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Biosorption of heavy metals onto different eco-friendly substrates

Eman M. Fawzy1*, Fatma F. Abdel-Motait2 and Soad A. El-zayat2

1Chemistry Department, Faculty of Science, Aswan University, Aswan, Egypt.
2Botany Department, Faculty of Science, Aswan University, Aswan, Egypt.

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Fungi play an important role in biosorption of heavy metals in heavily contaminated soil. Five metal-tolerant fungal species were isolated from two different contaminated soil (soils 1 and 2). The number of fungal colonies isolated from the contaminated soil 2 was higher than that of soil 1. The most resistant fungal species for the toxic studied metals (Pb, Cd, Cu and Zn) was Rhizopus stolonifer followed by Macrophomina phaseolina. It was established that the metal toxicity was related to the contamination levels, the physicochemical properties including pH, conductivity, organic matter and carbonate contents of the soils. This study confirmed the good ability of different chemicals (CaCO3, MO, Zeolite and phosphate) and biological fungal substrates (M. phaseolina and R. stolonifer) in bioremediation of polluted soils and reducing different heavy metals levels as compared to the control, especially for fungi. M. phaseolina amendment was superior in reducing the chemically available heavy metals in the studied soils.

Key words: Heavy metals, fungal adsorption, soil remediation, amendments, sequential extraction technique.

INTRODUCTION

The basic environmental elements constituting ecosystem is the soil, which is the important material basis of humans to survive and develop. The soil contaminated by heavy metals manifests as concealment, accumulation, reversibility and protraction. The major ways of heavy metal contamination are surface water and agricultural soil polluted by industrial effluent, chemical fertilizers and pesticides (Hezbullah et al., 2016), while sewage is the main source of water pollution used for irrigation (Salawu et al., 2015).

To prevent the heavy metal contamination, sources of contamination should be controlled and remediation of contaminated soil should be enhanced (Zhou et al., 2004). Recently, reducing the number of pollutants and improving the quality of the treated soils have been studied for the development of cheaper and more effective remediation technique. One of the most alternative treatments is adsorption. The adsorbents may be of mineral, organic or biological origin, zeolites, industrial byproducts, agricultural wastes, biomass and polymeric materials (Kurniawan et al., 2005). Microorganisms (bacteria, fungi and algae) are effective

*Corresponding author. E-mail: emanmohumed@hotmail.com. Tel: +20 1007292350. Fax: +20 97 3480 448.

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heavy metal sequestration for physicochemical methods (Brierley, 1990; Gadd, 1993; Niu et al., 1993; Wong and So, 1993; Wong et al., 1993). Removal of potentially toxic metals from polluted industrial and domestic effluents have already been used in large scale using certain microorganisms. These microorganisms have been shown to possess ability to survive by adapting or mutating at high concentrations of toxic heavy metals. To increase the tolerance of fungi for the bioleaching process, the adaptation of these fungi exposed to heavy metal ions has been examined and developed (Yang et al., 2009).

Generally, two mechanisms have been proposed for heavy metal tolerance in fungi. The first one is an extracellular (chelation and cell-wall binding) sequestration and the second is intracellular physical sequestration of metal by binding to proteins or other ligands to prevent it from damaging the metal sensitive cellular targets. Thus, extracellular mechanisms are mainly implied in the avoidance of metal entry, whereas intracellular systems aim to reduce the metal burden in the cytosol. In the first mechanism, different organic molecules that do not belong to the matrix of the cell wall are excreted by the fungal cell to chelate metal ions. Binding to the cell wall is called biosorption. The presence of various anionic structures, such as glucon and chitin gives negative charge to the cell surface of microorganisms (Maghsoudi et al., 2007; Bellion et al., 2006), which gives microorganisms the ability to bind metal cations. In the intracellular mechanism, metal transport proteins may be involved in metal tolerance, either by extruding toxic metal ions from the cytosol out of the cell or by allowing metal sequestration into the vacuolar compartment (Khan and Maqsood, 2007; Le et al., 2006).

In the present study, fungal-tolerant heavy metals will be screened in the two polluted soils. Moreover, although the separation of various chemical forms of heavy metals is very difficult, the use of sequential extraction methods provides an important approach and relevant environmental information on polluted samples. Therefore, the present study used sequential extraction scheme: (1) To predict the metal distribution among different fractions in two contaminated soils, determined periodically by a four-step chemical fractionation procedure. (2) To compare biosorption capacity of chemical and biological treatments to degrade available metals contaminated soils. (3) To evaluate the changes in the speciation of studied metals in amended soils after treatments.

MATERIALS AND METHODS

Instrumentation

The metal determination in the extract was carried out by means of atomic absorption spectrophotometry (Model Solaar 969, ATI Unicam Comp.) equipped with a digital direct concentration read out and an air-acetylene burner using single element hollow cathode lamps (ATI Unicam Comp.). When the concentrations were under the detection limit of flame, the AAS external standards in diluted acid were used to calibrate the accuracy of atomic absorption.

Reagents and glassware

All glassware and plastic materials used were previously treated for 24 h in 2 M nitric acid and rinsed with double distilled water and then with ultra-pure water. 50 ml of acid washed polyethylene centrifuge tubes were used for extraction, while 50 ml polyethylene vessels were used in the extracting solutions.

Samples collection

Two contaminated soils were selected for this study, one influenced by urban and wastewater (soil 1), and the other was influenced by industrial wastes (soil 2). The surface soils (0 to 20 cm) of the contaminated sites (ca 20 samples from each site using sterile polyethylene bags) were sampled, air-dried, and then hand-crushed using the mortar and sieved through 2 mm stainless steel. Samples were finally homogenized and stored until the analyses.

Analytical procedure

The pH-values and electrical conductivity (E.C.) of soil samples were measured in 1: 2.5 suspension of sample : bidistilled water using a pH-meter (Orion Research, Model SA520, U.S.A.) and conductivity meter (HANNA Instruments, HI 8033 Italy). Carbonates and organic matter were determined using standard procedures (APHA, 1992); (Nelson and Sommers, 1982).

Choice of reagents and leaching conditions

In the choice of extracting reagents, particular emphasis was placed on the selectivity, suitability and extracting efficiency of each leaching solution. The extraction methods, which are the most informative for environmental purposes are the total element, moderately (reducible and oxidizable) and easily extractable element extraction techniques. The former defines and includes both elements from the rock matrix and the non-residual elements (those adsorbed from the aqueous medium). The three other extraction techniques show no association with the type of rock forming the soil and give results only for the moderately and weakly hold elements, which include those originating from polluted waters.

Sequential extraction scheme

The Tessire et al., (1979) sequential extraction method was applied in triplicate to 2 g of soil samples (< 2 mm). The reagents and operating conditions for this method is summarized in Table 1. The procedure was conducted in five steps, assuming the forms of Cd, Pb, Zn and Cu extracted were (1) exchangeable (2) associated with Fe-Mn oxides (or reducible) (3) associated with organic matter (or oxidizable) (4) structurally bound in residual fraction.

Isolation of micro-organisms from polluted sites

The soil samples (10 g) were first suspended in 100 ml of sterilized water; the mixture was agitated for 30 min at room temperature and then diluted (10- to 10 000-fold). Aliquots of 100 µl of different
dilutions were placed on 2% malt extract agar (MEA) plates (three replicates) to make sure the growth of micro-organisms is present in samples. After at least 3 days of incubation at 25°C, developed colonies were randomly picked and isolated. Pure cultures of isolated micro-organisms were identified using the keys of Gilman (1957) and Domsch et al. (1980).

**Screening and choice of heavy metal-resistant microorganisms**

The purified isolates were screened on the basis of their tolerance to Cu, Pb, Zn and Cd. A disk of mycelium was inoculated aseptically onto MEA plates supplemented individually with 1, 10, 30, 50, 100, 300, 600 and 1000 ppm of heavy metals. The inoculated plates were incubated at 25°C for 14 days. The effect of the heavy metals on the growth of the isolates tested was estimated by measuring the radius of the colony extension (cm) when compared with the control (medium without metals). The minimum inhibitory concentration (MIC) was calculated which is defined as the lowest concentration of metals that inhibit visible growth of the isolate. The isolates which showed resistance to the studied heavy metals were selected for the following experiments.

**Different treatments for contaminated soil**

Six chemical and biological treatments were used to compare and evaluate the effectiveness of chemical remediation techniques. Seven different slurries of soil (1 g soil : 25 ml H₂O) were put in polyethylene bottles and treated according the following methods: (1) 1 g calcium carbonate (CaCO₃) added to increase soil pH to 7.0; (2) a high quantity of calcium phosphate (10 mg P); (3) 1% manganese oxide (5 g); (4) 1 g of *Macrophomina phaseolina* (5 g); (5) 1 g *Rhizopus stolonifer*; (6) 1% synthetic zeolite (5 g, Sigma Chemical Company, USA); & (7) kept as a control. Each treatment was performed in triplicate and incubated for two weeks at room temperature (25°C).

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**Table 1. Chemical extraction scheme for metal speciation in soil samples.**

| Reagent | Shaking time and temperature | Fraction |
|---------|-----------------------------|----------|
| 40 ml of 0.11 mol/l acetic acid | 16 h at room temperature | Water-soluble (available) |
| 40 ml of 0.1 mol/l hydroxylamine oxides hydrochloride (pH 2) | 16 h at room temperature | Occluded in Fe or Mn reducible |
| 10 ml 30% H₂O₂ (pH 2) | 1h at room temperature and 1h at 85°C | Organically bound and sulphides (oxidizable) |
| then 10 ml 30% H₂O₂ (pH 2) cool, add 50 ml mol/l ammonium acetate (pH 2) | 16 h at ambient temperature | Structurally bound (residual fractions) |

| Location | pH  | Conductivity (µS/cm) | Organic matter (mg/g) | CaCO₃ (Mg/g) | Total content (µg/gm dry soil) |
|----------|-----|----------------------|-----------------------|-------------|-------------------------------|
| Soil1    | 7.12| 78                   | 19                    | 21          | Pb 16.35, Cd 5.66, Cu 225.96, Zn 176.07 |
| Soil2    | 6.75| 912                  | 25                    | 2.1         | Pb 41.47, Cd 2.63, Cu 124.09, Zn 198.49 |

**Quality control and analysis**

The analyses of the sequential extractions procedure were replicated three times. A blank was also run at the same time. All glassware and plastic containers were previously soaked in supra pure nitric acid (Merck) overnight, and rinsed with de-ionized water.

**RESULTS AND DISCUSSION**

The monitoring of physicochemical properties of the studied contaminated soils showed that, a significantly higher organic matter content (25 mg/g) was observed in soil 2 (Table 2) caused by industrial wastes. This organic matter acts as a scavenger for metals and may provide sites for cations due to ligand or groups that form chelates and/or complexes with the metals (Fawzy, 2000). These findings tend to support the hypothesis that all geochemical processes leading to recycling and accumulation of trace metals in soils are associated with and influenced by organic matter.

The electrical conductivity values of the soil samples are 78 and 912 µS/cm for soils 1 and 2, respectively (Table 2). The highest conductivity value was observed at soil 2 (912 µS/cm), as a result of the effluent wastes from the factory. These effluents enriched with highly conducting materials, which can be adsorbed on the surface of the suspended matter and are deposited on the bottom and tend to increase the electrical conductance of the soil (Fawzy, 2000). On the other hand, there was pronounced decrease in the conductivity value obtained at soil 1 (78 µS/cm).

The pH values of the soil samples were 7.12 and 6.75...
(Table 2). A minimum pH value recorded at soil 2, may be ascribed to the increase of organic matter decomposition, which leads to release of CO₂ causing a drop of pH value. The hydrogen ion concentration (pH) is probably a single important factor influencing metal adsorption onto both inorganic and organic surface (Fawzy, 2008). The other major difference in soil properties of those contaminated soils was carbonate content (21 and 2.1 mg/g, for soils 1 and 2, respectively).

The total metal concentration provides little indication of metal specification bioavailability, mobility and reactivity in soil samples (Sánchez-Martin et al., 2007; Babel and Dacera, 2006). The total content is the predominant fraction for most of the studied metals (Table 2). Results in Table 2 reflect highest total Cd and Cu concentrations in soil 1 (5.66 and 225.96 µg/g, respectively), while soil 2 recorded the highest total Pb (41.47 µg/g) and Zn (198.49 µg/g).

**Fractionation of heavy metals before treatment**

In the sequential extraction scheme used in this study, the mobility and hence the possible bioavailability of metals decrease from readily exchangeable to residual. Figures 1 and 2 compare the mobility potential of heavy metals in different forms. It was noticed that Cu and Zn have the highest ability and susceptibility to be released

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**Figure 1.** Metal fraction concentrations (μg/g dry soil) of lead, cadmium, copper, zinc in the contaminated soil 1.

**Figure 2.** Metal fraction concentrations (μg/g dry soil) of lead, cadmium, copper, zinc in the contaminated soil 2.
Table 3. Mean values sequential fractionation (µg/gm dry soil) of Pb, Cd, Cu, Zn and recovery of summation percentage in the two contaminated soils (Soils 1 and 2).

| Soil   | Metal fractionation by different reagent (dry soil) (µg/g) | Sum of fractions µg/gm(dry soil) | Residual metal concentration (dry soil) (µg/g) | Recovery of a summation percentage |
|--------|-----------------------------------------------------------|----------------------------------|---------------------------------------------|----------------------------------|
|        | HOAC    | NH₂OH.HCl | H₂O₂+NH₄OAC |                                 |                                 |                                 |
| Lead   |         |           |             |                                 |                                 |                                 |
| Soil 1 | 1.7     | 1.3       | 3.1         | 6.1                            | 10.25                           | 17.9                           |
| Soil 2 | 1.6     | 1.08      | 4.75        | 7.43                           | 34.04                           | 37.3                           |
| Cadmium|         |           |             |                                 |                                 |                                 |
| Soil 1 | 0.16    | 0.19      | 0.57        | 1.08                           | 4.58                            | 19.1                           |
| Soil 2 | 0.4     | 0.36      | 1.47        | 2.23                           | 0.4                             | 84.8                           |
| Copper |         |           |             |                                 |                                 |                                 |
| Soil 1 | 1.44    | 2.11      | 76.74       | 80.29                          | 65.38                           | 0.44                           |
| Soil 2 | 3.9     | 31.17     | 48.23       | 83.3                           | 40.79                           | 67.13                          |
| Zinc   |         |           |             |                                 |                                 |                                 |
| Soil 1 | 13.49   | 4.76      | 96.49       | 114.74                         | 61.33                           | 65.17                          |
| Soil 2 | 40.44   | 54.26     | 67.92       | 162.62                         | 35.87                           | 81.93                          |

from the soil samples, while Pb and Cd have the lowest mobility.

The presence of an acid-soluble portion of Pb indicates its sensitivity to the acidic condition and tendency to leach easily. Metal accumulation in the residual fraction prevailed with Pb (10.25 and 34.04 µg/g) mostly present as a major chemical form in soils 1 and 2, respectively (Table 3). But the other three fractions (which are soluble, reducible and oxidisable forms) of Pb are almost equally important (1.7 to 1.6, 1.3 to 1.08, 3.1 to 4.75 µg/g of the total content for each fraction) in both contaminated soils 1 and 2, respectively (Table 3). This finding agreed with results reported by Chen et al. (2000).

Moreover, Cd showed a higher residual form concentration (4.58 µg/g) in the untreated soil 1. The same metal in soil 2 (Table 3) was found in highest concentration in oxidizable fraction (1.47 µg/g). Beside this, a remarkable reduction of the residual fraction observed in Cd content in soil 2 could be described (ascribed) as the dissolution and decomposition of inorganic and organic compounds, respectively of soil in this fraction leading to an increase in the concentration levels of the other fractions (Simth 1996). Jackson and Allowy (1992) reported that Cd and Pb may cause serious problems through food chains.

In both control soils, Cu and Zn displayed the highest potential mobility (Table, 3) with values of (0.44 to 67.13% for Cu) and (65.17 to 81.93% for Zn) for soils 1 and 2, respectively, with respect to total metal concentrations (Table 3). Higher concentration of heavy metals soil may increase uptake of these elements by crops and potentially affect human health via food chains (Chen et al., 2000)

Overall, Pb and Cd would not be expected to have a high toxicity potential. But the presence of these toxic metals in the environment can be harmful to humans and living species even in low concentration. Since toxic metals do not degrade into harmless end-products, they can accumulate in living bodies and get concentrated through the food chain (Singh et al., 2011). However, Zn and Cu although not usually with high toxicity potential (compared to Pb and Cd toxicity) they should be carefully monitored if these soils are to be reused for agricultural purposes.

Fungal tolerant to Pb, Cd, Cu and Zn isolated from the two studied soils

In this study, five fungal species were isolated from both soils 1 and 2. Emericilla quadrilinearata (100 colonies/g), Aspergillus niger (650 colonies/g) and Macrophomina phaseolina (50 colonies/g) were isolated from soil 1, while Aspergillus niger (2650 colonies/ g), Rhizopus stolonifer (200 colonies/g) and Aspergillus fumigatus (1350 colonies/g) were isolated from soil 2. The number of fungal colonies isolated from soil 2 contaminated sediment soils by industrial waste was higher than that from the soil 1 contaminated sediments urban and waste water. This variation would be referring to the high sugar content in the soil 2 which refers to sugar cane factory waste. This variation was clear with the number of A. niger colonies (650 and 2650 colonies/g) isolated from soils and soil 2, respectively.

Screening of the resistance of these fungal species to Pb, Cd, Cu and Zn has been studied. Generally, the most resistant fungal species to these elements up to 1000 ppm was R. stolonifer followed by M. phaseolina which showed resistance with all studied elements even with high concentrations expects cadmium at 1000 ppm recorded MIC$_{100}$. While the remaining studied fungal species (A. niger, A. fumigatus and E. quadrilinearata) were
Figure 3. Fungal growth rate exposed to different concentrations of toxic metals when compared with that of the control (without metals).

slightly sensitive to these heavy metals (Figures 3 and 4). E. quadrilineata recorded MIC100 with copper and cadmium at 600 and 1000 ppm, respectively, and MIC50 with zinc and lead at 10 and 1000 ppm respectively. Cadmium showed a similar effect on both Aspergillus species, whereas MIC90 was observed at 1000 ppm, and MIC50 of A. fumigatus and A. niger with zinc 100 and 300 ppm, respectively. A. fumigatus showed more sensitivity to copper than A. niger (MIC90 and MIC50 at 1000 ppm), respectively. In the case of lead, A. fumigatus completely inhibited with 1000 ppm. Despite that, only 50 ppm was enough to inhibit 50% of A. niger, the fungus showed stable growth rate up to 1000 ppm (Meizhen and Junfeng, 2014).

A similar observation on the toxic effect of higher concentration of heavy metals on the growth of fungi has been reported (Rao et al., 1997; Malik, 2004; Joshi et al., 2011). Ahmad et al. (2005) reported that the biomass of Rhizopus sp. had a higher adsorption capacity as compared to Aspergillus sp. biomass. The differences may be ascribed to the intrinsic ability of the organism, its chemical composition of cell wall leading to various types of interaction of metals with fungi (Gadd, 1993). According to the authors' knowledge, this is the first study on the resistance of E. quadrilineata for heavy metals.

Fractionation of heavy metals after treatment

Some chemical techniques for immobilizing metals in soils are application in polluted soils to reduce the soluble concentration of heavy metals in soils by precipitation, adsorption, or complexation (Chen et al., 2000). Recently, many industrial, agricultural and forestry sources are used as sorbents such as, red mud (Nadaroglu et al., 2010), sunflower stalks (Hussein, 2010), spent grain (Li et al., 2010), wheat bran (Yao et al., 2012), Aspergillus niger (George et al., 2012), Scyllymus hispanicus L. (Barka et al., 2010), eggshell and coral (Chakravarty et al., 2012), maize bran (Tofan et al., 2011), saw dust and neem bark (Naiya et al., 2009), citrus peels (Njikam and Schiewer, 2012), Rosa gruss and teplitz (Bhattia et al., 2005), Echornia speciosa (Abdel-Halim et al., 2003), Cupressus sempervirens,
Eucalyptus longifolia and Pinus halepensis (Al-Subu, 2002) and Pleurotus cornucopiae (Danis, 2010).

Fawzy (2008) used seven substrates to investigate their efficiency to reduce the extraction of heavy metals concentration in a heavily contaminated soil, and deduced that the most effective treatments in decreasing available metal concentrations were calcium carbonate, zeolite, manganese or iron oxide and phosphate. The present study was conducted to compare the efficiency of different substrates: calcium carbonate, zeolite, phosphate, manganese oxide with fungi isolated from the contaminated soils in immobilizing metals in polluted soils.

Table 4 shows the sequential extraction concentration of the measured metals (Pb, Cd, Cu and Zn) treated with working substrates in different fractions. It is noteworthy that the mobility of the studied metals in treated soils generally decreases. A significant decrease recorded in exchangeable Pb and Cd fractions of soil 1 treated with M. phaseolina, R. stolonifer, carbonate, zeolite, phosphate or manganese oxide (Table 4). The effective role of fungus in reducing the mobility of heavy metals is due to their ability to accumulate significant amount of metals (Iskandar et al., 2011). Say et al. (2001) studied the biosorption of Cd (II), Pb (II) and Cu (II) with the filamentous fungus Phanerochaete chrysosporium and reported that the fungal cell walls have a negative charge due to the arrangement of the carboxyl and phosphate groups of the cell walls.

The more significant function of M. phaseolina and R. stolonifer was observed to reduce Pb and Cd in both contaminated soils and Zn in soil 1 (Tables 4 and 5) as compared to the calcium carbonate which may be attributed to high capacities of metals binding to cell walls and may exhibit high values of intracellular accumulation (Blanquezet et al., 2004). Kapoor and Viraraghavan (1997) revealed that biosorption of heavy metal by fungi occurs as a result of ionic interaction and complex formation between metal ions and the functional group present on the fungal cell surface. These functional groups which may be involved in the biosorption of heavy metals include phosphate, carboxyl, amine and amide groups (Akhtar et al., 1996).

Table 5 showed a remarkable role of phosphate, manganese oxide, Macrophomina phaseolina and Rhizopus stolonifer in reducing Pb and Cd concentration in available fraction in soil 2. Application of manganese oxide, carbonate and zeolite reduced the mobility of Cd, Cu and Zn in two studied soils (Tables 4 and 5), this is in agreement with other studies reported by Mench et al. (1994) and Lee (1996). Liu et al. (1998) studied the effects of composts and calcium carbonate on the uptake of cadmium and lead by vegetables grown in polluted soils; they reported that application of calcium carbonate materials significantly reduces the solubility of heavy metals in contaminated soils. Many studied also indicated that application of manganese oxides mixed in contaminated soils could reduce the concentration of soluble Cd or Pb in soils (Mench et al., 1994). The mobile phase of Cu content into the contaminated soils (Tables 4 and 5) was can be transformed to unavailable form after amendment with manganese oxide, zeolite and carbonates.
Table 4. Distribution fractions of each studied metal (µg/g) in Soil 1 (influenced by urban and wastewater).

| Metals | Control | M. phaseolina | R. stolonifier | CaCO3 | MO | Zeolite | Phosphate |
|--------|---------|---------------|----------------|-------|----|---------|-----------|
| Pb     | F1      | 1.7           | 0.87           | 1.05  | 1.1| 1.3     | 1.1       | 1.2       |
|        | F2      | 1.3           | 7.62           | 2.04  | 2.8| 2.14    | 1.81      | 1.16      |
|        | F3      | 3.1           | 1.62           | 2.69  | 7.2| 2.03    | 7.4       | 8.3       |
|        | F4      | 10.25         | 11.22          | 10.63 | 6.85| 0.56    | 1.17      | 0.11      |
| Cd     | F1      | 0.16          | 0.01           | 0.02  | 0.02| 0.02    | 0.03      | 0.03      |
|        | F2      | 0.19          | 0.08           | 0.11  | 0.34| 0.22    | 0.26      | 1.58      |
|        | F3      | 0.57          | 0.17           | 0.20  | 11.73| 8.7     | 10.43     | 4.34      |
|        | F4      | 4.58          | 14.3           | 16.98 | 9.00| 1.43    | 2.00      | 5.00      |
| Cu     | F1      | 76.74         | 44.88          | 55.49 | 52.64| 30.03   | 42.68     | 52.00     |
|        | F2      | 2.11          | 2.12           | 1.41  | 7.76| 20.08   | 5.1       | 10.66     |
|        | F3      | 1.44          | 6.03           | 8.51  | 4.05| 6.17    | 23.26     | 22.2      |
|        | F4      | 65.38         | 13.39          | 37.98 | 34.05| 14.64   | 62.64     | 14.02     |
| Zn     | F1      | 96.49         | 73.72          | 86.67 | 78.9 | 94.02   | 94.51     | 83.41     |
|        | F2      | 4.76          | 30.18          | 19.20 | 8.51| 4.05    | 23.26     | 18.81     |
|        | F3      | 13.49         | 10.19          | 7.88  | 37.11| 36.86   | 71.99     | 1.46      |
|        | F4      | 61.33         | 24.86          | 18.27 | 34.05| 14.64   | 62.64     | 14.02     |

Soil1: Influenced by urban and wastewater.  F1: Water soluble and exchangeable; F2: Bound to Fe and Mn oxides; F3: Bound to organic matter; F4: Residual metal concentration.

Table 5. Distribution fractions of each studied metal (µg/gm) in Soil2 (influenced by industrial wastes).

| Metals | Control | M. phaseolina | R. stolonifier | CaCO3 | MO | Zeolite | Phosphate |
|--------|---------|---------------|----------------|-------|----|---------|-----------|
| Pb     | F1      | 1.6           | 0.9            | 1.1   | 1.4| 1.16    | 1.09      | 0.53      |
|        | F2      | 1.08          | 8.59           | 7.59  | 7.85| 2.14    | 7.71      | 0.95      |
|        | F3      | 4.75          | 2.28           | 4.52  | 3.7 | 5.14    | 15.11     | 6.23      |
|        | F4      | 34.04         | 12.17          | 12.48 | 15.74| 15.09   | 18.81     | 15.31     |
| Cd     | F1      | 0.4           | 0.23           | 0.23  | 0.34| 0.19    | 0.8       | 0.37      |
|        | F2      | 0.36          | 0.04           | 0.004 | 1.03| 1.03    | 1.64      | 1.13      |
|        | F3      | 1.47          | 2.02           | 3.02  | 0.04| 1.03    | 0.49      | 1.12      |
|        | F4      | 0.4           | 1.03           | 1.03  | 1.63| 0.043   | 0.18      | 0.23      |
| Cu     | F1      | 48.23         | 45.7           | 57.1  | 39.7| 43.7    | 32.7      | 39.9      |
|        | F2      | 31.17         | 42.06          | 51.13 | 39.63| 36.35   | 33.05     | 38.01     |
|        | F3      | 3.9           | 1.62           | 4.01  | 3.1 | 3.78    | 18.00     | 6.8       |
|        | F4      | 40.79         | 47.15          | 31.53 | 42.08| 61.36   | 22.30     | 25.11     |
| Zn     | F1      | 67.92         | 35.2           | 49.8  | 31.34| 46.01   | 38.92     | 46.65     |
|        | F2      | 54.26         | 32.72          | 26.92 | 54.05| 2.47    | 52.28     | 45.73     |
|        | F3      | 40.44         | 24.3           | 28.25 | 3.87| 46.35   | 28.13     | 13.64     |
|        | F4      | 135.87        | 143.98         | 138.01| 156.57| 175.52 | 128.78    | 130.1     |

Soil1: Influenced by urban and wastewater.  F1: Water soluble and exchangeable; F2: Bound to Fe and Mn oxides; F3: Bound to organic matter; F4: Residual metal concentration.
as substrates.

Conclusion

The application of sequential extraction method to study samples provides relevant information on possible toxicity of heavy metals in contaminated soils and gives valuable information on the mobility of these metals, helping in predicting their behavior to the ecosystem. *M. phaseolina* and *R. stolonifer* recorded significant roles as good biosorbent agents for Pb, Cd, Cu and Zn and showed better uptake capacity for Pb, Zn and Cd as compared to Cu. This uptake capacity increased in *M. phaseolina* as compared to *R. stolonifer*. The chemical remediation techniques, using calcium carbonate, manganese oxide, zeolite and phosphates can significantly reduce the availability of studied metals and then reducing their toxicity potential. However, these techniques certainly require intensive further improvements and studies in details to optimize the conditions for maximum biosorption of heavy metals of contaminated soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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