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The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers

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Abstract

Phytoextraction is an environmentally friendly in situ technique for cleaning up metal contaminated land. Unfortunately, efficient metal uptake by remediation plants is often limited by low phytoavailability of the targeted metals. Chelant assisted phytoextraction has been proposed to improve the efficiency of phytoextraction. Phytoremediation involves several subsequent steps: transfer of metals from the bulk soil to the root surfaces, uptake into the roots and translocation to the shoots. Nutrient solution experiments address the latter two steps. In this context we investigated the influence of the biodegradable chelating agent *SS*-EDDS on uptake of essential (Cu and Zn) and non-essential (Pb) metals by sunflowers from nutrient solution. EDDS was detected in shoots and xylem sap for the first time, proving that it is taken up into the above ground biomass of plants. The essential metals Cu and Zn were decreased in shoots in the presence of EDDS whereas uptake of the non-essential Pb was enhanced. We suggest that in the presence of EDDS all three metals were taken up by the non-selective apoplastic pathway as the EDDS complexes, whereas in the absence of EDDS essential metal uptake was primarily selective along the symplastic pathway. This shows that synthetic chelating agents do not necessarily increase uptake of heavy metals, when soluble concentrations are equal in the presence and absence of chelates.
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1. Introduction

Heavy metal pollution of soil is a widespread global problem. The clean up of metal contaminated land by traditional physiochemical methods can be very costly and also destructive to the soil. Phytoextraction is an environmentally friendly in situ technique for cleaning up metal contaminated land (Salt et al., 1998), which aims to preserve soil structure and fertility. Chelant (ligand) assisted phytoextraction has been proposed to improve the efficiency of conventional phytoextraction of metal polluted soils by solubilizing target metals from soil (Salt et al., 1995; Lasat, 2002) and making them more available for plant uptake and translocation to the shoots.

EDTA (ethylenediamine tetraacetic acid) has been the most commonly used chelating agent for this purpose (Blaylock et al., 1997; Huang et al., 1997; Epstein et al., 1999; Grcman et al., 2001; Collins et al., 2002; Shen et al., 2002; Wu et al., 2004), but due to its persistence in the environment (Bucheli-Witschel and Egli,

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2001; Nowack, 2002) it is now considered unsuitable for field use. Other synthetic chelating agents such as NTA (nitrilotriacetate) (Kulli et al., 1999; Kayser et al., 2000; Meers et al., 2004), have also occasionally been used.

(*S*,*S*)-*N*,*N*′-ethylenediamine disuccinic acid (EDDS) is a biodegradable structural isomer of EDTA (Nishikiori et al., 1984; Schowanek et al., 1997; Vandevivere et al., 2001). Other stereoisomers of ethylenediamine disuccinic acid are either non-biodegradable (*R*,*R*) or only partially degradable (*R*,*S*, *S*,*R*) so the *SS*-isomer is generally used (Schowanek et al., 1997). It is now used as a commercial substitute for EDTA in detergents (Jaworska et al., 1999; Knepper, 2003) and has the potential to be a substitute for EDTA in chelant assisted phytoremediation, as it is a strong chelator and unlike EDTA, it is easily biodegradable. EDDS can readily solubilize metals from soil and at pH 7 it was shown to be better at solubilizing Cu and Zn than EDTA at equimolar ratios of chelating agent to metals (Tandy et al., 2004). Several papers have recently been published on the use of EDDS in chelant enhanced phytoremediation of Pb, Zn, Cu and Cd in soil (Grcman et al., 2003; Kos and Lestan, 2003, 2004a,b; Luo et al., 2005; Meers et al., 2005).

Several processes are involved in phytoremediation: solubilization of the metals from soil and transfer to the roots, uptake of the metals into the roots and translocation to the above ground biomass. Ideally chelants would enhance all of these steps. Hydroponics experiments can be used to investigate the latter two processes.

Early work used hydroponics experiments to investigate trace element plant nutrition. Therefore low metal ($\leqslant$20 μM) and chelant concentrations were employed in these studies. It was found that EDTA or DPTA produced Zn deficiency in corn (*Zea mays*) and barley (*Hordeum vulgare*) (Barber and Lee, 1974; Halvorson and Lindsay, 1977). Also Ni uptake by beans (*Phaseolus vulgaris*) was not improved by DTPA (Wallace, 1980). These observations were seen as evidence that EDTA and DPTA complexes are not taken up by plants.

Other investigators have studied the influence of high concentrations of chelants ($\geqslant$50μM) on metal uptake, mainly on the phytoextraction of Pb. 0.25 mM EDTA was the threshold for rapid shoot uptake of Pb and at higher concentrations (0.75 mM) a maximum shoot Pb concentration of 56 mmol kg^{-1} could be reached in Indian mustard (*Brassica juncea*), a 400-fold increase on the control (Vassil et al., 1998). This led to drying of shoot matter and the formation of necrotic lesions however. Increases in shoot Pb uptake in the presence of chelants mostly fall in the range 2–10 times the control and 0.9–11 mmol kg^{-1} shoot Pb concentration (Wu et al., 1999; Hernandez-Allica et al., 2003; Piechalak et al., 2003).

Although chelating agents have been seen to increase plant shoot uptake of Pb in most cases, the uptake of Cu and Zn seems mainly to be unaffected or even decreased with chelants. Very little work has been carried out on chelant effects on Cu uptake and none on Zn at elevated metal concentrations in solution. In a toxicity study carried out using wheat (*Triticum aestivum*) it was found that at 30 μM Cu in hydroponic solution, leaf concentrations were similar in plants grown in chelated and non-chelated solutions (Taylor and Foy, 1985). When the concentration was increased to 50 μM for $CuSO_4$ and 60 μM for CuEDTA, however, the concentration was higher (43 mg kg^{-1}) in leaves from plants grown in the absence of chelates (chelate denotes the ligand–metal complex, chelant the ligand itself) than those grown in the presence of chelates (~30 mg kg^{-1}). On the other hand Cu shoot concentrations were found to increase when tobacco (*Nicotiana tabacum*) was grown in a solution of 0.126 mM Cu and 0.5 mM NTA compared to a solution of 0.038 mM free Cu in the absence of NTA (Wenger et al., 2003).

Direct methods have been used to measure EDTA inside plants, either as a complex (Sarret et al., 2001; Collins et al., 2001, 2002; Schaider and Sedlak, 2003) or as total EDTA (Vassil et al., 1998; Epstein et al., 1999; Bell et al., 2003). These investigations prove that the EDTA is taken up from solutions containing high concentrations.

There are indications that metal–chelant complexes are taken up along an apoplastic pathway (Tanton and Crowdy, 1971). This means that the complexes pass through the free space of the roots which is made up of root cell walls and water filled intercellular spaces in the root cortex and which is continuous with the surrounding soil solution (Marschner, 1986). Explanations of the translocation of synthetic chelating agents from roots to shoots are confounded with the problem that prior to reaching the xylem the complexes meet the Casparian strip, a highly suberized band that halts apoplastic flow and forces them to cross the cell membranes of the endodermis (Marschner, 1986). As most chelates are charged, large in size and have no known specific transporters, it is unlikely they can pass through the cell membrane. However the Casparian strip is not a perfect barrier. At the root tips it is not fully formed (Tanton and Crowdy, 1971; Haynes, 1980), and where lateral roots protrude from the main root system the Casparian strip can be disrupted. It has been found in fact that at such places components from the surrounding solution can enter the stele which houses the xylem without passing through a cell membrane (Haynes, 1980; Haussling et al., 1988). Furthermore some species have a small number of cells in the endodermis called passage cells which remain unsuberized. It is assumed that they allow entry of water and solutes into the xylem (Clarkson, 1996).

The aim of this work was to investigate the influence of the chelating agent EDDS on metal uptake of essential (Cu and Zn) and non-essential (Pb) metals by

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