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Research Article

The potential of *Lumbricus rubellus* as a bioaccumulator of excess Pb and Cd in organic media

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Abstract: Lead (Pb) and cadmium (Cd) are sources of serious problems in the environment due to their reactivity and toxicity. *Lumbricus rubellus* is an earthworm reared by people is expected to reduce Pb and Cd concentrations in the environments. The aim of this study was to explore the ability of *Lumbricus rubellus* in reducing excess of Pb and Cd in organic media generated from urban waste. Sixteen treatments (four levels of Pb concentration and four levels of Cd concentration) were arranged in a completely randomized design with three replications. Each treatment was placed in a wooden pot of 20 cm x 20 cm x 25 cm, and supplied with 40 *Lumbricus rubellus* for 30 days. Results of this study showed that 20 and 40% of the earthworm could survive until day 30 in organic media contaminated with Pb and Cd, respectively. Pb accumulated in the earthworm bodies ranged from 0.03 to 211.42 mg/kg, while the Cd accumulated in the earthworm body ranged from 0.57 to 22.11 mg/kg. The bioaccumulation factor for Pb was 46.98%, while that of Cd was 53.83%t. The content of Pb in vermicompost ranged from 0.04 to 19.41 mg/kg, while that of Cd ranged from 0.01 to 1.58 mg/kg.

***Keywords**: earthworm, heavy metals, metal accumulation, organic media*

Introduction

Lead (Pb) and cadmium (Cd) are sources of serious problems in the environment because these metals are not easily degraded (Sati et al., 2014). Lead is a toxic heavy metal that is often found in sewage of many industries such as electricity, battery manufacturing, mineral processing and paint formulations (Han et al., 2006). Lead accumulates mainly in the human bones, brain, kidneys, and muscles and can cause many serious disorders, including anemia, kidney disease, nerve disorders, and even death (Chua et al., 2012). Cadmium is a potentially toxic metal (Kuriakose and Prasad, 2008), which is classified as a source of carcinogens that cause cancer disease in humans (IARC, 1993).

Therefore, it is important to remove these heavy metals from urban waste (Kumar and Rao, 2011). There is also an urgent need to find alternative measures for the sustainable processing of municipal solid waste in the landfill (Sim and Wu, 2010). Methods used to remove Pb and Cd should be relatively efficient, and inexpensive. One promising alternative is to use biosorption organism as an adsorbent for removing heavy metals (Ozturk, 2007). The main advantage of biosorption technology is its effectiveness in reducing the concentration of heavy metal ions to a very low level (Wong et al., 2000). Rapid metal uptake and maximum loading capacity are some important factors to consider when choosing biosorption technology (Akhtar et al., 2007).

Earthworms can be employed as bio-indicators of environmental damage to the toxicity of a chemical compound, because earthworms are able to accumulate the chemical compounds in the environment into their body (Frund et al., 2011). Earthworms are solution to overcome the urban organic waste disposal and meet the needs of organic fertilizer for sustainable agriculture (Wani, 2002). Earthworms can also reduce significant concentrations of heavy metals (Suthar and Singh, 2009). The use of earthworms in waste management has long been documented. The organic material will undergo a process conducted by earthworm to produce vermicompost (Ansari and Ismail, 2008). Some epigeic earthworm species are often used as biological agents in the process of vermicompost (Ansari and Ismail, 2008). Vermicompost contains active microorganisms and enzymes that can increase fertility of biochemically degraded soils (Fernandez-Gomez et al., 2010). Therefore, the use of vermicompost as the organic amendment material is a promising alternative, suitable and inexpensive technology to remediate the heavy metal contaminated sites (Fernández-Gómez et al., 2012).

The aims of this study were (1) to explore the potential of *Lumbricus rubellus* earthworm as a bioaccumulator in reducing excess of Pb and Cd in organic media, and (2) to measure the Pb and Cd contents in the vermicompost produced by the earthworm.

Materials and Methods

The study was conducted in a greenhouse of UPN Veteran East Java in Surabaya from March to May 2015. The treatments tested were application of earthworm (*Lumbricus rubellus*) on urban organic waste supplied with 0, 150, 300, and 450 mg Pb / kg and 0, 15, 30, and 45 mg Cd / kg. Pb was supplied in the form of Pb $(C_2H_3O_2)_2$, and Cd was in the form of Cd $(NO_3)_2$. Sixteen treatments were arranged in a completely randomized design with three replications. The experiment was conducted in glass pots measuring 20 cm x 20 cm x 25 cm containing 1 kg of urban organic waste that previously had been incubated for 30 days. At each pot were applied 40 earthworms of known weight.

The experiment was conducted for 30 days. At the end of the experiment (30 days after application earthworms), the number and gross weight (weight + food in the body) of the earthworms that were still alive in the media were measured. The earthworms were placed in bottles for 24 hours under cold temperature (OECD, 2009). The earthworms were weighed and then dried in an oven at 60°C-70°C until a constant weight. The dried earthworms were then crushed and sieved to pass through 5 mesh sieve. A sample of 0.5 g was then mixed with 5 ml HNO_3 and 1 ml $HClO_4$ and left overnight. On the next day, the earthworm sample was destructed by slowly heating from 100°C to 200°C until yellow vapour discharged enhanced heat 200°C until white smoke was released. After cooling, 20 mL of distilled water was added into the bottle. The solution was then filtered through 42 Whatman filter paper and diluted to 50 ml. The contents of Pb and Cd in the 'earthworm' solution were measured using AAS (Atomic Absorption Spectrometer).

The remaining organic media (yield of vermicompost) was air-dried and sieved for measuring water, organic C, N, available P, available K, Pb and Cd contents in the remaining media. Water content was measured by gravimetric method. Pb and Cd contents were measured using a wet digestion method with 5 ml and 1 ml HNO_3 $HClO_4$. Organic-C content was measured using Walkey-Black method. Total-N content was measured by the Kjeldahl method. Available P content was measured by the method of Bray I. Available K content was measured using NH_4OAc spectrophotometry method. Statistical analysis was performed using the F test at a level of 95% (α = 5%), followed by the least significant difference test (LSD 5%) to see the difference in metal accumulation between treatments.

.Results and Discussion

The percentage of dead earthworms.

The increase of Pb and Cd doses applied to the media significantly affected the dead percentage of earthworms (Table 1). The highest percentage of dead earthworms (48.54%) due to application of Pb in the media was observed for P3 treatment. Application of Cd also significantly affected the percentage of dead earthworms. The highest percentage of dead earthworm (51.04%) due to application of Cd was observed for C3 treatment. Heavy metals in the environment will affect the lives of earthworms by accumulating heavy metals into the tissues of the body, which in turn will cause the death of the earthworms (Kizilkaya, 2004).

Lethal dosage-50.

Lethal dosage-50 (LD-50) is the concentration or dose causing the death of more that 50% of the organisms tested. Results of LD 50 of Pb and Cd doses are presented in Table 2. In general, the results of tests for dose of metal applied that caused the death of more than 50% (LD 50) decreased when it was combined with other metals. Data presented in Table 2 indicate that the toxicities of Pb which caused the death of more than 50% for Pb in C0, C1, C2 and C3 were 1069.26 mg/kg, 710.24 mg/kg, 461.08 mg/kg, and 381.68 mg/kg, respectively. The toxicities of Cd metal that caused the death of more than 50% for P0, P1, P2, and P3 were 68.79 mg/kg, 46.65

mg/kg, 38.61 mg/kg, and 35.37 mg/kg, respectively. The increase of dose of heavy metals would lead to the increase of toxicity that can kill the earthworms. Addition of other metals in the organic media would increase the toxicity that lead to the death of earthworms. The existence of some heavy metals in a biological medium can interact with organisms in the environment; so that the organism will suffer poisoning caused by some of these metals (Sati, et al. 2014).

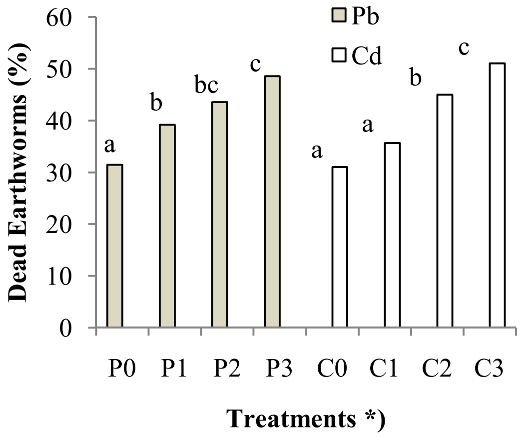

Figure 1. Lethal percentage of *Lumbricus rubellus* after 30 days

Table 2. LD-50 of Pb and Cd for *Lumbricus rubellus* after 30 days

Pb treatment over Cd	LD_{50}	R^2
C0	1069.26	0.993
C1	710.24	0.986
C2	461.08	0.996
C3	381.68	0.985
Cd treatment over Pb	LD_{50}	R^2
P0	68.79	0.995
P1	46.65	0.967
P2	38.61	0.941
P3	35.37	0.969

Earthworm biomass

The treatments that affected earthworm biomass were only doses of each applied Pb and Cd (Table 3). There was no significant interaction between Pb and Cd affecting the earthworm biomass (fresh weight and dry weight). Fresh weight of earthworms from P1, P2, and P3 treatments were 0.258 g/earthworm, 0.286 g/earthworm, and 0.291 g/earthworm, respectively. These figures were higher than that in the control treatment (P0) amounting to 0.334 g/earthworm. A similar trend was also noted for the dry weight of earthworms indicating that the increase of heavy metal dose would reduce fresh weight of earthworms.

The increase of Pb doses significantly affected the dry weight of earthworms, i.e. 0.055 g/earthworm, 0.058 g/earthworm, and 0.066 g/earthworm for P1, P2 and P3, respectively, compared with the dry weight of earthworms in the control treatment (P0) that amounting to 0.079 g/earthworm. The increase of dose of Cd applied also significantly affected the fresh weight and dry weight of earthworms compared to those in the control treatment (P0) of 0.364 g/earthworm. The dose of P3 yielded the lowest fresh weight (0.236 g/earthworm) that was not significantly different with the P2 treatment (0.273 g/earthworm), but was significantly different with the P1 treatment (0.296 g/earthworm). Similar trends were also observed for the earthworm dry weight.

Table 3.Weight of *Lumbricus rubellus* at 30 days

Treatment	Fresh weight (g/individual)		Dry weight (g/individu[1])	
P0	0.334	b	0.079	b
P1	0.291	a	0.066	a
P2	0.286	a	0.058	a
P3	0.258	a	0.055	a
LSD 5%	0.041		0.012	
C0	0.364	c	0.081	c
C1	0.296	b	0.065	b
C2	0.273	ab	0.064	b
C3	0.236	a	0.048	a
LSD 5%	0.041		0.012	

Remark: Figures accompanied by the same letter in the same column show no significant different at $\alpha = 0.05$

The increase of Cd dose also significantly reduced the earthworm dry weight. Application of Cd doses (C1, C2 and C3) yielded lower dry weight of earthworm than in the control treatment (C0), i.e. 0.079 g/earthworm. The lowest dry weight of earthworms (0.048 g/earthworm) was observed at a treatment supplied with 45 mg Cd/kg (C3 treatment). The P1 and P2 treatments yielded earthworm dry weight of 0.065 g/earthworm, and 0.065 g/earthworm, respectively. Quality of earthworm media and environmental conditions will lead to increased eating activity of earthworms, so there will be an increase in weight of earthworms during vermicomposting process that in turn affects the increase in biomass of earthworms (Suthar and Singh, 2009).

Pb and Cd accumulation by earthworms

The interaction of Pb and Cd doses significantly affected Pb and Cd contents in the body of earthworms (Table 4). The increase of Pb dose (P1, P2, and P3) significantly influenced Pb and Cd accumulation in earthworms. The highest accumulation of Pb (227.03 mg/kg) was found in P3C0. The increase of Cd doses also significantly influenced Cd accumulation by earthworms. The highest Cd accumulation (22.67 mg/kg) was observed for P3C3 treatment, although it was not significantly different from P1C3 treatment (22.67 mg/kg) and P2C3 treatment (22.67 mg/kg).

The increase of Pb and Cd contents in the body of the *Lumbricus rubellus* was in line with the increase of doses of Pb and Cd applied into the organic media. This indicates that the earthworms consumed Pb and Cd and accumulated them in their body tissues. The higher doses of metals in the media, the greater the amount of metal accumulation in the body of earthworms.

Table 4. Accumulation of Pb and Cd by *Lumbricus rubellus* after 30 days.

Treatment	Pb in earthworm (mg/kg)									
	C0		C1		C2		C3		Average	
P0	0.02	a	0.03	a	0.02	a	0.04	a	0.03	a
P1	78.03	b	57.67	b	44.73	b	36.70	b	54.28	b
P2	114.80	c	105.47	c	93.47	c	86.43	c	100.04	c
P3	227.03	d	214.00	d	203.97	d	200.67	d	211.42	d
LSD 5%	4.13		4.13		4.13		4.13		2.06	
Average	104.97	d	94.29	c	85.55	b	80.96	a	2.06	
Treatment	**Cd in earthworm (mg/kg)**									
	C0		**C1**		**C2**		**C3**		**Average**	
P0	0.51	a	8.03	b	14.57	a	21.38	a	11.12	a
P1	0.51	a	6.30	a	15.37	b	22.07	ab	11.07	a
P2	0.62	a	10.45	c	14.86	a	22.30	ab	12.06	b
P3	0.64	a	7.52	b	18.10	c	22.67	b	12.23	b
LSD 5%	1.14		1.14		1.14		1.14		0.57	
Average	0.57	a	8.08	b	15.72	c	22.11	d	0.57	

Remark: Figures accompanied by the same letter in the same column show no significant different at $\alpha = 0.05$

According to Butt and Lowe (2011), earthworms have properties of resistant and sensitive to pollutants, so that earthworms are able to accumulate chemical compounds at high concentrations in the environment. According to Langdon et al. (2003), *Lumbricus rubellus* can be used to detect the presence of heavy metals in the mining area by looking at the metal content in body tissue of the earthworms. Earthworms can serve as indicators of soil contaminated by heavy metals lead, cadmium, zinc and copper that are accumulated in the body of the earthworms (Spurgeon and Hopkins, 1999).

The treatment combinations showed that the increased doses of Pb decreased Pb accumulation in the body of the worms as Cd metal treatment dose increased. Instead, accumulation of Cd in the body of earthworms increased with increasing dose combination of Cd and Pb. According to Prasad (2004), the presence of Cd will reduce the disruption of transport of other heavy metals in the environment, so an increase in Cd dose will decrease the solubility of Pb, which in turn decreased the amount Pb consumed and accumulated by earthworms. There was a positive correlation between the increase in the dosage of Pb and Cd applied against the Pb and Cd contents in the body of the earthworm, which is expressed by the percentage of the relationship R> 0.90.

Bioaccumulation factor (BAF) of earthworms

Bioaccumulation factor (BAF) is the concentration of the chemical compounds in the earthworm's body compared to the amount of the chemical compounds in the soil (Frund et al., 2011). Experiments on earthworms in environments contaminated by heavy metals can be used to determine the ability of bioaccumulation of metals in the process of vermicomposting (Suthar and Singh, 2009). The value of bioaccumulation factors demonstrates the ability of earthworms in accumulating compounds in the environment. Data presented in Table 5 show that the value of BAF of earthworms to Pb increased with increasing doses of Pb applied into the organic media ranging from 0.36 (P1) to 0.47 (P3). Application of Cd to the organic media reduced the value of BAF of earthworms of Cd that ranged from 0.62 (P1C1) to 0.17 (P3C3). The increased application of Cd also reduced BAF of Cd by earthworms from 0.54 (C1) to 0.49 (C2). The addition of Pb sharply reduced the BAF value from 0.73 (P3 C3) to 0.27. Bioaccumulation factor of a metal will decrease with increasing concentration of the metals in the environment of earthworms, as well as any other metals that are also present in the environment (Zhao, et al., 2003).

Table 5. Bioaccumulation Factor (BAF) of *Lumbricus rubellus* after 30 days.

Pb				Cd			
Treatment	**BAF**	**Treatment**	**BAF**	**Treatment**	**BAF**	**Treatment**	**BAF**
P0	0.00	C0	0.00	C0	0.00	P0	0.00
P1	0.36	C1	0.63	C1	0.54	P1	0.74
P2	0.33	C2	0.29	C2	0.52	P2	0.40
P3	0.47	C3	0.18	C3	0.49	P3	0.27

Bioaccumulation ability of earthworms increased along with the increasing dose of Pb applied. However, addition of Cd at the media decreased in the ability of earthworms to accumulate Pb. Data presented in Table 5 show that the value of BAF of *Lumbricus rubellus* against Pb and Cd increased in the provision of these metals as a single metal. The toxicity of a metal on the organism can increase with increasing concentrations and the presence of other heavy metals in the environment (Kabata-Pendias and Pendias, 2001).

Vermicompost yield

The treatments that significantly affected the content of Pb in vermicompost were Pb doses, whereas treatments that significantly affected the content of Cd in vermicompost were Cd (Table 6). Application of 450 mg Pb/kg and 45 mg Cd /kg in the organic media produced vermicompost having the largest content of Pb (19.41 mg/kg) and Cd (11.13 mg/kg).

Table 6. Pb and Cd contents in vermicompost

Treat-ment	Pb (mg/kg)		Treat-ment	Cd (mg/kg)	
P0	0.04	a	C0	8.10	a
P1	5.36	b	C1	9.56	ab
P2	13.55	c	C2	9.56	ab
P3	19.41	d	C3	11.13	b
LSD 5%	2.44		LSD 5%	2.44	

Remark: Figures accompanied by the same letter in the same column show no significant different at $\alpha = 0.05$

The contents of Pb and Cd in the vermicompost produced did not exceed the minimum technical requirements of organic fertilizers and soil amendment (Decree of Agriculture Ministry no: 70/Permentan/SR.140 /B /2011).

At doses of Pb metal content results in the metal content of vermicompost (P1, P2 and P3) were significantly different from controls (P0). Pb metal content in the highest vermicompost contained in P3 of 19.41 mg/kg was significantly different from other treatments (P0, P1 and P2). Treatments that affected Cd content in vermicompost produced were C2 and C3 treatments. The highest content of Cd in the vermicompost was 1.58 mg/kg of the C3 treatment that was significantly different from other treatments (C0, C1 and C2).

Earthworms can affect the concentration of heavy metals in organic media by consuming and accumulating the metals into their body tissues (Kizilkaya, 2004; Karaca et al., 2010). In the process of consumption of contaminated metal organic media, earthworms reduce the heavy metals into their body the tissues and in vermicompost produced (Nahmani et al., 2007). Pb and Cd that given to organic media were consumed by earthworms to produce bio-products such as vermicompost. Heavy metals can be present in the body tissues of earthworms in high concentrations, while their residues can contain a lower amount of metal (Kizilkaya, 2004).

Conclusion

Earthworms have ability to accumulate 211.73 mg Pb/kg in the treatment of the provision of Pb 450 mg/kg (P3) and 22.11 mg Cd/kg in the treatment of 45 mg Cd/kg (C3). The produced vermicompost contained lower Pb but larger Cd than standard of organic fertilizer No. 70 / Permentan / SR.140 / 10/2011.

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References

Akhtar, K., Akhtar, M.W. and Khalid, A.M. 2007. Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum*. *Water Research* 41:1366–1378

Ansari, A.A. and Ismail, S.A. 2008. Reclamation of sodic soils through vermitechnology. *Pakistan Journal of Agriculture Research* 21: 92-97.

Butt, K.R. and Lowe, C.N. 2011. Controlled Cultivation of Endogeic and Anecic Earthworms. In : Karaca, A. 2011. Soil Biologi : Biology of Earthworms. ISBN 978-3-642-14635-0. p. 107-121.

Chua, L.W.H., Lam, K.H. and Bi, S.P. 2012. A comparative investigation on the biosorption of lead by filamentous fungal biomass. *Chemosphere* 39:2723–2736.

Fernández-Gómez, M.J., Romero, E. and Nogales, R. 2010. Feasibility of vermicomposting for vegetable greenhouse waste recycling. *Bioresource Technology* 101: 9654–9660.

Frund, H.C., Graefe, U. and Tischer, S. 2011. Earthworms as Bioindicators of Soil Quality. In Karaca, A. 2011. Biology of Earthworm. e-ISBN 978-3-642-14636-7. Soil Biology Vol. 24. pp. 261 – 278.

Han, R., Li, H.Y., Zhang, J., Xiao, H. and Shi, J. 2006. Biosorption of copper and lead ions by waste beer yeast. *Journal of Hazardous Materials* 137:1569–1576

IARC (International Agency of Research on Cancer). 1993. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. Volume 58

Kabata-Pendias, A. and Pendias, H. 2001. Trace Elements in Soils and Plants 3rd edition. ISBN 0-8493-1575-1. pp. 142-156, 202-218.

Karaca, A., Kizilkaya, R., Turgay, O.C. and Cetin, S.C. 2010. Effects of earthworms on the availability and removal of heavy metals in soils. In: Sherameti, I. dan A. Varma. (eds) Soil heavy metals, soil biology, vol 19. Springer, Berlin, pp 369–388

Kizilkaya, R. 2004. Cu and Zn accumulation in earthworm *Lumbricus terrestris* L. in sewage sludge amended soil and fractions of Cu and Zn in casts and surrounding soil. *Ecological Engineering* 22:141–151

Kumar, B.M. and Rao, V.G. 2011. Removal of Cu^{2+} and Pb^{2+} ions from aqueous solutions by free, immobilized and coimmobilized cells of *Saccharomyces cerevisiae* and *Lactobacillus sporogenes*. *International Journal of Science and Emerging Technology* 2

Kuriakose, S.V. and Prasad, M.N.V. 2008. Cadmium as an Environmetal Contaminant : Consequences to plant and Human Health. In : Prasad, M.N.V. 2008. Trace Elements as Contaminants and Nutrients : Consequences in Ecosystems and Human Health. ISBN 978-0-470-18095-2 (cloth). pp. 374-397.

Langdon, C.J., Piearce, T.G., Feldmann, J., Semple, K.T. and Meharg, A.A. 2003. Arsenic speciation in the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus*. *Environmental Toxicology and Chemistry* 22 (6): 1302–1308

Nahmani, J., Hodson, M.E. and Black, S. 2007. A review of studies performed to assess metal uptake by earthworms. *Environmental Pollution* 145:402–424.

OECD. 2009. Guidelines for the testing of chemicals. No. 207. Bioaccumulation in terrestrial oligochaetes, OECD, Paris, France. 45 p.

Ozturk, A. 2007. Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*. *Journal of Hazardous Materials* 147:518- 523

Prasad, M.N.V. 2004. Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, second edition. Heidelberg: Springer-Verlag, 462 pp

Sati, M., Verma, M. and Rai, J.P.N. 2014. Biosorption of Pb (ii) ions from aqueous solution into free and immobilized cells of bacillus megaterium. *International Journal of Recent Scientific Research* 5 (7) : 1286-1292.

Sim, E.Y.S. and Wu, T.Y. 2010. The potential reuse of biodegradable municipal solid wastes (MSW) as feedstocks in vermicomposting. *Journal of the Science of Food and Agriculture* 90 (13): 2153–2162.

Spurgeon, D.J., and Hopkin, S.P. 1999. Life-history patterns in reference and metal-exposed earthworm populations. *Ecotoxicology* 8:133-141.

Suthar, S. and Singh, S. 2009. Bioconcentrations of metals (Fe, Cu, Zn, Pb) in earthworms (*Eisenia fetida*), inoculated in municipal sewage sludge: Do earthworms pose a possible risk of terrestrial food chain contamination?. *Environmental Toxicology* 24 (1) : 25–32.

Wani, S.P. 2002. Improving the livelihoods: New partnerships for win-win solutions for natural resource management. Paper submitted in the 2nd International Agronomy Congress held at New Delhi, India during 26–30 November 2002.

Wong, J.P.K., Wong, Y.S. and Tam, N.F.Y. 2000. Nickel biosorption by two chlorella species *C. vulgaris* (a commercial species) and *C. miniata* (a local isolate). *Bioresource Technology* 73:133–137.

Zhao, F.J., Lombi, E. and McGrath, S.P. 2003. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. *Plant and Soil* 249: 37–43.