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# THE ROLE OF FUNGI ON ALLEVIATING THE STRESS INDUCED BY HEAVY METALS UPTAKE IN RYE PLANTS (*SECALE CEREALE* L.) CULTIVATED IN SOIL FROM A ROUMANIAN INDUSTRIAL AREA

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In this paper there are presented results from a laboratory experiment performed on a polluted agricultural soil. Three types of soil were used: unpolluted soil (as a reference), polluted soil and amended with expanded clay and polluted soil amended with expanded clay and inoculated with mycorrhizal fungi, in which there were inseminated two species of plants: sunflower and rye. In this article only results obtained on rye are presented. It was performed the characterization of the soil determining, pH, electrical conductivity, humidity, forms of mineral N, assimilable P, and the content of metals. Using the vegetable material (roots and leaves), the content of metals and the following parameters: lipid peroxides (LP), a and b chlorophyll, carotenoids and the content of proteins were determined. It was analysed the influence of inoculated fungi (*Glomus intraradices*) on the absorption of heavy metals by plants. The results confirmed an increase in the transfer coefficients from soil to plant under the influence of the mycorrhiza formed in the case of the following metals: Cd, Ni, V, Zn and a decrease in the case of As, Co,Cr, Cu, Mn, U. The conclusion of this study is that mycorrhizal fungi produce an increase in the biomass production as well as in the concentration of some of the elements (As, Co, Cd, Cr, Mn, Ni, Pb and U) at root level, a phenomenon known as phytostabilization.

## INTRODUCTION

Nowadays mobilized heavy metals present a big challenge because they cause considerable losses in plant productivity of economically important crops and a risk of toxic bioaccumulation along human food chain in industrially polluted regions. To cope with the metal contamination, different strategies of research activities have been developed including treatments for enhancing the plant resistance and tolerance.

For the particular area Slatina, where there is a factory for obtaining and processing aluminium, it can be said that the pollution is produced as a consequence of the existing working electrolysis section, capacities of processing, including a foundry, mills at cold and hot, and an extruded section. The pollutants evacuated from the aluminium factory's platform derive from wastewater: petroleum products and extractible substances with ether of petroleum, pollutants discharged in air, such as: powders, N oxides ($N_2O$, NO, $NO_2$), S oxides ($SO_2$), C oxides (CO), fluor ($F_2$, HF, $F^-$), chlorine ($Cl_2$), volatile organic compounds (VOCs): gasoline, ethers of petroleum, benzene, phenols, but also pollutants from soil. Studies were carried out establishing to what extent the pollution with fluorides extended to human and animal population.[1-3] Udrescu *et al.*[4] showed that the total content of F was found higher than the maximum admissible limit (MAL) in the polluted area. Research conducted in the year 2000 revealed that soil pollution with F was maintained in the ALRO Slatina area.[5] The same authors

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showed that some metals, such as Cu and Pb from soil did not exceed MAL; on the other, Cr exceeded two or three times the values corresponding to MAL. The factory is placed in the east peripheral area of the town[6] on the right of Slatina-Piteşti road, which is not affected by geological phenomenons of instability.

The purpose of this paper was on one hand, to establish the degree of pollution in soil and in the dominant vegetation from the agricultural ecosystems from around ALRO factory (large surfaces of agricultural field are frequently cultivated with corn (*Zea mays* L.), rye (*Secale cereale* L.) and sunflower (*Helianthus annuus* L.). On the other hand, the aim was to estimate the influence of microorganisms *(Glomus intraradices*) on the uptake of heavy metals by plants in order to find an optimum solution to remedy the polluted area.

## EXPERIMENTAL

### Materials and method

#### 1. Sampling area

The soil's physico-chemical characterization (pH, electrical conductivity, forms of mineral N, assimilable P and the content of metals: Cr, Mn, Ni, etc) was done by taking soil samples from two points situated across the source of pollution, in the industrial zone and populated with human settlements (C1) and in the vicinity of the pollution source, near the electric factory (C2) (Fig. 1) on a surface of approximately 4 $m^2$, with six replicates each one of them, till the depth of 20 cm, using a probe (for more details about the polluted area, see [6]).

For the characterization of the sampled soils (C1 and C2), table 1 can be checked, and for more details, see.[6]


Fig. 1 – The location of the soil sampling points.

*Table 1*

The characterization of the soils before carrying out the laboratory experiment

| Sample code | pH $\bar{x}$ /SD | H* $\bar{x}$ /SD | EC $\bar{x}$ /SD | $N\text{-}NH_4^+$ $\bar{x}$ /SD | $N\text{-}NO_3^-$ $\bar{x}$ /SD | $N\text{-}NO_2^-$ $\bar{x}$ /SD | $P\text{-}PO_4^{3-}$ $\bar{x}$ /SD |
|---|---|---|---|---|---|---|---|
| | | [%] | [µS/cm] | [µg/g d.w.] | | | |
| C1 | 5.84<br>*0.6* | 14.38<br>*1.92* | 35.33<br>*8.164* | 6.156<br>*0.229* | 3.587<br>*1.228* | 0<br>*0* | 30.16<br>*13.08* |
| C2 | 6.63<br>*0.27* | 15.94<br>*5.48* | 54.66<br>*19.32* | 6.826<br>*0.244* | 4.661<br>*3.01* | 0<br>*0* | 50.58<br>*30.4* |

*H = soil humidity

## 2. Experimental design

The laboratory experiment was carried out using one type of soil (C1, the most polluted), after having been very well manually homogenized. Three experimental variants were used, which were encoded as follows: R - reference soil (unpolluted), NM - polluted soil without mycorrhizal fungi and M - polluted soil and inoculated with mycorrhizal fungi, four replicates being used for each experimental variant. According to the studies performed by [7] (cited by [8]), in all the experimental variants, respectively R and NM, the soil was amended with 10% expanded clay, and for those type M, the soil was amended with 10% expanded clay which was inoculated with mycorrhizal fungi (AMF) 510 (160 spores/g soil) *Glomus intraradices*, the fungi being sold under the name of Blaethon "Tunesia, Collection of Microorganisms of the Institut fűr Pflanzenkrankenheiten und Pflanzenschutz" by the University from Hanovra. The plant studied was rye (*Secale cereale* L.), selected after conducting a germination test. Before the amendment, the polluted soil was autoclaved at 130°C for about 30 minutes. Similar to the characterization of the sampled soils (Table 1), after conducting the laboratory experiment, determinations on the soil samples were done, the same parameters being analysed. In addition, it was determined the soil's respiration after harvesting the plants. Determinations on the vegetable material were done, such as: microscopic view of the mycorrhiza, total content of proteins, lipid peroxides and the content of metals. What is more, it was determined the content of pigments (a and b chlorophyll and carotenoids) from the vegetable material.[6]

*Determinations performed on soil samples*

**pH determination**

An aqueous suspension is used (1:2.5 m/v). Before being measured it is shaken for about 15 minutes and then it is left to rest for an hour until balancing with carbon dioxide. pH is determined by using a pH-meter WTW Germany with a glass electrode.

*Determination of electrical conductivity*

It was done by using the aqueous solution from pH determination, as well as the multiparameter kit WTW Germany.

**The soil's humidity** content, expressed as a percentage, was calculated by finding out the ratio between the water mass lost by soil samples (through drying it out at 105° C) and the mass of the dry soil.

**Soil's respiration** was determined through the alkaline absorption method, using NaOH solution 100 mM and a soil sample in a vessel hermetically closed, for twelve hours. The soda resulted from the reaction is precipitated with barium chloride, and the excess of NaOH is titrimetric dosed with a HCl solution.[9]

*The determination of mineral nitrgen from soil's solution*

The content of mineral nitrogen ( $N-NO_2^-$, $N-NO_3^-$, $N-NH_4^+$ ) as well as of assimilable ($P-PO_4^{3-}$) was established through spectrophotometry, as follows:

**$N-NO_3^-$ determination**

Nitrate ion ($N-NO_3^-$) is the stable form of combined nitrogen for oxygenated systems, and is extracted quantitatively from the soil through ionic exchange using KCl. The most wide-spread method is based on the reaction of nitrates with sulfosalicylic acid using natrium hydroxide in order to forms a yellow colored complex.[10]

**$N-NH_4^+$ determination**

Ammonium ($N-NH_4^+$) is extracted quantitatively from the soil through ionic exchange using KCl. With a buffer of potassium sodium tartrate, sodium citrate interfering cations are bound. The most sensitive spectrophotometric procedure for the determination of ammonium is based on the conversion of ammonium into the intense blue indophenol complex (IPC) by means of salicylate and nitroprusside. Reagent concentration nitroprusside functions as a catalyst in the reaction between ammonium and salicylate and in the presence of Na dichlorisocyanurate forms a green-coloured complex.[11,12]

**$N-NO_2^-$ determination**

Nitrite ($N-NO_2^-$) is extracted quantitatively from the soil through ionic exchange using KCl. The mostly utilized spectrophotometric methods are based on the ability of nitrites to diazotize aromatic amino compounds in acidic medium producing diazonium salts which are copulated to create azo-dye. The most frequently diazotized aromatics are sulphanilic acid and/or its amids. Consequently, these diazonium salts are let to react with N-(1-naphtyl)-ethylene-diamine dihydrochloride that replaced the former 1-naphtylamine which was found to be carcinogenic and, forms a pink-collored complex.[13-15]

*The determination of assimilable phosphorus content*

Phosphate ($P-PO_4^{3-}$) is extracted quanitatively from the soil through ionic exchange using $NaHCO_3$. The determination is based on using of molid-ammonium and malachite green (1:3) to form the phospho molid ammoniu which is reduced and form a green-collored complex.[13,14,15]

*The determination of the total content of metals*

Metals' analysis was done after a digestion with 30% suprapur HCl and 65% suprapur $HNO_3$ (1:3, v:v) from Merck, using an instrument with a single collector, quadrupole inductively coupled plasma with mass spectrometry: ICP-MS Perkin-Elmer ELAN DRC-e with axial field technology for trace elements, rare earth elements and isotopic analyses. For each determination three analytical replicates were measured and standard solutions were prepared by diluting a 10 μg/ml multielement solution (Multielement ICP Calibration Standard 3, matrix 5% $HNO_3$, Perkin Elmer Pure Plus).

*Determinations performed on a vegetable material*

The following operations were done on the vegetable material obtained after harvesting: underground and above-ground portioning, washing the roots with water and then with distilled water, weighing to determine fresh underground and above-ground biomass, drying through lyophylization, grinding in a fine powder, and storage in polyethylene bottles up to -20°C until the analysis date. It was analysed the microscopic visualization of the mycorrhiza, the determination of the total content of protein, lipid peroxides, the content of metals, and the content of assimilating pigments (chlorophyll a and b and carotenoids) from the vegetable material.

*The microscopic visualization of the mycorrhiza formed by mycorrhizal fungi Glomus intraradices with the roots Secale Cereale* L.

The method described by [16] uses Lactophenol blue for colouring the roots preserved in a fixing solution (45.85%), ethanol (45.85%), formaldehyde (6%), and acetic acid (2.3%) (v/v). After colouring, the arbuscules formed in the mycorrhiza process could be visualized through a microscope (Fig. 2c).

*The determination of the total content of protein*

The vegetable material sample (100 mg) was mortar for 2 minutes with 2 ml of solution for extraction, which contains 2% polyvinylpyrrolidone, 2mM chelaplex III-EDTA and 2mM DTT (dithioerithritol) dissolved in 100mM $K_2HPO_4/KH_2PO_4$ buffer with pH 7.2; then, it was passed through a centrifuge tube and centrifuged for 10 minutes at 18000 rpm. The liquid fraction was subjected to dyalisis through a protein waterproof membrane, using a phosphate buffer with pH 7.2 for 8 hours at 4°C on a magnetic stirrer. Lowry method of determing the total content of proteins from the protein extract is based on their precipitation with trichloroacetic acid (10%) and solubilization with NaOH 1N, using bovine serum albumin (BSA) as a standard solution.[17]

*Lipid peroxides*

Malondialdehyde (MDA), resulted from the decomposition of peroxides' fat polyunsaturated acids, forms with the tiobarbituric acid a coloured adduct which can be measured spectrophotometrically at 440, 532 and 600 nm.[18]

*The determination of the content of assimilating pigments (chlorophylls and carotenoids)*

The sample of above-ground vegetable material (100mg) is homogenized with 3 ml of acetone solution (80% acetone: 15% ultrapure water, 5% ammonium, concentration of 25%, v/v), using an ultraturax at 75000 rpm, and then it is centrifuged at 4800 rpm for 20 minutes.

It is measured spectrophotometrically the liquid fraction's solution at 480, 645, 647, 652, 663, 664, 750 nm for chlorophyll *a* and *b* and carotenoids.[19]

*The determination of metals' content*

Metals' analysis was done after a digestion with 65% suprapur $HNO_3$ from Merck, using the same techniques as was described for the metal analysis in soil.

**3. Statistical analyses**

The data used to produce graphs and tables are arithmetic averages of the values of four replicates of each experimental variant, and processing was done using Microsoft Excel 2003 and Microsoft Word 2003. The metals' transfer coefficients (TC) from soil to plant were also calculated using the formula:

$$TC = \frac{conc.of \quad metal \quad in \quad plant}{conc. \quad of \quad metal \quad in \quad soil} \qquad (1)$$

According to[20] the transfer coefficients can be considered accumulation factors if TC <1, and concentration factors if TC>1. For lipid peroxides, assimilating pigments and protein content two parallel analytical replicates were measured. A Mann-Whitney test was used for comparison of change between the experimental variants at $p<0.05$. The program SPPS version no.17 for Windows was used for statistical evaluation.

## RESULTS AND DISCUSSION

Analysing the soil parameters before starting the experiment (Table 1) and at the end of it (Table 2), it can be noticed that the average of the soil's pH is within the acid class, in case of the six replicates taken across the source of pollution (C1), according to [21] and within the neutral class in case of those taken from the vicinity of the source (C2). Although it has higher values in case of C2 soil comparing with C1 (across the source), the electrical conductivity (EC) falls in the category of soils which are not saline (0 – 2 mS/cm, according to [22]) this having an insignificant effect on plants. At the end of the laboratory experiment, it can be observed an increase in the salinity, but without any vulnerability effects on plants. Also, it can be seen that at the end of this experiment, after harvesting the plants, pH dropped from 6.63 to 5.50 in case of the uninoculated variant (NM). As a consequence of the inoculation, it rose from 5.50 to 5.70. These variations which appear during the vegetation period are determined by the chemical and physico-chemical processes that happen in the soil. The values presented in Table 1 show that the soil is N poor and that it has a satisfactory content of P in bioavailable form ($P\text{-}PO_4^{3-}$). It was this fact that allowed the inoculation with mycoorhizal fungi. It is known that the fungi release metabolites which increase the solubility of the mineral ions bound into the soil, so that the anorganic ions are taken and translocated directly into the roots, a closed circuit of the nutrients being done.[23] In spite of all this, it can be noticed that in the case of mycorrhiza experimental variant, the mineral N concentration ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$) and the $P\text{-}PO_4^{3-}$ concentration decreased in comparison with the variant without mycorrhizal fungi (NM). That is because of a higher consumption of nutrients which are necessary for the plants' development (Table 2). The positive effect of the mycorrhizal fungi can be observed from the increase in the microbial activity, expressed by using the soil's respiration, which varies from 3.047 to 4.525 mg $CO_2$/g d.w. x 12h (Table 2). The difference between the phenological parameters of rye individuals in the three experimental variants can be noticed in Fig. 2a and 2b. Also, it can be observed that plants have developed vigorously, having a higher biomass in the variant with mycorrhiza than in the other one, without mycorrhiza. The symbiosis between the mycorrhizal fungi and the rye's roots was checked by looking through a microscope at fragments from the plants' roots, which were prepared according to the method described by.[16] Only arbuscles (Fig. 2c.), and not vesicles were identified, because the experiment lasted 45 days. They usually develop in the plants' first phase of growth. Concerning the consumption of plants by human population (according to[24]), it can be easily

seen from Table 3 that the elements As, Cr, Mn and Ni found in soil had higher concentrations than the MAL (values written in bold characters). Also, it can be observed the presence of some low uranium concentrations, (0.048 μg/g in reference soil, 0.204 μg/g in polluted one) which could be natural met as background concentration level. Regarding Cu, which is an essential element for the growth and development of the plants, it can be seen slight decrease in the concentration in soil comparing with the values it had before sowing (it dropped from 16.90 to 16.33 μg/g, both values indicating a deficiency in this element in soil). Cu's concentration fell from 16.33 to 16.04 μg/g in the mycorrhiza experimental variant (Fig. 3). Usually, an increase in Mn and Ca at the end of the experiment comparing with the values before sowing can be considered a positive result induced by mycorrhiza process, because these elements are nutrients for plants. If we were to compare the metals' concentrations in soil before the sowing and after harvesting the plants, it can be noticed that the majority of toxic elements for plants had a lower concentration in soil at the end of the experiment, with some exceptions (see Table 3). The increase of concentration to the rest of the elements could have been provoked by the modification of soil's pH during plants' development, which is possible to have induced a higher mobility of these elements in soil. Therefore, it can be concluded that we have to deal with polymetallic pollution. Also, from Table 3 it can be observed that the majority of elements which serve as nutrients for plants registered a slight increase in their concentration in soil (grey cells) at the end of the experiment. This is because the soil was homogenized with expanded clay, which brought an additional contribution of elements.[7]

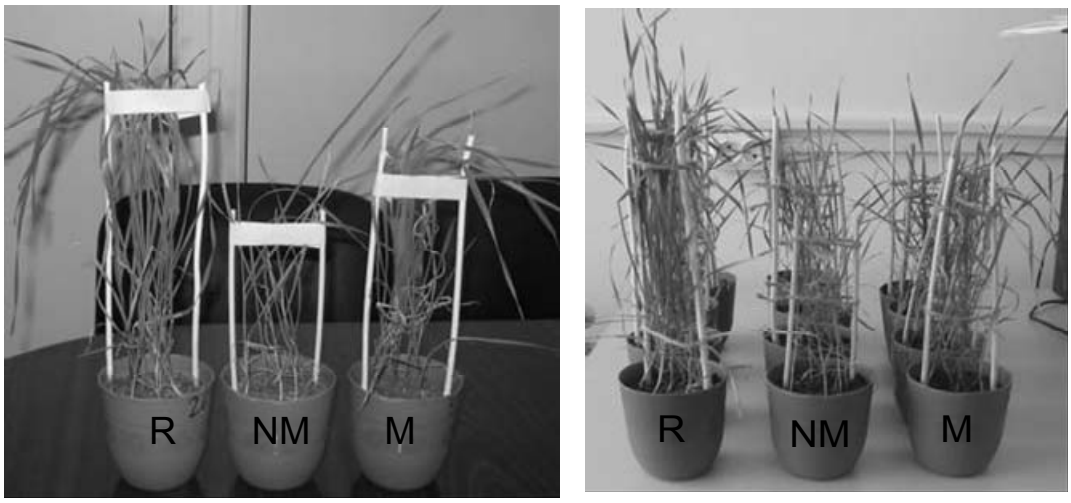


Fig. 2a – Differences between the phenological parameters of rye individuals (*Secale cereale* L.) in the three experimental variants (a replicate for each experimental variant can be seen in the left photo; the right photo presents the all four replicates from each experimental variant).

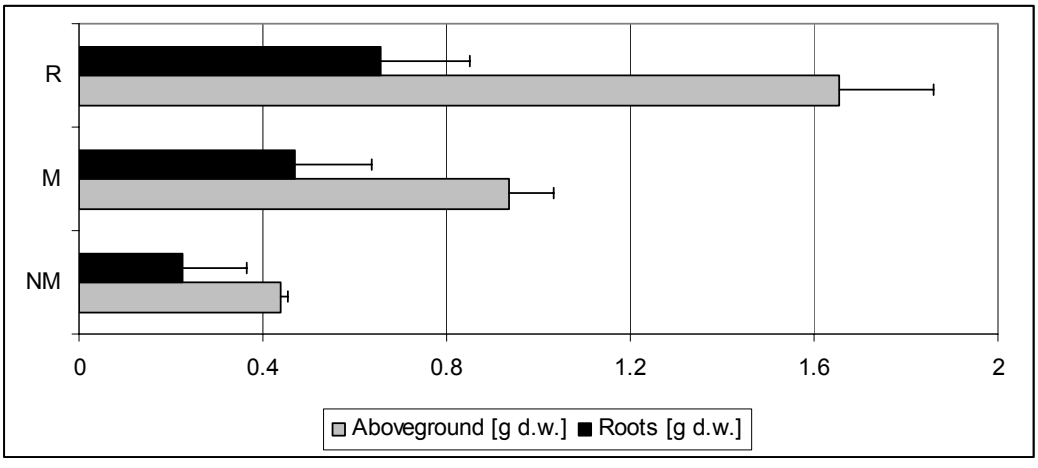


Fig. 2b – Biomass of roots and overground part of plants expressed in wet weight (w.w.) in all the three experimental variants.

*Table 2*

Parameters of the polluted soil after carrying out the laboratory experiment

| Sample code | pH | $\bar{x}$ /SD | H | $\bar{x}$ /SD | EC | $\bar{x}$ /SD | Soil respiration | $\bar{x}$ /SD | $N\text{-}NH_4^+$ | $\bar{x}$ /SD | $N\text{-}NO_3^-$ | $\bar{x}$ /SD | $N\text{-}NO_2^-$ | $\bar{x}$ /SD | $P\text{-}PO_4^{3-}$ | $\bar{x}$ /SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | | [%] | | [µS/cm] | | mg $CO_2$/g d.w.x12h | | [µg/g d.w.] | | | | | | | |
| R | 7.19 | 7.225 | 13.73 | 13.93 | 109 | 119.2 | 8.389 | 8.171 | 42.93 | 36.08 | 2.608 | 2.56 | 0.247 | 0.169 | 103.9 | 107.3 |
| R | 7.07 | 0.12 | 14.55 | 0.484 | 129 | 8.261 | 7.247 | 0.752 | 42.95 | 10.17 | 2.762 | 0.294 | 0.226 | 0.092 | 132.2 | 16.95 |
| R | 7.32 | | 14.05 | | 118 | | 9.047 | | 37.06 | | 2.131 | | 0.164 | | 96.22 | |
| R | 7.32 | | 13.42 | | 121 | | 8.004 | | 21.4 | | 2.741 | | 0.041 | | 96.89 | |
| NM1.1 | 5.51 | 5.502 | 13.36 | 15.4 | 107 | 113.2 | 2.617 | 3.047 | 16.94 | 18.33 | 3.963 | 3.995 | 0.061 | 0.041 | 22.6 | 26.44 |
| NM1.2 | 5.57 | 0.053 | 16.7 | 1.718 | 103 | 12.39 | 2.989 | 0.864 | 17.82 | 3.729 | 4.079 | 0.506 | 0.106 | 0.051 | 27.91 | 3.091 |
| NM1.3 | 5.49 | | 14.61 | | 112 | | 4.276 | | 14.95 | | 3.354 | | 0 | | 25.49 | |
| NM1.4 | 5.44 | | 16.94 | | 131 | | 2.309 | | 23.63 | | 4.586 | | 0 | | 29.74 | |
| M1.1 | 5.72 | | 17.13 | 14.72 | 108 | | 4.286 | 4.525 | 20.02 | 16.01 | 3.9 | 3.05 | 0.129 | 0.2 | 21.92 | 24.49 |
| M1.2 | 5.71 | | 12.08 | 2.068 | 109 | | 4.519 | 0.265 | 14.13 | 3.039 | 2.933 | 0.585 | 0.337 | 0.106 | 17.86 | 12.94 |
| M1.3 | 5.7 | 5.7 | 14.95 | | 95 | 112 | 4.4 | | 13.24 | | 2.798 | | 0.104 | | 14.79 | |
| M1.4 | 5.67 | 0.021 | 14.73 | | 136 | 17.22 | 4.897 | | 16.66 | | 2.57 | | 0.23 | | 43.41 | |

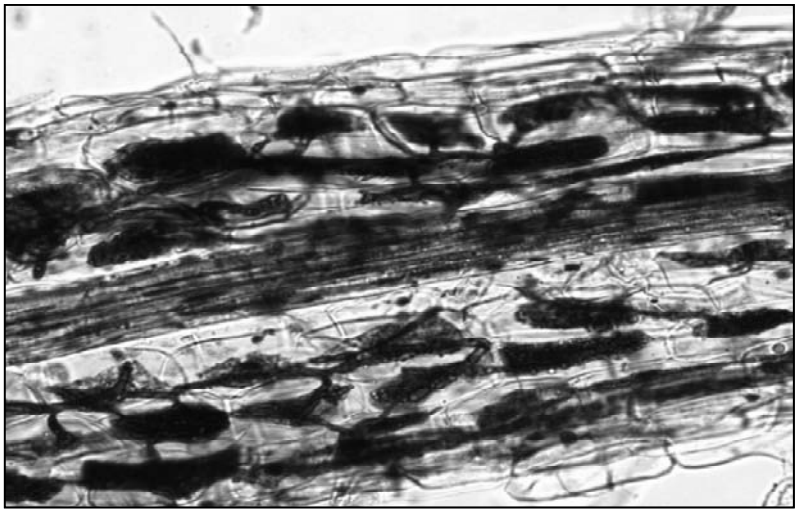

Fig. 2c – Microscopic image of the mycorrhiza formed as a result of the symbiosis between mycorrhizal fungi from *Glomus intraradices* species and *Secale cereale* L. roots.

*Table 3*

The elements' distribution in the soil samples sampled before ($C_{1i}$) and at the end ($C_{1f}$) of the laboratory experiment

| Element | $C_{1i}$ (NM), before | $C_{1f}$ (NM), at the end | Acceptable level in soil for plants that can be used by human population [24] |
|---|---|---|---|
| | $\bar{x}$ /SD, [µg/g d.w.] | | |
| As | **13.77**/*0.42* | **12.95**/*1.291* | 2 |
| Ca | 24670/*334.1* | 26520/*0.506* | - |
| Cd | 0.18/*0.11* | 0,166/*3.092* | 2 |
| Co | 11.78/*1.45* | 13.46/8.242 | 10-75 |
| Cr | **130.9**/*12.14* | **109.9**/*2.02* | 50-100 |
| Cu | 16.90/*1.38* | 16.33/*0.501* | 30-100 |
| Mn | **841.3**/*71.55* | **998.2**/*2.069* | 270-525 |
| Na | 555.9/*141.9* | 633.5/*3.688* | - |
| Ni | **72.67**/*46.96* | **52.88**/*76.26* | 35 |
| Pb | 22.89/*0.82* | 20.52/*11.19* | 2-60 |
| U | 0.31/*0.06* | 0.204/*445.3* | - |
| V | 88.29/*2.78* | 87.57/*16.53* | 18-115 |
| Zn | 76.65/*2.29* | 72.63/*6.59* | 17-125 |

Legend: the grey cells represent a slight increase in the nutrients' concentration in soil. The values written in bold characters represent the concentrations exceeding the acceptable limit

In Fig. 3 it can be seen that in the inoculated variant (M), the concentration of the elements As, Cd, Co, Cr, Mn, Ni, Pb and U found in soil was higher comparing with the ones from the non-inoculated variant (NM). This fact can be interpreted as a positive effect, because the modification of metals' disponibility and the prevention of them entering in the food chain, as a consequence of fungi inoculation, can produce the phenomenon of phytostabilization. A remarkable fact is that the concentration found in soil for the elements Ca, Cu, Na, V and Zn was lower in the inoculated variant. This could lead us to the conclusion that a phytoextraction was performed. However, taking into account the fact that these elements are essential for plants, and that physiologically talking, they have higher concentrations as a result of the installation of symbiosis between plants' roots and the mycorrhiza formed, we can state, without being certain though, because of the scale the experiment was carried out, that by using the mycorrhizal fungi, it was produced a phytostabilization of the metals at rizosphere level.

The values of the elements' transfer coefficients in the total biomass obtained in the case of every experimental variant can be seen in Table 4. The grey cells indicate a lower transfer factor for plants which were grown in an inoculated soil (M). These values confirm the higher concentrations of the elements found in soil after harvesting the plants, which were presented in Fig. 3. A notable fact is that more elements have a transfer coefficient (TC) >1, even very toxic elements, such as U.

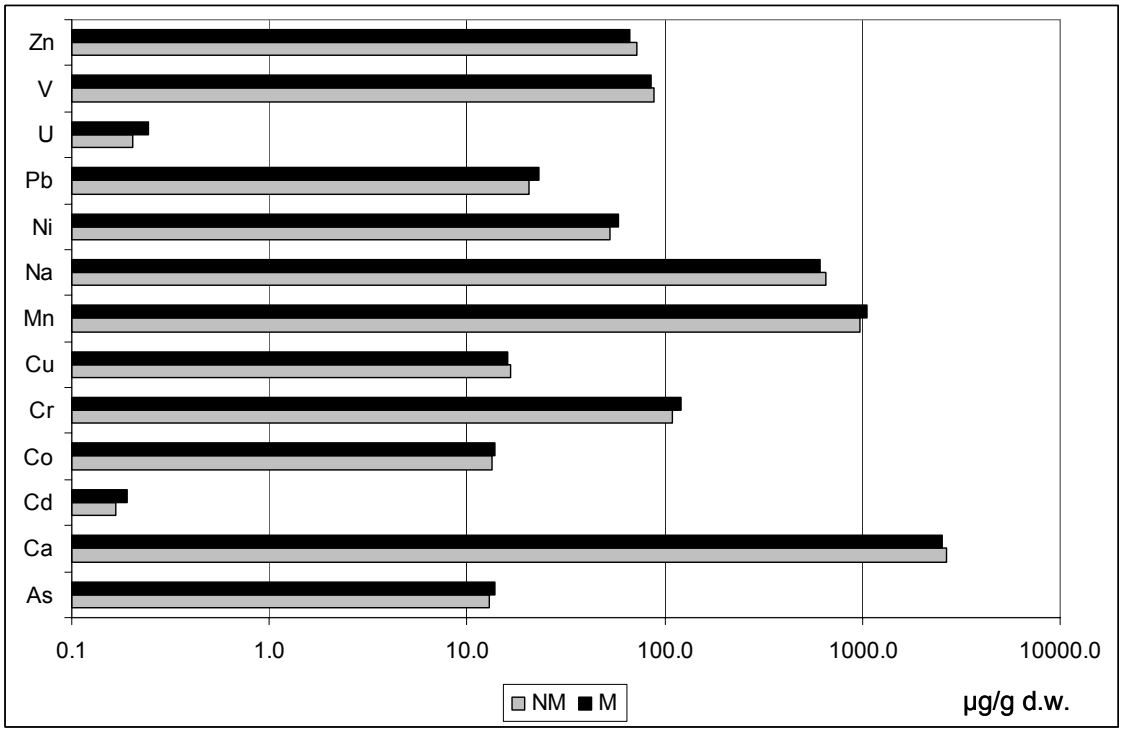


Fig. 3 – The elements' distribution in soil with (M) and without mycorrhizal fungi inoculation (non-mycorrhyza - NM).

*Table 4*

The transfer coefficients (TC) of the elements from soil to plant

| Element | TC of the elements from soil to plant | | |
|---|---|---|---|
| | R | C | CM |
| As | 0.234, acumulation | 1.216, concentration | 0.204, acumulation |
| Cd | 6.97, concentration | 25.36, concentration | 75.81,concentration |
| Co | 1.334, concentration | 3.667, concentration | 1.6, concentration |
| Cr | 0.461, acumulation | 0 .544, acumulation | 0.267, acumulation |
| Cu | 4.267, concentration | 9.662, concentration | 6.763, concentration |
| Mn | 1.506, concentration | 2.253, concentration | 2.081, concentration |
| Ni | 1.534, concentration | 1.001, concentration | 1.065, concentration |
| U | 52.08, concentration | 20.43, concentration | 14.30, concentration |
| Pb | 0.624, acumulation | 1.351, concentration | 0.809, acumulation |
| V | 0.164, acumulation | 0.169, acumulation | 0.179, acumulation |
| Zn | 12.74, concentration | 7.601, concentration | 23.96, concentration |

In the case of plants grown on contaminated soil without mycorrhiza (NM), it can be seen a reduction in the biomass production, especially in the aboveground part, when comparing with those from M variant (see Fig. 2b), which is reflected into a low protein content and assimilating pigments (Fig. 4). In case of roots, the protein content is significantly lower in NM (29.70 µg/g d.w.) as in M (45.9 µg/g d.w.) experimental variants. A significantly statistical variation can be noticed in the case of a and b chlorophyll (from 4.12 to 7.91 in the case of a chlorophyll and from 1.40 to 4.27 for b chlorophyll). It can be seen an intensification of the stress caused by metals pollution, which is revealed by the insignificant increase (from 0.50 µg/g d.w. in NM variant to 0.512 µg/g d.w. in M variant) of the MDA content, deriving from the decomposition of the peroxides of the polyunsaturated fatty acids, in case of the plants cultivated on polluted soil (NM). This has as a consequence an enhanced consumption of carbohydrates, which determines a reduction in the protein content, but also the increase in the concentration of oxygen's free radicals. This affects the main physiological processes of plants: photosynthesis, synthesis of chlorophyll pigments, respiration, root and foliar assimilation, etc. These values of the parameters mentioned confirm the positive effect of the mycorrhiza forming.

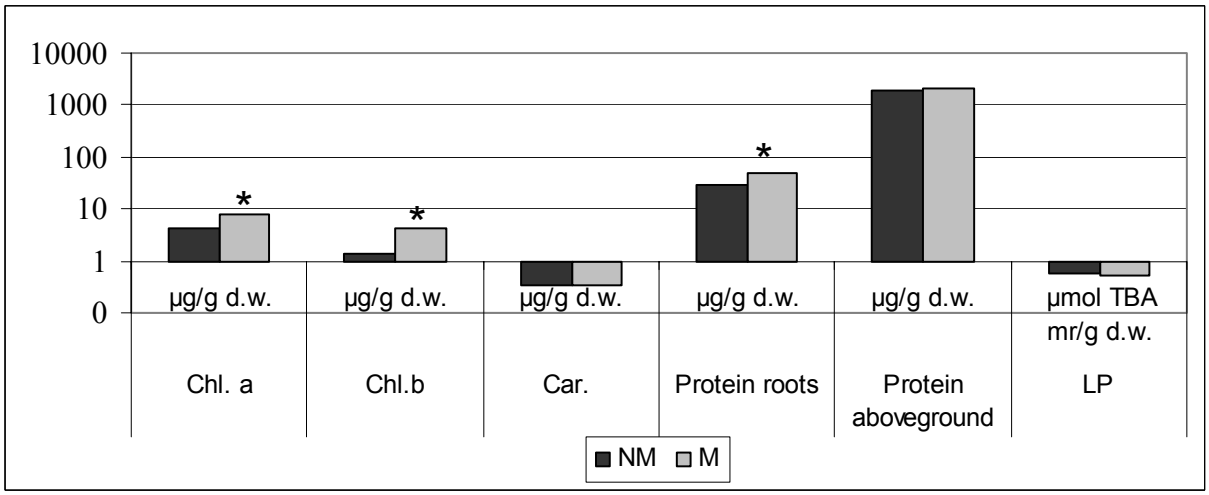


Fig. 4 – The assimilable pigments' variation in the aboveground part of the rye, the proteins content in roots and the aboveground part and lipid peroxides in aboveground part of this species of plant. Stars at $p<0.05$ indicates significant differences according to Mann-Whitney test.

## CONCLUSIONS

By analysing the results obtained after carrying out the laboratory experiment, we can conclude that the Slatina area is characterised not only by a polymetallic pollution (As, Cr, Mn, Ni), but also by a deficiency in Cu that has a harmful effect on crop plants' growth and development. Comparing the two experimental variants (NM and M), it can be observed that in NM variant the majority of toxic elements registered a decrease in the soil concentration at the end of the experiment compared with the values before sowing. The concentration of the elements: As, Cd, Co, Cr, Mn, Ni, Pb and U found in soil was higher in M variant than in NM variant, which can be interpreted as a positive effect of the mycorrhiza fungi inoculation that changes the disponibility and prevents the metals' penetration into the food chain. Under mycorrhiza influence, it was registered an improvement of some soil parameters, such as: pH and soil's respiration that led to the increase of the biomass production. By comparing the transfer coefficients from soil to plant in the two experimental variants, it can be confirmed an increase in the case of Cd, Ni, V, Zn and a decrease for As, Co, Cr, Cu, Mn, U under the influence of mycorrhiza formed. The increase in the transfer coefficients was possible due to the intensification of root assimilation of those ions, as a result of fungal hyphae being in action.

It can be concluded that mycoremediation, along with phytostabilization, immobilizes to a certain degree some of the metals from soil at root level and favours plants' development. After carrying out the laboratory experiment, it can be stated that the selection of this species of plant can be considered a real success and turning from a laboratory phase to a higher work scale, respectively experimental plots scale, is possible.

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## REFERENCES

1. D.F. Perkins, R.O. Millar and P.E. Neep, *Enviro. Poll.*, **1980** (Series A), *21*, 155-168.
2. O. L. Gilbert, *Environ. Poll. (Series A)*, **1985**, *39*, 293-302.
3. R. Fuge and M.J. Andrews, *Environ. Geochem. Health*, **1988**, *10*, 96-104.
4. S. Udrescu, A. Mori, C. Urzica, D. Hoza, E. Gament, C. Zelinschi and D. Popa, *Publicaţiile Societăţii Naţionale Române pentru Ştiinţa Solului*, **1997**, *29(B)*, 176-182.
5. M.A.P.M., "The Order of Water and Environmental Protection Ministry", 2000, *524,* http://apmot.anpm.ro/upload/27689_CAP_III%20SCHIMBARI%20CLIMATICE.pdf, scientific report.
6. A. Paun, A. Neagoe and I. Baciu, *Rev.Chim.*, **2011,** *in press*.
7. H. Von Alten, B.M. Blal, J.C. Dodd, F. Feldmann and M. Vosatka, "Mycorrhizal Technology in Agriculture", S.Gianinazzi, H. Schüepp, J.M. Barea and K. Haselwandter (Eds.), 2002, 281-296.
8. A. Neagoe, V. Iordache, R. Mascher, B. Knoch, E. Kothe and H. Bergmann, *Proceedings of Arbeitstagung in Jena Agricultural, Biological, Environmental, Nutritional and Medical Importance of Macro, Trace and Ultra Trace Elements, Friedrich Schiller Universität*, **2006**, *23*, 568-575.

9. F. Schinner, R. Öhlinger, E. Kandeler and R. Margesin, „Bodenbiologische Arbeitsmethoden“, Springer-Verlag, 1993, 134-136.
10. A. Neagoe, G. Ebenå and E. Carlsson, *Chem. Erde*, **2005**, *65*, 115-129.
11. R.V.E. Pym and P.J. Milham, *Anal. Chem*., **1976**, *48*, 1413-1415.
12. D. Scheiner, *Water Research*, **1976**, *10*, 31-36.
13. R. Bassler, “Handbuch der Landwirtschaftlichen Versuchs und Untersuchungsmethodik (Methodenbuch)”, Band I. VDLUFA Verlag, Darmstadt., 1991, p. 22.
14. D.J. Pietrzyk and W.C. Feank, “Analytical Chemistry”, Technical Publishing House, Bucharest, 1989.
15. S. Mănescu, M. Cucu and M.L. Diaconescu, “Sanitary Chemistry of the Environment”, Medical Publishing House, Bucharest, 1994.
16. M. Guether, “Untersuchungen zur differentiellen Genexpression in Medicago trunculata Wurzen mit Glomus Intraradices” Diplomarbeit, Friedrich Schiller Universität, Jena, Germany, 2005, p. 100.
17. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol., Chem.*, **1951**, *193*, 265-275.
18. L.M. Lagrimini, *Plant Physiol*, **1991**, *96*, 577-583.
19. P. Schöpfer, “Experimentelle Pflanzenphysiologie – Einführung in die Anwendung“, Band *2*, Springer, Berlin, 1989, p. 131.
20. N.Botnariuc and A.Vădineanu, “Ecologie”, Editura didactică şi pedagogică, Bucureşti, 1982, p. 439.
21. INRA, “Référentiel pédologique”, Association Française d’étude des sols, 1995, p. 332.
22. Research report of the fourth stage in the contract number 51-031 “Methodologies of evaluating the risk of agrophysical degradation”, responsible for the project- M. Dumitru, ICPA, **2007**, http://www.icpa.ro/proiecte/ RAMSOL/51-031_scientific raport.
23. R. M. Atlas and R. Bartha., “Microbial ecology-fundamentals and aplications”, third edition, The Benjamin / Cummings Publishing Company, 1993, p. 56-74.
24. A. Kabata-Pendias and H. Pendias, “Trace Elements in Soils and Plants”, 2nd edition, CRC Press, Boca Raton, Ann, Abor, London, 1992, p. 365.