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Allocation Plasticity & Plant-Metal Partitioning: Meta-Analytical Perspectives in Phytoremediation.

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ABSTRACT

In this meta-analysis of plant growth and metal uptake parameters, we selected 19 studies of heavy metal (HM) phytoremediation to evaluate trends of allocation plasticity and plant-metal partitioning in roots relative to shoots. We calculated indexes of biomass allocation and metal distribution for numerous metals and plant species among four families of interest for phytoremediation purposes (e.g. Brassicaceae, Fabaceae, Poaceae, and Solanaceae). We determined that plants shift their biomass and distribute metals more to roots than shoots possibly to circumvent the challenges of increasing soil-HM conditions. Although this shift is viewed as a stress-avoidance strategy complementing intrinsic stress-tolerance, our findings indicate that plants express different levels of allocation plasticity and metal partitioning depending on their overall growth strategy and status as 'fast-grower' or 'slow-grower' species. Accordingly, we propose a conceptual model of allocation plasticity and plant-metal partitioning comparing 'fast-grower' and 'slow-grower' strategies and outlining applications for remediation practices.

Capsule:

"This meta-analysis has revealed a shift in plant biomass and metal distribution from shoots to roots possibly to protect vital functions when subjected to metal stress"

Keywords: root/shoot partitioning; trace metal; metal toxicity.

1. INTRODUCTION

Environmental pollutants, such as heavy metals (HM), pose significant risks to ecosystems and human health. Through a process known as phytoremediation, plants are used to remove pollutants from contaminated environments despite inherent growth challenges, for example plant-HM toxicity and soil-pH changes (Salt et al., 1998). Under these increasingly challenging soil-HM conditions, recent studies have identified significant physiological compromises relating to plant growth, HM uptake, and HM tolerance (Audet and Charest, 2007a,b; Wilson, 1988a). It is postulated that plants adjust their relative biomass allocation and distribution to organ systems (e.g. roots or shoots) when subjected to environmental stress conditions, particularly nutrient deficiency: a phenomenon referred as allocation plasticity (Bell and Lechowicz 1994; Gedroc et al. 1996; Wilson, 1988a,b). In this regard, plants can be categorized in their stress-tolerance strategy as either 'slow-growers' or 'fast-growers relating to growth rate and HM uptake (Grime, 1979; Chapin, 1980). Extending from these findings, we investigated the current model of allocation plasticity in the context of HM phytoremediation implying soil-HM conditions ranging from low (trace) to high (toxic) levels. Using a meta-analytical approach and by fitting empirical models of biomass allocation, plant-HM distribution, and soil-HM or plant-HM levels, we evaluated the relationships of allocation plasticity and plant-metal partitioning among four selected plant families relevant to phytoremediation and representative of distinctive growth strategies: the Brassicaceae, Fabaceae, Poaceae, and Solanaceae.

2. METHODS

2.1. Meta-analysis

In this meta-analytical study, based on the methods of Hedges and Olkin (1985) and Lipsey and Wilson (2001), we investigated relationships between biomass allocation and plant-HM distribution in relation to soil-HM and plant-HM levels. More specifically, we calculated indexes of biomass partitioning and plant-HM concentration partitioning among four plant families: Brassicaceae, Fabaceae, Poaceae, and Solanaceae. These families were chosen as some of their species are of interest for phytoremediation purposes (more specifically metal phytoextraction), and, after meeting our selection criteria, provided sufficient degrees of freedom for robust statistical analysis. After a thorough scientific literature review, we selected 19 articles for having dealt with herbaceous plants among these four families, and for having provided measures of plant biomass and HM uptake for both roots and shoots. The selection criteria for inclusion in our analyses required that studies be greenhouse experiments having the soil mineral composition described and the data presented in tables. Overall, plants were grown in pots until maturity (ranging between 6 to 12 weeks depending on the species) and subjected to at least 4 weeks of metal-exposure. Key variables included plant organ dry mass (g), plant-HM concentration (mg kg^{-1} dry mass) and/or content (mg organ or plant dry mass-1), and total extractable soil-HM concentration (mg kg^{-1} dry soil). All the HM (e.g. Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and their soil concentration ranges, and plant species analyzed in this study are appended (Appendices 1 & 2 of the Supplementary Data).

2.2. Metrics

Indexes of biomass partitioning and plant-HM concentration ($[HM_{plant}]$) partitioning were used to assess the allocation biomass or metal distribution in roots relative to shoots and enable a relative measure of comparison between different plant species. The partition equations of biomass (1) and plant-HM concentration (2) are defined as:

(1) $$\frac{biomass_{root}}{biomass_{shoot}}$$

(2) $$\frac{[HM]_{root}}{[HM]_{shoot}}$$

Whereby values ≥ 1 indicate greater or equal allocation to root than shoot, and values <1 indicate greater allocation to shoot than root.

2.3. Statistical analyses

Based on the methods of Zar (1999), we fitted polynomial functions to all the plotted data and used a stepwise regression procedure to compare the maximum power of the polynomial that had statistical significance. We also examined the residual-fit spread to ensure the data meet normal distribution and homoscedasticity assumptions, and determined Cook's distances to test for outliers (data not shown). All the data were log-transformed to enhance the curvilinear relationships between each parameter and to meet the aforementioned statistical assumptions. Notably, we determined broadscale trends among each of the four families tested that met the selection criteria with the exception of the Solanaceae having low data resolution due to small sample size and narrow plant-HM or soil-HM distribution. The polynomial equations [*f(x)*], coefficients of determination [r^2], degrees of freedom [*df*], and *p*-value estimates were determined using S-Plus 8.0 (Insightful Corp., 2007).

3. Results

In all the figures, the plotted data are grouped by plant family and fitted with two polynomial smoothing curves derived by regression analysis. The upper solid line is fitted to the data representing the highest significant polynomials (e.g. 3rd degree polynomial), whereas the dashed line is fitted to the data representing the non-significant equations. In figure 1, the index of biomass partitioning is plotted as a function of the plant-HM content (Fig.1a) and the soil-HM concentration (Fig.1b) wherein the solid line is representative of the Fabaceae and Poaceae, in which case the biomass partitioning shifts from roots to shoots, and back to roots as plant-HM or soil-HM levels increase. By contrast, the biomass partitioning of the Brassicaceae, represented by the dashed line, did not vary significantly and remained relatively constant for this interval. As stated in the methods, the non-significant polynomial equations calculated for the Solanaceae are associated with small sample size and narrow plant-HM or soil-HM levels resulting in poor data resolution and narrow distribution. All regression summary statistics, including the derived equations, coefficients of determination, degrees of freedom, and estimates of p-value, are shown in Table 1.

In figure 2, the index of plant-HM uptake partitioning (e.g. plant-HM concentration) is plotted as a function of plant-HM content (Fig.2a) and soil-HM concentration (Fig.2b) wherein the solid line is generally representative of the Fabaceae, Poaceae, and Brassicaceae. The overall trend indicates a shift in plant-HM concentration more to roots than shoots as soil-HM concentration increases, as found for the biomass partitioning. However, we found no significant relationship among the Brassicaceae (Fig.2a) and attributed this to the poor data resolution and narrow distribution, as with the Solanaceae. All regression summary statistics are shown in Table 2.

Based on all of the empirical relationships determined, we propose a conceptual model of allocation plasticity and plant-metal partitioning outlining the 'fast-grower' versus the 'slow-grower' strategies. In this model (Fig.3), three growth zones are designated that represent low (a), intermediate (b), and high (c) HM levels. Typifying this model of allocation plasticity, the 'fast-growers' show a shift of biomass partitioning whereby their relative biomass allocation to roots is high under low, then decreasing at intermediate, and again increasing at high HM levels according to a parabolic pattern. Likewise, the 'slow-growers' follow a similar tendency although much less dramatically. As for plant-metal partitioning, both grower types show increasingly greater plant-HM partitioning to roots relative to shoots as plant-HM or soil-HM levels increase. Overall, the 'fast-growers' show a high degree of allocation plasticity in regards to biomass plasticity, whereas the 'slow-growers' show a high degree of metabolic plasticity in regards to metal-partitioning.

4. Discussion

Our meta-analytical findings have revealed a dynamic relationship between the plant biomass allocation and plant-HM distribution associated with the increase of plant-HM or soil-HM levels. First, we determined that the root to shoot biomass partitioning (an indicator of allocation plasticity) significantly shifts from a high allocation to roots at low soil-HM levels, then to shoots at intermediate levels, and finally to roots at high levels. In the context of phytoremediation, plants expressing a high level of allocation plasticity may shift their biomass allocation from shoots to roots to circumvent the challenges of increasing soil-HM conditions, notably HM toxicity and edaphic changes resulting in soil-nutrient imbalances. This relative shift in biomass is likely due to increasing requirements for nutrients or other limiting resources (Horst et al., 1990; Wilson, 1988b). As soil-HM reach potentially toxic levels, the rhizosphere may buffer the proximal soil-environment through the exudation of mucilage consisting of organic acids (e.g. polyuronic acids) involved in the regulation of soil-pH, soil-HM redox potential, and the mobilization of limiting mineral nutrients (Marschner, 1995; Mench et al., 1988; Neumann and Römheld, 2000; Ray et al., 1988). While root exudation has a general function of protecting the root apical zones from dessication, facilitating ion uptake, and improving soil-root contact and aggregation, it also contributes in developing microbial community profiles (St-Arnaud & Elsen, 2005; Yergeau et al., 2006). In this regard, the rhizospheric microbial community significantly affects soil-nutrient composition by immobilizing HM via bacterial and fungal ‘metal-binding’ (Joner et al., 2000; Mullen et al., 1989; Morel et al., 1986, 1991), then decreasing soil-HM bioavailability and plant-metal uptake (Audet and Charest, 2007b). This rhizospheric effect is believed to buffer the soil environment and reduce HM phytotoxicity in a stress-avoidance strategy. Although the overall trend of shifting biomass could represent a broad stress-tolerance

151 strategy, the patterns of allocation plasticity we observed among the four families tested were not
152 all the same. Among the Fabaceae and Poaceae, our findings show a significant and pronounced
153 shift in root to shoot biomass partitioning as either the plant-HM or soil-HM level increased, then
154 displaying a high level of allocation plasticity. By contrast, the Brassicaceae show no specific
155 pattern of biomass partitioning, nor any significant level of allocation plasticity. Hence, we
156 attribute these different patterns of biomass allocation among these families to their specific
157 growth strategies relating to their status as 'slow-grower' or 'fast-grower' types, as discussed
158 below. Moreover, the relationships tested among the Solanaceae show, in general, a low data
159 resolution as a result of the small sample size and narrow plant-HM or soil-HM distribution.
160 Consequently, the findings pertaining to the Solanaceae cannot be considered representative of
161 any biological trend until more data are available.

162 Similarly to the trend of shifting biomass allocation, the plant-HM concentration
163 partitioning (an indicator of plant-metal distribution) also shifts more to roots relative to shoots
164 and gradually increases as plant-HM or soil-HM levels increase. Although the overall distribution
165 of plant-HM in either shoots or roots was different among the families, for instance the
166 Brassicaceae having greater total plant-HM in shoots than roots compared to the Fabaceae and
167 Poaceae, we detected a general shift of plant-HM distribution to roots relative to shoots as plant-
168 HM or soil-HM levels increased. In plant cells, HM may lead to the production of superactive
169 radicals causing oxidative stress through binding to enzymes and prosthetic groups, thus
170 disrupting essential metabolic functions (Baccouch et al., 1998; Cho and Seo, 2005;
171 Schützendübel and Polle, 2002). In line with our findings, plants may have some metabolic
172 plasticity to regulate HM distribution more in roots than shoots, thereby reducing the incidence of
173 HM induced oxidative stress in photosynthetic organs. This perspective provides a nuance to the

174 'metal defence hypothesis' which postulates that plants mobilize and hyperaccumulate metals in
175 their shoots to deter insect herbivores (Behmer et al. 2005; Davis and Boyd, 2000; Pollard and
176 Baker, 1997). For this reason, more detailed experimental investigations are needed to verify this
177 aspect of our metal-partitioning hypothesis.

178 By integrating all of these meta-analytical findings and building from the studies of Grime
179 (1979) and Chapin (1980), we propose a general model of allocation plasticity and plant-metal
180 partitioning in which we compare 'slow-grower' and 'fast-grower' strategies in the context of
181 phytoremediation implying HM conditions from trace to excessive levels. Typifying our model,
182 the'fast-growers', owing to their high growth rates, show a cup-shaped pattern of biomass
183 partitioning thus indicating a high degree of allocation plasticity when subjected to stressful soil-
184 HM conditions; this is in contrast to the saucer-shaped pattern of the 'slow-growers' that show a
185 low degree of allocation plasticity. As previously proposed, the ability of plants to adjust their
186 relative biomass could enable them to buffer the proximal soil-environment and gain access to
187 limiting resources via rhizospheric processes (Marschner, 1995; Wilson, 1988b). By contrast, the
188 'slow-growers', owing to a high investment in intrinsic stress-resistance (e.g. phytochelatin
189 metabolism), show a higher level of metabolic plasticity compared with 'fast-growers' in regards
190 to their regulation of plant-metal partitioning. Taken as a whole, this relationship reflects a
191 functional equilibrium between the plant stress-tolerance strategies, particularly regarding
192 investment in intrinsic versus extrinsic strategies. From our results, we determined that the
193 Brassicaceae mostly express 'slow-grower' characteristics thus enabling them to tolerate
194 potentially toxic HM conditions and then partly contributing to their status as hyperaccumulators
195 (e.g. *Thlaspi* and *Brassica* spp.) (Freeman et al., 2004; Marchiol et al., 2004; Peer et al., 2003).
196 Furthermore, we have determined that the Fabaceae and Poaceae mostly express 'fast-grower'

197 characteristics thus enabling their rapid growth and adaptation in contaminated environments, as
198 in the case of *Trifolium* and *Lolium* spp. (Arienzo et al, 2004; Bidar et al., 2007). Another aspect
199 of 'slow' or 'fast' growth strategy in relation with HM stress concerns the investment in symbiotic
200 associations. One such example relates to the arbuscular mycorrhizal fungi and their dynamic
201 roles in enhancing the stress-tolerance of numerous herbaceous plant species (Audet and Charest,
202 2007b; Chen et al. 2007; Joner et al., 2000), in which they:

203 (1) Increase HM uptake via the extensive mycorrhizospheric network at low soil-HM
204 concentrations; and

205 (2) Reduce HM bioavailability by metal-binding processes at high soil-HM levels, then
206 increasing plant biomass and tolerance through a HM stress-avoidance.

207 Notably, this dynamic mycorrhizal effect at high soil-HM levels has been shown to decrease plant-
208 HM uptake and subsequently reduce cellular oxidative stress (Schützendübel and Polle, 2002).
209 The typically mycotrophic plant families (Smith and Read 1997), such as the Fabaceae, Poaceae
210 and Solanaceae evaluated in the current study, may invest more in mycorrhizal stress-avoidance as
211 an extrinsic tolerance strategy (Audet and Charest 2006). On the other hand, the typically non-
212 mycotrophic families, such as the Brassicaceae, must rely more on intrinsic plant stress-tolerance
213 mechanisms, for example phytochelatin production or HM sequestration (Cobbett, 2000; Cobbett
214 and Goldsborough, 2002; Meharg, 2005; Steffens, 1990). Consequently, it is most likely that a
215 dynamic compromise between biomass allocation and metal partitioning influences overall plant
216 growth strategy and investment toward 'intrinsic' or 'extrinsic' stress-tolerance mechanisms. This
217 being said, future investigations should question how these overall relationships are impacted
218 when considering exposure to other types of environmental pollutants not included in our analyses
219 (e.g. halogenated solvents, polycyclic aromatic hydrocarbons, and radionuclides); or whether the

relationships hold true for other plant families (e.g. Cannabaceae, Lamiaceae, Pteridaceae, etc). For instance, do similar relationships exist among aquatic or wetland families having different physiological adaptations to their ecosystems? Considering the particular physical characteristics of these environments, do the families respond differently to pollution exposure? How do their respective growth strategies enable them to circumvent such challenges? While the patterns of biomass allocation and metal-partitioning reported in our study pertain primarily to metal phytoextraction processes, it is interesting to explore the implications of these relationships among other branches of phytoremediation research, such as phytostabilization, phytovolatilization, and phytodegradation, to name a few. By integrating these aspects we may develop a more complete picture of plant and ecosystem function, and plant adaptations to environmental stress conditions.

5. Conclusion

By screening the current body of phytoremediation literature, our meta-analytical study has identified broad and dynamical trends relating to plant biomass allocation plasticity, metal partitioning, and metal stress-tolerance. Accordingly, the proposed conceptual models may stimulate thought and provide a framework for future investigations having possible implications for phytoremediation purposes. In addition, this framework may provide insights on overall processes of plant stress-tolerance and thereby be an asset for strategic environmental remediation practices

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Figure Captions

Figure 1. Biomass partitioning (root shoot^{-1}) as a function of plant-HM content (mg dry mass^{-1}) (a) and soil-HM concentration (mg kg^{-1} dry soil) (b). The upper solid line is fitted to data representing the highest significant polynomials, whereas the dashed line is fitted to the data representing the non-significant equations.

Figure 2. Plant-HM concentration partitioning (root shoot^{-1}) as a function of plant-HM content (mg dry mass^{-1}) (a) and soil-HM concentration (mg kg^{-1} dry soil) (b). The upper solid line is fitted to data representing the highest significant polynomials, whereas the dashed line is fitted to the data representing the non-significant equations.

Figure 3. Conceptual model of allocation plasticity and plant-metal partitioning for 'fast-grower' (solid line) and 'slow-grower' (dotted line) types. Designated are three growth zones representing low (a), intermediate (b), and high (c) plant-HM or soil-HM levels.

Table 1. Polynomial equations of biomass partitioning (root shoot^{-1}) as a function of plant-HM content (mg dry mass^{-1}) and soil-HM concentration (mg kg^{-1} dry soil).

Parameter	Family	Biomass partitioning			
		f(x)	r^2	*df*	*p*
Plant-HM Content	Brassicaceae	$0.01x^2-2.5$	0.18	15	0.22
	Fabaceae	$0.4x+0.2x^2-0.02x^3-1.4$	0.58	20	$>10^{-4}$
	Poaceae	$0.1x+0.05x^2-0.02x^3+0.5$	0.32	25	>0.05
	Solanaceae	-0.01x-0.9	0	6	0.79
Soil-HM Concentration	Brassicaceae	0.1x-2.5	0.11	24	0.09
	Fabaceae	$3.27x+0.5x^2-0.4x^3-6.4$	0.47	20	$>10^{-3}$
	Poaceae	0.3x-0.7	0.44	28	0.05
	Solanaceae	-0.01x-0.9	0.78	6	0.78

Polynomial equations [*f(x)*], coefficients of determination [r^2], degrees of freedom [*df*], and estimates of *p*-value are shown.

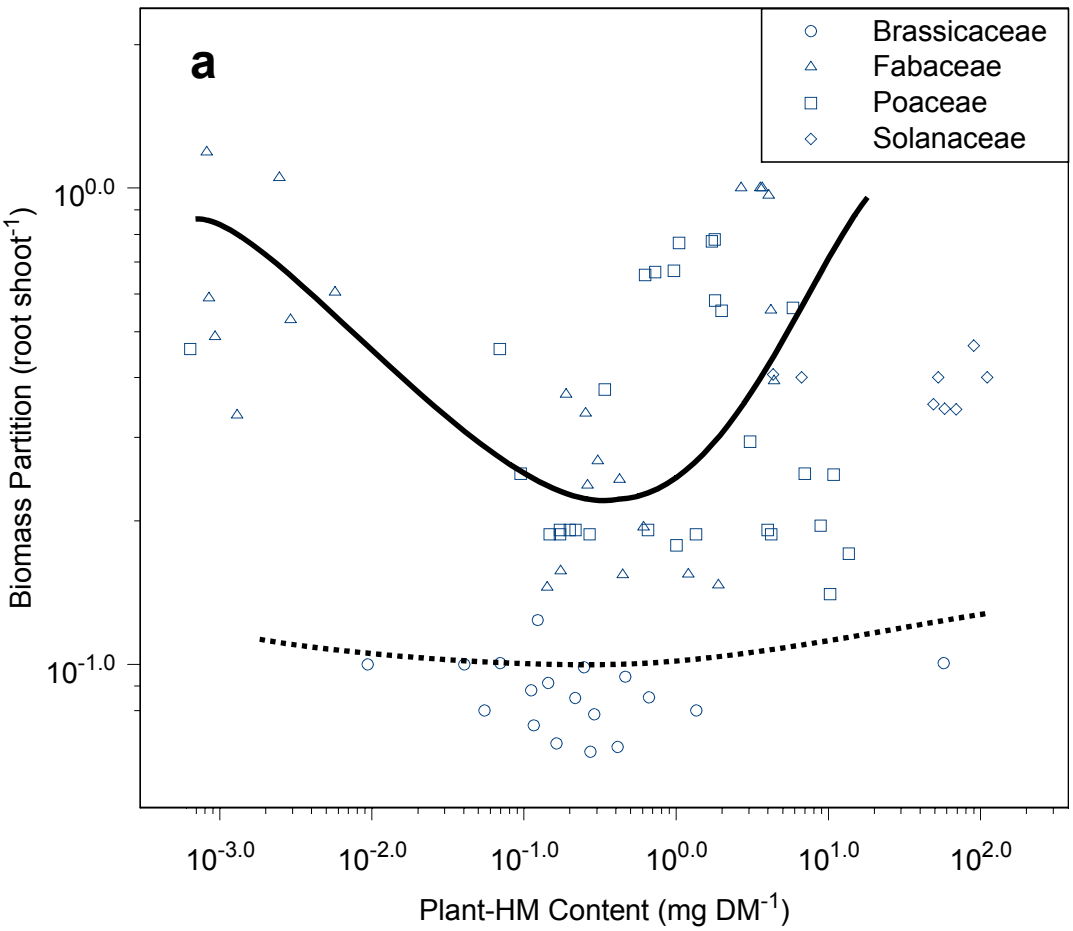
Table 2. Polynomial equations of plant-HM concentration partitioning (root shoot^{-1}) as a function of plant-HM content (mg dry mass^{-1}) and soil-HM concentration (mg kg^{-1} dry soil).

Parameter	Family	Plant-HM concentration partitioning			
		f(x)	r^2	*df*	*p*
Plant-HM Content	Brassicaceae	$-0.2x+0.03x^2-0.1$	0.34	19	>0.01
	Fabaceae	$x0.8+0.1x^2+1.7$	0.74	21	$>10^{-6}$
	Poaceae	$0.1x-0.2x^2+2.6$	0.42	8	0.1
	Solanaceae	$-0.28x+1.1$	0.49	6	0.05
Soil-HM Concentration	Brassicaceae	$0.4x+0.1x^2-0.1x^3+0.3$	0.1	95	>0.05
	Fabaceae	$2.6x+1.3x^2+0.01x^3-0.x^4+1.5$	0.43	19	>0.05
	Poaceae	$0.4x+0.2x^2+0.1x^3-0.01x^4+1.5$	0.41	25	$>10^{-3}$
	Solanaceae	$-0.2x+0.5$	0.1	20	0.3

Polynomial equations [*f(x)*], coefficients of determination [r^2], degrees of freedom [*df*], and estimates of *p*-value are shown.

Figures (3 in total)

Figure 1



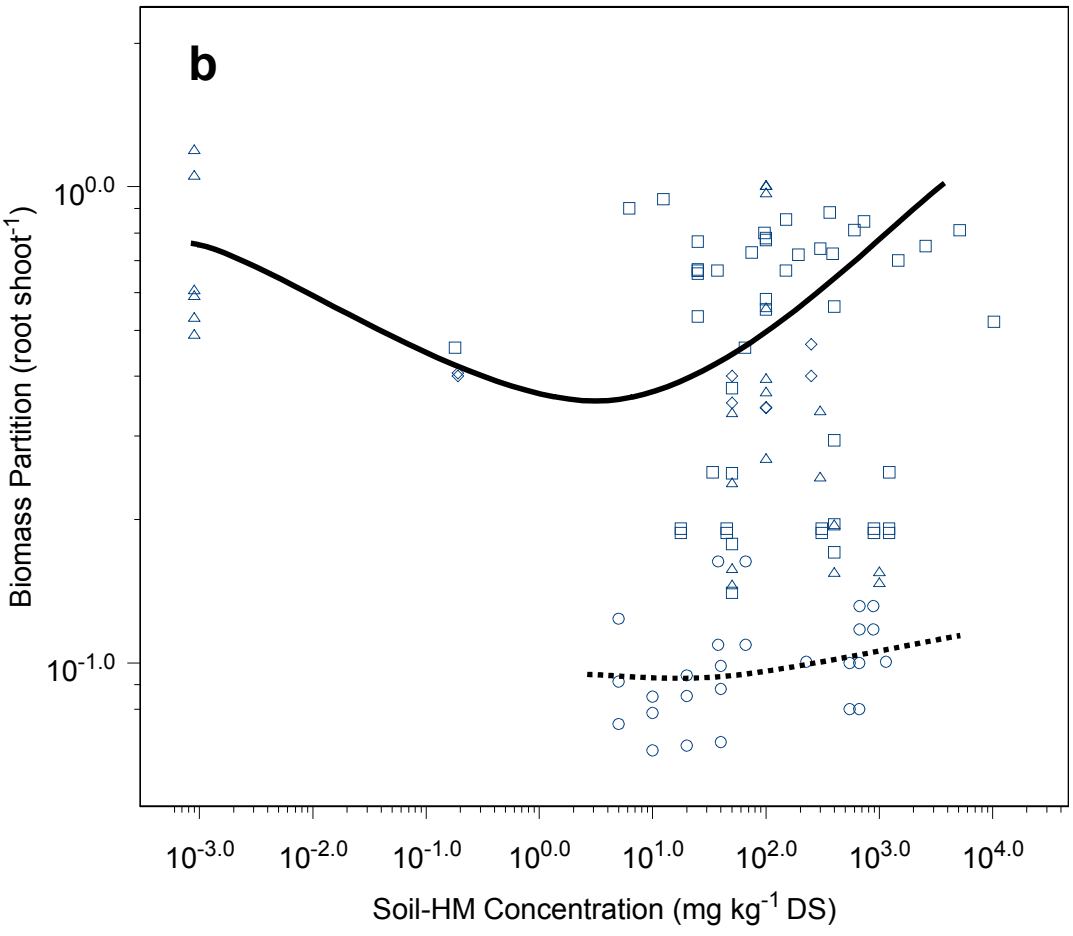
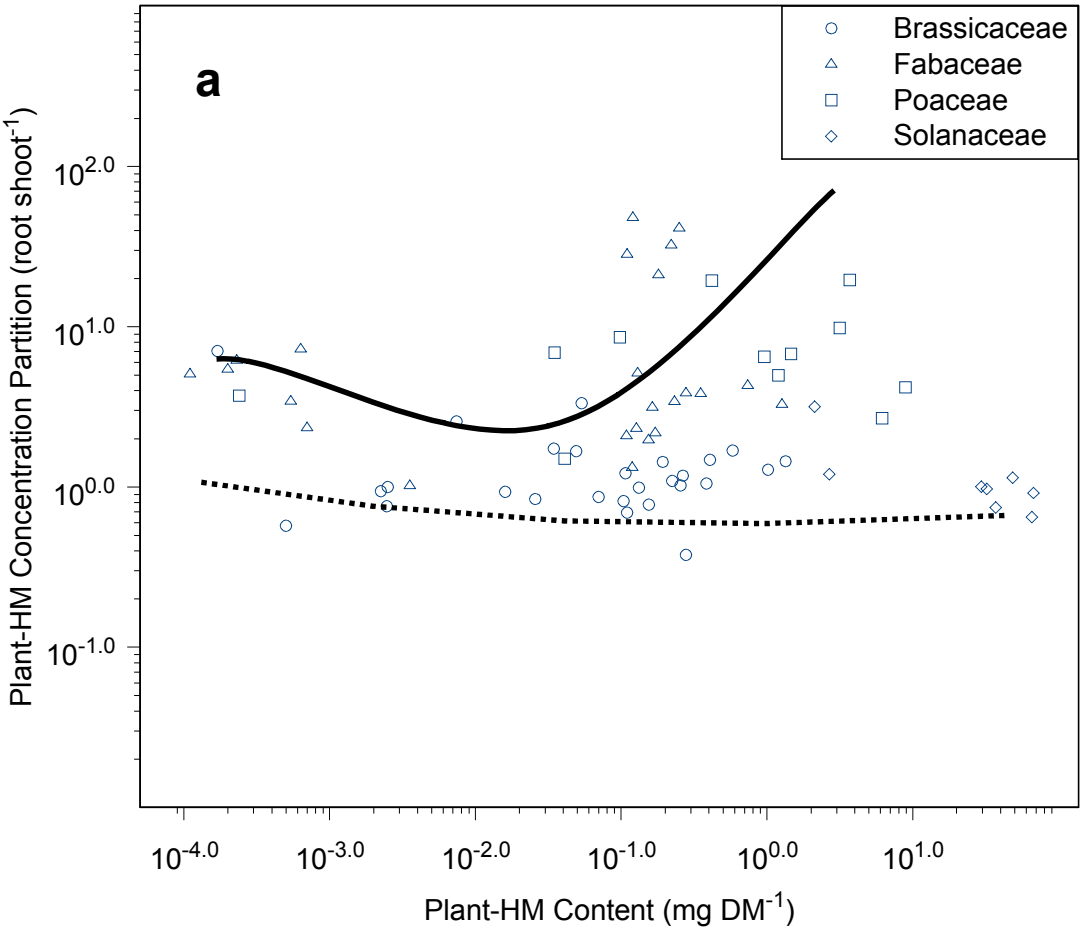


Figure 2


a
Brassicaceae
Fabaceae
Poaceae
Solanaceae
Plant-HM Concentration Partition (root shoot-1)
10^2.0
10^1.0
10^0.0
10^-1.0
10^-4.0
10^-3.0
10^-2.0
10^-1.0
10^0.0
10^1.0
Plant-HM Content (mg DM-1)

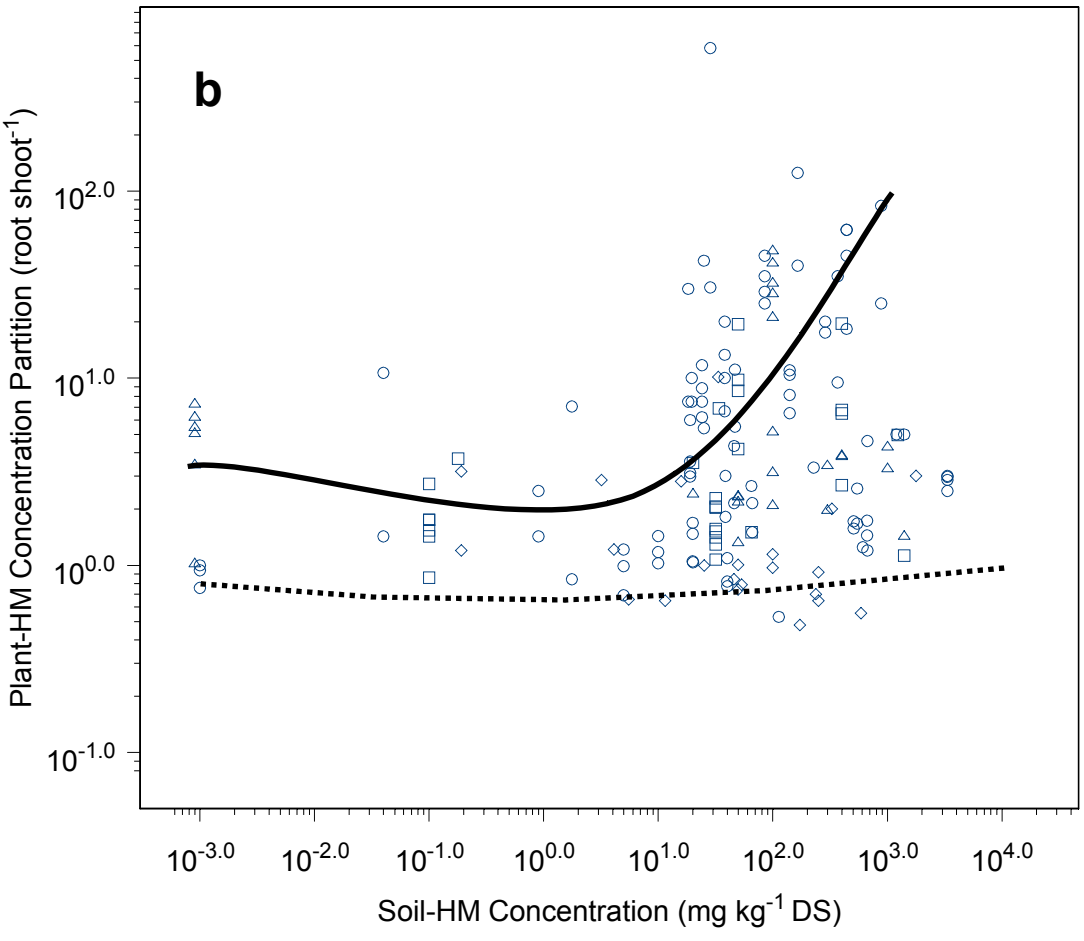
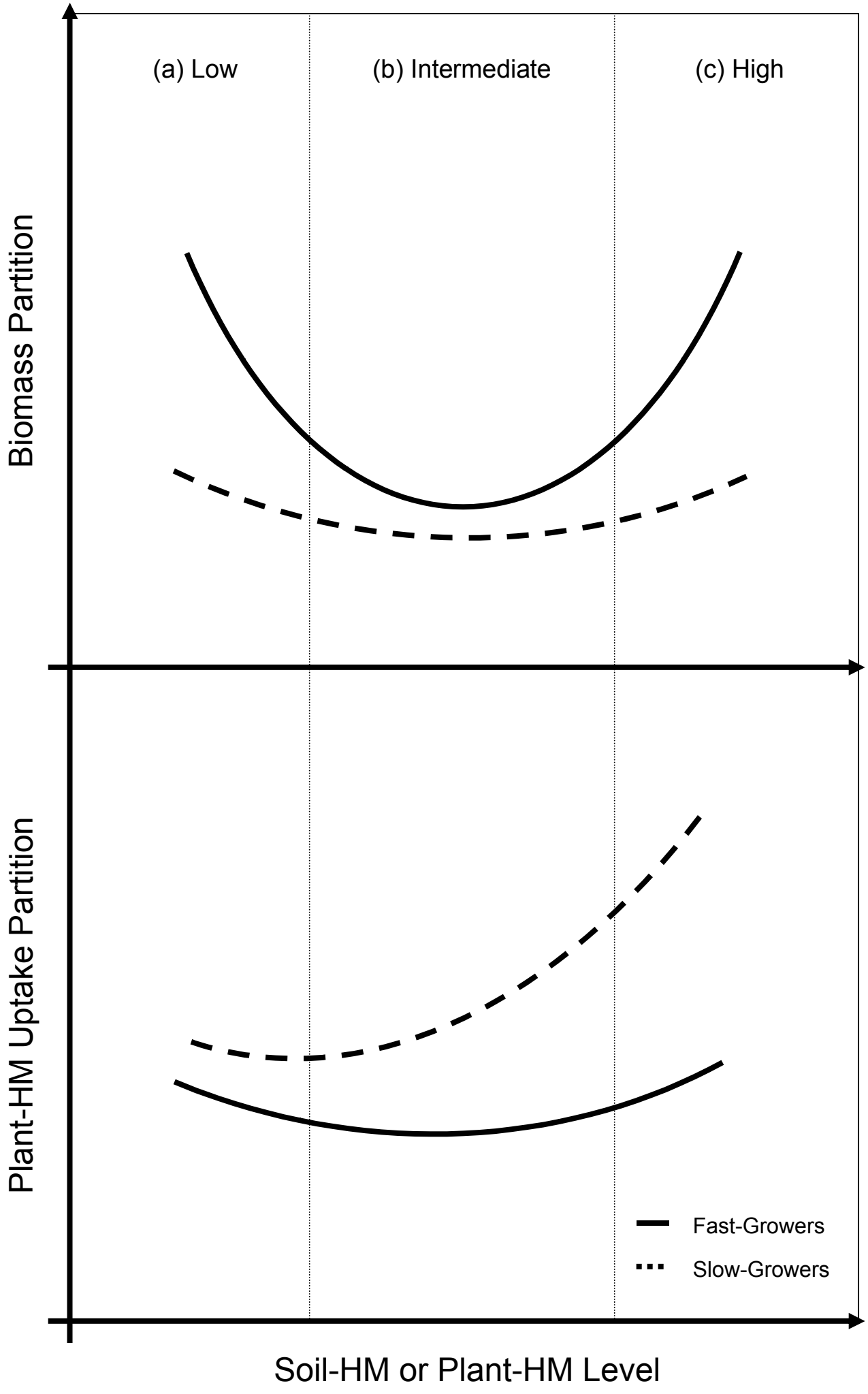

b
Plant-HM Concentration Partition (root shoot-1)
10^2.0
10^1.0
10^0.0
10^-1.0
10^-3.0
10^-2.0
10^-1.0
10^0.0
10^1.0
10^2.0
10^3.0
10^4.0
Soil-HM Concentration (mg kg-1 DS)

Figure 3


(a) Low
(b) Intermediate
(c) High
Biomass Partition
Plant-HM Uptake Partition
Fast-Growers
Slow-Growers
Soil-HM or Plant-HM Level

Appendix 1. Plant families & species comprised in the meta-analysis

Family	Species	Reference
Brassicaceae	*Brassica carinata*	Marchiol & al., 2004b
	Brassica napus	Marchiol & al., 2004a,b
	Brassica juncea	Blaylock & al., 1997; Marchiol & al., 2004b; Su & Wong, 2004
	Raphanus sativus	Marchiol & al., 2004a,b
	Thlaspi arvense	Hammer & Keller, 2002
	Thlaspi caerulescens	Ayoub & al., 2003; Hammer & Keller, 2002; Mcgrath & al., 1997
	Thlaspi ochroleucum	Mcgrath & al., 1997
Fabacceae	*Pisum sativum*	Rivera-Becerril & al., 2002; Zhu & al., 2001
	Trifolium pratense	Chen & al., 2003; Bi & al., 2003; Vivas & al., 2003
	Trifolium repense	Li & Christie, 2001; Zhu & al., 2001
Poaceae	*Andropogon virginicus*	Pichtel & al. 2000
	Hordeum vulgare	Ayoub & al., 2003
	Lolium perenne	Li & Christie, 2001; Zhu & al., 2001
	Triticum aestivum	Athar & Ahmad, 2002
	Zea mays	Chen & al., 2004a,b
Solanaceae	*Nicotiana glauca*	Barazani & al. 2004
	Nicotiana rustica	Audet & Charest, 2006

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

Soil-HM	**Concentration Range (mg kg^{-1} dry soil)**	**References**
Cd	0.001-100	Athar & Ahmad, 2002; Ayoub & al., 2003; Barazani & al., 2004; Chen et al., 2004a; Hammer & Keller, 2002; Marchiol & al., 2004a; McGrath & al., 1997; Pichtel & al. 2000; Rivera-Becerril et al., 2002; Su & Wong, 2004; Vivas et al., 2003b.
Cr	20-165	Athar & Ahmad, 2002; Barazani & al., 2004; Marchiol & al., 2004a,b; McGrath & al., 1997.
Cu	5-1470	Athar & Ahmad, 2002; Barazani & al. 2004; Hammer & Keller, 2002; Marchiol & al., 2004a,b; McGrath & al., 1997.
Mn	110-590	Athar & Ahmad, 2002; Barazani & al. 2004; Weissenhorn et al. 1995.
Ni	15-600	Athar & Ahmad, 2002; Barazani & al. 2004; Marchiol & al., 2004a,b; McGrath & al., 1997.
Pb	25-1400	Athar & Ahmad, 2002; Barazani & al., 2004; Marchiol & al., 2004a,b; McGrath & al., 1997; Pichtel & al. 2000; Vivas et al. 2003a.
Zn	0.1-10230	Athar & Ahmad, 2002; Audet and Charest 2006; Ayoub & al., 2003; Barazani & al. 2004; Bi et al. 2003; Chen et al. 2003, 2004b; Hammer & Keller, 2002; Li and Christie 2001; Marchiol & al., 2004a,b; McGrath & al., 1997; Su & Wong, 2004; Zhu et al. 2001.

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