Assessing the Extraction Efficiency of CaCl$_2$ and Rhizon Extraction Methods after the Application of Organic Matter and CaCl$_2$ as Soil Amendments to Enhance the Mobility of Cd and Zn

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Abstract

A pot experiment was conducted to study the extractability of cadmium and zinc by CaCl$_2$ and rhizon extraction methods after the application of organic matter and chloride as soil amendments. Two methods, Rhizon and CaCl$_2$ extraction methods were concurrently employed to study the effects of the various amendments on the mobility of Cd and Zn. Both CaCl$_2$ and Rhizon extraction methods generally extracted appreciable amounts of the heavy metals after the application of the amendments. However, the results from the experiment shows that the Rhizon samplers extracted higher concentrations of both Cd and Zn as compared to the CaCl$_2$ extraction method. The use of Rhizon soil moisture sampler is also non destructive to the soil and makes it possible to ascertain levels of heavy metals at equilibrium in the soil solution without changing the composition of the soil solution in the process of extracting it. Assessment of the soil pH in the soil samples and the Rhizon extracts after the application of the amendments showed no significant difference with the control. Comparatively, application of CaCl$_2$ had a significant mobilizing effect on the mobility of both Cd and Zn as a result of the combined effect of complexation of Cd and Zn by the chloride anion, and by the fact that Cd and Zn are preferentially absorbed in cation exchange positions. Therefore the Ca$^{2+}$ ion can displace these metals into the soil solution.

Keywords: CaCl$_2$ and Rhizon extraction; Organic matter; Amendments; Extraction efficiency

Introduction

Heavy metal contaminations in soils have been on the ascendancy and the consequences on the ecosystem and human health cannot be left unattended to. In particular, excess Cd and Zn in soil have been the cause of human health, animal fatalities and the disruption of natural ecosystems [1].

There are various pools or fractions of heavy metals in the soil, and the different chemical forms in which the metals occur in the soil matrix influence the mobility of these heavy metals, as well as their availability and toxicity. To carry out such determinations, many approaches based on desorption or adsorption phenomena have been designed, but extraction procedures are the most widely accepted and used method in soil science [2].

Soil solution is the medium through which nutrients or minerals are made available for their uptake by plants. Tye et al. [3] reported that the relationship between the availability of heavy metals in the soil matrix, toxicological impacts on soil flora and fauna, and the potential transfer of heavy metals through the food chain have focused on the soil solution phase. The most labile soil fractions of heavy metals can be estimated by soil moisture sampling or the use of water extracts [4]. Soil moisture extraction offers the possibility of ascertaining levels of metals at equilibrium in the soil solution without altering its composition in the process of extracting it [4].

Rhizon soil moisture samples have been tried and tested in various researches, and they are known to extract soil moisture without having a toll on the soil moisture composition [4]. The rhizon soil moisture samplers have been designed in such a way that, they have a small porous filter which consists of a synthetic polymer with 0.1µm diameter, and this makes it possible to extract soil moisture which is devoid of solid or colloidal materials. The fraction of heavy metals found in the extracts is the water extractable metal, and it is the most labile soil fraction of heavy metals [4]. Deprotonation of H$^+$ from active functional groups in the soil matrix or from the hydroxyl groups located at the periphery of soil colloids as a result of an increase in pH creates negative charges on the soil colloids. The negative charges created have the ability to attract positively charged cations, including heavy metals which can undergo cation exchange reactions and can therefore replenish the soil solution. The electro positively charged metal ions absorbed by the negatively charged soil colloids are considered to be labile and therefore, they are available to be taken up by plants.

Therefore, in order to determine the cation exchange process which these metals undergo, many authors have proposed the use of 0.01 M CaCl$_2$ as an extraction reagent to determine the exchangeable metals in oven or air dried soil samples [5]. According to Houba et al. [6], 0.01M CaCl$_2$ which is used as an extraction reagent has ionic strength which is synonymous to that of the soil solution and that, Ca is the predominant cation in the soil solution. 0.01M CaCl$_2$ is unbuffered and therefore, the requisite reactions and interactions occur at the pH of the soil. The Ca$^{2+}$ ion from the reagent also provides additional benefits of improving

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the aggregate stability of the soil as the soil colloidal material in the suspension is coagulated. This extraction procedure (0.01M CaCl₂) is used in the Dutch legislation for the assessment of nutrients and heavy metals in soils [2].

The liquid to soil ratio used in this extraction protocol is 5:1, as proposed by Van Ranst [7]. The solution extracted contains the heavy metals and these metals in the extraction solution are the exchangeable metals.

The solubility and therefore the bioavailability of heavy metal ions vary widely because many factors influence their concentration in soil solution. The overall combination of these soil physico-chemical properties controls the rate and extent of metals bioavailability and their uptake by plants. In this study, two of the factors were critically considered to know their effect on the availability of Cd and Zn. These factors are chloride ions and organic matter.

Chloride ions are found in soils as a result of poor irrigation practices (agriculture) in which saline water is used in irrigation schedules [8], or through the application of sewage sludge on soils prone to salinity. Chloride ions are known to induce the availability of heavy metals [4]. For instance, chloride has the ability to form complexes with metals like Cd in the form of CdCl₂, CdCl₃. These complexes have a very high solubility which renders Cd readily available in the soil solution [4].

Decomposition of organic matter releases humic acids which have a significant impact on the availability of metals. Heavy metals availability is significantly influenced by the presence of humic substances of the native soil [9]. High molecular weight humic acids are known to strongly bind metals which render metals unavailable in the soil solution. On the other hand, low molecular weight humic acids have the ability to chelate metals and prevent their adsorption from solid surfaces, thus enhancing the availability of metals. Thus, low molecular weight humic acids are highly active in the soil environment and have a positive impact on the binding abilities for metals [10]. Humic acids strongly depend on pH according to the following reaction:

\[
R–COOH \leftrightarrow R–COO^- + H^+ \tag{1}
\]

Where, R is the carbon chain of organic matter.

This charge acquisition by organic matter arises as a result of ionization, where H⁺ is dissociated from or onto the active functional group [10], and the charge generated strongly depends on soil pH. The deprotonated form (R-COO⁻) which is negatively charged, abounds and predominates at neutral to high soil pH and has the tendency to adsorb metal cations. At pH 6-8, humic acids develop the greater surface area and heavy metal retention reaches its peak [11]. Also, at high soil pH values, phosphate, hydroxides and carbonate anions control the solubility of heavy metals. Metals are soft acids and for that matter, they are able to form organo-metallic complexes with carboxyls, alcohols and phenols.

Several extraction techniques exist in literature relative to the study of the mobilization pattern of heavy metals in soil solution. However, the most appropriate and scientifically proven single extraction protocol with higher extraction efficiency has not yet been arguably recommended by scientists in this regard. Also, the use of organic amendment as an alternative to synthetic chelates in enhancing the mobility of metals in soil solution is not very clear in phytoextraction studies. A pot experiment was therefore conducted to assess and compare the effect of organic and inorganic amendments on the mobility of heavy metals in soil solution. The study also aimed at comparing the extraction efficiencies of both CaCl₂ and rhizon extractions protocols, and scientifically recommend the extraction protocol that is not destructive, laborious and time consuming, and that has the propensity to extract higher concentrations of metals in solution. The research was aimed at studying the effect of organic matter and chloride based amendments on the mobility of cadmium and zinc.

The main objectives of this work were to study the effect of various soil amendments (inorganic and organic) as alternative techniques to mobilize Cd and Zn in contaminated soil, and to assess the efficiency of these amendments by the use of single extraction methods such as CaCl₂ and rhizon sampler.

Material and Methods

Study area

Soil samples were taken from Lommel in the Campine region of Belgium. The Universal Transverse Mercator coordinates of the sampled plot is 31 656535 E and 56 75647 N.

Soil sources and characterization

Soil samples were taken from Lommel in the Campine region of Belgium. The Universal Transverse Mercator coordinates of the sampled plot is 31 656535 E and 56 75647 N. Soil was collected from a depth of 0-30 cm and stored in plastic bags for transport to the laboratory. The soil was air-dried for several weeks (4 weeks). The dried soil was passed through a 2 mm sieve mesh, and stored for further research and experiments.

Field capacity was estimated by adding excess water to 400 g dry soil. The soil was assumed to be at field capacity when formation of further droplets at the base of the pot after free percolation had completely stopped. From the increase in weight, the field capacity was calculated. The total carbon content was also determined by adding a known excess quantity of sulphuric acid and back titrate the excess with sodium hydroxide as described by Van Ranst [7]. pH-water was determined by allowing 5 g of air-dried soil to equilibrate in 25 ml of deionised water for 24 hours and subsequently measuring the pH of the supernatant with a pH glass electrode (Model 520, Orion, Boston, MA, USA). The electrodes were calibrated with pH 4 and 7 [4].

Determination of soil extractable metals (Cd and Zn) using CaCl₂

For soil extractable metals (Cd and Zn), 25 ml of 0.01 M CaCl₂ were added to 5 g of air dried soil. This mixture was shaken for 2 hours on a mechanical shaker and filtered through a white ribbon filter (Macherey-Nagel, Germany, 640µm, Ø 125 mm, Cat No. 203 210). Heavy metal analysis was performed with the filtrate using inductively coupled plasma-optical emission spectrometry (ICP-OES: Varian vista MPC, Varian, Palo Alto, California, USA).

Determination of soil extractable metals (Cd and Zn) using Rhizon extraction

Soil solution extraction was performed by inserting the inert porous tubing of the Rhizons–MOM type (Eijkelkamp Agrisearch Equipment, Geisebeek the Netherlands). At the end of the soil moisture sampler, a Luo-Lock was inserted into a vacuum tube (vacuette) to provide the necessary suction to extract soil solution over the porous filter material (-0.1µm diameter). The soil solution extracted by the Rhizons was diluted for the determination of both Cd and Zn using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES: Varian Vista MPX, Varian, Palo Alto, California, USA). Also, the pH of the extracts was measured using the pH meter [5].
Experimental Set Up

Treatment preparation

Small pots were used for the phytoextraction experiment. Pots where filled with 450 g of dry weight soil, together with the amendment, and were brought to 60% field capacity. The various treatments together with their respective dosages code are given in table 1. Each treatment was performed in triplicate. All the treatments were transported to a greenhouse and were frequently watered and sampled for the determination of the mobility of Cd and Zn, as well as soil pH determined in the extracts.

Study on the mobility

Soil samples in the pots were treated with the amendments and the soil solution was extracted at regular intervals (twice a week) continuously for 8 weeks. The soil solution extracted with the Rhizons was diluted for the determination of Cd and Zn using ICP-OES. Also the pH of the Rhizon extracts was determined using a pH meter after calibrating the electrode with pH 4 and pH 7 buffer solutions.

It must be emphasized that each treatment was replicated 3 times. Once a week, a soil sample of 10 g was taken from each replicate of each of the treatment for CaCl₂-extraction. This sample was air dried and the mobile fraction was determined using a CaCl₂-extraction. The soil solution extracted was ready for the analysis of Cd and Zn. The pH of the soil sample was also determined.

Statistical analysis

Statistical analyses were performed with S-plus 8.0 for windows. Normality and equality of variance of the samples was tested using a Kolmorogov-Smirnov-test (for normality) and a Modified Levene test (equality of variance).

For each sampling time and each group of amendments an analysis of variance (ANOVA) with consequent and a Tukey test was performed to investigate differences between the amendments. Also for each amendment in time this analysis was performed to investigate significant differences of an amendment in time. The results were evaluated on the basis of homogeneous groups at a given significant level (p<0.05).

Organic Matter-based Amendment

Rhizon extraction

The application of the organic matter based amendments (5% compost, 10% compost and silo maize) had a significantly lower effect on the mobility of both Cd and Zn apparently due to their formation of insoluble organic complexes with Cd and Zn (Figure 1). The observed concentrations of the metals mobilized by these amendments is 1.0 ± 0.5 mg/kg for 5% compost, 1.0 ± 0.5 mg/kg for 10% compost and 1.0 ± 0.8 mg/kg for silo maize. This decreases the metals unavailable in the soil solution.

From the results, it was obvious that the application of the organic matter based treatments did not enhance the mobility of both Cd and Zn into the soil solution. This may apparently be attributed to the formation of insoluble organ metallic complexes, hence making the metals unavailable in the soil solution.

Evolution of pH in time

There was no significant decrease between the evolution of pH in time after the application of the organic matter based amendments and that of the control. This observation was found to be similar in both the soil samples and the Rhizon extracts (Figure 2). During the decomposition of organic matter, H⁺ ions are released into the soil from the active functional groups which may result in a decrease in soil pH [10]. Contrary to this view, soil pH did not decrease following the application of the organic materials. Mc Cauley et al. [12] affirmed that, degradation of organic matter increases the cation exchange capacity, hence increasing the buffering capacity of the soil. The high buffering capacity of the soil resulting from the degradation of organic matter leads to an increase in pH as a result of consumption of H⁺ and liberation of OH⁻ and CO₃⁻[13]. Therefore the lack of effect of the organic materials on soil pH may probably be attributed to the findings made by Mc Cauley et al. and Gramss et al. [12,13].

CaCl₂ extraction

The effects of organic matter-based amendments namely, 5% compost, 10% compost and silo maize were assessed with respect to the mobility of Cd and Zn as a function of time. The range Cd concentrations mobilized by these amendments is 1.0 ± 0.5 mg/kg for 5% compost, 1.0 ± 0.5 mg/kg for 10% compost and 1.0 ± 0.8 mg/kg for silo maize. The concentrations of Cd mobilized by the various amendments were compared to that of the control (1.5 ± 1.0 mg Cd/kg soil) (Figure 3).
Similarly, the concentrations of Zn mobilized by the amendments (Figure 4) were found to be 48 ± 13 mg/kg for 5% compost, 48 ± 9 mg/kg for 10% compost and 48 ± 12 mg/kg for silo maize. The concentrations of Zn mobilized by these amendments over time were concurrently compared with that of the control (59 ± 14 mg Zn/kg). The concentrations of Zn mobilized by the application of the amendments (5% compost, 10% compost and silo maize) were significantly lower than that of the control. This observation may be attributed to the formation of insoluble organo-metallic complex, hence rendering the metal non-available in the soil solution.

**Application of the Chloride based Amendment**

**Rhizon extraction**

CaCl₂ dry and CaCl₂ liquid were applied as treatments to enhance the mobility of both Cd and Zn. Soil solution concentrations of the metals mobilized by the chloride salts were found to be in the range of (9.5 ± 2.1 mg Cd/kg, 161 ± 46 mg Zn/kg) for CaCl₂ powder and (8.1 ± 2.6 mg Cd/kg, 245 ± 32 mg Zn/kg) for adding CaCl₂ liquid (Figure 5).

The application of CaCl₂ powder and CaCl₂ liquid had significant effect on the mobility of Cd and Zn into the soil solution. Both CaCl₂ liquid and CaCl₂ powder reached their peak with regard to the mobility of Cd on the 5th day (week 1). With respect to the mobility of Zn after the application of CaCl₂ dry and CaCl₂ liquid, the critical periods at which both CaCl₂ liquid and CaCl₂ dry mobilized the highest concentration of Zn into the soil solution are 27th day (week 4) and 43rd day (week 6) respectively. The concentrations of both Cd and Zn mobilized by the chloride based treatments were significantly higher than that of the control.

**pH evolution in time**

There was no significant difference between the effect of the chloride based amendments on soil pH and that of the control in both the soil samples and the Rhizon extracts (Figure 6).

**CaCl₂ extraction**

The application of CaCl₂ dry and CaCl₂ liquid was employed to ascertain the mobilising effects of the form of CaCl₂ on the mobility of Cd and Zn. The range of concentrations of Cd mobilised by CaCl₂ powder was found to be 5.0 ± 1.0 mg/kg and 2.0 ± 1.4 mg/kg for CaCl₂ liquid (Figure 7). The range of concentrations of Zn mobilised by CaCl₂ dry was found to be 89 ± 48 mg/kg and 173 ± 59 mg/kg for CaCl₂ liquid. CaCl₂ liquid mobilised the highest concentration of Zn with the highest concentration of 1735 mg Zn/kg at week 4. However, the mobilising effect of CaCl₂ liquid dropped relative to CaCl₂ powder at week 6, whilst CaCl₂ powder also attained its highest effect on the mobility of Zn at week 6. Between week 7 and week 8, there was no significant difference (at 0.05 level) between CaCl₂ liquid, CaCl₂ powder and the control.

**Organic matter-based amendment**

From the results, it was explicitly manifested that the concentrations of Cd and Zn mobilised into the soil solution after the application of organic matter (5% compost, 10% compost and silo maize) were significantly lower than that of the control. The significantly lower amount of Cd and Zn in the soil by the organic ligands present in the organic materials, leading to the formation of insoluble organo-metallic complex, hence rendering them unavailable in the soil solution. Similar observation was made by Chani et al., Karaca [14], also observed that high organic matter content decreased the concentrations of Cd and Zn in the soil.
The effect is apparently explained by the ability of the organic matter to form insoluble chelate complexes with Cd and Zn. The relatively lower concentrations of Cd and Zn mobilised by the organic materials can also be ascribed probably to the increase in the Cation Exchange Capacity of the soil, resulting in the creation of sorption sites which have a high binding affinity for Cd and Zn, thereby reducing their respective concentrations in the soil solution. The amount of organic matter present in the soil has a significant effect on the binding of metals to the sorption sites. From the experiment, it was realized that 10% compost mobilised the least concentrations of Cd and Zn apparently due to the high organic matter content, with its resultant effect in increasing the CEC which resulted in the sorption of an appreciable amount of Cd and Zn to the sorption sites (Table 2).

Soil pH is considered to be a preponderant factor affecting the mobility and bioavailability of metals. However, in this study, the effect of pH on the mobility of metals after the application of the organic materials was not clearly manifested, and was therefore considered as a less important factor than the presence, amount and the nature of organic matter. This observation was in accordance with the findings of Sauve et al. [15], who also made similar observations.

From this study, it can be said that the application of the organic materials (5% compost, 10% compost and silo maize) did not enhance the mobility of Cd and Zn after the application of chloride base amendments.

**Table 2:** General properties of the soil in the study area.

<table>
<thead>
<tr>
<th>Property</th>
<th>EC (electric conductivity) (μS/cm)</th>
<th>pH-H₂O</th>
<th>CaCO₃ (%)</th>
<th>OM (%)</th>
<th>CEC (cmol (+)/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 ± 3.00</td>
<td>6.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.67 ± 0.06</td>
<td>2.50 ± 0.20</td>
</tr>
</tbody>
</table>

Intervals denote standard deviation (n=3)
the mobility of Cd and Zn. This observation is emanating from the fact, that the concentrations of Cd and Zn mobilized by these organic materials were statistically tested (at 0.05 level) to be lower than the control. It was also observed that organic matter content lowered the bioavailability of Cd and Zn in the soil solution. Karaca [14], found a negative correlation (r=0.950, p<0.01) between organic matter content and extractable Cd. It therefore implies that, the higher the organic matter content, the lower the concentration of Cd extracted from the soil solution, and vice versa. It can therefore be said that the application of organic matter results in low metal concentrations in the soil solution. Similar observation was made by Kashem and Singh [16], who found out that soluble metal contents in soils treated with organic matter lowered. Li et al. [17], related the availability of Cd in the soil directly to soil organic matter, affirming that plant Cd uptake increased with an increasing ratio Cd/organic C. This statement confirms the fact that the higher the organic matter content, the lower the ratio, and hence the lower the amount of Cd in the soil solution to be taken up by plants. The effect of compost and silo maize with respect to the mobility of Cd and Zn in phytoextraction exercises can not be predicted with certainty due to the poorly quantifiable heterogeneous effects of these organic materials.

**Chloride-based amendment**

It is explicit that the mobility of Cd by CaCl₂ dry was high during week one after the application of the amendment (CaCl₂ dry), and its mobilizing effect decreased appreciably with time. After week one, there was no significant difference (at 0.05 level) between CaCl₂ dry and the control till the end of the 8 weeks of the experiment as far as Cd mobility was concerned. Meers et al. [18], observed an increase in Cd uptake in the shoot of Zea mays after application of 2 mmol/kg CaCl₂ on contaminated dredged sediments. Although CaCl₂ dry mobilized the highest concentration of Cd as compared to CaCl₂ liquid week 1, the effect of CaCl₂ liquid on the mobility of Cd prevailed and became significantly higher as compared to CaCl₂ dry and the control between week 4 and week 6. After week 6, no difference could be found between the effects of CaCl₂ dry, CaCl₂ liquid and the control on the mobility of Cd into the soil solution.

Application of CaCl₂ had a significant mobilizing effect on the mobility of both Cd and Zn as a result of the combined effect of complexation of Cd and Zn by the chloride anion, and by the fact that Cd and Zn are preferentially absorbed in cation exchange positions. Therefore the Ca²⁺ ion can displace these metals into the soil solution [4].

Studies by Hahne and Krootje [19], comment on the enhancing effect of chloride on the uptake of heavy metals like Cd, Zn and Pb, as a result of the ability of these heavy metals to form soluble chloro complexes with chlorides.

**Extraction efficiency between rhizorn and CaCl₂ extraction methods in studying the mobility of Cd and Zn**

The heavy metal extraction efficiency obtained with both the CaCl₂ and Rhizorn extraction methods can be compared and contrasted from the results obtained in each case. The use of calcium chloride as an extraction solution in study the mobilization pattern of metals in soil solution has proved to be successful in this study. It can be said that the calcium chloride extraction method extracted an appreciable amount of Cd and Zn into the soil solution. This is apparently due to the combined effect of complexation by chloride and by the fact that Cd and Zn are normally absorbed in cation-exchange positions and, therefore, the divalent cation Ca²⁺ can remove these metals more easily and make them available in the soil solution. The only setback identified with the use of calcium chloride extraction method is that it is time consuming and quite laborious.

On the other hand, soil moisture extraction offers the possibility of ascertaining levels of metals at equilibrium in the soil solution without changing the composition of the soil solution in the extraction process [4]. Generally, the extractability of Cd and Zn with the rhizorn extraction method in this study was higher than with the calcium chloride extraction method. However, the critical periods at which the various treatments significantly enhanced the mobility of Cd and Zn into the soil solution after the application of each treatment were almost the same in both methods. The setback identified with the rhizorn extraction method was the irregularities with respect to the pattern of the mobility of the metals by the various amendments used in the pot experiment. Nonetheless, it is not time consuming and it is easier to use to study the mobility of metals with time, as compared to the calcium chloride extraction method. Arguably, it can be said that the use of rhizorn extraction method is more promising to be the most suitable for heavy metal assessment in comparison with CaCl₂ and other extraction protocols.

**Differences in pH measurement in the rhizons and in a soil sample**

The soil pH measured with both methods (soil sample extracts and Rhizorn extracts) followed the same pattern of evolution with time. Virtually there was no difference between the pH measurement in the Rhizorns and in the soil sample. Assessment of the soil pH in the soil samples and the Rhizorn extracts after the application of the amendments showed no significant difference with the control.

**Conclusion**

The application of the organic matter based amendments inhibited the mobility of both Cd and Zn apparently due to the formation of insoluble organo-metallic complexes, hence making the metals unavailable in the soil solution. The heterogeneous effect of organic matter based amendments makes it uncertain to be used for heavy metal mobilization purposes. Organic materials are therefore not suitable for the enhancement of heavy metal mobilization in the soil solution for onward phytoextraction, and will not be the next best economic alternative to synthetic chelating agents.

Application of the chloride salt treatments (CaCl₂) had a significant mobilizing effect on the mobility of both Cd and Zn as a result of the combined effect of complexation of Cd and Zn by the chloride anion, and by the fact that Cd and Zn are preferentially absorbed in cation exchange positions. Therefore, the Ca²⁺ ion can dissipate these metals into the soil solution, and hence make them available.

Though the 0.01M CaCl₂ extraction procedure seems to be suitable for the extraction of Cd and Zn since it uses the lowest salt concentration, the use of rhizorn extraction procedure is also very promising as it provides the possibility of studying levels of metals at equilibrium in the soil solution without changing the composition of the soil solution [4].

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References

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