

Mechanism of Phytoremediation Potential of Flax (*Linum usitatissimum* L.) to Pb, Cd and Zn

Mohammed Elanwar Hosman¹, Soad Soliman El-Feky¹, Mohamed Ismail Elshahawy² and Eman Mohamed Shaker²

¹Faculty of Science, Tanta University, Egypt

²Research Institute, Agricultural Research Center (ARC), Egypt

ABSTRACT

An experiment was conducted to study the efficiency of flax plant (*Linum usitatissimum* cv. Sakha) as phytoremediator for the contaminated metal soils with metals. The soil of experiment was enriched with different levels of Cd as (CdCl_2), (10, 20, and 40 mg/kg soil), Pb as [$\text{Pb}(\text{CH}_3\text{COOH})_2$], (150, 500 and 700 mg/kg soil) and Zn as (ZnSO_4), (400, 800 and 1000 mg/kg soil) in addition to control. Results revealed that, a negative relationship was observed between the heavy metals concentrations and heights In addition to weights of both shoot and root at all growth stages. Results indicated that, increasing in metal concentration in the soil, there was a gradual increase in metal uptake in Flax plant. The average ability of flax plant to reduce heavy metals from the soil (removal %) was 49% for Cd, 68.6% for Pb and 71.76% for Zn. Subsequently, the highest accumulation of Cd was detected in root whereas, the highest accumulation of Pb and Zn detected in capsule. According to Bioaccumulation factor (BCF) and the translocation factor (TF) measured in this study, Flax could be considered as a hyperaccumulator plant for both Pb and Zn that each BCF and $\text{TF} > 1$ and due to values of BCF which fairly close to 1 and TF which < 1 , Flax can therefore be considered a Cd excluder.

Keywords: Heavy metals, Flax, Phytoremediation

INTRODUCTION

Heavy metals stress is one of the major abiotic stresses that cause environmental pollution in recent decades [1]. These metals are unlike other organic pollutants are not degraded and converted into harmless compounds via biological processes. In addition, heavy metals can enter the food chain. Common feature of environmental stress is their ability for production of toxic oxygen derivatives. Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations [2]. However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and growth inhibition in most plants [3]. Based on their chemical and physical properties, three different molecular mechanisms of heavy metal toxicity can be distinguished: (i) production of reactive species by auto-oxidation and Fenton reaction (Fe, Cu), (ii) blocking of essential functional groups in biomolecules (Cd, Hg), and (iii) displacement of essential metal ions from biomolecules [4]. However, several conventional metal removal ways are useful for detecting the presence and the concentration of metals in the environment, but these methods do not come with an eco-friendly technique and need high operational and maintenance cost. This has drawn interest to develop a scientifically cost-effective remedial measure to remove heavy metals from contaminated sites. One of the established approaches was through phytoremediation [5].

Phytoremediation is a way for cleaning the polluted sites by using plants to extract the heavy metals from the contaminated soil and accumulate them in roots, stems and branches [6]. Logically, repeating cycles of planting and harvesting of plants accumulated with heavy metals will eventually reduce the concentration of toxic metals in soils to an acceptable level for other uses. The efficiency of phytoremediation differs between species, as different mechanisms of ion uptake are operative in each species, depended on their morphological, physiological and anatomical

characteristics [7]. Hyperaccumulator plants can play a key role in the fate of the pollutants of contaminated matrixes via their root systems [8] and capable of accumulating extra-ordinary high metal levels, demonstrates that plants have the genetic potential to clean up contaminated soil [9].

Flax (*Linum usitatissimum* L.) Fiber crops are dicotyledonous plant from the family Linaceae used for industrial purposes and potential of economic value after harvesting [10]. It is rich in polyunsaturated fatty acids; alpha-linolenic acid and linoleic acid which are essential for human, human bodies cannot manufacture them. These plants show a metal tolerance dependent on species so, is ideal for research [11].

AIM OF THE STUDY

The main objective of the current study is to investigate the effect of different concentrations of Cd, Pb and Zn on growth of *Linum usitatissimum* at different growth stages and the efficiency of the plant as phytoremediator for the contaminated soils with metal.

MATERIALS AND METHODS

Seeds of flax (*Linum usitatissimum*) cv. Sakha were obtained from Agricultural Research Centre, Sakha research Station Kafr El-Shaikh Governorate, Egypt. 20 flax seeds were sterilized and germinated in 30 cm depth and 25 cm diameter pots filled with 8 kg clay soil. Experiment was conducted during the winter season, 2012/2013 in Sakha research Station. The pots were divided into four groups, the first were used as the control, the second group were sublimated with different concentrations of Pb as $[Pb(CH_3COOH)_2]$, (150, 500 and 700 mg/kg soil), the third group of soil concentrations of Cd ($CdCl_2$), (10, 20 and 40 mg/kg soil), the fourth their soils were polluted with different concentrations of Zn ($ZnSO_4$), (400, 800 and 1000 mg/kg soil). All pots received recommended dose of NPK fertilizers (according to Ministry of agriculture and Land reclamation MALR), the first during seedling stage and the second at vegetative stage.

Flax seeds were cultivated and left to grow till the end of the season and samples were collected at vegetative (45 days old), flowering (100 days old) and harvesting (155 days old) stages and subjected to the following experiments.

Growth criteria

Samples were collected at the different growth stages and thoroughly washed with distilled water then separated into shoot and root, weighed to estimate their fresh weights, then, oven-dried at 70°C for 2 days and reweighed to estimate its dry weights. Both fresh and dry weights were estimated as g/plant. Length of root and shoot were estimated as cm/plant.

Determination the percentage of metal removal (%)

The percentage of metal removal (%) was calculated [12] using the following formula;

$$\% \text{ efficiency} = \frac{C_0 - C_1}{C_0} \times 100$$

Where, C_0 refer to the initial concentration and C_1 refer to the final concentration.

Determination the bioaccumulation and translocation factors

The Transfer factor (TF) was calculated to evaluate the rate of metal transfer between plant roots and other organs. The bioconcentration factor (BCF) indicates the ability of the selected plant for absorbing metal from the contaminated soil.

The bioaccumulation factor (BCF) and translocation factor (TF) were calculated [13] using the following formula;

$BCF = \text{Total metal concentration in plant biomass} / \text{Total metal concentration in soil}$

$TF = \text{Total metal concentration in plant organ} / \text{Total metal concentration in root}$

Soil analysis

Air-dried soil samples were ground passed through a 2 mm sieve and mixed thoroughly [14]. Chemical properties of the used soil were determined according to standard methods of Page [15] and Clark et al. [16]. The pH was measured

using a pH meter in soil water suspension (1:2.5), electrical conductivity (EC) to express total soluble salts were determined in the saturated soil paste. Cd, Zn and Pb were determined [17] using atomic absorption spectrophotometer, PERKIN ELIMER 3300.

Statistical analysis

Collected data were analyzed using analysis variance (ANOVA) in a complete randomized design using Anova: Two-Factor with Replication [18].

The soil used for this study was obtained from Agricultural Research Centre, Sakha research Station (Table 1).

RESULTS AND DISCUSSION

Growth criteria

The data represented in Figure 1, a negative relationship was observed between the tested heavy metals and Growth criteria of both shoot and root. As compared to control, the lowest value was recorded with soil treated with 1000 mg/kg soil of Zn where, the reduction was 50 and 61% for shoots fresh weights at vegetative and flowering stages respectively. The reduction in shoot dry weight reached to about 55 and 66% and shoot lengths reached to about 36 and 38%. But for root fresh weight, it reached to about 52 and 64% for vegetative and flowering stages, respectively, and reached to about 56 and 70% in root dry weight but, for root lengths it reached to about 35 and 41%.

The efficiency of flax plant in extracting heavy metals from soil and heavy metal accumulation.

Removal % and uptake

It would appear that also, by increasing in metal concentration in the soil, there was a gradual increase in metal uptake in flax plant. Metal concentrations in the soil decreased with time in the present study. Results in Table 2 stated that metal removal from the soil was increased due to accumulation in Flax plant, since the plant was able to remove

Table 1: Chemical analyses and heavy metals content of the soil

Soil Tested Characteristics	
pH (1:2.5 Soil:Water Suspension)	7.8
EC (Soil-Paste Extract), ds/m at 25°C	1.46
OM (Organic Matter), %	1.22
CaCO ₃ (Total Carbonates), %	2.46
CEC (Cation Exchange Capacity), meq/100 g soil	41.2
<u>Particle size distribution:</u>	
Sand %	18.83
Silt %	32.73
Clay %	48.44
Texture class	Clay
<u>DTPA-extractible metal (available), mg/kg:</u>	
Pb	4.2
Cd	0.43
Zn	7.6
<u>Aqua-Regia extracted metals (total), mg/kg:</u>	
Pb	27.3
Cd	1.5
Zn	31.5

between 44% and 68.6% of Pb, between 32% and 49% for Cd and between 22.4% and 71.76% for Zn. the potential of flax to accumulate heavy metals appear in the order of Zn>Pb>Cd.

Heavy metal accumulations in flax organs

Data presented in Figure 2 indicate that Cd accumulated in the roots of flax in amounts higher than those accumulated in their aboveground parts where, the highest detected value was 74% in samples treated with in 40 mg/kg soil of Cd in roots, but only lower levels were translocated into aboveground parts of Flax. The trend of Cd accumulation was: root>shoot>capsule>seed. While, the reverse was true for Pb and Zn as both metals accumulated in aboveground parts in levels higher than those accumulated in the roots. Where, the highest accumulation of Pb 55% detected in 700 mg/kg soil for capsule and the trend of Pb accumulation was: capsule>shoot>root>seed. The highest accumulation of Zn 59% detected in 800 mg/kg soil for capsule and the trend of Zn accumulation was: capsule>seed>root>shoot.

Quantification of accumulation efficiency

According to results in Table 3, TF in Pb treatments >1 for shoot and capsule but <1 for seeds. The TF for different organs could be arranged in the sequence: capsule>shoot>seed. This indicates the effective translocation of Pb from roots to capsule and shoot but seed had the lowest TF.

TF in all treatments of Cd was measured to be less than 1. The TF for different organs could be arranged in the sequence: shoot>capsule>seed. This demonstrated a lower ability to translocate Cd from the root to above-ground tissues and indicates the preference of flax in storing and accumulating Cd in its roots.

Data illustrate also that, TF in samples treated with different Zn concentrations >1 for capsule and seed but <1 for shoot. The TF for different organs could be arranged in the sequence: capsule>seed>shoot. This indicates that, the effective translocation of Zn from roots to capsule and seed but shoot had the lowest TF.

Data in Table 3 illustrate that, flax gave a BCF ranging from 2.1-0.8 for Pb treatments, 0.9-0.4 for Cd and 2.5-0.3 for Zn.

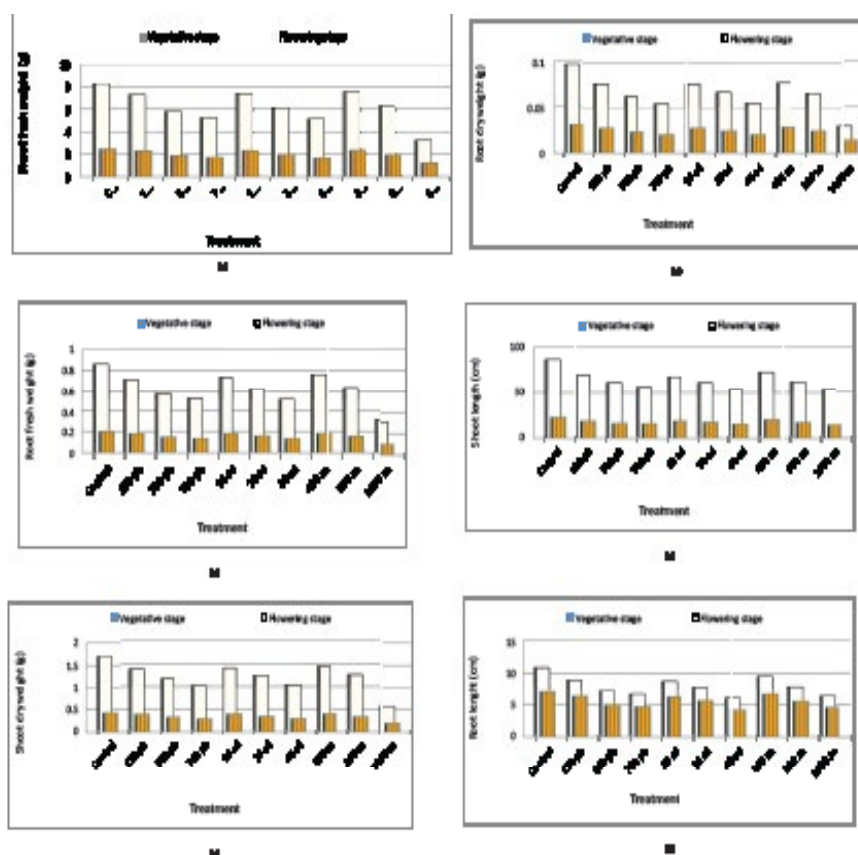


Figure 1: (a) Shoot fresh weight (g/plant), (b) root fresh weight (g/plant), (c) shoot dry weight (g/plant), (d) root dry weight (g/plant), (e) shoot length (cm) and (f) root length (cm) of Flax plant as affected by different concentrations of Pb, Cd and Zn (mg/kg soil) at vegetative and flowering stages

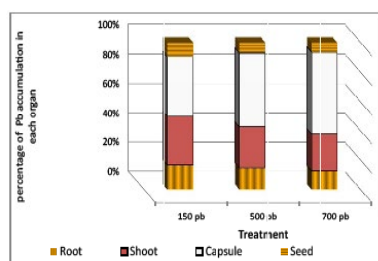
The efficiency of flax plant in extracting heavy metals from soil and heavy metal accumulation.

Table 2: The efficiency of Flax plant in extracting heavy metals (pb, cd and Zn mg/kg soil) from soil at harvesting stage

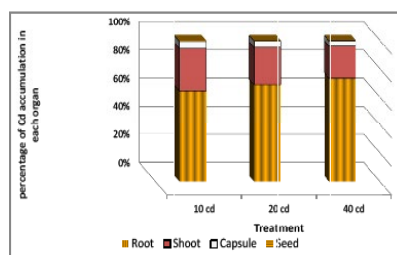
Treatments (Added conc.)	(Initial conc.) Available+Added	(Final conc.) After uptake	Uptake	Removal %
Pb control	4.2	4.190	0.01	0.47
150	154.2	48.40**	105.8**	68.6
500	504.2	264.2**	240.0**	47.6
700	704.2	393.64**	310.56**	44.1
L.S.D 0.05		16.32	22.65	
L.S.D 0.01		28.45	32.22	
Cd control	0.43	0.429	0.001	0.23
10	10.43	5.94**	4.49**	46.3
20	20.43	10.41**	10.02**	49.0
40	40.43	27.37**	13.06**	32.3
L.S.D 0.05		1.27	0.867	
L.S.D 0.01		2.19	1.17	
Zn control	7.6	7.54	0.06	0.78
400	407.6	115.10**	292.5**	71.76
800	807.6	318.19**	489.41**	60.6
1000	1007.6	781.89**	255.71**	22.4
L.S.D 0.05		26.43	89.08	
L.S.D 0.01		30.76	113.0	

* Results significantly different from control at (P<0.05)

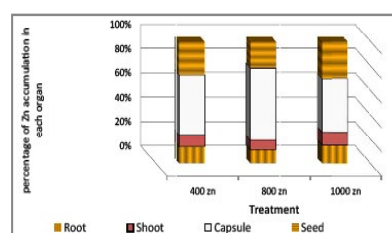
** Results significantly different from control at (P<0.01)



(a)



(b)



(c)

Figure 2: Accumulation of the tested heavy metals (mg/kg DW) in different organs of Flax plant as affected by (a) Pb treatments mg/kg soil, (b) Cd treatments mg/kg soil and (c) Zn treatments mg/kg soil

Table 3: Bioaccumulation factor (BCF) of Flax as affected by different heavy metals treatments (mg/kg) and Translocation factor (TF) of shoot, capsule and seed

Treatment	Shoot	Capsule	Seed	BCF
150 Pb	1.98	2.4	0.57	2.1
500	1.88	3.3	0.48	1
700	1.97	4.3	0.51	0.8
10 Cd	0.46	0.07	0.011	0.7
20	0.38	0.06	0.007	0.9
40	0.31	0.05	0.005	0.4
400 Zn	0.72	3.6	2.0	2.5
800	0.72	5.3	1.9	1.5
1000	0.73	2.9	2.0	0.3

DISCUSSION

Problems caused by heavy metals contamination in soils include phytotoxic effects of certain elements such as Cd, Pb and Zn, which are well known as micronutrient elements. They cause several phytotoxicity when endogenous levels are exceeded [19]. Another serious problem is posed by the uptake of potentially noxious elements by food or forage plant species and their transfer to the food chain [20]. Agricultural practices such as excessive application of phosphatic fertilizers for optimum crop production, extensive and injudicious usage of toxic pesticides, and use of sewage sludge can result in soil pollution with heavy metals [21]. One of the technologies for decontamination of polluted environment by using modern, non-destructive and environment-friendly technologies is called phytoremediation, which describes the treatment of environmental pollution using plants which capable of accumulating high levels of metals when grown in contaminated soils.

Data in the present study showed that, applied of high concentrations of the tested heavy metals induced reduction of flax growth started upon the addition of (150, 10 and 400 mg/kg dw of Pb, Cd and Zn, respectively) compared to control samples, resulted in a decrease in all measured growth criteria represented in measured growth parameters at both vegetative and flowering stages. Results of our study were corresponded with other researches. In Pb-exposed wheat (*Triticum aestivum* L.) [22], in Zn-stressed bean (*Phaseolus vulgaris* L.) [23], in Pb, Cd and Zn-exposed spinach (*Spinacia oleracea*) [24] and in Cd-exposed chickpea (*Cicer arietinum* L.) [25].

The reduction in plant growth has been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and/or decrease in many metabolic activities [9,26]. Besides, growth is a combination of cell division and elongation. In this context, a decrease in mitotic activity has been reported in several plant species after exposure to heavy metals where, metal binds to nucleic acids and causes aggregation and condensation of chromatin, as well as inhibiting the process of replication, transcription and ultimately affecting cell division which consequently results into a suppressed growth [27]. It has been suggested that slower growth could be considered an adaptive feature for plant survival under stress, because it allows plants to divert assimilates and energy, otherwise used for shoot growth, into protective molecules to fight stress [28].

In order to measure the potential of the tested species to accumulate a metal, this can be quantified by calculating bioconcentration factor and translocation factor. Bioconcentration factor (BCF) indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment [29]. Translocation Factor (TF) indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots [30]. Both BCF and TF are important in screening hyperaccumulators. The evaluation and selection of plants for phytoremediation purposes entirely depend on BCF and TF values [31].

Based on tolerance mechanisms, plant species have been divided into two types according to their BCF and TF:

(a) Metal excluders accumulate heavy metals from substrate into their roots but restrict their transport and entry into their aerial parts [32]. Such plants have a low potential for metal extraction but may be efficient for phytostabilization purposes, their BCF is greater than 1 but TF are lower than 1.033.

(b) Hyperaccumulators achieve BCF and TI greater than 1.034. However, TF cannot be used alone to define hyperaccumulation although it is a useful measure in supporting other evidence of hyperaccumulation [35].

TF in all treatments of Cd was measured to be less than 1 (Table 3). The different organs were arranged as follow; shoot>capsule>seed. This demonstrated a lower ability to translocate Cd from the root to above-ground tissues and indicates the preference of flax in storing and accumulating Cd in its roots. Cd content in the above-ground tissues of

flax showed that, the species could not be introduced as a hyper accumulator species for Cd. But due to accumulation of high amounts of Cd in its roots and considering the calculated values of BCF (Table 3) which fairly close to 1 and TF which <1, flax can therefore be considered a Cd-excluder. Plants that over-accumulate heavy metals in their roots, excluding or limiting translocation to above-ground tissues, can be regarded as efficient to phytostabilise heavy metals in soils [36] where, Phytostabilization defined as the process to reduce the mobility of contaminants in soil through adsorption onto roots, adsorption and accumulation by roots, or precipitation within the root zone [37]. Thus, flax plant could be a good candidate for phytostabilisation of Cd²⁺-contaminated soils with low Cd²⁺ bioavailability, to avoid high accumulation in above-ground tissues.

Several plants with the potential to exclude metals from aerial parts have been known. Both *Lemna gibba* and *Lemna minor* accumulated one and a half-fold more Cd in their roots than in their shoots. However, *L. minor* was shown to have a slightly better performance in removing cadmium and more tolerant, when compared with *L. gibba* [38]. The accumulation of Cd was about 2.40-fold in root as compared to shoot in Chickpea (*Cicer arietinum* L.) [25].

TF in Pb treatments >1 for shoot and capsule but <1 for seeds. The trend as follow capsule>shoot>seed. This indicates the effective translocation of Pb from roots to capsule and shoot but seed had the lowest TF. TF in Zn treatments >1 for capsule and seed but <1 for shoot. The trend as follow capsule>seed>shoot. This indicates the effective translocation of Zn from roots to capsule and seed but shoot had the lowest TF. According to results, Flax is considered a hyperaccumulator plant for both Pb and Zn by phytoextraction mechanism that each BCF and TF>1 except 1000 mg/kg soil of Zn that BCF<1. Phytoextraction is a mechanism in which plants absorb metals from the soils, transport and concentrate them in the above-ground parts of plants. The above-ground are harvested and can be carefully processed for dumping or recycling of metals [39]. Selection of suitable plant species is crucial for effective phytoextraction and biomass derived from shoot of a phytoremediator crop plant should be capable of depositing metal(oid) species at concentration 50-500 times higher than those in the contaminated soil substrate [40].

Some researchers introduced many species of plants as heavy metals phytoextraction. Some populations of *Noccaea caerulea* can accumulate Pb at >1,000 µg/g in leaf dry matter in the field and under hydroponic conditions [41]. Several plants could accumulate Pb in their tissue of more than 50 mg/g dry weight of plant. Among those species are species of *Brassica campestris* L., *Brassica carinata* A. Br., *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) Koch that could accumulate more than 100 mg Pb/g dry weight [42].

Physiological mechanisms of heavy metal tolerance and accumulation

The process of metal accumulation involves several steps, one or more of which are enhanced in hyper accumulators. Within the one plant species more than one mechanism could be in operation [43].

Phyto-extraction is a process to remove the contamination from soil without destroying soil structure and fertility [44]. Great metal accumulation may be attributed to well develop detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on appropriate ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of metal(oid)s [45] and metal exclusion strategies of plant species.

Solubilization of the metal from the soil matrix

Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal [46]. Plants have evolved these processes to liberate essential metals from the soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution. While no hyper accumulators have evolved to handle high concentrations of toxic metals if they are present in solution, phytoremediator plants could be modified to solubilize contaminants that are bound to the soil [47].

Uptake into the root

Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells [48]. While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called the xylem [49]. To enter the xylem, solutes must cross the Casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis [50]. Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel [51]. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. As Pb is not an essential element, plants do not have channels for Pb uptake. Instead, this element is bound to carboxylic groups of mucilage uronic acids on root surfaces [52].

Transport to the above ground organs

Once loaded into the xylem, the flow of the xylem sap will transport the metal to the above ground organs, where it must be loaded in to the cells. The cell types where the metals are deposited vary between hyper accumulator species [47]. In xylem sap, metal ions such as Zn^{2+} , Pb^{2+} , Ni^{2+} , Cu^{2+} , Fe^{2+} , etc. are transported mainly as metal complexes with asparagines, histidine, organic acids and nicotianamine. Once metal enters in phloem, further translocation to various plant organs. In phloem sap, Zn is thought to be transported either in ionic form or as Zn nicotianamine, Zn-malate, Zn-histidine complexes in phloem tissues. In *Sesbania drummondii* Pb is transported to stems and leaves in structures similar to Pb-acetate, Pb-nitrate and Pb-sulfide [53].

Detoxification/chelation

Metal could be converted to a less toxic form through chemical conversion or by complexation with ligands [54]. Organic acids and amino acids are suggested as ligands for chelation of heavy metal ions because of the presence of donor atoms (S, N and O) in their molecules [55]. As many chelators use thiol groups as ligands, the sulfur (S) biosynthetic pathways have been shown to be critical for hyperaccumulator function [56] and for possible phytoremediation strategies [47].

Sequestration

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal/metal-ligand (such as phytochelatins and metallothioneins) must be transported across the vacuolar membrane. In fact, two vacuolar proton pumps, a vacuolar proton-ATPase (VATPase) and vacuolar proton pyrophosphatase (VPPase), energize vacuolar uptake of most solutes. Uptake can be catalyzed by either channels or transporter proteins [49]. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge sites on the cell walls may interact with polyvalent cations [47].

Restriction of transport of heavy metals

Our results indicated that Cd accumulated in Flax root, this cellular exclusion of heavy metals is an important adaptive strategy for heavy metals tolerance in plants. In plants restraining Cd in roots, Cd is sequestered in the endoderm and the cortical regions of roots. When this filter fails, Cd can penetrate the xylem vessels and translocate to the shoot. Therefore, the specific composition of cell walls in the endoderm and cortex is critical [57]. Ultimately, the cell wall of roots is considered as an effective barrier against penetration of Cd and a relationship can be established between higher accumulations of Cd in the roots vs. lower accumulation in the leaves. This suggests that Cd-tolerance goes through the capacity of roots to limit the diffusion of Cd towards the entire plant.

CONCLUSION AND RECOMMENDATION

Due to accumulation of high amounts of Cd in its roots and considering the calculated values of BCF and TF, Flax can therefore be considered a Cd excluder by phytostabilization mechanism. Conversely, due to accumulation of high amounts of Pb and Zn in its above ground and considering the calculated values of BCF and TF Flax is considered an accumulator plant for both Pb and Zn by phytoextraction mechanism. The yielded results indicate that flax may potentially be useful for restoring heavy metal contaminated sites but due to flax accumulated Cd in its roots so, preferred Cd restoring, then Pb accumulated in capsule and very little move to seed finally, Zn that to some extent accumulated in seed.

Use of fiber crops like flax as heavy metal accumulators is recommended, as the ample amount of their product is used for non-food purposes. Industrial processing of biomass contaminated with heavy metals makes flax plant an economically remarkable crop for implementation of phytoextraction technology.

REFERENCES

- [1] Castro R, Caetano L, Ferreira G, Padilha P, Saeki M, et al. Banana peel applied to the solid phase extraction of copper and lead from river water. *Chem Res*, **2011**, 50: 3446-3451.
- [2] Nedelkoska TV, Doran PM. Characteristics of heavy metal uptake by plants species with potential for phytoremediation and phytomining. *Minerals Engineering*, **2000**, 13: 549-561.

- [3] Li Q, Cai S, Mo C, Chu B, Peng L, et al. Toxic effects of heavy metals and their accumulation in vegetables grown in a saline soil. *Ecotoxicol Environ Saf*, **2010**, 73: 84-88.
- [4] Schützendubel A, Polle A. Plant responses to abiotic stresses: Heavy metal induced oxidative stress and protection by mycorrhization. *J Exp Bot*, **2002**, 53: 1351-1365.
- [5] Aba-Alkhalil MS, Mofleh AE. Lead and cadmium pollution on some desert plants (Phytoremediation). *J Agric Vet Sci*, **2013**, 6: 25-32.
- [6] Hamzah A, Hapsari RI, Wisnubroto EI. Phytoremediation of Cadmium-contaminated agricultural land using indigenous plants. *Int J Environ Agric Res (IJOEAR)*, **2016**, 2: 2454-1850.
- [7] Rahman MA, Hasegawa H. Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere*, 2011, 83: 633-646.
- [8] Ibrahim MM, Alsahli AA, EL-gaaly G. Evaluation of phytoremediation potential of six wild plants for metal in a site polluted by industrial wastes: A field study in Riyadh, Saudi Arabia. *Pak J Bot*, **2013**, 42: 571-576.
- [9] Singh S, Parihar P, Singh R, Singh VP, Sheo MP. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics and ionomics. *Front Plant Sci*, **2016**, 6: 1143.
- [10] Griga M, Vrbova M, Horacek J, Smykal P. Flax (*Linum usitatissimum* L.) transformation with heavy metal binding protein genes. In: Sehnal F., Drobnik J. (Eds.) White Book of Genetically Modified Crops. EU regulations and research experience from the Czech Republic. Biology Centre AS CR Ceske Budejovice, **2009**, 57.
- [11] Bassuany FM, Hassanein RA, Baraka DM, Khalil RR. Role of stigmasterol treatment in alleviating the adverse effects of salt stress in flax plant. *J Agric Technol*, **2014**, 10: 1001-1020.
- [12] Tanhan P, Kruatrachue M, Pokethitiyook P. Uptake and accumulation of cadmium, lead and zinc by Siam weed (*Chromolaena odorata* (L.) King & Robinson). *Chemosphere*, **2007**, 68: 323-329.
- [13] Ait AN, Bernal MP, Ater M. Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant Soil*, **2002**, 239: 103-111.
- [14] Piper CS. Soil and plant analysis. Inter-science publishers Inc. New York, **1947**.
- [15] Page AL (ed). Methods of soil analysis. Part 2: chemical and microbiological properties. (2nd ed) Amer. Soc. Agro., In: Soil Sci. Soc. Amer. In: Madison, Wisconsin, USA, **1982**.
- [16] Clark FE, Evans DD, Ensinger LE, White JL. Methods of Soil Analysis. Amer Soc Agron Inc Ser. No. 9 Madison, Wisc, USA, **1986**.
- [17] Cottenie A, Verloo M, Kiekens L, Velgh G, Camerlynck R. Chemical analysis of plant and soils. Lab. Anal Agrochem, State Univ, Ghent, Belgium, **1982**.
- [18] Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd Ed., John Wiley and Sons, **1984**.
- [19] Wang Y, Xu L, Chen YL, Shen H, Gong YQ, et al. Transcriptome profiling of radish (*Raphanus sativus* L.) root and identification of genes involved in response to lead (Pb) stress with next generation sequencing. *PLoS ONE*, **2013**, 8: e66539.
- [20] Liu JG, Ma XM, Wang MX, Sun XW. Genotypic differences among rice cultivars in lead accumulation and translocation and the relation with grain Pb levels. *Ecotoxicol Environ Saf*, **2013**, 90: 35-40.
- [21] Oves M, Saghir KM, Qari AH, Felemban NM, Almeelbi T. Heavy metals: Biological importance and detoxification strategies. *J Bioremediat Biodegrad*, **2016**, 7.
- [22] Khizar HB, Anwar S, Nawaz K, Hussain K, Siddiqi EH, et al. Effect of heavy metal Lead (Pb) stress of different concentration on wheat (*Triticum aestivum* L.). *Middle-East Journal of Scientific Research*, **2013**, 14: 148-154.
- [23] Nejad RK, Najafi F, Arvin P, Firuzeh R. Study different levels of zinc sulphate (ZnSO₄) on fresh and dry weight, leaf area, relative water content and total protein in bean (*Phaseolus vulgaris* L.). *Plant Bull Env Pharmacol Life Sci*, **2014**, 3: 144-151.
- [24] Alia N, Sardar K, Said M, Salma K, Sadia A, et al. Toxicity and Bioaccumulation of Heavy Metals in Spinach (*Spinacia oleracea*) Grown in a Controlled Environment. *Int J Environ Res Public Health*, **2015**, 12: 7400-7416.

-
- [25] Parvaiz A, Arafat A, Abdel Latef EF, Abd-Allah AH, Sarwat M, et al. Calcium and potassium supplementation enhanced growth, osmolyte secondary metabolite production and enzymatic antioxidant machinery in cadmium-exposed chickpea (*Cicer arietinum* L.). *Front Plant Sci*, **2016**, 7.
- [26] Kumar S, Dhingra HR. Sexual reproduction and cadmium partitioning in two mung bean genotypes rose in soils contaminated with cadmium. *Indian J Plant Physiol*, **2005**, 10: 151–157.
- [27] Patil G, Umadevi M. Cadmium and lead effect on growth parameters of four Eucalyptus species. *Int J Biosci*, **2014**, 5: 72-79.
- [28] Zhu JK. Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol*, **2002**, 53: 247-273.
- [29] Ladislav S, El-Mufleh A, Gerente C, Chazarenc F, Andres Y, et al. Potential of aquatic macrophytes as bioindicators of heavy metal pollution in urban storm water run-off. *Water Air Soil Pollut*, **2012**, 223: 877-888.
- [30] Padmavathiamma PK, Li LY. Phytoremediation technology: Hyper-accumulation metals in plants. *Water Air Soil Pollut*, **2007**, 184: 105-126.
- [31] Wu Q, Wang S, Thangavel P, Li Q, Zheng H, et al. Phytostabilization potential of *Jatropha curcas* L. in polymetallic acid mine tailings. *Int J Phytorem*, **2011**, 13: 788-804.
- [32] Malik N, Biswas AK. Role of higher plants in remediation of metal contaminated sites. *Sci Rev Chem Commun*, **2012**, 2: 141-146.
- [33] Barcelo J and Poschenrieder C. Phytoremediation: principles and perspectives. *Contrib Sci*, **2003**, 2: 333-344.
- [34] Badr N, Fawzy M, Al-Qahtani KM. Phytoremediation: An economical solution to heavy-metal-polluted soil and evaluation of plant removal ability. *World Appl Sci J*, **2012**, 16: 1292-1301.
- [35] Van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant Soil*, **2013**, 362: 319–334.
- [36] Maestri E, Marmiroli M, Visiol G, Marmiroli N. Metal tolerance and hyperaccumulation: Costs and trade-offs between traits and environment. *Environ Exp Bot*, **2010**, 68: 1-13.
- [37] Gupta DK, Sandallo LM (eds). Metal toxicity in plants: perception, signaling and remediation. Springer, London, **2011**.
- [38] Dana AM. Comparative study of the toxicity and phyto-extraction capacity of *L. minor* and *L. gibba* in polluted water by cadmium. *Int J Plant Anim Environ Sci*, **2016**, 6.
- [39] Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals--concepts and applications. *US National Library of Medicine National Institutes of Health*, **2013**, 91: 869-881.
- [40] Kramer U. Metal hyperaccumulation in plants. *Annu Rev Plant Biol*, **2010**, 61: 517-534.
- [41] Mohtadi A, Ghaderian SM, Schat H. A comparison of lead accumulation and tolerance among heavy metal hyperaccumulating and non-hyperaccumulating metallophytes. *Plant Soil*, **2012**, 352: 267-276.
- [42] Tangahu BV, Abdullah SRS, Basri H, Idris M, Anuar N, et al. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int J Chem Eng*, **2011**.
- [43] Rajib D, Jayalekshmy VG. Mechanism of heavy metal tolerance and improvement of tolerance in crop plants. *J Glob Biosci*, **2015**, 4: 2678-2698.
- [44] Ghosh M, Singh SP. A review on phytoremediation of heavy metals and utilization of its byproducts. *Appl Ecol Environ Res*, **2005**, 3: 1-18.
- [45] Cui S, Zhou Q, Chao L. Potential hyperaccumulation of Pb, Zn, Cu and Cd in enduring plants distributed in an old smelter, northeast China. *Environ Geol*, **2007**, 51: 1043-1048.
- [46] Alberts B, Johnson A, Walter P, Lewis J, Raff M, et al. Molecular biology of the cell, 5th edn. Taylor & Francis, Garland Science, New York, **2007**.
- [47] Wendy AP, Baxter IR, Richards EL, Freeman JL, Murphy AS. Phytoremediation and hyperaccumulator plants, **2005**.
- [48] Zhu XF, Zheng C, Hu YT. Cadmium-induced oxalate secretion from root apex is associated with cadmium exclusion and resistance in *Lycopersicon esulentum*. *Plant Cell Environ*, **2011**, 34: 1055-1064.

-
- [49] Sunitha MSL, Prashant SA, Kumar S, Rao MLN, Kishor KPB. Cellular and molecular mechanisms of heavy metal tolerance in plants: A brief overview of transgenic plants overexpressing phytochelatin synthase and metallothionein genes. *Plant Cell Biotechnol Mol Biol*, **2013**, 14: 33-48.
- [50] Greipsson S. Phytoremediation. *Nat Educ Knowl*, **2011**, 2: 7.
- [51] Sharma P, Dubey R. Lead toxicity in plants. *Braz J Plant Physiol*, **2005**, 17: 35-52.
- [52] Sharma SS, Kaul S, Metwally A, Goyal KC, Finkemeier I, et al. Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status. *Plant Sci*, **2004**, 166: 1287-1295.
- [53] Revathi S, Venugopal S. Physiological and biochemical mechanisms of heavy metal tolerance. *Int J Environ Sci*, **2013**, 3.
- [54] Sheoran V, Sheoran A, Poonia P. Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: A review. *Crit Rev Environ Sci Technol*, **2011**, 41: 168-214.
- [55] Van Huysen T, Terry N, Pilon-Smits EAH. Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine- γ -synthase. *Int J Phytoremediation*, **2004**, 6: 1-8.
- [56] Akhter MF, Omelon CR, Gordon RA, Moser D, Macfie SM. Localization and chemical speciation of cadmium in the roots of barley and lettuce. *Environ Exp Bot*, **2014**, 100: 10-19.
- [57] Sun J, Cui J, Luo C, Gao L, Chen Y, et al. Contribution of cell walls, non-protein thiols and organic acids to cadmium resistance in two cabbage varieties. *Arch Environ Contam Toxicol*, **2013**, 64: 243-252.