

THE HEAVY METALS IMMOBILIZATION IN POLLUTED SOILS FROM ROMANIA BY THE NATURAL ZEOLITES USE

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Abstract. The effect of heavy metals' immobilization with natural zeolitic tuffs and with organo-zeolitic material in soils polluted by Pb, Zn, Cu and Cd affected by the nonferrous metallurgy industry it has been studied in an experiment with *Lolium perenne* in laboratory and greenhouse conditions. Surface soil samples (0-20 cm) of 100 kg each (as global sample) from four different types of soils in vicinity of Pb and Cu smelters in Baia Mare locality and Cu smelter in Zlatna locality were used in the experiment. The concentration of Pb, Zn, Cu and Cd in those four types of soils: (eutricambosol, BMR-O and luvosol, BMC-O in Baia Mare; dystricambosol, ZT-O and anthrosol-Z2T-O, in Zlatna) exceeds Romanian references limits. Within the experiment, from each global sample there have been realized two experimental options: first (I) treated experimental option of soil (polluted soil + zeolitic tuffs); second (II) treated experimental option of soil (polluted soil + organo-zeolitic material) in proportion of 82%-18%. Each experimental option of treatment was replicated three times. The mixtures of the two experimental options have been used to test the amendments effect for the plants growing, in comparison to the untreated soil (original soil). The experiment for the treatment of polluted soils, with natural zeolitic tuffs and with organo-zeolitic material was realized in pots of 6.5 kg, using as plant *Lolium perenne*. The experiment took place on a period of one year and six months. Natural zeolitic tuff was added as crushed with 2 mm in size, and also as an organo-zeolitic material (crushed tuff mixed with poultry manure). The zeolites used for the treatment of soils are characterized by high cation exchange capacity, high content of clinoptilolite >90%. Exchanged cations are represented by (Ca ± Mg, K and Na). The organo-zeolitic material determined the increase of pH from the untreated soil to the first and second treated experimental option of soil in all four types of soil used in the experiment. The growth of humus content is significant in the second treated experimental option of soil by passing from high content class to very high content over 5% for ZT-II sample and from low content class to moderate content for BMR-II and Z2T-II, with maintaining of the high content class, over 4%, for BMC-II sample. Both amendments have increased the cation exchange capacity of 2.54 times compared with the untreated soil. From the analysis of heavy metals concentrations from plants in three harvests, it has been demonstrated that Pb and Cd have reduced their contents in the treatment experimental options, which is associated with a bigger affinity of the zeolites structure for these two metals. In comparison with the aerial part of the plants, the roots extracted higher concentration of Pb and Cd. The concentration of heavy metal in roots is higher in the untreated soil than in both treated soil experimental options. Instead, Cu and Zn present higher contents in the aerial part, which would be a proof of selective accumulation of these metals, being used in the process of growing. The second experimental option of treatment for all soils ensured the conditions for the biomass growing and the roots development. In the EDX spectrum of the zeolitic tuff from the second treated experimental option of soil, there were obtained distinct peaks for Pb, Cu and Zn in the appropriate zones of Na, Ca and K peaks. The X-ray diffraction spectrum obtained on the zeolitic tuffs particles from the second treated soil experimental option showed the structural parameters change of the clinoptilolite. The reticular distance (*d*) from 8.9447 of the initial zeolitic tuff sample to 9.0916 of the zeolitic tuff used in the second treated experimental option of soil (BMR-II sample) demonstrated that the clinoptilolite cations were changed by Pb. These results can be considered a solution to reduce heavy metal uptake in plants due to the amendments based on the natural zeolites.

Keywords: soil pollution, heavy metal immobilization, X-ray diffraction, EDX, natural zeolite

1. INTRODUCTION

The soil pollution with heavy metal is associated with the mining and metallurgical industry from Baia Mare and Zlatna, two cities from Romania (Fig. 1). Heavy metals soil pollution occupies large areas in residential zones and around the cities, (Fig. 2, Fig. 3).

Heavy metal pollution affects the ability of soil to sustain the development of plants (Logan et al., 1997, Lai & Chen, 2004) and has negative effects on the activities of microorganisms in the nitrogen fixation (Smith, 1997). The effects of the heavy metals pollution of soil and plants from the affected areas in Romania were studied by Lăcătușu et al., 2001, 2008, Lăcătușu & Lăcătușu, 2008, Damian et al., 2010, Suciuc et al., 2008. Donisa et al., (2000) studied the heavy metals distribution within the soil profiles at a specific distance from the pollution sources in Baia Mare area. The heavy metals' behaviour, Pb, Cu, Zn, Cd, in the soil profile, nearby the smelters (Baia Mare-Romplumb, Baia Mare-Cuprom, and Zlatna) was realized by Damian et al., 2008a, Damian et al., 2008b.

Heavy metals bio-accessibility can be estimated related to the soil properties (Stewart et al., 2003, Poggio et al., 2009). The attempts to immobilize heavy metals in soil samples excessively contaminated with heavy metals in Baia Mare and Zlatna showed the efficiency of the treatment with natural zeolites and lime (Damian & Damian, 2007). By adding zeolites in soil, the pH growth is influenced (Lin et al., 1998). The negative effect of soil contamination with Cd on plants was reduced by

addition of zeolites (Eshghi et al., 2010). Application of zeolites has been demonstrated as an effective method of Pb immobilization in soil (Ponizovsky & Tsadilas, 2003). The reduction of Cd mobility in depth soil horizons was showed by using the zeolites (Mahabadi et al., 2007).

Many studies presented the effect of heavy metals immobilization in polluted soils of: limestone, (Tlustoš et al., 2006), phosphates, (McGowen 2001), lime, phosphate and compost (Padmavathiamma & Li, 2009a), ash and organic compost, (Nachtegaal et al., 2005), clay materials, (Zhang et al., 2011). Zinc sequestration in smectite structure was demonstrated by Vespa et al., (2010). The effects of the coal fly ash in reduce the plant available for heavy metals were studied by Sitarz-Palczak & Kalembkiewicz (2012).



Figure 1. Map of Romania with location of Baia Mare and Zlatna.



Figure 2. The vegetation development on luvisol nearby the copper smelter in Baia Mare.

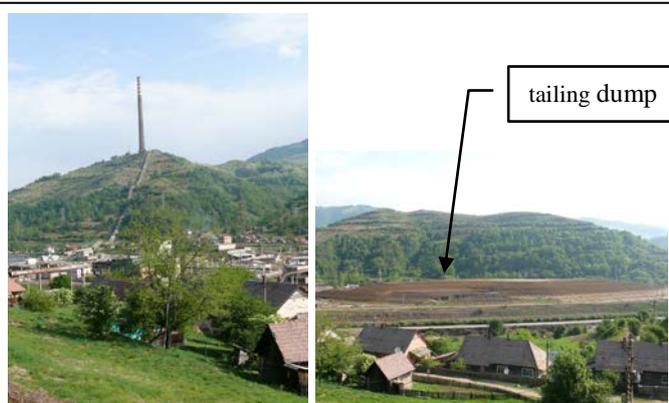


Figure 3. Dispersion furnace of gaseous emissions from copper smelter Zlatna (left); tailing dump in Zlatna city (right).

The cationic exchange of zeolites for lead was demonstrated by Gunter et al., (1994) and for NH_4^+ , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} by Langella et al., (2000). The structural characteristics and the chemical properties of zeolites are used in the soil remediation techniques (Gadepalle et al., 2007, Mumpton, 1999). Brannvall (2006) showed in an experimental study that the order for the heavy metals immobilization in soil is: $\text{Pb}^{2+} > \text{Zn}^{2+} > \text{Cu}^{2+}$, using rich in clinoptilolite natural zeolite from Ukrainian Transcarpathian region. The high cation exchange capacity of the clinoptilolite explains its affinity for the ammonium ion, (Hedström, 2001). The zeolite effects for soil microbial properties were verified by Mühlbachová & Šimon (2003) and relationships between availability of Cd, Pb and Zn with soil pH and microbial biomass in soil treated with natural clinoptilolite were evaluated by Mühlbachová et al., (2005). Peter et al., (2012) have demonstrated decreases of heavy metals accumulation in roots and leaves of *Hieracium piselloides* grown on a substrate treated with zeolites enrichment with ammonium ion.

2. MATERIALS AND METHODS

The soils used in this experiment were sampled from four sites of two areas, in Romania: Baia Mare and Zlatna localities. In Baia Mare, (Fig. 2) the soils were affected by the heavy metals pollution due to copper and lead smelters. The smelter from Zlatna (Fig. 3) produced the copper.

2.1. Areas selection and soil sampling

In Baia Mare, the soil was collected as two global samples: **BMR-O** (Baia Mare Romplumb Original), in the north-east part of the town Baia Mare, near the Romplumb lead smelter and **BMC-O** near the Cuprom copper smelter, (Baia Mare Cuprom Original). The soil type for sample (**BMR-O**) is eutricambosol and for sample **BMC-O** is luvosol. In Zlatna area, the soils from the global sample were collected from two sites: **ZT-O** (Zlatna Total-Original) (the northern slope of the Zlatna depression, represented by the dystricambosol), and **Z2T-O** (Zlatna 2Total-Original), represented by anthrosol (IUSS-International Union of Soil Science 2006) in the former of the copper metallurgical smelter location. The global samples from those four sites were sampled from the top horizon, 0-20 cm depth. Each of the soil global sample weighted 100 kg. After homogenization, of each sample were separated 300 g of soil, which were analyzed for the physical and chemical properties and the heavy metal content.

2.2. Volcanic Zeolitic tuffs

The rich in clinoptilolite zeolites source used in this experiment has been obtained from the natural deposits of volcanic zeolitic tuffs of Badenian age from the Northern-Western part of Romania, (Bârsana-Maramureş Basin) (Cochemé et al., 2003, Damian et al., 2007). The occurrence of the volcanic tuffs is represented by compact rocks with compositional homogeneity: vitroclasts, crystalloclasts and few lithoclasts, in a cineritic matrix. Mineralogical composition and the clinoptilolite content of the zeolitic tuffs samples were determined by electron microprobe, infrared and X-ray diffraction analysis. The volcanic glass, with a participation of over 90% includes crystalloclasts and lithoclasts, of which participation is under 10%. The volcanic tuffs used in the experiment contained > 90% clinoptilolite and a high cation exchange capacity: 40.34meq/g for Ca^{2+} , 27.78meq/g for K^+ and 69.52meq/g for Na, (Damian & Damian 2007). There have been selected the zeolitic tuffs with high content in K and Ca and great selection for the ammonium ion (NH_4^+), (Cocheme et al., 2003).

Vitroclasts are replaced with zeolites. The crystalloclasts are represented by plagioclase feldspar, sanidine, quartz, mica (biotite and muscovite). There are the following accessory minerals: leucoxen, rutile, apatite, zircon and sporadic orthite. Lithoclasts are represented by fragments of quartzite, eruptive rocks with pilotaxitic texture. Mainly, the diagenetic products are represented by zeolites through clinoptilolite, heulandite, and mordenite, associated with smectites (montmorillonite), hidromice and seladonite.

The average contents of the chemical composition of zeolites of the volcanic tuffs from Bârsana area determined by Cochemé et al., (2003) are represented by 67.86%- SiO_2 , 12.16%- Al_2O_3 , 0.10%- FeO , 0.12%- Fe_2O_3 , 0.24%- MgO , 2.99%- CaO , 0.98%- Na_2O , 2.51%- K_2O , 0.009%- TiO_2 , 0.01%- Cr_2O_3 .

2.3. The characteristics of the organo-zeolitic material

The organo-zeolitic material is a mixture of crushed volcanic zeolitic tuffs, with 2 mm in size and poultry waste. The organo-zeolitic material used in the experiment has been obtained after the method patented by Leggo (2004). The organo-zeolitic material composition used in our experiment contents: $\text{C}_{\text{org.}}$ = 5.01%, humus = 8.64%, pH = 9.17, N_t = 0.989%, C/N = 5.9, P = 4 968 mg kg^{-1} , K = 10046 mg kg^{-1} .

2.4. Experiments in the pots

From each global sample, 6.5 kg of soil have been separated in pots, without zeolites. These samples were used in the experiment as untreated soil. From each global sample there have been realized two experimental options of treatment: first experimental option (I) - polluted soil + zeolitic tuffs; second experimental option (II) - polluted soil + organo-zeolitic material, in proportion of 82% -18%.

Each experimental option of treatment was replicated three times. Thus, there have been obtained 24 pots with the same quantity of material as the original samples, (4 untreated soils with original polluted soil). The mixtures of the two experimental options have been used to test the amendments effect for the plants growing in comparison to the untreated soil. The mixture has been realized in solid state and was kept in laboratory for 4 weeks, being watered daily to ensure the humidity in proportion of 70%, from water soil capacity in pot. Conservation of the soil-amendment mixture in humid state has been realized with distilled water, with the purpose to equilibrate the pH and to realize the ionic exchange. All soil samples were sown with 50 seeds of *Lolium perenne*.

The germination and growth of *Lolium perenne* on untreated soil (original soil) were studied for one year and six months, in comparison to the soil treatment experimental options. The period of seeds germination and of plants growth up to 7-10 cm was studied in laboratory. The growth stages up to maturity were produced in greenhouses.

2.5. Analyses methods for soil, plant use in experiment

Soil samples before and after experiment have been analysed for the physical-chemical properties: pH, humus content, cation exchange capacity, C_{org} , base saturation, content of nutritive elements, C/N ratio, texture type. Determination of pH has been accomplished in watery suspension in report with the soil: water of 1:2.5. The sum of basic (SB) exchange cations has been achieved by the extraction with HCl 0.05 n, after Kappen method (me/100 g soil). The hydrolytic acidity (Ah) has been determined at equilibrium in solution of acetate of sodium 1 n, the soil report: solution of 1:2.5; by titration with NaOH, in presence of phenolphthalein of extracted acidity (me/100 g soil). The total cation exchange capacity (CEC) has been obtained by calculation: $CEC = SB + Ah$ (me/100 g soil). The humus volumetrically has been determined used through the wet oxidation method (Walkley-Black, 1934). The amount of total nitrogen has been

obtained by Kjeldahl method. Phosphorous and potassium soluble has been determined in solution of ammonium acetate lactate of at pH = 3.7.

Particle size distribution has been determined by sieving and sedimentation. Determination of the content of clay and silt has been effectuated by sedimentation with the Kubien pipette method.

As a prerequisite for heavy metals analyses, sample collecting and handling was carried out at all time without using metal instruments in order to prevent contamination.

Plant tissue was oven dried for 5 days at temperatures less than 60°C. After drying, plant tissue was finely ground using a non-contaminant mill (Planetary Micro Mill PULVERISETTE) with agate jar and grinding balls for two minutes at 600 rpm. If after grinding the samples were not sufficiently fine, this operation was repeated, until a fine powder was obtained.

After this initial treatment, samples were mineralized using ultrapure nitric acid in a Microwave-based Sample Digestion Unit „Multiwave“(Anton Paar). Approximately 0.2000 g of sample was weighed out in the reaction vessel (made of Teflon) and 5 ml of ultrapure nitric acid 65% were added. The vessels were allowed to react for approximately one minute prior to sealing the vessels. Samples were sealed, placed in the Multiwave, heated to 180°C and maintained at this temperature for 20 minutes to assure complete decomposition of sample matrices.

After cooling, the samples were filtered through MF-Millipore Membrane (mixed cellulose esters, Hydrophilic, 0.45 μ m pore diameter) and completed to a final volume of 25 ml with 2% (v/v) ultrapure nitric acid solution. The final solutions were stored for analysis in HDPE (High Density Polyethylene) bottles.

Soil samples were treated similarly before mineralization, but due to their complex mineral matrix, acidic digestion required two stages. First, approximately 0.5000 g of oven dried and ground soil sample (with SiO₂ jar and milling balls) were weighted in the reaction vessel and 4 ml of ultrapure hydrofluoric acid 40% were added. In this case the vessels were heated to 250°C for 30 minutes and after cooling, in each vessel 5 ml of ultrapure nitric acid 65% were added. The second stage is the same as the plant material digestion.

Heavy metal analyses were performed using both flame and graphite atomic absorption spectrophotometers (Perkin Elmer AAS 300 and AAS 600).

EDX spectrums for a microscopic zone of the zeolitic tuff particle after mixing with polluted soil

in the second soil treated experimental option were obtained using Scanning Electron Microscope (SEM) type JSM-5600 LV (JEOL Company) equipped with EDX spectrometer (Oxford Instruments) For the zeolitic tuffs samples before and after experiment were performed with the X-ray diffraction analyses using a Philips-Muller Diffractometer with PW 1050/25 goniometer and copper tube PW 1043/01 and the speed of registration of 1 degree per minute.

3. RESULTS

3.1. Soil properties and the heavy metals content before treatment

The soil types from the areas used in the experiment have been identified by the soil profiles (Damian et al., 2008a, Damian et al., 2008b). The soils used in the experiment are affected by different levels of heavy metals pollution from strong to moderate, caused by the gaseous emission from the cooper and lead smelters from the both towns. All the soil types presented high concentration levels for each area (Table 1). The soils from the two areas are characterized by an acid pH and a low content in humus and nutritive elements (Table 1).

The contents of heavy metals from the analyzed soils have been compared with maximum allowable limits for sensitive soils according to Romanian normative (Order 756/1997), (Table 1).

3.2. The effect of the treatment with zeolitic tuffs and organo-zeolitic material on the soil properties

Addition of amendments to soils modified the main soil properties, ensuring favourable conditions for plant growing (Table 2).

The changes in pH The mixture of soils with zeolites and organo-zeolitic material determined the pH increase in both experimental options of treatment. The pH in the untreated soil has an acid reaction and grows to weak-acid reaction, neutral and alkaline until the end of the experiment (between 5 and 8, favourable for plant growth) (Table 2). The increasing of pH in the treated soil is going to ensure the optimal conditions for plants development and at the same time will reduce the mobility of heavy metals from the soil solution, (Cárcamo et al., 2011). Therefore, by increasing the pH through reduction of H⁺ ions, their competition decreases for specific places of absorption of soil's components (the oxide phases of Fe and Mn, the loamy fractions and organic ones (Siegel 2002).

Gray et al., (2006) demonstrated that the pH increase determines an increase of the negative charge of colloids in soils (clays, organic matter and Fe and Al oxides), and this represents a possible mechanism for the heavy metals immobilization. Lombi et al., (2003) showed that the effect of immobilization of heavy metals in soil by increase of pH under the influence of amendments was not assured for a long time in remediation treatments.

Table 1. The main properties and the heavy metal content of the soils used in the experiment before treatment

Properties/Sample	BMR-O	BMC-O	ZT-O	Z2T-O	MAL
	Eutricambosol	Luvosol	Dystricambosol	Anthrosol	Order 756/1997
pH	4.24	4.20	3.49	7.64	
CEC me/100gsol	29.63	18.29	21.61	-	
Humus (%)	3.90	1.20	4.38	1.44	
Base saturation (%)	52.6	38.7	5.95	-	
C _{org}	2.262	0.696	2.54	0.83	
N (%)	0.176	0.056	0.180	0.050	
C/N	15.0	14.5	16.5	19.5	
P (mg·kg ⁻¹)	25.9	6.8	34.1	17.4	
K (mg·kg ⁻¹)	58	143	39	115	
Sand (%)	45.0	31.0	65.61	70.4	
Silt (%)	27.8	40.6	16.4	11.5	
Clay (%)	27.2	28.4	18.05	18.1	
Pb (mg·kg ⁻¹)	4330	1429	995	980	100
Cu (mg·kg ⁻¹)	50.2	286	99.7	1165	100
Zn (mg·kg ⁻¹)	377	264	127	1377	300
Cd (mg·kg ⁻¹)	27.27	4.32	0.50	5.05	3

BMR-O (Baia Mare Romplumb Original), BMC-O (Baia Mare Cuprom Original), ZT-O (Zlatna Total Original), Z2T-O (Zlatna 2Total Original): Original=untreated soil samples), MAL- maximum allowable limit.

Table 2. Nutrient content, physical and chemical properties of the soils samples used in the experiment before and after plants' growth

Soil sample	Soil type	pH _i	pH _f	Humus _i	Humus _f	Cation Exchange capacity _i	Cation Exchange capacity _f	C _{org} _i	C _{org} _f	Total Nitrogen _i	Total Nitrogen _f	C/N _i	C/N _f	P mobile	P mobile	K mobile	K mobile
														i	f	i	f
				%		me/100gsol		(%)				mg·kg ⁻¹					
BMR-O	Eutricambosol	4.25	4.47	1.20	1.56	29.63	29.06	0.696	0.905	0.056	0.075	14.5	11.7	6.8	9	143	130
BMR-I		5.26	5.54	1.14	1.26	31.44	29.28	0.66	0.731	0.102	0.061	7.6	9.5	9.6	7	1480	1198
BMR-II		6.45	7.01	2.34	2.88	51.71	carbonates grains	1.36	1.671	0.200	0.139	7.9	15.9	1262	802	3960	1471
BMC-O	Luvosol	4.20	4.50	3.90	3.84	18.29	21.50	2.262	2.227	0.176	0.188	15.0 0	16.8	25.9	20	58	78
BMC-I		5.12	6.02	3.24	3.78	25.53	25,25	1.88	2.193	0.158	0.176	13.9	17.6	30.5	12	1471	1181
BMC-II		6.43	7.43	4.38	4.44	46.53	carbonates grains	2.54	2.575	0.272	0.236	10.9	16.0	1398	814	3960	1584
ZT-O	Dystricambosol	3.49	3.69	4.38	4.38	21.61	20.02	2.541	2.541	0.180	0.184	16.5	20.1	34.1	32	39	69
ZT-I		5.22	5.16	2.76	4.14	25.10	23.09	1.60	2.401	0.134	0.156	13.9	22.1	28.3	17	1586	1198
ZT-II		6.66	7.47	5.16	4.98	52.31	carbonates grains	2.99	2.889	0.294	0.214	11.9	20.1	1816	856	4714	1447
Z2T-O	Anthrosol	7.63	7.51	1.44	1.32	-	carbonates grains	0.835	0.766	0.050	0.051	19.5	12.5	17.4	27	115	107
Z2T-I		7.86	8.90	1.14	1.08		carbonates grains	0.66	0.626	0.122	0.042	6.3	9.7	29.2	15	3507	1311
Z2T-II		8.50	7.88	2.82	2.82		carbonates grains	1.64	1.636	0.188	0.152	10.1	14.1	1442	1056	4639	3684

before (i-initial) and after plants' growth (f-final); (BMR-O, BMC-O, ZT-O, Z2T-O – See explanations in table 1 for Original-untreated soil samples), BMR-I, BMC-I, ZT-I, Z2T-I: first treated experimental option of soil (polluted soil + zeolitic tuffs); BMR-II, BMC-II, ZT-II, Z2T-II: the second treated experimental option of soil (polluted soil + organo-zeolitic material).

The humus content, in comparison with the original one, has been lower in all treated soils in the first experimental option with zeolites and increased with 0,48% to 1,38% in the second experimental option of treatment (with organo-zeolitic material) (Table 2). The decrease of the humus content in the first experimental option of treatment does not modify the content category. Instead, the growth of humus content is significant for the second experimental option of treatment, determining the transition from the high to the very high content category. Humus content grew in second experimental option of treatment and maintained itself at high values in the final (f) stage of the experiment, after the plants growth (Table 2).

The capacity of cationic exchange (CEC) has an influence on the retention processes of metallic contaminants (Picardal & Cooper, 2005). Adding the zeolites and organo-zeolitic material in the studied soil samples determined the increase of cationic exchange capacity from the untreated soil of 1.06 to 1.35 times for the first treated soil experimental option and of 1.74 to 2.54 times for the treated soil in the second experimental option (Table 2).

The content in nitrogen (N) decreased in the first treated experimental option towards the original sample but did not significantly modify the class content. Instead, towards the untreated soil sample, in the second experimental option, the class content has been modified from very low to medium in BMR sample, from the medium class content to high class content in BMC sample (Table 2). In the soil from Zlatna, the class content has been modified from medium to high class content in ZT-O sample, from very low to medium class content for the anthrosol, sample (Z2T-O) (Table 2). Nitrogen presents different contents between the initial and the final stages by decreasing in the both treated soil experimental options, where the plants growth has been greater during the experiment. The source of high nitrogen content in soil sample amended with organo-zeolites is due to the high selectivity of clinoptilolite for the ammonium ion from the manure waste (Leggo & Ledésert, 2009, Leggo et al., 2010).

The phosphor presents significant concentrations in the second treated experimental option of soil, amended with organo-zeolitic material, in all samples, maintaining its high value to the end of the experiments, after harvesting the plants (Table 2).

The content in mobile K of the all soil samples grows in both experimental options of treatment. The high content of the first experimental option is due to K^+ ions, released from the zeolites structure, and in the second experimental option the higher concentration is due to the organic material

from the organo-zeolitic mixture (Table 2). Potassium has been consumed in the developed plants, during the experiment, in the two experimental options of treatment. The potassium content was maintained at a comparable level at the end of the experiment, in the first experimental option (Table 2). In the second experimental option of soils treatment, the plants generated a high quantity of biomass (Fig. 5), which is associated with the reducing of the K content during the final stage of the plants development (Table 2). The high content of mobile K of soil samples, after the treatment in both of the experimental options, confirms the efficiency of the rich in clinoptilolite zeolitic material and of the organic material mixture. The potassium from zeolites is replaced by the ammonium ion from the organic material, being easily soluble in water, becoming therefore available for plants.

3.3. The concentration of heavy metals in soil sample before and after plants growth

The plants grown on the organo-zeolitic-polluted substrate system was studied by Leggo (2000), Leggo & Ledésert, (2001), Leggo et al., (2006).

The heavy metal concentrations were determined in soil before and after the plant growth in untreated soil and in treated experimental options of soil. The variation of heavy metals concentrations in soil samples are presented in figures 4a-h. Treated and untreated soil samples contain high concentration of Pb, Zn, Cu and Cd. By adding the zeolitic tuff and organo-zeolitic material in both experimental options of treatment, the total content of heavy metals has been lower compared to the untreated soil. In all types of studied soil, the heavy metal concentration decreased from the first to the second treated soil experimental option. The most significant effects of the amendments of the second experimental option of treatment were obtained for Pb and Cd decrease in soil samples. A weak decrease was obtained for Cu and Zn. After 1 year and six months the heavy metal concentrations in soils samples, after the plant growth, presents the different behavior.

The concentration of Pb and partially Cd in the entire soil samples decreased in treated experimental options of soil compared with the untreated soil. Is not about significant decrease from the initial content of the soil samples used in experiment. These results explain the low concentration of Pb in plants in I and II treatment experimental options, which sustain the role of zeolites in Pb immobilization.

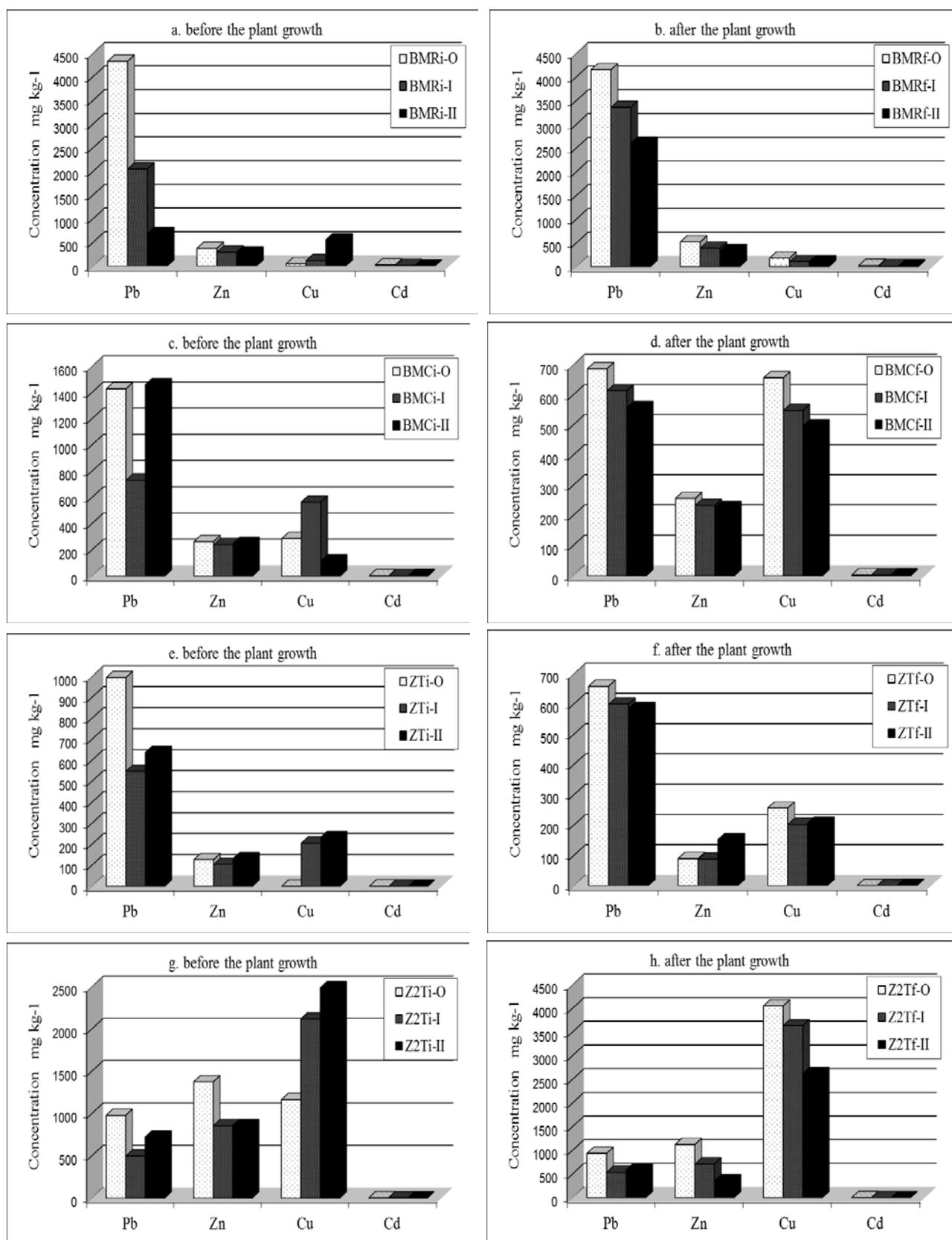


Figure 4a-h. Total content of heavy metals (mg kg^{-1}) in untreated and in treated soil samples before and after the plant growth; *Explanations for figure 4: BMR(i)-the initial soil sample before the plant growth; BMR(f)-the final soil sample after the plant growth; BMR-O, untreated soil (polluted soil); BMR-I, First treated experimental option of soil (polluted soil+zeolitic tuff); BMR-II, The second treated experimental option of soil (polluted soil+organo-zeolitic material), BMR-Baia Mare Romplumb, BMC-Baia Mare Cuprom, ZT and Z-2T-Zlatna.

Also the organo-zeolitic material in the second treated experimental option of soil ensured the increase of the organic matter content, which can determine decrease of bioavailability of heavy metal due to complexing of these, (Nachtegaal et al., 2005). In case of Zn and Cu in some soil samples obtained the increase of the concentration, especially after the plant growth. The increase was associated with the accumulation of heavy metals in the soil, which can be explained by the absence of water drainage of pots.

3.4. The plant development

Plants germination took place the quickest in the first treated soil experimental option, in all samples and in all three replicates, because by adding zeolitic tuff with particle size of 0.05-2 mm, it increased the sandy fraction. In these samples and in the untreated soil, plants had sprung after four days. In the second treated experimental option, at the same time, the plants had only germinated due to the organo-zeolitic material. This one has determined the soil compaction.

Plants growth was studied at intervals of seven days. In the first week, the plants growth was different in the three variants; maximum dimensions have been reached by plants in the first treated soil experimental option and in the untreated soil. After 40 days, plants from the second experimental option of treated soil, even if they grew in a slowly way, reached the maximum dimensions compared to the untreated soil and to the treated soil of the first experimental option.

In the further period of growth, at 72 days,

the plants development was superior in the second treated experimental option of soil, (Fig. 6 a-d). The plants from the untreated soil did not develop, and those from the first experimental option had a slow evolution.

3.5. Plant harvest

The aerial parts of the plants (*Lolium perenne*) have been harvested for three times in a year and six months. In this period of time, the plants have been harvested at 95 days, the first harvest - A, second harvest - B has been realized at 24 days after the first one, caused by the growth of the plants in the summer period. The final harvest (F), when plants got to their maturity, has been realized after a period of 11 months from the second harvest, which included the winter period. At the final harvest there have been extracted the plants' roots from the pots, too (Fig. 7 a-d).

3.6. The biomass quantity of the treated experimental options

In all harvests, the second treated experimental option of soil assured the biomass growth for all soil types, (Fig. 5). This is explained by the role of the organic part of the organo-zeolite material as source of nutrients (Chaiyarat et al., 2011) and by the zeolites structure which retain and release the ammonium ion gradually into the soil (Li, 2003, Ahmed et al., 2010, Latifah et al., 2011). Small amount of green and dry biomass of untreated soil can be explained by the effect of heavy metal toxicity according to Füleky & Barna (2008).

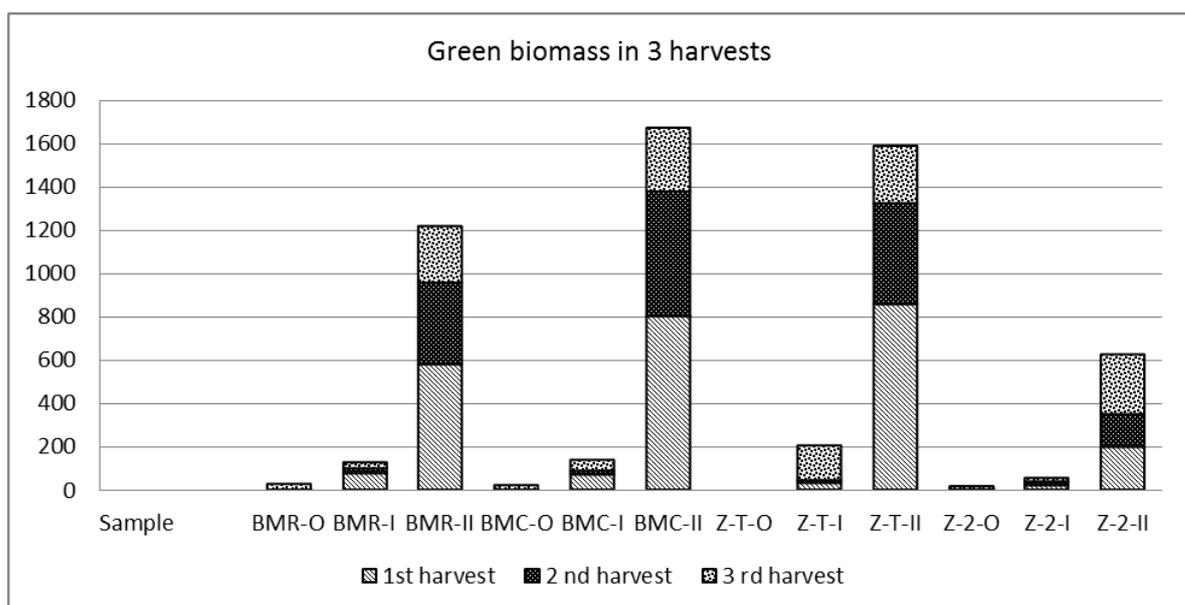
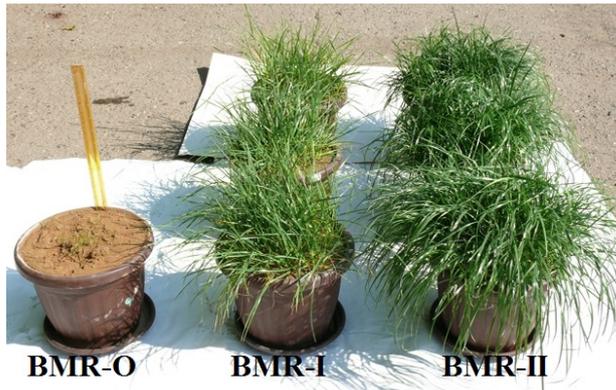


Figure 5. Green biomass of the aerial part of *Lolium perenne* in the three harvests.



a)



b)



c)



d)

Figure 6. Growth plants after 72 days; BMR-O, untreated soil (polluted soil); BMR-I, first treated experimental option of soil; BMR-II, the second treated experimental option of soil; a-BMR-Baia Mare Romplumb, b-BMC-Baia Mare Cuprom, c-ZT and d-Z-2T-Zlatna.



a)



b)



c)



d)

Figure 7. The root at the maturity stage of the plants

3.7. The heavy metals contents in the plants growth on the untreated and treated experimental options of soil

The heavy metals content has been analyzed in the aerial biomass of *Lolium perenne* in the three harvests. At the third harvest, when plants were mature, the concentration of heavy metals, Pb, Cu, Zn and Cd from the plants' roots has been determined (Table 3). In the second treated experimental option of soil there were obtained higher concentrations of Pb, Zn, and Cd in the second harvest than in the first harvest. This could be influenced by the decay of organic material (Antoniadis & Alloway, 2001) under the influence of higher temperatures in summer. The plants can concentrate different elements from soil through their roots (Yoon et al., 2006, Peralta-Videa et al., 2009).

Lead

Lead was accumulated more in plants grown on untreated soil, than in plants grown on the amended soil into both harvesting, A and B (Table 3). Pb is also concentrated more on roots than in the aerial part in all samples (Table 3). Generally, Pb is the less available metal for the grown plants on polluted soil (Intawongse & Dean, 2006). In the first treated experimental option, Pb shows a slight increase from the first harvest (A) to the second harvest (B) and the final harvest (F) for the Baia Mare zone. This type of Pb behaviour is associated with pH variation, from 5.26 to 5.54. In the second treated experimental option of soil, Pb content from plants is lower than in the first treated soil experimental option, from first to final harvest. In the second treated soil experimental options the lead content in the aerial part of the plants, decreased below typical toxicity concentration (Kabata Pendias & Pendias, 2001), (Table 3). These values of Pb concentration under 30 mg kg^{-1} correspond with the European Commission recommendations (2005/87/EC). Cd concentration decreased in the second experimental option of soil treatment, from the A to final harvest (F) in less than 1 mg kg^{-1} , (Table 3) which has been accepted as a maximum concentration in the green forages in 2005/87/EC regulations. Soil's reaction in the second treated soil experimental option varies from 6.46 to 7.01 and humus content grows from 2.34 to 2.88% (Table 3). The low Pb concentration from the second experimental option of soil treatment corresponds to the excessive growth of plants as a result to the nutritive elements added from the organo-zeolitic material and can be explained by the low translocation of Pb from roots in the aerial part

(Usman et al., 2006). The Pb concentration decreases in the biomass from harvests A, B and F compared to those from the untreated soil, (BMR) biomass. For each experimental option of treatment, from the first harvest to the last one, there can be observed an easy increase of Pb concentration. The zeolites ability to retain Pb in different pH conditions was demonstrated by Ponizovsky & Tsadilas (2003).

In the samples from Zlatna zone, the Pb content decreased in the aerial biomass from the treated experimental option comparative to the untreated soil sample in all harvest and presented very high concentrations in the root plants (Table 3). In the untreated soil of ZT-O sample, there has been developed only moss (Fig. 6c and Fig. 7c). At the end of the experiment, Pb concentration was $1323.43 \text{ mg kg}^{-1}$ in moss and in the two treated soil experimental options, Pb content increased from the first to the final harvest (Table 3).

Copper

In BMR and BMC samples, in all harvest stages, the content of Cu decreases in the first and second experimental options compared to the untreated soil, (Table 3). In BMR sample, in the first treated soil experimental option, there can be observed an increase of Cu content from A to F harvests, in conditions of variation for pH between 5.26 to 5.54. In the second treated soil experimental option, it can be observed a decrease of Cu content, from harvest A to the final one, associated with a pH variation, 6.46 to 7.01. In BMC soil sample, in the first treated soil experimental option, it is observed a low increase of Cu content from the first to the final harvest in conditions of variation of pH, from 5.26 to 6.02, and in the second experimental option it is observed a decrease of Cu content from harvest A to the final one in relation to the pH increase, from 6.45 to 7.43 (Table 2).

In ZT sample, concentration of Cu is lower in the biomass plant from the treated soil experimental options compared to the untreated soil. In the first treated soil experimental option it is observed an easy increase from the first harvest to the final one. In the second treated soil experimental option Cu concentration decreased from the first to the final harvest. In Z-2T sample, the Cu concentration increases in the biomass from harvests A-F in first experimental option, in conditions of pH growth from 7.86 to 8.90 (Table 2) and decreases in the second treated soil experimental option from harvest A to F, (Table 3).

Zinc

In BMR sample zinc presents lower concentrations in the biomass of the first and second treated soil experimental options than in the biomass from the untreated soil of the A and B harvests.

Table 3. Total contents of heavy metals in aerial and roots part of *Lolium perenne* grown on untreated soil (O-original) and treated experimental options of soil

Part plant/ harvest	Plants grown on soil sample (O-original untreated soil variant; I, and II treated experimental option of soil)											
	BMR-O	BMR-I	BMR-II	BMC-O	BMC-I	BMC-II	ZT-O (only moss)	ZT-I	ZT-II	Z2T-O	Z2T-I	Z2T-II
	Pb (mg·kg ⁻¹)											
aerial /A	116.64	43.50	15.50	29.00	5.31	3.86	-	19.64	6.92	8.26	6.95	4.41
aerial/B	p.n.g	42.66	16.88	p.n.g	6.44	4.51	-	23.87	7.29	p.n.g	p.n.g	21.83
aerial/F	184.90	67.33	9.72	48.11	18.23	14.94	1323.43	41.70	11.81	13.95	17.30	10.89
root/F	3632.83	75.09	627.47	728.68	438.13	495.02	-	328.96	1043.39	48.93	215.83	233.32
	Cu (mg·kg ⁻¹)											
aerial/A	43.43	13.65	24.36	46.95	19.29	37.31	-	21.16	30.02	14.27	36.54	44.57
aerial/B	p.n.g	18.75	23.69	p.n.g	22.22	35.55	-	22.58	27.33	p.n.g	p.n.g	25.59
aerial/F	34.03	24.96	8.44	52.21	28.03	12.09	715.08	27.42	28.96	19.59	60.02	17.84
root/F	222.43	113.92	57.30	507.29	408.99	425.87	-	122.28	180.79	232.69	780.08	1027.67
	Zn (mg·kg ⁻¹)											
aerial /A	219.97	183.81	219.82	256.89	139.58	158.98	-	103.10	74.54	158.12	376.41	229.80
aerial/B	p.n.g.	184.97	288.17	p.n.g	85.82	214.66	-	48.17	96.08	p.n.g	p.n.g	218.16
aerial/F	304.58	517.69	76.06	148.00	92.79	110.19	568.91	101.27	66.94	204.17	418.62	122.08
root/F	271.93	345.49	270.34	528.42	366.83	535.44	-	189.41	154.78	827.72	1299.27	1348.35
	Cd (mg·kg ⁻¹)											
aerial/A	7.69	3.35	3.72	2.62	1.17	1.78	-	0.20	0.17	0.31	0.57	0.55
aerial/B	p.n.g	5.05	3.95	p.n.g	1.46	1.76	-	0.18	0.23	p.n.g	p.n.g	0.52
aerial/F	14.47	30.98	0.52	2.90	4.41	0.50	4.59	0.24	0.07	4.09	0.38	0.20
root/F	262.16	35.22	15.35	46.64	27.68	7.53	-	0.65	0.79	2.26	10.09	6.25

(A-1st harvest, B-2nd harvest, F- harvest 3rd)

BMR-O untreated soil (Original soil); (I) First treated experimental option of soil -polluted soil+zeolites; (II) Second treated experimental option of soil-polluted soil+ organo-zeolitic material; p.n.g-plant not grow

In the final harvest, in the first treated soil experimental option, the Zn concentrations present an increase over the concentration value from the untreated soil, (Table 3). In the second treated experimental option, in the A harvest, the Zn content is comparable with the one from the original soil. In the B harvest, the Zn content increases to 288.17 mg kg⁻¹ and in the final harvest it decreases to 76.06 mg kg⁻¹. This decrease is followed by a pH modification, from 6.46 to 7.01. In the aerial part of the plants growth on the BMC sample, zinc concentration decreases in the first treated soil experimental option from A to F harvests. In the second treated soil experimental option it is maintained the same tendency of Zn concentration's variation.

In Z-T samples zinc presents high concentrations in the moss sample that had been developed in the untreated soil sample (Table 3). In the first treated soil experimental option Zn concentration is variable. In the second treated experimental option Zn concentration decreases from A to F harvest in the aerial part of the plants.

Zn concentration in Z-2T biomass sample in the first treated soil experimental option is higher than the one from the untreated soil, from A to F harvests (Table 3). In the second experimental option, the Zn concentration is also higher than the one from the untreated soil, A to F harvests. The decrease of Zn concentration was obtained in the final harvest of the second experimental option. After the researches results of Padmavathiamma & Li (2009b) the high concentrations of Zn in the tops of the *Lolium perenne* is related with the high content of Zn in soil. Zn and Cd are more soluble in soil and available for plants (Lasat, 2002).

Cadmium

In BMR sample, (Table 3) in the first treated soil experimental option, Cd has low concentrations, in the A harvest and in the B one, compared to the biomass from the untreated soil sample. In the F harvest of the first experimental option, cadmium concentration increases over the value from the biomass of the untreated soil. Cadmium is translocated to leaves due to their lower affinity for organic matter (Basta et al., 2005). The increase of the sandy fraction in soil texture by adding zeolitic tuffs could favour the cadmium migration. In the second treated experimental option the cadmium concentration decreases under the one from the biomass of the untreated soil, from A to F harvests. This decrease of cadmium concentration is in relation with the growth of pH from 6.46 to 7.01 and with 2.88% humus content. By contrast with the untreated soil, the soil + organo-zeolitic mixture

became more compact, which influenced the decrease of solution migration from soil. Lin et al., (1998) demonstrated that the soil-zeolites mixture leads to the increase of exchange capacity in conditions of high pH, which influenced the cadmium stability in soil.

Generally, in BMC sample (Table 3) the Cd concentration goes lower in the biomass from the first experimental option compared to the untreated soil, in the A harvest and in the B harvest, but suddenly increases in the final harvest. In the second treated soil experimental option, the cadmium content goes progressively lower in the untreated soil from A to F harvest. In Z-T and Z-2T samples (Table 3) the Cd concentration is lower in all harvests from the two treated soil experimental options compared to the one from the untreated soil. Kiran & Şahin in 2006 showed that the higher concentration of Cd leads to a lower development of the plant roots. In the roots development in soil samples from Zlatna area (Fig. 6 and Fig. 7) and from Baia Mare Romplumb (BMR) area, has been noticed variations between the treated and untreated soil experimental options.

A lack of supply with nutritive elements, especially nitrogen from the untreated and the first treated experimental options leads to a weak development of *Lolium perenne* plants in all soil types.

The plants roots retained a great quantity of heavy metals at the end of the experiment, which determined a lower accumulation in the aerial part of the plants, (Table 3).

The roots' cells, through specific mechanisms can function as "barrier", (Kabata-Pendias, 2011), against moving heavy metals in the aerial part of the plants (Wallace & Romney, 1977). Roots plants have the capacity to bind heavy metals in the cells' walls or through immobilization processes of heavy metals in vacuoles and do not allow their moving in the aerial part of plants (Lasat et al., 1998, Blaudez et al., 2000). At the same time, dissolution of organic components can determine heavy metals mobility in soil (Jordan et al., 1997; Geebelen et al., 2002), which leads to their undertake by roots (Albasel & Cottenie, 1985; Jin et al., 2005). Also, plants roots can acidify the rhizosphere by abstraction H⁺ through roots (Crowley et al., 1991) and can determine the heavy metals mobility (Mench et al., 1988).

Low concentrations of Pb from the aerial part of the plants sustain the reduced mobility of this metal in the soil-plant system and the immobilization in the root (Yoon et al., 2006). The great affinity of the zeolites structure for Pb would explain their potential to reduce the mobility of Pb (Gworek, 1992).

A part of cadmium has been undertaken by plants in concentrations of $7.69 \text{ mg}\cdot\text{kg}^{-1}$ in BMR-O. In the first treated experimental option it has been registered a growth of the cadmium content in the biomass from the A harvest ($3.35 \text{ mg}\cdot\text{kg}^{-1}$) to the B harvest ($5.05 \text{ mg}\cdot\text{kg}^{-1}$) and to the F harvest ($30.98 \text{ mg}\cdot\text{kg}^{-1}$). In the second treated experimental option, in the BMR sample biomass, the Cd and Pb concentrations decrease in all harvests compared to the one from the untreated soil.

Due to their use in the growth process, zinc and copper present a high content in the aerial part of the plants. Zinc is the element with an important role in certain metabolic processes of the plants in the synthesis of some proteins (Bonnet et al., 2000). Zinc and copper are micro-nutritive for plants and represent essential constituents of many enzymes and proteins in plant growth (Hall, 2002).

In our results, *Lolium perenne*, concentrations of Zn exceed the limit of $100 \text{ mg}\cdot\text{kg}^{-1}$ (Kabata-Pendias & Dudkas, 1991) for all soil samples and in all harvests for Baia Mare Romplumb (BMR) and for Zlatna zones, (Table 3). Values less than $100 \text{ mg}\cdot\text{kg}^{-1}$ have been registered in the biomass of the second treated soils experimental options from the BMR area and from the Z-T area, in the final harvest. The high contents of Zn and Cu in plants, suggest the existence of certain conditions that favoured the mobility of chemical forms for the two metals.

3.8. Stabilization of heavy metals in polluted soils treated with natural zeolites and organo-zeolitic material

The heavy metals soil pollution became inactive by immobilization in the zeolites structure (Mozgawa, 2000). The immobilization mechanisms in the zeolites structure are based on the exchange of alkaline and alkaline-loamy metals by heavy metals. The capacity of zeolites to immobilize Cu and Cd has been demonstrated by Terzano et al., 2005. Also the Pb and Cd ion immobilization on the zeolite structure was demonstrated by Mozgawa et al., (2009). After Lin et al., (1998), in conditions of high pH and cation exchange capacity of zeolite, Cd can be stabilized in soils. Immobilization studies, especially for Pb, have been performed using zeolites, phosphates and other materials by Castaldi et al., (2005), Moirou et al., (2001).

The zeolites role in stabilization of heavy metals from polluted soils has been verified by electron microprobe analyses. Particles of zeolitic tuff rich in clinoptilolite provided from the treated second experimental option of soil in BMR-II and ZT-II have been analyzed. Generally, in order to

emphasize the effects of some amendments in the heavy metals immobilization in soil, complex analysis methods are necessary (Panfili et al., 2005). To confirm the presence of the heavy metals in the zeolites structure, as a result of ionic exchange, there have been effectuated EDX analyses at electron microprobe (EDX micro-samples). The obtained spectrums are presented in Fig. 8 and Fig. 9 and contain Pb, Cu and Zn next to Na, Ca and K peaks. These results are in accordance to the cationic exchange between clinoptilolite and Pb^{2+} , Cd^{2+} and Cr^{2+} , which were confirmed by EDX electron microprobe by Mozgawa (2000). The presence of Pb, Cu and Zn in the EDX spectrum of the zeolitic tuffs particle can be explained by cationic change between heavy metals and Ca, Na from the zeolites composition (Moirou et al., 2001).

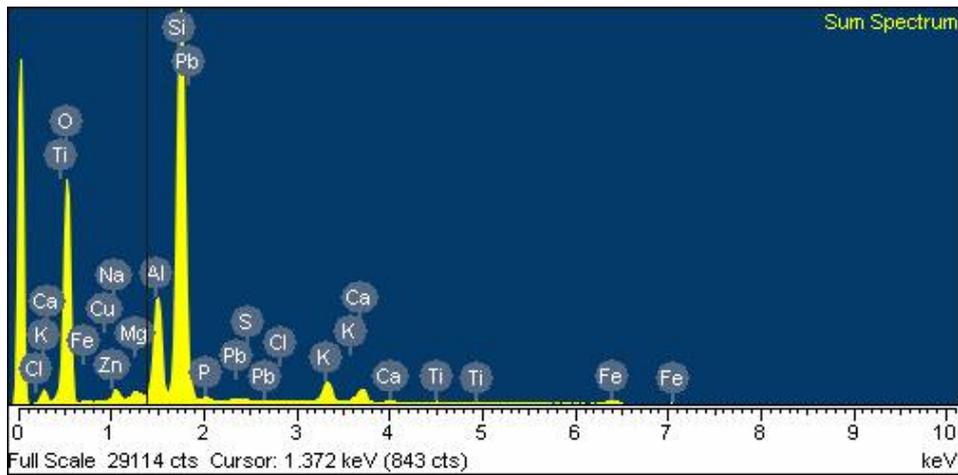
4. DISCUSSIONS

The investigated areas are poor in vegetation because the heavy metals present in high concentrations in soil inhibit the enzymatic activity, disrupt the vegetal metabolism and the proteins' oxidation, and also affect the cell membranes causing death of cells (Cheng, 2003).

The analytic data concerning the content of heavy metals of the studied global samples emphasize a wide variation of contents, especially for Pb, Zn, Cu and Cd. For these four heavy metals, the contents have been compared with maximum admissible values and with values of alert and intervention for sensitive soils (in Romanian Order 756/1997). The physical-chemical characteristics of soils from global samples of the two studied areas are favourable to heavy metals accumulation. Association of high concentrations of heavy metals of these two areas, with low values of pH and relative high contents of humus are probably due to the absorption of free ions of heavy metal by the organic material (Catlett et al., 2002).

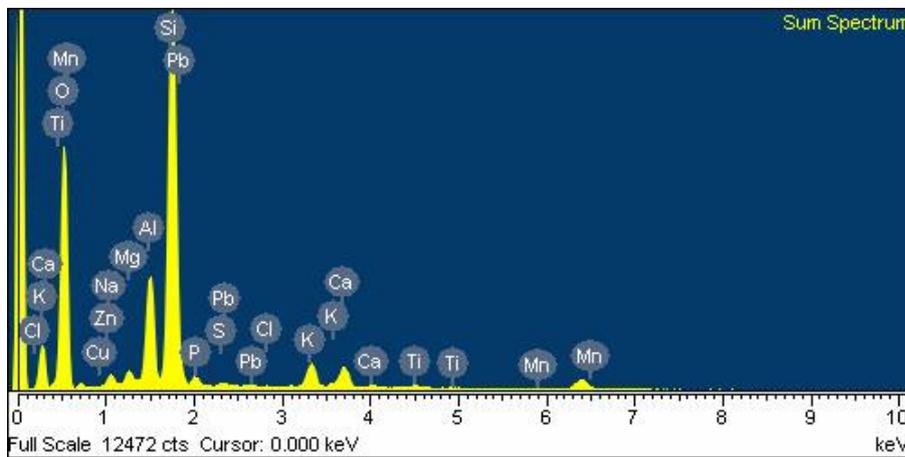
The representative soils for the four global samples have been analyzed for the main agro-chemical characteristics. The obtained results demonstrated the weak supply with nutritive elements: N, P and K.

Lolium perenne was used in order to check the capacity of the soil as a support for plant growth on the treated soil variants. In all period of harvests, plants grown on untreated soil have accumulated higher quantities of heavy metals than those grown on the treated soil experimental options. The heavy metals concentration was larger in the root of the plants in all of the treatment experimental options and in the untreated sample than in the aerial part of the plants.



Element	Weight%
O K	59.26
Na K	0.92
Mg K	0.21
Al K	6.24
Si K	27.45
P K	0.50
S K	0.12
Cl K	0.08
K K	2.01
Ca K	1.37
Ti K	0.14
Fe K	1.10
Cu K	0.02
Zn K	0.06
Pb M	0.51
Totals	100.00

Figure 8. EDX spectrum of the particle zeolitic tuff from BMR-II sample (left) and atomic percentages of elements in table (right).



Element	Weight%
O K	60.02
Na K	0.97
Mg K	0.65
Al K	6.47
Si K	25.57
P K	0.93
S K	0.23
Cl K	0.12
K K	2.20
Ca K	2.00
Ti K	0.31
Mn K	0.17
Cu K	0.04
Zn K	0.11
Pb M	0.19
Totals	100.00

Figure 9. EDX spectrum of the particle zeolitic tuff from ZT-II sample (left) and atomic percentages of elements in table (right).

Table 5. *d* values of the natural zeolites used in the experiment in comparison to the zeolitic tuffs particle at the end of the experiment (*BMR-II* and *ZT-II* samples)

hkl Koyama & Takéuchi, (1977)	Zeolitic volcanic tuff – Original sample d(Å)	Zeolitic volcanic tuffs from BMR-II d(Å)	Zeolitic volcanic tuffs from Z- II d(Å)
020	8.9447	9.0916	9.0359
131	3.9551	3.9725	3.9796
440	2.9740	3.0536	2.9838

Plants' roots retained a high quantity of heavy metals at the end of the experiment, which lead to a low accumulation in the aerial part. The roots' cells, through specific mechanisms, can function as barriers against moving heavy metals in the aerial part of plants (Wallace & Romney, 1977). Plants' roots have the capacity to bind heavy metals in their

cells' walls or through processes of immobilization of these heavy metals in cavities and do not allow their removal in the aerial part of the plants (Lasat et al., 1998).

From the analysis of heavy metals concentrations in plants it has been demonstrated that Pb and Cd have reduced contents in the treated

soil experimental options, which would relate with a bigger affinity of the zeolites structure for these two metals. Instead, Cu and Zn present higher contents in the aerial part of the plant, which would be a proof of selective accumulation of these metals being used in the process of growing.

Representations of Zn and Cu in plants, being marked by high contents, demonstrate the existence of some conditions that favoured the mobility of chemical forms for the two metals. From the four analyzed heavy metals, the less toxic for plants is Zn (Påhlsson, 1989). In general, Zn and Cu are involved in metabolic processes, being essential micro-elements. Zinc is the element with an important role in certain metabolic processes of plants and in some proteins' synthesis (Bonnet et al., 2000). In conditions of low pH, it has been registered a growth of the Zn, Cd and Cu concentrations in the aerial part of plants. It is possible that these metals could be present in the exchangeable metal forms in soil, (Ahumada et al., 2009), being easily undertaken by plants. In comparison to these metals, lead has a lower concentration in plants from the treated soil experimental options. At high values of pH, over 7, the analyzed concentrations of heavy metals decrease very much.

The Cd mobility is different, depending on the concentrations from plants in the untreated soil variant and of those treated with zeolites and organo-zeolitic material. Thus, in conditions of low pH of soils, between 3.69-4.50, from the untreated soil, in the stage of the final harvest, and between 5.16-6.02 of the soils from the first treated experimental option, concentration of Cd from plants is 4.59 mg·kg⁻¹ (Z-T-O) in moss, 14.47 mg·kg⁻¹ (BMR-O) and 46.64 mg·kg⁻¹ in BMC-O in original samples and it is 0.24 mg·kg⁻¹ in Z-T-I, of 4.41 mg·kg⁻¹ in BMC-I and of 30.98 mg·kg⁻¹ in BMR-I.

In the second experimental option of treatment, the content of Cd from plants suddenly decreased in all soil samples, being of 0.52 mg·kg⁻¹ in BMR, of 0.50 mg·kg⁻¹ in BMC and of 0.07 mg·kg⁻¹ in Z-T. These results highlight the pH influence on the Cd mobility and on the removal from soil to plants. The pH growth in soil may reduce the Cd biodisponibility and its absorption by the plants is due to the changing rhizosphere pH and Eh (Dong et al., 2007).

From the analysis of heavy metals concentrations from plants, it has been demonstrated that some metals as Zn and Cu did not negatively influence the developments of these ones in the treated soil experimental options up to concentrations between 66.95 mg·kg⁻¹-122.08 mg·kg⁻¹ for Zn and between 8.44 mg·kg⁻¹ - 28.96 mg·kg⁻¹ for Cu,

adequate to the final stage of harvest. These results are accordingly to those presented by Påhlsson (1989). Generally, those two metals have an important role in certain metabolic processes. Thus, Zn activates enzymes and has a role in the proteins and carbohydrates synthesis. Zinc forms constant complexes with DeoxyriboNucleic Acid (DNA) and RiboNucleic Acid (RNA) (Collins, 1981). Another explanation may be in relationship with the lower retaining or with the Zn and Cu desorption by the zeolites structures which have been demonstrated by Antoniadis et al., 2011, and Usman et al., 2006). In the case of Pb, the low concentrations from the aerial part of plants are generally limited due to Pb retention by the plants' roots. In the same stage of harvest of the second experimental option of treatment, plants' roots have retained the high concentrations of Pb (233.32 mg·kg⁻¹ - 1043.39 mg·kg⁻¹).

5. CONCLUSIONS

The used zeolitic tuffs are characterized by a high content in zeolites, by the presence of clinoptilolite and have as effect the mobility reduction, especially for Pb and Cd, subordinated for Cu and Zn. By adding zeolites and organo-zeolitic material in the polluted soils, the capacity of cationic exchange has been increased. The cationic exchange capacity has as effect the immobilization of heavy metals affecting their undertaken by plants. Also, due to the affinity of the clinoptilolite for ammonium ion, the organo-zeolitic mixture from the second treated soil experimental option ensured for a long period of time the azoth source necessary for plants growth, of which development is superior compared to the first treated soil experimental option. The pH increase and the contribution of nutritive substances, N, P and K, from the second treated soil experimental option, in all types of soils, led to plants growth and to the decrease of heavy metals content in the aerial part of the plants.

The active role of heavy metals immobilization in treated soils is obvious due to these analyses and is sustained by the reduced quantity of heavy metals moved from the soil in plants. The plants that have grown on the untreated soil in comparison to those grown on the treated soil experimental option have accumulated great quantities of Pb, Cd, Cu and Zn.

In respect to plants' growth, natural zeolites have had the capacity to modify the pH to values that are optimum for their development in both experimental treatment options. Generally, the pH increase in the polluted soils with heavy metals is very efficient for the reduction of heavy metals absorption from the soil by plants (Albasel &

Cottenie, 1985). Also, the organo-zeolitic material determined the growth of humus content and of the nutritive substances. Both amendments modified the cationic exchange capacity up to 2.54 times compared to the original untreated soil.

Acknowledgements

This research has been supported by Financial Assistance of National Council of Research from Romania, in Grant No.138/2006

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Received at: 30. 01. 2013

Revised at: 12. 10. 2013

Accepted for publication at: 25. 10. 2013

Published online at: 31. 10. 2013