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PHYTOREMEDIATION OF CADMIUM CONTAMINATED SOILS USING DIFFERENT HYPERACCUMULATING PLANTS

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ABSTRACT : The present study aimed at evaluating the capacity of three selected plant species i.e .*Luffa aegyptiaca*, *Brassica napus* and *Hibiscus cannabinus*, to accumulate and tolerate cadmium. The three plant species were planted under different treatments of cadmium (0, 25, 50, 100, 200, 400 and 800 ppm), after 150 days Cd concentration was determined in roots, stem and leaves of each species. Soil Cd was also determined in two forms; total and chemically extractable .The results of this study revealed that:

From the three plant species used ,*L. aegyptiaca* accumulated higher amounts of Cd compared to *B. napus* and *H. cannabinus*. Roots accumulated more Cd compared to stem and leaves. The results recommend the cultivation *of L. aegyptiaca* in soils contaminated with cadmium for its phytoremediation.

Key words: Phytoremediation, *Luffa aegyptica, Brassica napus, Hibiscus cannabinus*, Cd

INTRODUCTION

Heavy metals are difficult to remove from the environment and unlike many other pollutants can not be chemically or biologically degraded and are ultimately indestructible (Méjare and Bülow, 2001).

It is assumed that plants can be used for decontaminating waters and soils without any problems. The use of plants to accumulate toxic metals from polluted soils and waters specially heavy metals, as know as phytoremediation (Raskin *et al.*, 1997 and EPA, 1997). Phytoremediation offers a low cost and an environmentally friendly approach for decontaminating soils and waters of heavy metals. (Chaney *et al.*, 1997).

Cadmium is a metallic element with atomic number of 48; and relative atomic mass of 112.40. It is a widespread heavy metal, released into the environment by power stations, heating systems, metal-working industries, waste incinerators, urban traffic, cement factories and as a secondary product of phosphate fertilizers

The overexposure to cadmium may cause fatigue, headaches, nausea, vomiting, abdominal cramps, diarrhea, and fever. In addition progressive causes many dangerous diseases (National Organization of Rare Disorders, USA, report, 1998). Also , cadmium can cause serious problems to plants. It exerts adverse effects on most physiological processes. Under most environmental conditions Cd enters first to the plant roots: In root tip cells, Cd causes damage to nucleoli (Liu *et al.*, 1995). Cadmium also reduces the absorption of nitrate and its transport from roots to shoots, causes nitrate reductase activity in the leaves (Hernandez *et al.*, 1996).

Cadmium interacts with the water balance (Costa and Morel, 1994) and damages the photosynthetic apparatus, in particular the light harvesting complex II (Krupa, 1988), and the photosystem I and II (Siedlecka and Baszynsky, 1993).

In *Brassica napus* plants, Cd lowered total chlorophyll content, carotenoid content, and increased the non-photochemical quenching (Larsson *et al.*, 1998). Furthermore, Cd inhibited the oxidative mitochondrial phosphorylation, probably by increasing the passive permeability to H^+ of the mitochondrial inner membrane (Kessler and Brand, 1995).The phytoremediation method is one of the several methods for cleaning up heavy metals. However, the objectives of this study include evaluating the capacity of three selected different plants (*Luffa aegyptiaca*, *Brassica napus* and *Hibiscus cannabinus*) to accumulate cadmium.

MATERIALS AND METHODS

Plant Species

Three selected different plants representing the hyperaccumulating plants were selected for use in this study. These are Luffa aegyptiaca L., Hibiscus cannabinus L. and Brassica napus L.

The three plants were used for the phytoremediation experiments that involves measures of their capacity to accumulate cadmium.

The three plants were seeded in pots of (30 cm in diameter) filled with 8.25 kg soil. The soil used was a mixture of clay and sandy soils (1:2), respectively. The soil characteristics were determined according to the ordinary methods described by Piper (1950), Richard (1954) and Black *et al.*,(1982).

The experiment was set up under green house conditions at the Agriculture Genetic Engineering Research Institute (AGERI) - Agriculture Research Center (ARC), Giza during February to July, 2000.

pH, EC and Cd content were determined in irrigation water sample before the experiment.

The mechanical and chemical analysis of the study soil and chemical analysis of irrigation water are show in Table (1 and 2), respectively.

Table (1): Mechanical and chemical characteristics of the soil before planting.

PH	EC dS/m	CaCO3 %	O.M %	Particle size distribution %			
				Sand	Silt	Clay	Texture grade
7.6	1.16	16.77	0.24	74.14	8.06	17.8	Sandy loam

Table (2): Chemical analysis of water used for irrigation before the experiment.

pH	EC	Cd mg/l
7.35	0.51	0.001

Seeds of each of the three plants were germinated, but only five seedlings of equal size were grown, for one month, in each pot. The number of plants was then reduced to two in each pot. Pots were watered with tap water for one week and then were regularly irrigated with six different concentrations of cadmium nitrate Cd $(NO_3)_2 4H_2O$ (25,50,100,200, 400, 800 ppm) at 70% of the field capacity.Control plants were simultaneously irrigated with tap water. Each pot received a constant amount of the mineral fertilizer Crystalon at 1 g/liter after two and four months from the start of the experiment.

The experiment involved 105 pots arranged in a completely randomized pattern. These pots comprised 21 treatments in five replicates each.

After150 days samples of leaves, stem and roots were collected, washed, dried at 70°C, and the dry yield weight of each sample was recorded. Soil samples were collected from each pot after 2 days of plant harvesting. The soil and plant powdered materials were prepared and kept for analysis.

Total content of Cd in soil and plant samples was determined by digesting 0.5 g sample using (10 ml) a mixture of 1 ml

60% perchloric acid, 5 ml conc. nitric acid and 0.5 ml conc. sulfuric acid according to Stewart (1989) and chemically extractable Cd was determined according to Lindsay and Norvell (1978). The concentration of Cd was measured by atomic absorption spectrophotometer (AA-Scan 1 Thermo Jarrell Ash, USA).

RESULTS

Effect of Different Cadmium Concentrations in Irrigation Water on Dry Yield of Tested Plants

The effect of different concentrations of Cd in irrigation water on the mean value of dry yield of Luffa *aegyptiaca, Brassica napus and Hibiscus cannabinus* is shown in Table (3).

In *L. aegyptiaca* dry yield was gradually decreased as the Cd concentration increased. The dry yield of root plants exposed to highest Cd concentration (800 ppm) was reduced from a control value of 3.96 g to 2.06 g, respectively. Similar magnitudes of reduction in dry weight of stem and leaves were also recorded as dry weight decreased from 14.66 g in control plants to 9.90 g at the 800 ppm treatment. Dry weight of leaves decreased from 21.14 g in control plants to 12.70 g in plants exposed to the highest Cd concentration used, an abrupt reduction in root leaves dry weight was induced by the lowest concentration of Cd (25ppm).

The other two test plants *B. napus* and *H. cannabinus* were more sensitive to high concentration of Cd. The highest Cd concentration used (800 ppm) was lethal to both plants. Plants of these two species failed to survive at this concentration of Cd.

Data given in Table (3) show the effect of the applied treatments of Cd on the dry weight of the *Brassica napus.* An evident reduction in stem and root dry weight by the lowest the concentration of Cd (25ppm). The root dry weight of *Brassica* plants treated with (400 ppm) was reduced to 0.42 g compared to control values of 1.08 g. Similar results were recorded in stem and leaves of *B. napus*, where dry weight of stem decreased from 3.24 g, in control plants to 1.56 g in plants treated with the highest Cd concentration (400 ppm). Also, dry weight of leaves decreased from 3.00 g, in control plants to 1.36 g, of plants exposed to highest cadmium concentration.

Table (3): Effect of cadmium concentrations in irrigation water on mean* dry weight of the studied plants.

Treatment ppm	Luffa aegyptiaca						Brassica napus						Hibiscus cannabinus					
	Dry weight																	
	Root		Stem		Leaves		Root		Stem		Leaves		Root		Stem		Leaves	
	Value g	Dec.** %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %	Value g	Dec. %
0	3.96	...	14.66	...	21.14	...	1.08	...	3.24	...	3.00	...	8.62	...	30.50	...	24.40	...
25	3.54	10.6	12.88	12.1	15.68	25.8	0.88	18.5	2.48	23.5	2.84	5.3	7.94	7.9	24.86	18.5	17.12	29.8
50	2.98	24.7	12.22	16.6	15.16	28.3	0.84	22.2	2.18	32.7	2.66	11.3	6.40	25.7	24.52	19.6	13.02	46.6
100	2.58	34.8	10.88	25.8	14.78	30.1	0.68	37.0	1.76	45.7	2.08	30.7	5.28	38.7	21.68	28.9	12.86	47.3
200	2.54	35.9	10.16	30.7	13.90	34.2	0.54	50.0	1.74	46.3	1.98	34.0	5.26	39.0	18.30	40.0	12.00	50.8
400	2.42	38.9	10.06	31.4	13.42	36.5	0.42	61.1	1.56	51.7	1.36	54.7	5.02	41.8	15.52	49.1	11.70	52.0
800	2.06	48.0	9.90	32.5	12.70	39.9												

*Mean is average of five replicates

* * Dec. = Decrease % = ((control weight – treatment weight)/ control weight) X 100

Also, the effect of the treatments applied of Cd on the dry weight of *H. cannabinus* is shown in Table (3). An abrupt reduction in leaves and stem dry weight was recorded by the lowest concentration of Cd (25ppm).

The root dry weight of *Hibiscus* plants treated with 400 ppm were reduced to 5.02 g compared to control values of 8.62 g. Also dry weight of stem were reduced from 30.50 g in control plants to 15.52 g in plants treated with the highest cadmium treatment (400 ppm). Also, dry weight of leaves was gradually decreased to 11.70 g in plants treated with 400 ppm compared to control values of 24.20g.

Effect of Cd Concentrations in Irrigation Water on Soil Cd Content

Data given in Table (4) show the amount of chemically extractable and total Cd in the soil that was used to support the three test plants *L. aegyptiaca, B. napus* and *H. cannabinus*.

These data reveals that the increase in chemically extractable and total Cd in the soil samples was associated with Cd concentration in irrigation water. However, the amount of chemically extractable Cd and total Cd was also substantially affected by the type of plant species cultivated in the soil. The mean amount of the chemically extractable Cd varied widely from 0.2 mg/kg in control soil, cultivated with *L. aegyptiaca*, to 55.0 mg/kg in the treated soil with 400 ppm Cd and cultivated with *H. cannabinus*.

The amount of total Cd in the soil also varied widely. The lowest value (0.8 mg/kg) was recorded in the soil cultivated with Luffa and the highest amount was recorded in the soil treated with to 400 ppm Cd and cultivated with Hibiscus. These data may indicate that *L. aegyptiaca* absorb more Cd from soil than the other two test plants.

Cadmium Content of Plants Treated with Different Cadmium Concentrations in Irrigation Water

Data given in Table (5) show that mean values of Cd content (µg per g dw) of the investigated plants, *L. aegyptiaca*, *B. napus* and *H. cannabinus*. The amount of Cd in root samples increased from (4.9 to 2670 µg/g), (3.9 to 436 µg/g) and (1.6 to 355 µg/g) for *L. aegyptiaca*, *B. napus*

Table (4): Means of chemically extractable and total cadmium (mg/kg) in the investigated soil samples

Cultivated Soil	Cd Status	Cd concentration						
		0	25	50	100	200	400	800
Luffa aegyptiaca	Chem. Extractable	0.2	1.1	10.0	16.0	30.0	41.0	53.0
	Total	0.8	72.0	88.0	118.0	141.0	236.0	397.0
Brassica napus	Chem. Extractable	0.5	2.1	12.3	18.3	42.5	53.9	
	Total	1.2	83.0	120.0	146.0	300.0	390.0	
Hibiscus cannabinus	Chem. Extractable	0.7	2.2	14.0	20.1	45.0	55.0	
	Total	2.2	106.0	128.0	241.0	306.0	422.0	

Table (5): Mean values of cadmium content (µg/g) of root, stem and leaves samples of the investigated plants

Cd Treatment (ppm)	Cd concentration (µg/g)								
	Luffa aegyptiaca			Brassica napus			Hibiscus cannabinus		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
0	4.9	0.9	0.7	3.9	1.3	1.1	1.6	0.4	0.5
25	430.0	53.0	56.0	73.0	55.0	65.0	104.0	14.0	16.0
50	700.0	56.0	72.0	100.0	73.0	88.0	132.0	20.0	39.0
100	1020.0	79.0	112.0	207.0	115.0	120.0	161.0	37.0	49.0
200	1120.0	101.0	247.0	278.0	150.0	181.0	162.0	47.0	77.0
400	1190.0	147.0	415.0	436.0	250.0	320.0	355.0	56.0	103.0
800	2670.0	157.0	443.0	*F.S	F.S	F.S	F.S	F.S	F.S

* Failed to survive

and *H. cannabinus*, respectively. The highest mean value of Cd in root samples was found in *Luffa aegyptiaca* while, the lowest mean value was determined in *Hibiscus cannabinus* roots.

The concentration (mean values) of Cd in stem samples increased from (0.9 to 157 µg/g), (1.3 to 250 µg/g) and (0.4 to 56 µg/g) for *L. aegyptiaca*, *B. napus* and *H. cannabinus*, respectively. The highest mean value of Cd in stem samples was found in *B. napus* while, the lowest mean value was observed in *H. cannabinus* stem samples.

The amount of Cd in leaf samples increased from (0.7 to 443 µg/g), (1.1 to 320 µg/g) and (0.5 to 103 µg/g) for *L. aegyptiaca*, *B. napus* and *H. cannabinus*, respectively. The highest mean value of Cd in leaf samples was found in *L. aegyptiaca* leaf samples while, the lowest mean value was found in *H. cannabinus* leaf samples.

From the above-mentioned data it can be concluded that total Cd content in *L. aegyptiaca* was higher than that of the other two plants. The amount of Cd in the roots and leaves of this plant is also much higher than its amount in corresponding organs of the other two tested plants.

These results demonstrate that *L. aegyptiaca* has higher ability to take up Cd from Cd-polluted soils. However, it appears that Cd taken up by the investigated plants is accumulated in the roots.

DISCUSSION

In this study, three plant species known to accumulate heavy metals (*L. aegyptiaca*, *B. napus* and *H. cannabinus*) were grown for five months in soils supplied with different levels of cadmium in the irrigation water.

The biomass of the three plants, estimated as dry weight of roots, stem and leaves was decreased with the increase in the concentration of Cd. Plants of *B. napus* and *H. cannabinus* did not survive in soils supplied with 800 ppm cadmium.

The response of the metal accumulator *Brassica juncea* to cadmium and other metals was studied by Jiang *et al.*, (2000). Root biomass was significantly decreased in plants exposed to Cd compared to Zn, Cu and pb.

Reduction of biomass of plants exposed to Cd stress has been also recorded in many plants. For example in maize plants grown in hydroponics cultures (Root *et al.*, 1975). Growth of radish cv. Spring White Tip seedlings grown for 30 days in plastic planters in a silt loam soil containing Cd (0-1000 ppm) was also inhibited. The degree of inhibition varied with Cd concentration. In one experiment, plants treated with Cd at 500 or 1000 ppm did not survive (Zaman and Zereen, 1998).

The accumulation of Cd in the roots of the plants used in this study is congruent with the finding of Whiting *et al.* (2000) on *Thlaspi caerulescens.* The plants of this species from a population that accumulated Cd showed higher root biomass and root length allocation into the Cd-enriched soil. Plants from the population that did not accumulate Cd showed no such.

The decrease in dry weight of the three studied plants (*Luffa aegyptiaca*, *Brassica napus* and *Hibiscus cannabinus*) may be attributed to adverse effects of cadmium that involve actions on several metabolic processes in the plants exposed to Cd stress. Cadmium was reported to reduce the absorption of nitrate and its transport from roots to shoots, reducing nitrate reductase activity in the leaves (Hernandez *et al.*, 1996). Cadmium was also reported to inhibit root Fe (III) reductase induced by Cd leading to Fe (II) deficiency, and seriously affects photosynthesis (Alcantara *et al.*, 1994). In addition, Cd in plants causes leaf roll and chlorosis, and reduces cell growth, both in roots and stems. This last effect is partly due to the suppression of elongation, especially in the stem, because of an irreversible inhibition exerted by Cd on the proton pump responsible for this process (Aidid and Okamoto, 1992).

Cadmium interacts with the water balance (Costa and Morel, 1994) and damages the photosynthetic apparatus, in particular the light harvesting complex II (Krupa, 1988), and the photosystem I and II (Siedlecka and Baszynsky, 1993). Cadmium has been also inhibit reported to the stomatal opening, but how it does so has yet to be established. Probably the stomatal movements are not directly affected by Cd, but is rather influenced by strong interference of Cd with movements of K^{+}, Ca^{2+} and abscisic acid in the guard cells (Barceló *et al.*, 1986; Barceló and Poschenrieder, 1990).

In the three plants *L. aegyptiaca, B. napus* and *H. cannabinus,* cadmium was concentrated in roots than leaves and was lowest in stems. Generally the total cadmium concentration in *Luffa* was higher than *Brassica* and *Hibiscus*. Also *Brassica* and *Hibiscus* did not survive at the highest Cd concentration used (800 ppm).

Results obtained go with all available reference that insure the presence of some plants that are able to accumulate heavy metals. For example accumulation of certain heavy metals in some desert plants species was 2-260 times higher than the available heavy metal content of the soil. (Golan *et al.*, 2000).

Ambrosia artemisiifolia var. elatior, *Ambrosia trifida* and *Rumex crispus*, which were reported to have good phytoremediation qualities in different concentrations of Cu and Cd, were evaluated for growth responses in different heavy metal concentrations. Good growth rate for *A. trifida* and *A. artemisiifolia* var. elatior in Cu and Cd treatments and poor growth for *R. crispus* in Cd treatment were found. Although growth was retarded in all tested weeds up to 200 ppm for Cu and 50 ppm for Cd, the high amount of heavy metals uptake indicated that these weeds could be used for phytoremediation (Kang *et al.*, 1998).

Metal-accumulating plants is most needed in four areas: first, as a function of soil metal concentrations, physical and chemical soil properties, physiological state of the plant; second, the specificity of metal uptake, transport and accumula-tion; third, the physiological, biochemical and molecular mechan-isms of accumulation and hyperaccumulation; and fourth, the biological and evolutionary significance of metal accumulation (Raskin *et al.*, 1994). On the other hand, plants respond to heavy metal's toxicity in a variety of different ways. Such responses include immobilization, exclusion, compartmentalization and/or synthesis of metallothioneins, as well as the expression of more general stress response substances such as ethylene and stress proteins (Sanità di Toppi and Gabbrielli, 1999). On the other hand, the total cadmium concentration and the chemically extractable Cd in the soil of *Luffa* were lower than in *Brassica* and the *Hibiscus*. This indicates that the capacity of *Luffa* to accumulate cadmium is more than the other two plants.

The result of chemically-extractable Cd in soil has (more or less) a similar trend of variations as plant cadmium concentration, so the chemically-extractable Cd in soil of *Luffa* lower than *Brassica* and *Hibiscus* because the *Luffa* plant accumulate Cd from soil more than *Brassica* and *Hibiscus*. It may be concluded that of the three plants used in this study, *L. aegyptiaca* showed the highest capacity to accumulate cadmium. This species also has higher biomass and can survive with higher Cd concentration both in the soil and in its tissues.

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المعالجة النباتية للتربة الملوثة بالكادميوم باستخدام نباتات مختلفة فائقة الركم

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العناصر الثقيلة من الملوثات آلتي يصعب إزالتها من البيئة حيث لا تخضع للتكسير الكيميائي أو البيولوجي. ومع ذلك توجد عدة طرق لإزالة العناصر الثقيلة من التربة والماء ولكن أفضلها ما ظهر على ساحة العلم حديثا من استخدام بعض النباتات التى لها القدرة على تخليص التربة والماء من الملوثات المعدنية (phytoremediation).

لذلك فقد استهدفت هذه الدراسة قياس قدرة ثلاث أنواع مختلفة من النباتات هى اللوف والشلجم والتيل على تجميع وإزالة عنصر الكادميوم من التربة الملوثة بهذا العنصر.

ولتحقيق ذلك تم زراعة النباتات تحت معاملات مختلفة من تركيز الكادميوم (٠ , ٢٥ , ٥٠ , ١٠٠ , ٢٠٠ , ٤٠٠ و٨٠٠ جزء فى المليون) وبعد خمسة شهور تم حصاد النباتات وتقدير تركيز عنصر الكادميوم داخل الجذور, والسيقان والأوراق. كما تم أيضا تقدير عنصر الكادميوم فى التربة فى صورتين هما الصورة الكلية والصورة المستخلصة كيميائيا.

وأوضحت نتائج هذه الدراسة أن:

نبات اللوف أكثر قدرة على تجميع وإزالة عنصر الكادميوم من التربة الملوثة مقارنا بنباتي الشلجم والتيل.

تتراكم فى الجذور كمية أكبر من الكادميوم مقارنه بالسيقان والأوراق.

توصى الدراسة بملائمة نبات اللوف لمعالجة التربة الملوثة بعنصر الكادميوم (المعالجة النباتية).