmat de drojdia de bere *S.uvarum* A, sub acțiunea căreia, mustul prezintă un extract aparent egal cu 49,5% în raport cu *S.uvarum* B. Se remarcă și drojdiile de vin *S.ellipsoideus* și *S.oviformis*, ce prezintă, în ordinea enumerată, valori ale extractului aparent egale cu 54,5 și 60,7% în raport cu *S. uvarum* B.

Rezultatele obținute în studierea dinamicii gradului aparent de fermentare a mustului de bere, împreună cu valorile gradului real final de fermentare, sunt redate în Tabelul 2. Datele acestui tabel arată că deja

Tabel 2

Dinamica gradului aparent de fermentare a mustului de bere sub acțiunea celor 11 tulpini de drojdii industriale studiate, în comparație cu gradul real final de fermentare

Tulpini de drojdii (Saccharomyces)	Gradul aparent de fermentare (%)								Gradul real final de fermentare (%)
	Durata de fermentație (ore)								
	24	48	72	96	120	144	168	192	
S. uvarum A (bere)	66,04	74,30	85,27	86,61	87,02	88,58	90,10	90,66	91,28
S. uvarum B (bere)	52,59	72,27	75,65	76,32	78,00	78,65	80,12	80,17	81,02
S. uvarum 66 (bere)	45,37	72,65	76,18	77,16	80,20	83,02	84,12	84,27	84,50
S. uvarum T_{18} (bere)	58,45	69,64	80,44	82,04	85,00	86,77	87,11	87,52	87,90
S. uvarum $Tanc_{11}$ (bere)	49,13	70,02	79,11	81,44	82,10	82,79	86,19	88,27	88,50
S. uvarum 15 (bere)	61,53	66,41	68,51	77,53	82,15	86,17	86,29	86,52	86,92
S. cerevisiae CA (panificație)	52,70	67,01	69,27	75,43	80,17	83,77	85,31	87,60	87,52
S. cerevisiae CP (panificație)	55,97	72,65	78,36	83,92	84,51	85,04	86,12	87,52	87,15
S. cerevisiae CF (spirt)	72,59	74,15	79,48	80,09	85,12	88,42	90,15	91,48	91,66
S. oviformis (vin)	60,17	65,00	67,76	75,10	80,66	83,09	84,00	86,50	87,80
S. ellipsoideus (vin)	71,00	77,17	77,25	77,80	84,15	86,69	88,10	88,50	89,50

după 24 ore de fermentație se obțin grade aparente de fermentare ridicate, cu mici diferențe între ele. Drojdia de spirt *S.cerevisiae* CF prezintă cel mai mare grad aparent de fermentare, care este cu 62,5% mai mare decât la *S.uvarum* 66, drojdia cu cel mai mic grad aparent de fermentare. Se remarcă și drojdia de vin *S.ellipsoideus* și drojdia de bere *S.uvarum* A, care au, în ordinea enumerată, grade aparente de fermentare cu 56,5 și 45,6% mai mari decât *S.uvarum* 66.

Creşterea gradului aparent de fermentare a mustului de bere, sub acţiunea tulpinilor de drojdii studiate, continuă între 48 şi 144 ore de fermentaţie, după care rata de creştere a gradului aparent de fermentare este mai mică.

La sfârşitul fermentaţiei, la majoritatea tulpinilor de drojdii studiate, valorile gradului aparent de fermentare a mustului de bere sunt ridicate, cu mici diferenţe de la o tulpină la alta. Valorile cele mai mari ale gradului aparent de fermentare sunt înregistrate la drojdia de spirt *S.cerevisiae* CF, drojdia de bere *S.uvarum* A şi drojdia de vin *S.ellipsoideus*. Aceste valori sunt cu 14,1, 13,1 şi, respectiv, cu 10,4% mai mari decât la *S.uvarum* B, drojdia cu cel mai mic grad aparent de fermentare. Aceleaşi trei tulpini de drojdii se remarcă şi prin valorile maxime ale gradului final real de fermentare, care sunt cu 13,1, 12,7 şi, respectiv, cu 10,5% mai mari decât la *S.uvarum* B.

Comparând valorile extractului aparent cu valorile gradului final aparent şi real de fermentare, se constată că valori mici ale extractului aparent corespund unor valori mari ale gradelor finale de fermentare.

Concluzii. 1. La majoritatea tulpinilor de drojdii studiate, extractul aparent al mustului de bere se micşorează substanţial şi gradul aparent de fermentare prezintă valori ridicate chiar după primele 24 ore de fermentaţie.

2. La sfârşitul fermentaţiei, cele mai scăzute extracte aparente ale mustului de bere şi cele mai ridicate valori ale gradului aparent şi ale gradului real de fermentare se înregistrează la drojdia de spirt *S. cerevisiae CF*, drojdia de bere *S.uvarum* B şi drojdia de vin *S.ellipsoideus*.

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MICROBIAL COUNTS AND ACTIVITIES IN REPRESENTATIVE SOILS OF THE SATU MARE COUNTY

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SUMMARY. — Counts of aerobic heterotrophic bacteria, ammonifying bacteria and azotobacter cells, respiration, ammonifying capacity and N_2-fixing capacity as well as some chemical properties (humus, total N, NH_4^+-N, NO_3^--N, mobile P and mobile K contents, and pH) were determined in 11 soils. As expected, both microbiological and chemical properties varied depending on soil type, subtype and location. The most interesting finding was that these soils, although their pH is acid, contain azotobacter cells in a relatively great number and exhibit a considerable N_2-fixing capacity. Significant positive correlations were registered in these soils between total N content and count of azotobacter cells as well as between total N content and N_2-fixing capacity.

Ştefanic and his co-workers [4, 5] were the first to perform microbiological and enzymological studies on one of the representative soils of the Satu Mare county. They dealt with the influence of fertilisation, liming and ploughing depth on the total microflora and some enzyme activities in a pseudogleyed argillo-illuvial podzolic soil at the Agricultural Experimental Station in Livada. The aim of the investigations described in the present paper was to determine a series of chemical and microbiological properties in representative soils of this county that were selected according to [1].

Materials and methods. Eleven soils were studied. For chemical and microbiological analyses, the 0—25-cm layers of plots not affected by fertilisation were sampled during 1995. The soil types, subtypes, locations and sampling dates are listed in Table 1.

For chemical analyses, the soil samples were allowed to air-dry, then ground, whereas the microbiological analyses were carried out with field-moist soil samples.

The chemical properties (humus, total N, NH_4^+-N, NO_3^--N, mobile P and mobile K contents, and pH) were determined according to the methods recommended in [2].

The microbiological analyses comprised determination of the counts of aerobic heterotrophic bacteria, ammonifying bacteria and azotobacter cells as well as determination of respiration (CO_2 evolution), ammonifying capacity (NH_3 evolution) and N_2-fixing capacity.

For counting of bacteria, soil dilutions were prepared. Ten g of soil and 90 ml of a 3% (w/v) Na pyrophosphate solution in distilled water were mixed, then shaken for 3—4 hours [3]. The dilution obtained (10^{-1}) was submitted to further 10-fold dilutions with pyrophosphate solution up to the 10^{-9} dilution. Sterility of

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Table 1

List of soil types, subtypes, locations and sampling dates

Soil No.	Soil type and subtype	Location	Sampling date (1995)
	Chernozem		
1	— cambic	Petreşti	5.VII
2	— moderately leached argillo-illuvial	Chereuşa	16.VI.
	Brown soil		
3	— argillo-illuvial	Botiz	13.VI
4	— moderately pseudogleyed luvic	Răteşti	19.VII
5	— typical eu-mesobasic	Acâş	20.VI
6	— pseudogleyed eu-mesobasic	Gherţa Mare	6.X
	Albic luvisol		
7	— pseudogleyed	Mădăras	29.VI
8	— pseudogleyed	Ciuperceni	13.VI
9	— pseudogleyed	Certeze	26.IX
10	*Typical solonetz*	Căuaş	22.VI
11	*Forest soil*	Mujdeni	22.VI

the pyrophosphate solution was controlled by its inoculation and incubation on different nutrient media.

For determination of the count (colony-forming units — cfu) of the aerobic heterotrophic bacteria, the 10^{-8} soil dilution was inoculated on nutrient agar purchased from the Cantacuzino Institute, Bucharest. Volume of inoculum: 1 ml. Incubation: at 28°C for 48 hours.

For counting the ammonifying bacteria, the alkaline liquid peptone medium purchased from the Cantacuzino Institute was solidified with 2% agar-agar and sterilised by autoclaving at 120°C/30 minutes. The medium was inoculated with the 10^{-8} soil dilution. Volume of inoculum: 1 ml. Incubation: 28°C/48 hours.

The count (cfu) of azotobacter cells was established, as recommended in [3], on the Ashby medium, used in the following composition: glucose 20 g, K_2HPO_4 0.2 g, $MgSO \cdot 7H_2O$ 0.2 g, NaCl 0.2 g, $CaCO_3$ 5 g, agar-agar 20 g, distilled water 1000 ml. After liquefaction, the medium was distributed in Erlenmeyer flasks and autoclaved at 120°C/30 minutes. For inoculation, the 10^{-1} dilution was used. Volume of inoculum: 1 ml. Incubation: 28°/14 days.

Respiration (CO_2 evolution) and ammonifying capacity (NH_3 evolution) were determined with the *in vitro* methods recommended in [3]. Incubation: at room temperature for 7 days. They are expressed in mg of CO_2 and NH_3, respectively, produced by 100 g soil in 7 days.

The N_2-fixing capacity was assayed according to [3], in 100-g soil samples treated with 1 g glucose and incubated at 28°C for 30 days, assuring constant moisture content in soil during incubation. The N_2-fixing capacity (the difference between total N contents after and before incubation) is expressed in ppm N.

The chemical and microbiological data were submitted to correlation analysis.

Results. Table 2 shows that the chemical and microbiological properties varied in the studied soils depending not only on the soil type and subtype, but also on the location. Thus, the humus content is lowest in soil 4 (moderately pseudogleyed luvic brown soil) and in soil 10 (typical solonetz). The total N content is higher in soils 3—6 (brown

Table 2

Chemical and microbiological properties of the studied soils

Chemical and microbiological properties	Soils										
	1	2	3	4	5	6	7	8	9	10	11
Humus (%)	2.15	2.00	2.91	1.46	2.63	3.07	1.89	3.48	3.10	1.58	3.24
Total N (ppm)	370	381	409	549	517	431	231	269	381	185	269
NH_4^+—N (ppm)	25.5	1.21	1.42	53.2	0.02	19.3	2.41	1.24	39.0	4.50	1.78
NO_3^-—N (ppm)	13.5	1.71	1.02	1.73	29.1	1.13	21.2	1.29	1.61	1.94	1.50
Mobile P (ppm)	35.1	68.6	97.9	58.7	415	41.4	43.5	22.0	130	37.8	25.1
Mobile K (ppm)	169	179	403	125	619	70	43.4	79.8	26.8	36.6	25.6
pH	5.46	6.29	4.39	5.33	6.01	4.90	5.10	4.81	4.82	5.66	4.20
Count of aerobic heterotrophic bacteria (million cfu/g soil)	269	43	38	69	68	213	111	78	271	57	121
Count of ammonifying bacteria (million cfu/g soil)	168	63	54	51	43	113	63	71	109	22	56
Count of azotobacter cells (cfu/100 g soil)	1210	1532	842	1410	1327	546	380	560	1120	146	1230
Respiration (mg CO_2/100 g soil/7 days)	72.9	86.2	36.4	36.4	16.1	46.3	32.4	40.7	82.5	44.7	40.5
Ammonifying capacity (mg NH_3/100 g soil/7 days)	26.2	30.1	16.7	13.2	6.14	6.34	7.88	13.9	12.2	5.26	10.2
N_2-fixing capacity (ppm N/30 days)	417	423	479	614	739	517	508	353	626	213	370

soils) than in the other ones. The NH_4^+-N and NO_3^--N contents show great variations even within the same soil type (compare soil 1 with soil 2; soil 4 with soils 3, 5, 6, and soil 5 with soils 3, 4, 6). In soils 7—9 (pseudogleyed albic luvisols), the variation of NH_4^+-N and NO_3^--N contents is location-dependent (compare soil 9 with soils 7, 8, and soil 7 with soils 8, 9). The mobile P and K contents are less variable in the chernozems than in the brown soils. pH is acid in each soil; as expected, the lowest value (4.20) was registered in the forest soil.

It is not surprising that aerobic heterotrophic and ammonifying bacteria were cultivable from each soil, but the presence of azotobacter cells in a relatively great number in these acid soils, including the very acid forest soil, was not expected.

The studied microbial activities (respiration, ammonifying and N_2-fixing capacities) were present in each soil and did not show so pronounced soil-dependent variations as did the chemical properties. Capacity of these acid soils to fix N_2 is in good agreement with the finding, mentioned above, that they contain viable, *i.e.* potentially N_2-fixing azotobacter cells.

Table 3

Correlation matrix concerning chemical and microbiological properties of the studied soils
(the figures denote correlation coefficients)

Symbol	Chemical and microbiological properties	Chemical and microbiological properties												
		A	B	C	D	E	F	G	H	I	J	K	L	M
A	Humus	1												
B	Total N	−0.0415	1											
C	NH_4^+-N	−0.2532	0.5099	1										
D	NO_3^--N	−0.1992	0.1480	−0.2429	1									
E	Mobile P	0.0822	0.5313	−0.1344	0.6692*	1								
F	Mobile K	0.0439	0.5790	−0.2791	0.5541	0.8333**	1							
G	pH	−0.6265*	0.2254	−0.0353	0.4028	0.3913	0.3235	1						
H	Count of aerobic heterotrophic bacteria	0.2649	0.0361	0.4928	0.0061	−0.1451	−0.3557	−0.2198	1					
I	Count of ammonifying bacteria	0.2263	0.1368	0.3946	0.0075	−0.2233	−0.2017	−0.1062	0.8797***	1				
J	Count of azotobacter cells	−0.0216	0.6633*	0.3220	0.0710	0.3492	0.3644	0.2549	0.0501	−0.1062	1			
K	Respiration	−0.0322	−0.0864	0.2920	−0.4162	−0.3559	−0.4031	0.1957	0.4950	0.5564	0.3036	1		
L	Ammonifying capacity	−0.1373	0.1404	0.0625	−0.1974	−0.2424	0.0482	0.2907	0.0619	0.4218	0.5571	0.6932*	1	
M	N_2-fixing capacity	0.0726	0.7967**	0.3956	0.4825	0.7061*	0.5254	0.1228	0.1708	0.1708	0.4937	−0.1961	−0.1497	1

The asterisks indicate significance: * between 95 and 99%; ** between 99 and 99.9%; *** at>99.9%.

The results of correlation analysis are presented in Table 3. Only the significant correlations will be emphasised.

Among the chemical properties, three significant correlations were found, namely a negative one between humus and pH, and two positive ones between NO_3^--N and mobile P, and between mobile P and mobile K, respectively.

Similarly, three significant correlations were registered between the chemical and microbiological properties. These correlations (between total N and count of azotobacter cells, between total N and N_2-fixing capacity, and between mobile P and N_2-fixing capacity) are positive and suggest that *a)* the free-living N_2-fixing bacteria play a major role in the accumulation of N compounds in the studied acid soils and *b)* the P necessary for growth of these bacteria is available.

The microbiological properties correlated significantly and positively with each other in two cases: count of aerobic heterotrophic bacteria with count of ammonifying bacteria, and respiration with ammonifying capacity.

Conclusions. 1. Comparison of the chemical and microbiological properties in the 11 soils studied has revealed that the soil-dependent variations of chemical properties are larger than those of the microbiological properties.

2. These soils, although their pH is acid, contain a relatively great number of azotobacter cells and exhibit a considerable N_2-fixing capacity.

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APPLICATION OF THE RESAZURIN REDUCTION METHOD FOR DETERMINATION OF DEHYDROGENASE ACTIVITY IN SOIL

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SUMMARY. — According to literature data, reduction of resazurin was successfully applied for determination of dehydrogenase activity in activated sludges, lacustrine and marine sediments. We have tried to apply resazurin reduction for determining dehydrogenase activity in the *soil*. A procedure, comprising a short (1-hour) incubation of the reaction mixtures, was elaborated. It was found that in reaction mixtures containing small amounts of soil (100—300 mg), the reduction of resazurin is mostly a biotic (enzymatic) process and is due, to a much lesser extent, to abiotic (nonenzymatic) compounds.

Lenhard [4] was the first to apply in 1956, *i.e.* more than 40 years ago, the method of the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) for determination of dehydrogenase activity in soils, this activity being considered as a measure of the global biological activity of soil microorganisms. Lenhard [5] was also the first investigator using TTC for determination of dehydrogenase activity in composts. For the first time, dehydrogenase activity in river sediments and that in activated sludges were determined with the TTC method by Lenhard and his co-workers [6, 7]. The TTC method was also applied for determination of dehydrogenase activity in lacustrine and marine sediments for the first time by Ohle [11] and Wieser and Zech [15], respectively.

The TTC method has then become a basic method for studying global microbial activity in different terrestrial and aquatic ecosystems. In many journals and books published in different countries from Europe, America, Asia, Africa and Australia-New Zealand, there are hundreds of papers describing investigations in which the TTC method was also applied. For registering pesticides in the United States, which is conditioned by thorough studies of their side-effects on soil biota, the Environmental Proection Agency [14] proposed guidelines, according to which many biological methods, including the TTC method for determination of soil dehydrogenase activity, are recommended. The TTC method is dealt with in details in recent methodological books on soil biology and biochemistry [1, 13].

During application of the TTC method, the incubation time is relatively long, usually of 24 hours. In soils, composts, sludges, aquatic sediments, the reduction of TTC is catalysed by different dehydrogenase enzymes (biotic, enzymatic reduction), but some nonenzymatic reduction

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compounds (*e.g.* H_2S) can also contribute to the reduction of TTC (abiotic reduction).

It is well known that reduction of resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) is a routine method for estimating bacterial counts in milk. For determination of dehydrogenase activity, characterising the global microbial activity in lake sediments, Liu and Strachan [9, 10] have applied resazurin in place of TTC. Resazurin has also been used for determination of dehydrogenase activity in activated sludges [2, 3, 8] and in marine sediments [12].

Reduction of resazurin (a blue compound) takes place in two phases. In the first phase, a pink compound (resorufin) forms, which, in the second phase, is reduced to the colourless dihydroresorufin [9].

The resazurin reduction method presents the advantage of requiring a short incubation time, generally of one hour. This means that this method is more sensitive than the TTC method. Reduction of resazurin, like that of TTC, is attributed to dehydrogenases and nonenzymatic reducing compounds. In a reaction mixture, the total (biotic, enzymatic + abiotic, nonenzymatic) reduction is determined, whereas in another reaction mixture, treated with an antiseptic (*m*-cresol), the abiotic reduction is established. The difference between total and abiotic reductions is equal to the biotic (enzymatic) reduction.

The resazurin reduction method has a disadvantage, too. After incubation, the nonreduced resazurin is extracted with an organic solvent (*n*-amyl alcohol), then the extract is submitted to centrifugation for removal of colloids; otherwise the extract can not be examined photocolorimetrically.

No literature data are available concerning the application of resazurin reduction for determination of dehydrogenase activity of the *soil*. Taking into consideration the advantage of this method (short incubation time), we have tried to work out a technique for the soil in such a way that the disadvantage of the original method (extraction with *n*-amyl alcohol and centrifugation) be avoided. Therefore, we have added an acetic acid solution to the incubated reaction mixtures and this treatment has made it possible to extract the residual resazurin and its reduction products and also to precipitate the colloids.

As mentioned in [9], in acidic media, resazurin is present in undissociated, easily extractable form of red colour. The acetic acid extract has been treated with a NaOH solution, followed by photocolorimetric reading. In this alkaline solution, resazurin is dissociated forming the Na salt of blue colour. Absorption of light at 610 nm wave length by resazurin is nearly three times more intense in alkaline than in acidic media [9]. A reaction mixture without soil has served for comparison. From the optical density values, the total (biotic + abiotic) resazurin reduction can be established. For establishing the abiotic reduction of resazurin, we have used not *m*-cresol, but phenol, which is a more available and efficient antiseptic. In this case, too, a reaction mixture without soil has been the control. The difference between total and abiotic reductions gives the biotic reduction, *i.e.* dehydrogenase activity of

soil. Based on standard curves, dehydrogenase activity is expressed in μg of reduced resazurin.

Materials and methods. The resazurin reduction method was tested with air-dry samples of a leached chernozem (humus 4.17%, total N 1386 ppm, mobile P 47.7 ppm, mobile K 265.8 ppm, pH 6.44).

The reaction mixtures prepared in test tubes contained 1 ml of resazurin solution (65 mg resazurin/1000 ml distilled water) + 5 ml of distilled water (for determination of total reduction) or 1 ml of resazurin solution + 4.8 ml of distilled water + 0.2 ml of phenol (for determination of abiotic reduction). Then, the test tubes were placed on a water-bath at 37°C for 10 minutes, followed by addition of soil (100—1000 mg) and incubation at 37°C for one hour. After incubation, the reaction mixtures were treated with 0.1 ml of a 5% acetic acid solution. The soil particles in reaction mixtures were allowed to sediment. In the next step, 4 ml from the clear solution over the sedimented soil particles were transferred into a test tube, to which 0.8 ml of a N NaOH solution were pipetted. The solution obtained was examined photocolorimetrically at 610 nm.

To obtain the standard curves for the total and abiotic resazurin reductions, respectively, dilutions of the resazurin solution (1 ml dilution + 5 ml distilled water, and 1 ml dilution + 4.8 ml distilled water + 0.2 ml phenol, respectively) were submitted to the same procedure as that described above for the reaction mixtures. Based on the standard curves, the values of total (biotic + abiotic) and abiotic resazurin reductions were established. The difference gives the dehydrogenase activity of soil.

When field-moist soil samples were used, some difficulties appeared in the phenol-treated reaction mixtures. The solutions obtained after incubation of reaction mixtures, their extraction with acetic acid solution and their treatment with NaOH solution contained additional coloured compound(s) which was (were) not extractable from air-dry soil under similar conditions. Therefore, determination of the abiotic resazurin reduction in phenol-treated reaction mixtures with field-moist soil proved to be impracticable. This is why we replaced phenol with a heat treatment for inactivation of soil microorganisms, their dehdyrogenases. The heat treatment consisted of a 10—15-minute boiling of the soil suspension prepared from 1 g of field-moist soil and 9 ml of distilled water. After incubation of the reaction mixtures prepared with field-moist soil samples, sedimentation of soil particles was enhanced by addition of a drop from a 10% $CaCl_2$ solution.

Results and discussion. Table 1 shows the composition of reaction mixtures and the results obtained in total (biotic + abiotic) reduction of resazurin by air-dry samples of a leached chernozem. One can deduce

Table 1

Total (biotic + abiotic) reduction of resazurin by air-dry samples of a leached chernozem

Resazurin solution (ml)	Distilled water (ml)	Soil (mg)	5% Acetic acid (ml)	N NaOH* (ml)	Optical density	Non-reduced resazurin (μg)	Reduced resazurin (μg)
1	5	100	0.1	0.8	0.368	45.5	20.8
1	5	300	0.1	0.8	0.340	36.4	29.9
1	5	500	0.1	0.8	0.320	32.5	33.8
1	5	700	0.1	0.8	0.282	24.7	41.6
1	5	1000	0.1	0.8	0.253	22.1	43.2
1	5	0	0.1	0 8	0.402	66.3	—

* Added to 4 ml from the clear solution over the sedimented soil particles.

from this table that the elaborated procedure made it possible to estimate the activity of soil to reduce resazurin. The activity tended to increase with increasing amount of soil sample, but there was no linearity between the soil amounts and intensities of the resazurin-reducing activity.

It is evident from Table 2 that an abiotic reduction of resazurin also took place under the influence of air-dry samples of the studied

Table 2

Abiotic reduction of resazurin by air-dry samples of a leached chernozem

Resazurin solution (ml)	Distilled water (ml)	Phenol (ml)	Soil (mg)	5% Acetic acid (ml)	N NaOH* (ml)	Optical density	Nonreduced resazurin (μg)	Reduced resazurin (μg)
1	4.8	0.2	100	0.1	0.8	0.425	71.5	1.3
1	4.8	0.2	300	0.1	0.8	0.420	68.3	4.5
1	4.8	0.2	500	0.1	0.8	0.413	65.0	7.8
1	4.8	0.2	700	0.1	0.8	0.401	57.9	14.9
1	4.8	0.2	1000	0.1	0.8	0.389	45.5	27.3
1	4.8	0.2	0	0.1	0.8	0.430	72.8	—

* Added to 4 ml from the clear solution over the sedimented soil particles.

soil. In comparison with the total reduction (Table 1), the abiotic reduction was much lower, especially in reaction mixtures containing the smallest amounts (100—300 mg) of soil.

Using the data from Tables 1 and 2, the biotic (enzymatic) resazurin reduction, *i.e.* the dehydrogenase activity was calculated (Table 3). The activity showed a trend to increase with the soil amount up to

Table 3

Biotic (enzymatic) reduction of resazurin by air-dry samples of a leached chernozem

Soil (mg)	Enzymatic (dehydrogenase) activity: reduced resazurin (μg)
100	19.5
300	25.4
500	26.0
700	26.7
1000	15.9

700 mg soil/reaction mixture. At the highest soil amount (1000 mg), the enzymatic activity decreased as, in the total activity, the abiotic one became prevailing. To explain this observation, one can assume that the H (electron) donors for the dehydrogenase-catalysed reduction

of resazurin were dissolved more easily from smaller amounts of soil, whereas the resazurin-reducing nonenzymatic compounds were easily soluble also from higher amounts of soil when with each soil amount the aqueous phase of the reaction mixtures had the same volume.

Under the conditions of the elaborated procedure, small amounts of soil (100—300 mg/5 ml aqueous phase) are recommended.

Conclusions. 1. A rapid method requiring only one hour for incubation was elaborated for determination of soil dehydrogenase activity by using resazurin as acceptor of H (electrons) transferred from preexisting donors by the soil dehydrogenase enzymes.

2. The abiotic reduction of resazurin by soil was also demonstrated.

3. When under the conditions of the elaborated procedure the amount of soil was small in the reaction mixtures (100—300 mg air-dry soil/5 ml aqueous phase), the enzymatic resazurin reduction was much more intense than the abiotic one.

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INFLUENCE OF SUBSTRATE AND FINAL REACTION PRODUCT ON SYNTHESIS AND ACTIVITY OF PHOSPHATASE IN A SALT LAKE SEDIMENT

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SUMMARY. — The repressive effect of the final reaction product (inorganic phosphate) and the inducing effect of a substrate (ß-glycerophosphate) on the phosphatase synthesis and activity in a salt lake sediment were studied. Analyses were performed separately for acid, neutral and alkaline phosphatase activities, as well as in reaction mixtures without buffer, at the natural pH of the sediment. The repressive effect of inorganic orthophosphate was found to be stronger in the case of acid phosphatase synthesis. Also, synthesis of acid phosphatase was most strongly stimulated by the enzyme substrate, β -glycerophosphate. Supplementary N and C sources decreased the repressive effect of inorganic orthophosphate. In the case of acid phosphatase, the inducing effect of ß-glycerophosphate prevailed over the repressive effect of inorganic orthophosphate, and in the other three cases the repressive effect of the final reaction product was dominant.

The inhibitory effect of PO_4^{3-} on the activity of the acid, neutral and alkaline phosphatase, as well as on the phosphatase activity measured in reaction mixtures without buffer, at the natural pH in a salt lake sediment and in two soil types was presented in a previous paper [12]. In the present paper, we attempted to study the repressive effect of PO_4^{3-} (final reaction product) and the inducing effect of calcium β-glycerophosphate (phosphatase substrate), respectively, on the synthesis and activity of the four phosphatase types in the sediment of the salt lake Ursu (Sovata).

The literature data, concerning the constitutive or adaptive nature of the phosphatases in aquatic habitats, especially in sediments, are contradictory. It has been stated [9, 17] that the alkaline phosphatases, produced especially by the phytoplankton, are adaptive and dominant in the euphotic zone, and the acid phosphatases, produced especially by the bacteria, are constitutive and dominant in the profoundal zone of aquatic basins, including sediments.

Ayyakkannu and Chandramohan [1] found a direct relationship between phosphatase activity and concentration of total phosphorus in marine, estuarine and freshwater sediments, irrespective of the salinity variations, which suggested that the enzyme in sediments is of adaptive nature.

Kobori and Taga [9] established a positive correlation between alkaline phosphatase activity and concentration of total phospho-

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rus and inorganic phosphorus, both in neritic sediments of Tokyo, Sagami and Suruga Bays, and in sediments of seas from South-East Asia and from the Pacific Ocean. The positive relationship between alkaline phosphatase activity and inorganic phosphorus concentration is surprising, taking into account that majority of the subsequent researches established a negative correlation between the two parameters, which illustrates the repressive/inhibitive effect of inorganic phosphorus on the alkaline phosphatase, especially on the algal alkaline phosphatase, predominant in the water column of natural basins [4, 5, 11, 17, 18].

Karl and Craven [8] established the existence of an inhibitive effect of inorganic phosphate on the alkaline phosphatase activity in extracts of oceanic sediments or in extracts from a piscine sediment. They consider that the occurrence and regulation of alkaline phosphatase in nature is dependent upon microscale inorganic phosphate limitation of the autochthonous microbial communities.

On the other hand, Chróst *et al.* [6] found that alkaline phosphatase in the profoundal waters of the Glebokie lake (Poland) was independent of dissolved inorganic phosphate concentration. Also, Chappell and Goulder [3] did not find any evidence of end-product repression of native epilithic phosphatase activity on stones, downstream of the outfall from a sewage-work.

The influence of inorganic and organic phosphorus compounds on microbial synthesis and phosphatase stability in a salt lake sediment has been already studied in our country [16], but only phosphatase activity at the natural pH of the sediment in question has been analysed. It has been found that inorganic orthophosphate had a repressive effect on the synthesis of phosphatase, but this effect disappeared after a prolonged incubation period. β-Glycerophosphate had a persistent inducing effect on the synthesis of phosphatase analysed at the natural pH of sediment.

The present research shows that in the saline sediment studied, the four types of phosphatase are submitted to repression by the inorganic phosphate, and β-glycerophosphate has an inducing effect on the enzyme synthesis. The level of the induction or repression is conditioned by the concentration of the substrate and the final reaction product, respectively.

Material and method. The experiment was performed on a mean sample resulted by mixing three samples taken from different sites of the Ursu lake. The sediment (pH 8) is black, greasy, and it is characterized by a high enzymatic potential. We tested the constitutive or adaptive nature of phosphatases in the studied salt lake sediment, using $K_2HPO_4.3H_2O$ as a repressive agent, and calcium ß-glycerophosphate ($C_2H_7O_6PCa$) as an inducing agent, according to a scheme presented in Table 1. The supplementation of some of the experimental variants with glucose, urea and NH_4Cl maintained a C/N/P ratio of 20/5/1, considered to be optimum for the development of bacteria. Concentrations of 0.2 and 2 mg/ml PO_4-P, respectively, used for testing the repressive effect of PO_4^{3-} ion, were chosen after the test of the inhibitory effect of these concentrations in the first stage of the experiment [12].

One kg of sediment in 1-l graded cylinders was completed to 1 l with filtered lake water. The substances were added according to the scheme presented in

Table 1

Experimental variants for studying the repressive effect of PO_4, and the inducing effect of ß-glycerophosphate on the synthesis and activity of phosphatase

(For each variant 1 kg of sediment and lake water to 1 l were used)

Variant	Glucose (g)	$C_3H_7O_6PCa$ (g)	$K_2HPO_4 \cdot 3H_2O$ (g)	PO_4-P (mg/ml)	NH_4Cl (g)	Urea (g)	C (g)	N (g)	P (g)
V1	—	—	—	—	—	—	—	—	—
V2	—	—	1.5	0.2	—	—	—	—	0.203
V3	—	—	15	2	—	—	—	—	2.039
V4	10	—	—	—	0.8	0.5	4	0.442	—
V5	10	2	—	—	0.8	0.5	4.292	0.442	0.252
V6	10	4	—	—	0.8	0.5	4.585	0.442	0.504
V7	10	—	1.5	0.2	0.8	0.5	4	0.442	0.203
V8	10	—	15	2	0.8	0.5	4	0.442	2.039
V9	10	2	1,5	0.2	0.8	0.5	4.292	0.442	0.455
V10	10	2	15	2	0.8	0.5	4.292	0.442	2.291
V11	10	4	1,5	0.2	0.8	0.5	4.585	0.442	0.707
V12	10	4	15	2	0.8	0.5	4.585	0.442	2.543

Table 1 and the mixtures were incubated in the dark, at 28°C. The activity of the four types of phosphatases was determined after 3, 10, 20, 40, 100 and 200 days of incubation.

In the sediment used as inoculum, as well as in those of the experimental variants after 200 days of incubation, we measured the number of aerobic heterotrophic bacteria, PO_4^{3-} concentration (mobile phosphorus) according to the Egner-Riehm method [15], catalase activity and nonenzymatic catalytic H_2O_2-splitting [7], actual and potential dehydrogenase activity, nonenzymatic TTC reduction in reaction mixtures with or without glucose addition [2]. On the basis of the values of these activities, an enzymatic indicator of the sediment quality was calculated [13, 14], taking into account the values of phosphatase activity measured in reaction mixtures without buffer, at natural pH.

For each sample, acid (pH 5.5), neutral (pH 7) and alkaline (pH 10) phosphatase activity of the sediment was measured, as well as the phosphatase activity in reaction mixtures with distilled water, without buffer. Reaction mixtures were obtained using Tris universal buffer [19].

Phosphatase activity was measured using the Krámer and Erdei method [10]. Reaction mixtures consisted of 2.5 g sediment + 2 ml toluene + 5 ml buffer solution + 5 ml 1% disodium phenylphosphate solution. Comparatively, phosphatase activity was measured in reaction mixtures without buffer solution, this being replaced by the same quantity of distilled water, so that we obtained in all the reaction mixtures a 10-ml water phase volume and an 0.5% disodium phenylphosphate concentration. As controls, we used reaction mixtures with: sediment + buffer + toluene, without substrate; substrate + toluene and distilled water + toluene, without mud, respectively, at the same final volume. Incubation was carried out at 37°C for 24 hours. Phosphatase activity is expressed in mg phenol/2.5 g sediment (dry matter).

Results. The results of chemical, enzymological and microbiological analyses at the moment of initiation of the experiment and at the end of the incubation period are presented in Table 2.

Table 2

Results of chemical, enzymological and microbiological analyses in the sediment used for inoculation and in that of the experimental variants, after 200 days of incubation

Variant	PO_4—P (μg/ml)	Aerobic heterotrophic bacteria/g dry sediment ($\times 10^5$)	Enzymatic indicator of sediment quality
Sediment used for inoculation	1	14.06	0.555
V1	2	3.280	0.507
V2	9	2.761	0.487
V3	29	2.341	0.463
V4	1	371.5	0.908
V5	7	285.9	0.862
V6	10	314.5	0.958
V7	2	299.6	0.842
V8	32	341.2	0.790
V9	8	488.7	0.915
V10	32	342.6	0.802
V11	11	463.9	0.867
V12	28	272.4	0.900

The number of heterotrophic aerobic bacteria decreases by one order of magnitude in the cases of the variants with no C, N and P supplementation, compared to the sediment used as inoculum. The values of the enzymatic indicators of sediment quality in these variants are slightly decreased.

In variants V4—V12, to which glucose, urea and NH_4Cl were added, both the number of heterotrophic aerobic bacteria and enzymatic indicators of sediment quality are significantly increased.

In spite of its marked decrease, PO_4^{3-} concentration in the variants to which orthophosphate was initially added remains higher than in the other variants, maintaining a repressive effect on phosphatase synthesis.

Initial phosphatase activities, before the substances presented in Table 1 were added to the experimental variants, had the following mean values, expressed in mg phenol/2.5 g sediment (dry matter):

— acid phosphatase: 4.017;
— neutral phosphatase: 3.374;
— alkaline phosphatase: 2.426;
— in reaction mixtures without buffer: 2.880.

The influence of PO_4^{3-} ion on the synthesis and activity of phosphatase in the experimental variants to which no organic substances were added for the stimulation of microorganisms' development is presented in Fig. 1. After the first three days of incubation, a sudden decrease in all four activities occurred. This can be explained by the inhibitory effect of inorganic phosphate initially added to the experimental variants.

After an oscillatory evolution during the first 40 days of incubation, there is a relative uniform decrease in phosphatase activity in all four cases. With no exception, phosphatase activity in the control variant (V1) is higher than that of the variants supplemented with 0.2 and 2 mg/ml PO_4^{3-}-P. This confirms the repressive effect of the PO_4^{3-} ion on *de novo* synthesis of the enzyme. This is explained by the fact that at the end of the incubation period (after 200 days), PO_4-P concentration is too low (9 mg/ml PO_4-P in V2, 29 mg/ml PO_4-P in V3, respectively) to provide an inhibitory effect, which in obvious only at concentrations higher than 200 mg/ml PO_4-P [4]. Naturally, the repressive effect is stronger in variant V3, to which 2 mg/ml PO_4-P were added.

Enzymatic activity is lowest in the case of the acid phosphatase (37.54% in V3 compared to control). Acid phosphatase was the less inhibited by the PO_4^{3-} ion [4]. Thus, we can say that the acid phosphatase is less inhibited, while *de novo* synthesis of the enzyme is most strongly repressed by the added PO_4^{3-} ion.

Even if after 200 days of incubation the other indicators of biological activity in the studied sediments had a decreasing evolution (Table 2), comparison of variants V2 and V3 with the control (V1) after the whole incubation period is essential for the studied phenomenon, the repression of phosphatase synthesis, and the results confirm the existen-

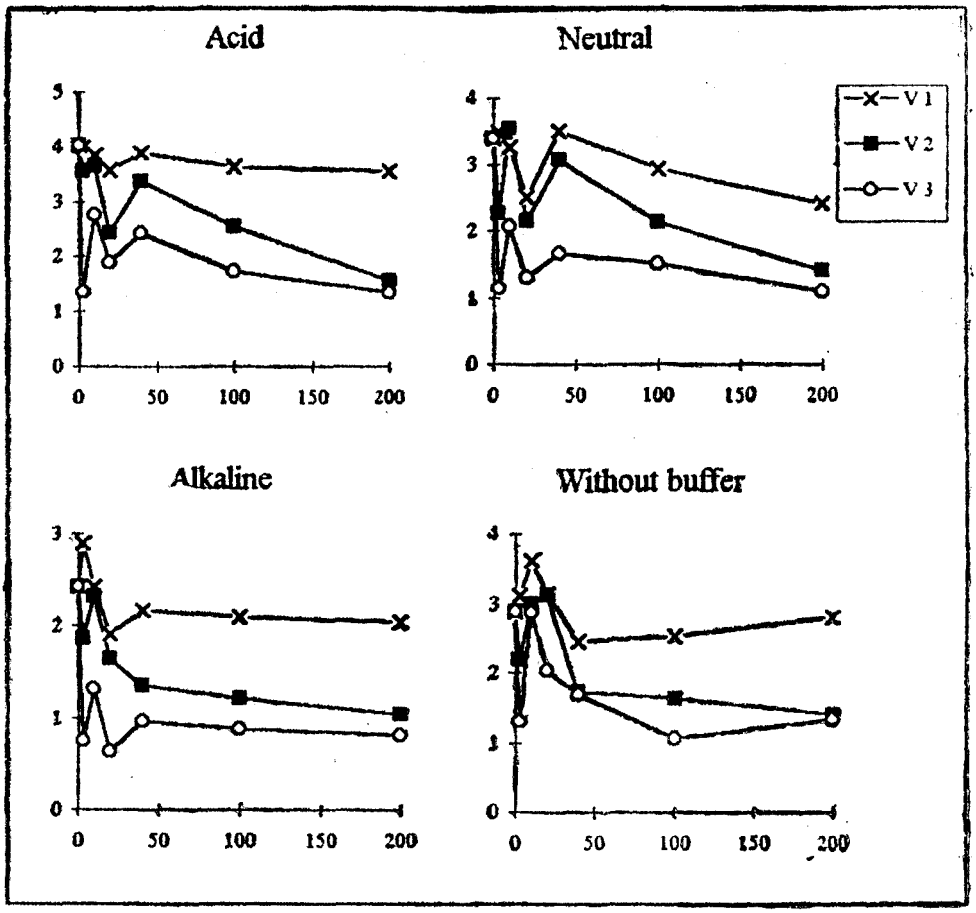

Fig. 1. *Influence of orthophosphate on the synthesis and activity of phosphatase in variants unsupplemented with C and N.*
Explanations: see *Table 1.*
X axes — Days of incubation. Y axes — mg phenol/2.5 g sediment (dry matter).

ce of this phenomenon, which is more marked in the case of acid phosphatase.

The repressive effect of inorganic orthophosphate can be found in the case of experimental variants V7 and V8 as well, to which C and N sources were added, as specified in Table 1 (Fig. 2).

We can conclude that nutrient substance supplementation led to a spectacular increase in phosphatase activity. After 200 days of incubation, phosphatase activity in the case of control variant (V1) is only 58.59% (acid), 32.53% (neutral), 28.94% (alkaline) and 41.15% (without

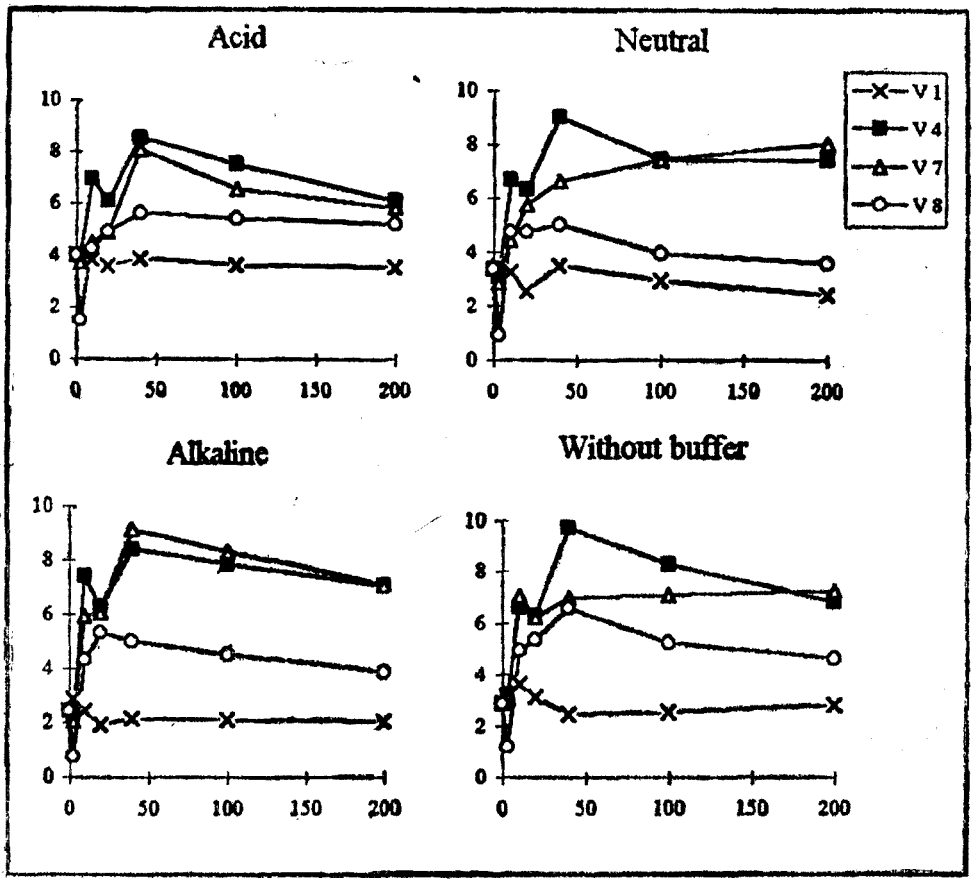

Fig. 2. *Influence of orthophosphate on the synthesis and activity of phosphatase in variants supplemented with C and N.*

Explanations: see *Table 1.*

X axes — Days of incubation. Y axes — mg phenol/2.5 g sediment (dry matter).

buffer), respectively, of that of the variant V4, to which supplementary C, N and P sources were added.

This incease is explained by the massive development of microflora, as a consequence of C, N and P source supplementation. If at the beginning of the experiment the number of heterotrophic aerobic bacteria was of the order of 10^6/g sediment (dry matter), after 200 days of incubation this number was 20—35 times higher (Table 2).

At the same time, the other studied enzymatic and nonenzymatic catalytic activities intensified as well, so that the enzymatic indicators of mud quality in experimental variants supplemented with C, N and P

sources had values obviously higher than that of the initial sediment used as inoculum (Table 2).

At a concentration of 0.2 mg/ml added PO_4-P, the repressive effect of orthophosphate is insignificant, enzymatic activity in variant V7, with the exception of acid phosphatase, slightly exceeding that of the control (V4).

In the case of variant V8, with 2 mg/ml PO_4-P initially added, the repressive effect is obvious in all cases. The intensity of neutral phosphatase activity decreases to less than half, while the intensity of acid phosphatase activity decreases by only 15% compared to the control (V4). Even if PO_4-P concentration decreases at the end of the incubation period to only 32 μg/ml, this concentration seems to be sufficient to maintain the synthesis rate of all phosphatase types at a low level.

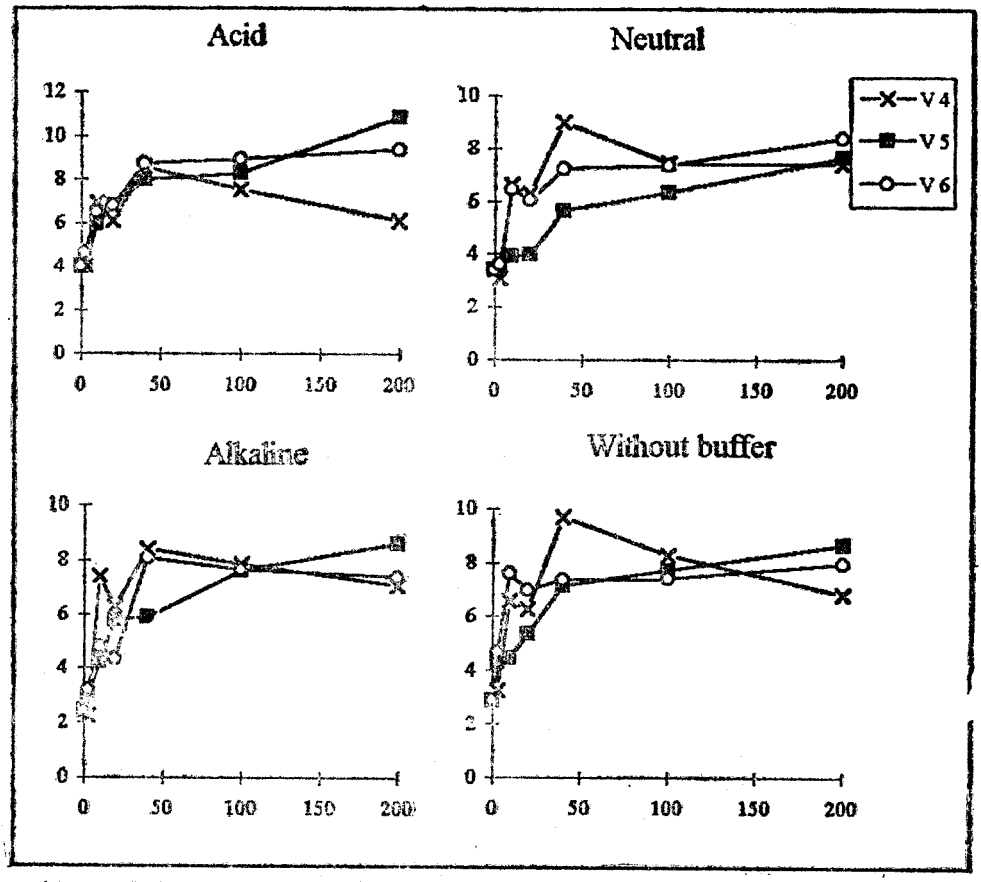

Fig. 3. *Influence of β-glycerophosphate on the synthesis and activity of phosphatase.* Explanations: see *Table 1.*

X axes — Days of incubation. Y axes — mg phenol/2.5 g sediment (dry matter).

The addition of β-glycerophosphate, the phosphatase substrate, to the initial mixtures of experimental variants led to an increase of the enzymatic activity, compared to the control variant V4 (Fig. 3). The induction is stronger in the case of acid phosphatase, but it is not correlated with the β-glycerophosphate concentration. The intensity of acid phosphatase activity increases by 53.54% in V6, and by 78.13% in V5, respectively, compared to the control (V4).

In the case of the other phosphatase types, the difference from the control is small and it is related to the substrate concentration only in the case of neutral phosphatase.

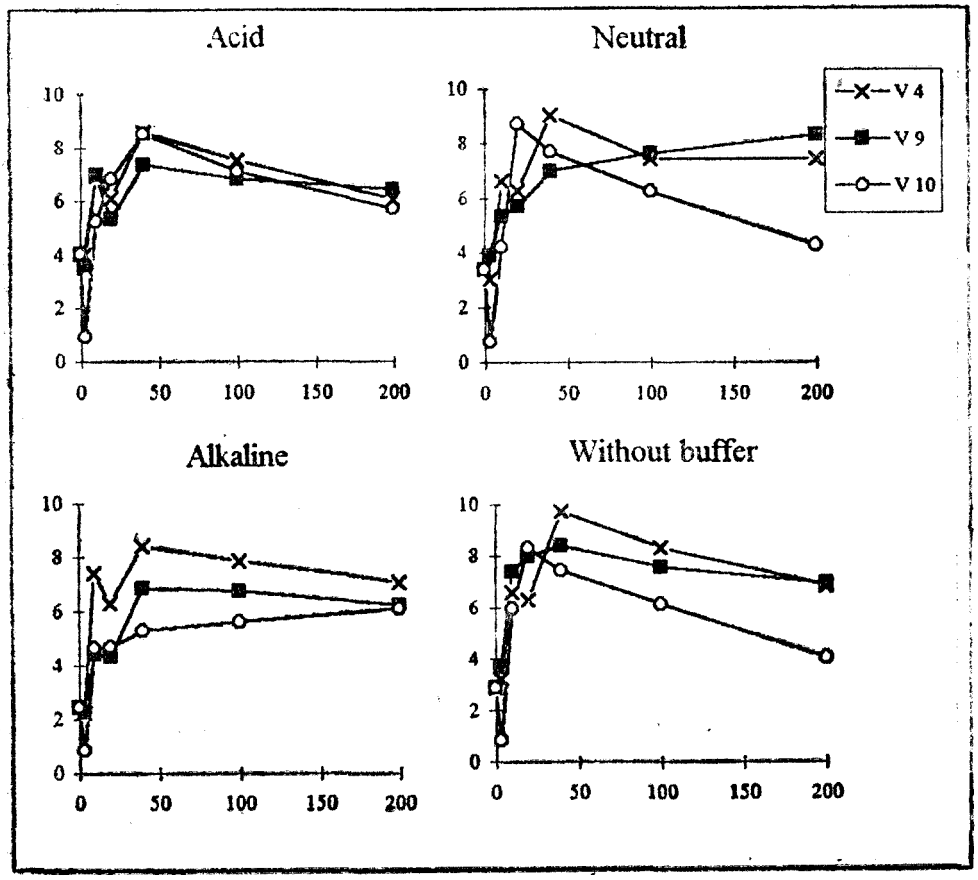

Fig. 4. *Effect of simultaneous addition of orthophosphate and β-glycerophosphate (2 mg/ml) on phosphatase synthesis and activity.*

Explanations: see *Table 1.*

X axes — Days of incubation. Y axes — mg phenol/2.5 g sediment (dry matter).

Only in the second part of the incubation period, after 100 days, the phosphatase activity level in the experimental variants exceeds that of the control. Thus, the manifestation of the inducing effect of β-glycerophosphate requires a lapse of time during which microorganisms adapt their enzymatic system of phosphatase synthesis. This interval is shorter in the case of acid phosphatase, in which induction is the strongest.

Figs. 4 and 5 show the effect of simultaneous supplementation of the experimental variants with both orthophosphate and β-glycerophosphate in the concentrations presented in Table 1. The analysis of the two figures shows that acid phosphatase reacts differently compared to

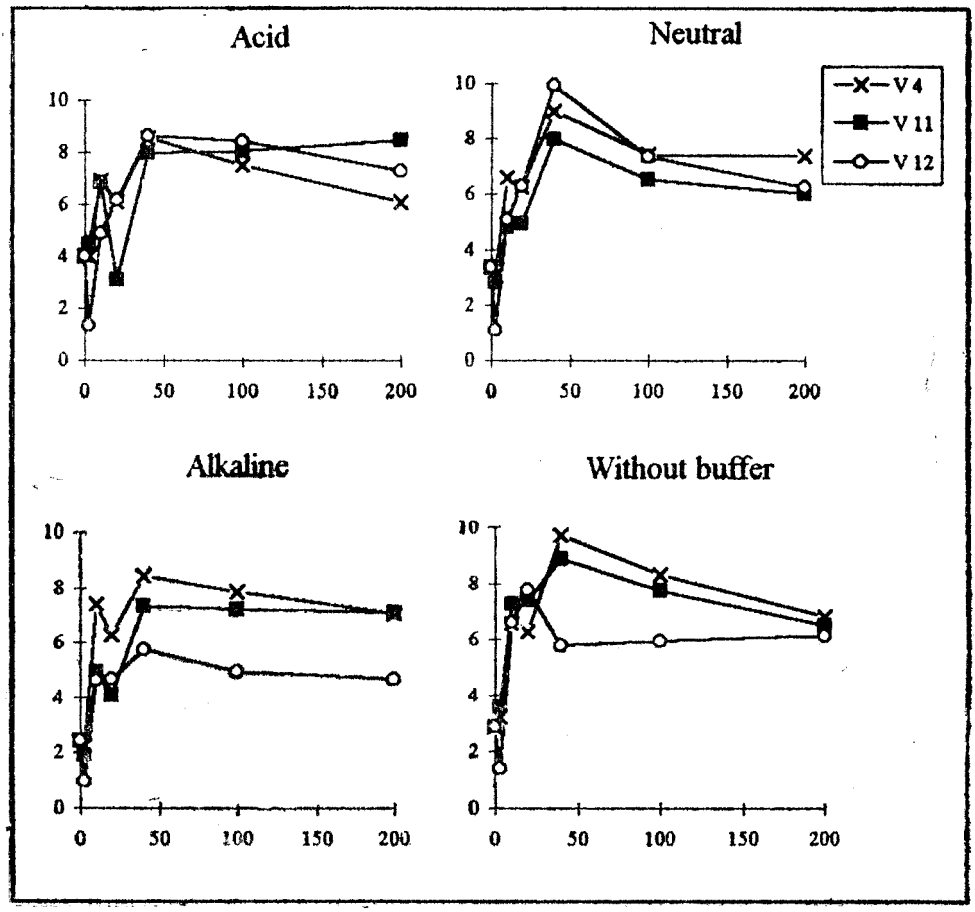

Fig. 5. *Effect of simultaneous addition of orthophosphate and β-glycerophosphate (4 mg/ml) on phosphatase synthesis and activity.*

Explanations: see *Table 1.*

X axes — Days of incubation. Y axes — mg phenol/2.5 g sediment (dry matter).

the other phosphatase types, confirming the results presented in connection with the two phenomena, induction and repression. In the case of acid phosphatase induction is stronger than repression, especially in the case of variants with an initial supplementation of 4 mg/ml β-glycerophosphate. In V11 the intensity of acid phosphatase activity increases by 39.49% and in V12 by 20.15% compared to the control (V4).

With a few exceptions, in the case of the other phosphatase types the repressive effect is stronger than the inducing effect. The strongest repression occurs in the case of neutral phosphatase and of phosphatase measured at the natural pH of the sediment, in variant V10, in which the activity is reduced after 200 days of incubation by over 40% compared to that of the control.

As it is shown in Figs. 4 and 5, after the first three days of incubation a marked decrease in the activity of all phosphatase types occurs. This is probably due in the first place to the inhibitory effect of inorganic orthophosphate, not to its repressive effect.

The subsequent evolutions are oscillatory, the values come close to the control (V4), which they exceed only rarely and insignificantly. With the exception of acid phosphatase, the repressive effect seems to appear at low concentrations as well, at the end of the 200 days of incubation, prevailing over the inducing effect of β-glycerophosphate. In the case of the acid phosphatase, the initial 4 mg/ml β-glycerophosphate addition has an inducing effect that overcomes the repressive effect of orthophosphate.

Conclusions. 1. The synthesis of the phosphatase is repressed by orthophosphate, after 200 days of incubation the level of enzymatic activity in all four cases being lower than 50% in the variant to which 2 mg/ml PO_4-P were initially added, and around this value in the variant initially supplemented whith 0.2 mg/ml PO_4-P.

2. When supplementary C, N and P sources are added to the experimental variants, the repressive effect of orthophosphate is substantially diminished and manifests itself only in the variant initially supplemented with 2 mg PO_4-P. The less affected is the acid phosphatase.

In the case of variants to which supplementary C, N and P sources were added, the manifestation of the repressive effect of orthophosphate may be diminished by the massive development of microflora, which provides a sufficiently high level of phosphatase synthesis, so that this effect seems to be lacking. However, at the initial 2 mg/ml PO_4-P concentration this effect is obvious, being more marked in the case of the neutral phosphatase and lower in that of the acid phosphatase.

3. The β-glycerophosphate has an inducing effect on the synthesis and activity of phosphatase, especially of the acid phosphatase, this effect being unrelated to the substance concentration. The effect manifests itself after 100 days of incubation, even earlier in the case of the acid phosphatase.

4. Simultaneous supplementation of the experimental variants with orthophosphate and β-glycerophosphate demonstrates that in the case of

the acid phosphatase the inducing effect prevails. In the case of the other phosphatase types, the inhibitory effect of the final reaction product prevails. Differences when compared to the control are small and they are not related to the concentration of the supplemented substance.

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RECENZII

Lutz-Arend Meyer-Reil und (and) Marion Köster (Herausgeber — Editors), **Mikrobiologie des Meeresbodens** *(Microbiology of the Sea Floor)*, Gustav Fischer Verlag, Jena, Stuttgart, New York, 1993, 292 pages with 90 figures and 12 tables in the text.

Microbiology of the Sea Floor is a comprehensive up-to-date treatise of a very important branch of aquatic microbiology, because the sea floor covers about 71% of the Earth surface and is the largest life and deposition space on Earth. Working in the field of environmental enzymology and microbiology, I should like to point out that — based on the outstanding investigations of the Editors and their associates — their dominating idea which, as Ariadne's thread, orientates the readers throughout the treatise, is that the free, particle- and cell-bound enzyme activities play a key role in all microbial transformations taking place on the sea floor and having a major contribution to the global carbon cycle.

The treatise consists of 8 chapters and two indices (index of taxa and subject index). Each chapter begins with Summary and Introduction, and ends with References. The Author(s) and titles of chapters as well as the titles of subchapters are listed below.

1. R. Gersonde and G. Kuhn: The Sea Floor, Structure and Sediments (Structure of the sea floor; Origin, composition and distribution of the deep-sea sediments).

2. L.—A. Meyer-Reil: Microbial Colonisation and Production (Determination of microbial cell number and biomass; Microbial colonisation of particles; Spatial distribution of cell number and biomass; Structure of microbial biomass; Cell number and biomass in relation to sediment parameters; Temporal development of cell number and biomass; Microbial production; Conclusions).

3. M. Köster: Microbial Activities at Interfaces (Significance of enzymatic decomposing activities for microbial substrate transformations; Microbial decomposition of organic material in the sediment/bottom water contact zone; Microbial activities at the oxic-anoxic interface; Influence of biogenic structures on decomposition and deposition of organic material).

4. H.—J. Rüger: Isolation and Identification of Benthic Bacteria (Isolation of benthic bacteria; Tests for the identification of marine bacteria; Identification of the most frequent sediment bacteria).

5. K. Schaumann: Marine Fungi (What are "marine fungi"?; Biology and ecology of benthic marine fungi; Characterisation of the marine-benthic mycoflora; Activity and role of fungi in the benthic ecosystem).

6. L. J. Stal: Microbial Mats (Structure of microbial mats; Nitrogen fixation; Interactions between phototrophic microorganisms; Interactions between purple sulphur bacteria and colourless sulphur bacteria; Interactions between sulphate-reducing bacteria and cyanobacteria; Conclusions).

7. R. Schmaljohann: Microbiological Aspects of Fluid and Gas Seeps (Basic types of submarine fluid and gas seeps; Occurrence and distribution of microorganisms at fluid and gas seeps; Characteristic microbial processes; Significance of chemoautotrophic and methanotrophic processes for the benthic food chain).

8. K. Lochte: Microbiology of Deep-Sea Sediments (Characterisation of the deep sea; Bacterial biomass; Bacterial activities; Role of the bacteria in the decomposition of organic material; Conclusions).

This treatise of exceptional value is addressed to a broad circle of readers, comprising experts and students in marine microbiology and, generally, in marine sciences, as well as those performing investigations in other fields of environmental science and technology.

ŞTEFAN KISS

Jürgen Friedel, **Einfluß von Bewirtschaftungsmaßnahmen auf mikrobielle Eigenschaften im C- und N-Kreislauf von Ackerböden** *(Influence of Cultivation Methods on Microbial Properties in the C and N Cycles of Arable Soils)*, Institut für Bodenkunde und Standortslehre, Universität Hohenheim, Stuttgart, 1993, 201 pages + Appendix (51 pages), including 83 figures and 59 tables.

Under the conditions of the agricultural experimental fields of the Hohenheim University (the fields being located at Ihinger Hof, Muttergarten, Roteklingengraben and Gondelsheim), the influence of long-term tillage, crop rotation (rape-cereals and legumes-cereals), long-term organic (farmyard manure, cattle slurry) and mineral-N fertilisation and single organic (farmyard manure, cattle slurry, green manure) and mineral-N fertilisation on a series of soil biochemical and microbiological properties was studied. These properties include: ATP content (as a measure of microbial biomass), enzyme (dehydrogenase, β-glucosidase, protease and urease) activities, Beck's "soil microbial index", respiration and C mineralisation, net N mineralisation, specific activity of microbial biomass and abundance of microbial populations (counts of viable microbial cells from physiological and taxonomic groups) (all determined in the laboratory), as well as cellulose decomposition (determined in the field). A model was elaborated for calculation of the amount of "decomposable young soil organic matter" from crop and root residues and organic fertilisers.

Some soil physical and chemical properties (gravimetric water content, maximum water capacity, particle size distribution, dry bulk density, aggregate stability, pH, organic C content, total N content, contents of water-soluble organic C compounds, and mineral N: NO_3^- + NH_4^+ content) were also determined.

The analytical data were evaluated by statistical methods.

Detailed description of the results is followed by discussion and conclusions. It is emphasised that the different cultivation methods do influence the biochemical and microbiological properties of soil mainly by changing the quantity and quality of its organic matter. Therefore, those biochemical and microbiological properties that are in close relation with the amount of "decomposable young soil organic matter" are suitable to reflect the cultivation-dependent changes. Such properties were found to be C mineralisation, urease and protease activities. The ATP content and β-glucosidase activity showed a stronger dependence on organic C content of soil, whereas dehydrogenase activity was largely dependent on soil pH, too. At the same time, contents of water-soluble organic C compounds did not reflect cultivation-induced changes in soil microbial properties and C availability. Soil-sparing tillage, crop rotations with high amounts of harvest and root residues and organic fertilisation have a positive influence on the microbial status of soils.

The investigations described in Dr. J. Friedel's book may serve as a model for studying biochemical and microbiological properties of agricultural soils submitted to different treatments.

ȘTEFAN KISS

G. A. Evdokimova, **Ekologo-mikrobiologicheskie osnovy okhrany pochv Krainego Severa** *(Ecological-Microbiological Bases of Soil Protection in the Far North)*, Rossiiskaya Akademiya Nauk, Kol'skii Nauchnyi Tsentr, Institut Problem Promyshlennoi Ekologii Severa (Russian Academy of Sciences, Kola Scientific Centre, Institute for Industrial Ecology Problems of the North), Apatity, 1995, 272 pages including 107 tables and 48 figures.

The area around the "Severonikel'" nickel-copper smelter complex, located in the northern taiga, near the town of Monchegorsk (Kola Peninsula, Russian Federation) was investigated in the 1976—1993 period. The smelter emissions contain mainly nickel, copper and cobalt (in form of sulphates, chlorides, sulphides, oxides), and SO_2. Due to their huge amounts, these pollutants affect large territories, within which four zones were selected for the investigations: the epicentre, the impact zone ("technogenic desert"), the buffer zone and the background zone. Not only the vir-

gin soils (already affected by the smelter emissions for many decades) were investigated, but experimental plots were also installed in the four zones, namely at 0.2—1 km (epicentre) and at 5, 15 and 50 km from the smelter. The surface (0—30—cm) layer of the plots was removed and replaced with unpolluted cultivated soil. The plots were then periodically fertilised with NPK or with NPK plus farmyard manure.

Complexity of the investigations can be deduced even from the part, chapter, subchapter and section titles. Part I, "Anthropogenic dynamics of the properties and micropopulation of soils", comprises Chapters 1—4, whereas Part II, "Restoration of cultivated soils", is formed of Chapters 5 and 6.

Chapter 1, "Heavy metals in soils in the impact zone of the nonferrous metallurgical enterprise", deals with heavy metals in natural soils (content and migration into the profile; forms of compounds) and with those in cultived soils (dynamics of the metal content in introduced soil; vertical migration of metals; forms of compounds; migration of metals in the soil-plant system; biological quality of plants).

Chapter 2, "Interactions between heavy metals and soil microorganisms", is dedicated to the following topics: Microbiological monitoring of cultivated soils (dynamics of the number and biomass of microorganisms); Structure of the microbial community; Microflora of forest soils; Separate action of copper and nickel ions on the soil microorganisms; Limits of the resistance of soil fungi to heavy metals and limits of the bioaccumulation of heavy metals by soil fungi; Genetic activity of metal-polluted soils in tests with microorganisms.

Chapter 3, "Biochemical activity of metal-polluted soils", comprises the subchapters entitled: Nitrogen-fixing activity and respiration of soil; Nitrification activity; Cellulolytic activity; Synthesis of free amino acids; Enzyme activity; Decomposition of plant residues.

In Chapter 4, "Influence of industrial emissions on the physicochemical properties and nutrient status of soil", the following studies are described: Content of the sulphate ions in soil; Changes in physicochemical soil properties; Changes in the nutrient status of soil (dynamics of the content and composition of organic matter; dynamics of the mobile forms of phosphorus and potassium compounds; dynamics of the mineral nitrogen compounds).

Chapter 5, "Restoration of the properties of cultivated soils after their chemical pollution", treats the following themes: Natural mechanisms of the self-purification of soil from heavy metals; Criteria of the self-restoration of soil; Methods of soil restoration; Dynamics of the processes of self-restoration of soil; Restoration of the chemical properties; Restoration of the physicochemical properties; Restoration of the microbial status of soils; Restoration of the biochemical functions of microorganisms; Reduction of the phytotoxicity of polluted soil.

Chapter 6, "Methods of soil regeneration", consists of two subchapters: Methods for determination of metal toxicity of soils; Methods for reduction of metal toxicity of soils.

The bibliographical list comprises nearly 400 titles.

The investigations performed by Dr. G. A. Evdokimova have convincingly shown that, even under the adverse climatic conditions of the studied areas, the soil microorganisms, their biochemical activities are sensitive indicators of soil pollution by industrial emissions and also of the efficiency of the methods applied for reclamation of such soils. In addition, bioaccumulation of heavy metals by some soil microorganisms directly leads, at least temporarily, to reduction of the phytotoxicity of polluted soils. However, as Dr. G. A. Evdokimova also points out, the best method is, everywhere, the prevention of pollution.

Dr. G. A. Evdokimova's valuable book presents much interest for the specialists working in the field of environmental science and technology.

ȘTEFAN KISS

Biologischer Landbau: Beitrag des DOK-Versuches *(Biological Agriculture: Contribution of the DOC Trial)*, Herausgeber (Editor): Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene (Federal Research Station for Agricultural Chemistry and

Environmental Hygiene), Liebefeld-Bern (Switzerland), 1995, 118 pages with 25 tables and 17 figures in the text.

The book comprises the Proceedings of a Conference held in October 1995 at the Research Station with the participation of 16 scientists from Switzerland and Germany, and includes 8 papers as well as 5 contributions to the round table discussions organised during the Conference.

The theme of the Conference was to comparatively evaluate three cropping systems: biodynamic, bioorganic and conventional (DOC), based on two 7-year crop rotations (1978—1984 and 1985—1991) on a 1.84-ha experimental field located at Therwil in the valley of the Birsig river (Switzerland). 96 experimental plots (each of 100 m^2) were installed.

In the biodynamic cropping system, only organic fertilisers (farmyard manure and compost) were applied to the plots together with special biodynamic preparations. The organic fertilisers represented 80 and 50% of the fertilisers used for the plots of the bioorganic and conventional cropping systems, respectively. Some plots received only mineral fertilisers and some others were not fertilised at all.

Several soil physical, chemical and biological parameters, the quality and quantity of harvested crops were compared in the different plots.

The low external input biological cropping systems, as compared to the high external input conventional cropping system, are environmentally sound, preserve the natural resources, thus the soil's fertility status — as indicated, for example, by higher microbial biomass (substrate-induced respiration, ATP), enzyme (dehydrogenase, alkaline phosphatase, protease, invertase) activities, biomass and abundance of earthworms, by stronger development of vesicular-arbuscular mycorrhizal fungi — is improved, the nutrient value of the harvested crops is better, but the quantity of crop yields is smaller (the difference is about 20—25%) and annually less stable. However, the DOC Trial has shown that the biological cropping systems are efficient from economical viewpoints, too. It is known that the bioproducts are marketed at higher prices than the products of the conventional agriculture.

It has been drawn the conclusion that the environmentally responsible, sustainable, durable, biological agriculture faces a good future, it will gain more ground, due to its present achievements and also to the possibilities for further development of its technologies.

ŞTEFAN KISS

Hubert de Jonge, **Sorption, Bioavailability and Mineralization of Hydrocarbons in Contaminated Soils,** University of Amsterdam, The Netherlands, 1996, 150 pages with 30 figures and 13 tables in the text.

Dr. de Jonge's work, based on original laboratory investigations, brings new data to the better understanding of the relation between the physicochemical properties of oil-contaminated soils and the bioavailability of the contaminating hydrocarbons to and their mineralization by the soil microorganisms.

The work comprises 9 chapters: General introduction; Identification of soil material sorption parameters from continuous stirred flow experiments: the use of transfer functions; Adsorption of CO_2 and N_2 on soil organic matter: nature of porosity, surface area, and diffusion mechanisms; Soil organic matter properties and naphthalene sorption. 1: Batch adsorption experiments; 2: Adsorption/desorption kinetics; Influence of sorption and solution chemistry on the bioavailability of naphthalene in two freshly contaminated soils; Increased bioavailability of mineral oil in soil slurries by monovalent cation-induced dispersion; The relation between bioavailability limitations and fuel oil hydrocarbon composition in contaminated soils; Sorption processes in soils and implications for the bioavailability of non-ionic organic contaminants.

One of Dr. de Jonge's findings of great importance for biotechnological remediation of oil-contaminated soils is that the monovalent cation-induced dispersion of soil particles enhances the bioavailability and, consequently, the microbial degradation of hydrocarbons in such soils.

The work presents much interest for experts in soil physicochemistry, soil and petroleum microbiology and environmental technologies.

ŞTEFAN KISS

Thomas Hintze, **Die Phosphatasen des Bodens und ihre Beeinflussung durch Zink und Kupfer. Ein enzymkinetischer Versuchsansatz** *(The Phosphatases of Soil and Their Influencing by Zinc and Copper. An Enzyme Kinetic Experimental Contribution)*, Shaker Verlag, Aachen, 1996, X + 80 pages with 28 figures, 18 tables and 1 map in the text and 6 enclosures on 21 pages.

Dr. Th. Hintze has studied the kinetics of acid and alkaline phosphatases in 5 arable and 5 forest soils from the Trier area (Germany), and has evidenced two distinguishable active forms of both enzymes is most soils and has distinguished four active forms of acid phosphatase in $ZnCl_2$- and $CuCl_2$-treated soil samples.

These findings are related to the deviation of the kinetic behaviour of both enzymes from the Michaelis-Menten kinetics valid for one molecular form of an enzyme.

As soil acid phosphatase could originate from many microbial and plant species and soil alkaline phosphatase from many microbial species, the soils should contain many species-specific molecular forms of both enzymes and also isoenzymes among the species-specific enzymes. Therefore, the small number (2 or 4) of the distinguished forms of these soil enzymes may indicate that activity of most molecular forms is overlapping.

Dr. Th. Hintze's book describes the most comprehensive studies, in which the existence of mulitple forms of phosphatases in soils was convincingly proved.

STEFAN KISS

Neue Konzepte in der Bodenbiologie *(New Concepts in the Soil Biology)*, Deutsche Bodenkundliche Gesellschaft (German Soil Science Society), Oldenburg, 1996, X + 384 pages with 163 figures and 70 tables in the text.

A conference on „New Concepts in the Soil Biology" was organised by the Biology Commission of the German Soil Science Society, the Austrian Society for Soil Biology and the Soil Biology and Applied Microbiology Group of the Union of the German Agricultural Research Stations, and held in Linz/Austria on 2—4th of October 1996.

The present volume compries the proceedings of this conference and has appeared as Band (Volume) 81 of the „Mitteilungen der Deutschen Bodenkundlichen Gesellschaft" (1996).

Ninety papers elaborated by 167 authors are included. The papers are grouped under 8 headings: Development and improvement of methods (25 papers); Interactions between soil microflora and fauna (11 papers); Substance fluxes, simulations of soil biological systems (7 papers); Carbon dynamics (6 papers); Soil ecological aspects (10 papers); Ecotoxicology (14 papers); Interactions between plant and soil microflora (9 papers), and Soil biological characterisation of stands (8 papers).

The papers taken as a whole clearly reflect the progress made in the recent years by both fundamental and applied soil biological research and precisely outline the growing role facing Soil Biology in solving such problems of mankind as the need to increase agricultural production at a high-quality level and without deteriorating the environment.

The volume will serve as a useful source of up-to-date information for experts and students whose professional activity is related to the (most) valuable natural resource called soil.

STEFAN KISS

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