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PAPER

Calcium- and ammonium ion-modification of zeolite amendments affects the metal-uptake of *Hieracium piloselloides* in a dose-dependent way

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The role of natural zeolite and of two types of modified zeolite (with ammonium and with calcium ions) in reducing the accumulation of ions of heavy metals in roots and leaves of *Hieracium piloselloides* grown on tailing ponds was investigated. The variation of the content of zeolite (5% w/w and 10% w/w) is another parameter that significantly and positively affects the accumulation of the metal ions in *Hieracium piloselloides*. The results showed that zeolite used as an amendment in the soil in tailing ponds significantly reduces the accumulation of heavy metal ions in *Hieracium piloselloides*. The highest concentrations of heavy metals were accumulated in plants grown on soil without zeolite, being followed by the plants grown on the substrate with natural zeolite. Moreover, the translocation factors of heavy metal ions uptake in roots and leaves grown on substrates with modified zeolites are lower than those calculated for the organs grown on the substrate amended with natural zeolite. This behaviour has demonstrated the positive effect of those changes of zeolite amendments in the potential phytoremediation practice.

1. Introduction

The mining industry is one of the biggest waste producers in the world. In many cases, the often hazardous waste is randomly stored on the surface or underground, representing important sources of potential environmental contamination.[1]

The toxicity of heavy metals and improving the water quality are currently two of the most challenging environmental problems faced by mankind.[2,3] The presence of heavy metals in the

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substrate usually triggers the development of metal-tolerant genotypes, often in a relatively short time span. Heavy metal tolerance has been the focus of intensive research and is one of the most studied examples of evolution in plants.

The species of the *Hieracium* genus are perennial members of the *Asteraceae* family, known as hyper-accumulators of heavy metals (lead, iron, copper) usually growing on tailing ponds.[2,4,5] The toxic effects of heavy metals have also been intensively studied at the level of biochemical-physiological processes such as photosynthesis, transpiration, enzyme activity[4,6] or metal accumulation in tissues. Zeolites are considered as important remediation agents for the immobilization of heavy metals in soils.[7–9] Zeolites can effectively decrease the quantity of heavy metals uptake by plants.[10–12] Many studies indicate that zeolites favor the formation of oxides, metal-carbonate precipitates, and complexes, which decrease metal solubility[9–11] and decrease the content of heavy metals in vegetables.[7] The modification of the zeolite structure with different ions increases its capacity to

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adsorb heavy metals because the modified ions induce the standardization of the ion-exchange surface from an energetic point of view.[13] The mechanism of zeolite activation with calcium or ammonium ions is well known.[13] The sodium, potassium, and magnesium ions of the unmodified zeolite structure are replaced with calcium or ammonium ions and the ion-exchange surface becomes more uniform and has a higher capacity for heavy metal adsorption.

Tica *et al.*[6] investigated the impact of the amendments such as: dolomite, diatomite, smectite, basaltic tuff, bentonite, alginate and zeolite (Slovakite) on Pb, Zn, Cu and Cd stabilization in contaminated soil, by measuring glucose-induced soil respiration, dehydrogenase, acid and alkaline phosphatase and β-glucosidase activities. They demonstrated that zeolite used as an amendment for the stabilization of the heavy metals in soil decreases the bioavailability of toxic metals to plants and reduces their accumulation in plants. The efficiency of stabilization is reflected in the respiration rate and in the enzymatic activity of the soil.

Ruggieri *et al.*[26] have demonstrated that the removal of arsenic from aqueous solution depends on the zeolitic content of the rock and mostly on the Si/Al ratio, because this ratio is the indicative of the occurrence of terminal Al–OH surface functional groups that lead to increase of the capacity of sorption. Thus, the overall effect is the better performance for removal of toxic heavy metal ions from wastewater. Moreover, they concluded that the activation of zeolites with cations such as Na, K, Ca, and Mg improves the removal efficiency.

The aim of this study is to establish the potential of the unmodified and modified zeolites in reducing the bioavailability of heavy metals (Pb, Cd, Co, Zn, Cu, Fe) and their accumulation in roots and leaves of *Hieracium piloselloides*.

2. Materials and methods

2.1. Preparation of the activated amendments

Zeolite originating from a deposit in Stoiana, a village in Cluj County, Romania (coordinates: 46°40′58″ N 23°55′45″ E), was used for this study. The mineral and chemical characteristics are presented in Table 1.[25]

The zeolite samples were milled 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 105 °C for 24 h to remove any adsorbed water. Finally, the zeolite samples were stored in a desiccator before performing the chemical activation in Ca-form or NH_4-form. The chemical treatment of the zeolite was performed by adding 1 L of the 2 M solutions of $CaCl_2$ and NH_4Cl to the samples of zeolite fractions of 0.5–1 mm (100 g), at room temperature, under stirring at 160 rotations per min, by using a Heidolph stirrer. After 24 h, the solid phase was separated from solution by washing with ultrapure water, until the removal of the Cl^- ions (controlled with $AgNO_3$ solution), and was dried at 105 °C using a Binder oven for 24 h.

2.2. The pot experiments

In April 2010, samples of *Hieracium piloselloides* surrounded by 1 kg soil were collected from the Bozanta Mare tailing pond, Maramures County (47°38.579 N 023°28.753 E).

Bozanta Mare tailing pond was built in 1977, having a surface of 1 050 000 m^2, depth higher than 40 m, and wave slope of 18–20°. The tailing pond was formed from the disposal of sewage plants, ferrous ore concentrated by flotation and cyanide from plant water extraction of Au and Ag. With an annual growth of over 2 m per year, the facility has accumulated over 150 million m^3 of wastewater–sterile mixture (3 : 1 vol). The huge quantities of wastewater and mine tailings in ponds represent a permanent threat by chemical pollution and the health threat to human communities. Slopes of these tailing ponds are formed resulting from ore processing that contain large quantities of unprocessed ferrous minerals (pyrite, chalcopyrite, galena, blend, *etc.*) and discharged wastewater containing heavy metals (Pb, Cu, Zn, Cd, Mn, Fe, *etc.*) and cyanide waste from extraction of Au and Ag.[24]

The mean total content of heavy metals in soil from the tailing pond is presented in Table 4.

The collected samples were brought to the laboratory and planted in plastic pots 400 mL in volume (three plants in each pot), containing soil from the tailing pond amended with natural and modified zeolites (chemically activated in Ca-form and NH_4-form). The proportion of the amendment in the soil was 5 and 10%. In each pot, the total quantity of soil–amendment mixture was 300 g. Soil without amendment was used as a control sample. The pots were watered with distilled water on a regular basis. The plants were allowed to grow for 38 days, at 20–22 °C. Every pot experiment was repeated three times and the standard deviation was calculated.

2.3. Structural and chemical analyses

The structures of the natural and modified zeolites were 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 program. Fourier Transformed InfraRed spectroscopy (FTIR) analyses of the natural and modified zeolites were performed. A Perkin Elmer Fourier Transform Infrared Spectrometer BXII was used. Sample wafers consisted of 10% sample in spectral quality KBr.

The concentrations of metal ions in the collected soil from the tailing pond and in the roots and leaves of *Hieracium piloselloides*

Table 1 Mineral and chemical characteristics of the natural zeolitic tuff[25]

Mineral composition	Composition (%)	Chemical composition	Composition (%)
Zeolite (clinoptilolite)	62	SiO_2	65.59
Volcanic glass	29.54	Al_2O_3	13.70
Feldspate	1–1.5	Fe_2O_3	1.47
Biotite	1–1.5	CaO	4.72
Limonite	0.5–1	MgO	0.92
Calcite	1–1.5	K_2O	1.65
Hornblend	<0.5	Na_2O	1.60
Montmorillonite	<0.5	TiO_2	0.14

(before and after 38 days of growth in pots) were measured by atomic absorption spectroscopy (AAS) in air–acetylene flame using a Perkin Elmer Analyst 800 spectrophotometer (Shelton, USA). A Retsch RM-100 grinding machine was used to prepare the samples for digestion. The digestion took place in a Berghof MWS-2 (Germany) microwave system. The parameters for vegetal material digestion were: in stage 1 – 145 °C, 5 minutes, power 75%; in stage 2 – 190 °C, 10 minutes, power 90%; and in stage 3 – 100 °C, 10 minutes, power 40%. For the digestion of soil, the following parameters: stage 1 – 180 °C, 25 minutes, power 99% and stage 2 – 100 °C, 10 minutes and power 99%. A mixture of 10 mL HNO_3 65% ($d = 1.4$ kg L^{-1}, Lach-Ner), 0.3 g plant powder and 1 g of dried soil was introduced into the microwave system. For the digestion of all samples, the authors have complied with the methodology provisions in the users' guide of the microwave oven. After digestion, the samples were brought to 100 mL in volumetric flasks with distilled water and analyzed by AAS.

The content of bioavailable components of the heavy metals in soil from the tailing pond was determined according to the Gichner et al.[27] method. Aliquots (10 g) of dried soil samples were extracted with 1 M solution of NH_4NO_3 in ratio 1 : 2.5 (w/v) for 2 h at 20 °C. The soil extracts were centrifuged (Hettich Elba 20) at 3000 rpm for 10 min and the supernatants were maintained at 6 °C. The metal content was determined by AAS.

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

The pH, conductivity and redox potential of the substrates at the start and end of the experiments were measured using an Inolab pH-meter pH 730 for pH measurements and a Hanna Multimeter Check HI991003 for conductivity and redox potential measurements.

2.4. Calculations and statistical analysis

The transfer capability of heavy metals from the substrate to the edible parts of *Hieracium piloselloides* (roots and leaves) was calculated using the translocation factor. The translocation factors (TF) of Pb, Cd, Co, Zn, Cu, and Fe were calculated as follows:[14–16]

$$TF = \frac{\text{metal concentration in edible part of vegetable (DW)}}{\text{metal concentration in substrate (DW) from where the plant was grown}}$$

where DW is the dry weight.

The Pearson correlation coefficients between the concentrations of the heavy metals in roots and leaves grown on different substrates were determined using the Microsoft Excel software.

Differences between individual means were compared using the paired samples t-test ($P < 0.05$) included in the SPSS 10.0 for Windows software. The following pair samples: substrate–metal concentration, percentage of amendment–metal concentration and plant organ–metal concentration were chosen to establish if the variation of the concentration of metal depending on the type of the substrate, percentage of the amendment and the organ of the plant is significant.

Table 2 Structural characteristics of natural and modified zeolites (D_{eff} – effective crystallite mean size, $\langle(\varepsilon^2)\rangle^{1/2}$ – root mean square of the microstrain size)

	D_{eff} (nm)	$\langle\varepsilon^2\rangle^{1/2}$
Zeolite	75.74	0.00232
Zeolite-NH_4^+	74.21	0.00122
Zeolite-Ca^{2+}	75.16	0.00156

3. Results and discussion

3.1. Characterization of the natural and modified zeolite amendments

Table 2 includes the structural characteristics of natural and modified zeolites. The average diameter of the zeolite particles, determined from X-ray diffraction analyses, is in the range 74.21–75.74 nm. No significant differences in the sizes of the three types of zeolite particles were obtained.

Additionally, the crystalline structures of the natural and modified zeolites were established from the X-ray diffraction pattern analyses (Fig. 1). The X-ray diffraction pattern of zeolites reveals the existence of peaks characteristic for clinoptilolite (θ between 10 and 35 degrees) and montmorillonite, but the predominant crystalline phase is clinoptilolite.

To establish if the zeolite structure is modified with calcium or ammonium ions, FTIR analyses were performed (Fig. 2). The peak localized at 1028.68 cm^{-1} in FTIR spectra of zeolites 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.[17] The introduction of non-tetrahedral cations into the alumino-silicate framework 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 bands 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 bond vibrations.

Table 3 includes the values of 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, corresponding to different vibration of the Si–O bond due to the connection with ammonium and calcium ions. The variation of the area and length of the peak of the three samples demonstrates 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).

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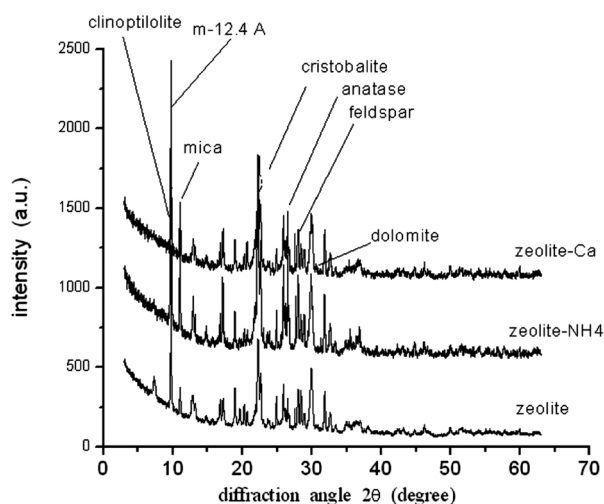*J. Environ. Monit.*, 2012, **14**, 2807–2814 | 2809

Fig. 1 X-ray diffraction patterns of natural zeolite, zeolite modified with calcium ions (zeolite-Ca), and zeolite modified with ammonium ions (zeolite-NH₄) (m-12.4 Å: Ca-montmorillonite, calcite, quartz, anatase, dolomite, pyrite, mica).

3.2. pH, electrical conductivity and redox potential of various growing substrates

In Table 4 are presented the values of pH, conductivity and redox potential of the investigated substrates at the beginning and at the end (after 38 days) of the pot experiments. At the beginning of the experiments, all the investigated substrates are slightly acidic (in the 4.45 to 5.37 range), due to the presence of pyrite, chalcopyrite, galena and blend, which on reaction with

oxygenated water generate sulphates and acidity. The pH of the soil–zeolite substrate is higher than that of the soil, due to the basic character of the zeolite. By modifying the zeolite with ammonium and calcium ions, the value of the initial pH increases, due to the basic character of the zeolite modified with ammonium and calcium ions. These results are in agreement with those of Leggo et al.[18] who studied the role of cliptilolite in organo-zeolitic-soil systems for phytoremediation. At the beginning of the experiments, the electrical conductivity of the soil–zeolite is lower than that of the soil. The zeolite acts to depress the electrical conductivity to a minimum limit of 50.7 mS cm⁻¹, a behaviour which is in agreement with that established by Leggo et al.[18] At the start of the experiments, the electrical conductivity of the substrates with modified zeolites is lower compared to that for the substrate with natural zeolite, due to the higher adsorption capacity of the modified zeolites, thus leading to a decrease in the amount of heavy metal ions in solution responsible for the increase in electrical conductivity.

Similar behaviour was observed in the case of redox potential, at the beginning of the experiments. The redox potential decreases in the following order: soil > soil–zeolite > soil–zeolite-NH_4^+ ≈ soil–zeolite-Ca^{2+}.

Table 3 Characteristics of the peak from FTIR spectra of the zeolite localized at 1030 cm⁻¹

Amendment	Area of the peak localized at 1028–1036 cm⁻¹ ($T\% \times$ 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-NH₄	27 427.21	53.35

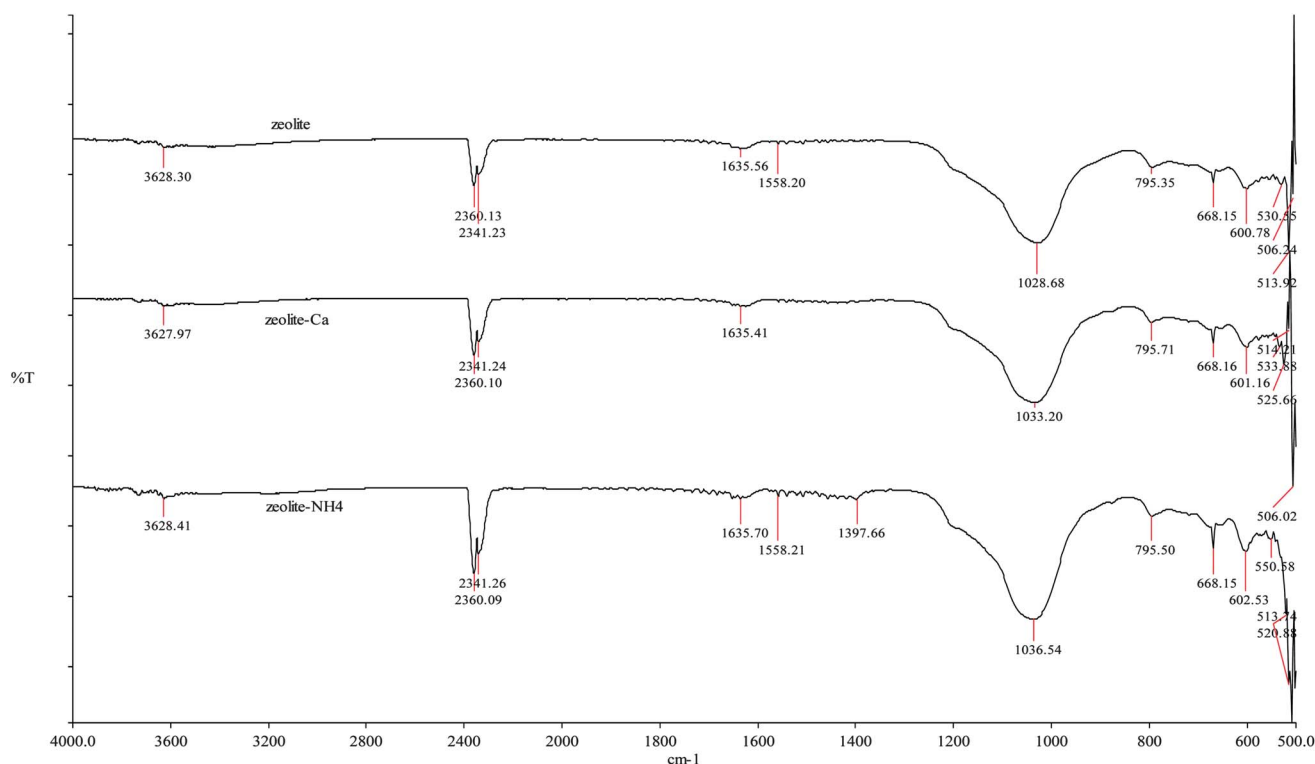**Fig. 2** FTIR spectra of zeolite, zeolite modified with calcium ions (zeolite-Ca) and zeolite modified with ammonium ions (zeolite-NH₄).

Table 4 Values of pH, conductivity and redox potential of the substrate at the beginning and at the end (after 38 days) of the pot experiments. The results are the average values of three replicates, for which the calculated standard deviation (SD) was lower than 5%

		pH ± SD	Electrical conductivity ± SD (mS cm^{-1})	Redox potential ± SD (mV)
Soil	Initial	4.45 ± 0.21	109.7 ± 5.4	356 ± 0.71
	Final	3.56 ± 0.17	125.4 ± 5.3	311 ± 0.70
Soil–zeolite 5%	Initial	4.96 ± 0.22	84.3 ± 4.9	243 ± 0.69
	Final	4.16 ± 0.21	97.5 ± 3.1	201 ± 0.60
Soil–zeolite 10%	Initial	5.11 ± 0.23	70.3 ± 2.8	239 ± 0.54
	Final	4.50 ± 0.19	77.3 ± 1.8	178 ± 0.65
Soil–zeolite-NH$_4^+$ 5%	Initial	5.07 ± 0.17	73.5 ± 1.5	240 ± 0.58
	Final	4.39 ± 0.13	66.4 ± 0.9	192 ± 0.54
Soil–zeolite-NH$_4^+$ 10%	Initial	5.23 ± 0.16	50.7 ± 0.8	228 ± 0.52
	Final	4.75 ± 0.16	21.8 ± 0.6	159 ± 0.49
Soil–zeolite-Ca^{2+} 5%	Initial	5.37 ± 0.15	74.5 ± 1.6	233 ± 0.61
	Final	4.96 ± 0.09	60.2 ± 0.8	178 ± 0.57
Soil–zeolite-Ca^{2+} 10%	Initial	5.49 ± 0.17	67.2 ± 0.4	211 ± 0.48
	Final	5.17 ± 0.15	39.5 ± 0.2	136 ± 0.46

The increase in the amount of zeolite leads to a decrease in the redox potential, due to the intensification of the adsorption capacity and, subsequently, to the decrease of the amount of metal ions available for redox reactions (Fe^{2+} and Fe^{3+}).

At the end of the experiments, the pH values of all the investigated substrates decreased (increase of the acidity). This behaviour is explained by the degradation of organic material and of the pyrite materials that leads to the generation of the humic acids and by the rhizosphere effect. The rhizosphere effect consists of the local acidification of the environment due to the adsorption of the heavy metal ions on the cell walls by replacing the protons from –COOH, –OH, –SH, and –NH$_2$ groups with heavy metal ions. The protons increase the acidity and, subsequently, lead to the decrease of the pH.[19] At the end of the experiments, the pH values corresponding to the substrates with zeolite are higher than that of the soil from the tailing pond. Moreover, the pH values of the substrates with modified zeolites are higher than that of the substrate with natural zeolite. The increase in the amount of zeolite leads to an increase in pH, due to the basic character of the amendment.

The values of electrical conductivity for the soil, soil–zeolite 5% and 10% and soil–zeolite-NH$_4^+$ 5% are higher at the end of the experiments as compared to the initial values, due to an increase in the concentrations of the humic acids and protons. In other cases, the electrical conductivity is lower at the end of the experiments as compared with the initial values.

The redox potential decreases, in all cases, at the end of the experiments as compared with the initial values, due to the decrease of the amount of the metal ions available for redox reactions.[20]

3.3. Heavy metal concentrations and metal translocation factors at various growing substrates

The concentrations and bioavailability of heavy metals in the soil collected together with the *Hieracium piloselloides* from the tailing pond are presented in Table 5. The highest concentration corresponds to iron (14 282 mg per kg DW) and the lowest concentrations correspond to cobalt (6.35 mg per kg DW) and gold (5.12 mg per kg DW). The bioavailable content for the investigated elements in the soil from the tailing pond is an important factor for their uptake by plants. It represents 0.778% of the total content of Pb, 1.968% of Cd, 0.961% of Co, 0.018% of Zn, 0.197% of Cu, 0.007% of Fe, 2.088% of Cr, 1.782% of Mn, 2.352% of Na and 0.778% of Ag. K and Au are not bioavailable for plants.

The concentrations of different heavy metals in roots and leaves of *Hieracium piloselloides* grown on different substrates are presented in Table 6.

The highest concentrations of heavy metals were accumulated in plants grown on soil without zeolite, being followed by the plants grown on the substrate with natural zeolite. Ruggieri *et al.*[26] have demonstrated that the zeolite rocks with the clinoptilolite content in the range 61–65% (similar to the content in zeolite of the zeolitic rock used in this study (Table 1)) showed a better removal for arsenic than the rocks with a higher

Table 5 Concentration and bioavailability of the heavy metals in the soil collected together with *Hieracium piloselloides* plants from the tailing pond. The results are the average values of three replications, for which the calculated standard deviation (SD) was lower than 5%

Pb ± SD	Cd ± SD	Co ± SD	Zn ± SD	Cu ± SD	Fe ± SD	Cr ± SD	Mn ± SD	Na ± SD	K ± SD	Au ± SD	Ag ± SD
mg per kg DW^a											
379.57 ± 17.97	77.52 ± 3.67	6.35 ± 0.30	104.93 ± 5.11	141.62 ± 7.08	14 282 ± 714.1	77.52 ± 12.47	258.53 ± 17.00	344.61 ± 60.3	948.32 ± 259	5.12 ± 3.2	64.84 ± 37.9
% bioavailable											
0.778 ± 0.002	1.968 ± 0.56	0.961 ± 0.04	0.018 ± 0.0004	0.197 ± 0.03	0.007 ± 0.0002	2.088 ± 0.67	1.782 ± 0.87	2.352 ± 0.93	0.000 ± 0.0	0.000 ± 0.0	0.778 ± 0.001

^{a} DW – dry weight.

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Table 6 Concentrations of the heavy metals in roots and leaves of *Hieracium piloselloides* grown for 38 days on different substrates containing 5% and 10% (w/w) zeolite and soil from the tailing pond. The results are the average values of three replications, for which the calculated standard deviation (SD) was lower than 5% (S – soil, Z – zeolite)

mg per kg DW[a]		Pb ± SD	Cd ± SD	Co ± SD	Zn ± SD	Cu ± SD	Fe ± SD
S	Roots	311.7 ± 15.85	70.54 ± 3.52	5.11 ± 0.25	95.14 ± 4.75	111.6 ± 5.58	10 462 ± 523
	Leaves	253.8 ± 12.69	60.23 ± 3.01	3.29 ± 0.16	62.01 ± 3.10	53.2 ± 2.66	8671 ± 433
S–Z 5%	Roots	231.8 ± 11.59	55.27 ± 2.76	4.32 ± 0.21	70.12 ± 3.50	73.2 ± 3.66	7221 ± 361
	Leaves	197.45 ± 9.87	41.94 ± 2.09	2.87 ± 0.14	32.44 ± 1.62	40.16 ± 2.00	3442 ± 172
S–Z 10%	Roots	168.7 ± 8.43	37.29 ± 1.86	3.51 ± 0.17	35.66 ± 1.78	47.81 ± 2.39	4321 ± 216
	Leaves	101.7 ± 5.08	25.27 ± 1.26	2.01 ± 0.10	14.43 ± 0.72	30.43 ± 1.52	2345 ± 117
S–Z-NH$_4^+$ 5%	Roots	111.8 ± 5.59	28.14 ± 1.40	2.11 ± 0.10	51.42 ± 2.57	59.4 ± 2.97	6179 ± 308
	Leaves	72.4 ± 3.62	18.45 ± 0.92	1.53 ± 0.07	22.43 ± 1.12	27.6 ± 1.38	2439 ± 121
S–Z-NH$_4^+$ 10%	Roots	73.21 ± 3.66	16.43 ± 0.82	0.85 ± 0.04	20.78 ± 1.03	21.53 ± 1.07	2471 ± 123
	Leaves	24.5 ± 1.22	5.74 ± 0.28	0.32 ± 0.01	8.71 ± 0.43	8.71 ± 0.43	1178 ± 58
S–Z-Ca^{2+} 5%	Roots	131.7 ± 6.58	30.47 ± 1.52	2.35 ± 0.11	31.42 ± 1.57	63.7 ± 3.18	6895 ± 344
	Leaves	93.4 ± 4.67	21.78 ± 1.08	1.17 ± 0.05	11.43 ± 0.57	44.3 ± 2.21	3011 ± 150
S–Z-Ca^{2+} 10%	Roots	85.2 ± 4.26	17.91 ± 0.89	1.33 ± 0.06	23.55 ± 1.17	33.11 ± 1.65	3145 ± 157
	Leaves	32.4 ± 1.62	6.74 ± 0.33	0.27 ± 0.01	9.11 ± 0.45	12.67 ± 0.63	1879 ± 93

[a] DW – dry weight.

clinoptilolite content. So, they concluded that the content of clinoptilolite in the zeolitic rock influences its adsorption capacity.

Lead, copper and iron were accumulated in the highest amounts, by the *Hieracium piloselloides* roots grown on soil (311.7, 111.6 and 10 462 mg kg^{-1}), thus demonstrating that the investigated specie is a hyper-accumulator of the three metals. The plants grown on substrates with modified zeolites have accumulated the lowest amount of heavy metals. This is the result of the high efficiency of the zeolite activated with calcium and ammonium cations to accumulate heavy metal ions, as the literature reports.[26]

Additionally, this behaviour could be explained by the variation of the pH in amended and non-amended soil. The amended substrate is more basic than the unmodified soil, thus leading to the increase of affinity for heavy metals and subsequently to the reduction of their accumulation in *Hieracium piloselloides*. The comparison of the amount of heavy metals accumulated in roots with the amount accumulated in leaves reveals that, in all the investigated cases, the leaves accumulated a lower amount of heavy metals than roots, due to the inhibition mechanism that almost all of the plants develop for protection against the destructive effect of the heavy metals.[6]

The concentrations of the heavy metal ions accumulated in organs of *Hieracium piloselloides* were used for the determination of the soil-to-plant translocation factors (Table 7). The translocation factors of the heavy metal ions in roots and leaves grown on soil are higher than those calculated for the samples grown on substrates with zeolite. Furthermore, the translocation factors of the metals in roots and leaves grown on substrates with zeolite decrease as the percentage of zeolite increases.

These results are confirmed by Shi *et al.*[7] who studied the effect of different doses of zeolite in reducing the lead accumulation in roots and aerial parts of grapes. The potential environmental availability of metals from contaminated soil may be controlled by soil amendments with exogenous zeolite or humic substances.

The translocation factors of cadmium were higher than those of lead, because of the better solubility and mobility of cadmium as compared to lead.[20] Therefore, cadmium was more easily taken up and transported from the roots to the leaves compared to lead.

The translocation factors of heavy metal ions uptake in roots and leaves grown on substrates with modified zeolites are lower than those calculated for organs grown on the substrate amended with natural zeolite. This behaviour has demonstrated the role of ammonium and calcium ions in heavy metal ions adsorption on

Table 7 Translocation factors of the heavy metals from the substrate in the edible parts of the *Hieracium piloselloides* grown on different substrates for 38 days

		Pb	Cd	Co	Zn	Cu	Fe
Soil	Roots	0.821	0.910	0.805	0.907	0.788	0.733
	Leaves	0.669	0.777	0.518	0.591	0.376	0.607
Soil–zeolite 5%	Roots	0.611	0.713	0.680	0.668	0.517	0.506
	Leaves	0.520	0.541	0.452	0.309	0.284	0.241
Soil–zeolite 10%	Roots	0.444	0.481	0.553	0.340	0.338	0.303
	Leaves	0.268	0.326	0.317	0.138	0.215	0.164
Soil–zeolite-NH$_4^+$ 5%	Roots	0.295	0.363	0.332	0.490	0.419	0.433
	Leaves	0.191	0.238	0.241	0.214	0.195	0.171
Soil–zeolite-NH$_4^+$ 10%	Roots	0.193	0.212	0.134	0.198	0.152	0.173
	Leaves	0.065	0.074	0.050	0.083	0.062	0.082
Soil–zeolite-Ca^{2+} 5%	Roots	0.347	0.393	0.370	0.299	0.450	0.483
	Leaves	0.246	0.281	0.184	0.109	0.313	0.211
Soil–zeolite-Ca^{2+} 10%	Roots	0.224	0.231	0.209	0.224	0.234	0.220
	Leaves	0.085	0.087	0.043	0.087	0.089	0.132

Table 8 Correlation coefficients between the concentrations of heavy metals in roots and leaves respectively, grown on different substrates, for 38 days

Metal pair	Pb–Cd	Pb–Co	Pb–Zn	Pb–Cu	Pb–Fe	Cd–Co	Cd–Zn	Cd–Cu	Cd–Fe	Co–Zn	Co–Cu	Co–Fe	Zn–Cu	Zn–Fe	Cu–Fe
Correlation coefficient	1.00	0.96	0.92	0.87	0.88	0.96	0.93	0.88	0.91	0.86	0.84	0.78	0.73	0.95	0.79

the modified zeolite, due the fact that the ammonium and calcium ions replaced with heavy metal ions by ionic exchange are useful for the plant metabolism. The difference between the adsorption capacities of the zeolite modified with ammonium ions and that modified with calcium ions is due to the lower hydration energy of the ammonium ions as compared with that of the calcium ions.[21–23] The values of the translocation factors corresponding to the uptake of heavy metals in roots and leaves grown on the substrate with zeolite-ammonium are lower than those corresponding to the substrate with zeolite-calcium (Table 7), for all the ions of heavy metal tested.

Table 8 shows the values of the correlation coefficients between the concentrations of the heavy metals investigated. All the values are positive, suggesting the change in the same direction of the concentrations of heavy metals (if the concentration of a heavy metal ion from the above-mentioned pair increases, an increase of the concentration of the other ion will occur). The highest values (those closest to 1) were obtained for the lead–cadmium, lead–cobalt, lead–zinc, cadmium–cobalt and cadmium–zinc pairs (generally the pairs containing lead or cadmium). This behavior can be explained taking into consideration the toxicity of lead and cadmium for the plant. A relatively high amount of lead and cadmium in plant organs will generate a detrimental effect on its metabolism by reducing the plant's defense system against the chemical aggression presented by the high bioavailability of metal ions. In this case the plant will accumulate high concentrations of metals.[5,7,16]

The lowest values of the correlation coefficients were obtained for the following metal pairs: lead–iron, lead–copper, cobalt–copper, cobalt–iron (generally pairs containing copper or iron). In this case, the bio-assimilation of each metal ion occurs independently from that of the other ion from the pair, a behaviour dictated by the plant's physiological metabolism.[7]

Paired sample *t*-test was performed to establish if the variation of heavy metal concentration depending on the type of the substrate, percentage of the amendment and the organ of the plant is significant. If the significance values are lower than 0.05, the variations of the heavy metal concentrations are significant. All the obtained values of significance (excepting that for the pair substrate–Co) are lower than 0.05, suggesting that the decrease of the metal concentration in plants grown on substrates amended with modified zeolites as compared with that measured in plants grown on soil is significant. The exception (pair: substrate–Co), having the value of the significance 0.154, suggests that the decrease of the concentration of Co depending on the substrate is random. Moreover, the decrease of the metal concentration in plants grown on the substrate containing 10% of amendment as compared with that measured in plants grown on the substrate amended with 5% unmodified or modified zeolite is also significant. Finally, the decrease of the concentration of Pb, Cd, Co, Zn, Cu and Fe from roots to leaves of *Hieracium piloselloides* is also significant.

4. Conclusions

The application of natural zeolite as an amendment in the soil from tailing ponds reduces the accumulation of ions of the heavy metals in the roots and leaves of *Hieracium piloselloides*. The zeolite modified with ammonium and calcium ions is more efficient in reducing the accumulation of ions of the heavy metals as compared with natural zeolite. This could be due to the fact that the ammonium ions have lower hydration energy than the calcium ions. The statistical analyses showed that the variation of the concentration of metals depending on the type of the substrate, percentage of the amendment and the organ of the plant is significant.

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