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Simulated Bioavailability of Heavy Metals (Cd, Cr, Cu, Pb, Zn) in Contaminated Soil Amended with Natural Zeolite Using Diffusive Gradients in Thin-Films (DGT) Technique

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Abstract: The aim of this study was to investigate the effect of natural zeolite amendment to contaminated soil on the heavy metal (Cd, Cr, Cu, Pb and Zn) simulated bioavailability over a three-month period of storage. Two pot experiments were created by amending soil with two different amounts of natural zeolite (3 and 6 wt.%). During the experiment, pH level, metal concentrations in soil solution (C_{sol}) and metal concentrations in soil by the diffusive gradients in thin-films technique (C_{DGT}) were determined. When the zeolite was added to the soil, a significant decrease ($p = 95\%$, $n = 3$) for Cd and Pb concentrations, both in C_{sol} and in C_{DGT} , was observed. The Cu, Cr and Zn concentrations also showed a decreasing trend, but the changes in their concentrations were not statistically significant. The R ratio between C_{DGT} and C_{sol} was calculated for each metal in order to assess the resupply of metals from the soil solid phase to soil solution. The R values significantly decreased for Cd and Pb, showing a low resupply from the solid phase in the samples with added zeolite.

Keywords: DGT; immobilization; toxic metals; inorganic amendments; soil remediation; in situ remediation



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1. Introduction

Metals originate in the soil environment from natural sources and different anthropogenic activities, such as mining and metallurgical processes, chemical industry, agriculture, waste disposal, etc. [1]. Metal pollution of soil is an increasingly pressing problem all over the world since, beside their toxicity, metals are almost not degradable, persist for a long period of time in the environment, and have a high bioaccumulation capacity [2–4]. Some metals, such as Cd, Cr, Pb, and Hg, are non-essential elements and become hazardous even at very low concentrations, while some essential elements, such as Cu and Zn, become toxic for the ecosystems and human health at high concentrations [5,6].

The presence of toxic elements in soil can disturb the normal functioning of soil biota, having a negative impact on the whole soil system. In different environmental conditions, the toxic elements can leach into the groundwater, affecting their quality [7–9]. The heavy metals have harmful effects on human health [10], e.g., Pb affects the nervous system, Cd has toxic effects on respiratory, reproductive, and skeletal organ systems, while Zn can become toxic at high concentrations [11]. High Cu concentration in the human body causes anemia and damage to capillaries, the liver, kidneys, and the stomach, while high Cr concentration can induce liver problems [12]. Moreover, above a certain dose, Cd, Cr and Pb are carcinogenic and can cause serious damage to human body health [13]. Because of metal toxicity, maximum admitted thresholds were established for total concentrations of toxic metals in soil [14]. The alert and intervention thresholds established by Romanian legislation are presented in Table 1.

Table 1. Physico-chemical properties of natural zeolite (NZ), control soil (CS) and mixtures with zeolites (NZS3 and NZS6) at the start of experiments (average \pm standard deviation, $n = 3$ parallel determinations).

	NZ	CS	NZS3	NZS6	Intervention Threshold Soil *	Maximum Threshold Zeolite **
pH	9.55 \pm 0.20	8.58 \pm 0.12	8.77 \pm 0.15	8.78 \pm 0.15	-	-
Cd (mg kg ⁻¹)	<1.0	34.7 \pm 1.7	33.6 \pm 1.8	31.4 \pm 1.2	5	10
Cr (mg kg ⁻¹)	4.25 \pm 0.23	15.2 \pm 0.7	14.7 \pm 0.5	14.7 \pm 0.6	300	500
Cu (mg kg ⁻¹)	1.16 \pm 0.12	553 \pm 24	522 \pm 20	516 \pm 15	200	500
Pb (mg kg ⁻¹)	6.33 \pm 0.43	392 \pm 25	378 \pm 18	366 \pm 19	100	300
Zn (mg kg ⁻¹)	4.40 \pm 0.38	2800 \pm 110	2779 \pm 87	2742 \pm 65	600	2000
CEC (meq/100 g)	129 \pm 6.5	60.2 \pm 4.1	62.4 \pm 3.5	61.5 \pm 3.1	-	-
C _T (%)	<0.01	2.65 \pm 0.14	2.58 \pm 0.21	2.52 \pm 0.17	-	-
N _T (%)	<0.01	1.10 \pm 0.06	1.04 \pm 0.06	1.05 \pm 0.10	-	-
SiO ₂ (%)	71.79 \pm 1.12	-	-	-	-	-
Al ₂ O ₃ (%)	11.19 \pm 0.35	-	-	-	-	-
CaO (%)	2.64 \pm 0.04	-	-	-	-	-
MgO (%)	0.66 \pm 0.02	-	-	-	-	-
K ₂ O (%)	2.50 \pm 0.10	-	-	-	-	-
Na ₂ O (%)	0.52 \pm 0.02	-	-	-	-	-
Fe ₂ O ₃ (%)	1.55 \pm 0.03	-	-	-	-	-
MnO (%)	0.03 \pm 0.003	-	-	-	-	-
Others (%)	9.12	-	-	-	-	-

* Threshold for sensitive use according to Romanian legislation [14]. ** Maximum levels in the sewage sludges intended for the application on agricultural soil (zeolite was associated in this study with a soil amendment) [15].

Different chemical and physical techniques have been proposed to remediate the metal-contaminated soils, some of them practiced *ex situ*, being highly expensive or invasive for the ecosystems [15,16]. The *in situ* techniques for the soil remediation are based on two main groups of approaches: metal removal from soil by phytoextraction using hyperaccumulators plants [17,18] and the immobilization of metals in soils using some plant species [19], or by adding different amendments to soil [1,20].

Since the bioavailability represents the capacity of a pollutant to reach the living organism [21], the scope of immobilization is to reduce their mobility in soil, and subsequent plant uptake and groundwater contamination [22,23]. The amendments can decrease the mobility and bioavailability of metals in soils through various mechanisms, such as adsorption, complexation, and precipitation [24,25]. The main advantages of *in situ* remediation are the relatively small cost and logistical requirements, and the possibility to be applied for large areas of contaminated soil [26]. Moreover, the remediation using amendment with reactive minerals (zeolites, phosphate rocks, carbonates, and clay minerals) is an effective and eco-friendly method, without significantly interfering with the natural soil functions [27].

Zeolites are aluminosilicates with porous crystalline structures that contain Al, Si and O in their regular framework, having well-defined channels or cavities. The zeolite structure determines the large surface area, high cation exchange capacity and excellent thermal properties such as thermal stability, temperature resistance and heat capacity [27]. The most prevalent naturally occurring zeolite is clinoptilolite with a strong affinity towards cations and a wide range of application areas, such as environmental protection, agriculture, industry, and medicine [28–31]. Clinoptilolite can immobilize metals by adsorption, as well as by slightly adjusting pH [32,33].

In order to evaluate the effectiveness of soil amendments for metal immobilization, the investigation of the metal bioavailability is required. Therefore, the development of reliable analytical methodologies to evaluate the metal bioavailability has been a hotspot in the field of agricultural environmental science [34,35]. The metal bioavailability is related to their uptake by plants. Single and sequential chemical extractions have been used to

evaluate the metal distribution in different soil fractions. Mostly, the metals present in water from soil pores were considered for determination of the available metal fraction in soil [34,36]. However, the capability of this extraction to predict the metal bioavailability with a good accuracy and their transfer to the plants is not clear, since the physical–chemical equilibrium depends on the pretreatment of soil samples and separation of the solid phase from the soil solution [37].

The difficulty in the accurate assessing of metal bioavailability in soil through chemical extractions is also given by the dynamic relationship between the metals in the soil solid phase, soil solution and uptake by plant roots. These limitations can be avoided using the Diffusive Gradient in Thin films (DGT) technique, a relatively simple tool which allows for simulating the plant uptake of metals from soil solution and metal resupply from the solid phase [38,39]. DGT is based on the diffusion of metals in solution through a diffusive gel layer to a binding phase, where they are retained [37,40]. The usefulness of this technique in the evaluation of metal bioavailability in soil and to predict plant uptake have been already demonstrated for several metals [38–45]. Some studies have employed DGT to assess the influence of soil amendments on metal bioavailability in contaminated soils [1,20,33,35,46–49]. However, the effectiveness of the DGT to predict the changes in metal bioavailability due to the zeolite addition needs further research.

The aim of this work was to evaluate the efficiency of addition of different amounts of natural zeolite as soil amendment to reduce the bioavailability of heavy metals present in a contaminated soil using the DGT technique. The metal (Cd, Cr, Cu, Pb, Zn) immobilization by zeolite was tested in pot experiments using different natural zeolite amounts (3 and 6 wt.%), after three months of storage. The decrease in R ratios between C_{DGT} and concentration in soil solution (C_{sol}) indicated the immobilization of metal in soil solution.

2. Materials and Methods

2.1. DGT Assembly and Reagents

The DGT tools by DGT Research Ltd. (Lancaster, UK) consists of a plastic shell enclosing a cellulose-acetate filter, a diffusive gel layer, and a resin gel (Chelex-100). A 0.45 μm pore-size filter membrane, 0.13 mm thick and with an exposed diffusion area of 3.14 cm^2 , is placed in the outermost layer. Until their deployment, the diffusive gels were stored in 0.01 M NaNO_3 , while the resin gels were kept in demineralized water (as supplied by the producer).

All reagents used were of analytical grade: 37% (*m/m*) HCl, 65% HNO_3 (*m/m*), HF 40% (*m/m*), NH_4Cl salt (Merck, Darmstadt, Germany). Calibration standards were prepared from ICP multi-element standard IV solution 1000 mg L^{-1} (Merck, Darmstadt, Germany) by appropriate dilutions. Ultrapure water was obtained from a Milli Q system (Millipore, France) and used for the dilutions. The accuracy of the analysis for total metal concentrations in soils was checked using certified reference material (CRM) ERM-CC141—Loam Soil (JRC-IRMM, Geel, Belgium). Percent recoveries (%) of metals in soil CRM, calculated using the average of five replicates, were in the range of 91–103%. The accuracy of the analysis for total metal concentrations in zeolite was assessed using CRM BCS-CRM 375/1 soda feldspar (Bureau of Analyzed Samples, Middlesbrough, UK), with satisfactory percent recoveries (%) of metals in the range of 86–104%.

2.2. Soil and Zeolitic Tuff Samples Characterization

The soil used for the experiments was sampled in July 2021 from the surface layer (0–10 cm) using a stainless-steel shovel from a former mining area in Iara, Cluj County, Romania (46°33′13″ N and 23°27′27″ E). The stones, roots, and litter were removed from the soil, and a composite sample from 10 sub-samples was obtained by homogenization. An aliquot of the soil sample was air-dried at room temperature, then grounded to pass through a 20-mesh sieve, then used for the chemical analyses.

Soil pH was determined by pH meter (Mettler Toledo, Schwerzenbach, Switzerland) at soil: water ratio (1:5 *w/v*). Soil cation exchange capacity (CEC) was determined according

to ISO 23470:2018 [50], by measuring the extractable major cations (K, Na, Ca, Mg, Al, Fe and Mn) in a hexaamminecobalt(III)chloride solution, using inductively coupled plasma optical emission spectrometry (ICP-OES Optima 5300 DV, Perkin-Elmer, Woodbridge, ON, Canada). The total carbon (C_T) and nitrogen content (N_T) were determined using a Flash 2000 CHNS/O analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Aqua regia extractable concentrations of metals in soil were measured using wet acid digestion (HNO_3 65%: HCl 37%, 1:3, *v:v*) according to ISO 11466:1995 [51], in a closed-vessel Speedwave Xpert microwave system (Berghof, Eningen, Germany), followed by ICP-OES determination. Water holding capacity (WHC) was determined gravimetrically, according to the method described by Muhammad et al. [52].

The samples of soil solution and DGT were analyzed for Cd, Cu, Cr, Pb and Zn concentrations using a graphite furnace atomic absorption spectrometer (GFAAS) PinAAcle 900T (Perkin Elmer, Norwalk, CT, USA). Six-point external calibration curves were plotted for each element, using calibration standards prepared by auto dilution of the highest concentrated standard solutions ($2 \mu\text{g L}^{-1}$ for Cd; $10 \mu\text{g L}^{-1}$ for Zn; and $20 \mu\text{g L}^{-1}$ for Cr, Cu and Pb) with the reagent blank, using the instrument autosampler. Sample aliquots of $20 \mu\text{L}$ were directly injected into the graphite tube, and then a volume of $5 \mu\text{L}$ of chemical modifier was added according to the recommendation of the instrument manufacturer (1% $NH_4H_2PO_4$ + 0.06% $Mg(NO_3)_2$ for Cd and Pb determination, 1% Pd + 0.06% $Mg(NO_3)_2$ for Cu determination, and 0.3% $Mg(NO_3)_2$ for Cr determination). Electrodeless discharge lamps (EDLs) were used for Cd and Pb, and hollow cathode lamps (HCLs) were used as light sources for Cu, Cr and Zn determination. The limits of detection (LODs), calculated using the $3s_y/x/m$ criterion, where s_y/x is the residual standard deviation of the calibration curve, y is the intercept and m is the slope of the calibration curve [53], were $0.14 \mu\text{g L}^{-1}$ for Cd, $0.22 \mu\text{g L}^{-1}$ for Cr, $0.40 \mu\text{g L}^{-1}$ for Cu, $0.37 \mu\text{g L}^{-1}$ for Pb, and $0.26 \mu\text{g L}^{-1}$ for Zn. These limits allowed for the determination of all studied trace metals in soil solution and DGT samples with satisfactory (<20%) accuracy and precision.

Natural zeolite (NZ) material obtained from a quarry located in Racos, Brasov County, Romania, was crushed and sieved to obtain a particle size <1 mm, and thermally activated at $200 \text{ }^\circ\text{C}$ for 2 h. The samples were characterized regarding chemical composition for major elements using ICP-OES after microwave-assisted digestion with a mixture of HNO_3 65%:HCl 37%:HF 40% (3:9:2, *v:v:v*). The measured concentrations of major elements (Al, Fe, Na, K, Ca, and Mg) were converted to oxides using atomic and molecular masses, while SiO_2 was determined gravimetrically [54]. The cation exchange capacity (CEC) was determined after the ammonium acetate saturation (AMAS) extraction method and measuring the extractable major cations (K, Na, Ca, and Mg) using ICP-OES. Three parallel determinations including digestion and instrumental measurements were carried out for each sample. Total surface area and pore radius were obtained from N_2 adsorption-desorption isotherms using the Brunauer-Emmett-Teller (BET) method for total surface area evaluation, and the Dollimore-Heal model for porosity data. The isotherms were obtained using a Sorptomatic 1990 apparatus (Thermo Electron Corporation, Waltham, MA, USA). The X-ray diffraction (XRD) patterns were recorded at room temperature using a D8 Advance (Bruker, Karlsruhe, Germany) diffractometer with $CuK\alpha$ radiation ($\lambda = 1.54060 \text{ \AA}$), operating at 40 kV and 40 mA.

2.3. DGT Experiments

The DGT determination of metals in the soils was performed according to a procedure that involved the following steps:

- Amendment of 485 g and, respectively, 470 g soil with 15 g and 30 g natural zeolite (NZ), carefully mixed in 1000 mL plastic containers. The obtained mixtures containing 3% and 6% zeolite (NZS3 and NZS6, respectively) and a control pot containing only soil (CS) were moistened until approx. 80–90% of maximum water holding capacity (MWHC) with distilled water and stored for three months. To maintain the soil moisture at a similar level, the pots were covered with a parafilm, watered and

homogenized weekly with distilled water. Each pot experiment was maintained at 20 ± 2 °C and was performed in triplicate.

- Determination of DGT metal concentration in initial soil (CS) and their mixtures (NZS3 and NZS6). Samples were watered to 80–90% MWHC, then the assembled DGT devices were gently placed on the mixture surface of each sample for 24 h at 20 ± 2 °C, for equilibration.
- DGT retrieval and elution: after 24 h deployment, the DGT devices were retrieved and carefully rinsed with distilled water. The binding gels were placed in polyethylene vials containing 1 mL of 1 M HNO₃ and kept 24 h for elution. The metal concentrations in the eluent were measured by GFAAS.
- DGT calculation, as described by Zhang and co-workers [38]. The metal concentrations accumulated by the DGT devices were calculated according to Equations (1) and (2):

$$C_{DGT} = M \cdot \Delta g / D \cdot A \cdot t \quad (1)$$

$$M = C \cdot (V_{acid} + V_{gel}) / f_e \quad (2)$$

where C_{DGT} represents the metal concentration in the soil solution and that re-supplied from soil solid phases (mg/L); C is the metal concentration ($\mu\text{g L}^{-1}$) in 1 M HNO₃; M is the accumulated mass of metal on the binding gel (mg); Δg is the thickness of the diffusion layer (0.0789 + 0.014 cm); D is the diffusion coefficient of metals at 20 °C ($\text{cm}^2 \text{s}^{-1}$); A is the area of the resin gel exposed to the diffusion flux (3.14 cm²); and t is the deployment time (s), V_{acid} and V_{gel} are the volume of the acid for elution (1 mL) and resin gel (0.14 mL), respectively, and f_e is the elution factor of metals from the resin gel (0.8).

- Determination of DGT metal concentration in samples from pot experiments after storage periods of 0, 1, 2 and 3 months (NZS3-I, NZS6-I; NZS3-1, NZS6-1; NZS3-2, NZS6-2; NZS3-3, NZS6-3). Assembled DGT devices were placed on the mixture surface of each pot experiment for 24 h at 20 ± 2 °C, for equilibration. DGT retrieval, elution, metal determination and calculation steps were similar with those used and described for the initial samples.

2.4. Soil Solution Metal Concentration

After completion of the DGT deployment, the soil and soil–zeolite mixtures were analyzed for metal content in soil solution (C_{sol}). The remaining slurries after DGT deployment were centrifugated at 4000 rpm for 20 min to extract the soil solution. Subsequently, the supernatants were filtered using 0.45 μm cellulose membrane and the metal concentrations in soil solution were quantified by GFAAS. These concentrations are important since the ratio (R value) of C_{DGT} to C_{sol} reflects the soil's ability to resupply a metal from the soil solid phase to soil solution. An R value closer to 1 indicates a very rapid resupply from the solid phase to the soil solution, whereas an R value close to 0.1 indicates a very slow resupply [39].

3. Results

3.1. Soil and Zeolite Physico-Chemical Characteristics

Table 1 shows the physico-chemical properties and aqua regia extractable metal concentration in soil, as well as in the soil with the added amounts of zeolite. The total Cd, Cu, Pb and Zn concentrations exceeded the Romanian intervention thresholds for sensitive soil use, presented in Table 1 [14].

The total Cd concentration in the composite soil was 34.7 mg kg^{-1} (6.94 times higher than the intervention threshold), the total Cu concentration was 553 mg kg^{-1} , the total Pb concentration was 392 mg kg^{-1} , while the total Zn concentration was 2800 mg kg^{-1} . Only the total Cr concentration (15.2 mg kg^{-1}) was lower than the intervention threshold (300 mg kg^{-1}).

The trace metal concentrations in zeolites ($<1.0 \text{ mg kg}^{-1}$ Cd, 4.25 mg kg^{-1} Cr, 1.16 mg kg^{-1} Cu, 6.33 mg kg^{-1} Pb, 4.40 mg kg^{-1} Zn) were much smaller than the maximum levels

allowed in the sewage sludges intended for the application on agricultural soil [15]. A small decreasing of the total trace metal concentrations in mixtures of soil with zeolite was observed, caused by the “dilution effect” of added zeolite containing much lower trace metal concentrations compared to the soil.

The CEC value of the used zeolite determined after the AMAS extraction method and measuring the extractable major cations was 129 meq/100 g. The contributions of each extractable cation were 33.6 meq K^+ /100 g, 15.4 meq Na^+ /100 g, 78.2 meq Ca^{2+} /100 g, and 1.9 meq Mg^{2+} /100 g. The theoretical CEC value calculated considering the total content of K, Na, Ca and Mg in zeolite after microwave-assisted digestion is 196.7 meq/100 g, which consists of 53.1 meq K^+ /100 g, 16.7 meq Na^+ /100 g, 94.4 meq Ca^{2+} /100 g, and 32.5 meq Mg^{2+} /100 g. The obtained results indicate that 92% of Na, 83% of Ca, 63% of K and 3% of Mg are exchangeable, and on average over 65% of sites can be used in cation exchange processes. The total surface area measured using the BET method was $52 \text{ m}^2 \text{ g}^{-1}$, while total pore volume and pore radius were $0.12 \text{ cm}^3 \text{ g}^{-1}$ and 21 \AA , respectively.

According to XRD analysis, the used zeolitic tuff contains clinoptilolite (PDF 00-047-1870) as the main crystalline phase, accompanied by minor quantities of albite (PDF 00-041-1480), muscovite (PDF 00-058-2034) and quartz (PDF 01-079-1910) (Figure 1). The non-crystalline components were not quantified by the XRD analysis, but the presence of amorphous volcanic glass in the zeolitic tuff is indicated by the broad diffraction hump between 18 and $32^\circ 2\theta$ [30].

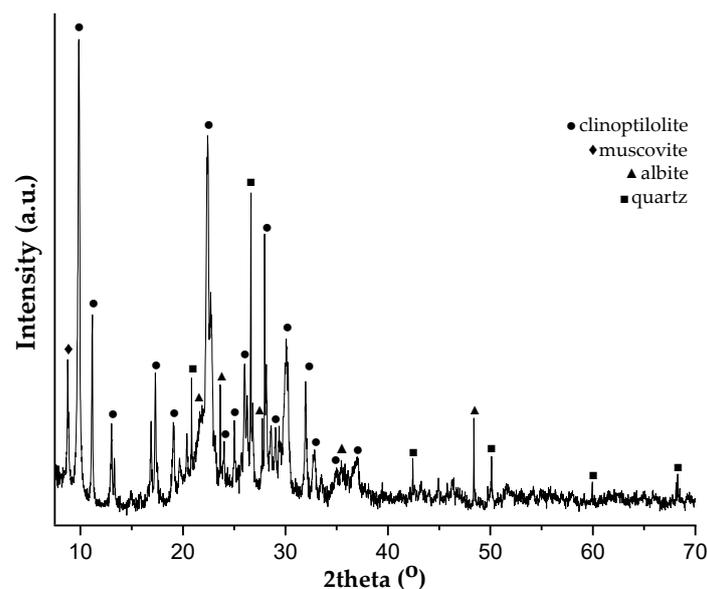


Figure 1. X-ray diffraction pattern of the zeolitic tuff sample.

The measured Si/Al ratio 5.56 (>4) and dominant alkaline cations ($Na + K > Ca$) in the zeolitic tuff sample also suggest the clinoptilolite as a major constituent [55]. The clinoptilolite mineral is considered an excellent ion exchanger and adsorption material for different cations. Its selectivity for the metals decreases in the following order: $Pb^{2+} > Cd^{2+} > Cu^{2+} > Cr^{3+} > Zn^{2+}$ [56]. The physico-chemical characteristics corroborated with its low content of toxic metals and make this material a suitable candidate for the remediation by immobilization of soils contaminated with toxic metals.

3.2. pH during the Storage Period

The pH levels of the different soil treatments during the storing periods are shown in Table 2. Even if the addition of zeolite slowly increased the pH average values of the soil-zeolite mixtures at the start of experiments due to the alkaline nature of zeolite ($pH = 9.55$), according to *t*-test ($p = 95\%$, and $n = 3$ determinations), these changes were not statistically significant. The pH of mixtures with zeolites during the equilibration periods remained also

almost similar to that from the first day of amendments (8.63–8.82 in NZS3 and 8.60–8.83 in NZS6).

Table 2. Heavy metal concentrations ($\mu\text{g L}^{-1}$) in soil solution (C_{sol}) and pH of control soil (CS) and mixtures with zeolite (NZS3 and NZS6) during the three-month storage (average \pm standard deviation, $n = 3$ parallel determinations).

	CS	t calc *	NZS3	t calc	NZS6	t calc
Initial						
pH	8.58 \pm 0.12	-	8.77 \pm 0.15	1.316	8.78 \pm 0.15	1.386
C_{sol} Cd	2.46 \pm 0.25	-	2.23 \pm 0.34	0.944	2.13 \pm 0.22	1.716
C_{sol} Cr	2.43 \pm 0.28	-	2.22 \pm 0.26	0.952	2.38 \pm 0.25	0.231
C_{sol} Cu	30.1 \pm 2.63	-	25.5 \pm 3.16	1.938	24.9 \pm 2.98	2.266
C_{sol} Pb	3.23 \pm 0.43	-	3.04 \pm 0.40	0.560	3.14 \pm 0.32	0.291
C_{sol} Zn	279 \pm 27.4	-	241 \pm 24.0	1.807	207 \pm 21.4	3.587
1 month storage						
pH	8.47 \pm 0.14	0.780	8.63 \pm 0.11	0.379	8.60 \pm 0.14	0.142
C_{sol} Cd	2.30 \pm 0.16	0.934	1.83 \pm 0.25	3.086	1.76 \pm 0.32	2.986
C_{sol} Cr	2.30 \pm 0.33	0.520	2.12 \pm 0.15	1.690	2.28 \pm 0.26	0.680
C_{sol} Cu	27.2 \pm 2.10	1.492	31.0 \pm 2.00	0.472	28.1 \pm 3.25	0.824
C_{sol} Pb	3.01 \pm 0.22	0.789	2.09 \pm 0.16	4.304	1.99 \pm 0.19	4.569
C_{sol} Zn	288 \pm 31.4	0.374	232 \pm 25.5	2.175	216 \pm 33.3	2.514
2 months storage						
pH	8.48 \pm 0.20	0.612	8.74 \pm 0.19	1.005	8.81 \pm 0.21	1.374
C_{sol} Cd	2.19 \pm 0.28	1.246	1.70 \pm 0.20	4.112	1.53 \pm 0.18	5.229
C_{sol} Cr	2.40 \pm 0.40	0.106	1.95 \pm 0.21	2.375	1.72 \pm 0.14	3.928
C_{sol} Cu	32.4 \pm 4.48	0.767	27.3 \pm 1.08	1.706	26.8 \pm 2.35	1.630
C_{sol} Pb	2.90 \pm 0.38	0.996	1.83 \pm 0.13	5.398	1.73 \pm 0.16	5.663
C_{sol} Zn	264 \pm 22.8	0.729	227 \pm 35.0	2.026	237 \pm 27.0	1.869
3 months storage						
pH	8.63 \pm 0.18	0.322	8.82 \pm 0.20	1.470	8.63 \pm 0.18	0.322
C_{sol} Cd	2.23 \pm 0.21	1.220	1.65 \pm 0.08	5.345	1.20 \pm 0.14	7.617
C_{sol} Cr	2.32 \pm 0.15	0.600	2.01 \pm 0.12	2.388	1.77 \pm 0.32	2.688
C_{sol} Cu	28.8 \pm 2.04	0.676	29.1 \pm 1.15	0.634	27.3 \pm 3.40	1.116
C_{sol} Pb	3.11 \pm 0.25	0.418	2.08 \pm 0.15	4.374	1.66 \pm 0.19	5.784
C_{sol} Zn	280 \pm 11.0	0.059	289 \pm 9.70	0.596	304 \pm 16.5	1.375

* t calc values in bold face indicates significant difference between the parameters in initial soil sample (CS initial) and in CS and mixtures during the 3-month period (t calc in the range 0–4.303 indicates no significant difference for $p = 95\%$).

3.3. Heavy Metal Concentrations in Soil Solution

The centrifugation of watered to MWHC of soils is a simple and fast method of assessing the concentration of metals in soil porewater. Throughout the three months of storage, the zeolite amendments generally influenced the heavy metal concentrations in the soil solution (Table 2). The average Cd, Cr, Cu, Pb and Zn concentrations in soil solution (CS) at the start of storage were of 2.46, 2.43, 30.1, 3.23, and 279 $\mu\text{g L}^{-1}$, respectively.

As an effect of zeolite amendment, after the 24 h of equilibration, the average concentrations in soil solutions slowly decreased with 9% for Cd, 9% for Cr, 15% for Cu, 6% for Pb and 14% for Zn when 3% zeolite was added, and with 13% for Cd, 2% for Cr, 17% for Cu, and 26% for Zn, when 6% zeolite was added. However, when the *t*-test was applied (*t*-test for $p = 95\%$, and $n = 3$ determinations), the changes in metal concentration on the first day of experiments were not significant.

Moreover, to find if the differences between the initial heavy metal's concentrations in the soil solutions and those extracted from mixtures of soil with zeolite during the three months of storage are statistically significant, a two-tailed *t*-test for $p = 95\%$ and $n = 3$ was calculated, and the *t* values are presented in Table 2. We used the two-tailed *t*-test since it

allows for the possibility of comparing the metal concentration in the initial sample and after the mixing with zeolite considering the confidence intervals of both datasets. The calculation of the t -values, both the average measured concentrations in soil solution and DGT, and the measurement errors (the standard deviations of the three determinations) thus allows for a better evaluation of the evolution of concentrations in the soil solution and in the DGT. In general, the metal content in soil solution remained unaffected in CS during the three months of experiments, for all the analyzed metals (the decreasing rate was below 10%, and t -values in the range 0–4.303).

3.4. DGT Measurement

Generally, both the C_{DGT} and C_{sol} of metals in soils increased with increasing total concentrations. As presented in Table 3, the highest DGT-measured concentrations (C_{DGT}) were found, as in soil solutions, for Zn. In all analyzed samples, the C_{DGT} was lower than the heavy metal concentration in the soil solution.

Table 3. DGT-measured concentrations (C_{DGT}) of heavy metals ($\mu\text{g L}^{-1}$) in soil slurries of control soil (CS) and mixtures with zeolite (NZS3 and NZS6) during the three-month storage (average \pm standard deviation, $n = 3$ parallel determinations).

	CS	t calc *	NZS3	t calc	NZS6	t calc
Initial						
C_{sol} Cd	1.01 \pm 0.20	-	0.90 \pm 0.15	0.759	0.88 \pm 0.13	0.960
C_{sol} Cr	0.92 \pm 0.17	-	0.84 \pm 0.12	0.645	0.81 \pm 0.14	0.896
C_{sol} Cu	15.5 \pm 2.35	-	14.5 \pm 1.41	0.647	15.1 \pm 1.05	0.298
C_{sol} Pb	1.25 \pm 0.29	-	1.26 \pm 0.21	0.082	1.27 \pm 0.24	0.092
C_{sol} Zn	155 \pm 21.2	-	148 \pm 17.2	0.445	131 \pm 20.3	1.397
1 month storage						
C_{sol} Cd	1.13 \pm 0.19	0.759	0.89 \pm 0.14	0.831	0.66 \pm 0.12	2.745
C_{sol} Cr	1.08 \pm 0.14	1.255	0.96 \pm 0.12	0.304	0.77 \pm 0.16	1.417
C_{sol} Cu	14.0 \pm 1.76	0.871	13.2 \pm 1.31	1.521	11.0 \pm 0.16	3.159
C_{sol} Pb	1.20 \pm 0.26	0.217	1.17 \pm 0.25	0.337	0.95 \pm 0.19	1.650
C_{sol} Zn	145 \pm 19.7	0.624	131 \pm 15.5	1.297	146 \pm 12.3	0.657
2 months storage						
C_{sol} Cd	0.96 \pm 0.18	0.332	0.41 \pm 0.10	4.830	0.40 \pm 0.09	4.891
C_{sol} Cr	0.73 \pm 0.09	1.502	0.83 \pm 0.10	0.801	0.79 \pm 0.07	1.219
C_{sol} Cu	19.2 \pm 2.33	1.544	12.6 \pm 1.08	1.919	10.0 \pm 0.75	3.945
C_{sol} Pb	1.37 \pm 0.22	0.610	0.58 \pm 0.11	3.605	0.39 \pm 0.09	4.606
C_{sol} Zn	146 \pm 23.3	0.589	135 \pm 19.0	1.376	112 \pm 13.6	3.202
3 months storage						
C_{sol} Cd	0.93 \pm 0.21	0.548	0.28 \pm 0.08	5.788	0.29 \pm 0.09	5.837
C_{sol} Cr	1.05 \pm 0.15	0.934	0.88 \pm 0.12	0.328	0.68 \pm 0.16	2.081
C_{sol} Cu	13.7 \pm 2.04	0.987	11.2 \pm 1.12	3.036	11.4 \pm 0.91	2.858
C_{sol} Pb	1.15 \pm 0.25	0.469	0.58 \pm 0.10	3.928	0.48 \pm 0.09	4.456
C_{sol} Zn	117 \pm 11.0	2.332	126 \pm 9.70	2.296	152 \pm 8.88	0.223

* t calc values in bold face indicates significant difference between the parameters in initial soil sample (CS initial) and in CS and mixtures during the 3 months period (t calc in the range 0–4.303 indicates no significant difference for $p = 95\%$).

After the 24 h of equilibration of soil with 3% NZ, the average C_{DGT} decreased with 11% for Cd, 8% for Cr, 7% for Cu and 5% for Zn, while no diminishing was observed for Pb. When 6% NZ was added, after 24 h of equilibration, the average C_{DGT} decreased with 13% for Cd, 12% for Cr, 3% for Cu and 15% for Zn, and also in this case no decrease was observed for Pb. According to the t -test values ($p = 95\%$, $n = 3$), these modifications were statistically significant (Table 3).

4. Discussion

The effect of zeolite-clinoptilolite addition to the contaminated soil on heavy metal (Cd, Cr, Cu, Pb, Zn) immobilization was demonstrated by assessing their simulated bioavailability decrease during a three-month experimentation period using the DGT technique.

The average concentrations in soil solution of NZS3 and NZS6 decreased after 3 months of storage with 33% for Cd, 17% for Cr, 3% for Cu, 36% for Pb, 51% for Cd, 27% for Cr, 9% for Cu, and 49% for Pb, compared to the initial soil sample. In both cases, no decrease in the Zn concentration was observed. A decreasing trend of metal concentrations in soil solution was generally observed during the storage period. However, the differences were significant only for Pb (in both NZS3 and NZS6) starting with 1 month of storage, and for Cd (in NSZ3 after 2 months of storage, and in both NZS3 and NZS6 after 3 months of storage).

The R value is commonly used to assess the capability of metal resupply released from the soil solid phase to soil solution [57]. In order to evaluate the influence of applying 3% and 6% NS on the simulated bioavailability of metals in soil, the R ratios between C_{DGT} to C_{sol} were considered. The R values in the control soil (CS) and soil amended with zeolite at 0, 1, 2 and 3 months after amendment (experiments 1, 2, 3, 4) are presented in Figure 2.

C_{DGT} generally showed a similar tendency with those observed in the soil solution. Thus, the average C_{DGT} measured in NZS3 decreased after 3 months with: 73% for Cd, 4% for Cr, 28% for Cu, 54% for Pb, and 13% for Zn, comparing with C_{DGT} in the initial soil sample. C_{DGT} in NZS6 diminished after 3 months with 71% for Cd, 26% for Cr, 27% for Cu, 61% for Pb, and with 2% in the case of Zn. As in the case of C_{sol} , the decrease in metal concentrations was significant only for Pb (in NZS6) and Cd (both in NSZ3 and NZS6), after 2 months of storage. The reducing (%) of the C_{DGT} over the time (3 months) due to the zeolite addition (6%) followed the order: Cd > Pb > Cu > Zn > Cr. This order is in line with the selectivity order reported for the clinoptilolite mineral— $Pb^{2+} > Cd^{2+} > Cu^{2+} > Cr^{3+} > Zn^{2+}$ [56]—which explains the significant reducing of bioavailable fractions mainly for Cd and Pb.

The R ratios at the start of experiments were in the range of 0.40–0.41 for Cd, 0.34–0.38 for Cr, 0.52–0.61 for Cu, 0.39–0.42 for Pb and 0.56–0.63 in the case of Zn (Figure 2). For all trace metals, R indicated a low-to-medium resupply from the solid phase. Since R ratios evaluate the soil ability to resupply a metal from the soil solid phase to soil solution, a decreasing value of R indicates the reduction in simulated bioavailability due to the metal immobilization in the soil solid phase. R values remained nearly unchanged in CS during the storage period, for all metals. Moreover, in the case of Cr, the R value was almost similar during the storage period, even if NZ was added to the soil. Slow decreases in R values were observed for Cu and Zn over the time, while in the case of Cd and Pb, the decreasing of R values was more obvious. The R values decreased until 0.23 for Pb and 0.17 for Cd, showing a very low resupply from the solid phase in samples where NZ was added.

Zeolite has also been previously used to reduce the metal bioavailability. Li et al. [58] used synthetic zeolite for the immobilization of Cd, Pb and As in soils and observed a decreasing of available concentrations with 10.2–96.8%, after 180 days. Zheng et al. [27] reported the use of synthetic zeolite for polluted soil remediation with the removal efficiency for Pb and Cu up to 90.7 and 81.4%. Moreover, the natural zeolite had a high potential to immobilize Pb, Cd, Zn and Cd in polluted soil [34,58]. Lee et al. [33] reported that the immobilization of Cu and Zn in soil was due to the zeolite application as a consequence of their binding onto negatively charged sites of amendment. Tica et al. [59] reported the decreasing of water-soluble Pb, Cd and Zn concentrations in soil following zeolite addition as an effect of pH increasing, but no effect was observed in the case of Cu. Li et al. [60] found that the Pb concentrations were lowered by 30% in the edible parts of rapeseed when natural zeolite was added to contaminated garden soil. Some studies revealed that the Cu, Zn, Cd and Ni sorption in soils amended by zeolite is improved by increased pH [61]. Garau et al. [3] confirmed that an increase in pH had an effect on the Pb, Cd and Zn immobilization in contaminated soil. Putwattana et al. [62] stated a 17% decrease in

Zn availability after the addition of 5% zeolite to contaminated soil. Shi et al. [63] showed that the addition of zeolite inhibits the uptake of Pb by plants by affecting rhizospheric behavior and concluded that CEC was the dominant factor for Pb immobilization.

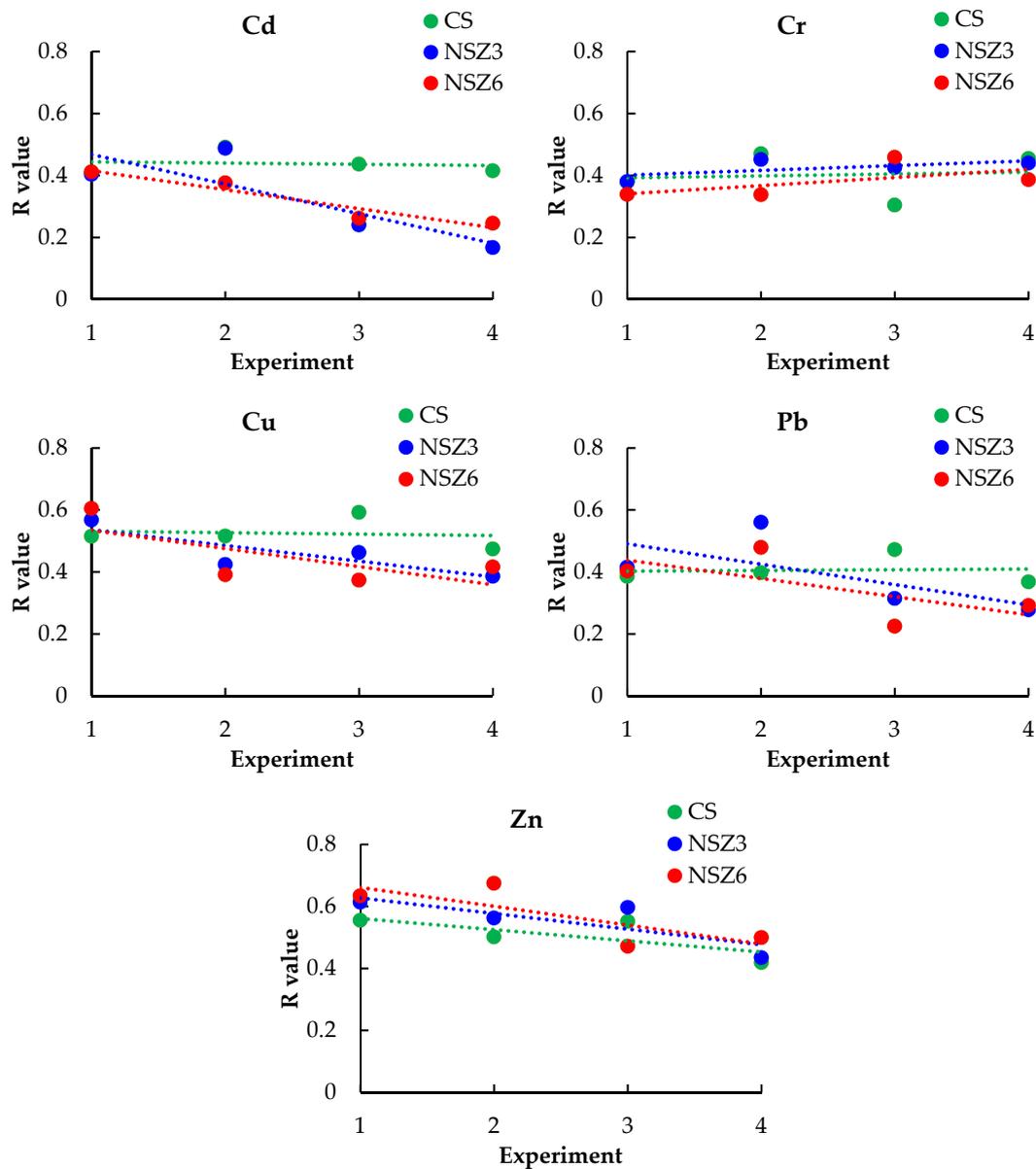


Figure 2. Ratio of C_{DGT} to C_{sol} (R value) in the control soil (CS) and soil amended with 3% (NZZ3) and 6% (NZZ6) zeolite at 0, 1, 2 and 3 months after amendment (experiments 1, 2, 3, 4).

No increase in the soil pH was observed after the zeolite addition, mainly because the soil used in experiments had a high pH value. Moreover, the added NZ did not contribute with organic matter ($C_T < 0.01\%$) to soil–zeolite mixtures. Therefore, the decrease in metal bioavailability can be attributed mainly by their adsorption in the zeolite structure. This finding is supported also by the correlation between clinoptilolite selectivity order [56] and the significant reduction in mobility for Cd and Pb.

Linear regression analysis was applied to evaluate the correlations between the DGT concentrations of the analyzed heavy metals. Strong positive correlations were found between C_{DGT} Cd and C_{DGT} Cr ($r = 0.947$), C_{DGT} Cd and C_{DGT} Pb ($r = 0.940$), C_{DGT} Cu and C_{DGT} Pb ($r = 0.940$), and C_{DGT} Cd and C_{DGT} Cu ($r = 0.714$), indicating a similar trend in their DGT concentrations as a result of zeolite addition.

The fate of the heavy metals in soil is related to the concentration of their bioavailable forms. The metal bioavailability is controlled by different physical and chemical processes, such as adsorption and desorption, ion exchange, complexation, precipitation and dissolution, oxidation-reduction, occlusion, diffusion and migration, immobilization and mobilization, and plant uptake [9,64]. The importance of each process varies for different metals, but in all cases the bioavailability is affected by soil pH. In general, at low pH values the dominant species in aqueous solution are the free aquo cations, enhancing their mobility. CEC is also an important factor in the immobilization of heavy metals in soil [64]. This parameter usually indicates the sum total of exchangeable cations that a soil can adsorb. The partitioning of metals between solid phase and aqueous solution is influenced by adsorption–desorption and precipitation–dissolution reactions. In the case of zeolitic materials added to the soil, metals are exchanged between solid and liquid phases by the ion exchange process.

Since no significant pH changes in soil were observed after zeolite addition, ion exchange can be considered the key process in soil that controls the bioavailability of metals in our study. The removal of metal ions from soil solution and its association with the soil solid fraction was found to be influenced by zeolite addition, mainly for Cd and Pb. The main advantage of the DGT technique used in this study is that it allowed one not only to evaluate the changes in metal concentration in soil solution but also the metal resupply from the solid phase, and it is an alternative for assessing plant uptake. Thus, comparing with the classical chemical extractions, it has a superior capability to predict the metal bioavailability to biota. The decreases in R values observed for some metals in our study provide useful information for establishing effective remediation strategies for contaminated soils.

Generally, the literature is scarce in presenting the assessment of metal immobilization in the soil solid phase by adding soil amendments. DGT was employed for this purpose in very few studies. Egene et al. [21] used DGT to assess the impact of organic amendments (biochar, compost and peat) on Cd and Zn mobility in contaminated soil, and reported a low resupply of the two metals from the solid phase (R ranged between 0.3 and 0.4), insufficient to fully sustain pore water concentrations. The 2% biochar addition resulted in a decrease in soil solution concentration of 40% for Cd and 48% for Zn, while the 4% biochar addition resulted in a decrease of 66% for Cd and 77% for Zn, over a three-year period [21]. Dai et al. [35] also reported the use of DGT compared with traditional extraction methods to assess the As bioavailability in soils to *Brassica chinensis*. The results showed that the DGT technique provided a better assessment of As bioavailability compared to traditional extraction methods, which are sensitive to soil pH and Feox content. The sequential extraction and DGT techniques were used to evaluate the efficacy of hydrochar and pyrochar for alleviating the bioavailability of Cd in soils. The obtained results revealed higher R values for Cd than in our study (R in the range of 0.88–0.96), but the R value was also lower in the amended soils compared with the initial soil [36]. In another study, the effect of two different organic amendments on trace metal transfer from soils to crop assessed by DGT was investigated [48]. R values calculated by DGT for all studied metals were <0.3, indicating a low resupply from the solid phase due to the amendment addition. Despite the fact that zeolite was reported as an effective amendment to reduce metal bioavailability in several previous studies [58–63], no literature report was found to present the assessment of metal resupply from the soil phase through R values measured by DGT, after the zeolite addition to the soil. Moreover, the soil remediation becomes a stringent necessity in the context of changing environments. The main climate change factors that affect soil properties are caused by the production of greenhouse gases, the continued increasing of air and soil temperature, extreme rainfall, and surface soil erosion [65]. Thus, it may be interesting to monitor the changes in metal resupply over an extended period to provide further insight into the mobility trend of metals in zeolite-amended soil. Our results may represent the first step in the elaboration of a soil remediation strategy based on metal stabilization in soil.

5. Conclusions

The efficiency of the addition of different amounts of natural zeolite in reducing the bioavailability of heavy metals in a contaminated soil was evaluated by the DGT technique. The mean trace metal concentrations in the initial soil were 34.7 mg kg^{-1} for Cd, 553 mg kg^{-1} for Cu, 392 mg kg^{-1} for Pb and 2800 mg kg^{-1} for Zn, higher than the regulated threshold for sensitive use according to the Romanian legislation. The metal (Cd, Cr, Cu, Pb, Zn) immobilization by zeolite was tested in pot experiments using different natural zeolite amounts (3 and 6 wt.%) after three months of storage. No increase in the soil pH was observed after the zeolite addition, and no supplementary organic matter was added to the soil. Excepting Zn, the average concentrations in the soil solution of soil mixed with natural zeolite decreased after 3 months of storage with 33% for Cd, 17% for Cr, 3% for Cu, 36% for Pb (Nzs3) and 51% for Cd, 27% for Cr, 9% for Cu, 49% for Pb (Nzs6). The DGT measured concentrations generally showed a similar trend with those observed in the soil solution. The R ratios between C_{DGT} and concentration in soil solution (C_{sol}) were calculated. The addition of natural zeolite to soil significantly reduced Cd and Pb simulated bioavailability in soil after two months of storage. The bioavailability of the other trace metals (Cu, Cr and Zn) also displayed a decreasing trend, but the change in bioavailability was not statistically significant. Based on the obtained results, it can be concluded that the decrease in metal bioavailability is mainly caused by their binding in the zeolite structure. Moreover, it appears that the natural zeolite is an environmentally friendly and efficient amendment to remediate by immobilization multi-metal-contaminated soils. C_{DGT} not only contains ions dissolved in soil solution, but also metals resupplied from the soil solid phase. The obtained results demonstrate the performance of the DGT technique to evaluate metal immobilization in soils, compared to that of traditional chemical extractions.

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