Heavy metal phytoremediation from a meta-analytical perspective.

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
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ABSTRACT

We conducted a literature survey and correlated heavy metal (HM) uptake and plant growth factors from published data to estimate the effectiveness of phytoextraction. The indicators of the actual plant HM uptake showed positive correlations with soil-HM concentrations, while the relative plant HM uptake showed negative correlations. Plant growth was negatively correlated with both the plant and soil-HM concentrations. These significant relationships were found for the majority of HM tested (e.g. Zn, Cd, Pb, Cu, Cr, and Fe) with a few exceptions (e.g. Ni, Co, and Mn). After fitting the correlation coefficients, the highest proportion of variance among the studies was mainly due to the experimental parameters or the plant species. When the metabolic costs of HM uptake are taken into account, the phytoextraction appears to be less effective beyond critical HM concentrations. Despite these constraints, it is emphasized that HM phytoextraction can play an important role in bioremediation.

“Capsule”: This meta-analytical approach has revealed a compromise between growth and HM uptake when plants are subjected to toxic soil-HM levels.

Key words: Bioconcentration factor; Specific extraction yield; Tolerance index.
1. **INTRODUCTION**

Phytoremediation is defined as the use of plants to remove pollutants from the environment (Cunningham et al. 1995; Salt et al. 1998). Inorganic pollutants, such as plant trace elements (e.g. Cr, Cu, Fe, Mn, Ni, and Zn) and non-essential elements (e.g. Cd, Co, and Pb), have been shown to be more difficult to remediate from contaminated soils as they cannot be degraded (Pilon-Smits 2005). Still, plants have been shown to take up and sequester heavy metals (HM) in roots and/or shoots and, therefore, to significantly contribute to their removal from the environment through a mechanism of phytoextraction. This mechanism occurs despite important growth challenges, including HM toxicity, changes to soil pH, and mineral imbalances.

For phytoextraction to be effective plants must take up HM from the soil, tolerate high plant or soil-HM levels, and produce sufficient harvestable biomass (Chaney et al. 1997; Meagher 2000; Pilon-Smits 2005; Salt et al. 1995, 1998). Using a meta-analytical approach, our objectives were (1) to describe the relationship between the levels of soil-HM and actual or relative plant HM uptake, (2) to describe the relationship between plant growth and plant HM uptake, and (3) to evaluate whether these relationships are the same for all the HM selected. Thus, we have tested the hypothesis that HM phytoextraction is an effective means of phytoremediation for highly polluted environments, using meta-analysis as a useful and meaningful approach for summarizing relationships from multiple studies (Lipsey and Wilson 2001). From the current body of phytoremediation literature, we have quantitatively evaluated the key plant physiological relationships influencing the effectiveness of HM phytoextraction and have detected, with high statistical power, broad-scale trends which have been underestimated by conventional or qualitative review analyses.
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 (1) the soil-HM concentration with actual or relative plant HM uptake and (2) the plant growth with actual plant HM uptake or soil-HM concentration, using combined results from multiple studies. The studies from which data were used have been selected through searching of library journal directories and online academic literature networks. In total, 36 articles were selected for having studied herbaceous plants and measured biomass and HM uptake, whether under laboratory or field conditions. All the HM with the soil concentration ranges and the 50 different plant species included in our study are appended (Supplementary Data). The distinguishing features required for inclusion in our analyses were that the soil mineral composition be described and the data be 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.

2.2. Metrics

Total plant HM content ($HM_{plant}$) and plant HM concentration ([HM$_{plant}$]) were used to measure the actual plant HM uptake. The specific extraction yield percentage (SEY$_{\%}$) and bioconcentration factor (BCF) were used to measure the plant HM uptake relative to the HM in soil. The SEY$_{\%}$, representing the percent ratio of plant HM content to soil-HM concentration ([HM$_{soil}$]) (adapted from Audet & Charest 2006), is defined as:

$$SEY_{\%} = \frac{HM_{plant}}{HM_{soil}}$$
The BCF, representing the ratio of plant HM concentration to soil-HM concentration (Dowdy and McKone 1997), is defined as:

\[
BCF = \frac{[HM_{plant}]}{[HM_{soil}]}
\]

Typically, the BCF is an indication of the magnification of contaminants from a lower to a higher trophic level (Newman and Unger 2003). For plants, the BCF has been used as a measure of HM accumulation efficiency, whereby values greater than 1 are an indication of potential HM phytohyperaccumulator species (Zhang et al. 2002). The tolerance index (TI), representing the ratio of biomass for plants grown in HM-soil to plants grown in non-HM control-soil for roots and shoots separately (Wilkins 1957, 1978), is defined as:

\[
TI = \frac{\text{biomass}_{HM}}{\text{biomass}_{control}}
\]

TI values lower than 1 indicate a net decrease in biomass and suggest that the plants are stressed, whereas TI values equal to 1 indicate no difference relative to non-HM control treatments. Also, TI values greater than 1 indicate a net increase in biomass and suggest that plants express a growth dilution effect.

2.3. Statistical analyses

The Pearson product-moment correlation test (Zar 1999) was used to calculate the significance and strength of the following correlations: between the total HM tissue content, HM tissue concentration, SEY%, BCF, or TI and soil-HM concentration, and between the TI and total HM tissue content or concentration. The correlation coefficients (r) were determined and fitted for variance attributable to differences in HM type, plant species, reference source, plant tissue type (either shoot or root), and study type (either field or laboratory study). This was done by adding these terms to the statistical model and solving for the coefficient of determination \(r^2\) (data not shown). The correlations were also calculated for each HM (e.g. Zn, Cd, Pb, Cu, Ni,
Cr, Co, Mn, and Fe) for all comparison variables representing the HM-specific correlation coefficients, with the exception of the comparisons involving the TI that did not show any significant HM-specific correlations (data not shown). The empty coefficient fields for the individual HM (Tables 2 and 3) indicate that the correlation model cannot be fitted with any variables since the data analyzed were taken from either one plant species, reference source, plant tissue type, or study type only. The Fisher correlation comparison test was used to compare coefficient values (Zar 1999). We applied logarithmic transformations to each of the variables in order to detect any linear relationship between the variables, and to meet normal distribution and homoscedasticity assumptions during analysis. We analyzed the residual rather than the raw values of SEY% and BCF in order to detect any trend underlying the negative relationship between [HMsoil] with SEY% or BCF. All of the p-values were determined using S-Plus® 7.0 (Insightful 2005).

3. RESULTS

The total plant HM content (Fig.1a) and plant HM concentration (Fig.1b) were plotted versus the soil-HM concentration; their correlation coefficients with [HMsoil] were 0.53 and 0.50, respectively (Table 1). The correlation coefficients for the total plant HM content and concentration were significantly increased once fitted for heavy metal type (0.65 and 0.61), and the strongest values were observed after fitting the reference source (0.75 and 0.73), or species type (0.76 and 0.82). The fitted coefficients for the study type showed no significant differences from the unfitted values. The HM-specific correlation coefficients for the total plant HM content and concentration were all significant and positive, with the exception of Cr and Mn showing no significant correlations (Table 2). Once fitted for reference source or species type, the strength of correlation significantly increased in all cases. Likewise, the HM-specific correlation
coefficients for total plant HM content and soil-HM concentration were all significant and positive, with the exception of Cr and Fe showing no significant correlations (Table 2). Again, the coefficients significantly increased in all cases once fitted for reference source and species type.

The residual values for the SEY\% (Fig.2a) and BCF (Fig.2b) were plotted against the soil-HM concentration; their correlation coefficients were -0.54 and -0.52, respectively (Table 3). The correlation coefficients for the SEY\% and BCF were significantly decreased once fitted for the HM type (-0.64 and -0.61), and the strongest once fitted for the reference source (-0.75 and -0.80) and the species type (-0.74 and -0.81). The fitted coefficients for the study type showed no significant differences from the unfitted values. The HM-specific correlation coefficients for residual SEY\% and residual BCF were mostly all significant and negative (Table 3). However, the SEY\% coefficients of Pb, Ni, Cr, and Mn indicated non-significant correlations, while Co showed a significant and positive correlation with soil-HM concentration. Furthermore, the BCF coefficients of Cr and Fe showed non-significant correlations, while Ni, Co, and Mn showed significant and positive correlations with soil-HM concentration. For the SEY\% and BCF correlations, the coefficients for nearly all the HM were the strongest once fitted for the reference source and species type.

The TI values plotted against the soil-HM concentration (Fig.3a), total plant HM content (Fig.3b), and plant HM concentration (Fig.3c) showed significant and negative correlations with soil-HM concentration (-0.21) or plant HM concentration (-0.19), whereas the soil-HM content showed no significant correlation (Table 4). All of these coefficients were significant and strongest once fitted for species type (-0.49, -0.44, and -0.49) and reference source (-0.55, -0.49, and -0.54). Only the correlation between plant HM content and TI showed non-significant coefficients when fitted for HM type, plant tissue type, or study type.
4. DISCUSSION

We observed that both plant HM content and concentration, which are indicators of actual plant HM uptake, strongly and significantly increase as soil-HM concentration increases; this trend being found for each individual HM studied. This is remarkable considering that some plants tolerate high tissue HM content or concentration, with levels as high as 325 mg [Ni] DM$^{-1}$ for *Alysum corsicum* (Li et al. 2003) and 125 000 mg [Pb] kg$^{-1}$ DM for *Raphanus sativus* (Chen et al. 2003a), while soil-HM concentration increased by approximately three to five orders of magnitude. In this regard, the predictive model for actual plant HM uptake from our meta-analytical results suggests that plants are able to accumulate heavy metals at higher soil-HM concentrations, and then an even greater potential for phytoremediation purposes than indicated in published reports so far. This is in agreement with the criterion that an effective HM phytoextraction requires that plants be increasingly tolerant to high plant-HM and soil-HM concentrations (Chaney et al. 1997; Meagher 2000; Pilon-Smits 2005; Salt et al. 1995, 1998).

On the other hand, both SEY$_\%$ and BCF, indicators of relative plant HM uptake, were shown to strongly and significantly decrease as soil-HM concentration increases; this trend being found for each HM studied, with few exceptions. Hence, HM phytoextraction was declining relative to increasing soil-HM concentration, even though the actual HM uptake was linearly increasing.

This response of decreased relative uptake is likely linked to the increased cost associated with tolerance to high plant HM levels, for example the cost of phytochelatin production or HM sequestration (Cobbett 2000; Maier et al. 2003; Wang et al. 2005). Furthermore, the decrease in relative plant HM uptake could also be the result of direct and/or indirect challenges such as HM toxicity causing plant poisoning, soil pH changes, and mineral imbalances (Foy et al. 1978; Marschner 1986), all of which similarly affecting the soil microbiota and their interactions with
plants (Giller et al. 1998; Hayman and Tavares 1985; Leyval et al. 1997; McGrath et al. 1995).

From our results, the predictive model for relative plant HM uptake suggests that HM phytoextraction becomes less effective as soil-HM concentration increases when the metabolic costs of HM uptake and sequestration are taken into consideration. This, therefore, challenges the aforementioned criteria for effective phytoextraction.

As for the individual HM studied, all share, at varying strengths, the general trend of positive correlation between plant HM content or concentration and soil-HM concentration, despite some non-significant correlations (e.g. Cr, Mn, and Fe with df < 28). In this case, non-significant correlations may be attributed to the small sample size or the narrow soil-HM concentrations less than one order of magnitude. For these reasons, the actual uptake correlation values for Cr, Mn, and Fe cannot be considered representative of any significant biological trends until more data are available. As for the correlations between residual values of SEY% or BCF with soil-HM concentration, all of the HM studied show negative correlations, with the exception of Ni, Co, and Mn. The non-significant correlations observed can be attributed to the effects of sample size or soil concentration range as previously stated, particularly for Co and Mn. However, any positive SEY% or BCF correlations imply that plants show higher relative uptake of Ni, Co, and Mn under increasing soil-HM concentrations, thus indicating their greater phytoremediation potential compared to the other HM studied. In this regard, the relative plant HM uptake may be affected by edaphic conditions, particularly soil-HM bioavailability (Walker et al. 2003). For example, a plant uptake likely increase as soil-pH becomes acidic, relating to increased HM bioavailability when colloidal sorption decreases (Apak 2002). Hence, plant HM levels may be higher when grown in more acidic soil conditions, then explaining differences in HM-specific uptake.
From our meta-analytical results, the TI values, which represent relative plant growth, decreased as the plant or soil-HM concentrations increased while there was no significant correlation between TI and total plant HM content. Accordingly, the potential for phytoextraction is likely affected by the rate of HM uptake rather than the level of HM tolerance, meaning that high biomass species may take up greater total HM content than low biomass species while possibly tolerating equal plant HM concentration. Nevertheless, we have provided evidence that plants subjected to soil-HM conditions are in the zone of nutrient toxicity and that any further HM uptake would eventually result in plant death; this being in agreement with the generalized relationship between plant growth and nutrient concentration (Epstein 1972). This is an indication that plants are becoming increasingly stressed, with their overall health declining under such soil-HM conditions. In our study, there is some incidence of increased plant growth under HM relative to non-HM conditions, yet this was not observed in conjunction with any decrease in plant HM concentration. Therefore, it may be interpreted that plants do not use any mechanism of growth dilution effect in tolerating soil-HM stress, a process in which the concentration of any compound decreases subsequent to its distribution in the growing biomass (Newman and Unger 2003). Instead, our findings strongly suggest a compromise with regard to plant resource allocation, this affecting plant capacity for HM uptake, tolerance, and growth under soil-HM conditions. In view of these metabolic costs, high biomass plants (e.g. Zea mays and Nicotiana sp.) have seldom been shown to take up higher HM concentration than some low biomass plants (e.g. Alysum corsicum and Raphanus sativus). Therefore, HM phytoextraction under increasing soil-HM concentrations would decline, and be limited to only better adapted or hyperaccumulator species (Xue et al. 2004; Yanai et al. 2006).

As for the fitted correlation coefficients, these values can also be interpreted as measures of variance between the different plant species chosen or the different experimental parameters.
(e.g. HM, plant tissue, or study type), as compared with the unfitted values. Notably, the
correlation values were significantly strongest once fitted for reference and plant species type;
therefore, the highest proportion of variance is attributable to the different methods or treatments
used in each study as well as the different plant species studied. It has been reported that
chelating agents enhance plant HM uptake (Blaylock et al. 1997; Chen et al. 2003b; Cui et al.
2004; Jiang and Yang 2004). It has also been shown that hyperaccumulators often tolerate and
take up higher HM levels compared to non-hyperaccumulator species (Delorme et al. 2001;
Marchiol et al. 2004; Shen et al. 2002). Moreover, the correlation values fitted for plant tissue or
study type were mostly not different from the unfitted values, and therefore account for only a
small proportion of the variance in our study. This has occurred even though plant HM uptake
levels were not necessarily the same in both shoots and roots for different plant species or HM
type (Chaney et al. 1997), and despite the fact that these plant HM levels differed between
laboratory and field conditions (Huang and Cunningham 1996).

5. CONCLUSION

Our meta-analytical study has quantified key relationships involving the physiology of
plants and the HM phytoextraction process. From these results, we should reject the assertion
that HM phytoextraction is an effective means of soil remediation under increasing soil-HM
levels. In this regard, it would be important to better understand the metabolic compromises in
plants between investing in HM tolerance and growth in a bioremediation perspective.

ACKNOWLEDGMENTS

The authors wish to thank the Editor-in-Chief and the two anonymous referees for this
publication. This research was funded by a grant from the Natural Sciences and Engineering
Research Council of Canada (NSERC) to Ch Ch.
REFERENCES


Figure 1. Plant HM content (mg DM$^{-1}$) (a) and concentration (mg kg$^{-1}$ DM) (b) in relation with soil-HM concentration (mg kg$^{-1}$ dry soil).

Figure 2. Residual SEY$\%$ (a) and residual BCF (b) in relation with soil-HM concentration (mg kg$^{-1}$ dry soil).

Figure 3. TI in relation with soil-HM concentration (mg kg$^{-1}$ dry soil) (a), plant HM content (mg DM$^{-1}$) (b), and plant HM concentration (mg kg$^{-1}$ DM) (c). The reference line indicates the TI value of 1.
Table 1. Correlation coefficients ($r$) for the plant HM content ($HM_{plant}$), plant HM concentration ([$HM_{plant}$]), residual specific extraction yield percentage (SEY$_{\%}$), and residual bioconcentration factor (BCF) in relation with the soil-HM concentration ([HM$_{soil}$]). The $r$ values for raw and fitted coefficients (heavy metal type, $r_{HM}$; reference, $r_r$; species, $r_s$; plant tissue type, $r_t$; study type, $r_{st}$) and degrees of freedom (df) are shown.

<table>
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<tr>
<th>Variables</th>
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<th>$r_{sp}$</th>
<th>$r_r$</th>
<th>$r_t$</th>
<th>$r_{st}$</th>
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<td>0.76$^a$</td>
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<td>0.55$^c$</td>
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<td>[$HM_{plant}$]</td>
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<tr>
<td>SEY$_{%}$</td>
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<td>-0.74$^a$</td>
<td>-0.55$^c$</td>
<td>-0.54$^c$</td>
</tr>
<tr>
<td>BCF</td>
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<td>-0.81$^a$</td>
<td>-0.52$^c$</td>
<td>-0.59$^c$</td>
</tr>
</tbody>
</table>

All p-values <0.01. Different letters within each row indicate significant differences between correlation coefficients according to Fisher’s comparison test at p<0.05.
Table 2. HM-specific correlation coefficients (r) for the plant HM content (HM$_{plant}$) and concentration ([HM$_{plant}$]) in relation with the soil-HM concentration (HM$_{soil}$). The r values for raw and fitted coefficients (reference, r$_r$; species, r$_s$; plant tissue type, r$_t$; study type, r$_st$) and degrees of freedom (df) are shown.

<table>
<thead>
<tr>
<th>HM</th>
<th>df</th>
<th>r</th>
<th>r$_r$</th>
<th>r$_s$</th>
<th>r$_t$</th>
<th>r$_st$</th>
<th>df</th>
<th>r</th>
<th>r$_r$</th>
<th>r$_s$</th>
<th>r$_t$</th>
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</table>

All p-values <0.01 except those indicated with ns, non-significant. Different letters within each row indicate significant differences between correlation coefficients according to Fisher’s comparison test at p<0.05. Empty coefficient fields indicate that the correlation model cannot be fitted with variables since the data analyzed were taken from either one plant species, reference source, plant tissue type, or study type only.
Table 3. HM-specific correlation coefficients for the residual specific extraction yield percentage (SEY $_{\%}$) and residual bioconcentration factor (BCF) in relation with the soil-HM concentration ([HM$_{\text{soil}}$]). The r values for raw and fitted coefficients (reference, $r_r$; species, $r_s$; plant tissue type, $r_t$; study type, $r_st$) and degrees of freedom (df) are shown.

<table>
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<tr>
<th>HM</th>
<th>SEY$_{%}$</th>
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<tr>
<td></td>
<td>df</td>
<td>r</td>
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<tr>
<td>Zn</td>
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<td>-0.73$^c$</td>
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<td>Cd</td>
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<td>Cu</td>
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All p-values <0.01 except those indicated with ns, non-significant. Different letters within each row indicate significant differences between correlation coefficients according to Fisher’s comparison test at p<0.05. Empty coefficient fields indicate that the correlation model cannot be fitted with variables since the data analyzed were taken from either one plant species, reference source, plant tissue type, or study type only.
Table 4. Correlation coefficients (r) for the soil-HM concentration ([HM\textsubscript{soil}]), plant HM content (HM\textsubscript{plant}), and plant HM concentration ([HM\textsubscript{plant}]) in relation with the TI. The r values for raw and fitted coefficients (heavy metal type, \(r_{HM} \); reference, \(r_{r} \); species, \(r_{s} \); plant tissue type, \(r_{t} \); study type, \(r_{st} \)) and degrees of freedom (df) are shown.

<table>
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<tr>
<th>Correlation comparison</th>
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<th>( r_{HM} )</th>
<th>( r_{sp} )</th>
<th>( r_{t} )</th>
<th>( r_{st} )</th>
</tr>
</thead>
<tbody>
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<td>-0.29\textsuperscript{b}</td>
<td>-0.49\textsuperscript{a}</td>
<td>-0.55\textsuperscript{a}</td>
<td>-0.27\textsuperscript{b}</td>
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<td>[HM\textsubscript{plant}]</td>
<td>220</td>
<td>-0.19\textsuperscript{b}</td>
<td>-0.32\textsuperscript{b}</td>
<td>-0.49\textsuperscript{a}</td>
<td>-0.54\textsuperscript{a}</td>
<td>-0.24\textsuperscript{b}</td>
</tr>
</tbody>
</table>

All p-values <0.01 except those indicated with ns, non-significant. Different letters within each row indicate significant differences between correlation coefficients according to Fisher’s comparison test at p<0.05.
### SUPPLEMENTARY DATA

Heavy metals and soil concentration ranges comprised in the meta-analysis.

<table>
<thead>
<tr>
<th>HM</th>
<th>soil HM range (mg kg(^{-1}) dry soil)</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Ni</td>
<td>5 - 2 570</td>
<td>Barazani et al. 2002; Citterio et al. 2003, 2005; Freeman et al. 2005; Gildon &amp; Tinker 1983; Marchiol et al. 2004a, b; McGrath et al. 1997; Li et al. 2003.</td>
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<tr>
<td>Cr</td>
<td>18 - 300</td>
<td>Citterio et al. 2003, 2005; Marchiol et al. 2004a, b.</td>
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<tr>
<td>Co</td>
<td>24 - 37</td>
<td>Li et al. 2003.</td>
</tr>
<tr>
<td>Species</td>
<td>Reference</td>
<td>Species</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>Pichtel et al. 2000</td>
<td><em>Pennisetum glaucum</em> x <em>P.</em></td>
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<tr>
<td><em>Aesculus glabra</em></td>
<td>Pichtel et al. 2000</td>
<td><em>Phytolacca acinosa</em></td>
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<td><em>Agrostis capillaris</em></td>
<td>Rydlova &amp; Vosatka 2003</td>
<td><em>P. pratense</em></td>
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<td><em>Allium cepa</em></td>
<td>Gildon &amp; Tinker 1983</td>
<td><em>Platanus occidentalis</em></td>
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<td><em>Alyssum corsicum</em></td>
<td>Li et al. 2003</td>
<td><em>Potentilla norvegica</em></td>
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<td><em>Ambrosia artemisiaefolia</em></td>
<td>Pichtel et al. 2000</td>
<td><em>Raphanus sativus</em></td>
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<td><em>Arabis gemmifera</em></td>
<td>Kubota &amp; Takenaka 2003</td>
<td><em>Sonchus oleraceus</em></td>
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<td><em>Brassica carinata</em></td>
<td>Marchiol et al. 2004a</td>
<td><em>Stenataphrum secundatum</em></td>
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<td><em>B. napus</em></td>
<td>Marchiol et al. 2004a, b</td>
<td><em>T. arvensis</em></td>
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<td>Pichtel et al. 2000</td>
<td><em>T. perfoliatum</em></td>
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<td>Ayoub et al. 2003</td>
<td><em>T. pratense</em></td>
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<td><em>Lepidium heterophyllum</em></td>
<td>Hutchinson et al. 2000</td>
<td><em>T. rosulare</em></td>
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<td>Pichtel et al. 2000</td>
<td><em>Trifolium pratense</em></td>
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<td>Pichtel et al. 2000</td>
<td><em>Triticum aestivum</em></td>
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<td><em>Vetiveria zizanoides</em></td>
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<td><em>N. rustica</em></td>
<td>Audet &amp; Charest 2006</td>
<td><em>Vigna radiata</em></td>
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<td><em>Paspalum notatum</em></td>
<td>Xia 2004</td>
<td><em>Zea mays</em></td>
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</tbody>
</table>
REFERENCES


Mereno, D.A., Villora, G., Hernández, J., Castilla, N., Romero, L., 2002 Accumulation of Zn,


