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EFFICIENCY OF FLAX (*Linum usitatissimum L.*) AS A PHYTOREMEDIATOR PLANT FOR THE CONTAMINATED SOILS WITH HEAVY METALS

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ABSTRACT

This study was conducted to study the efficiency of flax plant (*Linum usitatissimum* cv. Sakha 1) as a phytoremediator for the contaminated soils with heavy metals. The soil samples was supplemented with different levels of Cd as $CdCl_2$, (10, 20, and 40 mg/kg soil), Pb as $Pb(CH3COOH)_2$, (150, 500 and 700 mg/kg soil) and Zn as $ZnSO_4$ (400, 800 and 1000 mg/kg soil) in addition to control samples. Results show progressive reduction in the germination percentage as the concentration of the heavy metals was increased compared to control samples. Carbohydrate content increased by increasing Pb and Cd concentrations; while decrease by increasing Zn concentrations. In contrast to Carbohydrate; protein content decrease by increasing Pb and Cd concentrations; while increase by increasing Zn concentrations compared to control samples. Results also showed a progressive increase in metal uptake in response to the metal concentration in the soil. The average ability of flax plant to remove 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 an accumulator plant for both Pb and Zn by phytoextraction mechanism where each value of BCF and TF > 1 and due to values of BCF which fairly close to 1 and TF which < 1 , flax can be considered as Cd excluder by phytostabilization mechanism.

Keywords: Heavy metals, Flax, Phytoremediation, protein, Carbohydrate

INTRODUCTION

Heavy metals stress is one of the major abiotic stresses that cause environmental pollution in recent decades (Castro et al. , 2011). These metals, 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 (Liu et al., 2013). 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 trans-mission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Doran, 2000). 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 (Li et al., 2010). Based on their chemical and physical properties, three different molecular mechanisms of heavy metal toxicity can be distinguished: (i) production of reactive species by autooxidation and Fenton reaction (Fe, Cu), (ii) blocking of essential functional groups in biomolecules (Cd, Hg), and (iii) displacement of essential metal ions from biomolecules (Schutzendubel and Polle 2002). 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 (Aba-Alkhil and Moftah, 2013).

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 (Hamzah et al. 2016). 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 (Rahman and Hasegawa, 2011). Hyperaccumulator plants can play a key role in the fate of the pollutants of contaminated matrixes via their root systems (Ibrahim et al., 2013) and capable of accumulating extra-ordinary high metal levels, demonstrates that plants have the genetic potential to clean up contaminated soil (Singh et al., 2016). Maestri et al. (2010) and Malik et al. (2017) showed that, such metal accumulator plants having ability to accumulate in shoot 100 mg kg^{-1} of cadmium (Cd), 1000 mg kg^{-1} of arsenic (As), cobalt (Co), copper (Cu), lead (Pb) or nickel (Ni) or > 10,000 mg kg^{-1} of manganese (Mn) and zinc (Zn), are classified as hyper - accumulator plants.

Flax (*Linum usitatissimum L.*) is a Fiber crops plant from the family Linaceae. used for industrial purposes and potential of economic value after harvesting. (Griga et al., 2009). 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 (Bassuany, et al., 2014).

THE 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 germination percentage and some physiological parameters of *Linum usitatissimum* at different growth stages and the efficiency of the plant as a phytoremediator for soils contaminated with the tested heavy metals.

MATERIALS AND METHODS

Seeds of flax (*Linum usitatissimum*) cv. Sakha 1 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. The pots were divided into four groups, the first were used as the control, the second group was supplemented with different concentrations of pb as $Pb(CH3COOH)_2$ (150, 500 and 700 mg/kg soil), the third group of soil was supplemented with different concentrations of Cd as $CdCl_2$ (10, 20 and 40 mg/kg soil), the forth group was supplemented with different concentrations of Zn as $ZnSo_4$ (400, 800 and 1000 mg/kg soil). All pots received the recommended dose of NPK fertilizers (according to Ministry of agriculture and Land reclamation MALR), during seedling and vegetative stage.

Seeds were cultivated and left to grow till the end of the season, then samples were collected at seedling (21-d old), harvesting (155-d old) stages and subjected to the following experiments:

Germination percentage

Germination percentage at seedling stage was calculated using the following equation:

$$\text{Germinatin } (\%) = \frac{\text{No. of normal seedlings } \times 100}{\text{Total no. of seeds}}$$

Carbohydrates

Carbohydrate content was determined in plant samples shoots, root at harvesting stages according to Naguib (1962) using phenol - sulphuric acid colorimetric method.

Total soluble protein

Total soluble proteins were estimated in flax shoots, root at harvesting stages using borate extract according to Lowry method (1951).

Determination the percentage of metal removal (%)

The percentage of metal removal was calculated according to Tanhan et al. (2007) at harvesting stages using the following formula;

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

Where, C_0 refer to the initial concentration of a heavy metal and C_1 refer to its 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 according to Ait et al. (2002) using the following formula:

BCF = total metal concentration in plant biomass / total metal concentration in soil.

TF = total metal concentration in plant organ / total metal concentration in root.

Soil analysis

Air-dried soil samples were ground, passed through a 2 mm sieve and mixed thoroughly according to Piper (1947). Chemical properties of the used soil were determined according to standard methods of Page et al. (1982) and Clark et al. (1986). The pH was measured using a pH meter in soil water suspension (1: 2.5), electrical conductivity (EC) was also determined to express total soluble salts in the saturated soil paste. Cd , Zn and Pb were determined according to Cottenie et al., (1982) using atomic absorption spectrophotometer, PERKIN ELIMER 3300.

Statistical analysis

The collected data were analyzed using analysis of variance (ANOVA) in a complete randomized design using Anova: Two-Factor With Replication according to Gomez and Gomez (1984).

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

Table 1: chemical analyses and heavy metals content of the soil.

Soil Tested Characteristics	
pH (1:2.5 soil:water suspenstion)	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/100g soil.	41.2
Particle size distribution:	
Sand %	18.83
Silt %	32.73
Clay %	48.44
Texture class	Clay
DTPA-extractble metal (available), mg/kg^{-1}:	
Pb	4.2
Cd	0.43
Zn	7.6
Aqua-Regia extracted metals (total), mg/kg^{-1}:	
Pb	27.3
Cd	1.5

Zn	31.5

RESULTS

Germination percentage

Data presented in Fig.1 showed that, as compared to control , The results show progressive reduction in the germination percentage as the concentration of the heavy metal was increased. The lowest value was 61% reduction was recorded at 1000 mg/kg soil for Zn.

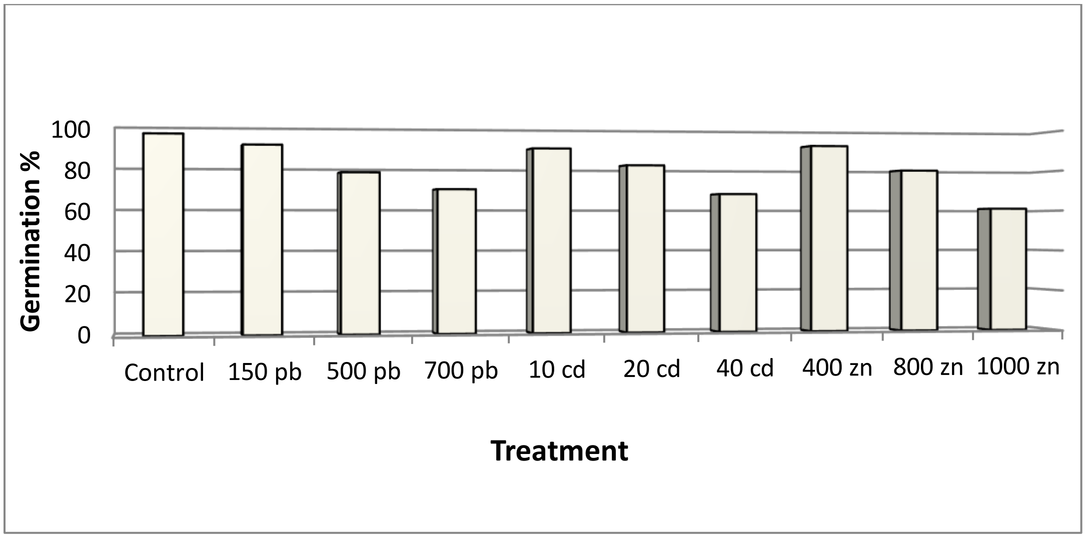


Fig 1. Germination percentage of flax plant as affected by different concentrations of Pb, Cd and Zn (mg/kg soil) at seedling stge.

Carbohydrates

The data presented in Fig. 2 indicate that, at vegetative stage, carbohydrate content were accumulated by increasing Pb and Cd concentrations in both roots and shoots. On contrary , under Zn treatments a reduction observed in carbohydrate . Likewise, sensitivity in roots than shoots has been recorded at all treatments. The highest value of carbohydrate content was recorded at 700 mg/kg soil of Pb where, the carbohydrate content increased by about 96% and 190% in comparison to control in shoots and roots respectively. While, the lowest value was recorded at 1000 mg/kg soil of Zn where, the carbohydrate content decreased by about 60% and 62% of shoots and roots respectively.

Protein content

Data presented in Fig. 3 revealed that at harvest stage, there was a negative relationship between metal concentration and protein content in Pb and Cd treatments in shoots and roots at. On contrary , under Zn stress protein content accumulate by increasing metal concentration. Likewise, sensitivity in roots than shoots has been recorded at all treatments. The highest value of protein content was also recorded at 800 mg/kg soil of Zn where, the protein content increased by about 77% and 115% in comparison to control in shoots and roots respectively. while, the lowest value was recorded at 40 mg/kg soil of Cd where, the protein content was reduced by about 46% and 58% of shoots and roots respectively.

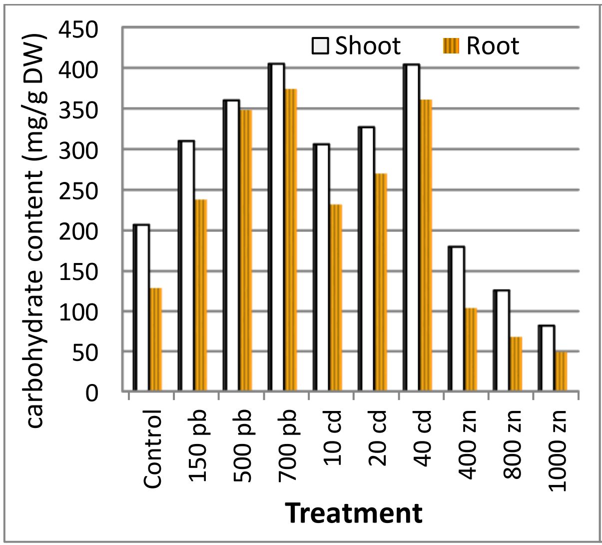


Fig.2

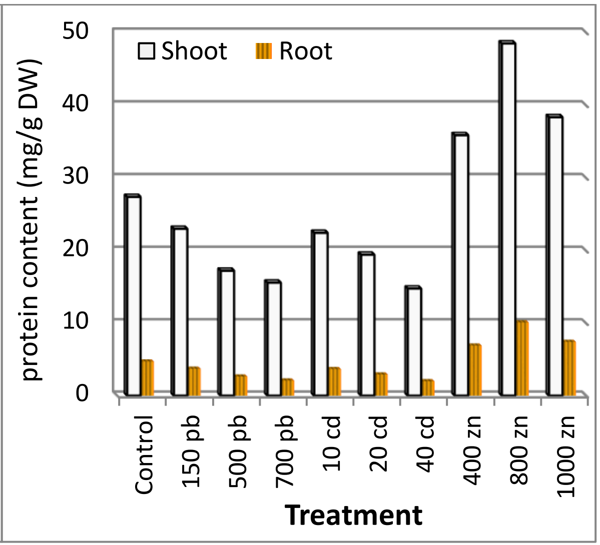


Fig.3

Fig.2. Total carbohydrate content (mg/g DW) of shoot and root of Flax plant by different concentrations of Pb, Cd and Zn (mg/kg soil) at harvesting stages.

Fig.3. The protein content (mg/g DW) of shoot and root of Flax plant affected by different concentrations of Pb, Cd and Zn (mg/kg soil) at harvesting stages.

The efficiency of Flax plant in extracting Heavy metals from soil and heavy metal accumulation

Removal % and uptake

Results in Table 2 showed that with increasing metal concentration in the soil, its uptake by the plant increased.

Metal removal from the soil was increased due to their uptake by flax plant, since the plant was able to remove between 44% and 68.6% of Pb, between 32% and 49% for Cd and between 22.4% and 71.76% for Zn.

The highest value of Pb removal was recorded in soil samples treated with 150 mg/kg soil, 20 mg/kg soil for Cd and 400 mg/kg soil for Zn.

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

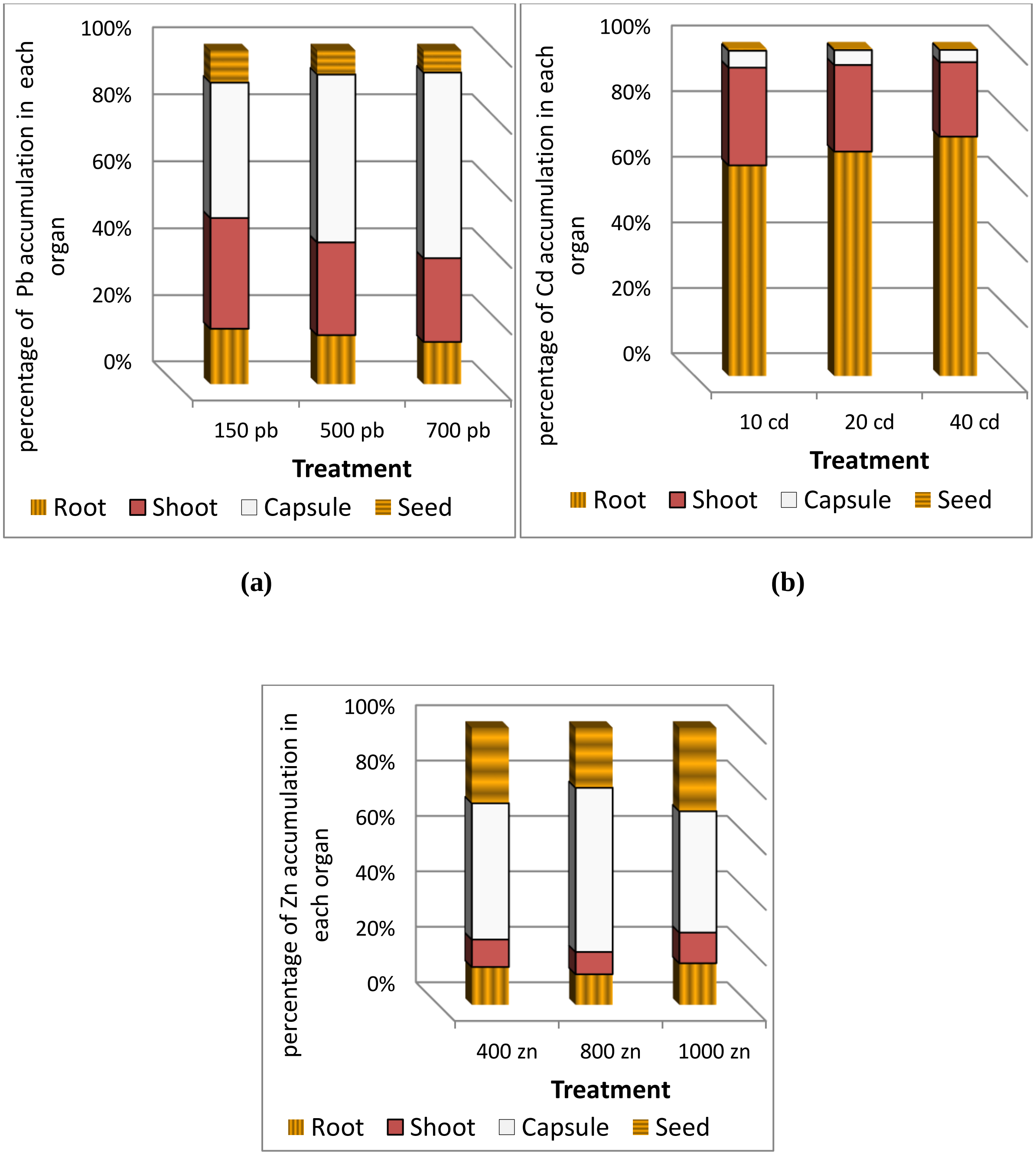
400	407.6	115.10**	292.5**	71.76
800	807.6	318.19**	489.41**	60.6
1000	1007.6	781.89**	255.71**	22.4
L.S.D 0.05		26.43	89.08	
L.S.D 0.01		30.76	113.0	

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

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

Heavy metal accumulations in Flax organs.

Data presented in Fig.4 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. In soil treated with 700 mg/kg, the highest accumulation of Pb was 55% in 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.



(a)

(b)

(c)

Fig. 4. 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.

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 following 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 following sequence: shoot > capsule > seed. These results 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 following 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.

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	TF			BCF
	Shoot	Capsule	Seed	
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 (wang et al., 2013). 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 (Liu et al., 2013). 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 (Oves et al., 2016). 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 (Hamzah et al., 2016).

The results of the present study (Fig.1) indicated that, reduction of seed germination with increasing metal concentration observed. Negative effects of metals exposure on seed germination have been often reported by several authors (Kavulicova et al, 2012) in Flax (*Linum usitatissimum*) and China aster (*Callistephus chinensis*) for Cd and Zn, and (Meher and Simeen, 2014) in *mung bean* for Pb and Cd.

Reduction in seed germination may be due to the interference and alterations in the cell membrane permeability properties by metal which resulted in decreased water absorption and transport as well as lowered water stress. The blockage of any one of the phases may inhibit the germination process (Jadia and Fulekar, 2008; Pattnaik et al., 2016).

Carbohydrate contents in the present study (Fig.2) increased with increasing Pb and Cd concentrations. These results are in agreement with the findings of El-khawaga (2014) who noticed increase in carbohydrate in flax under Pb and Cd stresses. Bouziani and Yssaad (2016) indicated an increase in total carbohydrate content in Broad Bean (*Vicia Faba L.*) exposed to high level of Pb. The authors indicated that, this increase accompanied with increased activity of acid invertase and sucrose synthesis. In addition to the role of sugars in osmoregulation, the increase in soluble sugars allow the plants to maximize sufficient carbohydrates storage reserves to support basal metaboslim under stressed environment (Dubey and singh, 1999; El-khawaga, 2014). Contrarly, under Zn treatments in our study, carbohydrate content decreased with increase Zn concentrations. These results in accordance with those of Manivasagaperumal et al. (2011) who reported that sugar and starch content showed a decreasing trend with progressive increase in zinc content in cluster. Borowiak et al. (2015) who reported that, increased Zn concentrations caused gradual decreased carbohydrate content in *hybrid Salix* . The decrease in total sugar content of stressed plant probably corresponded with the photosynthetic inhibition or stimulation of respiration rate (John et al., 2008) and interaction of heavy metals with the reactive centre of ribulosebisphosphate carboxylase (Kumar et al. (2012).

Data in our study (Fig.3) revealed that increasing Pb and Cd concentrations resulted in protein reduction. These result in accordance with those of Alia et al. (2015) who stated that Cd and Pb treatments induces a reduction in total protein content in Spinach, Bouziani and Yssaad (2016) indicated a reduction in protein content in Broad Bean (*Vicia Faba L.*) exposed to Pb stress and Dana (2016) figured out that exposure to higher concentrations of Cd resulted in a decrease in the level of protein in *Lemna gibba and Lemna minor*. The decrease in protein content may be attributed to interaction with thiol residues of proteins and replacement them with heavy metals in metalloproteins (Pal et al., 2006) and According to Auda and Ali (2010), heavy metals such as Cd and Pb disturb nitrogen metabolism, which further decreases the synthesis of protein.

Conversely, in the present study the protein content of flax exposed to different concentrations of Zn was found to be increased on the consequent increase in the concentration of Zn. This in agreement with the findings of Mishra and Prakash (2010) who reported an increase in protein in *Glycine max* L under Zn stress and Rastgoo et al. (2014) who showed that protein increased with Zn treatments in *Aeluropus littoralis*. increasing total protein could be related to boosting of some kind of protein with low molecular weight that synthesis of this kind of proteins was gained in stress condition so accumulation of proteins are happened at same time with accumulation of zinc in plant (Rout, 2003). This stress proteins such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins For protection of plant in heavy metal stress and reduce toxic effects of heavy metal (Namjooyan et al., 2012).

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 (Ladislas et al., 2012). Translocation Factor (TF) indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots (Padmavathiamma and Li, 2007). 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 (Wu et al., 2011).

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 (Malik and Biswas, 2012). 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 1.0. (Barcelo and Poschenrieder, 2003).

(b) Hyperaccumulators achieve BCF and TI greater than 1.0 (Badr et al., 2012). However, TF cannot be used alone to define hyperaccumulation although it is a useful measure in supporting other evidence of hyperaccumulation (van der Ent et al., 2013) .

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 demonstrate 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 hyperaccumulator species for Cd . But according to Yoon et al. (2006) who showed that plant species with BCF greater than 1 and TF less than 1 have the potential to be used for phytostabilization, so results in our study (table, 3) revealed that flax plant can therefore be considered a Cd-excluder by phytostabilization mechanism. 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 (Maestri et al. 2010) 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 (Gupta and Sandallo, 2011). Thus, flax plant could be a good candidate for phytostabilisation of Cd^{2+}-contaminated soils with low Cd^{2+} bioavailability, to avoid high accumulation in above-ground tissues.

TF in Pb treatments > 1 for shoot and capsule but < 1 for seeds . the trend show the following 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 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 value. According to these results and According to Yoon et al. (2006) who showed that plant species with both BCF and

TF greater than 1 have the potential to be used for phytoextraction, flax is considered an accumulator plant for both Pb and Zn by phytoextraction mechanism. 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 (Ali and Sajad, 2013).

Physiological mechanisms of heavy metal tolerance and accumulation

The process of metal accumulation involves several steps, one or more of which are enhanced in phytoremediation. Within the one plant species more than one mechanism could be in operation (Rajib and Jayalekshmy, 2015).

In our results, flax plant is considered as accumulator for Pb and Zn by phyto-extraction mechanism. this mechanism is undergoes by several steps as the following sequence:

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 (Alberts et al.2007). 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 hyperaccumulators 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 Wendy et al. (2005).

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 (Zhu et al., 2011). 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 (Sunitha et al., 2013). 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 (Yang and Jie, 2005) . Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel (Greipsson, 2011). 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 (Sharma and Dubey, 2005).

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 hyperaccumulator species. (Wendy, et al. 2005). 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 (Sharma et al., 2004).

Detoxification/Chelation

Metal could be converted to a less toxic form through chemical conversion or by complexation with ligands (Revathi and Venugopal, 2013). 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 (Sheoran et al., 2011). As many chelators use thiol groups as ligands, the sulfur (S) biosynthetic pathways have been shown to be critical for hyperaccumulator function (Van Huysen et al. 2004) and for possible phytoremediation strategies (Wendy et al. (2005).

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 (Sunitha et al., 2013). 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 (Wendy et al. (2005).

Our results indicated that Cd accumulated in Flax root, this cellular exclusion mechanism 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 (Akhter et al., 2014). Ultimately, the cell wall of roots is considered as an effective barrier against penetration of Cd and a relationship can be established between higher accumulation of Cd in the roots vs lower accumulation in the leaves (Sun et al., 2013). This suggests that Cd-tolerance goes through the capacity of roots to limit the diffusion of Cd towards the entire plant.

In Linum usitatissimum, the presence of an excess of Cd changes the ratio between low and high methylesterified pectins, causing the former to accumulate consistently in epidermal cells and determining the collapse of the subepidermal cell layer (Douchiche et al., 2007). This unbalance affects the primary walls and may cause swelling of hypocotyls tissues. It is not clear how Cd determines such effects, but it is likely that it alters the expression level of pectin-metabolizing enzymes such as pectin methyl-esterase (PME). Cd interferes with the process of cell wall structuring probably by altering the exact arrangement of pectins. In some experimental cases, exposure to soils contaminated with Cd induces a general increase of the levels of pectin and a reduction of methylesterified pectins (Astier et al., 2014). The reduced level of this enzyme enhances the ratio between low and high methyl-esterified pectins further, making a lower number of active sites available for Cd binding, thereby triggering a heavy metal exclusion mechanism (Zhu et al., 2012).

CONCLUSION AND RECOMMENDATION

Study concludes that *Linum usitatissimum L.* could be considered as a phytoremediator plant and classified as an accumulator for the tested heavy metals with different mechanisms and considered a good accumulator. Due to accumulation of high amounts of Cd in its roots and considering the calculated values of BCF and TF, Flax plant can therefore be considered a Cd accumulator 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 plant is considered an accumulator plant for both Pb and Zn by phytoextraction mechanism.

The present 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 then 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 and phytostabilization technology.

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