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# Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS)

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ABSTRACT

The use of plants for phytoextraction of heavy metals from contaminated soil is limited by the ability of the plants to grow on these soils and take up the target metals, as well as by the availability of the metals for plant uptake in the soil solution. The hypotheses of this study were that the growth-promoting phytohormone auxin (indole-3-acetic acid, IAA) can alleviate toxic effects of metals on plants and increase metal phytoextraction in combination with the biodegradable chelating agent ethylene diamine disuccinic acid (EDDS). To test these hypotheses we performed two sets of experiments with sunflowers (*Helianthus annuus* L.) in hydroponic solution. In the first set of experiments, five IAA concentrations (0, $10^{-12}$, $10^{-11}$, $10^{-10}$, $10^{-9}$ M) were applied in combination with Pb (2.5 μM) or Zn (15 μM). In the second set of experiments we applied combinations of IAA (0 or $10^{-10}$ M) and EDDS (0 or 500 μM) to Pb or Zn-stressed sunflowers.

Root and shoot growth of metal-stressed plants were most effectively increased with $10^{-10}$ M IAA, and also the extraction of both metals was significantly increased at this treatment level. IAA reduced the negative metal effects, such as reduced shoot and root dry weight, root length, root volume and root surface area. EDDS significantly decreased metal uptake by the plants, thus reducing metal stress and promoting plant growth. The combined application of IAA with EDDS significantly increased Zn uptake in comparison to EDDS only treated plants. The experiments indicate that IAA can alleviate toxic effects of Pb and Zn on plant root and shoot growth and can in combination with chelants such as EDDS increase the phytoextraction potential of these plants.

## 1. Introduction

Phytoextraction potentially is an attractive strategy to clean up metal-polluted agricultural soils, provided that sufficiently high extraction rates can be achieved. Restrictions on phytoextraction are given by the plant's ability to grow in the polluted soil, to take up the target metals and by the availability of the target metals in the soil for plant uptake. Only metals that are in the soil solution can be taken up by plants. The uptake takes place at the interface between soil solution and root tissue. The larger the contact area between roots and soil solution, the higher the potential uptake. Due to the formation of special tissue layers with suberin incrustations in their cell walls, in particular the epidermis layer with the well-known casparian strips between root cortex and stele, there is a gradient in the uptake of water and solutes along the root axis, declining from the apex to the basal zones. These layers act as an efficient barrier against the uptake of aqueous solutes via the apoplast (Marschner, 1995). These barriers are not fully developed at the root tips and also become disrupted in the basal zones where emerging lateral roots penetrate the cortex, forming leaks for uncontrolled apoplastic uptake of solutes into the xylem of the roots (Haynes, 1980; Clarkson, 1996; Tandy et al., 2006a).

Increasing root surface area, in particular of those zones with incomplete or leaky endo- and exodermis layers, may be helpful to increase metal uptake in phytoextraction. The growth-promoting phytohormone auxin is known to induce root growth by enhancing cell division, cell extension and inducing lateral root growth (Taiz and Zeiger, 2000). Rhizosphere bacteria, such as some strains of *Pseudomonas* and *Acinetobacter*, were found to produce indole-3-acetic acid (IAA), the most common auxin in plants, and thereby stimulate root elongation and lateral root production (Lippmann et al., 1995). Root inoculation with rhizosphere bacteria strains had similar effects on root morphology as exogenous IAA application (Lippmann et al., 1995). In particular IAA increased root and sometimes also shoot growth of plants that were stressed by salinity or heavy metals (Chaudhry and Rasheed, 2003; Sheng and Xia, 2006; Egamberdieva, 2009). Leinhos and Bergmann

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(1995) found that IAA alleviated drought stress and suggested that exogenously applied IAA may serve in mediating morphological reactions of plants in response to stresses, in particular by increasing root growth. The growth-promoting effect of auxin on stressed plants may be used for phytoextraction purposes.

In order to enhance phytoextraction it has been proposed to increase limited availability of polluting soil metals for plant uptake by adding chelating agents such as ethylene diamine tetraacetic acid (EDTA), nitrilotriacetic acid (NTA) or ethylene diamine disuccinic acid (EDDS) to the soil (Blaylock et al., 1997; Huang et al., 1997; Kulli et al., 1999; Tandy et al., 2006b). Increased metal availability may, however, also increase metal stress to plants. Tandy et al. (2006b) showed that the application of EDDS to soil, while increasing the uptake of Cu and Pb by sunflowers, decreased plant dry weight in pot experiments. Liu et al. (2007) found that the adverse effect of chelating agents on plant growth could be reduced by the application of IAA. López et al. (2005) furthermore found that the addition of IAA together with EDTA increased Pb uptake by alfalfa.

Based on these findings, the aim of this study was to test the hypotheses that: (1) auxin is capable of alleviating metal stress on plants, and (2) to test the potential of auxin to increase metal phytoextraction in combination with a chelant. As experimental system we chose sunflowers grown in hydroponic solution, treated with the heavy metals Pb and Zn, the auxin IAA and the chelating agent EDDS. Hydroponics were chosen because we were interested in the effects of these chemicals in solution on plant growth and metal uptake, separating them from effects of the treatments on metal partitioning between soil solution and solid phases.

## 2. Materials and methods

### 2.1. Experimental setup

In preliminary experiments we screened sunflower growth at Pb concentrations of 125, 62.5, 31, 15.5, 7.8, 3.9, 2.5 and 1 μM and Zn concentrations of 122, 61, 30.5 and 15 μM in nutrient solution to determine at which concentrations the metals stunted plant growth without killing them. On the basis of these experiments we chose treatments with 2.5 μM Pb and 15 μM Zn for this study.

We performed two sets of experiments (Table 1). In the first set of experiments we applied IAA concentrations between $10^{-9}$ and $10^{-12}$ M as well as no IAA (control), with and without metal stress (15 μM Zn, 2.5 μM Pb or no metal). The treatments were replicated four times. In the second set of experiments we grew seedlings in solutions with Zn, Pb or no metal, and applied IAA (0 or $10^{-10}$ M) and EDDS (0 or 500 μM) in four combinations to each metal treatment. Each treatment was replicated five times.

All experiments were carried out in a climate chamber at a 16 h (22 °C)/7 h (15 °C) day/night cycle with 0.5 h transition times between day and night phases. Seeds of *Helianthus annuus* L. (cv. Sanluca) were germinated in silica sand. Six days old seedlings were transferred into aerated brown 1-L bottles (one seedling per bottle) containing nutrient solution. Two different nutrient solutions were prepared. The first was a modified 10% Hoagland nutrient solution in which NaFe(II)EDTA was replaced by $FeSO_4 \cdot 7H_2O$ to avoid interference between EDTA and EDDS. The other solution was the treatment solution containing metal contaminant, auxin and chelant according to the before-mentioned treatments. Here, all micronutrients and $KH_2PO_4$ were omitted to avoid competition between metals and metal precipitation, in particular of Pb phosphate. To maintain a solution pH of 6, 2 mM 2-(N-morpholino)ethanesulfonic acid was added as buffer to both solutions. The solutions were always freshly prepared before application and alternately applied for periods of 3 d for a total of 18 d, starting with the Hoagland solution, so that the two solutions were each applied three times during the experiment. This alternation maximized the treatment time while avoiding P and micronutrient deficiency stress.

### 2.2. Root morphometry

At harvest, plants were immediately separated into roots and shoots and weighed.

**Table 1**

Root growth parameters. Mean values of root growth parameters (dry weight, volume, surface area, length, diameter and density) of sunflowers grown in the IAA screening experiment with Pb or Zn (set 1) and in the IAA + EDDS with Pb or Zn (set 2). Standard errors of the means are in parentheses (set 1: $n = 4$, set 2: $n = 5$). Different letters indicate significant differences between the treatments ($p < 0.05$).

| Experiment | Treatment | Volume (cm$^3$) | Surface area (cm$^2$) | Length (m) | Diameter (mm) | Density (mg cm$^{-3}$) |
|---|---|---|---|---|---|---|
| 1, Pb | Control | 3.26 (0.15) a | 303 (18) a | 2.27 (0.19) a | 0.45 (0.01) a | 35 (1) a |
| | 2.5 μM Pb | 1.92 (0.16) b | 188 (15) b | 1.48 (0.11) b | 0.42 (0.00) b | 40 (4) ab |
| | $10^{-10}$ M IAA | 5.03 (0.19) d | 530 (30) c | 4.49 (0.36) c | 0.39 (0.01) b | 35 (0) a |
| | 2.5 μM Pb + $10^{-10}$ M IAA | 2.58 (0.24) c | 245 (26) a | 1.87 (0.24) ab | 0.44 (0.02) a | 42 (1) b |
| 1, Zn | Control | 2.46 (0.16) ab | 394 (24) a | 5.08 (0.39) a | 0.26 (0.01) a | 76 (3) a |
| | 15 μM Zn | 1.63 (0.27) c | 260 (39) b | 3.32 (0.49) b | 0.25 (0.01) a | 63 (5) b |
| | $10^{-10}$ M IAA | 2.80 (0.34) a | 419 (63) a | 5.04 (0.91) a | 0.27 (0.01) a | 70 (0) ab |
| | 15 μM Zn + $10^{-10}$ M IAA | 1.75 (0.13) bc | 265 (17) b | 3.22 (0.19) b | 0.27 (0.00) a | 78 (2) a |
| 2, Pb | Control | 2.83 (0.30) ac | 283 (33) a | 2.27 (0.29) a | 0.41 (0.01) ad | 35 (1) abc |
| | 2.5 μM Pb | 2.35 (0.23) a | 230 (26) a | 1.79 (0.23) a | 0.41 (0.01) a | 38 (1) c |
| | $10^{-10}$ M IAA | 3.08 (0.27) ab | 300 (26) ac | 2.34 (0.21) a | 0.41 (0.00) a | 33 (1) a |
| | 2.5 μM Pb + $10^{-10}$ M IAA | 3.92 (0.48) bc | 414 (54) bc | 3.50 (0.50) b | 0.39 (0.01) cd | 37 (0) cb |
| | 500 μM EDDS | 4.13 (0.41) b | 492 (40) b | 4.70 (0.34) b | 0.34 (0.01) be | 35 (1) abc |
| | 500 μM EDDS + 2.5 μM Pb | 3.97 (0.48) b | 437 (42) b | 3.84 (0.27) b | 0.36 (0.01) cf | 33 (2) ab |
| | 500 μM EDDS + $10^{-10}$ M IAA | 3.61 (0.35) bc | 411 (34) b | 3.75 (0.25) b | 0.35 (0.01) ef | 36 (1) abc |
| | 500 μM EDDS + 2.5 μM Pb + $10^{-10}$ M IAA | 4.12 (0.56) b | 458 (58) b | 4.07 (0.48) b | 0.36 (0.01) c | 34 (1) abc |
| 2, Zn | Control | 3.78 (0.43) ac | 397 (48) ae | 3.34 (0.44) ad | 0.39 (0.00) a | 37 (1) ac |
| | 15 μM Zn | 2.78 (0.29) b | 286 (27) bd | 2.36 (0.19) b | 0.36 (0.03) a | 40 (1) c |
| | $10^{-10}$ M IAA | 2.52 (0.29) b | 255 (29) bd | 2.06 (0.24) b | 0.40 (0.00) a | 35 (1) ad |
| | 15 μM Zn + $10^{-10}$ M IAA | 2.85 (0.24) bc | 306 (28) ad | 2.62 (0.26) bd | 0.38 (0.00) a | 41 (1) c |
| | 500 μM EDDS | 4.34 (0.32) a | 535 (33) c | 5.26 (0.27) c | 0.33 (0.00) a | 34 (1) ad |
| | 500 μM EDDS + 15 μM Zn | 3.69 (0.41) ac | 411 (40) ae | 3.66 (0.32) a | 0.36 (0.01) a | 34 (1) ad |
| | 500 μM EDDS + $10^{-10}$ M IAA | 3.44 (0.35) ab | 399 (43) a | 3.69 (0.42) a | 0.35 (0.00) a | 35 (1) ad |
| | 500 μM EDDS + 15 μM Zn + $10^{-10}$ M IAA | 4.31 (0.27) a | 514 (25) ce | 4.88 (0.17) c | 0.24 (0.06) b | 33 (1) bd |

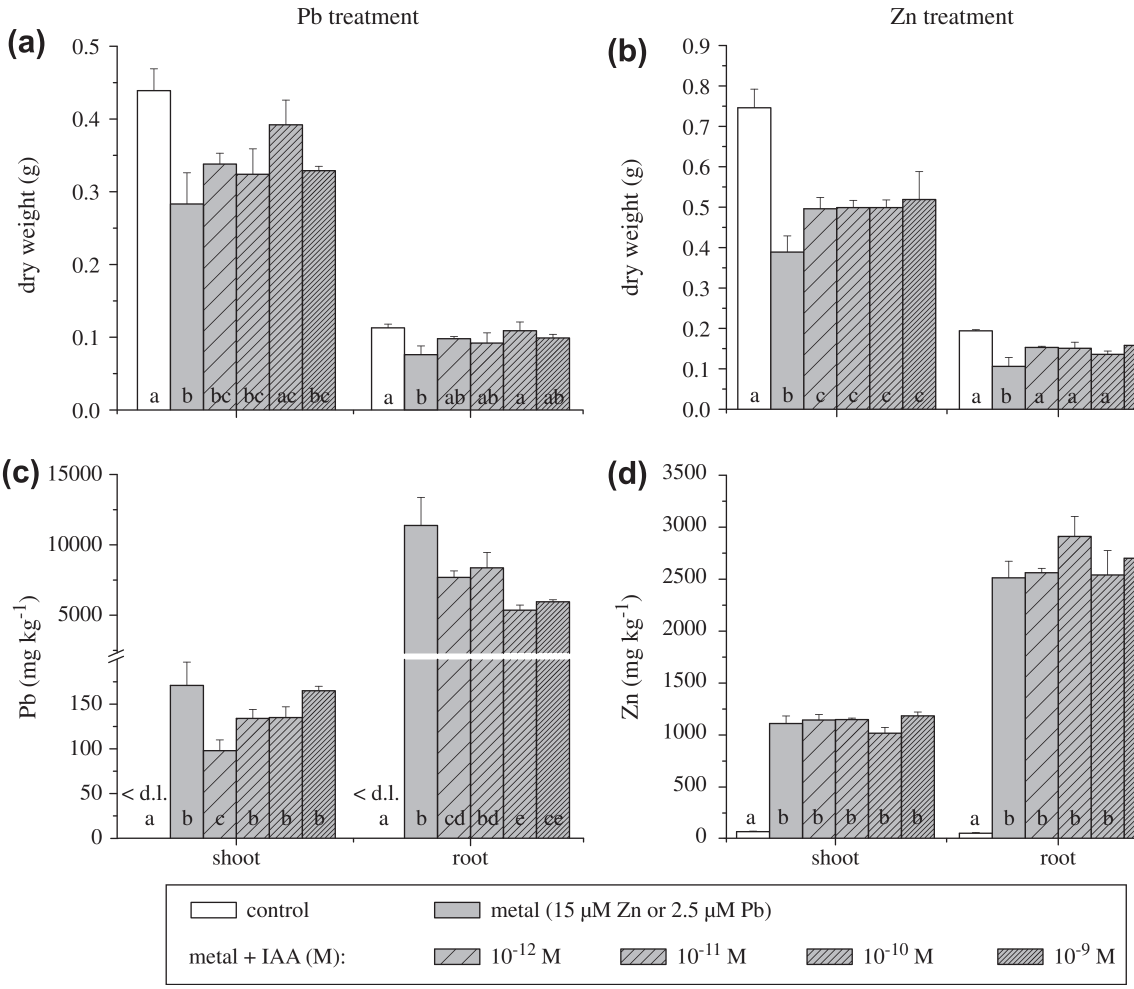

**Fig. 1.** Effects of different IAA concentrations. Shoot and root dry weight and metal concentrations of sunflowers grown in nutrient solution to which Pb (a, c) or Zn (b, d) were added with and without IAA. "$10^{-12}$ M" to "$10^{-9}$ M" represent the applied IAA concentrations in combination with the respective metal. Error bars give the standard errors of the means. Different letters indicate significant differences between treatments ($p < 0.05$).

All roots were scanned with a desktop scanner (Epson expression 10000XL, Epson, Nagano, Japan) equipped with a water tray, into which the roots were placed, and a positioning system. The water was needed to properly separate the roots from each other during the scanning process. Root morphology parameters (length, volume, diameter and surface area) were determined by means of the image analysis software WinRhizo (version Pro 2007d, Régents Instruments, Quebec, Canada). Root mass density was calculated dividing dry weight (see below) by volume.

### 2.3. Dry weight and metal concentrations of plant samples

After examination of root growth, the samples were dried at 60 °C for 3 d and weighed again to determine root and shoot biomass. Samples of about 0.25 g dry root and shoot of each plant (or less in cases where this amount of material was not available) were microwave-digested (lavis ETHOS, MLS GmbH, Leutkirch, Germany) in mixtures of 6 mL $HNO_3$ (65%), 2 mL $H_2O_2$ (30%), and 2 mL $H_2O$ for chemical analysis. The digested samples were filtered and diluted by adding Millipore water until an extract volume of 25 mL was obtained. The elements Ca, Cu, Fe, K, Mg, P and Zn were analysed by means of inductively coupled plasma optical emission spectrometry using a Varian, Vista MPX spectrometer (Palo Alto, USA) and Pb by means of stripping voltammetry using a mercury anode (Metrohm 797 VA Computrace, Herisau, Switzerland), according to Metrohm's "VA Application Note" No. V-28.

### 2.4. Statistics

Treatment differences were determined by analysis of variance followed by Fisher's least significant difference post hoc analysis (pair-wise multiple comparisons between each pair of means). The data were log-transformed in order to normalize frequency distributions. Differences were judged significant if the error probability was below 5% ($p < 0.05$).

## 3. Results and discussion

### 3.1. Effect of IAA on sunflowers grown without metal stress

In both sets of experiments we included treatments with IAA in the absence of metal stress. The IAA effects in absence of metals were quite variable (data not shown). In some cases IAA substantially increased, in others it decreased biomass production, independently of the applied IAA level. It is known that the range between beneficial and toxic effects of auxin can be quite narrow (Salisbury and Ross, 1992). Generally auxin is reported to increase plant root growth when applied at low concentrations, i.e. between

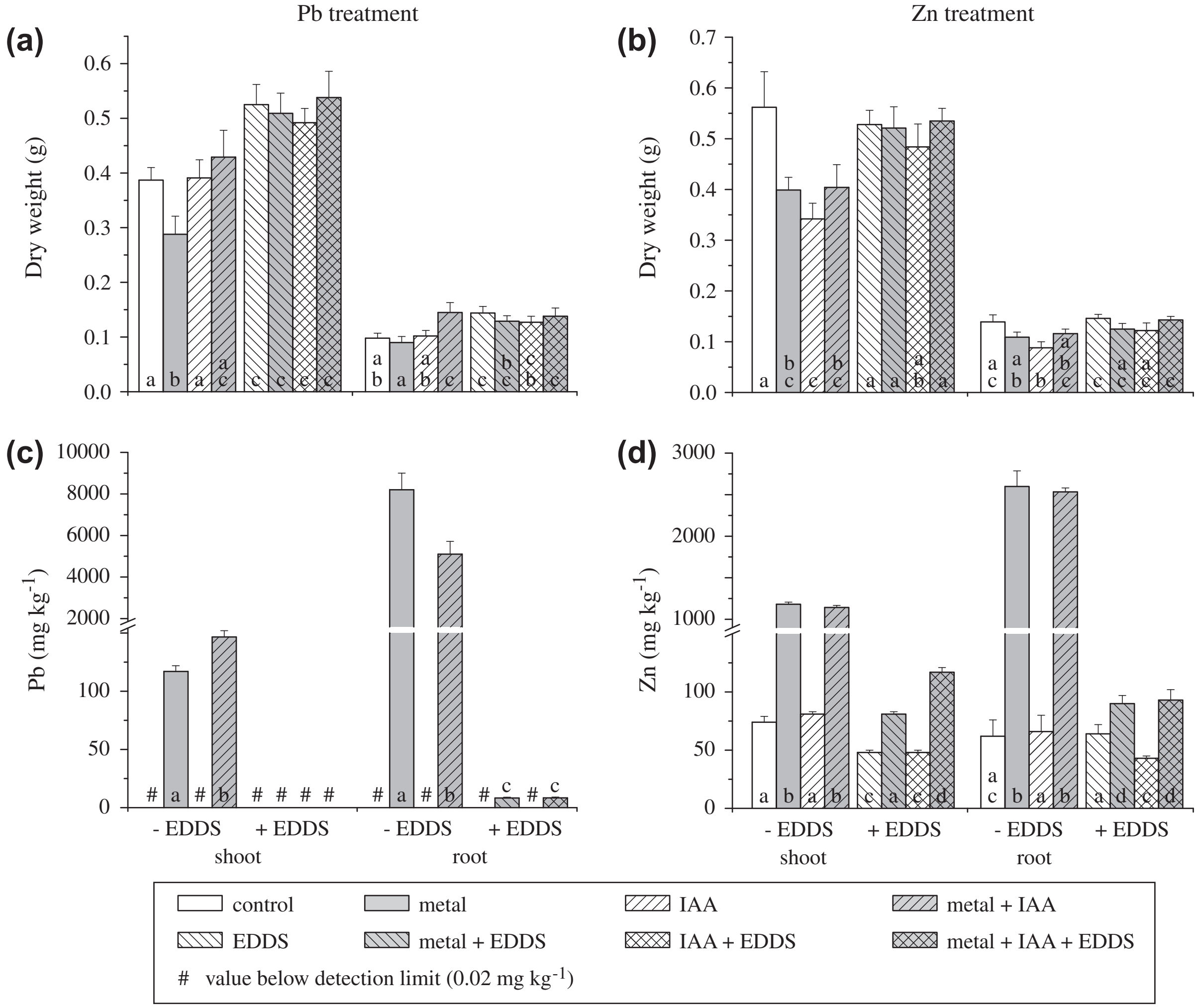

**Fig. 2.** Effects of IAA on EDDS-treated plants. Dry weight and metal concentration of sunflower plants grown in nutrient solutions to which (a, c) Pb and (b, d) Zn was added, with and without IAA ($10^{-10}$ M) and EDDS. Error bars show the standard errors of the means. Different letters in the respective plant parts indicate significant differences between the treatments ($p < 0.05$).

$10^{-7}$ and $10^{-13}$ M, and to inhibit growth at higher concentrations (Jagnow et al., 1991; Salisbury and Ross, 1992; Gaspar et al., 2002).

### 3.2. Effects of Pb and Zn on plant growth

The application of Zn decreased shoot dry weight by 30–50% in absence of IAA and EDDS (Figs. 1b and 2b). The application of Pb led to a similar but smaller effect (Figs. 1a and 2a). Also root dry weight was decreased by the metals, but the effect was only significant in the first set of experiments (Fig. 1a and b). The metal-induced decreases in root dry weight were associated with decreases in root volume, surface area and length (Table 1).

### 3.3. Effect of different IAA concentrations on growth and metal uptake of Pb or Zn stressed sunflowers

Toxic effects of Pb on shoot and root growth (dry weight) were alleviated by the addition of $10^{-10}$ M IAA in both experimental sets (Figs. 1a and 2a). Plant growth also increased at the other levels of applied IAA concentrations, but not significantly. Parallel to increasing root dry weight, the application of $10^{-10}$ M IAA to Pb-stressed plants also increased root volume, surface area and diameter in both experiments. Root length was not affected by IAA under Pb stress in the first experiment, but significantly increased in the second experiment. Root density of Pb-stressed plants remained unchanged in both experiments.

In the first experiment, the shoot Pb concentration of plants exposed to Pb was lower in combination with $10^{-12}$ M IAA than without IAA application (Fig. 1c). All other IAA concentrations had no significant effect on shoot Pb accumulation, although there was a trend for the lower IAA concentrations to decrease Pb accumulation. In contrast, shoot Pb concentration was increased by the application of $10^{-10}$ M IAA in the second experiment. Root Pb concentrations were decreased by IAA in both experiments. In the first experiment this effect was strongest at the two highest IAA concentrations (Fig. 1c). In the second experiment Pb concentrations of Pb-exposed roots were around 40% lower in the presence than in the absence of $10^{-10}$ M IAA. In contrast to our findings, López et al. (2005) had observed the opposite effect in hydroponically grown *Medicago sativa* L. treated with 200 µM Pb and 1, 10 and 100 µM IAA. In their study Pb concentrations increased in the roots with increasing IAA concentrations in the nutrient solution, while there was no trend in the shoots. They did not show data on plant growth, however, so that it is not clear whether the high Pb concentration applied may have been already in the phytotoxic range. Liu et al. (2007) on the other hand found that 100 µM IAA increased shoot Pb accumulation of *Sedum alfredii* Hance plants exposed to 200 µM Pb in nutrient solution. As in our experiment,

**Table 2**

General trend of metal and IAA effects. Effects on root growth, derived from both sets of experiments (treatments: control, metal only, metal + $10^{-10}$ M IAA).

| Treatment | Dry weight | | Volume | | Surface area | | Length | | Diameter | | Density | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | A + M | M | A + M | M | A + M | M | A + M | M | A + M | M | A + M |
| IAA + Pb | ↓ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↗ | ↓ | ↑ | → | ↗ |
| IAA + Pb + EDDS | → | ↑ | → | ↑ | → | ↑ | → | ↑ | → | ↓ | → | → |
| IAA + Zn | ↓ | ↑ | ↓ | ↗ | ↓ | ↗ | ↓ | → | → | → | ↓ | ↑ |
| IAA + Zn + EDDS | → | → | ↓ | ↗ | ↓ | ↗ | ↓ | ↗ | → | → | → | → |
| General trend | ↓ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↑ | → | ○ | → | ↑ |

↑, Increased; ↓, decreased; →, unchanged; ↗, trend to increase; ↘, trend to decrease; ○, ambiguous; M: metal vs. control treatment; A + M: IAA + metal vs. metal treatment. The combination "↓ ↗" means that there was neither a significant difference between A + M and M, nor between A + M and control, while there was a significant M effect (vs. control). The combination "→ ↗"means that there was a significant difference between A + M and control, but no significant M effect.

they observed increased dry weight, root surface area, root volume as well as root length by the application of IAA.

Similarly as in the case of Pb stress, IAA reduced the phytotoxic effects of Zn at all applied concentrations in the first experiment (Fig. 1b). Root dry weight was even fully restored at all IAA concentrations (Fig. 1b). Also root density was restored, whereas IAA had no effect on the other root parameters of Zn-stressed plants in the first experiment (Table 1). In the second experiment Zn toxicity effects were not alleviated by IAA addition. In contrast, IAA appeared to have a negative growth effect that was not observed in the Pb experiment, although the applied treatments were the same when no metal was added (Fig. 2b). Indole-3-acetic acid had no influence on Zn uptake in both experiments in the absence of EDDS.

Table 2 summarizes the observed effects of IAA ($10^{-10}$ M), Pb and Zn (derived from the histograms in Figs. 1 and 2 and from Table 1) on root growth in the two sets of experiments. It clearly shows that IAA has considerable potential to alleviate metal stress, although this did not become manifest in all cases in our experiments. The findings confirm a general role of IAA in stress alleviation also found by other authors (Chaudhry and Rasheed, 2003; Liu et al., 2007). In the presence of metals IAA generally had a positive effect on those root growth parameters that were negatively affected by the metals. In particular it compensated the metal-induced decreases in root dry weight, volume, surface area and length, which were reduced by both metals. Root diameter and density were much less sensitive to metal stress.

The fact that IAA-induced increases in shoot dry matter were not accompanied by decreased shoot Pb or Zn concentrations, but rather by increased metal accumulation, means that also total metal extraction was increased, indicating that IAA has some potential to enhance phytoextraction of polluting metals. However, one has to be aware that the stress-mitigating effect of IAA cannot

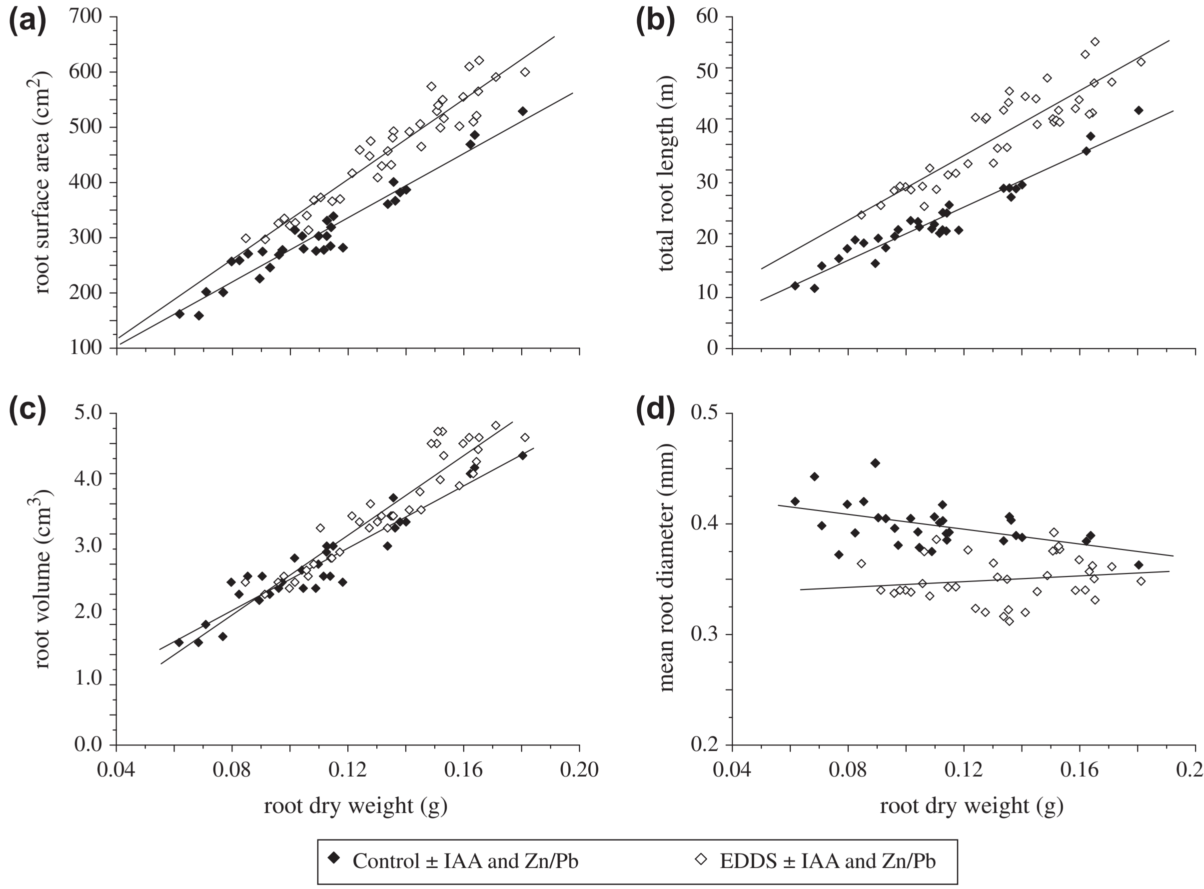

**Fig. 3.** Effects of EDDS on relationships between root growth parameters. Regression lines between root dry weight and (a) root surface area, (b) total root length, (c) root volume, and (d) mean root diameter of sunflower plants grown in nutrient solutions to which EDDS was applied or not, with and without IAA, Zn and Pb (pooled samples).

be expected to work at high levels of phytotoxicity. Liphadzi et al. (2006) grew sunflowers on a moderately and a highly contaminated soil. Adding IAA increased the biomass of the roots and stems in the moderately contaminated soil, but did not change growth on the highly contaminated soil, indicating an upper limit of metal stress that can be alleviated by IAA.

### 3.4. Effect of EDDS

The application of EDDS had a positive effect on plant growth independent of metal stress and IAA application (Fig. 2). The EDDS-treated plants generally (with or without IAA and heavy metals) looked healthier than the plants that were not exposed to EDDS. Plants treated with EDDS in general developed root systems of larger dry weight, volume, length and surface area than plants grown in solution without EDDS. They also showed a higher ratio between root surface area and root dry weight (Fig. 3a) and between root length and root dry weight (Fig. 3b), whereas the ratio between mean root diameter and root dry weight was decreased and root mass density was not affected (Fig. 3d). EDDS thus induced growth of longer and thinner roots, leading to an increased outer surface, per unit mass (and volume) of root tissue (Fig. 3c).

Metal uptake was strongly decreased by EDDS. Zinc accumulation by the roots of Zn-stressed plants was decreased by approximately a factor of 29 and shoot Zn by a factor of 15. Root Pb was decreased by three orders of magnitude and shoot Pb to a value below the detection limit (0.02 mg kg$^{-1}$). This decrease can be attributed to the formation of stable metal-EDDS complexes, reducing the free ion concentrations of the metals and thus their availability to be taken up by and to react with the roots. Alleviation of metal toxicity through the addition of EDDS was also found in Cu-stressed hydroponically grown *Chrysanthemum coronarium* L. by Wei et al. (2007), where the addition of EDDS increased biomass and decreased Cu concentration of roots and shoots. Tandy et al. (2006a) observed that EDDS alleviated metal stress on sunflowers grown in hydroponic solution with high concentrations of Cu, Zn and Pb.

The EDDS effects on plants that were not exposed to Pb or Zn contamination show that reduction of toxicity and plant uptake of these metals was not the only way in which EDDS had a positive effect on plant growth. The additional growth-promoting EDDS effect may have been due to enhanced Fe translocation to the shoots. Plants grown in absence of EDDS had a mean shoot Fe concentration of 75 ± 8 mg kg$^{-1}$, which is at the lower end of the range of sufficient Fe nutrition (50–250 mg kg$^{-1}$) of sunflowers (Bergmann, 1993). In the presence of EDDS the shoot Fe concentration increased to 119 ± 6 mg kg$^{-1}$. Root Fe concentration was decreased by the EDDS application from 6609 ± 308 to 143 ± 8 mg kg$^{-1}$. Whereas in the roots the metal mobilization effect of EDDS was the same for Fe as for the other metals, the EDDS effect on root–shoot translocation of Fe (i.e. increase) was opposite to that observed for Pb and Zn (i.e. decrease). The same contrast was also found between Fe and Cu. EDDS reduced the Cu concentration in the roots from 174 ± 6 to 35.4 ± 2.1 mg kg$^{-1}$ and in the shoots from 21.4 ± 0.5 to 12.6 ± 0.3 mg kg$^{-1}$.

### 3.5. Combination effect of EDDS and IAA

In absence of contaminating heavy metals, the application of IAA decreased root surface area and root length of EDDS-treated plants. In the presence of Zn IAA increased root length and decreased root diameter of EDDS-treated plants (Table 1). All other plant growth parameters remained unchanged. Growth of plants treated with Pb and EDDS remained unaffected by the application of IAA (Fig. 2 and Table 1). We did not find contradictions between the IAA effects on the growth of metal-treated plants between treatments with and without EDDS. The effects were just smaller or not significant in the presence of EDDS. The small impact of IAA on the growth of metal-treated plants in the presence of EDDS was probably due to the fact that the metal stress was already alleviated by the EDDS application. Thus there was little or nothing to alleviate anymore.

Shoot Zn concentration, however, was increased by IAA in EDDS-treated plants with Zn applications (Fig. 2d). As root length was the only root growth parameter found to increase after IAA application in the combined EDDS and Zn treatment, root length may be an important factor for Zn uptake. This is in line with the results of a modeling study carried out by Gardner (1960), suggesting that root length was more important than root diameter in determining uptake of water and elements. Lead concentrations were below the detection limit. Thus, it is unclear whether IAA also had an impact on Pb uptake by EDDS-treated plants. An increased plant metal accumulation induced by auxin added to the nutrient solution of metal-stressed plants in the presence of a chelating agent was also found by Israr and Sahi (2008). Moreover, Zhou et al. (2007) observed a similar effect also in corn (*Zea mays* L.) grown on metal-polluted soil. Treating the soil with IAA in combination with EDTA or NTA increased metal uptake in comparison to treatments with the chelating agents alone.

## 4. Conclusion

The experiments showed that the addition of IAA ($10^{-10}$ M) to nutrient solution can alleviate Zn and Pb stress in sunflowers by promoting root growth. In particular, IAA reduced negative metal effects on root and shoot dry weight, root length, root volume and root surface area. The application of EDDS promoted plant growth and alleviated the stress of the applied metals to a degree that no substantial stress remained to be alleviated by additional IAA application. No conclusion concerning IAA effects on Pb uptake in the presence of EDDS is possible, because plant Pb concentrations were below the detection limit in all EDDS treatments. Zinc uptake of plants exposed to Zn contamination of the hydroponic solution, however, was increased by IAA in the presence of EDDS. The combination of IAA with EDDS thus showed potential to enhance phytoextraction and should be further tested with contaminated soil.

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