The Effects of Arbuscular Mychorrizal Fungi on the Transfer of Heavy Metals and Oxidative Stress related Parameters in Sunflower Exposed to Multi-element Pollution

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This paper presents a laboratory experiment which aims to identify the main pollutants that appear to affect large areas of farmland around the aluminum plant in Slatina. After conducting a germination test using several species of plants, there were selected two of them, which developed more rigorously and produced a higher biomass. The experiments were made with sunflower (Helianthus annuus L.) and rye (Secale cereale L.), which were sown in pots with unpolluted reference soil, polluted soil amended with expanded clay and polluted soil amended with expanded clay inoculated with mychorriza fungi. This paper presents results on sunflower. The experiment was conducted under controlled conditions in a vegetation room and soil parameters were determined before and after running the experiment (pH, electrical conductivity, humidity, soil respiration, mineral forms of N, assimilable P and the metals content. In the plant material (roots, stems, leaves) the following parameters were determined: metal content, lipid peroxidation (LP), chlorophyll a and b, carotenoids and protein content. It was observed to what extent inoculated fungi (Glomus intraradices) influenced heavy metal absorption by plants. The study indicated an increase of the transfer coefficients from the soil in the plant under the influence of the formed mychorriza, in the case of metals V, Zn, Cu and a decrease in the case of Cr, Mn, Ni , Pb, U. The conclusion of this study was that mychorriza fungi have a role in the accumulation of heavy metals in the rhizosphere, a phenomenon known as phyto-stabilization.

Keywords: heavy metals, poly-element soil contamination, Helianthus annuus L., phyto-stabilization

This study was motivated by the presence of a large number of pollutants both in the free air [14] and on large areas of farmland around the plant. The harmful effects of pollutants have an impact on both human population and on plants and animals. The purpose of the research was to assess the influence of micro-organisms on the absorption of heavy metal by the plants, with the idea of selecting the most appropriate remediation technology for the contaminated area.

To achieve this purpose, three operational objectives have been identified:

- characterize the distribution of heavy metals in soil compartments and primary producers;
- characterize the effect of polluted soil inoculation with mychorriza fungi on primary producers, measured by assessment of oxidative stress parameters;
- proposing solutions to remedy the polluted area.

There were identified the dominant plant species in Slatina polluted area and a literature screening was conducted in order to identify an effective mechanism for protection of plants against heavy metal pollution. The dominant plant species in the area are: corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L). Previous studies conducted in Slatina polluted area, as a result of obtaining and processing aluminum, have established how much fluoride pollution has spread to human and animal population [16, 4, 5].

It is known [14] that the main pollutants discharged from the aluminum plant platform in Slatina are:

- pollutants from wastewater;

The main pollutants resulted from the technological processes and discharged in the surface waters are: petroleum products and extractable substances with petroleum ether.

- pollutants released into the air: dust, 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 (V.O.C.) such as: petrol, petroleum ether, benzene, phenols;
- pollutants from soil.

In the polluted area the total content of F is higher than the allowable limit (MAL), while Cu and Pb from soil do not exceed MAL, and Cr exceed 2-3 times the corresponding values [23].

Experimental part

Materials and methods

Description of the polluted area

Slatina is a town with 250000 inhabitants, situated about 150 km west of Bucharest. In the east peripheral area of the city is located ALRO factory, which produces aluminum and aluminum products. The perimeter of the factory, located northeast of Slatina (on the right side of the road Slatina-Pitesti), is situated in an area that is part of the high plains of the town of Slatina, and is not affected by geological phenomena of instability. In Figure 1 you can see the two soil sampling points selected to be located in an area with maximum pollution. Using a Corer probe, on each point 6 replicated from a surface of about 4 meters square have been taken, up to the depth of 20 cm. According to the obtained results, there will be made an

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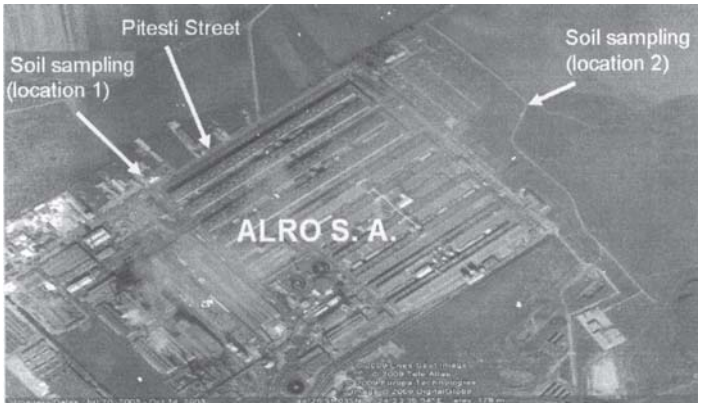

Fig. 1. The location of the sampling points of the soil

Sample number	Common name	Latin name	Number or grames of seeds
1	Corn	*Zea mays* L.	12
2	Sunflower	*Helianthus annuus* L.	12
3	Wheat	*Titricum aestivum* L.	20
4	Lupin	*Lupinus angustifolius* L.	12
5	Phacelia	*Phacelia tanacetifolia* Benth.	0.5 g
6	Clover	*Trifolium pratense* L.	0.5 g
7	Mustard	*Sinapis alba* L.	18
8	Barley	*Hordeum sativum* L.	16

Table 1
GERMINATION TEST USING EIGHT DIFFERENT PLANT SPECIES

extensive sampling on transects located at different distances from the source of pollution, so that they can capture an eventual wind transport of pollutants, which will be measured by analyzing atmospheric deposition both on soil and on vegetation.

Soil characterization and description of experimental design

The soil in the studied area is characterized by a horizon with organic matter of 5-8 cm thick, followed by yellow-brown clay layer with calcareous concretions, extended up to a depth of 6.2 to 10.4 m. Under the clay layer, the drillings from the center section of the electrolysis emplacement have revealed a clayey and sandy dust horizon, which was reddish, plastic, vigorous-hard, 3-9 cm thick [3]. In table 1 it can be seen that the soil in the immediate vicinity of the factory is weak acid, especially in sampling point 1, and the amount of nitrogen in the form available for the plants, is very low.

Germination test

A germination test was conducted with the view of selecting plant species capable to develop in that certain type of soil. A number of 8 species of plants have been tested: corn, sunflower, wheat, lupine, phacelia, shamrock, mustard, barley (table 1).

To accomplish the test, the soil was taken from an area situated near the pollution source (fig. 1), from the depth of 5-8 cm. The incubation was made in plastic pots, with 9 h of natural light, and 8 h of artificial light. In each pot approximately 100 mL of distilled water were added daily (depending on the initial soil water capacity). Based on the results of the germination test and the mychorriza potential of the species, sunflower and rye have been taken into consideration. Even if 2 plant species have been selected after the germination test, in the experimental section for this paper there will be presented only the researches made on sunflower.

The laboratory experiment

A number of 5 different experimental variants were taken into consideration, each of them having 4 replicates: uncontaminated reference soil (R), contaminated soil 1 (C1, situated opposite the pollution source), contaminated soil inoculated with mychorriza fungi (CM1), contaminated soil 2 (C2, on the same side of the pollution source, and situated nearby it), contaminated soil inoculated with mychorriza fungi (CM2), in which sunflower was cultivated (*Helianthus annuus* L.). In all experimental cases soil was amended with 10% expanded clay, inoculated (or not) with mychorriza fungi, according to the studies [15]. The contaminated soil, before being inoculated, was autoclaved for 30 min, at 130°C, and the inoculation was made with VAM 510 (160 spores/g of soil) *Glomus intraradices*, sold under the name of Blaethon "Tunesia, Collection of Microorganisms of the Institut fur Pflanzenkrankenheiten und Pflanzenschutz", by University from Hanovra. The experiment lasted 45 days, the plants growing in a growth chamber with constant microclimate, permanently monitored. A night and day alternating regimen was established, during the night the temperature being maintained at 16°C, and during the day keeping a temperature of 22°C with the luminous intensity of 5000lx. The relative humidity throughout the experiment was 60%. The contaminated soil was collected as described in 2.1, and before starting the experiment it was manually well homogenized.

Characterisation of soil samples

On soil samples the following parameters were determined: humidity, *p*H, electrical conductivity, mineral N content ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$), phosphorus content in available form by plants ($P\text{-}PO_4^{3-}$) and the pseudo-total content of metals. In addition, after harvesting the plants, the soil obtained respiration was also determined. Therefore, the *p*H was measured in aqueous suspension (ratio m:v, soil: distilled water, 1:2.5) after stirring in advance for 15 min and then leaving it to rest for an hour in order to balance the carbon dioxide and homogenize again before the measuring, using a WTW (Germany) pH-meter with glass electrode. The electrical conductivity and soluble salt content expression consists in determining the electrical conductivity (specific electrical conductivity) of aqueous extract of soil, caused by ions present in the extract, and estimating the total content of soluble salts by multiplying the total amount of electrical conductivity by a factor which

was experimentally determined. The electrical conductivity reading was made in the same aqueous solution used to determine *p*H, using the conductivity probe from the WTW multi-parameter instrument. Soil moisture was calculated after drying samples at a temperature of 105°C until reaching constant weight. Determination of soil respiration was performed as descriebed by [21]. The pseudo-total metals content it was done using aqua regia, according to the method [8]. The determination of mineral N forms ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$) was performed in fresh soil extract in a mixture of KCl 0.2M, by using colorimetric methods, in the following way: the method for determination of $N\text{-}NO_3^-$ was described by [15], for $N\text{-}NO_4^+$ it was done by [20, 18], for $N\text{-}NO_2^-$ according to [1, 17, 13] and the phosphate ($P\text{-}PO_4^{3-}$) was extracted quantitatively from the soil through ionic exchange using $NaHCO_3$. The determination is based on using of ammonium molibdate and malachite green (1:3) to form the phospho molid ammonia which is reduced and form a green complex [1, 17,13].

Determinations carried out on plant material

After harvesting the plants they were proportioned in both underground and above ground material, roots were rapidly washed with tap water and finally with distilled water and then, all plant material was weighed (in order to determine the underground and above-ground fresh biomass). After that, a part of the plant material was freeze-dried, and ground in a very fine powder (it was used a mill made of uncontaminated material and provided with cooling), placed in sealed polyethylene bottles, and kept at -20°C until the tests were conducted. Using plant material, the following determinations were made: mychorriza's microscopic view, total protein content, lipid peroxid and metal content. In addition, in the above ground plant material was determined the content of pigments (chlorophyll a and b and carotenoids).

Microscopic view of the mychorriza formed by Glomus intraradices mychorriza fungi with roots of Helianthus annuus L.

In order to view the microscopic vesicles and arbuscles that form the structures of haifa which appear during the mychorriza process, the Lactophenol blue staining method was used to paint the roots fragments, that were previously washed with tap water and distilled water subsequently. After washing, the fragments were preserved in a solution obtained from a mixture of distilled water (45.85%), ethanol (45.85%), formaldehyde (6%) and acetic acid (2.3%). Before the microscopic view, the roots kept in fixative solution were stained in Lactophenol blue solution using the method described by [6].

Protein content determination

This was done in the extract obtained as follows: the sample (approximately 100 mg) was ground in a cold mortar for 2 min with 2 ml extraction solution (100 mM K_2HPO_4/KH_2PO_4- buffer with *p*H 7.2; 2% Polyvinyl-pyrrolidone; 2mM chelaplex III-EDTA; 2mM DTT (dithioerithritol). After grinding the sample was placed in an Eppendorf tube and centrifuged 10 min at 18000rpm. The liquid fraction obtained was then subject to dialysis against phosphate buffer made at *p*H 7.2 (5 mM K_2HPO_4/KH_2PO_4) stirring on a magnetic stirrer for 8 h at 4°C (with phosphate buffer changed every 2 h). Total protein content was determined after their trichloroacetic acid precipitation and solubilization with NaOH by Lowry method using bovine serum albumin as standard (BSA) [12].

Lipid peroxidation

Polyunsaturated fats (e.g. unsaturated fatty acids) are degraded when cells are subject to oxidative stress, forming lipid peroxides. This method involves MDA determination, resulted by the decomposition of peroxides of polyunsaturated fatty acids, using thiobarbituric acid colorimetric method [11].

Determination of pigments (chlorophylls and carotenoids)

This was done by the method described by [22], which consists in mixing the sample of plant material (aprox. 100 mg) in acetone solution (80% acetone: 15% H_2O: 5%NH_3 solution, concentration 25% v/v) with an ultraturax at 75000 rpm, followed by centrifugation at 4800 rpm for 20 min. After that, the intensity of liquid fraction color was spectrophotometrically measured at: 480, 645, 647, 652, 663, 664, 750 nm for chlorophyll a, chlorophyll b and carotenoids.

The metal content

Determination of metals in plant material was made by mass spectrometry with inductively coupled plasma (ICP-MS, Elan DRC-e, Perkin Elmer) after digestion the sample in super-pure nitric acid.

Statistical analysis

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

Table 2
CHARACTERIZATION OF THE SOIL BEFORE CARRYING OUT THE LABORATORY EXPERIMENT; H = HUMIDITY OF SOIL

Sample code	pH	Average /SD	EC µS/cm	Average /SD	H %	Average /SD	$N\text{-}NH_4^+$ µg/g d.w.	Average /SD	$N\text{-}NO_3^-$ µg/g d.w.	Average /SD	$N\text{-}NO_2^-$ µg/g d.w.	Average /SD	$P\text{-}PO_4^{3-}$ µg/g d.w.	Average /SD
C1.1	5.38	5.84/	51	35.33	11.56	14.38	5.890	6.156	1.828	3.587	0	0	35.35	30.16
C1.2	6.71	*0.60*	36	*8.164*	16.63	*1.920*	5.843	*0.229*	5.365	*1.228*	0	*0*	10.86	*13.08*
C1.3	6.50		29		16.16		6.363		3.198		0		17.82	
C1.4	5.50		33		14.25		6.333		4.543		0		32.66	
C1.5	5.44		34		14.78		6.242		3.465		0		39.69	
C1.6	5.49		29		12.94		6.266		3.124		0		44.58	
C2.1	6.80	6.63	76	54.66	19.88	15.94	6.853	6.826	1.545	4.661	0	0	97.89	50.58
C2.2	6.12	*0.27*	43	*19.32*	6.716	*5.48*	6.842	*0.244*	3.233	*3.010*	0	*0*	33.58	*30.40*
C2.3	6.87		72		14.36		6.705		9.945		0		38.56	
C2.4	6.62		43		21.32		6.597		3.442		0		11.45	
C2.5	6.72		66		13.80		7.283		3.483		0		71.09	
C2.6	6.67		28		19.60		6.679		6.323		0		50.91	

transfer coefficients (TC) from soil to plant were also calculated using the formula:

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

The transfer coefficients can be considered accumulation factors if TC <1, and concentration factors if TC>1 [2].

Results and discussions

Analyzing soil parameters before starting the experiment (table 2) and at the end (table 3), it is apparent that in the 12 samples presented in table 2, the soil *p*H is within the neutral class according to the classification [9], when taken in the vicinity of the pollution source (C2.1.-C.2.6.), and acid grade for those across the source (C1.1 – C1.6). Electrical conductivity has higher values in the top polluted area (in the vicinity of the source) and lower values across the source (table 2), the values falling in the category of soils which are not saline (0-2 mS/cm according to [19]), considering the fact that soils are contaminated with metals. An increase in salinity is observed after the completion of laboratory experiment. It can be seen that at the end of the experiment, after harvesting the plants, uninoculated C1 *p*H increases from 5.84 to 6.38, and following the inoculation, from 6.38 to 6.56, while in C2 decreases from 6.63 to 5.3, and as a result of inoculation increases from 5.3 to 6.08. Such variations occur frequently after plant growth, and are determined by chemical and physico-chemical processes that occur in soil during the vegetation period. From the data presented in table 2 results that the soil is very low in nitrogen, and has a satisfactory phosphate content that allowed inoculation with mychorriza fungi. After inoculation (due to clay content, nitrogen and fungal hyphae action), and as a result of chemical and physico-chemical processes that occur in soil, nitrogen appears to be in large amounts at the end of the experiment (table 3), growing due to ammonium ion concentration, and in a very small measure due to nitrite ion, taking into account that the nitrate was found in smaller amounts at the end of the experiment, compared with the initial phase. Thus, it appears that plants have sufficient nitrogen and phosphorus amounts in the available form, their assimilation being enhanced by fungal hyphae, knowing that fungi provide an increased intake of nutrients for the plant [7, 24]. Thus, it can be explained why after inoculation there was a slightly lower content of nitrogen and phosphorus in the available form, compared to concentrations found in the experimental variants without inoculation (table 3). Positive effect of mychorriza fungi are seen in increased microbial activity, expressed through soil respiration, ranging from 2.83 to 5.57 mg CO_2 / g d.w.× 12 h in C1 soil and from 2.06 to 5.32 mg CO_2 / g d.w.×12 h (table 3).

Inspecting the phenological parameters of sunflower (fig. 2a) we could notice a big difference between sunflower individuals height, between the three experimental variants (from right to left: reference, contaminated, contaminated and inoculated). It was also noticed that during the experiment, the plants inoculated with mychorriza fungi were more vigorous compared to the uninoculated ones. To check the symbiosis between mychorriza fungi and the sunflower roots, a small part of the roots which were prepared as described in material and methods section was viewed under a microscope, and as shown in figure 2b, vesicles were identified, but not arbuscles, knowing that arbuscles form especially in the

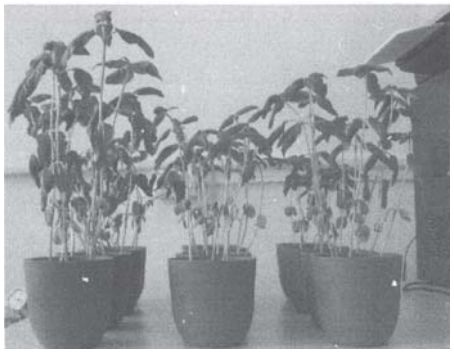

Fig.2.a The difference between phenological parameters of the individuals of sunflower (*Helianthus annuus* L.) in all the three experimental variants

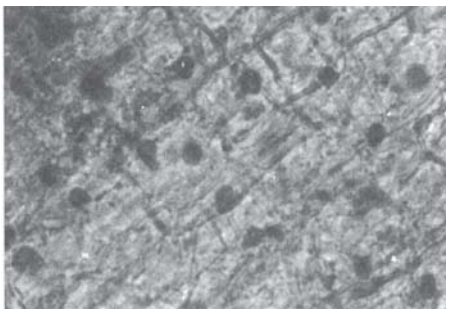

Fig. 2.b Microscopical image of the mychorriza formed as a result of the symbiosis between the mychorrizal fungi from *Glomus intraradices* species and the roots of *Helianthus annuus* L.

first stage of plant development, and in the case of this experiment, the harvesting being done after 45 days.

From table 4 we can see that the elements As, Cr, Mn and Ni determined in soil were found in concentrations above the allowable limit (values shown in bold), regarding the consumption of plants by human population, and according to [10], thus we can conclude that we are dealing with a multi-pollution. But if we compare metal concentrations before sowing with those obtained after harvesting the plants, we can see that most toxic elements at the end of the experiment show a decrease of the concentration in soil, except for As, which grew from 11.54 to 13.03 g/g in the case of C2 soil, and Cd, also in C2, which grew from 0.17 to 0.20 g/g, growth that can be interpreted in terms of soil *p*H change during plant development, which may have induced greater mobility of these elements in soil. It can also be seen in table 4 that most of the elements that work as a nutrient for plants showed a slight increase in their concentration in soil (underlined values), at the end of the experiment, and this was expected, considering that soil was homogenized with expanded clay, which brought an ample supply of items. In figure 3 we can see that in most cases, in the inoculated form in both CM1 and CM2, the concentration of the elements remained in soil is higher than the other uninoculated forms, like C1 and C2, which could be interpreted as a positive effect, because changing the availability of metals as a result of the fungi inoculation, can induce the phytostabilization phenomenon. It is compulsory to mention that the results should be interpreted with great caution as there have not been performed statistical tests yet, to confirm the above, and it is also necessary to work at a field scale, as the results obtained at laboratory scale cannot be extrapolated to field scale. Plant phenological parameters (individual height - figure 2, and biomass production - fig. 4) show the negative influence of soil pollution on plant growth and development. A slight decrease in biomass production (especially in the

Table 3
THE PARAMETERS OF THE POLLUTED SOIL AT THE END OF THE EXPERIMENT; H = HUMIDITY OF SOIL

Sample code	pH	Average / SD	EC µS/cm	Average / SD	H %	Average / SD	Soil respiration mg CO_2/ g d.w. x 12h	Average / SD	$N-NH_4^+$ µg/g d.w.	Average / SD	$N-NO_3^-$ µg/g d.w.	Average / SD	$N-NO_2^-$ µg/g d.w.	Average / SD	$P-PO_4^{3-}$ µg/g d.w.	Average / SD
R	7.16	7.042	97	102	10.86	12.00	8.977	8.593	48.35	49.67	0.471	0.719	0.258	0.089	4.00	99.39
R	7.22	0.263	107	9.128	11.73	0.940	8.063	0.575	52.94	2.478	0.879	0.208	0.020	0.117	121.2	65.83
R	7.14		92		12.37		9.194		50.13		0.905		0.000		117.1	
R	6.65		112		13.07		8.142		47.27		0.624		0.082		155.1	
C1.1	6.34	6.38	122	140.2	13.27	13.80	3.502	2.828	42.82	34.39	1.449	1.271	0.000	0.015	22.85	37.12
C1.2	6.43	0.037	160	20.56	14.16	0.443	3.284	0.660	34.73	6.140	0.509	0.856	0.041	0.019	38.60	9.828
C1.3	6.38		156		14.17		2.200		31.30		0.722		0.000		44.52	
C1.4	6.37		123		13.61		2.327		28.71		2.405		0.020		42.54	
CM1.1	6.47	6.557	132	120	12.65	12.31	6.788	5.572	25.41	26.82	0.159	0.646	0.000	0.035	29.88	33.04
CM1.2	6.57	0.074	117	8.831	11.72	1.748	6.690	1.351	27.83	1.079	0.476	0.484	0.000	0.059	28.73	4.811
CM1.3	6.54		120		14.53		4.280		27.48		1.308		0.124		34.25	
CM1.4	6.65		111		10.37		4.533		26.57		0.642		0.020		39.32	
C2.1	5.31	5.3	79	85	15.82	15.68	2.673	2.059	35.97	32.02	2.620	2.756	0.127	0.036	30.48	28.41
C2.2	5.27	0.021	82	7.527	16.38	1.654	1.881	0.426	28.60	3.688	2.682	0.266	0.021	0.060	24.32	6.442
C2.3	5.3		96		17.18		1.989		34.35		3.151		0.000		36.56	
C2.4	5.32		83		13.35		1.696		29.17		2.573		0.000		22.32	
CM2.1	5.48	6.075	88	98	10.37	11.76	5.570	5.318	31.43	27.63	1.290	0.772	0.000	0.010	27.47	28.09
CM2.2	6.15	0.406	94	9.380	13.20	1.203	5.231	0.407	28.03	2.843	0.669	0.386	0.000	0.020	35.65	5.479
CM2.3	6.35		110		11.33		4.782		24.86		0.361		0.000		26.69	
CM2.4	6.32		100		12.16		5.693		26.23		0.771		0.040		22.55	

Element (µg/g)	$C_{1,i}$ (7-12) Average/*SD*	$C_{1,f}$ (7-12) Average/*SD*	$C_{2,i}$ (1-6) Average/*SD*	$C_{2,f}$ (1-6) Average/*SD*	The accepted level in the soil for the plants which can be used by human *
As	**13.77**/ *0.42*	**12.73**/*2.46*	**11.54**/ *4.69*	**13.03**/ *0.75*	2
Ca	24670/*334.1*	2704/ *427.1*	2666/ *188.2*	2571/ *463.9*	-
Cd	0.18/ *0.11*	0.16/ *0.09*	0.17/ *0.05*	0.20/ *0.08*	2
Co	11.78/ *1.45*	11.57/ *1.46*	20.46/ *8.64*	10.52/ *2.94*	10-75
Cr	**130.9**/ *12.14*	**101.5**/ *13.64*	**116.7**/ *21.40*	**106.5**/ *2.69*	50-100
Cu	16.90/ *1.38*	16.68/ *0.76*	18.55/ *6.11*	17.50/ *2.98*	30-100
Mn	**841.3**/ *71.55*	**864.3**/ *89.64*	**1357**/ *597.1*	**717.2**/ *137.7*	270-525
Na	555.9/ *141.9*	598.2/ *123.1*	526.0/ *169.8*	663.2/ *104.2*	-
Ni	**72.67**/ *46.96*	**57.84**/ *8.48*	**51.9**/ *22.35*	**50.17**/ *3.69*	35
Pb	22.89/ *0.82*	19.21/ *2.72*	22.77/ *3.25*	19.99/ *3.69*	2-60
U	0.31/ *0.06*	0.18/ *0.04*	0.54/ *0.57*	0.21/ *0.11*	-
V	88.29/ *2.78*	85.92/ *0.04*	102.0/ *33.26*	86.20/ *0.84*	18-115
Zn	76.65/ *2.29*	80.01/ *2.30*	73.50/ *7.31*	88.54/ *2.07*	17-125

Table 4
THE DISTRIBUTION OF THE ELEMENTS IN THE SOIL SAMPLES WHICH WERE SAMPLED BEFORE (C_i) AND AT THE END OF THE EXPERIMENT (C_f)

**source:* [10]

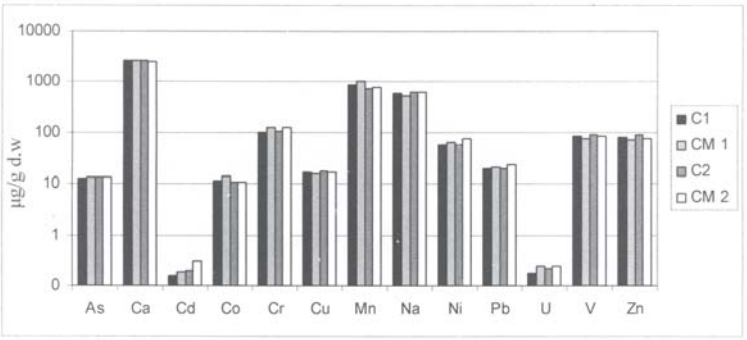

Fig. 3. The distribution of the elements in soil in the both versions: with the inoculation of mychorrizal fungi and without it

Element	The transfer coefficients (TC) of the elements from soil to plant				
	R	C1	CM1	C2	CM2
As	0.481 /acumulation	0.174 /acumulation	0.179 /acumulation	0.454 /acumulation	0.139 /acumulation
Co	4.646 /concentration	5.351 /concentration	1.863 /concentration	2.958 /concentration	3.032 /concentration
Cr	0.136 /acumulation	0.228 /acumulation	0.217 /acumulation	0.309 /acumulation	0.271 /acumulation
Cu	2.326 /concentration	4.026 /concentration	4.475 /concentration	2.822 /concentration	3.581 /concentration
Mn	0.547 /concentration	1.334 /concentration	0.742 /acumulation	2.296 /concentration	1.4 /concentration
Ni	0.783 /concentration	1.239 /concentration	1.083 /concentration	2.381 /concentration	1.044 /concentration
Pb	0.465 /acumulation	1.672 /concentration	1.071 /concentration	1.235 /concentration	0.997 /acumulation
U	2.594 / SDL*	23.1 /concentration	14.06 /concentration	16.2 /concentration	14.02 /concentration
V	0.133 /acumulation	0.123 /acumulation	0.164 /acumulation	0.094 /acumulation	0.209 /acumulation
Zn	2.346 /concentration	15.84 /concentration	21.98 /concentration	5.307 /concentration	24.17 /concentration

Table 5
THE TRANSFER COEFFICIENTS FROM SOIL TO PLANT FOR THE ELEMENTS WITH A HIGH LEVEL OF TOXICITY

* SDL = sub-detection limit

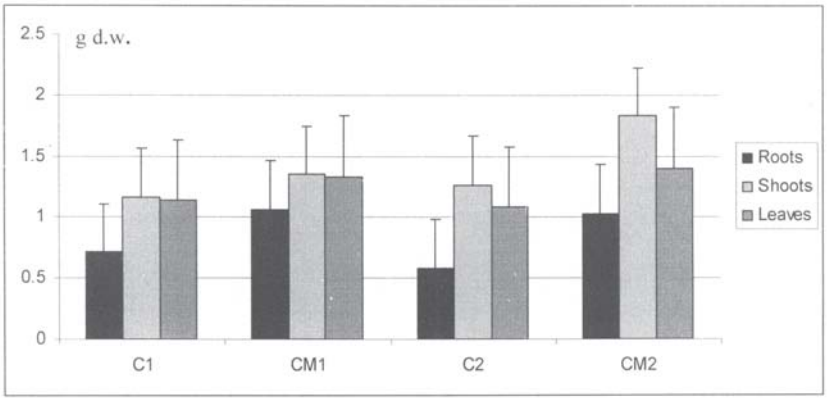

Fig. 4. The variation of plants biomass as an effect of soil inoculation with mychorrizal fungi

Table 6
THE PARAMETERS DETERMINED FROM THE VEGETABLE MATERIAL (PROTEIN CONTENT, LIPID PEROXIDATION (L.P.) AND PIGMENTS (CHLOROPHYLL a AND b, CAROTENOIDS)

Sample code	Proteins roots µg/g d.w.	Average / *SD*	Proteins leaves µg/g d.w.	Average / *SD*	Protein stems µg/g d.w.	Average / *SD*	Chl. *a* µg/g d.w.	Average / *SD*	Chl. *b* µg/g d.w.	Average / *SD*	Car. leaves µg/g d.w.	Average / SD	L.P. µmolTBA rm/g d.w.	Average / *SD*
R	107.2	111.6	6030	6270	2586	2534	13.07	13.3	3.409	4.9	0.456	0.4	0.451	0.5
R	108.1	*/ 4.5*	5998	*/ 357.2*	2544	*/ 72.7*	12.91	*/ 0.5*	2.919	*/ 2.2*	0.435	*/ 0.1*	0.461	*/ 0.1*
R	115.2		6281		2578		14.01		7.759		0.507		0.407	
R	115.9		6771		2429		13.13		5.434		0.336		0.535	
C1.1	21.72	26.2	2191	2329	1690	1450	6.631	6.4	5.050	4.9	0.382	0.4	0.488	0.5
C1.2	27.08	*/ 3.3*	2237	*/ 186.8*	1309	*/ 226.0*	6.335	*/ 0.4*	4.671	*/ 0.2*	0.393	*/ 0.0*	0.517	*/ 0.0*
C1.3	26.48		2285		1589		5.895		5.004		0.384		0.470	
C1.4	29.54		2603		1212		6.677		4.974		0.362		0.542	
CM1.1	35.30	36.4	2926	2703	2207	2487	9.797	9.3	4.581	5.594	0.477	0.5	0.666	0.6
CM1.2	39.53	*/ 2.5*	3022	*/ 327.8*	3504	*/ 692.7*	8.925	*/ 0.4*	5.994	*/ 0.4*	0.465	*/ 0.0*	0.653	*/ 0.0*
CM1.3	37.02		2320		1951		9.364		4.839		0.427		0.665	
CM1.4	33.73		2545		2289		9.082		6.561		0.454		0.586	
C2.1	44.38	38.5	944	999.5	1273	1419	7.078	6.8	1.372	1.5	0.154	0.1	0.539	0.5
C2.2	46.16	*/ 7.9*	946	*/ 83.2*	1265	*/ 178.2*	6.591	*/ 0.3*	1.536	*/ 0.1*	0.142	*/ 0.0*	0.542	*/ 0.0*
C2.3	30.67		987		1518		7.086		1.382		0.122		0.542	
C2.4	32.91		1121		1621		6.542		1.539		0.174		0.544	
CM2.1	51.97	52.4	895	1626	2384	1810	10.41	10.5	3.448	3.6	0.424	0.4	0.698	0.7
CM2.2	52.58	*/ 4.3*	1860	*/ 505.9*	1646	*/ 383.3*	9.96	*/ 0.5*	4.137	*/ 0.7*	0.369	*/ 0.0*	0.657	*/ 0.0*
CM2.3	57.77		2039		1595		11.06		4.244		0.464		0.726	
CM2.4	47.26		1710		1616		10.75		2.654		0.419		0.694	

ground) and height of individuals can be noticed, associated with a decrease in protein and pigment content (table 6) for plants grown on contaminated soil (C1 and C2). Increased oxidative stress, expressed by increased content of MDA derived from lipid peroxidation, in plants grown on contaminated soil, leads to higher consumption of carbohydrates, which causes a decrease in protein content. On the other hand, increasing the concentration of oxygen free radicals causes major disruption of plant physiological processes: photosynthesis, chlorophyll pigment synthesis, respiration, assimilation and foliar radiculitis, etc. The plants grown on inoculated soil (CM1, CM2) shows a significant decrease in oxidative stress, especially in the biomass above ground, probably generated by the reduction of metal content. We can assume that the oxidative stress in this experiment, expressed only by non-enzymatic parameters, whose values can be seen in table 6, decreased as a result of the positive effect of forming mychorriza, and also causing a significant increase in protein and pigment content.

It can be seen in table 5 the transfer factors values of elements in total biomass obtained for each experimental variant. The underlined values indicate a lower transfer factor in the plants grown on inoculated soil, like CM1 and CM2 variants. These values confirm the higher concentrations of elements remaining in the soil after harvesting the plants, shown in figure 3. Note that many elements show a concentration factor (TC > 1), even at highly toxic elements, such as, Ni, Pb, or U.

Conclusions

After conducting the study presented, one can conclude that the studied area is characterized by a multi-pollution that could affect primary productivity and consequently animal and human populations. This paper can highlight the beneficial role of polluted soil inoculation with mycorrhiza fungi, especially by increasing biomass production and reducing oxidative stress. It is not insignificant that in the experimental variants in which fungi were inoculated, a larger amount of metals in the soil remained, which can lead to the conclusion that plants grown with this inoculum are less toxic and, pollutant translocation on the Food chain might not be a problem, although the pollutant can migrate to groundwater. We consider it necessary to emphasize that, in the inoculated variants a larger quantity of metal at the root has been stored, compared with the uninoculated variants, which can lead to lower long-term migration of metals to groundwater.

Regarding the third objective proposed, concerning the optimal solutions for remediation of the polluted area, although the experiment carried out has clearly demonstrated the beneficial effect of mycoremediation, we believe it is premature to take such decisions, because it has been worked only on laboratory scale. Based on the success of this experiment, we believe it is necessary firstly to expand the study area, taking in work also sampling points located far from the pollution source, and on the other hand to pass on a higher working scale, knowing that such an experiment gives us information only on the

type of corrective procedure that deserves to be applied on a larger scale, respectively the scale of experimental plots, and only then to be able to make a final recommendation regarding the procedure of remediation of the entire polluted areas.

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