African Journal of Agricultural Research Vol. 6(21), pp. 5010-5023, 5 October, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1241
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Efficiency of amendments based on zeolite and bentonite in reducing the accumulation of heavy metals in tomato organs *(Lycopersicum esculentum)* grown in polluted soils

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Accepted 27 August, 2011

The aim of this study is to determine the way in which zeolite, zeolite modified with ammonium ions, zeolite modified with calcium ions and bentonite, influence the accumulation of copper, lead, iron, zinc and cadmium in different organs of tomatoes with each of the four tested at two different concentrations (5 and 10%). Moreover, the influence of the amendments on the content of chlorophyll and carotenoid was evaluated. The germination and biometric analyses demonstrated that the most vigorous plants were those grown on substrate containing zeolite-Ca and zeolite-NH_4. The accumulation of iron in the tomato plants can be reduced by using the zeolite modified with ammonium ions as amendment. All the investigated adsorbents are efficient to reduce the content of copper and lead in tomato organs. All the amendments tested are found to induce the increase of chlorophyll content in tomato leaves. The statistical analyses revealed that only the cooper concentration varies significantly between reference substrate (with no amendment) and each of those with amendments.

Key words: X-ray diffraction, heavy metals, chlorophyll, carotenoid, tomato, zeolite, bentonite, fourier transformed infrared spectroscopy (FTIR), statistical analyses.

INTRODUCTION

As in most cases metals outflow on surrounding soils, researching the toxic effect of heavy metals on plants is a topic of enormous interest. In polluted soils, such heavy metal ions as cooper, lead, cadmium, iron and zinc are either free ions or in different soluble forms, whose mobility depends on the pH (Lopez-Millan et al., 2009; Guala et al., 2010). These heavy metal ions are known to cause damage even at very low concentrations.

The physiological effects of the toxicity of these heavy metal ions are inhibition of seed germination, major reduction in growth rates (Larbi et al., 2002), changes in photosynthetic efficiency, respiration and transpiration and alterations in nutrient homeostasis (Larbi et al., 2002; Dong et al., 2006). At the cellular level, the toxicity of heavy metals is known to cause alterations such as membrane damage, disruption of electron transport, inhibition/activation of enzymes and interaction with nucleic acids (Leon et al., 2002; Chen et al., 2003a), production of reactive oxygen which are highly toxic and must be detoxified by cellular stress responses, if the plant is to survive and grow (Gratao et al., 2005).

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Gratao et al. (2008, 2009) have waste experience in researches focused on the studies of the morphological and biochemical changes in tomato hormonal mutants exposed to cadmium ions. They concluded that the detrimental effect of cadmium was significant observed after 20 days of exposure of the roots to the cadmium ions. At a relative high concentration, cadmium ions have penetrated the root-shoot barrier and have been accumulated in fruits which also exhibited reduced growth. At lower concentration of cadmium ions, the lipid peroxidation was pronounced in the roots, whereas in leaves, the lipid peroxidation activity increased at higher cadmium concentration. Moreover, cadmium ions caused changes in the intercellular spaces in leaves, depending on the tomato specie. Regarding to the enzymatic activity, the catalase activity was found to increase with the cadmium concentration, whereas the guaiacol peroxidase, glutathione reductase and superoxide dismutase activities in roots and leaves did not changed significantly as compared to the control. Roots of the mutant tomato were lower, in diameter, after exposure to the cadmium ions (Gratao et al., 2008, 2009).

Monteiro et al. (2011) have investigated the influence of cadmium and sodium ions on the biochemical changes of ethylene-insensitive "*Never ripe"* tomato mutant as compared to the control specie. They showed that the accumulation of sodium and cadmium in organs of "*Never ripe"* tomato reduced the leaf chlorophyll degradation. "*Never ripe"* fruits showed increased H_2O_2 production, reduced and enhanced ascorbate peroxidase activity in NaCl and $CdCl_2$, respectively, and enhanced guaiacol peroxidase activity in NaCl. Overall the results indicate that the ethylene signaling associated with "*Never ripe"* receptor can modulate the biochemical pathways of oxidative stress in a tissue dependent manner, and that this signaling may be different following Na and Cd exposure (Monteiro et al., 2011).

The zeolites have been shown to have great potential for a number of applications in various fields such as: absorption, separation, ion exchange and catalysis. They can be used as efficient adsorbents for heavy metals from polluted soils, their utilization being thus a solution to reduce the accumulation of heavy metals in plants, and particularly for those destined for human consumption (Castaldi et al., 2005; Shi et al., 2009). Zeolites are microporous crystalline solids with well defined structures consisting of a three-dimensional network of SiO_4 and AlO_4 tetrahedrally linked together by common oxygen atoms. The zeolites act as molecular sieve and as ionic exchanger, the cations such as sodium, potassium, calcium and magnesium, located at specific sites in the channel-void system of zeolite being replaced with the metal ions. This causes new chemical bonds to be formed and the initial structure to be deformed. These variations can be seen by infrared spectroscopy, a source of crucial information on the changes in zeolite structure caused by the exchange with cations of different ionic radius and charge. Moreover, the modification of zeolite with ammonium and calcium ions will improve the adsorption capacity, due to the increase of the exchange rate between the ammonium and calcium ions and heavy metal ions.

The relevance of tomato (*Lycopersicon esculentum*) in human nutrition is increasing, since it is generally considered as a healthy food because of the high content of lycopene and other health promoting natural compounds. However, tomato is a constituent of the Mediterranean diet ranking as one of the most important vegetables by size of the area cultivated and by volume of production (122.000 ha and 4,500,000 t/year) (Lopez-Millan et al., 2009).

The aim of this study is to investigate the efficiency of bentonite, and of zeolite in its unmodified and respectively modified forms, as amendments in reducing the concentration of heavy metals in tomatoes grown from seeds collected from unpolluted and polluted areas.

MATERIALS AND METHODS

Purchasing and preparation of the amendments based on zeolite

The natural zeolitic tuff was procured from Stoiana Parlisa, Cluj county, Romania (coordinates: 46°40' N 23°55' E).

The zeolite modified with calcium as well as the zeolite modified with ammonium was prepared by adding 100 g zeolite in 1 L solution 1 M $CaCl_2$ (Chemical Company, Iasi, Romania) or NH_4Cl (Chemical Company, Iasi, Romania), respectively. After 24 h the solid phases were separated from the solutions, washed using ultrapure water until removal the Cl^- ions (controlled with $AgNO_3$ solution) and dried at 105°C using a Binder oven for 24 h.

Bentonite was procured from Mediesul Aurit village bentonite processing plant, Satu Mare County (Romania), containing 75% montmorillonite, were used. Soil collected from a polluted area near Baia Mare (coordinates: 47°39' N 23°33' E), the largest city in Maramures County, was mixed with natural zeolite, with zeolite modified with calcium ions and respectively with zeolite modified with ammonium ions and with bentonite. Samples of soils were produced for 5 and 10% mass percentage of each of the afore-mentioned amendments, respectively (namely, zeolite, zeolite with calcium, zeolite with ammonium and bentonite), resulting in eighth soil samples with amendments.

Seeds of tomato (*L. esculentum)* collected from a polluted area (Baia Mare city) and respectively from an unpolluted area (Calinesti, a village in Satu Mare County, coordinates: 47°47' N, 22°53' E) (20 seeds from each type) were planted in plastic pots containing 300 g of each of the type of soil samples with zeolite and bentonite content as defined earlier. The pots were watered with distilled water on a regular basis. The plants were allowed to grow for 53 days, at 20 to 22°C.

Characterization of the amendments based on zeolite

The zeolite structure was determined from 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.

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).

Type	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

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 it was used. Sample wafers consisted in 10% sample in spectral quality KBr.

Germination and biometric measurements

The percentage of seeds germination and the rate of plant growth were calculated. The percentage of seeds germination was calculated as follows:

Seed germination (%) = (number of germinated seeds / total number of seeds) × 100 (Equation 1)

Determination of the metal concentration and of the photosynthetic pigments content

The content of heavy metal in soil used as base for the substrate was determined. After 53 days of growth, samples of roots, shoots and leaves were collected and used to determine the concentration of metal and the content of photosynthetic pigments.

Determination of the metal concentration

This includes three stages:

1. Drying for 5 days at 60°C for soils as well as for vegetal samples;
2. Mineralization in a Berghof MWS2 microwave system; 1 g soil was treated with 12 ml royal water (9 ml solution HCl 37%, Lach-Ner and 3 ml HNO_3 65%, Lach-Ner); 0.3 g of dried vegetal sample was treated with 10 ml nitric acid 63%; after 15 min necessary for the initiation of the mineralization process, the mixtures were introduced in the microwave system and the obtained liquid was diluted with ultrapure water up to 50 ml in the case of soils and up to 25 ml in the case of vegetal samples;
3. Analysis of metal concentration using AAS 800 Perkin Elmer spectrophotometer.

The results represent the average value of three determinations, for which the standard deviation, calculated using the Microsoft Excel software, was lower than 5%.

Determination of the translocation factor (TF)

This was done using the formula:

TF = content of metal in vegetal sample (mg/g) / content of metal in reference substrate (mg/g) (Equation 2)(Li et al., 2010).

Determination of the chlorophyll and carotenoid content

A sample of 0.05 g dried leaves was mixed with 4 ml solution containing 80% (mass percentage) acetone (Chemical Company, Iasi, Romania), 19.5% (mass percentage) ultrapure water, and 0.5% (mass percentage) ammonium solution with 25% concentration (Chemical Company Iasi, Romania). The mixture was stirred in a Hettich centrifugal device at 6 000 rotations/min, for 20 min. The aliquot part was diluted 1:3 (volume ratio) with ultrapure water. The obtained liquid was used to spectrometric measurements on T60U Spectrometer UV-Vis, PG Instruments. The absorbance at 645, 645 and 663 nm was determined. The content of chlorophyll (*a* and *b)* and carotene was determined using the formulas:

Chlorophyll *a* and *b* (mg/g sample) = (20.2 × Abs 645 + 8 x Abs 663) × FD × m (Equation 2)

Carotenoid (mg/g sample) = (Abs 480 + 0.114 × Abs 663 - 0.638 x Abs 645) × FD × m (Equation 3)

Where: Abs 480 is absorbance at 480 nm; Abs 645 is absorbance at 645 nm; Abs 663 is absorbance at 663 nm; FD is factor of dilution (FD = 3); and m is sample mass (g) (m = 0.05 g) (Li et al., 2010).

The results represent the average value of three determinations, for which the standard deviation, calculated using the Microsoft Excel software, was lower than 5%.

Statistical analyses

A one-way analysis of variance (one-way ANOVA) was carried out to compare the mean values measured for all the batches tested and to establish if there are significant differences between the concentration of heavy metal from control substrate and those containing zeolite and bentonite, respectively. Where significant *P*-value ($P < 0.05$) was obtained, differences between individual means were compared using a LSD and post-hoc Tukey`s HSD test ($P < 0.05$) (Bucea-Manea-Tonis et al., 2010).

The correlation coefficients between the values of chlorophyll and carotenoid were calculated using the Microsoft Excel software.

RESULTS AND DISCUSSION

Characterization of the amendments

Table 1 includes the morphological and structural characteristics of zeolite and bentonite. The structure of zeolite and bentonite was crystalline. The average diameter of the zeolite and bentonite particles was in the range 70.21 to 75.74 nm. The specific surface area of the

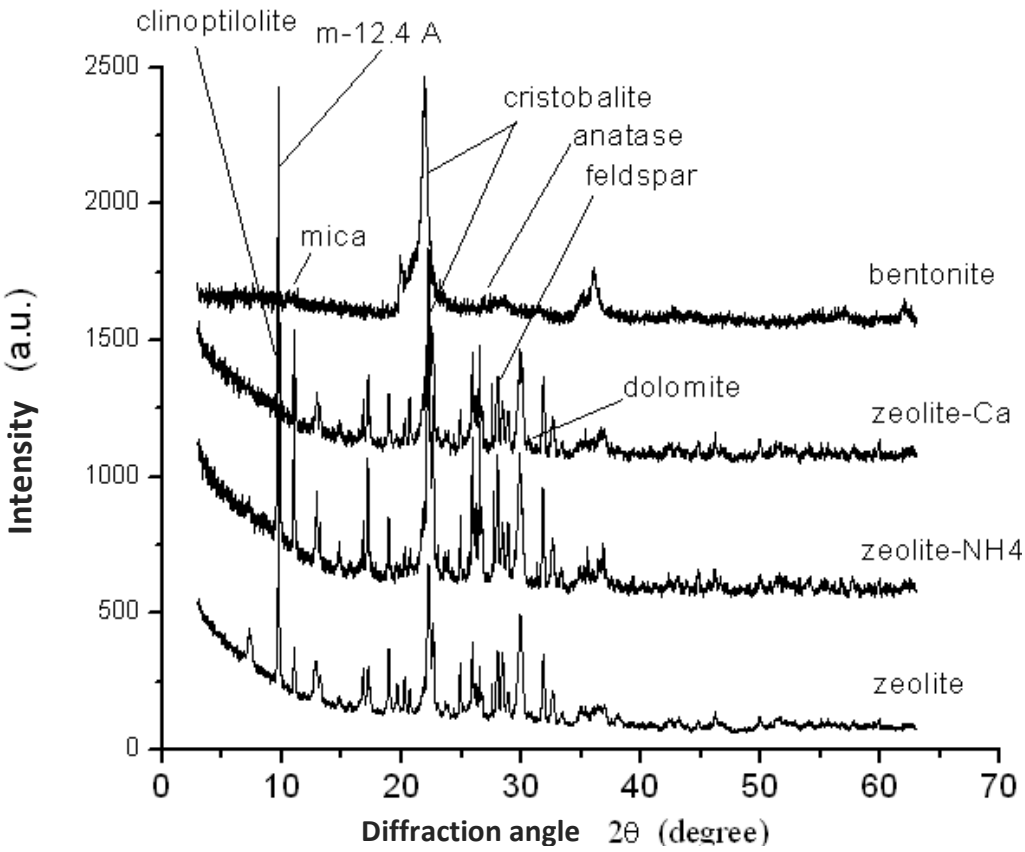

Figure 1. X-ray diffraction patterns of zeolite, zeolite modified with calcium ions (zeolite-Ca), zeolite modified with ammonium ions (zeolite-NH_4) and bentonite (m-12.4 Å: Ca-montmorrilonite, calcite, quartz, anatase, dolomite, pyrite, mica).

two materials was in the range 54.67 to 75.27 m^2/g. The diameter of particles was in inverse ratio to the specific surface area of the two materials.

Additionally, the crystalline structure of unmodified and modified zeolite and of bentonite could be observed by analyzing the X-ray diffraction patterns (Figure 1.) In X-ray diffraction pattern of the bentonite one observes a peak (at 10 theta degree) corresponding to mixture of Ca-montmorillonite, calcite, quartz, anatase, dolomite, pyrite and mica and separate peaks corresponding to cristobalite, anatase and mica. Different peaks, corresponding to clinoptilolite, mica, cristobalite, feldspar and dolomite could be noticed in the X-ray diffraction patterns of zeolite.

To establish if the zeolite structure was modified with calcium or ammonium ions, FTIR analyses were performed (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 tetrahedral or alumino- and silico-oxygen bridges (Castaldi, 2005). Introduction of non-tetrahedral cations into alumino-silicate frameworks can change their FTIR spectra in the range of pseudo-lattice vibrations located at about 1028 to 1036 cm^{-1} and 700 to 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 to 1036 cm^{-1} and at 600 to 602 cm^{-1}, which can be attributed to pseudo-lattice ring vibrations of SiO_4 or AlO_4 tetrahedral and particularly to the inter-tetrahedral bonds vibrations (Castaldi et al., 2005).

Table 2 includes details about 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 have demonstrated different intensities of vibration of certain bands, thus suggesting 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).

Germination and biometric measurements

The substrate on which the plant grows has an influence on the percentage of germination of tomato seeds

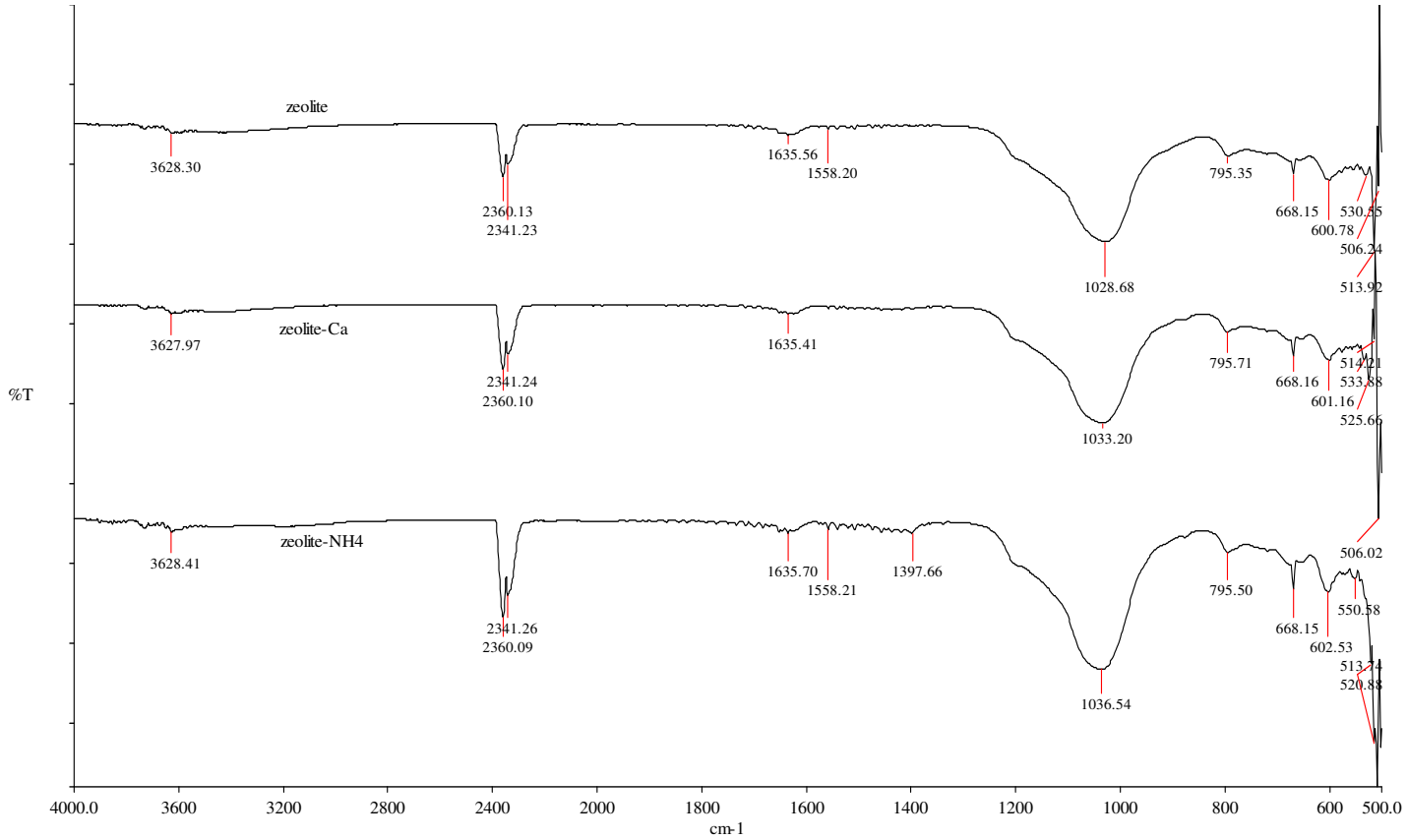

Figure 2. FTIR spectra of zeolite, zeolite modified with calcium ions (zeolite-Ca) and zeolite modified with ammonium ions (zeolite-NH_4).

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 % × 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

collected from a polluted area as revealed in Figure 3. The germination percentage varied between 50 and 90%. The germination percentage was highest for the seeds cultivated on soil with 5% zeolite and was lowest for seeds in soil with 10% bentonite.

The germination percentages of seeds from unpolluted area sowed on different substrates (Figure 4) were in the 15 to 65% range, lower than those observed in the case of seeds from polluted area. This behavior could be explained by the fact that the seeds collected from unpolluted area were more vulnerable to the presence of heavy metal ions from the substrate (soil collected from polluted area) than the seeds collected from the polluted area (Stefanov et al., 1995). The highest germination percentage was obtained when the tomato seeds collected from unpolluted area were sown on substrate with zeolite 5% (65%) and the lowest value was obtained on substrate with bentonite 10% (12%).

After 7 days, the tomato plantlets grew as shown in Figure 5; this graph reveals how the length of growth depends on the substrate used. The seeds originating from polluted soil have generated longer plantlets than the seeds originating from unpolluted soil, as a consequence of the adaptive capacity of those plants to environmental conditions (Stefanov et al., 1995). Zeolite represents a stimulus for the development of plants, as Figure 5 demonstrates: the number of plantlets reaching 1 cm in length was substantially higher in substrate with 5% calcium and respectively with 10% ammonium.

After 8 days of growth, the substrate with 5 and 10% zeolite generated the highest number of 5 cm-long plantlets, as shown in Figure 6. The tomato plantlets do

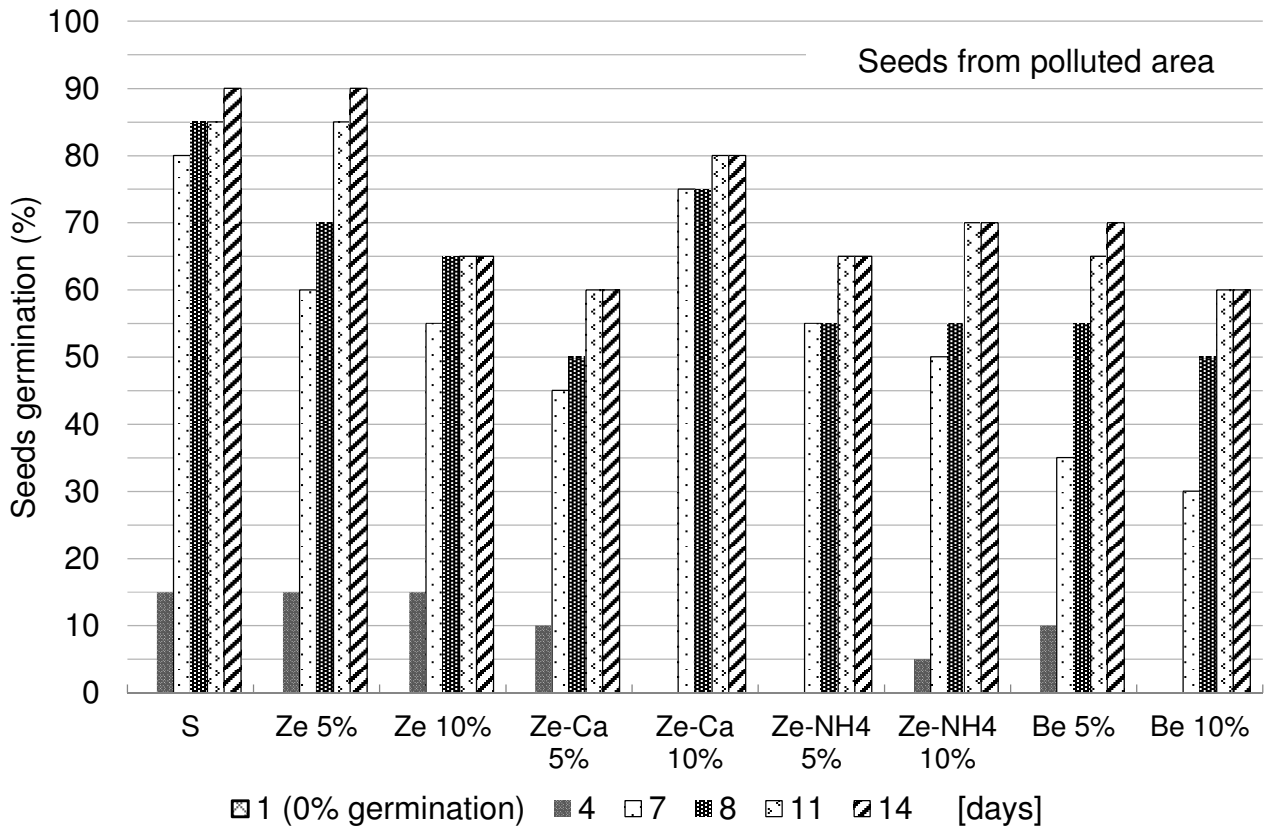

Figure 3. Germination percentage of tomato seeds collected from a polluted area sowed on different substrates (substrate, substrate with zeolite 5%, substrate with zeolite 10%, substrate with zeolite-Ca 5%, substrate with zeolite-Ca 10%, substrate with zeolite-NH_4 5%, substrate with zeolite-NH4 10%, substrate with bentonite 5%, and substrate with bentonite 10%).

not found the proper environment for growth in the substrate with bentonite.

After 11 days of growth, the number of tomato plantlets reaching 6 cm in length was highest in the substrate with zeolite-Ca 10%. Additionally, length of tomato plantlets grown on substrates with zeolite was higher than that of the plantlets grown on the other substrates (Figure 7).

After 14 days of growth (Figure 8), the growth pattern of plantlets was similar to the pattern remarked after 11 days of growth. The most developed plants have reached 8 cm in length and were those grown on substrate with zeolite-Ca10%. Only the substrate with zeolite was established as hosting 9 cm-long plantlets.

The developmental differences among the plantlets growing on different substrates are those photographically suggested in Figure 9. The substrate with zeolite-Ca and respectively with zeolite-NH_4 was host to the most vigorous plants, as a consequence of the involvement in the metabolic processes of those plants of the Ca and NH_4 ions.

Determination of the metal concentration

The contents of heavy metals in the soil used for the substrate preparation were: 45.79 mg/g iron, 18.65 mg/g copper, 15.95 mg/g lead, 18.98 mg/g zinc and 8 mg/g cadmium.

In Table 3 the percentages of the translocation factors of copper, iron, lead, zinc and cadmium ions from substrate in different parts of tomatoes grown on different substrates are presented. The TF values of copper and lead are lower than those of cadmium, zinc and iron. This was explained by the fact that lead ions present the lowest dehydration energy (1480 kJ/mol) as compared to the copper ions (2100 kJ/mol), iron ions (1920 kJ/mol), zinc ions (2044 kJ/mol) or cadmium ions (1806 kJ/mol) (Horwood, 1978). Low dehydration energy induced a higher capacity of exchange with cations from the zeolite or bentonite network (sodium, calcium, ammonium, etc).

The lower TF percentage of divalent cooper ions was explained by the fact that at pH was 4.76 (which was the average pH of the substrate) the copper ions are in the form, which presents very low hydration sphere. Thus, the migration rate of $Cu(OH)^+$ complex inside the zeolite or bentonite networks increased as well as the exchange rate.

The partitioning of metals in different parts of the plant was a common strategy to prevent the intoxication of the aerial parts, which was due to the binding of metals with

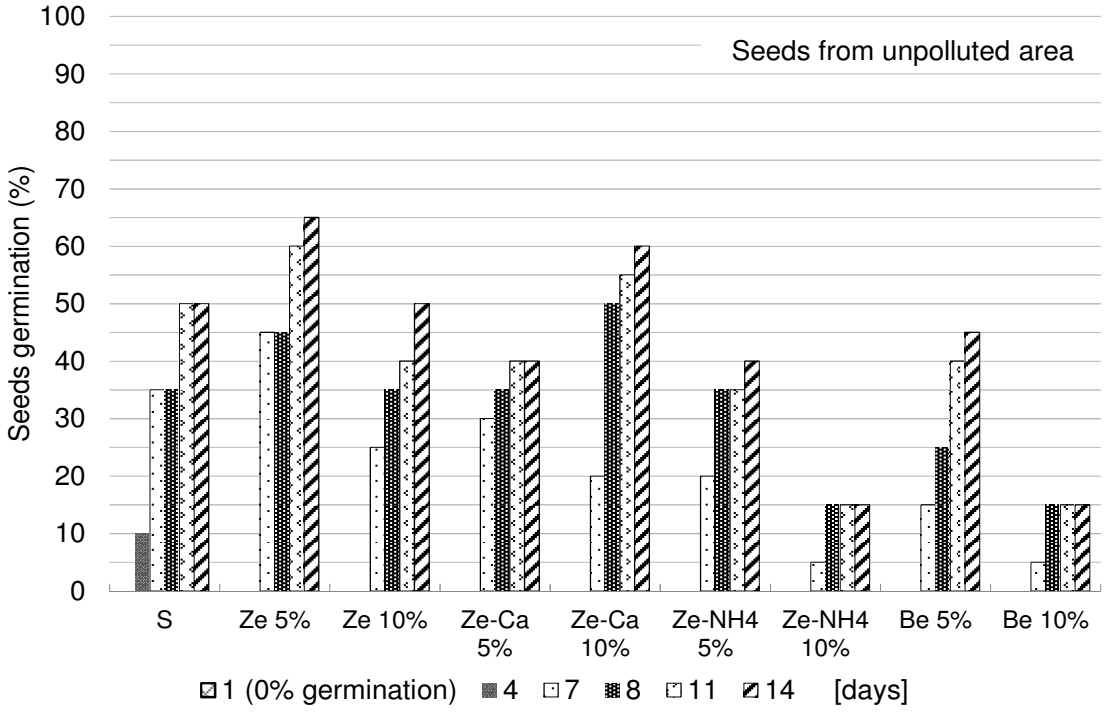

Figure 4. Germination percentage of tomato seeds collected from unpolluted area sowed on different substrates (substrate, substrate with zeolite 5%, substrate with zeolite 10%, substrate with zeolite-Ca 5%, substrate with zeolite-Ca 10%, substrate with zeolite-NH_4 5%, substrate with zeolite-NH4 10%, substrate with bentonite 5%, and substrate with bentonite 10%).

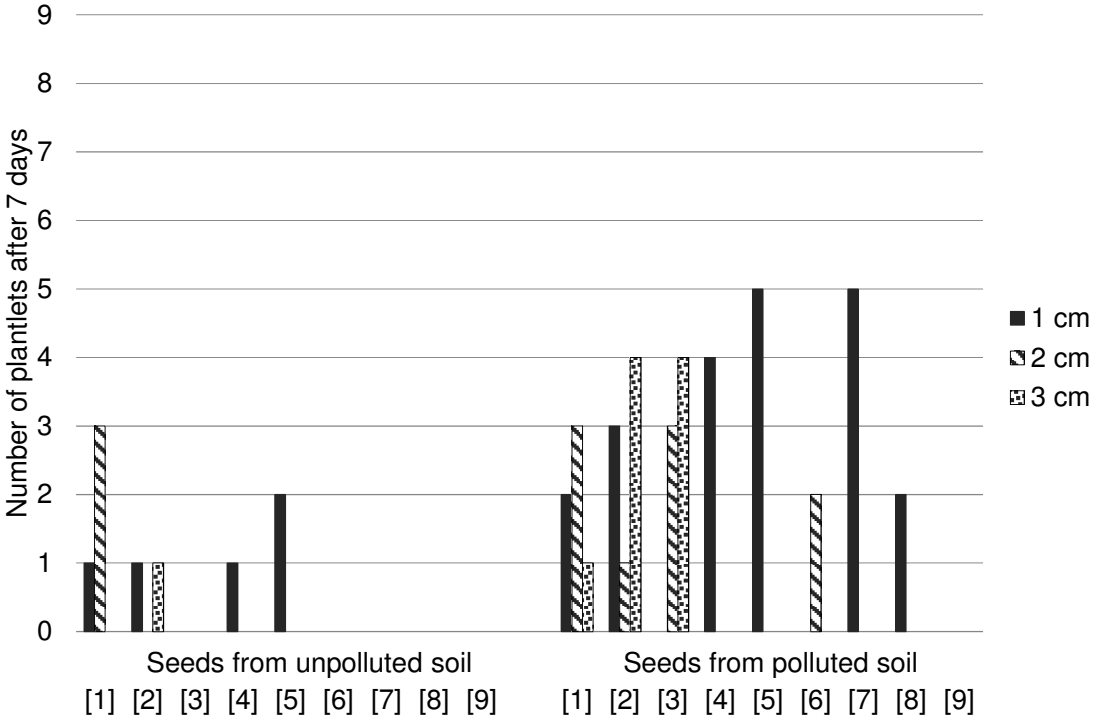

Figure 5. Variation of the number and length of the tomato plantlets grown on different substrates in 7 days ([1] – substrate, [2] – substrate with zeolite 5%, [3] – substrate with zeolite 10%, [4] – substrate with zeolite-Ca 5%, [5] – substrate with zeolite-Ca 10%, [6] – substrate with zeolite-NH_4 5%, [7] – substrate with zeolite-NH4 10%, [8] – substrate with bentonite 5%, [9] – substrate with bentonite 10%).

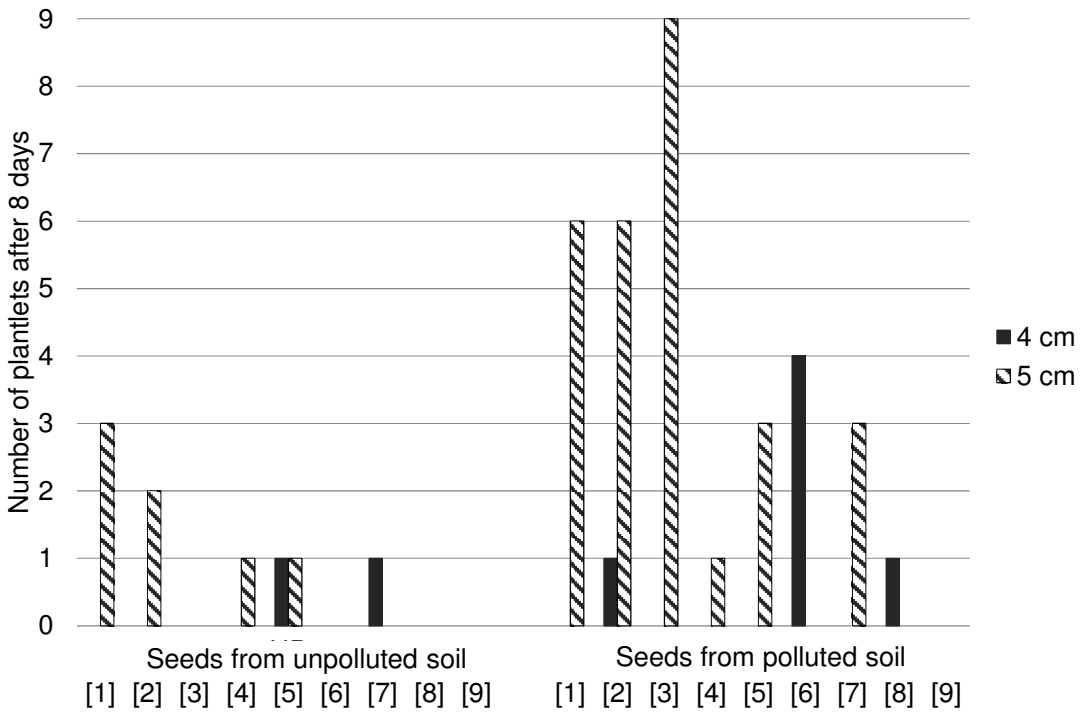

Figure 6. Variation of the number and length of the tomato plantlets grown on different substrates in 8 days ([1] – substrate, [2] – substrate with zeolite 5%, [3] – substrate with zeolite 10%, [4] – substrate with zeolite-Ca 5%, [5] – substrate with zeolite-Ca 10%, [6] – substrate with zeolite-NH4 5%, [7] – substrate with zeolite-NH4 10%, [8] – substrate with bentonite 5%, [9] – substrate with bentonite 10%).

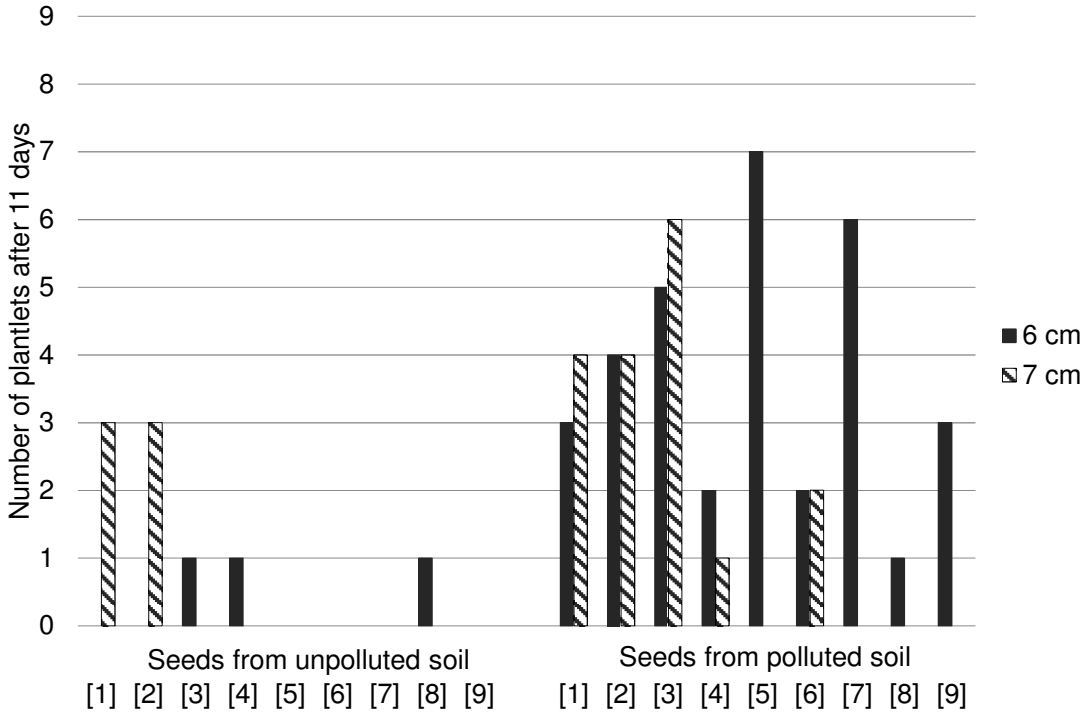

Figure 7. Variation of the number and length of the tomato plantlets grown on different substrates in 11 days ([1] – substrate, [2] – substrate with zeolite 5%, [3] – substrate with zeolite 10%, [4] – substrate with zeolite-Ca 5%, [5] – substrate with zeolite-Ca 10%, [6] – substrate with zeolite-NH4 5%, [7] – substrate with zeolite-NH4 10%, [8] – substrate with bentonite 5%, [9] – substrate with bentonite 10%).

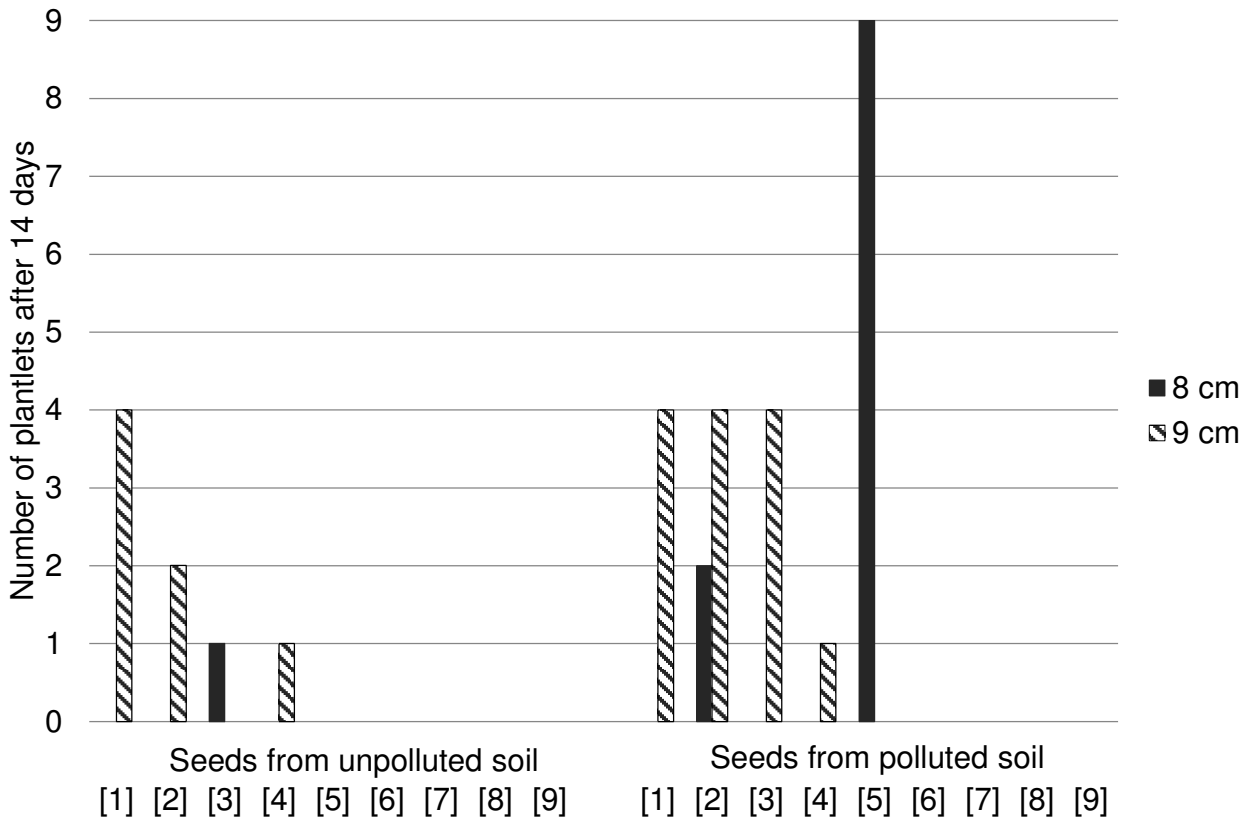

Figure 8. Variation of the number and length of the tomato plantlets grown on different substrates in 14 days ([1] – substrate, [2] – substrate with zeolite 5%, [3] – substrate with zeolite 10%, [4] – substrate with zeolite-Ca 5%, [5] – substrate with zeolite-Ca 10%, [6] – substrate with zeolite-NH4 5%, [7] – substrate with zeolite-NH4 10%, [8] – substrate with bentonite 5%, [9] – substrate with bentonite 10%).

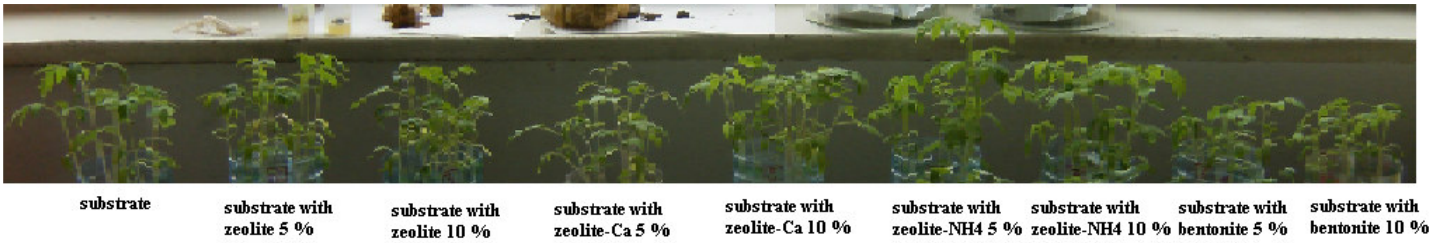

Figure 9. Macroscopic view of the tomato plantlets during 53 days of growing on different substrates (substrate, substrate with zeolite 5%, substrate with zeolite 10%, substrate with zeolite-Ca 5%, substrate with zeolite-Ca 10%, substrate with zeolite-NH_4 5%, substrate with zeolite-NH4 10%, substrate with bentonite 5%, substrate with bentonite 10%).

the ligands having high affinity for the metals. In general, the highest accumulation took place in the roots, followed by the aerial parts, a conclusion which was compliant with the findings of Singh et al. 2004 and Sinha et al. 1999.

The TF in roots generated by polluted seeds grown in substrate with no amendment was higher than that in roots originating from unpolluted seeds in the same substrate. However, the shoots and the leaves of plants grown in substrate with no amendment out of pollutes seeds accumulated less metal ions than the shoots and leaves grown out of unpolluted seeds cultivated in the same substrate.

The mechanism developed by plants grown in polluted areas to inhibit the movement of heavy metal ions from the root toward the shoot and afterwards toward leaves explained this behavior, whose effect is the attenuation of the destructive effect of heavy metals on the life of the

Table 3. The translocation factor of Cu, Fe, Pb, Zn and Cd from substrate in different parts of tomato grown on different substrates (Unpoll – tomato grown from seeds collected from unpolluted area, Poll – tomato grown from seeds collected from polluted area).

Amendment	Plant part	Fe (%)		Cu (%)		Pb (%)		Zn (%)		Cd (%)	
		Unpoll	Poll	Unpoll	Poll	Unpoll	Poll	Unpoll	Poll	Unpoll	Poll
No	Root	6.29	61.98	2.82	14.33	0.47	4.46	0.66	5.62	1.14	64.88
	Shoot	3.50	6.25	8.16	0.14	0.50	3.64	3.80	4.94	2.82	3.12
	Leave	3.29	10.51	9.41	0	5.84	0.48	2.87	3.27	3.16	5.13
Zeolite 5%	Root	72.37	65.98	0.14	0	5.19	4.39	8.59	8.56	11.34	12.96
	Shoot	16.86	11.16	0	0	0.12	1.99	4.45	4.68	4.18	3.36
	Leave	11.41	7.99	0.07	0	1.85	0.77	3.89	5.65	6.86	4.58
Zeolite 10%	Root	63.73	67.46	0	0	0.39	1.72	11.02	7.96	29.54	12.50
	Shoot	17.04	8.62	0	0	0.18	1.29	6.10	4.85	8.12	3.12
	Leave	6.66	7.38	0	0	0.07	1.54	5.13	2.68	13.58	5.59
Zeolite-Ca 5%	Root	61.88	67.36	0	0	1.32	6.54	8.11	6.81	18.82	12.36
	Shoot	6.24	8.64	0	0	1.28	0.56	5.17	5.21	7.76	4.82
	Leave	4.67	1.69	0	0	2.63	0	3.63	1.75	8.84	4.15
Zeolite-Ca 10%	Root	70.09	59.89	0	0	10.30	0	36.18	4.95	23.37	8.26
	Shoot	5.65	3.46	0	2.28	1.87	0.85	4.54	3.59	6.70	2.09
	Leave	7.24	2.06	0	0	0.25	0.11	2.98	1.61	6.72	2.79
Zeolite-NH4 5%	Root	43.60	23.18	0.63	2.90	0	0	17.39	7.66	26.03	5.50
	Shoot	17.01	3.82	0.52	1.92	9.49	0.16	9.47	6.02	6.81	1.93
	Leave	13.89	2.41	1.85	2.23	0	0	4.99	2.01	10.73	2.83
Zeolite-NH4 10%	Root	4.26	33.77	4.19	3.66	0	0	1.59	9.20	7.56	7.21
	Shoot	4.98	2.82	18	0.67	0	0	8.83	8.12	18.65	2.15
	Leave	3.71	2.77	12.42	0.88	0	0	4.82	2.66	19.78	3.73
Bentonite 5%	Root	47.06	42.57	1.69	0	0	0	10.11	7.30	33.20	25.87
	Shoot	7.12	4.49	0.21	0.22	0	0	5.86	5.60	12.93	7.03
	Leave	6.18	2.69	0	0	0	0	4.62	2.84	19.68	9.63
Bentonite 10%	Root	28.85	49.00	0	0	0	0	11.48	8.37	63.03	32.03

Table 3. Contd.

	Shoot	3.18	4.32	0	0	0	0	63.09	4.05	18.22	5.58
	Leave	5.35	4.12	0	0	0	0	4.24	4.51	16.69	5.16

plant.

The lower TF of iron in roots generated from unpolluted seeds was obtained by growing on zeolite-NH4 10% and in roots generated from polluted seeds was obtained by growing on zeolite-NH4 5%, thus the zeolite modified with ammonium ions is an efficient amendment to reduce the accumulation of iron in roots.

The percentage of TF of copper in roots generated from unpolluted and polluted seeds, grown on substrates with amendments was lower than that in roots grown on soil without amendment. This demonstrated that all the investigated absorbent systems are efficient in reduction of copper accumulation in tomato roots.

The TF values of lead in roots generated from unpolluted and polluted seeds grown on substrates with zeolite 10%, zeolite-NH4 5 and 10% and bentonite 5 and 10% were lower than those observed in the same roots grown on soil with no amendment, suggesting that the afore-mentioned materials are more efficient in decrease of lead accumulation in roots.

The TF values of zinc in roots generated from unpolluted and polluted seeds grown on substrates with amendments were higher than those observed in the same roots grown on soil with no amendment, suggesting that the afore mentioned materials are inefficient in reduction of zinc and cadmium accumulation in roots.

The TF values of cadmium in roots generated from unpolluted seeds grown on substrates with amendments were higher than those observed in the same roots grown on soil with no amendment, suggesting that the investigated amendments are inefficient in reduction of cadmium accumulation in roots grown from unpolluted seeds. In contrast, all the used amendments were efficient in reducing the cadmium accumulation in root grown from polluted seeds.

In Table 4 are presented the parameters of the one-way ANOVA statistical analyses performed for each heavy metal ion determined in roots, shoots and leaves of tomato grown in different substrates. The values of significance (*Sig.*) must be lower or equal to 0.05 for significant differences between the results obtained for control and those obtained in the case of amendments using. Value of significance lower than 0.05 was obtained only in the case of copper. For iron, lead, zinc and cadmium, the significant values were higher than 0.05, suggesting that the using of the investigated amendments is significant only for reducing the accumulation of copper in roots, shoots and leaves of tomato.

In Table 5 are presented the significance values of the differences between individual means compared using LSD and post-hoc Turkey`s HSD test ($P < 0.05$) for roots, shoots and leaves of tomato grown on different substrates. The significant values (lower than 0.05) were obtained for copper, as well as in the case of ANOVA statistical processing, excepting cases of zeolite-Ca^{2+}-10%, zeolite-$NH4^{+}$-5%, zeolite-$NH4^{+}$-10% and bentonite-5%. In the case of zeolite-Ca^{2+}-10% and bentonite-5% the values are close to 0.05, indicating the existence of significance.

Determination of the chlorophyll and carotenoid content

Table 6 includes details about the content of chlorophyll and carotenoid. There were higher content of chlorophyll in the leaves of tomatoes grown in substrate with zeolite 5%, or with zeolite-Ca 10%, or with zeolite-NH4 5% and bentonite than in the reference substrate. Except the substrate with zeolite-Ca 5%, the content of chlorophyll was higher in all the tomatoes originating from polluted seeds grown in substrate with amendment as compared to the reference substrate. This was in tune with the conclusion about the content of heavy metals in leaves. The content of chlorophyll increases as the concentration of heavy metals decreases, as a consequence of their participation to the biosynthesis of chlorophyll (Li et al., 2010). However, the reduction of chlorophyll content at a higher concentration of heavy metals could be attributed to the interference of heavy metals in the formation of chlorophyll through direct inhibition of an enzymatic step (Van Assche and Clijsters, 1990). A higher content of chlorophyll in tomatoes grown on substrate with amendments as compared to the content determined in leaves grown on substrate without amendment was a proof for the decrease of the concentration of heavy metals, as a consequence of the positive

Table 4. Parameters of the One-Way ANOVA statistical analyses performed for each metal ion found in roots, shoots and leaves of tomato grown in different substrates.

Metal	Sum of squares	df	Mean square	F	Sig.
Copper					
Between groups	337.126	8	42.141	4.49	0.000*
Within groups	422.373	45	9.386		
Total	759.500	53			
Iron					
Between groups	2461.323	8	307.665	0.523	0.833
Within groups	26449.278	45	587.764		
Total	28910.701	53			
Lead					
Between groups	52.137	8	6.517	1.239	0.299
Within groups	236.658	45	5.259		
Total	288.795	53			
Zinc					
Between groups	571.484	8	71.436	0.808	0.599
Within groups	3979.378	45	88.431		
Total	4550.862	53			
Cadmium					
Between groups	1356.517	8	169.565	0.989	0.457
Within groups	7712.98	45	171.400		
Total	9069.497	53			

* The mean difference is significant at the 0.05 level.

Table 5. Significance values of the differences between individual means compared using LSD and post-hoc Tukey`s HSD test ($P < 0.05$) for roots, shoots and leaves of tomato grown on different substrates.

Test		Amendment	Cu	Fe	Pb	Zn	Cd
Tukey HSD	No	Zeolite-5%	0.049*	0.970	1.000	1.000	0.996
		Zeolite-10%	0.047*	0.990	0.931	1.000	1.000
		Zeolite-Ca^2–5%	0.047*	0.999	1.000	1.000	1.000
		Zeolite-Ca^2–10%	0.079	0.999	1.000	0.984	0.999
		Zeolite-NH_4^+-5%	0.343	1.000	0.998	0.996	1.000
		Zeolite-NH_4^+-10%	1.000	1.000	0.596	1.000	1.000
		Bentonite – 5%	0.076	1.000	0.596	1.000	0.999
		Bentonite – 10%	0.047*	1.000	0.767	0.424	0.914
LSD	No	Zeolite-5%	0.002*	0.273	0.892	0.656	0.420
		Zeolite-10%	0.002*	0.861	0.206	0.614	0.864
		Zeolite-Ca^2–5%	0.002*	0.679	0.702	0.772	0.607
		Zeolite-Ca^2–10%	0.004*	0.661	0.800	0.323	0.507
		Zeolite-NH_4^+-5%	0.024*	0.338	0.474	0.423	0.563
		Zeolite-NH_4^+-10%	0.645	0.122	0.060	0.669	0.643
		Bentonite – 5%	0.003*	0.376	0.060	0.645	0.539
		Bentonite – 10%	0.002*	0.283	0.104	0.033	0.188

*The mean difference is significant at the 0.05 level.

Table 6. Effect on total chlorophyll and carotenoid contents (mg/g) in the leaves of tomato grown on different substrates (Unpoll – tomato grown from seeds collected from unpolluted area, Poll – tomato grown from seeds collected from polluted area).

Amendment	Chlorophyll (mg/g)		Carotenoid × 10^3 (mg/g)	
	Unpoll	Poll	Unpoll	Poll
No	0.050	0.440	0.96	9.46
Zeolite 5%	0.162	0.601	2.74	11.36
Zeolite 10%	0.039	1.277	0.74	25.51
Zeolite-Ca 5%	0.026	0.292	0.37	5.24
Zeolite-Ca 10%	0.087	1.306	1.81	24.42
Zeolite-NH4 5%	0.055	2.297	1.02	46.01
Zeolite-NH4 10%	0.004	1.502	0.07	28.9
Bentonite 5%	0.177	0.845	3.20	17.11
Bentonite 10%	0.156	0.835	3.31	17.43

Table 7. The coefficients obtained by correlate the content of heavy metal in leave (mg/g) and the content of chlorophyll (mg/g) and carotenoid (mg/g), respectively; (Unpoll – tomato grown from seeds collected from unpolluted area, Poll – tomato grown from seeds collected from polluted area).

	Fe		Cu		Pb		Zn		Cd	
	Unpoll	Poll	Unpoll	Poll	Unpoll	Poll	Unpoll	Poll	Unpoll	Poll
Chlorophyll	-0.07	-0.30	-0.32	0.83	-0.25	-0.51	0.03	-0.23	0.30	-0.29
Carotenoid	0.39	-0.23	-0.39	0.81	-0.06	-0.61	-0.12	-0.17	-0.09	-0.27

role of the zeolite and of the bentonite for an increased accumulation of chlorophyll.Carotenoid, a non-enzymatic antioxidant, is a part of photosynthetic pigments. The low content of carotenoid, namely one thousand times less than the content of chlorophyll was a proof of the stress conditions in leaves generated by the presence of heavy metals. Carotenoid plays an important role in the protection of chlorophyll pigment under stress conditions (Kenneth et al., 2000). An increase in the content of carotenoid was considered as a defense strategy of the plants to combat metal stress, as substantiated by tomatoes grown from polluted seeds in this research. The degeneration of chlorophyll and carotenoid was the most common response of the plants exposed to higher concentrations of various heavy metals (Moustakas et al., 1997).

In Table 7 the coefficients obtained by correlating the content of heavy metal in leave and the content of chlorophyll and carotenoid, respectively, are presented.

The negative values of the correlation coefficients suggest that the increase of heavy metal content in leaves induced the decrease of the content of chlorophyll or carotenoid. The most significant dependence, namely the highest values in modulus, was observed in the case of copper and lead in tomatoes grown from unpolluted seeds, suggesting the destructive role of these metals on the chlorophyll structure and biosynthesis (Singh et al., 2004). The positive values of correlations observed for zinc and cadmium in tomato generated from unpolluted seeds, have demonstrated that the increase of the content of these ions leads to the increase of the chlorophyll content. This could be explained by the requirement of these ions in the chlorophyll biosynthesis Singh et al., 2004). The correlation coefficients (between copper level and the chlorophyll and carotenoid content respectively, from leaves grown from polluted seeds were positive, suggesting the direct proportionality from the two pairs of parameters. The correlation coefficients between heavy metal content and carotenoid were negative, excepting the case of iron in tomato grown on unpolluted seeds and copper in tomato grown from polluted seeds, suggesting the increase of carotenoid content with decrease in the heavy metals level, results confirmed by literature (Singh et al., 2004).

Conclusions

Tomato plantlets grown on substrate containing zeolite modified with calcium and ammonium ions were more vigorous than those grown on substrate with zeolite or with bentonite.

The zeolite modified with ammonium ions is an efficient amendment to reduce the accumulation of iron in roots.

The copper and lead level from tomato organs can be reduced by amending the soil with all the investigated amendments.

The tested amendments were not efficient in reducing the level of zinc and cadmium from tomato.

All the tested amendments are found to induce the increase of chlorophyll content in tomato leaves.

The content of chlorophyll and carotenoid vary inverse proportionally with the content of heavy metals in leaves. The statistical analyses revealed that only the cooper concentration varies significant between reference substrate (with no amendment) and each of those with amendments.

ACKNOWLEDGEMENTS

We have conducted our research work and subsequent analyses within the framework of the projects: PN II RIVAM no. 32124/01.10.2008 and PN II BIOMEG no. 52144 / 2008, financed by UEFISCDI Romania and Bilateral Project Romania-South Africa ZEMIP 82AS/2008 financed by ANCS Romania.

REFERENCES

Bucea-Manea-Ţoniş R, Bucea-Manea-Ţoniş R, Epure M (2010). SPSS and EXCEL in the analysis of the statistiical data in economic, social and technical domains. Bucharest, Romania, AGIR Edition, p. 337.

Castaldi P, Santona L, Cozza C, Giuliano V, Abbruzzese C, Nastro V, Melis P (2005). Thermal and spectroscopic studies of zeolites exchanged with metal cations, J. Mol. Struct., 734: 99-105.

Chen YX, He YF, Luo YM, Yu YL, Lin Q, Wong MH (2003). Physiological mechanism of plant roots exposed to cadmium. Chemosphere, 50: 789-793.

Dong J, Wu FB, Zhang GP (2006). Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings *Lycopersicon esculentum*). Chemosphere, 64: 1659-1666.

Gratão PL, Monteiro CC, Antunes AM, Peres LEP, Azevedo LA (2008). Acquired tolerance of tomato (*Lycopersicon esculentum* cv. Micro-Tom) plants to cadmium-induced stress. Ann. Appl. Biol., 153(3): 321-333.

Gratão PL, Monteiro CC, Rossi ML, Martinelli AP, Peres LEP, Medici LO, Lea PJ, Azevedo RA (2009). Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium. Environ. Exp. Bot., 67(2): 387-394.

Gratão PL, Polle A, Lea PL, Azevedo RA (2005). Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol., 32(6): 481-494.

Guala SD, Vega FA, Covelo EF (2010). The dynamics of heavy metals in plant–soil interactions. Ecol. Model., 221: 1148-1152.

Horwood E (1978). Metal Ions in solution, Chichester, England, pp.182-183.

Kenneth E, Pallet KE, Young J (2000). Carotenoids. In: Ruth, G.A., Hess, J.L. (Eds.), Antioxidants in Higher Plants. CRC Press, Boca Roton, FL, pp. 60-81.

Larbi A, Morales F, Abadía A, Gogorcena Y, Lucena JJ, Abadía J (2002). Effects of Cd and Pb in sugar beet plants grown in nutrient solution: induced Fe deficiency and growth inhibition. Funct. Plant Biol., 29: 1453-1464.

Leon AM, Palma JM, Corpas FJ, Gomez M, Romero-Puertas MC, Chatterjee D, Mateos RM, del Rio LA, Sandalio LM (2002). Antioxidative enzymes in cultivars of pepper plants with different sensitivity to cadmium. Plant Physiol. Biochem., 40: 813–820.

Li Q, Cai S, Mo CH, Chu B, Peng LH, Yang FB (2010). Toxic effects of heavy metals and their accumulation in vegetables grown in a saline soil. Ecotoxicol. Environ. Saf., 73: 84-88.

Lopez-Milan A, Sagardoy R, Solanas M, Abadia A, Adadia J (2009). Cadmium toxicity in tomato (*Lycopersicon esculentum)* plants grown in hydroponics. Environ. Exp. Bot., 65: 376-385.

Monteiro CC, Carvalho RF, Gratão PL, Carvalho G, Tezotto T, Medici LO, Peres LEP, Azevedo RA (2011). Biochemical responses of the ethylene-insensitive *Never ripe* tomato mutant subjected to cadmium and sodium stresses. Environ. Exp. Bot., 71(2): 306-320.

Moustakas M, Lanaras T, Symeonidis L, Karataglis S (1997). Growth and some photosynthetic characteristics of field grown Avena sativa under copper and lead stress. Photosynthetica, 30: 389-396.

Sinha S (1999). Accumulation of Cu, Cd, Cr, Mn and Pb from artificially contaminated soil by *Bacopa monnieri*. Environ. Monit. Assess., 57: 253-264.

Singh S, Sinha S, Saxena R, Pandey K, Bhatt K (2004). Translocation of metals and its effects in the tomato plants grown on various amendments of tannery waste: evidence for involvement of antioxidants. Chemosphere, 57: 91-99

Stefanov K, Seizova K, Yanishlieva N, Marinova E, Popov S (1995). Accumulation of lead, zinc and cadmium in plant seeds growing in metalliferous habitats in Bulgaria. Food Chem., 54: 311-315.

Shi W, Shao H, Li H, Shao M, Du S (2009). Co-remediation of the lead-polluted garden soil by exogenous natural zeolite and humic acids, J. Hazard. Mat., 167: 136-140.

Van Assche F, Clijsters H (1990). Effect of metals on enzyme activity in plants. Plant Cell Environ., 13: 195-206.