1 # Food-chain transfer of zinc from contaminated *Urtica dioica* and *Acer*
2 # *pseudoplatanus* L. to the aphids *Microlophium carnosum* and
3 # *Drepanosiphum platanoidis* Schrank

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13 **Abstract**

14 This study examines the food-chain transfer of Zn from two plant species, *Urtica*
15 *dioica* (stinging nettle) and *Acer pseudoplatanus* (sycamore maple), into their corresponding
16 aphid species, *Microlophium carnosum* and *Drepanosiphum platanoidis*. The plants were
17 grown in a hydroponic system using solutions with increasing concentrations of Zn from 0.02
18 to 41.9 mg Zn/l. Above-ground tissue concentrations in *U. dioica* and *M. carnosum* increased
19 with increasing Zn exposure ($p$<0.001). Zn concentrations in *A. pseudoplatanus* also
20 increased with solution concentration from the control to the 9.8 mg Zn/l solution, above which
21 concentrations remained constant. Zn concentrations in both *D. platanoidis* and the phloem
22 tissue of *A. pseudoplatanus* were not affected by the Zn concentration in the watering
23 solution. It appears that *A. pseudoplatanus* was able to limit Zn transport in the phloem,
24 resulting in constant Zn exposure to the aphids. Zn concentrations in *D. platanoidis* were
25 around three times those in *M. carnosum*.

26 **Capsule**

27 Concentrations of Zn in two aphid species are dependant on species and exposure.

28 **Keywords**

29 Stinging nettle; Sycamore maple; Common nettle aphid; Sycamore aphid; Contaminated land

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30 **1. Introduction**

31 The importance of the impact of contaminated land on terrestrial ecological receptors
32 is increasingly being recognised in site investigation, risk assessment and remediation
33 processes. Practitioners commonly use an Ecological Risk Assessment (ERA) to determine
34 the potential for harm that a site may pose to ecological receptors and many countries have
35 produced frameworks and guidance for conducting such investigations (Bryns and Crane,
36 2002). The ERA process often makes use of a combination of field and laboratory analysis
37 and models to determine the risk to either ecological function or the food-chain transfer of
38 pollutants. The majority of the ecotoxicological tests used in ERA are based on ecological
39 function and use ecological endpoints such as mortality, reproduction and growth. In order to
40 estimate the risk to higher organisms from a contaminated site it is often necessary to use
41 models to predict the pollutant concentrations through the food-chain and relate these to
42 published toxicological endpoints for the species of interest. There are a variety of models
43 available to estimate the food-chain transfer of pollutants (USEPA, 2002; USEPA, 2003).
44 However, the models are often not species specific, may have been based on aquatic
45 organisms (that may not be appropriate for flying insects), or may only be applicable to a
46 certain group of contaminants (USEPA, 2002; USEPA, 2003). This has serious implications
47 for those using such models to estimate risk from contaminated land to ecological receptors.
48 At best it may result in significant gaps in the range of biological species for which such a risk
49 assessment can be conducted. At worst it may result in an over- or under-estimation of the
50 risk leading to either unnecessarily costly remediation or no remediation taking place where it
51 is needed.

52 *Urtica dioica* L. is prevalent in almost all urban ecosystems and is an early coloniser
53 of contaminated land (Barta and Cagán, 2003; Edwards et al., 1998). It is extremely
54 important in urban ecosystems as it provides a habitat and food source for a wide range of
55 invertebrates (Barta and Cagán, 2003; Davis, 1991). In addition, it is also relatively simple to
56 cultivate, widely available and fast growing (Davis, 1991), and as such, may be a useful
57 species for ecotoxicological testing (Sinnett et al., In press). *Acer pseudoplatanus* L. is a tree
58 species that has been introduced to the UK, but is commonly found in urban areas (Gilbert,
59 1991). It is an early coloniser (Dixon, 2005) and tolerant of a wide range of site conditions

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60 (Moffat and McNeill, 1994). *U. dioica* and *A. pseudoplatanus* both have extremely prevalent
61 species-specific aphids associated with them; *Microlophium carnosum* Buckton and
62 *Drepanosiphum platanoidis* Schrank respectively.

63 The food-chain transfer of metals to a variety of aphids have been assessed in a
64 number of studies (Crawford et al., 1995; Green et al., 2003; Merrington et al., 1997a),
65 although these studies have all concentrated on aphids whose hosts are agricultural plant
66 species. Aphids are an important source of food for a large number of other insects and
67 birds, either indirectly for their honeydew (e.g. ants) or directly (e.g. parasitoids, ladybirds and
68 blue tits; Braun and Flückiger, 1985; Gilbert, 1991). *M. carnosum* is a large aphid (3.3 to 3.8
69 mm) commonly found on *U. dioica*, primarily on the underside of the leaves and the stem
70 (Rotheray, 1989) during May to October (Barta and Cagán, 2003; Davis, 1991). *D.*
71 *platanoidis* is abundant on the underside of leaves of *A. pseudoplatanus*, during April to
72 October, with population peaks in June and October (Dixon, 2005). The importance of aphids
73 as a food source may mean that they could provide a pathway for metal contamination to
74 transfer to higher organisms. Elevated populations of some aphid species (*Aphis pomi*) have
75 also been reported close to roads, and it has been suggested that this may be, in part, due to
76 the increased concentrations of Zn in the host plant as this is an essential element for aphids
77 (Kean and Müller, 2004). Used in conjunction with *U. dioica* and *A. pseudoplatanus*, *M.*
78 *carnosum* and *D. platanoidis* have the potential to assess the risk of food-chain transfer of
79 metals in urban ecosystems.

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

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87 **2. Materials and methods**

88 *2.1. Transfer of Zn into* Microlophium carnosum *and* Drepanosiphum platanoidis

89 *U. dioica* were cultivated in the same way as described in Sinnett et al. (In press). *U.*
90 *dioica* cuttings, taken from Alice Holt Forest, Farnham, UK, and *A. pseudoplatanus* (bare
91 rooted 1+1 stock; Prees Heath Forest Nurseries, Shropshire, UK) were planted individually in
92 1 litre containers filled with perlite. Perlite was used as it has no inherent sorption capacity
93 that could influence Zn availability. Additionally, pores between individual perlite beads
94 ensure an aerobic environment. Pea shingle was placed on the perlite to a depth of 2 cm to
95 minimise evaporation.

96 A fully replicated randomised block experiment with five plants per Zn treatment was
97 set up in a glasshouse. Plants were grown under 16 h of artificial light and 8 h darkness per
98 day (photosynthetically active radiation = 0.37 mmol/m/s). The temperature of the glasshouse
99 was regulated to 20 $^{o}$C (±5 $^{o}$C) by heating and ventilation.

100 Each container was watered with one of five solutions: control (¼ strength Hoagland's
101 solution for *A. pseudoplatanus* and full strength for *U. dioica* (Hoagland and Arnon, 1941) or
102 one of four Zn treatments in Hoagland's solution. The Hoagland's formulation provided
103 background micronutrient concentrations of 0.02 or 0.08 mg Zn/l for the ¼ strength or full
104 strength solutions respectively. Zn amendments were added as $ZnSO_4.7H_2O$ to provide
105 concentrations of 0.02 (control), 5, 10, 20, and 50 mg Zn/l. The solution in each container
106 was replaced by mass when necessary to return to a given mass.

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

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116 *U. dioica* and *A. pseudoplatanus* were harvested 28 and 14 d respectively, after the
117 aphids had been added. *D. platanoidis* populations appeared to be declining on the *A.*
118 *pseudoplatanus* so these were harvested earlier than *U. dioica* in order to ensure that enough
119 aphid mass was available for analysis. Reproduction rates of *D. platanoidis* vary during the
120 season, being closely linked to the amino-nitrogen content of the leaves and this decline is
121 likely to have been a result of the leaves reaching maturity (Dixon, 2005). The netting was
122 loosened from around the pot and the stem cut, the netting was then closed at the bottom and
123 detached from the ceiling, the netting along with its contents were then placed in the freezer
124 at -20 $^{o}$C for 2 h. The plants were then removed from the freezer and the dead aphids
125 collected individually with a fine brush and tweezers. *A. pseudoplatanus* were divided into
126 their stem, petiole and leaf components. After thawing, the above-ground tissue of *U. dioica*
127 and leaf and shoot tissues of *A. pseudoplatanus* were washed in deionised water to remove
128 the honeydew, weighed and dried at 70 $^{o}$C for 24 h and reweighed. The stem tissues of *A.*
129 *pseudoplatanus* were discarded as the aphids only feed on the petioles and leaves. The
130 aphids were weighed, dried at 50 $^{o}$C for 24 h and re-weighed. The aphid and plant material
131 samples were then milled and analysed to determine their Zn concentrations.

132 *2.2. Determination of phloem or water extractable plant Zn concentrations*

133 In order to understand the Zn exposures to the aphids, a further experiment was set
134 up to determine the phloem Zn concentrations in *A. pseudoplatanus* and the water extractable
135 plant Zn concentrations in *U. dioica*. *U. dioica* cuttings and *A. pseudoplatanus* were planted
136 individually in 1 litre containers filled with perlite in the same way as for the aphid exposure
137 experiment. A fully replicated randomised block experiment with five replicates for both *U.*
138 *dioica* and five replicates for *A. pseudoplatanus* was set up in a glasshouse under the same
139 conditions as the aphid exposure experiment. Each container was watered with one of three
140 solutions: control (¼ strength Hoagland's solution for *A. pseudoplatanus* and full strength for
141 *U. dioica*) or one of two Zn treatments in Hoagland's solution of 0.02 (control), 5, and 20 mg
142 Zn/l.

143 *U. dioica* and *A. pseudoplatanus* were harvested after the same duration as the aphid
144 experiment in order to ensure that the plants had been exposed to the Zn solutions for the

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145 same time period. The method used to determine the concentration of Zn in the sycamore
146 phloem tissue was based on that of Thornber and Northcote (1961) which extracts the water-
147 soluble material within the phloem. The leaf and shoot tissues of *A. pseudoplatanus* were
148 removed from the stem tissue and discarded. The bark was carefully removed from the stem
149 tissue using a grafting knife and the phloem tissue was then removed, again with a grafting
150 knife. The phloem tissue was weighed and then boiled at 100 $^oC$ in 200 ml of deionised water
151 for 3 h. Following boiling, the samples were centrifuged and the solution removed and filtered
152 through a 0.45 µm Whatman filter. It was not possible to separate the phloem tissue of *U.*
153 *dioica* from the rest of the stem so the entire above-ground biomass was subjected to boiling
154 in order to derive the water-soluble fraction of the plant material. The above-ground tissue of
155 *U. dioica* was removed, weighed and boiled at 100 $^oC$ in 300 ml of deionised water for 3 h.
156 The solutions were then analysed to determine their Zn concentrations.

157 *2.3. Determination of Zn concentration*

158 The Zn solutions used for watering and the phloem extracts were analysed, with
159 yttrium as an internal standard, using a Spectro Flame Inductively Coupled Plasma – Optical
160 Emission Spectrometer (ICP-OES; Spectro Analytical Instruments, West Midlands, UK). The
161 target Zn concentrations in the solutions used for watering of 0.02, 5, 10, 20, and 50 mg Zn/l
162 were found to be 0.02, 4.7, 9.8, 18.0 and 41.9 mg Zn/l respectively.

163 Bush branches and leaves (NCS DC73349, China National Analysis Centre for Iron
164 and Steel) and oriental tobacco leaves (CTA-OTL-1, Commission for Trace Analysis of the
165 Committee for Analytical Chemistry of the Polish Academy of Sciences and Institute of
166 Nuclear Chemistry and Technology, Warsaw, Poland) were used as certified reference
167 materials (CRM) during the analysis of the plant tissue. Plant samples and the CRMs were
168 prepared for analysis by dry-ashing at 450 $^oC$ for 18 h and wet digestion (Chapman, 1967).
169 Wet digestion was achieved by incubating each sample for 1 h at 60 $^oC$ in 0.75 ml
170 concentrated $HNO_3$, followed by a further 14 h incubation with 2.25 ml concentrated HCl and
171 heating for 2 h at 110 $^oC$. After cooling, 0.15 ml of 30% $H_2O_2$ was added to each sample
172 followed by heating for 0.5 h at 110 $^oC$. To ensure complete oxidation of all organic matter
173 the $H_2O_2$ treatment was performed twice. The digested samples were analysed for Zn using

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174 the ICP-OES (Kilbride et al., 2006); the limit of detection was 0.67 µg/kg for Zn. Mean
175 recovery was 104.9 and 98.6% from the bush branches and leaves and oriental tobacco
176 respectively.

177 Mussel (CE278, European Commission, Geel, Belgium) and bovine liver (1577b, US
178 Department of Commerce, National Institute of Standards and Technology. Gaithersburg, MD
179 20899, USA) tissues were used as CRMs during analysis of the aphid samples. Aphid
180 samples and CRMs were digested in 1 ml concentrated $HNO_3$ at 180 °C for 1 h, after which 1
181 ml of deionised water was added and the sample further digested at 180 °C to dryness. A
182 further 0.01 ml of concentrated nitric acid was added and the sample digested at 60 °C for 1
183 h. The digested samples were analysed for Zn using the ICP-OES (Kilbride et al., 2006).
184 Mean recovery from mussel and bovine liver was 92.9 and 93.6% respectively for the *M.*
185 *carnosum* samples and 111.8 and 102.6% respectively for the *D. platanoidis* samples.

186 *2.4. Statistical analysis*

187 The plant and aphid Zn uptake data were subjected to general linear regression
188 analysis to assess the significance of changes in plant and aphid concentrations with
189 increasing Zn concentration in hydroponic solutions and plant material respectively, using
190 Genstat version 8.1 (Genstat, 2005). Mean values are reported with ± standard errors
191 throughout.

192 Linear and exponential models of Zn uptake into each of the plant and aphid tissue
193 types compared to that of the solution concentration and, in the case of aphids, the leaf
194 concentrations were fitted using Genstat version 8.1 (Genstat, 2005). A comparison of the
195 residual sum of squares of alternative models relative to the smallest residual mean square
196 was used to determine the most appropriate model. This comparison was used for nested
197 models and was referred to an F-distribution with 1, *n* degrees of freedom where *n* is the
198 residual degrees of freedom from the exponential model.

199 **3. Results**

200 Zn concentration in solution had a significant effect on the Zn uptake into the above
201 ground tissue ($F_{1,13}$=533.63; $p$<0.001) of *U. dioica*; no plants survived in the 41.9 mg Zn/l
202 solution treatment (Fig. 1). Zn concentration in both solution and nettle tissue had a

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203 significant effect on the Zn concentration in *M. carnosum* ($F_{1,13}$=107.95; $p$<0.001 and
204 $F_{1,13}$=77.38; $p$<0.001 respectively; Fig. 1). The concentration of water-soluble Zn in *U. dioica*
205 increased significantly with increasing Zn concentration in solution ($F_{1,13}$=138.89; $p$<0.001)
206 from 6.6 to 47.6 mg/kg (Fig. 2). The difference between the bulk tissue concentration of Zn
207 and that which was water-soluble increased with increasing Zn exposure; the water-soluble
208 fraction being 2 to 17 times smaller than the bulk tissue concentration.

209 Zn concentration in solution had a significant effect on the Zn uptake into the leaf
210 ($F_{2,22}$=3.57; $p$=0.046) and shoot ($F_{2,22}$=5.43; $p$=0.012) tissue of *A. pseudoplatanus*; this
211 relationship was exponential not linear (Fig. 3). There was no significant effect of the
212 concentration of Zn in solution or in the petiole or leaf tissue of *A. pseudoplatanus* on the
213 concentration in *D. platanoidis* using either the linear or exponential models (Fig. 3).
214 Similarly, the concentration of Zn in the phloem extract was not significantly related to the
215 concentration of Zn in solution (Fig. 2). Phloem concentrations in *A. pseudoplatanus* were
216 smaller than those in the leaf and shoot tissues. This difference increased with increasing Zn
217 concentrations; phloem concentrations being 11 to 25 times lower than leaf or shoot tissue
218 concentrations.

219 The concentration of Zn in the above-ground tissue of *U. dioica* were approximately
220 13 times that in the leaves of *A. pseudoplatanus* as a result of exposure to the 18.0 mg Zn/l
221 solution; 2153±68.7mg/kg compared with 163±20.6 mg/kg. Despite this, the Zn concentration
222 in *M. carnosum* was less than a third of that in *D. platanoidis*; 131.5±11.0 mg/kg compared
223 with 406±21.2 mg/kg.

224 **4. Discussion**

225 No *U. dioica* survived in the 41.9 mg/l solution, in contrast Sinnett et al. (In press)
226 found that *U. dioica* were able to survive, albeit with reduced growth, in this solution. This
227 difference is likely to be as a result of the additional stress exerted on the plants by the
228 introduction of the aphids. Zn concentrations in the above-ground tissue of *U. dioica*
229 increased with Zn exposure, reaching a mean of approximately 2100 mg/kg for the 18.0 mg
230 Zn/l solution. This is in the range that was reported in a separate experiment where *U. dioica*
231 were exposed to Zn under the same experimental conditions, but in the absence of aphids;

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232 where concentrations of Zn in the leaf, shoot and stem tissue were approximately 1800, 2800
233 and 2500 mg/kg respectively (Sinnett et al., In press). In *A. pseudoplatanus*, tissue
234 concentration increased up to the 9.8 mg Zn/l solution and then remained constant at
235 approximately 160 mg/kg despite the increasing Zn concentration in solution. Zn
236 concentrations in the above-ground tissue of *U. dioica* have been reported to range between
237 42 and 52 mg/kg in uncontaminated soils (Hobbelen et al., 2004). Leaf concentrations of
238 between 23 and 532 (mean 113 mg/kg) have been reported in *U. dioica* growing on dredged
239 sediments with a Zn concentration of between 149 and 1817 (mean 54 mg/kg; Tack and
240 Verloo, 1996). Zn concentrations in *U. dioica* around the Avonmouth smelter have been
241 found to be as high as 3000 mg/kg, although this is likely to have occurred from atmospheric
242 deposition as well as soil uptake (Jones, 1991). The substantial quantities of Zn that nettles
243 appear to be capable of taking up make this species an important pathway for Zn in the food-
244 chain. Mertens et al. (2004) found Zn concentrations with a mean of 74 mg/kg in *A.*
245 *pseudoplatanus* grown on dredged sediments with a Zn concentration of 359 mg/kg. The
246 normal range of Zn in plant tissue has been reported to be 27-150 mg/kg with an upper toxic
247 limit of 100-500 mg/kg (Kabata-Pendias and Pendias, 2001), which suggests that the
248 concentrations reported here for *A. pseudoplatanus* are unlikely to cause a toxic effect.

249 The Zn concentrations in the tissue of *U. dioica* and *A. pseudoplatanus* showed large
250 differences; at the lowest Zn solution concentration the tissue concentration of *A.*
251 *pseudoplatanus* is greater than that of *U. dioica*, but at higher concentrations the converse is
252 true, increasing from a 3 fold to a 13 fold difference at the highest solution concentration. The
253 relationships between solution and tissue concentration between the species were also
254 different; *U. dioica* having a steep linear relationship whilst for *A. pseudoplatanus* the Zn
255 tissue concentration reached a plateau at approximately 160 mg/kg. This suggests different
256 responses to Zn between the two species. *U dioica* continues to accumulate Zn until a toxic
257 concentration is reached and the plant can no longer survive. In the present experiment this
258 occurred to plants grown in the 41.9 mg Zn/l solution. In contrast, *A. pseudoplatanus* is able
259 to limit transport of Zn into its above-ground tissue and therefore survive in media containing
260 higher concentrations of Zn. However, the increasing difference between the water-soluble
261 concentrations in *U. dioica* or phloem concentrations in *A. pseudoplatanus* and their bulk

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262 tissue concentrations with increasing Zn exposure suggests that both species are able to limit
263 the transport of Zn in their above-ground tissues.

264 Previous studies investigating the transfer of metals into aphids have used wheat
265 grown in sewage sludge amended soils. In these studies the Zn concentrations in the plant
266 tissue were substantially lower (<150 mg/kg; Green et al., 2003; Green et al., 2006;
267 Merrington et al., 1997a. Merrington et al., 1997b; Winder et al., 1999) than those found in *U.*
268 *dioica* in the current study and more comparable to those in *A. pseudoplatanus*. Despite this,
269 the concentrations of Zn in *M. carnosum* reported in the current study are similar to those
270 found in these previous studies, which used different aphid species (Green et al., 2003;
271 Green et al., 2006; Merrington et al., 1997a. Merrington et al., 1997b; Winder et al., 1999). In
272 contrast, the concentrations in *D. platanoidis* were generally two to three times greater, even
273 at the lowest Zn solution concentration. All of these studies found that Zn accumulated in the
274 aphids *Rhopalosiphum padi* and *Sitobian avenae* feeding on wheat. In our study, from the
275 total plant concentrations it appeared that *M. carnosum* was not accumulating Zn as the *U.*
276 *dioica* bulk tissue concentration from the 18.0 mg Zn/l solution was approximately 2100 mg/kg
277 and the aphid concentration was 131 mg/kg. However, the analysis of the water-soluble
278 tissue extract suggests that *M. carnosum* may actually be exposed to much lower
279 concentrations. Zn concentrations in *D. platanoidis* were greater, at around 375 mg/kg, than
280 both the total plant and the phloem concentrations of 160 and 6.2 mg/kg respectively.

281 It has been reported that Zn is concentrated in the stem tissue as well as the roots
282 (Haslett et al., 2001) and is readily transported in the phloem of *A. pseudoplatanus* (Dollard
283 and Lepp, 1980) and wheat (Haslett et al., 2001; Riesen and Feller, 2005). Aphids feed
284 directly on the phloem sap (Dixon, 2005) and are therefore exposed to the Zn in this solution.
285 The chemical form that Zn takes in the phloem is not well understood (Grusak et al., 2007),
286 although it is likely to be in a soluble form, bound to chelators, amino acids and/or organic
287 acids and it is also unclear whether the Zn is transported apoplastically or symplastically
288 (Grusak et al., 2007). Studies on barley have shown that most of the Zn in the roots is in a
289 soluble form. However, Zn in the leaves is mainly located in the mesophyll cells and, to a
290 lesser extent the epidermal cells. The Zn in the apoplastic solution (97%) is mainly bound to
291 cell walls (Grusak et al., 2007). The ability of *A. pseudoplatanus* to limit Zn in its above-

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292 ground biomass, and because the transfer of Zn to the phloem is regulated by the
293 requirements of plants (Haslett et al., 2001), may explain why, in this species, the
294 concentrations in the phloem are similar regardless of the exposure to the plant or plant tissue
295 concentrations. Water-soluble concentrations of Zn in *U. dioica* are much lower than the total
296 plant concentrations, suggesting that the Zn within this species is also bound in the plant
297 tissue. However, in *U. dioica*, the water-soluble Zn concentration did increase with Zn
298 exposure which may suggest that this species is not as adept at limiting Zn transport or that it
299 has a greater Zn requirement.

300 Although it appears that both aphid species bioaccumulated Zn, the concentrations in
301 *M. carnosum* were smaller than those for *D. platanoidis* despite an increased level of
302 exposure. This may be because the duration of exposure of *D. platanoidis* was double that
303 for *M. carnosum.* Alternatively, *M. carnosum* may be able to regulate Zn; Crawford et al.
304 (1995) found that *Aphis fabae* on broad beans (*Vicia faba*) were able to regulate Cu by
305 excretion in honeydew. Unfortunately, it proved impossible to obtain sufficient quantities of
306 honey dew for analysis in the present study. The Zn concentrations in *M. carnosum* were
307 comparable with those found in other studies, whereas those in *D. platanoidis* were greater.
308 This, coupled with the fact that the concentrations in *D. platanoidis* were greater even when
309 *A. pseudoplatanus* was watered with the control solution suggest that this species may simply
310 have greater Zn concentrations (or requirements) than have previously been reported in other
311 aphid species.

312 The greater Zn concentration in *D. platanoidis* has important implications, both for the
313 estimation of risk to higher organisms and the modelling of food-chain transfer as it highlights
314 the, often substantial, differences between biological species. When the ladybird *Coccinella*
315 *septempunctata*, lacewing *Chysoperla carnae* and carabid bettle *Bembidion lampros* were fed
316 aphids with Zn concentrations ranging between 163-249, 104-188 and 60-116 mg/kg
317 respectively their corresponding tissue concentrations were between 184-217, 105-249 and
318 99-112 respectively (Green et al., 2003; Green et al., 2006; Winder et al., 1999). This
319 suggests that, although only in the lacewing was Zn accumulated, the tissue concentrations of
320 the predators of aphids are likely to reflect the tissue concentrations of their prey. Therefore
321 species feeding on *D. platanoidis* may be exposed to higher concentrations of Zn in their diet

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322 than those feeding on other species of aphid. This demonstrates the importance of species
323 specificity in modelling food-chain transfer in terrestrial ecosystems.

324 **5. Conclusions**

325 This study demonstrates the importance of species-specify in ERA and food-chain
326 transfer modelling. *A. pseudoplatanus* appears to be able to regulate the Zn concentration its
327 above-ground biomass which results in tissue concentrations that are unaffected by Zn
328 exposure at the concentrations tested. This results in a constant exposure to *D. platanoidis*.
329 *U. dioica*, however, continues to accumulate Zn in its above-ground tissue at concentrations
330 more than ten times greater than those measured in *A. pseudoplatanus*, resulting in an
331 increasing level of exposure to *M. carnosum*. Despite the greater plant Zn concentrations in
332 *U. dioica* compared with *A. pseudoplatanus, M. carnosum* had Zn concentrations that were
333 approximately one third of those in *D. platanoidis.*

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419 **Fig. 1.** Zn uptake models (solid lines), with 95% confidence intervals (dashed lines), and data
420 for the above-ground tissue of *Urtica dioica* (x; n=15; 54 d exposure) and the whole body of
421 *Microlophium carnosum* (o; n=15; 28 day exposure) after exposure to Zn. Where [ZnNettle],
422 [ZnAphid], [ZnSoln] is the concentration of Zn in the tissue of *U. dioica* (mg/kg dry weight), *M.*
423 *carnosum* (mg/kg dry weight) and the watering solution (mg Zn/l) respectively.

424 **Fig. 2.** Water-soluble Zn concentration in above-ground tissue of *Urtica dioica* (o; n=15;
425 mg/kg wet weight; 98 day exposure) and phloem tissue of *Acer pseudoplatanus* (x; n=15;
426 mg/kg wet weight; 98 day exposure) compared with the Zn concentrations of Hoagland's
427 solution in which the plants were grown.

428 **Fig. 3.** Zn concentration in leaf tissue of *Acer pseudoplatanus* (x; n=25; mg/kg; 98 day
429 exposure) and in the whole body tissue of *Drepanosiphum platanoidis* (o; n=23; mg/kg; 14
430 day exposure) compared with the Zn concentrations of Hoagland's solution in which the *A.*
431 *pseudoplatanus* were grown. Zn uptake model (solid lines) for concentration in the leaves of
432 *A. pseudoplatanus*, with 95% confidence intervals (dashed lines) where [ZnSyc] and [ZnSoln]
433 is the concentration of Zn in the leaf tissue of *A. pseudoplatanus* (mg/kg dry weight) and the
434 watering solution (mg Zn/l) respectively.

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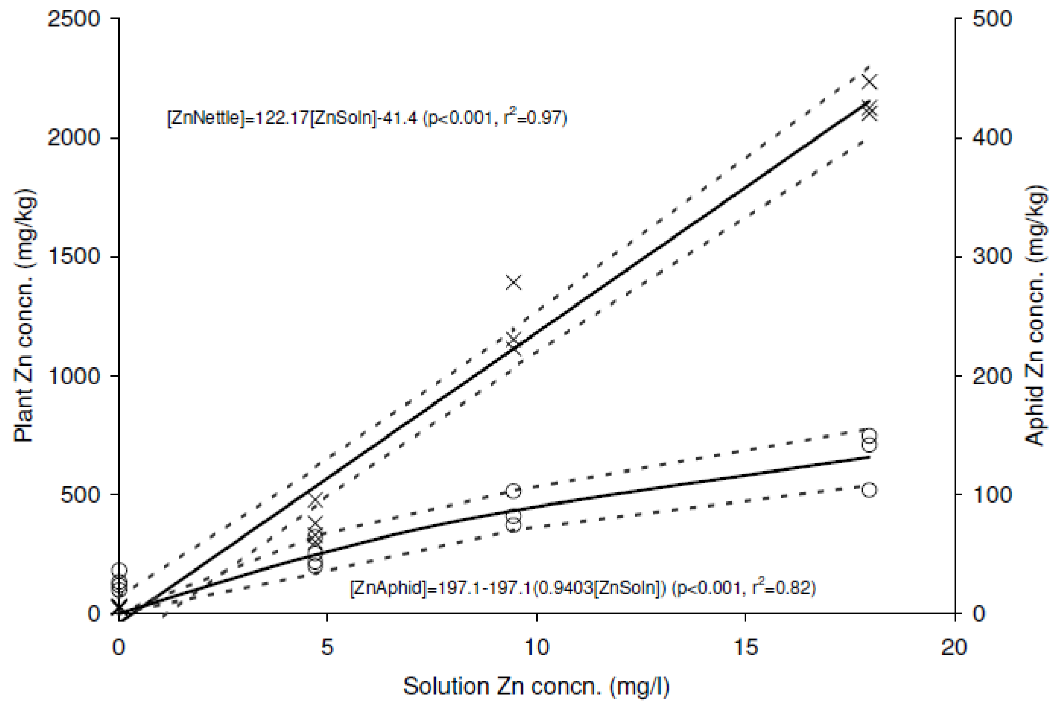
435 Figure 1

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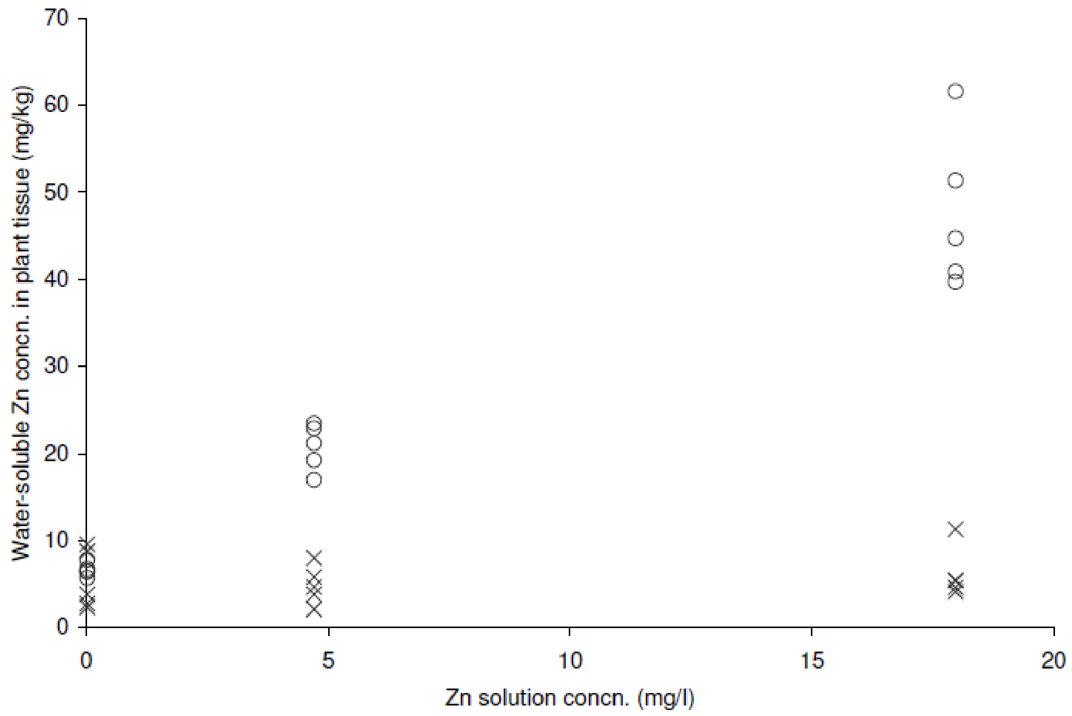
437 Figure 2

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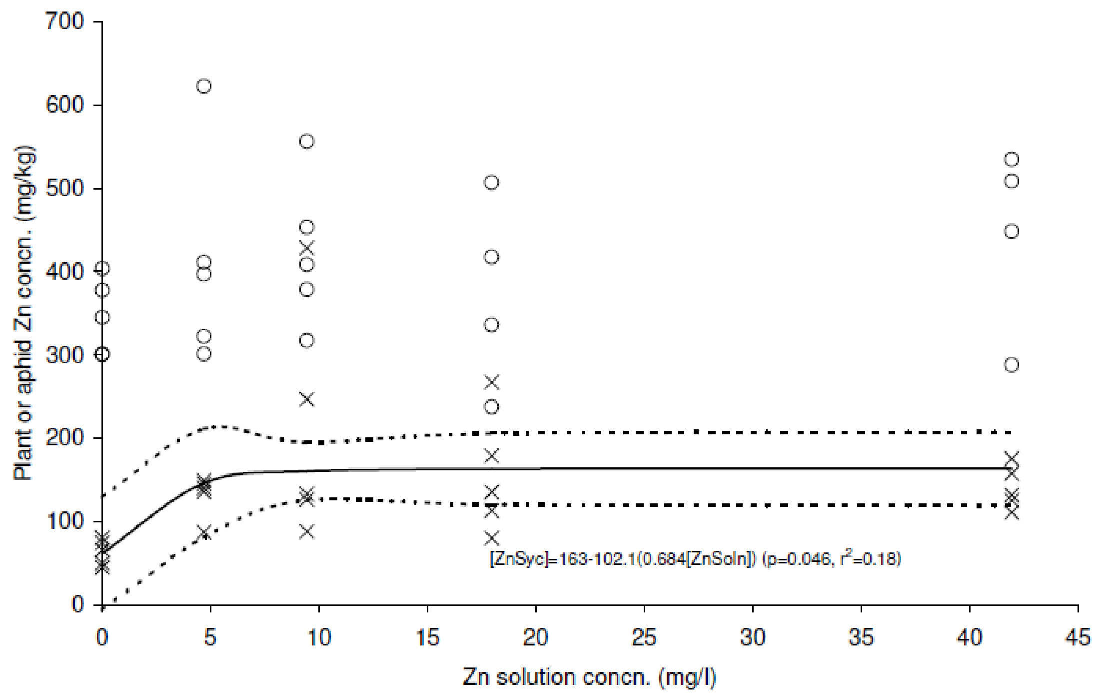
439 Figure 3

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