Food-chain transfer of zinc from contaminated Urtica dioica and Acer pseudoplatanus L. to Microlophium carnosum and Drepanosiphum platanoidis Schrank

Article

Accepted Version

Sinnet, D., Hutchings, T.R. and Hodson, M. E. (2010) Food-chain transfer of zinc from contaminated Urtica dioica and Acer pseudoplatanus L. to Microlophium carnosum and Drepanosiphum platanoidis Schrank. Environmental Pollution, 158 (1). pp. 267-271. ISSN 0269-7491 doi: https://doi.org/10.1016/j.envpol.2009.07.008 Available at https://centaur.reading.ac.uk/1683/

To link to this article DOI: http://dx.doi.org/10.1016/j.envpol.2009.07.008

Publisher: Elsevier

Food-chain transfer of zinc to aphids

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Number of words:

Number of references: 32

Number of tables: 0

Figure legends:

Figure 1: Zn concentration in a) the leaf tissue of *A. pseudoplatanus* (n=25) and above-ground tissue of *U. dioica* (n=15) exposed for 98 and 54 days respectively and b) *D. platinoidis* (n=23) and *M. carnosum* (n=15) exposed for 14 and 28 days respectively compared to the Zn concentration in Hoagland's solution and c) is the Zn concentration in *D. platinoidis* (n=23) and *M. carnosum* (n=15) compared with the Zn concentration in leaf tissue of *A. pseudoplatanus* and above-ground tissue of *U. dioica.* (Where [ZnNettle], [ZnLeaf], [ZnAphid] is the concentration of Zn in the tissue of *U. dioica* (mg/kg), the leaf tissue of *A.pseudoplatanus* (mg/kg), *M. carnosum* (mg/kg) and the watering solution (mg Zn/l) respectively).

Figure 2: Zn concentration in the phloem tissue of *U. dioica* (n=15) and *A. pseudoplatanus* (n=15) exposed for 54 and 98 days respectively to Zn in Hoagland's solution.

Food-chain transfer of zinc from contaminated *Urtica dioica* and *Acer pseudoplatanus* L. to *Microlophium carnosum* and *Drepanosiphum platanoidis* Schrank

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Abstract – This study examines the food-chain transfer of Zn from two plant species, *Urtica dioica* (stinging nettle) and *Acer pseudoplatanus* (sycamore maple), into their corresponding aphid species, *Microlophium carnosum* and *Drepanosiphum platanoidis*. The plants were grown in a hydroponic system using solutions with increasing concentrations of Zn from 0.017 to 42 mg Zn/l, although *U. dioica* only survived in solution containing up to 18 mg Zn/l. Above-ground tissue total and phloem concentrations in *U. dioica* and *M. carnosum* concentrations increased with increasing Zn exposure ($p<0.001$). When *U. dioica* were exposed to the 18 mg Zn/l solution the corresponding above-ground plant tissue, phloem and *M. carnosum* concentrations were around 2100, 50 and 131 mg/kg respectively. Although Zn concentrations in *M. carnosum* were lower than total plant concentrations bioaccumulation was taking place as concentrations were greater than those in the phloem which represents the Zn reservoir to which the aphids were exposed. Zn concentrations in *A. pseudoplatanus* also increased with solution concentration from the control to the 9 mg Zn/l solution, after which concentrations remained constant at around 160 mg/kg. Zn concentrations in both *D. platanoidis* and the phloem tissue of *A. pseudoplatanus* were not affected by the Zn concentration in the watering solution with concentrations of 6.2 and 375 mg/kg respectively for exposure to solutions of 18 mg Zn/l and above. It appears that *A. pseudoplatanus* is able to regulate Zn, whereas *U. dioica* is not resulting in increasing Zn exposure to the aphids on the latter species. Despite this Zn concentrations in *M. carnosum* were around a third of those in *D. platanoidis*, suggesting that the latter species may have naturally elevated Zn concentrations.

Keywords – stinging nettle, sycamore maple, common nettle aphid, sycamore aphid, contaminated land

INTRODUCTION

The importance of the impact of contaminated land on terrestrial ecological receptors is increasingly being recognised in the site investigation, risk assessment and remediation process. Many practitioners commonly use an Ecological Risk Assessment (ERA) to determine the potential for harm that a site may pose to ecological receptors and many countries have produced frameworks and guidance for conducting such investigations [1]. The ERA process often makes use of a combination of field and laboratory analysis and models to determine the risk to either ecological function or the food-chain transfer of pollutants. The majority of the ecotoxicological tests used in ERA are based on ecological function and use endpoints such as mortality, reproduction and growth. In order to estimate the risk to higher organisms from a contaminated site it is often necessary to use models to predict the pollutant concentrations through the food-chain and relate these to published toxicological endpoints for the species of interest. There are a variety of models available to estimate the food-chain transfer of pollutants (e.g. [2,3]). However, the models are often not species specific, may have been based on aquatic organisms (for example in the case of flying insects), or may only be applicable to a certain group of contaminants [2,3]. This has serious implications for those using such models to estimate risk from contaminated land to ecological receptors. At best it may result in significant gaps in the range of species for which such a risk assessment can be conducted, at worst it may result in an over or under-estimation of the risk leading to either unnecessarily costly remediation or no remediation taking place where it is needed.

Urtica dioica L. is prevalent in almost all urban ecosystems and is an early coloniser of contaminated land [4,5]. It is extremely important in urban ecosystems as it provides a habitat for a wide range of invertebrates [5,6]. In addition, it is also relatively simple to cultivate, widely available and fast growing [6], and as such, may be a useful species for ecotoxicological testing. *Acer pseudoplatanus* L. is a tree species that has been introduced to the UK, but is commonly found in urban areas [7]. It is an early coloniser [8] and tolerant of a wide range of site conditions [9]. *U. dioica* and *A. pseudoplatanus* both have extremely prevalent species-specific aphids associated with them; *Microlophium carnosum* Buckton and *Drepanosiphum platanoidis* Schrank respectively.

The food-chain transfer of metals to a variety of aphids have been assessed in a number of studies (e.g. [10-12]), although these studies have all concentrated on aphids whose hosts are agricultural plant species. Aphids are an important source of food for a large number of other insects, either indirectly for their honeydew (e.g. ants) or directly (e.g. parasitoids and ladybirds) [7,13]. *M. carnosum* is a large aphid (3.3 to 3.8 mm) commonly found on *U. dioica*, primarily on the underside of the leaves and the stem [14], during May to October [5,6]. *D. platanoidis* is abundant on the underside of leaves of *A. pseudoplatanus*, during April to October, with population peaks in June and October [8]. Used in conjunction with *U. dioica* and *A. pseudoplatanus*, *M. carnosum* and *D. platanoidis* have the potential to assess the risk of food-chain transfer of metals in urban ecosystems.

This study aims to assess the transfer of Zn to *M. carnosum* and *D. platanoidis* from *U. dioica* and *A. pseudoplatanus* grown under hydroponic conditions in order to determine the potential for Zn transfer to aphid predators in urban ecosystems. The study was originally carried out with Cd in addition to Zn, however the small masses of aphids combined with the smaller concentrations of Cd in their tissue meant that Cd concentrations in aphids were often below detection limits and therefore Cd data are not reported here due to the patchy nature of the dataset.

MATERIALS AND METHODS

Transfer of Zn into Microlophium carnosum and Drepanosiphum platanoidis

U. dioica cuttings, taken from Alice Holt Forest, Farnham, UK, and *A. pseudoplatanus* (bare rooted 1+1 stock; Prees Heath Forest Nurseries, Shropshire, UK) were planted individually in 1 litre containers filled with perlite. Perlite was used as it has no inherent sorption capacity that could influence Zn availability. Additionally, pores between individual perlite beads ensure an aerobic environment. Pea shingle was placed on the perlite to a depth of 2 cm to minimise evaporation.

A fully replicated randomised block experiment with five replicates was set up in a glasshouse. Plants were grown under 16 h of artificial light and 8 h darkness per day (PAR = 0.37 mmol/m/s). The temperature of the glasshouse was regulated to 20 ^{o}C (±5 ^{o}C).

Each container was watered with one of five solutions: control (¼ strength Hoagland's solution for *A. pseudoplatanus* and full strength for *U. dioica* [15]) or one of four Zn treatments in Hoagland's solution. The Hoagland's formulation provided background micronutrient concentrations of 0.02 or 0.08 mg Zn/l and 0.0008 or 0.0032 mg Cu/l for the ¼ strength or full strength solutions respectively. Zn amendments were added as $ZnSO_4.7H_2O$ to provide concentrations of 0.02 (control), 5, 10, 20 and 50 mg Zn/l. The solution in each container was replaced by mass when necessary.

M. carnosum were added to the *U. dioica* pots 26 days after planting whilst *D. platanoidis* were added to the *A. pseudoplatanus* pots 84 days after planting. Differences in timing were due to the availability of sufficient aphid populations in the field. Leaves with aphids on them were removed from Alice Holt Forest and placed at the base of each plant. Enough leaves were used so that at least 5 aphids were transplanted to each pot. Each pot was then covered individually with a fine mesh net suspended from the ceiling, this was tied securely around the lip at the top of the pot to prevent the aphids from moving to different plants. At each watering, the netting was loosened round the pot and lifted enough to add the appropriate solution and re-secured.

U. dioica and *A. pseudoplatanus* were harvested 28 and 14 days respectively, after the aphids had been added. *D. platanoidis* populations appeared to be declining on the *A. pseudoplatanus* so these were harvested earlier than *U. dioica* in order to ensure that enough aphid mass was available for analysis. Reproduction rates of *D. platanoidis* vary during the season, being closely linked to the amino-nitrogen content of the leaves and this decline is likely to have been a result of the leaves reaching maturity [8]. The netting was loosened from around the pot and the stem cut, the netting was then closed at the bottom and detached from the ceiling, the netting along with its contents were then placed in the freezer at -20 ^{o}C for 2 hours. The plants were then removed from the freezer and the dead aphids collected with a fine brush. *A. pseudoplatanus* were split into their stem, shoot and leaf components. The above-ground tissue of *U. dioica* and leaf and shoot tissues of *A. pseudoplatanus* were washed in deionised water to remove the honeydew, weighed and dried at 70 ^{o}C for 24 hours and reweighed. The stem tissues of *A. pseudoplatanus* were discarded as the aphids do not feed on this woody material. The aphids were weighed, dried at 50 ^{o}C for 24 hours and reweighed. The aphid and plant material samples were then milled and analysed to determine their Zn concentrations (see below).

Determination of phloem Zn concentrations

In order to understand the different Zn exposures to the aphids a further experiment was set up to determine the phloem Zn concentrations within *U. dioica* and *A. pseudoplatanu*. *U. dioica* cuttings and *A. pseudoplatanus* were planted individually in 1 litre containers filled with perlite in the same way as for the aphid exposure experiment. A fully replicated randomised block experiment with five replicates for *U. dioica* and five replicates for *A. pseudoplatanus* was set up in a glasshouse under the same conditions as the aphid exposure experiment. Each container was watered with one of three solutions: control (¼ strength Hoaglands solution for *A. pseudoplatanus* and full strength for *U. dioica* [15]) or one of two Zn treatments in Hoaglands solution. Zn amendments were added as $ZnSO_4.7H_2O$ to provide concentrations of 0.02 (control), 5 and 20 mg Zn/l. The solution in each container was replaced by mass when necessary.

U. dioica and *A. pseudoplatanus* were harvested after the same duration as the aphid experiment in order to ensure that the plants had been exposed to the Zn solutions for the same time. The method used to determine the concentration of Zn in the phloem tissue was based on that of Thornber and Northcote [16] which extracts the water-soluble material within the phloem. The leaf and shoot tissues of *A. pseudoplatanus* were removed from the stem tissue and discarded. The bark was carefully removed from the stem tissue using a grafting knife and the phloem tissue was then removed, again with a grafting knife. The phloem tissue was weighed and then boiled at 100 ^{o}C in 200 ml of deionised water for 3 hours. Following boiling, the samples were centrifuged and the solution removed and filtered through a 0.45 µm Whatman filter. It was not possible to separate the phloem tissue of *U. dioica* from the rest of the stem so the entire above-ground biomass was subjected to boiling under the assumption that the water-soluble fraction of the plant material will give an indication of the phloem concentration. The above-ground tissue of *U. dioica* was removed, weighed and boiled at 100 ^{o}C in 300 ml of deionised water for 3 hours. The solutions were then analysed to determine their Zn concentrations (see below).

Determination of Zn concentration

The Zn solutions used for watering and the phloem extracts were analysed using a Spectro Flame Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES; Spectro Analytical Instruments, West Midlands, UK). The target Zn concentrations in the solutions used for watering of 0.02, 5 and 20 mg Zn/l were found to be 0.017, 4.71 and 17.97 mg Zn/l respectively.

Plant samples were prepared for analysis by dry-ashing at 450 ^{o}C for 18 hours and wet digestion [17]. Wet digestion was achieved by incubating each sample for 1 hour at 60 ^{o}C in 0.75 ml concentrated HNO_3, followed by a further 14 hour incubation with 2.25 ml concentrated HCl and heating for 2 hours at 110 ^{o}C. After cooling, 0.15 ml of 30 % H_2O_2 was added to each sample followed by heating for 30 minutes at 110 ^{o}C. To ensure complete oxidation of all organic matter the H_2O_2 treatment was performed twice. The digested samples were analysed for Zn using the ICP-OES [18].

Aphid samples were digested in 1 ml concentrated HNO_3 at 180 ^{o}C for 1 hour, after which 1 ml of deionised water was added and the sample further digested at 180 ^{o}C to dryness. A further 0.01 ml of concentrated nitric acid was added and the sample digested at 60 ^{o}C for 1 hour. The digested samples were analysed for Zn using the ICP-OES [18].

The limit of detection was 0.67 µg/kg for Zn. Bush branches and leaves (NCS DC73349, China National Analysis Centre for Iron and Steel), oriental tobacco leaves (CTA-OTL-1, Commission for Trace Analysis of the Committee for Analytical Chemistry of the Polish Academy of Sciences and Institute of Nuclear Chemistry and Technology, Warsaw, Poland), mussel (CE278, European Commission, Geel, Belgium) and bovine liver (1577b, US Department of Commerce, National Institute of Standards and Technology. Gaithersburg, MD 20899, USA) tissues were used as Certified Reference Materials (CRM) with batches of plant and aphid samples as appropriate. Mean recovery from oriental tobacco leaves was 104.9 and 98.6 % from the bush branches and leaves and oriental tobacco respectively. Mean recovery from mussel and bovine liver was 92.9 and 93.6 % respectively for the *M. carnosum* samples and 111.8 and 102.6 % respectively for the *D. platanoidis* samples.

Statistical analysis

The plant and aphid Zn uptake data were subjected to general linear regression analysis to assess the significance of changes in plant and aphid concentrations with increasing Zn concentration in hydroponic solutions and plant material respectively, using Genstat version 8.1 [19]. Mean values are reported with ± standard errors throughout.

Linear and exponential models of Zn uptake into each of the plant and aphid tissue types compared to that of the solution concentration and, in the case of aphids, the leaf concentrations were fitted using Genstat version 8.1 [19]. A comparison of the residual sum of squares of alternative models relative to the smallest residual mean square was used to determine the most appropriate model. This comparison used for nested models and is referred to an F-distribution with 1, n degrees of freedom where n is the residual degrees of freedom from the exponential model.

RESULTS

Zn concentration in solution had a significant affect on the Zn uptake into the above ground tissue ($F_{1,13}$=533.63; p<0.001) of *U. dioica*; no plants survived in the 42 mg Zn/l solution treatment (Figure 1). Zn concentration in both solution and nettle tissue had a significant affect on the Zn concentration in *M. carnosum* ($F_{1,13}$=107.95; p<0.001 and $F_{1,13}$=77.38; p<0.001 respectively; Figure 1). The concentration of Zn in the phloem extracts from *U. dioica* increased significantly with increasing Zn concentration in solution ($F_{1,13}$=138.89; p<0.001; r^2=0.908).

Zn concentration in solution did not have a significant effect on the Zn uptake into either the leaf or shoot tissue of *A. pseudoplatanus*. This is because the Zn concentrations in the tissues reached a plateau between the 9 and 18 mg Zn/l solutions; the exponential model was, however, significant for both leaf ($F_{2,22}$=3.57; p=0.046) and shoot ($F_{2,22}$=5.43; p=0.012) tissue (Figure 1).

There was no significant effect of the concentration of Zn in solution or in the leaf or shoot tissue of *A. pseudoplatanus* on the concentration in *D. platanoidis* using either the linear or exponential models (Figure 1). Similarly, the concentration of Zn in the phloem extract was not significantly related to the concentration of Zn in solution.

The concentration of Zn in the above-ground tissue of *U. dioica* were approximately 13 times that in the *A. pseudoplatanus* as a result of exposure to the 18 mg Zn/l solution; 2153±68.7mg/kg compared with 163±20.6 mg/kg. The phloem extract concentrations at this 18 mg Zn/l exposure were 48.2±2.4 mg/kg in *U. dioica* and 6.1±1.2 mg/kg in *A. pseudoplatanus* (Figure 2). Despite this, the Zn concentration in *M. carnosum* was less than a third of that in *D. platanoidis*; 131.5±11.0 mg/kg compared with 406±21.2 mg/kg. Phloem concentrations of both species were lower than those in above-ground tissue in *U. dioica* or in the leaf and shoot tissues in *A. pseudoplatanus*, this difference increased with increasing Zn concentrations; from 3 up to 17 times lower and 11 up to 25 times lower in *U. dioica* and *A. pseudoplatanus* respectively.

DISCUSSION

Zn concentrations in the above-ground tissue of *U. dioica* increased with Zn exposure, reaching a mean of approximately 2100 mg/kg for the 18 mg Zn/L solution. In *A. pseudoplatanus* tissue concentration increased up to the 9 mg Zn/l solution and then remained constant at around 160 mg/kg despite the increasing Zn concentration in solution. Zn concentrations in the above-ground tissue of *U. dioica* have been reported to range between 42 and 52 mg/kg in uncontaminated soils [20]. Leaf concentrations of between 23 and 532 (mean 113 mg/kg) have been reported in *U. doica* growing on dredged sediments with a Zn concentration of between 149 and 1817 (mean 54 mg/kg) [21]. Zn concentrations in *U. dioica* around the Avonmouth smelter have been found to be as high as 3000 mg/kg, although this is likely to have occurred from atmospheric deposition as well as soil uptake [22]. The substantial quantities of Zn that nettles appear to be capable of accumulating make this species an important pathway for Zn in the food-chain. Mertens et al. [23] found Zn concentrations with a mean of 74 mg/kg in *A. pseudoplatanus* grown on dredged sediments with a Zn concentration of 359 mg/kg. The normal range of Zn in plant tissue has been reported to be 27-150 mg/kg with an upper toxic limit of 100-500 mg/kg [24], which suggests that the concentrations reported here for *A. pseudoplatanus* are unlikely to cause a toxic effect.

The Zn concentrations in the tissue of *U. dioica* and *A. pseudoplatanus* showed large differences; at the lowest Zn solution concentration the tissue concentration of *A. pseudoplatanus* is greater than that of *U. dioica*, but at higher concentrations the reverse is true, increasing from a 3 fold to a 13 fold difference at the highest solution concentration. The relationships between solution and tissue concentration between the species were also different; *U. dioica* having a steep linear relationship whilst for *A. pseudoplatanus* the relationship was exponential with the Zn tissue concentrations reaching a plateau at around 160 mg/kg. This suggests different responses to Zn between the two species. *U dioica* is unable to regulate Zn and continues to accumulate this metal until a toxic concentration is reached and the plant can no longer survive, in the present experiment this must have occurred to plants grown in the 42 mg Zn / L solution. In contrast *A. psuedoplatanus* is able to regulate the Zn concentration in its above-ground tissue and therefore survive in media containing higher concentrations of Zn.

Previous studies investigating the transfer of metals into aphids have used wheat grown in sewage sludge amended soils. In these studies the Zn concentrations in the plant tissue were substantially lower (<150 mg/kg) [11,12,25-27] than those found in *U. dioica* in the current study and more comparable to those in *A. pseudoplatanus*. Despite this, the concentrations of Zn in *M. carnosum* reported in the current study are similar to those found in these previous studies, which used different aphid species [11,12,25-27], whereas the concentrations in *D. platanoidis* were generally two to three times greater, even at the lowest Zn solution concentration. All of these studies found that Zn was bioaccumulated in the aphids *Rhopalosiphum padi* and *Sitobian avenae* feeding on wheat. In our study, from the total plant concentrations it appeared that *M. carnosum* was not accumulating Zn as the *U.dioica* bulk tissue concentration from the 18 mg Zn/l solution was around 2100 mg/kg and the aphid concentration was 131 mg/kg. However, the analysis of the phloem tissue of the nettle tissue revealed that *M. carnosum* were accumulating Zn as this concentration was around 50 mg/kg. Zn concentrations in *D. platanoidis* were greater, at around 375 mg/kg, than both the total plant and the phloem concentrations of 160 and 6.2 mg/kg respectively.

It has been reported that Zn is concentrated in the stem tissue as well as the roots [28] and is readily transported in the phloem of *A. pseudoplatanus* [29] and wheat [28,30]. Aphids feed directly on the phloem sap [8] and are therefore exposed to the Zn within this solution. The chemical form that Zn takes within the phloem is not well understood [31], although is likely to be in a soluble form, bound to chelators, amino acids and/or organic acids, it is also unclear whether the Zn is transported apoplastically or symplastically [31]. Studies on barley have shown that, whilst most of the Zn in the roots is soluble, that in the leaves is primarily located in the mesophyll cells and, to a lesser extent the epidermal cells; where is it present primarily in the cytoplasm, followed by the chloroplasts. The Zn in the apoplatsic solution is mainly (97 %) bound to cell walls [31]. The ability of *A. pseudoplatanus* to regulate Zn in its above-ground biomass, and because the transfer of Zn to the phloem is regulated by the requirements of the plant, may explain why, in this species, the concentrations in the phloem are similar regardless of the exposure to the plant or plant tissue concentrations. Water-soluble concentrations of Zn in *U. dioica* are much lower than the total plant concentrations, suggesting that the Zn within this species is also bound within the plant tissue and not readily transported in the phloem. However, the water-soluble concentrations increase with increasing Zn concentration in the watering solution and the plant tissue, further suggesting that *U. dioica* is not able to regulate Zn transport within the plant. This results in increased exposure to aphids as the concentration of Zn in the plant tissue increases.

Although it appears that both aphid species bioaccumulated Zn, the concentrations in *M. carnosum* were smaller than those for *D. platanoidis* despite an increased level of exposure. This may be because the duration of exposure of *D. platanoidis* was double that for *M. carnosum.* Alternatively, *M. carnosum* may be able to regulate Zn; Crawford et al. [10] found that *Aphis fabae* on broad beans (*Vicia faba*) were able to regulate Cu by excretion in honeydew. Unfortunately, it proved impossible to obtain sufficient quantities of honey dew for analysis in this study. The Zn concentrations in *M. carnosum* were comparable with those found in other studies, whereas those in *D. platanoidis* were elevated. This, coupled with the fact that the concentrations in *D. platanoidis* were elevated even when *A. pseudoplatanus* was watered with the control solution suggest that this species may simply have naturally greater Zn concentrations compared with other aphid species regardless of the concentration within the plant.

The greater Zn concentrations in *D. platanoidis* has important implications, both for the estimation of risk to higher organisms and the modelling of food-chain transfer, particularly given that the Zn tissue concentrations in *A. pseudoplatanus* were substantially lower than those in *U. dioica.* When the ladybird *Coccinella septempunctata*, lacewing *Chysoperla carnae* and carabid bettle *Bembidion lampros* were fed aphids with Zn concentrations ranging between 163-249, 104-188 and 60-116 mg/kg respectively their corresponding tissue concentrations were between 184-217, 105-249 and 99-112 respectively [12,26,27]. This suggests that, although only in the lacewing was Zn accumulated, the tissue concentrations of the predators of aphids are likely to reflect the tissue concentrations of their prey. Therefore species feeding on *D. platanoidis* may be exposed to higher concentrations of Zn in their diet than those feeding on other species of aphid. This demonstrates the importance of species specificity in modelling food-chain transfer in terrestrial ecosystems.

Acknowledgements – The authors wish to thank Sylvia Cowdry for assisting with experimental work and Alberto Morales from the Environmental and Human Science Division Laboratory, Forest Research for sample analysis. This work was funded by EPSRC as part of the Pollutants in the Urban Environment (PUrE) research consortium (Grant Number EP/C532651/2).

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Ref Type: Journal

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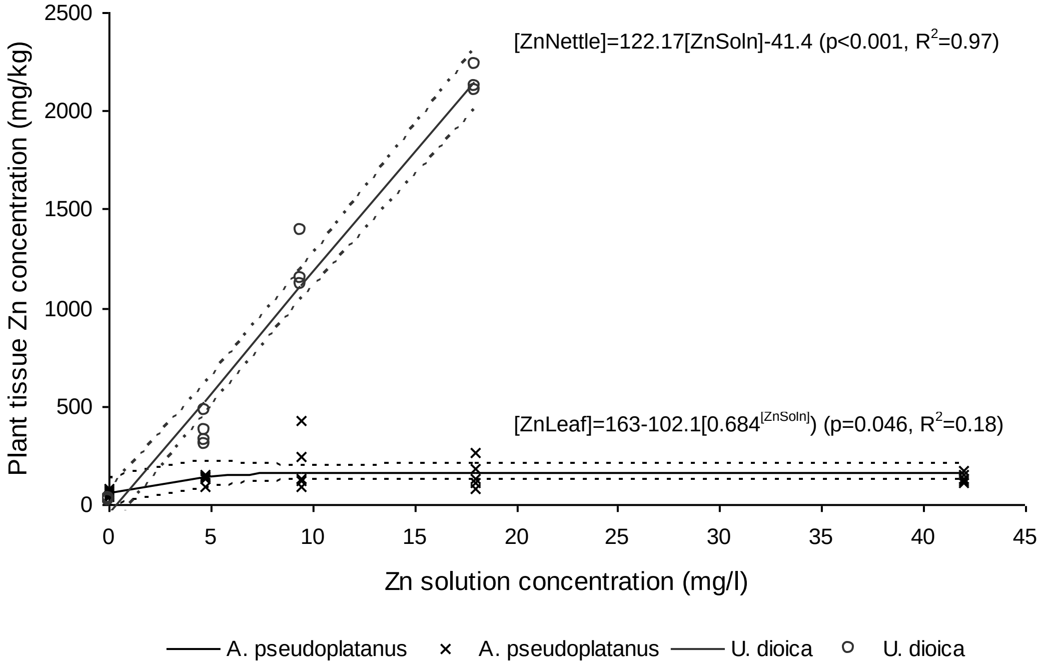
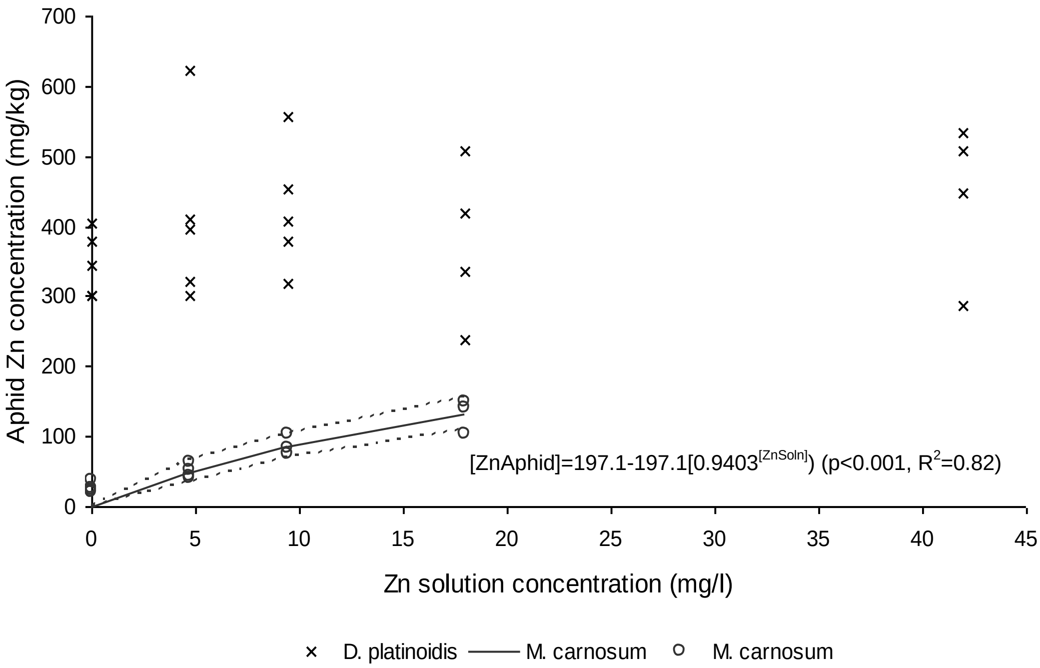
a)


[ZnNettle]=122.17[ZnSoln]-41.4 (p<0.001, R2=0.97)
[ZnLeaf]=163-102.1[0.684[ZnSoln]) (p=0.046, R2=0.18)
Plant tissue Zn concentration (mg/kg)
Zn solution concentration (mg/l)
0
500
1000
1500
2000
2500
0
5
10
15
20
25
30
35
40
45
A. pseudoplatanus
A. pseudoplatanus
U. dioica
U. dioica

b)


[ZnAphid]=197.1-197.1[0.9403[ZnSoln]) (p<0.001, R2=0.82)
Aphid Zn concentration (mg/kg)
Zn solution concentration (mg/l)
0
100
200
300
400
500
600
700
0
5
10
15
20
25
30
35
40
45
D. platinoidis
M. carnosum
M. carnosum

c)

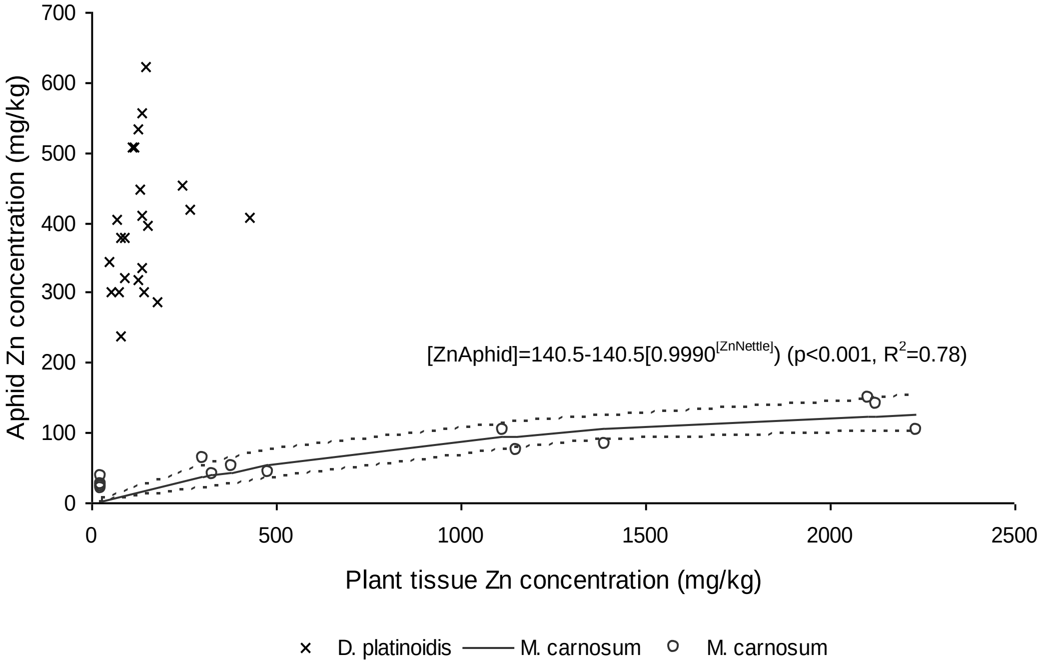


Figure 1: Zn concentration in a) the above-ground tissue of *U. dioica* (n=15) and the leaf tissue *A. pseudoplatanus* (n=25) exposed for 54 and 98 days respectively and b) *M. carnosum* (n=15) and *D. platinoidis* (n=23) exposed for 28 and 14 days respectively compared to the Zn concentration in Hoagland's solution in which the *U. dioica* and *A. pseudoplatanus* were grown and c) *M. carnosum* (n=15) and *D. platinoidis* (n=23) Zn concentrations compared with the Zn concentration in the above-ground tissue of *U. dioica* and the leaf tissue of *A. pseudoplatanus.* (Where [ZnNettle], [ZnLeaf], [ZnAphid], [ZnSoln] is the concentration of Zn in the tissue of *U. dioica* (mg/kg dry weight), the leaf tissue of *A.pseudoplatanus* (mg/kg dry weight), *M. carnosum* (mg/kg dry weight) and the watering solution (mg Zn/l) respectively).

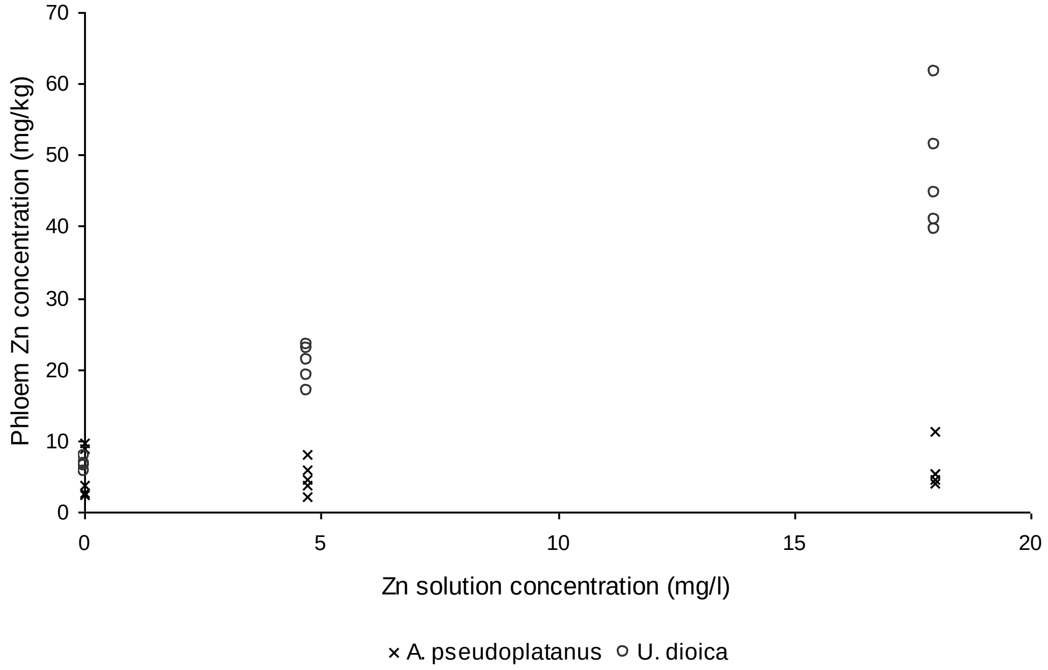


Figure 2: Zn concentration in the phloem tissue of *U. dioica* (n=15; mg/kg wet weight) and *A. pseudoplatanus* (n=15; mg/kg wet weight) exposed for 54 and 98 days respectively to Zn in Hoagland's solution.