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SCREENING OF VEGETABLES FOR PHYTOEXTRACTION OF HEAVY METALS

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ABSTRACT

To understand the phytoextraction of heavy metals from contaminated soil, eighteen seasonal vegetable crops were investigated. The soil was 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 metal (1600 mg kg^{-1}). Lowest copper (Cu) accumulation was recorded in *Brassica juncea* and highest in *Vicia faba*, while lowest lead (Pb) accumulation in *Solanum tuberosum*, and highest in *Allium fistulossum*; and lowest zinc (Zn) accumulation in both *Allium fistulossum* and *Solanum tuberosum,* and highest was in *Vicia faba* from the single metal contamination. Similarly, lowest Cu, Pb and Zn was recorded in *Brassica juncea, Brassica caulorapa* and *Allium fistulossum*, respectively; and highest accumulation of Cu and Pb was in *Spinacea oleracea* and Zn in *Spinacea oleracea* from their mixed metal treatments.

Key words: Phytoextraction, vegetables, heavy metals.

INTRODUCTION

The continuous increase of non-degradable heavy metals, such as cadmium (Cd), arsenic (As), mercury (Hg), silver (Ag) and lead (Pb) which are emitted via anthropogenic activities in the environment, have no biological function as nutrients (Nies 1999). Heavy metals from the industrial emissions, household waste, sewage sludge, pesticides and fertilizers are major global problems as ultimately contribute to the deposition on soil of urban and sub urban areas (Lagerwerff and Spect 1970, Goyer 1997). Heavy metal contaminated soils reduce plant species richness and increase biologically inactive land via destroying soil quality by the influences on the breakdown of organic matter with the negative activity of microorganisms and earthworms (McLean 1975).

With regard to uptake of metals and their accumulation, plants are excluders, indicators/ or accumulators and hyperaccumulator. The genus *Allyssum* and *Thlaspi*, (both species belonging to family Brassicaceae) which have effective capability in reducing heavy metal from the contaminated soil by accumulating high load of Zn, Ni, Cd and Pb in their body (Baker 1981, Baker *et al.* 1994, Chaney *et al.* 1997). Hyper-accumulator has generally slow growth with production of low biomass. Most plant species tend to exhibit higher heavy metal levels in the root and shoots in responses to increasing levels of heavy metals in the soil (Hutchinson and Whitby 1974, Pietz *et al.* 1978). Plants which develop tolerances mechanisms against phytotoxicity, constitute a risk factor for human health via food chain, create dangerous situation through direct consumption of edible plants or indirectly from animals coming from the heavy metal contaminated fields (Lantzy and Mackenzie 1979, Angelone and Bini 1992).

Copper and zinc, are the essential micronutrients, especially in the production of blood haemoglobin in human, but in high doses, cause anaemia, liver, kidney damage, stomach/intestinal irritation, sexual immaturity, grey hair, dehydration, damage of mucous membrane, (WHO 1996, VCI 2011). Lead (Pb) may cause various problems in human body part like gastrointestinal tract, kidneys, central nervous system and brain. Memory loss, nausea, insomnia, anorexia, damage in the red blood cells, and weakness of the joints are some of the symptoms of Pb toxicity in human. Lead influences the genetic structure and evolution of exposed plant and animal populations (Johnson 1998, Baldwin and Marshall 1999, NSC 2009).

Various bioremedial works have been conducted to reduce heavy metal contamination from contaminated soils. Heavy metal tolerance in cowpea (leguminous) plants was increased by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen–fixer *Rhizobium* bacterium (Saleh and Saleh 2006). Indian mustard (*Brassica juncea*) was an effective plants for phytoextracting Zn after the application of the synthetic chelates EDTA (ethylenediamine tetraacetic acid) when compared to the oat (*Avena sativa*) and barley (*Hordeum vulgare*) grown from the Zn contaminated soil (Ebbs and Kocchian 1998, Nowack *et al*. 2006). But EDTA has been found associated with high toxicity and persistence in the environment (Tandy *et al.* 2004, Evangelou 2007). *Sorghum* spp can be used in remediation process, because their fibrous root show the efficiency in accumulation of heavy metals (Jadia and Fulekar 2008). Further, the use of phytoextraction are simple, more economically viable and environment friendly (Garbisu and Alkorta 2001). A variety of plant species like *Brassica juncea*, *Hibiscus cannabinus*, *Abelmoschus esculentu*m, *Beta vulgaris* L., *Oryza sativa* L., *Arabidopsis halleri* were studied for extracting Cd from contaminated soil (Yanai *et al.* 2004, Ishikawa *et al.* 2006, Murakami *et al.* 2007).

Information about heavy metal accumulation among vegetables is important to understand the best accumulator. On this basis, planning can be made for cultivation of particular vegetable crops in heavy metal contaminated soil. The main objective of this study is to identify the suitable vegetable species for phytoextraction of heavy metals from heavy metal contaminated soil.

MATERIALS AND METHODS

Vegetables are valuable being the rich sources of minerals (Ca, Mg, Fe, P, K, S), amino acids, vitamins [Vit. A (antioxidant) Vit.C (ascorbic acid), Vit. E (tocopherol) and Vit. K (anti-haemorohagic factor)] and dietary fibers.

Some common vegetables which are cultivated in Kathmandu valley are selected for the present study. Seeds of vegetables such as *Beta vulgaris* L. (Beet root) Chukandar, *Beta vulgaris* var. cicla (Swiss Chard) Foliage beet, *Brassica juncea* L. (Broad leaf mustard) Rayo sag, *Brassica caulorapa* L. (Kohlrabi) Gyathgobi, *Brassica rapa* L. (Turnip) Gantemula, *Brassica rapa* L. var. purple top globe (Turnip) Salgam, *Lepidium sativum* L. (Pepper Cress) Chamssor, *Raphanus sativus* L. (Radish), Puthane Rato, *Raphanus sativus* L. (Radish), Puthane Seto mula, *Coriandrum sativum* L. (Coriander) Dhaniya, *Spinacia oleracea* L. (spinach) Patane palungo, *Spinacia oleracea* L. inermis (round seeded spinach) Deshi palungo, *Spinacia oleracea* L. (Prickly seeded spinach) Gobre, *Trigonella foenumgraecum* L. (Fenugreek) Methi and *Vicia faba* L. (Broad bean) Bakula were collected from reliable seed store (Annapurna Beez Bhandar, Ason). Some bulbets such as *Allium sativum* L (Garlic) Lahsun, *Allium fistulosum* L. (Cibol or Welsh onion) Chyapee, and tubers *Solanum* *tuberosum* L. (Potato) Aalu, were purchased from local market of Kathmandu.

The seeds/ bulbets / tuber of the vegetables were grown in polythene bags containing 3.5 kg of sandy loam soil which 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 with their mixed concentration (=1600 mg kg^{-1} soil). Triplicate bags of each vegetable per treatment for experiment were arranged in randomized block design.

The cultivated vegetables were harvested after 5 weeks. The harvested vegetables were cleaned thoroughly with tap water for the removal of outer contamination (soil particles and aerial) and were finally washed with de-ionized water. The vegetables were air dried for two to three days and finally oven dried at 60 to 70^0 C up to constant dry weight. The representative samples were made powder by using Mortar and pestle. Analysis of the representative samples (1 g dry weight) of each vegetables were used for the wet digestion process by using concentrated HNO_3 (Chettri *et al.* 1997). Metal concentration of Cu, Pb and Zn from triplicate samples were analyzed with the conduction of Atomic Absorption Spectrometry (AAS) using 324.7 nm for Cu, 283.3 nm for Pb and 213.9 nm for Zn (Welz, 1985). 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. 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 with their mixed concentration (=1600 mg kg^{-1} soil).

RESULTS

Among the tested vegetable crops for phytoextraction, copper accumulation <25 mg kg^{-1} from 300 mg $CuCl_2$ kg^{-1} soil treatments, were observed in *A. sativum*, *L. sativum* and white *R. sativus* but low Cu accumulation (20–22 mg kg^{-1} DW) were observed in *B. juncea* and *B. caulorapa*. Moderate Cu accumulation (ranging from 25 to 50 mg kg^{-1}) was recorded in *T. foenumgraecum*, *B. rapa* and *C. sativum*. High Cu accumulation (50 to 100 mg kg^{-1}) was observed in *A. fistulossum*, *S.* (Deshi), *S. oleracea* (Patane), *S. oleracea* (Gobre) and *Beta vulgaris* var. cicla (Swiss chard). Among studied vegetables, *V. faba* accumulated high concentration of Cu (432.5 mg kg^{-1}). *Solanum tuberosum*, *Beta vulgaris* (beet root), *R. sativus* (red radish) and *B. rapa* var. purple top globe (salgam) could not germinate on soil with 300 mg $CuCl_2$ kg^{-1} contamination (Fig. 1). In Figs. 1-4, *Spinacia oleracea* Deshi is indicated as inermis, *S. oleracea* Patanae as Shankhamul and *S. oleracea* Gobre as Thimi.

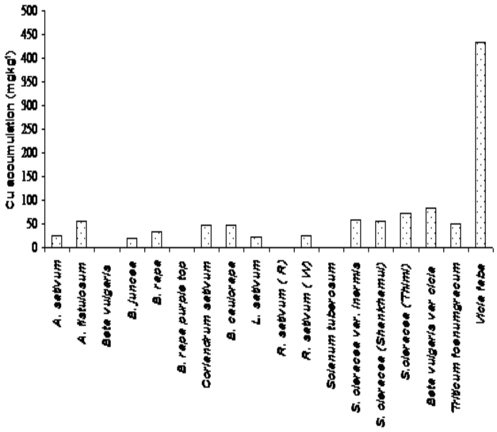

Fig. 1. Phytoextraction of copper by vegetable crops grown on soil with $CuCl_2$ treatment.

Among the vegetable crops for phytoextraction test, lead accumulation <50 mg kg^{-1} from 500 mg Pb $(NO_3)_2$ kg^{-1} soil treatments was observed in *Beta vulgaris* (beet root), *B. juncea*, *R. sativus* (red), *B. caulorapa*, *R. sativus* (W) and *S. tuberosum*. Lowest Pb accumulation was observed in *S. tuberosum*. Moderate accumulation of Pb (50 to 100 mg kg^{-1}) was recorded in *A. sativum*, *L.*

sativum, *S. oleracea* (Deshi), *S. oleracea* (Patane) and *Beta vulgaris* var. cicla (Swiss chard). Highest accumulation of Pb (512.5 mg kg^{-1}) was noticed in *A. fistulosum* followed by *C. sativum, V. faba, B. rapa* var. purple top globe and *S. oleracea* (Gobre). *Trigonella foenumgraecum* could not germinate in 500 mg Pb $(NO_3)_2$ kg^{-1} contaminated soil (Fig. 2).

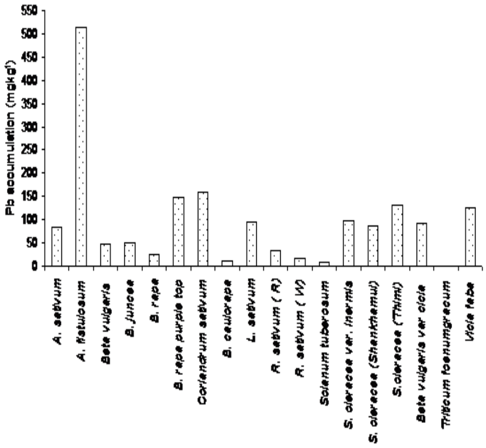

Fig. 2. Phytoextraction of lead by vegetable crops grown on soil with Pb $(No_2)_3$ treatment

Among the tested vegetable crops for phytoextraction of Zinc from 800 mg $ZnCl_2$ mg kg^{-1}, Zn accumulation was highest in *V. faba* (broad bean) (202 mg kg^{-1}) followed by *L. sativum.* Low Zn (<100 mg kg^{-1}) was accumulated in *A. sativum*, *A. fistulosum*, *B. caulorapa*, *T. foenumgraecum*, *Solanum tuberosum*, *R. sativus* (both red and white radish), *B. rapa* var. purple top globe, *S. oleracea* (Deshi), *S. oleracea* (Gobre). Moderate Zn accumulation, ranging from 100 to 200 mg kg^{-1} was recorded in *B. juncea, S. oleracea* (*patane*), *Beta vulgaris* var. cicla. *Coriandrum sativum* could not germinate in 800 mg $ZnCl_2$ kg^{-1} contaminated soil (Fig. 3).

Among the tested vegetable crops for phytoextraction of Cu, Pb and Zn, from their (1600 mg) mixed metal kg^{-1} soil treatments, Cu accumulation in *B. juncea, C. sativum, B. rapa* (both varieties), *S. tuberosum* and *T. foenumgraecum* have less than 15 mg kg^{-1}. *Allium fistulosum*, *V. faba*, *S. oleracea* (Patane), *Beta vulgaris* var. cicla and *S. oleracea* (Deshi) showed moderate Cu accumulation (15 – 29.5 mg kg^{-1} DW). Accumulation of Pb in most vegetables was <10 mg kg^{-1} from the mixed metal treated soil and were within the range of 5.83 (*S. tuberosum*) to 30.5 mg kg^{-1} (*S. oleracea* Deshi).

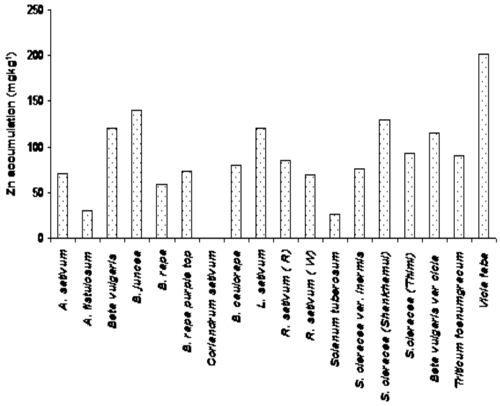

Fig. 3. Phytoextraction of zinc by vegetable crops grown on soil with $ZnCl_2$ treatment

Zinc accumulation ranged from 100–200 mg kg^{-1} in *Beta vulgaris* (beetroot), *L. sativum*, *T. foenumgraecum*, *R. sativus* (red), *S. oleracea* (Gobre) and *Beta vulgaris* var. cicla. More than 200 mg kg^{-1} Zn accumulation was noticed in *V. faba*, *B. caulorapa*, *B. rapa* var. purple top globe, *S. oleracea* (Deshi) and *S. oleracea* (Patane). Among these vegetables, *S. oleracea* (Patane) accumulated highest amount of Zn (335.5 mg kg^{-1}). Out of red and white varieties of radish, the former accumulated more Cu, Pb and Zn than the later. Similarly, among the three varieties of *S. oleracea*, Cu and Zn accumulation was low in Gobre, but Pb accumulation was more or less similar in all three varieties. Zinc accumulation was higher in *S. oleracea* (Patane) than in others two varieties (Fig. 4).

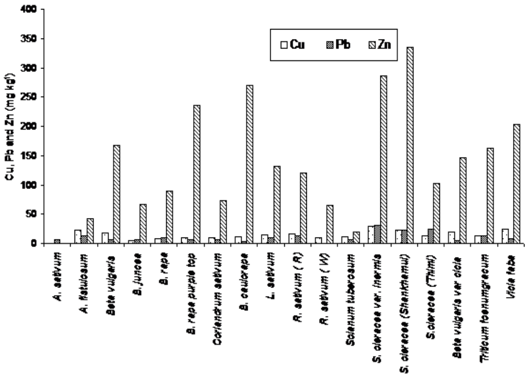

Fig. 4. Phytoextraction of Cu, Pb and Zn by vegetables grown on soil with mixed metals ($CuCl_2$ +Pb $(No_2)_3$ +$ZnCl_2$) treatments.

DISCUSSION

The accumulation of Cu and Zn in *V. faba* among vegetables tested for phytoextraction indicated that the responses of plants to the exposure to heavy metals are complicated due to variable tolerance as well as multivariable relationships between the concentration of soil metals and plants metals (Kabata-Pendais *et al.* 1993). High accumulation of Cu in *V. faba* is mainly due to presence of high proteins and bitter alkaloid compounds (Daniels *et al*. 1997, Jain 2007). Presence of alkaloid like lignin, phenols, etc along with $–NH_2$ group forms complex with accumulated Cu and this enhances Cu accumulation. In the present study potato could not grow in $CuCl_2$ contaminated soil but showed highly reduced accumulation of Pb and Zn from both single and their mixed metal treatments. Reduced uptake of heavy metals is one of the plant adaptation strategies to avoid metal toxicity (Baker and Walker 1990). Out of these tested vegetable crops, reduced uptake mechanism has been identified in potato tuber, which is possibly due to high starch content. Highest accumulation of Pb was observed in *A. fistulosum*. Possible reason for this may be due to presence of organosulphur, like cystein di- sulphide, methionine, glutamine which form complex with Pb because of being a border line metal (Streit and Stumm 1993, Kuiper 2005). Low accumulation of Pb less than lower range of critical tissue concentration of 20 mg kg^{-1} DW as suggested by Kabata-Pendais *et al.* (1993) was observed in *B. caulorapa*, *R. sativus* (white) and *S. tuberosum*. High accumulation of Zn in *B. juncea* supports the finding of Kumar *et al.* (1995) and Salt *et al.* (1995). They suggested *B. juncea*, to be more effective than *Thlaspi caerulescens* in phytoextracting Zn, as it removed 4 folds more Zn than *T. caerulescens* (Ebbs and Kochain 1998). This was due to the fact that *B. juncea* produced 10 times more biomass than *T. caerulescens*.

In the mixed metal condition, *S. oleracea* (Patane and Deshi), *B. caulorapa* showed high Zn accumulation which is in contrast to Zn accumulation from the single metal treatment. High accumulation of Pb and Cu was observed in *S. oleracea* (inermis which is also indicated as Deshi) from mixed metal treatment. High uptake of Zn, Pb or Cu from the mixed metal treatments in the plants may be due to competitive uptake among the supplied cations. This result is further justified from the monitoring experiment where the Zn accumulation in *S. oleracea* (in all species) is high and have tendency of hyperaccumulation (Sharma and Chettri 2005).

From single and mixed metal treatments, it is observed that heavy metal accumulation in vegetables is not only dependent on the concentration of supplied cations in the soil but also on the availability of other metal species at rhizospheric soil environment.

Present study identified some vegetables to be very sensitive, some as good accumulator/ or some as moderate or some as low accumulator of heavy metals. Vegetables like potato, beet root, turnip and radish could not germinate and grow in Cu contaminated soil. Fenugreek (*T. foenumgraecum*) and coriander (*C. sativum*) could not germinate and grow in Pb and Zn contaminated soil, respectively. Similarly, *A. fistulosum* could not germinate in mixed metal contaminated soil.

Best Cu accumulator are *V. faba* followed by *Beta vulgaris var.* cicla, *S. oeracea* (Thimi), *A fistulosum* when grown on Cu contaminated soil. *A fistulosum* followed by *Brassica rapa* purple top, *C. sativum*, *S. olearacea* (Thimi), *V. faba* are best Pb accumulator. *V. faba >S. olearaca* (Shankhamul), *Brassica juncea*, *L. sativum*, *Beta vulgaris* var cicla are the best Zn accumulator.

T. foenumgraecum, *B. rapa* and *C. sativum* are the moderate Cu accumulator. *A sativum*, *L sativum*, *S oleracea* (deshi) *S. olearacea* (patne) and *Beta vulgaris var.* cicla are moderate Pb accumulator. *B.juncea*, *S. oleracea* (patne), *B. vulgaris* var cicla are the moderate Zn accumulator.

B. juncea is low Cu accumulator. *Solanum tubersoum* is low accumulator of both Pb and Zn from single heavy metal treatment while *B. juncea*, *B. caulorapa* and *S. tubersoum* are the low accumulator of Cu, Pb and Zn, respectively, from mixed metal treated soil.

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