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# REMEDIAL MEASURES FOR IMMOBOLIZATION OF HEAVY METALS FROM CONTAMINATED SOIL

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## ABSTRACT

To understand the remedial measures of heavy metals from contaminated soil, three vegetable (*Brassica juncea, Brassica rapa* and *Lepidium sativum*) were grown on soil artificially contaminated with 300 mg $kg^{-1}$ $CuCl_2$, 500 mg $kg^{-1}$ $Pb(NO_3)_2$, 800 mg $kg^{-1}$ $ZnCl_2$ or their mixed metal (1600 mg $kg^{-1}$). This experiment showed that Cu and Pb accumulation in vegetables are higher in lime treatments than in 20% cowdung treatments. Zinc accumulation increased in *B. rapa* and *L. sativum* in both cow dung and lime treatments compared to control. Accumulation of Cu, Pb and Zn from mixed metal treatment was highest in *L. sativum* (at lime 9 g $kg^{-1}$ for Cu and Pb, and 20% cow dung for Zn). Morphological changes such as fresh weight, dry weight, shoot length, root length, mostly increased significantly ($P \leq 0.01$) with Zn and cow dung treatment, but none with lime treatment. Fresh and dry weight increased only in *L. sativum* grown in 3 g $kg^{-1}$ lime treatment. Immobilization of Cu, Pb and Zn in both single and mixed treatments was found to be high in cow dung amended soil. From this it can be ascertained that 20% cow dung treatment is suitable for immobilization of supplied heavy metals than lime treatments.

**Key words**: Vegetables, heavy metals, remedial measures.

## INTRODUCTION

The continuous increase of non-degradable toxic heavy metals in the environment emitted via anthropogenic activities like emissions, household waste, sewage sludge, pesticides and fertilizers are major global problems (Nriagu and Pacyna 1988, Goyer 1997, Sharma and Chettri 2005). These ultimately contribute to the deposition of heavy metals on soil of urban and sub urban area and may create dangerous situation on human health. From the contaminated soil heavy metals can enter in human body directly trough inhalation of dusty air of the city (EPA 1976) and ingestion of food, i.e. through edible plants/animals coming from such contaminated fields. Heavy metal contaminated soils reduce plant species richness (Whitton 1970, McLean 1975, Kuiper 1981, Rygg 1985) and increase biologically inactive land via destroying soil quality (McGrath *et al.* 2001).

Although various phytoextraction works by using plants have been conducted to reduce heavy metal content from contaminated soils (Baker 1981, Legittimo *et al.* 1995, Ebbs and Kocchian 1998), Batch and column tests for the development of an immobilization technology was studied for toxic heavy metals (Cu, Ni and Pb) in contaminated soils of closed mines (Jang *et al.* 1998).

From the study of biomonitoring of heavy metals in vegetables and their dietary intake estimation via vegetables (Sharma and Chettri 2004, 2005), it is observed that there is high accumulation of heavy metals (Pb and Cd) in soil and vegetables of Kathmandu valley as compared to normal plant value of Bowen, 1979. Heavy metal contaminated soil will certainly hamper production of vegetables and will invite toxicological problems among the human and animal health through food chain. Therefore, to reduce this problem, a suitable approach has been investigated in the present study.

The availability of heavy metal present in soil can be changed into the insoluble and benign forms (such as hydroxides and sulphides compounds) (Arocha *et al.* 1996). In agricultural fields, soil pH decreases with the use of chemical fertilizers like ammonium sulfate and diammonium phosphates, urea, etc (Brady and Well 2004). Therefore, in the present study some easily available and eco-friendly, organic material like cow dung and inorganic materials like agricultural lime were selected. It is hypothesized that organic matter like cow-dung in the soil will increase organic acids (like humic acid and fulvic acids) and proteins which will provide binding sites to excess heavy metal ions in the soil and this will reduce heavy metal accumulation in vegetable crops. Similarly, inorganic material agricultural lime was selected because the acidic soil is neutralized by it. Generally, toxic metals are available to plants under low pH condition, but addition of agricultural lime will increase soil pH to neutral or slightly alkaline condition. Therefore, It is hypothesized that adding agricultural lime will reduce heavy metal accumulation in vegetable crops.

## MATERIALS AND METHODS

Sandy loam soil (3.5 kg per bag) were artificially contaminated each with 300 mg $kg^{-1}$ $CuCl_2$ / 500 mg $kg^{-1}$ $Pb(NO_3)_2$ / 800 mg $kg^{-1}$ $ZnCl_2$ / or their mixed concentration (=1600 mg $kg^{-1}$ soil). All experiments were conducted in polyethylene bags and were designed in such a manner to understand the accumulation of Cu, Pb and Zn in vegetables after the uses of (I) cow dung as organic manure and (II) lime as inorganic treatments. All treatments were in triplicates and were arranged in randomized block design. The seeds of *B. juncea L.* (Broad leaf mustard), *B. rapa L.* (Turnip) and *L. sativum* L. (Pepper Cress) were collected from reliable seed store - Annapurna Biz Bhandar, Ason, Kathmandu. Artificially heavy metal contaminated soil were amended with cow-dung (5 and 20%) or agricultural lime (3 g and 9 g $kg^{-1}$ soil) and control for each treatment was set without cow-dung or lime treatment. Vegetable crops grown were harvested after 5 weeks. The harvested crops were cleaned thoroughly with tap water and finally washed with de-ionized water. Fresh weight (FW) was measured for individual plants (after half hour of washing) which were then oven dried at temperature of $60^0$-$70^0$ C for 48 h to 72 h to obtain the constant dry weight (DW) after air dried. Root length (RL) and SL were measured to study the effect of cow dung or lime. Ultimately a representative samples were prepared for the digestion process. Metal analysis of the representative samples were done after wet digestion according to Chettri *et al.* (1997). Metal (Cu, Pb and Zn) content in the filtrate was analyzed using Atomic Absorption Spectrometry (AAS) with their respective wavelength (Welz 1985). For each treatment, triplicate samples were tested. Two plant materials of NBS standards (National Bureau of Standards, USA) with Nos. 1573 (Tomato leaves) and 1575 (Pine needles) were also analyzed, following the same procedure and the metal recoveries ranged from 94 to 99 percent. Each soil sample was also analyzed for CU, Pb and Zn, following the same procedure. To understand the significant difference in growth parameters like FW, DW, DW% RL and SL, after growing on soil contaminated with single and mixed metals amended with cow dung or lime, data were statistically analyzed using One-way Anova followed by Duncan's multiple range test (with the help of SPSS 12.0 version).

## RESULTS

Accumulation of Cu, Pb and Zn in *B. juncea*, *L. sativum* and *B. rapa*, tested for their remedial measures by using cow dung and lime are presented in Fig. 1 A-C (from single metal treatments) and in Fig. 2 (from mixed metal treatments). Copper accumulation in *B. juncea*, *L. sativum* and *B. rapa* (grown on 300 mg $CuCl_2$ $kg^{-1}$ soil) was higher in lime (both 3 and 9 g $kg^{-1}$ treatments) than in cow-dung treatments. *B. juncea* and *B. rapa* showed highest accumulation of Cu in low dose of lime (3 g $kg^{-1}$), but *L. sativum* showed high accumulation in high dose of lime (9 g $kg^{-1}$). Lowest accumulation of Cu was observed at 20% cow-dung in all vegetables (Fig. 1A). Lead accumulation (from 500 mg $Pb(NO_3)_2$ $kg^{-1}$ soil) in *L. sativum* was significantly high in 9 g lime treatment than in both control and 3 g lime treatment (Fig. 1B). Lead accumulation in *B. rapa* also increased slightly in both 3 and 9 g $kg^{-1}$ lime treatments. No significant changes have been observed in *B. juncea* treated with both doses of lime than in control. Lowest accumulation of Pb was recorded in all vegetables grown on soil amended with 20% cow dung (Fig. 1B). Zinc accumulation (from 800 mg $ZnCl_2$ $kg^{-1}$ soil) in *B. rapa* and *L. sativum* was increased in both cow-dung and lime treatment than in control, but in *B. juncea* it decreased in both treatments than in control (Fig. 1C).

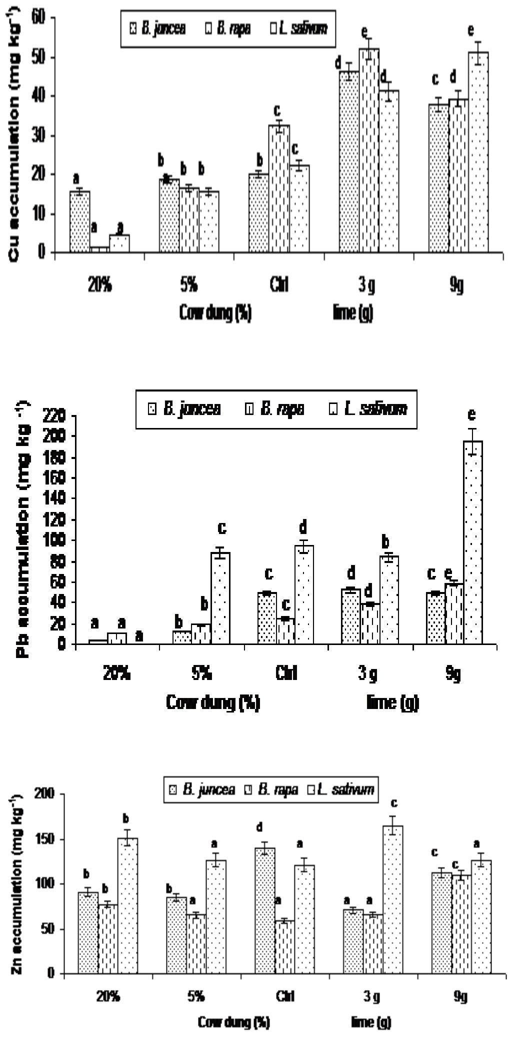


**Fig. 1 A-C. Accumulation of Cu, Pb and Zn in vegetables grown on soil treated with $CuCl_2$ $Pb(NO_3)_2$ and $ZnCl_2$ respectively; and cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

Accumulation of Cu, Pb and Zn (from mixed metal 1600 (Cu+Pb+Zn) mg $kg^{-1}$ soil) was highest in 3 g lime in *B. juncea* than in control or other treatments (Fig. 2 A-C). Lowest accumulation of Pb was observed in *B. juncea* and *L. sativum* grown on 5 and 20% cow-dung. Zn accumulation in *L. sativum* was quite high in 20% cow-dung treatment. Accumulation of Pb and Zn in *B. rapa* were mostly reduced in cow dung treatment compared to control but the plant could not grow in lime treated soil.

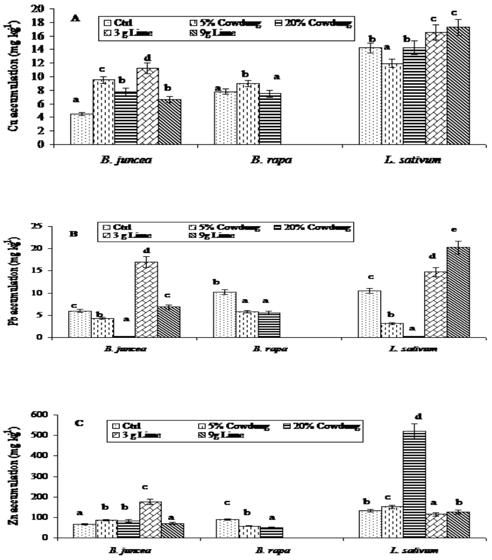

**Fig. 2 A-C. Accumulation of Cu (A), Pb (B) and Zn (C) in vegetable crops grown on soil treated with mixed metals ($CuCl_2$+ $Pb(NO_3)_2$ +$ZnCl_2$) along with cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

Morphological parameters such as FW, RL and SL increased in all tested vegetable grown on $CuCl_2$ treated and cow-dung amended soil but FW, DW, RL and SL decreased in all the cases of $CuCl_2$ treated and lime amended soil (Fig. 3A).

Fresh weight and SL increased significantly ($P<0.05$) in *B. juncea* and *B. rapa*, and RL increased significantly ($P<0.05$) in *L. sativum* grown on 20% cow-dung amended and Pb treated soil. Shoot length, FW, DW decreased in all grown on Pb-treated and lime amended soil (Figs. 3B)

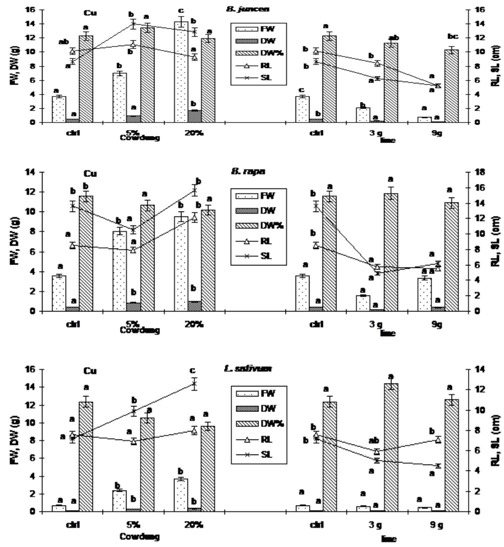

**Fig. 3A. Fresh weight, DW, DW%, RL and SL of the vegetables grown on soil treated with $CuCl_2$ and cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

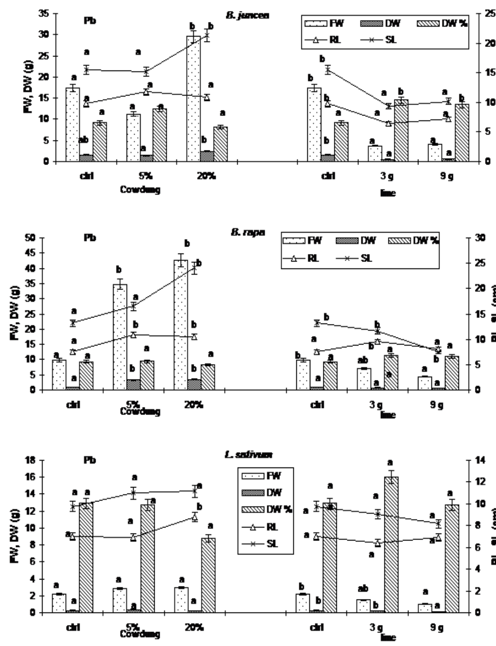

**Fig. 3B. Fresh weight, DW, DW%, RL and SL of the vegetables grown on soil treated with $Pb(NO_3)_2$ and cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

Fresh weight, DW, SL and RL (except RL in *B. rapa*) increased significantly ($P < 0.01$) in all vegetables grown on cow-dung amended and Zn treated soil, but no significant changes were observed with lime treatments (Figs. 3C). Fresh weight and DW increased only in *L. sativum* grown in 3 g lime treatment, whereas DW% increased significantly in *B. rapa* of both the doses of lime.

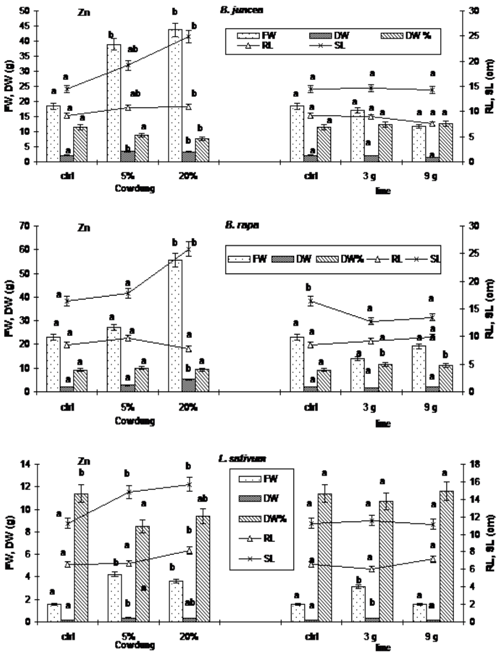


**Fig. 3C. Fresh weight, DW, DW%, RL and SL of the vegetables grown on soil treated with $ZnCl_2$ and cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

In vegetables grown on mixed metal treated soil and amended with cow-dung, SL and FW in *B. juncea* increased significantly; FW and DW in *L. sativa* increased significantly; and SL, RL, FW and DW in *B. rapa*, increased significantly (Fig. 3D). Vegetables grown on mixed metal and lime amended soil showed changes in RL, FW and DW, all of which decreased significantly but DW% increased significantly than in control. In *L. sativum*, only DW% increased significantly in lime amended conditions. *Brassica rapa* could not grow in lime amended soil.

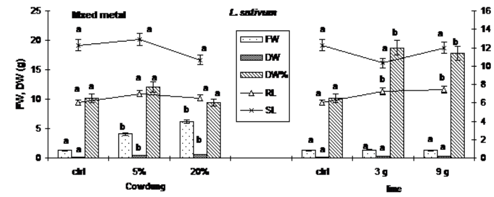


**Fig. 3D. Fresh weight, DW, DW%, RL and SL of the vegetables grown on soil treated with mixed metals and cow-dung or lime. Different letters on the bar of each treatment of a crop denote significant differences obtained from ANOVA followed by Duncan's multiple range test (at P = 0.05)**

Soil pH of the soil (treated with heavy metals - as single metal or their mixed metal) increased both with cow dung (at 5% and 20%) and lime (at 3 and 9 g $kg^{-1}$ soil) treatments. Soil pH was comparatively higher in cow-dung treatments than in lime treatments. Soil pH ranged from 6.12 to 6.49 in control condition, but it increased with 5% and 20% cow dung treatments and ranged from 7.53 to 7.86 and from 8.07 to 8.28, respectively.

Immobilization of each supplied heavy metals such as Cu, Pb and Zn in both single and mixed metal treatments were found to be high in cow-dung amended soil (Fig.4). It indicated that cow-dung helps in retaining toxic metals in the soil and reduces its mobilization to the plant body. But in soil treated with single metal salts such as $CuCl_2$ and $Pb(NO_3)_2$ with lime amendment, Cu and Pb retained in the soil were lower than in control, indicating their free mobility in the plants. In mixed metal treatments, immobilization of Cu, Pb and Zn mostly increased in both cow-dung and lime treatments. From this it can be ascertained that 20% cow-dung treatment is suitable for immobilization of supplied metals than lime treatments (Fig. 4).

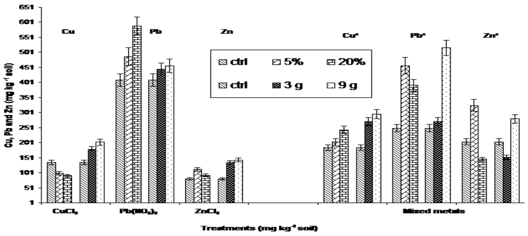


**Fig. 4. Concentration of Cu, Pb and Zn in soil treated with single and mixed metals with different quantities of cow dung (%) or lime (g). (*indicate metals retained in mixed metal treated soil).**

**DISCUSSION**

In the present study, the accumulation of toxic metals was low and FW was higher in most cases in cow dung amended soil than in lime amended soil, although the soil pH was increased more in cow dung amended soil. This may be due to sorption of toxic heavy metal by the protein, carbohydrate and phenolic compounds (Friedmann and Waiss 1972) contained in cow dung. These compounds have metal binding functional group such as carboxyl, hydroxyl and amino groups. Besides this, the presence of cation exchange capacity in cow-dung would have played the roles in the nutrient balance via supply of essential macronutrients (NPK) and micronutrients (Fe, Mn, Cu, B etc). The increased DW in increased soil pH via the addition of cow dung treatment may indicate the availability of essential nutrient in exchangeable fractions by the organic portions of the cow dung in one hand and the binding of excess of toxic metals in other side by the functional group. At high pH most of the Pb is retained in the hydroxide and carbonate phase (Yong *et al.* 1993).

Most of the vegetables grown on lime amended soil showed significant decrease in FW, DW and SL, indicating loss in yield. In lime treatments of 9g $kg^{-1}$ soil, accumulation of Pb, Cu and Zn increased in most vegetables. This is possibly due to unavailability of essential nutrient ions on one hand (Scotti *et al.* 1999) and availability of excessive supplied toxic ions in increased soil pH condition, which must have hampered their growth. The supplied lime ($CaCO_3$) probably regulated the root permeability (Jacobson *et al.* 1961) and possibly maintained the integrity of the cell structure (like cell membrane, cell wall). This probably helped in binding more toxic metals than in control condition.

Vegetable crops grown on soil with single metal treatment amended with cow dung showed increased FW, DW, RL and SL. This may be due to availability of moisture and nutrient from the organic material on one hand and unavailability of toxic metals to plants on the other hand. The supplied heavy metals in the soil may get bind with humic acid, fulvic acids and proteins present in the cow dung (Akio *et al.* 1993) and form complexes (Benes *et al.* 1976, Stevenson 1976, Cole 1977). Metals bound in humic acids are often difficult to remove and even in very sandy soils extraction can require vigorous procedures (Hodgson 1963).

The percent amount of each representative chemical species of metals differs not only according to pH, but also according to available ligands in soils solutions. The important characteristics are the stability constants of complexes in the soil and the plant system, and are determined by the ligand type (i.e. the matrix in soil and/or plant like humic acid, fulvic acids, simple organic acids, and organic materials in general) as well as pH and ionic strength (Streit and Stumm 1993). Typical ligands in soil solutions contain the carboxyl groups which are formic, acetic, oxalic and citric acid. Organic ligands that contain nitrogen or sulfur in the soil solution is the amino acids. A great variety of different ligands partners include those derived from the benzene ring to which carboxyl (to form benzene carboxylic acids) or hydroxyl groups (to form phenolic groups) are attached. Actually the majority of heavy metals in the soil system will usually be absorbed to some surface and not found in free solution, except at an excess of solved chelating agents (Streit and Stumm 1993).

Soil pH in lime amended soil is 7.6 to 7.9, which are mostly higher than in control (6.8-7.6). Generally, the availability of metals for uptake by plants is greater at low pH than at high pH (Sims 1986) and the net effect of an increase in soil pH value by liming of soil for example is a reduction in metal adsorption by plants (Davis and Coker 1980). As the organic matter was not provided in lime amended soil, there would be low available binding sites for soil heavy metal. So due to this reason the supplied heavy metals were easily available to plants.

## CONCLUSIONS

From the experiments of remedial approaches it can be concluded that cow dung provides ligands for binding heavy metals and reduced availability of dominant cations for the plant uptake. Therefore, cow dung treatment may have resulted good productivity than in the lime treatments. Cow dung (10-20% by weight) is recommended to use in orders to reduce heavy metal uptake in the vegetables when grown on contaminated soil. Lime alone is not recommended for the immobilization of heavy metals.

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