THE INFLUENCE OF THE TYPE AND CONCENTRATION OF AMENDMENTS ON THE GROWTH DYNAMICS OF *Phaseolus vulgaris* L.

Camelia NICULA[*], Anca PETER[*], Leonard MIHALY-COZMUTA[*], Anca MIHALY-COZMUTA[*], Emil INDREA[**], Virginia DANCIU[***]

[*] *North University of Baia Mare, Faculty of Sciences, Department of Chemistry-Biology*
[**] *National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103 Donath, 400293 Cluj-Napoca, Romania*
[***] *Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos 11, 400028, Cluj-Napoca, Romania*
Corresponding author: Camelia Nicula, North University of Baia Mare, Faculty of Science, Department of Chemistry-Biology, 76 Victoriei., 430122 Baia-Mare, Romania, tel.: 0040262430122, fax: 0040262276153, e-mail: vargacamelia@yahoo.com

Abstract. The experiments were performed to test the influence of amendments on the growth of *Phaseolus vulgaris* L. plants. The amendments tested were natural bentonite, natural zeolite modified with Ca^{2+} and natural zeolite modified with $NH4^{+}$, respectively. The seeds of beans were planted in polluted soil mixed with 5% and respectively 10% content of amendment. Mixes with the two above-mentioned concentrations were prepared with each type of amendment considered. Soil without amendment was used as control-soil. In each of the seven experimental series, 15 seeds were planted. The height of each plant was measured and recorded after a pre-established number of days, up to one calendar month. Data were analyzed using version 16.0 (for Windows) of the SPSS software package.

Keywords: *Phaseolus vulgaris* L., zeolite, bentonite, growth dynamics, statistical analysis.

INTRODUCTION

The contamination of soils with heavy metals is currently one of the most troublesome environmental problems faced by mankind. The plants exposed to heavy metals face the inhibition of the growth process, the leakage of ions from the membrane of cells, the destruction of pigments and even death [3, 21, 10]. The most common impact of the heavy metal is the accumulation of large amounts of peroxide compounds rapidly attacking several kinds of bio-molecules and disrupting the metabolism of the plant's cell [13, 12]. This increase in peroxide compounds, known as oxidative burst, increases the level of H_2O_2, and activates the defence mechanisms of the host against pathogens [6, 19, 15].

Zeolites are hydrated alumino-silicates with unique crystal structure, consisting of a three-dimensional network of $(SiO_4)^{4-}$ and $(AlO_4)^{5-}$ tetrahedrons [17, 11]. In the network of zeolites there are two types of cations: (a) "located" cations, bounded to certain positions by electrostatic forces; (b) "free" hydrated cations, distributed randomly in large cavities in zeolites.

Because of this structure, the surface of zeolites is negatively charged, which explains the absorption capacity of these materials. Zeolites have a high affinity towards metal cations (Zn^{2+} [17, 23], Pb^{2+} [1, 16, 25], Cd^{2+} [1, 25, 22], Cu^{2+} [17], NH_4^+ [22]), a low affinity for anions and a lack of reactivity for non-polar organic molecules [14]. The zeolitic structures can be recharged with other cations. This explains the capacity of natural and synthetic zeolites to act as ion exchangers.

The clinoptilolite, a compound with a complex formula, namely $(Na,K,Ca)_{2-3}Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12(H_2O)$, is one of the most effective types of zeolites in terms of the capacity to fix the ions of heavy metals [2].

In polluted soil, amendments determine a significant decrease of the degree of contamination in plants. By adding 10000 - 25000 kg zeolite per hectare up to 25 cm in depth from the surface of soil, most of the metals (Cd, Co, Cu, Ni, Zn) loose approximately 90 – 95% of their leaching properties. Zeolites induce the pH to increase, thus determining the immobilization of metals, which are adsorbed in pores, and also enabling the precipitation of metal hydroxides [18].

Zeolites can be used as antibacterial agents, growth promoters and agents stimulating the biological activity of soil. Also, the zeolites are effective in the recultivation of acidic land, by improving the nutritional balance of nitrogen and by decreasing the quantity of metal in polluted soil [20]. Zeolites remove and block a large part of ions potentially toxic to plants in their pores, avoiding their absorption by plants. Zeolites can be used as natural fertilizers for several reasons: they contain nontoxic natural compounds, are easily applicable at an early development stage of vegetation and a lasting effect throughout the vegetation season.

Besides their benefits if used as fertilizers and as natural bio-insecticides in agriculture, zeolites bring considerable advantages when used for environmental remediation, particularly on contaminated land. Thus, clinoptilolite zeolites administered in different experimental conditions have caused a reduction of the content of Cd in plants grown on soil contaminated with Cd. However, the quantity of biomass can decrease if the quantity of amendment exceeds a certain threshold. Therefore, additional research on each type of substrate in combination with each species of plant shall reveal at which concentration of zeolite a plant absorbs the lowest quantity of metal, thus maximizing the plant's capacity to survive and to best adapt to the particular conditions of a polluted site.

The zeolite added in contaminated soil has determined the ions of Fe, Cu and Pb to loose over 74% of their mobility, and the ions of Mn, Cd and Zn to loose between 8% and 37% of their mobility as compared to their initial extractable fraction. By adding zeolite, however, the mobility of such ions as Ca, Mg or Ba goes up, which is beneficial for the nutrition with minerals of the plants [8].

Experimental research demonstrated the ability of zeolites to increase the solubility of natural phosphates. In a zeolite-water-phosphate mix, the zeolite acts as absorbent for Ca, while the cations in the channels of zeolite (K^+ and Na^+) pass in the aqueous solution. By adding zeolite in the mix, as well as because of the light mechanical activation of the phosphate in raw state, the solubility of phosphates has increased [24].

The bentonite is a natural aluminosilicate akin to the zeolite. Montmorillonite, the key component of bentonite, is made up of crystals with the smallest diameter among all clay minerals. As for zeolite, the property of bentonite to trigger the exchange of ions is determined by the negative charge of its surface [9, 7]. The molecular formula for montmorillonite is usually given as $(M^+_x \cdot nH_2O)\ (Al_{2-y}Mg_x)Si_4O_{10}(OH)_2$, where $M^+ = Na^+, K^+, Mg^{2+}$, or Ca^{2+} [4].

MATERIALS AND METHODS

Preparation of activated amendments

Samples of zeolite rocks with a content of 62% clinoptilolite, rocks originating from a deposit in Stoiana, a village in Cluj County (Romania), were used for this study. The clinoptilolite samples were grinded in a mortar (Retsch RM 100, Germany) and sieved to separate the fraction ranging from 0.5 to 1 mm by means of mechanical sieves (Retsch AS200 basic). This fraction was washed with distilled water to remove any turbidity and was dried at 110°C for 24 h to remove any adsorbed water. Finally, the clinoptilolite samples were stored in a desiccator before performining the chemical activation in Ca-form and NH_4-form.

The chemical treatment of the zeolite (clinoptilolite) was performed by adding 1 L of the 2 M solutions of $CaCl_2$ and NH_4Cl respectively, to the samples of clinoptilolite fractions of 0.5 - 1 mm (100 g), at room temperature. After 24 h the solid phases were separated from solutions, washed until the removal of the Cl^- ions (controlled with $AgNO_3$ solution) and dried at 105^0C using a Binder oven for 24 h.

Samples of bentonite with a 75% content of montmorillonite and originating from a processing plant in Mediesul Aurit, a village in Satu Mare County (Romania), were used without any activation treatment.

Experimental batches

In April 2010, samples of soil were collected from an area contaminated with heavy metals, situated in Ferneziu (N 47º41`30.45``; E 23º37`36.40``), an outskirt of Baia Mare, in the west of Maramures County. The area is in the neighborhood of a former lead processing plant inaugurated in 1884 and a metallurgy plant inaugurated in 1907, both major sources of decades-long polution but currently with any activity discontinued. The samples were prelevated from a depth of 10 cm and sieved with a 20-mm sieve.

The seeds of beans originate from the crop collected in the fall of 2010 by farmers living in the area identified above. Before being planted, the seeds were washed with distilled water, then with alcohol and finally again with distilled water three times.

Batches of 15 seeds were planted in plastic pots of 250 mL in volume, containing soil amended with bentonite and zeolite chemicaly activated in Ca-form and NH_4-form, respectively. The proportion of the amendment in soil was 5 and 10%, respectively. In each pot, the total quantity of soil-amendment mixture was of 300 g. Soil without amendment was used as control-soil. The pots were watered with distilled water on a regular basis.

The seedlings were labeled for accurate identification, and growth caracteristics were measured and recorded for each seedling after 7, 14, 24 and 31 calendar days.

Chemical and structural analysis

The structure of the zeolite was determined based on the X-ray diffraction patterns recorded by means of a DRON X-ray powder diffractometer linked to a data acquisition and processing facility. CuKα radiation (λ = 1.540598 Å) and a graphite monochromator were used. The results were processed using the PCCELL programme.

The surface area and the pore size distribution of the unmodified zeolite and bentonite were determined using a Sorptomatic 1990 (Thermo electron Corp.) equipment and N_2 adsorption.

Fourier Transformed InfraRed Spectroscopy (FTIR) analyses of the unmodified and modified zeolite were performed. A Perkin Elmer Fourier Transform Infrared Spectrometer 2000 was used. Sample wafers consisted in 10% sample in spectral quality KBr.

The concentrations of metal ions in soil and seeds samples were measured by atomic absorption spectroscopy (AAS) in air-acetylene flame using a Perkin Elmer AAnalyst 800 spectrophotometer.

A Retsch RM-100 grinding machine was used to prepare the samples for mineralization. The mineralization took place in a Berghof MWS-2 microwave system. The parameters for seeds mineralization were: in stage 1 - 145^0C, 5 minutes, power 75%; in stage 2 - 190^0C, 10 minutes, power 90%; in stage 3 - 100^0C, 10 minutes, power 40%. For the mineralization of soil the authors have established the following parameters: stage 1 - 180^0C, 25 minutes, power 99%; stage 2 - 100^0C, 10 minutes, power 99%.

A mix of 10 mL HNO_3 65% (d = 1.4 kg/L, Lach-Ner) with 0.3 g plant powder, respectively with 1 g of dried soil was introduced in a microwave system. For the mineralization of all samples, the authors have complied with the methodology provisions in the users' guide of the microwave oven. After mineralization, the samples were brought to 100 ml volumetric flask with distilled water and subjected to AAS-analyse.

Statistical analysis

A one-way analysis of variance (one-way ANOVA) was carried out to compare the mean values measured for all the batches tested. Where significant *P*-value ($P<0.05$) were obtained, differences between individual means were compared using a post-hoc Turkey`s HSD test ($P<0.05$) [5].

RESULTS AND DISCUSSION

Characterization of amendments

Table 1 includes the morphological and structural characteristics of zeolite and bentonite. The structure of zeolite and bentonite is crystalline. The average diameter of the zeolite and bentonite particles is in the range between 70.21 and 75.74 nm. The specific surface area of the two materials is in the range between 54.67 and 75.27 m^2/g.

The diameter of particles is in inverse relationship with the specific surface area for both zeolite and bentonite.

Additionally, the crystalline structure of unmodified and modified zeolite and of bentonite is observed by the X-ray diffraction pattern analysis (Figure 1). The X-ray diffraction pattern of the bentonite reveals the existence of peaks at 22 and 35 degree, demonstrating the presence of montmorillonite. In bentonite, the predominant crystalline phase is montmorillonite. The X-ray diffraction pattern of zeolite reveals the existence of peaks characteristic for clinoptilolite (theta between 10 and 35 degrees) and montmorillonite, but the predominant crystalline phase is clinoptilolite.

Table 1. Morphological and structural characteristics of unmodified zeolite and bentonite (D_{eff} – effective crystallite mean size, $<(\varepsilon^2)>^{1/2}$ - root mean square of the microstrain size, S_{BET} – specific surface area).

	D_{eff} (nm)	$<\varepsilon^2>^{1/2}$	S_{BET} (m^2/g)
Zeolite	75.74	0.00232	54.67
Bentonite	70.21	0.00532	75.27

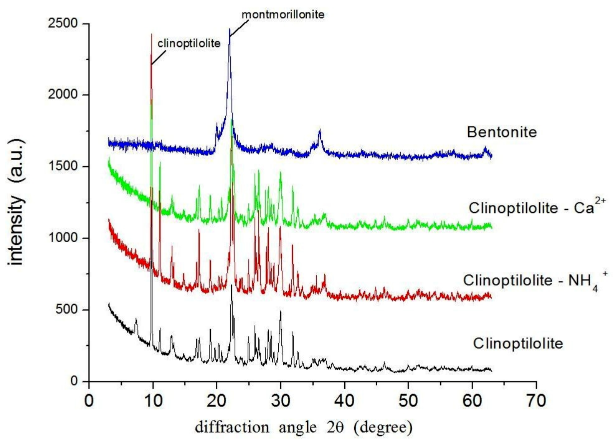

Figure 1. X-ray diffractogram of bentonite, natural zeolite and zeolite activated as Ca^{2+}-form and NH_4^+-form respectively.

FTIR analyses were performed, to establish if calcium or ammonium ions have modified the structure of the zeolite (Figure 2). The peak localized at 1028.68 cm^{-1} in FTIR spectra of zeolite corresponds to the vibration of the bands connected with the internal Si–O(Si) and Si–O(Al) vibrations in tetrahedra or alumino- and silico-oxygen bridges. The introduction of non-tetrahedral cations into the network of alumino-silicate can change their FTIR spectra in the range of pseudo-lattice vibrations located at about 1028-1036 cm^{-1} and 700–500 cm^{-1}. The changes in the FTIR spectra of zeolites exchanged did not result in a distinct shift of these band positions but in changes in their intensity. In this range, a weak but systematic variation was observed in the band at 1028-1036 cm^{-1} and at 600-602 cm^{-1}, which can be attributed to pseudo-lattice ring vibrations of SiO_4 or AlO_4 tetrahedra and particularly to the inter-tetrahedral bonds vibrations.

Table 2 gives the area and the length of the peak localized at 1028.68 cm^{-1} from the FTIR spectra of zeolite, 1033.2 cm^{-1} from the FTIR spectra of zeolite-Ca and 1036.54 cm^{-1} from the FTIR spectra of zeolite-NH_4. The variation of the area and length of the peak of the three samples demonstrates different intensities of vibration of certain bands, thus suggesting the generation of different bands (Si-O-Ca, in the case of zeolite modification with Ca and Si-O-NH_4, in the case of zeolite modification with NH_4).

The mineral and chemical composition of the natural zeolite and bentonite samples is indicated in Table 3.

Soil and seeds analysis

The mean concentrations of metallic ions in soil and seeds are those indicated in Table 4, the values in the table representing the means of triplicates. All the determinations have a 95% degree of confidence.

Assesment of plants'growth

Table 5 includes details about the height of plants, as measured at different time intervals and denominated in cm.

Figure 3 shows the mean values for growth after 7 days (a), 14 days (b), 24 days (c) and after 31 days (d) of growth for beans cultivated on control-soil, bentonite-soil 5%, bentonite-soil 10%, Ca^{2+}-zeolite-soil 5%, Ca^{2+}-zeolite-soil 10%, NH_4^+-zeolite-soil 5% and NH_4^+-zeolite-soil 10% respectively.

Table 2. Characteristics of the peak from FTIR spectra of the zeolite (Figure 2) localized at 1030 cm^{-1}.

Type	Area of the peak localized at 1028-1036 cm^{-1} (T % x cm^{-1})	Height of the peak at 1028-1036 cm^{-1} (T %)
Zeolite	23 502.36	33.28
Zeolite - Ca	26 774.18	50.23
Zeolite – NH_4	27 427.21	53.35

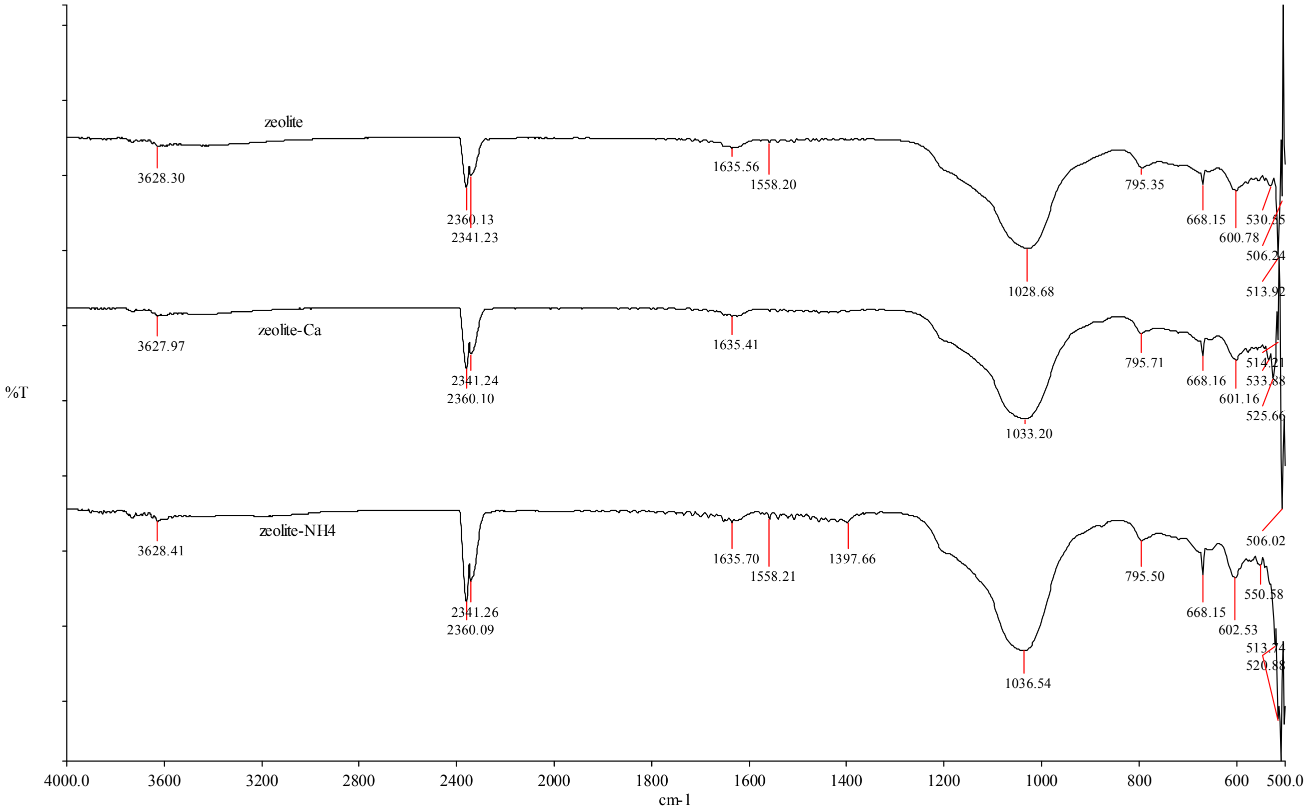

Figure 2. FTIR spectra of zeolite, zeolite modified with calcium ions (zeolite-Ca) and zeolite modified with ammonium ions (zeolite-NH_4)

Table 3. Mineral and chemical composition of the natural zeolite and bentonite.

Zeolite				**Bentonite**			
Mineral	***Content (%)***	***Chemical composition***	***Content (%)***	***Mineral***	***Content (%)***	***Chemical composition***	***Content (%)***
Clinoptilolite	62	SiO_2	65.59	Montmorillonite	75	SiO_2	56.17
Volcanic glass	34	Al_2O_3	13.70	Biotite/mica	12	Al_2O_3	19.39
Feldspar	1-1.5	Fe_2O_3	1.47	Dolomite	4-4.5	Fe_2O_3	4.19
Biotite/mica	1-1.5	CaO	4.72	Anatase	4-4.5	CaO	2.90
Calcite	1-1.5	MgO	0.92	Quartz	1.5-2	MgO	3.62
Limonite	0.5-1	K_2O	1.65	Feldspar	0.5-1	K_2O	1.38
Hornoblende	<0.5	Na_2O	1.60	Calcite	0.5-1	Na_2O	1.96
Montmorillonite	<0.5	TiO_2	0.14	Pyrite	<0.5	TiO_2	0.41
		LOI*	10.21			LOI*	9.98

* - loss on ignition

Table 4. Content (mean value) of heavy metals in seeds and soil samples.

	Cu (mg/g DW*)	**Pb (mg/g DW*)**	**Zn (mg/g DW*)**	**Cd (mg/g DW*)**	**Fe (mg/g DW*)**
Soil sample	0.794	1.543	0.697	0.016	13.543
Seeds sample	0.017	0.084	0.009	0.004	0.085

* DW = dry weight.

For the statistic analysis of data with one-way **ANOVA** (technique of analysing the data in terms of variation of sums of squares) were used the following steps:

Step 1. Test homogeneity of variance using the Levene statistic in SPSS (Table 6).

a. If the test statistic's significance is greater than 0.05, one may assume equal variances.

b. Otherwise, one may not assume equal variances.

Step 2. If we can assume equal variances, the F statistic is used to test the hypothesis (Table 7). If the test statistic's significance is below the desired alpha (typically alpha = 0.05), then at least one group is significantly different from another group.

Step 3. Once we have determined that differences exist among the means, post hoc pairwise and multiple comparisons can be used to determine which means differ. Pairwise multiple comparisons test the difference between each pair of means, and yield a matrix where asterisks indicate significantly different group means at an alpha level of 0.05.

When all pairs of means are being compared, Tukey's is the procedure of choice. *Tukey's HSD (Honestly Significant Difference)* is perhaps the most popular post hoc. It is appropriate to use this test when one desires all the possible comparisons between a large set of means (6 or more means). These analyses will allow us to determine which group means are significantly different from one or more other group means. The one-way Anova Outputs of the Tukey HSD test for growth after 14, 24 and 31 days, respectively, which represent the significantly different groups, are presented below (Table 8-10).

Table 5. Growth in height expressed in cm at different time intervals.

No	Growth after 7 days (cm)	Growth after 14 days (cm)	Growth after 24 days (cm)	Growth after 31 days (cm)	Series	No	Growth after 7 days (cm)	Growth after 7 days (cm)	Growth after 7 days (cm)	Growth after 7 days (cm)	Series
1	0.5	21.2	44.6	62.0	CS	31	0.5	5.4	17.8	19.9	NZ10%
2	0.2	5.5	16.0	25.0	CS	32	2.7	29.2	46.4	51.2	CaZ5%
3	0.2	3.2	12.4	16.5	CS	33	2.7	28.5	45.5	50.4	CaZ5%
4	0.2	2.6	4.2	6.5	CS	34	2.2	28.0	44.6	49.8	CaZ5%
5	0.3	8.0	19.3	27.5	CS	35	1.7	28.2	44.8	48.9	CaZ5%
6	4.0	27.0	37.0	37.5	Be5%	36	1.5	27.8	44.2	47.5	CaZ5%
7	3.5	26.0	34.2	36.5	Be5%	37	1.3	27.3	42.5	46.7	CaZ5%
8	3.5	26.2	30.2	31.0	Be5%	38	1.0	26.7	42.3	46.8	CaZ5%
9	3.0	24.2	26.0	30.0	Be5%	39	1.2	25.7	40.8	45.2	CaZ5%
10	2.5	22.0	24.6	28.8	Be5%	40	1.0	25.5	41.0	45.2	CaZ5%
11	1.5	19.2	23.5	24.8	Be5%	41	0.5	25.5	41.2	45.5	CaZ5%
12	0.3	13.0	19.0	17.5	Be5%	42	0.5	24.5	38.4	42.3	CaZ5%
13	0.3	13.0	17.2	18.0	Be5%	43	0.5	24.3	37.4	41.5	CaZ5%
14	0.2	7.5	10.0	12.5	Be5%	44	0.3	21.6	35.6	39.2	CaZ5%
15	0.2	4.0	7.2	11.8	Be5%	45	0.2	20.7	32.5	36.8	CaZ5%
16	6.5	24.2	46.8	58.2	Be10%	46	0.2	19.8	33.6	35.7	CaZ5%
17	1.5	23.5	39.3	45.7	Be10%	47	2.9	36.0	60.0	65.0	CaZ10%
18	0.5	21.5	35.3	44.0	Be10%	48	2.5	29.8	49.2	54.0	CaZ10%
19	4.5	31.2	61.6	76.0	NZ5%	49	2.4	28.8	47.6	52.0	CaZ10%
20	3.2	30.3	42.7	44.8	NZ5%	50	2.2	27.5	46.8	51.5	CaZ10%
21	1.5	18.4	35.2	42.7	NZ5%	51	2.0	27.2	45.6	50.0	CaZ10%
22	0.5	13.5	32.7	38.6	NZ5%	52	1.8	26.4	45.2	49.8	CaZ10%
23	0.3	9.6	29.8	36.5	NZ5%	53	1.7	24.8	42.8	47.2	CaZ10%
24	0.2	4.5	10.8	19.0	NZ5%	54	1.5	25.0	42.6	47.5	CaZ10%
25	2.9	25.7	43.2	68.2	NZ10%	55	1.5	24.5	42.0	46.5	CaZ10%
26	1.9	23.4	32.5	38.9	NZ10%	56	1.4	22.8	38.8	43.0	CaZ10%
27	1.5	20.5	28.5	33.4	NZ10%	57	1.3	21.2	35.6	39.8	CaZ10%
28	1.5	19.7	25.3	30.6	NZ10%	58	1.2	19.0	32.8	36.5	CaZ10%
29	1.0	11.8	21.3	25.2	NZ10%	59	1.0	17.5	31.5	34.8	CaZ10%
30	0.5	9.8	19.2	22.4	NZ10%						

*Legend: CS = control soil, Be5% = bentonite 5%, Be10% = bentonite 10%, CaZ5% = Ca-zeolite-soil 5%, CaZ10% = Ca-zeolite-soil 10%, NZ5% = NH_4-zeolite-soil 5%, NZ10% = NH_4-zeolite-soil 10%

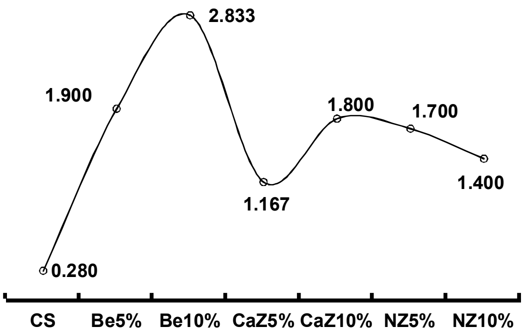

Figure 3a. Mean values for growth (cm) after 7 days.

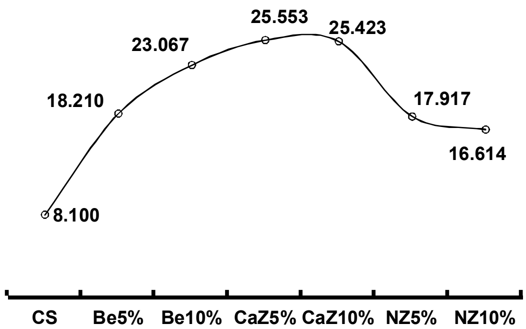

Figure 3b. Mean values for growth (cm) after 14 days.

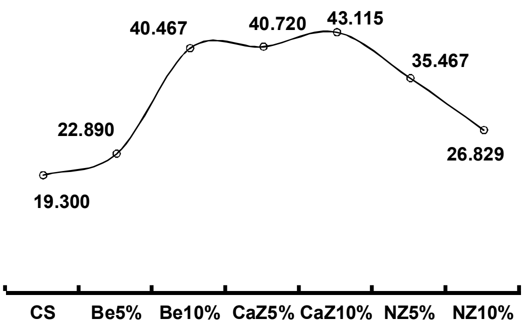

Figure 3c. Mean values for growth (cm) after 24 days.

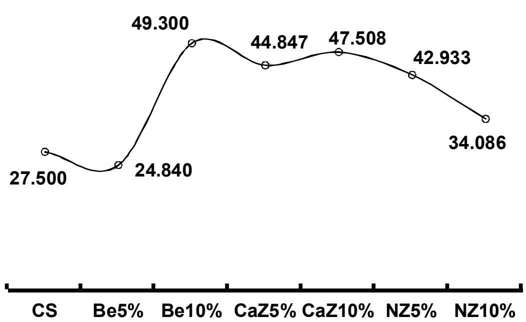

Figure 3d. Mean values for growth (cm) after 31 days.

Legend: CS = control soil, Be5% = bentonite 5%, Be10% = bentonite 10%, CaZ5% = Ca-zeolite-soil 5%, CaZ10% = Ca-zeolite-soil 10%, NZ5% = NH_4-zeolite-soil 5%, NZ10% = NH_4-zeolite-soil 10%

Figure 3. Mean values after 7 days (a), 14 days (b), 24 days (c) and 31 days (d) of growth respectively.

Table 6. Test of Homogeneity of Variances.

Growth (cm)	Levene Statistic	df1	df2	Sig.
after 7 days	10.850	6	52	0.000
after 14 days	4.902	6	52	0.000
after 21 days	1.722	6	52	0.134
after 31 days	1.805	6	52	0.116

Table 7. The F statistic test – ANOVA.

Growth (cm)		Sum of Squares	df	Mean Square	F	Sig.
after 7 days	Between Groups	17.493	6	2.916	1.968	0.087
	Within Groups	77.028	52	1.481	-	-
	Total	94.521	58	-	-	-
after 14 days	Between Groups	1,678.145	6	279.691	6.586	0.000
	Within Groups	2,208.193	52	42.465	-	-
	Total	3,886.338	58	-	-	-
after 21 days	Between Groups	4,567.952	6	761.325	8.500	0.000
	Within Groups	4,657.304	52	89.564	-	-
	Total	9,225.256	58	-	-	-
after 31 days	Between Groups	4,693.844	6	782.307	5.722	0.000
	Within Groups	7,108.792	52	136.708	-	-
	Total	11,802.637	58	-	-	-

Table 8. Multiple Comparisons - Tukey HSD: Growth (cm) after 14 days.

(I) Series	(J) Series	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control-soil	bentonite-soil 5%	-10.110	3.569	0.088	-21.056	0.836
	bentonite-soil 10 %	-14.967*	4.759	0.041	-29.561	-0.373
	Ca-zeolite-soil 5%	-17.453*	3.365	0.000	-27.773	-7.134
	Ca-zeolite-soil 10%	-17.323*	3.429	0.000	-27.839	-6.807
	NH_4-zeolite-soil 5%	-9.817	3.946	0.185	-21.917	2.284
	NH_4-zeolite-soil 10%	-8.514	3.816	0.297	-20.216	3.187
bentonite-soil 5%	bentonite-soil 5%	10.110	3.569	0.088	-0.836	21.056
	bentonite-soil 10 %	-4.857	4.290	0.915	-18.012	8.298
	Ca-zeolite-soil 5%	-7.343	2.660	0.104	-15.502	0.815
	Ca-zeolite-soil 10%	-7.213	2.741	0.138	-15.619	1.193
	NH_4-zeolite-soil 5%	0.293	3.365	1.000	-10.026	10.613
	NH_4-zeolite-soil 10%	1.596	3.211	0.999	-8.252	11.444
bentonite-soil 10 %	bentonite-soil 5%	14.967*	4.759	0.041	0.373	29.561
	bentonite-soil 10 %	4.857	4.290	0.915	-8.298	18.012
	Ca-zeolite-soil 5%	-2.487	4.121	0.996	-15.126	10.152
	Ca-zeolite-soil 10%	-2.356	4.174	0.998	-15.156	10.443
	NH_4-zeolite-soil 5%	5.150	4.608	0.920	-8.981	19.281
	NH_4-zeolite-soil 10%	6.452	4.497	0.781	-7.338	20.243
Ca-zeolite-soil 5%	bentonite-soil 5%	17.453*	3.365	0.000	7.134	27.773
	bentonite-soil 10 %	7.343	2.660	0.104	-0.815	15.502
	Ca-zeolite-soil 5%	2.487	4.121	0.996	-10.152	15.126
	Ca-zeolite-soil 10%	0.130	2.469	1.000	-7.442	7.703
	NH_4-zeolite-soil 5%	7.637	3.148	0.209	-2.016	17.290
	NH_4-zeolite-soil 10%	8.939	2.983	0.059	-0.208	18.086
Ca-zeolite-soil 10%	bentonite-soil 5%	17.323*	3.429	0.000	6.807	27.839
	bentonite-soil 10 %	7.213	2.741	0.138	-1.193	15.619
	Ca-zeolite-soil 5%	2.356	4.174	0.998	-10.443	15.156
	Ca-zeolite-soil 10%	-0.130	2.469	1.000	-7.703	7.442
	NH_4-zeolite-soil 5%	7.506	3.216	0.248	-2.357	17.369
	NH_4-zeolite-soil 10%	8.809	3.055	0.078	-0.560	18.177
NH_4-zeolite-soil 5%	bentonite-soil 5%	9.817	3.946	0.185	-2.284	21.917
	bentonite-soil 10 %	-0.293	3.365	1.000	-10.613	10.026
	Ca-zeolite-soil 5%	-5.150	4.608	0.920	-19.281	8.981
	Ca-zeolite-soil 10%	-7.637	3.148	0.209	-17.290	2.016
	NH_4-zeolite-soil 5%	-7.506	3.216	0.248	-17.369	2.357
	NH_4-zeolite-soil 10%	1.302	3.626	1.000	-9.816	12.420
NH_4-zeolite-soil 10%	bentonite-soil 5%	8.514	3.816	0.297	-3.187	20.216
	bentonite-soil 10 %	-1.596	3.211	0.999	-11.444	8.252
	Ca-zeolite-soil 5%	-6.452	4.497	0.781	-20.243	7.338
	Ca-zeolite-soil 10%	-8.939	2.983	0.059	-18.086	0.208
	NH_4-zeolite-soil 5%	-8.809	3.055	0.078	-18.177	0.560
	NH_4-zeolite-soil 10%	-1.302	3.626	1.000	-12.420	9.816

*. The mean difference is significant at the 0.05 level.

Table 9. Multiple Comparisons - Tukey HSD: Growth (cm) after 24 days.

(I) Series	(J) Series	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control-soil	bentonite-soil 5%	-3.590	5.184	0.992	-19.486	12.306
	bentonite-soil 10 %	-21.167	6.911	0.051	-42.361	0.028
	Ca-zeolite-soil 5%	-21.420*	4.887	0.001	-36.407	-6.433
	Ca-zeolite-soil 10%	-23.815*	4.980	0.000	-39.088	-8.543
	NH_4-zeolite-soil 5%	-16.167	5.731	0.090	-33.740	1.407
	NH_4-zeolite-soil 10%	-7.529	5.541	0.821	-24.522	9.465
bentonite-soil 5%	bentonite-soil 5%	3.590	5.184	0.992	-12.306	19.486
	bentonite-soil 10 %	-17.577	6.230	0.090	-36.681	1.528
	Ca-zeolite-soil 5%	-17.830*	3.864	0.000	-29.678	-5.982
	Ca-zeolite-soil 10%	-20.225*	3.981	0.000	-32.433	-8.018
	NH_4-zeolite-soil 5%	-12.577	4.887	0.156	-27.564	2.410
	NH_4-zeolite-soil 10%	-3.939	4.664	0.979	-18.241	10.364
bentonite-soil 10 %	bentonite-soil 5%	21.167	6.911	0.051	-0.028	42.361
	bentonite-soil 10 %	17.577	6.230	0.090	-1.528	36.681
	Ca-zeolite-soil 5%	-0.253	5.985	1.000	-18.608	18.102
	Ca-zeolite-soil 10%	-2.649	6.062	0.999	-21.238	15.940
	NH_4-zeolite-soil 5%	5.000	6.692	0.989	-15.522	25.522
	NH_4-zeolite-soil 10%	13.638	6.531	0.375	-6.389	33.665
Ca-zeolite-soil 5%	bentonite-soil 5%	21.420*	4.887	0.001	6.433	36.407
	bentonite-soil 10 %	17.830*	3.864	0.000	5.982	29.678
	Ca-zeolite-soil 5%	0.253	5.985	1.000	-18.102	18.608
	Ca-zeolite-soil 10%	-2.395	3.586	0.994	-13.393	8.602
	NH_4-zeolite-soil 5%	5.253	4.572	0.909	-8.766	19.272
	NH_4-zeolite-soil 10%	13.891*	4.332	0.035	0.607	27.176
Ca-zeolite-soil 10%	bentonite-soil 5%	23.815*	4.980	0.000	8.543	39.088
	bentonite-soil 10 %	20.225*	3.981	0.000	8.018	32.433
	Ca-zeolite-soil 5%	2.649	6.062	0.999	-15.940	21.238
	Ca-zeolite-soil 10%	2.395	3.586	0.994	-8.602	13.393
	NH_4-zeolite-soil 5%	7.649	4.671	0.659	-6.675	21.972
	NH_4-zeolite-soil 10%	16.287*	4.437	0.010	2.681	29.893
NH_4-zeolite-soil 5%	bentonite-soil 5%	16.167	5.731	0.090	-1.407	33.740
	bentonite-soil 10 %	12.577	4.887	0.156	-2.410	27.564
	Ca-zeolite-soil 5%	-5.000	6.692	0.989	-25.522	15.522
	Ca-zeolite-soil 10%	-5.253	4.572	0.909	-19.272	8.766
	NH_4-zeolite-soil 5%	-7.649	4.671	0.659	-21.972	6.675
	NH_4-zeolite-soil 10%	8.638	5.265	0.657	-7.508	24.784
NH_4-zeolite-soil 10%	bentonite-soil 5%	7.529	5.541	0.821	-9.465	24.522
	bentonite-soil 10 %	3.939	4.664	0.979	-10.364	18.241
	Ca-zeolite-soil 5%	-13.638	6.531	0.375	-33.665	6.389
	Ca-zeolite-soil 10%	-13.891*	4.332	0.035	-27.176	-0.607
	NH_4-zeolite-soil 5%	-16.287*	4.437	0.010	-29.893	-2.681
	NH_4-zeolite-soil 10%	-8.638	5.265	0.657	-24.784	7.508

*. The mean difference is significant at the 0.05 level.

The analysis of data included in the output listed above reveals that after 7 days the various types of amendments present in soil in various concentrations do not determine significant differences in growth.

After 14 days of growth, the beans cultivated on soil amended with 10% bentonite, with 5% zeolite activated with Ca and respectively 10% zeolite activated with Ca determine significant differences in growth. Therefore, 14 days after plantation there are no significant differences among the plants cultivated on soils with different amendments, but there are differences between the plants cultivated on control-sample and the plants cultivated on soil amended with bentonite and respectively zeolite activated with Ca.

After 24 days, the growth of beans cultivated on soil amended with zeolite activated with Ca at 5% concentration and respectively 10% concentration is significantly different as compared to the growth of beans cultivated on the control-soil, soil amended with 5% bentonite and soil amended with 10% zeolite activated with ammonium. This shows that after 24 days of growth the differences are significant not only among plants cultivated on control-soil as compared to those cultivated on soil with amendment, but also among the plants cultivated on soils with different amendments.

After 31 days, there are significant differences in growth among plants grown on 10% zeolite activated with Ca and both the plants grown on control-soil and on soil amended with 5% bentonite. The growth stimulated by zeolite activated with Ca at 5% concentration and the growth stimulated by bentonite at 10% concentration is also significantly different as compared to the growth stimulated by 5% bentonite. The conclusion is that after 31 days of growth significant differences in growth appear not only among the plants cultivated on control-soil and those on soils with various types of amendments, but also among the growth stimulated by the same amendment (e.g. bentonite) applied in different concentrations.

Table 10. Multiple Comparisons - Tukey HSD: Growth (cm) after 31 days.

(I) Series	(J) Series	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control-soil	bentonite-soil 5%	2.660	6.404	1.000	-16.979	22.299
	bentonite-soil 10 %	-21.800	8.539	0162	-47.985	4.385
	Ca-zeolite-soil 5%	-17.347	6.038	0.080	-35.862	1.169
	Ca-zeolite-soil 10%	-20.008*	6.153	0.031	-38.876	-1.139
	NH_4-zeolite-soil 5%	-15.433	7.080	0.324	-37.145	6.278
	NH_4-zeolite-soil 10%	-6.586	6.846	0.960	-27.581	14.409
bentonite-soil 5%	bentonite-soil 5%	-2.660	6.404	1.000	-22.299	16.979
	bentonite-soil 10 %	-24.460*	7.697	0.038	-48.063	-0.857
	Ca-zeolite-soil 5%	-20.007*	4.773	0.002	-34.645	-5.369
	Ca-zeolite-soil 10%	-22.668*	4.918	0.001	-37.749	-7.586
	NH_4-zeolite-soil 5%	-18.093	6.038	0.059	-36.609	0.422
	NH_4-zeolite-soil 10%	-9.246	5.762	0.680	-26.916	8.424
bentonite-soil 10 %	bentonite-soil 5%	21.800	8.539	0.162	-4.385	47.985
	bentonite-soil 10 %	24.460*	7.697	0.038	0.857	48.063
	Ca-zeolite-soil 5%	4.453	7.395	0.996	-18.224	27.130
	Ca-zeolite-soil 10%	1.792	7.489	1.000	-21.174	24.758
	NH_4-zeolite-soil 5%	6.367	8.268	0.987	-18.987	31.720
	NH_4-zeolite-soil 10%	15.214	8.068	0.499	-9.528	39.957
Ca-zeolite-soil 5%	bentonite-soil 5%	17.347	6.038	0.080	-1.169	35.862
	bentonite-soil 10 %	20.007*	4.773	0.002	5.369	34.645
	Ca-zeolite-soil 5%	-4.453	7.395	0.996	-27.130	18.224
	Ca-zeolite-soil 10%	-2.661	4.431	0.997	-16.248	10.926
	NH_4-zeolite-soil 5%	1.913	5.648	1.000	-15.407	19.233
	NH_4-zeolite-soil 10%	10.761	5.352	0.421	-5.652	27.173
Ca-zeolite-soil 10%	bentonite-soil 5%	20.008*	6.153	0.031	1.139	38.876
	bentonite-soil 10 %	22.668*	4.918	0.001	7.586	37.749
	Ca-zeolite-soil 5%	-1.792	7.489	1.000	-24.758	21.174
	Ca-zeolite-soil 10%	2.661	4.431	0.997	-10.926	16.248
	NH_4-zeolite-soil 5%	4.574	5.771	0.985	-13.122	22.271
	NH_4-zeolite-soil 10%	13.422	5.481	0.200	-3.387	30.231
NH_4-zeolite-soil 5%	bentonite-soil 5%	15.433	7.080	0.324	-6.278	37.145
	bentonite-soil 10 %	18.093	6.038	0.059	-0.422	36.609
	Ca-zeolite-soil 5%	-6.367	8.268	0.987	-31.720	18.987
	Ca-zeolite-soil 10%	-1.913	5.648	1.000	-19.233	15.407
	NH_4-zeolite-soil 5%	-4.574	5.771	0.985	-22.271	13.122
	NH_4-zeolite-soil 10%	8.848	6.505	0.820	-11.101	28.796
NH_4-zeolite-soil 10%	bentonite-soil 5%	6.586	6.846	0.960	-14.409	27.581
	bentonite-soil 10 %	9.246	5.762	0.680	-8.424	26.916
	Ca-zeolite-soil 5%	-15.214	8.068	0.499	-39.957	9.528
	Ca-zeolite-soil 10%	-10.761	5.352	0.421	-27.173	5.652
	NH_4-zeolite-soil 5%	-13.422	5.481	0.200	-30.231	3.387
	NH_4-zeolite-soil 10%	-8.848	6.505	0.820	-28.796	11.101

*. The mean difference is significant at the 0.05 level.

The experiments undertaken and the analysis with the SPSS 16.0 for Windows software lead to the following conclusions:

- the nature (type) and the concentration of amendments in soil do not determine significant differences after 7 days of growth;
- after 14 days of growth, there are no significant differences among the plants cultivated on soils with different amendments; differences occurred only between plants cultivated on the control-soil and plants cultivated on soil amended with bentonite and zeolite activated with Ca respectively;
- after 24 days of growth there are significant differences not only between plants cultivated on control-soil as compared to those cultivated on soil amended, but also between the plants cultivated on soils with different amendments;
- after 31 days of growth there are significant differences not only between the plants cultivated on control-soil and those cultivated on soils with different amendments, or among the plants cultivated on soil with different amendments, but also among the plants cultivated on soil with the same amendment but in different concentrations (e.g. bentonite 5% as compated to bentonite 10%);
- out of all the amendments tested, zeolite activated with Ca stimulates the highest growth rate of the beans cultivated on amended soil.

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REFERENCES

[1] Apiratikul, R., Pavasant, P., (2008): Sorption of Cu^{2+}, Cd^{2+}, and Pb^{2+} using modified zeolite from coal fly ash. Chemical Engineering Journal, 144: 245-258.

[2] Arriagada, R., Garcia, R., Cid, R., (2001): Retention of chromium and mercury with natural and synthetic zeolites. In: Blanco, J., Avila, P., (eds.): Catalizadores y

Adsorbentes para la Proteccion Ambiental en la region Iberoamericana. CYTED, Madrid, pp. 207-211.

[3] Baiker, A.J.M., (1981): Accumulators and excluders – strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3(1-4): 643-654.

[4] Brindley, G.W., Brown, G., (1980): Crystal structures of clay minerals and their X-ray identification. London, Mineralogical Society, (Monograph No. 5), 495 pp.

[5] Bucea-Manea-Ţoniş, R., Bucea-Manea-Ţoniş, R., Epure, M, (2010): SPSS şi EXCEL în analiza datelor statistice în domeniile economic, social, tehnic, Editura AGIR, Bucuresti, 337 p.

[6] Clemens, S., (2006): Toxic metal accumulations, responses to exposure and mechanisms of tolerance in plants. Biochimie 88: 1707-1719.

[7] Garau, G., Castaldi, P., Santona, L., Deiana, P., Melis, P., (2007): Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. Geoderma: 142: 47-57.

[8] Garcia-Sanchez, A., Alastuey, A., Querol, X., (1999): Heavy metal adsorption by different minerals: application to the remediation of polluted soils. The Science of the Total Environment, 242: 179-188.

[9] Inglezakis, V.J., Stylianou, M.A., Gkantzou, D., Loizidou, M.D., (2007): Removal of Pb(II) from aqueous solutions by using clinoptilolite and bentonite as absorbents. Desalination, 210: 248-256.

[10] Iskandar, I.K., Kirkham, M.B., (2001): Trace Elements in Soils: Bioavailability, Flux, and Transfer. CRC/Lewis Publishers, Boca Raton, 287 p.

[11] Kaya, A., Durukan, S., (2004): Utilization of bentonite embedded zeolite as clay liner. Applied Clay Science. 25: 83-91.

[12] Kotchoni, S.O., Gachomo, E.W., (2006): The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. Journal of Biosciences, 31(3): 389-404.

[13] Lin, C.C., Chen, L.M., Liu, Z.H., (2005): Rapid effect of copper on lignin biosynthesis in soybean roots. Plant Science, 168(3): 855-861.

[14] Mier, M.V., Callejas, R.G., Gear, R., Cisneros, B.E.J., Alvares, P.J.J., (2001): Heavy metal removal with Mexican clinoptilolite: multi-component ionic exchange. Water Research 35: 373-378.

[15] Mishra P.K., Prakash, V., (2009): Antioxidant modulation in response to zinc induced oxidative stress at different pH in *Glycine max* L. cv. Merrill. American-Eurasian Journal of Agriculture & Environmental Science 6(4): 485-493.

[16] Nah, I.W., Hwang, K., Shul, Y., (2007): A simple synthesis of magnetically modified zeolite. Powder Technology. 177: 99-101.

[17] Ören, A.H., Kaya, A., (2006): Factors affecting adsorption characteristics of Zn^{2+} on two natural zeolites. Journal of Hazardous Materials 131(1-3): 59-65.

[18] Querol, X., Alastuey, A., Moreno, N., Alvarez-Ayuso, E., Garcia-Sanchez, A., Cama, J., Ayora, C., Simon, M., (2006): Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. Chemosphere, 62(2): 171-180.

[19] Radic, S., Babic, M., Skobic, D., Roje, V., Pevalek-Kozlina, B., (2010): Ecotoxicological effects of aluminum and zinc on growth and antioxidants in *Lemna minor* L.. Ecotoxicology and Environmental Safety, 73(3): 336-342.

[20] Rehakova, M., Cuvanova, S, Dziva, M., Rimar, J., Gavalova, Z., (2004): Agricultural and agrochemical uses of natural zeolite, of the clinoptilolite type. Solid State and Materials Science, 8: 397-404.

[21] Silveira, M.L.A., Alleoni, L.R.F., Guilherme, L.R.G., (2003): Review: Biosolids and heavy metals in soils. Scientia Agricola 60: 793-806.

[22] Teutli-Sequeira, A., Solache-Rios, M., Olguin, M.T., (2009): Influence of Na^{+}, Ca^{2+}, $Mg^{2+}$and NH_4^{+} on the sorption behavior of Cd^{2+} from aqueous solutions by a Mexican zeolitic material. Hydrometallurgy, 97: 46-52.

[23] Trgo, M., Peric, J., Vukojevic Medvidovic, N., (2006): Investigations of different kinetic models for zinc ions uptake by a natural zeolitic tuff, Journal of Environment Management 79: 298-304.

[24] Yusupov, T.S., Shumskaya, L.G., (2002): Control of cation-exchange interaction between zeolites and phosphates on the basis and mechanochemical activation. Journal of Mining Science, 38(2): 177-181.

[25] Wingenfelder, U., Nowack, B., Furrer, G., Schulin, R., (2005): Adsorption of Pb and Cd by amine-modified zeolite. Water Research 39: 3287-3297.

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