PHYTO-REMEDIATION AND CHEMICAL METHOD OF Cd-CONTAMINATED WATER-CULTURE ANDCALCAREOUS SOIL

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ABSTRACT:

The objectives of the current investigation aimed to a) assess the ability of sorghum plants (Sorghum Bicolor L.) to accumulate cadmium in its tissues (phyto-remediation) and b) compare the efficiency of phyto-remediation and chemical method to extract Cd from either Cd-contaminated water-culture or sandy clay loam calcareous soil. To achieve these objectives, three experiments were conducted, i.e., a) nutri-culture experiment (water-culture), b) green pot experiment using a calcareous soil and c) sequential extraction experiment (laboratory incubation). Cd was applied at the rates of 0, 1 and 2 mg L^{-1} in the water-culture experiment; and 0, 100 and 200 mg kg^{-1} in the green pot experiment.

The obtained results indicated that dry matter yield of sorghum plants decreased in both the water-culture and pot experiments vs increasing its concentration and uptake in plant tissues with increasing the applied Cd rate. Sequential extraction showed that removal amounts of Cd by ammonium bicarbonate-DTPA (AB-DTPA) as well as ammonium acetate-EDTA progressively increased with increasing Cd rate from 100 up to 200 mg kg^{-1} soil. Percent removal of Cd by 1^{st} extraction was greater than the 2^{nd} one using either extractant. AB-DTPA extracted about 18 % which nearly double of contaminating Cd by AA-EDTA (about 7 %).

Key Words: Sorghum plants, cadmium, calcareous soil, hydroponics, sequential extraction, phyto- remediation and chemical method.

INTRODUCTION:

The accelerating industrialization in developing countries with an enormous and increasing demand for heavy metals causes a high anthropogenic emission of these pollutants into the biosphere. The area of land contaminated with heavy metals has increased during the last century due to mining, smelting and other activities. Most remediation technologies consist primarily of removal and replacement of contaminated soils **(Geiger *et al.*, 1993)**. The concept of using hyperaccumulator plants to extract metals from contaminated soils was assessed as a method for remediation of such soils **(Chaney 1983; Baker and Brooks 1989; Salt *et al.*, 1995 and Cunningham *et al.*, 1995)**.

A major attraction of the use of hyperaccumulators in phyto-remediation is the possibility of employing species that remove large amount of a particular element from the soil without significant chemical intervention, other than the application of conventional fertilizers **(Reeves and Baker, 2000)**.

Phyto-remediation involve capital and operating costs, which are lower than those of other technologies. Discounting the costs of monitoring the site, the major costs of phyto-remediation are the tiling and preparation of the soil, planting of seeds, weed and pest control and harvesting and disposal of the biomass **(Glass, 2000)**. **Khalil (1990)** found that contents of heavy metals (Pb, Cd, Co, Cr and Ni) in some soils of Abu-Rawash, Egypt, increased with prolonging irrigation with sewage waters.

In general, it is apparent that although a number of well-established extraction procedures with some useful predictive power exist, many of them are specific to one element and are relevant only to specific crops and may be restricted to particular soils. Perhaps the most generally useful for heavy metals analysis are 0.01 M or 0.05 M EDTA. In the ex-situ washing methods, chelating agents or acids are used to enhance heavy metal removal. Ethylenediaminetetraacetic acid (EDTA) is the most commonly used chelate because of its strong chelating ability for different heavy metals **(Norvell, 1991)**. Laboratory studies which were carried out by **Elliot and Brown (1989), Brown and Elliot (1992), Pichtel and Pichtel (1997), Elliot and Shastri (1999), Helli *et al.* (1999) and Papassiopi *et al.* (1999)** indicate that EDTA is effective in removing Pb, Zn, Cu and Cd from contaminated soils, although extraction efficiency depends on many factors such as the contents of such metals in soil, the strength of EDTA, electrolytes, pH and nature of soil matrix.

This study was specifically conducted to a) assess the ability of sorghum plants grown on a calcareous soil polluted with Cd, to accumulate cadmium in their tissues (phyto-remediation); b) determine to the extent sorghum can extract cadmium from such soil and c) compare the efficiency of such phyto-remediation and chemical method to decontaminate the Cd-polluted soil.

MATERIALS AND METHODS:

Three experiments were carried out in Soil, Water and Environment Research Institute, Giza Governorate. The first experiment was performed using nutri-culture technique (water-culture), while the others (green pot and laboratory incubation experiments) were carried out using a sandy clay loam calcareous soil in a green pot experiment.

a. Nutri-culture experiment (water-culture or nutrient solution):

The nutri-culture experiment, using a nutrient solution as a medium for plant growth, was conducted to evaluate accumulation of cadmium (Cd) by selected plants of sorghum (Sorghum Bicolor L.). Seeds of sorghum having tolerance to cadmium were obtained from Agricultural Research Center (A.R.C). Seeds were germinated for five days in a wetted-cotton, and then seedlings were transferred to containers and grown for 10 days in diluted nutrient solution. After that seedlings were transferred and transplanted into one liter-nutrient solution pots (at a rate of 2 plants /pot) containing complete nutrient solution. Pots were distributed in a randomized complete block (RCB) design with three replicates. The nutrient solution was weekly changed and the heavy metal of Cd was applied at rates of 0, 1 and 2 mg Cd/L as $CdSO_4.4H_2O$, plants were grown for 6 weeks (42 days). The nutrient solution, which used as a medium for plant growth, was a Hoagland solution that modified by **Johnson *et al.* (1957)**. Its chemical composition of nutrients is shown in Table (1). At end of experiment, plants were removed from the pots, their shoots and roots were separated, oven-dried (70 °C for h), weighed, ground and kept for analysis.

Table (1): Composition of the Hoagland nutrient solution used in nutri-culture experiment according to Johnson *et al.* (1957).

Nutrient sources	Concentration of stock solution (g L^{-1})	Needed volume from stock solution per liter of final solution (ml L^{-1})	Final concentration of nutrients	
			Element	mg L^{-1}
Macronutrients			N	232.40
KNO_3	101.10	5.00	K	257.40
$Ca(NO_3)_2.4H_2O$	236.16	5.00	Ca	160.00
$NH_4H_2PO_4$	115.08	1.00	P	62.00
$MgSO_4.7H_2O$	246.49	2.00	S	32.00
KCl	3.73	1.00	Mg	24.00
Micronutrients			Cl	1.77
HBO_3	1.55		B	0.27
$MnSO_4.H_2O$	0.34		Mn	0.11
$ZnSO_4.7H_2O$	0.58	1.00	Zn	0.13
$CuSO_4.5H_2O$	0.13		Cu	0.03
H_2MoO_4	0.08		Mo	0.05

Iron was added as Fe-EDDHA (6%) at the rate of 1 mlL^{-1} of the final solution

b. Green pot experiment:

A Pot experiment was executed to study to the extent sorghum plants to accumulate cadmium (Cd). A calcareous soil collected from the 0-30 cm surface soil at Nubaria region was selected, air-dried, crushed, sieved, weighed and a portion of 5 kg of each soil were placed in plastic polyethylene pots of 24 cm diameter and 19-cm height. Some chemical and physical characteristics of the studied soil are shown in Table (2). Seeds of sorghum, which were used in nutri-culture experiment, were planted in the pots, and thinned after two weeks of germination to five plants per pot. Pots received the recommended doses of N, P and K, and then were treated with Cd as CdSO4.4H2O at rates of 0, 100 and 200 mg Cd/kg. Shoots above-soil surface were cut after 60 days, and roots collected by soaking the pots in water and gently washing the soil out of the roots. The roots and shoots were rinsed in deionized water and air-dried at 70 °C for 72 h and dry matter yields of roots and shoots were determined.

c. Sequential extraction experiment:

Sequential extraction was done on the Cd-treated soil and incubated for 30 days. Two consecutive extractions using EDTA over a period of 2 h were done. The data of Cd chemical extraction from the soil was compared with those of phyto-remediation.

Table (2): Physical and chemical characteristics of the studied soil.

Soil characteristics	Value
Particle size distribution %:	
Coarse sand	17.50
Fine sand	31.10
Silt	22.40
Clay	29.00
Soil texture class	Sandy clay loam
Chemical analysis of soil paste extract:	
ECe (dS/m)	4.80
Cations and anions (mmolc L^{-1}):	
Ca^{2+}	11.73
Mg^{2+}	7.03
Na^{+}	28.43
K^{+}	1.20
CO_3^{2-}	0.00
HCO_3^{-}	3.12
Cl^{-}	26.36
SO_4^{2-}	18.91
Some chemical properties:	
:pH (1: 2.5 soil water suspension)	8.47
$CaCO_3$ %	27.00
Total Cd (μg kg^{-1} soil):	72.45
Available Cd-DTPA (μg kg^{-1} soil):	34.50

Methods of analysis:

Soil analysis:

Soil pH (1:2.5 soil water suspension), electrical conductivity (EC) and soluble ions in the soil paste extract and calcium carbonate using Collin's Calcimeter were determined according to **Jackson (1973)**. Mechanical analysis using the Pipette Method was determined according to **Piper (1950)**. Available heavy metal of cadmium in the studied soils was extracted using ammonium bicarbonate DTPA (AB_DTPA) according to **Soltanpour, (1991)** as well as using ammonium-acetate-EDTA (AA_EDTA) as described by **Lakenen and Ervio (1971)**. Total cadmium was determined using aqua regia as described by **Cottenie *et al.* (1982)** and determined using plasma emission spectrometry (ICP JY ULTIMA 2).

Plant analysis:

Plant samples were digested with a mixture of concentrated H_2SO_4 and $HClO_4$ (10:1) according to Chapman and Pratt (1961), and Cd in digested solution was measured using Inductively Coupled Plasma Emission Spectrometry.

RESULTS AND DISCUSION:

a. Nutri-culture experiment:

Dry matter yield of plants did not show significant change by applying up to 2 mg Cd L^{-1} (Table, 3). However, Cd content as well as uptake in sorghum plants increased considerably by Cd application, the increase was progressive with increasing Cd rate. Bio concentration factor (BCF) was calculated according the following equation:

BCF = Concentration in plan / Concentration in the growing media

A high BCF of 57 was noticed with the high Cd rate, while a low BCF of 33 occurred with the low Cd rate. Cadmium uptake by sorghum plants tended to increase with increasing the applied Cd rate.

Table (3): Effect of applied Cd rates on dry matter yield, Cd content and uptake in tissues of sorghum plants grown on nutri-culture (42 day growth period).

Cd rate (mg/L)	Sorghum plant parameters		
	Dry matter yield (g/pot)	Cd content (µg/g)	Cd uptake (µg/pot)
0	2.73	2.14	5.84
1	2.67	57.30	152.70
2	2.67	65.83	175.53
L.S.D. at 0.05	0.28	4.74	16.27

b. Green pot experiment:

Data of Table (4) reveal that dry matter yield of sorghum shoots grown on a sandy clay loam calcareous soil decreased by application of Cd. The decrease was greater at the higher rate. Using the low and high rates of cadmium (100 and 200 mg Cd/kg soil) decreased dry matter yield by 9.9 and 17.4 %, respectively, regarding shoots, while the decrease concerning roots was less. The decrease caused by Cd reflects of Cd toxicity. These results are in agreement with those of **Sun-YueBing *et al.* (2008)**.

Table (4): Effect of applied Cd rates on dry matter yields of sorghum shoots and roots grown on the studied soil.

Cd rate (mg/kg)	Dry matter (g/pot)		
	Shoots	Roots	Shoots + Roots
0	30.33	12.35	42.68
100	27.33	12.33	39.66
200	25.08	11.40	36.46
L.S.D. at 0.05	2.05	1.02	2.51

Concentration of cadmium in sorghum plants:

Data of Table (5) and Fig. (2) reveal that sorghum plants which received Cd contained considerable amounts of Cd in their roots than in their shoots, however, Cd content in roots reached 16-24 times of shoots.

Table (5): Effect of applied Cd rates on Cd concentrations in sorghum shoots and roots grown on the studied soil.

Cd rate (mg/kg)	Cd concentration (µg/gm)	
	Shoots	Roots
0	0.10	0.33
100	3.60	55.90
200	5.75	132.78
L.S.D. at 0.05	2.01	8.52

Such findings indicating a low mobility of cadmium within plant organs, taking into consideration that either the applied low or high Cd rates resulted in a considerable increase in Cd concentration in roots as well as shoots. The obtained results are in a full agreement with those of **Chaney *et al.* (2007)**.

Uptake of cadmium by sorghum plants:

Data of cadmium uptake by sorghum plants grown on the calcareous soil are presented in Table (6). Cadmium uptake by shoots was 0.03 mg/pot in case of the check treatment (without Cd application), increased three to four folds upon applying Cd. However, Cd was giving values of 0.10 and 0.13 mg Cd/pot upon applying 100 and 200 mg Cd kg^{-1} soil, respectively.

Table (6): Effect of applied Cd rates on Cd uptake by sorghum shoots and roots, following 60 days of growth.

Cd rate (mg/kg)	Cd uptake (µg/pot)	
	Shoots	Roots
0	0.03	0.04
100	0.10	0.69
200	0.13	1.51
L.S.D. at 0.05	0.02	0.73

Uptake of Cd by roots followed a pattern similar to that of shoots, however, the uptake increased by Cd application and this was progressive with increasing Cd rate. Cd uptake by roots recorded detectable values of 0.04, 0.69 and 1.51 mg Cd/pot for the applied Cd rates of 0, 100 and 200 mg Cd kg^{-1} soil, respectively. Thus, causing increases of 16 and 37 folds by applying the low and high Cd rates, respectively. Values of Cd uptake by roots were considerably higher than the corresponding ones taken up by shoots, particularly when Cd was applied at the highest rate.

c. Removal of cadmium using chemical extraction:

The extractability or removal of cadmium with ammonium bicarbonate-DTPA (AB-DTPA) from contaminated soils as affected by cadmium rate is shown in Table (7).

Table (7): Cd removed from Cd-contaminated soil by two successive extractions using AB-DTPA and DTPA.

Cd rate (mg/kg)	Cd removed from soil (mg/kg soil)	
	1st extraction	2nd extraction
0	0.50	0.48
100	12.65	5.16
200	22.65	11.09
L.S.D. at 0.05	1.50	0.57

The removed cadmium by the first AB-DTPA extraction was progressively and significantly increased with increasing cadmium rate. The greatest value was observed with the soil that received the highest rate of cadmium, while the lowest one was observed with the soil that did not receive cadmium. Extractable-cadmium increased about two folds with increasing cadmium rate from 100 to 200 mg Cd/kg soil. This trend was true in both the two extractions.

AB-DTPA extractable-cadmium recorded high values in the first extraction compared with those of the second one. This trend could be attributed to the nature of the action of the extractant solution on cadmium retained by soil. The extractant usually extracts most accessible forms of available Cd. The obtained results show that small amounts of cadmium could be extracted with AB-DTPA solution after a long-term (one month) of incubation with soil.

Table (8): Removal of Cd from Cd-contaminated soil by two successive extractions with EDTA.

Cd rate (mg/kg)	Removal Cd (mg/kg soil)	
	1st extraction	2nd extraction
0	0.70	0.18
100	3.38	2.58
200	6.35	4.87
L.S.D. at 0.05	1.14	1.92

The extractability of cadmium from contaminated soils using AA-EDTA showed a pattern similar to that of AB-DTPA. There was a marked increase with increasing cadmium rate (Table, 8). The highest release of cadmium was obtained from first extraction in case of the high rate of Cd application.

Comparison between phyto-remediation and chemical extraction:

The ability of each method to remedy-polluted soil is shown in Table (9). Phyto-remediation removed only a very small fraction of Cd contaminating the soil, less than 1 %. Thus decontamination of soil using sorghum plants would need long time to exert a much effect.

Table (9): Percentage of cadmium removed from the studied Cd-contaminated soil using phyto-remediation and chemical extraction methods.

Cd rate (mg/kg)	Phyto-remediation (sorghum)	Chemical extraction	
		AB-DTPA	FDTA
100	0.79	17.81	5.92
200	0.82	16.87	5.61

On the other hand, chemical method was more effective than phyto-remediation, however, values of AB-DTPA extractable-cadmium from contaminated soils ranged from about 17 to 18%. Comparing values for AA-EDTA extraction were much lower being about 6%. This shows the high extractability nature of the DTPA extracting soluble **Soltanpour (1991)**. It seems that chemical extraction is a short-term process for decontamination of Cd polluted soils. These results are in agreement with those of **Giannis *et al.* (2008)**.

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العلاج النباتى والكيميائى لمزرعة مائية وأرض جيرية ملوثة بعنصر الكادميوم

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تهدف الدراسة الى تحديد قدرة نباتات الذرة الرفيعة (Sorghum Bicolor L) على تراكم عنصر الكادميوم فى أنسجتها (Phyto-remediation)، مع مقارنة كفاءة العلاج النباتى والطريقة الكيميائية على استخلاص عنصر الكادميوم من كلا المزرعة المائية والأرض الجيرية الملوثة بهذا العنصر. ولتحقيق هذه الاهداف أجريت ثلاث تجارب هى:

أ) تجربة مزرعة مائية.

ب) تجربة أصص خضرية باستخدام تربة جيرية.

ج) تجربة الاستخلاص المتتالى من خلال تحضين معملى للتربة الجيرية.

وكانت معدلات الإضافة من عنصر الكادميوم هى ٠، ١، ٢ مجم Cd /لتر فى حالة تجربة المزرعة المائية، فى حين كانت ٠، ١٠٠، ٢٠٠ مجم Cd /كجم تربة فى حالة تجربة الأصص الخضرية.

وتشير النتائج المتحصل عليها إلى حدوث نقص فى الوزن الجاف لنباتات الذرة الرفيعة فى كلا تجربتى المزرعة المائية والأصص الخضرية مقابل زيادة تركيز عنصر الكادميوم وإمتصاصه فى أنسجة النباتات بزيادة معدلات إضافة الكادميوم. كما توضح نتائج الإستخلاص المتتالى أن كميات الكادميوم المستخلصة بـ Ammonium bicarbonate-DTPA (AB-DTPA) وكذلك Ammonium acetate-EDTA قد زادت بدرجة واضحة عند زيادة معدل إضافة الكادميوم من ١٠٠ حتى ٢٠٠ مجم Cd /كجم تربة. وقد كانت النسبة المئوية المزالة من الكادميوم فى المستخلص الأول أعلى بكثير منة فى حالة المستخلص الثانى وذلك باستخدام كلا محلولى مادتى الإستخلاص، حيث أن محلول AB-DTPA قد إستخلص حوالى ١٨٪ والتى تقارب ضعف الكمية المستخلصة من عنصر الكادميوم عن طريق محلول AA-EDTA (حوالى ٧ ٪).