The effect of EDDS and citrate on the uptake of lead in hydroponically grown *Matthiola flavida*

Abstract

Root and shoot lead concentrations and the impact of chelating agents on these were investigated in two populations of the novel metallophyte *Matthiola flavida*. Plants were exposed in hydroponics to Pb(NO$_3$)$_2$, supplied alone, or in combination with citric acid, or EDDS. When supplied at concentrations expected to bind about 95% of the Pb in a solution containing 1-µM Pb (1000 µM citrate or 3.1 µM EDDS, respectively), the root and shoot Pb concentrations were dramatically lowered, in comparison with a 1-µM free ionic Pb control exposure. A 1-mM EDDS + 1-µM Pb treatment decreased the plants’ Pb concentrations further, even to undetectable levels in one population. At 100 µM Pb in a 1-mM EDDS-amended solution the Pb concentration increased strongly in shoots, but barely in roots, in comparison with the 1-µM Pb + 1-mM EDDS treatment, without causing toxicity symptoms. Further increments of the Pb concentration in the 1-mM EDDS-amended solution, i.e. to 800 and 990 µM, caused Pb hyperaccumulation, both in roots and in shoots, associated with a complete arrest of root growth and foliar necrosis. *M. flavida* seemed to be devoid of constitutive mechanisms for uptake of Pb-citrate or Pb-EDDS complexes. Hyperaccumulation of Pb-EDDS occurred only at high exposure levels. Pb-EDDS was toxic, but is much less so than free Pb. Free EDDS did not seem to be toxic at the concentrations tested.

Key words: citric acid; EDDS; hydroponics; lead; *Matthiola flavida*.

1. Introduction

Heavy metal contamination of soils is a widespread global problem. The cleaning-up of metal contaminated land by traditional physico-chemical methods can be very costly and, moreover, destructive to the soil. Phytoextraction has been proposed as an environmentally friendly in situ remediation technology for soils contaminated with heavy metals (Salt et al., 1998; Zhao et al., 2010). Synthetic chelators have been used to supply
plants with micronutrients in both soil culture and hydroponic culture for more than 50 years (Evangelou et al. 2007). Chelator application has been proposed to improve the efficiency of phytoextraction (Lasat, 2002). Chelating soil additives that form water-soluble metal complexes can readily desorb metals from the soil matrix, thus enhancing their concentrations in the soil solution, which in turn increases their mobility (Schmidt, 2003). In cases where diffusion or convection to the root surface is limiting the metal’s uptake into the root, such additives are expected to enhance the metal accumulation in the plant body, provided that chelate splitting at the root surface occurs readily, or that the metal can (also) effectively be taken up in the chelated form. In the latter case, chelating agents can also be expected to enhance plant metal accumulation when a metal’s uptake is limited by its concentration in the soil solution, rather than by its transport from the bulk soil to the root surface. Chelating agents, such as organic acids, amino acids and synthetic chelators, as well as biosurfactants, have been previously studied for their potential to enhance metal accumulation in plants. It is beyond doubt that chelators can enhance the accumulation of particular heavy metals, in plants growing in soil, but also in hydroponics, which would imply that metals can be taken up as undissociated chelates, rather than as free ions, after chelate splitting at the root surface prior to uptake (Gunawardana et al., 2010). The mechanisms underlying the apparent uptake of metal chelates are elusive. At least some (natural) metal chelates can be transported across biological membranes. However, there is no evidence of the existence of a transporter that could mediate the transport of carboxylic or synthetic amino-carboxylic chelates across the plasma membrane of root cells. Alternatively, it has been proposed that metal chelates would be taken up via the apoplastic pathway, and pass through the endodermis via the cracks created by the lateral outgrowth of root primordia (Luo et al., 2006; Tandy et al., 2006). It has also been proposed that chelating agents, when applied at high dose, may kill transfer cells, which creates another apoplastic pathway for metal chelate entry into the vasculature (Niu et al., 2011).

The metal-chelating aminopolycarboxylic acid, ethylene diamine tetraacetate (EDTA), has been the most frequently used agent to enhance plant accumulation of heavy metals (Kos and Lestan, 2003; Meers et al., 2005). However, due to its high resistance to biological degradation and,
therefore, high environmental persistence, which may lead to secondary contamination and enhance the risk of leaching of metals to the groundwater (Tandy et al., 2006; Zhao et al., 2010), it is now considered unsuitable for field use. For this reason, the present study has focused on a synthetic, but biodegradable chelator, EDDS, and a natural one, citric acid.

(S,S)-N,N’-ethylenediamine disuccinic acid (EDDS) is a biodegradable structural isomer of EDTA (Vandevivere et al., 2001; Tandy et al., 2006), which has been shown to enhance the uptake of heavy metals such as Cu, Cd, Pb, Zn and Ni in various plant species (Luo et al., 2006). Citric acid is a low molecular weight organic acid present at high concentrations in the vacuoles of photosynthetic plant tissues (Gunawardana et al., 2010). It is also exuded from plant roots into the soil and, therefore, has been proposed as an alternative to synthetic chelators for use in chelator-assisted phytoextraction (Evangelou et al., 2006).

Matthiola flavida is a perennial herb of the Brassicaceae family. It is a novel facultative metallophyte from Iran, which seems to be capable of considerable foliar lead accumulation in nature (A. Mohtadi, unpublished results), and also has ability to grow and hyperaccumulate lead in controlled pot experiment (Mohtadi et al., 2012b), and thus potentially suitable for the phytoremediation of Pb-contaminated soils.

The primary aim of this work is to assess and explain the effects of EDDS and citrate acid on the uptake of lead in hydroponically grown Matthiola flavida. A second aim is to compare the Pb accumulation capacities of plants from a metallicolous population and a nearby non-metallicolous one in a controlled experiment.

2. Materials and methods

2.1. Plant materials and experimental conditions

Seeds of Matthiola flavida Boiss were collected at the Irankouh mining site and at a non-contaminated site at Mount Sofeh, both in Central Iran. The soil at the Irankouh mining site contains Pb at concentrations ranging from 4200 to 12000 ppm, as measured after nitric and perchloric acid extraction (Ghaderian et al., 2007). Seeds were sown in peat soil and after
three weeks seedlings of both populations were transferred to hydroponic culture, in 1-L polyethylene pots (three plants per pot) containing a modified half-strength Hoagland’s solution composed of 3 mM KNO₃, 2mM Ca(NO₃)₂, 1 mM NH₄H₂PO₄, 0.50 mM MgSO₄, 20µM Fe(Na)-EDTA, 1 µM KCl, 25 µM H₃BO₃, 2 µM MnSO₄, 2 µM ZnSO₄, 0.1 µM CuSO₄ and 0.1 µM (NH₄)₆Mo₇O₂₄, in demineralized water, buffered with 2 mM 2-(N-morpholino)ethanesulphonic acid (MES), pH 5.5, adjusted with KOH. Nutrient solutions were renewed weekly and plants were grown in a growth chamber (20/15 °C day/night; light intensity 200 µE m⁻² s⁻¹, 14 h day⁻¹, relative humidity 75%).

2.2. Experimental design

After 10 days of pre-culture, the plants were transferred to the test solution, which was composed as above, but with macronutrients supplied at 0.1-strength and micronutrients at full strength, and without NH₄H₂PO₄ and Fe(Na)-EDTA, to avoid precipitation of Pb phosphate and complexation of Pb with EDTA, owing to displacement of Fe(III). In the first experiment 1µM of Pb was added without chelating agents, as well as in combination with citrate (100, or 1000 µM, as C₆H₅Na₃O₇) and EDDS (3.1, or 1000 µM, as C₁₀H₁₃N₂Na₃O₈). Each treatment had four replicate pots with three plants each. These treatments were maintained for 1 week. In a second experiment, we also tested 100 µM, 800 µM and 990 µM Pb(NO₃)₂, in combination with 1000 µM EDDS. In both experiments root growth was measured by staining the roots with carbon powder at the start of the exposure, as described in Schat and Ten Bookum (1992). After one week the increase in root length was measured and the plants were harvested for analysis. Prior to harvest the roots were desorbed through rinsing with Na₂EDTA (20 mM) for 15 min.

2.3. Determination of Pb concentration

Pb concentrations were determined in shoots and desorbed roots (4 replicate samples of 3 pooled plants per population per concentration). Pb was determined by digesting 20-50 mg of oven-dried plant material in 1 ml of a 1 to 4 (v/v) mixture of 37% (v/v) HCl and 65% (v/v) HNO₃ in
Teflon cylinders for 7 h at 140 °C, after which the volume was adjusted to 5 ml with demineralized water. Pb was determined on a flame atomic absorption spectrophotometer (AAS, Perkin Elmer 100).

2.4. Statistical analysis

All the data were statistically analyzed using two-way model 1 ANOVA, with treatment and population as main factors. A posteriori comparisons of individual means were performed using Tukey’s test. In case of inhomogeneity of variances, data were subjected to logarithmic transformation prior to analysis.

3. Results

After one week of exposure to 1 μM free Pb, the shoot and root Pb concentrations were roughly the same in both populations, i.e. ±100 and ±3000 μg g⁻¹ d. w (Fig. 1). Addition of 1000 μM citrate or 3.1 μM EDDS to the 1-μM Pb(NO₃)₂-amended nutrient solution dramatically decreased the accumulation of Pb in roots (p<0.001 in Tukey’s), to a comparable degree in both populations (p>0.05 for population x treatment interaction in ANOVA), in comparison with the 1-μM free Pb control and the 100-μM citric acid treatment, the latter being without a significant effect (Fig. 1). The Pb concentrations in shoots were comparably decreased in the 3.1-μM EDDS treatment, or even more so in the 1000-μM citrate treatment (p<0.001), again to a comparable degree in both populations (p>0.05 for population x treatment interaction) (Fig. 1).

Adding EDDS at a 1-mM concentration caused a further decrease of the plant Pb concentration, particularly in the root. In the Sofeh population, the shoot to root Pb concentration ratio increased from 0.03 in the EDDS-free control to 0.12 and 0.40 in the 3.1- and 1000-μM EDDS treatments, respectively (Table 1).

In the Irankouh population the root and shoot Pb concentrations in the 1000-μM EDDS treatment even fell below the detection limit of the method. Both the 3.1- (data not shown) and 1000-μM EDDS treatments
(Table 2) were without significant effects on root growth and did not cause any other apparent toxicity symptoms. The 1-µM free Pb control, on the other hand, significantly inhibited root growth, but was without effects on shoot biomass (Table 2).

When supplying Pb at 100 µM, in combination with 1 mM EDDS, the Pb concentrations in shoots were higher than in the 1-µM free Pb controls, but those in roots were lower by more than one order of magnitude, leading to shoot to root Pb concentration ratios around 5 (Tables 1, 3). Further increments of the Pb concentration in the nutrient solution caused Pb hyperaccumulation, both in shoots and in roots, and progressively decreased the shoot to root concentration ratios again, to 1.0 and 0.3 at 800 and 990 µM, respectively (Tables 1, 3). The 100-µM Pb treatment was without apparent toxic effects (Table 2), but the 800- and 990-µM treatments, however, almost completely arrested root growth and caused heavy foliar necrosis and a partial die-back of the shoot (Table 2).

4. Discussion and conclusions

Our results obtained both with EDDS and citrate addition demonstrated that *M. flavida* barely or not took up Pb from chelator-amended solutions, when virtually all of the Pb is chelated, and the Pb-chelator complex concentration is low (1 µM). However, at high Pb concentrations in the nutrient solution (≥ 100 µM), there was considerable Pb accumulation, or even hyperaccumulation, from EDDS-amended solutions in which virtually all the Pb is chelated. These results show that *M. flavida* can accumulate Pb from EDDS-amended nutrient solutions. The uptake, translocation and toxicological kinetics of Pb accumulated from EDDS-amended solutions, however, were very different from those in non-amended ones. Taken together, our results clearly suggest that Pb was taken up as Pb-EDDS complex. The uptake of this complex is apparently a low-affinity phenomenon. Even when supplied at 100 µM, the uptake of the complex was, assuming a 4:1 shoot to root dry weight ratio, still lower than half of that of free Pb supplied at 1 µM. On the other hand, the root-to-shoot translocation of the complex was by far superior to that of Pb supplied in the free ionic form, i.e. a shoot to root concentration ratio of about 5 at 100 µM Pb + 1 mM EDDS, in comparison with 0.03 at 1
µM Pb without EDDS, leading to higher foliar Pb concentrations in the 1-
mM EDDS treatment. Further increases of the Pb supply, in the presence
of EDDS, even led to Pb hyperaccumulation in the leaves, but
considerably decreased the translocation factor again. We were unable to
assess whether Pb can also be hyperaccumulated from citrate-amended
solutions, since solutions with more than 1 mM citrate acidified quickly
owing to microbial infestation.

The mechanism of Pb-EDDS hyperaccumulation remains elusive. In any
case, complete chelation of Pb with either citrate or EDDS seemed to
decrease Pb uptake in the Irankouh population, as well as in a number of
the plants from Sofeh, to undetectable levels, when the concentration of
the complex was low. This may be taken to suggest that vital *M. flavida*
roots are virtually devoid of constitutive systems to take up Pb in
complexed form, regardless of whether the chelator is natural or
synthetic, suggesting that Pb-complex uptake may be triggered by one or
another toxic lesion in the roots, either owing to the complex, or to the
chelator itself. At high concentrations, whether under hydroponic culture
or in soil, passage cells—physiological barriers that control ion
absorption—are injured or killed, and offer additional channels of entry
to the root xylem for the complex and transfer to shoot (Gunawardana et
al., 2010; Niu et al., 2011). In our experiment it seems that the complex
itself was much more toxic than the free chelator, because toxicity was
not apparent at lower Pb concentrations (1 and 100 µM) combined with
1000 µM EDDS. In addition, Pb accumulation was higher at 990 µM Pb
than at 800 µM Pb, which suggests that free EDDS is not necessary, at
least not at high concentrations, for Pb-EDDS hyperaccumulation. So it
may be the complex itself that induces its own hyperaccumulation, as
soon as some critical exposure threshold has been exceeded. Given our
observation that there was no apparent toxicity in the 100 µM Pb + 1000
µM EDDS treatment, it seems that there could be a non-toxic
concentration range of Pb-EDDS exposure, where the Pb-EDDS
accumulation in the leaves exceeds that of free Pb. However, the
exposure time was only one week and toxic lesions could still develop in
a longer-term experiment. Moreover, under these circumstances the
complex did not accumulate in the root, which could have prevented root
growth inhibition.
We did not find considerable differences in Pb accumulation between the two populations. This is not in agreement with a previous experiment, in which the foliar Pb accumulation, under exposure to free Pb, was about two times higher in the metallicolous population, in comparison with the non-metallicolous one (Mohtadi et al., 2012a). We do not know how to explain this discrepancy, but it is possible that the difference in exposure time, i.e. one week in this, and two weeks in the previous experiment, might have played a role. In any case, in the present experiment we did not observe considerable inter-population differences, irrespective of the Pb speciation in the nutrient solution.

In conclusion, (1) *M. flavida* does not seem to possess constitutive mechanisms to take up Pb-citrate or Pb-EDDS complexes, (2) *M. flavida* can hyperaccumulate Pb-EDDS, but probably only under excessive, probably toxic exposure and, (3) although there might be a non-toxic concentration window for Pb-EDDS exposure which allows enhanced foliar Pb accumulation, EDDS-assisted Pb phytoextraction will probably kill the phytoremediator crop.
Fig. 1. Shoot and root Pb concentrations (µg g⁻¹ d. w.) in two populations of *M. flavida* (mean ± SE) after exposure to 1-µM Pb, at different citric acid and EDDS concentrations (µM) for 1 week. Pb was undetectable in the Irankouh population in the 1-mM EDDS treatment.

**Table 1.** Shoot/root ratio of Pb concentrations in two populations of *M. flavida* after 7 days of exposure to different concentrations of Pb and EDDS.

BD r/s = Pb concentrations below detection limits in roots and shoots

ND = not determined
Table 2. Root length growth (mm) after 5 days and total plant fresh weight (g) after 12 days of exposure to different concentrations of Pb in the presence of 1 mM EDDS in the Irankouh population of M. flavida (mean ± SE).

* = significantly different from control at p < 0.05; *** = significantly different from control at p < 0.001. N = shoot necrotic, largely dehydrated
<table>
<thead>
<tr>
<th></th>
<th>Root length growth</th>
<th>Total plant f. w.</th>
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<tbody>
<tr>
<td>Control (no Pb, no EDDS)</td>
<td>47.7±7.5</td>
<td>0.70±0.13</td>
</tr>
<tr>
<td>1 µM Pb, no EDDS</td>
<td>34.4±5.5*</td>
<td>0.67±0.08</td>
</tr>
<tr>
<td>1 µM Pb, 1 mM EDDS</td>
<td>53.6±9.1</td>
<td>0.91±0.30</td>
</tr>
<tr>
<td>100 µM Pb, 1 mM EDDS</td>
<td>51.8±11</td>
<td>0.88±0.21</td>
</tr>
<tr>
<td>800 µM Pb, 1 mM EDDS</td>
<td>1.7±0.8***</td>
<td>N</td>
</tr>
<tr>
<td>990 µM Pb, 1 mM EDDS</td>
<td>0.7±0.5***</td>
<td>N</td>
</tr>
</tbody>
</table>

**Table 3.** Pb concentrations (µg g⁻¹ d. w.) in two populations of *M. flavida* (mean ± SE) after exposure to different concentrations of Pb, in a 1-mM EDDS background, for 1 week.

NT = not tested

<table>
<thead>
<tr>
<th></th>
<th>Sofeh</th>
<th>Irankouh</th>
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<tr>
<td>shoot</td>
<td>root</td>
<td>shoot</td>
</tr>
<tr>
<td>100 µM Pb, 1 mM EDDS</td>
<td>283±63</td>
<td>48±10</td>
</tr>
<tr>
<td>800 µM Pb, 1 mM EDDS</td>
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<td>990 µM Pb, 1 mM EDDS</td>
<td>7126±779</td>
<td>17655±2530</td>
</tr>
</tbody>
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**References**


L.): anatomical, chemical and histochemical analysis. Plant and Soil 343, 303-312


