

1. INTRODUCTION

The increased urbanization and industrialization have made a profound effect on environmental destabilization by introducing heavy metal contamination. Heavy metals can neither be destroyed nor altered by chemical or physical means (Conner, 1994), but are transformed and circulated from one form to other in the ecosystem. There are strong interactions of metals with organic and inorganic materials of the soil (Adriano, 1986). All land plants depends on top soil for uptake of mineral nutrients. The limit concentrations of heavy metals if exceed in the agricultural soils affect the biosphere (Fergusson, 1990) and agricultural productivity by inhibiting the decomposition of organic matter and reducing the soil fertility (Baath, 1989; Chaudri *et al.*, 1993). Increased concentrations of heavy metals in the environment damage biomolecules at cellular level (Somashekaraiah *et al.*, 1992, Stoths and Bagchi, 1995; Schutzenubel and Polle, 2002), followed by some visible injuries (Wang and Schapp, 1988) and in extreme cases may change the vegetation (Ernst, 1998).

1.1. Heavy Metals

Heavy metals are the natural components of the geosphere and have also become the inseparable part of the soil, water, air and biota (Brummer *et al.*, 1991). Metal having a density higher than 5 g cm^{-3} are defined as heavy metals. Altogether 53 elements are heavy metals, but based on their solubility under physiological conditions, only seventeen are available to living cells. Hence, the metals are of importance for organisms and ecosystems (Weast, 1984). Among these Fe, Mo and Mn are important as micronutrients; Zn, Ni, Cu, V, Co and Cr are of low importance as trace elements, but are toxic beyond certain limit. Elements like V, Cr, Mo, Mn, Fe, Cu, Ni, Zn, Sn and Se are enzymatic elements and have catalytic function (Markert, 1994). Heavy metals like Cd, As, Hg, Ag, Sb, U and Pb have no biological function as nutrients and seem to be more or less toxic to living organisms (Godbold and Hüttermann, 1985; Nies, 1999).

1.1.1. Sources of Heavy Metals

Atmospheric pollution is one of the sources of heavy metals to the soil, as air borne heavy metals which fall upon, react with and are absorbed by plants and soil near the sites of

pollutant generation (Chamberlain, 1960; Lagerwerff, 1971; Bohn, 1972; Little, 1973; Buchauer, 1973). Similarly, the contaminated water with toxic metals may be the sources for soil contamination as such water is used in irrigation. Sources of As, Cr, Cu, Mn, Cd, Ni, Mo, Zn, Sb, Hg and Se in the water bodies are mainly through household waste, coal fired power stations, iron and steel production, metal smelters and other industrial discharges. It has been estimated that an average of 7,800 tones (t) As; 1,000 t Cd; 19,000 t Cu; 516,000 t Mn; 19,000 t Pb; 66,000 t V; 46,000 t Zn and 6,000 t Hg are emitted into the atmosphere each year (Nriagu and Pacyna, 1988), but estimation for Cd emission by Di Toppi *et al.* (1999) is quite high (30,000 t per year).

In agricultural practices, use of inorganic fertilizers is increasing day by day. Phosphorus fertilizers contain several heavy metals like Cd, which are not eliminated during the manufacturing process (Sharply and Menzel, 1987). Besides this, heavy applications of poultry manure increase the uptake of Cu and Zn in forages (Bomke and Lowel, 1991). Therefore, it can be generalized that there are two sources of heavy metals in the environment.

Natural sources: Soil and sediments are the final and usual fate of trace metals which are derived from parent soil matter or continental dusts, earthquakes and volcanoes.

Anthropogenic sources: It includes various industrial activities like metal smelters, blast furnace, electrolysis, cement industry, iron and steel production, vehicular traffic; house hold waste and sewage sludge; agricultural wastes (food additives, phosphate fertilizers, pesticides and herbicides); energy supply (coal fired power stations, petroleum combustion, nuclear power plants, high tension lines, heating systems); waste incinerations etc (Ernst and Joesse, 1983; Morselli *et al.*, 1999).

1. 1. 2. Soil as a Sink of Toxic Metal

All emitted heavy metals either of anthropogenic or natural origin ultimately accumulates in the soil. Heavy metal accumulation proceeds through specific adsorption onto or occlusion by oxides and hydroxides of Fe and Mn in soils. The accumulated heavy metals in soil may be present in different forms or complexes like exchangeable, Pb-displaceable, acid soluble, Mn-oxide occlude, organically bound, amorphous Fe oxide occluded, crystalline Fe oxide-occluded and residual fractions (Iawasaki *et al.*, 1993).

Background concentrations, the normal and critical concentrations of different heavy metals in soil and in plant body have been estimated from various researchers (Alloway, 1968;

Norrish, 1968; Beckett and Davis, 1977; Davis and Beckett, 1978; Nriagu, 1978; Bowen, 1979; MacNicol and Beckett, 1985; Page *et al.*, 1987; Pahlsson, 1989; Kabata-Pendias and Pendias, 1992) and is summarized in **Table 1**. The critical soil value is defined as the range of values above which toxicity is considered to be possible, and the critical plant value is the level above which toxicity effects are likely.

Table 1. Background, normal and critical concentrations of heavy metals in soil and plant tissues.

Metal	Background concentration $\mu\text{g/g}$	Normal soil value (mg kg^{-1})	Critical soil value (mg kg^{-1})	Normal plant value $\mu\text{g/g DW}$	Critical plant concentration $\mu\text{g/g DW}$
Pb	20-50 ^N 100 [@]	35*	400 ^{KP}	0.1-100* 0.1-10**	20-300 ^{KP} 25-85#
Cu	100 [@]	30*	125 ^{KP}	4-15**	20-100 ^{KP} 15-20#
Zn	300 [@]	90*	400 ^{KP}	15-100* 8-100**	200*** 60-900# 100-400 ^{KP}
Ni	-	50*	100 ^{KP}	1** 1-2.7	10-100 ^{KP}
Cd	0.1-1.0 ⁺ 2.0 [@]	0.35*	8 ^{KP}	0.1-02.4* 0.2-0.8**	5-30 ^{KP} 3-10#
Mn	-	1000	3000 ^{KP}		300-500 ^{KP}
Cr	-	70*	100 ^{KP}	0.2-1.0* 0.2-10**	5-30 ^{KP}
Fe	-	40000*	-	140**	-

Sources: ^{KP}K-Pendias and Pendias (1992); *Bowen (1979); **Alloway (1968); ***Davis and Beckett (1978); ⁺Page *et al.* (1987); [@]French regulation of agricultural soils (^NNorrish, 1968 and Nriagu, 1978); # (Beckett and Davis, 1977; MacNicol and Beckett, 1985; Pahlsson, 1989).

1.1.3. Negative Impact of Heavy Metals on Human Health

According to United States Environment Protection Agency (US EPA)- Pb, Cd, As, Mn, Ni, Co and Zn are carcinogenic, hence environmental problems are gaining importance in real life. The permissible level of some heavy metals for human consumption is given in **Table 2**.

Cadmium

Cd classified as carcinogen (MAK *et al.*, 1995) is a causal factor in cardiovascular diseases, hypertension, renal tubular dysfunctions, aminoaciduria, hypercalciurea, glucosuria, proteinuria and osteoporosis (Edmunds and Smeldy, 1996; WHO 1996). Cd toxicity links with reproduction as placenta retains Cd that restricts the transfer of the Zn and Cu to the foetus and reduces birth weight (Peerebome-Stegeman, 1987).

Copper

Copper toxicity causes Wilson's disease, hepatocellular and lenticular degeneration, and cirrhosis, neurological symptoms, Kayser-Fleisher ring, hemolytic anemia, and bone damage. Its effect includes nausea, vomiting, diarrhoea, convulsion and death. The inhalation of Cu dusts can lead to perforation of nasal septum. Pulmonary Cu deposition can lead to the development of liver and lung tumors (Scheinberg, 1979).

Lead

It is one of the 10 priority pollutants of US (Environmental Protection Act, 1986). A concentration of 250-550 $\mu\text{g L}^{-1}$ Pb in the blood of children indicates poisoning (WHO, 1996), at least 10 $\mu\text{g Pb per dl}$, a level high enough to adversely affect the intelligence, behavior, and development of children (CDC, 1997). Lead concentration of 1.4 $\mu\text{g/l}$ of blood may cause neurological effects, adverse effects to the human reproductive systems and possibly cancer (Apostoli *et al.*, 1998). Chronic Pb toxicity leads to anemia, kidney damage, sterility, abnormal foetus development and abnormal neurological development and function (Hammond, 1977; Moore and Goldberg, 1985). Lead also produce deficits in perception (sensitivity), distractibility, impulsivity, hyperactivity, non- persistent and language function, and has also been suggested as a factor in criminal behaviour (Silbergeld and Goldberg, 1975; Rice, 1996). Lead influences the genetic structure and further evolution of exposed plant and animal populations (Johnson, 1998). Most of the ingested Pb is initially present in the blood, but the excess amount next enters the soft tissues (brain), and ultimately deposits in bone by replacement with Ca^{++} (Colin, 1995).

Table 2. Permissible limit of heavy metals for human consumption

Metal	Permissible Range	Sources
Cu	2 mg/day	Fishben, 1987
Pb	430 $\mu\text{g/day}$ from food and water (a maximum chronic intake). Below 20 $\mu\text{g}/100\text{ ml}$ for pregnant women and children, but 3 mg per person (0.05 mg kg^{-1} body weight (b. w.) $7\text{ }\mu\text{g kg}^{-1}$ body weight (b. w.), 35-40 $\mu\text{g Pb}$ in 100 ml blood (max. level of adults) 250 $\mu\text{g/day}$ recommended daily allowances.	FAO/WHO, 1972 WHO, 1996 FAO/WHO, 1972
Cd	0.05 $\mu\text{g g}^{-1}$ DW (normal plants value), 10-25 $\mu\text{g/day}$ in most countries (intake from food). 400-500 μg weekly intake corresponding 57-71 $\mu\text{g d}^{-1}$ (tolerable range) 70 $\mu\text{g/day}$ recommended daily allowances.	Elinder <i>et al.</i> , 1988 FAO/WHO, 1992 WHO, 1996
Zn	15 mg/day (recommended daily intake)	Kiekens, 1995
Cr	0.05 mg l^{-1} in the drinking water (max. permissible level). 400 mg kg^{-1} is upper limit in UK. 50-200 $\mu\text{g/day}$ in food (recommended daily intake)	Anonymous, 1996; McGrath, 1995 WHO, 1996; Mertz, 1969.

Zinc

Sexual immaturity, skin lesions, grey hair, dehydration, electrolyte imbalance, dizziness, lethargy, muscular incardination, irritation and damage of mucous membrane are the symptoms of Zn toxicity (WHO 1996; Dara, 2004).

Chromium

Chromate poisoning causes liver and kidney disorder (Sitting, 1981). Major health hazards are digestive tract cancer; cutaneous contact dermatitis, ulcers and lung cancer. Cr (VI) is far more toxic than the Cr (III).

Cobalt

Ingestion of excessive amounts of Co causes intercellular hypoxia, polycythemia and chronic exposure leads to goiter (Sawyer *et al.*, 2003; Dara, 2004).

Nickel

Nickel is a toxic carcinogen (ATSDR, 1988; Goyer, 1991, 1997) and causes allergic contact dermatitis, pulmonary asthma (International Agency for Researches on cancer, 1990, 1993), conjunctivitis, inflammatory reactions and also damages the kidneys (Friberg *et al.*, 1986).

1.1.4. Negative Impact of Heavy Metals on Plants

In general, plants possess physiological mechanisms that enable them to resist elevated heavy metal concentration in their substrate (Antonovics *et al.*, 1971; Baker, 1981, 1987; Woolhouse, 1983). Plants do not always show visible symptoms morphologically but may have hidden injury due to pollutants or changes in metabolic pathways (Barman *et al.*, 1999). Plants show different types of responses as morphological, anatomical, physiological and behavioral (developing sensitive, tolerant and resistance varieties) when grown on contaminated soils with toxic metals. Cu and Zn are phytotoxic and large concentration in the soil has adverse effects on crops, livestock and man (Kiekens, 1995). The phytotoxic effect of Co, Cr and Cu is the reduction in biomass, Fe concentrations, chlorophyll-a and b content, activity of catalase in leaves of cauliflower (Chatterjee and Chatterjee, 2000). Excess heavy metals in soil cause internal water deficit in the plant body via reduced conductivity of stems and poor root system development (Lamoreaux and Chaney, 1977) which finally reduces biomass production and nutritional quality (Cottonie *et al.*, 1976).

1.2. Uptake of Heavy Metals

Increased concentrations of heavy metals such as Cd, Hg, Pb, Cu, Zn in the soil accumulate in the cellular parts such as epidermal layer, vacuoles or in between cell wall and cell membranes (Brune *et al.*, 1995; Chettri *et al.*, 2000) after their uptake. They affect the elasticity of cell walls (Matsumoto *et al.*, 1977) or result various physiological disorders on respiration, photosynthesis, growth, carbohydrate metabolism (Eleftheriou and Karataglis, 1989; Costa and Morel, 1994; Prasad, 1995; Wojcik and Tukendorf, 1999).

Excess uptake of any heavy metal induces a deficiency of essential nutrients, affecting the cation balance at the sub cellular level, e.g. excess Zn and/or Cu concentrations in the growing medium hamper the uptake of Fe and/or Mn (Ernst, 1974). The displacement of Ca^{2+} by Cd^{2+} (Rivetta *et al.*, 1997); Mg^{2+} by Mn^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} (Passow *et al.*, 1961; Wildner and Henkel, 1979) leading to altered activities of certain enzymes such as ribulose-1, 5-bisphosphate-carboxylase/oxygenase resulting mineral deficiency (Clarkson and Hanson, 1980; Van Assche and Clijsters, 1986; Siedlecka, 1995).

1.3. Impact of Heavy Metal on Chlorophyll

Plants growing in heavy metal contaminated soil may absorb and accumulate heavy metals in their body. Although Fe, Mo, Mn, Zn, Cu, Mg, Co, Se and possibly Ni have major role for the growth and development of plants body (Halpern, 1985; Baker and Brooks, 1989; Salt *et al.*, 1995; Clarke and Berg, 1998; Clemens, 2001) but may become toxic beyond certain level. Morphological or physiological changes in plants will not be visible unless there is certain metabolic change in cellular activities, like photosynthesis (Sempio *et al.*, 1971; Carlson and Bazzaz, 1977; Djingova and Kuleff, 1993). Magnesium of chlorophyll molecule is substituted by heavy metals (such as Hg, Cu, Cd, Ni, Zn, Pb) resulting HMs-chlorophylls (Watanabe *et al.*, 1985; Kupper *et al.*, 1998) which have much lower in-vitro florescence quantum yield compared to Mg-chlorophyll.

Van Assche and Clijsters (1986a, b) observed inhibition of photosynthesis in *Phaseolus vulgaris* (Dwarf beans) by toxic concentration of Zn via a) effect on electron transport and photophosphorylation, b) effect on ribulose-1, 5-bisphosphate carboxylase/oxygenase, grown under standard nutrient conditions. Positive linear relationship was observed between peroxidase activity and leaf tissue metal concentration (Cu, Zn and Pb) for all metals and negative linear relationship between photosynthetic pigments and increasing Cu and Zn concentration in leaf tissue in the *Avicennia marina* (Forsk.) *vierch* (grey mangrove) (MacFarlane and Burchett, 2001).

Empetrum nigrum L. (crowberry), a heavy metal tolerant species, growing near Cu-Ni smelter showed low concentration of chlorophyll, organic (citric and malic) acids and stem water potential but in contrast, ABA contents in stems and leaves were high near the pollution source (Monni *et al.*, 2001). Ultrastructural and morphological characteristics in wheat (*Triticum*

aestivum L. cv. *vergina*) grown in naturally Cu polluted fields (near Cu-mining area) showed poorly developed internal membrane system consisting of thylakoids arranged parallel to each other with only a few rudimentary grana (Eleftheriou and Karataglis, 1989).

1.4. Monitoring Heavy Metals in Soil and Vegetable Crops

Heavy metals in soil may enter from various sources like vehicular traffic or industrial emissions, mining activities, industrial effluents, etc. 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 (Lagerwerff and Spect, 1970, 1971; Page and Bingham 1973; Hutchinson and Whitby, 1974; Carlson *et al.*, 1975; Pietz *et al.*, 1978). High concentration of As, Cd, Cu, Mn, Pb, Zn were measured in sludge treated soil than in untreated control soils (McBride *et al.*, 1999). High accumulation of Zn, Cd, Cu and Pb in soils and plants were recorded near a metal smelters or mining sites (Little and Martin, 1972; Buchauer, 1973; Chettri *et al.*, 1997; Monni *et al.*, 2001). Similarly, high accumulation of Pb, Cd, As and Zn in the Iceberg lettuce, Cherry, Belly radish, Roma Bush beans and Better Boy tomatoes were recorded which were cultivated on mine wastes and on mixed mine waste-amended soils (Cobb *et al.*, 2000).

Study of different metals in roadside soil showed that Pb, Cd and Cu are good indicator for vehicular traffic pollution (Carlosena *et al.*, 1999). The soil and plant samples from roadsides contaminated with toxic by-products of vehicular traffic, showed a close correlation between the daily mean volume of traffic and metals accumulated in the soil samples (Daniels *et al.*, 1997).

Fields irrigated with mixed industrial effluent have been found to contain the potentially toxic elements such as Fe, Mn, Zn, Cu, Pb, Ni and Cr in the soil and vegetables, wheat and weed (Barman and Ray, 1999; Kisku *et al.*, 2000; Barman *et al.*, 2000). The uptake of heavy metals was found in different vegetables crops (carrot, lettuce, peas, potatoes, radish, sweet corn and tomatoes) cultivated in sludge treated soil in field conditions (Dowdy and Larson, 1975).

1.4.1. Monitoring Heavy Metals in Kathmandu

Biomonitoring of toxic heavy metals such as Pb, Cd and Cr in Kathmandu valley using lower plants indicated high amount of heavy metal pollution in the city (Devkota *et al.*, 1997; Chettri *et al.*, 2001; Shakya *et al.*, 2004). Every year large areas of land are contaminated with heavy metals because the sewage from the urban areas and various industries have contaminated

river system (Gautam and Agrawal, 1994), which are used for irrigation. Besides this, increasing use of pesticides in our agricultural system may also accelerate heavy metal contamination in vegetables crops. Fall out of toxic elements due to emissions from vehicles and industries have also contributed significantly in Kathmandu (Bhattarai and Shrestha, 1981; NESS, 1995; Devkota *et al.*, 1997; Chettri *et al.*, 2001).

1.5. Dietary Intake of Heavy Metals

Heavy metals such as Fe, Mo, Mn, Zn, Cu, Mg, Co, Se, possibly Ni and Cr are essential for growth and development of plants (Salt *et al.*, 1995; Plant *et al.*, 1996) but are toxic at higher concentration (Bowen, 1979). Cadmium and lead are known to be non-essential but are found increasing in soil due to emissions and use of phosphatic fertilizers (Sharply and Menzel, 1987). Plants generally absorb heavy metals through their roots and are accumulated in different plant parts such as epidermis, cell wall, between cell wall and cell membrane, or vacuoles in inactive chemical form like Cd- phytochelatin complex (Ortiz *et al.*, 1992; Brune *et al.*, 1995; Chardonnens *et al.*, 1998; Chettri *et al.*, 2000).

Some plants develop tolerance mechanisms against phytotoxicity which may constitute a risk factor for human health after entering the food chain. The Cd present in soil is taken up easily by crop plants and passes in our food. Increased Cd levels in soil are a potential health risk, safety margins are small (Buchet *et al.*, 1990). The repeated consumption of such exposed vegetables can lead to health damage as was shown in comparative studies of renal tissues of deceased adults from unexposed and Cd – exposed living areas (Anke *et al.*, 1979, 1994). The leafy vegetables accumulate high concentration of Cd even under normal condition of cultivation (Ellen *et al.*, 1990).

World Health Organization has recommended about 400 g of fresh fruits and vegetables per person per day. This may be approximately 25 % when compared to the total daily food intake, i.e. 1575 g/day (Kannan, 1997). Vegetables have high minerals (Ca, Mg, Fe, P etc), amino acids, vitamins, carbohydrate, cellulose etc and play vital role in neutralization of the acid substances produced in the course of digestion of meat, cheese and other foods (Thompson and Kelley, 1979). *Brassica* species are useful for anti-carcinogenic diet as it reduces coronary heart disease and many cancerous diseases via glucosinolate synthetic pathways (Graham, 2000).

Most of the heavy metals especially Hg, Co, Cd, Bi, Zn, Cu, Ag, Au, Pb, Pt, Sn, Ni, Rb and Te are very dangerous for a human body (Kabzinski, 1998). Binding of heavy metal with membrane can seriously block the charge separation property of the energy transducing membrane and result into metabolic energy crises (Singh, 2003). The interaction of heavy metals with sulphhydryl groups leads to destruction of both structural and functional properties of the proteins. Besides the sulphhydryl, amino group, carboxylic group and imidazol groups in the proteins also bind with these metals and result into altered functions of proteins and enzymes (Singh, 2003).

1.6. Phytoremediation

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 (Galloway *et al.*, 1982; Nriagu and Pacyna, 1988; Verkleij, 1993; Filipek, 1994; Goyer 1997; Di Toppi *et al.*, 1999). These ultimately contribute to the deposition of heavy metals on soil of urban and sub urban areas and may create dangerous situation on human health due to accumulation in roadside plants, soil and street dust, both through direct inhalation of contaminated air of the city (EPA, 1976) and through a consumption of edible plants / animals coming from the fields situated near the roads with a heavy traffic.

With regard to uptake of metals and their accumulation, plants are excluders, indicators/ or accumulators (Baker, 1981; Legittimo *et al.*, 1995). In the Brassicaceae family several hyperaccumulator have been identified in the genus *Allyssum* and *Thlaspi* which can hyperaccumulate Zn, Ni, Cd and Pb (Baker and Brook, 1989). *Thlaspi caerulescens* grown on calamine and serpentine soil can hyperaccumulate Cd, Ni and Zn (Reeves and Brooks, 1983; McGrath *et al.*, 1993; Lombi *et al.*, 2000). Hyperaccumulator plants have effective capability in reducing heavy metal from the contaminated soil by accumulating high load in their body (Baker *et al.*, 1994; Ernst, 1996; Chaney *et al.*, 1997).

Differences in heavy metal tolerance are found among the plants (Punz and Sieghardt, 1993; Malkowski *et al.*, 1996). 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). Various phytoextraction works have been

conducted to reduce heavy metal content from contaminated soils. Ebbs and Kocchian (1998) observed phytoextraction of Zn by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*) and suggested *B. juncea* as an effective plants for phytoextracting Zn particularly after application of the synthetic chelates EDTA to the Zn contaminated soil. Lombi *et al.* (2000) compared the Cd and Zn accumulation capacity in four populations of *Thlaspi caerulescens* and *T. goesingense*, cultivated on selected mine soil in bags and fields. Zn hyperaccumulation in all populations of *T. caerulescens*, showed same ability but were significantly different for Cd accumulation. Cadmium uptake was not decreased by the increased Zn concentration in the substrate and indicated that the mechanisms of Cd and Zn hyperaccumulation are not identical in these species.

1.7. Justification

Uptake, accumulation and detoxification of heavy metals vary with individual plant species. Binding of heavy metals to a variety of organic ligands can cause denaturation of proteins including enzymes, disruption of cell membranes and decomposition of essential metabolites; and many can act as anti-metabolites towards essentials nutrients (Bowen, 1966). Thus the normal physiological processes may change after heavy metal uptake. As morphology is the manifestation of physiology, it is speculated that heavy metal accumulation (Cd, Cu, Pb and Zn) in shoot and root will reflect its effect on morphological characters like living biomass i.e. fresh weight, dry weight, leaf area, shoot length and root length etc. As root is the major absorbing organ, it is hypothesized that root growth will be highly affected with most of the heavy metals. The bioaccumulation of toxic metals is possible by the replacement or displacement of similar metals, which may deplete the essential micronutrients (like Cu, Fe, Mn and Zn) from the plant body and may affect their growth and development. Therefore, to understand these facts, effects of Cd, Cu, Pb and Zn on morphology of some common vegetables of Kathmandu have been considered in the present study.

In higher plants it is speculated that only metals like Cd, Co, Cu, Mn, Mo, Ni and Zn have moderate to high mobility in plants (Streit and Stumm, 1993) and can reach up to the chlorenchyma cells. The excess bioaccumulation of heavy metals in these cells may affect the chlorophyll content which is directly related with productivity in terms of biomass and seed production. Excess availability of metals in soil will certainly increase uptake of these metals.

The excess accumulation of heavy metals forms complexes with ligands having –COOH, –SH, –NH₂ functional group (Singh, 2003) and this possibly replaces essential micro-nutrients (Wildner and Henkel, 1979; Van Assche and Clijsters, 1986; Rivetta *et al.*, 1997). Therefore, it is hypothesized that the excess accumulation of Cd, Cu, Pb and Zn causes deficiency or enrichments of essential micronutrients like Cu, Fe, Mn and Zn, all of which will have direct effect on chlorophyll content (Chl-a, Chl-b and total-chl).

Biomonitoring of toxic metals such as Cu, Pb, Ni, Co, Cr, Cd, Zn, Mn etc studied in ambient air by the use of lower plants (Devkota *et al.*, 1997; Chettri *et al.*, 2001; Shakya *et al.*, 2004) indicated high amount of heavy metal pollution in Kathmandu valley. More than 300 ppm of Pb in the air of municipal streets of Kathmandu valley was recorded (Bhattarai and Shrestha, 1981). The Pb content in the ambient air of the Kathmandu city varied from 0.18 µg/m³ in Maharajung to 0.53 µg/m³ around Royal Palace, 2.6 µg/m³ in Kalimati and 6.08 µg/m³ at Bhotahity (NESS, 1995). Beside this, concentrations of Cu, Cr and Pb ranged from 0.06-0.44, 0.07-0.21 and 0.13-0.40 ppm, respectively, in all water samples of Bagmati River, except at Guheswori and Pashupati area. Level of Cd from Tekudovan (8.25 ppb) and Nakkhu khola (6.07 ppb) were above WHO standard for human drinking water (Gautam and Agrawal, 1994). Bagmati water in urban areas has been highly polluted in last three decades. Water bodies from such polluted river system are heavily used in irrigation for vegetable production in Kathmandu. Based on these facts, it is hypothesized that toxic heavy metals are deposited in the soil of agricultural fields and also in vegetable crops grown on such fields. Therefore, this study was conducted in vegetable growing fields to reveal if the level of toxic heavy metals (such as Cd, Cu, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in soils and vegetables (grown on them) are within the range of critical plant tissue concentration.

Health of some hundreds of thousands of people is endangered only due to heavy metal pollution by increasing anthropogenic emissions which are dangerous because they enter food chain and risk human health (Lantzy and Mackenzie, 1979; Galloway *et al.* 1982; Angelone and Bini, 1992). Slight high concentration of Cu and Zn may pose little risk to human health abstractly, but the soil plant barrier protects the food chain from such element. By itself, Pb and Cd do not show strong phytotoxicity (Adriano, 1986), but consuming the plant having high concentration of Pb and Cd have many adverse effect upon animals. Hence, accumulation of toxic heavy metal in our body may exceed beyond the recommended doses. Therefore it is hypothesized that there is dietary intake of toxic metals (like Pb and Cd) in our daily food

through vegetable. Hence, to estimate the dietary intake of heavy metals through green vegetables, the present study was carried out to understand if the intake of heavy metals is within recommended dose of WHO (1993, 1996). or not.

Study of Sharma and Chettri (2004, 2005) showed high accumulation of heavy metals (Pb and Cd) in soil and vegetables of Kathmandu valley than the normal plant value (Bowen, 1979). Heavy metal contaminated soil will certainly hamper production of vegetables and will invite toxicological problems among the human and animals through food chain. Information about heavy metal accumulation potential among vegetables is important to understand because it will ascertain the best accumulator. On this basis, planning can be made for cultivation of particular vegetable crops in heavy metal contaminated soil. Therefore, to solve the problem of heavy metal contamination in food chain and also to identify the suitable vegetable crops to reduce their toxicity in agricultural fields, screening of vegetable for **phytoremediation and phytoextraction** were conducted in the present study, as the uptake rate vary not only among plant species but also among cultivars (Marschner, 1983; Metz and Kloeke, 1998).

To understand, how the heavy metal contaminated field can be corrected, some experiments were designed using organic and inorganic materials, which are easily available, ecofriendly and also very commonly used by the farmers. 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). It is speculated that the presence of organic matter like cow-dung in the soil will provide humic acid, which will provide binding sites to excess heavy metal ions of the soil. Therefore, it is hypothesized that addition of cow-dung will reduce uptake/accumulation of toxic heavy metal in the body of vegetable crops.

In agricultural fields, soil pH decreases with the use of chemical fertilizers like ammonium sulfate and diammonium phosphates, urea, etc (Brady and Well, 2004). Increased concentration of heavy metals in the soil decreases soil pH (personal observation). Generally plants cannot grow in highly acidic soil due to their root injury (Black, 1973). Soil pH can be neutralized by adding agricultural lime. Hence it was speculated that under increased soil pH condition, the availability of toxic metals will reduce. Therefore, it is hypothesized that addition of lime in heavy metal contaminated soil will reduce heavy metal uptake in plants.

1.8. Objectives

From the previous works of heavy metal monitoring in Kathmandu, it is speculated that the soil is contaminated with heavy metal via fall out. Therefore to understand the concentrations of heavy metals (Cd, Cu, Pb and Zn) in soil and vegetables crops growing on them, the work was carried out with the following objectives.

1. To evaluate uptake of different toxic heavy metals in some of the vegetable crops and its effect on their morphological parameters and chlorophyll content after growing them in soils having different concentrations of metals.
2. To identify which vegetables crops, either leafy ones or the one with modified root system (like fusiform, napiform or conical), will be suitable for biomonitoring metals in the soil.
3. To understand if the optimum concentration of metal which vegetable crops can retain in their body is within the tolerable range for human consumption or not.
4. To monitor the concentrations of different toxic heavy metals in vegetable crops, available in our market and our daily intake, coming from different fields around Kathmandu Valley.
5. To assess the possibilities of immobilization of heavy metal from soil to the vegetables crops by using cow dung and agricultural lime.
6. To assess suitable vegetable species for phytoextraction of heavy metals from heavy metal contaminated soil.

2. LITERATURE REVIEW

2.1. Heavy Metal Uptake and its effects

Kelly *et al.* (1979) studied the influence of soil Cd level on uptake and accumulation in seedlings of *Pinus strobus* L., *Pinus taeda* L., *Liriodendron tulipifera* L., and *Betula alleghaniensis* Britt. The seedlings grown for 17 weeks in field fine sand soil with pretreatment of Cd, Pb, Cu and Zn having concentrations 0.6, 11.4, 2.0 and 20.6 ppm, respectively. The seedlings were transplanted further in soil amended with CdCl₂ - 0, 15 and 100 ppm. All species exhibited increased Cd content in roots and shoots in response to increased soil Cd levels. Besides, shoot elongation and root and shoot dry weights were reduced with increasing levels of soil Cd.

Sawidis and Reiss (1995) studied the effect of heavy metals on pollen tube growth of *Lilium longiflorum* and its ultrastructure. The effects were observed with 3 µM and 100 µM of heavy metals (at 0, 3, 10, 30 and 100 µM), added as chlorides salts to the medium and greatest toxicity were found in Cd²⁺, Cu²⁺ and Hg²⁺, whereas pollen tube germination and growth rate was less affected by Mn²⁺ and the affected tubes showed swelling of the pollen tip region. It was suggested that Cd²⁺ is the only metal which produces effect at the intracellular level and organelle distribution within the tip region that appeared disorganized.

Nagoor (1999) observed the physiological and biochemical responses of cereal (maize) seedlings in graded levels of Cu, Cd and Hg. Altered protein metabolism in terms of protease activity, protein content, amino acid and proline were reported. Copper stimulated the protease activity, lower concentration was effective in embryo while higher concentration produced significant effect in endosperm. Cadmium and Hg decreased the protease activity in embryo and endosperm. The protein content in the embryo decreased during initial period, and then increased proteins in the seedlings after their treatment. Amino acids and proline were accumulated in embryo and endosperm.

Stresty and Rao (1999) studied the ultra structural alterations in response to Zn and Ni stress in the root cells of pigeon pea. The unusual deposition of electron dense globules were

observed in the vacuoles of the root cortical cells at the lower metal concentration and suggested it as a process involved in metal detoxification.

Tatar *et al.* (1999) studied the effect of Pb, Ni and V contamination on organic acid (citric, fumaric and malic) transport in xylem sap of cucumber. The cucumber plants were grown in (a) control nutrient solutions containing iron as Fe (III)- ethylene-diamine-tetracetate (Fe (III)-EDTA), Fe (III) –citrate or FeCl₃ and (b) in nutrient solutions contaminated with Ni, Pb or V in a concentration of 10⁻⁵ M. In the presence of Pb or Ni contamination, the transport of the organic acids became higher by factors 1.1-2.3 in nutrient solutions having Fe (III)–citrate or FeCl₃ compared to the control plants, but the transport of organic acids decreased by 30-40 % in the case of plants growing in solutions having Fe (III)-EDTA. Generally the deviation in the organic acid transport between the contaminated and the control plants were proportional to the heavy metal transport in the xylem in the sequence Ni > Pb > V. It was concluded that the element transport in the plants depend on the chemical form of Fe III used in nutrient solutions.

Scotti *et al.* (1999) observed the effect of fly ash on the availability of Zn, Cu, Ni and Cd to *Cichorium intybus* (chicory). Plant was grown in two soils at pH 5.7 and 7.0, respectively. The soils were augmented with fly-ash and/or metals as salts. In acid soil (pH 5.7) the fly ash addition caused a decrease in uptake of Zn, Cu, Cd and Ni concentration for all treatments. They suggested that added metals were more available to plants than those naturally occurring in soils.

Mihucz *et al.* (2000) investigated the transported of heavy metals ions in xylem sap of cucumber plants by size exclusion chromatography and atomic absorption spectrometry. Plants were grown in hydroponics containing iron as Fe (III)-EDTA, Fe (III) citrate or FeCl₃ and exposed to Pb, Ni and V contamination. The results indicated that Cu and Mn- added to the hydroponics as nutrient elements were determined in the collected fraction during the chromatographic runs and were found transported in the xylem vessels together with small inorganic ions like nitrate ions. In case of Ni, other low molecular weight compounds eluting earlier than the nitrate ions may take part in its transport toward the shoots. Lead was not detected in the above mentioned fractions.

Kozlov *et al.* (2000) studied the root versus canopy uptake of Ni and Cu by birch (*Betula pubescens* subsp. *Czerepanovi*) in an industrially polluted area. The seedlings were transplanted from a control site to clean and metal-contaminated soils to distinguish between aerial contaminations of leaf surfaces of *B. pubescens* by dust particles and root derived contamination in leaves by soluble metals close to Ni-Cu smelters on the Kola Peninsula, northwest Russia.

Patterns of leaf surface contamination and root uptake were similar for Ni and Cu; however, Ni but not Cu was effectively translocated from roots to shoots and leaves. The majority (80-95 %) of Ni and Cu found in birch foliage in the heavily contaminated site was due to deposition of dust particles on leaf surfaces; 32-40 % of foliar Ni and 9-19 % of foliar Cu were in water soluble forms.

Tandon *et al.* (2000) investigated the Cd induced effects on the germinating seeds of gram (*Cicer arietinum* L.). They observed the markedly depressed growth in the seeds receiving different doses of Cd (1, 2 and 5 mM) as compared to control ones and content of Cd in gram seeds increased but Zn content decreased with the increase in Cd dose.

Kupper *et al.* (2000) analyzed the cellular compartmentation of Cd and Zn in relation to other elements in the hyperaccumulator plant, *Arabidopsis halleri* L. Energy-dispersive X-ray microanalysis of frozen-hydrated tissues was used. *Arabidopsis halleri* was found to hyperaccumulate both Zn and Cd in the shoot biomass. Large concentrations of Zn and Cd were found in the leaves and roots, but were very little in flowers. Zinc and Cd were found accumulated in the cell wall of the rhizodermis (root epidermis) in roots (grown hydroponically) and were suggested due to the precipitation of Zn/Cd phosphates. In leaves, the trichomes had by far the largest concentrations of Zn and Cd. The epidermal cells other than trichomes were very small and contained lower concentrations of Zn and Cd than mesophyll cells. In particular, the concentrations of Cd and Zn in the mesophyll cells increased markedly in response to increasing Zn and Cd concentration in the nutrient solution. Mesophyll cells have been suggested to play an important role for the major storage of Zn and Cd hyperaccumulation in leaves of *A. halleri*.

Singh and Aggarwal (2005) studied the effect of heavy metal fertilizer on growth, yield and metal distribution in wheat. The application of heavy metals in soil before sowing caused varying extent of reduction in yields of wheat (*T. aestivum*) cv. HD2285. Hg caused maximum reduction in biological as well as economic yields followed by Cu, Pb and Cd, while Zn did not affect the growth and grain yield of wheat markedly. The number of spikes/pot and grains/ spike were reduced, while 1000 grain weight increased significantly by the application of Cu, Pb and Cd in soil. Accumulation of Zn was higher in grain than in straw. The content of metals in wheat shoots was in the order of Zn > Cu > Cd > Pb. Large proportion of both essential (Cu) and toxic metals (Pb and Cd) absorbed by wheat plants thus remained in straw and as small proportion of the same only transported to grain.

Ladislav *et al.* (2006) investigated the Cd induced inhibition of apoplastic ascorbate oxidase in barley roots. They observed the Cd induced root growth inhibition accompanied by a corresponding loss of plasma membrane integrity in root cells as evaluated by Evans blue uptake by using filter paper technique.

2. 2. Heavy Metals and Chlorophyll Content

Schmidt *et al.* (1997) studied the physiological effects of Cu on Fe acquisition processes in *Plantago lanceolata* L. In plants exposed to low (0.3-0.7 μM) Cu and suboptimal Fe levels, reduction activity at the root surface increased and associated with severe interveinal chlorosis than plants grown in Cu free medium. In Fe-sufficient plants, withholding Cu over a prolonged period slightly enhanced the reduction activity. The results indicated different mechanisms underlying Cu-induced alterations in iron nutrition. From the study it was concluded that Cu can affect Fe nutrition by 1) decreasing the availability of Fe in the medium by displacement of Fe from the chelate molecule, (2) inhibition of induction of Fe stress response and (3) inhibition of a cytoplasmic components or a subunit of the transplasma membrane electron transport chain. In addition blockage of either external or internal Fe binding sites might contribute to the inhibitory effect of Cu on iron nutrition.

Chettri *et al.* (1998) studied the effects of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *C. rangiformis*. The result showed that increased lichen Cu content (upto 1600 $\mu\text{g g}^{-1}$ DW) had no effect on the total chlorophyll content in *C. rangiformis*, but Cu concentrations exceeding 175 $\mu\text{g g}^{-1}$ DW decreased total chlorophyll content in *C. convoluta*. The chlorophyll-a/b ratio of *C. rangiformis* was unaffected by increasing thallus Zn content, whereas an increase in thallus Pb content caused a slight increase in the Chlorophyll a/b ratio. A marked decrease in the ratio of Chl-a/b occurred in Cu accumulated both lichen species. The effects of Cu on chlorophyll were reduced in the presence of Pb and Zn in both lichens, but to a less extent in *C. rangiformis*. Among the cations only Cu is taken up into photobiont cells and suggested that Cu may interfere with the biosynthesis of chlorophyll or cause lipid peroxidation processes in the photosynthetic membranes.

Kupper *et al.* (1998) studied *in-situ* detection of heavy metal substituted chlorophylls in water plants. The *in vivo* substitution of Mg, the central atom of chlorophyll by heavy metals (Hg, Cu, Cd, Ni, Zn and Pb) leads to a breakdown in photosynthesis and is an important damage

mechanism in heavy metal-stressed plants. Among the many methods, they observed a new method for in situ detection that an extreme heterogeneity in the reaction of cells in the same tissue upon heavy metal stress while some cells are already disintegrating, others still show normal fluorescence and photosynthetic activity.

Oncel *et al.* (2000) investigated the interactive effect of temperature and heavy metal stress on the growth and some biochemical compounds in wheat *Triticum aestivum* L.cv.Gerek-79 and Bolal-2973) seedlings. The seeds were treated with Pb and Cd (at 0, 50, 100, 250 and 500 mg l⁻¹) simultaneously applied at various temperatures (8/4, 25/18, 35/26⁰ C). Significant reduction in seedling length and dry weight, chlorophyll, total soluble phenolics, and free proline were observed at high Cd concentrations. Insignificant differences were observed with Pb treatments even at 500 mg l⁻¹. However, Cd showed toxic effect even at such low concentration as 50 mg l⁻¹.

Ernst *et al.* (2000) investigated the combination toxicology of metal (Zn and Cd) enriched soils: physiological responses of a Zn- and Cd- resistant ecotype of *Silene vulgaris* on polymetallic soils. *Silene vulgaris* were grown on 15 polymetallic soils originating from a Cd- Pb -Zn mine at Belgium for a full life-cycle. The degrees of regulation of the metal concentration in the young seedlings were a very reliable indicator of the subsequent plant performance. The Cu concentration in roots and shoots had no thresholds and showed the tendency to increase near linearly with the external Cu soil concentration. Similar behaviour was found for Cd, Mn and Pb. The metal concentration of seeds was the lowest of all plant parts; nevertheless it increased linearly with increasing concentration of Fe and Zn in the soil. From visible symptoms the degree of chlorosis was positively related with the concentration of Zn, but not with that of Cu and interrelated with Fe availability. Phytochelatins (PCs) were present in measurable amounts in leaves of plants grown on soils rich in Cu, but PCs amounts in the early vegetative phase were not be related to vegetative and seed biomass at the reproductive stage. Therefore, it was concluded that PCs were a less reliable indicator of metal toxicity during a full life cycle than the metal concentration of young seedlings.

Singh and Tiwari (2001) investigated that Cd toxicity induced changes plant water relations and oxidative metabolism of *Brassica juncea* L. Although lower and marginal levels of excess Cd (100 and 250 ppm) improved growth but higher levels (500 ppm) caused significant suppression. Significant accumulation of proline (indicator of water stress) occurred at higher level of Cd. Gradual increase in activities of certain antioxidant enzymes such as catalase and peroxidase along with increased lipid peroxidation are suggestive for disturbed oxidative

metabolism. The excess levels of Cd also decreased the concentration of soluble protein and chlorophylls and increased the ratio of chlorophyll a/b.

2.3. Heavy Metal Monitoring in Soil and Higher Plants

Rayappa and Singara Charya (1993) studied pollution tolerance index in some common plants around some major industries in Warangal city, Andhra Pradesh. *Calotropis*, *Jatropha* and *Ocimum* were recognized as very useful plants in reducing atmospheric pollution while *Psidium*, *Terminalia* and *Magnifera* plants were recommended as pollution indicators.

Exlund (1995) studied the Cd and Pb deposition around a Swedish battery plant as recorded in Oak tree rings. The tree ring analysis showed great potential as a tool for the study of both temporal and spatial dimensions of historical Cd and Pb emissions that occurred from 1726 to 1840 around a Swedish alum work. After World War II, the use of leaded petrol in Sweden accelerated the Pb emissions.

Lodenus (1995) studied the bioaccumulation of Cd and Hg in North European forest ecosystems. They suggested that the changes in chemical or biological conditions may increase the concentrations of heavy metals in some part of forest biota, even if the total anthropogenic input is decreasing.

Aksoy and Ozturk (1997) suggested that *Nerium oleander* L. can be used as a biomonitor of heavy metals. Concentrations of Pb, Cd, Zn and Cu were measured in unwashed and washed leaves and soils. Significant correlations between the heavy metal concentration in surface soil and washed leaf samples were observed.

Daniel *et al.* (1997) studied the heavy metal dispersion detected in soils and plants alongside roads in Hungary. The result showed a close correlation between the daily mean volume of traffic and metals accumulated in the soil samples. Plants, near the road, accumulated very high amounts of Pb, Cd and other heavy metals derived from vehicular traffic. Some plants such as *Echinocloa crus-galli*, *Clematis recta*, *Artemesia vulgaris*, *Crepis biennis*, *Portulaca oleracea* and *Lathyrus pratensis* were reported to be the good indicators, as they accumulated three or four different heavy metals in high concentrations.

Jonsson *et al.* (1997) studied the heavy metals of the 20th century recorded in Oak tree rings (*Quercus robur* L.). They analyzed the concentrations of Cd and Pb in the tree rings using AAS and constructed the chronologies from the results with calculated estimates of the

accumulation of these metals in soil. The conspicuous differences between the soil /wood ratios for Cd and Pb imply that a large proportion of the soil Cd is taken up by the tree than is the cases for Pb.

Chettri *et al.* (1997) studied lichens as a tool for biogeochemical prospecting. Content of five heavy metals (Cu, Mn, Pb, Zn and Cr) in lichens and vascular plants were compared from abandoned Cu mining area, Gerkario and Megali Panagia in North Greece. Out of five metals, four metal Cu, Pb, Mn and Cr in the epigeic lichen *Cladonia convoluta*, two (Cu and Pb) in both epilithic lichen, and one metal (Pb) in vascular plant *Minuartia* (root) were significantly ($P < 0.05$) correlated between the metal content in plant tissue and in soil. Further, discoloration of *C. convoluta* with higher concentrations adds a visible clue for the biogeochemical exploration.

Breulmann *et al.* (1998) studied the chemical characterization of Diptercarpaceae by use of chemical fingerprintings– a multielemental approach at Sarawak, Malaysia. Altogether 44 chemical elements were analyzed. The different compartments showed considerably different element concentrations with most elements showing the highest mean concentration in the leaves or twigs and the lowest mean concentration in the trunks. The leaves of saplings showed higher concentration for most elements (Co, Cr, Fe, Mn, Ni and Pb) than in the twigs, trunks and roots than of mature tree, but Fe and Mn had higher concentrations in mature roots than saplings.

Lee and Kim (1998) studied the heavy metals of rainfall and soil at industrial, urban and rural area. Positive correlation of heavy metals (Zn, Pb and Cu) between rainfall and soil contamination in the forest was reported.

Khasem and Singh (1999) studied the heavy metal contamination of soil and vegetation in the vicinity of industries in Bangladesh. They collected soils, grass (*Cynodon dactylon* L.), water hyacinth (*Eichhornia crassipes* L.), rice (*Oryza sativa* L.) and arum (*Alocasia esculenta* L.) from tannery, ceramic, textile, dying and sulphuric acid producing industrial sites. The concentrations of total Cu, Mn, Ni, Pb and Zn decreased with increasing distance from the disposal points of the tannery and the textile dying industries. Besides Cd, Cu, Mn, Ni, Pb and Zn showed highly significant ($P < 0.01$) positive correlation with their total and DTPA-extractable contents in soils. The concentrations of most of heavy metals were also higher in the vegetation samples of tannery and the content of Pb in grass samples exceeded the toxic limit.

Zalidis *et al.* (1999) studied the forms and distribution of heavy metals in soils of the Axios Delta of Northern, Greece. Very low exchangeable forms of the heavy metals in soils indicated the availability of the heavy metals to plants at a minimum. Most of the heavy metals occurred in

forms which were considered immobile constituents of inorganic minerals or carbonates compounds. Only Cu and Zn present in appreciable quantities as organically-based forms that can become potentially available under certain conditions. The significant relationships between the structural components of the various heavy metals (e.g. Mn-Cu; Mn-Zn; Mn-Cr; Mn -Pb) indicated close relationships of the immobile fractions of the Cu, Zn, Cr and Pb in the retention or adsorption on Mn-oxides.

Barman and Ray (1999) studied the uptake of Cd, Cu, Zn and Ni in different varieties of rice for a comparative study of the mature plants grown in polluted (Kalipur) and unpolluted (Sriniketan) fields. The concentration of Cd, Cu, Zn and Ni reached critical in plant tissues concentration and suggested no rice cultivation in polluted fields.

Egli *et al.* (1999) observed the changes in heavy metal contents in an acidic forest soil affected by depletion of soil organic matter within the time span 1969-1993 in a forested ecosystem near Mohlin, North Western-Switzerland. The Pb content primarily correlated with organic matter (with a significant decrease in the sub soil). Good correlations were also found with Fe and partially correlate with Al and Mn. Cd correlates well with pH, (earth) alkali ions, and generally to a lower degree with Mn, Fe and Al. Zinc showed an intermediate position having significant correlations with organic C, earth-alkali ions, Fe, Al, and Mn but were very weakly to soil pH. The main transportation mechanisms of Pb in the subsoil were believed to be primarily in colloidal form.

Gratton *et al.* (2000) observed the heavy metal accumulation in soil and *Pinus banksiana* (jack pine) needles from Sudbury, Ontario, Canada. The site was known for the mining and smelting of high sulphides ores containing Ni, Cu and Fe and precious metals. Significant positive correlations between Cu and Co ($r=0.77$, $P = 0.05$) and Ni and Co ($r = 0.74$, $P = 0.05$) were observed. Significant correlation between Cu and Ni concentration ($r = 0.82$, $P = 0.05$) suggested a uniform deposition and plant uptake patterns.

Han *et al.* (2000) studied the accumulation of heavy metals in a long-term poultry waste-amended soil. Cu and Zn accumulated close to the soil surface where the total amount of Cu and Zn in waste-amended soils were significantly higher than in non-amended soils. The total metal concentrations in amended soils were not critically high. Cu in the amended soil was present mostly in the organic matter fraction (46.9 %), whereas Zn was found in the easily reducible oxide (ERO) fraction (47.3 %). They suggested that Cu and Zn in this long term amended soil are potentially bioavailable and mobile.

Pyatt (2001) studied the Cu and Pb bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in sites contaminated as consequences of extensive ancient mining activities in Cyprus. Waste resultant from Cu mining activities of approximately two millennia ago continues to exert an important influence on organisms. The bioaccumulation and biomagnifications of Pb and S are particularly marked in *Acacia* and *Eucalyptus*. Pod of *Acacia* and the fruit capsule of *Eucalyptus* showed an enhanced metal loading; but the values in the seeds were much reduced. The seeds of *Acacia* differ chemically from those of *Eucalyptus*. The importance of these plants was realized as biomonitors to evaluate environmental quality.

Peng Kejean *et al.* (2006) studied the vegetation compositions and heavy metal uptake by wild plants at three contaminated sites in Xiangl area, China. The highest concentrations of Cd (287 mg kg^{-1}) in the leaves of *Lobelia chinensis* Lour. at the Datianwan site, followed by of *Solanum nigrum* L. with (99 mg kg^{-1} Cd) in the leaves and suggested these plants as potential Cd hyperaccumulators.

Kang Sing-Fen *et al.* (2006) studied the effects of traffic pollution on urban soils and plants. Soil samples from the road sides were significantly higher in electric conductivity and organic carbon content but lower in pH compared to those from the parks (away from the main trunk road in Lanzhou city of northwest China). Soil samples from the roadsides with significantly higher contents of Zn, Cd, Hg, Pb, Cu, Cr and As than those from the parks, and suggested a considerable accumulation of these elements due to traffic pollution. Leaf samples from the road sides with significantly higher contents of Zn, Cd, As, Hg, Pb Ni, Co, Cr and N than those from the parks, indicated that traffic pollution result in a considerable accumulation of these elements in leaves of *Sophora japonica* growing in the roadside soils.

El-Hasan *et al.* (2006) studied the distribution of heavy metals in urban street dusts of Karak city, Jordan. The distribution and higher concentration of heavy metal (Pb, Cu, Zn, Ni, Fe, Cr, Cd and Mn) in city urban areas, showed automobile originated main sources of pollution such as emissions, and wear and tear of automobiles.

2.4. Heavy Metals and Dietary Intake

Anke *et al.* (1994) studied Cd in feed and foodstuffs from Germany and observed that leafy vegetables (parsley, cabbage, lettuce) store particularly much Cd compared to bulbs, fruit and the tubers (potatoes) of root and scions (carrots, kohlrabi). The leafy vegetables, several thickened

stems and roots as well as mushrooms in particular delivered much Cd into the food chains of humans than relatively little Cd from fruit and tubers (potatoes).

Muller *et al.* (1996) studied the oral Cd exposure of adults in Germany. They analyzed the Cd contents of foodstuffs, typical of German eating habits. The investigated vegetables include potatoes which had Cd concentration $< 25 \text{ ng g}^{-1}$, however, individual samples of lettuce showed very high Cd levels.

Anke *et al.* (1998) suggested anthropogenic Cd exposure of a habitat transferred to herbivores and predatory animals and lifted to a higher trophic level. Cd exposures were registered between the sixties and eighties in several German regions.

Carlosena *et al.* (1999) classified the edible vegetables affected by different traffic intensities by using potential curves. Lead, Cd and Cu found were suggested to be the main metallic pollution tracers along the roadside soil pollution by the impact of motorized traffic in the vicinity of motorways. Other vegetables obtained were explained as a function of both the agricultural conditions (private versus commercial) and the different traffic intensities supported by the cultivated areas.

Huttner *et al.* (1999) surveyed the chromosomal aberrations in humans as genetic endpoints to assess the impact of pollution. The impact of pollution in two different populations using chromosomal aberrations was reported in human peripheral blood lymphocytes as a biomarker of chronic exposure to heavy metals. Significant increase in the frequency of chromosomal aberrations in human peripheral blood lymphocytes were observed in those that are exposed to heavy metals (Pb, Cu and Cd) pollution.

Mielke *et al.* (1999) evaluated the urban environment and children's health: soils as an integrator of Pb, Zn and Cd in New Orleans, Louisiana, USA. A significant association ($P=1.2 \times 10^{-23}$) was found between median blood Pb and medial soil Pb. They suggested soil Pb is a useful diagnostic tool, and curtailing soil Pb may complement primary Pb prevention for children.

2. 5. Phytoextraction and bioremediation

Gloaguen and Morvan (1997) studied the removal of heavy metals ions from aqueous solution by modified barks (*Picea*, *Pinus*, *Pseudotsuga*, *Larix*, *Tectona* and *Afzelia*). The use of

bark in removing Pb^{2+} , Zn^{2+} , Cr^{2+} , Fe^{2+} and Cu^{2+} suggested due to exchanges against protons on the bark substrate that presumably contain carboxyl groups in both pectin and tannin compounds.

Ebbs and Kochian (1998) observed phytoextraction of Zn by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*). The phytoextraction of Zn was effective particularly after the application of synthetic chelates EDTA to the Zn contaminated soil. A hydroponics screening of 22 grass species indicated that oat and barley tolerated the high Cu, Cd and Zn concentrations present in the solutions and also accumulated elevated concentration of these metals in the plant shoots. Hydroponic experiments comparing these 2 grasses to Indian mustard indicated that, although shoot Zn concentration were greater for Indian mustard, the grasses were considerably more tolerant. A pot experiments conducted using a Zn-contaminated soil showed that the addition of EDTA to the soil significantly increased Zn accumulation by *B. juncea* but not by oat or barley. Nevertheless, barley accumulated > 2 mg of Zn plant⁻¹, 2-4 times more Zn than in Indian mustard in the presence of EDTA. The results suggested that barley has a phytoremediation potential equal to, if not greater than that for *B. juncea*. The 10 times more biomass production by *B. juncea* than *T. caerulescens* suggested that a greater shoot biomass can extract more heavy metals from soil than the plants having lower biomass and the plant species suitable for phytoremediation may not be limited to hyperaccumulator.

Jang *et al.* (1998) studied the Batch and column tests for the development of an immobilization technology for toxic heavy metals in contaminated soils of closed mines. They evaluated the laboratory tests of various treatments for the immobilization of Cu, Ni and Pb in soils typically found at mine facilities. Based on their results of the column leaching test, it was found that the degree of heavy metal leaching was highly dependent on pH.

Ciba *et al.* (1999) removed metals like Cd, Co, Cu, Mn, Ni Pb and Zn from the compost, prepared from the organic fraction of municipal wastes. They determined the metal content in compost using Rudd's method of sequential extraction. The investigation also concerned the way of removing these metals by means of leaching with solutions of sulphuric and nitric acid, as well as by electrochemically separating the metals from the solution after the compost had been leached with sulphuric acid. The application of the electrochemical method with varying pH values of the leaching solution within the range of 6.8-2.8 allowed for the separation of 83.5 % Cd, 55.0 % Co, 65.4 % Cu, 59.4 % Mn, 70.3 % Ni, 90.5 % Pb and 56.2 % Zn.

Grimes *et al.* (1999) studied the availability and binding of heavy metals in compost derived from household waste. Leachability data were used to determine the environmental availability of Cd, Cu, Pb and Zn contained in natural compost while batch sorption data to determine uptake of additional Cd, Cu, Pb and Zn by compost and assess its potential use in remediation work, as an alternative to natural materials such as peat. The relative binding of these additional metals to compost was found to be in the order $Pb > Cd > Cu > Zn$. They suggested that the sorption of metals on compost took place, at least in parts, by exchange of Ca bound to the compost, with the evidence that the sorption occurred in both humic and non-humic sites in the compost.

Vulava and Seaman (2000) observed the mobilization of Pb from highly weathered porous material by extracting agents. To evaluate the influence of clay mineralogy and soil organic matter present in two highly weathered surface and sub surfaces, soil materials on Pb sorption and subsequent extraction using Ethylene-Diamine Tetra Acetic Acid (EDTA) and carboxymethyl -cyclodextrin (CMCD) were conducted by batch and dynamic leaching experiments. The presence of strongly adsorbing Pb^{2+} and excess H^+ increased the positive charge on amphoteric mineral surfaces and reduced the negative charge on clay minerals, which induced selective dispersion and transport of Fe oxides. Nearly 100 % of residual Pb was extracted from the surface soil by EDTA as compared to about 56 % removal by CMCD under similar conditions. About 80 % of residual Pb was removed by EDTA from the Pb contaminated subsurface material but less than 2 % of residual Pb was extracted by CMCD under similar conditions.

Theodoratos *et al.* (2000) investigated the use of the municipal sewage sludge for the stabilization of soil contaminated by mining activities in Lavrion Greece. Application of the US EPA toxicity characteristic leaching procedure (TCLP) on the stabilized mixtures proved that Pb, Zn and Cd solubility was reduced by 84 %, 64 % and 76 %, respectively, at 15 % w/w sludge addition, while 10 % w/w addition was sufficient to reduce Pb solubility below the US EPA TCLP regulatory limit. Dwarf beans when grown in the sewage treated soil showed a positive effect on their growth. Further, Pb and Zn uptake of plant leaves and roots were reduced, while Cd uptake was unaffected. The result supported that municipal sewage sludge was a potential effective stabilizing agent for contaminated soil containing Pb, Zn and Cd.

Lombi *et al.* (2001) investigated the physiological evidences for a high-affinity Cd transporter highly expressed in a *Thlaspi caerulescens* ecotype Ganges (Southern France) and

Prayan (Belgium). Uptake kinetics and translocation characteristics of Cd and Zn were presented for two contrasting ecotypes of the Cd/Zn hyperaccumulator. The results showed a considerable scope for selecting hyperaccumulator ecotypes to achieve higher phytoextraction efficiencies.

Vengris *et al.* (2001) investigated the electrokinetic remediation of Pb-, Zn- and Cd-contaminated soil (under laboratory-scale conditions). Soil extracts of heavy metals (by 1M HCl solution) when analyzed by optical emissions spectrometry, the efficiency of electrochemical remediation was partially dependent on the soil pH. With pH increase, the migration of heavy metals ions towards the cathode was limited. When acetic acid was added to the sandy soil, almost complete remediation was achieved. A clay layer inserted in the cathode area did enhance the remediation rate. The most effective clean up was achieved for Zn and Cd, with less effective clean up being achieved for Pb. They suggested that clay could be used as immobilizing media for heavy metal ions by electro-kinetic remediation of various soils.

Katia *et al.* (2005) investigated that nicotianamine over accumulation confers resistance to Ni in *Arabidopsis thaliana*. Nicotianamine is a methionine derivative involved in iron homeostasis, able to bind various other metals in vitro. The results opened new perceptives for the modulation of nicotianamine content in plants for phytoremediation.

McBride (2005) studied the Mo and Cu uptake by forage grasses and legumes grown on a metal-contaminated sludge site and concluded that Mo, Cu and sulfur (S) bioavailability remains elevated in the soil for several decades after sewage sludge application.

Polatajko *et al.* (2006) suggested that Se is present in *Allium* sps. and is an essential trace element (for human/animals) to play a putative role in the prevention of cancer and other illnesses.

Skorzynska-Polit, *et al.* (2006) studied the activity and localization of lipooxygenases in *Arabidopsis thaliana* under Cd and Cu stresses. The change in ultra-structure of the leaf parenchyma cells were more evident in plants treated with Cd than those treated with Cu.

Saleh Al-G *et al.* (2006) observed the increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium. They suggested it as a new approach to increase the heavy metal tolerance of legumes plants.

With the advancement of time, there have been a lot of research and publications in the field of responses of heavy metals. Some of the “**Review Articles**” are: “*Microorganisms and heavy metal toxicity*” (Gadd and Griffiths, 1978), “*Some aspects of interactions between heavy*

metals and plant mineral nutrients” (Sidlecka, 1995), “*Soil and human health*” (Oliver, 1997), “*The genetic effects of environmental lead*” (Johnson, 1998), “*Metal and micronutrients food safety issues*” (McLaughlin *et al.*, 1999), “*Plant and rhizosphere processes involved in phytoremediation of metal contaminated soil*” (McGrath *et al.*, 2001), “*Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization*” (Schutzendubel and Polle, 2002), “*Cellular mechanisms for heavy metals detoxification and tolerance*” (Hall, 2002), “*Salt tolerance and salinity effects on plants with emphasis on physiological biochemical and molecular mechanism*” (Parida and Das, 2005).

Similarly, several well-known “**Books**” as mentioned below were dealt during the research study: “**Soil and Plant Relationships**” (Black, 1973), “**Diagnostic Criteria for Plants and Soil**” (Chapman, 1975) “**Vegetable Crops**”, (Thompson and Kelly, 1979), “**Trace Elements in the Terrestrial Environments**” (Adriano, 1986), “**Plant as Biomonitors for Heavy Metals in the Terrestrial Environments**” (Markert, 1993), “**The Origin of Heavy Metals in Soil**” (Alloway, 1995), “**Mineral Nutrition of Higher Plants**” (Marschner, 1995), “**Fundamentals of Environmental Pollution**” (Kannan, 1997), “**Environmental Chemistry**” (De A. K., 2002), “**Chemistry for Environmental Engineering and Sciences**” (Sawyer *et al.*, 2003), “**Stress Physiology**” (Singh, 2003), “**A Text book of Environmental Chemistry and Pollution Control**” (Dara, 2004), “**The Nature and Properties of Soil**” (Brady and Well, 2004), “**Plant Ecology**” (Schulze *et al.*, 2005), “**Fundamentals of Plant Physiology**” (Jain, 2007).

3. MATERIALS AND METHODS

To understand responses of some vegetable crops to toxic heavy metals different experiments like uptake of heavy metals and its impact on morphological parameters and chlorophyll were performed. Vegetables like *Brassica juncea* var *cuneifolia* L. (broad leaf mustard), *Brassica rapa* L. (turnip), *Lactuca sativa* L. (lettuce), *Lepidium sativum* L. (cress leaf) and *Daucus carota* L. (carrot) were considered for these experiments. Virgin sandy loam soil were collected from Bhaktapur and were amended with heavy metal salts like CdCl_2 , CuCl_2 , $\text{Pb}(\text{NO}_3)_2$, and ZnCl_2 for the above experiments. All the experiments (except monitoring) were conducted in polyethylene bags (10X12") on the terrace of Amrit Campus, Thamel, Kathmandu during 2002/2003. For monitoring and dietary intake (of heavy metals) experiments, samples of soil and vegetables (grown on them) were collected from different biotops of Kathmandu valley. To evaluate phytoremediation, virgin sandy loam soil collected from Bhaktapur were contaminated with heavy metal salts like CuCl_2 , $\text{Pb}(\text{NO}_3)_2$, and ZnCl_2 . Altogether 18 different vegetables were tested for phytoremediation.

3. 1. Uptake of Heavy Metals and Morphological Parameters

3. 1.1. Soil Preparation and Metal Treatment

Sandy clay soil collected from Thimi area was mixed with poultry waste in 10:1 ratio and was amended one month before the seed sowing. Concentrations of heavy metals in the present study were based on the regulatory limit of heavy metals in soil (according to Hungarian limit of agricultural soils, see Kovacs *et al.*, 1993). Soil was treated with 1, 2, 10 and 20 mg kg^{-1} CdCl_2 ; 50, 100, 500 and 1000 mg kg^{-1} of CuCl_2 ; 50, 100, 500 and 1000 mg kg^{-1} of $\text{Pb}(\text{NO}_3)_2$ and 150, 300, 1500 and 3000 mg kg^{-1} of ZnCl_2 ; and 251 (1+50+50+150), 502 (2+100+100+300), 2510 (10+500+500+1500) and 5020 mg kg^{-1} (20+1000+1000+3000) of mixed metals salt ($\text{CdCl}_2 + \text{CuCl}_2 + \text{Pb}(\text{NO}_3)_2 + \text{ZnCl}_2$) concentration. Solution of each heavy metal salt in single and mixed treatments were mixed thoroughly in 3.5 kg soil per bag in all cases except *Daucus carota*. Due to presence of elongated modified taproot, 5 kg soil/bag was considered for the growth of *D. carota*.

3.1. 2. Vegetable Seed Selection and Experimental Design

Certified seeds of winter vegetable crops were purchased from local store Annapurna Beej Bhandar, Kathmandu and were sown in the month of September. The seeds of vegetable crops like *Brassica juncea* var *cuneifolia* L. (broad leaf mustard), *Brassica rapa* L. (turnip), *Lactuca sativa* L. (lettuce), *Lepidium sativum* L. (cress leaf) and *Daucus carota* L. (carrot) were selected due to their common cultivation in Kathmandu valley and their high demand for the consumption among Kathmandu dwellers.

The experimental bags were arranged in randomized block design with 5 replicates for each treatment. After 2 weeks of germination 3-4 plants per bag were maintained and were irrigated regularly with optimum amount of water (to avoid flooding and leaching of metals). Germination of vegetable seeds was recorded up to 15 days. Morphological parameters like root length (RL), shoot length (SL), fresh weight (FW), dry weight (DW), dry weight percent (DW %) and leaf area (or leaf length in case of *L. sativum*) were recorded after 3 weeks and 7 weeks of seed sowing. Plants collected after 3 weeks have been considered as young and those collected after 7 weeks i.e. on a normal harvest time are considered as matured. In case of *L. sativa* and *D. carota*, all plants were harvested after 7 weeks only.

3.1.3. Sample Preparation for Metal Analysis and Soil pH

All studied vegetables after harvest were washed thoroughly with tap water. Measurements like RL, SL, FW and DW of whole plant were recorded. Vegetable crops were air-dried for 2-3 days, and then oven dried at 60 to 70⁰ C up to constant DW. Root and shoot of dried vegetables were homogenized separately and stored for metal analysis. Similarly, soil samples collected from 5 replicate bags having same metal concentration were sieved and homogenized to obtain a representative samples for measuring soil pH and heavy metals. Soil pH was measured using Deluxe pH meter (Model 101). The ratio of water: soil used for pH measurements were 2.5:1 by volume.

3.1.4. Digestion and Metal Analysis

Representative samples (1 g DW) of shoot and root of each treatment were dipped in 8 ml concentrated HNO₃ (Merck). These were left overnight at room temperature. On the next day, the mixture was warmed for 2 h at 50⁰ C and subsequently heated at 160⁰ C for 4 h. The cooled extracts were filtered through Ashless filter paper (Whatman 589³) and were diluted to prepare

25 ml with double de-ionized water (Sawidis *et al.*, 1995). The filtrates were then analyzed for Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb and Zn by using Perkin Elmer (2380) Atomic Absorption Spectrometer (AAS) at wavelengths 228.8 nm for Cd, 240.7 nm for Co, 357.9 nm for Cr, 324.7 nm for Cu, 248.3 nm for Fe, 279.5 nm for Mn, 232.0 nm for Ni, 283.3 nm for Pb, and 213.9 nm for Zn (Welz, 1985). Two plant materials of National Bureau of Standards (USA) each with Nos. 1573 (Tomato leaves) and 1575 (Pine needles) were analyzed following the same procedure and the metal recoveries obtained were 92.7 %, 95.5 %, 94.2 % and 97.5 % for Cd, Cu, Pb and Zn respectively. The relative standard deviation of the measurements was 8.7 % for Cd, 2.5 % for Cu, 8.2 % for Pb and 3.6 % for Zn.

3.1.5. Statistical Analysis

To understand the significant effect of different concentrations of heavy metals in single and their mixed metals on morphological parameters, data of RL, SL, FW, DW, DW %, leaf area/or length were statistically analyzed by using non-parametric Kruskal- Wallis test (using SPSS 12.0 for windows version). This test was considered because of large variation of data within a group.

3. 2. Chlorophyll Content

The leaves of vegetable crops such as *B. juncea*, *B. rapa*, *L. sativa*, *L. sativum* and *D. carota* which were grown on soil treated with different concentrations of each CdCl_2 , CuCl_2 , $\text{Pb}(\text{NO}_3)_2$, ZnCl_2 in single and their mixed metal concentrations from uptake experiments were tested for the measurement of chlorophyll contents. Chlorophyll estimation from the leaf was conducted when the plants were 6 weeks old. Metal content in the shoot portion of the vegetable crops from the uptake experiments were also used for the investigation of impact of heavy metals (such as Cd, Cu, Pb and Zn) on the chlorophyll content, as shoot mainly consists of leaf only in these vegetables at this age.

3.2.1. Chlorophyll Extraction

Chlorophylls were extracted from 2nd top leaf from each vegetable crop (*B. juncea*, *B. rapa*, *L. sativa*, *L. sativum* and *D. carota*) of 6 weeks old age. Approximately 40 mg FW of leaf tip was dipped in 3 ml of Dimethyl Sulphoxide (DMSO) which was mixed with a pinch of

polyvinyl polypyrrolidone (PVPP). The triplicate test tubes were heated inside the water bath for continuous 2 h at a temperature of 70⁰ C in the dark. Maximum absorbance of chlorophyll a (Chl-a) and Chlorophyll b (Chl-b) were measured at 648.2 nm and 664.9 nm using spectrophotometer, respectively. Chlorophyll (Chl-a, Chl-b and total-chl) concentrations were calculated using the extinction coefficients and equations given by Barnes *et al.* (1992).

$$\text{Chl-a} = 14.85 A_{664.9} - 5.14 A_{648.2}$$

$$\text{Chl-b} = 25.48 A_{648.2} - 7.36 A_{664.9}$$

$$\text{Total-chl} = 7.49 A_{664.9} + 20.34 A_{648.2}$$

3.2.2. Statistical Analysis

To study the difference in the chlorophyll concentrations (Chl-a, Chl-b, total-chl and Chl-a/b) of vegetable crops after their growth in soil treated with different concentrations of heavy metals in single and mixed metals were compared using one way analysis of variance (ANOVA) followed by Duncan's multiple range test.

3.3. Monitoring of Vegetables and Soil Samples

3.3.1. Study Area

Kathmandu is a bowl shaped valley surrounded by high foot-hills above 2700 m elevations at its periphery. Hence, it restricts the sweeping off of anthropogenic emissions (emitted from heavy traffic, various factories and brick kilns) of heavy metals from the atmosphere of Kathmandu. Kathmandu is regarded as the hot spot from pollution point of view due to high atmospheric pollution (IUCN, 1991a, b; Jha and Lekhak, 2003).

Water of Bagmati River in urban areas has been polluted highly in last one decade due to draining of waste water from houses and industries into the river. The Kathmandu drains around 60 million liters of polluted water per day and only 7 % of the contamination is caused by industries (personnel communication).

The agricultural fields in Kathmandu valley are fertile with soil type- sandy loam. As the population of Kathmandu is increasing, demand of green vegetables is also increasing. To meet the demand, farmers are motivated to use more chemical fertilizers like sulphates, urea, complex

compounds, Di-ammonium phosphate, potash, Tri-nitrogen super-phosphate (CBS, 1990) and even the herbicides and pesticides (personnel communications).

The soil and vegetable samples were collected directly from 12 different sampling sites (i.e. fields) located near the main riverside of Kathmandu valley (**Fig. 1**). Sampling sites, such as Banasthali, Shovabhadrawati and Khusibun are located near Bishnumati River; Chapro, Thimi, Manohara near Manohara River; Shankhamul, Kusingal and Nakhu near Bagmati River; Kalopul and Ghattekulo near Dhobikhola; and Balkhu near Balkhu Khola.

3.3.2. Soil and Vegetables Samples

Soil and vegetable samples were collected from 2002/3/14 to 2002/5/29 from fields especially growing for commercial purpose. List of biotops and vegetable crops collected is given in **Table 3**. The vegetables and soil samples collected from field were kept in white polythene bags during transportation. Representative soil samples of each sampling site were obtained by mixing soil samples collected from 10 different points, each from horizontal slices of 10-cm depth and an area 10X10 cm². The soil samples were homogenized into representative samples of each field and oven dried at 70⁰ C – 100⁰ C for 72 h till constant DW and then sieved with 0.25 mm mesh to obtain uniform soil particles for metal analysis.

All collected vegetable samples were washed thoroughly with clean tap water, and lastly with de-ionized water to remove all the outer impurities. They were air-dried for 2-3 days at room temperature and then oven dried at 70⁰ C for 72-96 h till constant DW. The oven dried 10-20 plants of each vegetable crop from each site were separated in shoot (aerial portion) and root portion which were then homogenized for representative samples for each species/sampling sites.

3.3.3. Soil Parameters

Homogenized representative soil samples collected were tested in National Agricultural Research Center (NARC), Lalitpur for soil pH, organic matter, N, P, K and soil texture. Percentage of organic matter was measured by Walkey-Black method. Total nitrogen (N %) from soil was determined by Kjeldahl method. Available phosphorous (kg/ha) were extracted with 0.5 NaHCO₃, pH 8.5 (Olsen *et al.*, 1954), potassium (kg/ha) extracted with 1M CH₃COONH₄, pH 7.0 (Cahoon, 1974). Soil pH was measured by using electronic pH meter (ratio of water: soil used were 2.5:1 by volume). Soil texture was measured by sieving the soil particles from different size of the mesh.

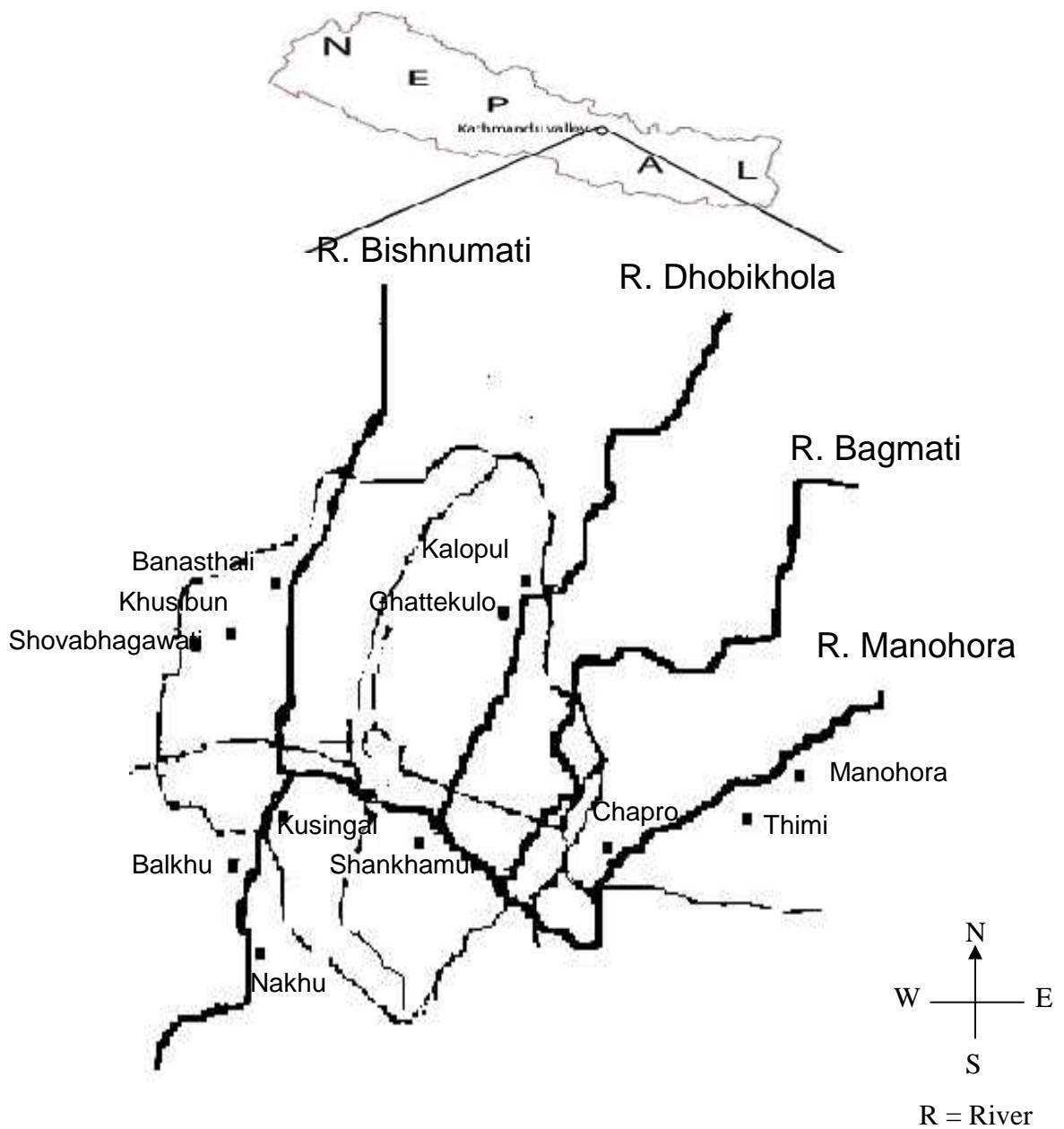


Fig. 1. Sampling sites in Kathmandu Valley for monitoring heavy metals in soil and vegetables

Table 3. Sampling sites and vegetable crops collected for monitoring heavy metals in Kathmandu valley.

Places	Biotops No.	Vegetable crops
Thimi	28	<i>Brassica juncea</i> var. <i>cuneifolia</i> (broad leaf mustard); <i>Brassica rapa</i> L. (turnip); <i>Lepidium sativum</i> L. (cress leaf); <i>Raphanus sativus</i> L. (radish); <i>Spinacia oleracea</i> L. (spinach Gobre); <i>Daucus carota</i> L. (carrot).
Manohora	12	<i>B. juncea</i> L.; <i>B. rapa</i> L.; <i>L. sativum</i> L.; <i>S. oleracea</i> L.; <i>Solanum tuberosum</i> L.; <i>D. carota</i> L.
Shankhamul	65	<i>B. juncea</i> L.; <i>S. oleracea</i> L. (Spinach Patane); <i>D. carota</i> L.
Ghattekulo	52	<i>B. juncea</i> L.; <i>R. sativus</i> L.; <i>D. carota</i> L.;
Kalopul	48	<i>B. juncea</i> L.
Banasthali	4	<i>B. juncea</i> L.; <i>D. carota</i> L.; <i>S. oleracea</i>
Kusingal	72	<i>B. juncea</i> L.; <i>B. rapa</i> L., <i>R. sativus</i> L.
Shovabhagawati	79	<i>B. juncea</i> L.; <i>R. sativus</i> L.; <i>S. oleracea</i> L.
Khusibun	88	<i>B. juncea</i> L.; <i>R. sativus</i> L.; <i>L. sativum</i> L.
Balkhu	95	<i>B. juncea</i> L.
Chapro	8	<i>D. carota</i> L, <i>S. oleracea</i> L. (spinach)
Panchkhal	-	<i>Solanum tuberosum</i> L.
Sankhu	-	<i>S. tuberosum</i> L.

3.3.4. Analysis of Heavy Metals

Metal analysis of representative soil and vegetable samples were performed as mentioned in uptake experiments. In the case of monitoring of heavy metals in soil and vegetables, contents of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were analyzed.

3.3.5. Statistical Analysis

To understand the relation between different heavy metals and the soil properties in collected soil samples, Pearson correlation coefficient test was conducted. Significance level was also calculated using paired t-test. Correlation between concentrations of heavy metals in soil and their respective accumulation in vegetable parts collected from the same biotops was also calculated.

3.4. Dietary Intake of Heavy Metal via Vegetables

The vegetable crops such as *B. juncea*, *B. rapa*, *L. sativum*, *R. sativus*, *S. oleracea*, *S. tuberosum* (tuber) and *D. carota* from different biotops as in monitoring study were collected from Kathmandu valley. To mimic the exact (household preparation) conditions before cooking, vegetables parts like leaves of *B. juncea*, *L. sativum* and *S. oleracea*; roots of *B. rapa*, *D. carota* and *R. sativus* and tuber of *S. tuberosum* were thoroughly washed with clean tap water and peeled off (if needed). Fresh weight of individual plant from each vegetable crop species were measured and then were air-dried for 2-3 days at room temperatures and then oven dried at 70⁰-80⁰ C for 72-96 h till constant DW. Dried parts of each vegetable samples were homogenized by using mortar and pestle, and representative samples were prepared for metal analysis. Metal analysis of each vegetable sample was performed as mentioned above in uptake experiment.

3.4.1. Estimation of Heavy Metal Intake

Daily dietary intake of heavy metals from vegetables was calculated on the basis of vegetables consumption survey among 100 households of Kathmandu valley. Daily intakes of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn concentration via fresh vegetables (*B. juncea*, *B. rapa*, *R. sativus*, *L. sativum*, *S. oleracea*, *D. carota* and *S. tuberosum*) were calculated on the basis of 110 g FW day. Concentrations of metals in the fresh vegetables were calculated on the basis of the DW and FW relation of each vegetable crop as performed by Voutsas and Samara (1998).

The estimated intake of potentially toxic elements (Cd, Co, Ni and Pb) were compared with WHO Provisional Tolerable Daily Intakes (PTDIs) (WHO 1993, 1996). The essential elements like Cu, Cr, Fe, Mn and Zn were compared with recommended daily intakes (RDIs) from Daily Dietary Allowances (RDAs) or the Estimated Safe and Adequate Daily Dietary Intakes as recommended by National Research Council (1989).

3. 5. Phytoremediation

3.5.1. Soil Preparation

To mitigate the problem of soil contamination by heavy metals such as CuCl_2 , $\text{Pb}(\text{NO}_3)_2$, and ZnCl_2 in single and their mixed metals contamination, 3 different sets of experiment were designed to understand the accumulation potential of Cu, Pb and Zn in 18 different vegetable crops as well as to reduce the uptake of heavy metal accumulation in edible plants from heavy metal contaminated soil.

a) To understand the accumulation ability of vegetable crops, screening of phytoextraction of heavy metals such as Cu, Pb and Zn, were performed in 1st sets of experimental bags which have sandy loam soil and were artificially contaminated each with $300 \text{ mg kg}^{-1} \text{ CuCl}_2$ / $500 \text{ mg kg}^{-1} \text{ Pb}(\text{NO}_3)_2$ / $800 \text{ mg kg}^{-1} \text{ ZnCl}_2$ / or their mixed concentration ($=1600 \text{ mg kg}^{-1} \text{ soil}$). This set was also considered as control for sets (b) and (c). Each bag was filled up with 3.5 kg soil.

b) For the remedial measures of heavy metals (Cu, Pb and Zn from their single and mixed metals) contaminated soils by using organic manure, 2nd sets of experimental bags were filled with sandy loam soil amended with cow dung (by weight) at 5 % and 20 % and were contaminated each with $300 \text{ mg kg}^{-1} \text{ CuCl}_2$ / $500 \text{ mg kg}^{-1} \text{ Pb}(\text{NO}_3)_2$ / $800 \text{ mg kg}^{-1} \text{ ZnCl}_2$ / their mixed metal concentrations ($=1600 \text{ mg kg}^{-1} \text{ soil}$).

c) Similarly, for remedial measures of heavy metal (Cu, Pb and Zn) contaminated soil by using inorganic substances, 3rd sets of experimental bags were filled with sandy loam soil amended with lime at 3 g and 9 g $\text{kg}^{-1} \text{ soil}$ and were further contaminated with $300 \text{ mg kg}^{-1} \text{ CuCl}_2$ / $500 \text{ mg kg}^{-1} \text{ Pb}(\text{NO}_3)_2$ / $800 \text{ mg kg}^{-1} \text{ ZnCl}_2$ / their mixed metal concentrations ($=1600 \text{ mg kg}^{-1} \text{ soil}$).

3.5.2. Phytoextraction

Major vegetable crops such as carrot, radish (red), radish (white), turnip, spinach, cress leaf, leaf mustard, fenugreek, garlic (dry and green), coriander green and potato are grown in the fields of Kathmandu (DoAMDD, 2002) due to a good source of nutrients on one hand and their short life span. So they have been investigated in the present study. Seeds of altogether 18 vegetable crops as mentioned in **Table 4** were collected from reliable seed store (Annapurna Biz Bhandar, Ason, Kathmandu), and bulblets (of *Allium sativum* and *A. fistulosum*) and tubers (of

Solanum tuberosum) from local market. The vegetable crops were collected, germinated and investigated for metal accumulation (Cu, Pb and Zn) after their growth of 5 weeks. Same procedure as mentioned in previous experiments was used for phytoextraction experiment.

Triplicates bags of each treatment/experimental sets were arranged in randomized block design. Vegetable crops like *B. juncea*, *B. rapa* and *L. sativum* were considered for the 2nd and 3rd sets of experiments for the changes in accumulation pattern of Cu, Pb and Zn in the vegetable species. Each soil sample was measured for soil pH to understand the reason for immobilization of heavy metals in cow dung and lime treated soil.

Table 4. List of vegetable crops investigated for phytoextraction of heavy metals

Botanical names	Common names	Local names	Family
<i>Allium sativum</i> L.	Garlic	Lahsun	Amaryllidaceae
<i>A. fistulosum</i> L.	Cibol or Welsh onion	Chyapee	Amaryllidaceae
<i>Beta vulgaris</i> L.	Beet root	-	Chenopodiaceae
<i>Beta vulgaris</i> var. <i>cicla</i>	Swiss Chard	Foliage beet	Chenopodiaceae
<i>Brassica juncea</i> L.	Broad leaf mustard	Rayo sag	Cruciferae
<i>Brassica caulorapa</i> L.	Kohlrabi	Gyathgobi	Cruciferae
<i>Brassica rapa</i> L.	Turnip	Gantemula	Cruciferae
<i>B. rapa</i> L. var. <i>purple top globe</i>	Turnip	Salgam	Cruciferae
<i>Lepidium sativum</i> L.	Pepper Cress	Chamssor	Cruciferae
<i>Raphanus sativus</i> L.	Radish	Puthane Rato mula	Cruciferae
<i>R. sativus</i> L.	Radish	Puthane Seto mula	Cruciferae
<i>Coriandrum sativum</i> L.	Coriander	Dhaniya.	Umbelliferae
<i>Spinacia oleracea</i> L.	Spinach	Patane palungo	Chenopodiaceae
<i>S. oleracea</i> L.	Round seeded spinach	Deshi palungo	Chenopodiaceae
<i>S. oleracea</i> L.	Prickly seeded spinach	Gobre palungo	Chenopodiaceae
<i>Solanum tuberosum</i> L.	Potato	Aalu	Solanaceae
<i>Trigonella foenumgraecum</i> L.	Fenu-greek	Methi	Papilionaceae
<i>Vicia faba</i> L.	Broad bean	Bakula -Simi	Papilionaceae

3.5.3. Remedial Measures

The soil prepared in set b) and c) were used for remedial measure experiments. Vegetable crops like *B. juncea*, *B.rapa* and *L. sativum* were grown and collected after the growth of 5 weeks when the leafy vegetables were ready for harvest. The collected samples were washed thoroughly with tap water. Root length (RL) and SL were measured along the FW and DW of whole plant to observe the effect of cow dung and lime on the morphological changes. Analysis of the metal content such as Cu, Pb and Zn were conducted as mentioned in previous experiments.

3.5.4. Statistical Analysis

To understand if there was significant difference in growth parameters like RL, SL, root/shoot ratio, FW, DW and DW % in vegetable crops after growing on soil contaminated with single and mixed metals amended with cow dung or lime, data were analyzed statistically using One-way Anova followed by Duncan's multiple range test (with the help of SPSS 12.0 version).

4. RESULTS

4.1. Uptake of Heavy Metals and Impact on Morphology

Concentrations of Cd, Cu, Pb and Zn were 0.25, 7.2, 8.5 and 54.75 mg kg⁻¹ respectively, in the non-amended soil. The soil amended with poultry waste has been considered as control which had concentrations 0.25, 7.2, 22.2 and 74.1 mg kg⁻¹ of Cd, Cu, Pb and Zn, respectively. After treatment of soils with different concentration of CuCl₂, Pb (NO₃)₂, ZnCl₂ and their mixed salts, soil pH decreased from 7.1 to 5.8, 6.6, 5.3 and 4.1, respectively, but no such change was observed in soil pH with CdCl₂ treated soil.

Vegetable crops were found to be growing on soil supplied with 1 to 20 mg kg⁻¹ of CdCl₂; 50 to 100 mg kg⁻¹ of CuCl₂; 50 to 1000 mg kg⁻¹ of Pb (NO₃)₂; and 150 to 300 mg kg⁻¹ of ZnCl₂. Most of the vegetables could not grow at high concentrations of CuCl₂ (at 500 and 1000 mg kg⁻¹) and ZnCl₂ (at 1500 and 3000 mg kg⁻¹). *Lactuca sativa* could not grow, even at 300 mg ZnCl₂, but was found to be growing on concentrations with 500 mg CuCl₂ kg⁻¹ soil. Among the tested vegetables, highest accumulation of Cd was observed in *Lepidium sativum* (mature) (Table 5), Cu in *B. juncea* (Table 8), Pb and Zn were observed in *B. rapa* (Tables 11 and 14). Lowest accumulations of all studied heavy metals were found in *D. carota*. Changes in morphological parameters like root lengths (RL), shoot lengths (SL), fresh weight (FW) and dry weight (DW) in young and mature vegetables after increased heavy metal (Cd, Cu, Pb and Zn) uptake, are given in Figs. 2, 5, 8, 11 and 15. Changes in leaf area or leaf length after heavy metal (Cd, Cu, Pb and Zn) uptake is given in Figs. 3, 6, 9, 12 and 16. Chi –square value obtained from data of RL, SL, FW, DW and leaf area/length of vegetable crops grown on different heavy metals (CdCl₂, CuCl₂, Pb(NO₃)₂ and ZnCl₂) with their significance level obtained from Kruskal Wallis, are presented in Tables 6, 9, 12, 15 and 18. Fluctuations in concentrations of micronutrients (Cu, Fe, Mn and Zn) in shoot and root of each vegetable after excessive accumulation of Cd, Cu, Pb and Zn are presented in Figs. 4, 7, 10, 13 and 17.

4.1.1. Cadmium

Cadmium accumulation was high in roots of *B. juncea* and *L. sativa* in comparison to their shoots. But in shoots (both young and mature) of *B. rapa*, *L. sativum* and *D. carota* Cd

accumulation is higher than in roots. Among studied vegetables, highest Cd accumulation was found in shoot and roots of young *B. rapa* and *B. juncea*, respectively. In mature condition highest Cd accumulation was observed in both shoots and roots of *L. sativum* (**Table 5**).

Table 5. Cadmium (mg kg⁻¹ DW) in shoots and roots of vegetable crops grown on soil treated with different concentrations of CdCl₂. (-) indicates insufficient samples to detect heavy metals

Vegetable crops	Treatment (mg kg ⁻¹ soil)	Young		Mature	
		shoot	root	shoot	root
<i>B. juncea</i>	ctrl	0.25	0.25	0.25	0.50
	1	0.25	2.25	1.50	2.50
	2	2.50	2.73	2.75	3.25
	10	5.75	8.22	6.75	8.75
	20	12.50	17.27	9.00	11.50
<i>B. rapa</i>	ctrl	0.75	0.50	0.25	0.50
	1	3.00	1.05	0.75	1.25
	2	5.50	2.75	2.75	3.25
	10	9.25	6.25	9.75	14.50
	20	17.75	15.75	17.00	15.50
<i>L. sativum</i>	ctrl	1.00	0.25	0.25	0.26
	1	1.28	0.75	1.25	1.50
	2	1.39	1.50	3.22	2.20
	10	9.80	6.25	12.00	0.25
	20	15.80	9.20	19.75	16.50
<i>L. sativa</i>	ctrl	-	-	0.25	0.52
	1	-	-	1.25	2.25
	2	-	-	2.25	3.38
	10	-	-	4.00	4.25
	20	-	-	5.75	10.50
<i>D. carota</i>	ctrl	-	-	0.25	0.28
	1	-	-	1.00	0.50
	2	-	-	1.25	0.87
	10	-	-	2.50	2.00
	20	-	-	5.25	2.25

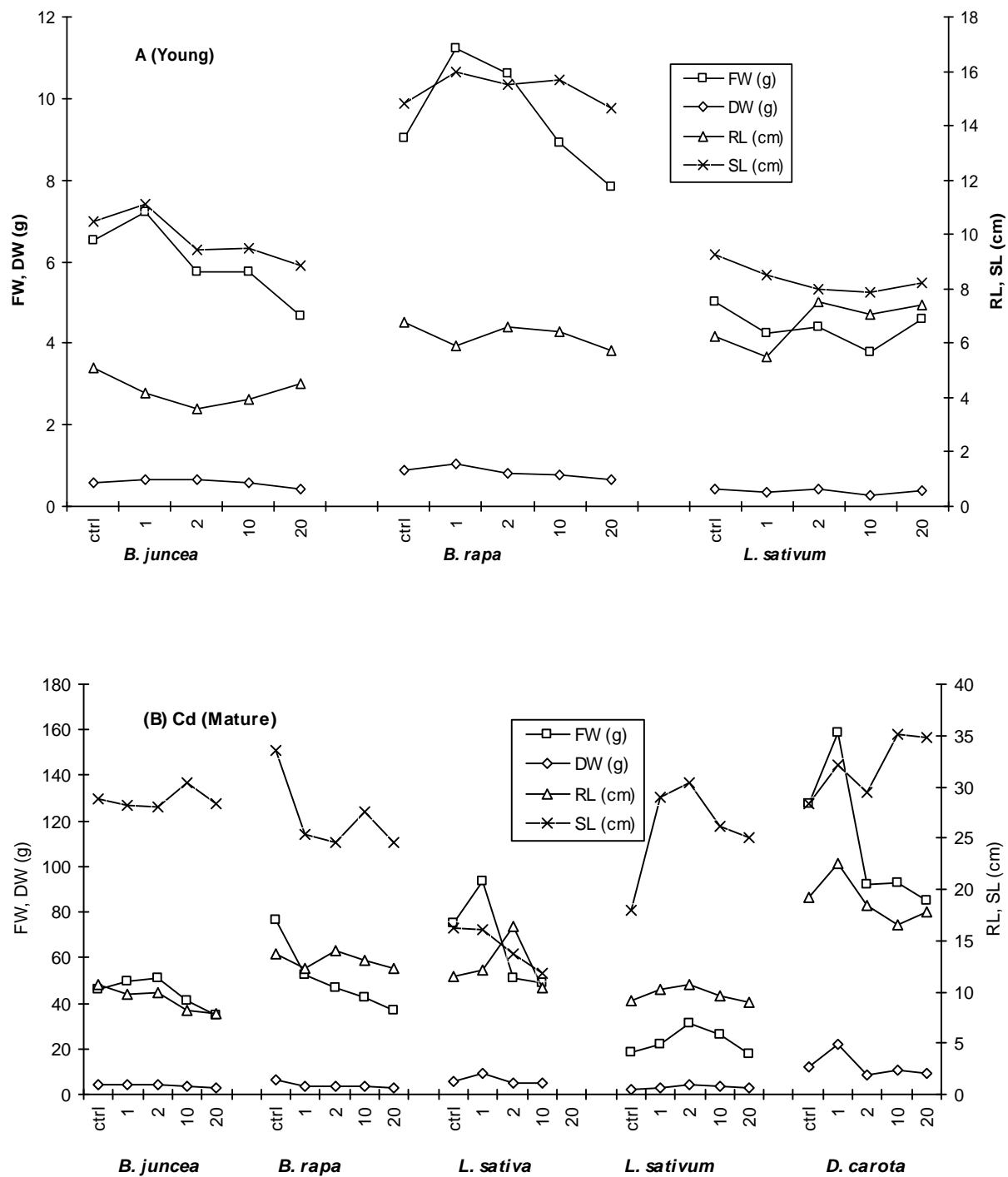


Fig. 2. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in young (A) and mature (B) vegetable crops grown on soil treated with different concentrations of CdCl₂.

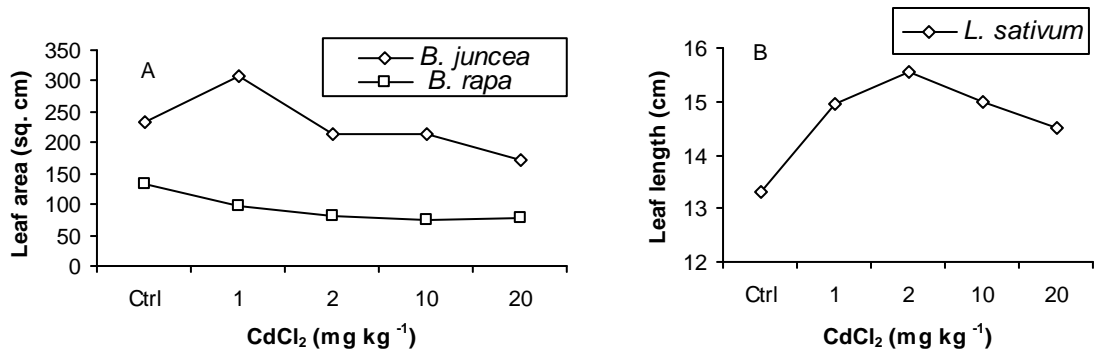


Fig. 3. Leaf area (A) and leaf length (B) of vegetable crops grown on soil treated with different concentrations of CdCl₂.

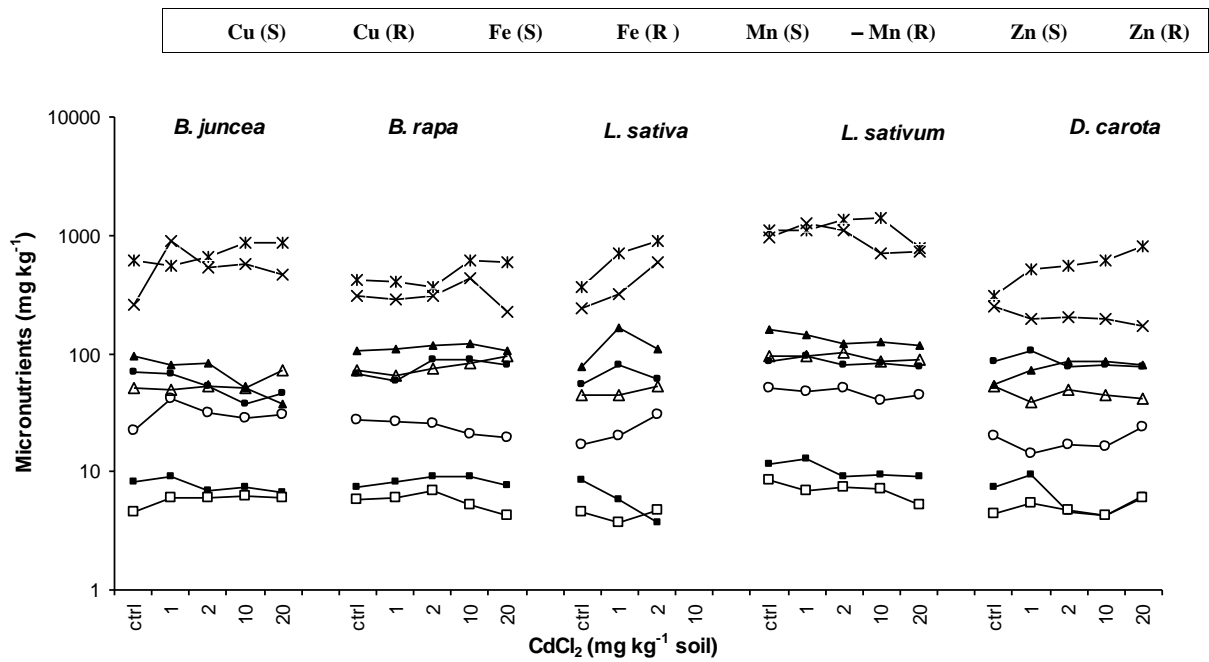


Fig. 4. Micronutrients (Cu, Fe, Mn and Zn) accumulation in shoots (S) and roots (R) of *Brassica juncea*, *Brassica rapa*, *Lactuca sativa*, *Lepidium sativum* and *Daucus carota* grown on soil treated with different concentrations of CdCl₂.

Table 6. Chi-square value obtained from Kruskal-Wallis test for morphological parameters of vegetables grown on soil treated with different concentrations of CdCl₂.

Vegetable crops	Observation (n)	FW	DW	RL	SL	Leaf area/ length
<i>B. juncea</i> ^Y	15	5.23	6.07	6.24	8.45	
<i>B. juncea</i> ^M	15	7.43	2.86	8.50	4.86	11.15* sq.cm
<i>B. rapa</i> ^Y	25	2.33	3.81	1.58	0.61	
<i>B. rapa</i> ^M	25	14.53**	12.52**	2.18	15.12**	15.27** sq.cm
<i>L. sativum</i> ^Y	15	0.76	1.6	5.79	9.35*	
<i>L. sativum</i> ^M	22,39 ^R , 23 ^L	11.59*	9.12*	10.57*	13.86**	3.14 cm
<i>L. sativa</i>	12	8.95*	6.90	8.10*	8.69*	
<i>D. carota</i>	22,25 ^R	12.02**	13.22**	10.92*	5.91	

Significance level * = P<0.05, ** = P<0.001, unmarked = not significant, ^R=root, ^L=leaf, ^Y=young, ^M=mature,

Table 7. Dry weight (%) of vegetable crops grown on soil treated with different concentrations of CdCl₂.

Treatments (mg kg ⁻¹ soil)	<i>B. juncea</i> ^Y	<i>B. juncea</i> ^M	<i>B. rapa</i> ^Y	<i>B. rapa</i> ^M	<i>L. sativum</i> ^Y	<i>L. sativum</i> ^M	<i>L. sativa</i> ^M	<i>D. carota</i> ^M
ctrl	8.92	8.48	9.75	8.38	8.55	11.75	6.09	9.14
1	9.23	9.31	9.50	6.47	7.85	12.15	8.03	11.35
2	10.97	7.58	7.64	7.08	9.25	13.33	9.64	9.18
10	10.00	8.84	8.55	8.31	8.24	13.27	9.86	11.53
20	9.27	8.57	8.45	8.01	7.93	14.07	9.90	10.13
Chi sq	8.4	2.77	15.75**	12.83**	5.51	4.46	2.59	11.71**

Significance level * = P<0.05, ** = P<0.001, unmarked = not significant, ^Y indicates young and ^M the matured

In *B. juncea*, leaf area reduced significantly (P<0.05) but FW, DW, RL, SL and DW % changed not significantly after increased Cd accumulation. In matured *B. rapa* FW, DW, SL, leaf area and DW % reduced more significantly (P<0.05) with high Cd accumulation in shoots and roots (**Figs. 2 and 3, Tables 6 and 7**). Although SL decreased significantly in young *L. sativum* (which have accumulated 15.8 and 9.2 mg Cd kg⁻¹ DW in leaf and root respectively), but in mature condition, its SL and RL increased significantly along with increase in FW and DW. Though Cd accumulation was low in *L. sativa*, RL and SL tend to decrease with 4.0 mg kg⁻¹ and 4.25 mg kg⁻¹ DW in leaf and in root respectively. Among the tested vegetable crops, accumulation of Cd in *D. carota* was lowest and RL, FW, DW reduced significantly but DW %

increased significantly (**Fig. 14 A**). Most vegetables grown with low doses of CdCl₂ (1 and 2 mg kg⁻¹ soil) enhanced FW and DW, but at high dose (20 mg kg⁻¹ soil) these parameters decreased (**Tables 5, 6 and 7, Figs. 2 and 3**). On the basis of FW of individual plant (mature) and increased accumulation of Cd in their shoots, threshold Cd concentration in *B. juncea*, *B. rapa*, *L. sativum*, *L. sativa* and *D. carota* were ascertained to be 2.75, 0.25, 3.22, 1.25 and 1.0 mg kg⁻¹ DW, respectively.

With increased accumulation of Cd, the micronutrients Cu decreased in leaves of most studied vegetables, except *B. rapa* (**Fig. 4**). Besides, this Zn and Mn decreased in shoots of *B. juncea*. Similarly there are decrease of Fe in root, Zn in shoot and Mn in both root and shoot of *L. sativum*; Fe in roots of *B. rapa* and *D. carota* (but there are increased accumulation of Fe in shoot of both).

4.1.2. Copper

Copper accumulation in roots and shoots of most vegetable crops after growing on CuCl₂ treated soil was almost similar in all cases showing good mobility of Cu from root to shoot. Highest Cu accumulation was observed in both shoots and roots of young *B. juncea*. Among matured plants highest accumulation of Cu was observed in shoots and roots of *L. sativum* and *L. sativa*, respectively (**Table 8**).

Most vegetables grown on soil treated with 50 mg CuCl₂ kg⁻¹ soil, enhanced FW and DW, but both these parameters reduced at medium doses (100 mg kg⁻¹). In both young and matured *B. juncea*, RL, SL, FW, DW and leaf area decreased but FW, DW and RL in matured ones were significantly (P<0.05) reduced with increased Cu accumulation. Root length, SL and DW reduced significantly in young *B. rapa*, and leaf area reduced significantly (P<0.05) in matured condition in it. Shoot length and DW in young *L. sativum* reduced significantly with Cu treatments. Root length, FW and DW in *L. sativa* significantly increased at lower concentrations up to 18 mg kg⁻¹ DW (at 100 mg CuCl₂ kg⁻¹ soil treatments) but afterwards RL tends to decrease. However, *L. sativa* was able to grow in 500 mg CuCl₂ kg⁻¹ soil while other vegetables could not germinate at this concentration. Shoot length and DW significantly (p<0.05) decreased in *D. carota* but DW % increased significantly (**Tables 9 and 10; Figs. 5, 6 and 14 B**). Toxic effect of Cu is seen mostly after 50 mg kg⁻¹ soil treatment (except in *L. sativa*). On the basis of FW of whole plants (matured) and increased accumulation of Cu in their shoots, threshold Cu

concentrations in *B. juncea*, *B. rapa*, *L. sativum*, *Lactuca sativa* and *D. carota* were ascertained to be 18, 17.75, 14.75, 17.25 and 10.75 mg kg⁻¹ DW, respectively.

Increased accumulation of Cu in shoots from the vegetables grown from CuCl₂-contaminated soil, showed the depletion of the micronutrients such as Fe and Zn in shoot of *B. juncea*; and Mn in shoot, Fe and Zn from root of *D. carota*. Similarly Fe and Mn in root, and Zn in both root and shoot of *B. rapa* decreased. In *L. sativum*, depletion of Zn was observed in both root and shoot (Fig. 7).

Table 8. Copper (mg kg⁻¹ DW) in shoots and roots of vegetable crops grown on soil treated with different concentrations of CuCl₂. (-) indicates insufficient sample to detect heavy metal.

Vegetable crops	Treatment (mg kg ⁻¹ soil)	Young		Mature	
		shoot	root	shoot	root
<i>B. juncea</i>	ctrl	8.25	7.08	8.5	6.5
	50	25.00	26.00	18.00	21.75
	100	51.50	55.00	22.75	32.50
<i>B. rapa</i>	ctrl	8.25	9.00	10.25	7.75
	50	16.00	15.00	17.75	12.75
	100	18.00	38.21	27.75	16.50
	500	-	-	-	-
<i>L. sativum</i>	ctrl	9.00	-	8.25	5.50
	50	16.00	-	14.75	11.79
	100	18.90	-	21.50	16.50
	500	22.00	-	33.50	19.00
<i>L. sativa</i>	ctrl	-	-	9.75	5.98
	50	-	-	13.50	10.25
	100	-	-	17.25	18.25
	500	-	-	25.25	37.00
<i>D. carota</i>	ctrl	-	-	7.50	4.48
	50	-	-	10.75	4.50
	100	-	-	15.75	6.00
	500	-	-	-	-

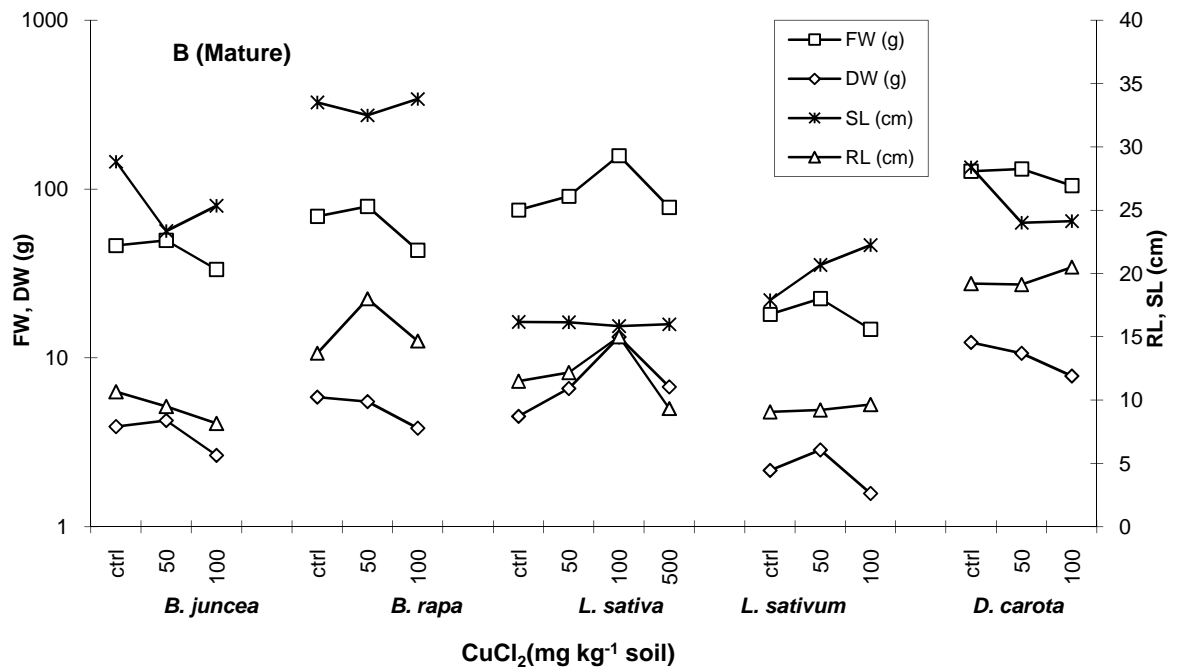
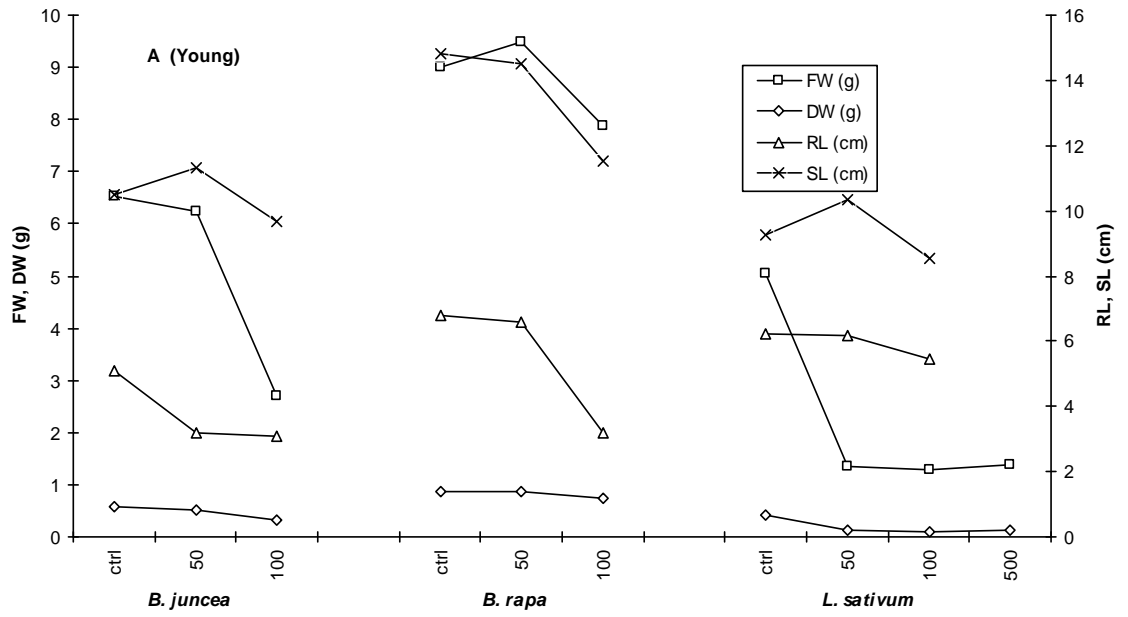


Fig. 5. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in young (A) and mature (B) vegetable crops grown on soil treated with different concentrations of CuCl_2 .

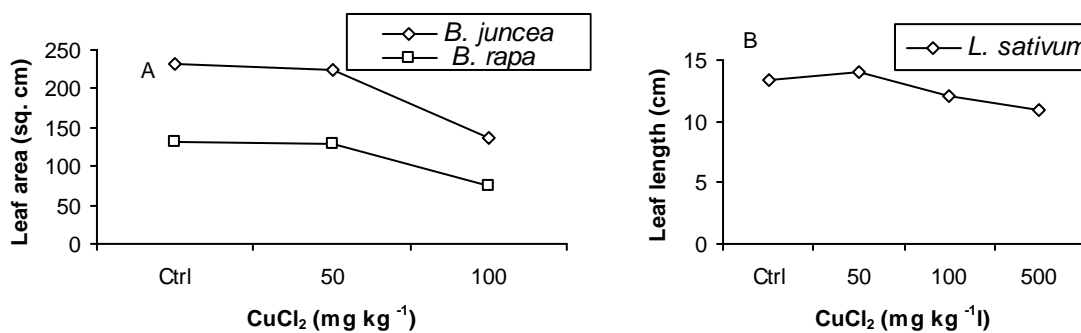


Fig. 6. Leaf area (A) and leaf length (B) of vegetable crops grown on soil treated with different concentrations of CuCl_2

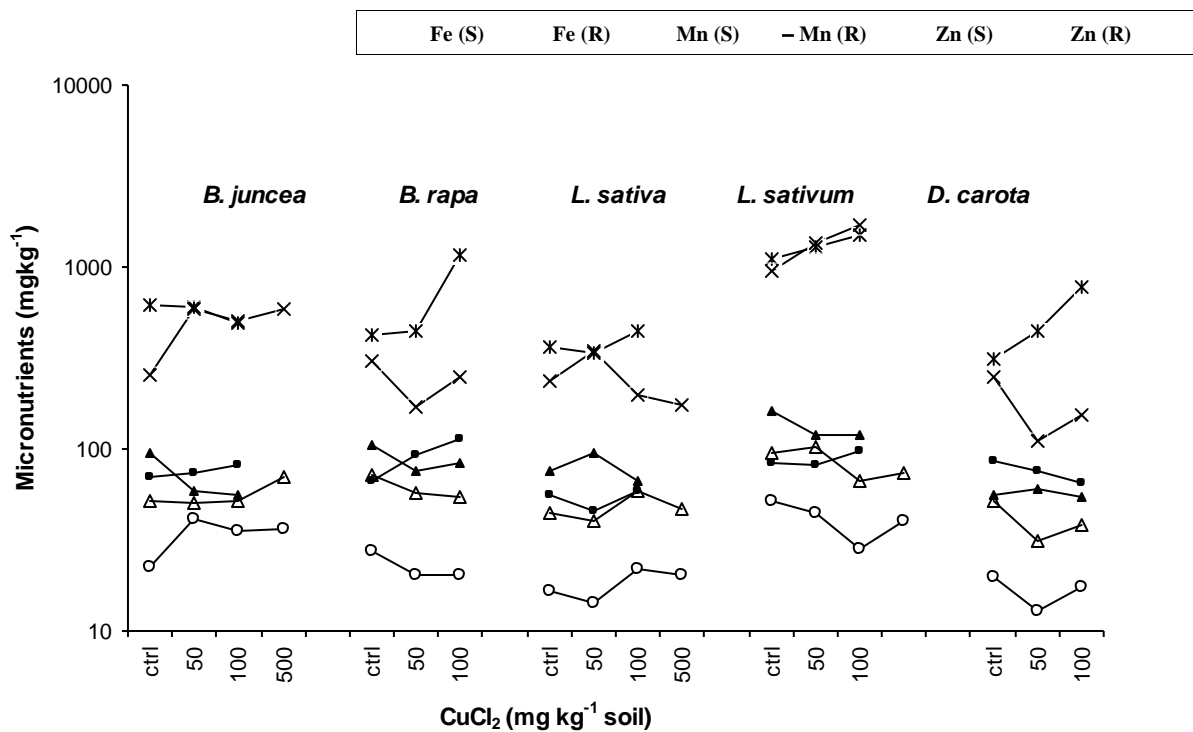


Fig. 7. Micronutrients (Fe, Mn and Zn) accumulation in shoots (S) and roots (R) of *Brassica juncea*, *Brassica rapa*, *Lactuca sativa*, *Lepidium sativum* and *Daucus carota* grown on soil treated with different concentrations of CuCl₂.

Table 9. Chi-square value obtained from Kruskal-Wallis test for morphological parameters of vegetable crops grown on soil treated with different concentrations of CuCl₂.

Vegetable crops	Observation (n)	FW	DW	RL	SL	Leaf area/length
<i>B. juncea</i> ^Y	25	5.42	5.60	5.42	3.95	
<i>B. juncea</i> ^M	24	5.96*	6.01*	6.39*	2.57	5.60 sq.cm
<i>B. rapa</i> ^Y	23	4.52	6.69*	10.8**	6.26*	
<i>B. rapa</i> ^M	23	2.72	2.79	1.33	0.19	6.01* sq.cm
<i>L. sativum</i> ^Y	15	6.29	7.94*	1.69	7.32*	
<i>L. sativum</i> ^M	25,39 ^R	4.81	6.07	6.59	2.27	6.1 cm
<i>L. sativa</i>	15,20 ^L	7.67*	8.44*	9.26*	0.87	
<i>D. carota</i>	22,24 ^R	7.21	8.40*	2.78	11.66*	

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^R=root, ^L=leaf, ^Y=young, ^M=mature

Table 10. Dry weight (%) of vegetable crops grown on soil treated with different concentrations of CuCl₂.

Treatments (mg kg ⁻¹ soil)	<i>B.juncea</i> ^Y	<i>B.juncea</i> ^M	<i>B.rapa</i> ^Y	<i>B.rapa</i> ^M	<i>L.sativum</i> ^Y	<i>L. sativum</i> ^M	<i>L.sativa</i> ^M	<i>D.carota</i> ^M
ctrl	8.92	8.48	9.75	8.41	8.55	11.75	6.09	9.75
50	8.55	8.67	9.07	7.13	9.79	10.91	8.39	8.07
100	12.46	7.91	9.28	9.09	9.05	12.71	8.73	7.48
500	-	-	-	-	9.58	12.59	7.25	10.71
Chi sq.	5.6	1.16	1.72	4.68	5.18	1.06	3.21	7.79*

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^Y indicates young and ^M the mature

4.1.3. Lead

Lead accumulation in most of the vegetable crops after growing on soil treated with different concentration of Pb(NO₃)₂ was higher in roots than in shoots (except *D. carota*). Among the studied vegetables highest accumulation of Pb was observed in shoots and roots of

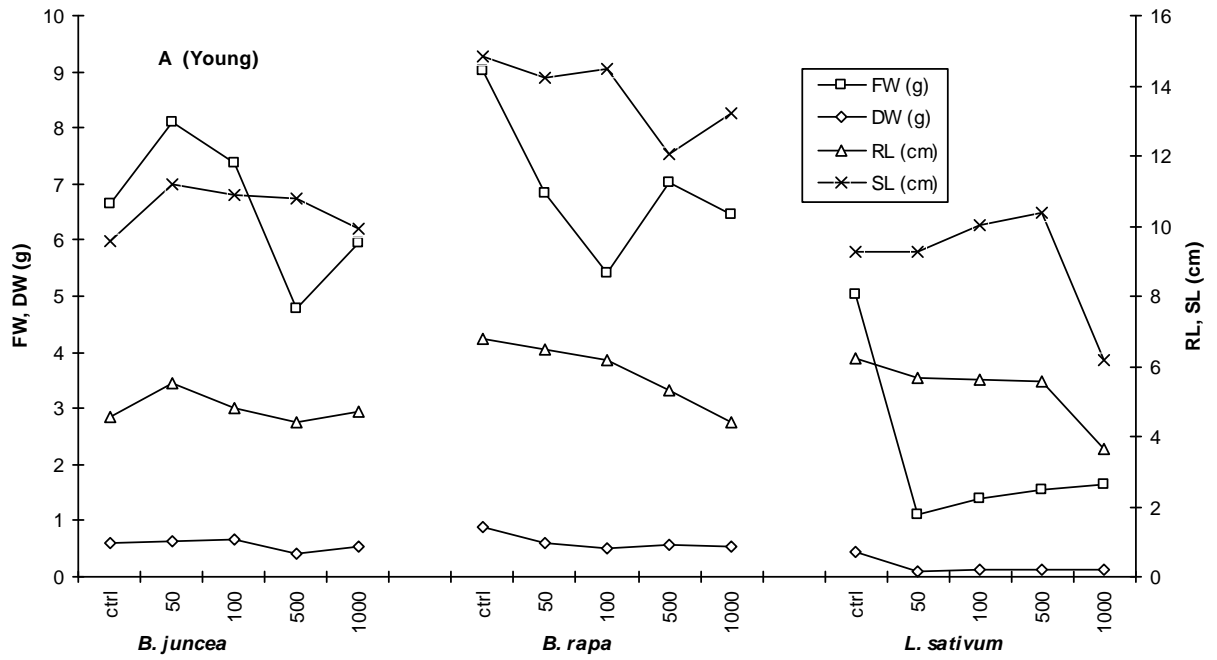
young *L. sativum* and *B. rapa*, respectively. In matured plants highest Pb accumulation were observed in both shoots and roots of *L. sativa* (Table 11).

Insignificant decrease in FW, DW, RL and SL was observed in both young and matured *B. juncea*. Slightly decreased DW % at 1000 mg Pb kg⁻¹ soil has been observed in young *B. juncea*. Root length and DW reduced significantly (P<0.05) in young *B. rapa* and resulted significant decreased DW % in young stage. But in mature stage, *B. rapa* showed significant decrease in FW and RL (Tables 11, 12 and 13; Figs. 8 and 9). In young *L. sativum* SL, FW and DW decreased, but SL increased with low concentration when it matured (Fig. 8, Table 12). Although FW, SL, RL increased in lower doses, RL significantly (P<0.001) decreased at 1000 mg Pb kg⁻¹ soil in *D. carota* (Fig. 14 C). On the basis of FW of whole plants (mature) and accumulation of Pb in their shoots, threshold Pb concentrations in *B. juncea*, *B. rapa*, *L. sativum*, *L. sativa* and *D. carota* were ascertained to be 46.5, 34.5, 52.7, 81.0 and 31.5 mg kg⁻¹, respectively.

Table 11. Lead (mg kg⁻¹ DW) in shoots and roots of vegetable crops grown on soil treated with different concentrations of Pb(NO₃)₂. (-) indicates insufficient sample to detect heavy metals.

Vegetable crops	Treatment (mg kg ⁻¹ soil)	Young		Mature	
		shoot	root	shoot	root
<i>B. juncea</i>	ctrl	28.75	27.08	22.00	1.25
	50	33.75	42.30	43.25	28.75
	100	80.5	86.47	46.50	35.75
	500	164.0	203.0	51.25	148.75
	1000	200.0	307.0	85.50	158.00
<i>B. rapa</i>	ctrl	29.50	33.3	26.75	27.5
	50	38.75	44.5	34.5	45.7
	100	48.25	67.5	48.0	53.7
	500	121.50	309.5	59.5	70.75
	1000	174.25	402.5	95.2	101.5
<i>L. sativum</i>	ctrl	16.5	18.2	16.5	20.8
	50	32.3	40.2	24.2	33.7
	100	60.37	65.3	52.7	63.0
	500	199.5	207.5	113.1	174.0
	1000	205.8	257.3	147.2	221.4
<i>L. sativa</i>	ctrl	-	-	18.0	22.5
	50	-	-	45.75	52.2
	100	-	-	81.0	112.3

	500	-	-	107.0	266.0
	1000	-	-	167.0	376.0
<i>D. carota</i>	ctrl	-	-	14.00	16.32
	50	-	-	23.75	13.25
	100	-	-	31.50	19.75
	500	-	-	51.25	24.50
	1000	-	-	64.25	58.00



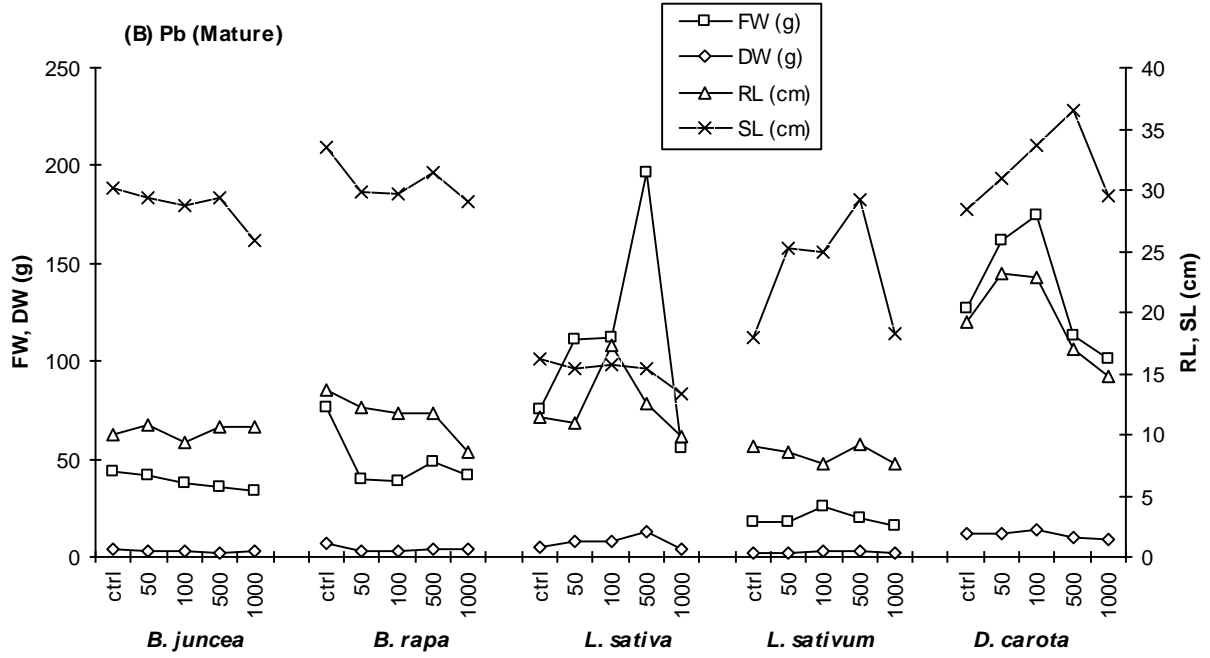


Fig. 8. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in young (A) and mature (B) vegetable crops grown on soil treated with different concentrations of $Pb(NO_3)_2$.

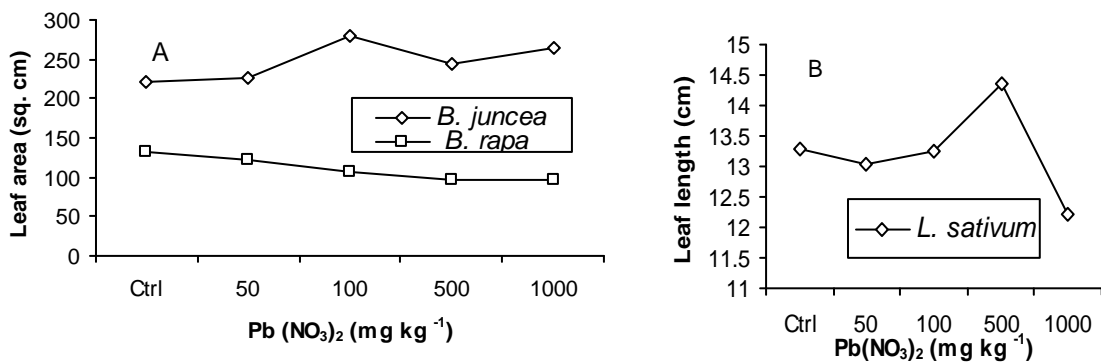


Fig. 9. Leaf area (A) and leaf length (B) of vegetable crops grown on soil treated with different concentrations of $Pb(NO_3)_2$.

Table 12. Chi-square value obtained from Kruskal-Wallis test for morphological parameters of vegetable crops grown on soil treated with different concentrations of Pb(NO₃)₂.

Vegetable crops	Observation(n)	FW	DW	RL	SL	Leaf area/ length
<i>B. juncea</i> ^Y	25	6.50	6.66	1.90	4.10	
<i>B. juncea</i> ^M	24	1.58	1.86	5.23	2.54	11.59* sq.cm
<i>B. rapa</i> ^Y	23	7.40	10.93*	11.98**	1.68	
<i>B. rapa</i> ^M	23	11.71**	8.59	11.73**	4.05	8.60 sq.cm
<i>L. sativum</i> ^Y	15	11.08*	9.5*	4.72	9.86*	
<i>L. sativum</i> ^M	25,39 ^R	7.89	6.22	6.82	10.37*	6.18 cm
<i>L. sativa</i>	15,20 ^L	7.63	7.57	8.35	3.34	
<i>D. carota</i>	22,24 ^R	4.30	3.65	16.17**	6.76	

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^R=root, ^L=leaf, ^Y=young, ^M=mature

Table 13. Dry weight (%) of vegetable crops grown on soil treated with different concentrations of Pb(NO₃)₂.

Treatments (mg kg ⁻¹ soil)	<i>B.</i> <i>juncea</i> ^Y	<i>B.</i> <i>juncea</i> ^M	<i>B.</i> <i>rapa</i> ^Y	<i>B.</i> <i>rapa</i> ^M	<i>L.</i> <i>sativum</i> ^Y	<i>L.</i> <i>sativum</i> ^M	<i>L.</i> <i>sativa</i> ^M	<i>D.</i> <i>carota</i> ^M
ctrl	9.15	7.94	9.75	8.38	8.55	11.75	6.09	9.75
50	8.69	7.98	8.76	8.33	9.46	12.39	6.89	7.67
100	8.97	6.34	9.47	8.63	9.49	11.96	7.32	8.19
500	9.18	6.83	8.27	8.99	8.64	13.09	6.48	8.45
1000	7.86	7.53	8.30	8.44	8.29	13.05	7.03	8.34
Chi sq.	14.89**	10.28*	10.41*	2.26	1.16	3.86	3.27	7.69

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^Yindicates young and ^M the mature.

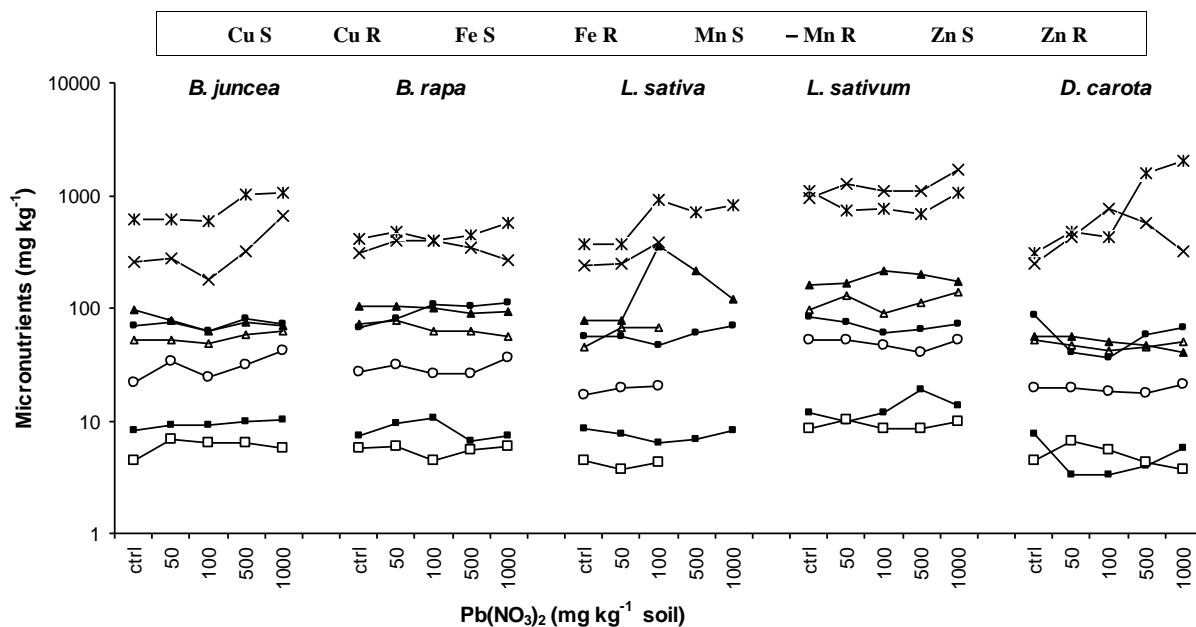


Fig. 10. Micronutrients (Cu, Fe, Mn and Zn) accumulation in shoots (S) and roots (R) of *Brassica juncea*, *Brassica rapa*, *Lactuca sativa*, *Lepidium sativum* and *Daucus carota* grown on soil treated with different concentrations of $Pb(NO_3)_2$.

With increased Pb accumulation in shoots, depletion of Zn in shoots of *B. juncea*, Fe and Mn in shoots of *L. sativum*, Cu, Mn and Zn in shoots of *D. carota* were observed. With excessive accumulation of Pb, slight increase in Fe and Cu in *B. juncea* (root and shoot), Fe and Zn in *L. sativa* (root and shoot) and Zn in *L. sativum* (root and shoot), Fe in *D. carota* (root and shoot) were observed (**Fig. 10**).

4.1.4. Zinc

Zinc accumulation was found to be higher in shoot than in their roots in all vegetable crops grown on soil treated with different concentrations of $ZnCl_2$. In young stage, highest Zn accumulation in shoots and roots were observed in *L. sativum* and *B. rapa*, respectively. In matured condition high accumulation of Zn were observed in shoots and roots of *B. rapa* and *B. juncea*, respectively (**Table 14**). Slight decrease in SL and RL was observed in *B. juncea* (matured) with high Zn accumulation (**Fig. 11**). In young stage, *B. rapa* showed decrease in FW, RL and SL but in matured condition only SL decreased significantly ($P < 0.05$). Shoot length and FW decreased in *L. sativum* (young) but in mature stage SL increased not significantly and DW

% increased significantly ($P < 0.05$). Root length and SL in *D. carota* reduced slightly but FW, DW and DW % decreased significantly (**Figs. 11, 12 and 14 D; Tables 15 and 16**).

Table 14. Zinc (mg kg^{-1} DW) in shoots and roots of vegetable crops grown on soil treated with different concentrations of ZnCl_2 . (-) indicates insufficient sample to detect heavy metals.

Vegetable crops	Treatment (mg kg^{-1} soil)	Young		Mature	
		shoot	root	shoot	root
<i>B. juncea</i>	ctrl	109.5	10.25	105.0	66.0
	150	188.0	145.0	173.5	139.5
	300	244.5	176.0	313.5	252.0
<i>B. rapa</i>	ctrl	126.0	98.0	192	76.5
	150	170.0	105.0	219	114
	300	277.0	201.2	505	171
<i>L. sativum</i>	ctrl	162.0	129	124.8	99.84
	150	193.5	173	172.5	155.0
	300	293.7	198	268.5	204.1
<i>L. sativa</i>	ctrl	-	-	93.0	95.16
	150	-	-	184.5	108.2
	300	-	-	-	-
<i>D. carota</i>	ctrl	-	-	55.5	52.08
	150	-	-	58.5	48.00
	300	-	-	85.5	48.36

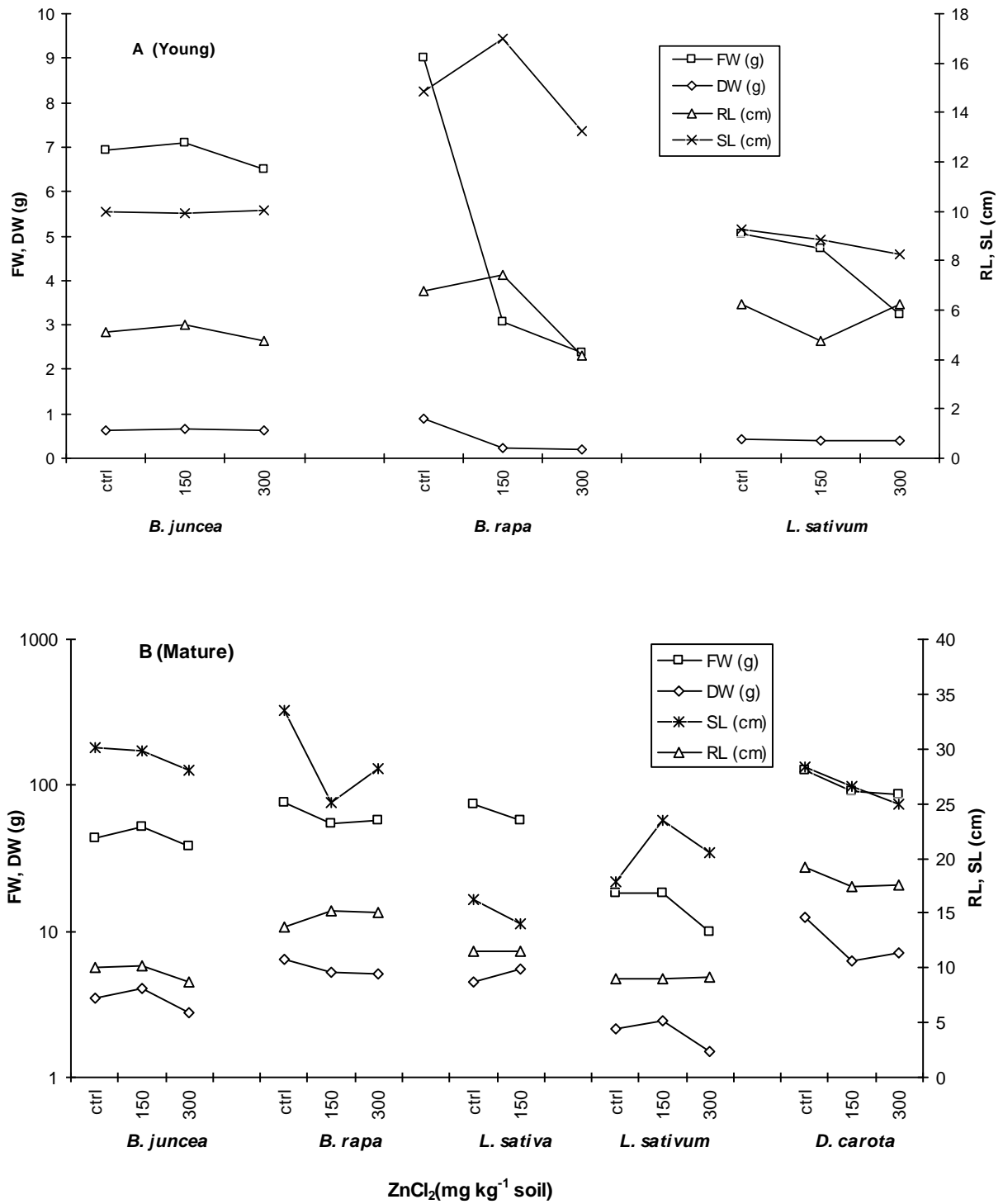


Fig. 11. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in young (A) and mature (B) vegetable crops grown on soil treated with different concentrations of ZnCl₂.

On the basis of FW of whole plants (mature) and accumulation of Zn in their shoots, threshold Zn concentrations in *B. juncea*, *B. rapa*, *L. sativum*, *L. sativa* and *D. carota* were ascertained to be 173.5, 192, 172.5, 93.0, and 55.5 mg kg⁻¹ DW, respectively.

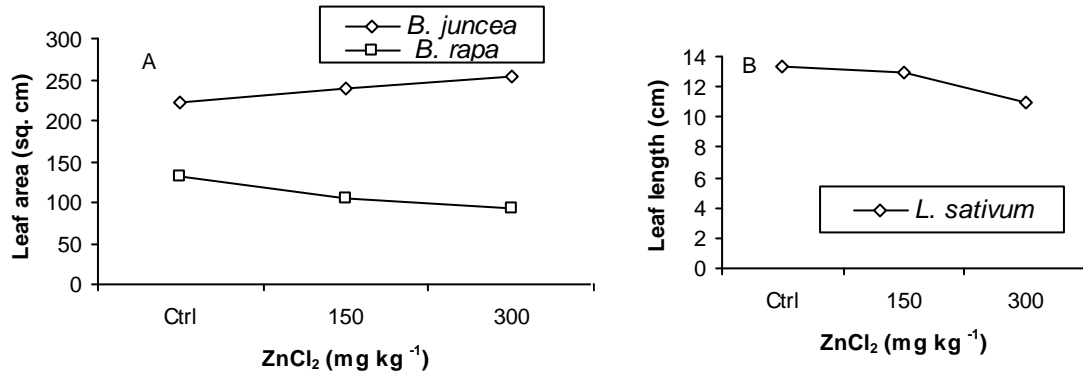


Fig. 12. Leaf area (A) and leaf length (B) of vegetable crops grown on soil treated with different concentrations of ZnCl₂

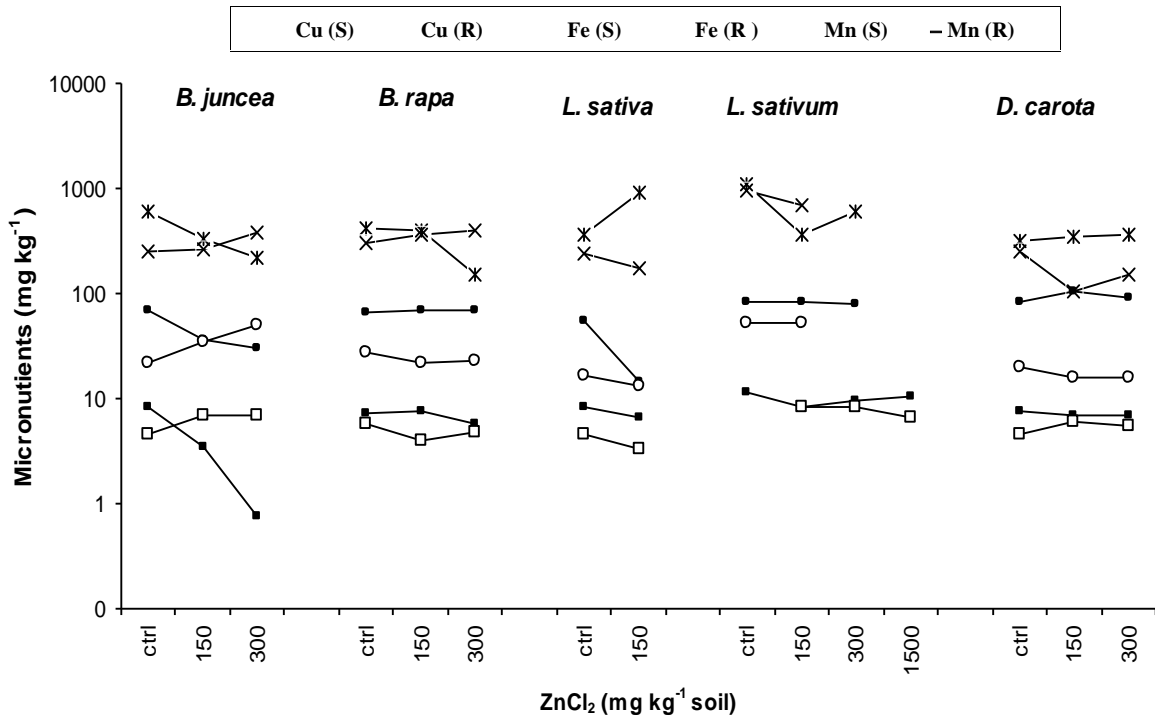


Fig. 13. Micronutrients (Cu, Fe and Mn) accumulation in shoots (S) and roots (R) of *Brassica juncea*, *Brassica rapa*, *Lactuca sativa*, *Lepidium sativum* and *Daucus carota* grown on soil treated with different concentrations of ZnCl₂.

The depletion of Cu, Fe and Mn in shoot of *B. juncea*, Fe in shoot and Mn in root of *B. rapa*, Cu and Mn in shoot, Fe and Mn in root of *L. sativa*; Cu and Fe in shoot and root of *L. sativum*, Cu in shoot and Fe in roots of *D. carota* were observed (**Fig. 13**).

Table 15. Chi-square value obtained from Kruskal-Wallis test for morphological parameters of vegetable crops grown on soil treated with different concentrations of ZnCl₂.

Vegetable crops	Observation (n)	FW	DW	RL	SL	Leaf area/ length
<i>B. juncea</i> ^Y	25	0.26	0.09	0.16	0.13	
<i>B. juncea</i> ^M	15,22 ^L	1.82	3.03	6.2*	0.7	3.98 sq.cm
<i>B. rapa</i> ^Y	14,17 ^R	10.91**	10.22	8.32**	6.58*	
<i>B. rapa</i> ^M	15	5.46	2.41	1.03	6.80*	3.47 sq.cm
<i>L. sativum</i> ^Y	9	7.53*	3.43	3.29	6.54*	
<i>L. sativum</i> ^M	13,23 ^R	4.77	1.02	0.12	0.59	4.75 cm
<i>L. sativa</i>	6,7 ^L	2.33	1.19	0.43	3.86*	
<i>D. carota</i>	13,14 ^R	8.57**	9.10**	4.77	1.99	

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^R=root, ^L=leaf, ^Y=young, ^M=mature

Table 16. Dry weight (%) of vegetable crops grown on soil treated with different concentrations of ZnCl₂.

Treatments (mg kg ⁻¹ soil)	<i>B. juncea</i> ^Y	<i>B. juncea</i> ^M	<i>B. rapa</i> ^Y	<i>B. rapa</i> ^M	<i>L. sativum</i> ^Y	<i>L. sativum</i> ^M	<i>L. sativa</i>	<i>D. carota</i> ^M
ctrl	9.13	7.94	9.75	8.38	0.68	11.75	6.09	9.75
150	9.18	8.07	7.96	9.59	0.58	13.07	9.49	6.88
300	9.42	7.04	8.58	8.66	0.71	15.09	-	8.28
Chi sq.	1.12	2.18	4.04	5.32	1.87	7.5*	3.86*	7.1*

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^Yindicates young and ^M the mature



20 10 2 1 ctrl
(A) CdCl₂ mg kg⁻¹soil



1000 500 100 50 ctrl
(B) CuCl₂ mg kg⁻¹soil



1000 500 100 50 ctrl
(C) Pb(NO₃)₂ mg kg⁻¹soil



3000 1500 300 150 ctrl
(D) ZnCl₂ mg kg⁻¹soil

Fig. 14. Morphological changes in *D. carota* grown on soil treated with different concentrations of heavy metals.

4.1.5. Uptake of Cd, Cu, Pb and Zn from Mixed Metal Treatments

Heavy metals (Cd, Cu, Pb and Zn) accumulation in shoots of *B. juncea*, *B. rapa* and *L. sativum* were higher than in their respective roots grown on soil treated with mixed metals (CdCl₂+CuCl₂+Pb (NO₃)₂+ZnCl₂) (**Table 17**). Accumulation of Cu and Pb in roots of mixed

Table 17. Cd, Cu, Pb and Zn concentrations (mg kg⁻¹ DW) in shoots and roots of vegetable crops grown on soil treated with different concentrations of mixed metals. (-) indicates insufficient sample to detect heavy metals.

Vegetable crops	Treatment (mg kg ⁻¹)	Cd		Cu		Pb		Zn	
		shoot	root	shoot	root	shoot	root	shoot	root
<i>B. juncea</i> ^Y	ctrl	0.25	-	8.25	-	19.75	-	108.5	-
	251	2.50	-	18.25	-	25.50	-	196.5	-
	502	4.00	-	45.25	-	114.5	-	366.0	-
<i>B. juncea</i> ^M	ctrl	1.00	1.00	8.75	14.25	13.00	17.00	85.50	53.77
	251	1.12	1.00	11.76	19.75	18.90	18.75	131.04	157.50
	502	1.75	1.25	13.00	11.25	36.50	27.25	226.5	189.00
<i>B. rapa</i> ^Y	ctrl	0.25	0.67	8.25	9.00	-	33.33	60.00	98.00
	251	1.75	2.50	25.00	17.75	-	62.00	160.50	250.00
	502	3.00	2.75	69.75	27.75	-	-	429.00	-
<i>B. rapa</i> ^M	ctrl	0.25	0.25	10.25	5.75	26.75	17.50	100.50	72.00
	251	1.00	0.50	10.50	8.00	36.75	43.75	163.50	96.00
	502	2.50	0.75	12.25	12.50	49.50	11.25	237.00	124.00
<i>L. sativum</i> ^Y	ctrl	-	-	-	-	-	-	-	-
	251	-	-	-	-	-	-	-	-
	502	-	-	-	-	-	-	-	-
<i>L. sativum</i> ^M	ctrl	0.50	0.26	11.50	8.84	16.75	20.80	124.50	99.84
	251	2.75	1.50	16.75	14.75	32.75	35.25	243.00	163.50
	502	2.75	2.00	27.25	24.50	53.25	17.50	339.00	195.00
<i>L. sativa</i> ^M	ctrl	1.25	0.52	8.50	5.96	17.00	9.75	93.00	45.00
	251	3.25	3.75	16.80	27.80	21.60	74.25	226.00	75.00
	502	7.00	3.92	21.87	28.60	42.19	115.0	759.0	557.14
<i>D. carota</i> ^M	ctrl	-	0.28	-	4.48	-	16.52	-	52.08
	251	-	1.96	-	14.28	-	19.32	-	110.88
	502	-	-	-	-	-	-	-	-

metals treated *L. sativa* was higher than in their shoots. Accumulation of Cd was mostly low in all vegetable crops grown in mixed metal treated soil. Highest accumulation of Cd and Zn were observed in shoot and roots of *L. sativa*. High Cu accumulation was noticed in both shoot and root of *B. rapa* (young) and root of *L. sativa* (mature). Lead accumulation was high in shoot of *L. sativum* (mature) and in root of *L. sativa*.

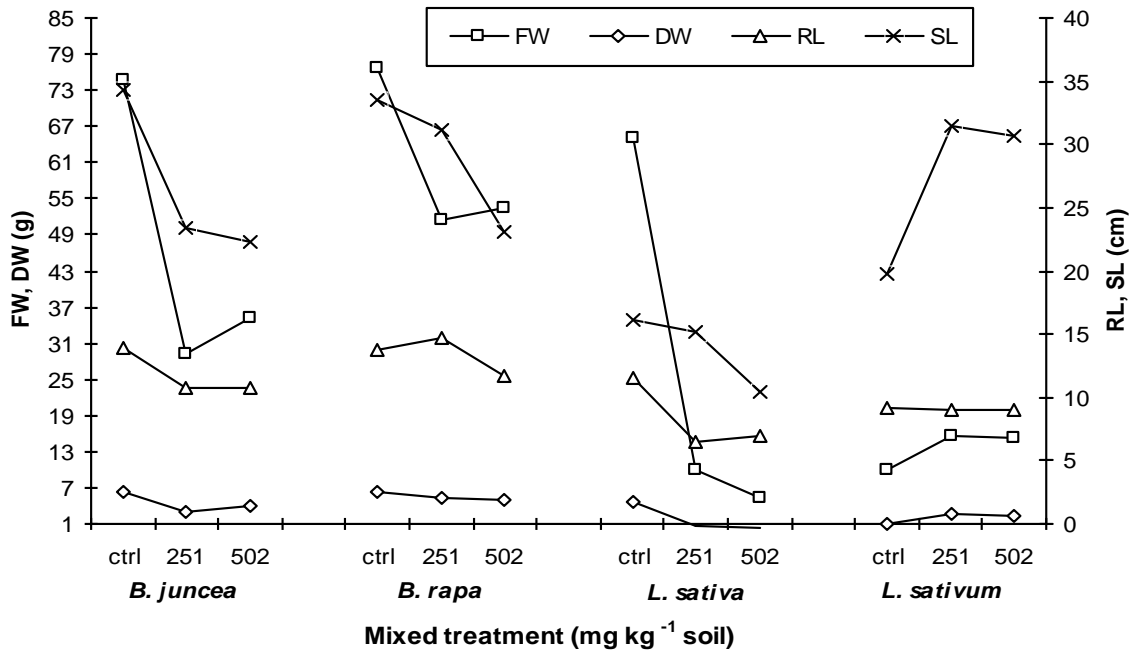


Fig. 15. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in matured *Brassica juncea*, *Brassica rapa*, *Lactuca sativa* and *Lepidium sativum* grown on soil treated with different concentrations of mixed heavy metals.

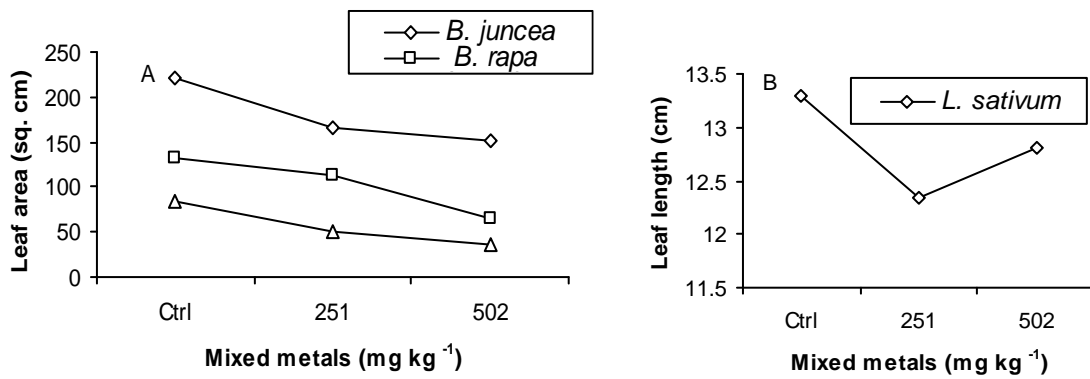


Fig. 16. Leaf area (A) and leaf length (B) of vegetable crops grown on soil treated with different concentrations of mixed heavy metals [$\text{CdCl}_2 + \text{CuCl}_2 + \text{Pb}(\text{NO}_3)_2 + \text{ZnCl}_2$].

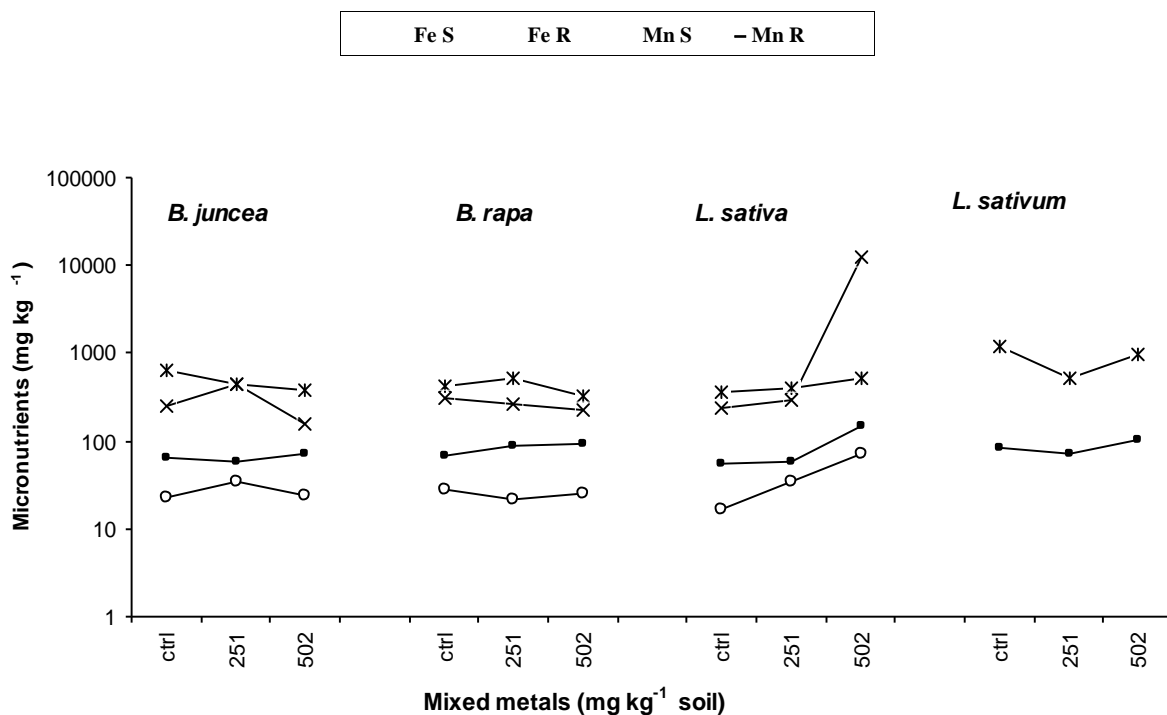


Fig. 17. Fe and Mn accumulation in shoot and root of *Brassica juncea*, *Brassica rapa*, *Lactuca sativa* and *Lepidium sativum* grown on soil treated with different concentrations of mixed heavy metals ($\text{CdCl}_2 + \text{CuCl}_2 + \text{Pb}(\text{NO}_3)_2 + \text{ZnCl}_2$)

Table 18. Chi-square value obtained from Kruskal-Wallis test for morphological parameters of vegetable crops grown on soil treated with different concentrations of mixed heavy metals.

Vegetable crops	Observation (n)	FW	DW	RL	SL
<i>B. juncea</i>	14	8.69**	9.65**	8.59*	2.46
<i>B. rapa</i>	15	9.50**	3.44	9.44**	6.18*
<i>L. sativum</i>	13	3.52	6.79*	5.42	3.36cm
<i>L. sativa</i>	13	8.73**	6.22*	6.81*	8.02**

Significance level * = $P < 0.05$, ** = $P < 0.001$, unmarked = not significant, ^R=root, ^L=leaf, ^Y=young, ^M=mature,

The RL and SL, FW and DW in *B. juncea* decreased significantly ($P < 0.05$) with increased accumulation of metals (Cd, Cu, Pb and Zn) from their mixed metal treatments. Shoot length, RL and FW significantly ($P < 0.05$) decreased in *B. rapa* but DW and DW % increased significantly ($P < 0.05$) in *L. sativum*. Root length, SL, FW, and DW decreased significantly in *L.*

sativa. There was significant increase in DW % in all vegetables except *L. sativa* (Figs. 15 and 16; Tables 18 and 19).

The Fe concentration decreased in both shoot and roots of *B. juncea*, Fe in roots of *B. rapa* but Fe and Mn both increased in *L. sativa* after mixed metal treatment (Fig. 17).

Table 19. Dry weight (%) of vegetable crops grown on soil treated with different concentrations of mixed heavy metals.

Treatments (mg kg ⁻¹ soil)	<i>B. juncea</i> ^Y	<i>B. rapa</i> ^Y	<i>L. sativum</i> ^Y	<i>L. sativa</i> ^M
ctrl	8.26	8.38	8.68	8.59
251 mg	10.62	10.32	16.39	5.71
502 mg	11.34	9.06	14.97	9.44
Chi sq.	6.62*	5.78*	6.75*	4.3

Significance level * = P<0.05, **= P<0.001, unmarked= not significant, ^Yindicates young and ^M the mature

4.2 Impact of Heavy Metals on the Chlorophyll Content

Studied vegetables were found to be growing in all treatments except at 500 and 1000 mg kg⁻¹ CuCl₂ and 1500 and 3000 mg kg⁻¹ ZnCl₂ treatments. *Lactuca sativa* could not grow even in 300 mg ZnCl₂/kg soil treatment but could grow in 500 mg CuCl₂ kg⁻¹ soil treatment. Accumulations of each Cd, Cu, Pb and Zn (from mixed metal treatments) in the leaves are given along the X-axis and their effect on Chl-a, Chl-b and total-chl along the Y-axis (Figs. 18 - 21) respectively. Chlorophyll ratio (Chl- a/b) in all vegetables grown with different concentrations of heavy metals is given in Table 20.

The mean total chlorophyll concentration ranged from 1.47 in *L. sativa* to 3.66 mg g⁻¹ FW in *L. sativum*. The Chl-a/b ratio was higher (0.713 mg g⁻¹) in *B. juncea* and were lower (0.53 mg g⁻¹) in *L. sativum* among the control vegetable crops (Table 20). The Chl-b in all vegetables was higher when compared to Chl-a (Figs. 18 - 21). Changes in Cu-, Fe-, Mn- and Zn-accumulation in leaves of vegetable crops grown on soil treated with different concentrations of CdCl₂, CuCl₂, Pb(NO₃)₂ and ZnCl₂ are summarized in Table 21.

4.2.1. Cadmium

Among the tested vegetables *L. sativum* accumulated more Cd than other vegetables (**Fig. 18**). All chlorophylls (total-chl, Chl-a and Chl-b) decreased significantly ($P < 0.05$) in both *B. juncea* and *L. sativum* with increased leaf Cd accumulation. Significant increase in Chl-a and total-chl was found in *D. carota*. The Chl-a/b insignificantly decreased (**Table 20**) in *L. sativum*, but it increased significantly in *D. carota*.

Table 20. Chlorophyll a/b ratio in leaves (n=4-6) of vegetable grown on soil treated with different concentrations of single heavy metals.

Heavy metals	Treatments (mg kg ⁻¹ soil)	<i>B. juncea</i>	<i>B. rapa</i>	<i>L. sativa</i>	<i>L. sativum</i>	<i>D. carota</i>
CdCl₂	Ctrl	0.713b	0.553a	0.665a	0.532b	0.598a
	1	0.733b	0.580a	0.664a	0.472ab	0.815b
	2	0.701b	0.639a	0.726a	0.461a	0.828bc
	10	0.601a	0.586a	0.671a	0.414a	0.977cd
	20	0.709b	0.626a	0.668a	0.481ab	0.670ab
CuCl₂	Ctrl	0.713b	0.553a	0.665a	0.532b	0.598a
	50	0.627a	0.557a	0.809b	0.484a	0.634a
	100	0.676a	0.585a	0.825b	0.493a	0.607a
	500	-	-	0.735a	-	-
Pb (NO₃)₂	Ctrl	0.713b	0.553a	0.665a	0.532a	0.598a
	50	0.574a	0.514ab	0.666a	0.739b	0.635bc
	100	0.603ab	0.496a	0.662a	0.813bc	0.614abc
	500	0.562a	0.590b	0.667a	0.947c	0.639c
	1000	0.639ab	0.489a	0.639a	-	0.587a
ZnCl₂	Ctrl	0.713b	0.553a	0.665a	0.532a	0.598a
	150	0.644a	0.532a	0.695a	0.547a	0.597a
	300	0.626a	0.575a	-	0.635b	0.623a

The letters denoted by same alphabet along the column do not differ significantly ($P < 0.05$) according to Duncan's Multiple Tests followed after one way ANOVA.

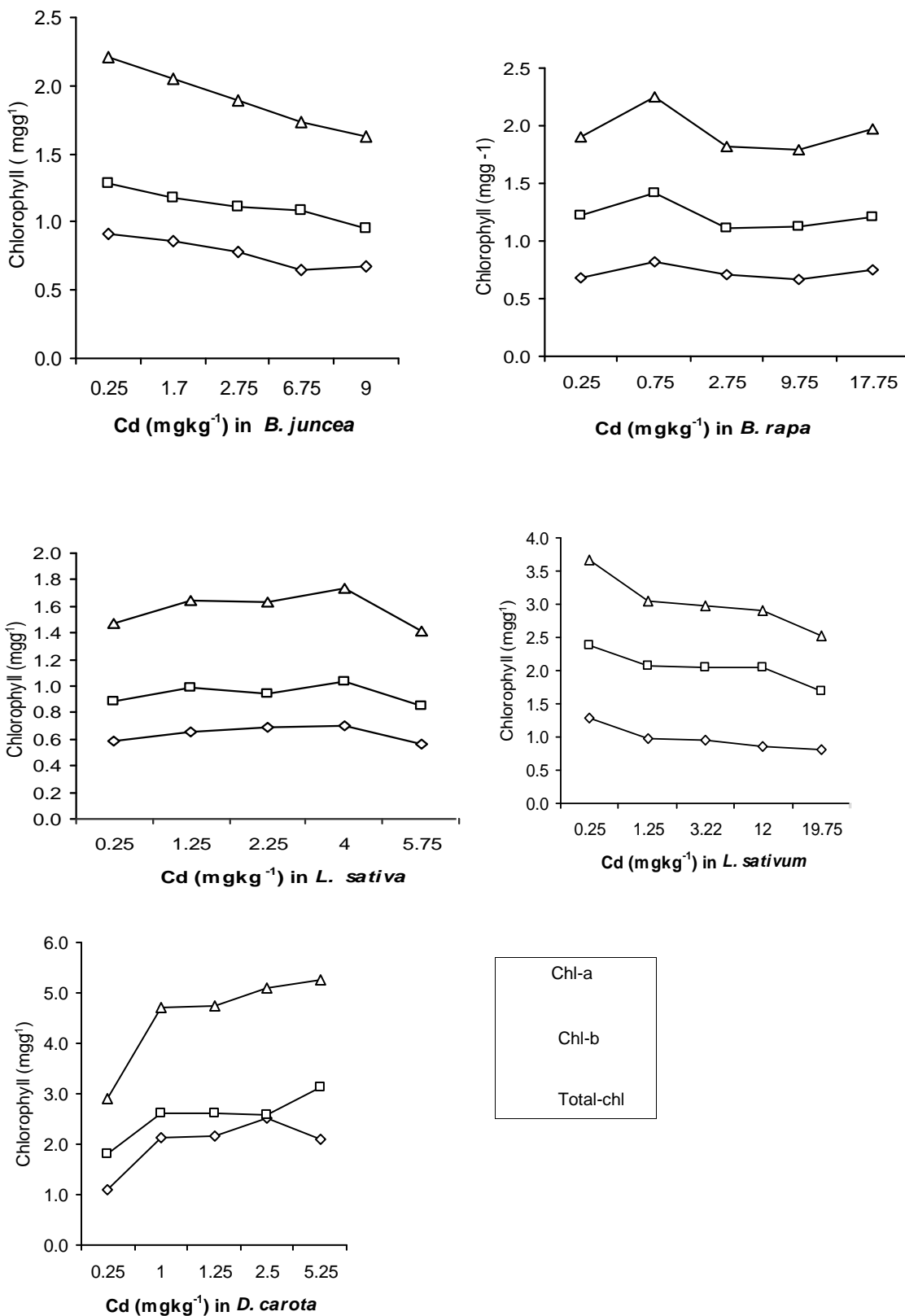


Fig. 18. Chlorophylls (mg g⁻¹) and Cd concentrations (mg kg⁻¹) in vegetable leaves after growing on soil treated with different concentrations of CdCl₂.

Mostly CdCl₂-treated vegetables showed Cu depletion and Fe enrichment. Depletion of Cu, Zn and Mn in *B. juncea*; and both Cu and Zn in *L. sativum* were observed after increased Cd uptake and its accumulation. With increased Cd accumulation, Cu decreased in leaves of both *L. sativa* and *D. carota*. There was no decrease of any essential micronutrients in *B. rapa* instead of increase of Cu, Mn, Fe after Cd uptake (**Table 21**).

4.2.2. Copper

Accumulation of Cu was more in leaves of *B. rapa* among the studied leaves of other vegetable crops. Copper toxicity on chlorophyll concentrations was not so prominent in most vegetable crops except in *B. juncea* (in which total-chl and Chl-a decreased significantly at $P < 0.05$) and *D. carota* where insignificant decrease of all chlorophyll was observed at 100 mg kg⁻¹ CuCl₂ soil treatments. Insignificant decrease of Chl-a was observed in *B. rapa*, although chlorophylls increased at 50 mg Cu kg⁻¹ soil (**Fig. 19**). Insignificant change in Chl-a/b was observed in all vegetables (**Table 20**). Iron accumulation increased but Zn decreased in most CuCl₂ treated vegetables. Decrease of both Zn and Fe in *B. juncea* and Mn decreased in *D. carota* was observed after CuCl₂ treatments. Similarly, Zinc decreased in leaves of both *B. rapa* and *L. sativum* but Fe and Mn increased in both the cases. In *L. sativa* there is no decrease of any micronutrients but only Fe increased along increased Cu uptake (**Table 21**).

4.2.3. Lead

Lead accumulation was more in *L. sativum* than in other tested vegetables (**Fig. 20**). The concentrations of total-chl, Chl-a and Chl-b insignificantly decreased in *D. carota* at only 1000 mg kg⁻¹ soil Pb(NO₃)₂ treatment. Total-chl increased significantly up to 500 mg Pb(NO₃)₂ kg⁻¹ soil treatment in *B. juncea*. The Chl-a insignificantly changed in most of the vegetables except in *L. sativum* in which Chl-a increased upto 500 mg kg⁻¹ soil Pb treatment resulting significantly increase in Chl-a/b (**Table 20**) ratio. Iron and Zn increased with increase of Pb in *B. rapa* and *L. sativa*. Similarly, Fe increased in *D. carota* shoots but slight depletion of Zn, Cu and Mn was noticed in it. With increased Pb uptake, Fe and Cu increased but slight by Zn decreased in *B. juncea* (**Table 21**).

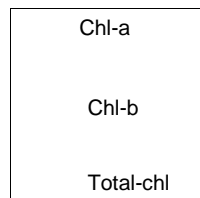
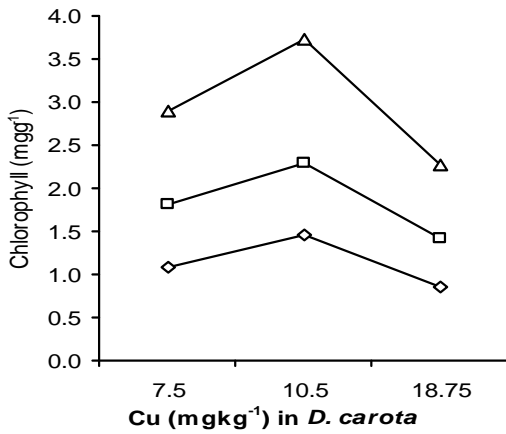
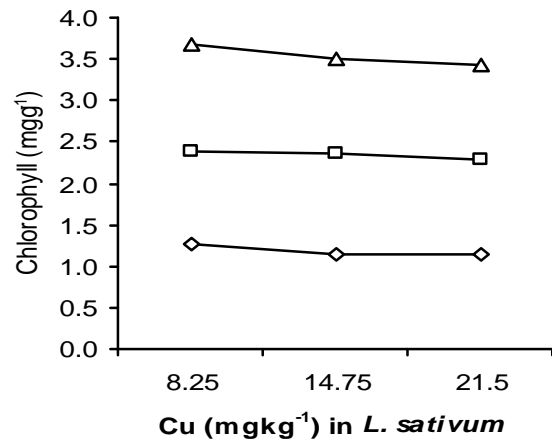
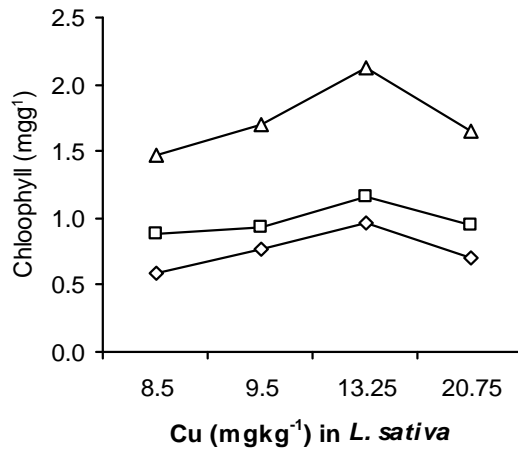
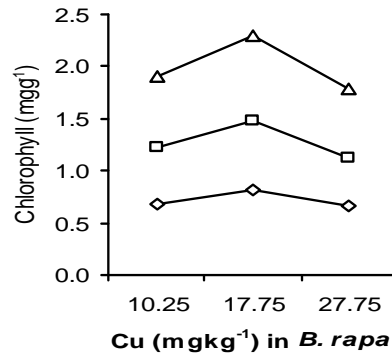
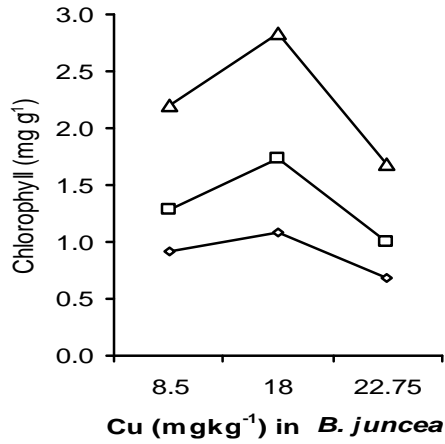


Fig. 19. Chlorophylls (mg g⁻¹) and Cu concentration (mg kg⁻¹) in vegetable leaves after growing on soil treated with different concentrations of CuCl₂.

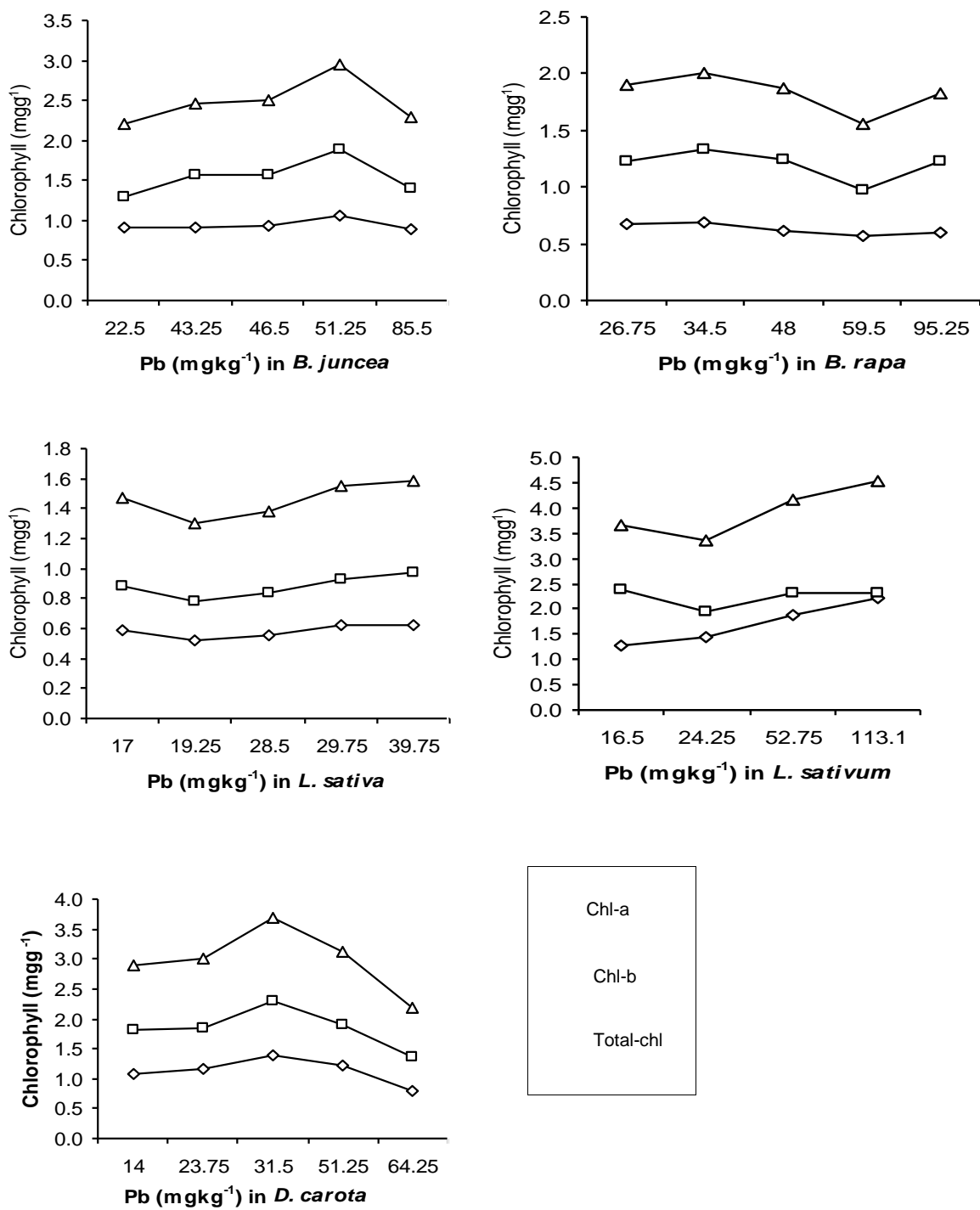


Fig. 20. Chlorophylls (mg g⁻¹) and Pb concentrations (mg kg⁻¹) in vegetable leaves after growing on soil treated with different concentrations of Pb(NO₃)₂.

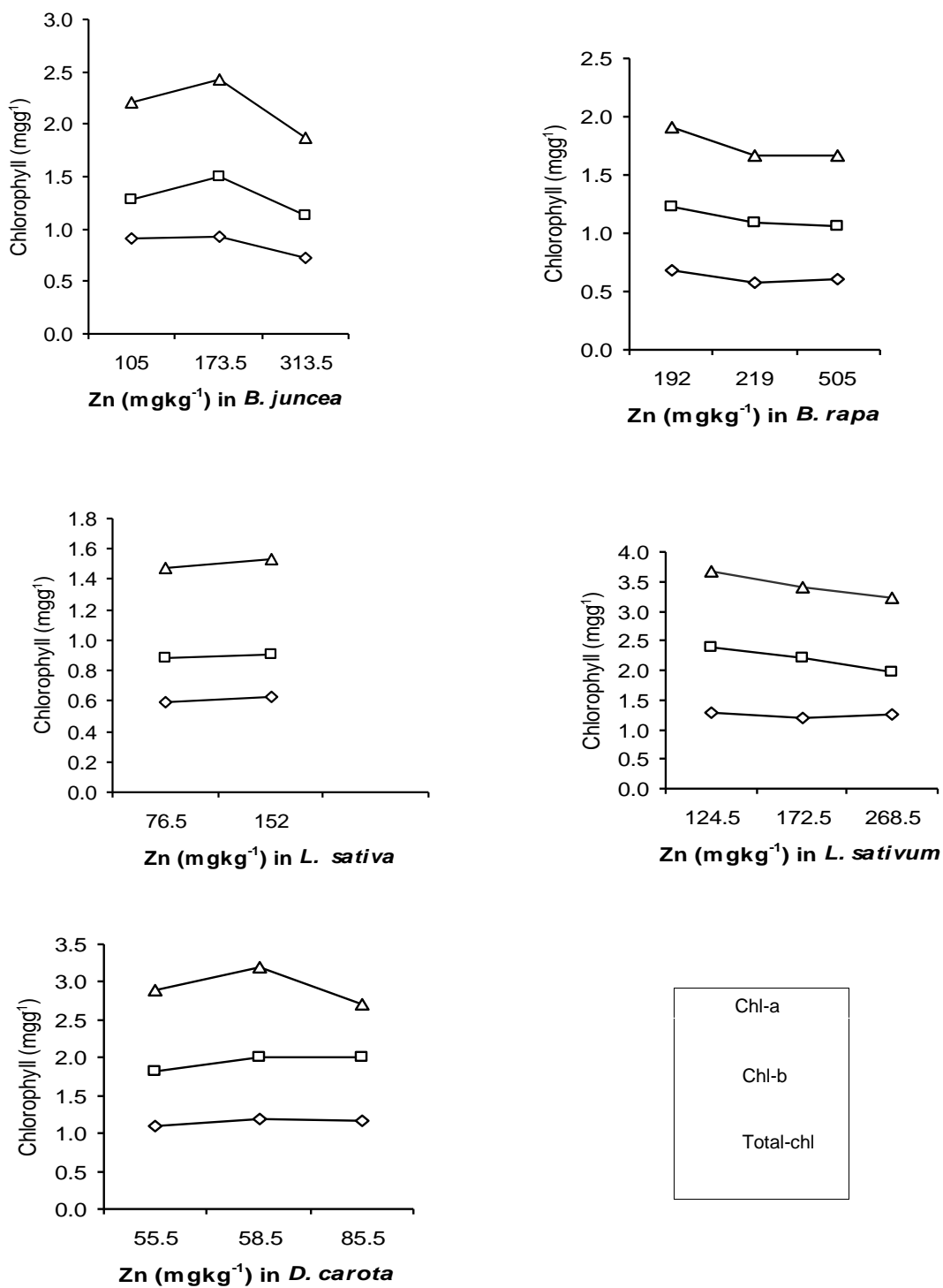


Fig. 21. Chlorophylls (mg g⁻¹) and Zn concentrations (mg kg⁻¹) in vegetable leaves after growing on soil treated with different concentrations of ZnCl₂.

4.2.4. Zinc

Zinc accumulation was highest in *B. rapa* and chlorophylls were not significantly affected in all, except in *L. sativum* (**Fig. 21**). Total-chl in *L. sativum* decreased insignificantly but Chl-b decreased significantly ($P < 0.05$) at 300 mg $ZnCl_2 \text{ kg}^{-1}$ soil treatment, resulting significant ($P < 0.05$) increase in Chl-a/b (**Table 20**). Significant decrease of Chl-a/b in *B. juncea* was observed at 150 mg $ZnCl_2 \text{ kg}^{-1}$ soil treatment. Increased Zn accumulation induced Cu-depletion in most cases, except in *B. rapa* and *D. carota*. Besides, depletion of Fe and Mn in *B. juncea*; Fe in *B. rapa* and *L. sativum*; and Mn in *L. sativa* (**Table 21**) were observed with increased Zn uptake in vegetables grown on $ZnCl_2$ contaminated soil. In *D. carota*, only Cu decreased but Fe and Mn increased slightly.

4.2.5. Mixed metal treatments

In *B. juncea*, total-chl and Chl-b increased significantly at low dose (251 mg kg^{-1}) treatment with mixed metals. Decrease in total- chl, Chl-a and Chl-b was observed in *L. sativum* and *L. sativa*. Insignificant increase in total -chl, Chl-b and Chl-a was observed in *B. rapa* at low dose treatments of mixed metals (**Fig. 22**).

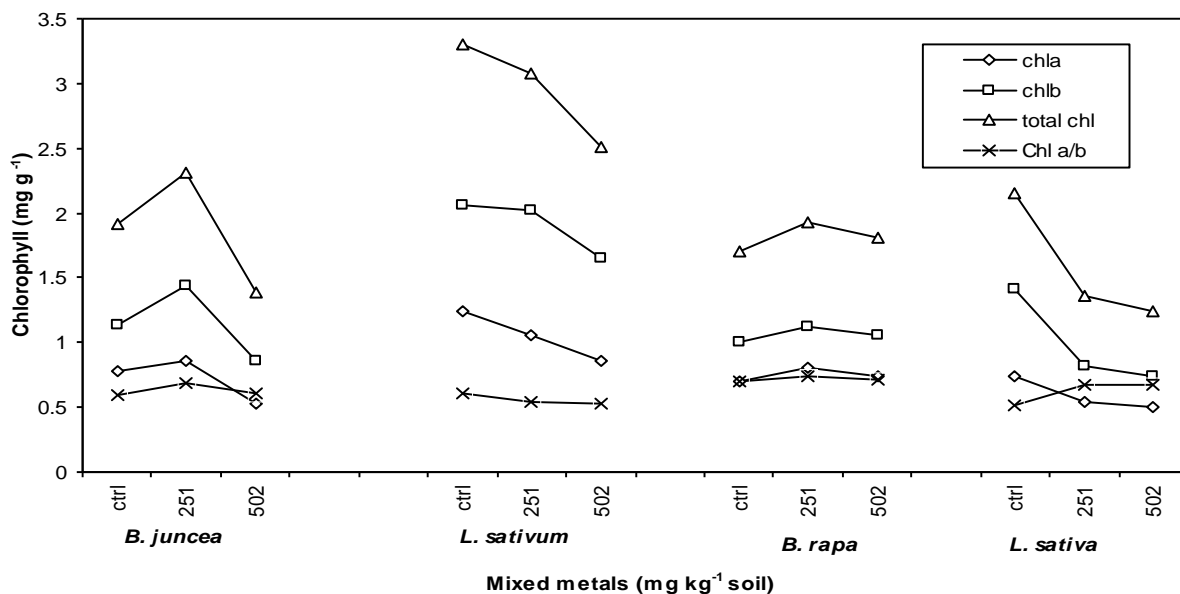


Fig. 22. Chlorophylls (mg g^{-1}) in different vegetables leaves after growing on soil treated with different concentrations of mixed metals.

Table 21. Changes in micronutrients in vegetable leaves after uptake of excess heavy metals

Heavy metals	Vegetable crops	Micronutrients		
		No change	Decrease	Increase
CdCl₂	<i>B. juncea</i>	-	Cu, Zn, Mn	Slight Fe
	<i>B. rapa</i>	Zn	-	Mn, Cu, Fe
	<i>L. sativa</i>	Mn	Cu	Zn, Fe
	<i>L. sativum</i>	Mn	Cu, Zn	Fe
	<i>D. carota</i>	Mn	Cu	Zn, Fe
CuCl₂	<i>B. juncea</i>	-	Zn, Fe	Mn
	<i>B. rapa</i>	-	Zn	Fe, Mn
	<i>L. sativa</i>	Mn, Zn	-	Fe
	<i>L. sativum</i>	-	Slight Zn	Fe, Mn
	<i>D. carota</i>	Zn	Mn	Fe
Pb(NO₃)₂	<i>B. juncea</i>	Mn	Slight Zn	Fe, Cu
	<i>B. rapa</i>	Cu, Zn	-	Fe, Mn
	<i>L. sativa</i>	-	Cu	Fe, Mn, Zn
	<i>L. sativum</i>	-	Mn, Fe	Cu, Zn
	<i>D. carota</i>	-	Mn, Cu, Zn	very high Fe
ZnCl₂	<i>B. juncea</i>	-	Fe, Mn, Cu	-
	<i>B. rapa</i>	-	Cu, Fe	Mn (slight)
	<i>L. sativa</i>	-	Mn, Cu	Fe
	<i>L. sativum</i>	Mn	Fe, Cu	-
	<i>D. carota</i>	-	Cu	Fe, Mn (slight)
Mixed metals	<i>B. juncea</i>	-	Fe	Mn
	<i>B. rapa</i>	Fe	-	Mn (slight)
	<i>L. sativa</i>	-	-	Fe, Mn
	<i>L. sativum</i>	Fe, Mn	-	-

4.3. Monitoring of Heavy Metals in Soil and Vegetables

4.3.1. Soil Parameters and Soil Heavy Metals

Soil properties in the soil samples from the vegetables growing fields of Kathmandu valley are given in **Table 22** and content of soil heavy metals are given in **Table 23**. Almost all soil samples were acidic in nature, pH ranging from 3.9 (at Khusibun) to 6.1 (at Chapro). Organic matter in the soil ranged from 0.54 % (at Banasthali) to 5.76 % (at Balkhu). Organic matter above 4.42% is found in Khusibun and Shankhamul. Nitrogen ranged from 0.065 % (Thimi and Ghattekulo) to 0.286 % (Banasthali, Shankhamul and Khusibun), phosphorus (kg/ha) ranged from 359 to 961 kg/ha. Phosphorus was high was in Manohora, Shankhamul and Khusibun. Potassium (kg/ha) ranged from 167 to 819 kg/ha and high in Shankhamul, Khusibun and Balkhu. Soil texture ranged from loamy to sandy loam at different plots from various agricultural fields. The clay percent above 10 were present in Shankhamul (11 %), Manohora (14 %), Balkhu (15 %) and Banasthali (16 %) (**Table 22**).

Although concentrations of Cu, Pb, Zn and Fe in soil of some field and Cd in all field, exceed the normal soil values as prescribed by Bowen (1979), but lie below critical soil value (Kabata-Pendias and Pendias, 1992). Cadmium accumulation in soil ranged from 0.25 (Kalopul) to 2.00 mg kg⁻¹ (Shankhamul). Soil Cu in Banasthali, Nakhu, Shankhamul, and Khusibun are slightly higher than normal soil value. Soil Cu ranged from 15 mg kg⁻¹ (Kusingal) to 65 mg kg⁻¹ (Shankhamul). Soil Pb in Banasthali, Manohora, Nakhu, Shankhamul, Kusingal, Shovabhagawati and Balkhu exceed normal soil value. Soil Pb ranged from 30.50 mg kg⁻¹ (Kalopul) to 46.75 mg kg⁻¹ (Shankhamul). The concentration of Ni, Co, Cr and Mn in soil are mostly within the normal soil value, except Cr at Banasthali. Soil Zn ranged from 70.50 mg kg⁻¹ (Kusingal) to 162 mg kg⁻¹ (Khusibun) (**Table 23**). Soil Fe ranged from 14157 (Kusingal) to 75636 mg kg⁻¹ (Khusibun).

Results of Pearson correlation coefficient (r) between different soil parameters and soil metal content are given in **Table 24**. Significant (P < 0.05) positive correlation was observed between Cu and Pb concentrations in soil. Similarly, significant positive correlation was also observed between soil Cu and N, P, K concentration in the soil. Cadmium showed poor correlation with all soil parameters. Soil pH showed significant negative correlation with P. Zinc concentration showed significant negative correlation with pH and significant positive

correlation with P and Fe. A significant correlation was also observed between Zn and Fe; N and K; K and Ni; K and Co; clay and Ni; clay and Co; Clay and Mn; Ni and Co; Ni and Mn, Co and Mn, organic matter (OM) and K content in the soil.

Table 22. Some of soil parameters in the soil samples collected from vegetable crops growing fields

Soil samples	pH	OM%	N%	P kg/ha	K kg/ha	Texture (%)			
						Sand	Silty	Clay	Class
Banasthali	5.6	0.54	0.286	532	405	43	41	16	L
Chapro	6.1	2.95	0.146	614	431	61	29	10	SL
Manohora	5.0	2.80	0.155	959	291	45	41	14	L
Thimi	4.5	1.60	0.065	793	176	67	27	6	SL
Ghattekulo	5.4	2.28	0.065	528	176	66	25	9	SL
Shankhamul	4.9	4.56	0.286	961	687	48	41	11	L
Kusingal	5.2	2.81	0.114	390	211	48	45	7	L(B)- SL
Shovabhagawati	5.3	2.81	0.147	359	247	52	39	9	L(B)- SL
Kalopul	5.4	3.48	0.188	557	193	74	19	7	SL
Khusibun	3.9	4.42	0.286	957	819	54	37	9	SL
Balkhu	5.2	5.76	0.18	511	634	38	47	15	L

Table 23. Concentrations (mg kg⁻¹) of heavy metals in the soil samples collected from different agricultural fields.

Biotops	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
^{NSV} B	0.35	1-40**	70	30	40000	1000	50	35	90
^{UCSV} KP	8.00	-	100	125	-	3000	100	400	400
Banasthali	1.50	12.00	73.75*	34.50*	26719	312	22.25	37.25*	70.50
Chapro	1.68	10.92	31.08	18.48	22500	142	17.36	32.76	72.24
Manohora	1.05	7.50	29.00	23.00	26928	48	14.25	37.25*	117.00*
Nakhu	1.25	8.75	32.00	34.50*	32670	443	29.25	36.50*	90.00
Thimi	1.04	6.25	29.12	21.32	18009	-	12.50	31.46	81.12
Kalopul	1.00	5.75	33.50	20.10	23661	127	12.00	30.50	141.00*
Ghattekulo	1.50	8.10	33.00	20.10	61322*	141	9.90	34.50	117.00*
Shankhamul	2.00	10.75	19.75	65.5*	-	283	19.50	46.75*	115.50*
Kusingal	2.00	7.50	15.25	15.00	14157	128	12.50	36.00*	70.50
Shovabhagawati	1.56	8.06	14.76	23.08	21900	247	15.60	37.44*	76.72
Khusibun	0.75	8.50	33.00	43.25*	75636*	-	14.75	33.00	162.00*
Balkhu	1.00	15.25	23.75	26.25	18711	675	27.50	39.25*	79.50

*exceeded normal soil values, **Normal soil value as proposed by Swaine (1955), ^{NSV}B Normal soil value as proposed by Bowen (1979), ^{UCSV}KP upper critical soil value as proposed by Kabata-Pendias and Pendias (1992).

Table 24. Correlation Coefficient (r) values among the soils heavy metals and the soil properties.

	Cu	Pb	Cr	Cd	Ni	Co	Zn	Fe	Mn	pH	OM	N	P	K	clay
Cu	1														
Pb	0.69*	1													
Cr	0.07	-0.19	1												
Cd	0.32	0.56	-0.53	1											
Ni	0.36	0.53	0.23	0.09	1										
Co	0.31	0.52	0.22	0.21	0.97**	1									
Zn	0.44	-0.06	-0.05	-0.15	-0.32	-0.29	1								
Fe	-0.22	-0.22	0.16	-0.09	0.27	0.47	0.83**	1							
Mn	0.37	0.37	0.49	-0.04	0.88**	0.67*	-0.17	0.34	1						
pH	-0.41	-0.01	0.20	0.14	0.17	0.27	-0.64*	-0.49	-0.34	1					
OM	0.35	0.34	-0.57	0.21	0.37	0.37	0.37	0.14	0.46	-0.28	1				
N	0.77**	0.43	0.39	-0.12	0.52	0.41	0.35	0.28	0.21	-0.21	0.28	1			
P	0.60*	0.18	0.02	0.14	-0.04	-0.10	0.66*	0.43	0.14	-0.61*	0.21	0.37	1		
K	0.74**	0.44	0.03	0.18	0.60*	0.60*	0.42	0.45	0.57	-0.40	0.64*	0.76**	0.49	1	
Clay	0.27	0.50	0.49	-0.03	0.88**	0.86**	-0.12	0.20	0.61*	0.24	0.07	0.48	0.09	0.40	1

*Significant at $p \leq 0.05$, ** significant at $p \leq 0.01$

4.3.2. Heavy Metal Accumulation in Vegetables

Heavy metal content in the vegetable crops collected from different biotops (as mentioned in material and methods) is given in **Table 25 and 26**. Results of correlation coefficient (r) between the heavy metals content in soil and their bioaccumulation in different parts of vegetable is given in **Table 27**.

Cadmium

Among the leafy vegetables, cress leaf showed high Cd accumulation where as turnip showed high accumulation among roots. Although most of leafy vegetables showed more Cd accumulation compared to roots, even bioaccumulation of Cd in root of *S. oleracea* var. Gobre is mostly greater than in soil (at Thimi and Shovabhadrawati) showing tendency of hyperaccumulation. In leaves of *B. juncea* and *S. oleracea* it ranged from 0.5 to 1.5 mg kg⁻¹. In roots of carrot and radish it ranged from 0.25 to 1.25 mg kg⁻¹. Cadmium accumulation in roots of *B. juncea* from Manohara and Kalopul were found to be higher than in leaves. In potato tuber, Cd concentration ranged from 0.25 mg kg⁻¹ (of Sankhu) to 0.75 mg kg⁻¹ (of Panchkhal). The plant Cd values are within the ranges of normal plant values (**Table 25**).

Copper

Copper concentration in *B. juncea* (17.75 mg kg⁻¹) from Khusibun and *S. oleracea* from most of the sampling sites were slightly above the normal plant value of Alloway (1968) but within range of critical plant tissue concentrations of Kabata- Pendias and Pendias (1992) as shown in **Table 25**. Among the leafy vegetables, highest Cu accumulation (25 mg kg⁻¹) was found in spinach leaves from Shankhamul area (**Table 25**). Bioaccumulation of Cu in edible part (either shoots/roots) was highest in spinach leaves > cress leaf > broad leaf mustard > turnip > carrot > red radish > white radish = potato tuber. Copper accumulation was less in root of carrot (4-6.5 mg kg⁻¹) and radish (3.5 mg kg⁻¹ - 5.75 mg kg⁻¹) than in most leafy vegetables. In some places accumulation of Cu in leaves of leafy vegetables is higher than in root indicating high mobility, such as in aerial portion of both carrot and radish than in their roots. In underground tuber of potato Cu accumulation was relatively low.

Cobalt and Chromium

Cobalt accumulation ranged from 0.25 to 6.0 mg kg⁻¹ in *B. juncea*. Highest mean value of Co was observed in *S. oleracea*, but highest concentration was observed in *B. rapa* (root) collected from Thimi and *S. oleracea* (both root and shoot) collected from Thimi and Chapro, *R. sativus* (shoot) from Khusibun and *D. carota* (shoot) collected from Manohara, Ghattekulo and Shankhamul (**Table 25**). Chromium accumulation in all vegetables was found within normal plant value and ranges from 0.25 mg kg⁻¹ to 4.75 mg kg⁻¹ in spinach (**Table 25**)

Iron and Manganese

Accumulation of Fe is extremely higher in studied vegetables than prescribed normal plant value (140 mg kg^{-1}), except potato and was high in the order of broad leaf mustard > spinach > cress leaf > turnip > radish > carrot > potato (**Table 25**). Among the studied vegetable crops, bioaccumulation of Mn was high (ranging from 48 to 187.2 mg kg^{-1}) in cress leaf and low in potato tuber (ranging from 8.25 to 18.25 mg kg^{-1}). In all vegetable crops, Mn concentration was found to be below the critical plant tissue concentration (**Table 26**).

Nickel

Although, Ni accumulation is higher than the normal plant value, but is below the value of critical plant concentration (10 mg kg^{-1}). High mean value of Ni content was seen in leafy portion than in the roots. High concentrations of Ni was observed in leaves of *B. juncea* collected from Thimi and Balkhu, and leaves of *S. oleracea* collected from Shankhamul (**Table 26**).

Lead

Highest accumulation of Pb was observed in red radish and lowest in potato. High Pb accumulation above 20 mg kg^{-1} was observed in all leafy vegetables. Concentrations of Pb in all vegetable plants exceed the normal plant values as given by Alloway (1968) but are within the range value of critical plant tissue concentration (**Table 26**). In broad leaf mustard of Kalopul, Banasthali, Shovabthagawati and Balkhu, higher accumulation of Pb have been found in roots than in their leaves. Lead accumulation in the leaves of *B. juncea* ranged from 15.00 (at Balkhu area) to 34.75 mg kg^{-1} (at Khusibun). Highest Pb concentration (37.50 mg kg^{-1}) was observed in spinach leaves at Thimi area. Concentrations of Pb are also mostly lower in roots than in leaves, as in the case of Cu. Carrot root grown at Ghattekulo area showed high Pb concentration up to 23.5 mg kg^{-1} . In radish, Pb concentrations ranged from 20 mg kg^{-1} at Kusingal to 39.05 mg kg^{-1} at Khusibun. In contrast to carrot, more accumulation of Pb was observed in roots of radish than in their leaves. Concentration of Pb in potato tuber ranged from 4.75 (Manohora) to 9.75 mg kg^{-1} (Panchkhal), and were very low in comparison to other vegetables.

Table 25. Concentrations (mg kg⁻¹) of heavy metals in shoots (S) and roots (R) of vegetable crops collected from different biotops in Kathmandu valley.

Places	Cd (S)	Cd (R)	Co (S)	Co (R)	Cr (S)	Cr (R)	Cu (S)	Cu (R)	Fe (S)	Fe (R)
^{NPV} A		0.1-2.4		-		0.2-10		5-15		140
^{RVC} KP		5-30		-		5-30		20-100		-
<i>B. juncea</i>										
Thimi	1.00	0.75	3.50	3.50	2.75	4.50	6.50	6.75	6284.0	1728.00
Manohora	1.00	1.04	1.25	3.90	4.25	6.24	7.75	13.26	573.0	151.32
Kalopul	0.50	0.94	6.00	2.81	1.75	1.25	8.00	9.69	4167.0	515.625
Bansthali	0.75	0.75	1.50	2.00	4.50	2.25	3.00	7.50	5859.0	603.00
Shankhamul	1.50	1.50	2.75	1.50	2.25	1.50	11.25	9.25	5463.0	511.50
Kusingal	1.50	0.75	3.75	1.33	2.25	0.80	9.25	5.83	6336.0	593.07
Shovabhagwati	1.25	0.53	2.25	0.25	3.25	3.25	9.75	8.75	4851.0	1006.50
Khusibun	0.75	0.50	0.25	2.25	2.25	0.50	17.75	8.53	8361.0	225.75
Balkhu	0.75	0.29	3.00	1.00	3.00	0.75	11.50	2.06	7866.0	157.50
Mean	1.00	0.78	2.69	2.06	2.92	2.34	9.42	7.96	5528	610
SD	±0.35	±0.35	±1.66	±1.19	±0.94	±1.97	±4.06	±3.06	±2282	±508.8
<i>B. rapa</i>										
Thimi	1.25	0.75	1.75	4.25	5.25	0.50	11.75	7.75	1138.5	390.00
Manohora	0.75	0.25	0.25	0.75	4.00	1.25	8.25	5.25	846.0	3429.00
Kusingal	0.50	1.00	3.25	1.75	2.25	0.25	5.25	3.00	1116.0	342.00
Mean	0.83	0.67	1.75	2.25	3.83	0.67	8.42	5.33	1033.50	1387.00
SD	±0.38	±0.38	±1.50	±1.80	±1.51	±0.52	±3.25	±2.38	±162.77	±1768.59
<i>L. sativum</i>										
Thimi	0.50	0.50	3.25	2.25	3.50	3.50	7.25	7.50	526.5	553.5
Manohora	1.50	0.75	2.00	3.25	1.50	3.75	9.50	13.25	1647.0	387.0
Khusibun	1.30	0.32	2.08	0.25	3.12	2.22	13.26	11.75	4062.0	749.01
Mean	1.10	0.52	2.44	1.92	2.71	3.16	10.00	10.83	2078.50	563.17
SD	±0.53	±0.22	±0.70	±1.53	±1.06	±0.82	±3.04	±2.98	±1806.82	±181.28
<i>S. oleracea</i>										
Thimi	1.25	1.25	4.00	4.25	4.75	4.50	14.75	17.25	3735.00	1773.00
Bansthali	0.75	0.50	3.00	0.75	2.75	1.00	13.25	13.50	5157.00	315.00
Chapro	1.10	0.75	4.40	1.50	1.93	3.00	14.85	16.75	15939.00	1389.00
Shankhamul	0.50	1.75	1.25	3.75	2.50	2.50	25.00	19.50	6993.00	546.00
Shovabhagwati	0.25	1.75	1.50	1.00	0.25	1.75	7.50	8.25	171.00	2493.75
Manohora	1.04	0.25	2.60	-	3.12	-	15.60	13.25	828.36	-
Mean	0.82	1.04	2.79	2.25	2.55	2.55	15.16	14.75	5470.56	1303.35
SD	±0.39	±0.64	±1.28	±1.63	±1.48	±1.33	±5.65	±2.65	±5738.5	±893.78
<i>D. carota</i>										
Chapro	0.75	0.25	3.00	0.75	2.50	1.25	9.50	6.50	547.5	220.5
Bansthali	0.50	0.25	1.75	2.00	2.25	0.25	10.50	4.00	307.5	210.0
Manohora	0.25	0.25	4.00	2.00	3.00	0.25	6.00	5.50	478.5	396.0
Ghattekulo	1.00	1.25	4.50	0.25	7.00	0.75	9.50	4.25	514.0	450.0
Shankhamul	0.25	1.10	4.25	2.20	1.00	0.28	10.50	5.50	507.0	2039.0
Mean	0.55	0.62	3.50	1.44	3.15	0.56	9.20	5.15	470.90	663.10
SD	±0.33	±0.51	±1.13	±0.88	±2.27	±0.44	±1.86	±1.02	±94.59	±776.38
<i>R. sativus (R)</i>										
Kusingal	0.25	0.25	2.25	2.25	2.50	0.75	5.50	3.75	222.5	612.0
Shovabhagwati	1.50	1.25	2.00	2.25	5.25	0.75	13.0	5.75	1082.5	1971.0
Khusibun	1.10	0.26	4.95	2.86	3.58	0.78	17.05	5.20	1725.9	1263.6
Mean	1.30	0.59	3.07	2.45	3.77	0.76	11.18	4.90	1010.3	1282.20
SD	±0.0.64	±0.57	±1.64	±0.34	±1.39	±0.02	±5.86	±1.03	±754.3	±679.69
<i>R. sativus (W)</i>										
Thimi	0.50	0.25	0.25	0.25	3.75	1.25	7.75	3.75	862.5	873.0
Ghattekulo	1.00	0.75	2.50	2.00	5.00	0.75	5.50	3.75	675.0	2331.0
Shovabhagwati	0.26	0.25	2.04	0.25	2.81	1.50	6.38	3.50	466.6	153.0
Mean	0.59	0.42	1.60	0.83	3.85	1.17	6.54	3.67	668.05	1119
SD	±0.38	±0.29	±1.19	±1.01	±1.10	±0.38	±1.13	±0.14	±198.04	±1109
Potato tuber										
Sankhu	-	0.25	-	1.50	-	1.25	-	4.00	-	87.0
Panchkhal	-	0.75	-	1.50	-	0.25	-	3.75	-	108.0
Manohora	-	0.50	-	2.50	-	1.25	-	3.25	-	87.0
Mean	-	0.50	-	1.83	-	0.92	-	3.67	-	94.0
SD	-	±0.25	-	±0.58	-	±0.58	-	±0.38	-	±12.12

* exceeded normal plant value, ^A Alloway (1968), ^B Bowen (1979), ^{KP} Kabata-Pendias and Pendias (1992)

Table 26. Concentrations (mg kg⁻¹) of heavy metals in shoot (S) and root (R) of vegetable crops collected from different biotops in Kathmandu valley.

Places	Mn (S)	Mn (R)	Ni (S)	Ni (R)	Pb (S)	Pb (R)	Zn (L)	Zn (R)
<i>NPV</i> ^A	-	-	-	1-2.7	-	0.1-10	-	20-400 ^B
<i>RVCP</i> ^{KP}	300-500	300-500	10-100	10-100	20-300	20-300	100-400	100-400
<i>B. juncea</i>								
Thimi	67.00	57.00	7.00	0.25	25.25	9.75	57.00	33.00
Manohora	42.25	78.52	4.25	1.56	23.00	19.76	49.50	70.20
Kalopul	25.75	26.56	3.25	1.25	16.50	21.56	51.00	69.38
Bansthali	27.25	102.00	4.75	3.75	17.75	24.75	37.50	25.50
Shankhamul	38.00	29.50	5.00	6.25	28.25	23.25	88.50	85.50
Kusingal	30.00	19.08	6.75	3.18	31.75	13.25	118.25	52.47
Shovabhagwati	37.25	35.50	4.25	5.00	28.75	30.50	54.00	33.00
Khusibun	74.50	25.25	4.50	1.50	34.75	20.05	105.00	112.75
Balkhu	73.75	31.00	7.00	1.00	15.00	22.00	75.00	31.76
Mean	46.19	44.93	5.19	2.64	24.56	20.54	70.64	57.06
SD	±19.19	±28.38	±1.38	±2.03	±7.00	±6.10	±27.79	±29.69
<i>B. rapa</i>								
Thimi	63.25	21.00	6.50	1.50	31.25	16.25	159.00	99.0
Manhora	74.25	27.27	5.75	4.25	29.50	24.75	63.00	48.0
Kusingal	43.25	8.75	3.25	0.50	7.00	25.25	52.50	73.50
Mean	60.25	19.01	5.17	2.08	22.58	22.08	91.50	73.50
SD	±15.72	±9.42	±1.70	±1.94	±13.52	±5.06	±58.69	±25.5
<i>L. sativum</i>								
Thimi	72.7	60.75	3.50	3.50	29.00	22.25	52.50	51.00
Manohora	48.0	40.75	2.75	1.25	22.00	24.00	84.00	106.50
Khusibun	187.2	97.20	6.24	5.08	31.72	29.86	363.48	251.58
Mean	102.65	66.23	4.16	3.28	27.57	25.37	166.66	136.36
SD	±74.27	±28.62	±1.84	±1.92	±5.01	±3.99	±171.18	±103.57
<i>Spinacia oleracea</i>								
Thimi	90.7	84.75	6.00	6.75	37.50	27.25	79.50	72.0
Bansthali	98.5	60.00	4.00	2.25	22.25	23.00	96.00	82.5
Chapro	111.1	67.00	3.85	4.50	17.05	2.75	247.50	145.5
Shankhamul	72.0	42.25	7.25	6.25	12.75	13.50	462.00	265.5
Shovabhagwati	5.0	42.25	2.50	2.00	7.00	19.00	150.00	69.0
Manohora	3.1	-	5.46	-	31.98	26.25	96.72	39.0
Mean	63.41	59.25	4.84	4.35	21.42	18.63	188.62	112.25
SD	±47.69	±17.95	±1.71	±2.2	±11.6	±9.27	±147.39	±82.86
<i>D. carota</i>								
Chapro	28.0	9.75	4.50	0.25	24.25	19.25	39.00	30
Bansthali	31.0	10.50	4.00	3.25	12.75	17.75	54.00	43.5
Manohora	53.7	11.75	3.50	3.50	23.75	20.50	36.00	34.5
Ghattekulo	53.5	127.80	3.50	3.00	25.75	23.50	76.50	57
Shankhamul	57.0	26.13	5.75	5.50	22.75	20.75	110.50	112.2
Mean	44.65	37.18	4.25	3.10	21.85	20.35	63.2	55.44
SD	±13.93	±51.10	±0.94	±1.88	±5.20	±2.13	±30.92	±33.36
<i>R. sativus (R)</i>								
Kusingal	12.7	9.00	1.50	0.50	12.50	20.00	112.75	102.0
Shovabhagwati	100.5	11.50	4.00	3.25	25.00	33.75	157.50	162.0
Khusibun	179.8	26.78	6.05	2.80	23.66	39.05	161.70	92.04
Mean	97.70	15.76	3.85	2.18	20.99	30.93	143.6	118.68
SD	±83.59	±9.63	±2.28	±1.48	±6.86	±9.83	±27.13	±37.85
<i>R. sativus (W)</i>								
Thimi	49.0	13.0	4.00	3.25	7.50	22.50	42.00	40.5
Ghattekulo	34.0	14.0	2.25	6.00	23.00	27.75	61.50	147.5
Shovabhagwati	30.3	14.0	4.08	2.00	26.50	28.82	38.25	49.5
Mean	37.78	13.67	3.44	3.75	19.00	26.36	47.25	79.17
SD	±9.00	±9.58	±1.03	±2.05	±10.11	±3.38	±12.48	±59.35
Potato tuber (underground stem)								
Sankhu	-	11.5	-	1.50	-	5.75	-	45.0
Panchkhal	-	8.25	-	0.25	-	9.75	-	52.5
Manohora	-	18.25	-	0.25	-	4.75	-	42.0
Mean	-	12.67	-	0.67	-	6.75	-	46.50
SD	-	±5.1	-	±0.72	-	±2.65	-	±5.41

* exceeded normal plant value, ^AAlloway (1968), ^BBowen (1979), ^{KP}K-Pendias and Pendias (1992)

Zinc

Hyperaccumulation (accumulation of higher concentration than the concentrations in soil i.e. >1) of Zn was observed in red radish collected from Kusingal, Shovabhagawati and Khusibun, but not observed in white radish. Similarly, hyperaccumulation of Zn was also observed in *B. juncea* collected from Kusingal, *L. sativum* from Khusibun and *S. oleracea* from Shankhamul. In most of the cases Zn hyperaccumulation was observed (**Table 26**) exceeding critical plant concentration of 200 mg kg^{-1} (Davis and Beckett, 1978). Zinc accumulation in spinach collected from Shankhamul showed 462 mg kg^{-1} which showed exceeding critical plant tissue concentration. *Brassica juncea* showed greater Zn accumulation in leaves than in their roots and ranged from 37.50 mg kg^{-1} (at Banasthali) to $118.00 \text{ mg kg}^{-1}$ (at Kusingal). Zinc accumulation in root of radish ranged from 40.5 mg kg^{-1} (at Thimi) to 162 mg kg^{-1} (at Shovabhagawati). In case of carrot, more Zn accumulation was observed in leaves than in roots. The level of Zn in potato ranged from 42 mg kg^{-1} to 52.5 mg kg^{-1} .

No significant correlation was observed between Pb, Cu, Mn content in soil and their respective accumulation in studied vegetable crops. Significant positive correlations were observed between Ni content in soil and Ni accumulation in leaves of broad leaf mustard, leaves of white radish, carrot leaves and roots of broad leaf mustard; Cd content in soil and Cd in leaves of broad leaf mustard; Cr content in soil and Cr in leaves of radish (W) and turnip; Zn content in soil and Zn in roots of broad leaf mustard and cress and also Zn in leaves of cress plants; Fe content in soil and Fe in roots of radish (W) and in leaves of carrot; Co content in soil and leaves cress. Significant negative correlation was observed between Co content in soil and Co in carrot leaves only (**Table 27**).

Table 27. Results of Correlation Coefficient (r) obtained between the soil heavy metals and their respective accumulation in vegetable parts collected from the same sampling sites.

Vegetables	Cu	Pb	Cr	Cd	Ni	Co	Zn	Fe	Mn
<i>B. juncea</i> (leaf)	0.373	0.06	0.573	0.814**	0.666*	-0.3	0.275	0.199	0.64
<i>B. juncea</i> (root)	0.147	0.403	0.056	-0.195	0.684*	-0.49	0.942**	-0.403	0.048
<i>L. sativum</i> (leaf)	0.845	-0.076	0.42	-0.034	0.25	0.773*	0.809*	0.631	-0.083
<i>L. sativum</i> (root)	0.287	-0.487	-0.147	0.301	0.01	0.069	0.872*	0.729	0.361
<i>R. sativus</i> (W) (leaf)	0.071	0.794	0.745*	0.316	0.893**	0.285	0.65	0.195	0.387
<i>R. sativus</i> (W) (root)	-0.65	0.737	-0.251	0.588	-0.476	-0.085	0.649	0.993**	0.29
<i>R. sativus</i> (R) (leaf)	0.708	-0.076	0.285	0.141	0.541	0.076	0.579	0.729	0.212
<i>R. sativus</i> (R) (root)	0.484	-0.236	0.448	0.265	0.154	0.081	-0.527	0.808	0.311
<i>S. oleracea</i> (leaf)	0.792	-0.51	0.269	-0.36	0.623	-0.055	0.422	-0.088	-0.283
<i>S. oleracea</i> (root)	0.492	-0.11	-0.396	0.66	-0.445	-0.217	0.317	-0.805	0.356
<i>B. rapa</i> (leaf)	-0.138	-0.489	0.839*	0.027	-0.116	0.016	-0.534	-0.772	0.3
<i>B. rapa</i> (root)	-0.103	0.848	-0.753	-0.06	0.182	-0.346	-0.503	0.859	0.303
<i>D. carota</i> (leaf)	0.467	-0.444	0.49	-0.156	0.919*	-0.817*	0.468	0.991**	-0.116
<i>D. carota</i> (root)	-0.023	0.14	-0.178	0.469	0.192	-0.279	0.486	-0.302	-0.578

*Significant at $p \leq 0.05$, ** significant at $p \leq 0.01$; W-white, R- red.

4.4. Estimation of Heavy Metals in Dietary Intake

4.4.1. Accumulation of Heavy Metals in Edible Portions

Concentrations of heavy metals in edible parts of vegetable crops are given in **Table 28**. The most of the heavy metals are within normal plant tissue concentration in most of the edible parts of vegetables but mean value of Pb and Ni exceeds in all vegetables except potato. Among the leafy vegetables, Cd accumulation was found high in broad leaf mustard, cress leaf and spinach. Among roots, Cd was high in carrot > turnip > radish > potato tuber.

Concentration of each Cu, Cd, Co, Zn and Fe (from mean value) in spinach was highest and above the normal plant value. Cobalt concentration ranged from 0.25 to 6.0 mg kg⁻¹ in broad leaf mustard indicating its high bioaccumulation ability. Chromium accumulation was found high in broad leaf mustard > cress leaf > spinach. Bioaccumulation of Cu was highest in spinach leaves > cress leaf > broad leaf mustard > carrot > turnip > radish > potato tuber.

Concentrations of Fe accumulation in all vegetables were high but without any visible symptoms. Concentration of Mn in cress leaf ranged from 40 to 187.2 mg kg⁻¹ indicating its high bioaccumulation ability. Normal plant tissues concentration of Ni ranged from 1-2.7 mg kg⁻¹, which was higher than the normal plant value but less than the lower range of plant critical concentrations (10 mg kg⁻¹). High accumulation of Pb was observed in shoots of cress leaf and broad leaf mustard. Zinc concentration in spinach leaf collected from Shankhamul showed 462 mg kg⁻¹ DW and exceeding critical plant tissues concentration (100-400 mg kg⁻¹). Hyperaccumulation of Zn i.e. more Zn accumulation in plant part than the surrounding soil was observed among spinach leaf from all sampling sites.

4.4.2. Estimation of Dietary Intakes of Heavy Metals

On the basis of household survey, average daily intake of total vegetables among adult has been estimated to be 225 g (FW) per person. Out of this, 6 studied vegetable species account for 48.9 % (or about 110 gm FW) of the total consumption of vegetables among Kathmandu dwellers. The mean daily availability of the examined vegetable species and daily intake of heavy metals via their consumption are given in **Table 29** and **Table 30**. Of these elements Cd, Co, Ni and Pb are considered as potentially toxic while Cr, Cu, Fe, Mn and Zn are considered as

essential heavy metals. $\Sigma_6\text{veg}$ represents the total element intake resulting from the consumption of the six studied vegetable species.

Table 28. Heavy metal concentrations (range and mean in mg kg⁻¹ DW) in edible parts of the vegetables (n=5-9) from the agricultural fields of Kathmandu valley

Vegetable crops	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
<i>B. juncea</i> (leaf)	0.50-1.5 1.00	0.25-6 2.69	0.75-4.5 2.75	3-17.75 9.42	573-1393 974.17	25.75-74.5 46.19	3.25-7.05 5.19	16.5-34.7 24.36	37.5-118 70.61
<i>D. carota</i> (root)	0.25-1.25 0.62	0.25-2.2 1.44	0.25-1.2 0.56	4.0-6.5 5.15	35-339.9 147.28	9.75-127.8 37.18	0.25-5.5 3.1	17.75-23 20.35	30-112 55.40
<i>L. sativum</i> (leaf)	0.50-1.50 1.10	2.0-3.25 2.44	1.50-3.5 2.71	7.25-13 10.0	274-677 492	48-187.2 102.65	2.75-6.24 4.16	22-31.72 25.37	52.5-363 166.67
<i>R. sativus</i> (root)	0.50-1.25 0.55	0.25-2.8 1.64	0.75-1.5 0.96	3.5-5.75 4.28	146-388 221.6	2-26.78 14.71	0.50-6 2.98	12.5-26.5 22.00	40.5-147 98.92
<i>S. oleracea</i> (leaf)	0.25-1.25 0.82	1.25-4.4 2.83	0.25-4.7 2.55	13.2-25 15.16	28-26565 5848.2	5-111 75.47	2.5-7.25 4.72	7.0-37.5 21.42	79.5-462 188.62
<i>B. rapa</i> (root)	0.25-1.00 0.56	0.2-4.25 1.75	0.5-2.25 1.13	3.0-7.75 5.00	57-874.5 473.25	8.7-27.6 19.25	0.5-4.25 2.56	16.25-25 23.38	48-73.5 70.50
<i>S. tuberosum</i> (tuber)	0.25-0.75 0.50	1.5-2.5 1.83	0.2-1.25 0.92	3.25-4 3.87	87-108 93	8.25-18.25 10.3	0.25-1.5 0.83	4.75-9.75 7.75	42-52.5 63.0

The total intakes of the potentially toxic heavy metals such as Cd, Ni and Pb via vegetables represents 33.43 %, 40.38 % and 251.64 %, of the provisional tolerable daily intake (PTDI), respectively. PTDI was calculated on the basis of recommended weekly doses as given by WHO (1996). On the other hand green vegetables consumption seem to contribute appreciably to the Recommended Daily Intake (RDI) percentage of essential heavy metals such as Cu (15.49-7.7 %), Cr (97.4-24.35 %), Fe (41 %), Mn (65.71-26.28 %) and Zn (21.23 %) (National Research Council, 1989). Intake of Pb via vegetables ($\Sigma_6\text{veg}$) is very high (**Table 29**)

and upon consumption of only 110 g/day fresh matter (FM), Pb intake is 307.56 µg/day, but reaches up to 629.10 µg when consumed 225 g (FM)/day. Consumption of Cd, Co and Ni is 23.40; 57.64 and 109.58 µg/day, respectively, on the basis of total vegetables (225 g FM) and are lying within the PTDI. Intake concentrations of essential heavy metals like Cu (232.42 µg), Cr (48.70 µg), Fe (7169.2 µg) and Mn (1314.29 µg) and Zn (3185.69 µg) through fresh vegetables was found within Recommended Daily Dietary Allowances (RDAs, 1989) (**Table 30**).

Table 29. Daily intakes^a of potentially toxic heavy metals via fresh vegetables (range and mean values in µg/day; n=5-9)

Vegetable species	Mean daily availability (g/day)	Mean daily availability (µg/day)			
		Cd	Co	Ni	Pb
<i>B. juncea</i> (leaf)	20	0.86-3.88	0.40-10.32	5.6-17.48	3.82-82.23
		2.09	5.34	10.49	45.98
<i>D. carota</i> (root)	10	0.28-1.55	0.19-4.49	2.32-7.77	18.14-39.86
		0.84	2.52	5.35	24.46
<i>L. sativum</i> (leaf)	20	2.37-5.85	3.79-7.80	10.72-11.38	57.85-85.8
		4.11	5.8	11.54	71.9
<i>R. sativus</i> (root)	20	0.36-2.23	0.36-5.95	0.74-17.85	10.92-68.5
		0.89	2.87	6.12	36.59
<i>S. oleracea</i> (leaf)	20	0.84-4.72	2.12-15.11	7.71-22.68	38.5-141.67
		2.14	6.72	12.70	67.70
<i>B. rapa</i> (root)	20	0.51-2.58	0.93-14.64	0.74-8.77	37.47-99.19
		1.37	4.93	7.37	60.93
6 veg	110	11.44	28.18	53.57	307.56
veg	225	23.40	57.64	109.58	629.10
PTDI (adult 70kg)	-	70 ^b	-	250 ^b	250 ^b
%PTDI		33.43		40.38	251.64

^acalculated by multiplying concentrations of elements (µg/g Fresh matter) by available weight of vegetables (daily availability in g/day).

^bcalculated from WHO Provisional Tolerable Weekly Intakes (WHO, 1996, 1993).

Table 30. Daily intakes^a of essential trace elements via fresh vegetables (range and mean values in µg/day; n=5-9)

Vegetable crops	Fresh weight (mean) (g/day)	Cu	Cr	Fe	Mn	Zn
<i>B. juncea</i> (leaf)	20	6.52-28.33 18.35	3.01-12.34 6.05	211.2-1672.2 1051.67	44.38-124-92 89.2	83.02-879 208
<i>D. carota</i> (root)	10	3.28-8.98 6.58	0.28-0.56 0.45	21-270 136.54	13.5-98.6 43.14	39.6-568.4 187.45
<i>L. sativum</i> (leaf)	20	24.18-37.1 30.61	5.69-5.85 5.77	329.4-812.4 570.92	187.2-341.4 264.33	328-663 495
<i>R. sativus</i> (root)	20	2.59-11.16 7.17	0.77-4.11 1.88	122.4-466.2 265.92	2.96-42.74 27.28	59-193 142
<i>S. oleracea</i> (leaf)	20	23.27-55.6 36.72	4.36-17.94 8.03	60-4560 912	4.64-342.8 164.85	144-783 353
<i>B. rapa</i> (root)	20	4.45-26.70 14.20	0.37-2.58 1.63	68.5-10495.4 567.90	12.98-72.36 53.73	20.03-341 172
6 veg	110	113.63	23.81	3504.95	642.54	1557.45
veg	225	232.42	48.70	7169.2	1314.29	3185.69
RDI (adult 70kg)	-	1500-3000 ^c	50-200 ^c	15000 ^b	2000-5000 ^c	15000 ^b
% RDI		15.49-7.7	97.4-24.35	41	65.71-26.28	21.23

^acalculated by multiplying concentrations of elements (µg/g FM) by available weight of vegetables (daily availability in g/day).

^bRecommended Daily Allowances (RDAs 1989).

^cEstimated safe and adequate Daily Dietary Intakes (RDA 1989).

4.5. Phytoremediation

4.5.1. Phytoextraction

Altogether 18 vegetable crops (*Allium sativum* L., *A. fistulosum* L., *Beta vulgaris* L., *Beta vulgaris* var. *cicla*, *B. juncea* L., *B. caulorapa* L., *Brassia rapa* L., *Brassica rapa* var. *purple top globe*, *Lepidium sativum* L., *Raphanus sativus* var. *Puthane* (red), *R. sativus* var. *Puthanae* (white), *Coriandrum sativum* L. *S. oleracea* var. *Patane*, *S. oleracea* var. *Deshi*, *S. oleracea* var. *Gobre*, *Solanum tubersum* L., *Trigonella foenumgracum* L., *Vicia faba* L.) as mentioned earlier in **Table 4**, were germinated on soil treated with their single (300 mg CuCl₂/ 500 mg Pb (NO₃)₂/ 800 mg ZnCl₂/ and their mixed metal (300 mg CuCl₂ + 500 mg Pb (NO₃)₂ + 800 mg ZnCl₂). Details of each metal accumulation from single and their mixed metal treatment have been given below (**Figs. 23-26**).

Copper

Among the studied vegetable crops grown on 300 mg CuCl₂ kg⁻¹ soil, Cu accumulation <25 mg kg⁻¹ was observed in *A. sativum*, *L. sativum* and white *R. sativus* (**Fig. 23**), but low Cu accumulation 20–22 mg kg⁻¹ DW was observed in *B. juncea* and *B. caulorapa*, respectively. Moderate Cu accumulation (ranging from 25 to 50 mg kg⁻¹) was recorded in *T. foenumgracum*, *B. rapa* and *C. sativum*. High Cu accumulation (50 to 100 mg kg⁻¹) was observed in *A. fistulosum*, *S. (Deshi)*, *S. oleracea* (*Patane*), *S. oleracea* (*Gobre*) and *Beta vulgaris* var. *cicla* (*Swiss chard*). Among studied vegetables, *V. faba* accumulate high concentration of Cu (432.5 mg kg⁻¹). *Solanum tuberosum*, *Beta vulgaris* (*beetroot*), *R. sativus* (*red radish*) and *B. rapa* var. *purple top globe* (*salgam*) could not germinate on soil contaminated with 300 mg CuCl₂ kg⁻¹.

Lead

Among the studied vegetables crops grown on soil treated with 500 mg Pb(NO₃)₂ kg⁻¹ treatments, low accumulation of Pb (<50 mg kg⁻¹) was observed in *Beta vulgaris* (*beet root*), *B. juncea*, *R. sativus* (*red*) (**Fig. 24**), but *B. caulorapa*, *R. sativus* (*W*) and *S. tuberosum* (*potato* have the lowest Pb accumulation) contain the Pb below the lower range of critical tissue concentration (20 mg kg⁻¹ DW). Moderate accumulation of Pb (50 to 100 mg kg⁻¹) was recorded

in *A. sativum*, *L. sativum*, *S. oleraceae* (Deshi), *S. oleraceae* (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. oleraceae* (Gobre). *T. foenumgracum* could not germinate in soil contaminated with 500 mg Pb(NO₃)₂ kg⁻¹.

Zinc

Among the studied vegetable species grown on 800 mg ZnCl₂ contaminated soil, low Zn accumulation (<100 mg kg⁻¹) was measured in *A. sativum*, *A. fistulosum*, *B. caulorapa*, *T. foenumgracum*, *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*. Among tested vegetables *V. faba* (broad bean) accumulated highest amount of Zn (202 mg kg⁻¹) (Fig. 25) and *L. sativum*, (accumulated quite high amount of Zn, after *V. faba*). *Coiandrum sativum* could not germinate in ZnCl₂ – treated soils.

Mixed metals

Among the studied vegetables crop species grown on 1600 mg mixed metal kg⁻¹ soil, accumulation of Cu in *B. juncea*, *C. sativum*, *B. rapa* (both varieties), *S. tuberosum* and *T. foenumgracum* were less than 15 mg kg⁻¹. But *A. 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) (Fig. 26).

Accumulation of Pb in most vegetables were <10 mg kg⁻¹ from the mixed (1600 mg kg⁻¹) metal treated soil and were within the range of 5.83 (*S. tuberosum*) to 30.5 mg kg⁻¹ (*S. oleracea* Deshi). *A. fistulosum* could not grow in soil treated with mixed metal

Among the vegetable crops grown from mixed metal treatments, Zn accumulation ranged from 100–200 mg kg⁻¹ in *Beta vulgaris* (beetroot), *L. sativum*, *T. foenumgracum*, *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 (up to 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 3 varieties of *S. oleracea*,

Cu and Zn accumulation was low in Gobre, but Pb accumulation was more or less similar in all 3 varieties. Zn accumulation was higher in Patane than in others 2 varieties (**Fig. 26**).

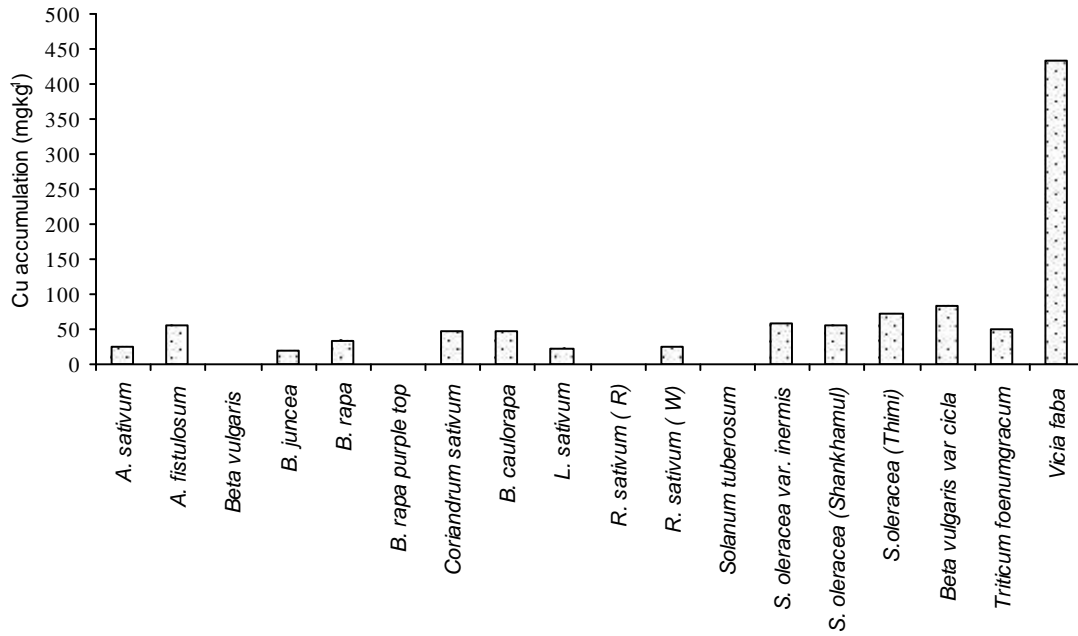


Fig. 23. Phytoextraction of Cu by different vegetable crops grown on soil treated with CuCl_2 .

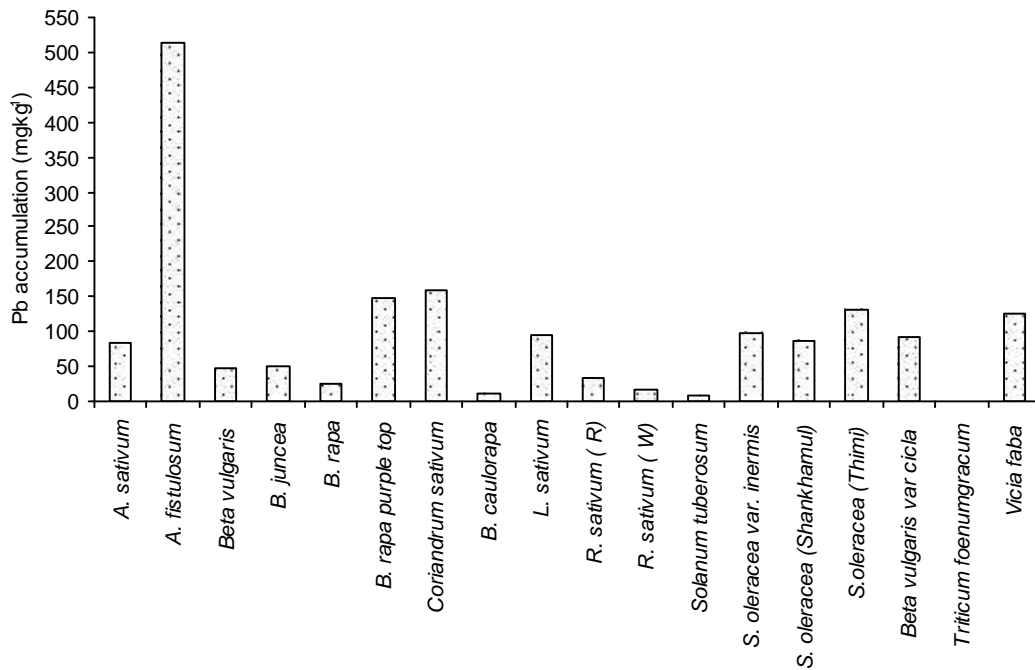


Fig. 24. Phytoextraction of Pb by different vegetable crops grown on soil treated with $\text{Pb}(\text{NO}_3)_2$.

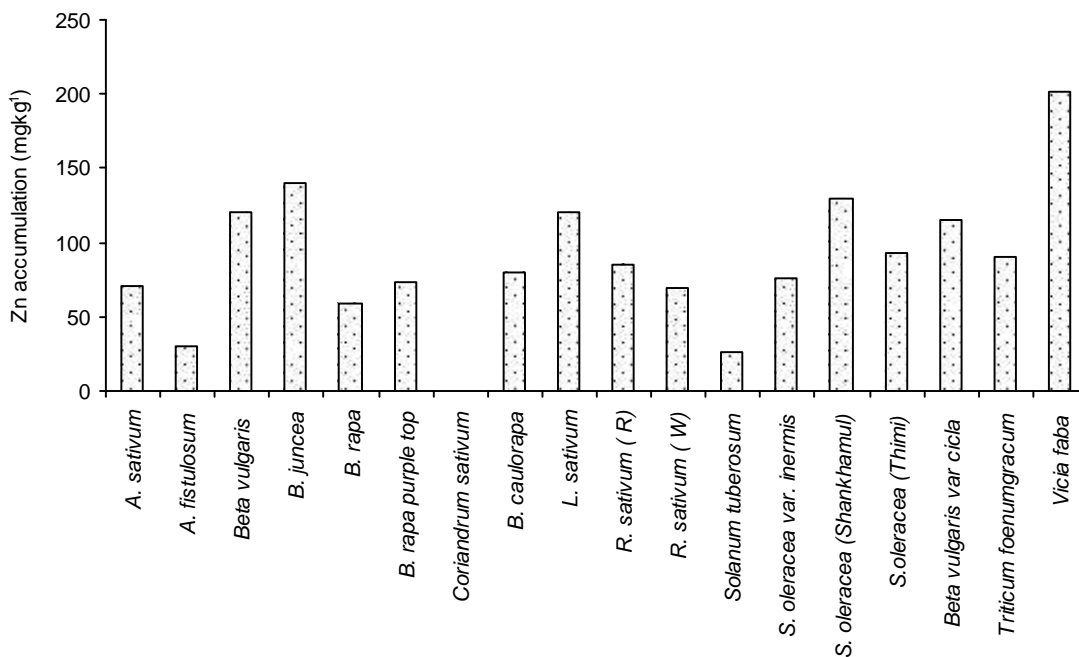


Fig. 25. Phytoextraction of Zn by different vegetable crops grown on soil treated with $ZnCl_2$

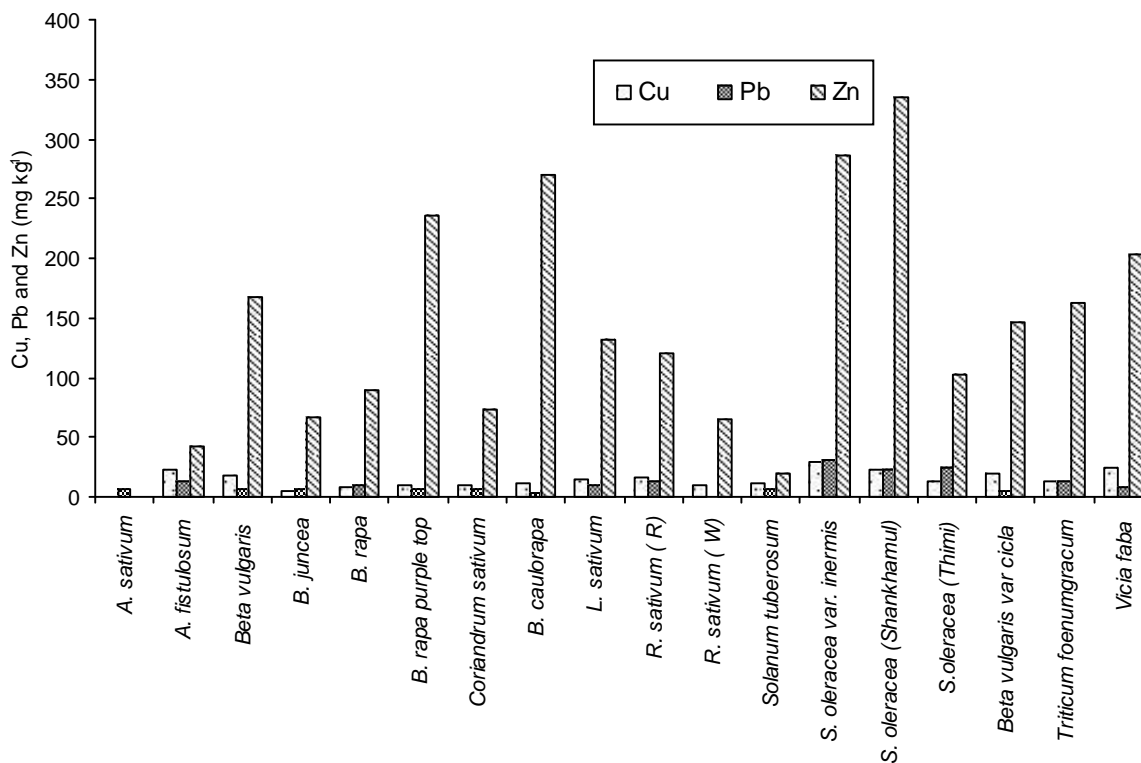


Fig. 26. Phytoextraction of Cu, Pb and Zn by different vegetables grown on soil treated with mixed heavy metals.

4.5.2. Remedial Measures using Cow dung and Lime

Accumulation of Cu, Pb and Zn in vegetable crops grown on i) control (soil treatment only with metals such as Cu / Pb/ Zn), ii) grown on cow-dung (5 and 20 %) amended and metal contaminated soil, and iii) grown on lime (3 and 9 g kg⁻¹ soil) amended and metal contaminated soil are given in **Figs. 27- 30**.

Copper

Cu accumulation, in all studied vegetables such as *B. juncea*, *L. sativum* and *B. rapa* when grown in CuCl₂ treated soil, was high in both 3 and 9g lime treatments than in cow-dung treatments. *B. juncea* and *B. rapa* showed highest accumulation of Cu in low dose of lime (3 g) treatment, but *L. sativum* showed high accumulation in high dose of lime (9 g). Lowest accumulation of Cu was observed at 20 % cow-dung treatment in all tested vegetables (**Fig. 27**).

Lead

Lead accumulation in *L. sativum* was significantly high in 9 g lime than in both control and 3 g lime treatment (**Fig. 28**). Lead accumulation in *B. rapa* also increased slightly in both 3 and 9 g lime treatments. Insignificant changes have been observed in *B. juncea* treated with both doses of lime than in control. Lowest accumulation of Pb was recorded in all three vegetables grown on soil amended with 20 % cow dung (**Fig. 28**).

Zinc

Zinc accumulation was increased in *B. rapa* and *L. sativum* than in control in cow-dung and lime treated soil. Zn accumulation decreased in *B. juncea*, in all treatments than in control (**Fig. 29**).

Mixed metals

Accumulation of Cu, Pb and Zn from supplied mixed metal (**Figs. 30 A, B, C**) were highest in 3 g lime in *B. juncea* than in control or other treatments. Lowest accumulation of Pb from mixed metal treatment was observed in *B. juncea* and *L. sativum* grown on 5 and 20 % cow-dung treatment. Zn accumulation in *L. sativum* was quite high in 20 % cow-dung treatment. Accumulation of Pb and Zn from supplied mixed metal in *B. rapa* were mostly reduced in cow dung treatment compared to control but the plant could not grown in lime treated soil.

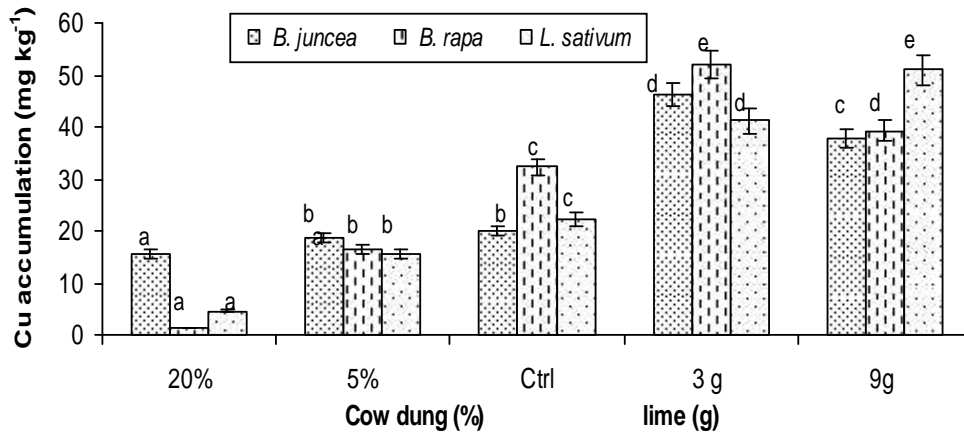


Fig. 27. Accumulation of Cu in vegetables grown on soil treated with CuCl_2 and cow-dung or lime. Different letters above the histogram in each species at different treatments denote significant difference at $P < 0.05$ according to Duncan's multiple range test followed by one way ANOVA.

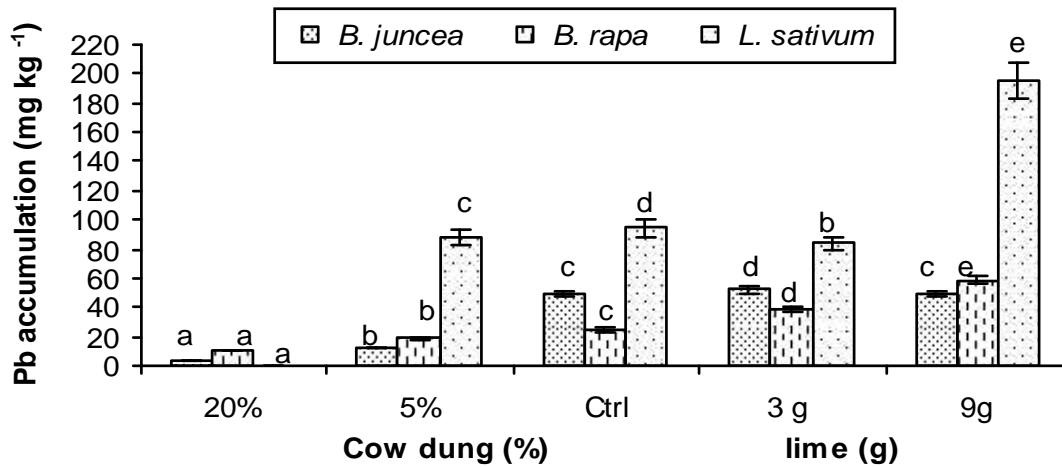


Fig. 28. Accumulation of Pb in vegetables grown on soil treated with $\text{PbNO}_3)_2$ and cow-dung or lime. Different letters above the histogram in each species at different treatments denote significant difference at $P < 0.05$ according to Duncan's multiple range test followed by one way ANOVA.

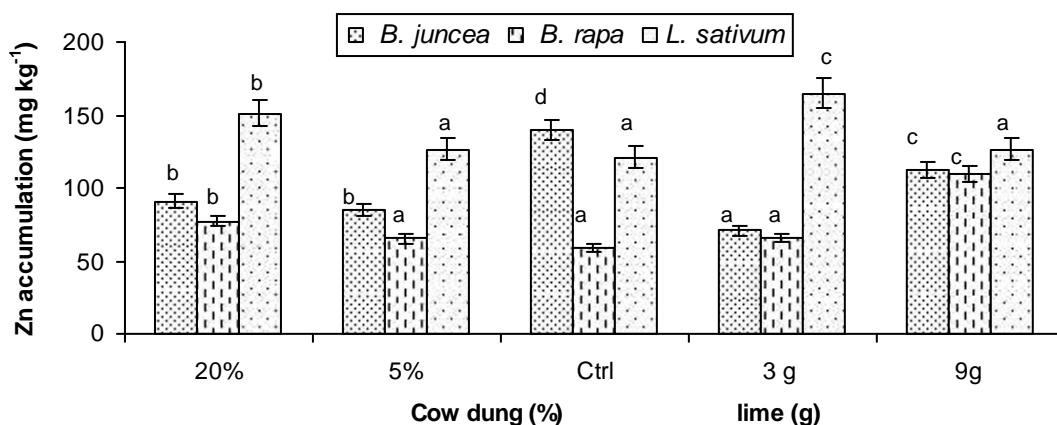


Fig. 29. Accumulation of Zn in vegetables grown on soil treated with ZnCl₂ and cow-dung or lime. Different letters above the histogram in each species at different treatments denote significant difference at P<0.05 according to Duncan's multiple range test followed by one way ANOVA.

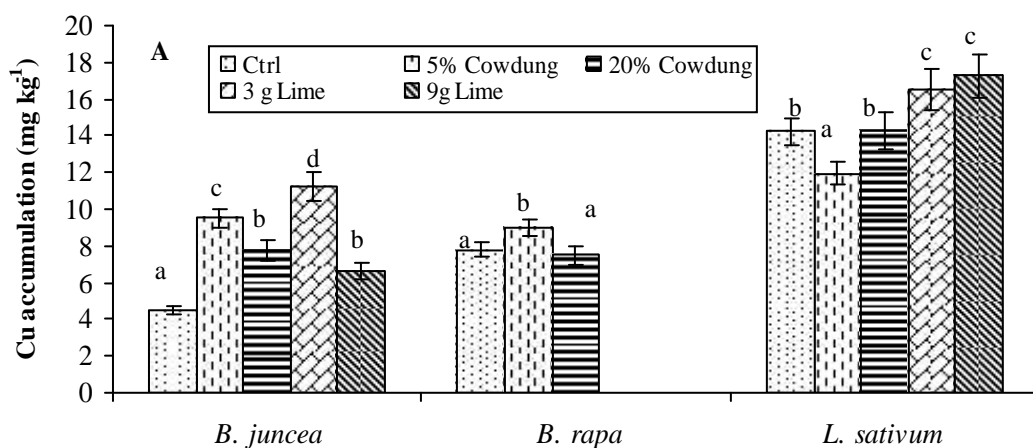


Fig. 30 A. Accumulation of Cu in vegetable crops grown on soil treated with mixed metals (CuCl₂+ Pb(NO₃)₂ +ZnCl₂) along with cow-dung or lime. Different letters above the histogram in each species at different treatments denote significantly difference at P<0.05 according to Duncan's Multiple Range test followed by one way ANOVA.

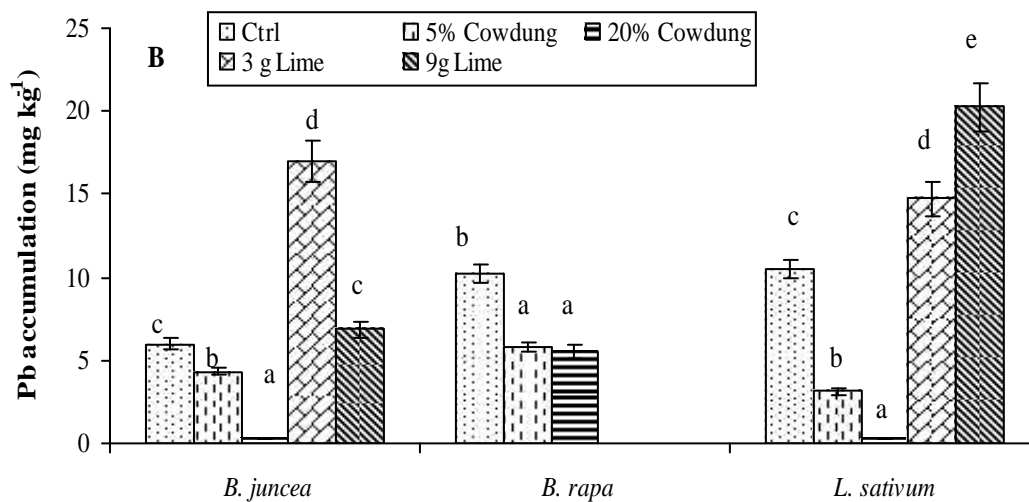


Fig. 30 B. Accumulation of Pb in vegetable crops grown on soil treated with mixed metals ($\text{CuCl}_2 + \text{Pb}(\text{NO}_3)_2 + \text{ZnCl}_2$) along with cow-dung or lime. Different letters above the histogram in each species at different treatments denote significantly difference at $P < 0.05$ according to Duncan's Multiple Range test followed by one way ANOVA.

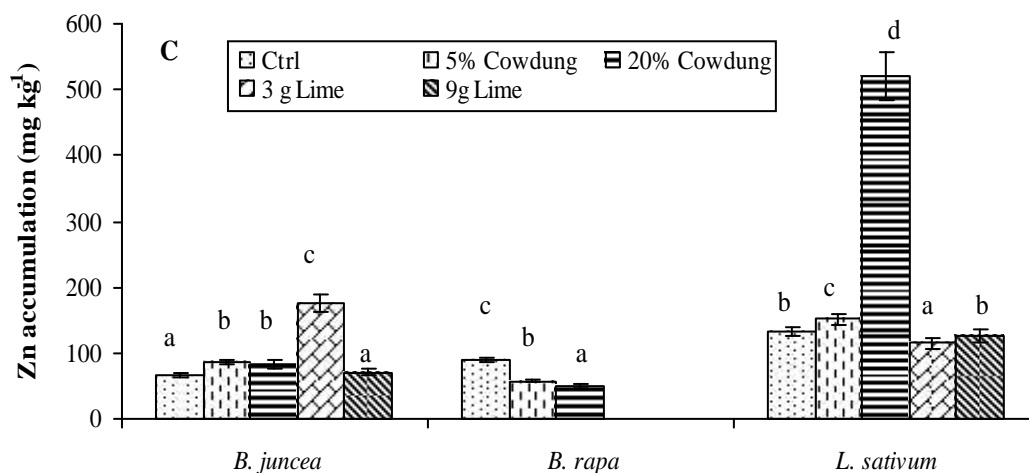


Fig. 30 C. Accumulation of Zn in vegetable crops grown on soil treated with mixed metals ($\text{CuCl}_2 + \text{Pb}(\text{NO}_3)_2 + \text{ZnCl}_2$) along with cow-dung or lime. Different letters above the histogram in each species at different treatments denote significantly difference at $P < 0.05$ according to Duncan's Multiple Range test followed after one way ANOVA

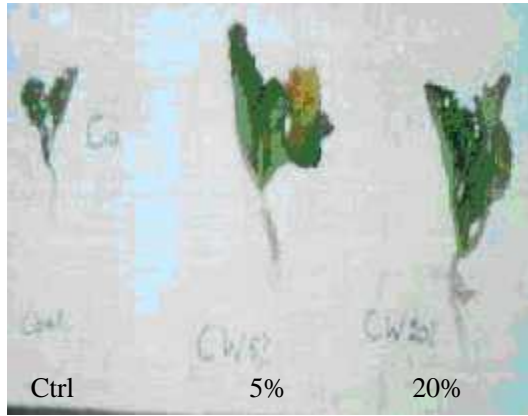
4.5.3. Morphological Parameters

All FW, DW, RL and SL increased in all studied vegetable crops grown on CuCl₂ treated and cow-dung amended soil. All FW, DW, RL and SL decreased in all cases in CuCl₂ treated and lime amended soil (**Figs. 31 A and B, 32**). Fresh weight (FW) 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 three species grown on Pb-treated and lime amended soil (**Figs. 31 C and D, 33**).

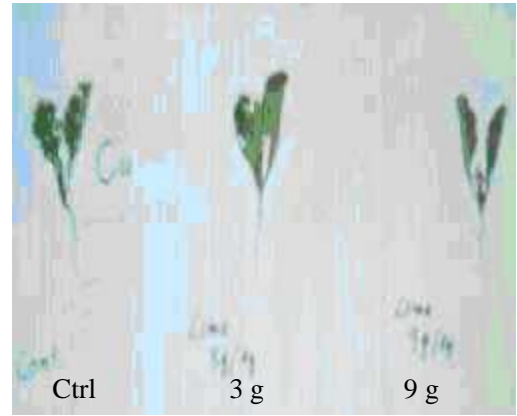
Fresh weight, DW, SL and RL (except RL in *B. rapa*) increased significantly (P < 0.01) in all vegetable crops in cow-dung amended and Zn treated soil, but no significant changes were observed with lime treatments (**Figs. 34 A and B, 35**). Fresh weight and DW increased only in *L. sativum* grown in 3 g lime treatment, whereas DW % increased significantly in *B. rapa* of both the doses of lime.

In vegetables grown on mixed metal treated soil and amended with cow-dung, parameters like SL and FW in *B. juncea* increased significantly (**Figs. 34 C and D**); FW and DW in *L. sativa* increased significantly; and SL, RL, FW and DW in *B. rapa*, increased significantly. Vegetables grown on mixed metal and lime amended soil showed changes in parameters like RL, FW and DW which all 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. 36**).

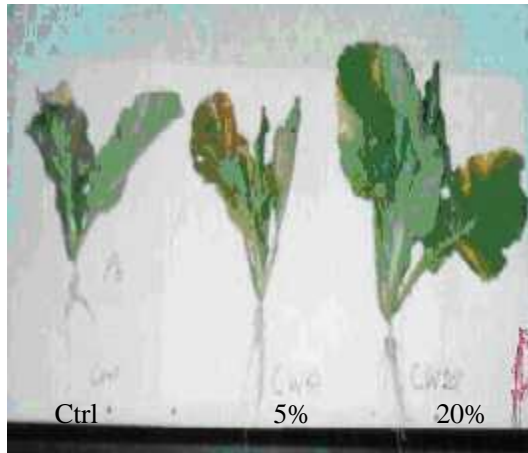
In remedial measures, root/shoot ratio decreased mostly in cow dung treatments but mostly increase in lime treatments. Root/ shoot ratio decreased in cow dung treatments in *B. juncea*, *L. sativum* (CuCl₂ treatments), *B. rapa* (Pb(NO₃)₂ treatments), *L. sativum* and *B. rapa* (ZnCl₂ treatments) , and *B. juncea* (mixed metal treatments). Only in *B. rapa* treated with mixed metals and cow dung root/shoot ratio increased (**Table 31**). Root/ shoot ratio increased mostly in lime treatments-like *L. sativum* and *B. rapa* (CuCl₂ treatments), *B. rapa* (Pb(NO₃)₂ and ZnCl₂ treatments) (**Table 31**)



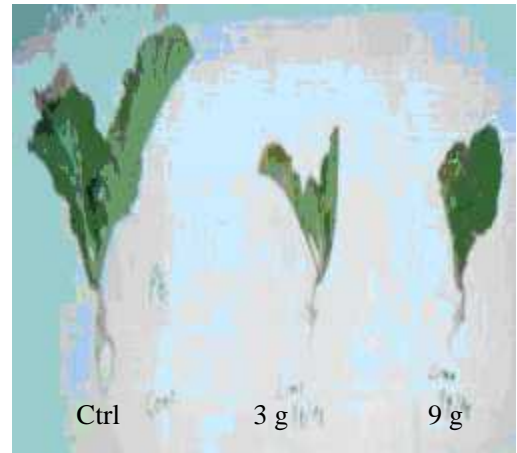
(A) CuCl_2 and cow dung (%) treatment



(B) CuCl_2 and lime (g) treatment

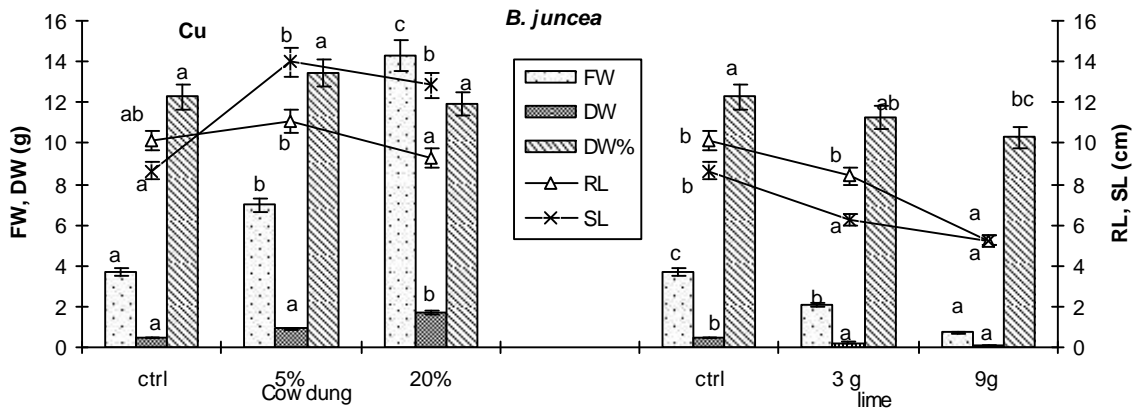


(C) $\text{Pb}(\text{NO}_3)_2$ and cow dung (%) treatment



(D) $\text{Pb}(\text{NO}_3)_2$ and lime (g) treatment

Fig. 31. Morphological changes in *B. juncea* grown on CuCl_2 or $\text{Pb}(\text{NO}_3)_2$ treated soil and mixed with different quantities of cow dung or lime.



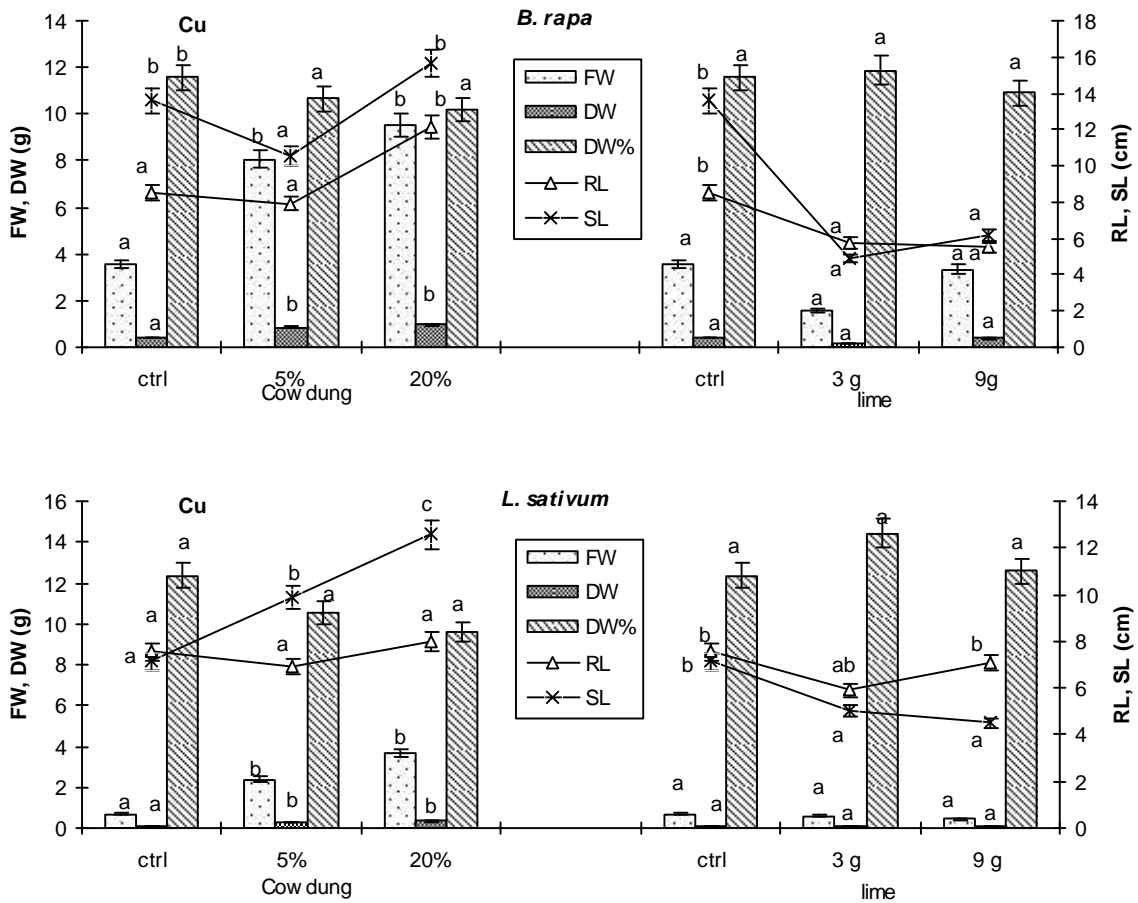
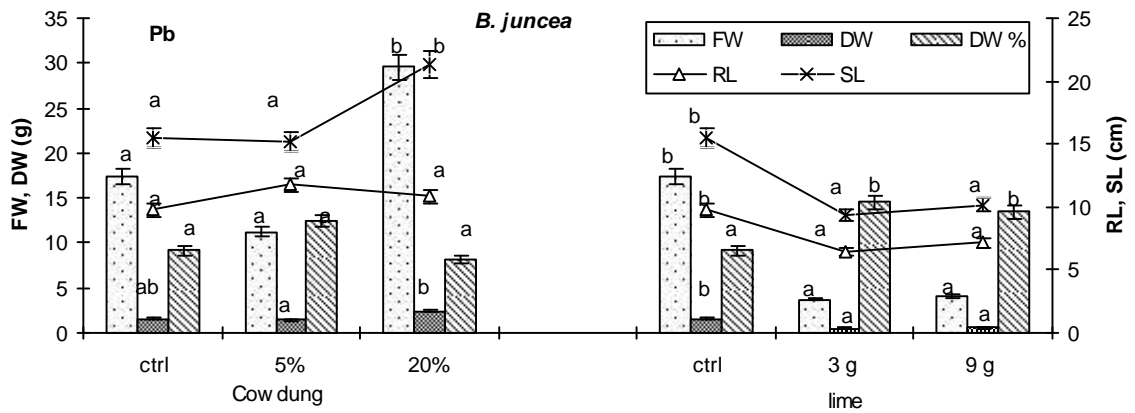


Fig. 32. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in vegetable crops grown on soil treated with CuCl_2 and different quantities of cow-dung or lime. Different letters for each parameter denote significant differences in mean value at $P < 0.05$ obtained from Duncan's multiple range test



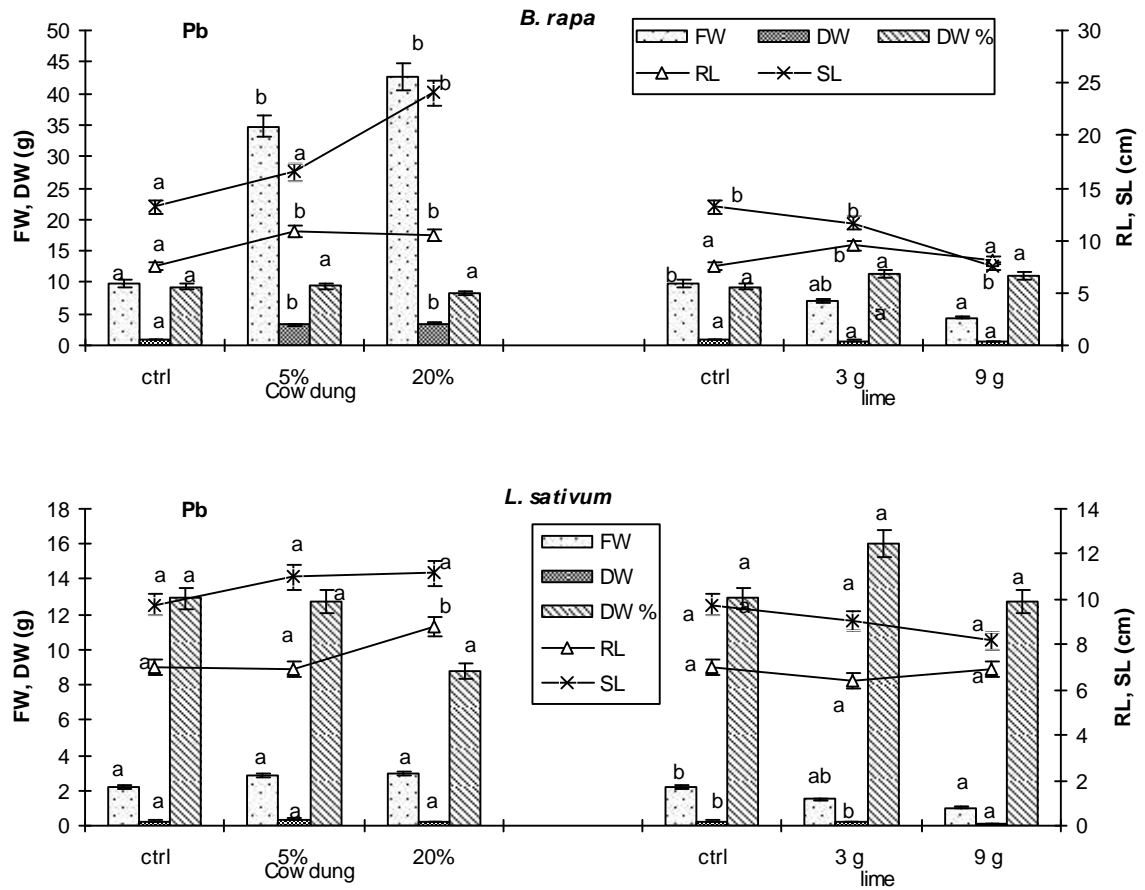
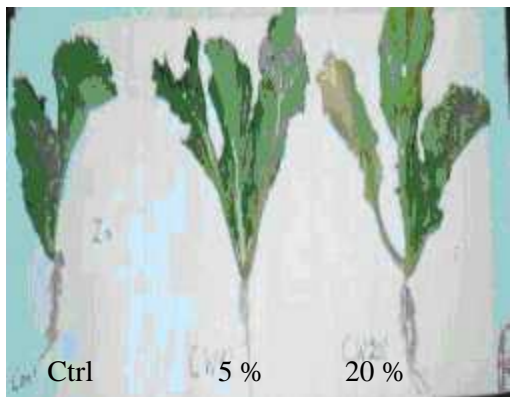
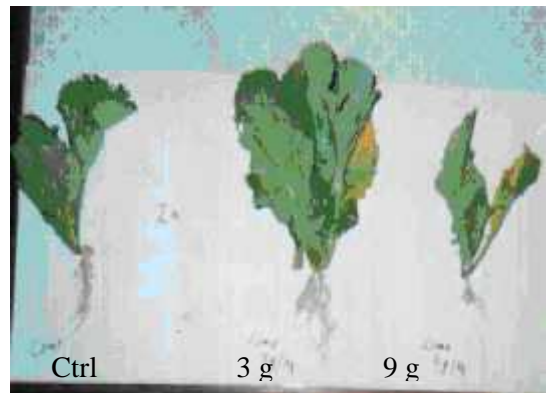


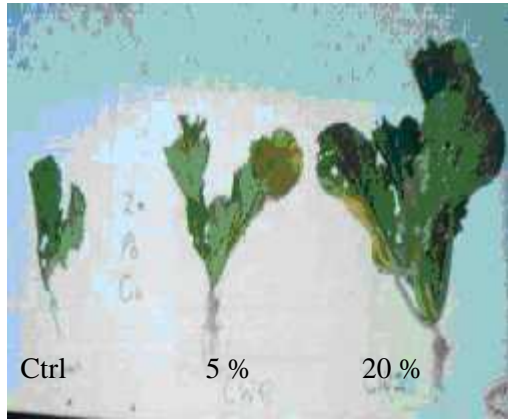
Fig. 33. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in vegetable crops grown on soil treated with $Pb(NO_3)_2$ and different quantities of cow-dung or lime. Different letters for each parameter denote significant differences in mean value at $P < 0.05$ obtained from Duncan's multiple range test.



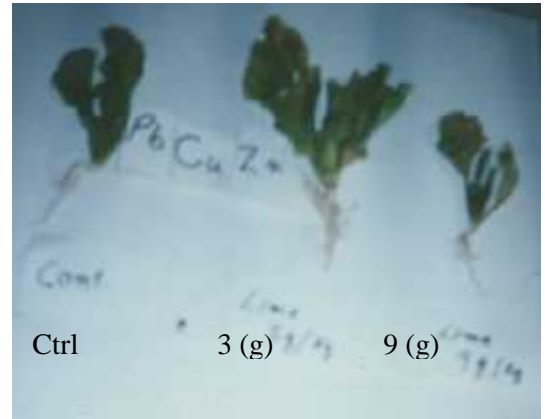
(A) $ZnCl_2$ and cow dung (%) treatment



(B) $ZnCl_2$ and lime (g) treatment

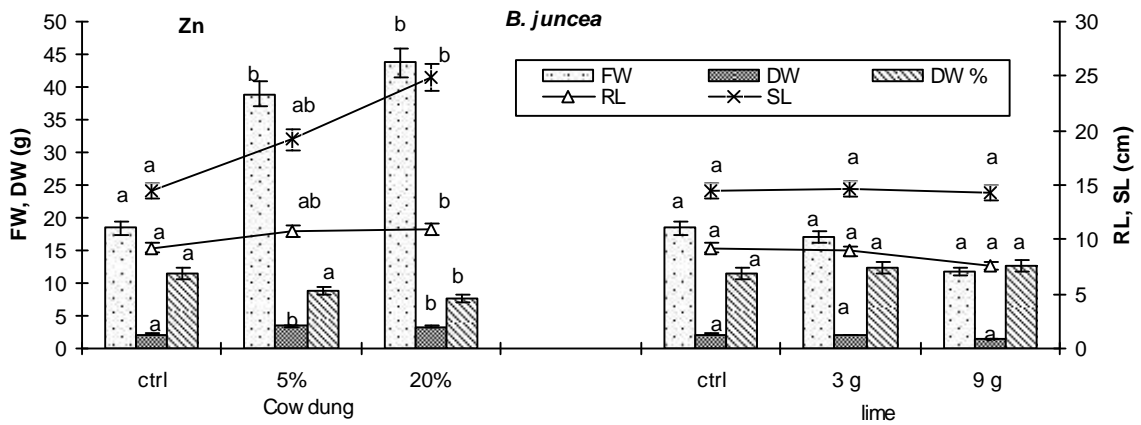


(C) Mixed metals and cow dung (%) treatment



(D) Mixed metals and lime (g) treatment

Fig. 34. Morphological changes in *B. juncea* grown on $ZnCl_2$ or mixed metals treated soil with different quantities of cow-dung or lime.



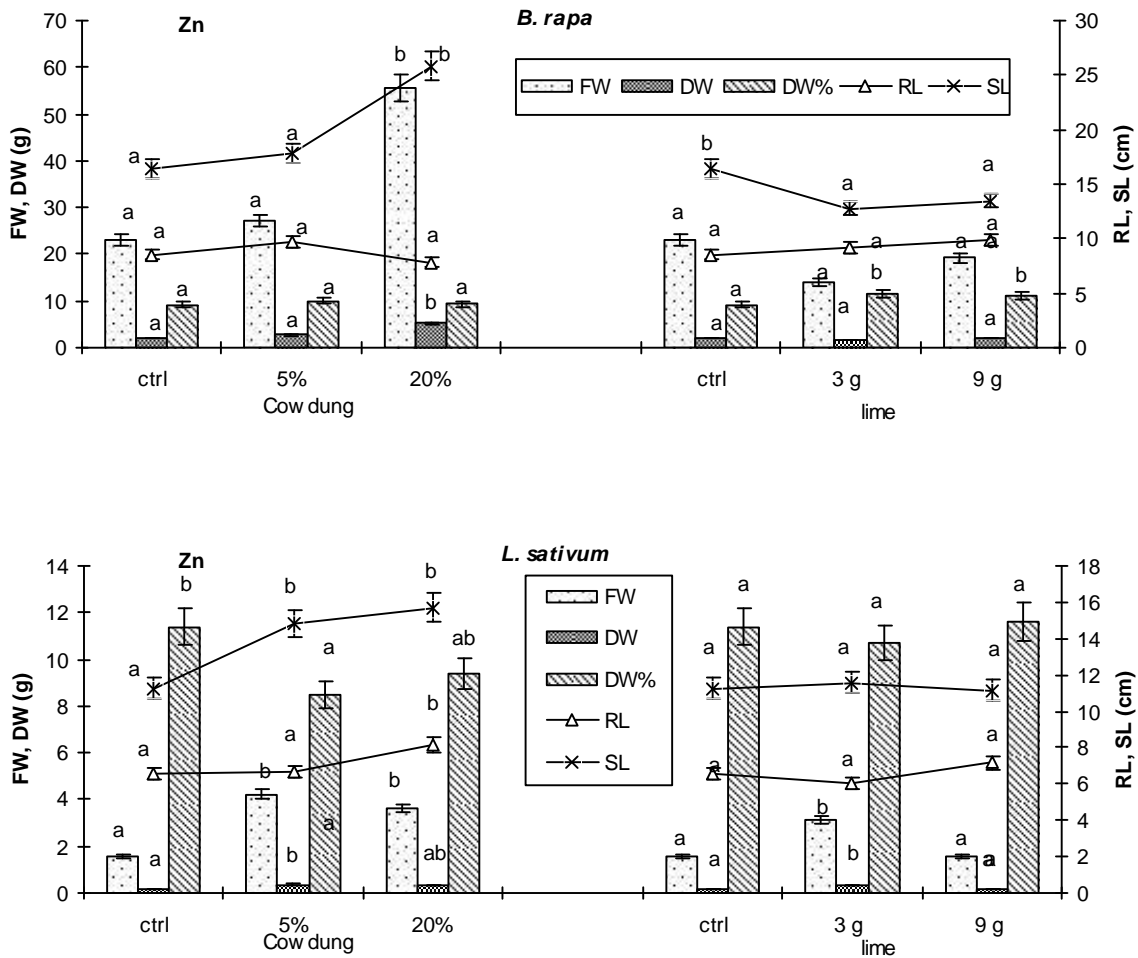
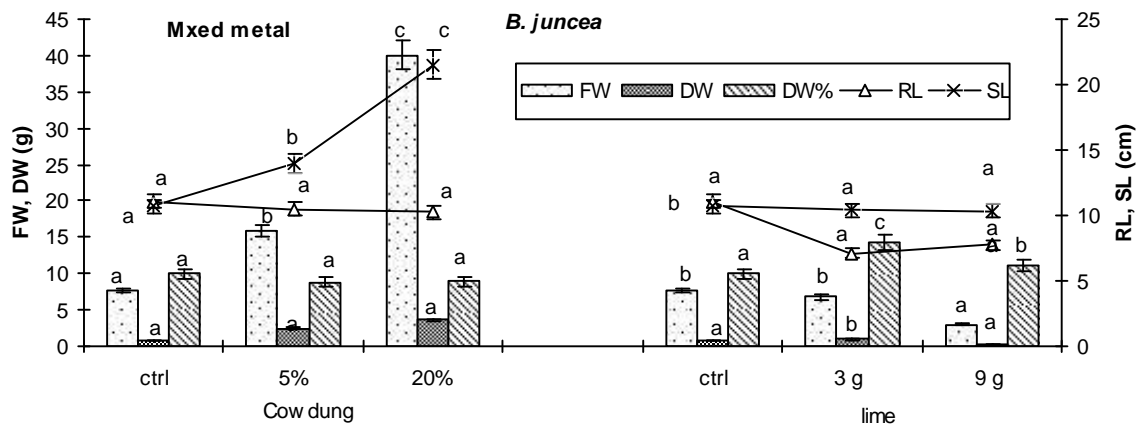


Fig. 35. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in vegetable crops grown on soil treated with ZnCl₂ and different quantities of cow-dung or lime. Different letters for each parameter denote significant differences in mean value at P<0.05 obtained from Duncan's multiple range test.



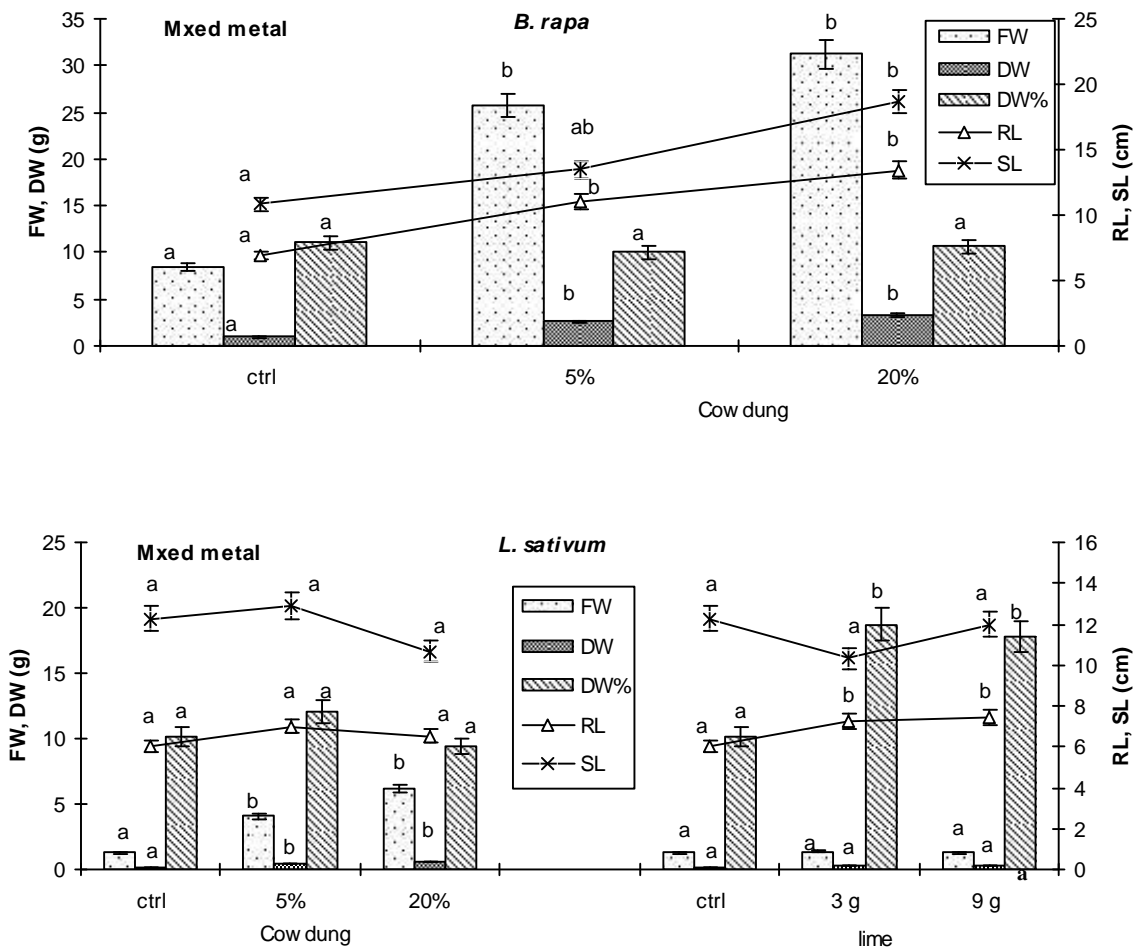


Fig.36. Fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) in vegetable crops grown on soil treated with mixed metals ($\text{CuCl}_2 + (\text{PbNO}_3)_2 + \text{ZnCl}_2$) and different quantities of cow-dung or lime. Different letters for each parameter denote significant differences in mean value at $P < 0.05$ obtained from Duncan's multiple range test

Table 31. Root/shoot ratio in vegetable crops grown on soil treated with different heavy metals and cow dung or lime.

Heavy metals and vegetable crops	Cow dung (%)			Lime (g)		
	Ctrl CuCl ₂	CuCl ₂ + 5 %	CuCl ₂ + 20 %	Ctrl CuCl ₂	CuCl ₂ + 3 g	CuCl ₂ + 9 g
CuCl₂ (300 mg kg⁻¹ soil)						

<i>B. juncea</i>	1.21 b	0.81 a	0.72 a	1.22 a	1.40 a	1.07 a
<i>L. sativum</i>	1.05 ab	0.70 a	0.64 a	1.06 a	1.21 a	1.59 b
<i>B. rapa</i>	0.63 a	0.77 a	0.79 a	0.63 a	1.18 b	0.94 b
Pb(NO₃)₂ (500 mg kg⁻¹ soil)						
<i>B. juncea</i>	0.63 a	0.78 b	0.51 a	0.63 a	0.70 a	0.71 a
<i>L. sativum</i>	0.80 a	0.65 a	0.79 a	0.81 a	0.72 a	0.87 a
<i>B. rapa</i>	0.59 b	0.66 b	0.45 a	0.60 a	0.82 a	1.11 b
ZnCl₂ (800 mg kg⁻¹ soil)						
<i>B. juncea</i>	0.65 a	0.75 a	0.46 a	0.65 a	0.64 a	0.53 a
<i>L. sativum</i>	0.58 b	0.45 a	0.53 ab	0.58 a	0.52 a	0.68 a
<i>B. rapa</i>	0.52 a	0.55 b	0.30 b	0.52 a	0.73 b	0.73 b
Mixed (CuCl₂ + Pb(NO₃)₂ + ZnCl₂) (1600 mg kg⁻¹ soil)						
<i>B. juncea</i>	1.05 c	0.74 b	0.48 a	1.05 b	0.69 a	0.81 ab
<i>L. sativum</i>	0.50 a	0.56 a	0.60 a	0.50 a	0.73 a	0.71 a
<i>B. rapa</i>	0.65 a	0.84 b	0.73 ab	-	-	-

Different letters for each treatment denotes significant differences in mean value at P<0.05 obtained from Duncan's multiple range test.

4.5.4. Soil pH of Cow dung and Lime Amended Soil

Soil pH increased in soil treatments with CuCl₂, Pb(NO₃)₂, ZnCl₂, their mixed metal and each with cow dung at 5 % and 20 %. Similarly soil pH increased in heavy metals and lime treatments at 3 and 9 g kg⁻¹ soil. Soil pH was comparatively higher in cow-dung treatments than in lime treatments (**Table 32**).

Table 32. pH of soil treated with heavy metals and cow dung or lime (mean ±sd)

Treatments	Cow-dung	Lime
------------	----------	------

(mg kg ⁻¹ soil)	Ctrl	5 (%)	20 (%)	Ctrl	3 (g)	9 (g)
300 mg CuCl ₂	6.15±0.12	7.53±0.26	8.22±0.21	6.15±0.12	7.57±0.52	7.6±0.62
500 mg Pb (NO ₃) ₂	6.49±0.43	7.79±0.29	8.18±0.22	6.49±0.43	7.63±0.23	7.73±0.45
800 mg ZnCl ₂	6.18±0.32	7.85±0.37	8.24±0.36	6.18±0.32	7.65±0.35	7.91±0.28
1600 mg mixed metals	6.12±0.36	7.86±0.46	8.07±0.36	6.12±0.36	7.78±0.46	7.65±0.57

4.5.5. Immobilization of Supplied Heavy Metals

Salts of heavy metals such as CuCl₂, Pb(NO₃)₂, ZnCl₂ in single or their mixed metal treatments were supplied in soil amended with cow dung (at 5 % and 20 %) and (lime at 3 g and 9 g). Immobilization of each supplied Cu, Pb and Zn in both single and mixed metal treatments were found to be high in cow-dung amended soil (**Fig. 37**). 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 CuCl₂ and Pb(NO₃)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. 37**).

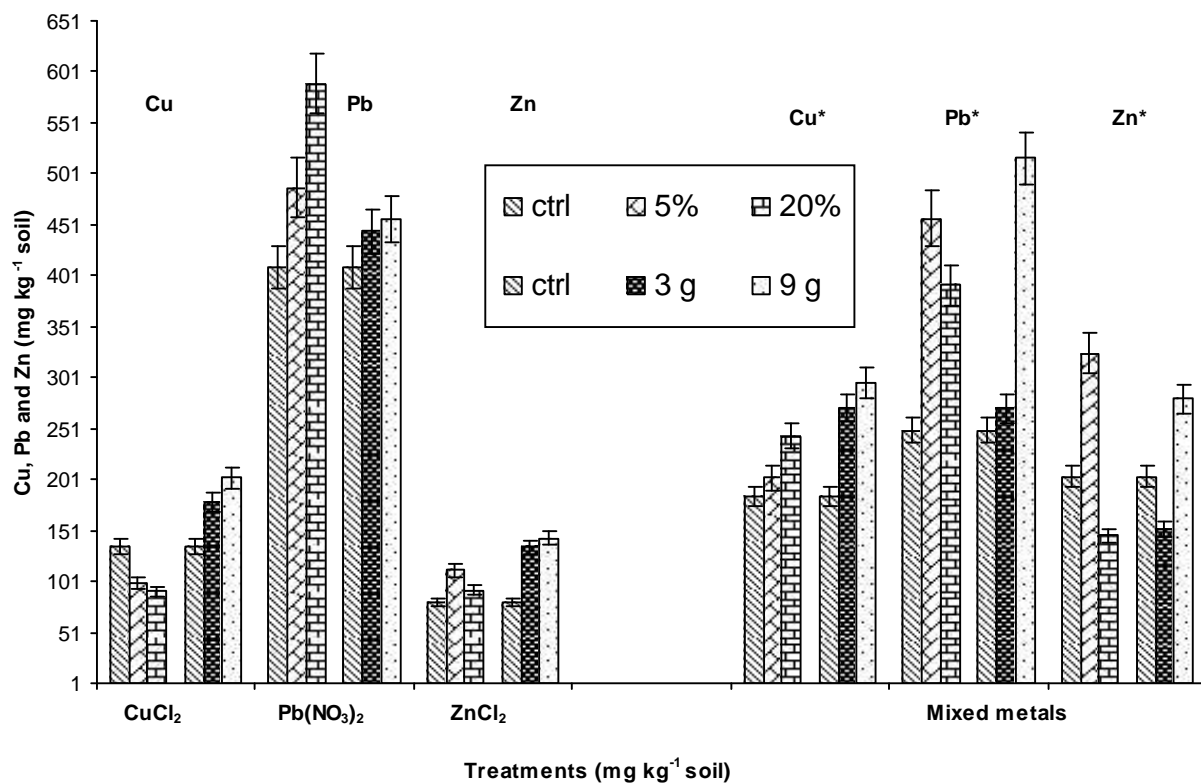


Fig. 37. Concentration of Cu, Pb and Zn in soil treated with single and mixed metals with different quantities of cow dung (%) or lime (g). (*indicate metals retained in mixed metal treated soil)

5. DISCUSSION

5.1. Uptake and Morphology

Uptake of heavy metals by plants from soil is quite complex, as it does not follow any particular pattern and varies with respect to metals, plant species and plant parts (Barman *et al.*, 2000). Most of the vegetable seeds (except *Lactuca sativa*) could not germinate at 500 and 1000 mg CuCl₂ kg⁻¹ soil and also at 1500 and 3000 mg ZnCl₂ kg⁻¹ soil treatments, which possibly is due to the more toxic effects of these metals on their physiology (Wong and Bradshaw, 1982; Fernandes and Henriques, 1991). There are also evidences that, as low as 0.5 mg Cu l⁻¹ is high enough to kill root meristem of sensitive plants (Karataglis, 1982). The *L. sativa* which have high cellulose content (Thompson and Kelley, 1979) may be the reason for the ability to grow on 500 mg kg⁻¹ CuCl₂- treated soil or may be due to higher degree of genetic plasticity (Larcher, 1995). Lowest accumulation of all supplied heavy metals in *Daucus carota* indicated the presence of certain avoidance mechanism to overcome heavy metal toxicity.

Cadmium: Accumulation of Cd ranged from 0.25 mg kg⁻¹ DW to 19.75 mg kg⁻¹ DW in the vegetables, which is within the critical tissue concentration (5 - 30 mg kg⁻¹ DW) as given by Kabata-Pendias and Pendias (1992). Significant decrease in fresh weight (FW), dry weight (DW), shoot length (SL) in *Brassica rapa* indicated Cd toxicity possibly due to high Cd accumulation in root (15.75 mg kg⁻¹ DW) and shoot (17.75 mg kg⁻¹ DW). Similarly, root length (RL), FW and DW decreased in *D. carota* even with Cd accumulation at 2.25 (roots) and 5.25 mg kg⁻¹ DW (shoots) grown on 20 mg CdCl₂ kg⁻¹ soils. This may be due to Cd-induced depletion of Cu in shoot, and Fe in root. Decreases in SL, leaf area, FW, DW, DW % in *B. rapa* grown on CdCl₂ treated soil may be possibly due to the fact that Cd is easily soluble, taken up both actively/and passively through simple diffusion, transported in aerial portion particularly bind to sulfhydryl groups (Hinsley, 1989). Cd uptake in plants have been reported to block the entry of essential macro- and micronutrients for pectin-calcium binding sites such as Cd for Ca (Barcelo *et al.*, 1988), and decrease the cell wall elasticity (Matsumoto *et al.*, 1977). As a result, it may affect growth i.e. differentiation and cell division. Mature (7 weeks old) *L. sativum* showed significant increase in SL, FW and DW up to 10 mg CdCl₂ kg⁻¹ soil treatment, which is possibly be due to low dose stressors, that generate non-specific alarm reactions and have changed the

hormonal balance (Nyitrai *et al.*, 2002). Increased leaf area or length with low concentrations of Cd in *B. juncea* and *L. sativum* may be either due to increased vacuolization or increase in elasticity. But the decrease in leaf area of *B. rapa* may be due to Cd stress which inhibits photosynthesis and transpiration (Bazzaz *et al.*, 1974).

Copper: The visible symptoms in decrease of root length (RL) when grown on CuCl₂ excess soil may be due to increased Cu accumulation, which were 55, 38 and 37 mg kg⁻¹ DW in roots of *B. juncea*, *B. rapa* and *L. sativa*, respectively. Generally Cu toxicity results visible symptoms when its accumulation in plant reaches 20-30 mg kg⁻¹ DW (Reuther and Labanauskas, 1966; Davis and Beckett, 1978). Copper toxicity in root was mainly due to the fact that roots are the first target organs for heavy metal accumulation (Ernst, 1993). Cu accumulation in roots leads to increased efflux of K⁺ by changing cell permeability, damage cell membranes (De Vos *et al.*, 1991), changes membrane proteins such as H⁺ ATPase or changes in the composition of fluidity of membrane lipids (Meharg, 1993). Thus efflux of K⁺ by root cells disturbs the intracellular K⁺ ion relation (Schulze *et al.*, 2005), which in turn control turgidity of the cell, and these possibly have reduced cell division and cell elongation. Decrease in RL in vegetable grown on CuCl₂ treated soil appear due to reduced percentage of dividing cells (Eleftheriou and Karataglis, 1989), decreased synthesis of the components of the cell walls, damaged golgi apparatus or changes in the metabolism of polysaccharides in the root cap (Punz and Sieghardt, 1993). All these changes may be due to low soil pH or decrease accumulation of micronutrients such as Cu, Fe, Mn and Zn in plants. Shoot length and leaf area are reduced in most cases like *B. rapa* (young), *B. juncea* (young), *L. sativum* (young), *D. carota* (mature) *L. sativum* (mature), *B. juncea* (mature) with 100 mg CuCl₂ treated soil after increased Cu accumulation and with depletion of Zn accumulation in their body. Low soil pH may be responsible for root injury (Black, 1973). The damaged root may decrease uptake of nutrients like Fe, Ca and Mn taken by young, unuberized root tips (Greger, 1989; Godbold and Kettner 1991). Reduced Zn accumulation with excess Cu may lower auxin and IAA metabolism (Singh, 2003), which may be related to stunted growth and smaller leaves of the plants.

Lead: Root length of *B. rapa* and *D. carota* decreased with 1000 mg Pb(NO₃)₂ kg⁻¹ soil treatment and may be due to greater accumulation of Pb in root system. Roots act as a barrier to restrict the transport of Pb to shoots (Jarvis and Jones, 1978; Adriano, 1986). Lead is mostly

deposited in cell walls or vacuoles (Chettri *et al.*, 1997; Kabata-Pendais and Pendias, 1992). Root length increased slightly with low doses of $\text{Pb}(\text{NO}_3)_2$ in *D. carota* and *L. sativa*, may be due to possibility of development of low dose stressors as in the case of Cd (Nyitrai *et al.*, 2002), and changes the hormonal balance which possibly accelerates cell division and elongation.

Shoot length (SL) although increased in *L. sativum* (young and mature) with most of the low concentrations (up to 199 and 207 mg kg^{-1} Pb in shoots, respectively), but is reduced at 1000 mg kg^{-1} $\text{Pb}(\text{NO}_3)_2$ treatment. Similarly, Pb toxicity was observed at accumulation of 31.5 mg Pb kg^{-1} in shoot (at 100 mg kg^{-1} treatment) in *D. carota*. Accumulation of Pb in plants has been reported to cause induction of enzymes like peroxidases (Lee *et al.*, 1976; Maier, 1978) or induced cell elongation (Lane *et al.*, 1978). Peroxidase activity under heavy metal stress has been reported to be higher in root than in shoot (Garcia *et al.*, 1996). Peroxidase participate in many reactions including lignifications, cross linking of cell wall, polysaccharides oxidation and endol-3-acetic acid, regulation of cell elongation, phenol oxidation (Gaspar *et al.*, 1991) and is responsible to protect the cell against oxidative damage (Garcia *et al.*, 1996). In the present study SL and RL also increased in *L. sativa* and *D. carota* at low doses $\text{Pb}(\text{NO}_3)_2$ treatments and this is possibly due to least disturbance in cellular metabolic activities on one hand and continued cell enlargement, cell division and elongation due to peroxidase activity on the other hand.

As Pb is not essential element for biological system, it is mostly localized extracellularly in small concentrations, but once they exceed the critical level, it enters the cell membrane and interfere with metabolic processes (Chettri *et al.*, 1997). This may be the reason for decrease of SL, FW, DW in all studied vegetables mostly grown at high doses (1000 $\text{mg Pb}(\text{NO}_3)_2 \text{ kg}^{-1}$ soil).

Zinc: Zinc accumulation in the studied vegetables after growing on ZnCl_2 treated soil, ranged from 85 to 505 mg kg^{-1} in shoot portion. Generally, plants are sensitive to Zn concentration above 200 mg kg^{-1} in leaf tissue (Davis and Beckett, 1978). The possible reason for decrease in RL of *B. rapa* (young) and *B. juncea* (mature); and SL of *B. rapa* (young and mature), *L. sativum* (young) and *L. sativa* may be due to Zn toxicity in one hand or Zn induced Cu- deficiency along with Mn or Fe or both in other hand. Cu deficiency lowers the content of soluble carbohydrate (Singh, 2003) in the cell. As soluble carbohydrates are source of energy for metabolic activity, its deficiency in the cell will certainly affect cell division and its elongation.

Zn toxicity emphasizes the complexity of interactions between Zn and the uptake and utilization of P, Fe and Mn (White *et al.*, 1979). Accumulation of excess Zn has effect on water

splitting enzyme and ultimately effect on photosynthesis (Van Assche and Clijsters, 1986). Zinc accumulation also disturbs water channel protein, present in plasma membrane and reduces water uptake (Lambers *et al.*, 1998). This probably reduces the physiological activity of the cell and ultimately showed the stunted growth in most studied vegetables.

Mixed metals: High accumulation of all supplied heavy metals (Cd, Cu, Pb and Zn) in shoots of *B. juncea*, *B. rapa* and *L. sativum* showed that these metals are not restricted to root regions but are easily translocated to aerial portion. But in the case of *L. sativa*, metals like Cu and Pb are mostly arrested in roots and were restricted in their transport to shoot. Decrease in RL, SL, FW in *B. juncea*, *B. rapa* and *L. sativa* grown on soil treated with different concentration of mixed metal (CdCl₂, CuCl₂, Pb(NO₃)₂ and ZnCl₂) is mainly due to the toxicity of accumulated metals in their body. Significant increase in DW and DW % of *L. sativum* grown on mixed metal treated soil is speculated due to tolerance of this vegetable crop to toxic heavy metals.

In single metal treatment, *L. sativum* showed significant increase in FW, DW, RL and SL in Cd treatments; SL in Pb(NO₃)₂ and ZnCl₂ treatment and insignificant change in CuCl₂ treatments. Besides this, depletion of micronutrients like Fe and Mn were not observed in mixed metal treatments. This indicated that *L. sativum* is highly tolerant to the stress of toxic heavy metals.

5.2. Heavy Metals and Chlorophyll

The variable chlorophyll concentrations among the control vegetable crops indicated the intrinsic property of chlorophyll biosynthesis. Presence of high Chl-b concentrations compared to Chl-a, in all vegetables might indicate the degradation of later either forming the HMs-chlorophylls (Cd-chl, Cu-chl, Pb-chl, Zn- Chl) by substitutions of Mg in chlorophylls (Kupper *et al.*, 1996) or by the oxidation of the methyl group II to the aldehyde (Bidwell, 1979). Chlorophyll degradations (phaeophytinisation) by heavy metals have been observed experimentally in *Ramalina duriaei* (Garty *et al.*, 1992). The decrease in Chl-a/b indicates the senescence characteristics (Dean *et al.*, 1993) and has been explained by the earlier structural loss of chloroplast stroma lamellae (containing photosystem I and most of the Chl-a) compared to the grana lamellae (containing photosystem II) (Bricker and Newman, 1982).

All chlorophylls decreased significantly in both *B. juncea* and *L. sativum* with increased Cd accumulation. Significantly decreased Chl-a/b (P < 0.05) only in *L. sativum* has been

speculated as the Cd inhibited the chlorophyll biosynthesis due to prevention of photoactive protochlorophyll reductase complex formation and δ -Aminolaevulinic acid (ALA) synthetase (Oncel *et al.*, 2000; Singh, 2003). Cadmium also blocks sulphhydryl (-SH) groups of this enzyme complex, which provides chlorophyll synthesis (Stobart *et al.*, 1985). Besides this, depletion of Cu, Zn and/or Mn by excess Cd, indicated the decreased resistance to toxic metals as well as loss of Cu- Zn /or Mn- superoxide dismutase (SOD), one of the antioxidative enzymes related to the inhibition of the oxidative damage by increasing resistance to toxic effects (Spsychalla and Desborough, 1990). Cadmium induced Fe-enrichment in the present study might be responsible for the production of $O_2^{\cdot-}$ and $HO^{\cdot-}$ radicals as suggested by Caro and Puntarulo (1996). $HO^{\cdot-}$ radicals might fragment the Cu/Zn- SOD enzyme (Casano *et al.*, 1997). But, Fe increased either with Zn or Mn probably might have masked the toxic effect of Cd on chlorophylls. Sometimes the increased Fe accumulations do not show toxic effects due to its important role in forming ferritin that protect against heavy metal induced oxidative injury (Fabisiak *et al.*, 1999). No significant loss of chlorophyll in *B. rapa* and *L. sativa* might be due to enrichment of both Mn and Fe, which might have associated with increased activity of either Mn-SOD enzymes present in cytosol or Fe-SOD present in chloroplast. Significantly increased chlorophyll in *D. carota* with low Cd accumulation indicated Cd tolerance as well as the enhanced antioxidative defenses in carrot cells as suggested by Kim *et al.* (2000). Carotenoids pigments in carrot have also controlled the oxidant level protecting against photo-inhibition damage (Foyer *et al.*, 1994; Parida and Das, 2005) as a result there may be increased chlorophyll synthesis.

No significant chlorophylls loss in Cd treated *L. sativa* and *B. rapa* may be due to protective and regulatory effect of Zn and Fe which have been increased after Cd treatments. Zinc is required in the enzymes ALA hydratase where as Fe as cofactors in δ -Aminolaevulinic acid (ALA) synthetase (Singh, 2003).

Total-chl and Chl-a decreased significantly in *B. juncea* at 100 mg $CuCl_2$ kg^{-1} soil have been accounted either due to its additional accumulation or induced deficiency of both Zn and Fe, which might have inhibited the biosynthetic enzymes of the chlorophyll pathway as suggested by Van Assche and Clijsters (1990). Copper induces inhibition of mobilization of Zn and Fe, uptake and/or translocation of other essential metals (Schmidt *et al.*, 1997). Enrichment of transition elements, like Cu and/or Fe inhibits the cell defense mechanisms leading to the production of free radicals and oxidative destruction of the membrane lipids (Sandmann and Boger, 1980; Halliwell and Gutteridge, 1986; De Vos *et al.*, 1992; Weckx and Clijsters 1996;

Mazhoudi *et al.*, 1997). Excess Cu may also react with sulphhydryl groups by lowering the intracellular thiol concentration or may interfere with electron transport (Stauber and Florence, 1987).

Although *B. rapa* accumulated higher Cu, but no prominent changes in chlorophyll (except on Chl-a), may be due to increased accumulation of Fe-Mn which might have regulatory effect on enzymes superoxide dismutase (SOD) of the chloroplast. Similarly, with CuCl_2 treatments, the slightly increased Fe along with no depletion of Mn and Zn in *L. sativa* must have prevented the loss of chlorophyll.

Daucus carota at only $1000 \text{ mg Pb(NO}_3)_2 \text{ kg}^{-1}$ soil treatment showed no significant loss of chlorophylls indicating toxicity which may be due to either depletion of Mn, Zn and Cu or due to highly increased Fe accumulation. Lead induced Cu and Zn deficiency that might have inhibited the enzymatic activities of Cu/Zn-SOD and the Mn-SOD enzymes for removing the toxic superoxide radicals (Singh, 2003). In general, there is no significant reduction in the chlorophylls of Pb accumulated leaves or might have been minimized by moderate increase of Zn-Fe or Mn-Fe accumulation. Increased Chl-a, total chlorophyll and Chl-a/b in Pb treated *L. sativum* may be due to increased peroxidase activity (MacFarlane and Burchett, 2001) on one hand and also increased micronutrients like Cu and Zn which are the cofactors of enzyme SOD. Lead possibly has helped *L. sativum* to enhance the tolerance of toxic metals in mixed metal treatments by producing several antioxidants. For the protection against oxidative stress, plant cells are equipped with several antioxidants like ascorbate, glutathione, cysteins and oxygen radical detoxifying enzymes such as catalase, peroxidases SOD, glutathione reductase and others (Chaoui *et al.*, 1997; Jimenez *et al.*, 1997).

In Zn treated *L. sativum*, Chl-b decreased significantly and total-chl decreased insignificantly, which may be due to Cu- and Fe-deficiency. Although Zn accumulation was highest in *B. rapa*, no significant effect on chlorophylls may be due to increased concentrations of non-supplied Fe (i.e. Zn and Fe may have certain major roles in protection of the chlorophylls loss which have resulted only insignificant changes in chlorophylls. The rate of - Aminolaevulinic acid (ALA), the precursor of heme- or chlorophyll synthesis is regulated by Fe which helps in the proliferation of the chloroplasts (Singh, 2003).

In mixed metals ($\text{CdCl}_2 + \text{CuCl}_2 + \text{Pb(NO}_3)_2 + \text{ZnCl}_2$) treatments, significant increase in total chlorophyll and Chl-b in *B. juncea* treated with low dose (251 mg kg^{-1} soil) is possibly due to synergistic effects of Cu, Pb and Zn. As these metals showed enhancement of total chlorophyll

and Chl-b at low dose with these single metal treatment. No significant change in total chlorophyll, Chl-a and Chl-b in *B. rapa* even in mixed metal treatment is possibly due to cumulative effects of Cu, Cd and Pb, as these metals protected chlorophylls at low doses in single metal treatment.

5.3. Monitoring Heavy Metals in Soil and Vegetables

In the present study, soil samples from agricultural fields of Kathmandu valley were monitored for heavy metals such as Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Root uptake is one of the general processes of accumulation of heavy metals in different parts of plant body from the soil, but is a complex process (Barman *et al.*, 2000). Highest concentrations of heavy metals were found in the agricultural fields such as presence of Cu 65.5 mg kg⁻¹, Pb 46.75 mg kg⁻¹, Cd 2.0 mg kg⁻¹ in the soil of Shankhamul, and Zn 162.00 mg kg⁻¹ in the soil of Khusibun. Similarly, higher concentrations of heavy metals compared to normal soil value (literature value) were found in different fields such as Cu, Pb and Cr in the soil of Banasthali; Pb and Zn in Manohora; Cu and Pb in Nakhu; Zn in Kalopul and Ghattekulo; Cu, Pb, Cd and Zn in Shankhamul; Pb in Kusingal, Shovabhadrawati and Balkhu; and Cu, Zn and Fe in Khusibun. This may be due to precipitation or fall out of atmospheric pollution, as well as by the use of heavy metal polluted river water in irrigation, or by use of chemical fertilizer. There is presence of heavy metals in the atmosphere and river water of Kathmandu as pointed out by the biomonitoring studies of lower plants (Devkota *et al.*, 1997; Chettri *et al.*, 2001; Shakya and Chettri, 2004) and river water (Gauatm and Agrawal, 1994). Similarly, the heavy metals present in chemical fertilizers (Galloway *et al.*, 1982; Sharply and Menzel, 1987) provide the evidences for the increase of heavy metals in the soil of Kathmandu.

In fields like Shankhamul, although concentrations of Cu, Pb and Cd were high in the soil but the accumulation of these metals were low in vegetable crops grown there compared to the vegetables collected from other fields. The possible reason for this can be the soil factors (soil pH, organic matter, clay, phosphate contents etc) which vary in different area. Increased clay particles can bind metal cations and become unavailable to plants and organisms in the soil (Hodgson, 1963). Increased organic matter in the soil bound the soil heavy metals strongly by their humic- and fulvic- acids and proteins, thus making less available to plants (Zimdahl and Foster, 1976; Gadd and Griffiths, 1978; Bassuk, 1986).

Concentration of less Cu accumulation in all vegetable crops (except Spinach) cultivated in the soil of Shankhamul, may be due to the fact that Cu was not in available form due to strongly binding of large fractions of Cu to inorganic and organic compounds. There is presence of high organic matter (4.56 %) in the field of Shankhamul as well as it is known that there is uses of domestic and municipal wastes in the fields by the farmers. It is supported by the fact that large fractions of Cu are immobile in the organic matter (McLaren and Crawford, 1973), and by the excessive use of municipal or domestic sewage (Alloway, 1995; Grimes *et al.*, 1999).

In Khusibun area, accumulation of more concentrations of Pb, Cd and Zn in vegetable parts compared to their soil concentrations may be due to their more solubility and availability via the presence of low soil pH value (3.9) and high concentration of K (819 kg/ha) prevailing in that field. The availability of Zn to plants depends on soil pH, concentration/sources of Zn. These metals such as Zn, Cd and Pb are generally mobile in acid soils. Similar results were also suggested by John *et al.* (1975) and Miller *et al.* (1976) when examined some vegetable crops with decreasing soil pH.

From the study of correlation among soil heavy metals and soil parameters, significant positive correlation observed between soil Cu and soil Pb may be due to the fact that both these elements are strongly bound to colloids of clay/and organic matter (McLaren and Crawford, 1973; McLaughlin *et al.*, 1999). Copper showed strong positive correlation with N, P, K in acid soil that may be due to their available form (Cu-org, Cu^{2+}) associated with exchangeable forms with colloids of clay in the soil solution (Brady, 2002).

Significant negative correlation observed between Zn and pH, and also between P and pH may be due to solubility of Zn and P which are dependent on the pH (Hornberg and Brummer, 1993). Significant positive correlation between Zn and Fe may be due to the fact that both (Zn II) and Fe (II) are in oxidation state. In acidic soils, Zn is present in the form of Zn^{2+} , ZnSO_4^0 or Zn-org in the order of decreasing availability. Similarly, Fe is present as Fe^{2+} , FeSO_4^0 , $\text{FeH}_2\text{PO}_4^+$ (Streit and Stumm, 1993). So in the acidic soil solution availability of these ions showed positive relationships between them and negative relationships with soil pH. Insignificant correlations of Cr with any other heavy metals or soil properties indicate that almost all Cr in soil solution is in insoluble form and are basically inert (Pais and Benton Jones, 1997).

The significant positive relationships shown by Ni with Co, Mn and clay; by Co with Mn and clay; by K with clay and organic matter; by Mn with clay indicate retention of these ions in

exchangeable form in clay particles of acid soils (Zalidis *et al.*, 1999) and also in organic matter (Streit and Stumm, 1993).

Different vegetable crops collected from same agricultural plot have different levels of different metal concentrations in their tissues. This may be due to differences in ligands (Rauser and Curvetto, 1980) at the binding sites like cell wall, middle lamella or cell organelles (Reilly, 1969; Barcelo and Poschenrieder, 1990; Bringezu *et al.* 1999; Chettri *et al.*, 2000). Environmental factors, association or interactions or competition (synergistic or antagonistic) with neighboring metals during mineral absorption process may be the reasons for differences in different metal accumulation.. Some plant species have heritable tolerance or resistance enabling them to grow and to reproduce on metal contaminated soil (Verkleij and Schat, 1990; Ernst *et al.*, 1992). Different crop varieties have different sensitivities to the toxic nature of effluents. The order of decreasing sensitivity is proteinaceous seed>oil seed >starchy seed (Thompson and Kelly, 1979). Additionally, heavy metal concentrations differs between plant species depending on morphology of shoots and roots (Foroughi *et al.*, 1981), formation of metabolically inactive complexes in roots (Baker and Walker, 1990), transpiration rate (Hardeman and Jacoby, 1984) and translocation from vegetative to generative plant parts (Kloke, 1981; Morghan, 1993). Plant roots also play an important active role in mobilizing metals from soil particles by the acidification of rhizospheric region (Kabata-Pendias and Pendias, 1992; Marschner, 1995). Anatomy, biochemistry and physiology may be involved in heavy metal accumulation and distribution in the aerial parts of plants in the case of soil contamination (Salt *et al.*, 1995; Xiong, 1998). It was also noted in the present work that within an individual plant, different parts like root, stem and leaf have variability in heavy metal accumulation. Physiological reasons for the uptake and accumulation of heavy metal concentrations in plant root and shoots differ with the type of organic acids in the plant body (Tatar *et al.*, 1999).

Although Pb is immobile in soil (Streit and Stumm, 1993), high Pb accumulation in both leafy and root vegetables clearly indicates that green and leafy vegetables of Kathmandu valley accumulate Pb through root uptake. Besides this, there may be atmospheric fall out which may directly deposits in the soil surface or indirectly in the leafy portion. So deposition of Pb in soil is directly related to the exhaust emissions of leaded gasoline on one hand and wear and tear of tires on the other hands (Djingova and Kuleff, 1993).

Roots of *D. carota*, *R. sativus* and *B. rapa* also accumulated different heavy metals (especially Pb) and supports the view of Witting (1992) that underground parts may also act as suitable bioindicators, and is possibly due to a moderate mobility of Fe, Mn, Zn, Cu and Mo (Ziegler, 1988) in the xylem and phloem of root and their accumulation in parenchymatous tissues of cortex layer (Kelepertsis and Andrulakis, 1983).

Although most leafy vegetables showed more Cd accumulation in leaves as compared to roots, even bioaccumulation of Cd in *S. oleracea* (spinach- Gobre) root (collected from Thimi and Shovabhagwati) is greater than in soil showing tendency of hyperaccumulation. Upper red portion of root of Spinach – Gobre are mostly used for vegetable along with its leafy portion. As this upper root portion along with entire root can accumulate high amount of Cd, it should be avoided for vegetable use.

Chromium accumulation in all vegetables was found within normal plant value and ranges from 0.25 mg kg⁻¹ to 4.75 mg kg⁻¹ in spinach. This might be due to very lower chromate uptake via roots and occur only in hexavalent form and is rapidly reduced to immobile trivalent form in the soil (Streit and Stumm, 1993).

Although high Cu concentration may be responsible for premature seedlings death of wheat plants (Lolkema, 1985), in the present study more Cu accumulation was in spinach collected at Shankhamul area. This is also supported by Baszynski *et al.* (1982), not having any changes in the inner structure of chloroplasts grown in excess Cu, may be due a strong tendency for accumulation in the vacuole (Ernst *et al.*, 1992). From this it was found that *S. oleracea* (Patane) showed high Cu tolerance when compared to other varieties/or species. High concentration of Cu, among the studied vegetables, only in the Patane spinach collected from the Shankhamul area indicated the variability rate of uptake (Kurz *et al.*, 1999).

In all vegetables Fe accumulation was high above the normal plant concentration. It is possibly due to inorganic deposits of H₂PO₄ and iron phosphate in the vascular bundles along the veins of a leaf to such an extent that the adjacent tissues become even markedly deficient in iron. Water logged condition and mobile organic complex, low organic matter and chelates increase the Fe uptake (Streit and Stumm, 1993). Mostly the roots of the broad leaf mustard and spinach have high Fe concentrations than in leaf which may be due to the formation of metabolically inactive complexes (Baker and Walker, 1990).

In the present study *S. oleracea* (spinach) and *R. sativus* (red radish) was found to be good hyperaccumulator of Zn at most biotops, where they were present. Some members of

Cruciferae like *B. juncea* and *B. rapa* also showed hyperaccumulation of Zn at Kusingal and Thimi, respectively. This may be due to the fact that Zn uptake not only depend upon species, age or parts of the plant, but also upon the mobility of Zn in the soil, water soluble and /or exchangeable forms. Zinc mobility in soil and its availability to plants is also controlled by pH of the soil, total amount of Zn in soil, source of Zn, organic matter and soil type (Chlopecka *et al.*, 1996). Hyperaccumulator have usually a more efficient transport of specific metals from the roots to the shoots in comparison to non-hyperaccumulator (Kupper *et al.*, 2000). Non hyperaccumulators do not translocate heavy metals very efficiently from roots to shoots (McGrath *et al.*, 2001).

From the study of correlation among the respective metal contents in soil and vegetable crops, insignificant positive relationships between soil Pb and the Pb in root /shoot most possibly is due to its least mobile nature (Fuller, 1997). Although Cu content in soil is high but insignificant correlation was observed between its concentration in soil and plant body. This may be possibly due to binding of Cu with soil components which makes it unavailable through the formation of Cu complexes and also being practically immobile (Kirkham, 1977; Fuller, 1997).

Result of correlation analysis between heavy metal content in soil and in vegetable parts indicated that in very few cases there is significant correlation. Uptake of heavy metal in plant is not only dependent on availability of the ions in the soil but are also regulated by various other soil properties like pH, organic matter, N, P, K and the clay content. From this it can be suggested that heavy metal content in the soil is not the single factor to reflect the concentration in plants (Ernst, 1974; Kisku *et al.* 2000), but various factors like abiotic (Baker, 1983), biological (Chang *et al.*, 1982), forms of the heavy metals such as dissolved, exchangeable, residual (Roberts and Johnson, 1978), present in soil might play major role in the absorption and uptake of heavy metals from the soil.

5.4. Dietary Intake of heavy metals through vegetables

Plants which develop heavy metal tolerance may constitute a risk factor for human health especially when they enter food chain. Although the studied vegetables are the good sources for essential elements like Cu, Cr, Zn, Fe and Mn, but when grown in contaminated areas the bioaccumulation of Cd and Pb are in alarming concentrations. The result of intake value of heavy metals via vegetables when compared with Provisional Tolerable Weekly Intake (PTDI) of

WHO (1993, 1996) indicated that the daily intake of most of them (except Cd and Pb) are within permissible limit. Lead intake through vegetables highly exceeds the maximum permissible level of 250 µg as calculated from WHO Tolerable Weekly intake (WHO 1993, 1996). Similarly, Cd intake through vegetables alone is 33.43 µg/day and can be considered quite high, as the recommended daily intake from the total food is only 70 µg/day (WHO, 1996).

The reason for very high intake of heavy metals via vegetables in Kathmandu valley is due to the increased contamination of heavy metal in vegetables. The quantitative estimation of the heavy metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in dietary intake through vegetables in Greece as reported by Voutsas and Samara (1998) was found to be less than daily dietary intake of similar heavy metals in Kathmandu valley through the different vegetables (Sharma and Chettri, 2004). It indicated the relatively enhanced values of Cu, Pb, Cd, Cr and Zn in all vegetable crops grown in Kathmandu valley. The variations in metal concentrations in vegetables may be due to difference in vegetable varieties, sampling periods, sampling locations, soil characteristics, degree/type of pollution, fertilizer application etc.

The intake of non-essential heavy metals such as Cd and Pb higher in the vegetables diet from the vegetable grown from agricultural fields of Kathmandu may be due to its high concentration in soil (greater than 0.35 mg kg⁻¹ as given by Bowen, 1979). The Cd content of agricultural soils has long been of great concern as it is easily taken up by crop plants and passes in our food. Increased Cd levels in soil are a potential health risk, safety margins are small (Buchet *et al.*, 1990). This is supported by the fact that leafy vegetables (especially spinach) accumulate high concentration of Cd even under normal condition of cultivation (Pfeilsticker and Markert, 1977; Ellen *et al.*, 1990). The repeated consumption of such exposed vegetables can lead to the health damage as was shown in comparative studies of renal tissues of deceased adults from unexposed and Cd-exposed areas (Anke *et al.*, 1979; 1994).

In the present investigation Pb accumulation in leafy vegetables like *B. juncea*, *S. oleracea* and *L. sativum*, collected from Thimi was found to be high indicating a prominent or leading source for Pb in our body. The high accumulation of Pb in these species may be through root uptake when grown on Pb contaminated soil. The deposition of heavy metals (Cd, Cu, Pb and Zn) in soil may have resulted from emissions of vehicular traffic or brick factories. There is evidence that atmosphere of Kathmandu have high level of heavy metal pollution (Bhattarai and Shrestha, 1981; Devkota *et al.*, 1997; Chettri *et al.*, 2001).

From the uptake experiments and heavy metal monitoring study it is found that the toxic metals (Pb and Cd) in *D. carrot* (root) and potato, respectively, are low, indicating the presence of certain avoidance mechanism to overcome heavy metal toxicity. Hence these vegetables do not contribute much in heavy metal circulation in human diet.

5.5. Phytoremediation

5.5.1. Phytoextraction

Among the 18 tested vegetable crops *V. faba* has been found to accumulate excessive amount of supplied Cu and Zn. High Cu accumulation (50 to 100 mg kg⁻¹) was observed in *A. fistulosum*, *S. oleracea* (Desi), *S. oleracea* (Patane), *S. oleracea* (Gobre) and *Beta vulgaris* var. *cicla*. But very low Cu accumulation was 20 – 22 mg kg⁻¹ DW in *B. juncea* and *B. caulorapa*, respectively and both species belong to the family cruciferae. 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 plant-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, 1997; Jain, 2007). Presence of alkaloids like lignin, phenols etc along with –NH₂ group forms complex with accumulated Cu and this enhances Cu accumulation.

In the present study potato could not grow in CuCl₂ 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 even lower range of critical tissue concentration (20 mg kg⁻¹ DW) 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 primarily to the fact that *B. juncea* produced 10 times more biomass than *T. caerulea*.

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 uptake of Zn from the mixed metal treatments 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 a tendency of hyperaccumulation.

5.5.2. Remedial measures

Most of the vegetable crops grown on organic matter (cow dung) amended and single metal contaminated soil 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).

In the remedial measures root/shoot length ratio decreased mostly in cow dung treatments and increased mostly in lime treatments. This result is mainly due to the fact that shoot length increased with cow dung treatments but shoot length decreased with lime treatment. The increase in shoot length in cow dung treatment must be due to unavailability of toxic metals and availability of essential nutrients as well as high water content.

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 are the amino acids. A great variety of different ligand partners include those derived from the benzene ring to which carboxyl (to form benzene carboxylic acids) or hydroxyl groups

(to form phenolic groups). 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).

In the present study, the accumulation of toxic metals was low and the yield production was higher in cow dung than in lime treatment, although the soil pH was increased more in former treatment. This may be due to sorption of toxic heavy metal by the protein, carbohydrate and phenolic compounds (Friedmann and Waiss, 1972) contained in them. 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).

In the present study, no increase in the DW in the vegetable crops grown on lime amended soil and further treated with single metal such as CuCl_2 , $\text{Pb}(\text{NO}_3)_2$, ZnCl_2 and their mixed metal concentration may be due to increased soil pH which makes metal less available to plants. Some combinations of metals have synergistic toxicity (Fern, 1971). 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 9 g 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.

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.

6. CONCLUSIONS AND RECOMMENDATIONS

From the uptake experiments it can be concluded that among the studied vegetables, *D. carota* showed lowest accumulation of all heavy metals in roots and shoots. High accumulations of each Cd and Pb in the shoot portion of leafy vegetables were observed in *L. sativum*; Cu and Zn in *B. juncea* and *B. rapa*, respectively. From this experiment it can be also concluded that accumulation of supplied heavy metals (Cd, Cu and Pb) are more rapid at initial phase (3 weeks) than in the matured condition. After accumulation of excess heavy metals, some changes were noticed in morphological parameters but adverse effects were noticed like reduction in FW, DW, SL, RL, leaf area and DW % after reaching their threshold concentrations. Due to reduction in RL, SL, leaf size, FW, DW and DW %, it can be concluded that accumulation of excess heavy metals are responsible to reduce crop productivity. Reduction in productivity has been speculated due to induced depletion of micronutrients like Cu, Fe, Mn and Zn. by supplied heavy metal.

Based on morphological parameters *L. sativum* has been identified as the tolerant vegetable crop, as the FW, DW, SL and RL increased in most treatments with both single and mixed metal treatments. Low concentrations of Pb are found to be responsible for such changes.

Based on chlorophyll content, it can be concluded that the excess accumulation of each heavy metals in each vegetables showed different responses on chlorophyll contents. The decreased concentrations of total-chl in CdCl₂ treated *B. juncea* and *L. sativum*; Cu treated *B. juncea*; and Zn treated *L. sativum*, the variable responses of heavy metals on different vegetables or responses of different doses on same vegetable species may be due to toxicity via their excess accumulation in the plant tissues or due to depletion of essential micronutrients, such as Cu- Zn and/or Mn in Cd –treatment; Fe and Zn in Cu –treatment; Cu, Zn and Mn in Pb –treatment; Fe and Cu in Zn –treatment.

The variable responses of excess heavy metal may be related to differences in their adsorption site or binding ligands on the cellular compartments/cell wall. Insignificant changes or enhanced chlorophyll concentration in most of the vegetables even after heavy metal accumulation must be due to moderate or slight increased accumulation of micronutrients such as Zn-Fe, or Cu- Zn, or Mn-Fe. Finally the induced deficiency of micronutrients may hamper chlorophyll biosynthesis probably by blocking the enzymatic steps. Increased micronutrients (Cu/Fe/Mn/Zn) after heavy metal accumulation probably increased their enzymatic activity of

SOD and enhanced antioxidative defenses against toxic effects of heavy metals and so reduced chlorophyll loss.

From the chlorophyll content experiments, it is clear that in Pb treated *L. sativum*, there is increase in chlorophyll-a and total chlorophyll indicating there must be acceleration of some enzymatic activities which must have helped to increase the biomass by enhancing the tolerance to stress of toxic metals in mixed metal treatments.

From the monitoring study it can be concluded that the toxic heavy metals (Cd and Pb) were recorded above the suggested normal soil value in most of the studied agricultural fields of Kathmandu valley. Measurements of heavy metals in vegetable (both leafy/underground modified root except potato tuber) collected from Khusibun, Shovabhagawati and Thimi showed high accumulation of most heavy metals. Accumulation of Pb (>10 mg/kg– a normal plant value) in both leafy and underground vegetables (except in potato tuber) collected from all biotops showed entry of Pb in our food chain through their accumulation in vegetable parts. The green vegetables as being the good sources of Zn, Cu, Fe etc, are necessary for our good health, but the accumulation of toxic metals like Pb and Cd, will certainly deplete the essential micronutrients in the vegetable diet.

From the monitoring experiments it can be said that *S. oleracea* (all variety of spinach) and *R. sativus* (red radish) are hyperaccumulator of Zn as their shoots showed more amount of Zn than in the soil where they were found growing. Similarly, *S. oleracea* (Spinach- Gobre) showed tendency of Cd hyperaccumulation in some sampling sites like Thimi and Shovabhagawati.

From the dietary estimation it can be concluded that the investigated vegetables like broad leaf mustard, cress leaf, radish carrot and turnip are responsible for contributing high dietary intake of essential elements like Cu, Cr, Fe, Mn and Zn. Similarly there is high intake of potentially toxic elements like Pb through the broad leaf mustard, cress leaf and spinach. Estimated Pb intake from vegetable source is about 151 % higher than the provisional tolerable daily intake as prescribed by World Health Organization. It also showed that most of our vegetables fields are highly contaminated with toxic metals.

From the phytoextraction study, it can be concluded that among 18 investigated vegetables, *V. faba* is the best accumulators for Cu and Zn; and *A. fistulosum* is the best Pb accumulator from the single metal treatment. But in the case of mixed metal treatments highest Pb, Cu and Zn accumulation was observed in *S. oleracea* (Patane and Deshi). Comparing results from single and mixed metal treatments, it can be concluded that heavy metal uptake is not only

dependent on the concentration of supplied cations in the soil but also on the available other metal species and the rhizosperic soil environment.

From the remedial measures 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.

RECOMMENDATIONS

1. It is recommended not to grow *L. sativum* in heavy metal contaminated soil as it can accumulate quite high amount of supplied heavy metals and does not show any morphological symptoms of heavy metal toxicity.
2. *Daucus carota* can be grown in heavy metal contaminated areas, as uptake of heavy metals in it is comparatively low than the leafy vegetables.
3. Potato tuber can be grown in heavy metal contaminated areas as its uptake is low, but commercial cultivation of other leafy vegetables should be avoided in such areas.
4. As *S. oleracea* and red radish are hyperaccumulator of Zn, they can be recommended for phytoextraction of Zn from Zn-contaminated soil.
5. Regular monitoring of heavy metals (Pb and Cd) in the agricultural fields is needed as it directly affects our food quality and health.
6. To check further heavy metal contamination in the agricultural fields, its sources need to be identified and accordingly preventive measures need to be taken.
7. Public awareness about heavy metal contamination and its consequences need to be imparted among the general public, especially farmers, house wives and school children through Environmental Educational Program. For example to reduce heavy metal concentration in our diet, we must discard older parts of the stem and leaves as they contain heavy load of some element than growing tissues, because trace metals such as Pb and Cd gets accumulated..
8. Washing vegetable is very important because it reduces external heavy metal contamination.
9. Peeling off of the epidermal layer of edible portion should be done as to remove outer contaminated layer.
10. 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.
11. Lime alone is not recommended for the immobilization of heavy metals.

7. SUMMARY

To understand the bioaccumulation of different heavy metals having different concentrations in contaminated soil and its impact on morphology of plants, 5 vegetables- *Brassica juncea* var. *cuneifolia* L., *Brassica rapa* L., *Lactuca sativa* L., *Lepidium sativum* L. and *Daucus carota* L., were investigated under pot condition. They were grown on soils having different concentrations of CdCl₂ (1, 2, 10 and 20 mg kg⁻¹ soil), CuCl₂ (50, 100, 500 and 1000 mg kg⁻¹ soil), Pb(NO₃)₂ (50, 100, 500 and 1000 mg kg⁻¹ soil) and ZnCl₂ (150, 300, 1500 and 3000 mg kg⁻¹ soil) and also their mixed metals (251, 502, 2510 and 5020 mg kg⁻¹ soil). For each treatment control was maintained by growing each vegetable in uncontaminated soil. The experiment was designed in randomized block design with 5 replicates for each treatment.

Morphological parameters such as root length (RL), shoot length (SL)), fresh weight (FW), dry weight (DW), dry weight % and leaf size (leaf area/length) were examined in potted plants grown on soil, artificially contaminated with heavy metal. Bioaccumulations of non-supplied micronutrients (Cu, Fe, Mn and Zn) after treatment with heavy metals were also observed in these vegetables. On the basis of fresh weight (FW) of whole plant (matured), threshold concentration for Cd in *B. juncea*, *B. rapa*, *L. sativum*, *L. sativa* and *D. carota* were ascertained to be as 2.75, 0.25, 3.22, 1.25 and 1.0 mg kg⁻¹ DW, respectively; for Cu were 18, 17.75, 14.75, 17.25 and 10.75 mg kg⁻¹ DW; for Pb to be 46.5, 34.5, 52.7, 81.0 and 31.5 mg kg⁻¹ DW; and for Zn were 173.5, 192, 172.5, 93.0, and 55.5 mg kg⁻¹, respectively. Among the studied vegetables, *D. carota* showed lowest accumulation of all heavy metals (Cd, Cu, Pb and Zn) in roots and shoots. High accumulations of each Cd and Pb in the shoot portion of leafy vegetables were observed in *L. sativum*; Cu and Zn in *B. juncea* and *B. rapa*, respectively. Accumulations of supplied heavy metals (Cd, Cu and Pb) are more rapid at initial phase (3 weeks old) than in the matured condition. After accumulation of heavy metals some changes were noticed in morphological parameters but adverse effects were noticed like reduction in FW, DW, SL, RL, leaf area and DW % after reaching their threshold concentrations. Reduction in FW, SL and RL in *B. rapa*, *L. sativa* and *D. carota* have been recorded as symptoms of Cd toxicity; and reduction in FW and RL in *B. juncea*, *B. rapa* and *L. sativa* as symptom of Cu-toxicity. Similarly, reduction in FW in *B. rapa*, *L. sativum* and RL in *B. rapa*, *D. carota* have been

observed as Pb-toxicity; reduction in FW and RL in *B. juncea* and *B. rapa*, and SL in *B. rapa*, *L. sativum* and *L. sativa* as symptoms of Zn- toxicity.

Changes in bioaccumulation of non-supplied essential micronutrients (Cu, Fe, Mn and Zn) were observed in vegetable shoots with increased Cd, Cu, Pb and Zn accumulation. With increased Cd accumulation, there is decreased Cu, Mn and Zn in *B. juncea*, Mn and Cu decreased in *B. rapa* root, Cu decreased in *L. sativa*, Mn decrease but slight Cu and Zn decreased in shoots of *L. sativum* with slight Cu and Mn decreased in shoots of *D. carota*. With increased Pb accumulation, there was depletion of Zn in shoots of *B. juncea*, Fe and Mn in shoots of *L. sativum*, Cu, Mn and Zn in shoots of *D. carota*. With excessive accumulation of Pb, slight increase in Fe and Cu in *B. juncea* (root and shoot), Fe and Zn in *L. sativa* (root and shoot) and Zn in *L. sativum* (root and shoot), Fe in *D. carota* (root and shoot) were observed. The depletion of Cu, Fe and Mn in shoot of *B. juncea*, Fe in shoot and Mn in root of *B. rapa*, Cu and Mn shoot, Fe and Mn root of *L. sativa*; Cu and Fe in *L. sativum*, Cu in shoot and Fe in roots of *D. carota* were observed after excess Zn accumulation. The concentration of micronutrient Fe decreased in both shoot and roots of *B. juncea*, Fe in roots of *B. rapa* but Fe and Mn both increased in *L. sativa* after mixed heavy metal treatment. Thus depletion of essential micronutrients from the plants has been speculated for the loss in productivity due to heavy metals stress.

Based on morphological parameters *L. sativum* has been identified as the tolerant vegetable crop, as the FW, DW, SL and RL increased in most treatments with both single and mixed metal treatments. Low concentrations of $\text{Pb}(\text{NO}_3)_2$ are found to be responsible for such changes.

To understand the impact of accumulation of heavy metals (Cd, Cu, Pb and Zn) on **chlorophyll content**, *B. juncea*, *B. rapa*, *L. sativa*, *L. sativum* and *D. carota*, grown for uptake experiments were considered. A portion of tip of 2nd leaf of vegetables crops (6 weeks old) which were grown on heavy metal contaminated soil were collected for chlorophyll measurements. Chlorophyll was extracted from 40 mg fresh leaf using DMSO mixed with a pinch of PVPP. The total chlorophyll, Chl-a and Chl-b was calculated using the equations given by Barnes *et al.* (1992).

Increasing leaf Cd content, up to 9 mg kg⁻¹ and 19.75 mg kg⁻¹, in *B. juncea* and *L. sativum*, respectively, showed significant loss of Chl-a, Chl-b and total chlorophyll. But in *B. rapa* and *L. sativa* up to 17.75 mg kg⁻¹ and 5.75 mg kg⁻¹ respectively, insignificant changes was noticed. Increasing leaf Cu content up to 27.25 mg kg⁻¹ showed significant loss of Chl-a, and

total chlorophyll at $P < 0.05$ in *B. juncea* and up to 18.75 mg kg^{-1} in *D. carota* showed insignificant decrease. But no significant losses in chlorophylls were observed in *B. rapa*, *L. sativa* and *L. sativum* in Cu treatments. Increasing leaf Pb content up to 85.5, 95.25, 39.75, 113.1 mg kg^{-1} respectively in *B. juncea*, *B. rapa*, *L. sativa* and *L. sativum* showed insignificant loss of chlorophylls but were rather increased at lower doses. Lead accumulation (64.25 mg kg^{-1}) in *D. carota* at $1000 \text{ mg Pb kg}^{-1}$ soil treatments showed significant loss in total chlorophyll. Increasing leaf Zn content up to 313.5, 505, 268.5, 85.5 mg kg^{-1} respectively in *B. juncea*, *B. rapa*, *L. sativum* and *D. carota* showed insignificant changes in chlorophylls.

Depletion of Cu, Zn and/or Mn was observed in *B. juncea* and *L. sativum* due to increased Cd accumulation. Similarly, depletion of Zn, Fe in *B. juncea* and Mn in *D. carota* were observed after increased Cu accumulation. There was highly increased Fe accumulation in Pb-treated *D. carota* with depletion of Zn, Cu and Mn. There was depletion of Fe, Mn and Cu in *B. juncea*, Cu and Fe in *B. rapa*, Mn and Cu in *L. sativa* and Cu in *D. carota* with increased Zn accumulation. Present studies suggest that depletion of essential micronutrients from the plant body after accumulation of increased toxic heavy metal may be one of the reasons for chlorophyll loss, because the micronutrients have catalytic roles in the enzymatic activities as well as protective role against the oxidative damages.

In the case of $\text{Pb}(\text{NO}_3)_2$ treated *L. sativum* it was noticed that Chl-a increased significantly up to 500 mg kg^{-1} soil Pb treatments resulting significantly increased Chl-a/b. This may be the reasons for the no negative effect on the morphological parameters. Finally it can be concluded that low doses (up to 500 mg kg^{-1} Pb) if freshly added to the soil have no harmful effects but may enhance enzymatic activities.

To understand the level of heavy metal pollution in soil and vegetables, **monitoring of heavy metals in soil and vegetables** from 12 different agricultural fields of Kathmandu valley were conducted. Main objectives of this study was to identify the suitable vegetable species (either leafy vegetables or the one with modified root system) for monitoring heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the environment.

Highest accumulations of Cd, Cu and Pb (2 , 65.5 and 46.75 mg kg^{-1} soil, respectively) from Shankhamul; Ni (29.25 mg kg^{-1} soil) from Nakhu; Co and Mn (15.25 and 675 mg kg^{-1} soil, respectively) from Balkhu; Cr (73.75 mg kg^{-1} soil) from Banasthali, Zn and Fe (162 and 75636

mg kg⁻¹ soil respectively) from Khusibun reflected the deposition of heavy metals in the soil of agricultural fields.

Soil pH ranged from 3.9 - 6.1 in Khusibun and Chapro, respectively. Organic matter (%) ranged from 0.54 (at Banasthali) - 5.76 (at Balkhu), nitrogen (%) ranged from 0.065 (Thimi/Ghattekulo) to 0.286 (Banasthali and Khusibun), P (kg/ha) ranged from 359 (Shovabhagawati) - 961 (Shankhamul), Clay content (%) ranged from 6 (Thimi) to 16 (Banasthali), in soil samples collected from different vegetable growing fields.

Soil Cu showed positive relationships with soil Pb, and also with soil properties such as N P K. Soil Ni showed significant positive relationships with soil Co, Mn, K and clay. Soil Co showed positive relationships with Mn, K and clay. Soil Zn showed positive relationships with P and Fe, but showed negative relationships with soil pH. Soil Mn showed positive relationships with soil Ni, Co and clay. On the basis of mean values, highest Cd was accumulated in cress leaf, where as highest in the turnip root among the roots of other vegetables. Highest accumulation of Cu, Co and Zn were recorded in spinach leaf and Pb in red radish leaf; Ni, Cr and Fe were recorded in *B. juncea* and Mn in cress leaf. Concentration of non-essential but toxic metal like Pb was higher than the normal plant value (0.1-10 mg kg⁻¹) in all the vegetables (except potato) collected from different sampling sites. Low Cu accumulation was observed in all vegetable crops (except Spinach) cultivated in the soil of Shankhamul when compared to soil Cu concentration, which indicated the low availability of Cu to plants possibly due to binding of Cu with inorganic and organic compounds. In Khusibun area more accumulation of Pb, Cd and Zn observed in vegetable crops compared to soil heavy metals may be due to their more solubility and availability via the presence of low pH value (3.9) and high concentration of K (819 kg/ha) prevailing in that field. High Pb was accumulated in both leafy and root vegetables, and clearly indicates that green and leafy vegetables of Kathmandu valley accumulate Pb through root uptake. Besides this, there may be direct atmospheric fall out in the soil and in the leafy portion by exhaust emissions of leaded gasoline and wear and tear of tires. The bioaccumulation different heavy metals (especially Pb) in roots of *D. carota*, *R. sativus* and *B. rapa* is possibly due to their moderate mobility to the storage organs. Though most leafy vegetables showed more Cd accumulation in leaves as compared to roots, even bioaccumulation of Cd in *S. oleracea* (spinach- Gobre) root (collected from Thimi and Shovabhagwati) is greater than in soil showing tendency of hyperaccumulation. Upper red portion of root of Spinach – Gobre are mostly used for vegetable along with its leafy portion. As this upper root portion along

with entire root can accumulate high amount of Cd, it should be avoided for vegetable use. In the present study *S. oleracea* (spinach) and *R. sativus* (red radish) was found to be good hyperaccumulator of Zn at most biotops. Some members of Brassicaceae like *B. juncea* and *B. rapa* also showed hyperaccumulation of Zn at Kusingal and Thimi, respectively

The quantitative estimations of heavy metals through **daily dietary intake** via green vegetables, was carried out to understand if the intake of heavy metals are within recommended dose or not For this dietary intake through vegetables *B. juncea* var. *cuneifolia* (leaf mustard), *B. rapa* L. (turnip), *L. sativum* L. (cress leaf); *R. sativus* L. (radish), *S. oleracea* L. (spinach), *D. carota* L. (carrot) were evaluated and compared with provisional tolerable daily intakes as recommended by WHO (1993, 1996). Intake estimation was based on vegetable availability data and analysis of heavy metals in vegetables which were found to contribute significantly to the recommended daily intake of essential elements such as Cr (97.4-24.35 %), Cu (15.45-7.7 %), Fe (41 %), Mn (65.71-26.28 %), and Zn (21.23 %) within the limits. The mean daily intake of potentially toxic metals was 33.4% (for Cd), 40.38 % (for Ni) and 251.6 % (for Pb) of the provisional tolerable daily intakes for adults. The daily intake of Pb is about 151 % higher than the maximum provisional tolerable daily intake through leafy vegetables like *B. juncea*, *L. sativum* and *S. oleracea*.

To mitigate the problem of heavy metal contamination in soil, **Phytoremediation** study includes screening of vegetables for phytoextraction of heavy metals and remedial measures using cow dung and lime treatments. The objective of phytoextraction and remedial measures respectively were to assess suitable vegetable species for removing heavy metals from contaminated soil and the possibilities of treating heavy metal contaminated soil by amending with cow dung and agricultural lime, which possibly will reduce mobilization of heavy metal from soil to the vegetables. Cow dung and lime were selected for remedial measures because they eco-friendly, easily affordable and cost-effective. About 18 vegetable crops like *Allium sativum* L., *Allium fistulosum* L., *Beta vulgaris* L., *Beta vulgaris* var. *cicla*, *B. juncea* L., *B. caulorapa* L., *B. rapa* L., *B. rapa* var. *purple top globe*, *L. sativum* L., *R. sativus* var. *Puthane* (red), *R. sativus* var. *Puthanae* (white), *Coriandrum sativum* L., *S. oleracea* (Patane), *S. oleracea* (Deshi), *S. oleracea* (Gobre), *S. tubersum* L., *T. foenumgracum* L., *V. faba* L., were selected for screening of phytoextraction and were grown on soil contaminated with 300 mg CuCl₂ kg⁻¹ soil,

500 mg $\text{Pb}(\text{NO}_3)_2 \text{ kg}^{-1}$ soil, 800 mg $\text{ZnCl}_2 \text{ kg}^{-1}$ soil or their mixed metal (1600 mg kg^{-1} soil). Vegetables for phytoextraction of heavy metals was selected and conducted in the present study based on the uptake rate vary among the plant species and also among cultivars.

From the **phytoextraction test**, vegetables grown in the single metal treatment (CuCl_2 , $\text{Pb}(\text{NO}_3)_2$ and ZnCl_2) showed that Cu accumulation was low in *B. juncea* (20 mg kg^{-1} DW), and *B. caulorapa*, (22 mg kg^{-1} DW), where as highest (432.5 mg kg^{-1}) was in *V. faba*. But *S. tuberosum*, *B. vulgaris*, *R. sativus* (red radish) and *B. rapa* var. *purple top globe* (salgam) could not germinate on 300 mg CuCl_2 -treated soil. Lead accumulation was low in *S. tuberosum*, where as highest (512.5 mg kg^{-1}) was observed in *A. fistulosum*. *Trigonella foenumgracum* could not germinate in $\text{Pb}(\text{NO}_3)_2$ contaminated soils. Among the studied vegetable species grown on 800 mg ZnCl_2 contaminated soil. Zinc accumulation was low in *A. fistulosum* and *S. tuberosum* and $<100 \text{ mg kg}^{-1}$ was measured in *A. sativum*, *A. fistulosum*, *B. caulorapa*, *T. foenumgracum*, *Solanum tuberosum*, *R. sativus* (both red and white radish), *B. rapa* var. *purple top globe*, *S. oleracea* (Deshi), *S. oleracea* (Gobre), where as *V. faba* (broad bean) accumulated highest (202 mg kg^{-1}).

From mixed metal (1600 mg mixed metal kg^{-1} soil), low Cu was accumulated in *B. juncea* and high 29.5 mg kg^{-1} DW in *S. oleracea* (Deshi) == *V. faba*. Accumulation of Pb ranged from 5.83 (*S. tuberosum*) to 30.5 mg kg^{-1} (*S. oleracea* Deshi).

Zn accumulation was low in *S. tuberosum* and *A. fistulosum* but was highest (more than 335.5 mg kg^{-1}) in *S. oleracea* (Patane) > *S. oleracea* (Deshi) = *B. caulorapa*, >*B. rapa* purple top globe > *V. faba*. All studied vegetables accumulated more than 200 mg Zn kg^{-1} DW.

Out of red and white varieties of radish, the former accumulated more Cu, Pb and Zn than the later. Similarly, among the 3 varieties of *S. oleracea*, Cu and Zn accumulation was low in Gobre, but Pb accumulation was more or less similar in all 3 varieties of spinach. Zn accumulation was higher in Patane than in others 2 varieties of spinach.

For **the remedial measures**, *B. juncea*, *L. sativum* and *B. rapa*, were grown on soil treated heavy metals as i) control (soil treatment only with heavy metals), ii) soil treatments with heavy metals along the cow-dung (5 and 20 %), and iii) soil treatments with heavy metals along the lime (3 and 9 g kg^{-1} soil). Copper and Pb accumulation in vegetables, harvested at 5 weeks was higher in lime treatment than in 20 % cow dung treatments. Zinc accumulation increased in *B. rapa* and *L. sativum* in both cow dung and lime than in control. In *B. juncea*, Zn accumulation decreased in both treatments of cow dung compared to lime and control.

Accumulation of Cu, Pb and Zn from mixed metal treatment was highest in 3g lime in *B. juncea* than in control or other treatments. Lowest accumulation of Pb from mixed metal treatment was observed in *B. juncea* and *L. sativum* grown on 5 and 20 % cow-dung treatment. Zn accumulation in *L. sativum* was quite high in 20 % cow-dung treatment. Accumulation of Pb and Zn in *B. rapa* were almost reduced in cow dung treatment compared to control but the plant could not grow in lime treated soil.

Morphological changes with single metals such as FW, DW, RL and SL increased in all vegetables grown soil treated 300 mg $\text{CuCl}_2 \text{ kg}^{-1}$ soil and cow dung but all FW, DW, RL and SL decreased in all cases in 300 mg $\text{CuCl}_2 \text{ kg}^{-1}$ soil and lime treatment. Similarly, FW and SL increased significantly in *B. juncea* and *B. rapa*, and RL increased significantly in *L. sativum* when grown on $\text{Pb}(\text{NO}_3)_2$ and 20 % cow dung treatment. Morphological changes such as FW, DW, SL and RL (except RL in *B. rapa*) increased significantly ($P=0.01$) in all vegetables in ZnCl_2 and cow dung treated soil, but insignificant changes were observed with lime treatment. Similarly, FW and DW increased only in *L. sativum* grown in 3 g lime treatment, whereas DW % increased significantly in *B. rapa* at both the doses of lime.

In vegetables grown on mixed metal and cow dung treated soil, 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. In vegetables grown on mixed metal and lime amended soil, 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. In remedial measures root/shoot length ratio decreased in cow dung but increased in lime treatment. This is mainly due to increased shoot length in cow dung treatment and decreased shoot length in lime treatments.

Immobilization of Cu, Pb and Zn in both single and mixed metal treatments was found to be high in cow-dung amended soil. But in lime amended soil treated with single metal salts such as CuCl_2 and $\text{Pb}(\text{NO}_3)_2$, the concentration of Cu and Pb retained in the soil was lower than in control, indicating their free mobility in the plants. From this it can be ascertained that 20 % cow dung treatment is suitable for immobilization of supplied metals than lime treatment.

8. REFERENCES

- Adriano, D. C. 1986. *Trace elements in the Terrestrial environments*. Springer, New York, 533 pp.
- Akio, S., Nobufumi, M. and Yosio, U. 1993. Effects of heavy application of fresh cow dung on to the humus composition of an Ando soil, Southern Kyusyu. *Bulletin of the Faculty of Agriculture*, Kagoshima University, **43**: 1-10.
- Aksoy, A. and Ozturk, M. A. 1997. *Nerium oleander* L. as a biomonitor of lead and other heavy metal pollution in Mediterranean environments. *Science of the Total Environment* **205**:145-150.
- Alloway, B. J. 1995. Cadmium. In: *The origin of heavy metals in soil*, (Ed) Alloway B J. Blackie Academic and professional, Glasgow, pp. 122-125.
- Alloway, W. H. 1968. Agronomic controls over environmental cycling of trace elements. *Advances in Agronomy*, **20**: 235-274.
- Angelone, M. and Bini, C. 1992. Trace elements concentrations in soils and plants of Western Europe. In: *Biogeochemistry of trace metals*, (Ed) Adriano D C. Boca Raton. Florida, Lewis Publishers, pp. 19-60.
- Anke, M., Groppe, B., Grun, M. and Kronemann-J. H. 1994. Relations between the cadmium content of soil, plants, animals and humans. In: *Trace elements in man and animals 7*. Momcilovic (Zagreb: University of Zagreb), pp. 2610-2611.
- Anke, M., Gleis, M., Arnhold, M., Muller, M., Seifert, M., Anke S., Hartmann, E. and Gunstheimer, G. 1998. Environmental cadmium pollution and its health effect on people in Germany. In: *International symposium on itai-itai disease, environmental cadmium pollution and countermeasures*, (Ed) Nogakawa K. Kanazawa, Medical university, 1-1 Daigaku, Uchinda, Ishikawa 920-0293, Japan, 36-37.
- Anke, M., Muller, M. and Hella Kronemann-Jena 1994. Cadmium in feed and foodstuffs. *Proc. Soc. Nutr. Physiol.* **2**: 10-16.
- Anke, M., Kronemann, H., Groppe, B. and Grun, M. 1979. Die Kadmiumbelastung der landwirtschaftlichen Nutztiere, Wildwiederkauer and Bevölkerung in der DDR. *Emissionen and Immissionen von Schadstoffen in der Tierproduktion*, (Ed) Mehlhorn G. (Leipzig: Karl-Marx-Universität Leipzig), pp. 457-474.
- Anonymous, 1996. *Table of US heavy metal legal limits*. Ceramics Industry, July: 82-213.

- Antonovics, J., Bradshaw, A. D. and Turner, R. G. 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res.*, **7**: 1-85.
- Apostoli, P., Kiss, P., Porru, S., Bonde, J. P. and Vanhoorne, M. 1998. Male reproductive toxicity of lead in animals and humans. *Occup. Environ. Med.* **53**: 364-374.
- Arocha, M. A. 1996. Voc immobilization in soil by adsorption, absorption and encapsulation. *J. of Hazardous materials*, **51**:131-149.
- ATSDR (Agency for toxic substance and diseases registry). 1988. Toxicological profile for nickel, ATSDR/U.S. *Public Health Service*, ATSDR/TPP, 1988:-88/19.
- Baath, E. 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water, Air, Soil Poll.* **47**: 335-379.
- Baker, A. J. M. 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. *J. Plant Nutr.* **3**: 643-654.
- Baker, A. J. M. 1987. Metal tolerance. *New Phytologists*, **106**: 93-111.
- Baker, A. J. M. and Brooks, R. R. 1989. Terrestrial higher plants which accumulate metallic elements-a review of their distribution, ecology and phytochemistry. *BioRecovery*, **1**: 81-126.
- Baker, A. J. M. and Walker, P. L. 1990. Ecophysiology of metal uptake by tolerant plants. In: *Heavy metal tolerance in plants: evolutionary aspects*, (Ed) Shaw A J. CRC Press, Inc; Boca Raton, Florida, pp. 155-178.
- Baker, A. J. M., Reeves, R. D. and Hajar, A. S. M. 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte, *Thlaspi caerulescens* J. and C. Presl (Brassicaceae). *New phytol.* **127**: 61-88.
- Baker, D. A. 1983. Uptake of cations and their transport within the plants, In: *Metals and micronutrients, uptake and utilization by plants*, (Eds) Robb D A and Pierpoint W S. Academic Press London, pp. 3-19.
- Barcelo, J. and Poschenrieder, Ch. 1990. Plant water relations as affected by heavy metal stress: a review. *J. Plant Nutr.* **13**:1-37.
- Barcelo, J., Vazquez, M. D. and Poschenrieder, Ch. 1988. Cadmium induced structural and ultra-structural changes in the vascular systems of bush bean stems. *Bot. Acta*, **101**: 254-261.
- Barnes, J. D., Balaguer, L., Maurique, E., Elvira, S. and Davidson, A. W. 1992. A reappraisal of the use of Dimethyl Sulphoxide (DMSO) for the extraction of chlorophylls a and b in lichens and higher plants. *Environ. Exp. Bot.* **32**: 85-100.

- Barman, S. C. and Ray, M. 1999. Uptake of heavy metals (Cd, Cu, Zn and Ni) and a comparative study of mature plants grown in polluted and unpolluted fields, using different varieties of rice. *J. of Sci. and Eng. Res.* **1**: 13-17.
- Barman, S. C., Kisku G. C. and Bhargava S. K. 1999. Accumulation of heavy metals in vegetables, pulse and wheat grown in fly ash amended soil. *J. Environ. Bio.* **20**:15-18.
- Barman, S. C., Sahu, R. K., Bhargava, S. K. and Chatterjee, C. 2000. Distribution of heavy metals in wheat, mustard and weed grown in field irrigated with industrial effluents. *Bulletin Environ. Contam. Toxicol.* **64** (4): 489-496.
- Bassuk, N. L. 1986. Reducing lead uptake in lettuce. *Hort. Sci.* **21**: 993-995.
- Baszynski, T., Krol, M., Krupa, Z., Ruskowska, M., Wojcieszka, U. and Wolinska, D. 1982. Photosynthetic apparatus of spinach exposed to excess copper. *Z. Pflanzenphysiol.* **108**: 385-395.
- Bazzaz, F.A., Rolfe, G. L. and Carlson, R. W. 1974. Effects of cadmium on photosynthesis and transpiration of excised leaves of corn and sunflower. *Physiol. Plant*, **32**: 373-376.
- Beckett, P. H. T. and Davis, R. D. 1977. Upper critical levels at toxic elements in plants. *New Phytol.* **79**: 95-106.
- Benes, P., Gjessing, E. T. and Steinnes, E. 1976. Interactions between humus and trace elements in fresh water. *Water Res.* **10**: 711-716.
- Bhattarai, D. R. and Shrestha, P. R. 1981. Lead contents in the dust of Kathmandu city road. *Nepal Chemical Society Proc. I*, 47-50.
- Bidwell, R. G. S. 1979. *Plant physiology*, 2nd edition. Collier MacMillan Publishers. London.
- Black, C. A. 1973. *Soil plant relationships*, 2nd edition. Wiley Eastern Private Limited, New Delhi, 792p.
- Bohn, H. L. 1972. Soil adsorptions of air pollutants. *J. Environ. Qual.* **1**: 372-377.
- Bomke, A. A. and Lowel, L. E. 1991. Trace element uptake by two British Columbia forages as affected by poultry manure application. *Can. J. Soil Sci.* **71**: 305-312.
- Bowen, H. J. M. 1966. *Trace Elements in Biochemistry*. Academic Press, London.
- Bowen, H. J. M. 1979. *Environmental Chemistry of the Element*. Academic Press, London.
- Brady, N. C. 2002. *The nature and properties of soils*, 10th edition. Printice Hall of India, Private limited, New Delhi 110 001. 621p.
- Brady, N. C. and Well, R. R. 2004. *The Nature and Properties of Soils*, 13th edition. Pearson education (Singapore) Pte, Ltd.

- Breulmann, G. , Ogino, K., Ninomiya, I., Asthon, P. S., Li-Frankie, I. V., Leffler, U. S., Weckert, V., Lieth, K., Korschak, R. and Markert, B. 1998. Chemical characterization of Dipterocarpaceae by use of chemical fingerprintings- a multielemental approach at Sarawak, Malaysia. *The Science of the Total Environment*. **215**: 85-100.
- Bricker, T. M. and Newman, D. W. 1982. Changes in the chlorophylls, proteins and electron transports activities of soybean cotyledon chloroplasts during senescence. *Photosynthesis*, **16**: 239-244.
- Bringezu, K., Lichtenberger, O., Leopold, I. and Neumann, D. 1999. Heavy metal tolerance of *Silene vulgaris*. *J. Plant Physiol.* **154**: 536-546.
- Brummer, G. W., Hornburg, V. and Hiller, D. A. 1991. Schwermetallbelastung von Boden. *Mitteilgn Dtsch. Bodenkundal. Gesellsch.* **63**: 31-42.
- Brune, A., Urbach, W. and Dietz, K. J. 1995. Differential toxicity of heavy metals is partly related to a loss of preferential extraplasmic compartmentation: a comparison of Cd, Mo, Ni and Zn stress. *New Phytol.* **129**: 403-409.
- Buchauer, M. J. 1973. Contamination of soil and vegetation near a zinc-smelter by zinc, cadmium, copper and lead. *Environ. Sci. Technol.* **7**: 131-135.
- Buchet, J. P., Lauwerys, R., Roels, H., Bernard, A., Bruaux, P., Claeys, F., Ducoffre, G., de Plaen, P., Staessen, J., Amery, A., Lijnen, P., Thijs, L., Rondia, D., Sartor, F., Saint, R. A. and Nick, L. 1990. Renal effects of cadmium body burden of the general population. *Lancet*, **336**: 699-702.
- Cahoon, G. A. 1974. *Handbook of reference methods for soil testing*. The council of soil testing and plant analysis. Athens, Georgia, 101 p.
- Carlosena, A., Andrade, J. M., Tomas, X., Fernandez, E. and Prada, D. 1999. Classification of edible vegetables affected by different traffic intensities using potential curves. *Talanta*, **48**: 795-802.
- Carlson, R. W., Bazzaz, F. A. and Rolfe, G. L. 1975. The effects of heavy metals on plants. *Environ. Res.* **10**:113-120,
- Carlson, R. W. and Bazzaz, F. A. 1977. Growth reduction in American sycamora (*Platanus occidentalis* L.) caused by Pb- Cd interaction. *Environ. Pollut.* **12**: 243 - 253.
- Caro, A. and Puntarulo, S. 1996. Effect of in vivo iron supplementation on oxygen radical production by soybeans roots. *Biochemica et Biophysica Acta.* **1291**: 245-251

- Casano, L. M., Gomez, L. D., Lascano, H. R., Gonzales, C. and Trippi, V. S. 1997. Inactivation and degradation of Cu/Zn-SOD by active oxygen species in wheat chloroplasts exposed to photo-oxidative stress. *Plant Cell Physiol.* **38**: 433-440.
- CBS, 1990. *Statistical pocket book*. Central Bureau of Statistics, National Planning Commission, Secretariat, HMG, Kathmandu, Nepal.
- CDC 1997. Screening young children for lead poisoning: Centers for Disease Control and Prevention, Guidance for state and local public health officials. Atlanta, GA.
- Chamberlain, A. C. 1960. Aspects of the deposition of radioactive and other gases and particles. *I. J. Air Pollut.* **3**: 63-88.
- Chaney, R. L., Malik, M., Li, Y. M., Brown, S. L., Brewer, E. P., Angle, J. S. and Baker, A. J. M. 1997. Phytoremediation of soil metals. *Cur. Opi. Biotechnol.* **8**: 279-284
- Chang, A. C., Page, A. L., Foster, K. W. and Jones, T. E. 1982. Comparison of cadmium and zinc accumulation by four cultivars of barley grown in sludge amended soils. *J. Environ. Qual.* **II**, 409.
- Chaoui, A., Mazhoudhi, S., Ghorbal, M. H. and Ferjani, E. E. 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidants enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Sci.* **127**: 139-147.
- Chapman, H. D. 1975. *Diagnostic criteria for plants and soils*. 1st Indian Reprint, Eurasia Publishing house (P) limited Ram Nagar, New Delhi.
- Chardonnens, A. N., Ten Bookum, W. M., Kuijper, L. D. J., Verkleij, J. A. C. and Ernst W. H. O. 1998. Distribution of cadmium in leaves of cadmium tolerant and sensitive ecotypes of *Silene vulgaris*. *Physiol. Plant*, **104**: 75-80.
- Chatterjee, J. and Chatterjee C. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ. Poll.* **109** (1): 69-74.
- Chaudri, A. M., McGrath, S. P., Giller, K. E., Rietze, E. and Sauerbeck, D. R. 1993. Enumeration of indigenous *Rhizobium Leguminosarum* biovar *Trifoli* in soil previously treated with metal contaminated sewage sludge. *Soil Bio. Chem.* **25**:301-309.
- Chettri, M. K., Cook, C. M., Vardaka, E., Sawidis, T. and Lanaras, T. 1998. The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *C. rangiformies*. *Environ. Exp. Bot.* **39**: 1-10.
- Chettri, M. K., Sawidis, T. and Karataglis, S. 1997. Lichens as a tool for biogeochemical prospecting. *Ecotox. Environ. Safety*, **38** (1): 322-335.

- Chettri, M. K., Sawidis, T., Zachariadis, G. A. and Stratis, J. A. 1997. Uptake of heavy metals by living and dead *Cladonia* thalli. *Environ. Exp. Bot.* **37** (1): 39-52.
- Chettri, M. K., Thapa, K. B., Paudel, K. and Acharya, B. D. 2001. Biomonitoring of toxic heavy metals in Kathmandu valley using Lichens. *Ecoprint*, **8**(1): 69-76.
- Chettri, M. K., Sawidis, T. and Chmielewska, E. W. 2000. Localization of heavy metals in lichen thalli: an ultrastructural approach. *Bios* (Macedonia, Greece) **5**: 61-75.
- Chlopecka, A. and Adriano, D. C. 1996. Mimicked in situ stabilization of metals in a cropped soil: Bioavailability and chemical form of zinc. *Environ. Sci. Technol.* **30**: 3294-3303.
- Ciba, J., Korolewicz T. And Turek, M. 1999. The occurrence of metals in composted municipal wastes and their removal. *Water, Air and Soil Poll.* **111**: 159-170.
- Clarke, N. D. and Berg, J. M. 1998. Zinc fingers in *Caenorhabditis elegans*: finding families and probing pathways. *Science*, **282**: 2018-2022.
- Clarkson, P. T. and Hanson, J. B. 1980. The mineral nutrition of higher plants. *Ann Rev. Plant Physiol.* **31**: 239-298.
- Clemens, S. 2001. Molecular mechanism of plant metal tolerance and homeostasis. *Planta*, **212**: 475-486.
- Cobb, G. P., Waters, S. M., Wixson, B. G. and Dorward-K. E. 2000. Accumulation of heavy metals by vegetables grown in mine wastes. *Environ. Toxicol. Chem.* **19** (3): 600-607.
- Cole, M. 1977. Lead inhibition of enzyme synthesis in soil. *Applied Environ. Microbiol.* **33**: 262-268.
- Colin, B. 1995. *Environmental Chemistry*, W. H. Freeman and Company, New York.
- Conner, J. R. 1994. Chemical stabilization of contaminated soils. In: *Hazardous waste site soil remediation theory and application of innovative technologies*, (Eds) David, J. W. and Clarke A. N. pp81-169.
- Costa, G. and Morel, J-L. 1994. Water relation, gas exchange and amino acid content in Cd treated lettuce. *Plant Physiol. Biochem.* **32**:561-570.
- Cottonie, A., Dhaese, A. and Camerlynck, R. 1976. Plant quality response to uptake of polluting elements. *Qual. Plant-Pl. Fds. Hum. Nutr.* **26** (1/3): 293-319.
- Daniels, P., Kovacs, B., Prokish, J. and Gyori, Z. 1997. Heavy metal dispersion detected in soils and plants along side roads in Hungary. *Chemical Speciation and Bioavailability*, **9**(3): 83-93.

- Dara, S. S. 2004. *A text book of Environmental Chemistry and Pollution control*. Chand S and Company Ltd. Ram Nagar, New Delhi. 354 p.
- Davis, R. D. and Beckett, P. H. T. 1978. Upper Critical levels of toxic elements in plant. 11. Critical levels of Cu in young barley, wheat, rape, lettuce and rye grass and of Ni and Zn in young barley and rye grass. *New Phytol.* **80**: 23-32.
- Davis, R. D. and Coker, E. G. 1980. Cadmium in agriculture with special reference to the utilization of sewage sludge on land. *Technical Report TR 139*. WRC Medmenham, Marlow.
- De Vos, C. H. R., Schat, H., De Waal, M. A. M., Vooijs, R. and Ernst, W. H. O. 1991. Increased resistance to copper induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiologia Plantarum*, **82**: 523-528.
- De Vos, C. H. R., Vonk, M. J., Vooijs, R. and Schat, H. 1992. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiol.* **98**: 853-858.
- De, A. K. 2002. *Environmental Chemistry* (reprint of 4th edn, 2000) New Age International (P) limited, New Delhi.
- Dean, M. A., Letner, C. A. and Eley, J. H. 1993. Effect of autumn foliar senescence on chlorophyll a/b ratio and respiratory enzymes of *Populus tremuloides*. *Bull Torrey Bot. Club*, **120**: 269-274.
- Devkota, B., Bania, C. and Ghimere, G. P. S. 1997. Studies on air pollution due to heavy metals (Cd and Pb) using lichens as biomonitors. *Ecoprint* **4**: 61-68.
- Di Toppi, L. S., Lambardi, M., Pazzagli, L., Cappugi, G., Durante, M. and Gabbrielli, R. 1999. Response to cadmium in carrot in vitro plants and cell suspension cultures. *Plant Sci.* **137**: 119-129.
- Djingova, R. and Kuleff, I. 1993. Monitoring of heavy metal pollution by *Taraxaccum officinale*. In: *Plants as biomonitors-indicators for heavy metals in the terrestrial environment*. (Ed) Markert B. VCH Weinheim. New York. Basel. Cambridge, pp 435-460
- DoAMDD, 2002. Agricultural Marketing Information Bulletin (Special Issue). Department of Agricultural Marketing Development Directorate, Ministry of Agricultural and Cooperatives, Lalitpur, HMG, Nepal, 131 p
- Dowdy, R. H. and Larson, W. E. 1975. Availability of sludge borne metals to various vegetables crop. *J. Environ. Qual.* **4**: 278-282.

- Ebbs, S. D. and Kochian, L. V. 1998. Phytoexcretion of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*) and Indian mustard (*Brassica juncea*). *Environ. Sci and Technol.* **32**: 802-806.
- Edmunds, W. M. and Smedley, P. L. 1996. Ground water geochemistry and health: an overview. In: *Environmental Geochemistry and Health* (Eds) Appleton J D, Fuge R. and Mc Call G J H), Special publication, No. 113, Geological Society, London. pp 91-105.
- Egli, M., Fitze, P. and Oswald, M. 1999. Changes in heavy metals content in an acidic forest soil affected by depletion of soil organic matter within the time span 1969-1993. *Environ. Poll.* **105**: 367-379.
- Eleftheriou, E. P. and Karataglis, S. 1989. Ultrastructural and morphological characteristics of cultivated wheat growing on copper-polluted fields. *Botanica Acta.* **102**:134-140.
- El-Hasan T., Mufeed, B., Hamez, Al-Omari, H., Anf Z., EL-Alali, A., Al-Naser, F., Bruce W. B., and Anwar, J. 2006. The distribution of heavy metals in urban street dusts of Karak City, Jordan. *J. Soil and Sediments Contam.* **15**(4):357-365.
- Elinder, C. G., Gerhardson, L. and Oberadaeter, G. 1988. Biological monitoring of cadmium, In: *Biological monitoring of toxic metal.* (Eds) Clarkson T. W., Friberg, L., Mordberg, G. F. and Sager, P.R., Rochester series on Environmental Toxicity, Plenum Press, pp 145-147.
- Ellen, G., VanLoon, J. W. and Tolsma, K. 1990. Heavy metals in vegetables grown in the Netherlands and in domestic and imported fruits. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung*, **190**: 34-39.
- Environmental Protection Act (EPA) 1986. Method 3505, Acid digestion of sediments, sludge and soils. *Test methods for evaluation Solid waste*, Sw 846, 3rd edition. US Government Printing Offices, Washington D C.
- Environmental Protection Act (EPA) 1976. EPA 68-02-1344, EPA RES. Triangle Park North Carolina
- Ernst, W. H. O. 1974. *Schwermetallvegetation der Erde*. G. Fisher verag , Stuttgart, Germany.
- Ernst, W. H. O. 1993. Geobotanical and biogeochemical prospecting for heavy metals deposits in Europe and Africa. In: *Plants as biomonitors-indicators for heavy metals in the terrestrial environment* (Ed.) Markert B., VCH Weinheim /New York /basel Cambridge. pp 107-126.
- Ernst, W. H. O. 1996. Phytotoxicity of heavy metals. In: *Fertilizers and environment*, (Ed) Rodriguez-Barrueco C. Kluwer Academic Publ, Dordrecht. pp 423-430.

- Ernst, W. H. O. 1998. Evolution and ecophysiology of metallophytes in Africa and Europe. In: (Eds) Breckle S W, Schweizer B and Arndt U. *Results of world wide ecological studies. First symposium*, Schimer A F W, Foundation (Walter E. and H.). Stuttgart 1998. Heimbach Verlag G., Stuttgart, 2000.
- Ernst, W. H. O. and Joosse, E. N. G. 1983. *Unnwellbelastung autchminerals toffee, Biologische effekte*. Jena: VEB Gustav, Fischer Verlag.
- Ernst, W. H. O., Nelissen, J. M. and Ten Bookum, W. H. 2000. Combination of toxicology of metal enriched soils: physiological responses of a Zn- and Cd- resistant ecotypes of *Silene vulgaris* on polymettalic soils. *Environ. Exp. Bot.* **43**: 55-71.
- Ernst, W. H. O., Verkleij, J. A. C. and Schat, H. 1992. Metal tolerance in plants. *Acta Bot. Neerl.*, **41**: 229-248.
- Exlund, M. 1995. Cadmium and lead deposition around a Swedish battery plant as recorded in oak tree rings. *J. Environ. Qual.* **24**: 126-131.
- Fabisiak, J. P., Pearce, L. L., Borisenko, G. G., Tyhurina, Y.Y., Tyurin, V. A., Razzack, J., Lazo, J. S., Pitt, B. R. and Kagan, V. E. 1999. Bifunctional antipro-oxidant potential of metallothionein: redox signaling of copper binding and release. *Antioxidant Redox Signal*, **1**: 349-364.
- Fergusson, J. E. 1990. *The heavy elements: chemistry, environmental impact and effects*. Oxford UK, Pergamon Press.
- Fern, V. H. 1971. The synergistic effect of lead and cadmium. *Experientia*, **25**:56-57.
- Fernandez, J. C. and Henriques, F. S. 1991. Biochemical, physiological and structural effects of excess copper in plants. *Bot. Rev.* **57**(3): 246-273.
- Filipek, T. 1994. Degradacja chemiczna gleb. In: *Diagnoza stanu srodowiska przyrodniczego wojweodztwie lubelskim*, (Eds) Wilgata, T. and Chmielewski, T., Lublin, Poland. pp. 81-86.
- Fishbein, L. 1987. Trace and ultra trace elements in nutrition: An overview. 1. Zinc, copper, chromium, vanadium and nickel. *Toxicol. Environ. Chem.* **14**: 73-99.
- Foroughi, M., Venter, F. und Teicher, K. 1981. Wirkung von steigenden Mull- Klarschlamm- Kompost- Gaben auf den Schwermetallgehalt von Tomaten, Gurken und Bohnen im Gefa Bversuch., *Landw. Forsch*, SH 37, KongreBb and Braunschweig. pp 254-266.
- Foyer, C. H., Lelandais, M. and Kunert, K. J. 1994. Photo-oxidative stress in plants. *Physiol. Planta*, **92**: 696-717.

- Friberg, L., Nordberg, G. F. and Vouk, V. B. 1986. *Handbook on the toxicology of metals: Specific Metals*, Elsevier, Amsterdam.
- Friedman, M. and Waiss, A. C. 1972. Mercury uptake by selected agricultural products and by-products. *Environ. Sci. Technol.* **6**:457-458.
- Fuller, W. H. 1997. *Movement of selected metals, Asbestos and cyanide in soil: Application in waste disposal* Proble, EPA-600/2-77-020. Solid and Hazardous Waste Research Division. US Environmental Protection Agency, Cincinnati, OH
- Gadd, G. M. and Griffiths, A. J. 1978. Microorganisms and heavy metal toxicity. *Microbial Ecology*, **4**: 303-317.
- Galloway, J. N., Thornton, J. D., Norton, S.A., Volchok, H. L. and McLean, R. A. 1982. Trace metal in atmospheric deposition: a review and assessment. *Atmosph. Environ.* **16**: 1677-1700.
- Garcia, A., Navarro, P. and Castillo, P. 1996. Peroxidase and NADPH oxidase activities in leaves and roots of sunflower plants, as markers of heavy metal toxicity. In: *Plant peroxidases: Biochemistry and Physiology*, (Eds) Obniger C, Bruner U, Ebermann R, Penel C and Greppin H, University of Geneva, Geneva, pp. 369-373.
- Garty, J., Karary, Y. and Harel, J. 1992. Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen *Ramalina Duriaei* (De Not.) Bagl. *Environ. and Exp. Bot.*, **32** (3): 229 -241.
- Gaspar, T., Penel, C., Hagege, D. and Greppin, H. 1991. Peroxidase in plant growth, differentiation and development processes. In: *Biochemical, molecular and physiological aspects of plant peroxidases*. (Eds) Lobarzewski J, Greppin H, Penel H and Gaspar T, University of Geneva, Geneva, pp. 249-280.
- Gautam, S. K. and Agrawal, V. P. 1994. Measurements of heavy metals in Bagmati River and its tributaries flowing through Kathmandu valley. In: *Abstracts (Scientific session), Env22, 2nd National Conference of Sci. and Technol.* June, 8-11. Nepal Academy of Science and Technology, Kathmandu, Nepal.
- Gloaguen, V. and Morvan, H. 1997. Removal of heavy metal ions from aqueous solutions by modified barks. *J. Environ. Sci. Health, A* (**32**) 901-912.
- Godbold, D. L. and Huttermann, A. 1985. Effect of zinc, cadmium and mercury on root elongation of *Picea abies* (Karst) seedlings, and the significance of these metals to forest die back. *Environ. Poll. Ser. A* **38**: 375-381.

- Godbold, D. L. and Kettner, C. 1991. Lead influences root growth and mineral nutrition of *Picea abies* seedlings. *J. Plant Physiol.* **139**: 95-99.
- Goyer, R. A. 1991. Toxic effects of metals. In: *Casarett and Doull's toxicity*. (Eds), Amdur M O, Doull J D and Klaasse C D, Pergamon Press, New York, pp. 623-680.
- Goyer, R. A. 1997. Toxic and essential metal interactions. *Annu. Rev. Biochem* **17**: 37-50.
- Graham, K. 2000. Gardening in lead-contaminated soils, UK health and wealth from *Brassica* BBSRC, News and Events, Features, April.
- Gratton, W. S., Nkongolo, K. K. and Spiers, G. A. 2000. Heavy metal accumulation in soil and *Pinus banksiana* (jack pine) needles in Sudbury, Ontario, Canada. *Bull. Environ. Contam. toxicol.* **64**: 550-557.
- Greger, M. 1989. Cadmium effects on carbohydrate metabolism in sugar beet (*Beta vulgaris*). Doctoral dissertation. Academityck A B, Edsbruk, pp. 9-11.
- Grimes, S. M., Taylor, G. H. and Cooper, J. 1999. The availability and binding of heavy metals in compost derived from household waste. *J. Chem. Technol. and Biotechnol.* **74**: 1125-1130.
- Hall, J. L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.* **53** (366):1-11.
- Halliwell, B. and Gutteridge, J. M. C. 1986. Iron and free radical reactions: two aspects of antioxidant protection. *Trends Biochemical Sci.* **11**, 375.
- Halpern, J. 1985. Mechanisms of Co-enzyme B₁₂- dependent rearrangements. *Science*, 227: 879-885.
- Hammond, P. B. 1977. Exposures of humans to lead. *Annu Rev. Pharmacol. Toxicol.* **17**: 197-214.
- Han, F. X., Kingery, W. L., Selim H. M. and Gerard, P. D. 2000. Accumulation of heavy metals in a long term poultry waste amended soil. *Soil Science.* **165**: 260-268.
- Hardeman, R. T. and Jacoby, B. 1984. Absorption and translocation of Cd in bush beans (*Phaseolus vulgaris*). *Physiol. Plant*, **61**: 670-674.
- Hinsley, T. D. 1989. Uptake of cadmium by crop plants grown on sludge- amended soil. In: *cadmium 79- d proceedings*, 2nd International Cadmium Conference, Cannes, France, pp 83-90.
- Hodgson, J. F. 1963. Chemistry of the micronutrient elements in soils. *Adv. Agron.* **15**: 119-159.

- Hornberg, V. and Brummer, G. W. 1993. Verhalten von Schwemetallen in Boden:1. Untersuchungen zur Schwermetallmobilitat. *Zeitschrift fur Pflanzenernahrung und Bodenkunde*, **156**: 467-477.
- Hutchinson, T. C. and Whitby, L. M. 1974. Heavy metal pollution in the Sudbury mining and smelting region of Canada. I. Soil and vegetation contamination by Ni, Cu and other metal. *Environ. Consvr.* **1**: 123-132.
- Huttner, E., Gotze, A. and Nikolova, T. 1999. Chromosomal aberrations in humans as genetic end points to assess the impact of pollution. *Mutation Research*, **445**: 251-257.
- International Agency for Research on cancer (IARC), 1993. Cadmium and cadmium compounds. In: *IARC monographs on the evaluation of carcinogenic risks to humans. Beryllium, cadmium, mercury and exposure in the glass manufacturing industry*, **58**: 119-237.
- International Agency for Research on cancer, 1990. IARC monographs on the evaluation of carcinogenic risks to humans. *Chromium, Nickel and Welding*, **49**: 449-525
- IUCN, 1991a. *Sources of industrial pollution in Nepal—A national survey*, HMG, Nepal, National planning commission in collaboration with IUCN (The World Conservation Union) Kathmandu, Nepal.
- IUCN. 1991b. *Environmental pollution in Nepal-A review of studies*, HMG Nepal, National Planning Commission in collaboration with IUCN, Kathmandu, Nepal
- Iwasaki, K., Yoshikawa, G. and Sakurai, K. 1993. Fractionation of zinc in greenhouse soils. *Soil Sci. Plant Nutr.* **39** (3): 507- 515.
- Jacobson, L., Hannapel, R. J., Schaedle, M. and Moore, D. P. 1961. Effects of root to solution ratio in ion absorption experiments. *Plant Pysiol.* **36**: 62-65.
- Jarvis, S. C. and Jones, L. H. P.1978. Uptake and transport of cadmium by perennial rye grass from flowing solution culture with a constant concentration of cadmium. *Plant and Soil*, **49**: 333-342.
- Jha, P. K. and Lekhak, H. D. 2003. Air pollution studies and management efforts in Nepal. *Pure Appli. Geophys.* **160**:341-348.
- Jain, V. K. 2007. *Fundamentals of plant physiology*. Chand S. and Company Ltd, New Delhi, 594 p.
- Jang, A., Choi, Y. S. and Kim, I. S. 1998. Batch and column tests for the development of an immobilization technology for toxic heavy metals in contaminated soils of closed mines. PII: S0273-1223(98)00237-6. *Water Sci.* **37** (8): 81-88.

- Jimenez, A., Hernaendez, J. A., Del Rio, L. A. and Sevilla, F. 1997. Evidences for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiol.* **114**: 275-284.
- John, M. K., Van Laerhoven, C. J. and Chuah, H. H. 1975. Factors affecting plant uptake and phytotoxicity of cadmium added to soils. *Environ. Sci. Technol.* **6**:1005-1009.
- Johnson, F. M. 1998. The genetic effects of environmental lead. *Mutation Research*, **410**:123-140.
- Jonsson, A., Exlund, M. and Hakansson, K. 1997. Heavy metal of the 20th century recorded in oak tree rings. *J. Environ. Qual.* **26**: 1638-1643.
- Kabata - Pendias, A. and Pendias, H. 1992. *Trace elements in soils and plants*. 2nd edition, CRC Press, Boca Raton, Florida, USA.
- Kabata-Pendias, A., Piotrowska, M. and Dudka, S. 1993. Trace metals in legumes and monocotyledons and their suitability for the assessment of soil contamination. In: *Plants as biomonitors, Indicators for heavy metals in the terrestrial environment* (Ed) Markert B, VCH-Publishers, Weinheim, New York. Pp. 485-494,
- Kabzinski, A. K. M. 1998. Application of covalent affinity chromatography with thiol–disulphid interchanges for determination of environmental exposure to heavy metals based on the quantitative isolation of Cd-thionein from human breast milk. *Biomedical chromatography*, **12**: 217-225.
- Kang S-Fen, Li Feng –Rui, Zhang Ai- Shong, Tan Jian–An, Yang Fa-Way, Hei Wen–Long and Liu Jiangbin 2006. Effects of traffic pollution on urban soils and plants. *I. Huanjing Kexu,e* **27**(3): 556-560.
- Kannan, K. 1997. *Fundamentals of Environmental Pollution*. Chand, S. and Co. Ltd. Ram Nagar, New Delhi India.
- Karataglis, S. 1982. Combined tolerance to copper, zinc and lead by pollution of *Agrostis tenuis*. *Oikos*. **38**: 234-241.
- Khasem- M D, A. and Singh, B. R. 1999. Heavy metal contamination of soil and vegetation in the vicinity of industries in Bangladesh. *Water, Air and Soil Poll.* **115**: 347-361.
- Katia, P., Stephane, M., Laurence, M., Michen, L. and Pierre, C. 2005. Nicotianamine over - accumulation confers resistance to Ni in *Arabidopsis thaliana*. *Transgenic Research*, **14**: 739-748.

- Kelepertsis, A. E. and Andrulakis, I. 1983. Geobotany-biochemistry for mineral exploration of sulphide deposits in Northern Greece-heavy metal accumulation by *Rumex acetocella* L. and *Minuartia verna* (L.) Hiern. *J. Geochem. Explore.* **18**: 267-274.
- Kelly, J. M., Parker, G. R. and McFee, W. W. 1979. Heavy metal accumulation and growth of seedlings of five forest species as influenced by soil cadmium level. *J. Environ. Qual.* **8**: 361-364.
- Kiekens, L. 1995. Zinc. In: *heavy metals in soil*, 2nd edition (Ed) Alloway B J., Blackie Academic and professional, Glasgow, pp. 284-305.
- Kim, Y.-Ok, Takeuchi, F., Hara, M. and Kuboi, T. 2000. Characterization of cadmium –tolerant carrot cells in responses to cadmium stress. *Soil Sci. Plant Nutr.* **46**(4): 807-814.
- Kirkham, M. B. 1977. Trace elements in sludge on land: Effect on plants, soils and ground water. In: *Land as a waste management alternative*, (Ed) Lacher R C., Ann Arbor Sci. Publishers, Ann Arbor, MI, pp. 209-247.
- Kisku, G. C., Barman, S. C. and Bhargava, S. K. 2000. Contamination of soil and plants with potentially toxic elements irrigated with mixed industrial effluent and its impact on the environment. *Water, Air and Soil Poll.* **120**:121-137.
- Kloke, A. 1981. Aufnahme umweltrelevanter Elements durch die Pflanzen, Deutsche Gesellschaft Qualitätsforschung (Pflanzliche Nahrungsmittel) eV; XVII. Vortragstagung Siedlungsabfall-Verwertung und Nahrungsqualität, Speyer.
- Kovacs, M., Turcsanyi, G., Penksza, K. and Kaszab, L. 1993. Heavy metal accumulation by ruderal and cultivated plants in a heavily polluted district of Budapest. In: *Plants as biomonitors, Indicators for heavy metals in the terrestrial environment*, (Ed) Markert B., VCH, Weinheim, New York, Tokyo, pp. 495-505.
- Kozlov, M. V., Haukioja, E., Bakhtiarov, A. V., Stroganov, D. N. and Zimina, S. N. 2000. Root versus canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. *Environ. Poll.* **107**: 413-420.
- Kuiper, J. 1981. Fate and effects of cadmium in marine plankton communities in experiments enclosures. *Mar. Ecol. Prog. Ser.* **6**:161-174.
- Kuiper, K. M. 2005. *S- allyl mercaptocysteine prodrugs and methods treatments*. (WIPO) World intellectual property organization, 2005.
- Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol.* **29**: 1232-1238.

- Kupper, H., Kupper, F. and Spiller, M. 1996. Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *J. Exp. Bot.* **47**:259-266.
- Kupper, H., Kupper, F. and Spiller, M. 1998. In: situ detection of heavy metal substituted chlorophylls in water plants. *Photosynthesis Research*, **58**: 123-133.
- Kupper, H., Lombi, E., Zhao, F. J. and McGrath, S. P. 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta*, **212**:75-84.
- Kurz, H., Schulz, R. and Romheld, V. 1999. Selection of cultivars to reduce the concentration of cadmium and thallium in food and fodder plants. *J. Plant Nutr. Soil Sci.* **162**: 325-328.
- Ladislav, T., Bocova, B., Hottova, J., Mistrik, I. and Olle, M. 2006. Cadmium induced inhibition of apoplastic ascorbate oxidase in barley roots. *Plant Growth Regulation* **48**(1): 41-49.
- Lagerwerff, J. V. 1971. Uptake of cadmium, lead and zinc by radish from soil and air. *Soil Sci.* **3**:129-133.
- Lagerwerff, J. V. and Spect, A. W. 1970. Contamination of road side soil and vegetation with cadmium, nickel, lead and zinc. *Environ. Sci. Tech.* **4**: 583-586.
- Lambers, H., Chapin I I I, F. S. and Pons, T. I. 1998. *Plant physiological ecology*. Springer - Verlag, New York Inc.
- Lamoreaux, R. J. and Chaney, W. R. 1977. Growth and water movement in silver maple seedlings affected by cadmium. *J. Environ. Quality*, **6**: 201-205.
- Lane, S. D., Martin, E. S. and Garrod, J. F. 1978. Lead toxicity effects on indole -3-ylacetic acid-induced cell elongation. *Planta*, **144**:79-84.
- Lantzy, R. J. and MacKenzie, F. T. 1979. Atmospheric trace metals global cycles and assessment of man's impact. *Geochimica of Cosmochimica Acta*, **43**, 511.
- Larcher, W. 1995. *Physiological plant ecology*. Springer -Verlag, Berlin Germany.
- Lee, C- K and Kim, J. K. 1998. Studies on the relation of heavy metals between rainfall and soil in the forest. *J. of Korean Forestry Soc.* **87**: 584-599.
- Lee, K. C., Cunningham, B.V. A., Chung, K. H., Paulsen, G. M. and Liang, G. H. 1976. Lead effects on several enzymes and nitrogenous compounds in soybean leaf. *J. Environ. Qual.* **5**: 357-359.
- Legittimo, P. C., Ducceschi, L. and Martini, M. 1995. Plant species as indicators of geochemical anomalies: Experiences on *Hex aquifolium* (holly). *Environ. Geol. (Berlin)* **25**:114-118.

- Little, P. 1973. A study of heavy metal contamination of lead surfaces. *Environ. Poll.* **5**:159-172.
- Little, P. and Martin, M. H. 1972. A survey of zinc, lead and cadmium in soil and natural vegetation around a smelting complex. *Environ. Poll.* **3**: 241-254.
- Lodenus, M. 1995. Cadmium and Mercury in north European forests ecosystems. The contaminants in the Nordic ecosystems. *Dynamics, Processes and Fate*, 25-32.
- Lolkema, P. C. 1985. Copper resistance in higher plants. Ph.D. Thesis 128p. Vrije University, Amsterdam.
- Lombi, E., Zhao, F. J., Dunham, S. J. and McGrath, S. P. 2000. Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. *Research New Phytol.* **145**: 11-20.
- Lombi, E., Zhao, F. J., McGrath, S. P., Young, S. D. and Sacchi, G. A. 2001. Physiological evidence for a high affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytol.* **149**: 53-60.
- MAK-und BAT Werte- Liste, Deutsche Forschungsgemeinschaft 1995. Senatskommission zur Prufung gesundheitsschadlicher Arbeitsstoffe, Mitteilung 28, VCH Verlagsgesellschaft
- MacFarlane, G. R. and Burchett, M. D. 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove *Avicennia marina* (Forsk.) Vierch. *Marine Pollution Bulletin* **2**(3): 233-240.
- MacNicol, R. D. and Beckett, P. H. T. 1985. Critical tissues concentration of potentially toxic elements. *Plant and Soil*, **85**:107-129.
- Maier, R. 1978. Aktivitat und multiple formen der peroxydase in unverbleiten pflanzen von *Zea mays* und *Medicago sativa*. *Phyton.* **19**: 83-96.
- Malkowski, E., Stolarek, J. and Karcz, W. 1996. Toxic effects of Pb^{2+} ions on extension growth of cereal plants. *Polish J. Environ Studies*, **5**: 41-45.
- Markert, B. 1993. *Plants as biomonitors*. Indicators for heavy metals in the terrestrial environment. VCH-Publishers, Weinheim, New York, 644.
- Markert, B. 1994. The biological system of the elements (BSE) for terrestrial plants (glycophytes). *Sci. of the Total Environ.* **155**: 221-228.
- Marschner, H. 1983. General introduction to the mineral nutrition of plants. In: *Inorganic plant nutrition. Encyclopedia of plant physiology*, (Eds) Lauchi A and Bielecki R L, Springer Verlag, New York, Berlin, Heidelberg, Tokyo, New Ser vol (15), pp. 5-60.

- Marschner, H. 1995. *Mineral nutrition of higher plants*, 2nd edition. Academic Press, London. 889 p.
- Matsumoto, H., Morimura, S. and Takahashi, E. 1977. Less involvement of pectin in the precipitation of aluminum in pea roots. *Plant Cell Physiol.* **18**: 325-335.
- Mazhoudi, S., Chaoui, A., Ghorbal, M. H. and EI -Ferjani, E. 1997. Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill). *Plant Sci.* **127**: 129-137.
- McBride, M. B., Richards, B. K., Steenhuis, T. and Spirs, G. 1999. Long term leaching of trace elements in a heavily sludge –amended silty clay loam soil. *Soil Sci.* **164**(9):613-623.
- McBride, M. D. 2005. Molybdenum and copper uptake by forage grasses and legumes grown on a metal-contaminated sludge site. *I. Communications in Soil Sci. of plant analysis*, **36**: 17-81.
- McGrath, S. P. 1995. Chromium and Nickel. In: *Heavy metals in soils*, 2nd edition (Ed) Alloway B J., Blackie Academic and professional, Glasgow, pp.152-178.
- McGrath, S. P., Jhao, F. and Lombi, E. 2001. Plant and rhizosphere processes involved in metal contaminated soil. *Plant and Soil*, **232**: 207-214.
- McGrath, S. P., Sidoli, C. M. D., Baker, A. J. M. and Reeves, R. D. 1993. The potential for the use of metal–accumulating plants for the in situ decontamination of metal polluted soils. In: *Integrated soil and sediments research: A basis for proper protection*, (Eds) Eijsackers H J P and Hamers T., Kluwer Academic Publishers, Dordrecht, pp. 673-676.
- McLaren, R. C. and Crawford, D. V. 1973. Studies on soil copper. 1. The fractionation of Cu in soils. *J. Soil Sci.* **24**: 172-181.
- McLaughlin, M. J., Parker, D. R. and Clarke, J. M. 1999. Metals and micronutrients-food safety issues. *Field Crop Rresearch*, **60**:143-163.
- McLean, R. O. 1975. Zinc tolerance of *Hormidium rivulare* Kutz. *Br. Phycol. J.* **10**, 313.
- Meharg, A. A. 1993. The role of the plasmalemma in metal tolerance in angiosperms. *Physiologia Plantarum*, **88**:191-198.
- Mertz, W. 1969. Chromium occurrence and function in biological systems. *Physiological review*, **49**: 169-239.
- Metz, R. and Kloeke, A. 1998. EinfluB der Sortenwahl auf den cadmium transfer Boden - Pflanze, Landw. *Forsch*; SH **49**, KongreB band GieBen, 139-142.

- Mielke, H. W., Gonzales, C. R., Smith, M. K. and Mielke, P. W. 1999. *The urban environment and children's health: Soils as an integrator of lead, zinc and cadmium in new Orleans, Louisiana, U.S.A.* Environmental Research Section A, **81**: 117-129.
- Mihucz, V. G., Tatar, E., Kmethy, B., Zaray, G. and Cseh, E. 2000. Investigation of the transported heavy metal ions in xylem sap of cucumber plants by size exclusion chromatography and atomic absorption spectrometry. *J. of Inorganic Biochem.* **81**: 81-87.
- Miller, J. E., Hassett, J. J. and Koeppe, D. E. 1976. Uptake of cadmium by soybeans as influenced by soil cation exchange capacity, pH and available phosphorus. *J. Environ. Qual.* **5**: 157-160.
- Monni, S., Uhlig, C., Hansen, E. and Mage, E. 2001. Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. *Environ. Poll.* **112**:121-129.
- Moore, M. R. and Goldberg, A. 1985. Health implications of the hematopoietic effects of lead. In: *Dietary and environmental lead: Human health effects*, (Ed) Mahaffey K R, Elsevier, Amsterdam, pp. 260-314.
- Morghan, J. T. 1993. Accumulation of cadmium and selected elements in flax seed grown on a calcareous soil. *Plant Soil*, **150**: 61-68.
- Morselli, L., Barilli, L., Olivieri, P., Cechini, M., Aromolo, R., Dicarolo, V., Rosa, F. and Gata, L. L. 1999. Heavy metal determination in dry surrogate depositions, characterization of an urban and a natural site. *Annali di Chimica*, **89**:739-755.
- Muller, M., Anke, M., Hartmann, E. and IL-Lling-G. H. 1996. Oral cadmium exposure of adults in Germany. 1: Cadmium content of foodstuffs and beverages. *Food additives and contaminants*, **13**(3): 359-378.
- Naggor, S. 1999. Physiological and biochemical responses of cereal seedlings in graded levels of heavy metals. H. Effects on protein metabolism in maize seedlings. *Advances in Plant Sci.* **12**: 425-433.
- National Research Council 1989. *Recommended Dietary Allowances*. 10th ed, Washington D C: Academic Press.
- NESS, 1995. *Research on Environmental pollution and management*. Nepal Environment and Scientific Services, Thapathali, Kathmandu.
- Nies, D. H. 1999. Microbial heavy metal resistance. *Applied microbial. Biotechnol.* **51**: 730-750.
- Norrish, K. 1968. Some phosphate minerals in soils. Trans 9th Int. Congr. Soil Sci; Adelaide **11**: 713-723.

- Nriagu, J. O. 1978. Lead in soils, sediments and major rock types. In: *The Biochemistry of Lead in the Environment*, (Ed) Nriagu J O, Part A, Elsevier, Amsterdam, pp. 15-72.
- Nriagu, J. O. and Pacyna, J. M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, **333**:134-139.
- Nyitrai, P., Boka, K., Sarvari, E. and Keresztes, A. 2002. Characterization of the stimulating effect of low dose stressors in maize seedlings. *Proceedings of the 7th Hungarian Congress on Plant physiology*, **46**: 117-118.
- Oliver, M. A. 1997. Soil and human health: a review. *European J. of Soil Sci.* **48**: 573-592.
- Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. 1954. *Estimation of available phosphorus in soil by extraction with sodium carbonate*. No. 939 USDA. Government printing office, Washington D C.
- Oncel, I., Keles, Y. and Ustun, A. S. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Poll.* **107**:315-320.
- Ortiz, D. F., Kreppel, L., Speiser, D. M., Schell, G., Medonald, G. and Ow, D. W. 1992. Heavy metal tolerance in fission yeast requires an ATP-binding cassette-type vacuolar membrane transport. *EMBO J.* **11**:3491-3499.
- Pais, I. and Benton, J. Jr. 1997. *The hand book of trace elements*. St Lucie Press, Florida, USA
- Page, A. L. and Bingham, F. T. 1973. Cadmium residues in the environment. *Residue Rev.* **48**:1-44.
- Page, A. L., Chang, A. C. and El -Amamy, M., 1987. Cadmium levels in soil and crops of the United States. In: *Lead, Mercury, Cadmium and Arsenic in the Environment*, (Eds) Hutichinson T C and Meeama K M, John Wiley and Sons, New York, pp.119-146.
- Pahlsson, A. M. B. 1989. Toxicity of heavy metals (Zn, Cu, Cd and Pb) to vascular plants. *Water, Air and Soil Poll.* **47**: 287-319.
- Parida, A. K. and Das, A. B. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. and Environ. Safety*, **60**: 324-349.
- Passow, H., Rothstein, A. and Clarkson, T. W. 1961. The general pharmacology of the heavy metals. *Pharmacol. Rev.* **13**: 185-224.
- Peereboom-Stegeman, J. H. J. C. 1987. Toxic trace elements and reproduction. *Toxicol and Environl. Chem.* **15**: 273-292.

- Peng, K., Li, X., Luo, C., and Shen, Z. 2006. I. Toxic-hazardous substances and environmental engineering. *J. Environ. Sci. and Health part A*. **41**(1):65-76.
- Pfeilsticker, K. and Markard, C. 1977. Kadmiumgehalte pflanzlicher Lebensmittel aus Kleingarteneines Industriegebietes. *Kadmium symposium*, (Eds) Anke M and Schneider H J, Jena: Friedrich-Schiller-Universität, pp. 224-230.
- Pietz, R. I., Vetter, R. J., Masarik, D. and McFee W. W. 1978. Zinc and Cadmium contents of agricultural soils and corn in northwestern Indiana. *J. Environ. Qual.* **7**:381-385.
- Plant, J. A., Baldock, J. W. and Smith. B. 1996. The role of geochemistry in environmental and epidemiological studies in developing countries: a review. In: *Environmental Geochemistry and health*, (Eds) Appleton J D, Fuge R and Mc (Call) G J H, Special Publication No 113 Geological Society, London, pp. 7-22.
- Polatajko, A., Jakubowski, N. and Szpunar, J. 2006. State of the art report of Selenium speciation in biological samples. *J. Anal At. Spectrom.* **21**: 639-654.
- Prasad, M. N. V. 1995. Cadmium toxicity and tolerance in vascular plants. *Environ. Exp. Bot.* **35**: 525-545.
- Punz, W. F. and Sieghardt, H. 1993. The response of roots of herbaceous plant species to heavy metals. *Environ. Exp. Bot.* **33**: 85-98.
- Pyatt, F. B. 2001. Copper and lead bioaccumulation by *Acacia retinoides* and *Eucalyptus torquat* in sites contaminated as a consequences of extensive ancient mining activities in Cyprus. *Ecotoxicol. Environ. Safety*, **50**: 60-64.
- Rausser, W. E. and Curvetto, N. R. 1980. Metallthionein occurs in roots of *Agrostis* tolerant to excess copper. *Nature*, **287**: 563-564.
- Rayappa, M. and Singaracharya, M. A. 1993. Pollution tolerance index in some common plants around some major industries in Warangal City, Andhra Pradesh. *Poll. Res.* **12**(1): 57-59.
- Reeves, R. D. and Brooks, R. R. 1983. European species of *Thlaspi* L.(cruciferae) as indicators of Ni and Zn. *J. of geochemical Exploration* **18**: 275-283.
- Reilly, C. 1969. The uptake and accumulation of copper by *Becium homblei* (De Wild) Duvig and Plancke. *New phytol.* **68**: 1081-1087.
- Reuther, W. and Labanauskas, C. K. 1966. Copper. In: *Diagnostic criteria for plants and soil*. . (Ed) Chapman H D, Abilene, TX, Quality Printing, pp.157-179..
- Rice, D. C. 1996. Behavioral effects of lead: Commonalties between experimental and epidemiologic data. *Environ. Health Perspect*, **104**: 337-351.

- Rivetta, A., Negrini, N. and Cocucci, M. 1997. Involvement of Ca²⁺-calmodulin in Cd²⁺ toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination. *Plant Cell and Environ.* **20**: 600-608.
- Roberts, R. D. and Johnson, M. S. 1978. Dispersal of heavy metals from abandoned mine workings and their transference through terrestrial food chains. *Environ Pollu.* **16**, 293.
- Rygg, B. 1985. Effect of sediment copper on benthic fauna. *Mar. Ecol. Prog. Ser.* **25**: 83-89.
- Saleh, Al-G. and Saleh, M. I. 2006. Increased heavy metal tolerance of cow pea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen fixer Rhizobium bacterium. *African J. of Biotechnol.* **16**:133-142.
- Salt, D. E., Blaylock, M., Kumar, P. B., Dushenkov, A. N., Ensley, V., Chet, B. D. and Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol.* **13**: 468-474.
- Sandmann, G. D. and Boger, P. 1980. Copper mediated lipid peroxidation processes in photosynthetic membranes. *Plant physiol.* **66**:797-800.
- Sawidis, T. and Reiss, H. D. 1995. Effects of heavy metals on pollen tube growth and ultrastructure. *Protoplasma*, **185**:113-122.
- Sawidis, T., Chettri, M. K., Zachariadis, G., Straits, J. and Seaward, M. R. D. 1995. Heavy metals bioaccumulation in lichens from Macedonia in North Greece. *Toxicol. Environ. Chem.* **50**: 157-166.
- Sawyer, C. N., McCarty, P. L. and Parkin, G. F. 2003. *Chemistry for environmental engineering and Sciences* (5th edition) Tata McGraw-Hill Publishing Company, Ltd, New Delhi, pp. 752
- Scheinberg, H. 1979. Occurrence, analysis and biological relevance. In: *Metals and their compounds in the environment*, (Ed) Merian E, New York: VCH, pp. 17-39.
- Schmidt, W., Bartels, M., Tittel, J. and Fuhner, C. 1997. Physiological effects of copper on iron acquisition processes in plantago. *New Phytol.* **135**: 659-666.
- Schulze, E. D., Beck, E. and Muller- Hohenstein, 2005. *Plant Ecology*. Springer-Verlag, Berlin Heidelberg, New York, pp. 679.
- Schutzendubel, A. and Polle, A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. of Exp. Bot.* **53** (372): 1351-1365.
- Scotti, A. I., Silva, S. and Botteschi, G. 1999. Effects of fly ash on the availability of Zn, Cu, Ni and Cd to chicory. *Agriculture, Ecosystem, and Environ.* **72**: 159-163.

- Sempio, C., Raggi, V., Barberini, B. and Draoli, R. 1971. Action of cadmium on the resistance of Frassineto wheat M to powdery mildew. *Phytopathol. Z.* **70**: 281-294.
- Shakya, K., Chettri, M. K. and Sawidis, T. 2004. Appraisal of some mosses for biomonitoring air borne heavy metals in Kathmandu valley. *Ecoprint*, **11**: 35-49.
- Sharma, B. and Chettri, M. K. 2004. Dietary Intake of trace elements via vegetables grown in Kathmandu valley. *Proceedings of IV conferences on Sci. and Technol.* Nepal Academy of Science and Technology, March, 23-26. Vol. **1**: 980-988pp.
- Sharma, B. and Chettri, M. K. 2005. Monitoring of heavy metals in vegetables and soil of agricultural fields of Kathmandu Valley. *Ecoprint*, **12**: 1-9.
- Sharply, A. N. and Menzel, R. G. 1987. The impact of soil and fertilizers phosphorus on the environment. *Adv. Agron.* **41**: 297-324.
- Sidlecka, A. 1995. Some aspects of interactions between heavy metals and plant mineral nutrients. *Acta Societatis Botanicorum Poloniae*, **64**: 265-272.
- Silbergeld, E. K. and Goldberg, A. M. 1975. Pharmacological and neurological investigations of lead induced hyperactivity. *Nueropharmacology* **14**, 431.
- Sims, J. T. 1986. Soil pH effects on the distribution and plant availability of manganese, copper and zinc. *Soil Sci. Soc. Am. J.* **50**:367-373.
- Singh, D. P. 2003. *Stress Physiology*, New age international (P) Ltd, Publishers, Ansari road, New Delhi 110 002, pp.169.
- Singh, P. K. and Tiwari, R. K. 2001. Cadmium toxicity induced changes in plant relations and oxidative metabolism of *Brassica juncea* L. plants. [http://www. Geocites. Com/j-environ –biol/abstracts/Jan. 2003/abs16.html](http://www.Geocites.Com/j-environ-biol/abstracts/Jan.2003/abs16.html).
- Singh, S. and Aggarwal, P. K. 2005. Effect of heavy metal fertilizer on growth, yield and metal distribution in wheat. I. *Indian J. of Plant Physiol.* **10**(3): 302-305.
- Sitting, M. 1981. *Handbook of toxic and hazardous chemicals*. Park Ridge N J: Noyes Publications, pp.119-120; 185-186.
- Skorzynska-Polit, E., Drqzkiewicz, M., Wianowska, D., Maksymiec W. M., Dawidowicz, A. L. and Tukiendorf, A. 2006. The influence of heavy metal stress on the levels of some flavols in the primary leaves of *Phaseolus coccineus*. *Acta physiologiae plantarum* **26**(3): 247-254.

- Somashekaraiah, B. V., Padmaja, K. and Prasad, A. R. K. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. *Physiol Planta*, **85**: 85-89.
- Spychalla, J. P. and Desborough, S. L. 1990. Superoxide dismutase, catalase and α -tocopherol content of stored potato tubers. *Plant Physiol.* **94**:1214-1218.
- Stauber, J. L. and Florence, T. M. 1987. Mechanisms of toxicity of ionic copper and copper complexes to algae. *Mar Biol.* **94**: 511-519.
- Stevenson, F. J. 1976. Binding of metal ions by humic acids. In: *Environmental Biogeochemistry*, (Ed) Nriagu J O., Ann. Arbor Sci. Ann. Arbor Mich. (Vol 2) pp. 519-540.
- Stobart, A. R., Griffiths, W. T., Ameen-Bukhari, J. and Shewood, R. P. 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiol. Plant*, **63**: 293-298.
- Stoiths, S. J. and Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. and Med.* **18**:321-336.
- Streit, B. and Stumm, W. 1993. Chemical properties of metals and the process of bioaccumulation in terrestrial plants. In: *Plants as biomonitors-indicators for heavy metals in the terrestrial environment*, (Ed) Markert B, VCH, Weinheim. New York. Basel, Cambridge, pp. 415-424.
- Stresty, T. V. S. and Rao, K. V. M. 1999. Ultra structural alterations in response to zinc and nickel stress in the root cells of pigeon pea. *Environ. Exp. Bot.* **41**: 3-13.
- Swaine, D. J. 1955. *The trace element content of soil*. Common wealth Bull. Soil Sci. Tech. Comm., No 48, London.
- Tandon, P. K., Soni, A. K. and Rai V. K. 2000. Cadmium induced effects on germinating seeds of gram. (*Cicer arietinum*). *Biol. Memoirs*, **26** (2): 62-64.
- Tatar, E., Mihucz, V. G., Varga, A., Zaray, G. and Cseh, E. 1999. Effect of lead, nickel and vanadium contamination on organic acid transport in xylem sap of cucumber. *J. Inorganic Biochem.* **75**:219-223.
- Theodoratos, P., Moirou, A., Xenidis, A. and Paspaliaris, I. 2000. The use of municipal sewage sludge for the stabilization of soil contaminated by mining activities. *J. of hazardous materials*, **B77**:177-191.
- Thompson, H. C. and Kelly, C. W. 1979. *Vegetable Crops*, (5th edition) Tata McGraw Hill Book Company Inc New York.

- Van Assche, F. and Clijsters, H. 1986a. Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentrations of zinc: effects on electron transport and photophosphorylation. *Physiol. Planta*, **66**: 717-721.
- Van Assche, F. and Clijsters, H. 1986b. Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc; Effect on Ribulose-1, 5 -Biphosphate carboxylase\ oxygenase. *J. Plant Physiol.* **125**: 355-360.
- Van Assche, F. and Clijsters, H. 1990. Effects of metal on enzyme activity in plants. *Plant Cell and Environ.* **13**: 195-206.
- Vengris, T., Binkiene, R. and Sveikauskaite, A. 2001. Electro-kinetic remediation of lead, Zn and Cd -contaminated soil. *J. of Chemical Technol. Biotechnol.* **76**:1165-1170.
- Verkleij, J. A. C. 1993. The effects of heavy metals stress on higher plants and their use as biomonitors. In: *Plants as biomonitors, Indicators for heavy metals in the terrestrial environment* (Ed) Markert B, VCH-Publishers, Weinheim, New York, pp. 415-424.
- Verkleij, J. A. C. and Schat, H. 1990. Mechanisms of metal tolerance in higher plants. In: *Heavy metal tolerance in plants: evolutionary aspects*, (Ed) Shaw A J. CRC, Press, Inc., Boca Raton, Florida. pp. 179-193.
- Voutsas, D. and Samara C. 1998. Dietary intake of trace elements and polycyclic aromatic hydrocarbons via vegetables grown in an industrial Greek area. *The Sci. of the Total Environ.* **218**: 203-216.
- Vulava, V. M. and Seaman, J. C. S. 2000. Mobilization of lead from highly weathered porous material by extracting agents. *Environ. Sci. Technol.* **34**: 4828-4834.
- Wang, D. and Schapp, W. 1988. Air pollution impacts on plants: current research challenges. I. S. I. Atlas. *Sci, Anim. Plant Sci.* **1**(1): 33-39.
- Watanabe, T., Machida, K., Suzuki, H., Kobayashi, M. and Honda, K. 1985. Photoelectro-chemistry of metallo-chlorophylls. *Coordination Chemistry Rev.*, **64**: 207-224.
- Weast, R. C. 1984. *Handbook of chemistry and physics* (64th edition), Boca Raton, CRC Press.
- Weckx, J. E. J. and Clijster, H. M. M., 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. *Physiologia Plantarum*, **96**: 506-512.
- Welz, B. 1985. *Atomic Absorption Spectrometry*. VCH, Weinheim, Germany.
- White, M. C., Chaney, R. L. and Decker, A. M. 1979. Role of roots and shoots of soyabean in tolerance to excess soil zinc. *Crop Sci.* **19**: 126-128.

- Whitton, B. A. 1970. Toxicity of heavy metals to freshwater algae. A review: *Phykos*. **9**: 116-125.
- WHO 1972. 16th Report of joint FAO-WHO, Expert committee on food additives, Report No. 505, Geneva, 1972.
- WHO 1992. *Cadmium. Environmental health Criteria* 134. World Health Organization, Geneva.
- WHO 1993. *Evaluation of certain food additives and contaminants*. Forty first report of the Joint FAO/WHO, Geneva: Expert committee on Food Additives, Technical Report Series 837.
- WHO 1996. *Trace elements in Human nutrition and Health*. World Health Organization, Geneva.
- Wildner, G. F. and Henkel, J. 1979. The effect of divalent metal ion on the activity of Mg²⁺-depleted ribulose-1.5-biphosphate oxygenase. *Planta*, **146**: 223-228.
- Witting, R. 1992. Die Eignung der Krautschicht von Waldern Veroffentl. *Naturschutz Landschaftspflege*, Beiheft **64**: 134-145.
- Wong, H. M. and Bradshaw, A. D.1982. A comparison of toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*. *New Phytol.* **91**:255-261.
- Woolhouse, H. W. 1983. Toxicity and tolerance in the responses of plants to metals. In: *Encyclopedia of plant physiology*, (Eds) Lange O L, Nobel P S, Osmond C B and Ziegler H, Springer -Verlag, Berlin, pp. 245-300.
- Xiong, Z-T. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica peknensis* Rupr. *Bull Environ. Contam. Toxicol.* **60**: 285-291.
- Yong, R. N., Galvez-Cloutier, R. and Phadungchewit, Y. 1993. Selective sequential extraction analysis of heavy metal retention in soil. *Canadian Geotechnical J.* **30**: 834-847.
- Zalidis, G., Barbayiarinis, N. and Matsi, T. 1999. Forms and distribution of heavy metals in soils of the Axios Delta of Northen Greece. *Commun. Soil Sci. Plant Anal*, **30** (5 and 6): 817-827.
- Ziegler, H. 1988. Weg der Schadstoffe in der Pflanze, In: *Schadwirkungen and PflanzenAuf* (Eds) Hock B and Elstner E, Wissenschaftaveslag, Mannheim, BI 2, pp.35-46..
- Zimdahl, R. L. and Foster, J. M. 1976.The influences of applied phosphorous, manure or lime on uptake of lead from soil. *J. Environ. Qual.* **5**: 31-34.

Appendix

Published papers based on the Present Study:

1. Dietary Intake of trace elements via vegetables grown in Kathmandu valley. 2004. *Proceedings of IV conferences on Sci. and Technol.* Vol. **1**. 980-988.
2. Monitoring of heavy metals in vegetables and soil of agricultural fields of Kathmandu Valley.2005. *Ecoprint*, **12**: 1-9.
3. Impacts of Heavy metals on accumulation of essential Micronutrients and Chlorophylls in some Vegetables.2008. *Poll. Res.* **27**(2):355-363