BIOCONCENTRATION OF SOME HEAVY METALS(Cd,Ni,Co) BY SOME EDIBLE PLANTS UNDER GREENHOUSE CONDITION

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ABSTRACT

A pot experiment was conducted using refined sand. Three tested plants were used, i.e. French beans (*Vigna unguiculata*), sesame *(Sesamum indicum) and* sorghum *(Sorghum vulgare var saccharatum).* Plants were collected at 30 and 60 days and prepared to analysis.

Dry matter yield (DMY) and Tolerance index (TI) of the tested plants (shoots and roots) under different applied treatments, at second growth period were higher than at the first growth period. The low levels of added Cd and Ni (5, 10 and 12.5, 25 mg/kg, respectively) have a positive effect on growth of french beans and sesame. The highest rates of added Cd and Ni (20 and 50mg/kg, respectively) caused inhibition of plant growth, especially sorghum plants.

The contents of Cd, Ni and Co (mg/kg) and its uptake (mg/pot) by the tested plants at second growth period were higher than of the first one. Moreover, accumulation in roots was high compared with shoots. French bean plants more affected to Cd accumulation compared with other plants. Sorghum plants showed pronounced sensitive to Ni treatment. The values of the bioconcentration ratio (BCR) decreased with increasing of added Cd, Ni & Co. Values of BCR in roots were higher than in shoots.

The ability of the tested plants to accumulate Co can be follow this order: sesame > french beans > sorghum. At the two growth periods, the value of shoots BCR for french beans and sorghum plants were higher than the BCR of roots. Except french beans plants (shoots and roots), the values of BCR at the second growth period were higher than at first growth period. In general, the BCR values decreased with increasing Co addation.

Keywords: Accumulate, contamination, Edible Plants, Heavy metals, Pollution, Tolerance

INTRODUCTION

Total content of Cd in the soils was high significant correlation with Cd content in the edible portions of cabbage, carrot, lettuce and radish which grown in 50 different soils polluted from various sources (***Holmgren et al., 1993 and Alloway, 1995***). In addition, ***Aboulroos et al. (1996)*** found a high correlation between the concentrations of Ni, Pb, Zn, Cd and Co in corn leaves and their contents in the soil. Cirtain plants can concentrate biogenic and non- biogenic heavy metals in their roots and shoots to levels for exceeding those present in the soil. ***Alloway (1995)*** showed that lettuce, spinach, celery and cabbage tended to accumulate relatively high concentrations of Cd, while potato tubers, maize, french beans and peas accumulated only small amounts of Cd. Also, he gave the following decreasing order of sensitivity to Cd toxicity, based on the element concentration in the soil causing a 25% decrease in yield: spinach > soybean > curly cress > lettuce > maize > carrot > turnip > field bean > wheat > radish > tomato > squash > cabbage > Swiss chard > upland rice.

Therefore, the aim of this study is to identify the bioconcentration of tested heavy metals (Cd, Ni and Co) by french beans (*Vigna unguiculata*), Sesame (*Sesamum indicum*) and Sorghum (*Sorghum vulgare var saccharatum*) under greenhouse conditions.

MATERIALS AND METHODS

The used pure sand was taken from Quweisna region, Minufiy Governorate and sieved through a 2 mm sieve, washed by tap water, treated by dillute HCl (6%) and H_2O_2 (30%) to remove the carbonate and oxidize the organic matter, respectively. The treated sand was washed several times with tap water followed by distilled water until free from Cl^- ions. The refined sand was air-dried.

Three heavy metals (Cd, Ni and Co) on the form of acetate at four application levels were added to the washed sand before planting. These levels are: (1) Cadmium (Cd): 0, 5, 10 and 20 mg Cd/kg. (2) Nickel (Ni): 0, 12.5, 25 and 50 mg Ni/kg. (3) Cobalt (Co): 0, 0.5, 1.0 and 2.0 mg Co/kg. The pots were filled with 3 Kg refined sand and irrigated every two days using the complete nutrient solution ***(Agarwala and Chatterjee, 1996)*** to obtain the moisture content at 60% of water holding capacity. Completely block randomized design was used in this experiment. The pots were classified into three main groups. Each pot of the first main group was cultivated by 1.12g (8 seeds) of french beans (*Vigna unguiculata*) of family Leguminosae, the second was planted by 0.05g of Sesame (*Sesamum indicum*) of family Pedoliaceae and the third was cultivated by 0.3g of Sorghum (*Sorghum vulgare var saccharatum*) of family Gramineaefor each pot. In 17 May 2003, the cultivated pots were irrigated using complete nutrient solution ***(Agarwala and Chatterjee, 1996)*** at 60 % of water holding capacity. This experiment was carried out in 8 replicates. After 8 days of planting, the plants were thinned to 4, 6 and 4 plants for the first, second and third group, respectively. Each main group was divided to 3 subgroups. These 3 subgroups were treated with heavy metals at four concentrations before planting. Four replicates of each treatment were taken after 30 days from planting and the others were taken after 60 days from planting. Whole plants were taken. The harvested plants (for two periods of growth) were washed three times by tap water and two times by distilled water. The samples were divided into shoots and roots, except sesame taken as whole plants. The plant samples (shoots and roots) were oven-dried at 70 °C for 48 hours until the weight become constant. The plant samples were digested by wet ashing according to ***Chapman and Pratt (1961).*** The contents of the tested heavy metals were measured using Perkin Elmer atomic absorption, Spectrophotometer model 2830. The tested parameters were assessing as follow:

(1) Tolerance index (TI) = Dry matter yield of treated plants / dry matter yield of untreated plants, ***(Abdel-Sabour et al., 1996).***

(2)The bioconcentration ratio (BCR) = Element in plant (µg/g dry weight) / Element in soil as available. (µg/g soil), ***Blum (1997).*** The obtained data were analyzed statistically according to ***Gomez and Gomez (1984).***

RESULTS AND DISCUSSION

Cadmium (Cd)

Data in Table (1) show that, the obtained dry matter yield (DMY) of the tested plants (g/pot) varied widely from plant to another. The DMY for the tested plants (french beans, sesame and sorghum) in the second period of growth were higher than those found in the first period at different concentrations of added Cd. This trend was found in shoots and roots. The DMY of sorghum shoot (Table 1) was reduced at the high Cd (20 mg/kg) rate compare with control, where it was decreased from 0.97 to 0.63 and from 1.73 to 1.32 g/pot at the first and second growth periods, respectively. Similar trend was obtained by El-Shikha (2000) and El-Kassas et al. (2002).

Generally, the highest biomass values of french beans (shoot and roots) and sesame plants were found under low Cd addition (10 mg Cd/kg). The lowest biomass values were found under high Cd addition (20 mg Cd/kg) in the all tested plants and both growth periods. The trend of results was agreed with the obtained data of Salem (2002), who reported that the DMY of kenaf plants (shoots and roots) were progressively decreased with increasing Cd concentration up to 20 mg/kg (Table 1).

Table (1): Dry matter yield (DMY) , tolerance index (TI) and bioconcentration ratio (BCR) of the tested plants as affected by added Cd (mg kg^{-1} sand) at two growth periods.

Plant		Cd addition, mg / pot									
		0	15			30			60		
Type	Part	DMY g/pot	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR
First period											
Fb	Shoots	1.19	1.25	1.05	5.67	1.50	1.27	3.03	1.21	1.02	1.70
	Roots	0.28	0.33	1.19	12.54	0.33	1.21	6.53	0.27	0.99	3.73
Se	Whole	0.60	1.38	2.28	5.44	1.40	2.32	4.07	0.86	1.42	2.16
S	Shoots	0.97	0.76	0.79	5.05	0.76	0.78	2.85	0.63	0.65	2.04
	Roots	0.61	0.43	0.71	8.25	0.36	0.60	4.15	0.36	0.59	2.325
Second period											
Fb	Shoots	2.39	2.99	1.25	3.29	3.42	1.43	1.89	3.24	1.36	2.08
	Roots	1.03	0.80	0.78	7.05	1.24	1.21	4.37	0.87	0.85	3.33
Se	whole	0.79	1.61	2.03	8.65	1.94	2.45	5.18	1.34	1.70	3.01
S	Shoots	1.73	1.65	0.95	2.65	1.63	0.94	1.39	1.32	0.76	1.39
	Roots	1.04	0.89	0.85	6.95	0.75	0.72	3.74	0.69	0.66	2.64

L.S.D at 0.05	Plant part	Growth period	Added Cd (mg kg-1)
French beans (Fb)	0.0274	0.0177	0.0306
Sesame (Se)	-	0.0123	0.0183
Sorghum (S)	0.0135	0.0716	0.0532

Tolerance index (TI) was used to evaluate the effect of added Cd on growth of the tested plants. In most treatments (Table 1) the values of TI for DMY of the tested plants at the second growth period were higher than the first growth period. The highest values of TI were found for sesame followed by french beans. Similar trend was obtained by EL-Kassas *et al.* (2002). It is noticed from Table (1) that the values of TI were decreased with increasing Cd concentration addition, where the high levels of added Cd can influence on the tolerance of the tested plants .

The content of Cd (mg/kg dry matter) and its uptake (mg/pot) by french beans, sesame and sorghum plants at two growth periods were recorded in Table (2). The presented data showed that, the Cd content in the sesame plants was increased in the second growth period. In general, the content of Cd in roots was more than in the shoots at the two growth periods. These results were in agreement with those obtained by Eissa and El-Kassas, (1999). In the most treatments under this study, the high values of Cd uptake were found at the high concentration of added Cd (20 mg/kg) at the second growth period specially with french beans plants followed by sorghum plants.

The bioconcentration ratio (BCR) was an adequate measure to compare the efficiency of different plant species for metal absorption, translocation from roots to shoots. The high values of BCR were found with french beans plants followed by sorghum plants Also, these values in the for roots were higher than shoots for french beans and sorghum plants in most treatments under study. El-Sokkary and Sharaf (1996) obtained similar trends.

Table (2): Cadmium content (mg kg^{-1} dry weight) and uptake (mg/pot) by tested plants as affected by added Cd (mg / pot) at two growth periods.

Plant		Added concentration of cadmium (mg / pot)							
		0		15		30		60	
Type	Part	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot
First period									
Fb	Shoots	1.75	0.0021	28.33	0.0354	30.33	0.0456	34.00	0.0410
	Roots	12.50	0.0034	62.68	0.0265	65.33	0.0217	74.58	0.0202
Se	Whole	13.25	0.0080	27.43	0.0378	40.68	0.0571	43.18	0.0369
S	Shoots	2.50	0.0024	25.25	0.0193	28.50	0.0215	40.75	0.0255
	Roots	3.00	0.0018	41.25	0.0179	41.50	0.0152	46.50	0.0167
Second period									
Fb	Shoots	15.50	0.0370	16.43	0.0492	18.88	0.0645	41.50	0.1343
	Roots	18.75	0.0193	35.25	0.0282	43.70	0.0544	66.58	0.0581
Se	whole	28.75	0.0228	43.25	0.0696	51.83	0.1007	60.25	0.0793
S	Shoots	12.00	0.0208	13.25	0.0219	13.93	0.0227	27.88	0.0367
	Roots	29.75	0.0281	34.75	0.0309	37.38	0.0310	52.83	0.0366

French beans (Fb) Sesame (Se) Sorghum (S)

Nickel (Ni)

The data in Table (3) show that, the effect of different concentrations of added Ni on biomass of the tested plants have a wide variations. The obtained DMY of the tested plants were increased with increase the growth periods. This trend was found at all concentrations of added Ni. According to the absolute values of dry matter yield of the cultivated plants at different concentrations of added Ni, the tested plants can be arranged as follows: french beans > sorghum > sesame. In the most treatments under this study, the obtained DMY of shoots were higher than roots at the same concentration of applied Ni.

The low concentrations of added Ni (12.5 and 25 mg/kg) resulted in an increase of DMY (shoots and roots) of both french beans and sesame at the first growth period. Also, these results indicate that the high concentrations (50 mg/kg) resulted in a decrease of both french beans and sesame plants compared with zero treatment. On the other hand, the low concentrations of Ni addition decreased the obtained DMY of sorghum plants (shoots and roots) compared with control treatment. Also, from the previous discussion it can be suggested that, the tested plants can be arranged according to their sensitivity to pollution with Ni as follows: sorghum > sesame > french beans. The highest values of TI were found for french beans followed by sesame plants in the second growth period (Table, 3).

Table (3): Dry matter yield (DMY), tolerance index (TI) and bioconcentration ratio(BCR) of the tested plants as affected by added Ni (mg / pot) at two growth periods.

Plant type	Plant part	Ni addition, mg / pot									
		0	37.5			75			150		
		DMY g/pot	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR
First period											
Fb	Shoots	1.19	1.33	1.12	5.44	1.70	1.43	2.75	1.20	1.01	1.74
	Roots	0.28	0.28	1.01	8.32	0.36	1.31	4.45	0.24	0.87	2.34
Se	Whole	0.60	0.76	1.26	4.06	0.90	1.49	2.44	0.63	1.04	1.28
S	Shoots	0.97	0.90	0.93	4.62	0.86	0.89	2.57	0.79	0.82	1.46
	Roots	0.61	0.50	0.82	3.77	0.49	0.81	1.96	0.45	0.74	1.01
Second period											
Fb	Shoots	2.39	3.56	1.50	10.32	3.75	1.57	5.51	4.03	1.69	3.34
	Roots	1.03	1.09	1.06	9.92	1.11	1.08	5.76	1.17	1.14	2.29
Se	whole	0.79	0.80	1.01	9.79	0.81	1.03	6.77	1.04	1.32	3.71
S	Shoots	1.73	1.68	0.97	8.89	1.63	0.94	5.66	1.53	0.88	3.39
	Roots	1.04	1.04	0.99	12.14	1.02	0.98	6.64	1.01	0.97	4.11

L.S.D at 0.05	Plant part	Growth period	Added Ni (mg kg-1)
French beans (Fb)	0.0141	0.0972	0.0223
Sesame (Se)	-	0.0727	0.0133
Sorghum (S)	0.0516	0.0516	0.0833

The Ni content (mg / Kg dry matter) in the tested plants (Table 4) at second growth period was higher than the first growth one. This trend was found with both shoots and roots for french beans and sorghum plants. These results indicate that, the tested plants were more sensitive for Ni applications in the earlier growth periods where the ability to Ni absorption was lower. Also, Ni concentration in the tested plants was increased with the increase of added Ni, but this increase was not in parallel with the added Ni. Similar trends were obtained by Salem (2002).

The uptake of Ni by the tested plants for shoots and roots at second growth period was higher than the first growth one. According to the values of Ni uptake, the cultivated plants can be arranged as follows: french beans > sorghum > sesame. From the absolute values of Ni uptake, it can be concluded that, french beans plants were more tolerance for high Ni concentrations compared with sesame and sorghum plants.

The data in Table (3) show that, BCR values were greatly decreased with the increasing of Ni addition in all treatments. Also, the BCR values in the second growth period were higher than the first growth one. The obtained values of BCR in french beans shoots were lower than in the roots at the first and second growth periods. On the other hand, the BCR values of sorghum shoots at the first growth period were higher than in the roots, while at the second growth period the obtained values of roots were greater than shoots. It can be concluded from the previous discussion of BCR that, the translocation and accumulation Ni from roots to shoots in french beans plants was greater than of sorghum plants.

Table (4): Nickel content (mg kg^{-1}, dry matter) and uptake (mg/pot) by tested plants as affected by added Ni (mg / pot) at two growth periods.

Plant type	Plant part	Added concentration of Nickel (mg kg^{-1})							
		0		37.5		75		150	
		Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Con. mg kg^{-1}	Uptake mg/pot
First period									
Fb	Shoots	42.44	0.0503	68.00	0.0906	68.83	0.1170	86.92	0.1046
	Roots	63.25	0.0174	104.00	0.0289	111.25	0.0399	116.75	0.0280
Se	Whole	35.81	0.0216	50.75	0.0385	60.86	0.0547	63.75	0.0400
S	Shoots	46.50	0.0449	57.750	0.0520	64.25	0.0554	73.13	0.0578
	Roots	37.63	0.0229	47.125	0.0236	49.00	0.0241	50.50	0.0228
Second period									
Fb	Shoots	66.08	0.1578	129.04	0.4626	145.13	0.5445	167.19	0.6741
	Roots	79.17	0.0813	124.00	0.1348	143.88	0.1596	164.38	0.1923
Se	whole	72.67	0.0576	122.31	0.0977	169.13	0.1373	185.44	0.1932
S	Shoots	69.58	0.1207	111.06	0.1870	141.44	0.2303	169.63	0.2597
	Roots	57.17	0.0596	151.75	0.1578	166.00	0.1698	205.25	0.2075

French beans (Fb) Sesame (Se) Sorghum (S)

Cobalt (Co)

From Table (5) it can be noticed that, 1) The DMY of french beans plants (shoots and roots) increased with increase of Co addition up to 2 mg/kg sand compared with control treatment, 2) All the applied concentrations of Co resulted in an increase of DMY of sesame plants and 3) The DMY of sorghum plants (shoots and roots) decreased with increase of added Co, at the both growth periods. It can be concluded that, the application of Co had a positive effects on sesame plant growth and negative effects on sorghum plant growth. The values of TI in the tested plants as affected by added Co were presented in Table (5). Where these values indicate that : 1) French beans and sesame plants were more tolerant for Co compared with sorghum plants, 2) In french beans, TI values of roots were higher than shoots at first growth period and 3) The TI of the sorghum plants for shoots and roots which found at the second growth period were higher than the first growth period at the same concentration of applied Co.

Data in table (6) show that, Co content in both shoots and roots of the tested plants increased as effected by addition of different amounts of Co, where this increase was found at first and second growth periods. Regarding to the values of Co content it can be noticed that, Co content in shoots of french beans and sorghum plants was higher than that found in the r oots o f t hem. T he french beans plants were more tolerant for high doses of applied Co in the first growth period while sesame plants was high tolerance at the second growth period. Similar trend were obtained by Mohamed and Abdel-Sabour (2000), they reported that the sesame plants showed higher accumulation of Co than corn plants, and they suggested that sesame plants have a higher affinity to accumulate cobalt than corn plants.

Table (5): Dry matter yield (DMY), tolerance index (TI) and bioconcentration ratio (BCR) of the tested plants as affected by added Co (mg / pot) at two growth periods.

Plant type	Plant part	Co addition, mg / pot									
		0	1.5			3.0			6.0		
		DMY g/pot	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR	DMY g/pot	TI	BCR
First period											
Fb	Shoots	1.19	1.24	1.05	204.00	1.32	1.11	131.44	1.72	1.45	70.26
	Roots	0.28	0.34	1.23	189.38	0.35	1.26	118.81	0.44	1.59	69.94
Se	Whole	0.60	0.71	1.17	82.50	0.77	1.27	42.03	0.78	1.29	32.21
S	Shoots	0.97	0.79	0.82	93.50	0.76	0.78	56.44	0.73	0.76	32.33
	Roots	0.608	0.48	0.79	87.13	0.44	0.72	48.67	0.42	0.70	31.67
Second period											
Fb	Shoots	2.39	3.26	1.36	195.88	3.36	1.41	101.75	3.68	1.54	52.21
	Roots	1.03	1.09	1.06	182.75	1.12	1.09	92.67	1.35	1.32	48.37
Se	whole	0.79	0.80	1.01	204.33	0.82	1.03	106.19	0.82	1.04	59.44
S	Shoots	1.73	1.71	0.99	173.33	1.69	0.97	90.67	1.66	0.95	48.13
	Roots	1.04	1.03	0.99	158.25	1.029	0.99	85.75	1.02	0.98	43.54

L.S.D at 0.05	Plant part	Growth period	Added Co (mg kg-1)
French beans (Fb)	0.2411	0.1510	0.2455
Sesame (Se)	-	0.0762	0.0197
Sorghum (S)	0.0160	0.0536	0.0938

Table (6): Cobalt content (mg kg^{-1}) and uptake (mg/pot) by tested plants as affected by added Co (mg / pot) at two growth periods.

Plant type	Plant part	Added concentration of Cobalt (mg kg^{-1})							
		0		1.5		3.0		6.0	
		Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot	Cont. mg kg^{-1}	Uptake mg/pot
First period									
Fb	Shoots	69.75	0.0827	102.00	0.1268	131.44	0.1730	140.52	0.2411
	Roots	61.50	0.0169	94.69	0.0320	118.81	0.0412	139.88	0.0610
Se	Whole	37.08	0.0224	41.25	0.0292	42.08	0.0322	64.42	0.0502
S	Shoots	43.63	0.0421	46.75	0.0369	56.44	0.0427	64. 67	0.0475
	Roots	42.125	0.0256	43.56	0.0209	48.67	0.0212	63.33	0.0268
Second period									
Fb	Shoots	94.31	0.2252	97.94	0.3190	101.75	0.3417	104.42	0.3839
	Roots	74.75	0.0768	91.38	0.0992	92.67	0.1036	96.75	0.1310
Se	whole	100.58	0.0797	102.17	0.0816	106.19	0.1531	118.88	0.0975
S	Shoots	84.75	0.1470	86.67	0.1482	90.67	0.0882	96.25	0.1593
	Roots	68.63	0.0716	79.13	0.0817	85.75	0.2413	87.08	0.0890

French beans (Fb) Sesame (Se) Sorghum (S)

Cobalt uptake by the tested plants have a wide variations (Table, 6). The obtained values of Co-uptake by shoots were higher than of roots. The sesame plants were more sensitive for high Co concentrations while french beans plants were more tolerant and more suitable to planting in the soils polluted by Co. The presented data in Table (6) show that the values of BCR for shoots of french beans and sorghum plants were higher than roots at first and second growth periods. Also, in most treatments under study, the values of BCR were decreased with the increase of Co addition.

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التركيز الحيوي لبعض العناصر الثقيلة ببعض النباتات المأكولة تحت البيوت المحمية

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أجـريت تجربة أصص باستخدام الرمل النقي وذلك لاختبار كفاءة ثلاث أنـوع نباتـية مخـتلفة هي اللوبيا والسمسم والذرة الرفيعة علي تراكم بعض العناصـر الثقـيلة.وحصدت النباتات وجهزت للتحليل بعد عمر ٣٠ و ٦٠ يوم وكانت أهم النتائج هي :

١- كـان محصول المادة الجافة ودليل التحمل بكل المعاملات للنباتات المختبرة سواء للمجموع الخضري أو الجذري اعلي بمرحلة النمو الثانية مقارنة بالأولي. وكان للمعاملات بالكادميوم و النيكل بمستوياتها المنخفضة (٥ ، ١٠ و ١٢,٥ ، ٢٥ ملليجـرام / كجم علي التوالي) تأثير موجب علي السمسم واللوبيا بينما التركيزات العالية منهما (٢٠ و ٥٠ ملليجرام / كجم علي التوالي) سببت تثبيط لنمو النباتات المدروسة وبخاصة الذرة الرفيعة.

٢- بكـل النـباتات تحـت الدراسـة كـان تركيــز الكادمـيوم والنـيكل و الكـوبالت(ملليجرام / كجم) وكذلك الكمية الممتصة (ملليجرام / أصيص) اعلي بمـرحلة النمو الثانية مقارنة بالأولي. وكانت معدلات التراكم بالجذور اعلي من المجموع الخضري وذاد التراكم بزيادة معدلات الإضافة من تلك العناصر. كما أوضـحت النتائج أن اللوبيا حساسة لتراكم الكادميوم مقارنة بباقي النباتات تحت الدراسـة بيـنما اظهـر الذرة الرفيعة حساسية للنيكل. وانخفضت نسبة التراكم الحـيوي (BCR) بـزيادة الإضافة من العناصر المدروسة وكانت تلك القيمة بالمجموع الجذري اعلي من الخضري.

٣- مقدرة النباتات تحت الدراسة لتراكم الكوبالت أخذت الترتيب التالي : السمسم > اللوبـيا > الـذرة الـرفيعة. وكانـت قيمة BCR بمرحلتي النمو للمجموع الخضـري للذرة الرفيعة أكبر من الجذري.وكانت تلك القيمة اعلي بمرحلة النمو الثانية لكل النباتات المدروسة مقارنة بالأولى ماعدا اللوبيا وعموما تنخفض تلك القيمة مع زيادة الإضافة من الكوبالت.