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Minimizing the Health Hazard of Lettuce Cultivated in Some Heavy Metals Affected Soils

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Abstract: Heavy metal (HM) cations could be found in agricultural soils by application of fertilizers, conditioner materials, or applied of sewage sludge, composts, and other industrial and urban waste materials. The sorption reactions or release of these pollutants in such competitive systems, are important to be determined to understand their mobility and subsequently bioavailability to plants. This study was conducted to evaluate the selective sequence and estimating the competitive bioavailability of several heavy metals i.e. Lead (Pb), Cadmium (Cd), Nickel (Ni), and Iron (Fe) by Lettuce plant (*Lactuca Sativa*) grown in two light texture soils as affected by cobalt (Co) treatments applied at different rates. These soils have different chemical and mineralogical characteristics and were chosen for its exposed to different conditions of contamination such as industrial contamination (Helwan) or application of sewage sludge (El-Gabal El-Asfar). The obtained results indicated that addition of Co in the studied soils, significantly led to decrease Pb, Cd, Ni and even Fe content in lettuce plant by increasing Co at any concentration applied without any exceptions, however, a reverse trend was observed for Co. In contrast, results indicated that the addition of cobalt led to increase HM residual in soil samples. From the obtained results we assumed that application of 10 to 12.5 ppm Co for contaminated soils were the best treatments should be applied to have healthy plants. The remediation effects of Co through competition phenomenon takes place were discussed. The obtained results in this study showed that cobalt application as an amendment may help in minimizing the hazardous of heavy metals in soils.

Key words: Cobalt, Lettuce, heavy metals, remediation

INTRODUCTION

The increasing consumption, production, and exponential of the earth's raw materials (fossil fuels and minerals), coupled with the growth of the world's population over the past 200 years, have resulted in environmental buildup of waste products, of which heavy metals are of particular concern (Adriano, 1986; Zaghloul and Abou-Seeda, 2005). Soils, however, are an important sink for these metals due to their soils' high metal retention capacities. Heavy metal pollution of soils has become a widespread problem and often possesses serious long-term risks for cultivated soils and ecosystem health. Important heavy metals pose threats to soil quality and human health such as Ni, Cd and Pb etc. They are used for a wide variety of industrial, urban, and agricultural applications and can be toxic to humans (Adriano, 1986; Kabata-Pendias and Pendias, 1992; Forstner, 1995; Zaghloul and Abou-Seeda, 2005). People exposed to low levels of Cd, for example, over time may incur kidney damage as well as lung, bone, cardiovascular system, liver, and reproductive system damage (USEPA, 1992; Hrudey *et al.*, 1995). Under such conditions, techniques are needed to recover the excavated soils. These problems created the importance of applying new technologies for minimizing the hazards of these pollutants.

Remediation technologies for soils/sediments can be grouped into three general categories: (1) extraction/removal, (2) destruction after separation, and (3) in situ processes. For third process, several technologies can be employed to clean up the soils and the mining wastes contaminated by toxic metals, this process including thermal, biological, and physical/chemical procedures, or their appropriate combinations. These technologies usually require the removal of contaminants from polluted soils, such as Phytoremediation. Recently, more attention has been focused on the development of in situ (in place) immobilization methods of metals in soils, which are generally non-disruptive for the natural landscape, than the conventional

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excavation, treatment and disposal methods. The in situ immobilization of metals in soils, using amendments, such as apatite, zeolite clay minerals (Zaghloul and El-Hady, 2007 and Mourad and Zaghloul 2007) or waste by-products (e.g. steel shot, beringite, iron-rich biosolids or cement dust (Zaghloul and Abou-Seeda, 2005) is considered as a promising alternative to the currently available remediation methods.

The application of these techniques is mostly relies on the fundamental understanding of natural geochemical processes, governing the speciation, migration and bioavailability of metals in the soil or groundwater environment. It is noticed that these processes are also important for the detoxification of highly toxic heavy metal-loaded industrial wastes. The main advantage of the stabilization approach is the simple mixing of amendments with soil, using common agricultural facilities, or placing it as a liner around the contaminated location. However, as economic evaluation point of view, such treatment/removal technologies are generally costly to practice and destructive to the application sites.

Cobalt (Co) as will as many heavy metals, considered being a global environmental hazard when high concentration arises mainly from mining and smelting activities, dispersal of sewage sludge and the use of soil fertilizers. On the other hand Co as a transition element is an essential component that plays a key role in several enzymes and co-enzymes (Nadia Gad, 1997 and Nadia Gad and Zaghloul, 2006). Also, it has been shown to influence growth and metabolism of plants, in different degrees, depending on the concentration and status of cobalt in rhizosphere and soil system. Moreover, the need for Co in ruminant nutrition and fixation of N_2 by legumes and the presence of nodules of non-legumes has long been recognized (Marschener, 1995). In addition, Co may play other roles in relation with other HM found in soil system.

From the biological prospective, heavy metals can conveniently be assigned to four operationally distinct classes. Class A represented in this study by iron (Fe), which is essential for life in relatively high concentrations. Class B contains metals for which no biological role has been established, and which is low or medium concentrations exhibit little or high toxicity; they include lead (Pb) and cadmium (Cd) and Nickel (Ni). A third class C, consists of elements like cobalt (Co), essential in trace amounts and at higher concentrations, class C elements can become toxic or very toxic. This study tries to evaluate the relationships between C class represents by cobalt and other reported elements from different classes in both soil and plant. In the case of having significant detecting of antagonistic phenomenon between class C and other classes, the remediation goal of HM in soil by Co addition or the aim of this research will be achieved.

From this point of view, the main goal of this work is to evaluate the interaction between Co and some heavy metals such as Pb, Cd, Ni and Fe.

MATERIALS AND METHODS

Soils:

In this study, two soil types polluted from different sources were used. The 1st one was selected from Helwan site (S_1) polluted from industrial wastes and the 2nd was selected from El-Gabal El-Asfar region (**S_2**) polluted from effluents of human activities. The collected soils were air-dried and screened through a 2-mm sieve. Some physical and chemical properties of the investigated soils were presented in Table (1).

Table 1: Some chemical and physical properties of the studied soils.

Properties		Helwan	El-gable El-Asfar
Soluble Cations meq L^{-1}	Ca^{++}	103	10
	Mg^{++}	34	40
	Na^{+}	46.5	3.5
	K^{+}	325	41.9
Soluble Anions meq L^{-1}	CO_3^{--}	-	-
	HCO_3^{-}	6.51	4.65
	CL^{-}	143	62.5
	SO_4^{--}	120	45
EC (dsm)		2.82	1.15
Organic matter (%)		0.43	3.34
$CaCO_3$ (%)		22.97	0.71
Macronutrients (mg $100g^{-1}$)	N	2.11	2.79
	P	0.06	0.09
	K	0.17	0.12
Particle size distribution (%)	Sand	74.5	91.7
	Silt	15.3	4.05
	Clay	10.2	4.25
Soil texture		Sandy Loam	Sandy

Experiment Procedure:

A green house experiment conducted in the National Research Centre (NRC), Cairo, a randomized complete block design with three replications was conducted using Lettuce plants (*Lactuca Sativa*) as an indicator plant grown in 10 kg plastic pots filled with the air dried soil samples. Cobalt as $CoSO_4$ was applied to the pots at six levels i.e. 0, 7.5, 10, 12.5, 15 and 20 mg kg^{-1} soil. All pots received 60, 50 and 40 mg kg^{-1} N, P_2O_5 and K_2O as urea, super phosphate and potassium sulphate, respectively. Noteworthy that each value represented in this study was the mean value of three replicates and other agricultural practices for the indicator plant were applied. After three month, plants were harvested, and analyzed for macronutrients N, P, K and heavy metals Pb, Cd, Ni, Co and Fe as described by Cottenie *et al.* (1982) and Ma and Rao (1997).

Statistical Analysis:

Statistical analysis applied in this study to test the significance of using Co in remediation of contaminated soils, were conducted by SAS software program (SAS institute, 1985). Also, the same software program was used to test the significant differences in rate coefficients and cumulative quantity of Co transformation.

RESULTS AND DISCUSSIONS

Heavy Metals Statues in Used Soils:

Data in table (2) show the available concentration of Pb, Cd, Ni and Co in used soils and plant in addition to critical levels of these pollutants for both soil and plant. According to these critical levels documented by (Aboul Roos, *et al.*, 1996 and Alloway, 1995). The obtained results indicated that these values are quite higher than the world accepted concentrations in soils to be planted by edible crops especially for Pb, Cd and Ni.

Moreover, values of these pollutants in Lettuce plant were higher than the safe values to be used in human foods. In this concern, Greger (1999) showed the transfer of heavy metals from soils to plants is dependent on three factors: the total amount of potentially available elements (quantity factor), the activity as well as the ionic ratios of elements in the soil solution (intensity factor), and the rate of element transfer from solid to liquid phases and to plant roots (reaction kinetics).

Significant correlations had been found between the soluble heavy metal concentrations in used soil and in cultivated plants, several countries passed legislation establishing quality- standards based on soluble heavy metal concentrations in the soil. These concentrations must be reduced below maximum threshold levels to avoid having HM contaminated plants, reducing of plant growth or bad nutritional quality. On the other hand, the leaching of metals into the ground water should be stopped to decrease water pollution. Under this experimental condition, we try to apply save remediation technique to minimize the hazardous of heavy metals pollutants in both soils and water.

Table 2: Available HM concentration (mg kg^{-1}) in used soils and lettuce and critical levels of these pollutants.

Soil				
Soil Location	Pb	Cd	Ni	Fe
Helwan	4.8	3.4	6.8	210
El-gable El-Asfar	5.5	4.5	7.9	260
Critical level	1.88	0.55	0.75	150
Lettuce Plant				
Helwan	47.9	34.1	68.4	210.7
El-gable El-Asfar	55.4	45.8	78.5	262.5
Critical level	24.0	16	0.5-4.0	73

Effect of Cobalt Application Rates on the Content of Pb, Cd, Ni and Co, Fe on Lettuce Grown in Used Soils:

The present work intensively discuses the effect of cobalt applied at different rates on both non-nutritive and nutritive heavy metals content in Lettuce plant. For non-nutritive HM, we studied Pb, Cd and Ni, the most common pollutants found in these soils created from human activities. These pollutants, in addition, represent the most toxic heavy metals could be found in soils under Egyptian conditions created from different sources of contamination. Concerning nutritive HM, we studied Fe content.

Moreover this study examined the relationship between these heavy metals in lettuce and the presence of Co in tested plant species which represents the expected competition between these elements or remediation technique tested. Results presented in table 3 showed that all studied heavy metals were

Table 3: Heavy metals content (ppm) in lettuce plant indicator as affected by added cobalt application.

Co Conc. App.	Helwan						El-Gabal El-Asfar					
	Pb	% dec.	Cd	% dec.	Ni	%dec.	Pb	%dec.	Cd	%dec.	Ni	% dec.
Conl.	**47.9**		**34.1**		**68.4**		**55.4**		**45.8**		**78.5**	
7.5	40.1	16	25.4	25	53.7	21	46.7	16	41.6	9	59.2	25
10	31.5	34	22.1	35	40.4	41	41.1	26	35.5	23	50.3	36
12.5	24.4	48	16.2	52	30.5	55	26.1	53	28.7	38	39.6	50
15	19.4	60	11.8	65	20.3	70	22.2	60	23.8	48	30.1	62
20	12.7	74	9.7	71	12.4	81	20.6	63	17.5	62	21.2	73

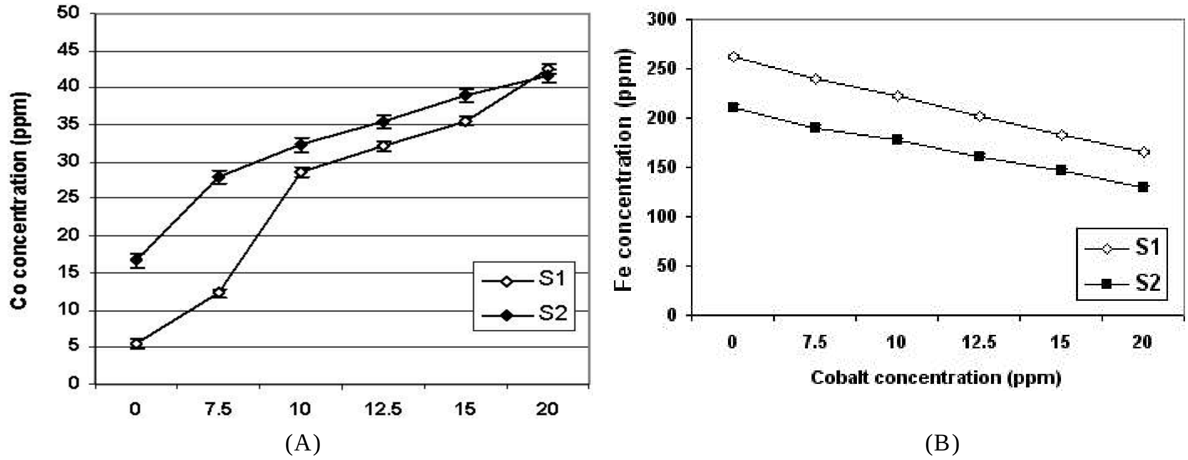

Fig. 1: Content of Co and Fe in Lettuce plant as affected by cobalt application.

drastically and significantly influenced by cobalt treatments applied in contaminated soil media at different rates with different criteria controlled by type and properties of soil used, rate of Co applied and type of heavy metal studied.

In Helwan soil, data represented in table 3 showed that the reduction of Pb content in lettuce plants was ranged between 16.5 and 74% by increasing Co concentration applied from 7.5 to 20 ppm respectively, the corresponding values were 16 and 63% for El-Gabal El-Asfar (S_2). Between 10 and 12.5 ppm Co application, the reduction in Pb uptake was ranged between about 35, 50% of in both soils.

Concerning cadmium content, data show that the reduction of Cd content in lettuce plant varied between soil samples used, results showed, however, that 7.5 ppm Co, reduced about 25% of Cd content under control. By increasing the concentration to 20 ppm, the reduction of Cd reached to 71.5 %. In El-Gabal El-Asfar soil (S_2), the same trend was noticed but with different magnitude. In general, the addition of Co led to reduction in Cd absorption in Lettuce plants ranged up to 60% depending on the rate of Co addition. The optimal concentrations of Co (i.e. 10 to 12.5 ppm), led to a reduction of Cd content to about 43, 52 and 23, 38 % of total Cd content in S_1 and S_2 respectively.

Regarding the status of Ni under the presence of cobalt, the results in table 3 indicated that the reduction of Co content was more observed compared to the other heavy metals. By applying high Co concentration i.e. 20 ppm, the reduction of Ni content reached about 82 and 72% in S1 and S2 respectively. Here it worth to mentioned that application of Co in the range of 12.5 ppm exerted a beneficial reduction in most of the studied heavy metals reaching to 50% of the control plant.

Concerning the nutritive heavy metals studied, data showed that increasing of Co concentration applied, led to reduce Fe content Fe content have the higher reduction in its concentration observed compared to other heavy metals studied. The relationship between applied cobalt and cobalt content in lettuce was also calculated. Like other non-nutritive HM, increasing the application rate of Co to soils, led to increase Co content in Lettuce grown on both soils (Fig. 1). By increasing the application rate of Co to 7.5 and 10 ppm, Co content in lettuce were increased from 5 ppm in control to 12 and 29 ppm in S_1 and from 16 to 28 and 32 ppm in S_2 respectively. The optimum available Co (~ 100% over control) was almost observed again at 12.5 ppm for both soils of having 33 and 37 ppm Co content in S_1 and S_2 respectively.

Effect of Co Applied at Different Rates on Residual of Pb, Cd and Ni Heavy Metals in the Studied Soil Samples:

Table (4) presents the status of Non-nutritive Pb, Cd and Ni accumulation in the Helwan and El-Gabal El-Asfar soils as affected by rate of Co applied in used soil samples. Generally, data indicate that graduation of increasing Co applied to soils was barreled with increasing the percent of HM accumulation in these soils compared with initial concentration (HM concentrations before plantation of lettuce) of these heavy metals. Moreover, regardless the soil type used or type of HM studied, the 20 ppm application rate showed the highest accumulation of HM in soils reached to more than 94% over control treatment.

In Helwan soil, the comparison study between treated and untreated soils indicated that the accumulation of Ni reached to about 40 and 55% over control (initial concentration) by increasing the application rate of Co from 7.5 to 10 ppm. These values increased to about 98% by increasing the application rate to 20 ppm in soil media. The respective values observed in S_2 were 24, 40 and 95%. Concerning the variation between different soils, data showed that there is a significant difference between used soils in their capacities to minimize the available form or increasing the accumulation of these pollutants in soil. Results also indicate that more or less, the 50-60% of total Ni accumulated in both soils was detected between 10 and 12.5 ppm Co applied.

For Cd pollutant accumulation, data indicated that, in S_1 the increasing of Cd in soil was more pronounced at any rate of cobalt applied. In this respect, data showed that at 10 and 12.5 application rates of Co, the accumulation of Cd in soil was increased from 51 to 70% of total Cd represents in this soil; meanwhile the respective values were 35 and 47% of available Cd in El-Gabal El-Asfar soil. The same result was observed at other rates applied with one exception observed at 20 ppm where the accumulation was increased to 95% in S_2 against 94% in S_1. It is worth to mention that, again the 50% accumulation of Cd in soils was observed at the rate of 10 and 12.5 ppm Co. Although the Pb take the same trend of other non-nutritive heavy metals studied i.e. Cd and Ni, data showed that in most cases the accumulation of Pb as affected by Co applied was less than the accumulation of Cd and Ni especially in S_1.

Data in table 3 showed that the rate of Pb accumulation was increased from 16 to 74 % under control by Appling 7.5 and 15 ppm Co in S_1. Using the same rates of application in El-Gabal El-Asfar soil, the accumulation of Pb decreased to 15.5 and 62%. Increasing the application rate to 20 ppm, the accumulation of Pb increased to about 95% in both soils.

Concerning the 50% accumulation, minimizing in available form of Pb or remediation effect of Co, data in table (4) showed that using of 10 and 12.5 ppm led to have this value in S_1 but not observed in S_2. Numerically, by applying 10 and 12.5 ppm Co, the accumulation of Pb increased from 45 to 58% compared to control, under the same conditions, the accumulation of Pb in S_2 had an acceptable values reached to about 36 and 48% under control treatment.

Although there is a variation between different soils in their available form concentration of non-nutritive HM in the beginning of the experiment, the comparison between these soils indicated that, the amelioration effects of Co take a reverse trend through the entire reaction time of plant growth and rate of Co concentration applied. Fig (2) showed that higher concentrations of the studied HM were observed in S_2 compared to S_1 as shown in Table 1. For example, the available form of Ni, Cd and Pb concentrations were 67, 33 and 55 ppm in S_1 against 77, 44 and 54 ppm in S_2 respectively. Nevertheless as previously mentioned through out the explanation of the available data, in all cases the accumulation values of these metals or the beneficial remediation for the tested plant were higher in that grown on S_1 than which grown in S_2.

Data in the same table also showed that by increasing rate of Co applied to studied soils, the Fe concentration was increased in soil system. In Helwan soil, for example, increasing the application rate from 7.5 to 10 ppm cobalt, led to increase Fe concentration in soil from 18 to 31.5 ppm. Moreover, the gradual increasing of the application rate of Co to 12.5, 15 and 20 ppm, led to increase Fe concentration in the same soil to 52, 61 and 81 ppm respectively. Although the same trend was also observed in S_2, the higher Fe concentration values were observed in this soil compared with S_1.

Unlike Fe, data in the same Fig showed that the residual cobalt showed decreasing order in soils by increasing applied Co in soils. In El-Gabal El-Asfar soil, data showed that increasing of Co application from 0 to 7.5 ppm led to decrease Co concentration from 59 ppm to 32 ppm and reached to 13.5 ppm by applying 20 ppm in soil.

The respective values for S_1 were 16.5 for control decreased to14 and 2 ppm by applying 7.5 and 20 ppm Co. It is worth to mentioned that this result in reverse with that obtained for all non-nutritive HM or even with that of Fe.

Prior to discuss our results, it will be useful to reemphasize here several important characteristic of heavy metals and their reactions in soil and plant systems. According to the ionic radius of the studied metals which could be arranged as follow: Co $\leq$ Ni < Cd< Fe< Pb, it may explain the highest competition takes place between Co and Ni in their uptake by lettuce compared with other HM of easiest Co uptake against highest Ni accumulation in soil system in one hand and between Co and Pb, Cd and Fe in other hand. The same observation concerning the influence of ionic radius on the competition of metals uptake by plants at soil-plant interface was reported by Naidu *et al.* (1994), he also mentioned that

Table 4: Percent increase in residual Pb, Cd and Ni in the studied soils as affected by cobalt concentration applied.

Co Concentration applied	Helwan			EL-Gabal El-Asfar		
	Pb	Cd	Ni	Pb	Cd	Ni
0	16.1	23.2	20.8	16.2	9.6	23.6
7.5	32.1	35.6	40.3	26.4	23.5	39.3
10	45.6	51.7	54.6	36.3	35.2	48.9
12.5	58.6	69.9	69.2	48.5	46.6	59.9
15	71.6	68.8	82.4	61.8	64.4	72.7
20	94.8	94.3	97.8	95.3	95.1	95.1

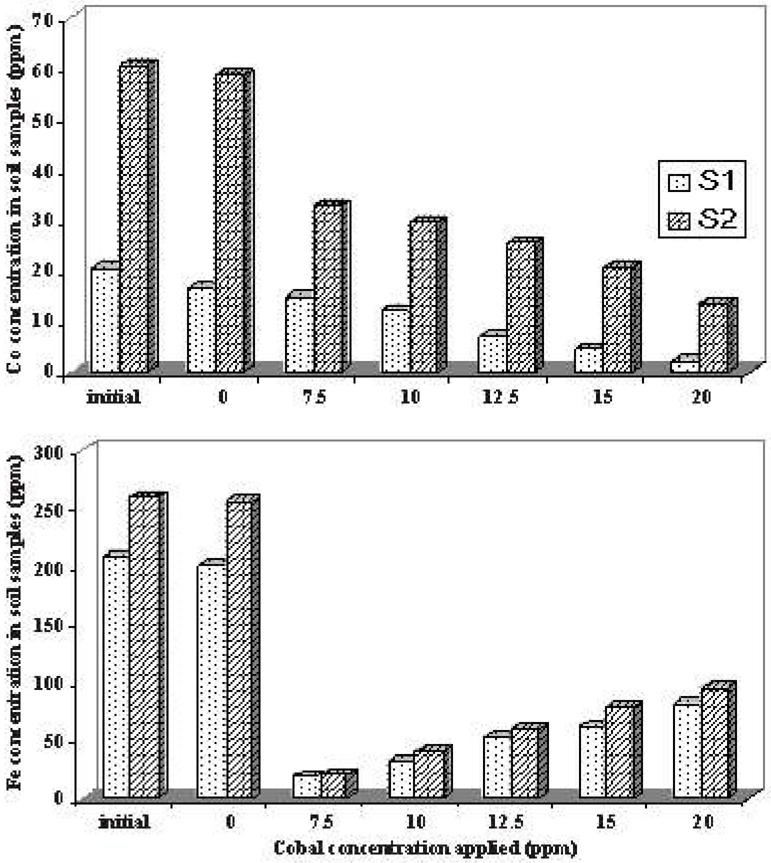

Fig. 2: Effect of Co applied on residual Co and Fe in the used soils.

the dynamic equilibrium between metals in solution and soil-solid phase is determined by the properties of the soil and composition of the soil solution.

From the obtained results, the presence and the percent of active $CaCO_3$ also is important factor under Egyptian condition controlling HM availability in soil. Also Zaghloul and Abou Seeda, (2005), concluded that the major soil factors controlling the equilibrium are ionic radius, soil pH, and presence of cations in soil solution that may compete for sorption, presence of ligands in soil solution that may affect sorption, soil organic matter and dissolved organic material. Also, Sposito (1989) defined the tendency of the metals to form covalent bonds on the basis of the ionic radius and the ionization potential quantified by the Misono softness parameter. This parameter measures the ability of the metal cations to form strong complexes. According to their ability to form covalent bonds, HM could be take the following order: Pb > Cd > Cu > Ni> Co > Zn. In this study the stability constant of Pb, Cd, Ni or Fe with other ions will be higher than that which associated with Co and accordingly the availability of Co will be more pronounced.

To emphasized the abovementioned result, McBride, 1994, McBride, *et al.* 1997 and 1999) reported that electronigativity is an important factor in determining which of the trace metals chemisorbed with the highest preference and, on this basis, the predicted order of bonding preference would be Cu > Co > Ni > Pb > Cd > Zn > Mg > Sr. On the other hand, according to this author, if the ability of the metals to chemosorb were based on only electrostatics, the strongest bond should be formed by the metal with the greatest charge-to-radius ratio, which would produce a different order for the same metals, that is, Mg >

Cu > Co> Ni > Zn > Cd > Pb.

Also, Garcia-Miragaya and Page 1976 found that Sorption of cations by soils is a competitive process. Cadmium and Pb all form divalent cations so the presence of other divalent cations (e.g. Ca^{2+} and Co^{2+}) will increased the sorption of the contaminants of concern due to competition and hence decreased their phytoavailability depending on their ionic radius (Harter, 1992)., in contrast the cobalt content will be increased in the studied plant. As a general conclusion, cobalt as a nutrient found in soils can be used effectively in minimizing the hazardous of some heavy metals could be found in soil systems.

Concerning the uptake of heavy metals by lettuce, as a point of view, results indicated that the uptake of heavy metals in plants directly affected by competition, since nutrient cations compete with the metal for uptake sites (Greger, 1999). Thus, the uptake of the studied metals may increased by decreasing the ionic radius of these toxic heavy metal. On the other hand, a generous availability of nutrients promotes plant growth, which in turn creates an increasing number of uptake sites for metals in the plants. This may increase the uptake (Ekvall and Greger, 2002) and metal concentrations in plants may be expected to increase, decrease, or to be stable, depending on the relative responses of metal uptake and growth rate.

The remediation effect of Co may also referred to the beneficial effect of Co applied at rates ranged between 10 and 12.5 ppm, in having healthy plants represented by increasing the fresh weight (FW) and dry weight (DW) of lettuce compared to other concentrations applied (data not shown), perhaps this increase of plant growth under contaminated conditions, led to increase lettuce carry out the injury of pollutants toxic effects. However, data indicted that although Pb, Cd and Ni contents in lettuce were decreased by increasing Co concentration applied in system, the continues increasing of Co applied in the growing media over 12.5 ppm, led to decrease FW and DW parameters of lettuce.

The comparison between used soil samples showed that the remediation effect of Co was more pronounced in S_1 than in S_2, this result perhaps mainly due to the presence of $CaCO_3$ in S_1 and its capacity to build stable complex with HM beside the higher concentration of soluble Ca^{2+} observed in S_1 compared to other used soil. Zaghloul and Abou Seeda, 2005 reported that under any conditions the mobility of some HM like Pb decreased by increasing of the presence of $CaCO_3$ in soil. Moreover, the retention capacity of HM mainly depends on the diameter of $CaCO_3$ fraction in soil.

As a conclusion, it will be useful to reemphasize that using such investigated soils in cultivation of edible crops for human consumption will be very critical and it will be expected to have health hazards in the long run. Results indicated that, in most cases, the presence of Pb, Cd and Ni in soils used to produce lettuce, led to have higher concentrations of these elements than recommended levels for human consumption even after application of Co. However, if these contaminated soils should to be used in such activities, perhaps application of Co to these soils has a benefit in reducing the hazardous of pollutant heavy metals. In addition, our results indicated that application of cobalt to soil system affected with toxic HM such as Pb, Cd and Ni at rates ranged between 10 and 12.5 ppm could be a good procedure in minimizing the uptake of these pollutants. It should be taking into consideration the deficiency of some important trace elements like Fe when this technique applied.

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