**QUEEN'S
UNIVERSITY
BELFAST**

Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus

Bi, Y. L., Li, X. L., & Christie, P. (2003). Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus. *Chemosphere*, 50(6)(6), 831-837. [https://doi.org/10.1016/S0045-6535\(02\)00227-8](https://doi.org/10.1016/S0045-6535(02)00227-8)

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.

Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus

Y.L. Bi ^a, X.L. Li ^{a,*}, P. 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, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK

Abstract

In a pot experiment, red clover (*Trifolium pratense*) was grown in sterilized Zn-amended low available P soil (0, 50 or 400 mg Zn kg⁻¹) with or without 100 mg kg⁻¹ added P and with or without inoculation with the arbuscular mycorrhizal (AM) fungus *G. mosseae*. When the plants were harvested after 40 days, AM colonization of the roots was still at an early stage, with only 14–38% of total root length colonized on average. AM colonization was highest in low-P soil, and was lowest in soil amended with 400 mg Zn kg⁻¹. Shoot yields were highest in AM plants with added P, but root yields were unaffected by AM inoculation. Shoot and root yields were higher with 100 mg added P kg⁻¹ soil, but lower with 400 mg Zn kg⁻¹ than 50 mg Zn kg⁻¹ or controls unamended with Zn. Shoot and root P concentrations were seldom higher in AM plants, but shoot P offtakes were higher in AM plants with added P.

Concentrations of Zn and Cu were much higher in the roots than in the shoots. Shoot and root Zn and shoot Cu were lower, but root Cu was higher, in AM plants. Soil residual pH after plant growth was higher in AM treatments, and residual total Zn was also higher, indicating lower Zn uptake by AM plants. Soil solution pH was higher in AM treatments, and soil solution Zn was lower in the presence of mycorrhiza. The results are discussed in terms of AM protection of the plants against excessive shoot Zn uptake.

© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Arbuscular mycorrhiza; Early colonization; Phosphorus uptake; Zinc uptake; Red clover

1. Introduction

Arbuscular mycorrhizal (AM) fungi form ubiquitous symbiotic associations with higher plant roots. These associations have the capability of increasing host plant uptake of immobile elements such as P and Zn when they are in short supply (Thompson, 1996), but are also capable of lowering plant uptake of various heavy metals when they are available in excessive quantities in the soil (Leyval et al., 1997; Zhu et al., 2001). The

mechanisms involved in decreased uptake of metals by AM plants are largely unknown. Selective (lower) fungal transfer of non-essential metals has been suggested for Cd, as indicated by enhanced root/shoot Cd ratios in AM plants (Joner and Leyval, 1997). Similarly, Zhu et al. (2001) observed increased immobilization of Zn in or near the roots of AM white clover with increasing application of ZnSO₄ to the soil. The mechanism involved could be direct intracellular precipitation of metal cations with phosphate, or adsorption of metal ions on hyphal walls. Chen et al. (2001), using a glass bead compartment cultivation system, found that Zn accumulated to concentrations of over 1200 mg kg⁻¹ (dry matter basis) in *G. mosseae* mycelium associated with maize plants, and Joner et al. (2000) found that excised

* Corresponding author. Tel.: +86-10-6289-1325; fax: +86-10-6289-1016.

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

mycelium of several *Glomus* spp. had large capacities for rapid passive adsorption of Zn and Cd. Li and Christie (2001) found that soil solution Zn concentrations were lower and pH values were higher in AM red clover than in non-mycorrhizal controls. They suggested that the protective effects of AM inoculation for plants against excessive Zn uptake may have been due to decreasing Zn bioavailability in the soil solution or immobilization of Zn in the host plant roots.

Indirect mechanisms deriving from the effects of AM fungi on rhizosphere properties have also been suggested. These include changes in pH (Li et al., 1991), microbial populations (Olsson et al., 1998) and root exudation patterns (Laheurte et al., 1990). These are all factors known to influence metal mobility or bioavailability.

The objective of the present study was to assess the influence of early stages of AM fungal colonization of red clover on plant growth and Zn uptake at varying soil zinc concentrations with and without low-P stress.

2. Materials and methods

The soil used was a sandy clay loam derived from Silurian shale 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). Moist samples removed from the top 10 cm of the soil profile were passed through a 0.5-cm sieve, large stones and plant root debris were removed, and the soil was air dried. 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 a 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. Sterilized soil (equivalent to 1200 g oven dried) was placed in 13-cm diameter plastic pots.

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 pot on 16 July 1998. There were 12 treatments consisting of three Zn addition levels (as Zn(NO₃)₂) of 0, 50 and 400 mg Zn kg⁻¹ soil, two added P levels (as Ca(H₂PO₄)₂) of 0 and 100 mg P kg⁻¹, and inoculation with the AM fungus *G. mosseae* or uninoculated. The mycorrhizal fungus used was *Glomus 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.

Table 1
Mean AM colonization rates, and shoot and root dry matter (DM) yields of mycorrhizal and non-mycorrhizal red clover

Mycorrhizal status	Added P (mg kg ⁻¹)	Added Zn (mg kg ⁻¹)	Root length colonized (%)	Shoot yield (g pot ⁻¹)	Root yield (g pot ⁻¹)
Non-mycorrhizal	0	0	ND ^a	3.1	1.16
		50	ND	2.6	0.83
		400	ND	1.0	0.44
	100	0	ND	11.8	2.14
		50	ND	12.2	2.17
		400	ND	4.3	0.84
Mycorrhizal	0	0	35.0	2.0	0.68
		50	38.0	2.2	0.65
		400	13.9	1.5	0.56
	100	0	22.3	13.4	2.15
		50	15.2	13.4	1.88
		400	19.6	8.2	1.40
Standard error			2.35	0.30	0.096
Significance ^b due to:					
Mycorrhiza			ND	***	NS
Added P			***	***	***
Added Zn			***	***	***
Mycorrhiza × added P			ND	***	*
Mycorrhiza × added Zn rate			ND	***	***
Addec P × added Zn rate			***	***	***
Mycorrhiza × added P × added Zn rate			ND	NS	NS

^a ND, not determined (no mycorrhiza observed in uninoculated pots).

^b By ANOVA; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, not significant.

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 all of the soil in the pot before the seeds were sown. Ten ml of a suspension of *Rhizobium leguminosarum* biovar. *trifolii* were added to each pot to ensure effective nodulation of the roots. There were eight replicates, giving a total of 96 pots.

Mineral nutrients were added in solution to each pot at rates of 300 mg K (KNO₃), 108 mg N (KNO₃) and 100 mg Mg (MgSO₄) kg⁻¹ soil as a basal application. After 4 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 each 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 20/16 °C. Soil solution samples

were taken using the soil moisture samplers on three occasions 12, 23 and 40 days after sowing in order to monitor the Zn concentration and pH of the soil solution. The plants were harvested after 40 days 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 fresh roots from each pot was stained with acid fuchsin and the proportion of root length infected was measured using the gridline intersect method at ×200 magnification with a compound microscope. The weights of shoots and bulk 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. Plant P was determined colorimetrically, and Zn by atomic absorption spectroscopy using standard methods (Ministry of Agriculture, Fisheries and Food, 1986). Other elements were analysed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Soil pH, Olsen P, exchangeable K and aqua-regia-extractable Zn and Cu (with ICP-AES) were determined on samples of the residual soil after plant growth. Soil solution samples were analysed for pH and for Zn using ICP-AES.

Table 2

Mean concentrations and offtakes of P, Zn and Cu in shoots of mycorrhizal and non-mycorrhizal red clover

Added P (mg kg ⁻¹)	Added Zn (mg kg ⁻¹)	Shoot concentration			Shoot offtake			
		P (%)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	P (mg pot ⁻¹)	Zn (µg pot ⁻¹)	Cu (µg pot ⁻¹)	
<i>Non-mycorrhizal</i>								
0	0	0.10	19	17.5	3.2	59	55	
	50	0.10	122	18.5	2.6	316	48	
	400	0.11	1430	26.1	1.1	1462	28	
	100	0	0.23	17	12.0	27.2	191	142
		50	0.23	73	15.9	28.2	894	194
		400	0.29	1418	33.9	12.5	6165	148
<i>Mycorrhizal</i>								
0	0	0.13	27	16.0	2.6	52	32	
	50	0.11	44	16.8	2.5	98	38	
	400	0.10	632	23.6	1.5	964	37	
	100	0	0.22	13	11.3	29.8	167	150
		50	0.22	32	13.4	29.2	423	182
		400	0.24	461	15.8	19.3	3728	127
Standard error		0.006	30.7	2.58	0.65	150.4	15.2	
Significance ^a due to:								
Mycorrhiza		*	***	**	***	***	NS	
Added P		***	*	NS	***	***	***	
Added Zn		***	***	***	***	***	*	
Mycorrhiza × added P		***	NS	NS	***	***	NS	
Mycorrhiza × Zn rate		***	***	*	**	***	NS	
Added P × Zn rate		***	NS	NS	***	***	NS	
Mycorrhiza × added P × Zn rate		NS	NS	NS	*	***	NS	

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

Data were subjected to analysis of variance (ANOVA) using the GENSTAT package (GENSTAT Committee, 1994). Soil solution pH and Zn concentrations were tested by repeated measures ANOVA to compare the three sampling occasions.

3. Results

No AM colonization was observed in any of the uninoculated control pots. The highest colonization rates (35–38% of root length) occurred in the absence of added P with no Zn addition or 50 mg added Zn kg⁻¹ soil (Table 1). The highest application rate of Zn lowered colonization to only 14%. Except at the highest Zn application rate, addition of P decreased AM colonization. Shoot yield was increased by AM but root yield was unaffected (Table 1). Shoot and root yields were depressed by the highest application rate of Zn.

Shoot and root P and Zn concentrations were lowered by AM colonization (Tables 2 and 3), and were increased by the high application rate of Zn. Shoot Cu showed similar trends, but root Cu concentrations were

increased by AM and decreased with increasing Zn application rate. Shoot P offtakes were increased by AM with added P, but root offtakes were decreased by AM. Both shoot and root Zn offtakes were decreased by AM. Shoot Cu offtakes were unaffected by AM, but root offtakes increased with AM colonization. Root concentrations of Zn and Cu were much higher than shoot concentrations (Tables 2 and 3).

At the end of the experiment, residual soil pH was higher in AM treatments (6.1 on average) than in controls (pH 5.5) (Table 4), and soil solution pH was higher in AM pots than in non-mycorrhizal controls during plant growth (Table 5). Soil and soil solution pH decreased with increasing Zn application rate.

Residual soil Olsen P was lower in AM pots (27 vs. 31 on average), but there was no AM effect on residual soil exchangeable K (Table 4). Residual soil total Zn was higher in AM pots, but total Cu was unaffected by AM colonization. Soil solution Zn was consistently lower in AM pots than in non-mycorrhizal controls throughout the experiment, and was unaffected by added Zn (Table 5).

Table 3

Mean concentrations and offtakes of P, Zn and Cu in roots of mycorrhizal and non-mycorrhizal red clover

Added P (mg kg ⁻¹)	Added Zn (mg kg ⁻¹)	Root concentration			Root offtake		
		P (%)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	P (mg pot ⁻¹)	Zn (µg pot ⁻¹)	Cu (µg pot ⁻¹)
<i>Non-mycorrhizal</i>							
0	0	0.13	93	84	1.5	108	95
	50	0.13	948	50	1.1	768	42
	400	0.16	9791	51	0.7	4395	18
100	0	0.36	107	68	7.7	222	145
	50	0.36	621	60	7.7	1350	128
	400	0.44	8991	63	3.7	7488	53
<i>Mycorrhizal</i>							
0	0	0.15	200	117	1.0	130	74
	50	0.15	377	98	1.0	242	63
	400	0.14	4101	79	0.8	2162	49
100	0	0.29	91	101	6.1	206	230
	50	0.29	313	83	5.5	586	154
	400	0.32	3756	85	4.5	5230	122
Standard error		0.007	302.1	10.0	0.26	273.1	19.3
Significance ^a due to:							
Mycorrhiza		***	***	***	***	***	**
Added P		***	NS	NS	***	***	***
Added Zn		***	***	**	***	***	***
Mycorrhiza × added P		***	NS	NS	**	NS	*
Mycorrhiza × Zn rate		***	***	NS	***	***	NS
Added P × Zn rate		***	NS	NS	***	***	NS
Mycorrhiza × added P × Zn rate		NS	NS	NS	***	NS	NS

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

Table 4
Residual soil analysis after plant harvest

Added P (mg kg ⁻¹)	Added Zn (mg kg ⁻¹)	pH (units)	Olsen P (mg kg ⁻¹)	Exch. K (mg kg ⁻¹)	Total Zn (mg kg ⁻¹)	Total Cu (mg kg ⁻¹)
<i>Non-mycorrhizal</i>						
0	0	5.6	12	57	35	7.7
	50	5.3	13	68	99	7.6
	400	5.3	12	146	432	7.3
100	0	5.7	48	17	34	7.1
	50	5.7	47	18	93	7.4
	400	5.3	52	55	432	7.6
<i>Mycorrhizal</i>						
0	0	6.1	10	84	37	7.9
	50	6.2	10	74	111	7.8
	400	5.9	10	120	471	7.5
100	0	6.3	45	18	34	6.9
	50	6.2	44	17	102	7.6
	400	6.0	46	29	432	7.2
Standard error		0.03	0.6	5.6	5.2	0.18
Significance ^a due to:						
Mycorrhiza		***	***	NS	***	NS
Added P		***	***	***	**	**
Zn rate		***	***	***	***	NS
Mycorrhiza × added P		NS	**	NS	*	NS
Mycorrhiza × Zn rate		***	NS	***	NS	NS
Added P × Zn rate		NS	***	***	NS	**
Mycorrhiza × P × Zn		***	NS	NS	*	NS

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

4. Discussion

In general, our present results agree with those of Li and Christie (2001), who studied AM red clover under similar conditions. For example, the decreased plant uptake of Zn, and lower concentrations of Zn and higher pH values in the soil solution of AM pots are in agreement. However, some of the results differ, and this may be related to differences in the experimental conditions. Firstly, Li and Christie (2001) used three-compartment pots ('rhizoboxes'). The plants grew in a central compartment separated by 30- μ m-mesh barriers from the two outer compartments. Extraradical hyphae (but not plant roots) could penetrate into the outer compartments, and the Zn applications were made to one of the outer compartments. The highest Zn addition was 1000 mg kg⁻¹, but only the external mycelium of the AM pots could penetrate the soil containing the added Zn. As a consequence, there was no significant depression of plant yield by applied Zn, even at an application rate of 1000 mg kg⁻¹. In contrast, in the present study, the roots of the plants were exploiting the same single soil volume as the external mycelium. Our results show that plant yields were depressed by application of 400 mg Zn kg⁻¹. In fact, application of 1000 mg Zn kg⁻¹ was

included as a treatment in the present experiment, but all of the seedlings (both AM and non-mycorrhizal) died in this high-Zn treatment and the treatment has not been included in this paper. On the other hand, Zhu et al. (2001) grew white clover plants in the same soil mixed with sand and amended with various rates of Zn up to 400 mg kg⁻¹. The highest application rate led to a small decrease ($P < 0.05$) in plant yield, but the plants had grown for 20 weeks, three times longer than in the present study. Thus, on balance it would seem that we can interpret our plant growth responses to applied Zn as responses of young plants at an early stage of AM colonization.

One new aspect investigated in the present work was the chemical analysis of the residual soil in the pots after plant growth. The higher residual soil pH observed in the AM treatments agrees with the higher soil solution pH values noted by Li and Christie (2001). The lower plant-available P found in the AM pots also supports the higher P offtakes detected, and the higher residual soil total Zn measured in the AM pots is in accord with the lower Zn uptakes observed in AM plants. Thus, the AM fungus seems to protect the host plant from moderate Zn pollution by immobilizing Zn in the mycelium. This may lower soil solution Zn concentrations, lower

Table 5

Mean soil solution pH and Zn concentration on three sampling occasions during the growth of AM and non-mycorrhizal red clover

Added Zn (mg kg ⁻¹)	Added P (mg kg ⁻¹)	pH			Zn (mg l ⁻¹)		
		28 July	8 Aug	28 Aug	28 July	8 Aug	28 Aug
<i>Non-mycorrhizal</i>							
0	0	6.6	6.5	5.8	0.2	0.1	0.2
	50	6.1	5.8	5.2	1.9	3.2	4.6
	400	5.4	5.6	5.4	77.8	80.2	91.6
100	0	6.4	5.4	6.7	0.3	0.7	0.2
	50	6.0	5.2	6.6	2.3	5.5	1.1
	400	5.5	5.3	5.5	59.6	105.1	83.1
<i>Mycorrhizal</i>							
0	0	6.8	6.7	6.4	0.2	0.3	0.2
	50	6.8	6.8	6.5	0.4	0.6	0.7
	400	6.3	6.6	6.3	31.4	26.4	40.4
100	0	7.0	6.9	6.6	0.9	0.3	0.2
	50	6.9	6.9	6.8	0.4	1.4	0.4
	400	6.3	6.5	6.8	30.6	58.0	7.1
Standard error			0.03		1.78		
Significance ^a							
Mycorrhiza			***		***		
Zn rate			***		**		
P rate			**		NS		
Date			**		**		
Myc × added P			NS		NS		
Myc × added Zn rate			***		***		
Myc × P × Zn rate			NS		NS		

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

root and shoot Zn concentrations, and leave more residual Zn in the soil.

Another difference between Li and Christie's (2001) experiment and the present work is the shorter growth period in the present experiment (40 vs. 63 days). We attribute the lower AM colonization rates in the present experiment to the shorter plant growth period. However, we cannot be certain that our results reflect an earlier stage of AM colonization. Because the roots of the plants were exploiting the same soil compartment as were the external hyphae (as noted above), we cannot be sure that the lower colonization rates found in the highest added Zn treatment would not persist if the experiment were continued until 60 days as a result of Zn toxicity to the roots. This aspect requires further investigation.

5. Conclusions

Our results are consistent with the suggestion (Chen et al., 2001; Li and Christie, 2001; Zhu et al., 2001) that AM fungi immobilize heavy metals such as Zn in moderately polluted soils. This decreases soil solution Zn concentration and root and shoot Zn uptake, and

increases residual Zn remaining in the soil after plant growth. This is in contrast to their tendency to decrease residual soil available P in low-P soils. The fungi increase soil pH, and this could also contribute to lower plant Zn uptake by making soil Zn less bioavailable. Joner et al. (2000) showed that excised mycelium of several *Glomus* spp. previously exposed to heavy metals had rapid and high metal-binding capacities. Use of metabolic inhibitors indicated that fungal sorption of metals was passive. However, AM fungi can also increase plant uptake of soil trace metal nutrients (such as Zn and Cu) when these are in short supply. There remains the question of which mechanisms are involved in enhanced plant trace metal nutrition.

Acknowledgements

We thank the Major State Basic Research Development Programme of the People's Republic of China (Project G1999011808), the National Natural Science Foundation of China (Project 40071050), the Laboratory of Material Cycling in Pedosphere, Institute of Soil Science, Chinese Academy of Sciences, the Royal

Society and the British Council (Project PEK/0992/314) for generous financial support. Ms E.A. Wasson gave excellent technical assistance and Dr David J. Kilpatrick, Biometrics Department, Queen's University Belfast, performed the statistical analysis of the data.

References

- Chen, B.D., Christie, P., Li, X.L., 2001. A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. *Chemosphere* 42, 185–192.
- GENSTAT Committee, 1994. GENSTAT 5 Reference Manual. Clarendon Press, Oxford.
- Joner, E.J., Leyval, C., 1997. Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytol.* 135, 353–360.
- Joner, E.J., Briones, R., Leyval, C., 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant Soil* 226, 227–234.
- Laheurte, F., Leyval, C., Berthelin, J., 1990. Root exudates of maize, pine and beech seedlings influenced by mycorrhizal and bacterial inoculation. *Symbiosis* 9, 111–116.
- Leyval, C., Turnau, K., Haselwandter, K., 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7, 139–153.
- 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.
- Li, X.L., George, E., Marschner, H., 1991. Phosphorus depletion and pH decrease at the root–soil and hyphae–soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New Phytol.* 119, 397–404.
- Ministry of Agriculture, Fisheries and Food, 1986. The Analysis of Agricultural Materials. In: Reference Book 427, third ed. HMSO, London.
- Olsson, P.A., Francis, R., Read, D.J., Söderström, B., 1998. Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids. *Plant Soil* 201, 9–16.
- Thompson, J.P., 1996. Correction of dual phosphorus and zinc deficiencies of linseed (*Linum usitatissimum* L.) with cultures of vesicular–arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 28, 941–951.
- Zhu, Y.G., Christie, P., Laidlaw, A.S., 2001. Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemosphere* 42, 193–199.