POLISH JOURNAL OF SOIL SCIENCE
VOL. L/2 2017 PL ISSN 0079-2985

**DOI:** 10.17951/pjss/2017.50.2.141

MAŁGORZATA MAJEWSKA*

# MOBILIZATION OF CADMIUM FROM *FESTUCA OVINA* ROOTS AND ITS DISTRIBUTION BETWEEN OPERATIONAL FRACTIONS IN SOIL

*Received: 28.05.2017*
*Accepted: 30.10.2017*

*Abstract. Festuca ovina* (L.) was grown hydroponically on the Hoagland medium supplemented with $CdCl_2$ (10 µg Cd $ml^{-1}$). Next, the plants were transplanted into pots (100 plants in pots) with uncontaminated soil and incubated under controlled conditions for 12 months. Approximately 420 µg Cd were introduced into 200 g of soil via the plant roots, the released cadmium being distributed between fractions with varying stability and extractability. After 2 months, the pool of Cd exchangeable and bound to Fe and Mn oxides was 16% and 75%, respectively. After the end of the pot cultivation, the content of Cd in these fractions had decreased to 5% and 53%, respectively. In contrast, the percentage of Cd defined as organically bound increased from 6% (after 2 months) to 43% (after 12 months). The residual fraction was 2% of the metal present in the soil and was constant during plant cultivation. The results obtained indicate that the Cd stabilization by roots was dependent on time. Additionally, the distribution of Cd among the tested fractions was seen to have changed during the experiment. The amount of Cd bound to soil organic matter increased, lowering the amount of the bioavailable Cd form and Cd fraction bound to the oxide minerals.

**Keywords:** heavy metals, sequential extraction, Cd extractability, Cd stability, phytoremediation

---

* Department of Environmental Microbiology, Institute of Microbiology and Biotechnology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, 19 Akademicka St., 20-033 Lublin, Poland, e-mail: majewska@poczta.umcs.lublin.pl

## INTRODUCTION

It is well known that metals occurring in terrestrial ecosystems can exist in different chemical forms. Hence, measurements of total metal concentrations are not enough to specify their mobility, bioavailability and toxicity (Conesa *et al*. 2010, Bolan *et al.* 2014). Based on ways of their binding by soil constituents and the strength of the created bounds, seven geochemical fractions of metals can be described: (1) a mobile fraction containing soluble and non-specifically sorbed metals, as well as metal-organic complexes, (2) an easily mobilizable fraction including metals specifically sorbed on the particle surface and bound to carbonates, (3) occluded in manganese oxides, (4) bound to soil organic matter, (5) occluded in amorphous iron oxides, (6) occluded in crystalline iron oxides, and (7) structurally bound in soil minerals (Conesa *et al*. 2010, Stritsis *et al*. 2014). Chemical speciation of metals determined in soil changes over time, and their redistribution between operational fractions is possible (Majewska and Kurek 2007, Kabata-Pendias 2011, Oves *et al.* 2012). However, a dynamic equilibrium between the processes of mobilization and immobilization of metals comes about (Bolan *et al.* 2014). This is influenced by the physico-chemical properties of the soil (e.g. pH, cation exchange capacity, particle size, content and type of soil organic matter), activity and diversity of soil microorganisms, and growth of plants (Stritsis *et al.* 2014, Li *et al.* 2016).

The roots of the plants inhabiting contaminated soil create an encouraging environment for existing microorganisms (rhizosphere), and, together, perform an important function in the mobilization of less active fractions of metals. On the other hand, natural processes in the plant rhizosphere (e.g. bioaccumulation, biosorption, complexation, precipitation, redox reactions) may cause the immobilization of the previously released metals (Lou *et al*. 2013). In generating a shift in the equilibrium within the rhizosphere-based processes towards the retention of the metals, we are able to increase the efficiency of phytostabilization of various pollutants. Of note: according to Prasad (2003), phytostabilization is not only the sequestering of the metals in the rhizosphere and tissues of plants, but also the immobilization by the humus formed during decomposition and humification of the plant residues.

Roots are an important component of the topsoil (Ping *et al.* 2010). They increase the resistance of the upper soil layer to water and wind erosions (Ernst 2005, Beats *et al*. 2006), and together with rhizospheric microorganisms, act as factors in accelerating the rates of plant residue degradation and in encouraging humus accumulation (Abakumov *et al*. 2013). The water-soluble root exudates and mucilage of fully functional roots, the sloughing of root cells, as well as the accumulation of dead roots are sources of organic carbon and energy for rhizospheric microorganisms (Hütsch *et al.* 2002). Furthermore, the aforementioned act as substrates for humification (Abakumov *et al*. 2013). Humic substances, in addition to the stable products of humification such as humus acids (fulvic and humic acids) and humin, also contain easy degradable proteins, polysaccharides, fatty acids, alcohols, esters and others substances originating from microorganism and plant metabolism (Semenov *et al.* 2013). According to Semenov *et al*. (2013), the structure of soil organic matter is (1) dynamic, because it undergoes continuous modifications; (2) polyfunctional, in view of its amphiphilic and hydrophobic nature; and (3) heterogeneous, due to the presence of the carboxyl, alcohol, phenol, peptide, amide and other functional groups (Ernst 2005, Semenov *et al*. 2013). Humic substances are formed by the polycondensation and polymerization of various monomers. Monomers are released from biodegradable polymers and are polymerizated *de novo* or are successively added to the active functional group at the end of the existing chain e.g. the lignin or polyphenols that are the main blocks of the humic skeleton. Soil organic matter is, hence, a multicomponent continuum of partially decomposed biota residues, biomass of microorganisms, rhizodeposits, biomolecules and humus (Semenov *et al*. 2013). In this context, transformation of dead roots and rhizodeposits contaminated with metals can lead to the creation of metal-containing humic substances, and, thus, metal stabilization in soil organic matter. The phenomenon of stabilization and destabilization of organic matter can, therefore, significantly change the mobility of metals and their distribution between geochemical fractions.

The aim of this study was to evaluate the amount of Cd released from the roots of contaminated plants growing in uncontaminated soil, and to assess its distribution between four operational fractions as determined by sequential extraction. In this study, the amount of total soil organic carbon (TOC), as well as three of its fractions (water-dissolved, base-dissolved and remaining in soil after extraction) was measured. Moreover, the amount of microorganisms and soil pH were investigated.

## MATERIALS AND METHODS

### *Hydroponic and pot growth of plants (two-step cultivation of plants)*

Seeds of *Festuca ovina* var. Bornito C1 (600 ± 25) were arranged in plastic sieves with a surface area of 169 cm$^2$ which were then placed in plastic containers holding 700 ml of the Hoagland medium (Hoagland and Arnon 1950). Plants were grown in the hydroponic culture for 3 weeks. After this, the medium was changed to a fresh medium supplemented with 10 µg Cd ml$^{-1}$ (as $CdCl_2 \cdot 2.5H_2O$) and incubation of plants was continued over the next 3 weeks. During plant growth, the volume of the evaporated growth medium was systematically replenished with uncontaminated Hoagland medium (Majewska and Słomka 2016). After this period, the plants were removed from the Hoagland medium and their roots were gently immersed in deionized water to remove droplets of the Hoagland medium, but not the Cd immobilized on the surface of roots. Subsequently, the plants were transplanted to pots containing 200 g of soil noncontaminated with Cd (100 plants in pots). The moisture of the soil was held at 60% of the water-holding capacity during the 12 month long experiment. The utilized brown soil originated from a cultivated field located in the region of Puławy (51°25′23″N and 22°05′45″E). Based on texture composition, this soil was defined as slightly loamy sand (88% sand, 11% silt, 1% clay). Soil total carbon content was 7.2 $g \cdot kg^{-1}$; total nitrogen content was 0.6 $g \cdot kg^{-1}$; $Na^+$, $K^+$, $Mg^{+2}$ and $Ca^+$ exchange capacity was 5.1, 9.0, 10.0, 170.0 mg $kg^{-1}$, respectively. The properties of the soil were analyzed using standard methods described by Alef and Nannipieri (1995). The plants, when under hydroponic cultivation, as well as when potted, were located inside a growth chamber (Biogenet, FD147 Inox) that allowed automatic climatic control (day/night temperatures of 24/18°C and 70% air moisture) and controlled light conditions (250 µmol $m^{-2}$ $s^{-1}$ irradiance and a 12/12 photoperiod) for 12 months.

Randomly chosen pots at time zero (day of plant transplantation) and after 2, 4, 6, 8, 10 and 12 months of the plant cultivation were assessed for (1) the concentration of Cd in plant biomass; (2) distribution of Cd between the four operational fractions determined by sequential extraction; (3) pH and number of soil microorganisms; (4) TOC and its three fractions with differential solubilization (dissolved in water, alkali and non-dissolved organic carbon).

### *Determination of Cd concentration in plants and soil*

After each 8-week period, plants were collected, separated into roots and shoots, rinsed three times with deionized water and dried at 105°C until constant weight. The dry plant material (20 mg) was then digested with 20 ml concentrated $HNO_3$ for 12 hours at 20°C. Next, the acid was evaporated to dryness, and then the residue was dissolved in 1M $HNO_3$. Concentrations of Cd in the mineralizates were determined according to the dithizone method described by Fiedler *et al.* (2004) and Majewska and Słomka (2016). Standard curves from 2 to 10 µg Cd $ml^{-1}$ were prepared from stock solution ($CdCl_2 \cdot 2.5\ H_2O$ in 1M $HNO_3$), and the absorbance of the samples was determined at $\lambda$=549 nm using spectrophotometer UV-Vis (Varian, UV-visible spectrophotometer).

After 2, 4, 6, 8, 10 and 12 months of plant cultivation, soil samples taken from randomly chosen pots (1 g) were sequentially extracted utilizing four types of solutions (Majewska *et al.* 2011), in the following order: 10 ml 0.1M $NaNO_3$, 20 ml 1M $NH_3OH \cdot HCl$ in 25% acetic acid, 8 ml in a mixture of 30% $H_2O_2$ with 0.02M $HNO_3$ in a 5:3 ratio, and 5 ml in a mixture of concentrated HCl with $HNO_3$ in a 3:2 ratio. After each extraction step, the extracts were separated from the soil by centrifugation at 9000 × *g* for 15 minutes. Afterwards, Cd concentrations in the collected supernatants were measured after mineralization in concentrated $HNO_3$ as described above. The percentage of the Cd in each individual fraction was calculated in relation to the sum of all determined fractions.

### *Determination of soil pH and the number of microorganisms*

The numbers of microorganisms inhabiting the soils were estimated using the plate count method. The soil sample (10 g) was suspended in 90 ml of sterile distilled water and shaken (250 rotation per minute) at 20°C for 30 minutes. After preparing a 10-fold dilution series, 0.1 ml of the appropriate dilution was pipetted on an agar surface and spread with a sterile glass rod. Fungi were cultivated on the Martin medium (Martin 1950) and bacteria on PYS (Peptone–Yeast extract–Soil extract) agar (Alef and Nannipieri 1995) for 7 days at 28°C. The number of microorganisms was expressed as log10 colony forming units (CFU) per gram of dry weight soil mass. In addition, at the same time, the pH of soil samples as suspended in distilled water in a ratio of 1:2.5 (w/v) were measured (Alef and Nannipieri 1995).

### *Estimation of various fractions of the total soil organic carbon*

Fractions of soil organic matter were analyzed using a threestep sequential extraction procedure. This method makes it possible to fractionate TOC into fractions defined as the water-soluble organic carbon (W-SOC, extracted with deionized water), base-soluble organic carbon (B-SOC, extracted with 0.1M NaOH) and non-extractable fraction of soil organic carbon (N-SOC, not removed during sequential extraction). Soil samples (3 g) were suspended in 30 ml of each extractant and shaken (150 rotations per minute) at 20°C for 24h in 50-ml centrifuge tubes. The tubes were then centrifuged for 15 min at 10 000 × *g* and the supernatants were collected. The soil sample was subsequently washed with 20 ml of deionized water, shaken for 30 min and again centrifuged. The main extractant and supernatant after washing were mixed. Concentrations of organic carbon in W-SOC, B-SOC and N-SOC fraction were determined using Tiurin's method with detection by titration (Lityński *et al.* 1976). Tiurin's method was also used to analyze the content of TOC in each soil sample.

### *Statistical analysis of data*

Statistical analysis was performed on four or eight replicates from each samples (soil or plant biomass). Standard deviations were determined, and differences between the individual means were compared using Student's t test (Microsoft® Excel 2010). The level of significance was set at $P<0.05$ (Brandt

1999). The values of correlations coefficients between concentration of Cd in each tested fraction and (1) pH, (2) numbers of microorganisms (bacteria and fungi), (3) TOC, (4) B-SOC and (5) N-SOC were also determined using Microsoft® Excel 2010.

## RESULTS AND DISCUSSION

### *Plant growth and Cd concentration in roots and shoots*

The addition of 10 µg Cd ml$^{-1}$ inhibited the growth of plants of *F. ovina* (L.) when hydroponically cultivated, and no significant increase of the length of the roots and shoots was found. After 3 weeks in the hydroponic medium contaminated with this metal, the concentration of Cd immobilized by roots was approximately 30 times higher than in shoots (Table 1). In the next step of the experiment, the Cd-contaminated plants were transferred to the pots containing uncontaminated soil (0.57 µg Cd g$^{-1}$DW) and were cultivated under controlled conditions for 12 months. During the first two months of pot cultivation, the dry mass of the shoots and roots increased about six-fold, in comparison to time 0 (the day on which the plants were transferred to pots). Moreover, the biomass of the shoots significantly increased (by an average of 14-fold) during the full 12-month cultivation (Table 1), while the dry mass of the roots systematically increased up to the eight month of incubation, and after this time, a decrease in the root biomass was noted.

Approximately 420 µg Cd were introduced into 200 g of soil via the roots of 100 plants (Table 1). About 90% of the Cd immobilized in such roots during the hydroponic cultural regime was released to the soil, while the heavy metal remaining in the root was sequestrated in their tissues or translocated to the shoots of growing plants. After a 2 month pot cultivation, the initial concentration of Cd was decreased in the roots and shoots, to 128 and 34 µg Cd g$^{-1}$DW, respectively. During further cultivation, the concentrations of Cd in the roots and shoots changed, and, from the sixth to the tenth month, it was significantly higher in the shoots than in the roots with similar values obtained at the end of this experiment (Table 1). In contrast, the total amount of Cd in the grass biomass (the sum of Cd accumulated in roots and shoots) did not increase from the second to the twelfth month of cultivation. According to the “dilution hypothesis” presented by Overdieck (2016), metal content could be diluted in the tissues of roots and shoots during plant growth; this is observed as Cd concentration decreases in plant biomass. The decreasing total amount of Cd in the roots and the increasing of the same in shoots can suggest that the metal was translocated from roots to shoots, and that the metal released to soil in the first months of plant cultivation was made unavailable. Overall, it can be said that the contained

TABLE 1.THE PARAMETERS OF PLANTS AND SOIL DETERMINED IN EACH TIME INTERVAL DURING POT EXPERIMENT. IN THE LAST COLUMN ARE SHOWN THE STATISTICAL LEVELS OF SIGNIFICANCE ANALYZED USING ONE-WAY ANOVA TEST. HASH (#) AND ASTERISK (*) INDICATE PARAMETERS OF SOIL AND PLANTS DETERMINED BEFORE TRANSFER OF PLANTS TO POTS. CARET (^) INDICATES THE SIGNIFICANTLY ($P \leq 0.050$) LOWER VALUES FOR TIME 0 THAN FOR NEXT TIME INTERVALS.

| Parameters | | Incubation time (months) | | | | | | | ANOVA *P* |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | |
| Number of plants in pot | | *100 ± 5 | 102 ± 4 | 85 ± 11 | 92 ± 9 | 94 ± 8 | 96 ± 5 | *68 ± 13 | P = 0.048 |
| Dry weight of plants [mg] | Shoots | *129 ± 31 | 819 ± 26 | 1003 ± 2 | 1453 ± 10 | 1536 ± 37 | 1942 ± 139 | 1999 ± 96 | P < 0.001 |
| | Roots | *58 ± 12 | 342 ± 51 | 350 ± 86 | 591 ± 10 | 844 ± 248 | 597 ± 72 | 468 ± 23 | P = 0.029 |
| Total Cd in biomass [μg] | Shoots | *29 ± 5 | 28 ± 4 | 47 ± 5 | 59 ± 9 | 39 ± 9 | 64 ± 17 | 47 ± 8 | P < 0.001 |
| | Roots | *417 ± 35 | 43 ± 1 | 21 ± 3 | 14 ± 5 | 15 ± 10 | 11 ± 4 | 4 ± 2 | P < 0.001 |
| Cd concentration $[\mu g\ g^{-1}]$ | Shoots | *225 ± 40 | 34 ± 5 | 47 ± 5 | 46 ± 8 | 26 ± 5 | 33 ± 8 | 11 ± 1 | P < 0.001 |
| | Roots | *7 237 ± 616 | 128 ± 4 | 58 ± 9 | 24 ± 9 | 17 ± 11 | 18 ± 6 | 9 ± 5 | P < 0.001 |
| Total Cd in soil [μg] | | #^114 ± 17 | 516 ± 100 | 472 ± 118 | 584 ± 71 | 611 ± 44 | 467 ± 124 | 507 ± 106 | P = 0.629 |
| Cd concentration in soil $[\mu g\ g^{-1}]$ | | #^0.57 ± 0.09 | 2.58 ± 1.01 | 2.36 ± 0.88 | 2.92 ± 0.35 | 3.06 ± 0.27 | 2.33 ± 0.69 | 2.53 ± 0.56 | P = 0.423 |
| TOC in soil [%] | | #^0.72 ± 0.10 | 1.06 ± 0.08 | 1.15 ±0.12 | 1.18 ± 0.02 | 1.26 ± 0.05 | 1.15 ± 0.13 | 1.09 ±0.15 | P = 0.071 |
| pH in $H_2O$ | | #5.65 ± 0.04 | 5.48 ± 0.24 | 4.84 ±0.07 | 5.70 ± 0.09 | 6.33 ± 012 | 6.44 ± 0.06 | 6.74 ± 0.04 | P < 0.001 |
| CFU (log10) | Bacteria | #6.51 ± 0.08 | 7.41 ± 0.15 | 7.51 ± 1.14 | 6.89 ± 0.08 | 7.36 ± 0.17 | 7.71 ± 0.24 | 7.39 ± 0.07 | P < 0.001 |
| | Fungi | #5.50 ± 0.07 | 5.07 ± 0.11 | 4.86 ±0.09 | 5.12 ± 0.07 | 4.93 ± 0.09 | 5.54 ± 0.13 | 5.23 ± 0.16 | P < 0.001 |

soil minerals and organic components immobilized Cd (Li *et al*. 2016, Tahervand and Jalali 2016), preventing its translocation from soil to roots.

### *Organic carbon concentration in soil*

The soil contained about 0.72% of TOC. The transplanting of the grass to pots induced an increase in the amount of TOC by about 50% after 2 months of grass cultivation. From this 2 month period onwards, the content of TOC remained at a similar level up to the end of incubation, and the observed changes at each time interval were statistically insignificant (Table 1). This fact suggests that the input of organic matter in the start of plant growth in pots (organic compounds released from root surfaces and dead roots) was significant in regard to the increase of TOC amounts in the soil, and the further cultivation of plants did not induce a significant enrichment of the soil with organic matter. There is a high probability that the Cd accumulated in roots (in the hydroponic cultivation stage) could have accelerated the aging and dying of the root material. Root exudates and dead roots are known to be good sources of carbon and energy for soil microorganisms, and, together with microbial metabolites, are substrates for humification, raising the amounts of soil organic carbon (Abakumov *et al.* 2013). In such cases, during humus accumulation, metal-organic complexes can also be incorporated into soil organic matter (Renella *et al*. 2004, Semenov *et al*. 2013).

The 2-month period time seems to be enough to start the transformation leading to humus accumulation. The process of humification of organic matter in the soil is reflected in the amounts of organic compounds extracted with NaOH (e.g. humic and fulvic acids). The fraction of humic substances not extractable with NaOH (e.g. humins and ulmins) was considered to be a stable soil organic matter (Abakumov *et al.* 2013, Semenov *et al*. 2013). What is more, non-humificated plant residues (e.g. fine roots which stayed in soil after removing of plants) were found to be non-extractable matter. The water soluble fraction of organic carbon (W-SOC) did not exceed 0.01–0.02% of TOC during the plant cultivation (Fig. 1), whereas, significant increase of base soluble (B-SOC) and non-extractable fraction of organic carbon (N-SOC) were measured. During the first eight months, B-SOC and N-SOC were an average of 53% and 47% of TOC, respectively. After this time, the proportion between these fractions changed significantly. The percentage of B-SOC and R-SOC was 26% and 71% after 10 months, and 12% and 87% at the end of the experiment, respectively (Fig. 1).

### *Concentration and fractions of Cd in the soil*

The soil used in these studies contained 0.57 mg Cd kg DW (Table 1) distributed between four operational fractions (Fig. 2): ion exchangeable (8%), bound to Fe and Mn oxides (42%), organically bound (30%), and residual

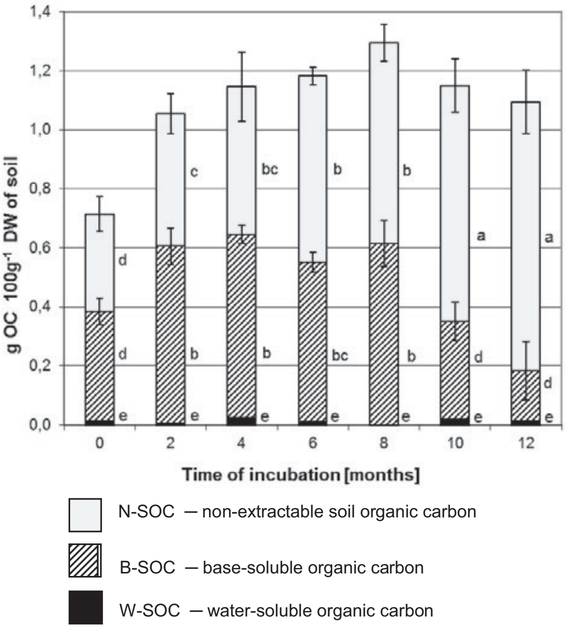

Fig. 1. Three-step sequential extraction of organic carbon (OC) from soil

The amounts of the OC are expressed as g OC · 100 g dry weight of soil. Standard deviations are shown as deviation bars (*n* = 8). Different letters indicate significant differences among time periods and OC fractions ($P \leq 0.05$).

(20%). The distribution of Cd between tested fractions resembled its distribution as found in the uncontaminated soil (sandy loam, 1 µg Cd $g^{-1}$DW) as investigated in previous studies (Majewska and Kurek 2007). On the day of pot transplanting, about 420 µg Cd was found to be present in the grass roots, and this was introduced into 200 g DW of soil. After a 2-month cultivation, a significant increase of total Cd amount was found in the soil when sampled. Indeed, the Cd concentration in the soil was 4.5 times higher than at time 0 (Table 1). However, its total concentration was similar during the entire period of the study (an average of 2.63 ± 0.30 µg $g^{-1}$). The grass roots were the principal source of Cd in the soil. The layer of mucilaginous material secreted and stored on root surface (Uren 1993) acted as matrix: accumulating Cd during hydroponical cultivation, and stimulating microorganism proliferation under the pot soil conditions (Massaccesi *et al.* 2015). This Cd stress was noted to induce the production and accumulation of various organic compounds on the root surfaces of *Secale cereale* (L.) (Majewska and Kurek 2011) and *F. ovina* (L.) (Majewska and Słomka 2016). These compounds act as an external barrier protecting roots against Cd. Such a protective mechanism under Cd stress was also found in *Triticum aestivum* (L.) (Li *et al*. 2014).

It was seen that cadmium released from roots was immobilized by the soil constituents, as its distribution between fractions was different from the distribution of native Cd (Fig. 2). In our work, the residual fraction was 2% of the metal present in the soil and was constant during plant cultivation. The largest amount of soluble and exchangeable Cd ($NaNO_3$-extracted), about 17% of soil Cd, was found in soil samples after 2 months of plant growth in pots. A further cultivation of plants caused the pool of Cd in this fraction to fall to 10% in the fourth month and 5% in the subsequent analyses, respectively. The changes in the bioavailable pool of Cd negatively correlated with the changes of soil pH, TOC and N-SOC (Table 2). In their review, Shaheen *et al*. (2013) mention studies which demonstrate that soil pH is a major factor affecting the mobility and bioavailability of Cd and Pb. They concluded that the increase of the soil pH enhances the metal cations retention to soil components via sorption, complexation and precipitation (hydroxides, carbonates and phosphates). Also, Cd removed from roots in the form of difficult-degraded organic complexes (Renella *et al*. 2004) is thought to be gradually immobilized by solid soil components and removed from soluble and exchangeable fraction of Cd.

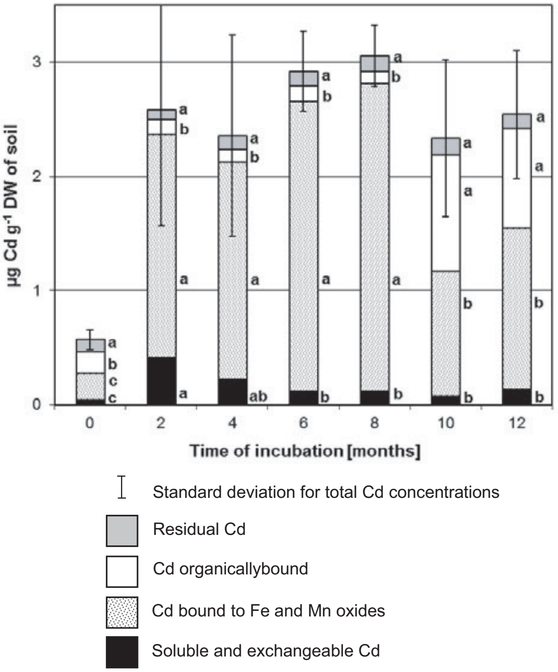

Fig. 2. Sequential extraction of Cd immobilized by soil

The concentration of Cd in the individual fractions are expressed as µg Cd · $g^{-1}$ dry weight of soil. Standard deviations for total Cd concentrations are shown as deviation bars ($n = 4$). Different letters indicate significant differences among time periods for the same fraction ($P \leq 0.05$).

It was noted that the highest amount of Cd mobilized from roots was bound to the soil Fe and Mn oxides. Indeed, during the first eight months, an average 80% of all soil Cd was immobilized in this fraction. These observations follow that of previous studies (Majewska *et al.* 2006) describing the Fe-Mn oxides fraction as dominant in soils contaminated with various forms of Cd ($CdCl_2$, Cd immobilized by microbial biomass, montmorillonite and humic acids). Other researchers (Shaheen *et al*. 2013) have documented that Fe, Mn and Al oxides have an about 30-fold higher sorption capacity than clays, and that they bind the metal ions by chemisorption. This type of sorption is a rapid reaction preferentially up-taking metal cations that can be replaced only by chemically similar cation or protons (Shaheen *et al*. 2013). What is more, there is a known high adsorption affinity of metal-organic complexes to soil minerals (Oburger *et al.* 2011, Karimzadeh *et al.* 2013, Ahmet and Homström 2014).The heavy metals are, hence, strongly bound to the soil Fe and Mn oxides, but are thermodynamically unstable in anoxic conditions (Fuentes *et al*. 2004).

TABLE 2. THE VALUES OF CORRELATION COEFFICIENTS BETWEEN CONCENTRATIONS OF Cd IN EACH OPERATIONAL FRACTION AND pH, NUMBERS OF BACTERIA AND FUNGI, AMOUNTS OF TOTAL ORGANIC CARBON (TOC), BASE-SOLUBLE ORGANIC CARBON (BSOC) AND NON-EXTRACTABLE SOIL ORGANIC CARBON (N-SOC).

| Operational fraction | Parameter | | | | | |
|---|---|---|---|---|---|---|
| | pH | Bacteria | Fungi | TOC | B-SOC | N-SOC |
| Soluble and exchangeable Cd | -0.67 | -0.06 | -0.42 | -0.55 | 0.37 | -0.68 |
| Cd bound to Mn/Fe oxides | -0.11 | -0.33 | -0.70 | 0.76 | 0.72 | -0.41 |
| Cd organically bound | 0.71 | 0.52 | 0.88 | -0.32 | -0.72 | 0.65 |
| Residual Cd | -0.27 | 0.03 | -0.02 | 0.65 | 0.41 | -0.23 |

Two months before the end of the pot cultivation, the content of Cd bound to Fe and Mn oxides decreased up to 53%, and the percentage of Cd defined as organically bound increased from 6% (after 2 months) to 43% (after 12 months). The increase of Cd amount in this fraction was connected with the stabilization of humus in the N-SOC fraction (Table 2). At the same time interval, the numbers of bacteria and fungi were found to be the highest. The analysis of correlation (Table 2) supported the negative effect of microbial activity on the amounts of Cd in the Fe/Mn oxides fraction. Moreover, positive values of correlation were determined between the amounts of organically bound Cd and bacterial and fungal numbers (Table 2). This observation confirmed that of earlier studies (Majewska and Kurek 2007) which also showed the significant effect of microbial activity on the changes in distribution of Cd in soils. Thus, in accordance with earlier work, soil organic matter, besides generating bound metal oxides, are an important factor in immobilizing toxic metal in soil (Shaheen *et al.* 2013, Bolan *et al*. 2014).

Kurek and Majewska (1998) found significant differences between retention of Cd immobilized to humic acid by adsorption and Cd immobilized during humification of contaminated plant residues. Presumably, Cd was entrapped in the structure of formed humic acids (Semenov *et al.* 2013), and extractant access to it was difficult, hence, in vitro and under soil condition Cd was more resistant to extraction.

Retention of Cd during phytoremediation has been studied for many years now, but the metal-stabilizing effect is certainly not always clear cut. On the one hand, plant roots are known to immobilize metals present in the soil. On the other hand, the decomposition of their exudates and organic residues consequently mobilize the metals accumulated previously. The diverse and multifaceted processes in the soil give rise to the question of how long a metal accumulated in plant roots can remain stable. Thus, it is important to understand how it is possible to encourage the long-term accumulation of the recalcitrant humified carbon compounds that become a slow-cycling storage carbon.

## CONCLUSIONS

1. In standard soil conditions, Cd stabilization by roots resulting from biosorption and bioaccumulation is dependent on time.
2. Cadmium released from roots is distributed between fractions with varying stability and extractability: the fraction of soluble and exchangeable Cd, Cd bound to oxides or organics and residual fraction of Cd.
3. Soil organic matter and soil oxides play very important roles in modulating the bioavailability of Cd. The distribution of Cd among the tested fractions changed during the experiment. The amount of Cd bound to soil organic matter increased, lowering the amount of soluble and exchangeable Cd and the fraction of Cd bound to oxide minerals.
4. Cd released from roots was stabilized in soil through being organically bound, and this fact gives hope that heavy metals contamination will be stabilized in soil, not only in root tissues, but also in the humus that becomes a slow-cycling storage of soil organic matter.

## REFERENCES

[1] Abakumov, E.V., Cajthaml, T., Brus, J., Frouz, J., 2013. *Humus accumulation, humification, and humic acid composition in soils of two post-mining chronosequences after coal mining*. Journal of Soil and Sediments, 13: 491–500, DOI: 10.1007/s11368-012-0579-9.

[2] Ahmet, E., Homström, S.J.M, 2014. *The effect of soil horizon and mineral type on the distribution of siderophores in soil*. Geochimica et Cosmochimica Acta, 131: 184–195, DOI: 10.1016/j.gca.2014.01.031.

[3] Alef, K., Nannipieri, P., 1995. *Methods in applied soil microbiology and biotechnology*. Academic Press, London, Great Britain.

[4] Beats, S.D., Poesen, J., Gyssels, G., Knapen, A., 2006. *Effect of grass roots on the erodibility of topsoil during concentrated flow.* Geomorphology, 76: 54–67, DOI: 10.1016/j.geomorph.2005.10.002.

[5] Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. *Remediation of heavy metals(loid)s contaminated soils – to mobilize or to immobilize?* Journal of Hazardous Materials, 266: 141–166, DOI: 10.1016/j.jhazmat.2013.12.018.

[6] Brandt, S., 1999. *Date analysis. Statistical and computational methods for scientist and engineers.* 3rd ed., Springer Verlag, New York.

[7] Conesa , H.M., Wieser, M., Gasser, M., Hockmann, K., Evangelou, M.W.H., Studer, B., Schulin, R., 2010. *Effects of three amendments on extractability and fractionation of Pb, Cu, Ni and Sb in two shooting range soils.* Journal of Hazardous Materials, 181: 845–850, DOI: 10.1016/j.jhazmat.2010.05.090.

[8] Ernst, W.H.O.,2005. *Phytoextraction of mine wastes – option and impossibilities*. Chemie Der Erde-Geochemistry, 65, (S1): 29–49, DOI: 10.1016/j.chemer.2005.06.001.

[9] Fiedler, H.D., Westrup, J.L., Souza, A.J., Pavei, A.D., Chagas, C.U., Nome, F., 2004. *Cd(II) determination in the presence of aqueous micellar solutions*. Talanta 64: 190–195, DOI: 10.1016/j.talanta.2004.02.008.

[10] Fuentes, A., Lloréns, M., Sáez, J., Soler, A., Aguilar, M.I., Ortuño, J.F., Meseguer, V.F., 2004. *Simple and sequential extractions of heavy metals from different sewage sludges.* Chemosphere, 54: 1039–1047, DOI: 10.1016/j.chemosphere.2003.10.029.

[11] Hoagland, D.R., Arnon, D.J., 1950. *The water culture method for growing plants without soil.* California Agricultural Experiment Station. University of California, Station Circular, 347: 1–39.

[12] Hütsch, B.W., Augustin, J., Merbach, W., 2002. *Plant rhizodeposition – an important source for carbon turnover in soils.* Journal of Plant Nutrition and Soil Science, 165: 397–407.

[13] Kabata-Pendias, A., 2011. *Trace elements in soil and plants*. CRC Press, Taylor & Francis Group, LLC, Boca Raton USA.

[14] Karimzadeh, L., Nair, S., Merkel, B.J., 2013. *Effect of microbial siderophore DFOB on Pb, Zn, and Cd sorption onto zeolite*. Aquatic Geochemistry, 19: 25–37, DOI: 10.1007/s10498-012-9176-1.

[15] Kurek, E., Majewska, M., 1998. *Release of Cd immobilized by soil constituents and its bioavailability.* Toxicological and Environmental Chemistry, 67: 237–249, DOI: 10.1080/02772249809358617.

[16] Li, Y., Wang, L., Yang, L., Li, H., 2014. *Dynamics of rhizosphere properties and antioxidative responses in wheat (Triticum aestivum L.) under cadmium stress*. Ecotoxicology and Environmental Safety, 102: 55–61, DOI: 10.1016/j.ecoenv.2014.01.004.

[17] Li, Z., Jia, M., Christie, P., Luo, Y., 2016. *Changes in metals availability, desorption kinetics and speciation in contaminated soils during repeated phytoextraction with the Zn/Cd hyperaccumulator Sedum plumbizincicola*. Environmental Pollution, 209: 123–131, DOI: 10.1016/j.envpol.2015.11.015.

[18] Lityński, T., Jurkowska, H. Gorlach, E., 1976. *Chemical and agricultural analysis. Methodological guide to soil and fertilizer analysis* (in Polish). PWN, Warszawa.

[19] Lou, Y., Luo, H., Hu, T., Li, H., Fu, J., 2013. *Toxic effects, uptake, and translocation of Cd and Pb in perennial ryegrass.* Ecotoxicology, 22: 207–214, DOI: 10.1007/s10646-012-1017-x.

[20] Majewska, M., Kurek, E., 2007. *Microbially mediated cadmium sorption/desorption processes in soil amended with sewage sludge*. Chemosphere, 67: 724–730, DOI: 10.1016/j.chemosphere.2006.10.051.

[21] Majewska, M., Kurek, E., 2011. *Effect of Cd concentration in growth media on Secale cereale roots and Cd interaction with rhizosphere microorganisms originating from different parts of the grain*. European Journal of Soil Biology, 47: 95–101, DOI: 10.1016/j.ejsobi.2010.12.005.

[22] Majewska, M., Kurek, E., Słomka, A., 2011. *Effect of plant growth on total concentrations of Zn, Pb, and Cd, and their distribution between operational fractions in the upper layer of a 100-year-old zinc-lead waste heap*. Polish Journal of Environmental Studies, 20: 591–597.

[23] Majewska, M., Kurek, E., Szlachetka, D., 2006. *Microbial activity – factor increasing retention of Cd added to soil.* Polish Journal of Environmental Studies 15, 2a: 127–134.

[24] Majewska, M., Słomka, A., 2016. *Levels of Organic Compounds, Number of Microorganisms and Cadmium Accumulation in Festucaovina Hydroponic Culture*. Polish Journal of Microbiology, 65, 2: 191–200, DOI: 10.1080/15226514.2017.1284744.

[25] Martin, J.P., 1950. *Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi.* Soil Sciences, 38: 215–220.

[26] Massaccesi , L., Benucci, G.M.N., Gigliotti, G., Cocco, S., Corti, G., Agnelli, A., 2015. *Rhizosphere effect of three plant species of environment under periglacial conditions (Majella Massif, central Italy).* Soil Biology & Biochemistry, 89: 184–195, DOI: 10.1016/j.soilbio.2015.07.010.

[27] Oburger, E., Leitner, D., Jones, D.L., Zygalakis, K.C., Schnepf, A., Roose, T., 2011. *Adsorption and desorption dynamics of citric acid anions in soil.* European Journal of Soil Science, 62: 733–742, DOI: 10.1111/j.1365-2389.2011.01384.x.

[28] Overdieck, D., 2016. *Macro- and Micronutrients.* In: D. Overdieck (ed.), *$CO_2$, Temperature, and Trees*. Springer, Singapore, pp. 89–117, DOI:0.1007/978-981-10-1860-2_8.

[29] Oves, M., Khan, M.S., Zaidi, A., Ahmad, E., 2012. *Soil contamination, nutritive values, and human health risk assessment of heavy metals: An overview.* [In:] A. Zaidi, P.A. Wani, M.S. Khan (eds.), *Toxicity of heavy metals to legumes and bioremediation.* Springer Verlag, Wien, pp. 1–27, DOI: 10.1007/978-3-7091-0730-0_1.

[30] Ping, X., Zhou, G., Zhuang, Q., Wang, Y., Zou, W., Shi, G., Lin, X., Wang, Y., 2010. *Effect of sample size and position from monolith and core methods on the estimation of total root biomass in a temperate grassland ecosystem in Inner Mongolia.* Geoderma, 155: 262–268, DOI: 10.1016/j.geoderma.2009.12.009.

[31] Prasad, M.N.V., 2003. *Phytoremediation of metal-polluted ecosystems: Hype for commercialization.* Russian Journal of Plant Physiology, 50, 5: 686–700, DOI: 10.1023/A:1025604627496.

[32] Renella, G., Landi, L., Nannipieri, P., 2004. *Degradation of low molecular weight organic acids complexed with heavy metals in soil.* Geoderma, 122: 311–315, DOI: 10.1016/j.geoderma.2004.01.018.

[33] Semenov, V.M., Tulina, A.S., Semenova, N.A., Ivannikova, LA., 2013. *Humification and non-humification pathways of the organic matter stabilization in soil: A review*. Euroasian Soil Science, 46, 4: 355–368, DOI: 10.1134/S106422931304011X.

[34] Shaheen, S.M., Tsadilas, C.D., Rinklebe, J., 2013. *A review of the distribution coefficients of trace elements in soils: Influence of sorption system, element characteristics, and soil colloidal properties*. Advances in Colloid and Interface Science, 201–202: 43–56, DOI: 10.1016/j.cis.2013.10.005.

[35] Stritsis, C., Steingrobe, B., Claassen, N., 2014. *Cadmium fractions in an acid sandy soil and in soil solution as affected by plant growth.* Journal of Plant Nutrition and Soil Science, 177: 431–437, DOI: 0.1002/jpln.201200627.

[36] Tahervand, S., Jalali, M., 2016. *Sorption, desorption, and speciation of Cd, Ni, and Fe by four calcareous soils as affected by pH*. Environmental Monitoring and Assessment, 188: 322, DOI:10.1007/s10661-016-5313-4.

[37] Uren, N.C., 1993. *Mucilage secretion and its interaction with soil, and contact reduction.* Plant and Soil, 155/156: 79–82, DOI: 10.1007/BF00024988.