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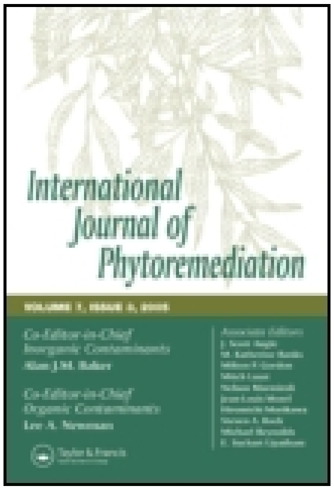

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The Optimal Root Length for Vetiveria zizanioides When Transplanted to Cd Polluted Soil

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The Optimal Root Length for *Vetiveria zizanioides* When Transplanted to Cd Polluted Soil

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In order to facilitate transportation and accelerate growth, roots of *Vetiveria zizanioides* must be pruned before transplanting. The present research is aimed to investigate the best root length for vetiver grown in cadmium (Cd) polluted soil. The results indicated that 6 cm root-length plant (RLP) was the best candidate in phytoremediation of Cd-contaminated soil for its stronger tolerance and better growth promoting activities.

Keywords: Cd, indole-3-acetic acid, root elongation, root length, toxicity

Introduction

Cd has been considered as an extremely significant pollutant widely distributed in aquatic ecosystems due to its high toxicity and great solubility in water. The majority of Cd enter the residential soil environment through weathering, chipping, scraping, sanding and so on. Moreover, the bioaccessibility of Cd and the mobility of fine particles are high in soil (Zhang *et al.* 2013). Thus it poses potential risks to human health through food chain as well as to ecosystem safety (Li *et al.* 2011). Generally, to remediate Cd pollution, phytoremediation is environmental-friendly, low-cost, and visually unobtrusive in comparison with microbial and physical-chemical remediation.

Vetiver fulfills the requirements of an accumulator plant with virtues like fast growth, high biomass, extensive root system, ease of harvest, strong resistance to the execrable environment (including temperature between –10 and 48°C and soil pH values from 3.0 to 10.5) and ability to accumulate high levels of Cd. Andra *et al.* (2009) showed that vetiver could tolerate high concentration of Pb in soil. Vetiver grows very well in soils highly contaminated by Cd, Zn, Pb and Cu (Lai and Chen 2004; Chiu *et al.* 2006) . Thus, vetiver grass has been widely considered to be a species of great potential for phytoextraction purposes in heavy metal pollutant soils.

For vetiver, root pruning is a necessary step before transplantation. This process can not only be benefit to decrease water loss resulting from transpiration, but also help to stimulate the generation of new roots which are necessary to sustain growth (Yashiroda 1960). However, little information is available on the relationship between root length and Cd accumulation as well as vetiver growth in Cd-polluted medium.

The aims of this study were 1) to further understand the response of Cd stress on vetiver; 2) to affirm the root length effect on Cd accumulation; 3) to find the relatively optimal transplanting root length with which vetiver can accumulate maximum Cd and grow more healthily.

Materials and Methods

Pot Experiments

One-month old tillers of vetivers were collected from the garden of the company of Shenbo water in Changsha. Plant roots were surface sterilized with 0.1% (v/v) sodium hypochlorite, rinsed with distilled water and then acclimatized in a hydroponic system with 12.5% (v/v) Hoagland nutrient solution for two weeks. Afterwards, vetiver taproots were pruned to original length (no root pruning), 9, 6, and 3 cm below the base. Simultaneously, the shoots were cut to remain 15 cm above the base. After soil equilibrium (The chemical and physical properties of soil are presented in Table 1), each RLP were transplanted to Cd-treated soils (Cd was added as $Cd(NO_3)_2{\cdot}4H_2O$ at a rate of 0 (E0), 10 (E10), 20 (E20), and 40 (E40) mg kg^{-1}.). Two tillers of vetiver were planted separately in pots and the pots were in a completely random block design. The pots were watered daily with deionized water under natural light (13–14 h day length, 20–30°C, 80% relative humidity).

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Table 1. Physico-chemical characteristics of the soil

pH	Org. C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Total P ($g\ kg^{-1}$)	Total K ($g\ kg^{-1}$)	CEC ($cmol\ kg^{-1}$)	Texture (%) Sand	Silt	Clay	Cd ($mg\ kg^{-1}$)
5.15	12.3	0.890	0.262	14.7	17.1	39.8	48.1	12.1	ND

ND: not detected (detection limit is 0.002 mg L^{-1}).

Sample Analysis

After 50 days' cultivation, vetivers under each treatment were harvested, separated into roots and shoots. The root length was measured with ruler. Then roots were soaked in 0.01 M $CaCl_2$ for 10 min and subsequently washed with deionized water for 3 times. The plant samples for Cd determination were dried at 68°C for 72 h and samples for indole-3-acetic acid determination were frozen in liquid nitrogen and stored at –80°C without exposure of light.

Determination of Cd concentration in Plants

Cd contents of all samples were determined by atomic absorption spectroscopy (PEAA700, Perkin Elmer, USA) after digesting the dried materials with HNO_3-$HClO_4$ (3:1, v/v).

Determination of auxin- indole-3-acetic acid (IAA)

The sample of grounded new-grown root apex (1 cm, 250 mg) was soaked in 4 mL 80% (v/v) methanol (including 0.3 mM L^{-1} DDTC) for one night at –20°C, followed by centrifugation at 15,000 *g* for 20 min at 4°C. Supernatant was separated and the sediment was soaked (4 mL 80% methanol, 2 h), centrifuged, and separated as before. Incorporated supernatant was injected into Waters Sep-Pak C_{18} solid-phase column (200 mg, 3 cc) to purify auxin-indole. Collected eluate was directly injected into the HPLC (Agilent 1100, Agilent, USA) with ultraviolet detection at 254 nm wavelength (25°C). Separation was carried out using 20% (v/v) acetonitrile and 80% (v/v) sodium acetate (pH 3.5). The flow rate was 1 mL min^{-1}, and the sample size was 20 μL.

Statistical Analysis

The responses of vetivers to the Cd stress were analyzed by a one-way ANOVA. If the statistical test was found to be significant at $p < 0.05$, a Tukey test was then employed to observe where the difference occurred (Zar 1996). All the tests were carried out using the Statistical Package for Social Science (SPSS 20.0 for Mac OS X). Each of the results shown in the figures represents the average of the three independent replicate treatments. The results were considered to be significantly different only if the probability (p) was less than 0.05.

Results and Discussion

Relationship Between Root Length and Cd Absorption

During the whole experiment, there were no visual symptoms of phytotoxicity in any of the treatments. This indicated that vetiver could tolerate 40 mg kg^{-1} Cd. It is well documented that high level of Cd leads to biomass reduction and phytotoxicity. But there is no visual phototoxicity in present research. Presumably, this could be associated with relatively low Cd^{2+} concentrations in soil or the short-term of cultivation. Moreover, it's difficult to identify metal toxicity which is characterized by invisibility and posterity (Liu *et al.* 2007). Cd contents in plants increased rapidly as Cd concentration increased from 10 to 40 mg kg^{-1} regardless of root length, with about minimum 710% increase and maximum 1770% increase than control (E0) in shoots while roots Cd increased from 130% to 740%. Therefore, Cd concentration of vetivers in present study was dose-dependent, which was early observed by Daud *et al.* (2009).

As shown in Table 2, the maximum shoots Cd acquisition was in original RLP and the maximum roots Cd absorption was in 6 cm RLP (Cd $\geq$ 10 mg kg^{-1}). Obviously, shorter taproots and fewer root hairs were observed in 6 cm RLP in comparison with original RLP. Thus, this research could confirm the result that root hairs contribute 8–45% to the acquisition of shoots Cd in the wild-type cultivar of barley (*Hordeum vulgare*) recorded by Zheng *et al.* (2011). Generally, the number of root hairs (Parker *et al.* 2000) and the most active uptake zone of cations-root apices (Boominathan and Doran 2003) could be factors of enhanced Cd absorption. 6 cm RLP devoid of root hairs retained more Cd than the others in roots, which may be attributed to the greatest value of IAA (see 3.2) that can stimulate the activities of elongating zone at sub-apical region (Sreevidya *et al.* 2010). Auxin has been reported to play an essential role in the initiation and subsequent growth of lateral roots (Peret *et al.* 2009). Taken together, high activities of cations-root apices and new lateral roots in 6 cm RLP might partly compensate for the shorter of taproots and fewer root hairs. Furthermore, these results may also be relative to the earlier maturation of apoplastic barriers (Redjala *et al.* 2011) in all RLP apart from 6 cm RLP.

With Cd addition, the highest Cd concentration in plants (shoots Cd + roots Cd) was observed in 6 cm RLP. The opposite phenomenon was observed in plants without Cd addition. It was shown repeatedly that Cd accumulation didn't increase with the increase of root length. These observations are tempting to speculate that 6 cm RLP has stronger tolerance under Cd stress. Compared with Indian mustard cultivated in

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Table 2. Cd concentrations (mg kg^{-1} DW) of vetiver seedlings which roots were pruned to different length grown in 0 mg kg^{-1} Cd (E0), 10 mg kg^{-1} Cd (E10), 20 mg kg^{-1} Cd (E20), 40 mg kg^{-1} Cd (E40) treated soils

	Cd concentration							
	root				shoot			
Root length	E0	E10	E20	E40	E0	E10	E20	E40
Original length	9.44 ± 0.822^{a}	16.67 ± 0.601^{b}	20.94 ± 0.255^{c}	24.72 ± 1.072^{d}	1.28 ± 0.096^{a}	13.94 ± 0.096^{b}	17.00 ± 0.928^{c}	22.78 ± 0.585^{d}
9 cm	10.61 ± 0.255^{a}	14.61 ± 0.918^{b}	16.44 ± 2.110^{bc}	22.11 ± 0.585^{d}	1.00 ± 0.167^{a}	10.83 ± 0.726^{b}	12.67 ± 0.441^{c}	11.78 ± 0.419^{bcd}
6 cm	6.72 ± 0.419^{a}	23.89 ± 0.918^{b}	49.83 ± 4.419^{c}	36.83 ± 0.601^{d}	0.89 ± 0.096^{a}	13.56 ± 0.855^{b}	15.28 ± 0.419^{c}	20.00 ± 0.726^{d}
3 cm	7.72 ± 0.347^{a}	18.61 ± 0.536^{b}	21.83 ± 0.601^{c}	25.11 ± 0.419^{d}	1.06 ± 0.096^{a}	10.11 ± 0.347^{b}	11.17 ± 0.333^{bc}	7.61 ± 1.084^{d}

For each treatment, different letters indicate significantly different values following Turkey's test ($P = 0.05$).
Data are mean ± SD of 3 replicates.

Table 3. Root elongation (cm) of *vetiver* seedlings which roots were pruned to different length grown in 0 mg kg^{-1} Cd (E0), 10 mg kg^{-1} Cd (E10), 20 mg kg^{-1} Cd (E20), 40 mg kg^{-1} Cd (E40) treated soils

	Root elongation			
Root length	E0	E10	E20	E40
Original length	8.98 ± 0.052^{a}	9.87 ± 0.027^{b}	9.160 ± 0.035^{c}	6.47 ± 0.086^{d}
9 cm	7.15 ± 0.103^{a}	8.42 ± 0.041^{b}	7.28 ± 0.079^{a}	6.89 ± 0.074^{d}
6 cm	11.24 ± 0.063^{a}	13.88 ± 0.051^{b}	13.23 ± 0.116^{c}	9.38 ± 0.083^{d}
3 cm	10.01 ± 0.074^{a}	11.64 ± 0.062^{b}	10.54 ± 0.217^{c}	9.29 ± 0.077^{d}

For each treatment, different letters indicate significantly different values following Turkey's test (P = 0.05).
Data are mean ± SD of 3 replicates.

nutrient solution, Cd accumulation in this study reaches no less than 9.7 and 22.7 times in roots and shoots respectively in the experiment done by Mohamed *et al.* (2012).

Endogeous Auxin- IAA

Zhao *et al.* (2008) have convincingly demonstrated that auxin can be synthesized both in shoot and root. Moreover, local auxin plays a crucial role in plant growth and development. In higher plants, IAA is the most common auxin which could promote organ formation. Correspondingly, root formation in plants is mediated by the hormone auxin-IAA (Sreevidya *et al.* 2010). Peret *et al.* (2009) have reported that IAA is essential for lateral roots initiation and its subsequent development. The accumulation of IAA, which is the endogenous auxin, promotes root initiation (Casimiro *et al.* 2003). In addition, root system is vitally significant for plants to absorb minerals and water. Consequently, it could be clearly shown that IAA is an optimal indicator for plant growth and flourish. In a threshold level of IAA, larger accumulation of IAA leads to faster speed of growth and greater biomass. During current

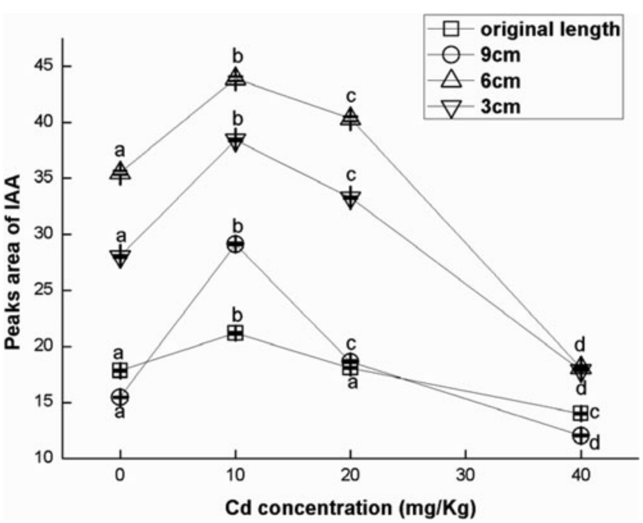

Fig. 1. Peaks area of indole-3-acetic acid (IAA) in root tips of different root length vetivers. Error bars indicate standard deviations. For each treatment, different letters indicate significantly different values following Turkey's test (P = 0.05).

experiment, 6 cm RLP secreted the maximum IAA of all RLP (Figure 1), suggesting that vetiver accompanied with 6 cm root length would be the optimal seedling when transplanting. In wheat roots, a hormesis phenomenon appeared at the low-dosed Cd (0.1 μM L^{-1}) (Li *et al.* 2011).The same result was observed in Figure 1 that IAA acquired the top value at low-dosed Cd (20 mg kg^{-1}) which would stimulate root elongation.

Root Elongation

Under Cd stress, root elongation was increased in low-dosed Cd (Cd $\leq$ 20 mg kg^{-1}) and was decreased in high-level Cd (40 mg kg^{-1}) than controls (Table 3), suggesting that low-dosed Cd stimulates root elongation while high-dosed Cd inhibits root elongation. It is hypothesized that root cell elongation and cell production could be enhanced or inhibited by certain concentration of Cd. In present study, the highest root elongation was located in 6 cm RLP and the lowest was located in 9 cm RLP (apart from 40 mg kg^{-1}) regardless of Cd concentration. These results indicate that root pruning regulates the length of root elongation, whereas the role of Cd concentration is negligible when the seedlings were grown in slightly Cd-polluted soil. This may be due to the auxin responses which coordinate root growth and development. Kawashima *et al.* (2004) performed root length to describe metal toxicity in plants. Commonly, root growth is inhibited by Cd treatment. In contrast with the vetivers grown on the control medium, root elongation was inhibited only in the treatment of 40 mg kg^{-1} Cd. These suggested that vetivers could resist to a certain level of Cd toxicity and slightly Cd would stimulate the plant growth which is consistent with Aibibu *et al.* (2010).

Conclusions

It was clearly demonstrated in our study that 6 cm RLP could grow fast, accumulate large amount of Cd and endure high-level of Cd. 6 cm RLP showed strong tolerance against high-toxic Cd and could grow healthily, therefore, 6 cm root length is the optimal root length for vetiver when transplanted to Cd-contaminated soil. In fact, this study focused only on the cellular level. Further research is also needed to explore the mechanistic details of ultrastructural alteration in 6 cm RLP.

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References

Aibibu N, Liu YG, Zeng GM, Wang X, Chen BB, Song HX, Xu L. 2010. Cadmium accumulation in *vetiveria zizanioides* and its effects on growth, physiological and biochemical characters. Bioresour Technol 101(16):6297–6303.

Andra SS, Datta R, Sarkar D, Saminathan SKM, Mullens CP, Bach SBH. 2009. Analysis of phytochelatin complexes in the lead tolerant vetiver grass *[vetiveria zizanioides (L)]* using liquid chromatography and mass spectrometry. Environ Pollut 157(7):2171–2183.

Boominathan R, Doran PM. 2003. Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. Biotechnol Bioeng 83:158–167.

Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett MJ. 2003. Dissecting Arabidopsis lateral root development. Trends Plant Sci 8:165–171.

Chiu KK, Ye ZH, Wong MH. 2006. Growth of *vetiveria zizanioides* and *phragmities australis* on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge: A greenhouse study, Bioresour. Technol 97(1):158–170.

Daud MK, Sun YQ, Dawood M, Hayat Y, Variath MT, Wu YX, Raziuddin, Mishkat U, Salahuddin, Najeeb U, Zhu SJ. 2009. Cadmium-induced functional and ultrastructural alterations in roots of two transgenic cotton cultivars. J Hazard Mater 161:463–473.

Kawashima CG, Noji M, Nakamura M, Ogra Y, Suzuk KT, Saito S. 2004. Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. Biotechnol Lett 26:153–157.

Lai HY, Chen ZS. 2004. Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. Chemosphere 55(3):421–430.

Li DD, Zhou DM, Wang P, Weng NY, Zhu XD. 2011. Subcellular Cd distribution and its correlation with antioxidant enzymatic activities in wheat roots. Ecotoxicol Environ Saf 74(4):874–881.

Liu YG, Wang X, Zeng GM, Qu D, Gu JJ, Zhou M, Chai LY. 2007. Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea (L.) Gaud*. Chemosphere 69(1):99–107.

Mohamed AA, Castagna A, Ranieri A, Sanità di Toppi L. 2012. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiol Biochem 57:15–22.

Parker JS, Cavell AC, Dolan L, Roberts K, Grierson CS. 2000. Genetic interactions during root hair morphogenesis in *Arabidopsis*. Plant Cell 12:1961–1974.

Peret B, De Rybel B, Casimiro I, Benkova E, Swarup R, Laplaze L, Beeckman T, Bennett MJ. 2009. Arabidopsis lateral root development: an emerging store. Trends Plant Sci 14:399–408.

Redjala T, Zelko I, Sterckeman T, Legué V, Lux A. 2011. Relationship between root structure and root cadmium uptake in maize. Environ. Exp. Bot. 71:241–248.

Sreevidya VS, Hernandez-Oane RJ, Gyaneshwar P, Lara-Flores M, Ladha JK, Reddy PM. 2010. Changes in auxin distribution patterns during lateral root development in rice. Plant Sci 178:531–553.

Yashiroda K. 1960. Bonsai Japanese Miniature trees: their style, cultivation, and training. London (UK): Faber & Faber Ltd.

Zar J. 1196. *Biostatistical analysis*. Upper Saddle River (NJ): Prentice-Hall.

Zhang HB, Luo YM, Makino T, Wu LH, Nanzyo M. 2013. The heavy metal partition in size-fractions of the fine particles in agricultural soils contaminated by waste water and smelter dust. J Hazard Mater 248-249:303–312.

Zhao Y. 2008. The role of local biosynthesis of auxin and cytokinin in plant development. Curr Opin Plant Biol 11:16–22.

Zheng RL, Li HF, Jiang RF, Römheld V, Zhang FS, Zhao FJ. 2011. The role of root hairs in cadmium acquisition by barley. Environ Pollut 159(2):408–415.

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