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GROWTH, PROTEIN EXPRESSION AND HEAVY METAL UPTAKE BY TOBACCO UNDER HEAVY METALS CONTAMINATED SOIL

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Abstract

This paper investigates heavy metal uptake, growth and protein expression of two tobacco cultivars (Flue cured Virginia, Dark sun cured) when exposed to heavy metals (Cd, Cr and Pb) and EDTA. Statistically analysis of the data revealed that growth parameters and heavy metal accumulation were significantly (p<0.05) affected by heavy metal and EDTA application. Minimum leaf fresh weight, dry weight and shoot length was measured by the application of Cr (500 mg kg^{-1}) as compared to other heavy metals and controls. Maximum reduction in leaf fresh weight, dry weight and shoot length was noted with the exposure of tobacco plants to 5 mM EDTA compared with control (0 mM EDTA). Heavy metals uptake was more by Flue cured Virginia than Dark sun cured. Tobacco cultivars exposed to heavy metal and EDTA resulted in the expression of several proteins of different molecular weight.

Key words: Heavy metal, EDTA, Tobacco, Protein.

Introduction

Worldwide industrialization has resulted in the contamination of our ecosystem. The sustainability of agriculture mainly depend on soil and water which are under sever treat due to different human activities (Nriagu & Pacyna,1988). Environmentally unfriendly activities of human beings exert different adverse effects on plants, animals and human health (Garbisu & Alkorta, 2001; Gisbert *et al*., 2003). Most of metals are very essential; however, they are toxic at high concentration and causes oxidative stress. Metal toxicity also disrupts the activity of enzyme and pigment by removal of essential metals (Henry, 2000). Therefore, metal containments destroy biodiversity and make the soil unsuitable for the growth of plants.

Several remediation methods such as acid leaching, land fill, thermal treatment, excavation and electro reclamation have been used. However, these methods are costly, had low efficiency and cause destruction of different soil properties. Phyto-remediation is an emerging technology that employs plants to clean up heavy metals contaminated sites (Chaney *et al*., 2000; Sheoran *et al*., 2016). In recent times, phyto-remediation technique got great attention as it is environmental friendly and cost-effective (Salt *et al*., 1998; Ali *et al*., 2013). This technique is also accepted by the public because it has less adverse effect over environment and human health (Fayiga *et al*., 2004). It has been reported that the bioavailability of heavy metals in plants may by increased by the presence of high concentration of pollutants in the rooting zone. Plants can detoxify pollutants through biological, chemical, and physical processes. A number of synthetic chelants (EDTA, DTPA, NTA, PDA etc.) are reported to enhance the availability of heavy metals containments in soils and its movement from root to shoot. These synthetic and organic chelants makes the pollutants to be easily absorbed by plants (Kayser *et al*., 2000; Meers *et al*., 2005). Keeping in view the importance of heavy metal in plant growth and human health, the present research was carried out to investigate (1). The impact of Cd, Cr and Pb on the growth and development of tobacco cultivars applied in different concentration; (2). The phyto-accumulating capacity of tobacco for Cd, Cr and Pb (3). To investigate the role of EDTA in the availability of Cd, Cr and Pb in the soil and (4). To monitor protein expression in tobacco cultivars under different levels of Cd, Cr and Pb and EDTA.

Materials and Methods

Plant materials and growth conditions: A green house conditions experiment was carried out in pot using factorial completely randomized design (CRD) with three replications at Institute of Biotecnology and Genetic Engineering, The University of Agricultural University Peshawar Pakistan. Tobacco plantlets were transplanted in pots (20 cm diameter and 19 cm depth) and grown for 30 days having artificially contaminated soil with different concentrations of heavy metal. Cadmium (30, 50 and 70 mg kg^{-1}), chromium (300, 400 and 500 mg kg^{-1}) and lead (200, 300 and 400 mg kg^{-1}) was applied in the form of nitrate. After establishment of seedlings in the pots, thinning was done at 3 plants pot^{-1} (having 4 kg of soil) for data collection. Thirty days after transplantation, 5 mM EDTA was added to half number of pots of each treatment. Forty five and sixty days after transplantation, plant samples were analyzed for shoot length, leaf fresh weight, leaf dry weight and heavy metal (Cd, Cr and Pb) concentrations. A composite soil sample (before the addition of heavy metals) was analyzed for heavy metal concentration. The methods of Madiha *et al*., (2012) were used for growth parameters and heavy metal analysis. Protein analysis was performed by SDS-PAGE after sixty days of transplantation (Bakht *et al*., 2016).

Statistical analysis

Data were analyzed statistically (ANOVA) as described by Gomez & Gomaz (1984). MSTATC computer software was used for statistical analysis (Russel & Eisensmith, 1983). LSD test was used for comparison means (Steel *et al.*, 1997).

Results and Discussion

Growth and development: Exposure of tobacco plants to heavy metals and EDTA had significantly (p<0.05) reduced shoot length with its increasing concentrations at 45 and 60 days after transplantation (Tables 1 and 5). Among heavy metals, maximum reduction in shoot length was attained when tobacco cultivars were treated with highest concentrations of Cd compared with other treatments. Maximum reduction in shoot length was noted with the exposure of tobacco plants to 5 mM EDTA compared with control (0 mM EDTA). Among cultivars, highest reduction in shoot length was observed in Dark sun curd variety compared to Flue cured Virginia. The result agree with Ahmad *et al*., (2012) and Mahmood *et al*., (2007) who demonstrated significant reduction in plant growth and development with increasing concentrations of cadmium. Similarly, Qadir *et al*., (2004) revealed reduction in shoot length by the addition of heavy metal. The results also suggested that increasing levels of heavy metals and EDTA application had significantly (p<0.05) affected leaf fresh weight of tobacco cultivars when recorded 45 and 60 days after transplantation (Tables 2 and 6). Maximum decrease in leaf fresh weight (g) was observed when tobacco cultivars were treated with highest concentrations of Cr compared with other heavy metals and controls. Application of 5 mM EDTA had significantly (p<0.05) reduced leaf fresh weight compared with cultivars treated with 0 mM EDTA. Maximum reduction in leaf fresh weight was observed in Dark sun curd variety compared to Flue cured Virginia variety. Qadir *et al*., (2004) and Odjegba & Fasidi (2007) reported subsequent decrease in biomass by the addition of heavy metal (cadmium, chromium and lead). Leaf dry weight (g) of tobacco cultivars was significantly (p<0.05) affected by different concentrations of heavy metals, cultivars and EDTA application and 60 days after transplantation (Tables 3 and 7). Increasing concentrations of heavy metals significantly (p<0.05) reduced leaf dry weight of tobacco cultivars as compared to controls. Treatment of plants with 5 mM EDTA resulted in maximum reduction in leaf dry weight compared with plants receiving no EDTA (0 mM). Among heavy metals treatments, highest reduction in leaf dry weight (g) was noted when tobacco cultivars were treated with highest concentrations of Pb as compared to other heavy metals and controls. Among cultivars, highest reduction in leaf dry weight was noted in Dark sun curd variety. These results agree with Qadir *et al*., (2004) and Odjegba & Fasidi (2007).

Heavy metals accumulation: Heavy metals accumulation (mg/kg) by tobacco cultivars was significantly (p<0.05) affected by cultivars, heavy metals and EDTA application at 45 and 60 days after transplantation (Tables 4 and 8). EDTA application (5 mM) had significantly (p<0.05) increased the phyto accumulation capacity of tobacco cultivars when compared treatments without EDTA (0 mM) resulting in dose dependent accumulation of heavy metals (Cd, Cr and Pb). Highest accumulation was measured in tobacco cultivars exposed to highest concentrations of Pb as compared to other treatments. Among cultivars, maximum heavy metal uptake (mg/kg) was noted in Flue cured Virginia variety as compared to Dark sun curd. These results correlates with Athar *et al*., (2002) who demonstrated reduced dry biomass due to increasing concentration of Cd. Similar results were also reported by Lo´pez *et al*., (2005) in alfalfa, Usman & Mohamed (2009) in corn, Ullah *et al*., (2010) and Bakht *et al*., (2020) in sunflower and Madiha *et al*., (2011) in brassica. Odjegba & Fasidi (2007) demonstrated that increasing concentration of heavy metal treatment and long exposure time accelerated the accumulation heavy metal in plant tissues, which agree with our results.

Table 1. Shoot length (cm) of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 45 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	27.81	25.60	26.30	24.05	25.94a
Cd 50	25.32	24.15	24.41	22.46	24.08b
Cd 70	22.53	22.75	21.66	21.09	22.08c
	25.22a(24.16c)		24.12b(23.50)		
Control	30.48b(29c)				29.74a
Cr 300	28.310	26.73	26.93	25.35	26.83a
Cr 400	27.000	25.00	25.02	24.01	25.25b
Cr 500	24.15	22.79	23.00	21.97	22.97c
	26.48b(24.84c)		24.98a(23.77d)		
Control	30.48b(29c)				29.74a
Pb 200	28.00	26.05	26.13	25.00	26.29a
Pb 300	26.03	24.95	25.00	23.10	24.71b
Pb 400	24.74	23.81	23.19	21.95	23.22c
	26.33d(24.71c)		24.77a(23.36b)		
Control	30.48b(29c)				29.74a

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia Means followed by different letter are statistically significant at p<0.05

Table 2. Leaf fresh weight of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 45 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	6.95	5.45	6.37	4.87	5.91 a
Cd 50	6.00	4.28	5.95	4.10	5.08 b
Cd 70	5.25	3.99	5.05	3.13	4.35 c
	6.06a(4.57b)		5.79c(4.03d)		
Control	6.85b(6.11c)				6.48a
Cr 300	6.68	5.87	6.17	5.21	5.9825a
Cr 400	6.19	5.10	6.00	4.84	5.5325b
Cr 500	5.57	4.88	5.43	3.77	4.9142c
	6.14c(5.28a)		5.86d(4.60b)		
Control	6.85b(6.11c)				6.48a
Pb 200	6.97	5.94	5.95	4.68	5.8850a
Pb 300	6.11	4.39	5.31	4.00	4.9525b
Pb 400	5.12	3.19	4.87	3.10	4.0700c
	6.06b(4.50c)		5.37a(3.92d)		
Control	6.85b(6.11c)				6.48a

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia Means followed by different letter are statistically significant at $p<0.05$

Table 3. Leaf dry weight of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 45 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	0.43	0.38	0.41	0.36	0.3950a
Cd 50	0.39	0.31	0.37	0.30	0.3425b
Cd 70	0.35	0.28	0.34	0.23	0.3000b
	0.39b(.32c)		0.37d(.29a)		
Control	0.47b(0.46c)				0.46a
Cr 300	0.44	0.39	0.41	0.37	0.4025a
Cr 400	0.40	0.36	0.38	0.34	0.3700b
Cr 500	0.36	0.33	0.35	0.32	o.3400b
	0.40a(0.36c)		0.38b(0.34d)		
Control	0.47b(0.46c)				0.46a
Pb 200	0.45	0.39	0.39	0.35	0.3950a
Pb 300	0.40	0.34	0.37	0.32	0.3575a
Pb 400	0.36	0.21	0.34	0.20	0.2775b
	0.40c(0.31b)		0.36a(0.29d)		
Control	0.47b(0.46c)				0.46a

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia. Means followed by different letter are statistically significant at $p<0.050$

Protein expression: Protein expression of the treated and control plants were monitored by SDS PAGE. Analysis of the protein profiling revealed that exposure of tobacco cultivars to heavy metals and EDTA resulted in the induction of new polypeptides, whereas some protein bands disappeared at 60 days after transplantation. Summary of the protein pattern of both cultivars are summarized in Tables 9 and 10. Protein profiling revealed that bands of 180, 170,140,100, 90 kDa were absent in Flue cured Virginia cultivar when Cd was applied at 30 mg kg^{-1} with or without EDTA. Protein bands of 120, 90 and 60 kDa were absent when exposed to Pb 200 mg kg^{-1} without EDTA. Polypeptides bands of 180,150,100 kDa were absent in treatments of Cr (300 mg kg^{-1}) without EDTA. Protein bands of 200 and 60 kDa polypeptides were absent at 400 mg kg^{-1} Cr exposure with EDTA. The data also suggested that protein bands of 180,150,130 kDa were absent when Cr was applied at 400 mg kg^{-1} without EDTA.

In case of Dark sun cured variety, repression of 200, 180, 100 kDa polypeptides were seen at 30 mg kg^{-1} of Cd without EDTA. Protein bands of 150, 130, 80 kDa polypeptides were absent at Cd (50 mg kg^{-1}) with EDTA treatment. Analysis of the data also suggested that 130, 70, 40 kDa protein bands were absent when Cd without

EDTA was applied at 70 mg kg^{1}. Moreover, 200 and 90 kDa proteins were absent when plants were exposed to Cd (70 mg kg^{-1}) with EDTA. Similarly, protein bands of 130 and 80 kDa were absent at 200 mg kg^{-1} Pb with EDTA. The data also suggested that 170, 130, 120, 70 kDa polypeptides were absent at 300 mg kg^{-1} Pb exposure without EDTA. Protein bands of 200, 180, 130, 80 kDa were absent at Pb 400 mg kg^{-1} with EDTA. Protein bands of 170, 130, 100, 80 kDa polypeptides were newly expressed at 400 mg kg^{-1} Cr with EDTA. Exposure of plants to Cr (500 mg kg^{-1}) with EDTA resulted in the disappearance of 130, 80 and 70 kDa proteins and the same treatment without EDTA showed disappearance of 200 and 170 kDa protein. From these results it can be concluded that these proteins may be involved in heavy metal tolerance of tobacco plants. Further investigation is required before the function of these proteins can be inferred. Toppi & Gabbrielli (1999) and Bakht *et al*., (2016) reported expression of different proteins by heavy metal application.

Table 4. Heavy metal up take of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 45 and 60 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	1.95	1.15	2.75	1.95	1.9500c
Cd 50	2.90	2.05	3.45	2.05	2.800 b
Cd 70	3.21	3.00	4.80	3.0	3.5025a
	2.68 a (2.06 b)		3.66 c (2.33 d)		
Control	0.050a(.045c)				0.047b
Cr 300	6.95	5.43	8.00	6.99	6.843 c
Cr 400	8.40	7.47	10.87	8.40	8.785 b
Cr 500	10.39	9.31	13.69	11.86	11.312a
	8.58 c (7.40 a)		10.85b(9.08d)		
Control	0.050a(.045c)				0.047b
Pb 200	7.99	6.41	9.45	7.89	7.935 c
Pb 300	9.14	7.95	11.67	9.31	9.518 b
Pb 400	12.05	10.21	14.82	11.26	12.086a
	9.72b(8.19c)		11.98d(9.48a)		
Control	0.050a(.045c)				0.047b

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia. Means followed by different letter are statistically significant at $p<0.050$

Table 5. Shoot length (cm) of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 60 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	31.25	29.40	29.00	26.05	29.710a
Cd 50	30.0	27.10	27.75	24.10	26.781b
Cd 70	27.81	24.95	25.42	23.15	24.348c
	29.66a(27c)		27.39c(24.43d)		
Control	33.24b(30.11c)				31.67a
Cr 300	32.000	29.00	30.01	27.83	26.295a
Cr 400	28.65	26.15	26.4	25.89	24.781 b
Cr 500	25.99	24.19	24.000	23.21	23.422c
	28.88b(26.44c)		26.82a(25.64d)		
Control	33.24b(30.11c)				31.67a
Pb 200	31.330	28.85	29.180	27.55	29.223a
Pb 300	29.05	26.30	26.95	24.95	26.812b
Pb 400	27.12	25.00	25.35	23.05	25.130c
	29.05c(26.74a)		27.16b(25.18d)		
Control	33.24b(30.11c)				31.67a

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia. Means followed by different letter are statistically significant at $p<0.050$

Table 6. Leaf fresh weight of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 60 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	7.02	5.73	6.83	4.94	6.1300a
Cd 50	6.44	4.81	6.05	4.20	5.3750b
Cd 70	5.95	4.11	5.56	3.33	4.7375c
	6.47a(4.88d)		6.14c(4.15b)		
Control	7.76b(6.56c)				7.205a
Cr 300	6.88	6.40	6.37	6.00	5.8850a
Cr 400	6.38	5.49	6.41	5.04	4.9525b
Cr 500	5.89	5.05	5.89	3.99	4.0700c
	6.40b(5.64a)		6.22c(5.10d)		
Control	7.76b(6.56c)				7.205a
Pb 200	7.29	6.25	6.49	5.29	6.3300a
Pb 300	6.00	5.01	5.75	4.48	5.3100b
Pb 400	5.05	4.25	4.99	3.50	4.4475c
	6.11c(5.18a)		5.74b(4.42d)		
Control	7.76b(6.56c)				7.205a

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia. Means followed by different letter are statistically significant at $p<0.05$

Table 7. Leaf dry weight of tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 60 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	0.47	0.40	0.45	0.37	0.4225a
Cd 50	0.41	0.33	0.42	0.32	0.3708b
Cd 70	0.38	0.29	0.37	0.28	0.3300b
	0.42b(0.34a)		0.41d(0.32c)		
Control	0.49c(0.47d)				0.48b
Cr 300	0.46	0.42	0.43	0.39	0.4250a
Cr 400	0.42	0.38	0.40	0.36	0.3900b
Cr 500	0.38	0.35	0.37	0.34	0.3600b
	0.42c(0.38a)		0.40d(0.36b)		
Control	0.49c(0.47d)				0.48b
Pb 200	0.47	0.40	0.43	0.38	0.4208a
Pb 300	0.42	0.36	0.39	0.34	0.3775b
Pb 400	0.38	0.32	0.35	0.26	0.3275b
	0.42d(0.36c)		0.39a(0.32b)		
Control	0.49c(0.47d)				0.48b

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia Means followed by different letter are statistically significant at $p<0.050$

Conclusion

Heavy metal (Cd, Cr and Pb) application showed significant effect on all studied growth parameters. Application of EDTA played a significant role in the accumulation of heavy metals by tobacco cultivars. The phyto-accumulation of heavy metals increased with the increasing dose of applied heavy metals and EDTA. The phyto-accumulation capacity of heavy metals of Flue cured Virginia was better than Dark sun cured variety. Exposure of tobacco cultivars to varying concentrations of heavy metals and EDTA resulted in the expression of various polypeptides. Some of these proteins were newly synthesized and few disappeared by heavy metals and or EDTA.

Table 8. Heavy metal up take by tobacco cultivars as affected by Cd, Cr, Pb and EDTA application at 60 days after transplantation.

Concentration of heavy metal (mg kg^{-1})	EDTA (0mM)		EDTA (5mM)		Mean
	Flue cured virginia	Dark sun cured	Flue cured virginia	Dark sun cured	
Cd 30	2.35	2.00	3.450	2.65	6.843 c
Cd 50	3.25	3.15	4.210	3.87	8.785 b
Cd 70	4.0	4.11	5.106	4.97	11.312 a
	3.2b(3.08a)		4.27c(3.83d)		
Control	0.055a(0.051b)				0.053c
Cr 300	9.98	8.00	12.12	9.99	10.022 c
Cr 400	12.0	9.94	14.86	12.16	12.240 b
Cr 500	14.07	12.60	16.93	14.44	14.511 a
	11.99c(10.18b)		14.63d(12.19a)		
Control	0.055a(0.051b)				0.053c
Pb 200	10.88	8.41	12.45	9.65	10.347 c
Pb 300	12.75	10.23	14.31	11.95	12.300 b
Pb 400	14.95	13.00	17.00	14.40	14.837 a
	12.83b(10.54a)		14.5d(12c)		
Control	0.055a(0.051b)				0.053c

Means in parenthesis are representing variety Dark sun cured whereas means outside parenthesis represents variety Flue cured Virginia. Means followed by different letter are statistically significant at $p<0.050$

Table 9. Protein profile of Flue cured Virginia as affected by heavy metals and EDTA application at 60 days after transplantation.

Treatments	Protein bands (kDa)			
	Newly expressed	Absent	More abundant	Repressed
Cd 30 (EDTA 0, 5 mM)		180, 170,140, 100, 90		
Cd 50 (EDTA, 5 mM)				200, 80,160,70
Cd 70 (EDTA, 5 mM)		150, 130,100		
Pb 200 (EDTA 0 mM)				170, 130, 90
Pb 200 (EDTA 0 mM)				200, 130, 100
Pb 300 (EDTA 5 mM)		120,90, 60		180, 130, 70
Pb 400 (EDTA 0 mM)				180, 120, 70
Pb 400 (EDTA 5 mM)				
Cr 300 (EDTA 0 mM)				
Cr 300 (EDTA 5 mM)		180,150, 100		170, 90, 60
Cr 400 (EDTA 0 mM)				200, 130,70, 60
Cr 400 (EDTA 5 mM)		180,150, 130		180, 120, 100, 80
Cr 500 (EDTA 0 mM)		200, 60		
Cr 500 (EDTA 5 mM)				

Key:
Cd 30, 50, 70 = 30, 50, 70 mg kg^{-1}
Cadmium; Pb 200, 300, 400= 200, 300, 400 mg kg^{-1} lead
Cr 300, 400, 500 = 300, 400, 500 kg^{-1} chromium

Table 10. Protein profile (kDa) of Dark sun cured Virginia as affected by heavy metals and EDTA application at 60 days after transplantation.

Treatments	Protein bands (kDa)			
	Newly expressed	Absent	More abundant	Repressed
Cd 30 (EDTA 0 mM)				
Cd 30 (EDTA 5 mM)				
Cd 50 (EDTA, 0 mM)	200, 180, 100	150,130, 80		180, 140, 120, 90
Cd 50 (EDTA, 5 mM)		130,70,40		
Cd 70 (EDTA, 0 mM)		200,90		170,140,100
Cd 70 (EDTA, 0 mM)				
Pb 200 (EDTA 0 mM)				
Pb 200 (EDTA 5 mM)	180,130			180,140
Pb 300 (EDTA 0 mM)	170,130			
Pb 300 (EDTA 5 mM)	120,70			200, 140, 80
Pb 400 (EDTA 0 mM)	200,180			150, 90, 60
Pb 400 (EDTA 5 mM)	130, 80			
Cr 300 (EDTA 5 mM)				
Cr 400 (EDTA 5 mM)	170,130			
Cr 500 (EDTA 0 mM)	100,80			
Cr 500 (EDTA 5 mM)	130,80			200, 120, 80
	70			
	200,170			

Key:
Cd 30, 50, 70 = 30, 50, 70 mg kg^{-1}
Cadmium; Pb 200, 300, 400= 200, 300, 400 mg kg^{-1}lead
Cr 300, 400, 500 = 300, 400, 500 kg^{-1} chromium

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