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PHYTO-AND-CHEMICAL-REMEDIATION OF COBALT-CONTAMINATED DESERTIC SANDY SOIL

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ABSTRACT

This study was executed to: (i) evaluate the ability of sunflower (*Helianthus annuus*), sorghum (*Sorghum bicolor*) and elephant grass (*Pennisetum purpureum Schum*) to accumulate Co in their tissues. (ii) and define to what extent these plants can clean-up a Co-contaminated sandy soil. Two experiments were conducted, the first was nutriculture (hydroponics) experiment and a pot sandy soil one. Sunflower absorbed appreciable quantities of cobalt in both experiments. Elephant grass was superior in accumulation of cobalt to the other plants particularly in the soil experiment. Sunflower and elephant grass could be considered hyperaccumnulator plants for cobalt and could be used to remedy Co-contaminated soils. Using phytoremediation may be of less cost as compared with other remediation techniques. Using chemical extraction in sequences may remove high amounts of cobalt and be most effective as compared with phytoremediation. Extraction of heavy metals with EDTA was more effective than using AB-DTPA extraction.

Keywords: Phytoremediation – chemical remediation – cobalt – sandy soil – sunflower – sorghum – elephant grass plant

INTRODUCTION

The area of land contaminated with heavy metals has increased during the last century, due to mining , smelting, using sewage water in irrigation, sewage sludge and other industrial activities (Geiger *et al.*, 1993). The clean-up of soils contaminated with heavy metals (HMs) is one of the most difficult tasks for environmental engineering. A number of techniques have been developed that aim to remove HMs from contaminated soil, including ex-situ washing with physical-chemical methods (Anderson, 1993) and in-situ phytoextraction (McGrath, 1998 and Salt *et al.*, 1998). Phytoremediation is defined as using plants to make soil contaminants non toxic (Chaney *et al.*, 1997) and has been categorized into five techniques: phytoextraction, phytovoltalization, rhizofiltration, phytodegradation of organic compound by rhizosohere biodegradation and phytostabilization (Flathman and Lanza, 1998). There is a small number of plant species endemic to metaliferous soils that can tolerate and accumulate high levels of toxic metals. These plants (termed metal hyperaccumulators) can accumulate more than 0.1 % Co, Cr, Pb and As or more than 1 % of Mn, Ni and Zn in plant shoots when grown in their natural habitat (Brooks *et al.*, 1977 and Baker and Brooks, 1989).

Although heavy metals are ubiquitous in soil parent materials, the major anthropogenic source of metals to soils and the environment are: metalliferous mining and smelting, agricultural and horticultural materials, sewage sludge, fossil fuel combustion, metallurgical industries-manufacture, use and disposal of metal commodities. For soils under Egyptian condition, Khalil (1990) found that the heavy metals (Pb, Cd, Co, Cr and Ni) content increased with prolonging periods of irrigation with sewage waters in Abo Rawash soil.

Khalil (1995) reported that Co uptake by sorghum was progressively increased with increasing the rate of applied Co up to 50 mg Co/kg for both roots and whole plants. It may be important to observe that the rate of increasing Co uptake by sorghum roots was very much higher than the rate of Co uptake by shoots as a result of Co application. Hence the translocation of Co was clearly reduced with Co application. Such results may lead to a general statement that the rate and magnitude of Co accumulation in roots and translocation up to the shoots in sorghum plants is a phenomenon very much dependent on soil characteristics.

In general, for extracting heavy metals a number of well-established extraction procedures with some useful predictive power exist. However, many of them are specific to one element, relevant only to specific crops and may be restricted in use to particular soil types. Perhaps the most generally useful for heavy metals analysis are 0.01 M or 0.05 M EDTA and 0.005 M DTPA. In the ex-situ washing methods, chelating agents or acids are used to enhance HM removals. Ethylenediaminetetraacetic acid (EDTA) is the most commonly used chelate because its strong chelating ability for different HMs (Norvell, 1991). Laboratory studies have shown that EDTA is effective in removing Pb, Zn, Cu and Cd from contaminated soils, although extraction efficiency depends on many factors such as the solubility of HMs in soil, the strength of EDTA, electrolytes, pH and soil matrix (Elliot and Brown, 1989; Brown and Elliot, 1992; Pichtel and Pichtel, 1997; Elliot and Shastri, 1999; Heil *et al.*, 1999; Papassiopi *et al.*, 1999).

The current investigation was executed to: (i) evaluate the ability of sunflower, sorghum and elephant grass to accumulate cobalt in their tissues (ii) define to which extent these plants can clean up Co-contaminated soil and (iii) compare the efficiency of phytoremediation and chemical extraction to decontaminate Co-polluted soil.

MATERIALS AND METHODS

Two experiments were carried out in the Soils, Water & Environment Res. Inst., ARC., Giza Governorate. The first experiment was performed using a nutriculture technique, while the other experiment was carried out using a sandy soil in pots.

Nutriculture (nutrient solution) experiment :

The nutriculture experiment, using a nutrient solution as a medium for plant growth, was conducted to evaluate Co accumulation by selected plant species such as sunflower "*Helianthus annuus*" and sorghum "*Sorghum bicolor.*". Seeds of plant species which have tolerance to cobalt were obtained from the agricultural research center (A.R.C). Seeds were germinated for five days in a wetted-cotton, then seedlings were transferred to containers and grown for 10 days in diluted nutrient solutions. Then 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. The studied heavy metal (Co) was at rates of 0 , 1 and 2 mg/L as $CoSO_4$, Plants were grown for 6 weeks (42 days). The nutrient solution which was used as a medium for plant growth was that of Hoagland which was modified by Johnson *et al.* (1957). Its 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, weighed, grouned and kept for analysis.

Table (1): Composition of the hoagland nutrient solution used in nutriculture experiment (according to Johnson *et al.*, 1957).

Compound	concentration of stock solution g/L	needed volume from stock solution for litre final solution, (ml/L)	final concentration of nutrients	
			element	mg/L
KNO_3	101.10	5.00	N	232.40
			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
			Mg	24.00
KCl	3.73	1.00	Cl	1.77
HBO_3	1.55	1.00	B	0.27
$MnSO_4.H_2O$	0.34		Mn	0.11
$ZnSO_4.7H_2O$	0.58		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%Fe) at a rate of 1 ml/L of the final solution.

Soil pot experiment :

A pot experiment was executed to study to which extent some plants can accumulate cobalt. A sandy soil was selected, air-dried, weighed and 5 kg of soil were placed in plastic polyethylene pots of 24-cm diameter and 19-cm height. Chemical and physical characteristics of the soil are shown in Table (2). Seeds of the same species of plant which was used in the nutriculture experiment in addition to seeds of elephant grass (*Pennisetum purpureum Schum*) were sown 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 were treated with Co as $CoSO_4$ at rates of 0 , 400 and 800 mg/kg.

Plant above-soil parts were cut after 60 days, and roots were 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 dried at 70°C for 72 hours and dry matter of roots and shoots was determined.

Sequential extraction experiment:

Sequential extraction was done on soil previously treated with cobalt and incubated for 30 days. Two consecutive extractions using EDTA or AB-DTPA over a period of 2 h were done. The results of chemical extraction of cobalt from previously contaminated soil was compared with results of phytoremediation.

Methods of analysis :

Soil analysis :

- Soil reaction (pH) was measured in 1 : 2.5 soil water suspension with pH meter; electrical conductivity (EC) and soluble ions were done in the soil paste-extract and calcium carbonate was determined using a calcimeter; all methods were according to Jackson (1973). Mechanical analysis was determined using the pipette method according to Piper (1950). Available cobalt was extracted using ammonium bicarbonate-DTPA according to Soltanpour (1991); as well as using Ammonium-Acetate-EDTA as described by Lakenen and Ervio (1971). Total cobalt was extracted using aqua regia as described by Cottenie *et al.* (1982) and determined by using Plasma Emission Spectrometry (ICP JY ULTIMA 2) .

Plant analysis :

Plant materials samples were digested with a concentrated mixture of H_2SO_4 + $HClO_4$ (10:1) acids according to Chapman and Pratt (1961). Cobalt concentrations were determined by Inductively Coupled Plasma Emission Spectrometry.

Table (2): Physical and chemical characteristics of the sandy soil of the current experiment.

Parameter		Sandy soil
Particle-size distribution :		
Coarse-sand	(%)	73.30
Fine-sand	(%)	18.90
Silt	(%)	2.50
Clay	(%)	5.30
Soil texture		Sand
EC (paste- extracted)	dS/m	3.00
pH	1: 2.5 (w/v)	8.73
Cations and anions (Soil paste extract):		
Ca^{2+}	m mol_c/l	7.02
Mg^{2+}	"	2.76
Na^+	"	17.22
K^+	"	1.00
CO_3^{2-}	"	0.00
HCO_3^-	"	3.88
Cl^-	"	13.86
SO_4^{2-}	"	10.26
$CaCO_3$	%	0.80
Available Co (µg/kg soil):		3.71
Total Co (µg/kg soil):		13.12

RESULTS AND DISCUSION

Nutriculture experiment:

Data presented in Table (3) show that dry weight of sunflower plants was not significantly affected by increasing cobalt rate up to 2 mg Co /L. However, a positive and significant increase in cobalt concentration occurred with increasing cobalt rate. The highest cobalt concentration (55.43 µg/g) was recorded with treatment which received 1 mg Co/L. The bioconcentration factor (BCF) relates concentration in plant to concentration in the growing medium, as follows:-

$$BCF = \frac{\text{Concentration in plant}}{\text{Concentration in growing medium}}$$

The BCF in sunflower plants were 55.43 and 14.1 with the second and third rates of cobalt, respectively. As, BCF decreased with increasing the medium concentration of cobalt, this may reflect disturbance of cobalt uptake when existed in the growth media at high concentrations. Sunflower plants took up more cobalt with increasing its concentration in the nutrient solution (Table 3). There was a sharp increase in cobalt uptake by sunflower plants when treated with the second level of cobalt (1 mg/l) and then it was sharply decreased to be almost 50% with using the third cobalt rate (2 mg/l).

Table(3): Effect of cobalt on dry weight, cobalt concentration and cobalt uptake of sunflower and sorghum plants grown in nutriculture.

Sunflower plants				Sorghum plants			
Cobalt rate (mg/L)	Plant parameter			Cobalt rate (mg/L	Plant parameter		
	Dry weight (g/pot)	Cobalt concentration (µg/g)	Cobalt uptake (µg/pot)		Dry weight (g/pot)	Cobalt concentration (µg/g)	Cobalt uptake (µg/pot)
0	2.53 A	0.72 C	1.82 C	0	2.73 AB	0.92 C	2.52 C
1	2.60 A	55.43 A	143.97 A	1	2.83 A	35.40 B	100.10 B
2	2.53 A	28.2 B	71.40 B	2	2.50 B	59.33 A	148.33 A
L.S.D.(0.05)	n.s	2.75	7.35	L.S.D.(0.05)	0.32	1.56	9.44

Sorghum plants produced slightly higher dry matter by 1 mg Co/L and significant decrease by 2 mg Co/L, (Table 3). Cobalt concentration and uptake by sorghum plants significantly and progressively increased with increasing cobalt rate. The highest concentration and uptake of cobalt were associated with the third rate (2mg/L). The BCF values of cobalt were 35.4 and 30 with the second and third rates, respectively. Sorghum plants reveal a good capability to survive in high cobalt concentration as their BCF values were almost constant (30 and 34) with the second and third rates of cobalt.

Soil experiment :

Data of Table (4) and Fig. (1) show that dry weight of sunflower shoots and roots was significantly decreased with increasing cobalt rate from 0 to 400 and 800 mg/kg soil. Significant differences were noticed between plants grown with no cobalt application and those which received either 400 or 800 mg Co/kg soil. The reduction in dry matter yield which accompanied cobalt application may be ascribed to the toxic effects of such high cobalt levels on the growing plants. Regarding cobalt concentration in roots and shoots of sunflower plants grown on sandy soil, the results obtained reveal that the lowest cobalt concentrations were found in roots and shoots of plants which did not receive cobalt.

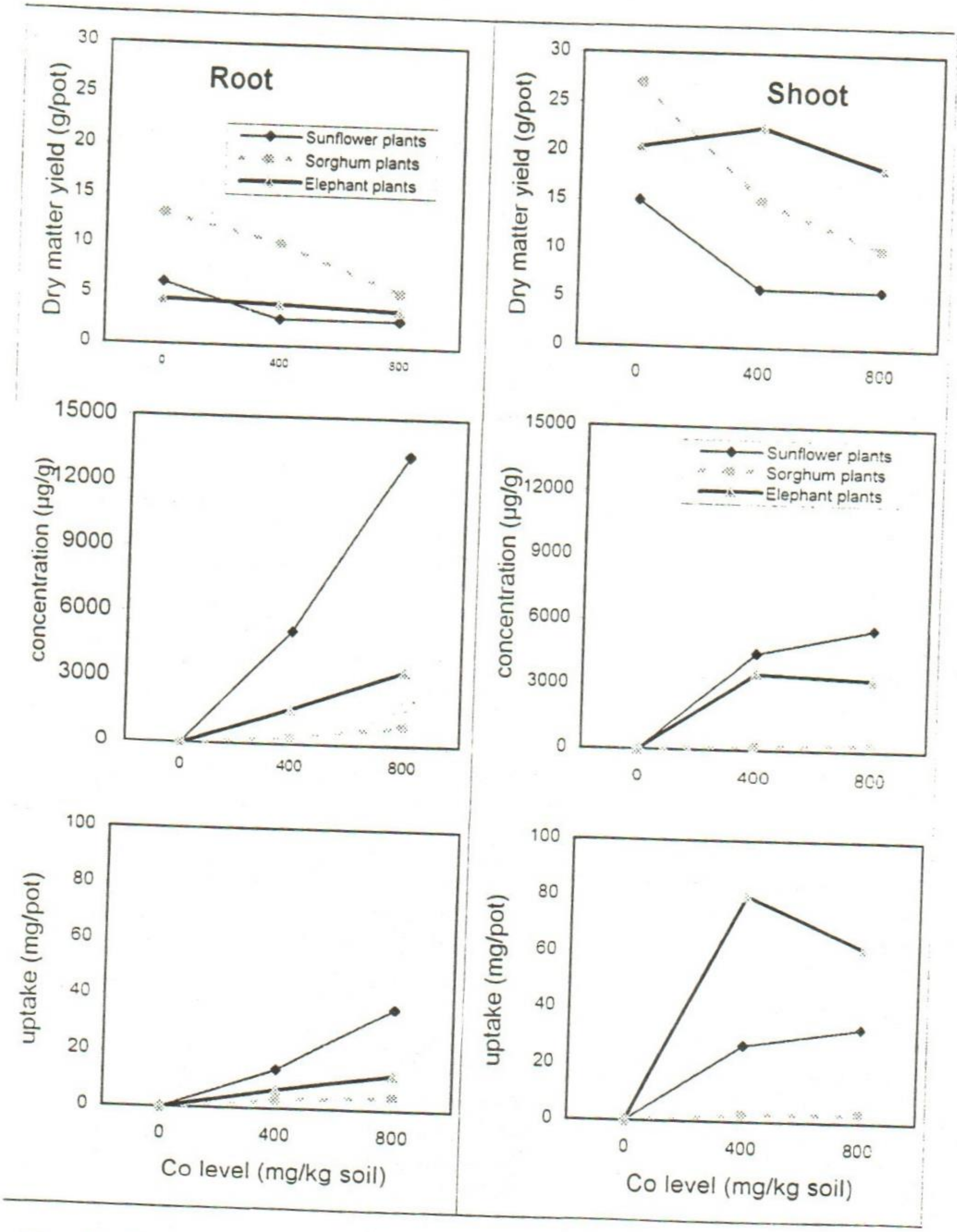

Fig. (1): Effect of Co rate on dry matter yield, Co concentration and Co uptake of sunflower, sorgham and elephant grass plants grown in sandy soil

Cobalt concentration in roots with using the second level of cobalt (400 mg/kg) amounted to 5151.6 µg cobalt/g, the corresponding value of cobalt concentration in shoots was 4997.5. In case of using the highest cobalt rate, cobalt concentration in roots and shoots recorded 13289 and 5656.5 µg/g, respectively. The obtained results show a considerable and effective cobalt translocation from roots to shoots, particularly with using the second cobalt level (400 mg cobalt/kg) and this was indicated by the trend of cobalt uptake by roots as well as shoots. In this concern, plant roots contain 13.91 mg cobalt/pot when grown in soil treated with 400 mg cobalt/kg. The corresponding uptake value of cobalt by shoots was increased to be 26.96 mg/pot. With 400 mg cobalt/kg, roots contained almost half of cobalt that took up by shoots. With using the 800 mg cobalt rate, cobalt uptake by roots and shoots recorded 35.9 and 33.15 mg cobalt/pot, respectively. Therefore increasing the cobalt rate from 400 to 800 mg cobalt/kg, resulted in an increase which is rather comparable to that caused by the 400 mg cobalt/kg in roots and shoots. The obtained results indicate that sunflower plants have an efficient ability to accumulate cobalt in their tissues, and could be considered a hyperaccumulator plant.

A trend similar to that obtained with dry matter yield of sunflower roots and shoots was noticed with sorghum plants, which showed the highest root weight with the treatment receiving no cobalt and the lowest root weight with that receiving cobalt 800 mg Co/kg. Similar results to that obtained with cobalt concentration in roots and shoots of sunflower plants were achieved with sorghum plants with a decrease of cobalt concentration in shoots and roots as compared with sunflower plants. The differences between these two plants may be attributed to their differences in genetic composition. Increasing cobalt rate from 0.0 to 800 mg Co/kg resulted in significant increases in cobalt uptake by shoots of sorghum plants grown on sandy soil. Such results may lead to a general statement that the rate and magnitude of cobalt accumulation in roots and translocated up to the shoots in the plants is a phenomenon very much dependent on soil characteristics. These results are in agreement with those of Khalil (1995) in which values of cobalt uptake by sorghum were progressively increased with increasing the rate of applied cobalt up to 50 mg Co/kg soil. Cobalt uptake by roots was higher than by shoots with a similar trend to that obtained with sunflower plants.

In case of dry matter yield of shoots of the elephant grass plants, an increase was noticed with using 400 mg Co/kg, followed by a decrease with adding 800 mg Co/kg, but in case of roots there was a reduction with increasing cobalt rate from 0.0 to 800 mg Co/kg soil. Cobalt concentration in shoots as well as roots was sharply and significantly increased with increasing cobalt rate from 0.0 up to 800 mg Co/kg. The mobility of cobalt from roots to shoots was obvious and is almost similar to that occurred with sunflower plants. Thus, elephant grass plants are considered to be hyperaccumulator for cobalt. Cobalt uptake of elephant grass was significantly and gradually increased with increasing cobalt rate either in shoots or roots as compared with treatment which received no cobalt. In case of shoots, there was a slight reduction with adding the 800 mg rate of cobalt. Cobalt uptake by shoots was much higher than that taken up by roots indicating that absorbed cobalt by roots is quickly translocated to shoots. This result emphasizes that elephant grass is one of hyperaccumulator plants for cobalt.

Table (4): Effect of cobalt rate on dry weight, Co concentration and Co uptake of sunflower, sorghum and elephant grass plants grown in sandy soil

Rate	Root			Shoot		
mg/kg	Dry weight	Concentration	Uptake	Dry weight	Concentration	Uptake
	g/pot	µg/g	mg/pot	g/pot	µg/g	mg/pot
Sunflower plants						
0	6.23	1.02	0.006	15.03	0.11	0.02
400	2.70	5151.00	13.91	6.00	4497.00	26.96
800	2.70	13289.00	35.9	5.86	5656.00	33.15
L.S.D	0.38	221.0	1.20	0.92	318.8	2.00
Sorghum plants						
0	13.16	4.50	0.06	27.00	3.70	0.10
400	10.3	288.50	2.97	15.10	168.00	2.53
800	5.50	868.10	4.76	10.20	309.00	3.15
L.S.D	1.02	23.09	0.66	1.95	9.03	0.22
Elephant grass plants						
0	4.53	1.60	0.01	20.53	0.44	0.01
400	4.20	1572.00	6.61	22.60	3559.00	80.41
800	3.70	3365.00	12.46	18.50	3338.00	62.03
L.S.D	0.38	62.72	0.47	2.19	75.1	5.26

Removal of cobalt by chemical extraction:

Table (5) shows the removal of cobalt from the sandy soil with ABDTPA and EDTA extractants. Results obtained reveal that removal of cobalt was markedly increased with increasing cobalt rate. It is worthy to note that ABDTPA extractable cobalt was increased two folds with increasing cobalt rate from 400 to 800 mg Co/kg soil, this trend was also true in the second extraction. ABDTPA and EDTA extractable-cobalt 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 on cobalt retained by soil, as the extractant usually extracts the most accessible forms of the adsorbate (cobalt), and followed by the less accessible ones.

Table (5):Removal of cobalt from contaminated soil by two successive extractions with ABDTPA and EDTA

Rate	ABDTPA-extractable Co (mg/kg)			EDTA-extractable Co (mg/kg)		
mg/kg	First extraction	Second extraction	Total	First extraction	Second extraction	Total
0	0.1	0.22	0.32	0.51	0.77	1.28
400	39.74	19.65	59.39	57.99	26.17	84.16
800	71.5	41.51	113.01	95.59	40.4	135.99
L.S.D	3.53	2.32		6.61	4.08	

Comparison between phytoremediation and chemical extraction:

Ability of both methods of remedy the polluted soil was studied and results of the removed heavy metals are shown in Table (6).

Table (6): Percentage of cobalt removed from contaminated soils by phytoremediation and chemical extraction.

Cobalt rate mg/k soil	Phytoremediation			Chemical extraction	
	Sunflower	Sorghum	Elephant grass	AB-DTPA	EDTA
400	2.04	0.27	4.35	14.84	21.03
800	1.73	0.20	1.86	14.12	17.00

Cobalt phytoremediation

Phytoremediation of cobalt using sunflower plants showed good ability to decontaminate sandy soil as it removed 2.04% and 1.73% from the first and second rates of cobalt, respectively. Elephant grass showed superiority in cobalt removal over the other two plant types when grown on sandy soil as it removed 4.35% and 1.86% from the first and second rates of cobalt, respectively.

Chemical extraction

Values of ABDTPA extractable-cobalt from the contaminated soil ranged from 14.84% and 14.12% from the first and second rates of cobalt, respectively. With the EDTA extractant, the percentages of removed cobalt were almost higher than the corresponding values of cobalt extracted with ABDTPA extractant, almost 21% of added cobalt was removed. It seems that chemical extraction is a short-term process of decontamination of heavy metals from polluted soils. This indicates that chemical extraction is more efficient and faster than phytoremdiation. These results are in agreement with those of Cunningham *et al.* (1995) who stated that phytoremediation is also frequently slower than physico-chemical processes, and may be considered as a long-term remediation process. Despite these limitation, in cases where large surface area of relatively immobile contaminants exist in the surface soils, phytoremediation may be appropriate.

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العلاج النباتي والكيميائي لأرض رملية صحراوية ملوثة بالكوبلت

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زادت مساحة الأرض الملوثة خلال العقد الأخير نتيجة أعمال التنقيب والانصهار وكذلك استخدام ماء المجاري في الري وكذلك استخدام وحل المجاري والأنشطة الأخرى. ولقد أجريت هذه الدراسة بهدف تقييم مقدرة بعض النباتات مثل عباد الشمس والذرة الرفيعة وعلف الفيل على تجميع الكوبلت في أنسجتها وتحديد الي أي مدي تستطيع هذه النباتات علاج الأرض الرملية الصحراوية الملوثة بالكوبلت. ولإنجاز هذه الدراسة تم إجراء تجربتين الأولي أجريت في محلول مغذي والثانية في تربة رملية صحراوية ملوثة بالكوبلت. ولقد أوضحت النتائج المتحصل عليها أن عباد الشمس استطاع امتصاص كميات كبيرة من الكوبلت سواء في مزرعة المحاليل المغذية أو التربة. وأظهرت نباتات علف الفيل تفوقا في قدرتها علي تجميع الكوبلت مقارنة بالنباتات الأخرى خاصة تحت ظروف الأرض. ممكن اعتبار كل من عباد الشمس وعلف الفيل نباتات ذات قدرة عالية علي تجميع الكوبلت وممكن استخدامها في علاج الأرض الملوثة بالكوبلت لو نمت فترة أطول. وتعتبر تكلفة العلاج النباتي منخفضة مقارنة بأي وسيلة علاج أخري. استخلص العلاج الكيميائي المتتالي كميات كبيرة من الكوبلت ويعتبر أكثر طرق العلاج فعالية مقارنة بالعلاج النباتي. وكان استخلاص الكوبلت باستخدام ال EDTA أكثر فعالية عن الاستخلاص بال AB-DTPA.