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THE TREATMENT OF SOIL POLLUTED WITH HEAVY METALS USING THE *SINAPIS ALBA* L. AND ORGANO ZEOLITIC AMENDMENT

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Abstract. The capacity of organo-zeolitic material was evaluated as an amendment for the treatment of soils polluted with heavy metals. Three soil experimental variants were used: experimental variant (sample I), untreated, polluted soil with Pb (4330ppm), Zn (377ppm), Cd (27.29ppm) and Cu (50.2ppm), experimental variant (sample II), polluted soil treated with organo-zeolitic material and experimental soil control variant (sample III). *Sinapis alba* L. was used as the test plant. The application of the organo-zeolitic material increased significant the production of biomass (795g) compared to the control soil (108.63g) and untreated soil (27g). Growth of plants on the untreated polluted soil was strongly inhibited by high metal content, low pH, lack of nutrients. The best evolution happened for the plants of the experimental variant (II sample). The accumulation of heavy metals is significant in all vegetative organs of the plant from experimental variant II. However, the plants did not show toxic effects of aerial vegetative organs. In the root of plants from experimental variant II, a very high content of Pb (1.107.1 mg/kg) and Zn (505.3 mg/kg) followed by Cu (49.7) mg/kg and Cd (9.33mg/kg) was observed. In the shoot (25.7mg/kg) and in the leaves (17.8mg/kg) the content of Pb decreases below the toxicity limit. The Zn concentration in the shoot and leaves is high (95.2 and 116.1mg/kg). The content of Cd in the shoots and leaves of plants grown on polluted soil treated with organo-zeolite exceeds the maximum permissible content of 1mg/kg and falls within the range specific to the toxicity range. Applying organo-zeolite treatment to heavy metal polluted soil has favored the accumulation of high amounts of metals in the roots but a poor translocation in shoots and leaves. Zn and Cu were accumulated the most and Pb the least. The value of the transfer factor in mustard leaves was higher in plants from the control soil than in mustard plants from the experimental variant treated with organo-zeolitic material. Spectrophotometric analysis of the mustard leaves extract indicated a significantly higher content of photosynthetic pigment (chlorophyll **a, b** and carotenoids) for the plants grown on the soil treated with organo- zeolite compared to the plants grown on the control soil.

Key words: Heavy metals, soil pollution, organo-zeolitic material, phytoremediation, *Sinapis alba* L.

1. INTRODUCTION

The abundance and persistence of heavy metals in the soils of the metallurgical locations in the Baia Mare area are demonstrated by the results of many researches. The studies carried out aimed at: highlighting the influence of pollution sources on the area around the development soil (Răuţă et al., 1997), the effects of heavy metals on soil, plants and animal organs (Lăcătuşu et al., 1998); evaluation of heavy metal abundance in urban soil using the geochemical, pedogeochemical and anthropogenic indexes, (Lăcătușu et al., 2008a); the distribution of heavy metals in surface and deep horizons in soil profiles (Damian et al., 2008); the relationship between soil pollution with heavy metals and the quality of plants and fruits from this area (Lăcătuşu et al., 2008b). Jelea et al., (2015, 2016) studied the effects of heavy metals for growth of differents plants specie that can be used for phytoextraction.

The transfer of heavy metals from soil into plants depends on the chemical character of the metal, the plant species (Big et al., 2012; Mihali et al., 2013), as well as the soil characteristics controlling the retention of metals (Uchimiya et al., 2011). Plant species naturally installed on the surface of mine waste dumps reflect the chemical composition of the substrate and can be used to rehabilitate them (Damian & Damian, 2006).

The bioavailability of toxic metals for plants is affected by the combined effect of soil properties and soil type (Olaniran et al., 2013), the extraction fractions from the soil solution (extractable metal fractions in soil solution) and the plant species according to the model developed by Wang et al., (2004).

Plants used for phytoremediation have developed strategies for resistance to the toxicity of various metals: cation efflux pumps (van Hoof et al., 2001) or heavy metals complexation within cells with strong ligands such as phytochelatins (Cobbett & Goldsbrough, 2002). A combined effect of the use of a plant species and a natural amendment in the soil sample could provide the necessary conditions for the phytoremediation of these soils. Applying the natural amendment has the role of increasing the pH and nutritive elements. Natural amendments can retain heavy metals in the soil through various sorption mechanisms that need to be understood and studied, (Uchimiya et al., 2011). Heavy metals in soil have a potential toxic effect on germination, growth and development in plants (Solanki & Dhankhar, 2011). In an experiment in which the pH of the soil was modified in a wide range of values from 3 to 13, Adamczyk-Szabela et al., (2015) demonstrated that the germination of plant seeds is strongly dependent on the pH value. The low pH value and low organic matter content of polluted soils reduce their natural barrier for heavy metals role, (Kwiatkowska, 2018).

2. MATERIALS AND METHODS

2.1 Location, type and physical and chemical characteristics of the soil

The investigated area is located in the north-east part of the city Baia Mare. The soil sample used in the experiment was collected from the depth of 0-20 cm from the land surface around the metallurgical plant. The area investigated corresponds to the area of development of the eutricambosol soil type having the coordinates 47° 41.190' N, 23° 37.232' E, (Fig. 1).

Eutricambosols occur in restricted areas on the slopes of the northern part of Baia Mare, in its eastern part, at the confluence of the Firiza Valley with the Săsar River and on narrow surfaces on the Firiza, the Red and the Borcut Valleys (Big et al., 2012).

In study zone the land is without vegetation and the soil structure is destroyed, (Fig. 1). The pollution with heavy metals of the soils in the adjacent areas of metallurgical plants in the Baia Mare area is persistent even after closing of the mining activities. Revegetation of areas affected by the metallurgical industry is difficult because of the presence of heavy metals (Pb, Cd, Zn, Cu) (Damian et al., 2008) and the acidic pH that will affect the germination and growth of plants. The control soil sample was harvested from an uncontaminated area located at 26 km from Baia Mare.

A B

Figure 1. Gaseous emissions tower nonferrous metallurgical plant (A); Soil sampling area (B).

The purpose of this paper is to use a natural organo-mineral (organo-zeolitic material) amendment to revegetate the heavy metal polluted soil from the metallurgical plant Romplumb Baia Mare. In order to verify the influence of this amendment on plants, *Sinapis alba* has been used because of its capacity to grow on heavy metal polluted substrates which has been confirmed by many research (Dar et al., 2003; Jankowski et al., 2014).

2.2 Source and properties of organo-zeolitic material

Volcanic rocks from Neogene tuffs in the Maramureș Basin were used as sources of zeolites. The presence of zeolites as tabular crystals formed by the pseudomorphic replacement of the vitroclasts of these rocks was demonstrated by Cochemé et al., (2003). Among the minerals specific to the zeolite class were identified: mordenite $(Ca, Na_2, K_2)Al_2Si_{10}O_{24}$ $7H_2O$, heulandite $(Ca, Na)_{2-3}Al_3(Al,Si)_2Si_{13}O_{36}$ $12H_2O$, clinoptilolite $(Na,K,Ca)_{2-3}Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12(H_2O)$.

The structure of clinoptilolite can be used for nitrogen adsorption due their porosity, according Mansouri et al., (2013) which demonstrated that after the nitrogen adsorption the total pore volume and specific surface area were increased.

Zeolites can modify pH and cationic exchange capacity, which are two factors that can act concurrently but with different effects in the remediation of soils polluted with heavy metals (Mahabadi et al., 2007; Shi et al., 2009). Reactions favored by organic and inorganic amendaments, complexation and adsorption may favor immobilization of heavy metals and may limit their transport and bioavailability (Li et al., 2015; Khalid et al., 2017). The role of amendments in polluted soils is to reduce leaching of contaminants, (Burlakovs et al., 2012).

The organo-zeolitic material was obtained from the volcanic tuff sample that were crushed to obtain particle to sizes of 2mm mixed with chicken waste and affected by a fermentation process applied after the method of Leggo (2004).

2.3 Experimental variants of soil used

The soil sample collected from the soil surface horizon was homogenized and prepared for mixing with organo-zeolitic material and for experimental variants of substrate for plants growth. The texture of the soil from the experiment is clay-loam. From the sample of the collected soil were made two experimental variants of the substrate: an experimental variant from the untreated original soil (sample I), an experimental variant of polluted soil mixed with organo-zeolitic material - 200g organo-zeolite was mixed with 800g soil (sample II).

A non-pollutant variant was used as a control soil (sample III) for comparison. Each of the three experimental variants was multiplied into 3 pots of 3 kg each, (Fig. 2):

- experimental variant (sample I) - untreated polluted soil,
- experimental variant (sample II) - polluted soil treated with organo-zeolitic material,
- experimental soil control variant (sample III).

The three experimental variants were watered daily with distilled water at 70% of the soil water capacity, in the equilibration period during the 14-days.

2.3.1 Sinapis alba used as the test plant

The seeds of *Sinapis alba* L from *Brasicaceae* family were used. The seeds are produced by Agroseem Seed Center Iasi, Romania. The seeds were germinated in the three experimental variants. In selecting the plant specie have been taken into account several plant-specific characteristics used for phytoremediation in accordance with Sarma (2011): high growth rate and high biomass production, tolerance to heavy metals (Pan et al., 2018).

Sinapis alba is ideal for the study of polluted soils (OECD Guideline 208 for Testing of Chemicals, 2003; Gerencsér et al., 2010). White mustard is a sensitive plant that can be used as a bioindicator of heavy metals (Adamcová et al., 2016). Another mustard species, *Brassica juncea*, is referred to as the phytoremediation plant, Novo & González (2014), on a pollutant substrate treated with amendments to ensure germination conditions. Hyperaccumulative plants possess characteristics that are based on tolerance to high metal concentrations (Mahar et al., 2016).

2.3.2.Experiment in pot

A triplicate of each soil experimental variant was prepared. In each pot were seeded 9 points with 2 seeds each point, with a total of 162 seeds in the 9 pots. On the day of sowing and the period of germination, the characteristic climatic factors registred at the weather station were favorable for plant development, temperature 18º C, humidity 40%, air pressure 763 mm Hg, no precipitation in the first days. Ten days after sowing, the plants are removed from each pot to ensure a proper density for growth (9 plants/pot). During the experiment (April-May 2017), the air temperature ranged between 12° C and 28° C, the air humidity was between 70-90% and the photoperiod of 13-14 hours.

The possible toxicological effect was assessed according to CSN EN 13 432 on growth of dicotyledonous plants.

2.3.3 Seedling and growth of plants

After 10 days was calculated the number of seedlings (OECD 2006). The evolution of the seedlings stage and growth of plants was followed at 7 days. The vegetative organ growth was assessed after 42 days (IDT ISO 11269-1: 1993). The vegetative organs were detached and were determined their fresh weight, the dry weight and physiological ratios (dry weight/fresh weight, humidity, dry weight amount to 100 g of fresh weight) for individual of white mustard. Dry weight was determined after the vegetative organs were stored for 3 days at the oven set at 60°C in Microbiologic thermostat incubator model LE-549 Scilab Instruments Ltd. Biometric measurements of seedlings from the treatment variants were compared with the control variant.

The tolerance index (T.I.) was determined using method of Shaw (1989).

2.3.4. Photosynthetic pigments

Determination of the content of photosynthetic pigments was performed after the Schöpfer method (1989) using 0.05g of dry sample. To distinguish chlorophyll pigments, the absorbance of the supernatant obtained from the extraction of the plant material was read at several wavelengths: 480 nm, 645 nm, 647 nm, 652 nm, 663 nm, 664 nm and 750 nm.

Reading at 750 nm is done to ensure that pigments do not absorb and do not lead to erroneous results. Absorbance measurement was performed on the Perkin-Elmer Lambda 25 UV-VIS spectrophotometer. The extract was diluted with solvent to obtain values of optic absorbance in the range 0.2-0.8. The content of chlorophyll and carotenoids pigments expressed in mg/g of dry matter was calculated based on the values of absorbance at the wavelengths mentioned previously by the formula given by Schöpfer (1989) taking into account the dilutions made to obtain values of absorbance to be less than 0.8, (Rouessac &Rouessac 2007).

2.3.5. Concentrations of heavy metals in soil and in plants

The physicochemical properties of the soil experimental variants have been analysed before the growth of the plants. Soil pH was measured in aqueous solution in a ratio with the soil to water of 1:2.5. The humus content has been obtained by the wet oxidation method (Walkley-Black 1934). Determination of total nitrogen was realised by Kjeldahl method. Phosphorous and potassium were obtained in solution of ammonium acetate lactate at pH=3.7. Total content of heavy metal were obtained from hydrochloric acid solution after soil mineralization with $HClO_4$ and HNO_3. The heavy metals in soil were analyzed using the atomic absorption spectrometry. The content of metals: Zn, Cu, Pb and Cd were determined from the vegetative organs of mature plants. Metal dosing was performed by the acetylene-air flame of spectrophotometer UNICAM (England) type Solaar S.

2.3.6. Data processing

Data processing has been accomplished through various mathematical methods. In some cases the percentages were calculated, and in other cases the results were statistically processed with the help of Student's T test. The Student's T test allows the elimination of aberrant values based on the Chavennet criterion (Snedecor & Cochram 1978, Weber, 1980). The statistical processing was performed with the Excel application in the Microsoft Office 2003 package.

2.3.7. Bioconcentration and translocation factors

Transfer factors from soil to plant or bioconcentration factor that quantify the ability of hyperaccumulators plant to accumulate a certain metal in its tissues was calculated by the equation (1) (Cui et al., 2004; Selvaraj et al., 2015):

BCF(Metal) soil to plant=Metal concentration in plant/Metal concentration in soil **(1)**

Concentration of the metals are expressed on dry weight of plant and soil. For the hyperaccumulator plants, the transfer factor quantify the effectiveness of the plant ability to accumulate a certain metal in their tissues, (Selvaraj et al., 2015).

Translocation factor of a metal from roots to shoots or from roots to leaves calculated by the equations 2-3 shows the ratio of the metals in the considered organs of the plants and is a measure of the translocation degree of metal and the also its mobility.

TF (Roots to shoots)= Metal concentration in shoots/Metal concentration in roots **(2)**

TF (Roots to leaves)=Metal concentration in leaves/Metal concentration in roots **(3).**

3. RESULTS AND DISCUSSIONS

3.1. Heavy metal content from the soil in experimental variants of substrat

Table 1 presents physicochemical characteristics and heavy metals contents of soil samples used as experimental variants and the chemical characteristics of organo-zeolite material. The concentrations of heavy metals in the studied soil were compared with the Romanian standard levels, Order 756/1997.

The soil from experimental variant (sample I) with untreated polluted soil has low nitrogen and phosphorus content and moderate potassium content. The pH values for the soil used in the experiment fall within the highly acidic reaction class. The humus content is very low.

The modifications of experimental variant soil treated with organo-zeolitic material (sample II) compared to the experimental variant of untreated polluted soil (sample I) are noticeable. These changes consist in increasing the pH that may decrease the phytoavailability of the soil, (Sun et al., 2014). It has also increased the humus content and nutrient elements content. The content of the heavy metals analyzed is excessive especially for Pb and Cd and high for Zn. For these metals, the maximum admissible limits are exceeded according to Order 756/1997.

Table 1. Physicochemical characteristics and heavy metal content of soil samples used as experimental variants.

Properties	Sample I (polluted soil-untreated)	Sample II (polluted soil treated with organo-zeolite)	Sample III (control soil)	Organo-zeolite material	Heavy metal Maximum available limit 756/1997
Humus (%)	1.20	2.34	3.90	8.64	
N_{total}(%)	0.05	0.20	0.27	0,98	
P(mg/kg)	6.8	1262	85	4968	
K(mg/kg)	143	4010	117	10046	
pH	4.88	6.70	6.56	9.17	
Cd (mg/kg)	27.27	15	1.50	0.33	3
Zn (mg/kg)	377	280	162.8	74	300
Cu (mg/kg)	50.2	39	29.3	15	100
Pb (mg/kg)	4330	3592	243.8	33	100

The organo-zeolitic material used in experimental variant II meets the necessary conditions for a natural change that ensures the source of macronutrients (Jakkula et al., 2018) necessary for plants growth and immobilization of heavy metals by various mechanisms, (Gadepalle et al., 2007). Nutrients are generated by decomposing chicken litter from the organic component (Leggo, 2017). Clinoptilolite in the tuff composition has an ion-exchange property with high affinity for ammonia, (Leggo et al., 2010).

3.2 Plants growth

In the seedling stage in the experimental variant of untreated polluted soil (sample I) have manifested effects of heavy metal toxicity combined with low pH values and poor supply of humus and nutrient elements. Thus, in all three pots of this sample the percentage of plant growth (55%) was the lowest compared to the 100% percentage obtained in experimental variant II. No changes occurred at 17 days. After 24 days, the percentage of seedlings in experimental variant I increased, the average being of 81.2%.

Significant growth differences are observed in each experimental variant and at each time interval (Figs. 2, 3, 4a). The best evolution had plants from the experimental variant treated with organo-zeolitic material (sample II). Di Giuseppe et al., (2018) have shown that zeolites have positive effects on seed germination. In the untreated experimental sample (I), the plants did not grow throughout the growing period and did not produce the biomass needed for the analysis, (Fig. 5b). Plant leaves in this experimental variant showed brownish yellow spots, uneven development, apparent chlorophyll deficiencies. Leaf chlorosis is the most common symptom in the case of heavy metals phytotoxicity (Lăcătuşu, 2006). The poor plant growth in the experimental variant is due to low germination of the seeds.

According to data presented by Padmavathiamma & Li (2009) in a soil with high concentration of heavy metals, germination is affected. When extracting the plants from pots, in case of experimental variant II, we observed a well-developed radicular system which is more than the height of the pot, which is 16 cm (Fig. 4b). These roots could not be recovered because of their fragility. The amount of green biomass at the end of the growing period was: 795g in sample II, 108.63g in sample III, and 27g in sample I (Fig. 5a).

The aerial part of the plants from experimental variant II did not exceed the height of the control sample, but there are significant differences in the aspect of shoot thickness, the size of the leaves and even the color (Fig. 4a). All plants of this variant have matured, producing inflorescences, (Fig. 4a, Fig. 7).

In figures 4a and 5a, *Sinapis alba* plants are shown at the end of the experiment after 42 days when have been determined biometric parameters such as roots and shoots length, roots, shoots, leaves weight as green and dry biomass for 6 plants in sample II and sample III.

The weight of vegetative organs shows the intensity of growth and development processes, being a physiological indicator of the plant. Table 2 presents the results obtained by determining the weight of the vegetative organs (root, shoot, leaves). The roots of the batch of plants in the control soil (sample III) had the medium value ± statistical estimate of 8.65 ± 0.23 cm, and in case of the experimental variant II of 9.78 ± 0.23 cm. The percentage difference from experimental variant III (control soil) was higher by 13.06%. The organo-zeolite in sample II allowed the development of the root system, (Fig. 4b). Data from literature (Treacy & Higgins, 2001; Polat et al., 2004) argues that the porous structure of the organo-zeolite provides a permanent water reservoir in the roots area, improving the horizontal spread of water. The contact surface of the roots with the soil solution is greatly increased by the branches (Fig. 4b) and the extremely large number of the absorbent hairs.

The length of the shoot (Table 2) is 78.14 ± 2.26 cm in control soil (sample III) and 68.16 ± 1.94 cm ($p<0.01$) in experimental variant II. In experimental variant II, a significant decrease in shoot height was observed with 12.78% compared to plants in control soil. Regarding the inflorescences,

Figure 2. *Sinapis alba* after 7 days.

Figure 3. *Sinapis alba* after 14 days.

Figure 4a. *Sinapis alba* after 42 days.

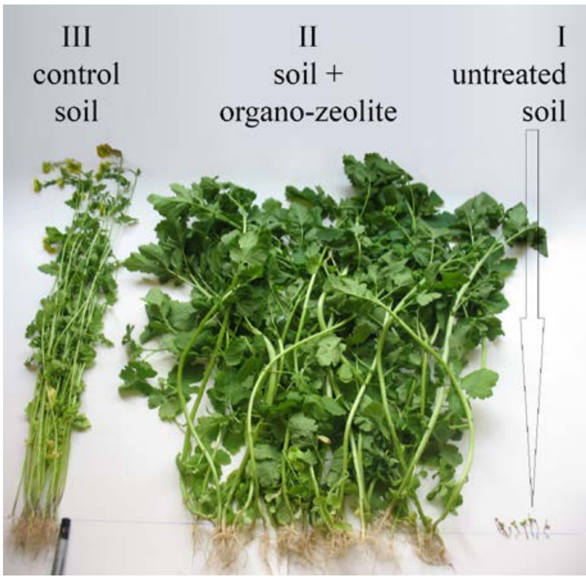

Figure 5a. Green biomass of the plants at the end of the experiment .

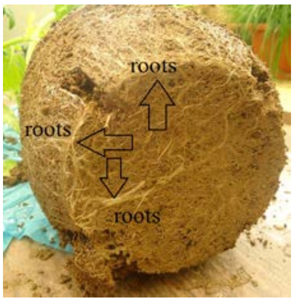

Figure 4b. Roots of the plants grown in soil treated with organo-zeolitic material.

Figure 5b. Plants in untreated soil at the end of the growing period.

Table 2. Influence of organo-zeolite treatment on the length plants of *Sinapis alba*

Sample		Root (cm)	Shoot (cm)	Inflorescence (cm)
Control variant (III)	$\overline{X}$ ± ES	8.65 ± 0.23	78.14 ± 2.26	2.90 ± 0.78
Experimental variant I	$\overline{X}$ ± ES	NG	NG	NG
Experimental variant II	$\overline{X}$ ± ES	9.78 ± 0.60	68.16 ± 1.94	2.31 ± 0.40
	p	<0.50	<0.01	<0.01
	D%	113.06	87.22	78.65

Note: The table includes the average ± the standard error ($\overline{X}$ ± ES), percentage differences as compared to the control (D%) and statistic significance considered from p<0.05. NG - no growth.

Table 3. Mass (g/plant) of vegetative organs (root, shoot, leaves) to the experimental variants

Vegetative organs		Average ± the standard error	Control variant (III)	Experimental variant I	Experimental variant II
Root	FW	$\overline{X}$ ± ES	0.245 ± 0.018	NG	1.003 ± 0.082
		p			<0.001
		D%			409.38
	DW	$\overline{X}$ ± ES	0.078 ± 0.007	NG	0.228 ± 0.014
		p			<0.001
		D%			292.31
	H%	$\overline{X}$ ± ES	67.79 ± 1.880	NG	78.29 ± 1.550
		p			<0.001
		D%			115.48
Shoot	FW	$\overline{X}$ ± ES	2.41 ± 0.152	NG	5.072 ± 0.023
		p			<0.001
		D%			210.45
	DW	$\overline{X}$ ± ES	0.515 ± 0.043	NG	0.368 ± 0.031
		p			<0.01
		D%			71.45
	H%	$\overline{X}$ ± ES	78.63 ± 1.260	NG	92.74 ± 0.629
		p			<0.50
		D%			117.94
Leaves	FW	$\overline{X}$ ± ES	0.415 ± 0.058	NG	1.833 ± 0.063
		p			<0.001
		D%			441.68
	DW	$\overline{X}$ ± ES	0.090 ± 0.080		0.278 ± 0.029
		p		NG	<0.10
		D%			308.88
	H%	$\overline{X}$ ± ES	78.31 ± 2.928	NG	84.83 ± 1. 900
		p			<0.10
		D%			108.32

Explication at table 2. FW – fresh weight (g/plant); DW – dry weight (g/plant); H% - humidity (%/plant).

the results obtained in the experimental variant II revealed a significant decrease by 21.35% compared to the experimental variant of the control soil. The root tolerance index was 113.06%, and the shoot tolerance index was 87.22%.

Table 3 shows the results on the weight of fresh and dry of vegetative organs. The results revealed an increase in the weight of the vegetative organs in experimental variant II. The dry weight (DW) content in experimental variant II was increased to the root (0.228 ± 0.014g) and leaves (0.278 ± 0.029g).

The dry weight/fresh weight ratio provides some information about the intensity of metabolic processes (Table 4). The plants root from experimental variant III (control soil), the dry weight/fresh weight ratio ranged from 0.259 to 0.409 with an average of 0.321. In case of experimental variant II, the ratio was between 0.178 and 0.311 with an average of 0.231 g. The dry weight/fresh weight leaf ratio had an average of 0.266 for sample III and 0.233 for sample II.

The availability of groundwater in the sample II is reflected in the plant water content and the intensity of all physiological processes. Milică et al., (1977) indicated that for the photosynthetic processes the leaves water content must be 87%; higher or lower concentrations decrease the intensity of the phenomenon. In experimental variant II, the leaves water content was around 84.83%. The water content of vegetative parts of the sample II was higher than in plants grown in soil control (Table 3). As a consequence, the dry biomass weight in sample II is lower than those in soil control. Zeolites are known for their water holding capacity, (Jakkula et al., 2018).

The second physiological parameter, dry substance (Ds) expresses the amount of dry weight in 100 g of fresh weight. The percentage of dried substance of roots decreased from 32.21 g% (control sample III) to 23.17 g% (sample II). In case of the shoots dry biomass quantity from 100 g fresh biomass decreases from 21.28 g% (control sample III) to 7.26 g% (sample II).

The results obtained in the case of the plants from experimental variant II, indicated the reduction of the quantity of dry substance from 100 grams of fresh biomass and an increase of the quantity of water from the vegetative organs (Table 4). This decrease was 9.04g% for the root, 14.02g% for the shoot and 11.34g% for the leaves.

In figures 6 and 7 were observed the morphological differences between the leaves of experimental variant III of the control soil and those of the experimental variant II.

Table 4. Values of physiological ratio in samples III and II

No.	Control variant III						Experimental variants II					
	root		shoot		leaf		root		shoot		leaf	
	DW/ FW	Ds	DW/ FW	Ds	DW/ FW	Ds	DW/ FW	Ds	DW/ FW	Ds	DW/ Fw	Ds
1	0.259	25.93	0.221	22.06	0.188	18.86	0.283	28.33	0.081	8.18	0.245	24.55
2	0.375	37.50	0.203	20.26	0.180	18.00	0.178	17.82	0.055	5.56	0.123	12.36
3	0.312	31.25	0.244	24.43	0.173	17.31	0.311	31.13	0.095	9.52	0.151	14.35
4	0.409	40.91	0.190	19.02	0.155	15.55	0.215	21.55	0.078	7.88	0.127	15.10
5	0.315	31.58	0.192	19.24	0.588	58.82	0.203	20.37	0.062	6.22	0.126	12.79
6	0.260	26.09	0.227	22.70	0.312	31.25	0.198	19.82	0.062	6.22	0.627	12.64
A	0.321	32.21	0.212	21.28	0.266	26.63	0.231	23.17	0.072	7.26	0.233	15.29

FW – fresh weight (g/plant); DW – dry weight (g/plant); A – average (g/plant); Ds – dry substance (g%).

Figure 6. Leaves and inflorescences of plants in the control soil variant.

Figure 7. Leaves and inflorescences of plants in the experimental variant II.

Figure 8. Pivotal roots of *Sinapsis alba* (left – control variant III; right - experimental variant II).

Figure 9. Pivotal roots of *Sinapsis alba* in experimental variant II.

Leaves from experimental variant II are 5-15 cm long, pinnately lobed with 3-5 irregular lobes, of which the terminal is much larger.

Morphological differences explain the increased content of chlorophyll and carotenoid pigments. Productivity in any crop depends on the photosynthesis process, which also depends on the chlorophyll content of the leaves in plants (Mondal et al., 2017).

The roots of the plants from experimental variant III show normal morphology (Fig. 8-left). In the case of plants from experimental variant II, the main root was vigorous and secondary roots were well-developed (Fig. 8-right, and Fig. 9). Roots are very rigid as a result of intense structural remodeling of the cell wall. Defense responses are concretized by enhancing enzymatic activities aimed at stiffening and impregnating the cell wall of the root. Some studies support (Sharma & Dubey 2005) that the modification of cell walls in roots is related with tolerance against excessive heavy metals content in soil.

The shoot is cylindrical, vigorous and well developed in experimental variant II. Flowers are arranged in dense racemes that stretch with flowering. The raceme are higher in plants in experimental variant III of the control soil.

3.3. The content of chlorophyll

Chlorophyll pigments give information about the photosynthetic apparatus and thus provide information on plant physiological productivity and physiological status (Curran et al., 1990, Filella et al., 1995, Oz et al., 2015).

The total chlorophyll concentration and the ratio of chlorophyll **a** and chlorophyll **b** can be used as early warning systems for the toxic effect of metal accumulation in plants, (Manios et al., 2003). The data obtained are shown in Table 5.

The content of chlorophyll **a** from experimental variant III (control variant) ranged from 0.628 mg/g to 0.855 mg/g with an average of 0.718 mg/g. In the experimental variant II the content of chlorophyll ranged from 1.879 mg/g to 1.922 mg/g with an average of 1.918 mg/g.

Concerning the chlorophyll **b** content, it had values between 0.330 mg/g and 0.484 mg/g with an average of 0.398 mg/g at the control variant and between 0.857 mg/g and 0.909 mg/g at experimental variant II with an average of 0.884 mg/g.

The increase in the content of chlorophyll and carotenoid pigments in experimental variant of soil treated with organo-zeolite was due to the nutrients necessary for their formation (N, Mg, K) as well as to limiting the toxic effects of heavy metals on chlorophyll. Heavy metals cause photoinhibition and oxidative stress (Küpper et al., 2002). But other authors show that this ratio may decrease or increase depending on the metal tolerance of the cultivated plant species (Ralph & Burchett, 1998) and the balance of Mg in the substitution reaction of chlorophylls with the toxic metals (affinity of the respective metals for the porphyrin nucleus).

The molecule of chlorophyll is a complex combination with Mg^{2+} divalent ion in the center of a tetrapyrole ring. The presence of other divalent cations such as Cu, Mn, Pb and Zn may displace Mg from the chlorophyll molecule affecting its structure and functions (Küpper et al., 2002). In hidroponical experiments (Paunov et al., 2018) was showed that Cd and Zn disturb photosyntetic perfomances of plants affecting chlorophyll fluorescence.

The ratio between chlorophyll **a** and chlorophyll **b** is 2.17/1 in experimental variant II versus 1.8/1 in experimental variant III. Data from literature (Jia et al., 2010) show that the ratio of chlorophyll **a** to chlorophyll **b** tends to decrease for plants growing in contaminated soils.

Carotenoid pigments had values between 0.049 mg/g and 0.068 mg/g in sample III, soil control. In experimental variant II, the results were between 0.066 mg/g and 0.093 mg/g. The content of carotenoid pigments had a mean and a statistical estimation of 0.061±0.005 mg/g in experimental variant III-soil control and 0.079±0.007 mg/g in experimental variant II. The content of carotenoid pigments increased compared to soil control (sample III) by 25.50%. Carotenoid pigments are protective agents against oxidative stress due to their antioxidant effects (Zakar et al., 2016).

Table 5. Content of chlorophyll **a, b** and carotenoid pigments (mg/g) in the mustard leaves

Sample	Average ± the standard error	Chlorophll **a**	Chlorophyll **b**	Carotenoid pigments
Control variant (III)	$\bar{X}$ ± ES	0.718 ± 0.068	0.398 ± 0.078	0.061 ± 0.005
Experimental variant I	$\bar{X}$ ± ES	NG	NG	NG
Experimental variant II	$\bar{X}$ ± ES	1.918 ± 0.012	0.884 ± 0.014	0.079 ± 0.007
	p	<0.001	<0.01	<0.25
	D%	267.13	222.11	125.50

Explication at table 2.

3.4. Heavy metals of vegetative part of the plants

At the end of the stage of plants growth, after 42 days were determinated heavy metals contents. Table 6 presents the content of metals determined from vegetative organs. The accumulation of heavy metals is significant in all vegetative organs of the plants from experimental variant II. However, the plants in this experimental variant did not show any toxicity effects of aerial vegetative organs.

Root of the plants uptake heavy metal amount higer than shoot of the plants. This results are in accord with other results obtained by Turan & Esringü (2007).

Similar results for Cd and Zn were obtained by Sridhar et al., (2005). But the authors mentioned structural changes of the roots, shoots and leaves caused by higher concentrations of Zn and Cd. In our experiment, the highest amount of Pb was accumulated in the plants root of experimental variant II. Pb can be retained in the roots related with exchangeable sites on the cell wall, (Sharma & Dubey 2005). The roots ability of lead retaining is due to accumulation in the endodermis, which acts as a barrier to the movement of root lead in the shoot (Sharma & Dubey, 2005). The absorption of the various cations is in a reverse relationship with the soil's ability to retain these ions. Roots absorb significant quantities of metals from soil (Shparyk & Parpan, 2004, Mihali et al., 2013).

This corresponds with the highest amount of Pb in the untreated polluted soil used as growth substrate. Pb is generally soil-bound and not easily transported over the soil (Kabata-Pendias, 2011). In the shoot and leaves Pb is (25.7mg/kg) and (17.8mg/kg) and falls below the toxicity limit after Kabata-Pendias (2011) and corresponds to the maximum content in green forage according to Eurpoean Commission Recommendations 2005/87/EC. Zinc has accumulated in significant quantities in the shoot and leaves near the root. Zinc has the role of micronutrient in plant growth processes, (Tripathi et al., 2015). Zn accumulates more in the growth zones with an important role as a metal component of enzymes or as a functional, structural or regulatory cofactor of many enzymes, (Nasiri & Najafi 2015). Zinc and cadmium are very mobile in soil and can be easily taken up by plants (Kabata-Pendias, 2011). The Zn concentrations in the shoot and plant leaves of the experimental variant of soil treated with organo-zeolitic material (sample II) (Table 5) fall within the limits of normal content in leaves (Kabata-Pendias, 2011).

Cadmium can be extracted from the soil by the plants and transported from the root to the upper parts by xylem, the vascular tissue in plants that conducts water and dissolved nutrients (Ghosh & Singh 2005). The highest concentration of Cd was obtained in the plant root of experimental variant II. In this variant, Cd was translocated more in leaves than in the shoot. Similar results were obtained by (Ghosh & Singh 2005) for *Brassica juncea* at the end of plant growth stages. Compared to the maximum content values in green forage according to the Commission Directive 2005/87/EC recommendations and those reported in Kabata-Pendias (2011), the Cd content of the shoot and leaves of the plants grown on the experimental soil sample treated with organo-zeolite (sample II) exceed the maximum admissible content of 1 mg/kg and fall within the range specific to the toxicity range.

Table 6. The content of metals in vegetative parts of plants and bioconcentration and translocation factors

Vegetative parts of plants	**Zn**	**Cu**	**Pb**	**Cd**
	mg/kg	mg/kg	mg/kg	mg/kg
Root experimental variant III	161.9	6.7	1.2	1.10
Shoot experimental variant III	84.5	5.1	1.1	0.75
Leaf experimental variant III	45.3	5.4	8.3	0.33
BCF soil to roots	0.99	0.23	0.004	0.73
TF roots to shoots	0.52	0.76	0.92	0.68
TF roots to leaves	0.28	0.81	6.92	0.30
Root experimental variant II	505.3	49.7	1.107,1	9.33
Shoot experimental variant II	95.2	10.5	25.7	1.30
Leaf experimental variant II	116.1	36.4	17.8	4.85
BCF soil to roots	1.80	1.27	0.31	0.62
TF roots to shoots	0.19	0.21	0.02	0.14
TF roots to leaves	0.23	0.73	0.02	0.52

BCF-Bioconcentration factor; TF- Translocation factor

3.5 Bioconcentration and tranlocation factors

According the results obtained by Subhashini et al., (2013), plants are good accumulators if the concentration ratio of the element in the plant to that in the soil is >1. Also plants have the potential for phytoextraction if the translocation factor and bioconcentration factor >1 and for phytostabilization if translocation factor <1 and bioconcentration factor >1.From the analysis of the metal bioconcentration factors from soil to the root, it is observed that *Sinapis alba* is a metal accumulator. Mustard is great at accumulating Zn and Cu which are essential metals, and it accumulates less Pb. By comparing translocation factors root-shoot and root-to-leaf, they are closely related to Zn and Cu which are essential metals. In the case of Pb and Cd, toxic metals, translocation factors roots-shoot and leaf-root are higher in control soil (sample III) compared with the polluted soil treated with organo-zeolite (sample II). Applying organo-zeolite treatment to soil polluted with heavy metals has favored the accumulation of high amounts of metals in the roots but a poor translocation in shoots and leaves. This is due to the high content of metals in the soil treated with organo-zeolite and the ion exchange properties of the zeolite (Shi et al., 2009).

The largest translocation factor root-to-leaf was for Cu in both experimental variants where the plants grew (Table 6) followed by the Cd translocation factor in the experimental variant II plants of 0.52. These values of the translocation factor <1 mean that the analyzed metals can not be translocated from the roots to the shoots and the leaves.

4. CONCLUSIONS

A test of phytoremediation was carried out on a soil sampled around the Pb and Zn metallurgical plant in the Baia Mare area with *Sinapis alba.* The soil sample affected by heavy metal pollution, characterised by acide reaction and low contents of the nutritive elements was treated with an natural amendment represented by organo-zeolitic material. The increase of the biomass from the experimental variant II compared to the control soil is due to the zeolite amendment (Khan et al., 2008). Six plants from each replication were used to analyze the heavy metals content.

The heavy metals content was compared between vegetative parts of plants, root, shoot and leaves, from experimental variant II and experimental variant III. Values obtained confirmed that plant roots retained the largest amount of heavy metals Pb, Zn, Cu and Cd. These results are in agreement with Angelova & Ivanov (2009) and are due to the high abilities of absorption of the root system specific for mustard. The highest concentration is in the case of Pb but is also due to the high concentration of Pb in the soil. However, due to Pb insolubility (Blaylock et al., 1997) it is not transferred to aerial parts of plants. The other metals have a more uniform distribution in all vegetative organs of plants. For Cu, Zn and Cd, the highest content after root is in the leaves followed by the one in the shoot. Application of zeolite and organic compounds in soil determined pH, humus and cation exchange capacity increase. These parameters influenced the heavy metals retention in high concentrations by plant roots and their decrease concentrations in shoot and leaves especially for Pb (Damian et al., 2018) and Cd (Sun et al., 2014). Toxic heavy metals can be sequestered by mechanisms involving their binding to the cell wall, (Solanki & Dhankhar 2011).

Sinapis alba L. and the treatment applied in soil with organo-zeolite material can be used for revegetation of heavy metals polluted land by demonstrating the increase of biomass at 795g compared to biomass in control soil 108.63g and 27g in polluted soil untreated. The organo-zeolite contains a sufficient amount of humus that has a favorable influence on the absorption of mineral substances. Experimental variant II showed an increased content of phosphorus and nitrogen and a pH of 6.56.

Providing soil with nutrients also explains the increase in the content of chlorophyll and carotenoid pigments in the plants grown on the experimental variant treated with organo-zeolite (sample II). The results of the experiment showed that *Sinapis alba* has tolerance and can be used as heavy metals accumulators in conditions of increasing biomass with the help of the organo-zeolitic amendment.

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