Microbial associated plant growth and heavy metal accumulation to improve phytoextraction of contaminated soils

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Abstract

Utilizing plants to remediate heavy metal contaminated soils, a process known as phytoextraction, offers many advantages but has yet to reach levels of efficiency that would make the strategy economically viable. Inoculation of the plant rhizosphere with microorganisms is an established route to improving phytoextraction efficiency. In general, microorganisms can improve phytoextraction by increasing the availability of heavy metals to the plant and by increasing plant biomass. This review uses a meta-analysis of the results from 103 microbial-augmented phytoextraction studies to examine if one of these microbial mechanisms has a greater potential to positively impact phytoextraction. Trends surrounding the use of heavy metal-accumulating versus non-heavy-metal-accumulating plants in phytoextraction are discussed. Microbially induced improvements in the accumulation of heavy metals in plant biomass, a focus of several studies, are always coincident with enhanced net phytoextraction. However, microbial treatments that improved plant biomass are more prevalent in the literature and account for a larger number of studies that reported improved phytoextraction, particularly in non-heavy-metal-accumulating plants. The experimental findings emerging from the literature that implicate specific microbial processes in improving phytoextraction are briefly reviewed and used to underline trends observed from the meta-analysis that indicate future directions regarding the use of microorganisms to improve phytoextraction efficiency.

1. Introduction

Phytoextraction, is a low-cost, environmentally friendly remediation technique that utilizes specialized metal-accumulating plants, known as heavy metal hyperaccumulators (HMHs), or metal-tolerant high biomass plants (non-HMH) to extract heavy metals from contaminated soils (Vamerali et al. 2010). In contrast to traditional heavy-metal remediation strategies, which involve the physical removal of contaminated soil, chemical washing and reburial, it is estimated that phytoextraction could reduce operational costs as much as 30-fold and reduce environmental harm (Gerhardt et al. 2009). While holding considerable promise, phytoextraction is not sufficiently efficient to be considered economically viable.

A frequently utilized strategy to improve phytoextraction is the inoculation of beneficial microorganisms into the plant rhizosphere (Abhilash et al. 2012, Sessitsch et al. 2013). Microbial candidates that improve heavy-metal phytoextraction are commonly sourced from contaminated soils and microbial communities present at the plant root-soil interface (rhizosphere communities) (Lodewyckx et al. 2002, Park et al. 2011, Zhang et al. 2011). These communities are metabolically and taxonomically diverse, containing microorganisms that are pre-adapted to conditions *in situ* and are capable of performing metabolic activities that can alter heavy metal-bioavailability and promote plant growth (Bai et al. 2014, Dell'Amico et al. 2008, Ma et al. 2009, Sumi et al. 2014). To date, multiple studies have evidenced that the addition of microorganisms to the plant rhizosphere can improve heavy metal accumulation in plants (Abou-Shanab et al. 2003, Abou-Shanab et al. 2003, Amprayn et al. 2012, De Souza et al. 1999, Ma et al. 2013, Malekzadeh et al. 2012, Whiting et al. 2001).

There are multiple mechanisms by which microorganisms may improve the accumulation of heavy metals in plants and hence phytoextraction. Broadly, these mechanisms assist phytoextraction by increasing the bioavailability of heavy metals in the soil and/or promoting plant growth (Abhilash et al. 2012, Mulligan 2005, Sessitsch et al. 2013), whereas increased metal bioavailability can facilitate plant uptake and increase the concentration of heavy metals in plant tissue, and plant-growth-promotion increases the amount of heavy metal-containing biomass. The total amount of heavy metal extracted is a product of both concentration and biomass production. Among the phytoextraction studies, there is often a trade-off between the concentrations of heavy metals that plants can tolerate and the biomass produced (i.e., HMHs versus non-HMHs).

The purpose of this review is to examine emerging trends and evidence from the literature regarding how microorganisms may be assisting phytoextraction. We summarize and discuss the outcomes of 103 microbial-augmented phytoextraction studies from approximately the last decade to assess broadly which microbial mechanisms have the greatest potential to further develop phytoextraction and whether HMHs or non-HMHs will best facilitate these advances. In addition to discussing broadly microbial metal-mobilization and plant growth promotion (PGP) to improve phytoextraction, we discuss cases from the literature that highlight the role of specific microbial processes in improving phytoextraction in light of trends observed in the meta-analysis.

2. Trends in phytoextraction research: microbial activities hypothesized to improve phytoextraction

To examine how microorganisms improve phytoextraction and trends in successful outcomes, we performed a meta-analysis of 28 phytoextraction papers containing a total of 103 individual phytoextraction studies utilizing either heavy metal hyperaccumulating plants (HMHs) or fast-growing high biomass plant species (non-HMHs). Studies are defined in this report as experiments that vary in plant species, microbial treatment or heavy metal application. For each study, the effect of microbial inoculation on plant biomass (PB), concentration of heavy metal per unit of plant tissue ([HM]), and net heavy metal extracted per *plant* (HM_{net}) was calculated from the difference between the microbially treated and control samples as a percentage of the control, as previously described (Kloepper et al. 1989) using the following equation:

$$\text{Microbial effect} = (\text{treatment value} - \text{control value})/\text{control value}$$

The studies were grouped based on the response, of PB- and [HM]- parameters to microbial inoculation. The change in HM_{net} due to microbial inoculation was used as a measure to indicate the success of the microbial treatments in improving phytoextraction.

Over one quarter of the studies reported improvements in phytoextraction (HM_{net}) through improvements in both [HM] and PB following microbial inoculation. An increase in HM_{net} was reported in all cases where microbial treatments increased [HM], even when PB was not affected (Figure 1).

Although increases in [HM] were always associated with improvements in HM_{net}, the percentage of studies that reported microbial-induced increases in [HM] was considerably lower (35%) than the percentage of studies that reported microbial-induced increases in PB (70%).

Of the studies that reported increases in HM_{net}, 39% reported improvements in both PB and [HM], 43% reported improvements in PB alone, while only 11% were able to be attributed to improvements in [HM]. Thus, given the current data, compared to improvements in [HM], improvements in HM_{net} are 1.6 times as likely to be associated with plant-growth promotion. By comparing the scenarios whereby improvements in HM_{net} could be attributed to either PB *or* [HM], we observe that improvements in HM_{net} are 3.9 times as likely to be reported as associated with improvements in PB compared to [HM].

Thirty-five studies included in this meta-analysis used HMHs with no historical agricultural use, and among these were the model HMHs, *Noccaea caerulescens* and *Alyssum murale* (Table 1). Most studies (66) used high biomass agriculturally developed species, some of which have been shown to hyperaccumulate metals under contaminated conditions, but are not HMHs, as ultimately such conditions are toxic to the plant (van der Ent et al. 2015). Two studies used species which are neither HMHs nor have a history of agricultural use, but have been reported as agricultural weeds (Table 1; (Mitich 1996)).

Figure 2 shows the association of improvements in HM_{net} with improvements in PB or [HM] for HMHs and non-HMHs. Improvements in [HM] were associated with HMHs and non-HMHs at roughly the same frequency in this meta-analysis. However, improvements in PB were more frequently associated with non-HMHs. Of the successful cases using non-HMHs, 50% were due to improvements in PB alone, 8% were due to an increase in [HM] and 38% to a combination of PB and [HM] (Figure 2).

HMHs actively sequester and store heavy metals in their aerial tissues and are able to obtain concentrations of heavy metals in their tissues 100-1000 times higher than concentrations found in non-accumulator plants (Alford et al. 2010, Rascio and Navari-Izzo 2011, van der Ent et al. 2013). However, HMHs have a number of properties that are not conducive to applied phytoextraction. Many HMHs tend to be slow growing with shallow root systems that are insufficient at permeating contaminated soils and extracting heavy metals to any great depth (Brewer et al. 1999, Krämer 2005, Słomka et al. 2012). When we examined the net amount of metals extracted per plant in individual studies, we found that non-HMHs generally extracted more heavy metals (mg/plant) than HMHs, particularly for Cd and Ni (Figure 3). Compared to non-HMHs, these trends most likely reflect the small size and slow growth of many HMHs. To account for the variation in the duration of plant-growth experiments (ranging from 14 to 150 days), we also calculate the rate of metal extracted per plant per day. Largely, the trends observed for total metal extracted were conserved when the duration of phytoextraction trials were accounted for. These data suggest that despite the high foliar concentration of metals that HMHs achieve, non-HMHs with high biomass production may be a more appropriate choice for developing phytoextraction in the immediate future. Additionally, the growth habits of many well-studied HMHs, such as the rosette form of *Noccaea caerulescens*, are not amenable to mechanical harvesting, which would increase the cost of phytoextraction (Brewer et al. 1999). However, HMH research will remain paramount for further understanding the uptake and sequestration of heavy metals. Conceivably, the knowledge gained by unravelling how these plants sequester such high concentrations of toxic metals will assist in the refinement of phytoextraction in the future.

The observation that non-HMHs tend to extract larger quantities of heavy metals per plant than HMHs and the high frequency of improved phytoextraction via PGP indicate that non-HMHs may be an appropriate choice of plant for improving phytoextraction and microbial PGP (as opposed to microbial mobilization of heavy metals) may be a superior strategy for improving phytoextraction. A point to be considered, though, is that usually only positive findings are routinely published, which may have skewed the outcome for this meta-analysis. The combined use of these two strategies is supported by the observed high frequency of microbial PGP in conjunction with non-HMHs (Figure 2). However, it is noteworthy that improvements in [HM] *always* translated to improvements in HM_{net}, suggesting that a targeted search for microorganisms that increase [HM], rather than improve plant biomass, is also a worthy research direction.

3. Linking Microbial Processes to Improvements in Phytoextraction

3.1 *Microbial improvement of phytoextraction*

Multiple microbial processes exist that can stimulate plant growth or increase heavy metal bioavailability or both. Detailed reviews on how microbial processes affect phytoextraction can be found elsewhere (Abhilash et al. 2012, Lebeau et al. 2008, Mulligan 2005, Sessitsch et al. 2013). The following discussion highlights the experimental evidence emerging from the literature that implicates specific microbial processes in improving phytoextraction and how they relate to the trends observed in the meta-analysis. In an experimental setting, specific microbial processes can rarely be identified, as the causative agent behind microbial-induced improvements in phytoextraction, even when the PGP and/or metal-mobilizing ability of an inoculum is known. This uncertainty is due to confounding factors, such as indigenous microorganisms and soil physicochemistry, that make it difficult to determine the processes being carried out by the inoculum *in situ*. As such, we will limit our discussion to work where a strong cause and effect can be established between a specific microbial process and improved phytoextraction.

3.2 Improving plant nutrition and mobilizing metals to enhance phytoextraction: Siderophores and Phosphate solubilization

To solubilize inorganic phosphates (P), microorganisms can produce and secrete an array of organic acids, such as gluconic acid, 2-ketogluconic acid, lactic acid and acetic acid (Rodríguez and Fraga 1999). The associated decrease in soil pH can also increase the solubility of some heavy metals (Kim et al. 2013). Thus, P-solubilizing microorganisms are believed to increase plant biomass by supporting plant health *and* mobilize heavy metals making them an attractive strategy for improving phytoextraction.

Correlations between increased plant P uptake and increased plant biomass have been observed under Cu stress following inoculation with the endophyte, *Penicillium funiculosum* (Khan and Lee 2013). The endophyte has previously been reported as having P-solubilization activity and is able to alleviate plant stress responses to Cu contamination, possibly via the secretion of gibberellins. Despite the increase in plant biomass, the inoculum decreased Cu concentration in the plant. A reduction in the amount of Cu accumulated in plant roots, in the presence of the endophyte, suggested that free metal ions were being absorbed by the fungus, rather than being transported into the plant (Khan and Lee 2013).

Improvements in phytoextraction due to the PGP ability of P-solubilizing microorganisms have been demonstrated in experiments that decoupled the PGP and metal-mobilizing activity of a P-solubilizing *Burkholderia cepacia* using a hydroponic experimental design in which heavy metals are necessarily mobile (Li et al. 2007). Using the Cd/Zn hyperaccumulator, *Sedum alfredii* growing in a nutrient solution with either 80 mg Zn L^{-1} or 8 mg Cd L^{-1}, Li et al. (2007) reported an increase in plant biomass that correlated with an increase in P uptake in the plants. The microbial treatment had negligible or negative effects on the concentrations of Zn and Cd in the plants, but due to the increased biomass, the total amount of Zn and Cd extracted was increased by 116% and 46%, respectively (Li et al. 2007).

P-solubilizing microorganisms that improve phytoextraction by increasing both PGP and heavy metal-mobilization have been reported. Compared to un-inoculated controls, the inoculation of *Brassica juncea* with a P-solubilizing *Bacillus* spp. induced a 349% increase in plant dry weight after 8 weeks and a 148% increase in Cd concentration (Jeong et al. 2013). However, reported increases in IAA content in the soil and the presence of a native soil microbial community make it difficult to attribute the experimental outcomes to P-solubilization alone.

Where organic acids improve P acquisition, microbial siderophores chelate and solubilize Fe^{3+} in soil and improve iron acquisition by plants (Rajkumar et al. 2010). The mobilization of toxic heavy metals by siderophores has also been demonstrated using the microbial siderophore desferrioxamine-B (DFO-B). In the presence of 10 μM Cd, DFO-B, the application improved Cd accumulation in *Noccaea caerulescens* by 37% and increased root to shoot translocation by 27% (Karimzadeh et al. 2012).

Evidence that siderophores produced by microorganisms *in situ* mobilize heavy metals and improve phytoextraction comes from studies investigating Zn accumulation in *N. caerulescens*. The addition of active rhizosphere communities to *N. caerulescens* affected a 4-fold increase in net Zn hyperaccumulation due to the microbial mobilization of non-labile Zn pools (Whiting et al. 2001). The increase in net Zn accumulation was a product of increases in plant biomass and Zn concentration. Following a lack of evidence to support Zn mobilization via mechanisms that alter soil pH (such as organic acid production), it was concluded that siderophores were most likely responsible for the increase in labile Zn.

Even though the secretion of siderophores is a clear strategy for improving plant growth in iron-limiting situations, there is little evidence of their ability to improve plant growth in the presence of other heavy metals. The increases in Cd concentration in the aforementioned DFO-B treatment were not linked to improvements in plant growth; the inoculum that increased *N. caerulescens* biomass may have had additional PGP activities that were not measured (Karimzadeh et al. 2012, Whiting et al. 2001).

3.3 Improving plant nutrition to enhance phytoextraction: N_2 fixation

Microbial-induced increases in plant nitrogen (N) availability have also been linked to improvements in phytoextraction. In pot trials, a heavy metal resistant, N_2-fixing *Bradyrhizobium* sp. (*vigna*), RN8 was able to increase the dry weight of green gram (*Vigna radiata* L. *wilczek*) by 28% in a soil containing 9,780 mg Zn kg^{-1} and by 24% in a soil containing 580 mg Ni kg^{-1}. Compared to un-inoculated controls, the increases in plant biomass were accompanied by increases in total N content in the plant (Wani et al. 2007). Similar to the study by Li et al. (2007) using P-solubilizing bacteria and *Sedum alfredii*, RN8 decreased concentrations of Zn and Ni in shoots of green gram. However, in both cases the increase in plant biomass created a net positive contribution to the total amount of heavy metals extracted per plant (Li et al. 2007, Wani et al. 2007).

3.4 Other microbial mechanisms of plant growth promotion to improve phytoextraction

In addition to increasing plant growth by improving plant nutrition, microorganisms can improve plant growth directly via the production of hormones, such as auxins (indole-3-acetic acid; IAA), cytokinins and gibberellins or indirectly via stress inhibiting enzymes, such as 1-

aminocyclopropane-1-carboxylic acid (ACC)-deaminase (Badri et al. 2009, García de Salamone et al. 2001, Glick 2003, Usha Rani et al. 2011). In plants, the expression of IAA-producing genes can be negatively regulated by the presence of heavy metals, such as Cd^{2+} (Elobeid et al. 2012). The microbial secretion of IAA can counteract the inhibitory effects of heavy metals on plant IAA production, enabling sustained plant growth (Elobeid et al. 2012). The direct relationship between microbial IAA production and improved plant growth, in the absence of heavy metals, has been demonstrated using *Azospirillum brasilense* strain SM and its IAA over- and under-expressing mutants (Kochar and Srivastava 2012). Although the microbial production of IAA has been repeatedly cited in the literature as a major factor contributing to hyperaccumulation of heavy metals via PGP, the presence of other PGP-processes makes it difficult to clearly establish cause and effect (Glick 2010, Lampis et al. 2015, Ma et al. 2011, Ma et al. 2009). Microbial use of IAA as a carbon source is an additional confounding factor in establishing whether IAA produced by an inoculum influences plant growth (Duca et al. 2014). Nevertheless, multiple reports demonstrated that foliar application of IAA or other phytohormones can improve the phytoextraction of metals, including Ni, Pb and Cd (Cabello-Conejo et al. 2014, Hadi et al. 2010).

3.5 The potential of improved plant biomass in enhancing phytoextraction

The evidence that improved plant nutrition (P or N) increases phytoextraction by improving plant biomass, even when concentrations of heavy metals in plant tissues are reduced, lends weight to the notion of targeting PGP strategies over metal-mobilization strategies to improve phytoextraction. Non-biological methods of increasing plant growth, such as fertilizer application, have been shown to improve Ni extraction in *Alyssum bertolonii* by increasing plant biomass as much as 300% with no appreciable reduction in Ni concentration (Robinson et al. 1997). There are also numerous examples in the literature of PGP-microorganisms improving phytoextraction, even if the mechanism of PGP action cannot be identified (Belimov et al. 2004, He et al. 2009, Li et al. 2007, Liu et al. 2015, Ma et al. 2009, Malekzadeh et al. 2012, Rani et al. 2013). For instance, increases in canola biomass caused by inoculation of *Pseudomonas fluorescens* and *P. tolaasii* increased total Cd accumulation by 72% and 107%, respectively, despite Cd concentrations in plant tissues remaining constant (Dell'Amico et al. 2008). Similarly, increases in *Salix dasyclados* biomass following inoculation with the ectomycorrhizal fungi, *Amanita muscaria*, increased total Pb accumulation by 85% without increasing Pb concentrations in plant tissues (Hrynkiewicz and Baum 2013).

The contrasting ways in which PGP-microorganisms and heavy-metal-solubilizing microorganisms improve heavy-metal accumulation were highlighted in research by Ma et al. (2009a). The work used three microbial strains that improved Ni phytoextraction by *Brassica juncea* in opposing ways. Two stains, SRA1 and SRA10 exhibited the high rates of siderophore production (hydroxamate and catechol type) and P-solubilization, and the ability to mobilize Ni in the soil, whilst a third strain, SRA2, exhibited the highest levels of IAA production and possessed other PGP attributes. The opposing biochemical attributes of the two groups of microorganisms corresponded well with the manner in which they influenced plant growth: The metal-mobilizing strains elicited minor significant improvements in plant growth, but considerably increased plant Ni concentration. Conversely, SRA2 had no impact on Ni concentration but improved plant biomass by 285% (Ma et al. 2009). Surprisingly, the net increase in Ni removed per plant (i.e., phytoextraction ability) was highest in the SRA2 treatment. Although both SRA1 and SRA10 improved plant biomass and Ni concentration, resulting in increases in net Ni extraction of 76% and 122%, respectively, the large improvements in plant biomass alone, caused by SRA2, which exhibited the highest levels of IAA production, improved total Ni extraction by 388% (Ma et al. 2009).

The observation that improved plant growth alone can be more effective at improving phytoextraction than a combination of plant growth and metal mobilization reinforces the notion that attempts to improve plant growth, as opposed to plant heavy metal-concentrations, are likely to have a more significant impact on phytoremediation optimization in the immediate future. However, combinations of metal-mobilization and PGP activities that work synergistically have been reported and should also be considered.

4. Conclusions and Future Perspectives

Much is known regarding the avenues by which microorganisms are able to improve phytoextraction efficiency. From the perspective of the plant, microorganisms can improve phytoextraction by increasing plant biomass or by increasing the availability of heavy metals to the plant. Our assessment of 103 phytoextraction studies indicates that the employment of microbial mechanisms to improve plant biomass is more likely to lead to improvement of phytoextraction and that these outcomes occur more frequently in association with non-HMH plants. Closer inspection of the literature confirms that PGP microorganisms constitute a feasible strategy for improving phytoextraction. The use of microorganisms to improve plant biomass via improved N or P nutrition can have a significant positive impact on phytoextraction, even when heavy metal-concentration in the plant is unchanged.

Microbial processes that mobilize heavy metals may not be the most efficient strategy for improving phytoextraction on their own. However, there is substantial scope for research into the use of metal-mobilization processes in a synergistic fashion with plant-growth promotion to improve phytoextraction.

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Figures

Figure 1. Summary of the main outcomes across all microbial-mediated phytoextraction studies. Studies are subdivided into six classes based on the behavior of the response variables *plant biomass* (PB)* and *concentrations of heavy metal in plant tissues* ([HM]). The behavior of the response variables was classified as increased (↑), decreased (↓) or unchanged (nil-). Within each response, variable-category studies were subdivided based on whether microbial treatments were successful (blues) or unsuccessful (oranges) at improving the net amount of heavy metals extracted (HM_{net}) per plant (Abou-Shanab et al. 2006, Belimov et al. 2004, Dell'Amico et al. 2008, Gao et al. 2010, He et al. 2009, Jeong et al. 2013, Khan and Lee 2013, Lampis et al. 2015, Li et al. 2007, Liu et al. 2015, Ma et al. 2009, Ma et al. 2009, Ma et al. 2011, Ma et al. 2013, Malekzadeh et al. 2012, Marques et al. 2013, Płociniczak et al. 2013, Prapagdee et al. 2013,

Rajkumar and Freitas 2008, Rajkumar et al. 2013, Rani et al. 2013, Sheng et al. 2008, Sheng and Xia 2006, Wani and Khan 2013, Wani et al. 2007, Whiting et al. 2001, Yang et al. 2012, Zaidi et al. 2006, Zhang et al. 2012). *Wherever possible, dry plant biomass was used.

Figure 2. Association of increased heavy-metal extraction per plant (HM_{net}) with increases in plant biomass (PB; green), increases in concentrations of heavy metals in plant tissues ([HM]; blue) or both (overlap) for studies using heavy metal hyperaccumulating plants (HMH) and studies using non-hyperaccumulating plants (non-HMH).

Figure 3. Distribution of heavy metals accumulated by heavy metal hyperaccumulators and non-hyperaccumulators in microbial-augmented phytoextraction studies.

Table 1. Heavy metal hyperaccumulator (HMH) and non-HMH plant species used in phytoextraction studies included in the meta-analysis and the metals used in phytoextraction.

Plant species	**Family**	**Common name**	**Target metal**	**Reference**
Heavy metal hyperaccumulator				
Alyssum murale	Brassicaceae	Yellowtuft	Ni	Abou-Shanab et al, 2006
Alyssum serpyllifolium	Brassicaceae		Ni	Ma et al, 2011
Noccaea	Brassicaceae	Alpine penny-	Cd, Zn	Karimzadeh et al, 2012;

caerulescens		cress		Whiting et al, 2001
Pteris vittata	Pteridaceae	Chinese brake fern	As	Lampis et al, 2015; Yang et al, 2012
Sedum alfredii	Crassulaceae		Cd, Zn	Li et al, 2007; Zhang et al, 2012
Sedum plumbizincicola	Crassulaceae		Cd, Pb, Zn	Liu et al, 2014; Ma et al, 2013
Non-heavy metal hyperaccumulator				
Brassica juncea	Brassicaceae	Indian mustard	Ni, Cu	Rajkumar et al, 2013; Ma et al, 2011, Ma et al, 2009a; Zaidi et al, 2006
Brassica napus	Brassicaceae	Canola	Cd	Dell'Amico et al, 2008; Sheng et at, 2006; Sheng et at, 2008
*Brassica oxyrrhina**	Brassicaceae	Smooth-stemmed turnip	Ni	Ma et al. 2009a
Glycine max	Fabaceae	Soybean	Cu	Khan & Lee, 2013
Helianthus annuus	Asteraceae	Sunflower	Cd, Zn	Marques et al, 2013;

				Prapagdee et al, 2013
Hordeum vulgare	Poaceae	Barley	Cd, Pb	Belimov et al, 2004
Lens culinaris	Fabaceae	Lentil	Ni	Wani & Khan, 2013
Luffa cylindrica	Cucurbitaceae	Sponge gourd	Ni	Rajkumar et al, 2013
Lycopersicon esculentum	Solanaceae	Tomato	Cd, Pb	He et al, 2009; Sheng et al, 2008
Ricinus communis	Euphorbiaceae	Castor oil plant	Cu, Ni, Zn	Rajkumar & Freitas, 2008
Sinapis alba	Brassicaceae	White mustard	Cd, Cu, Zn	Plociniczak et al, 2013
Solanum nigrum	Solanaceae	Black nightshade	Cd	Gao et al, 2010
Sorghum halepense	Poaceae	Sorghum	Cd, Ni	Rajkumar et al, 2013; Sheng et at, 2008
*Thlaspi arvense**	Brassicaceae	Field penny cress	Zn	Whiting et al, 2001
Vigna radiata	Fabaceae	Mung bean	Cd, Ni, Zn	Rani et al, 2013; Wani et al, 2007
Zea mays	Poaceae	Corn	Cd	Malekzadeh et al, 2012; Sheng et at, 2008

**agricultural weed*

Figure captions:

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Lens culinaris	Fabaceae	Lentil	Ni	Wani & Khan, 2013
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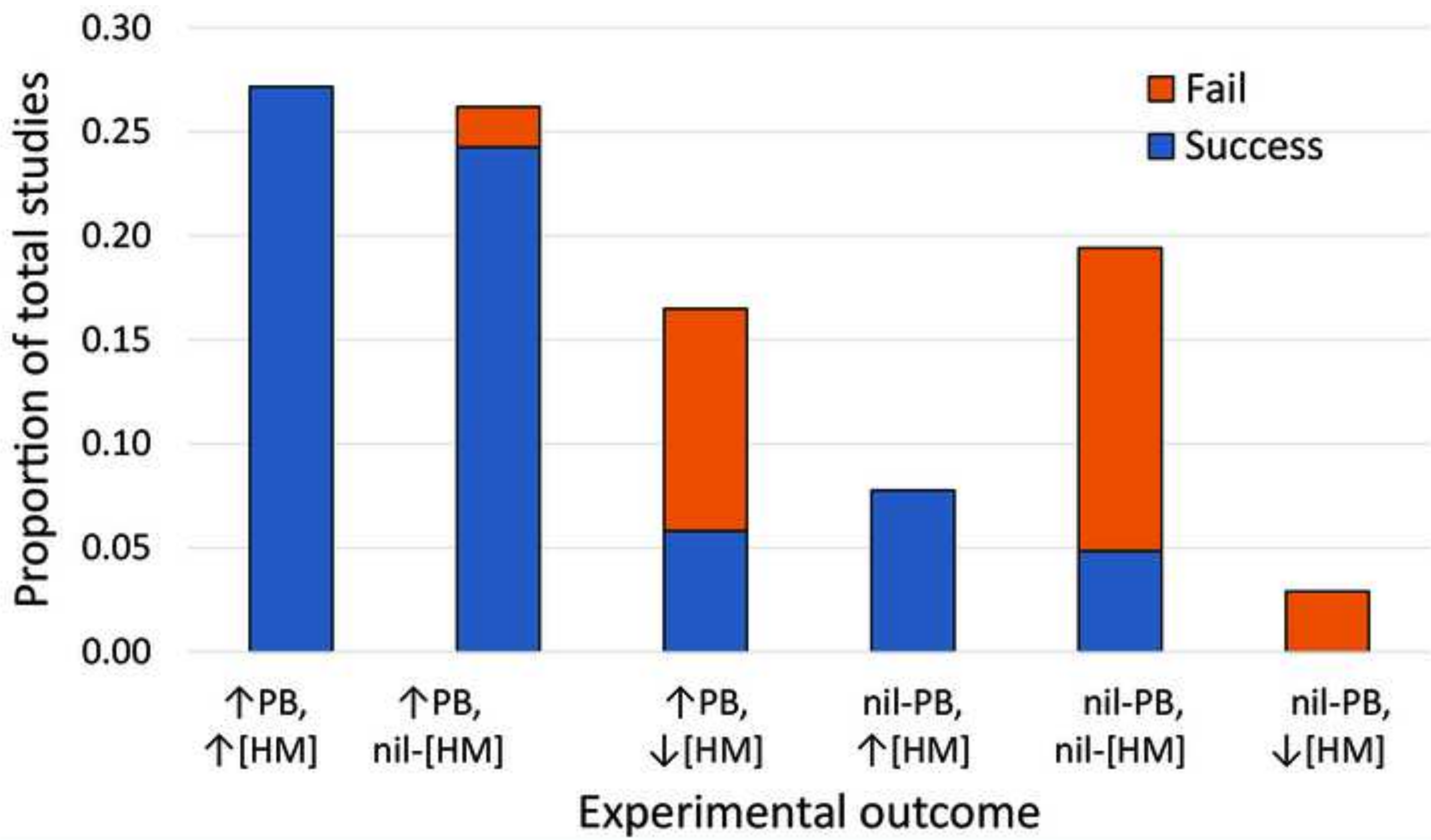
Sorghum halepense	Poaceae	Sorghum	Cd, Ni	Rajkumar et al, 2013; Sheng et at, 2008

*Thlaspi arvense**	Brassicaceae	Field penny cress	Zn	Whiting et al, 2001
Vigna radiata	Fabaceae	Mung bean	Cd, Ni, Zn	Rani et al, 2013; Wani et al, 2007
Zea mays	Poaceae	Corn	Cd	Malekzadeh et al, 2012; Sheng et at, 2008

**agricultural weed*

Figure1



0.30
0.25
0.20
0.15
0.10
0.05
0.00
Proportion of total studies
Fail
Success
↑PB, ↑[HM]
↑PB, nil-[HM]
↑PB, ↓[HM]
nil-PB, ↑[HM]
nil-PB, nil-[HM]
nil-PB, ↓[HM]
Experimental outcome

Figure2

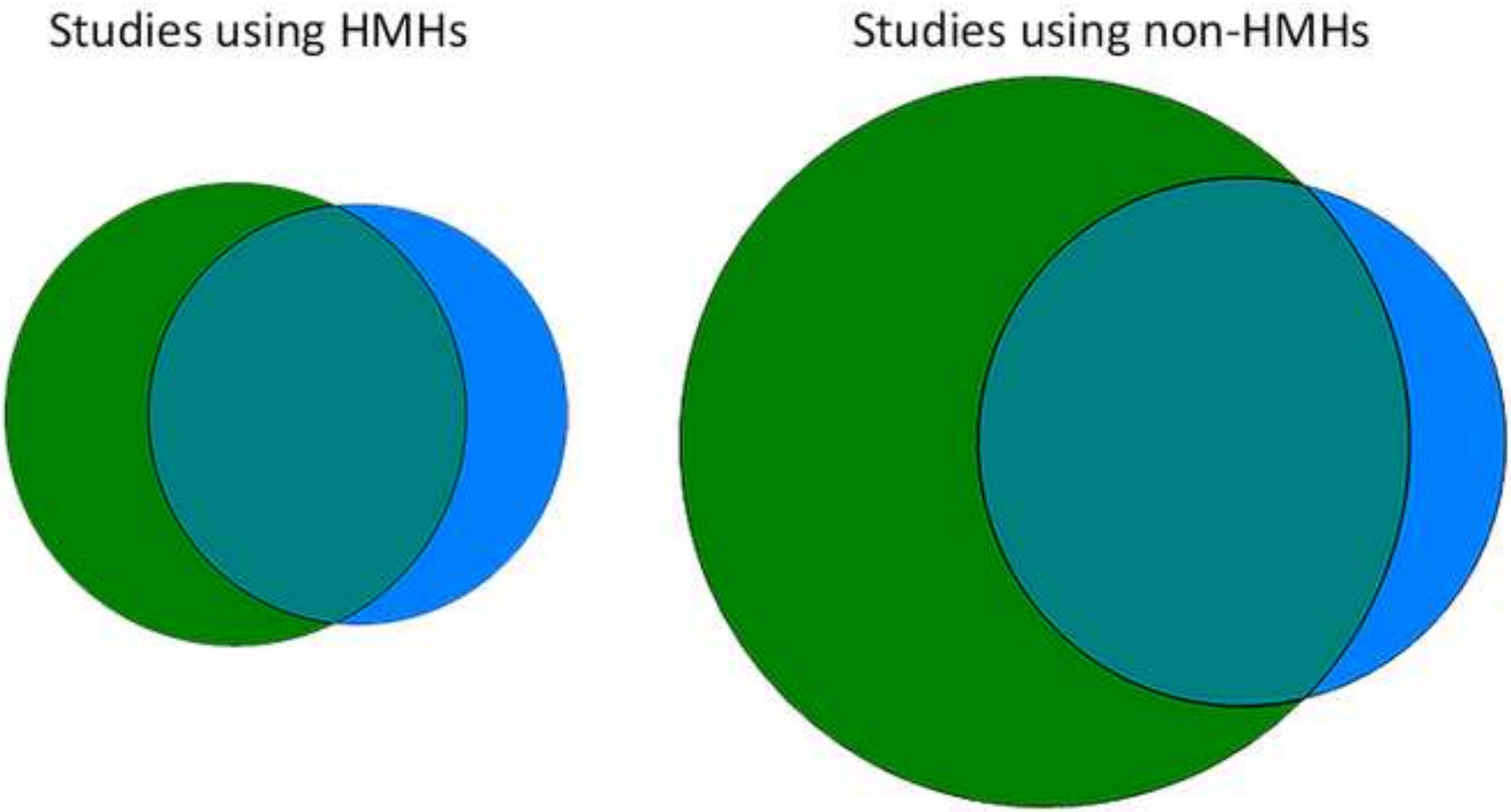
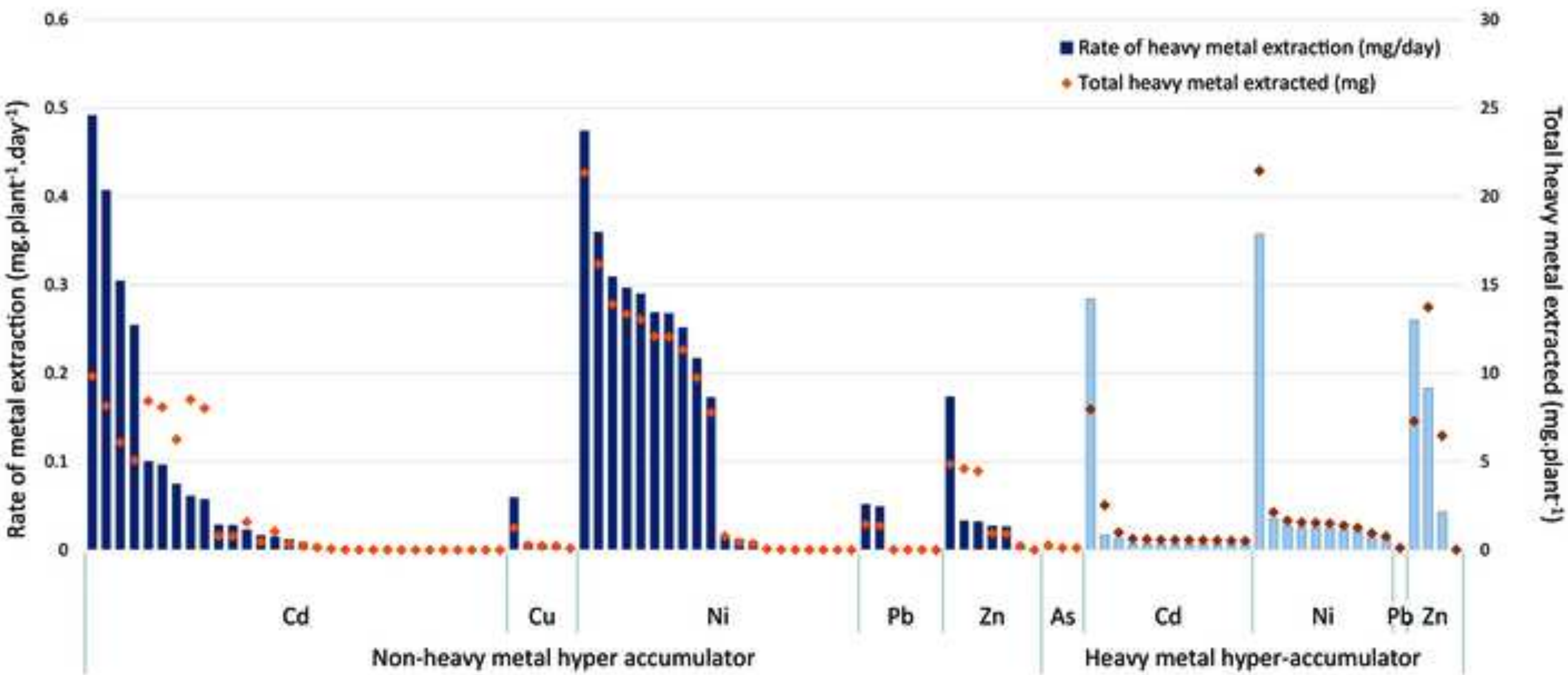


Figure3



Rate of heavy metal extraction (mg/day)
Total heavy metal extracted (mg)
Rate of metal extraction (mg.plant-1.day-1)
Total heavy metal extracted (mg.plant-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
30
25
20
15
10
5
0
Cd
Cu
Ni
Pb
Zn
As
Cd
Ni
Pb
Zn
Non-heavy metal hyper accumulator
Heavy metal hyper-accumulator