DISTRIBUTION AND EFFICIENCY OF THE PHYTOEXTRACTION OF CADMIUM BY DIFFERENT ORGANIC CHELATES

Distribución y Eficiencia de la Fitoextracción de Cadmio por Diferentes Quelatos Orgánicos

Juan M. Ruiz[1‡], Begoña Blasco[1], Juan J. Ríos[1], Luis M. Cervilla[1], Miguel A. Rosales[1], Mª Mar Rubio-Wilhelmi[1], Eva Sánchez-Rodríguez[1], Rosa Castellano[1] and Luis Romero[1]

SUMMARY

Cadmium (Cd), a heavy metal that causes widespread environmental contamination, is toxic to living beings and therefore at present its decontamination is a major environmental concern. One decontamination method offering an alternative to traditional methods is phytoextraction, which can be aided by chelates via enhanced Cd bioavailability in soils. Different organic chelates (alternatives to ethylene diamine tetra acetic acid, EDTA) were used: ethylene diamine disuccinate (EDDS), ammonium thiocyanate, citric acid, oxalic acid. Citric acid was used as a chelate because it improves the phytostability of Cd and, like EDDS, it is biodegradable and thus of low environmental impact. In this study we assess different organic chelates for their efficiency in Cd extraction, measuring the production of plant biomass as well as the distribution and concentration of Cd in chinese cabbage *Brassica rapa* L. cv. Onekilo, seeking an effective alternative to the application of EDTA.

Index words: Brassica rapa, *Cd distribution, Cd phytoaccumulation, EDDS.*

RESUMEN

El Cadmio (Cd) es un metal pesado causante de una amplia contaminación ambiental que en elevadas concentraciones resulta tóxico para los seres vivos, por lo que en la actualidad su descontaminación es un aspecto ambiental de gran importancia. Una de las tecnologías de descontaminación alternativas a los métodos tradicionales es la fitoextracción, que puede ser incrementada a través de la utilización de quelatos, al favorecer la biodisponibilidad del Cd en el suelo. Diferentes quelatos orgánicos como alternativa al tetraacetato de etilendiamina (EDTA) se utilizaron en este trabajo: disuccinato etilendiamina (EDDS), tiocinato de amonio, ácido cítrico y ácido oxálico. El ácido cítrico se utilizó puesto que mejora la fitoestabilidad del Cd, y al igual que el EDDS es biodegradable y por lo tanto muestran un bajo impacto ambiental. En este trabajo se evalúa el efecto de diferentes quelatos orgánicos sobre la fitoextracción de Cd, analizando la producción de biomasa y la distribución y concentración de este metal pesado en plantas de *Brassica rapa* L. cv. Onekilo.

Palabras clave: Brassica rapa, *distribución de Cd, fitoacumulación de Cd, EDDS.*

INTRODUCTION

Cadmium (Cd), a non-essential element in plants, appears in trace quantities in nature. Currently, this heavy metal is found in high concentrations in the environment, with some 38,900 tonnes being released annually (Gadd and White, 1993) from diverse sources such as electric plants, heating systems, metal industries, waste combustion, urban traffic, cement factories, and derivatives of phosphate fertilizers (Nriagu and Pacyna, 1998; Hasan *et al*., 2009). The toxicity of such pollution is aggravated by its long persistence in the environment, the half-life of this metal being estimated at more than 20 years. Therefore it is listed by the US-EPA as one of 126 priority pollutants (Nordberg, 2009). In addition to its persistence, it has been defined as a carcinogen in humans by the International Agency for Research on Cancer (IARC, 1993; Nordberg, 2009); it is involved in lung cancer (inhalation by smokers) and itai-itai disease in individuals chronically exposed to high dietary concentrations of Cd (Waisberg *et al*., 2004).

Because of these dangers, the decontamination of this trace element has become an environmental priority. Currently, one of the best-developed decontamination

[1] Departamento de Fisiología Vegetal, Facultad de Ciencias, Universidad de Granada. 1807 - Granada, España.
[‡] Autor responsable (jmrs@ugr.es)

Recibido: abril de 2009. Aceptado: septiembre de 2009.
Publicado en Terra Latinoamericana 27: 295-301.

technologies is phytoremediation, whereby plants eliminate heavy-metal soil contamination in a low-cost, non-intrusive way without scarring the natural landscape (Alkorta *et al*., 2004). In addition, the fact that phytoremediation is usually carried out *in situ* reduces exposure of the polluted substrate to humans, wildlife and other areas of the environment (Pilon-Smits, 2005).

Within phytoremediation, the most commonly used method is phytoextraction, requiring plants that: (i) are efficient accumulators of the target contaminant, (ii) exhibit little or no phytotoxic effects, and (iii) present rapid growth rates for speedy decontamination (Schnoor *et al*., 1995; Pilon-Smits, 2005).

One way of enhancing efficient accumulation of Cd is the application of diverse chelates, which displace the heavy metals of the soil and thus promote their uptake by plants and their translocation towards the aerial parts (Lombi *et al*., 2001; Shen *et al*., 2002). The most widely used chelate in phytoextraction is ethylenediamine tetraacetic acid (EDTA), although its use should be limited as much as possible, as it resists biodegradation and persists in the environment (Meers *et al*., 2004; Turgut *et al*., 2005). In addition, its prolonged presence in the environment, combined with its capacity for chelating and mobilizing heavy metals dramatically increases the risk of leaching, not only of Cd but also of such elements as Ni, Cu, Fe, Zn, and Pb (Meers *et al*., 2005). The formation of these complexes together with different toxic heavy metals for plants as well as for microorganisms damage complete ecosystems because elements move quickly through the food chain (Römkens *et al*., 2002).

In phytoremediation, the chelates most commonly used as alternatives to EDTA include persistent aminopolycarboxylic acid (APACs), specifically ethylendiamine disuccinate (EDDS), which is produced naturally by many microorganisms (Fodor *et al*., 1995; Jhan *et al*., 1998; Dixit *et al*., 2001) and shows a good degree of biodegradation that, together with its chelating capacity, makes it valuable as a substrate for induced heavy-metal phytoextraction (Meers *et al*., 2004).

Other organic chelates include citric and oxalic acid, the former increasing Cd uptake as well as diminishing its phytotoxicity by immobilizing the element in the cell vacuoles (Sun *et al*., 2005). Other agents, such as ammonium thiocyanate, can chelate heavy metals in the soil and protects roots (e.g. of *Brassica juncea*) from heavy-metal toxicity (Vara and de Oliveira, 2003).

Given the scarcity of works on this topic, in this study we assess different organic chelates for their efficiency in Cd extraction, measuring the production of plant biomass as well as the distribution and concentration of Cd in chinese cabbage *Brassica rapa* L. cv. Onekilo, seeking an effective alternative to the application of EDTA.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of the chinese cabbage (*Brassica rapa* L. cv. Onekilo) were germinated and grown for 30 days in cell flats (cell size 3 x 3 x 10 cm) filled with peat-lite mixture, and the flats were placed on benches under an experimental greenhouse in southern Spain (Granada, Saliplant S. L.). The 30-day-old seedling were transferred to a cultivation chamber under controlled environmental conditions with relative humidity of 60-80%, temperature 25/15 ºC (day/night) and 16/8 h photoperiod at a PPFD (photosynthetic photon-flux density) of 350 µmol m^{-2} s^{-1} (measured at the top of the plants with a 190 SB quantum sensor, LI-COR Inc., Lincoln, NE, USA). The plants were grown in vermiculite-filled pots (25 cm upper diameter, 17 cm lower diameter, 25 cm in height), each containing one plant. Over the entire experiment, plants were irrigated with a nutrient solution consisting of: 4 mM KNO_3, 1 mM KH_2PO_4, 2 mM $Ca(NO_3)_2$, 0.5 mM NaH_2PO_4, 2.5 mM $MgSO_4$, 5 µM $FeSO_4$, 0.025 mM H_3BO_3, 2 µM $MnCl_2$, 1 µM $ZnSO_4$, 0.1 µM $(NH_4)_6Na_7MoO_{24}$ and 0.25 µM $CuSO_4$. The nutrient solution (pH 4.5 to increase the solubility of Cd) was renewed every three days and the vermiculite partially rinsed with Millipore-filtered water in order to avoid nutrient accumulation.

At 45 days after sowing, we began to apply the different treatments, consisting of the addition of chelates + Cd to the nutrient solution with. The treatments were: ethylene diamine disuccinate (EDDS), citric acid, oxalic acid, and ammonium thiocyanate, all at a concentration of 0.5 mM (Sun *et al*., 2005), cadmium chloride ($CdCl_2$) at 0.25 mM and a control treatment without Cd added to the nutrient solution. The Cd was used at a concentration that causes toxicity in the model plant chinese cabbage *Brassica rapa* L. cv. Onekilo (0.25 mM de $CdCl_2$) (Moreno *et al*., 2003). The different treatments were maintained for 16 days, when most of the plants showed clear symptoms of Cd toxicity (chlorosis and slow growth).

The experimental design was a randomized complete block with three replicates of six plants each. The experiment was completely repeated 3 times (n = 9) under the same conditions as above.

Plant Sampling and Analysis

Half of the plants were sampled on day 45 after sowing (T_i), before beginning the treatment with $CdCl_2$ and chelators, while the rest of the plants were sampled at harvested (T_f), 62 days after sowing, at the end of the $CdCl_2$ and chelators treatment. Leaf samples were standardized by using only fully expanded leaves from the middle part of plants in each replicate, as these reflect most clearly, nutritionally and metabolically, the effects of the treatment applied.

The plant material was rinsed three times in distilled water after disinfection with non-ionic 1% detergent (Wolf, 1982), then blotted on filter paper. The samples were dried in a forced-air oven at 70 ºC for 24 h, ground in a Wiley mill. Dry weight (DW) was recorded and expressed as grams of DW. Relative growth rate (RGR) was calculated from the increase in DW of plants at the beginning (T_i) and at the end of treatments (T_f), using the equation $RGR = (\ln DW_f - \ln DW_i) / (T_F - T_i)$ (Gutschick and Kay, 1995).

In order to determine the total amount of Cd taken up by plant tissues, 0.15 g of dry plant material was mill and digested with a mixture of $HNO_3/HClO_4$ (50/50, v/v). The concentrations of Cd were determined by atomic absorption spectrophotometry (Hitachi Z-81001) (Moreno *et al*., 2003).

Statistical Analysis

Analysis of variance was used to assess the significance of treatment means. Differences between treatment means were compared using the least significant difference (LSD) at the 0.05 probability level. Levels of significance were represented by $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ and NS, not significant.

RESULTS AND DISCUSSION

Biomass Production and Relative Growth Rate

Despite not being an essential element for plants, Cd is taken up and distributed rapidly through the vascular system, causing a general growth depression and even death. Cd toxicity in plants has variable symptoms, often altering photosynthetic processes, respiration, and N metabolism, as well as reducing the uptake of water and essential nutrients (Chijtsters *et al*., 1985; Barceló and Poschenrieder, 1990; Hart *et al*., 1998; Sanita di Toppi and Gabbrielli, 1999; Schützendübel *et al*., 2001). To assess the condition of *Brassica rapa* L. cv. Onekilo growth for all treatments, we measured shoot and root dry weights, since plant biomass and yield are the most widely used indexes to define stress tolerance (Juan *et al*., 2005). As shown in Table 1, biomass production significantly declined in the different treatments so that the lowest values of root, shoot and complete plant biomass were found in plants treated with $CdCl_2$ and oxalic acid + Cd (Table 1), presenting 30% decreases regarding to control plants for root biomass and 50% for shoot and whole-plant biomass (Table 1).

The biomass reduction after the application of oxalic acid together with Cd may have been due to the increased Cd uptake, because the plant failed to induce any mechanism of tolerance against this heavy metal.

The treatment with EDDS + Cd registered the highest biomass-production values and an increase in growth with respect to control plants (Table 1). These results agree with those of Grěman *et al*. (2003), who did not find a decline in plant biomass in chinese cabbage treated with EDDS + Pb, with respect to control. Meanwhile, Meers *et al*. (2005) found no significant differences with respect to biomass production after treating with EEDS soils contaminated with different heavy metals. The positive effect on biomass triggered by the EDDS + Cd application could be explained by a reduction of Cd uptake by plants, or that this treatment induced a mechanism to counter act the toxicity of Cd.

In terms of biomass production, citric acid + Cd, and ammonium thiocyanate + Cd had a reduction of 16% in root biomass with respect to the control treatment (Table 1). According to Hue (1986), citric acid is a strong detoxifying agent, and in our experiment, it lowered Cd toxicity more than did oxalic acid, probably forming complexes with Cd and immobilizing them in inert zones such as root vacuoles (Dixit *et al*., 2001). This would explain the greater root and whole-plant biomass recorded (Table 1).

With ammonium thyocianate + Cd, biomass significantly increased with respect to plants treated with $CdCl_2$ (Table 1), perhaps because this compound, a synthetic polyacrylate, forms a hydrogel at the root level with the capacity to chelate metals and reduces heavy-metal uptake and thereby protects the plant (Vara and de Oliveira, 2003).

In addition to biomass production, another highly effective parameter to define stress tolerance in plants is RGR (Gutschick and Kay, 1995). In Table 1, the RGR data referring to root, shoot and whole plant, suggests similar trends to those discussed for biomass production. The treatment with EDDS + Cd presented RGR values higher than the control; greatest decreases in PGR (with respect to control) were registered in the treatments with $CdCl_2$ and oxalic acid + Cd (Table 1).

Cd Distribution

The study of the concentration and distribution of Cd in the different parts of the plant is essential to determine whether the chelates used in our experiment induce greater heavy-metal uptake and favour the accumulation of this metal in a certain part of the plant, these being key factors in phytoextraction programmes.

In general, the data reflected in Table 2 indicate that none of the chelates applied, except ammonium thiocyanate, altered the proportion of Cd in the plant with respect to the Cd treatment, given that for all of the treatments, Cd was distributed roughly half in the root zone and half in the shoot. Our results contrast with those of Cataldo *et al*. (1983), who reported that Cd ions were retained mainly in the roots, with only small amounts transported to the shoots. This discrepancy implies that the plant species influences Cd uptake and distribution.

Regarding the shoot, Cd appeared in all categories of leaves analysed, reflecting the pronounced mobility of this heavy metal, although the highest concentration was found in totally mature expanded leaves (Table 2), indicating that mobility to the shoot depends largely on the transpiration rate of the leaves. In this category of leaves, the citric acid + Cd and EDDS + Cd treatments increased the Cd concentration with respect to plants treated with $CdCl_2$, and thus the application of these chelates in this plant could promote phytoextraction of this metal, given that chinese cabbage leaves represent a major portion of the total biomass.

The application of ammonium thiocyanate + Cd gave the highest Cd concentration in root, representing 74% of the total Cd concentration (Table 2). As indicated above, the fact that ammonium thiocyanate is a synthetic polyacrylate that forms a hydrogel in the root zone, could explain such high Cd levels found in the roots of plants treated with this chelate. This response may be due to the definitive existence of the uptake process. This hypothesis is supported by the data on biomass and root RGR (Table 1), given that these parameters were not visibly affected with respect to the $CdCl_2$ treatment, despite the high Cd concentration.

Finally, in relation to the Cd concentration in the whole plant (Table 2), the highest values were registered with thiocyanate + Cd, possibly due to the uptake processes of this heavy metal into the root, while the lowest concentrations were found in the oxalic acid + Cd treatment (Table 2). These results indicate that the phytotoxic effect of this latter treatment were not owed to increased Cd uptake or accumulation in plants but to

Table 1. Effects of the different treatments on the production of dry matter and relative growth rate in *Brassica rapa* L. cv. Onekilo plants.

Treatments	Dry matter			Relative growth rate		
	Shoot	Root	Entire plant	Entire plant	Root	Shoot
	- - - - - - - - - - - - g - - - - - - - - - - - -					
Control	6.04	0.38	7.03	0.101	0.048	0.1
$CdCl_2$	3.06 (-49%)	0.28 (-26%)	3.34 (-52%)	0.058 (-43%)	0.028 (-42%)	0.057 (-43%)
Citric + Cd	3.81 (-37%)	0.32 (-16%)	4.13 (-41%)	0.072 (-29%)	0.036 (-25%)	0.071 (-29%)
EDDS + Cd	7.68 (+27%)	0.43 (+13%)	8.11 (+15%)	0.114 (+13%)	0.055 (+15%)	0.115 (+15%)
Thiocyanate + Cd	5.16 (-15%)	0.32 (-16%)	5.48 (-22%)	0.089 (-12%)	0.036 (-25%)	0.09 (-10%)
Oxálic + Cd	3.36 (-44%)	0.25 (-34%)	3.61 (-49%)	0.063 (-38%)	0.022 (-54%)	0.063 (-37%)
Significance†	***	***	***	***	***	***
LSD	0.72	0.01	0.55	0.009	0.005	0.009

† Levels of significance are represented by (*) at $P < 0.05$, (**) at $P < 0.01$, (***) at $P < 0.001$, and NS: not significant. ($n = 9$). EDDS = ethylene diamine disuccinate, LSD = least significant difference.

Table 2. Effects of the different treatments on concentration and distribution of Cd in *Brassica rapa* L. cv. Onekilo plants.

Treatments	Root	Basal leaf	Old leaf	Young leaf	Shoot	Entire plant
	- - - - - - - - - - - -	- - - - - - - - - - - -	- - - - - - - - - - - - mg kg^{-1}	- - - - - - - - - - - -	- - - - - - - - - - - -	- - - - - - - - - - - -
$CdCl_2$	7.59 (53)	2.18 (15)	2.76 (19)	1.81 (13)	6.75 (47)	14.34
Citric + Cd	7.48 (51)	1.97 (13)	3.46 (24)	1.71 (12)	7.14 (49)	14.62
EDDS + Cd	6.45 (46)	2.10 (15)	3.61 (26)	1.88 (13)	7.60 (54)	14.06
Thiocyanate + Cd	14.4 (74)	0.97 (5)	2.88 (15)	1.30 (6)	5.14 (26)	19.59
Oxalic + Cd	5.12 (47)	1.65 (15)	2.56 (24)	1.56 (14)	5.77 (53)	10.9
Significance†	***	***	***	***	***	***
$LSD_{0.05}$	0.68	0.19	0.35	0.22	0.74	1.81

† Levels of significance are represented by (*) at $P < 0.05$, (**) at $P < 0.01$, (***) at $P < 0.001$, and NS: not significant. ($n = 9$), % = value inside parenthesis, LSD = least significant difference.

toxic effect of oxalic acid, as described elsewhere (Grěman *et al.*, 2003).

Translocation and Phytoextraction Efficiency

The factor of translocation (FT) is used to evaluate the capacity of heavy-metal transport from the root to the shoot (Turgut *et al.*, 2005), the latter being the zone of the plant with the greatest biomass production in this species, and therefore the most effective part for phytoextracton. This makes FT a key index in selecting and appraising the different chelates applied. The highest FT value was registered in the EDDS + Cd treatment, this chelate being the most effective in stimulating this translocation (Table 3). The next highest FT corresponded to citric acid, although with far lower values (Table 3), due perhaps to Cd immobilization in the root-cell vacuoles. In the ammonium thiocyanate + Cd treatment, values significant fell with respect to the other treatments (Table 3), given that Cd is immobilized in the roots possibly by uptake processes, preventing its translocation to the shoot.

Another important index for selecting and evaluating different chelates is the phytoextraction efficiency, which indicates the quantity of Cd that can be accumulated in the shoot. As with FT, EDDS + Cd were the treatment registering the highest phytoextraction capacity (Table 3), given that it had the greatest biomass production (Table 1), and thus the highest Cd accumulation (Table 2). On the contrary, oxalic acid + Cd presented the lowest phytoextraction efficiency (Table 3) due both to the reduction in shoot-biomass production (Table 1) as well as to the lowest Cd concentration accumulated in this part of the plant.

Table 3. Effects of different treatments on the factor of translocation and on efficiency of phytoextraction of Cd in *Brassica rapa* L. cv. Onekilo plants.

Treatments	Factor of translocation	Efficiency of phytoextraction
		mg $leaf^{-1}$
$CdCl_2$	0.89	20.71
Citric + Cd	0.96	27.26
EDDS + Cd	1.18	58.44
Thiocyanate + Cd	0.26	26.57
Oxalic + Cd	0.13	19.4
Significance†	***	**
$LSD_{0.05}$	0.27	12.27

† Levels of significance are represented by (*) at $P < 0.05$, (**) at $P < 0.01$, (***) at $P < 0.001$, and NS: not significant. ($n = 9$). EDDS = ethylene diamine disuccinate, LSD = least significant difference.

CONCLUSIONS

- In conclusion, according to the data obtained in the present work, the compound most suitable for inducing phytoextraction of cadmium (Cd) in soils is ethylene diamine disuccinate (EDDS), given that, in addition to promoting the accumulation of Cd in the shoot, it prevents Cd toxicity in the whole plant.
- Our results with respect to EDDS are furthermore novel and useful given that they open a line of research on the physiological mechanisms underlying Cd-toxicity resistance induced in plants treated with this chelate.
- Finally, it bears highlighting the effects of citric acid, which improve the phytoextraction of Cd, as well as its biodegradability, which offer a good alternative for its lower environmental impact. Also, although more

research is needed in this regard, ammonium thiocyanate deserves attention, as it could be used successfully in programmes of root phytostabilization against heavy metals.

ACKNOWLEDGMENTS

This work was supported by PAI (Plan Andaluz de Investigación, AGR-161).

CITED LITERATURE

Alkorta, I., J. Hernández-Allica, J. M. Becerril, I. Amezaga, I. Albizu, and C. Garbisu. 2004. Recent findings on the phitoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. Rev. Environ. Sci. Biotech. 3: 71-90.

Barceló, J. and C. Poschenrieder. 1990. Plant water relations as affected by heavy metals: a review. J. Plant Nutr. 13: 1-37.

Cataldo, D. A., T. R. Garland, and R. E. Wildung. 1983. Cadmium uptake kinetics in intact soybean plants. Plant Physiol. 73: 844-848.

Clijtsters, H. and F. Van Assche. 1985. Inhibition of photosynthesis by metals. Photosynth. Res. 7: 31-40.

Dixit, V., V. Pandey, and R. Shyam, 2001. Differential oxidative responses to Cd in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J. Exp. Bot. 52: 1101-1109.

Fodor, E., A. Szabò-Nahy, and L. Erdei. 1995. The effects of cadmium on the fluidity and H^+ATPase activity of plasma membrane from sunflowers and wheat roots. J. Plant Physiol. 147: 87-92.

Gadd, G. M. and C. White. 1993. Microbial treatment of metal pollution-a working biotechnology. Trends Biotech. 11: 353-359.

Grěman, H., D. Vodnik, Š. Velikonja-Bolta, and D. Leštan. 2003. Heavy metals in the environment. Ethylenediaminedissuccionate as a new chelate for environmentally safe enhanced lead phytoextraction. J. Environ. Qual. 32: 500-506.

Gustschick, V. P. and L. E. Kay. 1995. Nutrient-limited growth rates: quantitative benefits of stress responses and some aspects of regulation. J. Exp. Bot. 46: 995-1009.

Hart, J. J., R. M. Welch, W. A. Norvell, L. A. Sullivan, and L. V. Kochian. 1998. Characterization of Cd binding, uptake and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol. 116: 1314-1420.

Hasan, S. A., Q. Fariduddin, B. Ali, S. Hayat, and A Ahmad. 2009. Cadmium: toxicity and tolerance in plants. J. Environ. Biol. 30: 165-174.

Hue, N. V. 1986. Effects of organic acids on aluminium toxicity. Soil Sci. Soc. Am. J. 50: 28-34.

IARC (International Agency for Research on Cancer). 1993. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. Monographs on the evaluation of carcinogenic risks to humans vol. 58. Lyon, France.

Jhan, T., F. Baluska, W. Michalke, J. F. Harper, and D. Volkmann. 1998. Plasma membrane H^+ATPase in the root apex: evidence for strong expression in xylem parenchyma and asymmetric localization within cortical and epidermal cell. Physiol. Plant. 104: 311-316.

Juan, M., R. M. Rivero, L. Romero, and J. M. Ruiz. 2005. Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. Environ. Exp. Bot. 54: 193-201.

Lombi, E., F. J. Zhao, S. J. Dunham, and S. P. McGrath. 2001. Phytoremediation of heavy-metal contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J. Environ. Qual. 30: 1919-1926.

Meers, E., M. Hopgood, E. Lesage, P. Vervaeke, F. M. G. Tack, and M. G. Verloo. 2004. Enhancement phytoextraction: in search of EDTA alternatives. Int. J. Phytoremediation 6: 95-109.

Meers, E., A. Ruttens, M. J. Hopgood, D. Samson, and F. M. G. Tack. 2005. Comparation of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58: 1011-1022.

Moreno, D. A, G. Villora, J. M. Ruiz, and L. Romero. 2003. Growth conditions, elemental accumulation and induced physiological changes in Chinese cabbage. Chemosphere 52: 1031-1040.

Nordberg, G. F. 2009. Historical perspectives on cadmium toxicology. Toxicol. Appl. Pharmacol. 283: 192-200.

Nriagu, J. O. and J. M. Pacyna. 1988. Quantitative assement of worldwide contamination of air, water and soil with trace metals. Nature 333: 134-139.

Pilon-Smits, E. 2005. Phytoremediation. Annu. Rev. Plant Biol. 56: 15-39.

Römkens, P., L. Bouwman, J. Japenga, and C. Draaisma. 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. Environ. Pollut. 116: 109-121.

Sanita di Toppi, L. and R. Gabbrielli. 1999. Response to cadmium in higher plant. Environ. Exp. Bot. 41: 105-130.

Schnoor, J. L., L. A. Licht, S. C. McCutcheon, N. L. Wolf, and L. H. Carreira. 1995. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol. 29: 318-323.

Schützendübel, A., P. Schwanz, T. Teichman, and K. Gross. 2001. Cd-induced changes in antioxidante systems, hydrogen peroxide content and differentiation in Scots pine roots. Plant Physiol. 127: 887-898.

Shen, Z. G., X. D. Li, C. C. Wang, H. M. Chen, and H. Chua. 2002. Lead phytoextraction from contaminated soil with high biomass plant species. J. Environ. Qual. 31: 1893-1900.

Sun, Q., X. R. Wang, S. M. Ding, and X. F. Yuan. 2005. Effects of exogenous organic chelators on phytochelatins production and its relationship with cadmium toxicity in wheat (*Triticum aestivum* L.) under cadmium stress. Chemosphere 60: 22-31.

Turgut, C., M. Katie Pepe, and T. J. Cutright. 2005. The effect of EDTA on *Helianthus annuus* uptake, selectivity, and translocation of heavy metals when grown in Ohio, New Mexico and Colombia soil. Chemosphere 58: 1087-1095.

Vara Prasad, M. N. and H. M. de Oliveira Freitas. 2003. Metal hyperaccumulation in plants. Biodiversity prospecting for phytoremediation technology. Electron. J. Biotechnol. 6: 285-321.

Waisberg, M., W. D. Black, C. M. Waisberg, and B. Hale. 2004. The effect of pH, time and dietary source of cadmium on the bioaccessibility and adsorption of cadmium to/from lettuce *Lactuva sativa* L. cv. Ostinata. Food Chem. Toxicol. 42: 835-842.

Wolf, B. 1982. A comprehensive system of lead analysis and its use for diagnosing crop nutrients status. Commun. Soil Sci. Plant Anal. 13: 1035-1059.