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Innate Zinc Tolerance in the Wetland Grass *Glyceria fluitans*?

By

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With 1 Figure

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Summary

MORAN B. M. & OTTE M. L. 2004. Innate zinc tolerance in the wetland grass *Glyceria fluitans*? – Phyton (Horn, Austria) 44 (1): 95–108, with 1 figure. – English with German summary.

Many wetland plants appear to be tolerant to zinc without the development of specific zinc tolerant populations. Zinc tolerance of two populations of *Glyceria fluitans* (*Poaceae*), one from an abandoned lead-zinc mine site (Glendalough) and one from a non-contaminated site (Lough Dan), was investigated. Plants were grown in sand culture on a wide range of zinc concentrations (2–5000 μmol Zn L^{-1}) in a greenhouse experiment and analyzed for growth (weight, leaf length) and zinc concentrations. Plants from Glendalough were smaller than plants from Lough Dan, but no differences in growth were observed between populations in response to zinc exposure. However, plants from the two populations differed in the way zinc was allocated to various plant parts. Differences in allocation of zinc within the plants point towards some adaptation of the mine site plants to zinc, but plants from that population are not more tolerant then plants from non-contaminated sites. This supports observations that wetland plants appear to have an innate ability to grow in the presence of high levels of metals in their environment.

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Zusammenfassung

MORAN B. M. & OTTE M. L. 2004. Angeborene Zinktoleranz im Sumpfgras *Glyceria fluitans?* – Phyton (Horn, Austria) 44 (1): 95–108, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Viele Pflanzen der Feuchtgebiete scheinen gegenüber Zink tolerant zu sein, ohne eigentliche zinktolerante Populationen zu entwickeln. Die Zinktoleranz wurde an zwei Populationen von *Glyceria fluitans* (*Poaceae*) untersucht; eine stammte von einer aufgelassenen Zinkmine (Glendalough) und die andere von einem nicht kontaminierten Standort (Lough Dan). Die Pflanzen wuchsen im Glashaus in Sandkultur in einem weiten Konzentrationsbereich von Zink (2-5000 µmol Zn L^{-1}). Sie wurden auf Wachstum (Gewicht, Blattlänge) und Zinkgehalt untersucht. Die Pflanzen von Glendalough waren kleiner als die von Lough Dan, aber es konnten keine Unterschiede im Wachstum zwischen den Populationen in Hinsicht auf die Zinkexposition beobachtet werden. Die Pflanzen der beiden Standorte unterschieden sich jedoch dadurch, wie Zink in die verschiedenen Pflanzenteile transportiert wurde. Die Unterschiede in der Zinkverteilung innerhalb der Pflanzen deuteten zwar auf gewisse Anpassungen an Zink am Minenstandort, jedoch sind die Pflanzen dieser Population nicht toleranter als die Pflanzen vom nicht kontaminierten Standort. Dies unterstützt Beobachtungen, dass Feuchtgebietspflanzen anscheinend eine angeborene Fähigkeit besitzen, in einer Umgebung mit hoher Metallkonzentration zu wachsen.

Introduction

Most studies on metal tolerance in plants have focused on dryland (i.e. non-wetland) plants (ERNST 1974, VERKLEIJ & SCHAT 1990), while metal tolerance in wetland plants has had very little attention. This recently changed with the increasing interest in using wetland cover for rehabilitation of mine wastes (WILLIANEN & al. 1998, MCCABE & OTTE 2000). Surprisingly, the few investigations into metal tolerance of wetland plants have been unable to identify differences in sensitivity between populations that had been exposed to various levels of metal concentrations in their respective habitats. Populations from non-polluted and metal-enriched habitats showed similar growth performance in the presence of high concentrations of metals, suggesting that innate tolerance to metals is a common trait of wetland plants (MCCABE & al. 2001). MCNAUGHTON & al. 1974 were the first to report on metal tolerance in *Typha latifolia* and observed that this trait existed in this species without the development of specific metal-tolerant populations. More recently, YE & al. 1997a confirmed the findings for *T. latifolia* and the same research team made similar observations on *Phragmites australis* (YE & al. 1997b, 1998). Then MCCABE & OTTE 2000 reported that floating sweetgrass, *Glyceria fluitans*, from populations that had not previously been exposed to zinc, were capable of growing well in zinc-lead mine tailings containing highly elevated levels of metals. MCCABE & al. 2001 reported some data from a small-scale pilot experiment showing no differences in tolerance to zinc when comparing a population from a zinc-lead tailings pond with one that had not been exposed to elevated levels of zinc. The pilot study also showed that the plants were able to grow in zinc concentrations that were much higher (up to 5,000 μmol L^{-1} than those observed in the field situation at Glendalough (about 50 μmol L^{-1}, BEINING & OTTE 1996). However, that experiment used insufficient replication, and so the observations must be treated with caution. Here we report on a detailed study of zinc tolerance in *G. fluitans* which aimed to confirm the findings from the earlier pilot study and to further investigate allocation of zinc at the organ level. Plants originating from the same populations as used in the pilot study were grown in a greenhouse experiment under similar conditions, but with an appropriate number of replications. The hypothesis was that plants from populations with a different history regarding exposure to zinc (plants from a mine site compared to plants from a site with no known mining history) would be equally tolerant of zinc.

Material and Methods

Glyceria fluitans (L.) R.Br. plants were obtained from the same populations as those used by MCCABE & al. 2001, i.e. from the tailings pond of an abandoned lead-zinc mine at Glendalough, (53°00'N, 6°23'W , grid reference T 095 962, elevation 140 m) and from a non-contaminated site at Lough Dan (53°05'N , 6°18'W , grid reference O 142 043, elevation 200 m) , both in Co. Wicklow, Ireland. For further description of the sites of origin of the populations, see BEINING & OTTE 1996 and MCCABE & al. 2001.

G. fluitans is an amphibious grass, usually rooting in submerged sediments (up to 60 cm depth) with upper leaves floating and inflorescences emerging above the water. However, it can also be found completely emerged. The plants were originally collected from Lough Dan and Glendalough on 30 November 1998. Plants were acclimatized in the greenhouse in the following manner. The tillers were planted in sand in pots (13 cm diameter, 1 L volume), which were then placed in tied bags that were perforated for gas exchange. Over the next two weeks the bags were gradually opened, in order to acclimatize the plants to the relatively low humidity (70-80%) of the greenhouse compared to that of their original, submerged, water-saturated environment. New leaves and shoots appeared after 4–5 days. The stocks were maintained on MES- buffered (1 mM, pH 5.0) ¹/₄ strength Long-Ashton nutrient solution (HEWITT & SMITH 1975) containing K_2SO_4 (0.5 mM), $CaCl_2$ (1.0 mM), $MgSO_4$ (0.375 mM), Na_2HPO_4 (0.325 mM), FeEDTA (0.25 mM), $NaNO_3$ (1.0 mM), $NH_4)_2SO_4$ (1.0 mM), $MnSO_4$ (2.5 μM), $CuSO_4$ (2.5 μM), $ZnSO_4$ (0.25 μM), H_3BO_3 (125 μM), $NaMoO_4$ (1.25 μM) and $CoSO_4$ (0.05 μM)

The experiment started on 14 June 1999. Green tillers of similar size and physiological age were taken from stock plants and transferred to pots with holes in the bottom (13 cm diameter, 1L) containing sand, which were in turn placed in closed pots (15.5 cm, 1.5 L volume) to hold nutrient solution. The plants were watered and allowed to establish for two weeks. On 28 June 1999, water was drained from the pots and replaced with 200 ml of nutrient solution (the amount required to saturate the sand) supplemented with $ZnSO_4$ to achieve the following treatments: 2, 500, 1250, 2500 and 5000 µmol Zn L^{-1}. This range of zinc concentrations was based on the preliminary observations by McCabe & al. 2001 and was similar to those used for short-term root elongation experiments by Harmens & al. 1993. Nutrient solution was replaced on days 7 and 42. Otherwise plants were watered when necessary. There were twelve replicates per treatment and pots were placed in a fully randomized manner. The experiment was carried out in a greenhouse at ambient temperatures (range 10–30 °C, average 18 °C), light at 90–1200 µmol PAR cm^{-2} s^{-1}, which typically was about 75% of that outside the greenhouse and a minimum humidity of 70% .

Previous research on *G. fluitans* (McCabe 1998, McCabe & Otte 2000, McCabe & al. 2001) had shown that the sum of the lengths of all leaves per plant was a good growth indicator. Leaf lengths of green leaves were measured on 1 July (Day 3), 5 August (Day 36) and 7 September (Day 65) 1999. The plants were harvested on 8 September 1999, thoroughly washed with deionised water and separated into live leaves, dead leaves, stems and roots. Plant parts were then dried at 60 °C until constant weight.

Dried plant parts were homogenized using liquid nitrogen. A 100 mg sample was then digested in 2 ml HNO_3: HCl (4:1) in Teflon digestion bombs at 150 °C for 8 hours. Digests were then diluted to a final volume of 10 ml with deionized water and filtered using 0.45 µm Gelman syringe filters. Samples were analyzed for zinc using an atomic absorption spectrophotometer (Unicam 929 Solaar). For quality control, an inter-laboratory standard reference of 'oriental tobacco leaves' CTA-OTL-1 (Glen Spectra Reference Materials, England) was also analysed and values on average deviated no more than 7% from certified values.

Statistical analysis was carried out following Sokal & Rohlf 1995. Data were log-transformed to obtain homogeneity of variance before analysis of variance.

Results

Leaf Length and Biomass

The mean sum of leaf lengths did not change over the course of the experiment, neither did it differ between populations, nor between zinc treatments (Table 1). However, plant biomass at the end of the experiment varied significantly between populations, but not between zinc treatments, as measured by fresh weights of dead leaves and stems and as dry weights of all plant parts: live leaves, dead leaves, stems and roots (Table 1, Fig. 1A, Table 2). Plants from the Lough Dan population tended to be more robust, showing greater biomass both as fresh and dry weight for most plant parts. The interactions between populations and zinc treatments were not significant for any of the biomass measurements. When expressed as a fraction relative to total biomass, the Glendalough plants allocated significantly more biomass to roots but less to the stems compared to plants from Lough Dan (Fig. 1A, Table 2). Plants from the two populations allocated similar amounts of biomass to dead and live leaves. Allocation of biomass to stems and roots was also affected by zinc treatment, as plants

Table 1.

Mean sum of leaf lengths, sum LL (cm), on three dates during the experiment, and fresh weights, FW (g), upon harvest of plant parts (standard deviations between brackets) of *Glyceria fluitans* originating from populations of Lough Dan (LDan) and Glendalough (Glen) grown on different zinc concentrations. Significance of differences (Probability P) between populations (Pop) and treatments (Treat) and due to the population x treatment (Pop X Treat) interaction as tested by 2-Way Analysis of Variance of log-transformed data is also given. Number of replications = 12.

Treatment		Sum LL (cm)			FW (g)			
Population	µmol Zn L^{-1}	Day 3	Day 36	Day 65	Live leaves	Dead leaves	Stems	Roots
LDan	2	45 (24)	48 (27)	45 (23)	0.14 (0.07)	0.22 (0.10)	0.60 (0.16)	3.4 (1.2)
	500	39 (26)	44 (34)	48 (36)	0.21 (0.13)	0.35 (0.25)	0.52 (0.33)	2.9 (1.5)
	1250	41 (33)	39 (30)	31 (24)	0.17 (0.09)	0.26 (0.12)	0.76 (0.66)	3.0 (1.8)
	2500	39 (32)	34 (32)	36 (34)	0.12 (0.08)	0.23 (0.08)	0.57 (0.39)	2.8 (1.4)
	5000	27 (29)	32 (29)	29 (26)	0.13 (0.07)	0.19 (0.06)	0.48 (0.24)	2.5 (1.9)
Glen	2	40 (24)	41 (34)	42 (31)	0.10 (0.07)	0.16 (0.11)	0.44 (0.44)	2.9 (1.5)
	500	24 (23)	19 (21)	33 (43)	0.14 (0.08)	0.17 (0.23)	0.28 (0.09)	1.8 (1.1)
	1250	20 (22)	22 (24)	25 (26)	0.13 (0.04)	0.23 (0.19)	0.28 (0.16)	1.9 (1.3)
	2500	39 (15)	63 (21)	66 (24)	0.15 (0.06)	0.17 (0.09)	0.33 (0.18)	2.8 (1.8)
	5000	28 (16)	33 (24)	38 (33)	0.10 (0.05)	0.12 (0.08)	0.33 (0.26)	2.7 (1.8)
Significance	Source				P			
	Pop.	0.189	0.259	0.653	0.175	0.000	0.000	0.187
	Treat.	0.596	0.391	0.818	0.341	0.349	0.257	0.317
	Pop x Treat	0.557	0.180	0.179	0.212	0.459	0.693	0.222

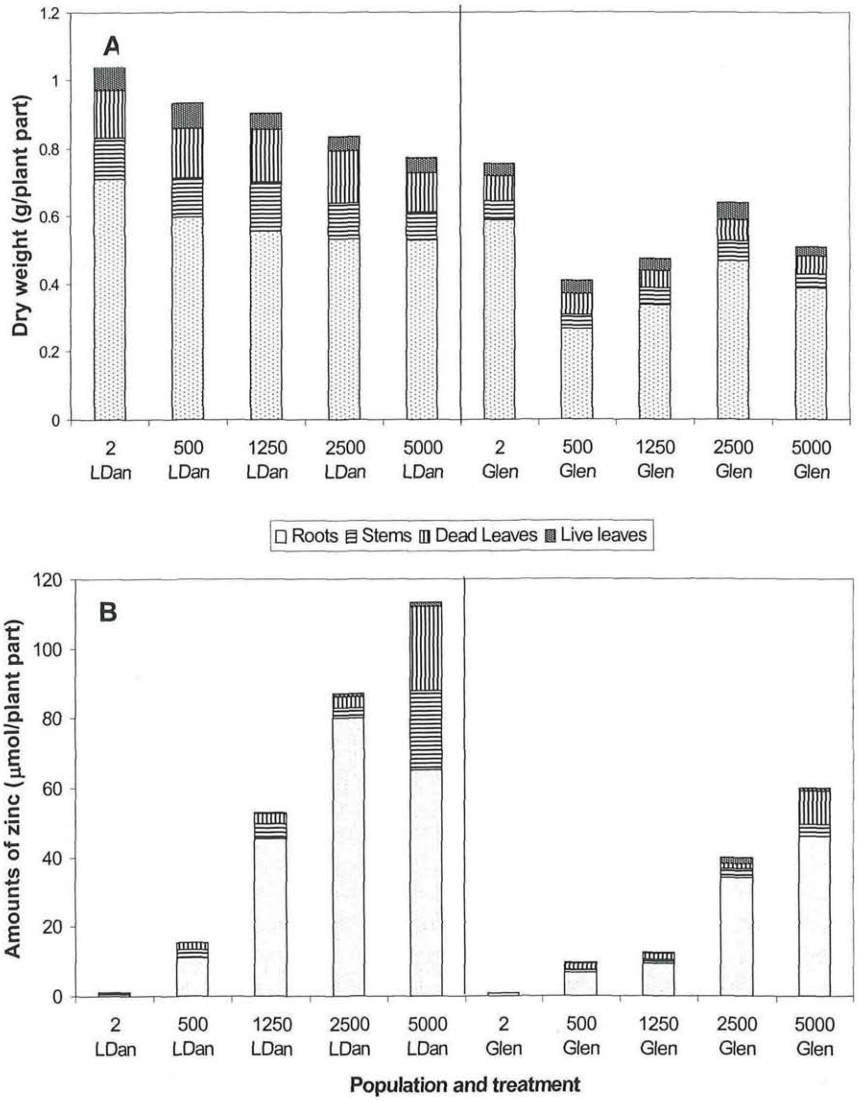

Fig. 1. Average dry weight per plant part (g) (1A) and average amounts of zinc (µmol) per plant part (1B) upon harvest of *Glyceria fluitans* originating from populations of Lough Dan (LDan) and Glendalough (Glen) grown on different zinc concentrations.

Table 2.
Significance of differences (Probablility, P) in dry weight, and allocation (relative to total) of biomass and zinc to plant parts between populations (Pop) and treatments (Treat) and due to the population x treatment (pop x treat) interaction as tested by 2-Way Analysis of Variance of arcsine-transformed data. Number of replications = 12. See also Fig. 1.

	Source	Live leaves	Dead Leaves	Stems	Roots
Dry weight	Pop	0.009	0.000	0.000	0.046
	Treat	0.413	0.692	0.513	0.207
	Pop x Treat	0.076	0.839	0.587	0.509
Biomass allocation	Pop	0.736	0.058	0.032	0.004
	Treat	0.350	0.247	0.040	0.066
	Pop x Treat	0.003	0.187	0.867	0.826
Zinc allocation	Pop	0.649	0.108	0.004	0.925
	Treat	0.000	0.000	0.010	0.000
	Pop x Treat	0.042	0.403	0.022	0.104

from both populations allocated less to both roots and stems when grown at 500 and 1250 μmol Zn L^{-1} than at other zinc concentrations.

Zinc Concentrations in Plant Parts

In the control zinc treatment of 2 μmol L^{-1}, zinc concentrations in live leaves of plants from both populations were higher then those in the roots. In all other treatments, the live leaves contained much lower concentrations of zinc than the roots (Table 3). Zinc concentrations in all plant parts increased with increasing zinc treatment. Zinc concentrations in live leaves of Glendalough plants were higher than those in the Lough Dan plants at all zinc treatments, except at 5000 μmol L^{-1} where the reverse was observed. This interaction between population and zinc treatment was significant. Zinc concentrations in dead leaves were higher in Glendalough plants than in Lough Dan plants at 2, 500 and 1250 μmol Zn L^{-1}, but similar at higher concentrations. No consistent differences were observed between the concentrations of zinc in stems of the two populations, as indicated by the significant population x treatment interaction. In the roots no differences between plants from the two populations were observed.

Allocation of Zinc between Plant Parts

The amounts of zinc accumulated in plants from the Lough Dan population ranged from about 2 to 114 μmoles $plant^{-1}$ (calculated as the concentration of Zn in a plant part multiplied by the dry weight of that plant part), while the plants from the Glendalough population accumulated about half that, i.e. 2 to 60 μmoles $plant^{-1}$ (Fig. 1B). When expressed

Table 3.

Mean concentrations of zinc (μmol g^{-1}) upon harvest of plant parts (standard deviations between brackets) of *Glyceria fluitans* originating from populations of Lough Dan (LDan) and Glendalough (Glen) grown on different zinc concentrations. Significance of differences (Probability, P) between populations (Pop) and treatments (Treat) and due to the population x treatment (Pop x Treat) interaction as tested by 2-Way Analysis of Variance of log-transformed data is also given. Number of replications = 12.

	Treatment	Zinc concentrations (μmol g^{-1})			
Population	μmol Zn L^{-1}	Live leaves	Dead Leaves	Stems	Roots
LDan	2	1.3 (1.2)	0.8 (0.5)	2.5 (1.6)	0.9 (0.7)
LDan	500	1.8 (2.3)	15.1 (5.9)	23.0 (11.7)	18.8 (6.3)
LDan	1250	8.4 (11.9)	18.0 (9.3)	29.8 (19.5)	84.4 (20.8)
LDan	2500	17.1 (8.7)	24.9 (12.7)	26.5 (7.7)	166 (42)
LDan	5000	39.2 (35.8)	190 (105)	243 (75)	131 (33)
Glen	2	2.5 (2.7)	1.4 (0.9)	2.2 (2.4)	1.3 (0.9)
Glen	500	8.6 (8.8)	27.0 (11.4)	28.6 (17.8)	25.4 (9.7)
Glen	1250	15.4 (4.2)	34.9 (56.3)	23.6 (8.8)	30.8 (16.6)
Glen	2500	33.3 (18.0)	22.7 (13.6)	46.6 (17.8)	75.5 (16.3)
Glen	5000	26.3 (19.1)	185 (36)	113 (88)	131 (76)

Significance		P			
	Source	Live leaves	Dead Leaves	Stems	Roots
	Pop	0.000	0.027	0.119	0.005
	Treat	0.000	0.000	0.000	0.000
	Pop x Treat	0.043	0.106	0.002	0.000

as a fraction relative to the total amount of zinc per plant, the allocation of zinc to the stems only varied between the populations (Table 2). Plants from Glendalough tended to allocate less to the stems compared with plants from Lough Dan, but a significant population x treatment interaction indicated that this pattern was not consistent for all treatments. For all plant parts, allocation of zinc varied between treatments, but not consistently, as indicated by significant interactions for live leaves and stems. With zinc concentrations increasing between 2 and 2500 μmol Zn L^{-1}, plants from Lough Dan increasingly allocated zinc to the roots, but at the highest treatment of 5000 μmol Zn L^{-1} allocation was similar to the 2 μmol Zn L^{-1} treatment. While the pattern was similar in plants from Glendalough, allocation of zinc in plants from this population was less variable with treatment compared to plants from Lough Dan.

Discussion

Although not immediately apparent from the sum of leaf lengths, the plants did grow over the course of the experiment. Plants had no dead

Table 4.

Comparison of zinc concentrations in plant parts of *G. fluitans* from the Lough Dan and Glendalough populations from field studies (concentrations in porewater: BEINING & OTTE 1996, concentrations in plants: MCCABE & al. 1997), a pilot experiment (MCCABE & al. 2001) and this study.

Study	Zinc concentration (µmol L^{-1}) in growth medium	Zinc concentrations in *G. fluitans* (µmol g^{-1} dw)							
		Lough Dan				Glendalough			
Field	Porewater	roots	stems	live leaves	dead leaves	roots	stems	live leaves	dead leaves
	<0.8	2.1	0.45	0.84	0.75				
	up to 50					380	150	94	290
Pilot Experiment	Treatment								
	2	1.7	4.8	0.5	1.8	1.9	3.3	0.9	0.8
	500	27	25	5.6	17	32	24	9	21
	5000	101	206	45.6	229	103	128	41.7	149
This study	Treatment								
	2	0.9	2.5	1.3	0.8	1.3	2.2	2.5	1.4
	500	18.8	23	1.8	15.1	25.4	28.6	8.6	27
	5000	131	243	39.2	190	131	113	26.3	185

plant material at the start of the experiment, but towards the end dead biomass accounted for up to 20%. This was not reflected in the sum of (live) leaf lengths, as leaves died off at the same rate as new leaves were formed. This was also observed in a previous outdoor transplantation experiment (McCabe & Otte 2000) in which dead aboveground dry biomass production (ca. 0.1-0.25 g plant^{-1} month^{-1}) was of the same order as live aboveground dry biomass production (ca. 0.05-0.9 g plant^{-1} month^{-1}). A high turnover of organic matter is relevant to the use of wetland plants for revegetation of mine tailings, because organic matter enrichment of these otherwise organic matter-poor substrates is vital for the success of phytoremediation efforts. It was observed in the field that *G. fluitans*-covered mine tailings at Glendalough were much higher in organic matter content than non-vegetated tailings (Jacob 2003).

In addition to investigating the zinc uptake and tolerance of two populations *G. fluitans* with different exposure histories, the experiment presented here was designed in part to confirm previous observations from a pilot experiment (McCabe & al. 2001) and the results were very similar. Although the experiments were carried out in different greenhouses, in different years and carried out by different operators, total biomass produced was the same and allocation of biomass to plant parts was very similar between both studies too, with most being allocated to the roots. Comparison of zinc concentrations also confirmed the previous findings (Table 4). As in plants collected from the field (Beining & Otte 1996, Table 4), a wide, 100-fold range of zinc concentrations was found in the various plant parts in the greenhouse experiments depending on exposure to zinc, and patterns between plant parts were consistent. When exposed to elevated levels of zinc, the live leaves always contained lower concentrations of zinc then the other plant parts and the lowest amounts of zinc per plant part were allocated to green leaves. This is a pattern often found in grasses and is seen as a mechanism to avoid the accumulation of toxic levels of metals in the plant parts most vital to survival and reproduction. Accumulation in the roots may be part of a mechanism to prevent increased levels in the shoots while accumulation in dead leaves may be a mechanism to remove zinc from the plants (Ernst & al. 1992). Only when exposed to the highest zinc concentrations of 5000 μmol L^{-1} did the plants from the unpolluted control site of Lough Dan take up zinc in aboveground biomass to higher concentrations than the plants from the metal-enriched mine site at Glendalough.

A further observation (Table 4) is that the zinc concentrations in roots and leaves of plants collected from the field in Glendalough and exposed to a maximum of 50 μmol Zn L^{-1} were higher than those of plants in the greenhouse experiments exposed to concentrations 10-fold or even 100-fold higher. This could be due to a number of factors. One explanation is that the plants in the field were exposed to elevated zinc concentrations over much longer periods than those in the greenhouse experiments. Another explanation is that even though the plants in both field and greenhouse experiments were thoroughly washed with deionized water before analysis, zinc would still have been present externally – associated with iron plaque on the roots (OTTE & al. 1989), adsorbed onto the outer surface and in the apoplast (HARMENS & al. 1993) and associated with phylloplane flora (NEWMAN & MCINTOSH 1989). The plants from the field study did not have any visible iron plaque on roots and none would have formed under the conditions of growth in the greenhouse experiments. Externally adsorbed and apoplastic zinc can account for up to 15% of the total associated with roots (HARMENS & al. 1993) and this may have been a more important fraction in the field situation due to the longer exposure period. The amount of zinc associated with phylloplane flora is likely to have been more important under field conditions than under greenhouse conditions as well, and this could therefore explain the difference in observations. Accurate assessment of the amount of zinc associated with the phylloplane flora is however very difficult.

In relation to the assessment of allocation of zinc within the plants in the study reported here, it is unlikely that the formation of iron plaque on the roots or phylloplane flora on the leaves would have formed a significant contribution to the overall plant zinc content, because the growth conditions were not amenable to formation of iron plaque or phylloplane flora. And because externally bound zinc accounts for a maximum of only 15% of the total associated with the roots (HARMENS & al. 1993) the observed allocation patterns would not have been much different from that found if that fraction had been removed. We are currently investigating allocation of zinc in *G. fluitans* in more detail at the tissue and cell level.

In 'classical' studies on zinc tolerance in non-wetland plants, tolerant populations from metal-contaminated sites had consistently lower zinc concentrations in aboveground biomass than non-tolerant plants from non-contaminated sites, when exposed to the same level of zinc in the growth medium. For example, MATHYS 1973 showed that zinc concentrations in shoots of zinc-tolerant *Agrostis tenuis* were lower compared to those of zinc-sensitive plants. Similar observations were reported for *Agrostis stolonifera* by WU & ANTONOVICS 1975. In this study, the two populations of *Glyceria fluitans* did not show consistently different patterns of metal accumulation. This was also found for other wetland plants, such as *Typha latifolia* (YE & al. 1997a) and *Phragmites australis* (YE & al. 1997b).

The findings of this study were consistent with earlier greenhouse experiments and outdoor transplantation experiments (MCCABE & OTTE 2000, MCCABE & al. 2001). Based on growth measurements, it appears that the plants from the two populations are equally tolerant. However, the populations did behave differently in other ways. Plants from Glendalough tended to be smaller than those from Lough Dan and allocation of zinc within these plants was different between populations. The Lough Dan plants appeared to retain increasing amounts of zinc in the roots with zinc concentrations in the medium increasing from 2 to 2500 μmol L^{-1}, thus preventing zinc from accumulating in the aboveground plant parts. However, this mechanism no longer seemed to work at 5000 μmol L^{-1} and preliminary studies had shown that this level of exposure is close to the highest these plants can survive. The Glendalough plants, on the other hand, appeared to allocate zinc similarly at all treatment levels, though they too cannot survive exposures higher than the highest treatment used in this study. It thus appears that although the two populations differ in terms of internal allocation of zinc, they are equally tolerant when measured by growth.

A question arising from this study is why the plants were able to grow for nearly three months in zinc concentrations much higher than those found under field conditions. The porewater zinc concentrations in Glendalough mine tailings are not higher than 100 μmol L^{-1} (BEINING & OTTE 1996, JACOB 2003) and under natural conditions in highly mineralized soils do not normally exceed 700 μmol L^{-1} (FÖRSTNER & WITTMANN 1981). One possible explanation is that the plants on the high concentrations used in these experiments would not be able to complete their lifecycle and so under such conditions in the field the population would not survive.

This study confirms previous studies which suggested that wetland plants show an innate ability to grow in the presence of high concentrations of zinc without having developed separate metal-tolerant populations (MCNAUGHTON & al. 1974, YE & al. 1997a,b). The apparent tolerance is not due to exclusion of zinc from uptake, as concentrations in the plants increased with increasing concentrations in the external medium, both under experimental conditions and in the field. Lack of differentiation between populations in relation to metal exposure has also been observed for the non-wetland grass *Andropogon virginicus* (GIBSON & RISSER 1982). It must however be noted that the two populations in this study were from locations within relatively close proximity to each other, not more than 20 km, and so could have originated from the same, possibly zinc-tolerant ancestors. On the other hand, 'classic' studies on metal tolerance typically concerned metal-tolerant and metal-sensitive populations originating from locations right beside each other (e.g. GODBOLD & al. 1984) and their differentiation into distinct populations with different sensitivities to metals is explained by assuming they were derived from the same zinc-sensitive population (ANTONOVICS & al. 1971). Why then does this not seem to occur in wetland plants? We are currently investigating this further. It also remains to be seen whether the apparent zinc tolerance of these plants is comparable to that of previously identified zinc-tolerant populations of other, non-wetland species. This too is currently being investigated by our research group.

Finally, there is a strong relationship between exposure to zinc and concentrations in the various plant parts, both under experimental conditions and in the field, and the results of this experiment proved to be highly reproducible (Table 4). These are two characteristics required of plants to be used for biomonitoring purposes (MARKERT 1993). Therefore *G. fluitans* may be a suitable candidate for monitoring of bioavailable zinc concentrations in its natural environment.

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