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Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives.

Patrick Audet & Christiane Charest

Ottawa-Carleton Institute of Biology
Department of Biology
University of Ottawa
30 Marie-Curie St.
Ottawa, ON, K1N 6N5 Canada

E-mail: ccharest@science.uottawa.ca ✉ (corresponding author)
paude086@uottawa.ca

Tel: (613) 562-5800 Ext. 6359
Fax: (613) 562-5486

ABSTRACT

To estimate dynamics of arbuscular mycorrhizal (AM) symbiosis in heavy metal (HM) phytoremediation, we conducted a literature survey and correlated HM uptake and relative plant growth parameters from published data. After estimating AM feedback responses for these parameters at low and high soil-HM concentration intervals, we determined that the roles of AM symbiosis are characterized by (1) an increased HM phytoextraction via mycorrhizospheric 'Enhanced Uptake' at low soil-HM concentrations, and (2) a reduced HM bioavailability via AM fungal 'Metal-Binding' processes at high soil-HM levels, hence resulting in increased plant biomass and enhanced plant tolerance through HM stress-avoidance**.** We present two conceptual models which illustrate the important compromise between plant growth, plant HM uptake and HM tolerance, and further emphasize the importance of AM symbiosis in buffering the soil environment for plants under such stress conditions.

"**Capsule**": *This meta-analysis has revealed a transition role of the AM symbiosis in phytoremediation shifting from 'Enhanced Uptake' to 'Metal-Binding' beyond critical soil-HM levels.*

Key words: AM feedback; HM bioavailability; HM phytotoxicity; stress-avoidance.

Abbreviations: arbuscular mycorrhizal (AM); heavy metal (HM)

1. INTRODUCTION

The arbuscular mycorrhizal (AM) symbiosis, an ancient interaction between plant roots and zygomycetous fungi (Morton & Benny, 1990), is recognized to benefit plants under environmental stress conditions such as nutrient deficiency, drought, and heavy metal (HM) pollution (Audet & Charest, 2006a; Charest et al*.,* 1997; Subramanian & Charest, 1998). Two antithetical hypotheses have been proposed as for the role of AM symbiosis in HM phytoremediation: (1) Increased HM phytoextraction via an enhanced mycorrhizosphere (Davies et al*.,* 2001, 2002; Díaz et al., 1996; Hovsepyan & Greipsson, 2004); and (2) Increased plant HM tolerance by a reduced HM bioavailability via fungal metal-binding processes (Audet and Charest, 2006a; Chen et al., 2004; Joner et al*.,* 2000; Weissenhorn et al*.,* 1995). The derived predictions for the first hypothesis, which we have designated as 'Enhanced Uptake', are that plant HM uptake is increased whereas HM phytotoxicity is reached at lower soil-HM concentrations in AM than non-AM plants. By contrast, the predictions for the 'Metal-Binding' hypothesis are that plant HM uptake is decreased whereas HM phytotoxicity is reached at higher soil-HM concentrations in AM than non-AM plants. We have determined in a previous meta-analysis (Audet & Charest, 2006b) that there is an important compromise between plant growth and HM uptake specifically relating to HM tolerance versus production of biomass under soil-HM conditions. To extend these observations, we have evaluated the impact of AM symbiosis in phytoremediation by testing for the first time the 'Enhanced Uptake' and 'Metal-Binding' hypotheses using meta-analytical approaches. Furthermore, we present conceptual models of HM uptake and plant growth that illustrate the dynamic roles of AM symbiosis in phytoremediation**.**

2. METHODS

2.1. Meta-analysis

In this meta-analytical study, based on the methods of Hedges & Olkin (1985) and Lipsey & Wilson (2001), we have tested the correlations between the AM feedback on plant HM uptake, the AM feedback on plant biomass, and the AM root colonization in relation to soil-HM concentrations by using combined results from multiple studies. After a thorough scientific literature review, we selected 20 articles for having dealt with herbaceous plants and AM fungi, and for having provided measures of plant biomass and HM uptake. For inclusion in our analyses, the selected studies consisted of greenhouse experiments having AM and non-AM inoculated treatments with the soil mineral composition described, and the data presented in tables. Key variables included soil HM concentration (mg kg^{-1} dry soil), plant HM concentration (mg kg^{-1} dry mass) and/or content (mg $plant^{-1}$) for shoots and/or roots, and plant dry mass (g) for shoots and/or roots. The data of AM root colonization were taken from studies having estimated the percent (%) colonized root length according to the method of Giovannetti & Mosse (1980). All the HM (e.g. As, Cd, Co, Cr, Cu, Fe, Mn, Pb, U, and Zn) with their soil concentration ranges along with plant and AM fungal species analyzed in our study are appended (Supplementary Data).

2.2. Metrics

The plant HM concentration ($[HM_{plant}]$) or content (HM_{plant}) for shoots and/or roots was used to measure the plant HM uptake, whereas the biomass for shoots and/or roots was used to measure plant growth. From these measures, we calculated the AM feedback percentage (%) as an estimate of the relative contribution of AM symbiosis to these plant parameters (modified from Plenchette et al., 1983). The equations of AM feedback on plant HM concentration (1),

plant HM content (2), and plant biomass (3), estimating the differences in AM relative to non-AM colonized plants, are defined as:

$$(1) \qquad \frac{\left([HM]_{AM} - [HM]_{nonAM}\right)}{[HM]_{nonAM}} \times 100\%$$

$$(2) \qquad \frac{\left(HM_{AM} - HM_{nonAM}\right)}{HM_{nonAM}} \times 100\%$$

$$(3) \qquad \frac{\left(biomass_{AM} - biomass_{nonAM}\right)}{biomass_{nonAM}} \times 100\%$$

2.3. Statistical analyses

The Pearson product-moment correlation test (Zar, 1999) was used to calculate the strength and significance of the following correlations: between the AM feedback on plant HM uptake, the AM feedback on plant biomass, or the AM root colonization in relation with the soil-HM concentration. We applied logarithmic transformations to each of these variables to enhance the relationship linearity, and meet normal distribution and homoscedasticity assumptions for all the analyses. We calculated coefficients for all the parameters at the low (10^{-3} to 1 mg kg^{-1} dry soil) and high (1 to 10^{4} mg kg^{-1} dry soil) soil-HM concentration ranges separately, since the linear relationships differed between these two intervals. We have detected broadscale trends for these two intervals despite the lower statistical power at the low than the high soil-HM interval given that there were fewer available data for the former than the latter. The low soil-HM interval refers to the 'control' type soils, whereas the high soil-HM interval refers to the 'treatment' soils from the studies included in our analyses. All of the p-values were determined using S-Plus® 7.0

(Insightful, 2005).

3. RESULTS

The AM feedback percentages (%) on plant HM concentration (Fig.1a) and plant HM content (Fig.1b) are plotted versus the soil-HM concentration. Their correlation coefficients (Table 1) at the low soil-HM interval were significantly positive (0.83 for both), ranging from 100% lower to 200% higher HM uptake in AM than non-AM plants as soil-HM concentration increased. Conversely, at the high soil-HM interval, the correlation coefficients were significantly negative (-0.38 and -0.25) at the high soil-HM interval, ranging from 150% higher to 100% lower HM uptake in AM than non-AM plants as soil-HM concentration increased.

The AM feedback % on biomass (Fig.2) is plotted versus the soil-HM concentration. There was no correlation at the low soil-HM interval, but a significant positive correlation (0.24) at the high soil-HM interval (Table 1). The AM feedback % ranged between 25% lower and 25% higher biomass at the low soil-HM interval, except a few outliers, whereas it ranged from 50% lower to 200% higher biomass at the high soil-HM interval.

The AM root colonization % is plotted versus the soil-HM concentration (Fig.3). There was a significantly positive correlation (0.43) at the low soil-HM interval, but no correlation at the high soil-HM interval (Table 1). The root colonization ranged between 20% and 80% colonized root length at the low soil-HM interval, whereas it ranged between 15% and 90% at the high soil-HM interval.

Our conceptual model of plant HM uptake in relation to soil-HM level (Fig.4) shows a positive and linear curve that tends to reach a plateau at the high soil-HM level. We have designated zones of ‘Enhanced Uptake’ and ‘Metal-Binding’ which show greater HM uptake in AM than non-AM plants at the low soil-HM level, and the reverse at the high soil-HM level. We also refer to the transition zone shifting from ‘Enhanced Uptake’ to ‘Metal-Binding’ as the area

of kinetic equilibrium without any detectable difference between the AM and non-AM plants.

Our conceptual model of relative plant biomass (% of maximum) in relation to soil-HM level (Fig.5) shows a parabolic curve of relative plant growth characterized by zones of deficiency (a), optimum (b), and toxicity (c). Plant growth is greater for AM than non-AM plants within the 'Metal-Binding' zone at the high soil-HM level, whereas there is no different response between the AM and non-AM plants within the 'Enhanced Uptake' and transition zone at the low to intermediate soil-HM level.

4. DISCUSSION

Our meta-analytical findings have revealed that AM symbiosis plays dynamic roles for plants as soil-HM levels increase. In fact, the AM feedback on plant HM uptake was shown to increase up to three-fold at the low soil-HM interval, while decreasing by the same factor at the high soil-HM interval. As predicted by the 'Enhanced Uptake' hypothesis, the greater volume of the mycorrhizosphere, compared to the rhizosphere alone, provides an increased access to soil resources, including macro-, micro-, and even non-essential elements. In our study, the mycorrhizospheric impact, accounting for nearly 200% greater HM uptake in AM than non-AM plants at the low soil-HM interval, is likely the result of active soil-HM transport to the roots via the extraradical hyphal network (Burleigh et al., 2003; González-Guerrero et al., 2005; Rosewarne et al., 1999). Our results also showed that the AM feedback decreases and eventually reaches negative values at the high soil-HM interval, accounting for nearly 100% lower HM uptake in AM than non-AM plants. As predicted by the 'Metal-Binding' hypothesis, the AM fungi are expected to reduce the soil-HM bioavailability since metals are sequestered in extraradical hyphae (Joner et al., 2000; Rufyikiri et al., 2003), therefore resulting in lower HM uptake in AM than non-AM plants. This sequestration process likely occurs in two phases in

which metals bind first to the hyphal wall, then diffusing into hyphal cells (Gadd, 1993; Gonzalez-Chavez et al., 2002). Considering all our metal-analytical results, both the 'Enhanced Uptake' and 'Metal-Binding' hypotheses are supported in that plant HM uptake is enhanced at low soil-HM concentrations, yet reduced at high soil-HM concentrations. Fittingly, our conceptual model of plant HM uptake incorporates both the 'Enhanced Uptake' and 'Metal-Binding' in the context of nutrient acquisition kinetics (Kirk, 2002; Marschner, 1995), in which plant HM uptake should be limited by the bioavailability of soil-HM and the maximum root uptake capacity. By integrating the AM symbiosis into our model, we revealed that the mycorrhizosphere provides 'Enhanced Uptake' via increased extraradical uptake sites, thus increasing the maximum root uptake capacity and resulting in higher HM uptake in AM than non-AM plants at low soil-HM level. In addition, the mycorrhizosphere also comprises more 'Metal-Binding' sites involved in the immobilization of soil-HM, hence resulting in decreased soil-HM bioavailability and lower HM uptake in AM than non-AM plants at high soil-HM levels. Therefore, we propose that the transition from 'Enhanced Uptake' to 'Metal-Binding' reflects a kinetic equilibrium between these two phenomena whereby their effects offset one another, resulting in no detectable difference in HM uptake between AM and non-AM plants at the intermediate soil-HM level.

Moreover, we measured an increasingly positive AM feedback on plant biomass at the high soil-HM interval, whereas no correlation was detected at the low soil-HM interval. This implies that the biomass in AM plants increases up to two-fold higher than non-AM plants, a response corresponding to a two-fold lower plant HM level then characterizing some plant stress-avoidance via hyphal 'Metal-Binding'. Consistent with this hypothesis, the AM fungi have been shown to buffer the soil environment by immobilizing soil-HM and reducing their bioavailability (Audet and Charest, 2006a; Chen et al., 2004; Joner et al., 2000; Weissenhorn et al., 1995).

Considering the compromise between plant growth and HM tolerance (Audet & Charest, 2006b), the AM plants most likely invest more in a stress-avoidance strategy via 'Metal-Binding' rather than in metabolically more costly stress-resistance alternatives, such as HM phytochelation and phytosequestration (Cobbett, 2000; Maier et al., 2003). In view of all our observations, we propose a conceptual model as to the role of AM symbiosis on relative plant biomass as influenced by the soil-HM bioavailability and plant HM uptake. In our model, AM symbiosis enhances plant biomass at high soil-HM levels via 'Metal-Binding' by decreasing HM bioavailability and subsequently reducing potential phytotoxic effects. Although it is well known that the 'Enhanced Uptake' of any limiting elements usually enhances plant growth (Kothari et al., 1990; Marschner, 1995), our meta-analytical findings rather favour the stress-avoidance scenario via hyphal 'Metal-Binding'.

Finally, the fact that the percent colonized root length is significantly increased at low soil-HM concentrations suggests that plants invest increasingly more in AM symbiosis at low soil-HM levels. Although no significant trend was detected at the high soil-HM levels, the studies included in our analysis reported AM colonization values as high as 90% total root length despite soil-HM concentrations reaching up to 10^3 mg kg^{-1} dry soil. This is remarkable considering that highly toxic soil-HM conditions have been shown in some cases to adversely affect AM root colonization, such as by reducing spore germination or hyphal development (Del Val et al., 1999; Leyval et al., 1997; Pawlowska & Charvat, 2004; Weissenhorn et al., 1995). However, other studies also showed that plants invest more in AM symbiosis under increasing soil-HM conditions, as indicated by increasing AM root colonization (Audet & Charest, 2006a). These contrasting results may be attributed to differences in plant or mycorrhizal HM tolerance as well as specific edaphic conditions, such as soil-HM concentration, HM speciation, and soil-pH (Giller et al., 1998; Hayman & Tavares, 1985; Leyval et al., 1997). Nevertheless, according to our two

proposed models of plant HM uptake and relative plant biomass, the AM feedback must be affected by the mycorrhizospheric volume. Besides the 'Enhanced Uptake' and 'Metal-Binding' phenomena, the mycorrhizosphere was also shown to change soil structure by stabilizing aggregates (Augé et al., 2001; Bearden & Peterson, 2000; Miller & Jastrow, 1990), thereby enhancing soil-HM retention capacity. Furthermore, since metal speciation is greatly influenced by pH (Apak, 2002), soil-HM bioavailability could be affected by mycorrhiza-induced substrate-pH modifications (Rufyikiri et al., 2003). Taking all of these factors into consideration, the AM symbiosis, by enhancing soil-HM retention either directly via fungal 'Metal-Binding' or indirectly via soil-aggregate HM sorption, could buffer the soil environment by reducing HM bioavailability.

5. CONCLUSION

In this meta-analytical survey, we have focused on the dynamic roles of the AM symbiosis in HM phytoremediation as characterized by the 'Enhanced Uptake' and 'Metal-Binding' hypotheses, the latter being associated with an enhanced HM tolerance in AM plants via stress-avoidance at high soil-HM levels. We also recognized the compromise between plant growth and HM tolerance, which points to the importance of 'Metal-Binding' processes in buffering the soil environment. Hence, a comprehensive survey of the mycorrhizosphere would be valuable to further understand plant tolerance mechanisms under various environmental stress conditions, especially with respect to bioremediation.

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Figure 1. AM feedback percentage (%) on plant HM concentration (a) and plant HM content (b) in relation to soil-HM concentration. The vertical reference line separates the low (10^{-3} to 1 mg kg^{-1} dry soil) and high (1 to 10^{4} mg kg^{-1} dry soil) soil-HM intervals.

Figure 2. AM feedback percentage (%) on plant biomass in relation to soil-HM concentration. The vertical reference line separates the low (10^{-3} to 1 mg kg^{-1} dry soil) and high (1 to 10^{4} mg kg^{-1} dry soil) soil-HM intervals.

Figure 3. AM root colonization (% colonized root length) in relation to soil-HM concentration. The vertical reference line separates low (10^{-3} to 1 mg kg^{-1} dry soil) and high (1 to 10^{4} mg kg^{-1} dry soil) soil-HM intervals.

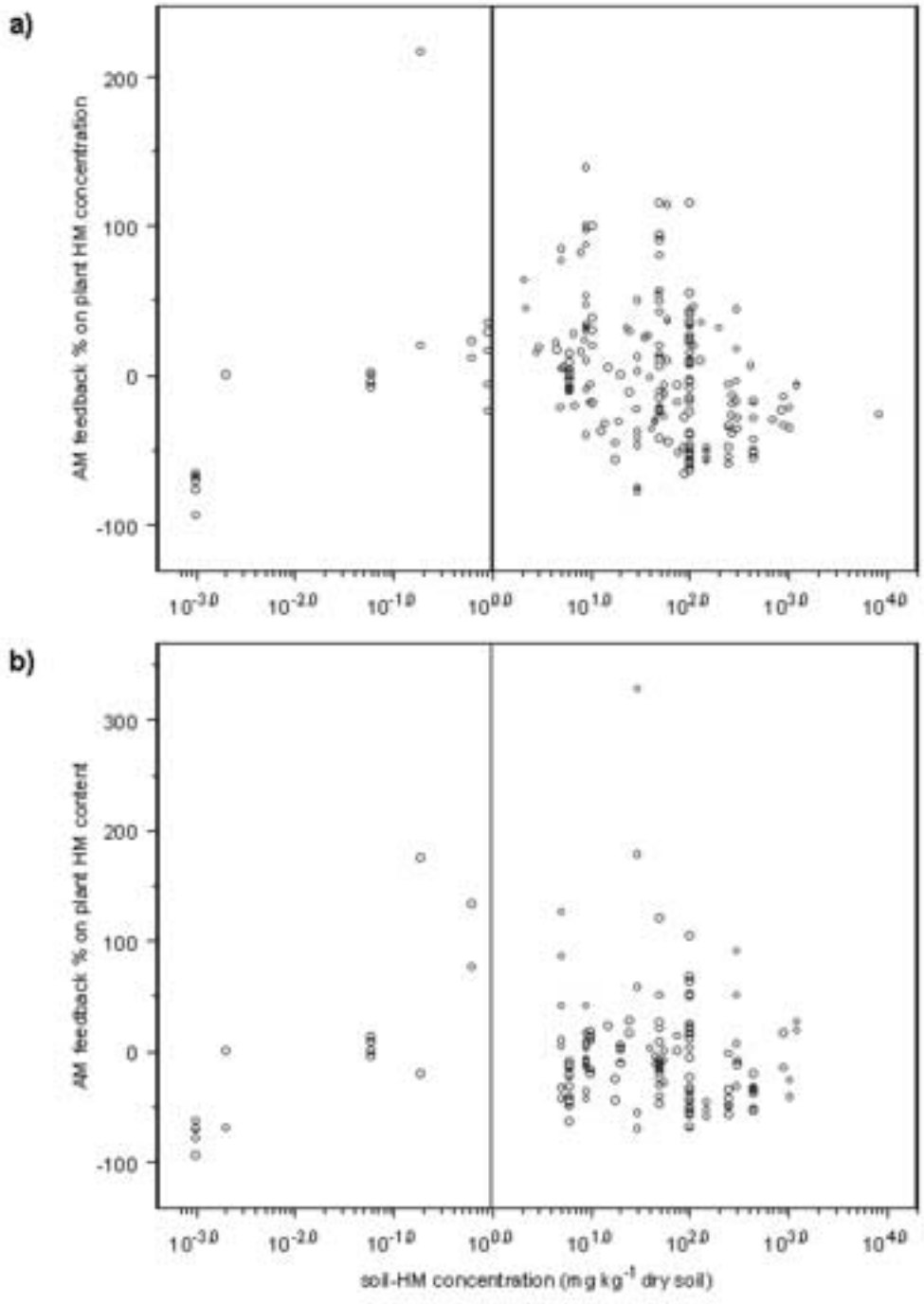
Figure 4. Conceptual model of plant HM uptake in relation to soil-HM concentration. Designated are zones of 'Enhanced Uptake' and 'Metal-Binding' showing greater HM uptake for AM than non-AM plants at low soil-HM levels (1), and lower AM plant HM uptake at high soil-HM level (2). The transition zone switching from 'Enhanced Uptake' to 'Metal-Binding' as the area of kinetic equilibrium showing no detectable difference between the AM and non-AM plants.

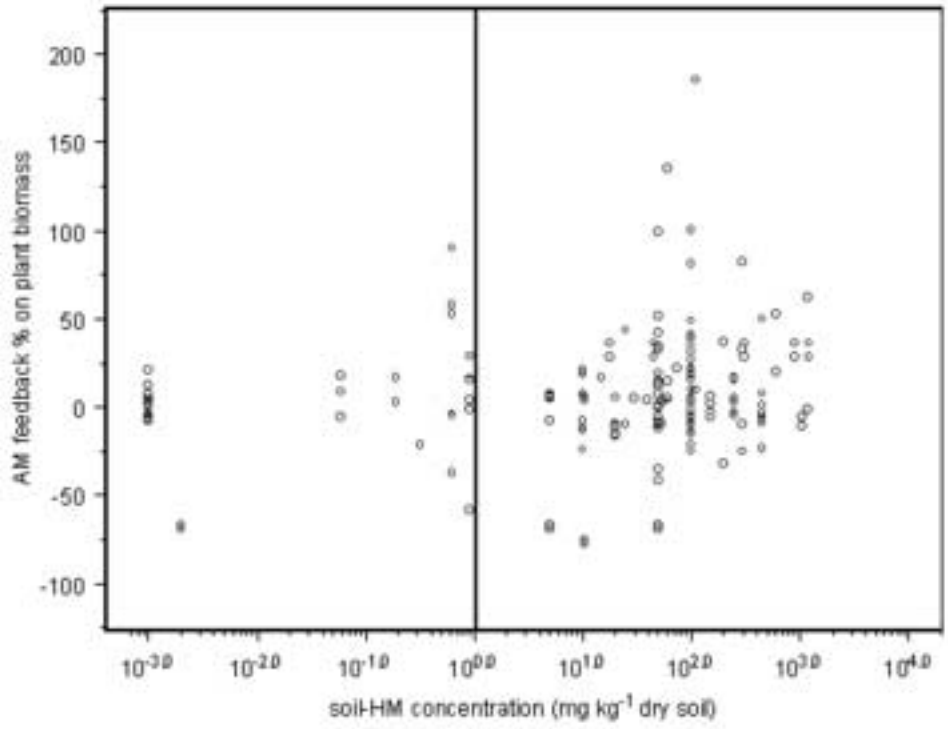
Figure 5. Conceptual model of relative plant growth (% of maximum) in relation to soil-HM concentration. Indicated are zones of deficiency (a) at low soil-HM, optimum (b) at intermediate soil-HM, and toxicity (c) at high soil-HM levels. Designated are zones of 'Enhanced Uptake' and 'Metal-Binding' showing greater biomass for AM than non-AM plants at high soil-HM levels (1), and no growth response associated with the 'Enhanced

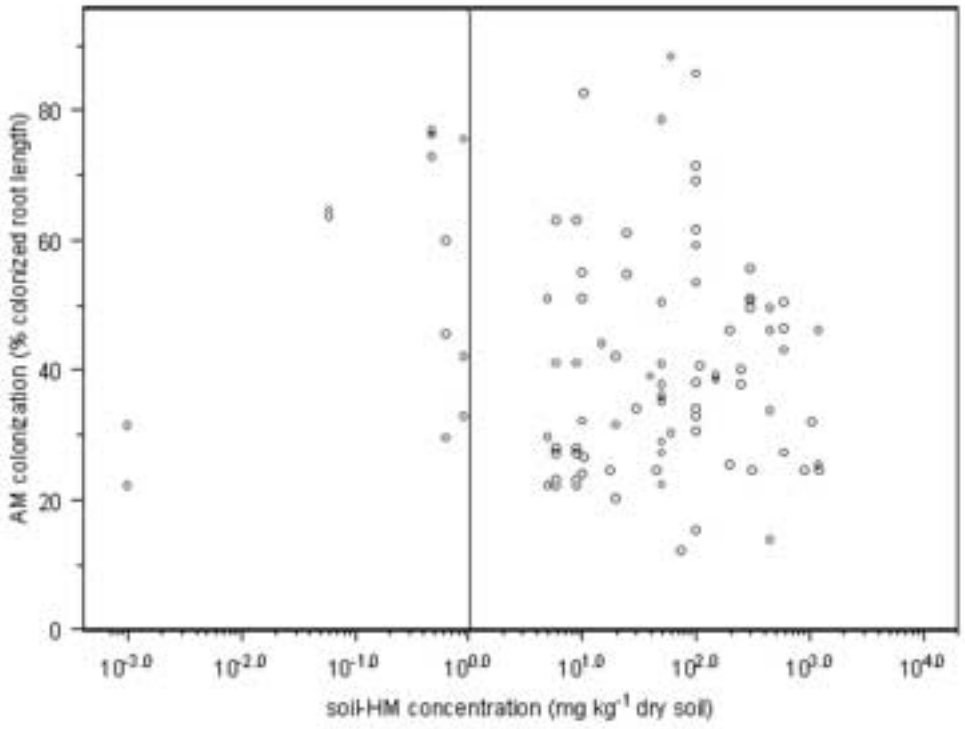
Uptake' and transition zones at low to intermediate soil-HM levels.

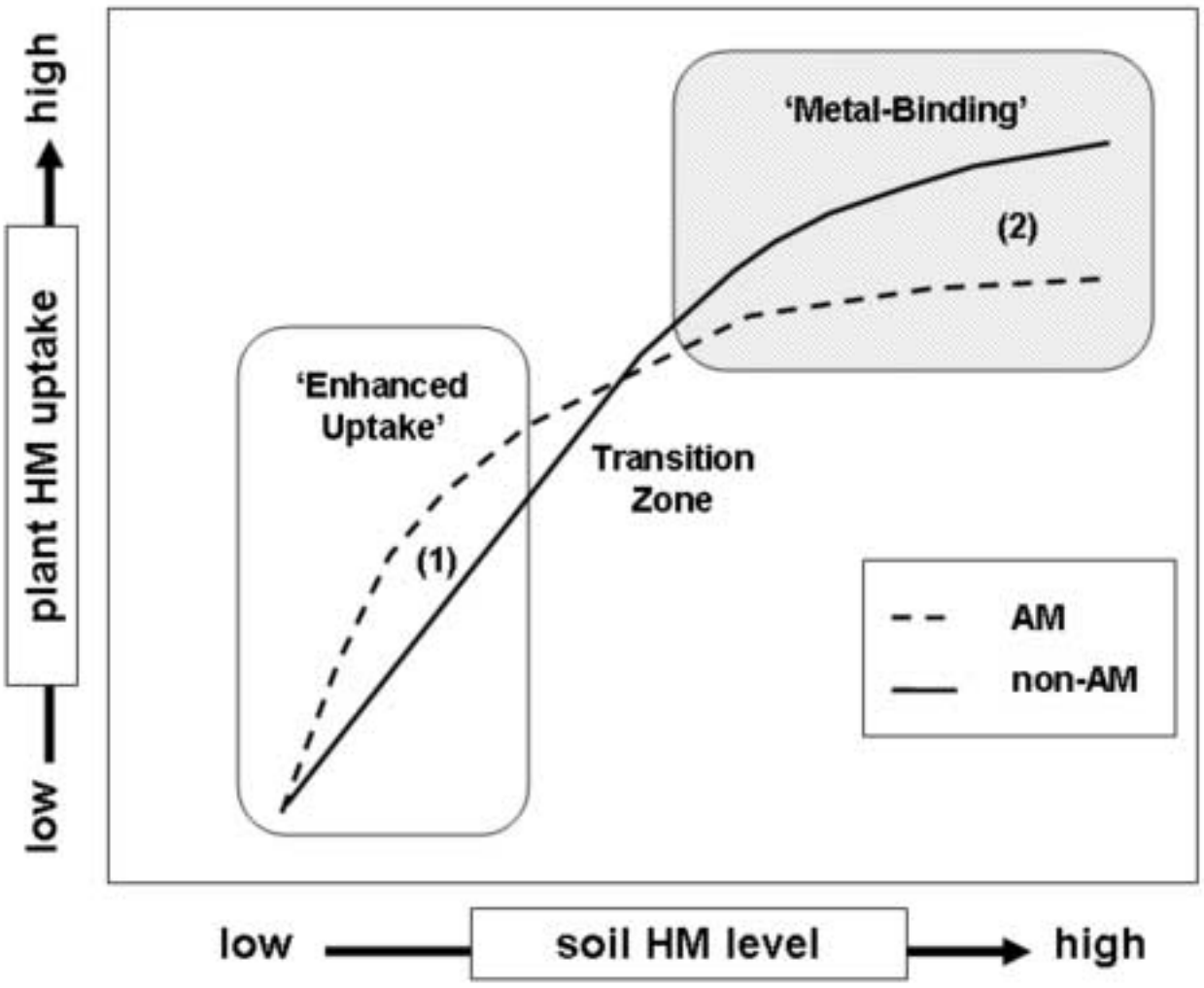
Table 1. Correlation coefficients (r) for AM feedback percentages (%) on plant HM content, plant HM concentration, biomass, and colonized root length in relation to the soil HM concentration. The r values, degrees of freedom (df), and p-values are shown.

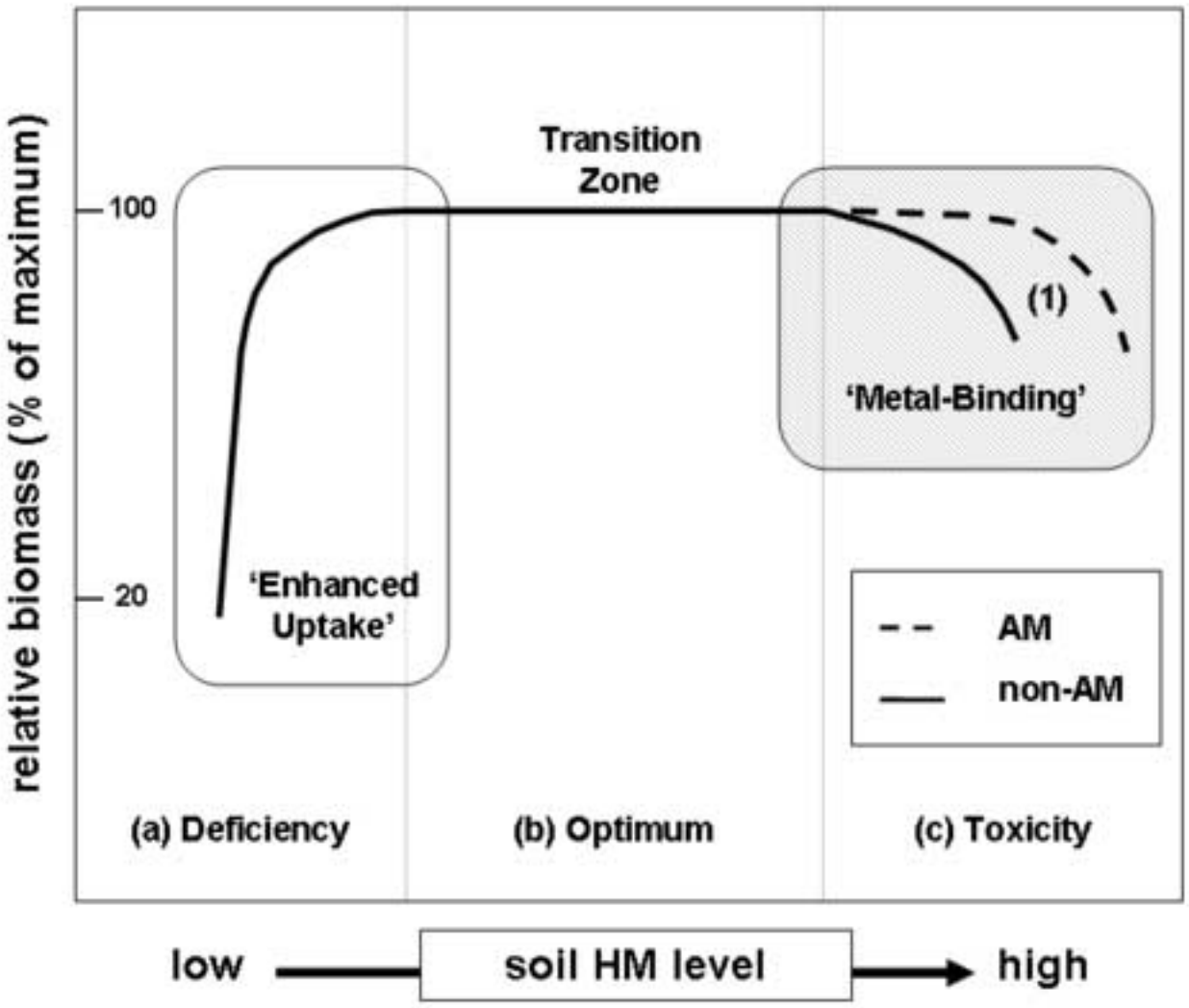
Parameter	**soil-HM concentration**					
	low soil-HM interval (10^{-3} - 1 mg kg^{-1} dry soil)			high soil-HM interval (1 - 10^{4} mg kg^{-1} dry soil)		
	r	*df*	p	r	*df*	p
plant HM concentration	0.83	22	$<10^{-7}$	-0.38	177	$<10^{-4}$
plant HM content	0.83	14	$<10^{-4}$	-0.25	131	$<10^{-3}$
plant biomass	0.24	30	0.19	0.24	130	<0.01
AM colonized root length	0.43	21	<0.05	-0.1	172	0.21


a)
AM feedback % on plant HM concentration
200
100
0
-100
$10^{-3.0}$
$10^{-2.0}$
$10^{-1.0}$
$10^{0.0}$
$10^{1.0}$
$10^{2.0}$
$10^{3.0}$
$10^{4.0}$
b)
AM feedback % on plant HM content
300
200
100
0
-100
$10^{-3.0}$
$10^{-2.0}$
$10^{-1.0}$
$10^{0.0}$
$10^{1.0}$
$10^{2.0}$
$10^{3.0}$
$10^{4.0}$
soil-HM concentration (mg kg^{-1} dry soil)


AM feedback % on plant biomass
200
150
100
50
0
-50
-100
$10^{-3.0}$
$10^{-2.0}$
$10^{-1.0}$
$10^{0.0}$
$10^{1.0}$
$10^{2.0}$
$10^{3.0}$
$10^{4.0}$
soil-HM concentration (mg kg^{-1} dry soil)


AM colonization (% colonized root length)
0
20
40
60
80
$10^{-3.0}$
$10^{-2.0}$
$10^{-1.0}$
$10^{0.0}$
$10^{1.0}$
$10^{2.0}$
$10^{3.0}$
$10^{4.0}$
soil-HM concentration (mg kg^{-1} dry soil)


high
plant HM uptake
low
'Metal-Binding'
(2)
'Enhanced Uptake'
Transition Zone
(1)
AM
non-AM
low
soil HM level
high


relative biomass (% of maximum)
100
20
Transition Zone
(1)
'Metal-Binding'
'Enhanced Uptake'
AM
non-AM
(a) Deficiency
(b) Optimum
(c) Toxicity
low
soil HM level
high

Appendix 1. Arbuscular mycorrhizal (AM) and plant species included in the meta-analysis.

AM species	Plant species	Soil HM	References
Glomus caledonium (Nicol. & Gerd.) Gerdemann & Trappe	*Zea mays* L.	Zn	Chen et al. 2004b
	Pteris vittata L.	As, U	Chen et al. 2006
Glomus intraradices Schenck & Smith	*Nicotiana rustica* L.	Zn	Audet and Charest 2006
	Nicotiana tabacum L.	Cd	Janouskova et al. 2005
	Pisum sativum L.	Cd	Rivera-Becerril et al. 2002
	Pteris vittata L.	As, U	Chen et al. 2006
Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe	*Allium cepa* L.	Zn, Co	Gildon and Tinker 1983
	Cannabis sativa L.	Cd , Cr, Ni	Citterio et al. 2005
	Pteris vittata L.	As, U	Chen et al. 2006
	Trifolium pratense L.	Cd, Pb, Zn	Bi et al. 2003; Chen et al. 2003; Li and Christie 2001; Vivas et al. 2003a
	Trifolium repens L.	Cd, Zn	Vivas et al. 2003b; Zhu et al. 2001
	Trifolium subterraneum L.	Cd	Joner and Leyval 1997
	Zea mays L.	Cd, Cu, Mn, Pb, Zn	Chen et al. 2004a; Weissenhorn et al. 1995
Glomus sp.[a]	*Cynodon dactylon* (L.) Pers.	As	Leung et al. 2006
	Glycine max L.	Cd, Cu, Fe, Mn, Zn	Heggo et al. 1990
	Lolium perenne multiflorum (Lam.) Parnell.	Cd	Yu et al. 2005
	Pteris vittata L.	As, U	Leung et al. 2006

[a] Consortium of *Glomus* sp.

Appendix 2. Heavy metals (HM) and soil concentration ranges included in the meta-analysis

HM	Soil HM range (mg kg^{-1} dry soil)	References
As	1 - 106	Chen et al. 2006; Leung et al. 2006
Cd	0.001 - 8 371	Chen et al. 2004a; Citterio et al. 2005; Heggo et al. 1990; Janouskova et al. 2005; Joner and Leyval 1997; Rivera-Becerril et al. 2002; Vivas et al. 2003b; Weissenhorn et al. 1995; Yu et al. 2005
Co	5 - 75	Gildon and Tinker 1983
Cr	50 - 300	Citterio et al. 2005
Cu	0.91 - 45	Heggo et al. 1990; Weissenhorn et al. 1995
Fe	7.9 - 77.4	Heggo et al. 1990
Mn	2.2 - 310	Heggo et al. 1990; Weissenhorn et al. 1995
Ni	5 - 100	Citterio et al. 2005
Pb	30 - 895	Vivas et al. 2003a; Weissenhorn et al. 1995
U	106	Chen et al. 2006
Zn	0.19 - 1 220	Audet and Charest 2006; Bi et al. 2003; Chen et al. 2003, 2004b; Gildon and Tinker 1983; Heggo et al. 1990; Li and Christie 2001; Weissenhorn et al. 1995; Zhu et al. 2001

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