Bull. Environ. Contam. Toxicol. (2006) 76:538–544

DOI: 10.1007/s00128-006-0953-7

Bulletin of Environmental Contamination and Toxicology

Absorption of Zinc and Lead by *Dittrichia viscosa* Grown in a Contaminated Soil Amended with Olive-Derived Wastes

R. Nogales, E. Benítez

Experiment Station of Zaidín, C.S.I.C., Profesor Albareda 1, Post Office Box 419, 18008-Granada, Spain

Received: 1 August 2005/Accepted: 9 January 2006

Remediation technologies available for reducing the harmful effects in heavy-metal-contaminated soils include excavation (physical removal of the contaminated material) and later replacement with unpolluted soil, stabilization of the metals in the soil on site, and the growing of plants (phytoremediation) to stop the spread of contamination or to extract the metals from the soil. One phytoremediation process is phytostabilization, which consists of combining plant growth and root development with soil amendments in order to decrease the bioavailability and solubility of metals in soils (Wong, 2003) For phytostabilization, plants should develop a strong root system and substantial aerial biomass, should accumulate heavy metals within the roots, and should have restricted translocations of metals from the roots to shoots (De Vos et al. 1991). In addition, natural and synthetic inorganic and organic amendments can be added to the soil to reduce contaminant exposure or to enhance geochemical processes such as precipitation, sorption (Mench et al, 2000).

Dittrichia viscosa, a ruderal plant species of the Compositae family, has a wide distribution in the Mediterranean region. This shrub has a large biomass, and it can be considered, according to Baker (1987), to be pseudo-metalophyte, because it grows in soils that are non-contaminated or contaminated by heavy metals (Melendo et al. 2002). Its use for phytostabilization purposes is unknown.

Olive-oil production is one of the foremost industries in the Mediterranean countries. In recent years, the implementation of a continuous biphasic olive-oil pressing system generates huge amounts of a new solid olive-mill waste called *alperujo* or biphasic olive pomace (OP). This olive waste contains up to 60% water as well as high concentrations of sugars, pectins, salts, acids, polyalcohols, and polyphenols (Benitez et al. 2004), and, therefore, its disposal poses a serious environmental problem. Proposals for the efficient elimination of this olive waste include its agricultural use after bioremediation via composting or vermicomposting (Benitez et al. 2002; Mari et al. 2003). The potential for using of biphasic olive pomace, natural, composted or vermicomposted, as organic amendments for phytostabilization of contaminated soils with heavy metals remains to be investigated.

Correspondence to: R. Nogales

The objective of this study was to investigate, in an artificially contaminated soil, the effect of the application of three olive-derived wastes (biphasic olive pomace, and compost and vermicompost from biphasic olive pomace) on growth and concentration of Zn and Pb in *Dittrichia viscosa*, as well as on the extractability of these metals in the soil after harvesting. The possible use of combining this shrub and olive-derived wastes can serve to develop phytostabilization processes of Mediterranean contaminated soils.

MATERIAL AND METHODS

Topsoil (0-20 cm) from a Typic Calcixeroll was collected near Granada, Spain. The soil was dried at room temperature for a week and sieved through an 8-mm sieve prior to use. Some chemical and physical characteristics of the natural soil are as follows: clay: 219 g kg^{-1}, silt 314 g kg^{-1}, sand: 467 g kg^{-1}, $CaCO_3$: 426 g kg^{-1}, pH: 7.3, organic carbon: 15 g kg^{-1}, N: 1.6 g kg^{-1}, cation exchange capacity:11.5 cmol(+) kg^{-1}. The soil was contaminated by adding 4000 mg Zn kg^{-1} soil in the form of $ZnCl_2$ and 3200 mg Pb kg^{-1}soil in the form of $PbCl_2$ using saturated aqueous solution and allowing the soil to incubate at 28°C for 2 months. Three olive-derived wastes were used: biphasic olive pomace (OP) obtained from a commercial olive-oil manufacturer (Romeroliva, Deifontes, Granada, Spain); a mature compost (COP) obtained from a mixture of biphasic olive pomace, olive leaves, and manure (total composting time, including maturation period, being 9 months); and a mature vermicompost (VOP) from a mixture of biphasic olive cake and biosolids (8:1 dw:dw) inoculated with earthworms of the specie *Eisenia andrei* (total vermicomposting time being 6 months). Some characteristics of those organic amendments are given in Table 1.

Table 1. Some properties of biphasic olive pomace (OP), compost from biphasic olive pomace (COP) and vermicompost from biphasic olive pomace (VOP)

	OP	COP	VOP
pH (H_2O)	5.2	8.3	7.4
Total organic carbon g kg^{-1}	480	233	351
Humic acid carbon g kg^{-1}	6	49	14
Total polyphenols g kg^{-1}	36	nd	nd
C/N	59	16	24
Total N g kg^{-1}	8.1	15	15
Total Pb mg kg^{-1}	5	6	73
Total Zn mg kg^{-1}	10	44	483

nd: not detected

One kilogram of the natural soil (S) or artificially contaminated soil, unamended (CS) or amended with 250 Mg ha^{-1} of biphasic olive pomace (CS+COP), compost from biphasic olive pomace (CS+COP) and vermicompost from biphasic olive pomace (CS+VOP) were placed in plastic pots (16 cm diameter, 15 cm height). Four replicates per treatment were prepared and placed in a controlled greenhouse. Five seeds of *Dittrichia viscosa* (L) Greuter were sown to each pot, and one month after emergence, the plants were thinned to three per pot. The pots were watered to about 80% of field capacity throughout the experimental period of twelve months. There were four replicates for each treatment. The plants were harvested and separated into shoots (stems and leaves) and roots. Roots and shoots were washed with deionised water, oven-dried at 60 ºC, weighed and stored in plastic vials. Total Zn and Pb contents of roots and shoots were tested by AAS after digestion with HNO_3-$HClO_4$ (2:1) until clear.

After the plant material was harvested, soil samples from each pot were collected for analysis of total, soluble and extractable Pb and Zn, organic carbon, humic acids and nitrogen. The total Zn and Pb concentrations were measured by AAS after the samples were digested in concentrated aqua regia (McGrath and Cunliffe, 1985). Pb and Zn were extracted using two different extractants: a) deionized water (water soluble) using a 1:10 sample:water ratio (Guisquiani et al. 1992), b) 0.005 M DTPA-0.005 M $CaCl_2$ + 0.1 M Triethanolamine, pH 7.3 (DTPA) using a 1:2 sample:extractant ratio (Lindsay and Norwell, 1978). The heavy-metal concentrations in the extracts were measured by AAS.Total organic carbon, total humic acids were determined according to MAPA (1986).

All results are the means of four replicates. Data were subjected to an analysis of variance (ANOVA) using STATGRAPHICS Plus 5.1 statistical software (Statistical Graphics Corp., Princeton, NJ), and Duncan's Multiple Range Test was used to separate the means.

RESULTS AND DISCUSSION

Table 2. Shoot dry weight (g/pot), Zn and Pb concentrations (mg kg^{-1}) in roots and shoots, and translocation factor (TF).

	S	CS	CS+COP	CS+VOP
Shoots (dw)	19.96±1.45^a	11.58±1.43^d	14.41±0.58^c	16.88±0.89^b
		Zn		
Root	100±9^c	1374±46^a	1087±82^b	1146±83^b
Shoot	17±2^c	173±5^a	154±5^b	138±8^b
TF	0.17±0.018^a	0.13±0.007^{bc}	0.14±0.013^b	0.12±0.005^c
		Pb		
Root	77±14^d	2161±60^a	1239±95^b	1089±50^c
Shoot	nd	4±0.46^a	3±0.38^a	2±20.39^a
TF	nd	0.002	0.002	0.002

Key: S: untreated soil, CS, contaminated soil, CS+COP: contaminated soil amended with olive pomace compost and CS+VOP: contaminated soil amended with olive pomace vermicompost.

Values are means±SD (n=4). Means of the some row followed by different letters differ significantly ($p<0.05$).

TF: metal concentration shoots/ metal concentration root

The application of biphasic olive pomace to the contaminated soil (CS+OC) drastically inhibited the germination of seeds of *D. viscosa*, and after two months the scarce pregerminated plants had died (without having grown). This dramatic negative effect could be due to the known high toxicity of this olive waste (Benitez et al. 2004), as consequence mainly of its high content in polyphenols (Table 1). The high toxicity of the biphasic olive pomace prevents its use as soil organic amendment for remediation and agricultural activities.

In the other treatments, the aerial biomass of *D. viscosa* grown in the artificially contaminated soil (CS) was a 42% lower than those of *D. viscosa* grown in the untreated soil (S) (Table 2). The application of compost and vermicompost from olive pomace to the contaminated soil improved significantly the yield of *D. viscosa*, especially when vermicompost was used as a soil amendment. In this treatment (CS+VOP), the aerial biomass of *D. viscosa* was some 45% higher than that found for contaminated soil and only a 15% lower than that found in the natural soil.

In the contaminated soil (CS), *D. viscosa* showed high concentrations of Zn and Pb in roots (Table 2). In the aerial part, the Zn concentration was appreciably lower than in roots, and the Pb concentration was very low. Application of compost and vermicompost from biphasic olive pomace to the contaminated soil significantly decreased Zn and Pb concentrations in roots and shoots. Decreases of Zn and Pb concentrations in plants have been reported when humified organic wastes were applied to contaminated soils (Shuman et al. 2001). The transfer factor (TF), defined as the ratio of Zn or Pb concentrations in shoots to that in root, is a good index of such translocation in a plant. TF was very low (< 0.5) in *D. viscosa* in all treatments, and the application of compost and vermicomposts scarcely changed this factor. The low TF values recorded are particularly interesting if *D. viscosa* is used for phytostabilization, because it would allow its harvest without restrictions.

Table 3. DTPA-extractable and water-soluble Zn and Pb (mg kg^{-1}) in post-harvest soil samples.

	DTPA-Zn	Soluble-Zn	DTPA-Pb	Soluble-Pb
S	22±2^{d}	0.2±0.03^{c}	11±1^{d}	0
CS	955±32^{a}	9.8±0.1^{a}	217±10^{a}	0.55±0.04^{a}
CS+COP	849±20^{b}	4.6±0.4^{b}	120±8^{b}	0.51±0.03^{a}
CS+VOP	773±23^{c}	5.8±0.7^{b}	98±8^{c}	0.43±0.03^{b}

Values are means±SD (n=4). Means of the some column followed by different letters differ significantly ($p<0.05$).

Likewise, the addition of compost and vermicompost from biphasic olive pomace significantly decreased the amounts of water-soluble and DTPA-extractable Zn and Pb in the contaminated soil after harvesting (Table 3). Comparatively, the greatest decreases occurred when the contaminated soil was amended with vermicompost (CS+VOP). However, total Zn and Pb concentrations in soil were scarcely affected (Table 4). Closely positive correlations (P <0.05) were recorded between the concentrations of Zn and Pb in the roots and shoots of the plant and the extractable forms of both metals in the soil after harvesting. Contrasting results have been found on the effect of organic amendments on the extractability and availability of heavy metals in soils (Ebbs et al. 1997; Karaca 2004). These contradictory results are because soil-metal availability is strongly influenced by the humic quality and content in metals and dissolved organic matter of the amendment as well as by the intrinsic properties of the soils (pH, organic matter, clay and iron oxide content, Eh, carbonates) (Kabata-Pendias, 2001; McBride, 1989). In our study, the reductions in extractable forms of Zn and Pb in the soil must be related to chelation, complexation and adsorption between metals in soil and the most humified organic-matter content in both olive-derived amendments, in addition to the possible dilution effect produced when these wastes were mixed with soil (Shuman et al. 2001). In addition, the calcareous nature of the contaminated soil would inhibit the formation of soluble organo-metallic complexes (Jahiruddin et al. 1985), which probably contributed to the inability of the compost and vermicompost from biphasic olive pomace to increase DTPA-extractable Zn and Pb concentrations.

Table 4. Total Zn and Pb concentrations, organic carbon, humic acids, nitrogen, in post-harvest soil samples

	S	CS	CS+COP	CS+VOP
Total Zn mg kg^{-1}	$100±9^{b}$	$3379±119^{a}$	$3110±210^{a}$	$3134±227^{a}$
Total Pb mg kg^{-1}	$77±14^{b}$	$2163±138^{a}$	$1989±213^{a}$	$1975±190^{a}$
Organic carbon g kg^{-1}	$11.9±0.9^{b}$	$10.1±0.7^{b}$	$28.3±2.9^{a}$	$28.4±1.8^{a}$
Humic acids mg kg^{-1}	$2073±118^{b}$	$2223±194^{b}$	$5272±451^{a}$	$5177±9117^{a}$
Nitrogen g kg^{-1}	$2.1±0.1^{b}$	$2.0±0.1^{b}$	$3.79±0.2^{a}$	$3.84±0.1^{a}$

Values are means±SD (n=4). Means of the some row followed by different letters differ significantly (*p<0.05*).

In conclusion, availability of Zn and Pb in an artificially contaminated soil was significantly reduced after application of compost or vermicompost from biphasic olive pomace. In addition, those new organic amendments augmented the amounts of humic acids and nitrogen in the soil, thereby implying improved fertility and quality of the artificially contaminated soil. As consequence, the aerial biomass of the shrub *D. viscosa* was significantly improved, maintaining, in addition, a high capacity to accumulate Zn and Pb in the roots. Therefore, the combined use of biostabilized olive-derived wastes and *D. viscosa* could constitute an alternative and appropriate tool in phytostabilization processes in soils contaminated by heavy metals, especially in the Mediterranean area, where that shrub is distributed widely and olive waste is produced in huge quantities.

Acknowledgments This study was financed by the Comisión Interministerial de Ciencia y Tecnología (CICYT) through project AGL2000-1424-CO2-01. E. Benitez thank to Science and Technology Ministry "Programa Ramon y Cajal" for funding their research. We would also like to thank David Nesbitt for assisting in the translation of the original manuscript into English.

REFERENCES

Baker AJM (1987) Metal tolerance. New Phytol 106:93-111.

Benitez E, Sainz H, Melgar R, Nogales R (2002) Vermicomposting of a lignocellulosic by-product from olive oil industry: a pilot scale study. Waste Manag Res 20:134-142

Benitez E, Melgar R, Nogales R (2004) Estimating soil resilience to a toxic organic waste by measuring enzyme activities. Soil Biol. Biochem 36: 1615-1623

Dabin B (1971) Etude d`une méthode d`extraction de la matière humique du sol. Sci Sol 1: 47-48

De Vos CHR, Schat H, De Waal MAM, Voojs R, Ernst WHO (1991) Increased resistance to copper induced damage of root cell plasmalemma in copper tolerant *Silene cucubalus*. Physiol Plant. 82:523-528.

Ebbs SD, Lasat MM, Brady DJ, Cornish J, Gordon R, Kochian LV (1997) Phytoextraction of cadmium and zinc from a contaminated soil. J Environ Qual 26:1424-1430

Guisquiani PL, Giglioti G, Businelli D (1992) Mobility of heavy metals in urban waste-amended soils. J Environ Qual 21:330-335.

Jahiruddin M, Livesey NT, Cresser MS (1985) Observations on the effect of soil pH upon zinc absorption by soils. Commun Soil Sci Plant Anal 16:909–922.

Kabata-Pendias A (2001) Trace Elements in Soils and Plants, third ed. CRC Press, Boca Raton, USA.

Karaca A (2004) Effect of organic wastes on the extractability of cadmium, copper, nickel, and zinc in soil. Geoderma 122:297-303

Lindsay WL, Norvell WA (1978) Development of DTPA soil test for zinc, iron, manganese and copper. Soil Sci Soc Am J 42:421–428.

MAPA (1986) Métodos oficiales de análisis. Tomo III. Plantas, productos orgánicos fertilizantes, suelos, agua, productos fitosanitarios y fertilizantes inorgánicos, Publicaciones del Ministerio de Agricultura, Pesca y Alimentación, Madrid.

Mari I, Ehaliotis C, Kotsou M, Balis C, Georgakakis D (2003) Respiration profiles in monitoring the composting of by-products from the olive oil agro-industry, Biores Technol 87:331-336.

McBride MB (1989) Reactions controlling heavy metal solubility in soils. Adv Soil Sci 10:1– 56.

McGrath SP, Cunliffe CH (1985) A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludge. J Sci Food Agric 36:794-798.

Melendo M, Benítez E, Nogales R (2002) Assessment of the feasibility of endogenous mediterranean species for phytoremediating lead-contaminates areas. Fresenius Environ Bull 11:1105-1109

Mench M, Vangronsveld J, Clijsters H, Lepp NW, Edwards R (2000). In situ metalimmobilisation and phytostabilisation of contaminated soils. In: Terry N, Bañuelos G (eds) Phytoremediation of contaminated soil and water, Lewis Publishers, Boca Raton, p 323

Shuman LM, Dudka S, Das K (2001) Zinc forms and plant availability in a compost amended soil Water Air Soil Poll 128:1-11

Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50: 775–780.