Effectiveness Of Roots In Preventing Metal Leaching In EDDS-Assisted Phytoextraction With *Brassica carinata* A. Braun. And *Raphanus sativus* L. var. *Oleiformis*

Marianna Bandiera[1], *Giuliano Mosca*[1] *and Teofilo Vamerali*[2]

[1] Department of Environmental Agronomy and Crop Sciences, University of Padova, Viale dell'Università 16, 35020 Legnaro – Padova (Italy)
[2] Department of Environmental Sciences, University of Parma, Viale G.P. Usberti 11/A, 43100 Parma (Italy)
Contact: Marianna Bandiera, Tel. +39.049.8272861, Fax +39.049.8272839, E-mail marianna.bandiera@unipd.it

ABSTRACT

In metal phytoextraction, soil amendment with chelators can improve metal uptake and translocation (to shoots) by increased mobilization. Due to their persistence, chelates may also cause metal leaching, increasing the risk of groundwater contamination.

In this study, the influence on metal leaching of dose and application time of EDDS, a more recent and less persistent chelator, was evaluated at pot level in Ethiopian mustard (*Brassica carinata* A. Braun) and fodder radish (*Raphanus sativus* L. var. *oleiformis*) by cultivating plants in severely metal-contaminated pyrite wastes (As, Co, Cu, Pb, Zn). Plant growth parameters were analysed in relation to water percolation and its metal content.

Compared with untreated controls, four EDDS treatments were examined: doses of 2.5 and 5 mmol EDDS kg^{-1} soil applied one week before harvest, and 1 mmol EDDS kg^{-1} soil repeated five times at 5- and 10-day intervals. To increase root growth, fodder radish treated with 1 mmol at the 5-day interval was also added with 1 mg IBA (indole-3-butyric acid) per kg of soil.

Shoot biomass, leaf area and root length, which were generally reduced in EDDS-treated plants of both species, were negatively correlated with volumes of percolated water. Roots played an important role in reducing metal concentrations in percolation water, due to increased uptake and retention of pollutants. These results suggest that, compared with controls, EDDS applied at harvest increases neither water percolation nor metal leaching, regardless of the dose used, unlike earlier low-dosage treatments.

KEYWORDS: ethylene diamine disuccinic acid (EDDS), Ethiopian mustard, fodder radish, heavy metals, metal leaching, root growth.

INTRODUCTIONS

Phytoextraction is an emerging technology that exploits plant ability to uptake pollutants, such as heavy metals, from the soil for land reclamation purposes. Although many studies have been carried out in the last few years to improve efficiency, there are still some limitations to its applicability, due to scarce metal availability in the soil for uptake and modest translocation from roots to the harvestable biomass.

Aiming at achieving better phytoextraction efficiency, the application of various kind of chelators to increase heavy metal mobilization from the soil solid phase to the bulk pore water has already been proposed in the past (Blaylock et al., 1997), but it may be associated with increased risk of metal leaching into groundwater due to slow plant uptake. For this reason, the choice of chelator and dose is an important task. Among various available compounds, ethylene diamine disuccinc acid (EDDS), a synthetic isomer of EDTA, has recently received increased attention. EDTA was intensively studied in phytoextraction, but its persistence in the environment and toxic effects on soil life (Grčman et al., 2001) may greatly limit its application. Conversely, EDDS is an aminopolycarboxylic acid naturally produced by some microorganisms (Goodfellow et al., 1997), with low persistence in soil. Schowanek et al. (1997) found that the [S,S]-EDDS stereoisomer is rapidly and completed mineralized, with a half-life ranging from 2.5 to 4.6 days, depending on type of medium and associated metal (Vandevivere et al., 2001). Efficient application of EDDS was reported in *Brassica carinata* compared with NTA (nitrilotriacetic acid) (Quartacci et al., 2007), but little information is available on environmental side-effects.

This experiment aims at studying the role of the root system in preventing groundwater metal contamination in EDDS-assisted phytoextraction of pyrite wastes with Ethiopian mustard and fodder radish.

METHODS

Fodder radish (*Raphanus sativus* L. var. *oleiformis*) and Ethiopian mustard (*Brassica carinata* A. Braun) were cultivated in greenhouse for 75 days. Cylindrical opaque PVC pots, with an inner diameter of 57 mm and height of 520 mm, were caulked with pipe insulation sheath. Pots were filled up to a depth of 490 mm with a mixture of metal(loid)-polluted pyrite cinders (Table 1) and sand (1:1 w/w) and their bottom was connected with black tubes to opaque PVC bottles (1 L) in order to collect leachate. One plant per pot was sown at the end of May and regularly watered with 1:2 diluted Hoagland solution. The cinders had been dumped in the past as the by-product of pyrite ore roasting for sulphur extraction at Torviscosa (Udine, north-east Italy) within an area which is part of the site of national interest for reclamation called 'Lagoon of Grado and Marano and adjacent rivers'. The cinders were severely contaminated by As (886 mg kg^{-1}) and trace metals, i.e., Co, Cu, Pb and Zn (100, 1,735, 493 and 2,404 mg kg^{-1}, respectively), exceeding Italian legal limits for agricultural uses several times (Table 1). Organic matter content was very low, pH was 7.3, and electrical conductivity 0.3 S m^{-1}.

Table 1. Metal(loid) concentrations in pyrite wastes, in comparison with Italian legal limits (Italian legislative decree 152/2006, limits for public, private and residential areas).

Metal(loid)	Total content (mg kg^{-1}) (A)	Legal limit (mg kg^{-1}) (B)	(A)/(B)
As	886	20	44/1
Zn	2404	150	16/1
Cu	1735	120	14/1
Co	100	20	5/1
Pb	493	100	5/1
Cd	0.46	2	1/4
Ni	3.1	120	1/38
Cr (total)	2.5	150	1/61
Mn	108	-	-

Compared with untreated controls (C), four EDDS treatments were examined: a low dose of 2.5 mmol kg^{-1} soil (named 2.5) and a high dose of 5 mmol kg^{-1} soil (named 5) applied one week before harvest, and a dose of 1 mmol kg^{-1} soil repeated five times at 5- (named 1x5-5d) and 10-day (named 1x5-10d) intervals, starting from 48 and 28 days after sowing (DAS), respectively. To increase root growth, fodder radish treated with 1 mmol EDDS at the 5-day interval was also added with 1 mg IBA (indole-3-butyric acid) kg^{-1} soil at 10-day intervals (EDDS+IBA).

During the growth period, leaf expansion, leaf chlorophyll content (measured on a Minolta SPAD-502 Chlorophyll Meter) and electrical capacitance (EC, by a capacimeter) of roots (Preston et al., 2004) were monitored at weekly intervals, starting from 11 July (47 DAS). At harvest, total root length (live and dead roots) was assessed by automatic analysis of root digital images acquired through a flatbed scanner. Shoot dry weight was measured after drying at 105°C, and concentrations of metals in shoots and percolated water were determined by ICP-OES.

RESULTS AND DISCUSSIONS

Harvestable biomass of plants treated with EDDS was generally reduced compared with untreated controls, especially for repeated applications, although this effect was less evident in mustard than in radish (-42% vs. -61%, average of all EDDS treatments). Root activity, expressed as the integral of EC curves, also decreased as a consequence of EDDS: the effect was more marked when EDDS application started earlier, regardless of soil addition with IBA (Figure 1). There was a good correlation between root EC and length (R^2: mustard 0.39, radish: 0.58), suggesting that EC may be used as an alternative way of non-destructive root observation. For the 1x5-5d in mustard only, EDDS enhanced root growth (length), although this was not evident from the EC values. As this treatment led to the highest concentration of various metals (summation) in the shoot, EC may not be representative of root uptake potential. However, this effect may be due to greater root turnover.

Water percolation was negatively correlated ($P \leq 0.01$) with shoot weight (R^2=0.63 and 0.78 for *B. carinata* and *R. sativus*, respectively) and leaf expansion (R^2=0.64 and 0.68). Poorer root EC and length were also important causes of water percolation (EC: R^2=0.89 and 0.65; root length: R^2=0.72 and 0.41) and metal leaching (EC: R^2=0.74 and 0.28; root length: R^2=0.67 and 0.14). Repeated EDDS applications caused significant loss of metal(loid)s from the wastes into percolated water (Figure 1) as a consequence of impaired growth and metal mobilization. Most of the leachate was accounted for by zinc (about 60%) in the case of EDDS application at harvest, and by copper (up to 99%) in repeated treatments, which confirms the high affinity of EDDS for Cu.

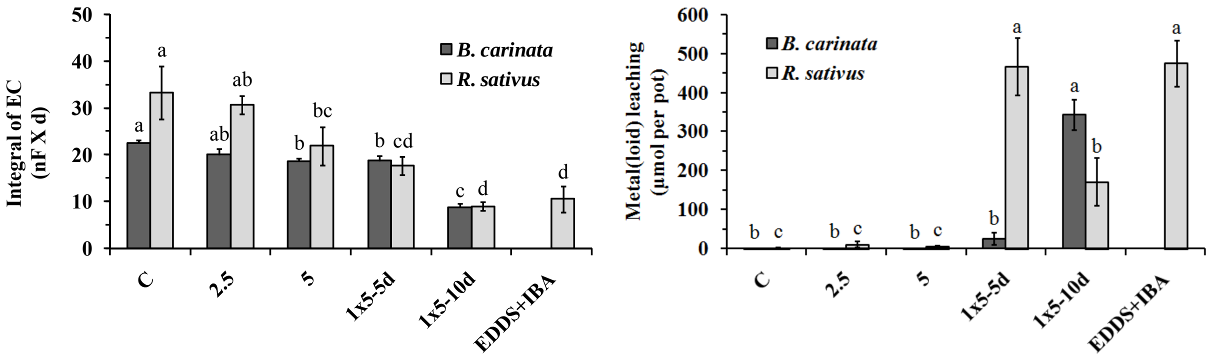

Figure 1. Integral over time of root capacitance curves (left) and metal(loid) leached (right) (±S.E). Letters: differences among treatments within same species (LSD test, $P \leq 0.05$).

EDDS always allowed higher metal concentrations in shoot (Figure 2), especially for Cu, Co, Pb, but not for Zn and arsenic. Removals of metal(loid)s, as a result of biomass and metal concentration, were increased by the lowest dose at harvest (2.5 mmol) of mustard only (Figure 2).

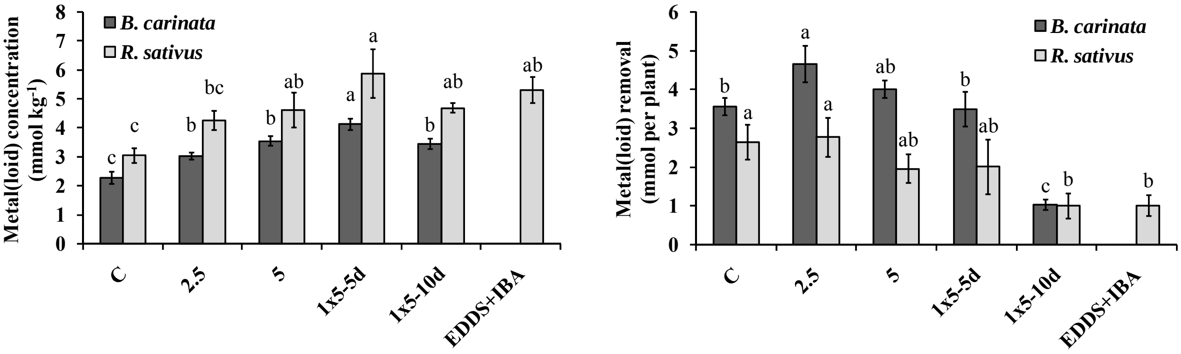

Figure 2. Concentration (summation of moles) and total removals of various metal(loid)s (Co, Cu, Pb, Zn, Cd, Cr, Mn, Ni, As) (±S.E.). Letters: differences among treatments within same species (LSD test, P≤0.05).

Root growth was directly involved in enhancing phytoextraction, since a positive correlation was highlighted between root length and removals of single metal(loid)s (As, Co, Cu, Zn, Cd, Mn, Ni), except Pb and Cr, or their summation. Analysis of metal(loid)s content in root tissues, currently in progress, will define the contribution of the root system in retaining metals, which has recently been found of great importance (Vamerali et al., 2009).

Our results show that *Brassica carinata* has a better adaptability in pyrite wastes and less sensitivity to EDDS than *Raphanus sativus* var. *oleiformis*. In mustard a single moderate dose of EDDS (2.5 mmol) applied before harvest is suggested, to induce higher phytoavailability of HM without creating risks of metal leaching into groundwater.

REFERENCES

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. 31, 860-865.

Goodfellow, M., Brown, A.B., Cai, J.P., Chun, J.S., Collins, M.D. 1997. Amycolatopsis japonicum sp. nov, an actinomycete producing (S,S)-N,N0-ethylenediaminedisuccinic acid. Syst. Appl. Microbiol. 20, 78-84.

Grčman, H., Velikonja-Bolta, Š, Vodnik, D., Kos, B., Leštan, D. 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil 235: 105-114.

Preston, G.M., McBride, R.A., Bryan, J., Candido, M. 2004. Estimating root mass in young hybrid poplar trees using the electrical capacitance method. Agrof Syst 60 (3), 305-309.

Quartacci, M.F., Irtelli, B., Baker, A.J.M., Navari-Izzo, F. 2007. The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica carinata*. Chemosphere 68, 1920-1928.

Schowanek, D., Feijtel, T.C.J., Perkins, C.M., Hartman, Federle, T.W., Larson, R.J. 1997. Biodegradation of [S,S], [R,R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere. 34, 2375-2391.

Vamerali, T., Bandiera, M., Coletto, L., Zanetti, F., Dickinson, N.M., Mosca, G. 2009. Phytoremediation trials on metal- and arsenic-contaminated pyrite wastes (Torviscosa, Italy). Environ Poll 157, 887-894.

Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C.J., Schowanek, D.R. 2001. Biodegradation of metal-[S,S]-EDDS complexes. Environ. Sci. Technol. 35, 1765-1770.