**QUEEN'S
UNIVERSITY
BELFAST**

Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil

Li, X. L., & Christie, P. (2001). Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. *Chemosphere*, 42, 201-207.

Published in:
Chemosphere

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil

Xiaolin Li ^{a,*}, Peter Christie ^{a,b}

^a Department of Plant Nutrition, College of Agricultural Resources and Environmental Sciences, China Agricultural University, Beijing 100094, People's Republic of China

^b Department of Agricultural and Environmental Science, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, UK

Abstract

Red clover plants inoculated with *Glomus mosseae* were grown in a sterile pasture soil containing 50 mg Zn kg⁻¹ in 'Plexiglas' (acrylic) containers with nylon net partitions (30 µm mesh) designed to separate the soil into a central root zone and two outer zones for hyphal growth with no root penetration. Two porous plastic soil moisture samplers were installed in each pot, one in the root compartment and the other in one of the hyphal compartments. The soil in the outer compartments was amended with one of the four application rates of Zn (as ZnSO₄) ranging from 0 to 1000 mg kg⁻¹. Non-mycorrhizal controls were included, and there were five replicates of each treatment in a randomised block in a glasshouse. Uninoculated plants received supplementary P to avoid yield limitation due to low soil P status. Plants grew in the central compartment for nine weeks. Soil moisture samples were collected 4, 24 and 62 days after sowing to monitor changes in the Zn concentration and pH of the soil solution.

At harvest, the mean mycorrhizal infection rate of inoculated plants ranged from 29% to 34% of total root length and was little affected by Zn application. Root and shoot yields were not affected by mycorrhizal infection. Plant Zn concentration and uptake were lower in mycorrhizal plants than non-mycorrhizal controls, and this effect was more pronounced with increasing Zn application rate to the soil. Soil solution Zn concentrations were lower and pH values were higher in mycorrhizal treatments than non-mycorrhizal controls and the mycorrhiza effect was more pronounced at higher Zn application rates. The protective effect of mycorrhiza against plant Zn uptake may have been associated with changes in Zn solubility mediated by changes in the soil solution pH, or by immobilisation of Zn in the extra-radical mycelium. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Arbuscular mycorrhiza; Red clover; Soil pollution; Soil solution Zn; Soil solution pH

1. Introduction

Elevated concentrations of trace metals such as zinc exist in many agricultural soils from management practices including application of sewage sludge or animal manure and from mining activities, and this may represent a risk to environmental quality and sustain-

able food production. Some studies have shown a positive impact of infection of plants by arbuscular mycorrhizal fungi on the resistance of the host plants to heavy metal contamination of soil (Heggo et al., 1990; Hetrick et al., 1994; Zhu et al., this issue). Leaf concentrations of the metals are correlated with metal concentrations in the soil while mycorrhizal plants take up less metal than non-mycorrhizal controls (Heggo et al., 1990; Zhu et al., this issue). There may be mechanisms involved that retard the uptake of heavy metals by the host plant with a resulting decrease in heavy

* Corresponding author.

E-mail address: lixl@mx.cei.gov.cn (X. Li).

metal concentration in the plant tissues. The binding of heavy metals on extramatrical mycelium of mycorrhizal fungi has been proposed to be a contributing factor for decreasing plant uptake of heavy metals in ectomycorrhizal associations. In addition, the delivery of heavy metals from soil to the mycorrhizal root governs to large extent the intake amount of heavy metals by roots, which is largely affected by the solubility of the heavy metal in the soil solution. Little is known of the impact of arbuscular mycorrhizal hyphae on Zn solubility and on subsequent plant uptake. The purpose of this study was to investigate the influence of arbuscular mycorrhizal hyphae on the alleviation of Zn toxicity to red clover through their effects on Zn solubility in the soil solution. This work extended the study by Zhu et al. (this issue) on white clover by monitoring changes in Zn concentration during growth of red clover and by increasing the maximum application rate of Zn to the soil up to 1000 mg kg⁻¹.

2. Materials and methods

Plexiglas pots were designed to permit separation of soil zones for root and hyphal growth. The pots were rectangular boxes 8 cm high and 8 cm deep. They had three compartments: a central one 2 cm wide separated from two outer ones each 5 cm wide by a nylon net of 30 µm mesh size, which allowed penetration by hyphae but not by roots. There were eight treatments comprising four application rates of Zn to the outer compartments in combination with inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae* at each Zn rate. The element zinc (as ZnSO₄ solution) was added to the outer compartments to give Zn addition levels of 0, 50, 400 or 1000 mg Zn kg⁻¹ soil. All non-mycorrhizal treatments received in the root compartment 300 mg P kg⁻¹ soil as Ca(H₂PO₄)₂, in order to supply non-mycorrhizal plants with sufficient P for adequate growth. Each treatment had five replicates.

Soil was collected from a mixed sward of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) in a long-term field experiment at the Agricultural Research Institute of Northern Ireland at Hillsborough, County Down (Irish Grid Reference J244577). The soil was a sandy clay loam derived from Silurian shale and is considered typical of a large area of County Down. Moist samples removed from the top 10 cm of the soil profile were passed through a 0.5 cm sieve, and large stones and plant root debris were removed. The soil contained 50 mg kg⁻¹ 'total' (aqua-regia – extractable) Zn, with 13 mg kg⁻¹ Olsen-P, 82.7 mg kg⁻¹ exchangeable K, 68 mg kg⁻¹ exchangeable Mg (all oven dry basis), and pH (soil:water ratio 1:2.5) value of 5.7 before sterilization. Soil was thoroughly mixed and then sterilized with 10 kGy γ-radiation using a ⁶⁰Co source.

The soil bulk density was adjusted to 1.1 g cm⁻³. In all treatments mineral nutrients were added in solution to each compartment at rates of 300 mg K(KNO₃), 108 mg N(KNO₃) and 100 mg Mg(MgSO₄) kg⁻¹ soil as a basal application. After four weeks of growth, a nitrogen dressing at the rate of 200 mg N(Ca(NO₃)₂) was made to each pot to prevent N limitation to plant growth. A soil moisture sampler (Rhizon SMS: Rhizosphere Research Products, Doderstraat 62, 6706 JG Wageningen, The Netherlands) was installed in the root compartment and in one of the hyphal compartments of each pot for collection of soil solution samples during the experiment.

The mycorrhizal fungus used was *G. mosseae*, which had been propagated on white clover grown in a glasshouse for two months. Colonized root segments and rhizosphere soil were used as inoculum. Mycorrhizal treatments received 13 g of fresh inoculum, while non-mycorrhizal plants received an equivalent amount of sterilized inoculum together with the filtrate (0.25 µm pore size) of unsterilized soil to provide a similar microflora except for the absence of the mycorrhizal fungus. The inoculum was mixed uniformly with the soil in the root compartment.

Red clover (*Trifolium pratense* L. cv. Merwe) seeds were surface-sterilized with 10% v/v H₂O₂ for 10 min and washed with deionized water. Thirty seeds were sown in each root compartment on 6 January 1998. 10 ml of a suspension of *Rhizobium leguminosarum* biovar. *trifolii* were added to the central compartment of each pot to ensure effective nodulation of the roots.

Each pot was placed on a piece of sponge in a 10 cm diameter saucer so that the sponge made good contact with the holes on the bottom of the root compartment. Water was added to the saucer and was then absorbed through the sponge into the soil in the pot. Soil moisture was regularly maintained at 70–90% of field capacity by weight. The experiment was conducted in a glasshouse with a day/night regime of 16/8 h and a temperature regime of 20°C/16°C.

Soil solution samples were taken three times, namely 4, 24 and 62 days after sowing, in order to monitor the Zn concentration and pH of the soil solution. The plants were harvested after nine weeks of growth. The roots were carefully washed free of soil with tap water, and cut into segments about 1 cm long. A randomly selected subsample of 1 g root fresh weight from each pot was stained with acid fuchsin. The proportion of root length infected was measured using the gridline intersect method. The weights of shoots and roots were determined after drying at 70°C for 48 h. Subsamples of roots and shoots were ground to pass a 0.5 mm sieve and dry ashed at 500°C for mineral nutrient analysis. Phosphorus was determined colorimetrically, and Zn and Cu by atomic absorption spectrophotometry using standard methods (Ministry of Agriculture and Fisheries, 1986).

3. Results and discussion

The results for mycorrhizal infection and plant growth (Table 1) show that no root infection was detected in the non-inoculated plants, while inoculated plants had infection rates of around 30% of root length irrespective of the Zn levels in the outer compartments. No significant difference was recorded in yield between mycorrhizal and non-mycorrhizal plants at any Zn level in the outer compartment. This was achieved by applying supplementary P fertilizer to the root compartments of the non-mycorrhizal plants, to eliminate the effect of improvement of plant P status exerted by mycorrhizal infection on elevation of Zn toxicity. Phosphorus concentrations in the shoots and roots were above 0.15%, sufficient for adequate growth of the host plant under these experimental conditions. There were also no large differences in P concentration between mycorrhizal and non-mycorrhizal control plants except at soil Zn levels of 0 and 50 mg kg⁻¹ in which mycorrhizal plants had slightly higher P than the controls. This indicates that the mycorrhizal hyphae in the outer compartments were able to grow well even at the highest Zn level of 1000 mg kg⁻¹, and furthermore they were active and efficient at absorbing P from the hyphosphere soil and transferring it to the plant, as evidenced by the comparatively high P acquisition by the mycorrhizal plants compared with non-mycorrhizal controls receiving 300 mg kg⁻¹ of additional P. Good mycorrhizal formation at relevant high level of Zn addition as in the present experiment has also been reported in other studies both in pot experiments (Diaz et al., 1996) and under field conditions (Koomen and McGrath, 1990).

The Zn concentrations in shoots and roots of red clover are shown in Table 2. In general, Zn concentration increased markedly with the increasing Zn application rate in the outer compartments irrespective of mycorrhizal treatment. However, the extent of the increase was different for mycorrhizal plants in comparison with non-mycorrhizal controls, with a smaller increase for mycorrhizal treatment than the non-mycorrhizal ones. This difference became more evident at higher Zn addition levels. The impact of mycorrhizal infection on lowering Zn concentration in host plants at high levels of Zn in the growing medium has been well documented (Shetty et al., 1995; Diaz et al., 1996). Comparing the Zn concentrations in the shoots with those in the roots, the latter had higher values, indicating that more Zn was located in the underground parts of the plants. However, even the highest Zn concentration found in the plant tissues of non-mycorrhizal controls at the highest soil Zn level may not have reached levels toxic to red clover in this experiment since there was no difference in plant yield in different soil Zn levels. Zinc uptake by mycorrhizal

Table 1
Mycorrhiza infection rates (percentage of total root length infected) and effect of mycorrhizal infection and Zn application rate on red clover shoot and root yield (g DM pot⁻¹)

Added Zn (mg kg ⁻¹)	Mycorrhiza infection		Shoots		Roots	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
0	0.0	28.9	5.50	5.20	0.89	0.80
50	0.0	30.4	5.35	5.24	0.84	0.76
400	0.0	33.6	5.46	5.26	1.06	0.81
1000	0.0	32.0	5.93	5.30	0.87	0.82
S.E.	1.92		0.220		0.046	
Significance ^a						
Mycorrhiza	***		NS		***	
Zn rate	NS		NS		*	
Myc × Zn rate	NS		NS		NS	

^a By analysis of variance; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

Fig. 1. (a) Zinc concentration and (b) pH value in the soil solution of the hyphal compartments of different treatments 62 days after sowing.

plants showed a similar pattern to that of Zn concentration as shown in Table 3. At lower levels of Zn addition the mycorrhizal plants showed similar Zn uptake to the corresponding non-mycorrhizal controls in both shoots and roots. However, at higher levels of Zn addition (ranging from 400 to 1000 mg kg⁻¹) mycorrhizal infection markedly decreased Zn uptake by shoots and roots. This indicates that the mycorrhizal fungus used was able to retard the transfer of Zn from the soil into the plant tissue.

One possible mechanism for this could be an induction of changes in the chemical behavior of Zn in the soil by mycorrhizal hyphal activity, resulting in a decline in Zn activity in the soil solution. This possibility was tested by measuring the Zn concentrations in the soil solution of the root and hyphal compartments during the course of the experiment. Overall, the soil solution Zn concentration in the root compartment was relatively low, ranging from 0.06 to 0.23 mg l⁻¹ depending on the total Zn levels in the hyphal compartment. However, no obvious difference was recorded either between mycorrhizal and non-mycorrhizal treatments or between different times for a given Zn level. In contrast to the root compartment, soil solution Zn concentration increased markedly as the Zn level increased throughout the whole range of Zn levels studied. For a particular Zn level it tended to decrease with time and this may have been due to progressive chemical adsorption of Zn on to soil solid constituents.

In terms of mycorrhizal impact, there was no difference in Zn concentration between mycorrhizal and non-mycorrhizal treatments at the first two times of measurement. However, at the last measurement the solution Zn was found to be substantially lower in mycorrhizal treatments than in non-mycorrhizal controls for the two higher Zn levels as shown in Fig. 1(a). This

may indicate the potential ability of mycorrhizal hyphae to decrease Zn solubility and subsequently to reduce Zn uptake by hyphae or roots.

This larger decline in solution Zn concentration in mycorrhizal treatments was associated with lower uptake of Zn by mycorrhizal compared with non-mycorrhizal plants at the higher Zn levels. One could speculate that more Zn had moved from the hyphal compartment with a higher Zn concentration in the soil solution into the root compartment in the case of non-mycorrhizal plants. As a result, more Zn was taken up passively by the roots, leading to a higher Zn concentration in non-mycorrhizal plants. Several mechanisms might be involved in the decline in the soil solution Zn concentration in mycorrhizal treatments. Using solution culture, Denny and Ridge (1995) found that the ericaceous mycorrhizal fungi they studied could produce slimes capable of binding substantial amounts of Zn. As a result, the Zn concentration in the solution declined. pH is one of the key factors affecting solution Zn concentration.

A small rise in soil pH can bring about a substantial decline in Zn concentration in the soil solution. In the present experiment the pH value of the soil solution was monitored together with Zn concentration. The pH in the root compartments did not change substantially across all treatments and ranged from 5.8 to 6.0. In the hyphal compartments, however, the soil solution pH changed with both the level of Zn addition and mycorrhizal treatment. The decrease in pH was proportional to the increase in Zn level in the hyphal compartments, and may have been induced by increasing SO₄²⁻ derived from the addition of ZnSO₄. Interestingly, mycorrhizal hyphae were associated with an increased pH compared with that of the corresponding non-mycorrhizal plants, and this was

especially evident at Zn levels above 50 mg kg⁻¹. This increase in pH corresponded with a decrease in solution Zn concentration in the mycorrhizal treatments, and may have resulted from the activities of mycorrhizal hyphae, as the presence of these was the only difference between mycorrhizal and the corresponding non-mycorrhizal treatments in the hyphal compartments. It could be argued that arbuscular mycorrhizal hyphae may behave in a similar fashion to plant roots in terms of utilization of NO₃-N as shown in other experiments (Johansen et al., 1993). They could release OH⁻ or other materials with a consequent increase in pH of the growing medium when they utilise NO₃-N. This is supported experimentally by the study of Bago et al. (1996), who demonstrated in sterile culture with *Glomus intraradices* the utilisation of NO₃-N by extraradical mycelium and increasing pH of the medium by up to one unit. In the present study, N was supplied in form of NO₃-N and NO₃-N would have been the dominant N form in the soil. Thus, the increase in soil solution pH of mycorrhizal treatments in the present study could have resulted, at least in part, from hyphal utilisation of NO₃-N from the soil solution.

Another possible explanation for the lower soil solution Zn concentrations in the mycorrhizal treatments is accumulation and immobilisation of Zn by the extraradical hyphae. The binding capacity of fungal hyphae to metals has been proposed as the mechanism responsible for tolerance to heavy metals in ericaceous mycorrhiza and ectomycorrhizal plants (Bradley et al., 1981; Brown and Wilkins, 1985; Wasserman et al., 1987; Chen et al., this issue). Chen et al. (this issue) grew two AM fungi, *G. mosseae* and *G. versiforme*, in a glass bead compartment cultivation system with maize and red clover as host plants. Although the plants grew in a soil-sand mixture that was not polluted with Zn, the fungal tissue harvested had up to about 10 times the Zn concentration of the host plant tissues and reached over 1200 mg Zn kg⁻¹ DM. This indicates a high capacity of the AM fungi to accumulate Zn in the mycelium. In the present work the depletion of soil solution Zn was more pronounced at later stages of plant growth. At this stage of the experiment, AM fungal biomass would have been at maximum and presumably binding more Zn than at the start of the experiment.

In conclusion, the mycorrhizal treatments tended to have lower Zn concentrations in the soil solution compared with non-mycorrhizal controls, especially at high Zn levels. These results indicate that the protective effect of mycorrhiza could be linked to changes in Zn solubility resulting from changes in soil solution pH that have been induced in turn by the activities of mycorrhizal hyphae in the soil. Alternatively, the effect could have been due to the binding and immobilisation of the metal in the extraradical mycelium. Clearly, more

research is required to elucidate the mechanisms by which AM infection increases plant resistance to heavy metal contamination.

Acknowledgements

This work was supported financially by The Royal Society, the Major State Basic Research Development Program of PR China (project G1999011807) and The National Natural Science Foundation of China (39790100). We thank Ms E. A. Wasson for excellent technical assistance.

References

- Bago, B., Vierheilig, H., Piche, Y., Azcon-Aguilar, C., 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. *New Phytol.* 133, 273–280.
- Bradley, R., Burt, A.J., Read, D.J., 1981. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. *Nature* 292, 335–337.
- Brown, M.T., Wilkins, D.A., 1985. Zinc tolerance of mycorrhizal *Betula*. *New Phytol.* 99, 101–106.
- Chen, B.D., Christie, P., Li, X.L., 2000. A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. *Chemosphere*, 42, 185–192.
- Denny, H.J., Ridge, I., 1995. Fungal slime and its role in the mycorrhizal amelioration of zinc toxicity to higher plants. *New Phytol.* 130, 251–257.
- Diaz, G., Azcon-Aguilar, C., Honrubia, M., 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant Soil* 180, 241–249.
- Heggo, A., Angle, J.S., Chaney, R.L., 1990. Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. *Soil Biol. Biochem.* 22, 865–869.
- Hetrick, B.A.D., Wilson, G.W.T., Figge, D.A.H., 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil. *Environ. Pollut.* 86, 171–179.
- Johansen, A., Jakobsen, I., Jensen, E.S., 1993. Hyphal transport by a vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. *Biol. Fertil. Soils* 16, 66–70.
- Koomen, I., McGrath, S.P., 1990. Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. *Soil Biol. Biochem.* 22, 871–873.
- Ministry of Agriculture, Fisheries and Food, 1986. The Analysis of Agricultural Materials, third ed. Reference Book 427, HMSO, London.
- Shetty, K.G., Hetrick, B.A.D., Schwab, A.P., 1995. Effects of mycorrhizae fertilizer amendments on zinc tolerance of plants. *Environ. Pollut.* 88, 307–314.

- Wasserman, J.L., Mineo, L., Majumdar, S.K., Vantyne, C., 1987. Detection of heavy metals in oak mycorrhizae of northeastern Pennsylvania forests, using X-ray micro-analysis. *Can. J. Bot.* 65, 2622–2627.
- Zhu, Y., Christie, P., Laidlaw, A.S., 2000. Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere*, 42, 193–199.