

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

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## 1 **Food-chain transfer of zinc to aphids**

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

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

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

24

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

52

53 **Keywords** – stinging nettle, sycamore maple, common nettle aphid, sycamore aphid,  
54 contaminated land

55

## INTRODUCTION

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

76 *Urtica dioica* L. is prevalent in almost all urban ecosystems and is an early coloniser of  
77 contaminated land [4,5]. It is extremely important in urban ecosystems as it provides a habitat  
78 for a wide range of invertebrates [5,6]. In addition, it is also relatively simple to cultivate,  
79 widely available and fast growing [6], and as such, may be a useful species for  
80 ecotoxicological testing. *Acer pseudoplatanus* L. is a tree species that has been introduced to  
81 the UK, but is commonly found in urban areas [7]. It is an early coloniser [8] and tolerant of a  
82 wide range of site conditions [9]. *U. dioica* and *A. pseudoplatanus* both have extremely

83 prevalent species-specific aphids associated with them; *Microlophium carnosum* Buckton and  
84 *Drepanosiphum platanoidis* Schrank respectively.

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

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

102

## MATERIALS AND METHODS

103 *Transfer of Zn into Microlophium carnosum and Drepanosiphum platanoidis*

104 *U. dioica* cuttings, taken from Alice Holt Forest, Farnham, UK, and *A. pseudoplatanus* (bare  
105 rooted 1+1 stock; Prees Heath Forest Nurseries, Shropshire, UK) were planted individually in  
106 1 litre containers filled with perlite. Perlite was used as it has no inherent sorption capacity  
107 that could influence Zn availability. Additionally, pores between individual perlite beads  
108 ensure an aerobic environment. Pea shingle was placed on the perlite to a depth of 2 cm to  
109 minimise evaporation.



110 A fully replicated randomised block experiment with five replicates was set up in a  
111 glasshouse. Plants were grown under 16 h of artificial light and 8 h darkness per day (PAR =  
112 0.37 mmol/m/s). The temperature of the glasshouse was regulated to 20 °C ( $\pm 5$  °C).

113 Each container was watered with one of five solutions: control ( $\frac{1}{4}$  strength Hoagland's  
114 solution for *A. pseudoplatanus* and full strength for *U. dioica* [15]) or one of four Zn treatments  
115 in Hoagland's solution. The Hoagland's formulation provided background micronutrient  
116 concentrations of 0.02 or 0.08 mg Zn/l and 0.0008 or 0.0032 mg Cu/l for the  $\frac{1}{4}$  strength or full  
117 strength solutions respectively. Zn amendments were added as  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  to provide  
118 concentrations of 0.02 (control), 5, 10, 20 and 50 mg Zn/l. The solution in each container was  
119 replaced by mass when necessary.

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

129 *U. dioica* and *A. pseudoplatanus* were harvested 28 and 14 days respectively, after the  
130 aphids had been added. *D. platanoidis* populations appeared to be declining on the *A.*  
131 *pseudoplatanus* so these were harvested earlier than *U. dioica* in order to ensure that enough  
132 aphid mass was available for analysis. Reproduction rates of *D. platanoidis* vary during the  
133 season, being closely linked to the amino-nitrogen content of the leaves and this decline is  
134 likely to have been a result of the leaves reaching maturity [8]. The netting was loosened  
135 from around the pot and the stem cut, the netting was then closed at the bottom and detached  
136 from the ceiling, the netting along with its contents were then placed in the freezer at -20 °C  
137 for 2 hours. The plants were then removed from the freezer and the dead aphids collected  
138 with a fine brush. *A. pseudoplatanus* were split into their stem, shoot and leaf components.  
139 The above-ground tissue of *U. dioica* and leaf and shoot tissues of *A. pseudoplatanus* were

140 washed in deionised water to remove the honeydew, weighed and dried at 70 °C for 24 hours  
141 and reweighed. The stem tissues of *A. pseudoplatanus* were discarded as the aphids do not  
142 feed on this woody material. The aphids were weighed, dried at 50 °C for 24 hours and  
143 reweighed. The aphid and plant material samples were then milled and analysed to  
144 determine their Zn concentrations (see below).

#### 145 *Determination of phloem Zn concentrations*

146 In order to understand the different Zn exposures to the aphids a further experiment was set  
147 up to determine the phloem Zn concentrations within *U. dioica* and *A. pseudoplatanus*. *U.*  
148 *dioica* cuttings and *A. pseudoplatanus* were planted individually in 1 litre containers filled with  
149 perlite in the same way as for the aphid exposure experiment. A fully replicated randomised  
150 block experiment with five replicates for *U. dioica* and five replicates for *A. pseudoplatanus*  
151 was set up in a glasshouse under the same conditions as the aphid exposure experiment.  
152 Each container was watered with one of three solutions: control (¼ strength Hoaglands  
153 solution for *A. pseudoplatanus* and full strength for *U. dioica* [15]) or one of two Zn treatments  
154 in Hoaglands solution. Zn amendments were added as ZnSO<sub>4</sub>·7H<sub>2</sub>O to provide  
155 concentrations of 0.02 (control), 5 and 20 mg Zn/l. The solution in each container was  
156 replaced by mass when necessary.

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

169 phloem concentration. The above-ground tissue of *U. dioica* was removed, weighed and  
170 boiled at 100 °C in 300 ml of deionised water for 3 hours. The solutions were then analysed  
171 to determine their Zn concentrations (see below).

#### 172 *Determination of Zn concentration*

173 The Zn solutions used for watering and the phloem extracts were analysed using a Spectro  
174 Flame Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES; Spectro  
175 Analytical Instruments, West Midlands, UK). The target Zn concentrations in the solutions  
176 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  
177 respectively.

178 Plant samples were prepared for analysis by dry-ashing at 450 °C for 18 hours and wet  
179 digestion [17]. Wet digestion was achieved by incubating each sample for 1 hour at 60 °C in  
180 0.75 ml concentrated HNO<sub>3</sub>, followed by a further 14 hour incubation with 2.25 ml  
181 concentrated HCl and heating for 2 hours at 110 °C. After cooling, 0.15 ml of 30 % H<sub>2</sub>O<sub>2</sub> was  
182 added to each sample followed by heating for 30 minutes at 110 °C. To ensure complete  
183 oxidation of all organic matter the H<sub>2</sub>O<sub>2</sub> treatment was performed twice. The digested  
184 samples were analysed for Zn using the ICP-OES [18].

185 Aphid samples were digested in 1 ml concentrated HNO<sub>3</sub> at 180 °C for 1 hour, after which 1  
186 ml of deionised water was added and the sample further digested at 180 °C to dryness. A  
187 further 0.01 ml of concentrated nitric acid was added and the sample digested at 60 °C for 1  
188 hour. The digested samples were analysed for Zn using the ICP-OES [18].

189 The limit of detection was 0.67 µg/kg for Zn. Bush branches and leaves (NCS DC73349,  
190 China National Analysis Centre for Iron and Steel), oriental tobacco leaves (CTA-OTL-1,  
191 Commission for Trace Analysis of the Committee for Analytical Chemistry of the Polish  
192 Academy of Sciences and Institute of Nuclear Chemistry and Technology, Warsaw, Poland),  
193 mussel (CE278, European Commission, Geel, Belgium) and bovine liver (1577b, US  
194 Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD  
195 20899, USA) tissues were used as Certified Reference Materials (CRM) with batches of plant  
196 and aphid samples as appropriate. Mean recovery from oriental tobacco leaves was 104.9  
197 and 98.6 % from the bush branches and leaves and oriental tobacco respectively. Mean

198 recovery from mussel and bovine liver was 92.9 and 93.6 % respectively for the *M. carnosum*  
199 samples and 111.8 and 102.6 % respectively for the *D. platanoidis* samples.

#### 200 *Statistical analysis*

201 The plant and aphid Zn uptake data were subjected to general linear regression analysis to  
202 assess the significance of changes in plant and aphid concentrations with increasing Zn  
203 concentration in hydroponic solutions and plant material respectively, using Genstat version  
204 8.1 [19]. Mean values are reported with  $\pm$  standard errors throughout.

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

## 212 **RESULTS**

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

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

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

229 The concentration of Zn in the above-ground tissue of *U. dioica* were approximately 13 times  
230 that in the *A. pseudoplatanus* as a result of exposure to the 18 mg Zn/l solution;  
231  $2153 \pm 68.7$  mg/kg compared with  $163 \pm 20.6$  mg/kg. The phloem extract concentrations at this  
232 18 mg Zn/l exposure were  $48.2 \pm 2.4$  mg/kg in *U. dioica* and  $6.1 \pm 1.2$  mg/kg in *A.*  
233 *pseudoplatanus* (Figure 2). Despite this, the Zn concentration in *M. carnosum* was less than  
234 a third of that in *D. platanoidis*;  $131.5 \pm 11.0$  mg/kg compared with  $406 \pm 21.2$  mg/kg. Phloem  
235 concentrations of both species were lower than those in above-ground tissue in *U. dioica* or in  
236 the leaf and shoot tissues in *A. pseudoplatanus*, this difference increased with increasing Zn  
237 concentrations; from 3 up to 17 times lower and 11 up to 25 times lower in *U. dioica* and *A.*  
238 *pseudoplatanus* respectively.

239

## DISCUSSION

240 Zn concentrations in the above-ground tissue of *U. dioica* increased with Zn exposure,  
241 reaching a mean of approximately 2100 mg/kg for the 18 mg Zn/L solution. In *A.*  
242 *pseudoplatanus* tissue concentration increased up to the 9 mg Zn/l solution and then  
243 remained constant at around 160 mg/kg despite the increasing Zn concentration in solution.  
244 Zn concentrations in the above-ground tissue of *U. dioica* have been reported to range  
245 between 42 and 52 mg/kg in uncontaminated soils [20]. Leaf concentrations of between 23  
246 and 532 (mean 113 mg/kg) have been reported in *U. dioica* growing on dredged sediments  
247 with a Zn concentration of between 149 and 1817 (mean 54 mg/kg) [21]. Zn concentrations in  
248 *U. dioica* around the Avonmouth smelter have been found to be as high as 3000 mg/kg,  
249 although this is likely to have occurred from atmospheric deposition as well as soil uptake  
250 [22]. The substantial quantities of Zn that nettles appear to be capable of accumulating make  
251 this species an important pathway for Zn in the food-chain. Mertens et al. [23] found Zn  
252 concentrations with a mean of 74 mg/kg in *A. pseudoplatanus* grown on dredged sediments  
253 with a Zn concentration of 359 mg/kg. The normal range of Zn in plant tissue has been

254 reported to be 27-150 mg/kg with an upper toxic limit of 100-500 mg/kg [24], which suggests  
255 that the concentrations reported here for *A. pseudoplatanus* are unlikely to cause a toxic  
256 effect.

257 The Zn concentrations in the tissue of *U. dioica* and *A. pseudoplatanus* showed large  
258 differences; at the lowest Zn solution concentration the tissue concentration of *A.*  
259 *pseudoplatanus* is greater than that of *U. dioica*, but at higher concentrations the reverse is  
260 true, increasing from a 3 fold to a 13 fold difference at the highest solution concentration. The  
261 relationships between solution and tissue concentration between the species were also  
262 different; *U. dioica* having a steep linear relationship whilst for *A. pseudoplatanus* the  
263 relationship was exponential with the Zn tissue concentrations reaching a plateau at around  
264 160 mg/kg. This suggests different responses to Zn between the two species. *U. dioica* is  
265 unable to regulate Zn and continues to accumulate this metal until a toxic concentration is  
266 reached and the plant can no longer survive, in the present experiment this must have  
267 occurred to plants grown in the 42 mg Zn / L solution. In contrast *A. psuedoplatanus* is able to  
268 regulate the Zn concentration in its above-ground tissue and therefore survive in media  
269 containing higher concentrations of Zn.

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

283 around 50 mg/kg. Zn concentrations in *D. platanoidis* were greater, at around 375 mg/kg,  
284 than both the total plant and the phloem concentrations of 160 and 6.2 mg/kg respectively.

285 It has been reported that Zn is concentrated in the stem tissue as well as the roots [28] and is  
286 readily transported in the phloem of *A. pseudoplatanus* [29] and wheat [28,30]. Aphids feed  
287 directly on the phloem sap [8] and are therefore exposed to the Zn within this solution. The  
288 chemical form that Zn takes within the phloem is not well understood [31], although is likely to  
289 be in a soluble form, bound to chelators, amino acids and/or organic acids, it is also unclear  
290 whether the Zn is transported apoplastically or symplastically [31]. Studies on barley have  
291 shown that, whilst most of the Zn in the roots is soluble, that in the leaves is primarily located  
292 in the mesophyll cells and, to a lesser extent the epidermal cells; where it is present primarily  
293 in the cytoplasm, followed by the chloroplasts. The Zn in the apoplatic solution is mainly (97  
294 %) bound to cell walls [31]. The ability of *A. pseudoplatanus* to regulate Zn in its above-  
295 ground biomass, and because the transfer of Zn to the phloem is regulated by the  
296 requirements of the plant, may explain why, in this species, the concentrations in the phloem  
297 are similar regardless of the exposure to the plant or plant tissue concentrations. Water-  
298 soluble concentrations of Zn in *U. dioica* are much lower than the total plant concentrations,  
299 suggesting that the Zn within this species is also bound within the plant tissue and not readily  
300 transported in the phloem. However, the water-soluble concentrations increase with  
301 increasing Zn concentration in the watering solution and the plant tissue, further suggesting  
302 that *U. dioica* is not able to regulate Zn transport within the plant. This results in increased  
303 exposure to aphids as the concentration of Zn in the plant tissue increases.

304 Although it appears that both aphid species bioaccumulated Zn, the concentrations in *M.*  
305 *carosum* were smaller than those for *D. platanoidis* despite an increased level of exposure.  
306 This may be because the duration of exposure of *D. platanoidis* was double that for *M.*  
307 *carosum*. Alternatively, *M. carosum* may be able to regulate Zn; Crawford et al. [10] found  
308 that *Aphis fabae* on broad beans (*Vicia faba*) were able to regulate Cu by excretion in  
309 honeydew. Unfortunately, it proved impossible to obtain sufficient quantities of honey dew for  
310 analysis in this study. The Zn concentrations in *M. carosum* were comparable with those  
311 found in other studies, whereas those in *D. platanoidis* were elevated. This, coupled with the  
312 fact that the concentrations in *D. platanoidis* were elevated even when *A. pseudoplatanus*

313 was watered with the control solution suggest that this species may simply have naturally  
314 greater Zn concentrations compared with other aphid species regardless of the concentration  
315 within the plant.

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

328

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334

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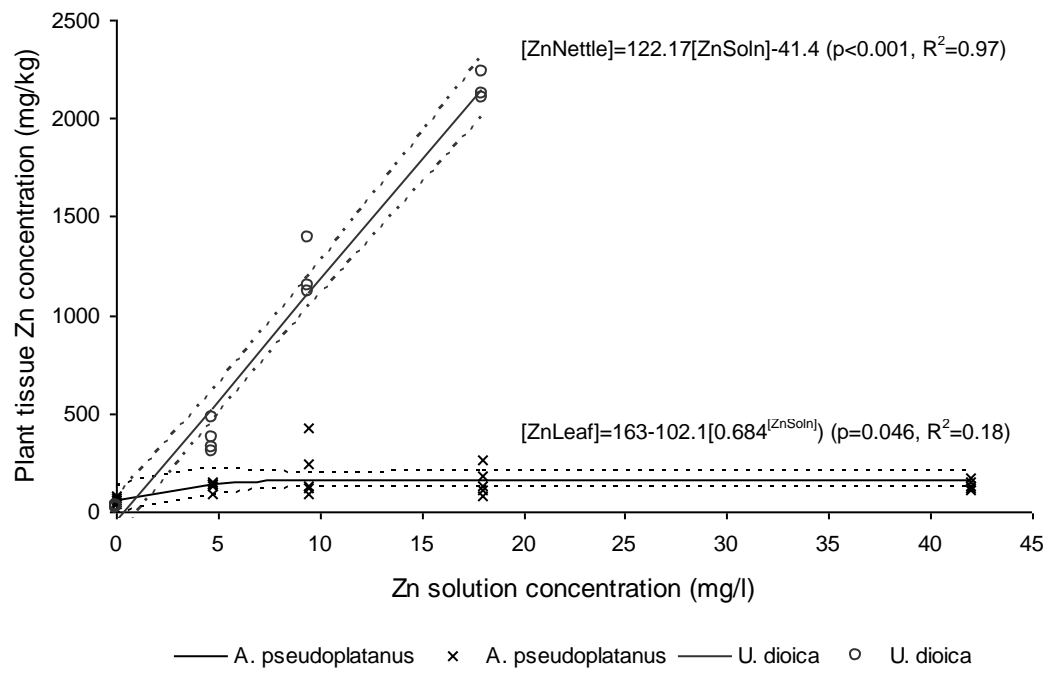
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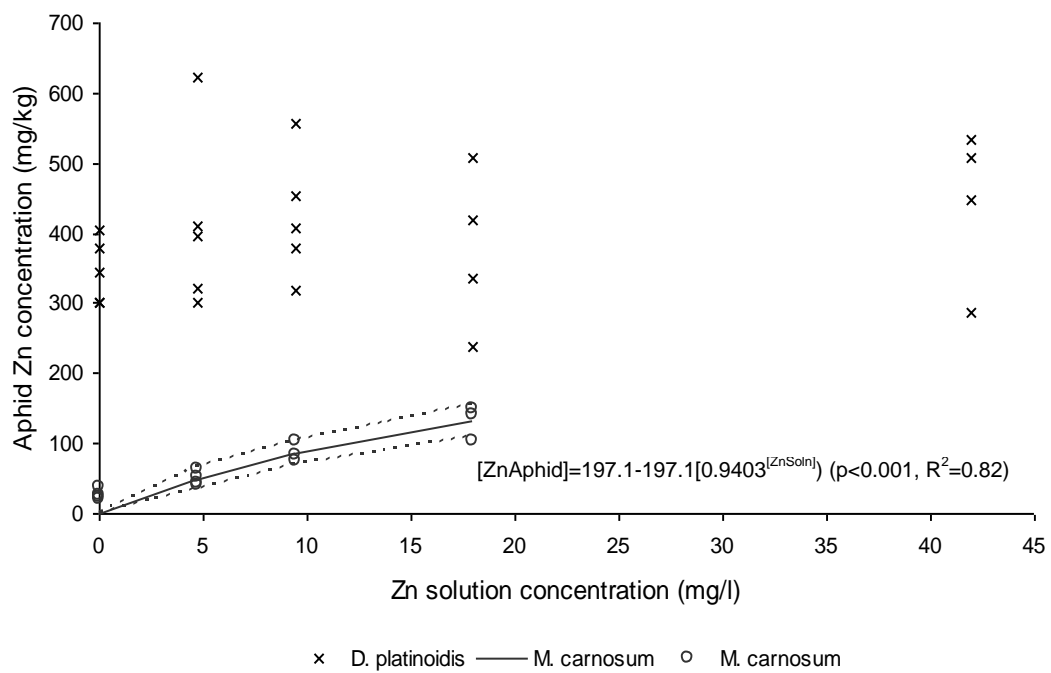
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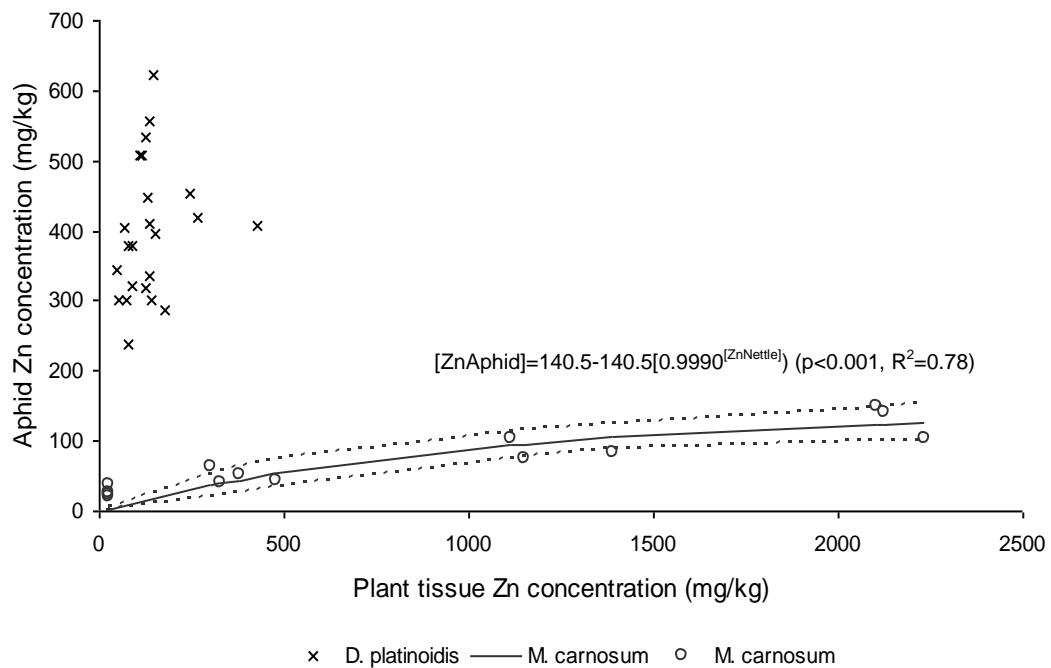
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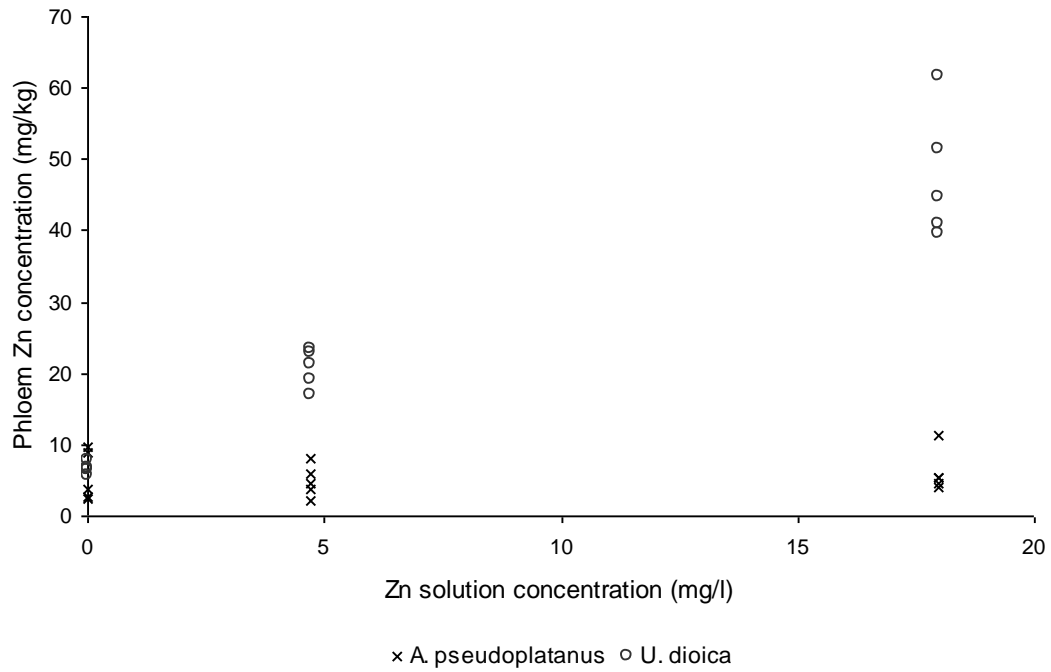
b)



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.**