

CONTAMINATION OF FOOD PLANTS WITH HEAVY METALS AND THEIR IMPACTS ON NUTRITIONAL VALUES

(PhD Thesis)



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Abstract

Heavy metals are considered as global environmental pollutants that are present everywhere in the environment. Urbanization, high population growth, construction and development and high demand for food are the major factors resulting in discharge of heavy metals to environment which ultimately lead to food chain contamination. Vegetables are the main source of diet and contamination of these vital sources may severely affect human health. Toxic metals have both direct and indirect effects on human health, while the effect on nutritional parameters is one of the indirect effects of heavy metals contamination.

This research work is divided into different parts and several experiments were conducted to achieve the objectives. The first study was conducted to evaluate the effects of heavy metals (cadmium (Cd), lead (Pb) and Cd-Pb mix) on bioaccumulation of different nutrients. Three plant species including potato (*Solanum tuberosum* L.), tomato (*lycopersicon esculentum*) and lettuce (*Lactuca sativa* L.) were grown in pots containing soil contaminated with Cd, Pb and Cd-Pb mix at four different levels. The edible portions of each plant were analyzed for Cd, Pb and different macro- and micro- nutrients including protein, vitamin C, nitrogen (N), phosphorous (P), potassium (K), iron (Fe), manganese (Mn), calcium (Ca) and magnesium (Mg). Results indicated significant variations in selected elemental concentrations in all the three plants grown in different treatments. The projected daily dietary intake values of selected metals were significant ($P < 0.001$) for Fe, Mn, Ca and Mg but not significant for protein, vitamin C, N and P. The elemental contribution to Recommended Dietary Allowance (RDA) was significant for Mn. Similarly, Fe and Mg also showed substantial contribution to RDA, while Ca, N, P, K, protein and vitamin C showed the minimal contribution for different age groups. This study suggests that vegetables cultivated on Cd and Pb contaminated soil may significantly affect their quality, and

the consumption of such vegetables may result in substantial negative effects on nutritional composition of the consumer body. Long term and continuous use of contaminated vegetables may result in malnutrition.

The second study was focused on arsenic (As), being a toxic metalloid, ubiquitously present in the environment and severely affects the health of plants and humans. Pot experiments were also conducted to evaluate the toxic effects of As on plant nutritional parameters. Three nutritious plant species such as potato, tomato and lettuce were selected to investigate the possible toxic impacts on macro- and micro- nutrients concentrations. 'As' was added to the soil at three different concentrations with and without Cd and Pb. The edible parts of each plant were analyzed for As and essential macro- (vitamin C, protein, N, P, K) and micro- nutrients (Ca, Mg, Fe and Mn). The concentrations of macro- and micro- nutrients showed significant variation ($P < 0.001$) in contaminated soil than in the control. 'As' amendments significantly ($p < 0.01$) increased the concentrations of Ca, N and protein, while P and K concentrations were decreased. As concentrations were both negatively and positively correlated with Mn, Mg and vitamin C depended upon the type of vegetable and the contamination level. The values of contribution to RDA through consumption of contaminated vegetables were significant ($P < 0.01$) for Mn, less significant ($p = 0.05$) for Fe, Mg, P and K, while non-significant for Ca. N and protein for different age groups. It is concluded that the consumption of As contaminated vegetables may have both direct and indirect effects on human health. As contamination severely affected the quality and nutritional balance in contaminated vegetables and their consumption may result in to severe malnutrition.

The third study was conducted to evaluate the potential effects of As, Cd and Pb on bioavailability, uptake and bioaccumulation of chromium (Cr), copper (Cu), nickel (Ni) and zinc

(Zn) by lettuce, potato and tomato. The concentrations of selected metals were determined using Atomic Absorption Spectrophotometer. The results indicated that plants cultivated in contaminated soil observed substantial changes in essential and trace element concentrations and the effects were more significant with increasing concentration of heavy metals in the soil. Both synergistic and antagonistic effects were observed depending upon type of vegetables, concentration of pollutants in the soil and metal mixture used. Among the selected vegetables the effects were more prominent on lettuce. The projected daily dietary intake of Cr, Ni and Zn was higher for vegetables grown in metals contaminated soil than in control. The contribution to RDA was significant for Cu and Ni and less significant for Zn, although great variation was observed in RDA for different treatment levels. Consumption of metals contaminated vegetables have provided 400% Ni RDA and >50% of Cu RDA for selected vegetables. The results indicate that the food quality and elemental composition of vegetables cultivated in metals soil was significantly affected, and their consumption may severely affect human health. From the findings of these studies, it is suggested that consumption of heavy metals contaminated vegetables have substantial effects on plant quality and nutritional balance that ultimately lead to malnutrition in consuming population.

CHAPTER 1

INTRODUCTION

1.1 Background Study

Heavy metals contamination of soil and vegetables is of great environmental concern (Khan et al. 2015a, b). Heavy metals are environmental pollutants that found ubiquitously in the environment. Soil contamination with heavy metals have become global issue and environmental threat because these metals are found in excess in soil and accumulate in plant tissues and enter the food chain (Gill et al. 2012). Soil pollution is the topic of universal interest for scientists and researchers regarding environmental crises (Nanda and Abraham, 2013). In recent years excessive use of fertilizers, pesticides and wastewater irrigation, mining activities and discharge of industrial effluents are the major causes of soil pollution (Kuo et al. 2006). Heavy metals are generally found in dissolved, exchangeable and insoluble form in soil (Aydinalp and Marinova, 2003).

Soil properties and soil load can significantly affect the bioaccumulation and transfer of heavy metals to plants. Soil properties like, cation exchange capacity (CEC), pH, organic matter (OM) contents, and concentration of different essential and non essential elements may severely affect the bioavailability of heavy metals (Zeng et al. 2011; Tyler and Olsson, 2006). Besides the above mentioned properties, heavy metals bioavailability and toxicity also depend upon redox potential and soil texture because these elements are attached with different soil fractions (Tracy and Sheila, 2006). Similarly, the adsorption and desorption capacity of soil have strong influence on heavy metals mobility and bioavailability (Krishnamurti et al. 1999) which is associated with soil physiochemical properties (Antoniadis et al. 2008). Moreover, the bioavailability of heavy metals varies from species to species and within the same species among different cultivars (Miteva et al. 2001). Soil pH and heavy metal concentrations have negative correlation (Bang

and Hesterberg, 2004), with decreasing pH the mobility, bioavailability, solubility and speciation of heavy metals increase (Zhao et al. 2010; Du Laing et al. 2007) with exception of Cr which shows a positive correlation with soil pH (Sahibin et al. 2002). Similarly, OM is one of the major contributing factor that influence heavy metals mobility and bioavailability. Soil OM and heavy metals have linear correlation as it affect heavy metals mobility and accumulation by plants (Impellitteri et al. 2002). Natural OM can form complex with heavy metals that control mobility and bioavailability of heavy metals (Wong et al. 2002a). The biochemical condition of rhizosphere may affect the heavy metals speciation and bioavailability in soil (Dessureault-Rompre' et al. 2008; Tao et al. 2003).

Among the non essential toxic metals Cd is considered to be the most important one because of its mobility in the rhizospheric environment and its bioavailability to plants growing on metal contaminated soil. Soil physiochemical properties have strong influence on metal uptake by plants. Cd is more bioavailable in non calcareous soil than calcareous (Thornton, 1992), soil pH also has strong influence on Cd uptake by food plants (Smith, 1994). Cd has the capability to move through the food chain (Rojas et al. 1999). Excessive Cd uptake from contaminated soil severely affects plant metabolism and productivity (Gill et al. 2012). Heavy metals are toxic to plants, animals and humans and cause toxic and carcinogenic effects in experimental animals and humans (Jin et al. 2003; Dalton et al. 2000). Among food plants the highest Cd concentration was found in leafy vegetables while the As and Pb concentration were found in pulses and fruits, respectively (Beccaloni et al. 2013). Food plants, particularly green leafy vegetables, are the good accumulator of Cd as compare to other food crops cultivated in metals contaminated soil (Alloway et al. 1990), where the order of contamination is leafy vegetables>root vegetables>grain crops (He and Singh, 1994). Similarly, the mobility and bioavailability of Cd is

low in clay soil than sandy soil (He and Singh, 1994).

Different plant species have differential uptake capacity and mechanism, on the basis of metals uptake and bioaccumulation the plants are divided in three major groups including, excluders, indicators and accumulators. Excluders are those plants having limited bioaccumulation capability, while indicators have linear correlation with increasing available metal concentrations in soil and accumulator can accumulate higher concentration of toxic metals (Bhargava et al. 2012). Based on ecological and physiological factors some plants have the ability to acquire resistance against heavy metals (Ray et al. 1988; Antonovics et al. 1967).

Food plants cultivated in metals contaminated soil can absorb these toxic metals from soil and transport it to aerial parts of plants, this transport from roots to shoot may occur through xylem (Uraguchi et al. 2009). The transport from soil to root is very fast and after 1 h of treatment substantial amount of toxic metals like Cd can be detected in the xylem, which determine the shoot metal accumulation. Similarly, remobilization of metals in leaf blades is another source of metal accumulation in plants (Rodda et al. 2011). The capability of paddy soil to form iron plaque have varying effect on heavy metals uptake based on the root surface iron plaque amount (Hu et al. 2007; Chen et al. 2005).

Several studies have been conducted on essential and non essential metals interaction and the results shown that their interactions greatly affect the bioavailability and supply of toxic and essential elements (Fan et al. 2010; Hassan et al. 2005). Cd has the capability to replace the essential elements in enzymes and affect the photosynthetic activities and protein function (Kučera et al. 2008).

As is a toxic metalloid occurs in both organic and inorganic forms in the environment derived from natural as well as anthropogenic sources (European Food Safety Authority, 2009). Plants

uptake As through their root system from the soil and leaves from air as air born As (USEPA, 1982). Some studies reveal that the As concentration is comparatively low in plants cultivated in highly contaminated soil (Pitten et al. 1999; Gebel et al. 1998). Pb is one of the most toxic environmental pollutants that accumulate in plants and soil, in soil environment it can be accumulate through Pb paint, pipes and gasoline (Eick et al. 1999).

Accumulation of heavy metals, like As, in food plants depend upon soil metal concentrations, soil (OM) and clay contents which have the potential to absorb heavy metals reducing phytoavailability (Silva Gonzaga et al. 2012). The influence of heavy metals on plant nutritional components depends on soil environment, type of food crop, exposure time and plant organs (Gonçalves et al. 2009).

The consumption of heavy metals contaminated food may cause serious health hazards (Jarup, 2003). Human beings are exposed to metals toxicity through variety of ways; however, food intake is the most important route of human exposure to heavy metals (Kabata-Pendias and Mukherjee, 2007). The higher concentration of heavy metals in the roots and aerial parts of food plants inhibit the accumulation and distribution of essential macro- and micro- nutrients elicit nutritional deficiency (Gomes et al. 2013; Dan et al. 2008) and metabolic dysfunctioning (Marshner, 2012; Dan et al. 2008). Similarly, higher concentration has significant negative impacts on metabolic activities and cause osmotic changes in cellular plants (Gill et al. 2011; Gill and Tuteja, 2010).

Among the food plants tomatoes are the most important one, they are rich source of essential macro- and micro- nutrients required for human health. These nutrients include vitamins, organic acid, carbohydrates and essential minerals (Giovannelli and Paradiso, 2002). Tomato is salt tolerant and heavy metal resistive crop specie among the fruit vegetables (Li et al. 2010).

Food chain contamination with heavy metals is a global environmental issue causing serious health hazards that threaten human lives (Rafiq et al. 2014) and animal health (Hossain et al. 2010). Vegetables and fruits are the main dietary source of human beings that provide the essential macro- and micro- nutrients required for healthy life. Research shows that high intake of fruits and vegetables significantly reduce the cancer risk, but the contamination of these dietary supplements with heavy metals may damage the DNA and cause serious health risks (Donma and Donma, 2003; Hadley et al. 2002; Goyer, 1995;; Dabeka et al. 1987). Similarly, chronic exposure to heavy metals may cause various health problems including kidney damage, mental disorders (Lamb et al. 2008), oxidative stress, DNA damage (Khan et al. 2012) and cancer (Ng et al. 1999).

Soil act as a medium to provide essential macro- and micro- nutrients for plants and a potential scavenger of heavy metals, however, this vital source is subjected to various organic and inorganic environmental pollutants (Dragun, 1998). Soil contamination with heavy metals and their transfer to food chain result their bioaccumulation in the food products (Grant and Sheppard, 2008). Literature shows that in China, in a single year, about 1.46×10^8 kg of food products are polluted by Cd (Gu and Zhou, 2002)

It has been revealed that heavy metal concentrations in soil and vegetables grown in metals contaminated soil were higher than non contaminated soil (Singh et al. 2010a,b). Total metal concentrations in soil is not a good indicator to assess phytoavailablility and toxicity to human beings (Adriano, 2001), but due to limited information available the NEQs of heavy metals in soil is still based on total metals concentration rather on bioavailable concentration (Shentu et al. 2008). Heavy metals accumulation, distribution and their effects on plants metabolic processes are affected by a number of biological and biochemical factors like variety and nutritional status

of plant species (Barceló & Poschenrieder, 1990; Page et al. 1972).

The present study was conducted to assess the bioaccumulation of heavy metals by food plants. The effects of heavy metals (As, Cd and Pb) on micro- and macro- nutrients concentration. Heavy metals effects on mobility and bioavailability of trace metal concentrations, their effects on plant nutritional values and their effects on the daily dietary nutrient requirement of an individual.

1.2 Study Objectives

- ❖ To determine the concentrations of heavy metals (As, Cd and Pb) in soil contaminated from different sources and their bioaccumulation in food plants (Lettuce, Potato, Tomato).
- ❖ To investigate the effects of heavy metals on plant nutritional values of Vitamins C, proteins, Zn, Fe, Mn, N, P, K, Ca, and Mg.
- ❖ To assess the human health effects through ingestion of selected plants.

CHAPTER 2

Literature Review

2.1. Heavy metals

Heavy metals have high density and mostly toxic in nature for human, plants and animals regardless of their concentrations (LWTAP, 2004) and have high atomic density 5 times greater than water or more than 4 g cm^{-3} (Hawkes, 1997) or more than 5 g cm^{-3} (Saxena and Shekhawat, 2013; Weast, 1984). The non essential metals are part of earth crust that enters the upper soil horizon and food chain through biogeochemical cycles (Tinsley, 1979). Metals and metalloids such as Cadmium (Cd), Lead (Pb), Mercury (Hg) and Zinc (Zn) are called heavy metals because of their high densities (Oves et al. 2012), while Arsenic (As) is included in this list because of similar properties (Chen et al. 1999). Essential and non essential trace elements, when exceed the threshold limits, can cause different physiological, morphological and genetical anomalies including reduced growth, mutagenic effects and increased mortality (Khan et al. 2010a; Li et al. 2010; Luo et al. 2011).

Food crops are one of the important parts of our diet and they may contain a number of essential and toxic metals (Yang et al. 2011; Waqas et al. 2015) depending on growing media characteristics. Vegetables are the major source of human exposure to heavy metal and contribute about 90% of the total metal intake, while the rest 10% intake occurs through dermal contacts and inhalation of contaminated dust (Khan et al. 2014; Martorell et al. 2011; Kim et al. 2009; Ferré-Huguet et al. 2008). Food safety is a burning issue regarding human health in the recent decades because of the high demand for food. This scenario lead to stimulate researchers and scientists to work on health risk associated with consumption of heavy metals, pesticides and toxin contaminated food (D'Mello, 2003).

Essential and non essential elements are regularly added to our food chain through excessive use of agrochemicals, municipal wastewater, industrial effluents and raw sewage for irrigation (Tongesayi et al. 2013). The Agency for Toxic Substances and Diseases Registry (ATSDR, 2011) has classified heavy metals and metalloids such as As, Pb and Cd found in the environment as 1, 2, and 7 on the basis of toxicity.

Some elements like Cu, Cr, Fe, Mn and Zn are essential for animals and human beings because they play an important role in different metabolic functions, enzymatic activities, sites for receptors, hormonal function and protein transport at specific concentrations (Antoine et al. 2012; Apostoli, 2002). Other elements like As, Cd, and Pb are non essential and have no beneficial role in plants, animals and humans (Chang, 2000) and have no nutritional function as they are highly toxic (Nies, 1999; Goldbold and Huttermann, 1985). It is necessary to characterize the sources and contents of heavy metals in soil in order to establish quality standards and to determine the threats to human health and food safety (Sun et al. 2013). Environmental pollution with heavy metals is unrelenting, covert, and permanent in nature (Wang et al. 2001). Biological organisms are incapable to degrade metals because of their non biodegradable nature and long half life, and they persist in their body parts and environment leading to health hazards (Nabulo et al. 2011; Amaral and Rodrigues, 2005). Heavy metal have the capability to move from contaminated soil and water and bioaccumulate in vegetables causing health risks (Stasinou and Zabetakis, 2013; Rattan et al. 2005).

Soil characteristics play an important role in terms of food production, the contamination of this vital resource with heavy metals and their ultimate uptake and bioaccumulation in food crops poses major environmental and health problems (Fig. 2.1), particularly in developing countries (Lin et al. 2007). Soil type and plant genotype and their interaction have significant

effect on heavy metal concentrations (Ding et al. 2013). Mineral fertilizers have higher heavy metal concentrations as compare to organic manure, therefore the application of mineral fertilizers result in soil heavy metals pollution (Hu et al. 2013).

So far, a number of experiments (pots and field) have been conducted to study the effect of soil parameters and other elements on mobility and bioavailability of heavy metals from soil to crop system (Khan et al. 2013a,b; Li et al. 2007; Hart et al. 2006). The mobility and bioavailability of heavy metals in contaminated soil is affected by a number of biological processes and physiochemical properties like soil pH, OM (Ahmad and Goni, 2010; Ernst, 1996; Alloway, 1995), CEC, soil texture and soil microbiota. Soil pH has significant effects on availability and accumulation of heavy metals in the edible parts of plants (Hu et al. 2013; Wang et al. 2013a). Similarly, CEC and OM have negative impact on the mobility and bioavailability of heavy metals like Pb (Ding et al. 2013; McLaughlin et al. 2011; Arshad et al. 2008). Cd toxicity to the soil environment is well known, it is a toxic heavy metal and causes toxicity even at low concentration. The bioaccumulation rate of Cd is higher in the field crops as compared to other elements (Arao and Ae, 2003; Moustakas et al. 2001).

For the improvement of nutritional composition of soil different agricultural strategies like biofortification and proper use of fertilizers have been suggested (Bonierbale et al. 2007; Graham et al. 2006).

In recent years most of the studies have focused on As contamination of food chain (Huang et al. 2012; Gilbert-Diamond et al. 2011; Kim et al. 2009) along with other heavy metals and metalloids that were found above the recommended limits (Tufuor et al. 2011). Food crop irrigated with industrial effluents and wastewater is the major source of soil and crop contamination with heavy metals and metalloids (Tiwari et al. 2011; Lee et al. 2008; Sipter et al.

2008).

The associated health hazards of toxic metal depend on concentrations of these metals in specific media and exposure time. Long time and chronic exposure can cause health hazards even at low concentrations of toxic metal (Mahalakshmi et al. 2012). Among the abiotic stresses to plants heavy metals toxicity is one of the major stresses and the toxicity is based on physiochemical properties of heavy metals (Saxena and Shekhawat, 2013; Zhuang et al. 2009).

The aim of this review is to summarize the literature about the heavy metals as a major environmental issue and critically discusses the information about heavy metals (As, Cd, Cr, Cu, Ni and Pb) contamination in soil and the grown food plants, metal bioaccumulation, soil to plants transfer, nutritional effects and health risks.

2.2 Heavy metals in soil

Soil contamination with heavy metals is a serious global environmental problem (Solgi et al. 2012; Facchinelli et al. 2001; Wang et al. 2001) posing risks to human, animals, microbes and plants and contaminating surface and groundwater. Heavy metals and other pollutants enter to the soil ecosystem through natural processes and anthropogenic activities. Trace element concentrations in soil environment are mainly dependent upon the geology of the area (Carral et al. 1995), while anthropogenic activities like solid waste disposal, wastewater irrigation, sludge application, automobile exhaust, mining and smelting processes, urbanization, agricultural activities and industrialization also contribute heavy metals into the soil environment (Wei and Yang, 2010; Lim et al. 2008, Montagne et al. 2007; Shi et al. 2005; Facchinelli et al. 2001; Tsai et al. 2001; Chen et al. 1999). Similarly, the physiochemical characteristics of soil have substantial effects on heavy metals concentration and its availability to plants (Fig. 2.1).

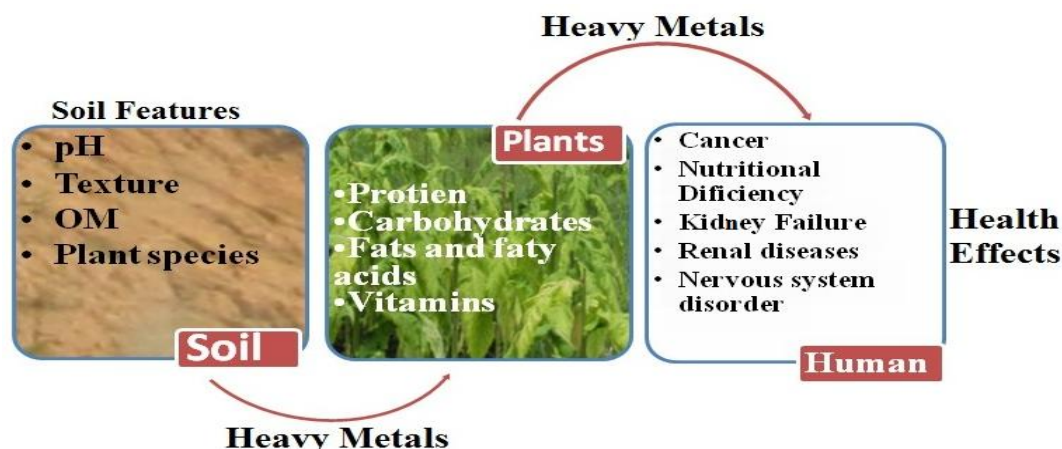


Fig. 2.1. The diagrammatic presentation of heavy metal sources, plant uptake and health effects.

Heavy metals toxicity is mainly associated with metals speciation in soil (Allen et al. 1980; Liu et al. 1996). The heavy metal concentrations in agriculture soil were in range of 0.1–40, 0.01–0.7, 5–3,000, 2–100, 10 and 2–200 mg kg⁻¹ for As, Cd, Cr, Cu, Ni and Pb, respectively (O'Neill 1995; Allaway 1968). While, the heavy metal concentrations in garden soil were in range of 3.06-15.89, 0.27-2.86, 14.08-53.97, 18.51-579.84, 10.04-35.60 and 5.11-60.85 mg kg⁻¹ for As, Cd, Cr, Cu, Ni and Pb, respectively (Szolnoki et al. 2013). Similarly, the concentrations of As, Cd, Cr, Cu, Ni and Pb in sediments were ranged from 0.2–13.8, 0.3–8.4, 2-1,000, 4-200, 2-500 and 7–150 mg kg⁻¹, respectively (Cannon et al. 1978). Among toxic elements, As is highly carcinogenic and present in the soil of both developed and developing countries (Fig. 2.2).

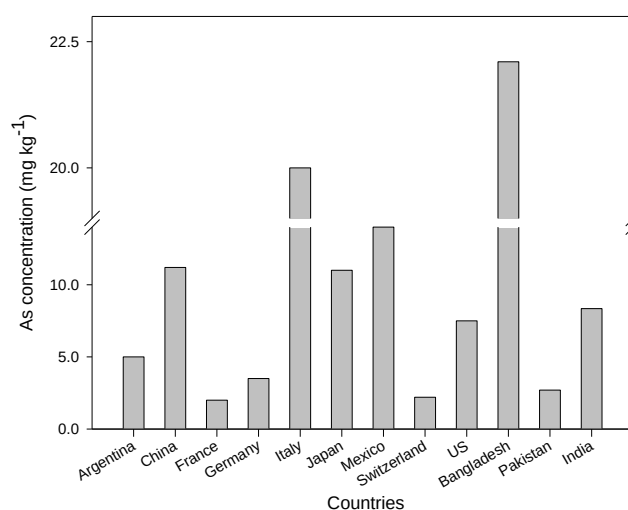


Fig. 2.2. Concentrations of As in the soils of different regions of the world: sources include Biswas et al. (2013); Arian et al. (2009) and Mandal and Suzuki (2002).

Table 2.1 summarizes the heavy metal concentrations in different soils, while their permissible limits in soil are given in Table 2.2. The permissible limits of different countries and organizations show variation in concentrations for different heavy metals. These differences may be due to different strategies adopted by those countries and organizations to set permissible limits. The variation in permissible limit may be based on soil characteristics and type. The permissible limits for As were 75, 30 and 20 mg kg⁻¹ set by USEPA, SEPA China and FAO/WHO, respectively, while, the permissible limits for Cu and Ni were similar for almost all countries and organizations. Other heavy metals like Cd, Cr and Pb show great variations in their respective permissible limits set by different organizations/countries. The concentrations of all the selected heavy metals were observed above the permissible limit set by different countries and International organizations (WHO, USEPA, SEPA etc) for heavy metals in soils from different regions of the world (Table 2.2).

Table 2.1. Heavy metal concentrations (mg kg⁻¹) in soil

References	Heavy metals				
	Cd	Cr	Cu	Ni	Pb
Waterlot et al. 2013	16.7 ^{acde}	N.A	74.1	N.A	1122 ^{acde}
Khan et al. 2010a	0.99 ^{ce}	N.A	88.8	40	54.4
Castro et al. 2009	N.A	8.5	11.0	15.5	<0.2
Noor-ul-Amin et al. 2013	N.A	0.121	0.225	0.123	0.225
Xu et al. 2013b	1.2 ^{ce}	N.A	107 ^{cde}	N.A	66
Zhuang et al. 2009	3 ^{cde}	N.A	449 ^{acde}	N.A	282 ^{ade}
Luo et al. 2011	0.90-17.1 ^{acd e}	6.41-68.9	72.4-11,140 ^{acde}	8.83-60.1 ^{cde}	52.2-4500 ^{acde}
Khan et al. 2013a, b	0.09- 0.11	0.44-0.51	0.33-0.50	0.46-0.55	N.A
Hu et al. 2013	1.7 ^{cde}	N.A	41	N.A	83
Khan et al. 2008b	0.84 ^{ce}	60.9	32.8	24.9	49.4
Piotrowska and Kabata-Pendias 1997	0.30 ^e	N.A	N.A	N.A	N.A
Yu et al. 2006	7.43 ^{adce}	47.07	132.82 ^{cde}	N.A	223.22 ^{de}
Gebrekidan et al. 2013	0.75 ^{ce}	31.02	25.25	26.00	3.27
Khan et al. 2008a	0.80-2.58 ^{cde}	64.2-77.5	22.8-61.8	22.8-38.5	29.7-97.9
Gichner et al. 2006	0.14	N.A	19	N.A	47.6
Singh et al. 2010b	2.98 ^{cde}	6.41	30.14	8.96	26.48
Kar et al. 2013	N.A	N.A	N.A	N.A	N.A
Kim et al. 2009	0.325 ^e	35.1	26.25	N.A	1448 ^{acde}
Biswas et al. 2013	N.A	N.A	N.A	N.A	N.A
Bigdeli and Seilsepour 2008	0.67 ^{ce}	70	54	32	60
Aremu et al. 2010	N.A	4.31	0.73	0.47	1.21
Meers et al. 2005	7.61 ^{acde}	204.25 ^{cde}	144.10 ^{cde}	23.15	262.16 ^{ade}

Liu et al. 2011	0.19	46.98	40.77	53.65 ^{cde}	50.11
Chang et al. 2014	0.17	46.7	N.A	N.A	42.5
Shi et al. 2013	N.A	N.A	37.7	N.A	N.A
Yang et al. 2007	≥0.28	≥48	≥20	≥33	≥35
Mishra et al. 2009	3.54 ^{cde}	42.60	57.57	47.10	N.A
Kachenko and Singh 2006	5.54 ^{acde}	N.A	78	N.A	363 ^{acde}
Brahman et al. 2014	N.A	N.A	N.A	N.A	N.A

^a Above the permissible limit of India;

^b Above the permissible limit of USEPA

^c Above the permissible limit of China EPA;

^d Above the permissible limit of EU

^e Above the permissible limit of FAO/WHO

Table 2.2. International standards for different heavy metals (mg kg⁻¹) in soil and plants

Heavy metals	Commission Regulation EU, (2006)		US EPA (2005)		FAO/WHO (1984, 2001a)		SEPA China (1995, 2005)		Indian standards (Awashthi, 2000)	
	Soil	Plants	Soil	Plants	Soil	Plants	Soil	Plants	Soil	Plants
As	N.A	N.A	75	N.A	20 ^a	0.1	30	0.5	N/A	1.1
Cd	3	0.2	85	N.A	0.3	0.1	0.6	0.1-0.2	3-6	1.5
Cu	100	20	N.A	N.A	100 ^a	73	100	20	135–270	30
Cr	100	1	3000	N.A	100 ^a	2.3	200	0.5	N.A	20
Ni	50	N.A	N.A	N.A	50 ^a	66.9	50	10	75–150	1.5
Pb	100	0.30	420	N.A	100 ^a	0.3	300	9	250-500	2.5

^a Ewers, 1991

Heavy metal concentrations are greatly affected by soil organic contents. Soils with high organic waste concentrations are generally confined to heavy metal concentrations of less than 1000 mg kg⁻¹ soil, while industrial waste contaminated soil contains more than 10,000 mg kg⁻¹ soil (Bader et al. 1999). Increasing concentrations and variation in distribution of heavy metal in metal amended soil generally augment the heavy metal concentrations in plants (Castro et al. 2009). Soil is the main source of contamination of food chain with heavy metals because soil is used as an important tool for waste management and waste dumping (Rogival et al. 2007; Zhuang et al. 2009).

The prime route of heavy metals intake into the human body is through soil-crop system in agriculture area (Liu et al. 2007), where the anthropogenic activities are the primary sources of contaminations (Wei and Yang, 2010; Yang et al. 2009a; Lim et al. 2008; Li et al. 2008; Montange et al. 2007). The main route of exposure to heavy metals in urban environment is through ingestion, absorption through skin and inhalation of dust particles (Lim et al. 2008; De Miguel et al. 2007; Sindern et al. 2007; Ahmed and Ishiga 2006; Ferreira-Baptista and De Miguel, 2005), whereas the primary source of pollution in urban soil is anthropogenic one which includes vehicular emission, power plant, tire wear particles, auto repair shops, car wash centers, brake lining, coal combustion, chemical plants, weathering of building, atmospheric deposition and house hold solid waste (Amato et al. 2009; Lu et al. 2009; Morton-Bermea et al. 2009; Zhou et al. 2008; Oliva and Espinosa, 2007; Duzgoren-Aydin et al. 2006; Han et al. 2006; Kartal et al. 2006; Sezgin et al. 2003; Madrid et al. 2002; De Miguel et al. 1997).

Ecotoxicological assessment of contaminated soil is very difficult due to complex nature and diversity of soil microbiology (COM, 2006). However, these test methods are considered as a good tool for obtaining information regarding bioavailability of contaminants to some specific

organisms (ISO/DIS, 2006).

Ecological risk assessment approach is generally applicable for representing metal contaminated sites (Jensen et al. 2006; US EPA 2001) for this purpose soil quality values also called guideline values are used (Niemeyer et al. 2010; CCME 2005; USEPA, 2005; National Environment Protection Council (NEPC), 1999). These guideline values are based on dose response data derived from laboratory tests on ecological process or single plant or animal specie (Jones, 2006). Finally statistical applications are applied to derive these guideline values (O'Halloran 2006). Soil quality values are established on the basis of total metal concentrations in the soil. However data of total metal concentrations is not sufficient for risk assessment, other qualities like chemical form, electric potential and ion activity of metals are to be considered while predicting metals toxicity (Wang et al. 2013b; Chapman et al. 2003; Stumm and Morgan, 1996). Soluble metals and free metal ions are more bioavailable and toxic (Lund, 1990) than other form of heavy metals.

2.3 Factors affecting mobility and bioavailability of heavy metals

Heavy metal accumulation in plants is strongly influenced by different soil parameters (Wilson et al. 2014; Wang et al 2013a; Ahmad and Goni, 2010) such as soil pH, OM (Fig. 2.1), redox potential, total metal contents and CEC (Wang et al. 2012a; Imai et al. 2002; Chlopecka, 1996). Heavy metals like Cu and Zn show significant negative co-relation with pH (Wang et al. 2013a; García et al. 2009), while soil OM effects vary from metal to metal (Wang et al. 2013a). The aging factor and other soil properties are the main factors affecting the bioavailability of heavy metals (Ahmad and Goni, 2010; Smolders et al. 2009). The sensitivity of heavy metals in soil is greatly affected by soil CEC and pH (Lock and Janssen, 2001).

The pedotransfer function assessment is now commonly used to understand heavy metal mobility and availability in soil environment. The following regression equations are used to forecast the values of those parameters which are difficult to measure from the easily measured soil properties such as soil particle size, pH, OM and CEC (Martin et al. 2005; Perfect, 2003; Bouma, 1989). The Freundlich equation is used as pedotransfer function assessment tool to assess metals availability in soil. The original model was proposed for Cd bioavailability. Springob et al. (2001) used the equation for assessment of Cd bioavailability. However the modified Freundlich equation can be used for other metal availability in soil environment. The modified Freundlich equation is as under

$$S = C_{\text{metal}}^M \quad \text{Eq 1}$$

Where S represents the sorbed fraction, C_{metals} represents soil metal concentrations and M (shape) represents the Freundlich parameters. M has an average value of 0.8 (Springob et al. 2001), while k value can be calculated through multiple regression techniques (Eq 2, 3). For assessment of k value the Springob et al. (2001) modified the Freundlich function in simple and log domain respectively.

The modified equations are as under:

$$S = k * x_1^a x_2^b \dots \dots x_n^z C_{\text{metal}}^M \quad \text{Eq 2}$$

$$S = k * + a \log x_1 + b \log x_2 \dots \dots + z \log x_n + M \log C_{\text{metal}} \quad \text{Eq 3}$$

Where x_1 to x_n are the soil variables used to predict the value of k, while a, b ... z are exponents allowing nonlinear contribution of different variables. Similarly Eq 2 can be expressed in log domain by multiple linear regression analysis (Eq 3). The ability of soil to accumulate metals depend upon soil OM which act as main sorbent for metals (Ge and Hendershot, 2005; Sauvé et al. 2003).

2.3.1 Soil organic matter (OM)

Soil OM has the capability to bind toxic metals to control heavy metals behavior and alleviate toxicity in soil (Datta et al. 2001). Wu et al. (2014) mentioned that the sequential analysis shows that Pb availability in soil gradually decreased while Cd availability increased. Similarly, the soil OM bounded fraction of Pb increased, while that of Cd decreased. This shows that Pb has higher affinity and stability towards OM than Cd (Winter et al. 2012). This difference in relative binding affinity of Pb and Cd with soil OM is because of metal chemistry differences (Wu et al. 2014).

The heavy metals binded with dissolved OM are easily available to soil flora (Krishnamurti et al. 2004). Similarly, humic acid is an important part of organic carbon having phenolic and carboxylic functional groups which increases the heavy metal mobility and availability to plants (Khan et al. 2006; Halim et al. 2003; Marschner and Kalbitz, 2003; Senesia et al. 2003; Evangelou and Marsi, 2001; Lagier et al. 2000; Fox and Comerfield, 1990). Therefore, soil with high humic acid concentration is not suitable for agricultural activities.

2.3.2 pH

Soil pH strongly influences the bioavailability of heavy metals in soil and their toxicity on living organisms (Amini et al. 2005; Basta et al. 2005; Seuntjens et al. 2004; Nigam et al. 2001; Prasad, 1999; Li and Shuman, 1996). At low pH the mobility and bioavailability of metals are greater as compared to high pH (Tsadilas et al. 2005; Kuo et al. 2004; Merry, 2001). The soil pH reduces the metals uptake by plants and the availability of metals increase when the pH is below the critical value of 4. The geology, distance from roadside, industries and type of irrigation affect the pH values in soil. Wastewater irrigation brings changes in the pH of soils (Zhuang et al. 2009). Like mobility and bioavailability of metals, the pH plays an important role

in the metal speciation in soil (Luo et al. 2011). Along with other physiochemical characteristic pH can significantly effect the heavy metal removal from contaminated soils (Di Palma et al. 2003). Heavy metal like Cu has the tendency of making Cu-organic matter complexes (Li et al. 2008) which leads to affect its availability. Cu bioaccumulation in plants are significantly affected by soil pH and negatively correlated with each other (García et al. 2009; Wang et al. 2006a, 2013a). Toxicological effects of free metal ions on ecological resources are the functions of soil pH (Lofts et al. 2004), therefore, it is necessary to have a basic knowledge of soil pH used for cultivation purposes. The soil with low pH should not be used for agriculture purposes due to highly availability of metals in it.

2.3.3 Soil Texture

Soil texture along with other factor is one of the important factors that induces metal availability in soil. Clay contents can significantly affect the availability of heavy metals and their subsequent toxicity to living organisms (Beyer and Cromartie, 1987). Crops grown on sandy soil are metal deficient, particularly Zn, as compared to loamy texture (Rashid and Ryan, 2004; Martens and Reed, 1991) this may be due to large pore size and the low retention capacity of sandy soil to retain metals.

Soil to plant transfer of heavy metals is strongly influenced by the soil texture. Treder and Cieslinski (2005) stated that plants cultivated on sandy soil have higher concentrations of heavy metals than those grown on clay loamy soil. The high bioaccumulation in plants is linked with higher mobility of metals in sandy soil as compared to clay soil.

Microorganisms and heavy metals have significant negative relationship. Effects of high heavy metal concentrations on the microbial community structure, activity and abundance have been reported (Khan et al. 2010b; Tsezos, 2009; Sandaa et al. 1999). Similarly, there is a strong

association between soil microbiota and soil texture, most of microorganisms occurs in association with clay content (Sessitsch et al. 2001; Alexander, 1977). Furthermore, the heavy metal movement from upper to lower soil horizon is also affected by soil texture. In sandy textured soil heavy metals can easily move from one horizon to other as compared to clay soil. Pore size has also effects the metal mobility and bioavailability.

2.3.4 Plant species

The bioaccumulation of heavy metal is different for different plant species reflected by their growth, reproduction, occurrence and survival in the metal contaminated soil. It is notable that different plant species show different toxicity to the same pollutant and in the same environmental condition, because the mechanisms of elemental uptake by plants are not the same for all plant species (Zechmeister et al. 2003; Garty, 2001). Heavy metal accumulation in food plants depends on metal concentrations as well as phyto-availability and phyto-variety, as different plants have different uptake rates (Yang et al. 2009b; Medina et al. 2005). The capacity of plants to uptake heavy metals is different for different heavy metals and the same heavy metal can be accumulated at different ratio in different plant species (Singh et al. 2010b). Metal bioavailability is also affected by the presence of organic compounds of that metal in plants. As species bioavailability is considerably reduced by organo-arsenic compounds present in plant tissues (Juhasz et al. 2006). Generally, the leafy vegetables metal uptake rates are higher and more contaminated than non leafy vegetables (Yu et al. 2006).

2.4 Heavy metals in plants

Vegetables are important source of human diets and their contamination can cause serious health problems (Khan et al. 2008a; Lim et al. 2008; Bi et al. 2006; Pruvot et al. 2006; Radwan and Salama, 2006; Liu et al. 2005a). Leafy vegetables like lettuce are considered as potentially

hyperaccumulators of heavy metals (Ramos et al. 2002). One of the properties of green leafy vegetable is the accumulation of heavy metals in their tissues without exhibiting any toxicity symptoms (Intawongse and Dean, 2006). Monteiro et al. (2007) reported that with increasing exposure duration the concentrations of heavy metals in lettuce roots and shoots increased. Heavy metals can cause changes in physiology and growth of tomato at variable concentrations and result in chlorosis and necrotic symptoms on leaves (López-Millán et al. 2009). The plants grown on wastewater irrigated soil contain high concentrations of metals in their vegetative and non vegetative parts (Khan et al. 2008b). Garate et al. (1993) described that lettuce have higher capacity to accumulate heavy metal in different tissues. Heavy metals uptake is also affected by different plants species (Fig. 2.1) and within the same specie by different cultivars (John and Van Laerhoven, 1976). Like other food crops potato is an important food crop grown throughout the world. It is rich with energy, dietary fibers, vitamins, carbohydrates and essential elements such as Fe, Ca, Zn and K (Finglas and Faulks, 1984). Mineral concentrations and photosynthetic activities of plants can also be affected by toxic metals (López-Millán et al. 2009).

According to Swedish National Food Administration, (1984) consumption of potato (200 g) provides an carbohydrate, protein, energy, Ca, Cu, P, Fe, Mg and Zn (36.6 g, 3.8 g, 172 kcal, 0.44 mg, 18 mg, 90 mg, 1.2 mg, 48 mg and 0.82 mg, respectively). The soil pH substantially affects the supply of these essential nutrients to plants, low pH means more supply of nutrients (Lutz et al. 1972). Moreover, the nutrient supply to plants is affected by a number of factors including soil type, climate, cultivation practices and storage condition (Srikumar and Ockerman, 1990). Concentrations of heavy metals vary in different organs of the same plant. Xu et al. (2013a) reported the heavy metal accumulation in order of leaf>root≈stem>tuber. However, other scientists conveyed that the root concentrations are higher than shoot (Van Assche and Clijsters,

1990).

In fruit plants, like tomato, the translocation rate of heavy metals to the fruit is rather low (Table 2.3 and Fig. 2.3), hence characterized as low rate translocation fruit vegetable (Angelova et al. 2009). Accumulation and distribution of heavy metals like Cd have been found in different parts of tomato (Donma and Donma, 2005). Tomato is an important food plant from economical as well as nutritional point of view (FAOSTAT, 2007). Tomato is rich source of minerals, vitamins and other nutrients (Giovanelli and Paradiso, 2002) consumed both in raw and processed form (Martinez-Valvercle et al. 2002).

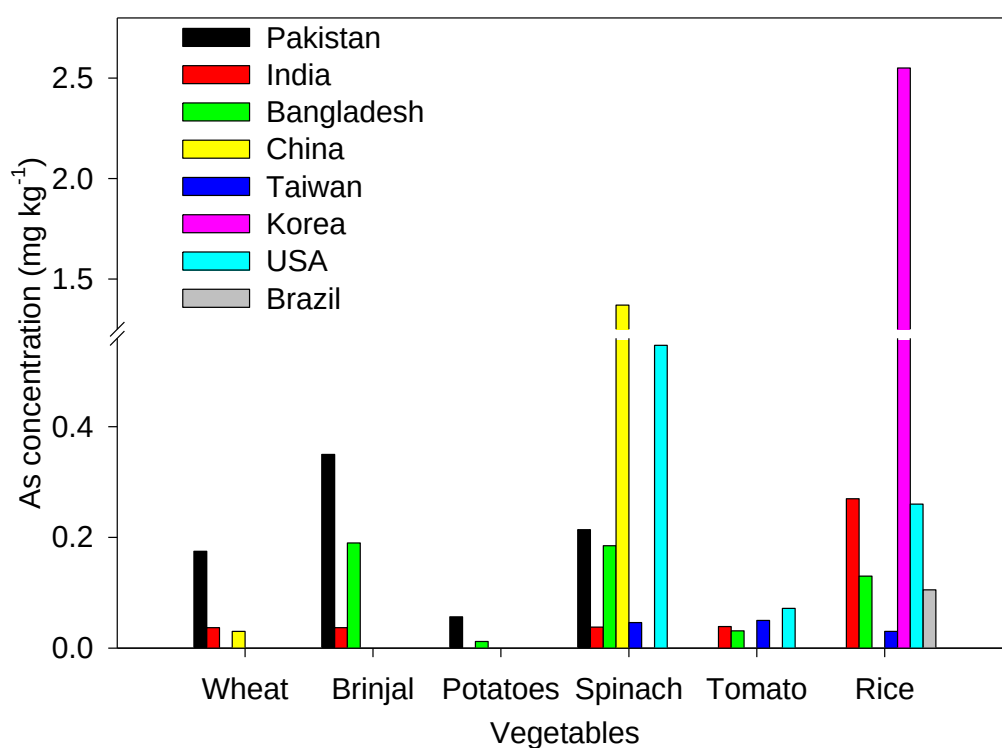


Fig. 2.3. The concentrations of As in different vegetable grown in different countries. Sources: Chang et al. (2014); Shi et al. (2013); Kar et al. (2013) and Ramirez-Andreotta et al. (2013); Batista et al. (2010); Arian et al. (2009); Yu et al. (2006); Williams et al. (2005); Patel et al. (2005); Owens et al. (2004); Alam et al. (2003); Sekhar et al. (2003)

Table 2.3. Heavy metal concentrations (mg kg⁻¹) in plants

Plants	Cd	Cr	Cu	Ni	Pb	References
Lettuce	14.98±0.53 ^{acde}	N.A	8.15±0.18	N.A	3.64±0.55 ^{ade}	Waterlot et al. 2013
Mustard	0.62±0.32 ^{cde}	N.A	17±9	4±1.3 ^a	17±1.7 ^{acde}	Khan et al. 2010a
Lettuce	0.84±0.02 ^{cde}	N.A	17±1.7	24±2.5 ^a	15±0.43 ^{acde}	
Spinach	2.10±0.75 ^{acde}	N.A	11±1.1	7±5.7 ^a	18±10 ^{acde}	
Lettuce	<0.2 ^e	<0.2	8.67±2.03	<0.2	<0.2	Castro et al. 2009
Tomato	N.A	6.1 ^{cde}	10.5	1.6 ^a	4.45 ^{ade}	Noor-ul-Amin et al. 2013
Onion	N.A	1.05 ^{cd}	6.05	2 ^a	2.7 ^{ade}	
Brinjal	N.A	7.5 ^{cde}	6.85	3 ^a	4.35 ^{ade}	
Lettuce	0.13±0.11 ^e	N.A	0.84±0.31	N.A	0.15±0.09	Xu et al. 2013b
Garlic	0.02±0.02	N.A	0.42±0.13	N.A	0.02±0.01	
Cabbage	0.04±0.04	N.A	0.6±0.29	N.A	0.11±0.08	
Lettuce	0.27±0.02 ^{cde}	N.A	0.98±0.10	N.A	0.13±0.01	Zhuang et al. 2009
Carrot	0.14±0.02 ^e	N.A	0.85±0.16	N.A	0.18±0.01	
Mustard	0.08±0.00	N.A	0.75±0.06	N.A	0.37±0.14 ^{de}	
Lettuce	4.22±0.51 ^{acde}	N.A	23.2±2.5 ^{cd}	N.A	8.59±0.9 ^{ade}	Luo et al. 2011
Rice	0.43 ±0.21 ^{cde}	N.A	42.3±5.6 ^{acd}	N.A	13.6±2.8 ^{acde}	
Cauliflow er	2.74±0.3 ^{acde}	N.A	15.8±2.5	N.A	8.82±1.5 ^{ade}	
Potato	0.09±0.01	0.11±0.06	0.06±0.04	0.06±0.05	N.A	Khan et al. 2013a, b
Tomato	0.09±0.01	0.12±0.06	0.03 ±0.02	0.07±0.05	N.A	
Rice	0.08 ±0.04	BDL	0.19 ±0.02	0.23± 0.21	N.A	
Tomato	0.03±0.02	N.A	0.91±0.15	N.A	0.02±0.02	Hu et al. 2013
Lettuce	0.06±0.01	N.A	0.42±0.03	N.A	0.04±0.01	

Spinach	0.52 ^{cde}	8.7 ^{cde}	14.3	8.6 ^a	4.5 ^{ade}	Khan et al.
Lettuce	0.9 ^{cde}	7.5 ^{cde}	14.6	14 ^{ac}	5.5 ^{ade}	2008b
Potato	0.11	N.A	N.A	N.A	N.A	Piotrowska and Kabata-Pendias 1997
Cucumber	0.66 ^{cde}	60.53 ^{acde}	10.3	N.A	21.26 ^{acde}	
Spinach	1.06 ^{cde}	109.44 ^{acde}	15.64	N.A	31.95 ^{acde}	Yu et al. 2006
Cabbage	0.71 ^{cde}	55.97 ^{acde}	10.97	13.41 ^{ac}	1.21 ^{de}	
Potato	0.18±0.04 ^e	0.39±0.06	2.52±0.13	0.25 ±0.06	2.58±0.36 ^{ade}	Gebrekidan et al.
Tomato	0.38±0.02 ^{cde}	0.60±0.06 ^c	2.43±0.15	0.73±0.06	2.50±0.24 ^{de}	2013
Cabbage	0.18±0.01 ^e	0.43±0.02	4.10±0.05	0.75±0.09	3.82±0.15 ^{ade}	
Lettuce	0.40-0.91 ^{cde}	3.4-4.0 ^{cde}	N.A	12-15 ^{ac}	2.25-5.3 ^{ade}	Khan et al. 2008a
Rice	0.23 ^{cde}	8.8 ^{cde}	63.3 ^{acd}	N.A	8.115 ^{ade}	Kim et al. 2009
Potato	0.7 ^{cde}	N.A	15.0	N.A	6.9 ^{ade}	Gichner et al.
Potato	6.3 ^{acde}	N.A	24.4 ^{cd}	N.A	51.2 ^{acde}	2006
Mustard	0.28 ^{cde}	1.25 ^{cd}	9.98	3.02 ^a	2.58 ^{ade}	Singh et al. 2010b
Spinach	0.20 ^{cde}	N.A	22.74 ^{cd}	0.00	2.57 ^{ade}	Bigdeli and
Tomato	0.01	N.A	39.99 ^{acd}	0.03	1.94 ^{de}	Seilsepour 2008
Maize	N.A	1.36±0.01 ^{cd}	2.05±0.2	1.04±0.5	0.32±0.01	Aremu et al. 2010
Bean	0.24±0.20 ^{cde}	5.31 ±1.80 ^{cde}	15.87 ±4.12	5.65 ±1.54 ^a	1.49 ±1.09 ^{de}	Meers et al. 2005
Rice	0.03±0.02	0.97±0.31 ^c	4.88±1.70	2.31±0.75 ^a	0.22±0.05	Liu et al. 2011
Lettuce	0.049±0.052	0.13±0.09	N.A	N.A	0.18±0.20	Chang et al.
Chinese cabbage	0.027±0.012	0.23±0.10	N.A	N.A	0.11±0.12	2014
Wheat	N.A	N.A	6.68±0.97	N.A	N.A	Shi et al. 2013

Vegetable	≥0.20	≥0.13	<1	≥0.20	≥0.10	Yang et al. 2007
Lettuce	0.08 ±0.01	0.18 ±0.02	2.02 ±1.76	0.38 ±0.30	N.A	Mishra et al.
Bringal	0.15 ±0.07 ^{ce}	0.21 ±0.01	3.76 ±0.34	1.03 ±0.11	N.A	2009
Spinach	0.34 ±0.13 ^{cde}	0.37 ±0.20	3.25 ±2.81	2.32 ±0.25 ^a	N.A	
Radish	0.24 ±0.04 ^{cde}	0.24 ±0.02	2.57 ±2.37	1.87 ±0.33 ^a	N.A	
Pudina	9.39±1.66 ^{acde}	65.60±2.66 ^{acde}	25.02±1.97 ^{cd}	21.64–30.11 ^{ac}	20.92±2.31 ^{acde}	Gupta et al. 2012
Cauliflow	12.46±1.03 ^{acde}	86.11±1.44 ^{acde}	15.26±0.80	58.95±2.00 ^{ac}	29.69±1.93 ^{acde}	
er						
Spinach	12.97±0.88 ^{acde}	95.79±1.21 ^{acde}	32.11±2.08 ^{acd}	68.66±1.36 ^{ace}	47.69±3.44 ^{acde}	
Radish	16.16±1.04 ^{acde}	76.26±2.17 ^{acde}	27.00±1.70 ^{cd}	60.12±2.18 ^{ac}	51.78±4.16 ^{acde}	
Lettuce	0.213±0.134 ^{cde}	N.A	0.533±0.171	N.A	2.49±1.57 ^{de}	Kachenko and
Spinach	0.361±0.185 ^{cde}	N.A	1.01±0.345	N.A	4.31±3.08 ^{ade}	Singh 2006
Lettuce	3.11	N.A	18.9	N.A	4.34	Luo et al. 2011
Cabbage	2.74	N.A	15.8	N.A	8.82	
Rice	0.43	N.A	42.3	N.A	13.6	
Rice	0.013	N.A	2.4	N.A	N.A	Batista et al.
						2010

^a Above the permissible limit of India;

^c Above the permissible limit of China EPA;

^e Above the permissible limit of FAO/WHO

^b Above the permissible limit of USEPA

^d Above the permissible limit of EU

Vegetables are vulnerable to heavy metals at high concentrations and large scale irrigation with wastewater and application of fertilizers for commercial production increase the risk of heavy metal contamination (Gil et al. 2004). Heavy metals have significant negative effects on plant growth (López-Millán et al. 2009), other toxic effects may include root browning, alteration of mineral concentrations and changes in photosynthesis (López-Millán et al. 2009). At higher concentration, metal moves from roots to shoots of the plant (Rodríguez-

Celma et al. 2010). Misra and Mani (1991) have suggested that metal concentrations in plant tissues were in the range of 0.02–7, 0.1–2.4, 0.2–1.0, 4–15, 1.0 and 1–13 mg kg⁻¹ for As, Cd, Cr, Cu, Ni and Pb, respectively.

The heavy metal concentrations in soil are given in Table 2.1, while their concentrations in plants are shown in Table 2.3 and Fig. 2.2 and 2.3. The Cd concentrations in most of the selected plants were above the permissible limits set by SEPA, EU, India and FAO. Different vegetables including leafy, ground stem and fruits were selected to study the effects of heavy metals. The maximum concentration was found in leafy vegetables such as lettuce. The order of contaminations were leafy>fruit>ground stem vegetables (Table 2.3 and Fig. 2.3). Cr concentrations were found above the safe limit of EU (1.0 mg kg⁻¹) and FAO (0.05 mg kg⁻¹). Similarly, the concentrations of Pb were also above the EU (0.3 mg kg⁻¹), FAO (0.3 mg kg⁻¹), Indian standard (2.5 mg kg⁻¹) and SEPA (9.0 mg kg⁻¹) in most of the selected plants. The order of contamination was potato>spinach>cucumber>lettuce>mustard>rice (Table 2.3). The permissible limits for heavy metals in vegetables are summarized in Table 2.2. Like soil, the permissible limits set by different countries and organizations for individual metal show great variation in their respective concentrations.

2.5 Metals uptake by plants

Vegetables (leafy and non leafy) grown on contaminated soil are considered as the major source of heavy metals. Based on heavy metal uptake the plants are classified as accumulator, hyper accumulator and excluders. Plants contamination with heavy metals may occur through soil-plant, water-plant as well as air-plant interfaces, however soil-plant interface is the major source of plants metal accumulation. Literature shows that there is strong relationship between heavy metals in soil and food crops (Bini et al. 2012). In general, the bioavailability of heavy

metals depends on amount of exchangeable metals in soil. Carbonate bound and exchangeable metals are more bioavailable than other fractions (Wong et al. 2002b). The bioavailability of heavy metals in plant varies for different plant organs, the absorption and bioaccumulation rate is highest for roots as compared to other parts (Verma and Dubey 2003). Similarly, solubility and soil type also affect the metals uptake by plants (Castro et al. 2009). The mean heavy metal uptake by plants increases as the contents of these metals increase in the soil environment (Chaves et al. 2011). Galal and Shehata (2015) reported that the bioavailability of heavy metals depends upon the distance from the source, they observed that the bioavailability rate was higher in heavy-traffic roadside plants. Sludge and compost amended soil reduce availability of metals to plants avoiding food chain contamination (Smith, 2009). Liu et al. (2005a) mentioned that the bioavailability of Cd is the highest, while that of As is the lowest for crop cultivated in heavy metal contaminated soil. *Amaranthus dubius* have the capability to remove heavy metals from soil through their root system, but shoot absorption is negligible for a number of heavy metals like As, Cr, Cu, Ni and Pb (Mellem et al. 2009).

2.6 Accumulation, distribution and chemical status of heavy metals in plants

Assessment of heavy metal concentrations in food crops is done through accumulation factor calculation (Table 2.4), which is the expression of metal concentrations in soil in relation with concentrations in plant (Eq. 4) (Li et al. 2012; Khan et al. 2010a). Heavy metals pass through a series of processes including bioaccumulation, transformation and biomagnification after entering into food chain, therefore it is very difficult to remove heavy metals from living organisms (Widowati, 2012).

Soil to plant transfer and accumulation of heavy metals is a matter of concern and has been presented in a number of articles (Table 2.4) and a key component for health risk

assessment (Khan et al. 2008a; Wang et al. 2006b; Liu et al. 2005c; Mapanda et al. 2005; Rattan et al. 2005; Cui et al. 2004; Fismes et al. 2002; Huq et al. 2001). Higher soil to plant transfer of heavy metals means strong accumulation of these metals in plant tissues (Khan et al. 2008a). Wang et al. (2006b) and Khan et al. (2008a) described that there is inverse relationship between soil total metal concentrations and soil to plant transfer factor. However, the soil to plant transfer is calculated on the basis of total metal concentrations in soil (Hooda et al. 1997). The bioaccumulation factor of heavy metals is measured by the ratio of metal concentrations in plants to metal concentrations in that soil (Eq. 4) (Mattina et al. 2002).

Food plants have the tendency to accumulate heavy metals in their tissues. Non essential elements entering our environment may lead to bioaccumulation by plants (Kashif et al. 2009). Trace elements have the capability to interact with roots through absorption in polluted soil environment and increasing risk of toxic effects on plants and animals (Rosselli et al. 2006). Plant analysis, both wild and cultivated plants, for heavy metals is one of the major sources for assessment of contaminated soil (Blaylock et al. 2003; Ernst, 1996; Wenzel et al. 1993), these plants act as bio-indicators for local and large scale soil pollution (Zupan et al. 2003; Baker et al. 2000; Baker and Brooks, 1989).

The accumulation factors (Table 2.4) and daily intake of heavy metals (Table 2.5) commonly calculated using the following formulae.

$$PTF = \frac{C_{plant}}{C_{soil}} \quad \text{Eq. 4}$$

Where C_{plant} and C_{soil} are the concentrations of heavy metal in plants and soil respectively (Khan et al. 2010a; Cui et al. 2005; Mohamed et al. 2003). Table 2.4 summarizes the soil to plant transfer factor of heavy metals. Results show that tomato, in general, has high bioaccumulation factor among vegetables (Noor-ul-Amin et al. 2013).

$$DIM = \frac{C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{food intake}}}{BW_{\text{average weight}}} \quad \text{Eq. 5}$$

DIM is daily intake of metals, C_{metal} represents metal concentrations in plant; C_{factor} is conversion factor (fresh weight to dry weight); $D_{\text{food intake}}$ shows daily vegetables intake and $BW_{\text{average weight}}$ is average body weight. According to FAO (2000) average daily intake of vegetables for adults and children are 0.345 and 0.232 kg person⁻¹ day⁻¹ respectively, Whereas the average body weight for adults and children were considered as 73 (FAO, 2000) and 32.7 kg respectively (Khan et al. 2010a; Wang et al. 2005; Ge, 1992). The daily food intake by a single individual is 55, 105 and 445 g day⁻¹ for pulses, vegetables and cereals, respectively (Tripathi et al. 1997).

Generally heavy metals have the capability to migrate from polluted soil to plant tissues (Stasinou and Zabetakis, 2013). Toxic metals can accumulate in different plant organs, however the accumulation rate varies from organ to organ (McLaughlin et al. 1999; Wagner, 1993) some organs accumulate more than others. Bioaccumulation and transportation of heavy metals in plants is the major human exposure pathway to soil contamination through food chain (Khan et al. 2014; Yu et al. 2006; Cui et al. 2004; Watanabe et al. 2000; Lacatutsu et al. 1996; Ryan et al. 1982). Bunzl et al. (1999) reported that information regarding total metal concentrations in polluted soil is insufficient to know about the mobility and availability of heavy metals to living organism.

Heavy metals need some specific media for mobility from soil to plants and within the plants from one organ to another organ, for this purpose they make bond with metal sulfur ligands and organic acids. Lugon-Moulin et al. (2004) and Grill et al. (1989) conveyed that heavy metals have the capability to bind with phytochelatins and glutathione and accumulate in different plant organs.

Table 2.4. Soil to plant transfer of heavy metals

Plants	As	Cd	Cr	Cu	Ni	Pb	Reference
Tomato	N.A	N.A	11	22	9	14	Noor-ul-Amin et al.
Onion	N.A	N.A	10	15.5	1	12.5	2013
Tomato	0.0070	N.A	N.A	N.A	N.A	N.A	Kar et al. 2013
Spinach	0.0065	N.A	N.A	N.A	N.A	N.A	
Cabbage	0.0022	N.A	N.A	N.A	N.A	N.A	
Lettuce	N.A	1.5	N.A	0.26	N.A	0.05	Xu et al. 2013b
Cabbage	N.A	0.4	N.A	0.12	N.A	0.03	
Lettuce	N.A	0.59	N.A	0.090	N.A	0.002	Waterlot et al. 2013
Bean	N.A	N.A	N.A	0.07	N.A	N.A	Ebbs et al. 2006
Tomato	N.A	N.A	N.A	0.06	N.A	N.A	
Carrot	N.A	N.A	N.A	0.06	N.A	N.A	
Rice	N.A	0.2		0.013	N.A	0.005	Zhuang et al. 2009
Slender amaranth (Ghinri)	N.A	1.135	0.326	0.677	N.A	0.399	Abbasi et al. 2013
Glossy nightshade (Kach mach)	N.A	1.776	0.385	0.735	N.A	0.573	
Lettuce	N.A	0.239	N.A	0.004	N.A	0.003	Luo et al. 2011
Brassica oleracea	N.A	0.943	N.A	0.007	N.A	0.011	
Potato	0.46	0.40	0.39	0.40	0.41	0.40	Cheraghi et a. 2013
Maiz	N.A	1.14	0.07	0.43	0.56	N.A	Khan et al. 2013 a,
Wheat	N.A	1.08	0.04	0.58	0.63	N.A	b
Rice	N.A	0.85	N.A	0.45	0.45	N.A	
Potato	N.A	0.98	0.25	0.08	0.13	N.A	
Rice	0.01	0.17	N.A	0.1	N.A	0.02	Liu et al. 2005a
Cabbage	N.A	0.26	0.01	0.16	0.03	1.27	Gebrekidan et al.
Tomato	N.A	0.23	0.01	0.09	0.01	1.26	2013
Onion	N.A	0.22	0.01	0.09	0.02	1.23	
Potato	N.A	0.26	0.01	0.1	0.01	0.78	
Ladies finger	0.001	N.A	<1.0	<1.0	<1.0	<1.0	Alam et al. 2003
Brinjal	0.014	N.A	<1.0	<1.0	<1.0	<1.0	
Potato	0.006	N.A	<1.0	<1.0	<1.0	<1.0	
Spinach	N.A	N.A	0.67	0.23	0.14	0.99	Chary et al. 2008
Brinjal	N.A	N.A	0.48	0.05	3.1	0.54	
Ladies finger	N.A	N.A	0.59	0.06	0.02	0.61	

Cabbage	0.02–0.05	0.47–4.69	0.02–0.06	0.23–0.53	N.A	0.07–0.29	Wang et al. 2012b
Lettuce	0.05–0.11	1.76–4.10	0.00–0.07	0.29–0.53	N.A	0.09–0.25	
Radish	0.02–0.05	0.30–2.01	0.00–0.02	0.15–0.28	N.A	0.00–0.06	

Table 2.5. Daily dietary intake of heavy metals via consumption of contaminated food (mg kg⁻¹ day⁻¹)

Plants	Individual	As	Cd	Cr	Cu	Ni	Pb	Reference
MAL/RDI*	Adults	0.015**	0.050a	0.02-0.03b	0.75-0.90b	0.7–1b	0.050a	^a EC 2006
							0.01-0.02b	^b FNB 2004
MAL/RDI*	Adults	0.58a	0.072b	N.A	1.5–3.0b	N.A	0.429b	^a Warren et al. 2003
								^b Iyengar and Nair 2000.
RfD*****	Adults + Children	3.0 x10 ⁻⁴	1 x10 ⁻³	3.0 x10 ⁻³	4.0x10 ⁻⁴	2.0 x10 ⁻²	N.A	USEPA 2012
Spinach	Adults	N.A	8.4x10 ⁻⁴	N.A	4.5x10 ⁻³	2.8x10 ⁻³	7.3x10 ⁻³	Khan et al. 2010a
	Children	N.A	1.3x10 ⁻³	N.A	6.7x10 ⁻³	4.2x10 ³	1.1x10 ⁻²	
Lettuce	Adults	N.A	3.4x10 ⁻⁴	N.A	9.6x10 ⁻³	4.2x10 ⁻³	5.9x10 ⁻³	
	Children	N.A	5.1x10 ⁻⁴	N.A	1.4x10 ⁻²	6.3x10 ⁻³	8.9x10 ⁻³	
Tomato	Adults	N.A	N.A	3.0	10.0	3.0	6.5	Noor-ul-Amin et al. 2013
	Children	N.A	N.A	3.7	12.0	3.4	7.5	
Onion	Adults	N.A	N.A	2.5	7.0	0.25	5.9	
	Children	N.A	N.A	3.0	8.0	0.3	6.9	
Rice	Adults	N.A	3.16 x10 ⁻³	N.A	N.A	N.A	N.A	Minh et al. 2012
	Children	N.A	2.27 x10 ⁻³	N.A	N.A	N.A	N.A	
Vegetables***	Adults	N.A	1.9 x10 ⁻²		3.62 x10 ⁻¹		6.2 x10 ⁻²	Xu et al. 2013b
	Children	N.A	N.A	N.A	N.A	N.A	N.A	

Vegetables	Adults	N.A	3.8×10^{-4}	1.7×10^{-3}	1.3×10^{-2}	5.5×10^{-3}	1.1×10^{-3}	Hu et al. 2013
	Children	N.A	N.A	N.A	N.A	N.A	N.A	
Rice	Adults	1.2×10^{-2}	4.0×10^{-3}	6.0×10^{-3}	1.08×10^{-1}	N.A	N.A	Antoine et al. 2012
	Children	N.A	N.A	N.A	N.A	N.A	N.A	
Rice	Adults	N.A	1.7×10^{-1}	N.A	1.72	N.A	0.43	Zhuang et al. 2009
Vegetables	Adults	N.A	8.0×10^{-2}	N.A	6.3×10^{-1}	N.A	1.8×10^{-1}	Luo et al. 2011
Maize	Adults	N.A	4.3×10^{-5}	1.3×10^{-5}	7.5×10^{-5}	1.1×10^{-4}	N.A	Khan et al. 2013a, b
	Children	N.A	6.4×10^{-5}	1.9×10^{-5}	1.1×10^{-4}	1.7×10^{-4}	N.A	
Wheat	Adults	N.A	4.0×10^{-5}	7.4×10^{-6}	1.0×10^{-4}	1.3×10^{-4}	N.A	
	Children	N.A	6.1×10^{-5}	1.1×10^{-5}	1.5×10^{-4}	1.9×10^{-4}	N.A	
Rice	Adults	N.A	3.2×10^{-5}	N.A	7.8×10^{-5}	9.1×10^{-5}	N.A	
	Children	N.A	4.8×10^{-5}	N.A	1.2×10^{-4}	1.4×10^{-4}	N.A	
Potato	Adults	N.A	3.7×10^{-5}	4.8×10^{-5}	1.3×10^{-5}	2.7×10^{-5}	N.A	
	Children	N.A	5.5×10^{-5}	7.1×10^{-5}	2.0×10^{-5}	4.0×10^{-5}	N.A	
Vegetables***	Adults	0.14	0.10	N.A	1.61	N.A	0.41	Liu et al. 2005a
Cereals	Adults	0.21	0.09	N.A	146	N.A	0.08	
Pulses	Adults	0.02	0.01	N.A	0.57	N.A	0.01	
Potato	Adults	2.0×10^{-2}	4.5×10^{-4}	8.1×10^{-3}	5.6×10^{-3}	6.8×10^{-3}	2.0×10^{-3}	Cheraghi et al. 2013
	Children	2.0×10^{-2}	5.3×10^{-4}	9.5×10^{-3}	6.5×10^{-3}	7.9×10^{-3}	2.0×10^{-3}	
Rice	Adults	0.174	N.A	N.A	2.633	0.488	N.A	Roychowdhury et al. 2003
	Children	0.093	N.A	N.A	1.404	0.26	N.A	
Vegetable	Adults	0.0104	N.A	N.A	0.795	0.18	N.A	

	Children	0.0209	N.A	N.A	0.475	0.108	N.A	
Radish	Adults	N.A	5.1×10^{-4}	1.1×10^{-3}	9.4×10^{-3}	1.1×10^{-3}	N.A	Khillare et al. 2012
Spinach	Adults	N.A	8.1×10^{-4}	2.3×10^{-3}	1.3×10^{-2}	6.4×10^{-3}	N.A	
Potato	Adults	N.A	5.0×10^{-5}	N.A	2.0×10^{-3}	N.A	6.0×10^{-4}	Mansour et al. 2009
Tomato	Adults	1.7×10^{-4}	N.A	N.A	N.A	N.A	N.A	Madeira et al. 2012
	Children	2.12×10^{-4}	N.A	N.A	N.A	N.A	N.A	
Vegetables	Adults	N.A	N.A	3.3×10^{-4}	1.12×10^{-3}	8.1×10^{-4}	6.1×10^{-4}	Chary et al. 2008
Lettuce	Adults	2.70×10^{-4}	2.50×10^{-4}	7.10×10^{-4}	5.40×10^{-3}	N.A	4.90×10^{-4}	Wang et al. 2012b
	Children	2.70×10^{-4}	2.50×10^{-4}	7.10×10^{-4}	5.40×10^{-3}	N.A	4.90×10^{-4}	
Cabbage	Adults	1.50×10^{-4}	9.30×10^{-5}	4.70×10^{-4}	4.80×10^{-3}	N.A	2.80×10^{-4}	
	Children	1.70×10^{-4}	1.10×10^{-4}	5.40×10^{-4}	5.60×10^{-3}	N.A	3.20×10^{-4}	
Radish	Adults	1.10×10^{-4}	7.60×10^{-5}	2.80×10^{-4}	2.40×10^{-3}	N.A	1.10×10^{-4}	
	Children	1.30×10^{-4}	8.70×10^{-5}	3.30×10^{-4}	2.80×10^{-3}	N.A	1.30×10^{-4}	
Vegetables	Adults	N.A	1.33×10^{-2}	N.A	3.3×10^{-4}	2.31×10^{-5}	2.5×10^{-2}	Elbagermi et al. 2012
Fruits	Adults	N.A	5.54×10^{-3}	N.A	2.05×10^{-4}	1.16×10^{-4}	3.7×10^{-2}	
Rice	Adult	9.3×10^{-3}	2.4×10^{-3}	N.A	0.2	N.A	N.A	Batista et al. 2010
Rice	Adult	20.85×10^{-3}	N.A	N.A	N.A	N.A	N.A	Batista et al. 2011

*MAL/RDA; Maximum allowable limit/ recommended dietary allowance

**Provisional Tolerable Weekly Intake; Codex 1995

*** Value calculated for group of vegetables

****Oral reference dose

2.7 Metals toxicity and tolerance

Heavy metals are shown toxicological effects on plants and animals and their degree of toxicities vary from species to species and from metal to metal. It is known from the literature that metal toxicity is associated with their speciation in soil; however it is difficult to determine some specific species because of their complex nature, functioning and distribution in soil environment (Czupyrán and Levy, 1989). The bioavailability and toxicity of heavy metals to living organism is dependent on soil physiochemical parameters and microbiology (Alam et al. 2003; Islam et al. 2000). Prasad (1999) discussed that the heavy metals toxicity may be influenced by changes in its characteristics such as pH.

Heavy metals interact with essential micro- nutrients and affect their uptake and transport by plants (Hernández et al. 1998; Thys et al. 1991) effecting plant growth and their physiological functions. Hernández et al. (1995) observed that at high Cd concentrations plant elicited toxic symptoms and caused the growth retardation. Similarly, Cd even at low concentration can exert toxicological effects on seed germination and growth (Li et al. 2005a) and Cu is also toxic to seedling growth (Munzuroglu and Geckil, 2002).

The genotoxicity of heavy metals to living organisms involves interaction of heavy metals with genetic material via binding with DNA basis (Hossain and Huq, 2002). A number of transgenic and bioassay techniques have been recommended for assessing the genotoxic effects of environmental pollutants on plants including RAPDs (random-amplified polymorphic DNAs), micronucleus (MCN) induction or Comet assay (Liu et al. 2005b; DeWolf et al. 2004; Kovalchuk et al. 2001; Angelis et al. 2000; Arkhipchuk et al. 2000; Steinkellner et al. 1998).

For heavy metals toxicity assessment various plant species are recommended depending on soil properties. Lettuce is used for toxicity assessment in moderate pH environment (pH 6-8)

(Chapman et al. 2010, 2012; ISO, 2005b; Sheppard et al. 1993) because it is not a good acid tolerant specie (Chapman et al. 2012; Environment Canada, 2005). Among higher plants Oat is recommended for toxicity tests of heavy metals (ISO, 2005a; Chang et al. 1997) under low pH conditions (Loureiro et al. 2006; Bilski and Foy, 1987; Small and Jackson, 1949), while *Allium cepa* is Pb tolerant in acidic environment (Dang et al. 1990). Heavy metals tolerance capability of plants may be associated with their chemical form (Yang and He, 1995). Similarly, metal induced reactive oxygen species (ROS) are responsible for peroxidation of fatty acid. To avoid the toxic effect of ROS plants adopt different defense strategies including antioxidant enzymes (Gill and Tuteja, 2010). Nitrogen and sulfur are essential nutrients responsible for protein and glutathione synthesis providing defensive mechanisms for heavy metals tolerance (Liedschulte et al. 2010; Sarwar et al. 2010; Zechmann and Müller, 2010; Anjum et al. 2008). The recent studies conducted on plant tolerance mechanism to environmental pollutants showed that heavy metals have strong influence on leaf size and structure and the first toxicity symptoms appear on leaves, thus measuring length to width ratio can be very effective in evaluating plant tolerance to heavy metals (Zhang et al. 2014).

Plants can evolve an advance and complex detoxification mechanism to avoid metal toxicity which comprise of selective uptake, chelation, compartmentalization and secretion of toxic metals (Pourrut et al. 2013). Phytochelatins play a key role in metal detoxification (Cobbett, 2000). The *Brassica juncea* (BjCdR15) and *Arabidopsis* TGA3 are the good tolerants to high metal concentrations because of the mechanisms such as the regulation of synthesis of phytochelatin by BjCdR15/TGA3 (Farinati et al. 2010). Similarly, different genes are involved in metal tolerance by mediating metal–glutathione conjugates transport (Kim et al. 2006). Plant protein actively involved in heavy metal homeostasis and metal tolerance (Suzuki et al. 2002).

2.8 Effects on plants growth

Heavy metals are phytotoxic in nature and have significant negative impacts on plant growth and even at lower concentrations they inhibit plant growth (Di Salvatore et al. 2008; Fjällborg et al. 2005; Li et al. 2005a). Under high concentrations of heavy metals the plant growth drastically reduces (Chaves et al. 2011; Gopal and Khurana, 2011; Kumari et al. 2011; Manivasagaperumal et al. 2011). High concentrations of heavy metals in the soil greatly affect the growth and metabolic processes of cultivated crops (John et al. 2009; Sinha et al. 2005). Heavy metals induced oxidative stresses, affect the photosynthetic process and cause growth retardation (Le Guédard et al. 2012; Bibi and Hussain, 2005; Wani et al. 2006). Elevated concentrations of heavy metals may result in stunted growth by hampering the photosynthetic machinery and disturbing the coordination mechanism between essential elements (Gill et al. 2012; Astolfi et al. 2004) ultimately causing plant death (Sanita di Toppi and Gabbrielli, 1999).

Cu is an essential element that plays an important role in various physiological functions and plant growth, however at higher concentration it becomes toxic and affects plant growth and interferes with normal physiological functions (Bouazizi et al. 2010; Hansch and Mendel, 2009; Upadhyay and Panda, 2009). Excess of heavy metals like Cu may cause root growth inhibition and damage the plasma membrane resulting in ion leakage from the cells (Bouazizi et al. 2010). Soares et al. (2001) presented that heavy metals greatly affect the plant growth, developmental processes and cell division. A reduction from 18-77% has been observed in plant height cultivated in metal contaminated soil (Chaves et al. 2011; Gopal and Khurana, 2011). A significant decrease was observed in the leaf area of plants grown on metal contaminated soil. This is because of changes in protein synthesis, photosynthetic activities and respiration (Chaves et al. 2011). High concentration of Cd has significantly negative impact on lettuce shoot growth

(Monteiro et al. 2007). This decrease may be due to metals induced chromosomal aberration (Seregin and Kozhevnikova, 2006).

2.9 Effects on plant structure

The effects of heavy metals on plant structure are well known. Bini et al. (2012) stated that high metal concentrations in plants have strong effect on plant morphology. Plants, under high metal stress, show clear symptoms of structural changes including absence of palisade structure, reduce leaf thickness and structural changes in mitochondria (Bini et al. 2012). Heavy metals strongly influence the structure of plant components and cause toxicological symptoms (Lindsey and Lineberger, 1981). The toxicity on cellular structure and function of organisms varies according to toxic potential of heavy metal (Saraf and Samant 2013). Trace elements elicit toxicity symptoms on plants root, shoot and leaf structure (Mangabeira et al. 2001; Vasquez et al. 1991). Plant cellular metabolism disruption due to heavy metals, may cause structural changes in cell membrane (Prasad, 1995) and chloroplast and results in reduced photosynthesis (Mahmood et al. 2010; Li et al. 2005b; Ramos et al. 2002). Similarly, high concentration of Fe produces free radicals which may damage cell membrane, protein and DNA and alter cellular structure (de Dorlodot et al. 2005; Arora et al. 2002).

2.10 Metal exposure and human effects

Toxicological effects to human beings from consumption of metals contaminated food are based on various factors including chemical forms of heavy metals, dose, exposure route, time, frequency, age, gender, nutritional source and biological species (Tchounwou et al. 2004; Causy et al. 2003). Fig. 2.1 shows the heavy metals interaction with human health effects. Similarly, personal and environmental hygiene are also play important roles in subsequent exposure to metal toxicity. Human health and soil quality are strongly related with each other especially to the

degree of pollution (Velea et al. 2009). The tendency of food plants to accumulate heavy metals in their tissues is a public health concern, because they are toxic to human health and plant tissues. Some metals are required for human body, while others (As, Cd, Pb etc) are toxic in nature even at trace concentrations (Gebrekidan et al. 2013; Khan et al. 2010a; Mitra et al. 2009; Yargholi and Azimi, 2008) and associated with carcinogenic health risks (Abbasi et al. 2013). Similarly, consumption of As contaminated vegetables can cause serious health risks (Batista et al. 2011). Many heavy metals are potential carcinogens (Kim et al. 2009; Lim et al. 2008; Lee et al. 2006) and can cause organ dysfunction and damage. World Health Organization, International Agency for Cancer Research (2012), USEPA /IRIS (2012) California EPA (2011) and U.S. Department of health and human services (2011) have declared As, Cd, Cr, Ni and Pb as human carcinogens.

Food plants contamination with heavy metals and their potential impacts on plant nutritional values is an emerging health issue because of their strong links with each other (Sharma et al. 2009; Zaidi et al. 2005). Toxicological effects of metal contaminated vegetables mainly associated with mineral contents due to their toxicological and nutritional characteristics (Fig. 2.1) (Weldegebriel et al. 2012; Yang et al. 2011; Chary et al. 2008).

The common route of entrance of heavy metals to human body is through ingestion, inhalation and dermal contacts (Kim et al. 2009), which result in minor to major health problems including diarrhea, nausea, lung diseases, anemia, kidney disorders, stomach problems, skin diseases, neurological disorders and cancer (NLM/HSDB, 2012; Chary et al. 2008). Some of the disorders are caused by acute toxicity, while others through chronic exposure.

Heavy metals have the capability to accumulate in different body parts, and cause adverse health impact regardless of their concentrations (Duruibe et al. 2007; Ikeda et al. 2000). The

toxicological effects of toxic metals may vary from person to person. Higher concentrations of non essential elements have effects on the reproductive capability of living organisms (Oetken et al. 2004). For health risk assessment associated with heavy metals contamination of soil a mathematical model should be established to predict the bioaccumulation and transformation of these toxic metals (Hough et al. 2003).

Health risk is the associated health hazard of some specific chemicals and/or phenomenon in a specific environment and/or environmental conditions depend upon exposure duration and availability of receptors (Alexander, 1995). Human health risk assessment is generally done through evaluating the health risk index (HRI), daily intake of metal, reference dose and general body weight of children and adults (Eq 6) (Li et al. 2012; Pandey et al. 2012; Luo et al. 2011; Singh et al. 2010a; Cui et al. 2004). Other techniques used for assessing carcinogenic and non carcinogenic health risks associated with heavy metals are target cancer risk (TCR) (Eq 9), hazard index (HI) (Eq 8) and target hazard quotient (THQ) (Eq 7), respectively (Yang et al. 2011; USEPA, 2006, 2010). The different equations used for calculating the health risks are given as under:

$$HRI = \frac{\sum n(C_n \times D_n)}{RfD - B_w} \quad \text{Eq 6}$$

$$THQ = \frac{(MC \times FI \times EFr \times ED)}{RfD_o \times BW \times AT} \times 10^{-3} \quad \text{Eq 7}$$

$$HI = THQ_1 + THQ_2 + \dots + THQ_n \quad \text{Eq 8}$$

$$TCR = \frac{(Cb \times I \times 10^{-3} \times CPSO \times EFr \times ED_{tot})}{(BW_a \times AT_c)} \quad \text{Eq 9}$$

Where Eq 6 C_n represented heavy metal concentrations in vegetables (mg kg^{-1} , fresh

weight), D_n daily intake of vegetables per year, RfD means reference oral dose (Table 2.5) and B_w is body weight for children and adults (Eq5). In Eq 7 C is metal concentrations in vegetables, I is the ingestion rate ($255 \text{ g day}^{-1} \text{ person}^{-1}$), EF_r is the exposure frequency ($350 \text{ days}^{-1} \text{ year}$), ED is the total exposure duration (70 years), BW is the average body weight and AT_n is the non-carcinogen's averaging time, $(ED \times 365 \text{ day}^{-1} \text{ year})$ (USEPA, 2006; Chien et al. 2002). THQ is target hazard quotient for heavy metals (Eq 8). CPSo represent the carcinogenic potency slope, oral ($\mu\text{g g}^{-1} \text{ day}^{-1}$); ATc represent the carcinogens averaging time ($70 \times 365 \text{ days}$) (Eq.6). The human health risk index for selected heavy metals and vegetables and different age groups are summarized in Table 2.6.

Studies on health risk assessment shows that children are more susceptible to heavy metal pollution than adults because of their physiological and behavioral characteristics (DHAenC, Department of Health and Aging and enHealth Council, 2012; Qu et al. 2012; Zota et al. 2011; Man et al. 2010). Health risk assessment is the evaluation of potential health effects to target population exposed to certain toxic media. It is the key procedure for hazardous substance management, designing remediation policies and taking control measures (McGraph et al. 2004; Khan and Husain, 2001).

Table 2.6. Human health risk index of heavy metal through consumption of vegetables

Plants	Individuals	As	Cd	Cr	Cu	Ni	Pb	Reference
Tomato	Adults	7.97×10^{-2}	N.A	N.A	N.A	N.A	N.A	Kar et al. 2013
Spinach	Adults	6.06×10^{-2}	N.A	N.A	N.A	N.A	N.A	
Cabbage	Adults	3.08×10^{-2}	N.A	N.A	N.A	N.A	N.A	
Lettuce	Adults	N.A	3.4×10^{-1}	N.A	2.4×10^{-1}	2.1×10^{-1}	1.7	Khan et al. 2010a
	Children	N.A	5.1×10^{-1}	N.A	3.6×10^{-1}	3.2×10^{-1}	2.5	
Spincach	Adults	N.A	8.4×10^{-1}	N.A	1.1×10^{-1}	1.4×10^{-1}	2.1	
	Children	N.A	1.3	N.A	1.7×10^{-1}	2.1×10^{-1}	3.1	
Vegetables	Adults	N.A	6.33×10^{-1}	N.A	1.51×10^{-1}	N.A	2.95×10^{-1}	Xu et al. 2013b
Vegetables	Adults	N.A	3.6×10^{-1}	1.1×10^{-3}	3.2×10^{-1}	2.7×10^{-1}	2.9×10^{-1}	Hu et al. 2013
Rice	Adults	N.A	2.5	N.A	1.5×10^{-1}	N.A	1.0×10^{-1}	Zhuang et al. 2009
Potato	Adults	N.A	4.5×10^{-1}	N.A	1.4×10^{-1}	3.4×10^{-2}	5.7×10^{-1}	Cheraghi et al. 2013
	Children	N.A	5.3×10^{-1}	N.A	1.6×10^{-1}	3.9×10^{-2}	5.7×10^{-1}	
Glossy nightshade (Kach mach)	Adults	N.A	4.6×10^{-2}	2.63×10^{-1}	9.0×10^{-3}	N.A	1.78×10^{-1}	Abbasi et al. 2013
Slender amaranth (Ghinri)	Adults	N.A	4.0×10^{-2}	1.76×10^{-1}	7.0×10^{-3}	N.A	2.36×10^{-1}	
Vegetables	Adults	N.A	2.71	N.A	2.6×10^{-1}	N.A	8.6×10^{-1}	Luo et al. 2011
Maize	Adults	N.A	8.5×10^{-2}	8.4×10^{-6}	2.0×10^{-3}	5.6×10^{-3}	N.A	Khan et al. 2013a, b

Wheat	Children	N.A	1.3×10^{-1}	1.3×10^{-5}	3.0×10^{-3}	8.4×10^{-3}	N.A	
	Adults	N.A	8.1×10^{-2}	5.0×10^{-6}	2.7×10^{-3}	6.3×10^{-3}	N.A	
Rice	Children	N.A	1.2×10^{-1}	7.4×10^{-6}	4.1×10^{-3}	9.5×10^{-3}	N.A	
	Adults	N.A	6.4×10^{-2}	NC	2.1×10^{-3}	4.6×10^{-3}	N.A	
Potato	Children	N.A	9.5×10^{-2}	NC	3.2×10^{-3}	6.9×10^{-3}	N.A	
	Adults	N.A	7.3×10^{-2}	3.2×10^{-5}	3.5×10^{-4}	1.3×10^{-3}	N.A	
Radish	Children	N.A	1.1×10^{-1}	4.8×10^{-5}	5.3×10^{-4}	2.0×10^{-3}	N.A	
	Adults	N.A	5.1×10^{-1}	7.3×10^{-4}	2.4×10^{-1}	5.5×10^{-2}	N.A	
Spinach	Adults	N.A	8.1×10^{-1}	1.5×10^{-3}	3.3×10^{-1}	3.2×10^{-1}	N.A	Khillare et al. 2012
Potato	Adults	N.A	7.0×10^{-4}	N.A	1.0×10^{-4}	N.A	1.0×10^{-3}	Mansour et al. 2009
Tomato	Adults	6.0×10^{-1}	N.A	N.A	N.A	N.A	N.A	Madeira et al. 2012
	Children	7.0×10^{-1}	N.A	N.A	N.A	N.A	N.A	
Rice	Adults	N.A	3.16	N.A	N.A	N.A	N.A	Minh et al. 2012
	Children	N.A	2.27	N.A	N.A	N.A	N.A	
Lettuce	Adults	9.10×10^{-1}	2.50×10^{-1}	4.70×10^{-4}	1.40×10^{-1}	N.A	1.40×10^{-1}	Wang et al. 2012b
	Children	9.10×10^{-1}	2.50×10^{-1}	4.70×10^{-4}	1.40×10^{-1}	N.A	1.40×10^{-1}	
Cabbage	Adults	4.80×10^{-1}	9.30×10^{-2}	3.10×10^{-4}	1.20×10^{-1}	N.A	7.90×10^{-2}	
	Children	5.60×10^{-1}	1.10×10^{-1}	3.60×10^{-4}	1.40×10^{-1}	N.A	9.10×10^{-2}	
Radish	Adults	3.80×10^{-1}	7.60×10^{-2}	1.90×10^{-4}	6.10×10^{-2}	N.A	3.30×10^{-2}	
	Children	4.40×10^{-1}	8.70×10^{-2}	2.20×10^{-4}	7.00×10^{-2}	N.A	3.80×10^{-2}	

2.11 Heavy metals effect on nutritional values

Balance diet is the key requirement of good health. Vegetables are good sources of macro- and micro- nutrients that meet daily nutrient requirements. However, no single vegetable has all nutrients to fulfill the dietary requirements. Therefore, for a balance diet a diversified nutritional regime is required to meet daily nutrient requirements (Grusak and Dellapenna 1999). FAO/WHO has set nutritional standards for children and adults at different age groups which are summarized in Table 2.7. Heavy metals at high concentrations in food chains and their consumption can cause depletion of essential nutrients in the body leading to serious health hazards (Arora et al. 2008; US Department of health and human services, 2005).

Table 2.7. Daily nutrient requirements ((mg-day⁻¹)^a, (µg day⁻¹)^b) for male, female and different age groups (FAO/WHO 2001b)

Sex/ Age group	Vitamin A (RE) ^b	Vitamin C ^a	Thiamin ^a	Riboflavin ^a	Folate ^b	Iron ^a	Zn ^a	Ca ^a	Mg ^a
Infant (0-12 month)	375-400	25-30	0.2-0.3	0.3-0.4	80	9.3	6.6-8.4	300-400	26-54
Children (1-9 y)	400-500	30-35	0.5-0.9	0.5-0.9	160-300	5.8-8.9	8.3-11.2	500-700	60-100
Male (10-18 y)	600	40	1.2	1.3	400	14.6-18.8	17.1	1300	230
Female (10-18 y)	600	40	1.1	1.0	400	14-32.7	14.4	1300	220
Male (19-65 y)	600	45	1.2	1.3	400	13.7	14.0	1000	260
Female (19-65 y)	500	45	1.1	1.1	400	29.4	9.8	1000	220
Pregnancy	800	55	1.4	1.4	600	N.A	11-20	1200	N.A
Lactation	850	70	1.5	1.6	500	15	14.4-19	1000	N.A

It is agreed upon that ROS is responsible, in part, for metals contamination of plants resulting in oxidative damages of proteins, lipids and nucleic acid, which in turn are responsible for various physiological disorders such as growth retardation, nutrient deficiency, reduced transport of nutrients, genotoxicity and retarded photosynthesis (Le Guédard et al. 2012; Nagajyoti et al. 2010; Upadhyay and Panda, 2009). Similarly, along with the above mentioned disorders the ROS may result in enzyme inactivation and DNA damages (Pourrut et al. 2013; Saxena and Shekhawat, 2013). Heavy metals alter the functions of antioxidative enzymes as well as its contents in plant cells and affect the oxidative stresses (Sharma and Dietz, 2009; Gallego et al. 1999) that may lead to plant death. Heavy metals are responsible for genetic instability in plants and result in DNA damage ((Liu et al. 2005b; Gichner et al. 2004; Steinkellner et al. 1998). Like other physiological disorders toxic metals strongly influence the genetic characteristics like point mutation and recombination of plants at variable concentrations (Kovalchuk et al. 2001, 2005). It is impossible, to a specific portion of leafy vegetables, for the determination of nutritional components because the serving size varies greatly depending upon people's knowledge, seasons, food tradition and habits, availability of specific vegetables and economic condition of the community (Stangeland et al. 2009). Consumption of heavy metal contaminated food can lead to serious depletion of nutritional components like Fe and vitamins that may cause a number of physiological and pathological disorders (Iyengar and Nair, 2000). The influence of heavy metals on plant nutritional components depends on soil environment, type of food crops, exposure time and plants organs (Gonçalves et al. 2009). Literature shows that metal contaminated food consuming population were observed to be deficient of macro- and micro- nutrients including fats, proteins, vitamins and minerals like Ca, Fe and Zn (Nordberg, 1996; Fox, 1988). Similarly, heavy metals like Cd and micro- nutrients have strong interaction in

gastrointestinal absorption. Fe and Zn are the most important micro- nutrients involved in these interactions. This absorption involves intestinal Fe transporters (Ryu et al. 2004; Park et al. 2002). Women and children have higher body Cd than men because their Fe requirements are greater than men (Nishijo et al. 2004). Therefore adequate nutrition is vital for young children and pregnant women in the earlier stages to have optimal development and growth conditions (Christian, 2010).

2.11.1 Effects on contents of carbohydrates

Heavy metals have strong influence on carbohydrate contents of vegetables. Excessive accumulation of toxic elements may inhibit carbohydrate synthesis by destroying the photosynthetic electron transport chain and production of reactive oxygen species (Sandalio et al. 2001). High Pb concentration results a decrease in the sucrose content of vegetables and affects the taste of rooted vegetables (Gawęda, 2007). Kratovalieva and Cvetanowska (2001) reported that tomato plants cultivated in heavy metal polluted soil showed an increase in the total sugar contents.

Heavy metals affect the carbohydrate metabolism by different ways and produce changes in their contents, which may be due to inactivation and impairment of certain enzymes participating in carbohydrate synthesis (Gawęda, 2007; Vikas et al. 2002; Nagor and Vyas, 1997). At high Cd concentration a general decrease occurs in carbohydrate metabolism, while glycolytic pathway observes an up regulation under heavy metal stresses (Rodríguez-Celma et al. 2010). Similarly, elevated Cd concentration significantly alters plant physiology that results in low carbohydrate metabolism (Chaffei et al. 2004). At lower concentration trace metal interrupts with photosynthetic activities, carbohydrate metabolism, assimilation of essential macro-nutrient and enzyme activities (Sanita di Toppi and Gabbrielli, 1999; Van Assche and Clijsters, 1990).

Heavy metals have the capability to replace chlorophyll Mg and alter its function (Kowalewska et al. 1987). From the existing literature it is clear that heavy metals have strong influence on carbohydrates of both leafy and non leafy vegetables and consumption of such contaminated food can lead to a decrease in net carbohydrate contents that may result in malnutrition.

2.11.2 Effects on proteins and amino acids

Proteins are the basic dietary and functional component of food plants having various structural, functional and nutritional properties and consider as essential nutrients for human beings. Nitrogen and sulfur are essential nutrients required for synthesis of proteins and amino acids (Hesse et al. 2004). The deprivation of essential nitrogen and sulfur in plants may affect the metabolic process (Carfagna et al. 2010). The total protein contents may range from 1 to 7 g 100 g⁻¹ of leafy vegetables (Uusiku et al. 2010). The crude protein contents of different leafy vegetables vary greatly from specie to specie (Odhav et al. 2007; Mosha and Gaga, 1999) and the variation may be due to climatic conditions (Odhav et al. 2007) and structural and physiological characteristic. Heavy metals are responsible for low protein in plants grown on metal contaminated soil (Widowati, 2012). Heavy metals can significantly reduce protein synthesis as a result of physiological changes in plants (Chaffei et al. 2004). High metal concentration may hamper protein metabolism by altering physiological functions and synthetic activities (Sandalio et al. 2001). Heavy metals have the capability to interact with proteins and DNA which produce oxidative damages in plant molecules (Leonard et al. 2004). Non essential toxic elements, due to oxidative stress, can affect the thylakoid and protein complex resulting a decrease in photosynthesis (Linger et al. 2005). Higher concentrations of toxic metals have effects on the decomposition of protein contents (Wu et al. 2014). Heavy metals induced membrane damage due to changes in functions and composition of membrane lipids and proteins (Meharg, 1993;

Van Assche and Clijsters, 1986). Similarly, high metal concentrations inhibit protein synthesis by altering the pigment–lipoprotein complex accumulation in photo-system 1 (Wang et al. 2009) II and effect ribulose-1, 5-bisphosphate carboxylase/oxygenase enzymes (Krantev et al. 2008).

Reports regarding heavy metal induced changes in protein concentrations have been contradictory. A number of proteins (fructokinase, enolase, GADPH and PGM) show decrease in concentrations in some plants species (*B. juncea* and poplar), while the same proteins show increase in other plants (*A. thaliana*) (Alvarez et al. 2009; Kieffer et al. 2009; Roth et al. 2006; Sarry et al. 2006).

2.11.3. Effects on fats and fatty acids

Literature shows that most of the studies regarding adverse impacts of heavy metals on food crop are focused upon bioaccumulation, transfer and health effects. Little knowledge is available on heavy metals impact on nutritional components, particularly lipids (Upchurch, 2008).

Heavy metal induced oxidative stress results in chloroplast degradation and lipid peroxidation (Khanna-Chopra, 2012), affecting the nutritional status of the contaminated plant. The photosynthetic activities of plants are also greatly affected by the contents of heavy metals (He et al. 2011; Velikova et al. 2011; Solti et al. 2008; Hermle et al. 2006). High Cd concentration results in lipid peroxidation (Monteiro et al. 2004) which is caused by metal induced lipoxygenase activity resulting in displacement of metal ions and altering the enzymatic activities (Wildner and Henkel, 1979).

Le Guédard et al. (2008) reported that the concentrations of tri-unsaturated fatty acids decreased when plant cultivated in heavy metal contaminated soil. In the same experiment they observed that the percentage of other fatty acids (C18:0, C18:1 and C18:2) was increased to

various levels. Thus they concluded that fatty acids can be used as a bio-indicator. Heavy metals show variable effects on different fatty acids as Djebali et al. (2005) and Ben Youssef et al. (2005) have observed that heavy metals have negative impact on C18:3 percentages, while other fatty acids were affected positively.

2.11.4. Effects on vitamins

Green leafy vegetables are considered as a rich source of nutrients like vitamins (Gupta and Bains, 2006). Vitamins are the essential nutrients for all living organisms and for plant microorganism association. Vitamin concentrations in soil have significant effects on plant growth (Bergner, 1997). The vitamin contents of plants show great variation in food crops provided with organic fertilizers to those with nitrogen fertilizers, plants grown with commercial fertilizers show significant reduction in vitamin contents (Price, 1945) which may be due to the presence of some trace elements.

Environment (physical, chemical and biological) has strong influence on vitamin contents and in extreme environment with high heavy metal concentrations, temperature and pH the vitamin contents are drastically reduced (Ipek et al. 2005). Vitamins are essential nutrients that play an important role in maintaining good health and providing protection against infectious diseases, augmenting immune system functions and provide protection against certain malignancies. However heavy metals have significant negative impacts on vitamin contents and functions (Pleasants et al. 1992; Sugawara et al. 1981). Similarly, metals induced lipid peroxidation which may reduce the vitamin contents (Tatli Seven et al. 2012) in the cultivated crops. There is negative correlation between the heavy metals and vitamins that is when heavy metal concentrations increased the vitamin contents decreased and vice versa (Widowati, 2012; Munzuroglu et al. 2005).

CHAPTER 3

Materials and Methods

3.1. Soil sampling, preparation and analyses

Soil samples were collected from a depth of 0-30 cm of the top soil, using stainless steel clean auger, air dried, sieved and then passed through a <2 mm screen. Basic soil physicochemical properties (pH, EC, OM, bulk density and soil texture) were investigated. The soil texture was determined using Soil Texture Triangle Bulk Density Calculator (Saxton et al. 1986). Soil pH and EC was measured at 1:2.5 soil to water ratio (Mendoza et al. 2015).

The total metal concentrations in soil samples were determined according to the standard procedure (Nezhad et al. 2014). Briefly, 0.5 g air dried soil samples were digested using concentrated HCl and HNO₃ at 3:1. The extracts were transferred to 50 ml tube and diluted with ultra pure water and the concentrations of elements were determined by Atomic Absorption Spectrophotometer (AAS, Perkin-Elmer model 2380). Soil bioavailable metals were extracted with 0.05 M EDTA (Merry et al. 1981) and analyzed using AAS-MODEL 2380.

3.2. Experimental design

Soil was spiked with selected toxic metals at different concentrations (Cd: 0, 1.0, 2.5 and 5.0 mg kg⁻¹, Pb: 0, 200, 300 and 400 mg kg⁻¹, As: 0, 0.5, 1.0 and 1.5 mg kg⁻¹, and Cd-Pb mix: 1.0/200, 2.5/300, and 5.0/400 mg kg⁻¹, As-Cd mix: 0.5/1.0, 1.0/2.5, and 2.5/5.0 mg kg⁻¹, As-Pb mix: 0.5/200, 1.0/300, and 2.5/400 mg kg⁻¹ and tri mixture, As-Cd-Pb: 0.5/1.0/200, 1.0/2.5/300, and 2.5/5.0/400 mg kg⁻¹) using CdSO₄, Pb(NO₃)₂ and Na₃AsO₄ salts, respectively. These treatments are represented by the symbols of elements and the figures after them show the level of contamination (e.g. Cd1 refers to level one contaminated with 1.0 mg kg⁻¹). The Cd, Pb and As salts were added to pots as single metals and binary and tri mixture following the order Cd as

Cd1 (1.0 mg kg⁻¹), Cd2 (2.5 mg kg⁻¹) and Cd3 (5.0 mg kg⁻¹), Pb as Pb1 (200 mg kg⁻¹), Pb2 (300 mg kg⁻¹) and Pb3 (400 mg kg⁻¹), As as As1 (0.5 mg kg⁻¹), As2 (1.0 mg kg⁻¹) and As3 (2.5 mg kg⁻¹) and binary mixture as Cd1+ Pb1 (CdPb1), Cd2+Pb2 (CdPb2), Cd3+Pb3 (CdPb3), As1+Cd1 (AsCd1), As2 + Cd2 (AsCd2), As3 + Cd3 (AsCd3), As1+ Pb1 (AsPb1), As2 + Pb2 (AsPb2) and As3 + Pb3 (AsPb3) and tri mixture as As1 +.Cd1 + Pb1 (Mix1), As2 + Cd2 + Pb2 (Mix2) and As3 + Cd3 + Pb3 (Mix3). A total mass of 4-8 kg of soil was added in to each pot depending on the type of vegetable. These treatments were performed in triplicates. Before seed sowing pots were irrigated with distilled water to 70% water holding capacity of soil. Basal NPK doses of 60, 35 and 40 mg kg⁻¹ were applied to each pot before cultivation. For NPK application, NH₄NO₃ and K₂HPO₄ were added to the soil. Irrigation was performed evenly to all pots when needed. All pots were randomly arranged and changed their position time to time to insure the equal amount of light reach to each pot.

Plant seeds (tomato and lettuce) were purchased from the local market, washed with deionized water and H₂O₂ and germinated in clean petri dishes. The seedlings were kept in control environment for one week with an average room temperature of 23±2 °C and a photoperiod of 12/12 (light/dark). After germination, equal numbers of fresh and healthy seedlings were transferred to the selected pots. Potato seeds were directly introduced to pots. The pots were kept in greenhouse under desired environmental conditions (Table 3.1).

Table 3.1. Environmental conditions during crop cultivation in greenhouse.

Crop	Plantation	Harvesting	Crop duration (days)	Light duration (h)	Max Temp °C *	Min Temp °C	Relative humidity (%)
Lettuce	5 th March 2013	10 th May 2013	66	745±12	26±3.5	15.5±3	37±7
Tomato	5 th March 2013	10 th July 2013	127	1703±19	29.5±5.7	17±2.7	29±8
Potato	3 rd Sept. 2013	27 th Nov. 2013	86	992±12	21±7	12±3	45±11

3.3. Vegetables preparation and extraction

The vegetable samples were washed with deionized water and oven dried at 70 °C to constant weight (Jordão et al. 2007). After grinding, 0.5 g sample was digested with 5 ml conc. HNO₃ for 1 h at 80 °C and further for 20 h at 120-130 °C using digestion block at the Department of Environmental Sciences, University of Peshawar. The extracts were transferred to 50 ml plastic tubes and the volume was raised to 50 ml by adding deionized water. The metal (Cd, Pb, Fe, Mn, Ca and Mg) concentrations were measured using AAS (Perkin-Elmer 2380). The Phosphorous (P) concentration was measured using UV-spectrophotometer, while potassium (K) concentration was measured using flame photometer.

3.4. Macro- nutrient analyses

The vitamin C contents were determined by Association of Analytical Chemist (AOAC, 1980) titrimetric procedure using 2,6 dichlorophenol indolephenol (Osunde and Makama, 2007). Total nitrogen (N) and protein contents were determined using Kjeldahl method. Briefly, 5 g of dried vegetable sample was macerated with a 15 ml glacial acetic acid and metaphosphoric mixture and 1 g activated charcoal. The samples were digested for 10 min, filtered and 10 ml of the concentrated acid mixture was added and the volume was adjusted to 100 ml with deionized

water. The vitamin C contents were calculated as;

$$\text{mg ascorbic acid / g sample} = V \times S \times D \quad (1)$$

Where V, S and D represent the volume of dye, standardization value in mg ascorbic acid and dilution factor (Osunde and Makama, 2007)

The total nitrogen content and total protein content (conversion factor: 5.70) were obtained according to the Kjeldahl method (Sovrani et al. 2012), using Kjeltex system I (Foss Tecator AB, Hoganas, Sweden). The pre digested 5 ml sample and 4 ml of NaOH solution was added in wolf bottle, and 5 ml of reagent mixture was taken in 100 ml beaker. The final volume was titrated against 1M HCl till pink color appears. The nitrogen contents were measured using formula.

$$\%N = \frac{(S - B) \times 0.005 \times 0.014 \times 100}{\text{Sample weight} \times \text{volume taken}} \quad (2)$$

3.5 Daily Dietary intake of metals

The daily dietary intake (mg d^{-1}) of macro- and micro- nutrients were determined on dry weight (d.w) basis.

$$\text{DIM} = C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{food intake}} \quad (3)$$

Where C_{metal} , C_{factor} and $D_{\text{food intake}}$ are the concentration of metal, conversion factor and daily food intake, respectively. A factor of 0.085 was used for conversion of d.w in to fresh weight (Khan et al. 2008a), while the daily average vegetables intake for children and adults were considered as 0.232 and 0.345 $\text{kg person}^{-1} \text{ day}^{-1}$ respectively (Khan et al. 2010a). The cumulative dietary intake for consumption of all three vegetables was also calculated by summing the dietary intake of metal (DIM) values for a single element. Furthermore, the projected dietary intakes for adults and children were compared with RDA values for each element and were presented as percentage of the RDA (NAS, 2001) to clearly illustrate the impact of these elements on human nutrition. FAO/WHO (2001a) and NAS (1989) recommended nutrient intake

(RNI) values were used to determine RDA for Ca, Mg and K due to unavailability of specific RDA values by NAS (Table 3.2). A factor of 1.2 (1.2 x RNI) was used to convert RNI to RDA (NAS, 2001). For toxicological assessment of selected heavy metals, the dietary intake of single plant species and cumulative intake for three plants were calculated on individual body weight basis, for this purpose the daily dietary intake values were divided by body weight of each age group (NAS, 2001).

$$DIM = \frac{C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{foodintake}}}{BW_{\text{average weight}}} \quad (4)$$

Table 3.2. Body weight and RDA (mgd⁻¹) for individuals of different age groups (NAS, 2001^a, 1989^b; FAO/WHO^c 2001a)

Age	Body weight		Fe ^a		Ca ^c		Mn ^a		Mg ^c		P ^a (g d ⁻¹)		K ^b (g d ⁻¹)		N ^b (g d ⁻¹)		Protein ^b (g d ⁻¹)		Vitamin C ^a
	M	F	M	F	M	F	M	F	M	F	M+F	M+F	M	F	M	F	M	F	M+F
1-13 y	25.00	25.00	8.330	8.330	960.0	960.0	1.530	1.430	146.4	142.4	0.736	1.800	4.520	4.560	28.25	28.50			45.00
14-18 y	64.00	57.00	11.00	15.00	1560	1560	2.200	1.600	276.0	264.0	1.250	2.400	9.440	7.040	59.00	44.00			60.00
>19 y	76.00	61.00	8.000	18.00	1200	1200	2.300	1.800	312.0	240.0	0.700	2.400	9.810	7.790	61.33	48.67			60.00
M; male, F; Female																			

3.6 Quality control

The soil (GBW07406-GSS-6) and plant (GBW07603-GSV-2) certified reference materials were used to check the accuracy of the measurements. The percent recovery for Cd and Pb was 93% and 91%, respectively.

3.7 Statistical analysis

The data were presented as mean $\pm$ standard deviation (SD) of three replicates. The differences between mean values were analyzed by one way ANOVA using statistical software SPSS 21.0. The graphs were prepared on Sigma plot 10.0. The significance levels $P < 0.05$ and $P < 0.01$ were used for determination of significant differences between different treatments.

CHAPTER 4

Results and Discussions

4.1 Cd and Pb interaction

4.1.1 Soil physiochemical characteristics

The soil selected for the pot experiment was a sandy clay loam with a bulk density of 1.48 g cm⁻³ (Table 4.1). The wilting point, field capacity, saturation and available water concentrations were 0.137, 0.212, 0.443 and 0.076 cm³ water cm⁻³ soil, respectively. The soil was neutral to slightly alkaline (pH 7.99) and rich with OM content. Cd and Pb concentrations were 3.4±0.02 and 2.02±0.01 mg kg⁻¹ respectively. The concentrations of Fe, Mn and Pb were below the permissible limits set by USEPA, FAO and SEPA China except Cd (Table 4.1). The results showed that the soil used for pot experiments was suitable for cultivation.

Table 4.1 Initial physicochemical properties of the soil used for pot experiments.

Parameters	pH	EC ^a	MC ^b	SOM ^c	Soil texture	Bulk density (g cm ⁻³)
	7.99	447.7	13.9	3.45	sandy clay loam	1.48
Elements	Total	Bioavailable	% bioavailable of total	USEPA (2005)	FAO (2001a)	SEPA (1995, 2005)
Cd (mg kg ⁻¹)	3.38±0.02	0.07±0.006	1.95	85	0.3	0.6
Pb (mg kg ⁻¹)	2.02±0.02	0.45±0.05	22.17	420	100	300
Mn (mg kg ⁻¹)	26.95±0.64	3.21±0.07	23.32	N.A	50	50
Fe (µg kg ⁻¹)	0.39±0.02	0.011±0.002	0.04	N.A	N.A	N.A
Ca (µg kg ⁻¹)	9.28±0.12	0.005±0.0001	0.001	N.A	N.A	N.A
Mg (µg kg ⁻¹)	13.01±0.21	0.001±0.0002	11.89	N.A	N.A	N.A

^a Electrical conductivity (µS cm⁻¹)

^b Moisture content

^c Soil organic matter

4.1.2 Heavy metals in plants

The contents of heavy metals in plants depend on their concentrations in soil. The results indicated that the concentrations of Cd in three different plant species at four concentration levels showed substantial variation (Fig. Anx. 1.1). Among the three plant species the bioaccumulation of Cd was highest for lettuce followed by tomato and potato. The effects of different treatment levels on Cd accumulation by plants were significant at $P < 0.0001$. These results are in agreement with the statements that lettuce is potentially hyperaccumulator of toxic metals (Monteiro et al. 2007). The effects on Pb concentrations were significant for Cd1, Cd2 and CdPb3 at $P < 0.001$ (Fig. Anx. 1.1). Cd-Pb mix have significantly reduced the Cd and Pb concentrations of lettuce at treatment level CdPb2 ($P < 0.001$), while significant increase was observed at CdPb1 for potato, however, the effects of other treatments were less significant. According to Angelova et al. (2009), tomato is a low rate translocation vegetable and the same results were also observed in the current experiment.

4.1.3 Cd-Pb effects on micro- nutrients

Vegetable grown in toxic metal contaminated soil are deficient of essential nutrients like Fe, Mg and Mn (Van Assche and Clijsters, 1990). The essential element concentrations in plants grown on different treatments were significantly ($P < 0.001$) varied (Fig. 4.1). Ca concentrations showed significant increase with the treatment of Cd1, Pb2, Pb3 and CdPb1, while a decrease was observed at Cd3, Pb1 and CdPb3 for potato. Regarding tomato, total Ca concentration was decreased except for Pb3 and CdPb3. Similarly, a significant decrease in Ca concentrations was observed at all three levels of Cd-Pb mix, Cd1 and Pb3 (Fig. 4.1). The different responses at different metal treatments are due to synergistic and antagonistic effects, as the same metal responded differently at variable concentration. Similarly, the effects also varied among the plant

species which may be linked with their uptake mechanisms.

The concentrations of Fe were significantly ($P < 0.01$) increased in potato grown on Cd1, Pb1 and CdPb1 treatments, while in lettuce grown on Pb1. Similarly, tomato showed a decrease in Fe concentration at different treatment levels except for Cd2 and Pb1. A positive correlationship between Cd and Fe in potato was obvious as Cd follow the same transporter (IRT1) specified for Fe as previously reported by Legay et al. (2012). Unlike Ca and Fe, the Mg concentrations were increased in tomato grown on Cd, Pb and CdPb mix treatments. However, the change in Mg concentration showed significant variation and the maximum increase (83%) was observed at Pb3. In potato, the Mg concentrations at Cd1 and Pb3 treatments were increased by 30 and 31%, respectively, while the rest of treatments showed antagonistic effects. The bioaccumulation of Mg in lettuce was also affected by Cd and Pb at different concentrations; under Cd stress the concentration of Mg increased with the increase of Cd concentration, while exhibited inverse relationship with Pb concentrations. Like other micro- nutrients, Mn is also an essential element and a decrease in its concentration may affect the plant growth and their metabolic activities. In this study, we observed that Pb and Cd have synergistic effects on Mn concentrations in potato, while antagonistic effects in tomato and lettuce, but these effects were inconsistent with increasing the contamination levels. However, these results are not consistent with the findings of Ramos et al. (2002) who observed a significant decrease in Fe but increase in Mn concentrations in lettuce. The contradictions in the results are obvious as the bioaccumulation and transport mechanism vary greatly among different plant species, metal characteristics and concentrations and differential interactions between metals and plant as previously described in various studies (Angelova et al. 2009; Ramos et al. 2002).

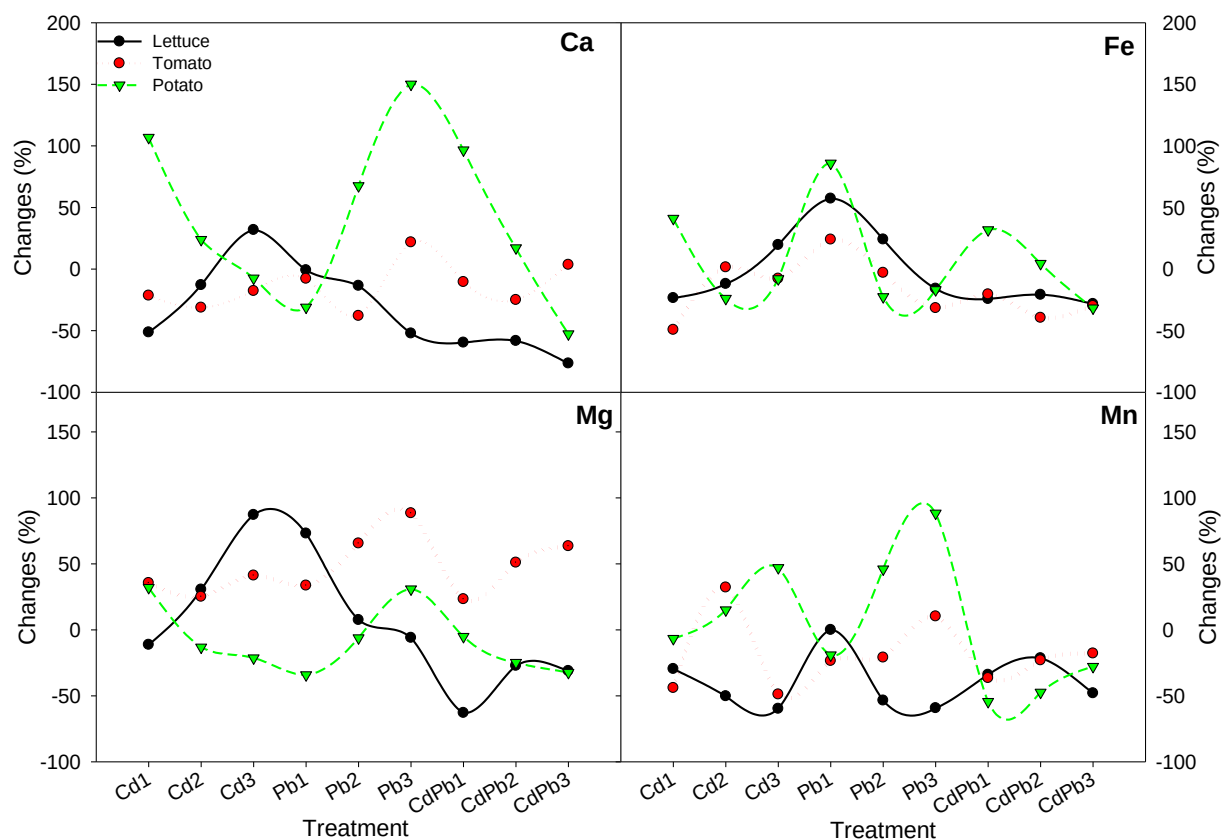


Fig. 4.1. Changes (%) in micro- nutrient concentrations in potato, tomato and lettuce grown on different treatment levels of Cd, Pb and Cd-Pb mix. The Cd1, Cd2, Cd3, Pb1, Pb2, Pb3, CdPb1 CdPb2 and CdPb3 represent the concentrations of Cd 1.0, 2.5 and 5.0 mg kg⁻¹, Pb 200, 300 and 400 mg kg⁻¹ and Cd/Pb of 1.0/200, 2.5/300 and 5.0/400 mg kg⁻¹, respectively.

4.1.4 Cd-Pb effects on macro- nutrients

Different treatment levels showed variable effects on macro- nutrient concentrations (Fig. 4.2). N is one of the essential elements that is required for plant growth and metabolism, it is an important part of different genetic, metabolic and structural components (Hassan et al. 2008). N concentrations under different Cd contamination levels showed significant ($P < 0.01$) variation. In case of tomato, the N contents were changed by -70, -9 and -34% at Cd1, Cd2 and Cd3 treatments, respectively, while for lettuce and potato both synergistic and antagonistic changes

were observed (Fig. 4.2). Previously, Gojon and Gaymard (2010) observed that N contents of *Arabidopsis thaliana* increased with increasing the dose of contaminants. Similarly, Dražić et al. (2004) reported that soybean grown in hydroponic condition under Cd stress showed a decrease in N concentration but these changes were not consistent with increasing Cd stress. The positive correlation between Cd and N is essential for the synthesis of Cd-detoxifying chelator like phytochelatins and glutathione (Gojon and Gaymard, 2010). These statements indicated that toxicity effects of same heavy metal varied from specie to specie and also at different concentrations. In lettuce and tomato, the effects of Pb on N metabolism were similar as Cd, while potato showed different effects. However, the effects decreased with increasing dose of Pb like 103, 95 and 47% for Pb1, Pb2 and Pb3, respectively. The effects of binary mixture were more significant for potato ($P < 0.001$) and tomato ($P < 0.01$) than lettuce ($P < 0.05$). The N contents increased for lettuce and potato, while significantly ($P < 0.01$) decrease for tomato.

The concentrations of P in plants were severely affected by Cd and Pb contamination. Cd and Pb at different contamination levels showed antagonistic effects on P bioavailability. All the three plant species showed progressive decrease with the increase of metal doses. The highest ($P < 0.001$) decrease was observed at Cd3 for lettuce. Among the different plant species, the effects were in order of lettuce>tomato>potato (Fig. 4.2). Previously, several studies have been conducted on effects of heavy metals on P bioavailability, however, the results are contradictory depending upon type of plant species and metal concentrations. A decrease in soybean P contents under hydroponic condition (Dražić et al. 2004), while an increase in *faffia glomerata* grown in nutrients solution (Gomes et al. 2013) were also observed.

K is an essential element required for plant growth, metabolism and synthesis of essential protein, however, exposure to high heavy metal concentrations may be severely affected its

bioavailability (Gupta et al. 2013). The results indicated that the K concentration in the edible parts of selected vegetables decreased with different application doses of Cd and Pb. The effect of Cd on potato and tomato was significant ($P<0.001$) at Cd1 and Cd2, while for lettuce the effect was significant ($P<0.01$) only at Cd2 and the same pattern of effect was followed by Pb at all treatment levels (Fig. 4.2). Similarly, the effect of binary mixture on K availability also varied from species to species. K concentrations in different vegetables showed a reduction pattern of potato>tomato>lettuce (Fig. 4.2). These effects on K bioavailability and translocation to shoot may be associated with physiological changes in plant structure like alteration of xylem and vascular system (Ouzonidou et al. 1994).

Vegetables and fruits are rich sources of vitamin C. Vitamin C is very labile and their quantities in vegetables are strongly influenced by the environmental condition, exposure to other elements and soil quality (FAO/WHO, 2001b). The concentrations of vitamin C in tomato and lettuce were increased for all the treatment levels, however, a decrease was observed in potato. The vitamin C contents significantly increased with increasing the dose of Cd for both lettuce and tomato. The decrease in potato was not consistent with increasing the dose of Cd and the highest decrease was observed for Cd2 at $P<0.001$ (Fig. 4.2). The effects of Pb on vitamin C were significantly different as compared to Cd, such as with increasing the dose of Pb the vitamin C contents were decreased progressively in all the three plant species. However, when compared with control (Fig. Anx. 1.1), the concentrations of Pb were increased the vitamin C contents by 47, 53 and 37% and 94, 50 and 22% in lettuce and tomato, respectively (Fig. 4.2). The binary mixture has almost similar effects for the three different plant species but the effects were less significant except for potato ($P<0.001$). Previous studies revealed that high Pb concentration results in an increase in vitamin C contents of vegetables (Mishra and Choudhuri,

1999). Cd and Pb have significantly affected the protein contents of lettuce, potato and tomato, however, the effects were not consistent for all the selected vegetables and different treatments. Data revealed that Cd and Pb have both synergistic and antagonistic effects on protein contents depending on its concentration and type of vegetable (Fig. 4.2), such as protein contents were inversely related to Cd and Pb concentrations in tomato, while direct correlation was observed with lettuce and potato with few exceptions. Pb concentrations have negative impacts on plant protein contents. Tiwari et al. (2013) reported a decrease in protein contents of wheat. Similarly, Cd bioaccumulation may result in alteration of different physiological functions affecting N metabolism (Chaffei et al. 2004), which ultimately affecting protein synthesis. This reduction in protein may result from Cd bonding with free peptides to form high molecular weight complexes with Cd decreasing free peptides and consequently inhibiting protein synthesis (Winfried, 1995).

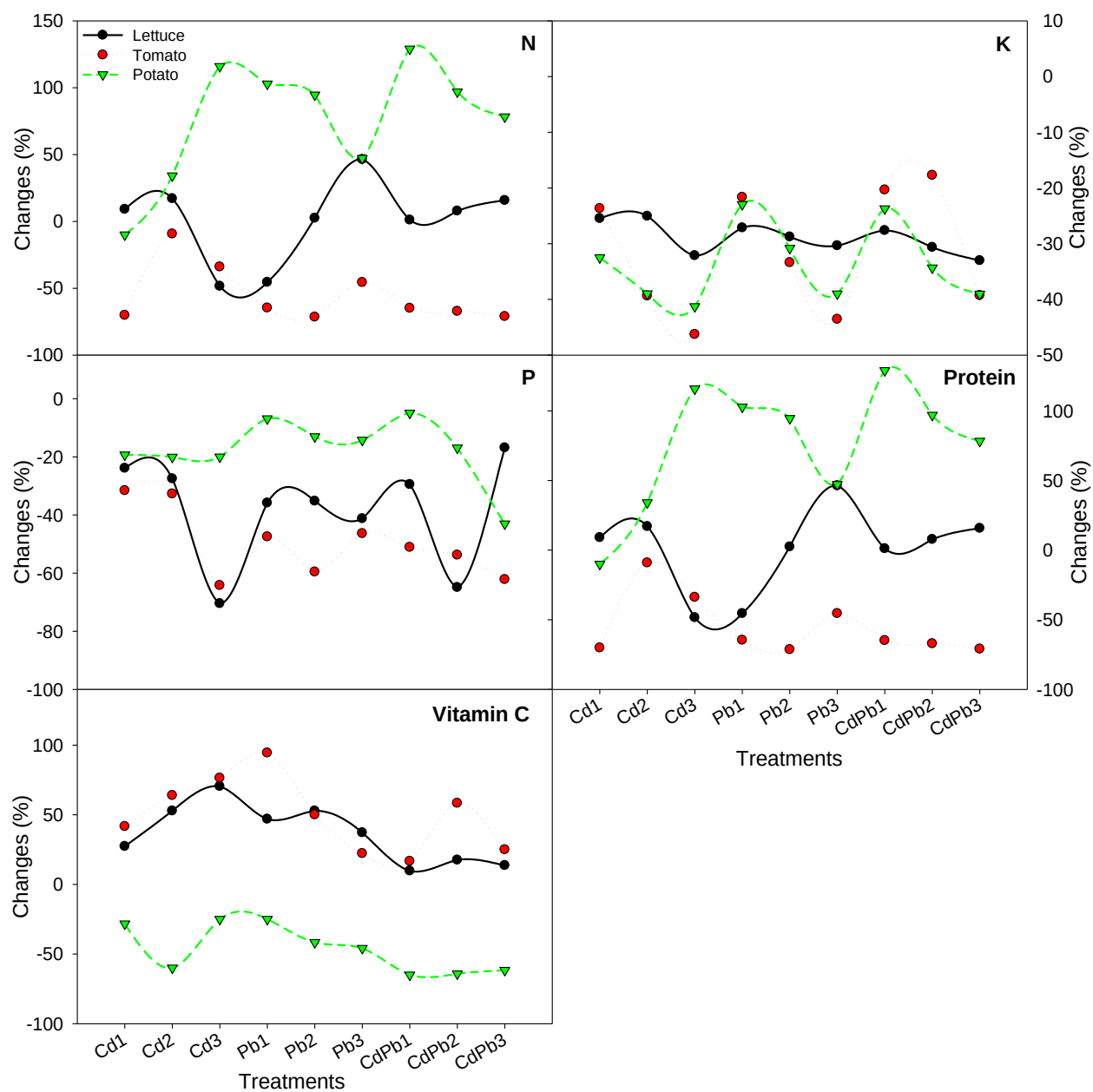


Fig. 4.2. Changes (%) in macro- nutrients concentrations of potato, tomato and lettuce under different treatments. The Cd1, Cd2, Cd3, Pb1, Pb2, Pb3, CdPb1 CdPb2 and CdPb3 represent the concentrations of Cd 1.0, 2.5 and 5.0 mg kg⁻¹, Pb 200, 300 and 400 mg kg⁻¹ and Cd/Pb of 1.0/200, 2.5/300 and 5.0/400 mg kg⁻¹, respectively.

4.1.5 Projected daily dietary intake of metals

Vegetables are rich sources of essential nutrients that meet daily dietary nutrient requirements of an individual. However, soil contamination with environmental pollutants has substantial effects on micro-nutrients that may affect both plant and animal health. The DIM values for children and adults were calculated for both essential and toxic metals (Table Anx. 1.1-1.3). The daily dietary requirements of essential elements depend on various factors like balance diet, excretion mechanism and routes and their concentrations in different body media like blood, plasma and urine (NAS, 2001). The daily intake (mg d^{-1}) of essential and potentially toxic elements for adults and children by consumption of vegetables cultivated at Cd, Pb and Cd-Pb mix contaminated soil show significant variation for different treatment levels and age groups (Table Anx. 1.1-1.3). The dietary doses of Cd from consumption of vegetables were above the maximum tolerable intake level ($1\mu\text{g kg}^{-1} \text{d}^{-1}$) (WHO, 1996) for all the treatment levels except for Pb1 and Pb2 for children (1-13y) and adults (>19y) and for all the three vegetables at individual and cumulative intake (Table 4.2 and Anx. 1.3). Dietary exposure to Pb was not significant for different age groups, however, the projected dietary dose for children exceeded the maximum allowable limit for Pb ($3.6 \mu\text{g kg}^{-1}\text{d}^{-1}$) at treatment levels CdPb1 (potato) and Cd2, Cd3, Pb3 and CdPb1(tomato). With exception of Pb3 for children, the cumulative dietary dose was well below the maximum allowable limit of WHO (1996) (Table 4.2 and Anx. 1.3). Chen and Gao (1993) reported that among different food sources vegetables are the major source of Cd and Pb intake that contribute about 23 and 28% to daily dietary intake of Cd and Pb respectively, with an average dietary exposure of 21.2 and $81.2 \mu\text{g d}^{-1}$. The dietary intake of essential and non-essential elements is affected by the presence of that particular element (Turnlund et al. 1998) and other essential and toxic metals (Morais et al. 1994) in the body. The amount of essential

nutrients like vitamins (Davis et al. 1995), carbohydrates (Seaborn and Stoecker, 1989) and medication (Kamath et al. 1997) also have substantial effects on dietary intake of metals.

The daily dietary intake values ($\text{mg kg}^{-1} \text{ d}^{-1}$) for Fe were significantly higher for vegetables cultivated on contaminated soil than control. Other micro- nutrients like Mn follow almost the same pattern of dietary intake except Ca and Mg (Table 4.2 and Anx. 1.4).

Tables 4.2. Cumulative values of DIM of Cd, Pb and micro- ($\text{mg kg}^{-1} \text{d}^{-1}$) and macro- ($\text{g kg}^{-1} \text{d}^{-1}$) nutrients

	Cd	Pb	Fe	Mn	Ca	Mg	N	P	K	Protein	Vitamin C*
Children											
Control	2.58E-03**	1.77E-03	1.00E-01	5.22E-02	3.97E-01	8.79E-01	2.46E-03	1.99E-03	3.21E-03	1.40E-03	1.04E-02
Cd1	3.35E-03	2.82E-03	8.11E-02	3.76E-02	3.65E-01	1.03E+00	1.62E-03	1.48E-03	2.31E-03	9.24E-04	1.17E-02
Cd2	4.39E-03	2.28E-03	8.91E-02	4.75E-02	3.71E-01	9.92E-01	2.66E-03	1.45E-03	2.09E-03	1.52E-03	1.19E-02
Cd3	5.46E-03	3.17E-03	1.12E-01	3.68E-02	4.56E-01	1.18E+00	2.37E-03	9.71E-04	1.93E-03	1.35E-03	1.42E-02
Pb1	8.44E-04	2.43E-03	1.56E-01	4.60E-02	3.60E-01	1.08E+00	1.94E-03	1.38E-03	2.44E-03	1.11E-03	1.46E-02
Pb2	6.26E-04	2.80E-03	1.14E-01	4.25E-02	4.08E-01	1.03E+00	2.15E-03	1.25E-03	2.22E-03	1.22E-03	1.20E-02
Pb3	3.95E-03	4.21E-03	8.18E-02	5.16E-02	4.32E-01	1.17E+00	2.50E-03	1.31E-03	2.00E-03	1.42E-03	1.02E-02
CdPb1	4.17E-03	3.47E-03	8.44E-02	3.14E-02	3.42E-01	7.16E-01	2.41E-03	1.40E-03	2.44E-03	1.37E-03	8.74E-03
CdPb2	3.65E-03	2.27E-03	7.96E-02	3.73E-02	2.59E-01	8.28E-01	2.25E-03	1.10E-03	2.29E-03	1.28E-03	1.09E-02
CdPb3	4.24E-03	2.71E-03	7.14E-02	3.45E-02	1.65E-01	8.21E-01	2.15E-03	1.13E-03	2.01E-03	1.23E-03	9.33E-03
Adolescent											
Control	1.01E-03	6.92E-04	3.93E-02	2.04E-02	1.55E-01	3.44E-01	9.60E-04	7.75E-04	1.25E-03	5.47E-04	4.04E-03
Cd1	1.31E-03	1.10E-03	3.17E-02	1.47E-02	1.43E-01	4.02E-01	6.33E-04	5.80E-04	9.02E-04	3.61E-04	4.56E-03
Cd2	1.71E-03	8.91E-04	3.49E-02	1.85E-02	1.45E-01	3.87E-01	1.04E-03	5.66E-04	8.16E-04	5.92E-04	4.65E-03

Cd3	2.13E-03	1.24E-03	4.36E-02	1.44E-02	1.78E-01	4.62E-01	9.25E-04	3.79E-04	7.53E-04	5.28E-04	5.53E-03
Pb1	3.30E-04	9.51E-04	6.11E-02	1.80E-02	1.41E-01	4.21E-01	7.60E-04	5.39E-04	9.53E-04	4.33E-04	5.71E-03
Pb2	2.45E-04	1.09E-03	4.43E-02	1.66E-02	1.59E-01	4.04E-01	8.39E-04	4.89E-04	8.65E-04	4.78E-04	4.67E-03
Pb3	1.54E-03	1.64E-03	3.19E-02	2.02E-02	1.68E-01	4.55E-01	9.75E-04	5.11E-04	7.82E-04	5.56E-04	3.99E-03
CdPb1	1.63E-03	1.35E-03	3.30E-02	1.23E-02	1.34E-01	2.80E-01	9.42E-04	5.47E-04	9.51E-04	5.37E-04	3.42E-03
CdPb2	1.43E-03	8.89E-04	3.11E-02	1.46E-02	1.01E-01	3.24E-01	8.79E-04	4.30E-04	8.93E-04	5.01E-04	4.25E-03
CdPb3	1.66E-03	1.06E-03	2.79E-02	1.35E-02	6.44E-02	3.21E-01	8.41E-04	4.43E-04	7.86E-04	4.79E-04	3.65E-03
Adults											
Control	1.26E-03	8.68E-04	4.92E-02	2.55E-02	1.95E-01	4.30E-01	1.20E-03	9.71E-04	1.57E-03	6.85E-04	5.06E-03
Cd1	1.64E-03	1.38E-03	3.97E-02	1.84E-02	1.79E-01	5.04E-01	7.93E-04	7.26E-04	1.13E-03	4.52E-04	5.71E-03
Cd2	2.14E-03	1.11E-03	4.37E-02	2.32E-02	1.81E-01	4.85E-01	1.30E-03	7.09E-04	1.02E-03	7.41E-04	5.82E-03
Cd3	2.67E-03	1.55E-03	5.47E-02	1.80E-02	2.23E-01	5.78E-01	1.16E-03	4.75E-04	9.43E-04	6.61E-04	6.93E-03
Pb1	4.13E-04	1.19E-03	7.64E-02	2.25E-02	1.76E-01	5.27E-01	9.51E-04	6.75E-04	1.19E-03	5.42E-04	7.15E-03
Pb2	3.06E-04	1.37E-03	5.54E-02	2.08E-02	1.99E-01	5.06E-01	1.05E-03	6.12E-04	1.08E-03	5.99E-04	5.85E-03
Pb3	1.93E-03	2.06E-03	3.99E-02	2.53E-02	2.11E-01	5.71E-01	1.22E-03	6.40E-04	9.79E-04	6.96E-04	5.00E-03
CdPb1	2.04E-03	1.70E-03	4.14E-02	1.54E-02	1.68E-01	3.50E-01	1.18E-03	6.85E-04	1.19E-03	6.72E-04	4.28E-03
CdPb2	1.79E-03	1.11E-03	3.90E-02	1.83E-02	1.27E-01	4.06E-01	1.10E-03	5.39E-04	1.12E-03	6.27E-04	5.32E-03
CdPb3	2.07E-03	1.32E-03	3.49E-02	1.69E-02	8.06E-02	4.02E-01	1.05E-03	5.55E-04	9.84E-04	6.00E-04	4.57E-03
*Cumulative DIM (mg kg⁻¹ d⁻¹) **E-03: represent 1x 10⁻³.											

Among the three vegetables, lettuce showed the highest intake of all the elements, while tomato showed the lowest intake for different age groups. This result was obvious and expected, since lettuce has foliar sorption, and tomato fruit is excluder tissue.

The DIM of macro- nutrients was of great importance because these nutrients are essential part of our diet and any change in their concentrations may significantly affect human health (Table Anx. 1.2, 1.3 and 1.5). The highest DIM value was observed for N for different age groups and treatment levels followed by P and protein, while vitamin C showed less contribution to daily dietary intake through vegetables consumption (Table 4.2 and Anx. 1.5). Among different vegetables potato showed the highest DIM for N, P, K and protein followed by lettuce and tomato except for vitamin C which followed the order of tomato>potato>lettuce for different age groups. The variation in DIM contribution by macro- nutrients at different treatment levels and plants species is due to the changes in bioavailability under different treatment levels, as prescribed earlier. The cumulative DIM of vegetables contributed significant intake of N, P and protein for children followed by adults and adolescents (Table 4.2).

The consumption of metals contaminated vegetables provided a dietary intake of >30% of RDA for Mn with a highest consumption by female under age of 19 y. Although all the three plants grown on Cd and Pb contaminated soils have contributed <5% of RDA for Ca, but lettuce provided sufficient dietary intake for both male and female and different age groups (Table Anx. 1.6). Similarly, Zn have <10% contribution to RDA for children and <20% for adults and adolescents with few exceptions and showed a great variation in RDA for different age groups and gender under different contamination levels.

The contribution to RDA by macro- nutrients was <20% for all the macro- nutrients (Table 4.3 and Anx. 1.7), however, P contributed more than other nutrients. Although the contribution

to %RDA by macro- nutrients was <20 and showed significant variation ($P<0.01$) among different treatment levels and age groups. Similarly, different plant species showed the order of contribution as potato>lettuce>tomato, except for vitamin C which followed the order of tomato>potato>lettuce for adults and children (Table Anx. 1.7). Previously, it is revealed that Cd interaction with essential nutrients is well known. Cd interferes with essential elements and hampers their activities, such as high Cd concentration may affect Cu and Zn metabolism, decrease Fe absorption and Ca transport (Peraza et al. 1998). Generally, the RDA was modest for Fe and Mg, lowest for Zn and Ca, while Cu and Mn were observed with highest RDA for different plant species and age groups.

Table 4.3. Cumulative RDA (%) of macro- and micro- nutrients provided by dietary intake of contaminated vegetables

	Fe		Mn		Ca	Mg		N		P	K	Protein		Vitamin C
	M	F	M	F	M+F	M	F	M	F	M+F	M+F	M	F	M+F
Children														
Control	30.3	30.3	87.1	87.1	1.66	28.9	28.9	1.36	1.35	6.74	4.45	0.12	0.12	0.58
Cd1	24.4	24.4	62.6	62.6	1.52	33.9	33.9	0.90	0.89	5.04	3.21	0.08	0.08	0.65
Cd2	26.9	26.9	79.1	79.1	1.55	32.6	32.6	1.47	1.46	4.92	2.90	0.13	0.13	0.66
Cd3	33.6	33.6	61.4	61.4	1.90	38.9	38.9	1.31	1.30	3.29	2.68	0.12	0.12	0.79
Pb1	47.0	47.0	76.7	76.7	1.50	35.4	35.4	1.08	1.07	4.68	3.39	0.10	0.10	0.81
Pb2	34.2	34.2	70.9	70.9	1.70	34.0	34.0	1.19	1.18	4.25	3.08	0.11	0.11	0.66
Pb3	24.6	24.6	86.0	86.0	1.80	38.4	38.4	1.38	1.37	4.44	2.78	0.13	0.12	0.57
CdPb1	25.5	25.5	52.3	52.3	1.43	23.5	23.5	1.33	1.32	4.76	3.38	0.12	0.12	0.49

CdPb2	24.0	24.0	62.2	62.2	1.08	27.3	27.3	1.24	1.23	3.74	3.18	0.11	0.11	0.60
CdPb3	21.5	21.5	57.5	57.5	0.69	27.0	27.0	1.19	1.18	3.85	2.79	0.11	0.11	0.52
Adolescent														
Control	34.0	24.9	88.3	121.4	0.95	11.8	12.4	0.97	1.30	5.90	4.97	0.09	0.12	0.64
Cd1	27.4	20.1	63.4	87.2	0.87	13.9	14.5	0.64	0.86	4.41	3.58	0.06	0.08	0.72
Cd2	30.2	22.1	80.2	110.3	0.88	13.4	14.0	1.05	1.40	4.31	3.24	0.10	0.13	0.74
Cd3	37.7	27.7	62.2	85.6	1.09	15.9	16.6	0.93	1.25	2.89	2.99	0.09	0.11	0.88
Pb1	52.7	38.7	77.8	106.9	0.86	14.5	15.2	0.77	1.03	4.10	3.78	0.07	0.09	0.91
Pb2	38.4	28.1	71.8	98.8	0.97	13.9	14.6	0.85	1.13	3.72	3.43	0.08	0.10	0.74
Pb3	27.6	20.2	87.2	120.0	1.03	15.7	16.4	0.98	1.32	3.89	3.10	0.09	0.12	0.63
CdPb1	28.6	20.9	53.0	72.9	0.82	9.6	10.1	0.95	1.27	4.17	3.77	0.09	0.12	0.54
CdPb2	26.9	19.8	63.1	86.7	0.62	11.2	11.7	0.89	1.19	3.27	3.54	0.08	0.11	0.67
CdPb3	24.1	17.7	58.3	80.2	0.39	11.1	11.6	0.85	1.14	3.38	3.12	0.08	0.10	0.58
Adults														
Control	46.7	20.8	84.4	107.9	0.95	11.8	12.4	0.93	1.17	10.54	4.97	0.08	0.11	0.64
Cd1	37.7	16.7	60.7	77.5	0.87	13.9	14.5	0.61	0.77	7.88	3.58	0.06	0.07	0.72
Cd2	41.5	18.4	76.7	98.0	0.88	13.4	14.0	1.01	1.27	7.70	3.24	0.09	0.12	0.74
Cd3	51.9	23.1	59.5	76.1	1.09	15.9	16.6	0.90	1.13	5.15	2.99	0.08	0.10	0.88
Pb1	72.5	32.2	74.4	95.0	0.86	14.5	15.2	0.74	0.93	7.33	3.78	0.07	0.08	0.91
Pb2	52.7	23.4	68.7	87.8	0.97	13.9	14.6	0.81	1.03	6.65	3.43	0.07	0.09	0.74
Pb3	37.9	16.9	83.4	106.6	1.03	15.7	16.4	0.95	1.19	6.95	3.10	0.09	0.11	0.63
CdPb1	39.3	17.5	50.7	64.8	0.82	9.6	10.1	0.91	1.15	7.44	3.77	0.08	0.10	0.54

CdPb2	37.0	16.5	60.3	77.1	0.62	11.2	11.7	0.85	1.07	5.85	3.54	0.08	0.10	0.67
CdPb3	33.2	14.7	55.8	71.3	0.39	11.1	11.6	0.82	1.03	6.03	3.12	0.07	0.09	0.58

M; Male. F; Female

4.2 As toxicity

4.2.1. As concentrations in vegetables

Most of the vegetables are hyperaccumulators of heavy metals and have the capability to accumulate As in sufficient quantity in both underground and aboveground tissues (Rahman et al. 2007). The mean As concentrations in the edible portion of lettuce, potato and tomato were 0.76, 0.63, 0.57 mg kg⁻¹ respectively, in control pots (Fig. Anx. 2.1). The concentrations of As were increased with increasing its concentration in soil. As in binary and tri-elements mixture forms showed variable effects on its bioaccumulated concentrations in plant tissues. Vegetables grown under binary mixture stress showed an increase with increasing the concentration of Cd (As-Cd), while decreased with increasing the concentrations of Pb (As-Pb). Similarly, the tri mixtures showed antagonistic effects on lettuce, synergistic effects on potato and both synergistic and antagonistic effects were observed on tomato As concentrations. As bioaccumulation and distribution are strongly depend on its excretion, metabolism and methylation capacity (Vahter, 2007). Many other factors including pH, redox condition, soil OM, soil texture, oxides of Al, Fe and Mn, concentrations of P, S, and As in soil, land use pattern and growth seasons can affect and control As uptake and its transfer into above ground parts of the plants (Waqas et al. 2015; Williams et al. 2007; Song et al. 2006;)

4.2.2 As interaction with Cd and Pb in vegetables

Vegetable contamination with heavy metals is strongly influenced by their concentrations in soil (Huang et al. 2006), while metal (As, Cd and Pb) availability is controlled by their interactions with each other in the soil environment (Marwa et al. 2012). The results showed that Cd concentrations in different treatment levels were ranged from 1.3-6.4 mg kg⁻¹ in lettuce, from 1.5-6.8 mg kg⁻¹ in potato and from 1.8-4.2 mg kg⁻¹ in tomato (Fig. Anx. 2.1). The As effects on Cd bioaccumulation were significant ($P < 0.01$) for the treatments of AsPb2, AsCd3, AsPb1 and AsCd2, while non-significant for other treatment levels. Generally, As and Cd were negatively correlated, therefore the increases in As concentration in soil led to decreases in bioaccumulation of Cd in lettuce, while increased in potato and tomato. However, these changes are not consistent among all treatment levels. These results confirmed that the bioaccumulation of heavy metals vary among the plant species (Liu et al. 2003). Pb has the capability to interact with other heavy metals and its phytoavailability is strongly influenced by other metal concentrations (Wu et al. 2014). The Pb concentration in the edible parts of lettuce, potato and tomato were ranged from 0.25-3.65 mg kg⁻¹, 0.6-4.8 mg kg⁻¹ and 0.54-3.0 mg kg⁻¹, respectively (Fig. Anx. 1). The results showed that Pb concentration in selected vegetables gradually decreased with increasing As concentration, however the effects on potato were not consistent for all treatments. The combine treatment of As and Cd has no significant effect on Pb concentration in lettuce while a significant ($p < 0.01$) decrease was observed in potato and tomato. Similarly, when treated as binary mixture with As, lettuce Pb concentrations gradually increased with increasing the concentrations of both the elements in binary mixture, while a decrease was observed at treatment level AsPb1 (70%) and AsPb2 (89%) but a significant ($P < 0.01$) increase was observed at AsPb3. Similarly, combine treatment of the three metal mixture (As, Cd and Pb) showed variable effects on Pb

concentrations in vegetables. No significant effects were observed at treatment Mix1, while changes of 33% and 49% were observed in lettuce Pb concentration at Mix2 and Mix3. Similarly, a gradual decrease in potato Pb concentration was observed with increasing the concentrations of tri-element mixture. Unlike lettuce and potato, the Pb concentration in binary and tri mixture increased than the control except AsCd3 treatment (Fig Anx. 2.1).

4.2.3. As effects on essential nutrients

4.2.3.1. *Micro- nutrients*

Figure 4.3 showed the effects of As on micro- nutrient concentrations in the selected plant species. As has strong interactions capability with other essential macro- and micro- nutrients and significantly effect their concentrations in food crops (Gusman et al. 2013). The concentrations of Fe showed great variation in different plant species and at different treatment levels. The highest concentration (544 mg kg^{-1}) was observed in lettuce at Mix2 followed by AsCd3 and AsCd2. As has synergistic effects on Fe concentration in lettuce, while potato and tomato showed both antagonistic and synergistic effects, depend upon the level of contamination and metal mixture used (Fig. 4.3). Unlike Fe, a progressive decrease was observed in Mn concentration with increasing As stress. In lettuce, the highest decrease (69%) was observed in lettuce at As3, while the lowest (6%) was observed at Mix1. However, potato and tomato were not followed the same order of decreases for Mn concentrations, rather, the two plants showed both synergistic and antagonistic effects under As stress. Similar, results were also reported by Najmowicz et al. (2010) and they concluded that the As effects on Mn bioavailability varied from plant to plant. Similarly, antagonistic effects on Fe and Mn have also been observed by Parsons et al. (2008). This interaction may be due to the formation of Fe and Mn hydroxides

complexes in the root system (Rosas-Castor et al. 2014) through complexation reaction and surface absorption (Hsu et al. 2012).

For Mg concentrations, both synergistic and antagonistic effects under different treatment levels of As were observed. Lettuce and tomato showed synergistic effects for Mg concentration except Mix3, while potato showed both synergistic and antagonistic effects. Ca concentrations in the edible tissues of potato and lettuce showed almost similar results, however these results are not consistent with those observed in tomato. These changes were significant ($P < 0.05$) for potato and lettuce but non-significant for tomato except As2, As3 treatments. Similar changes in Mg and Ca concentrations were also observed by Rosas-Castor et al. (2014). Previously, the indirect effects of different heavy metals on macro- and micro- nutrients such as P, K, Ca and Mg concentrations have also been reported (Maathuis, 2009).

For a balance diet and healthy food, the nutritional value of the food crop is a key component. Plant nutritional values depend on several factors like the presence and absence of essential and toxic elements, their nutrient concentrations and digestibility (Alam et al. 2003). These interaction may affect the phytoavailability of these essential nutrients, resulting in over as well as under nutrition depend upon the type of nutrient and interaction. In general perception it can be revealed that the interaction between heavy metals and essential nutrients are very complex and vital (Kordas et al. 2007). Essential elements when exceed their threshold limits may also exert toxicity symptoms (Kordas et al. 2007).

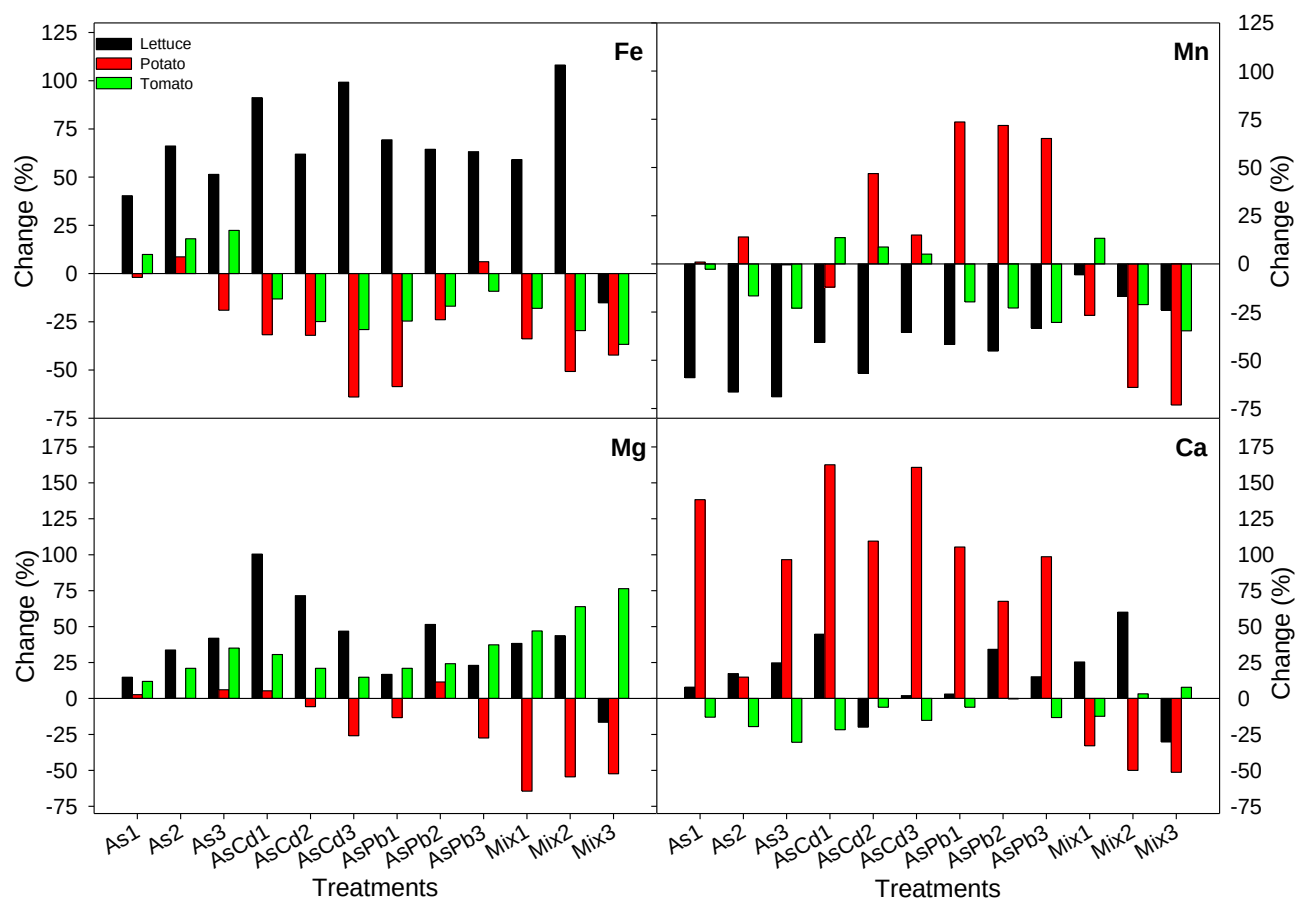


Fig. 4.3. Changes (%) in micro- nutrient concentrations of vegetables under different treatment levels of As

4.2.3.2. Macro- nutrients

As and other toxic elements lead to severely affect the macro- nutrient concentrations in food plant and ultimately result in malnutrition (Iyengar and Nair, 2000). The associated disabilities and anomalies of malnutrition may include psychological and social disorders, growth retardation and immune system problems (Iyengar and Nair, 2000). As effects on macro- nutrient concentrations were varied from nutrient to nutrient and also for different species, even for the same nutrient and same species, a great variation was observed at different contamination

levels (Fig. 4.4). N is an essential macro- nutrients that has a significant role in plant growth and metabolism (Hesse et al. 2004) and considered as the basic structural and functional unit of different genetic components (Hassan et al. 2008). N concentrations showed both synergistic and antagonistic effects in different treatment levels and its concentration increased with increasing As stress, however, this increment was not consistent with binary and tri-element mixture where both increases (AsCd2, AsCd3 AsPb2, AsPb3 and Mix3) and decreases (AsCd1, AsPb1, Mix1 and Mix2) were observed (Fig. 4.4). In potato, N showed an increment at low and moderate As stress, while at high concentration a decrement was occurred. The effects of As stress on N concentrations were significant ($p < 0.001$) for potato, while non-significant for lettuce. In tomato, a decrement in N concentrations was observed with increasing As stress in all treatments (individual, binary and tri-element mix forms). Similar effects were also observed in protein concentrations in the selected vegetables.

Amino acids are essential components of protein and As toxicological effects on these essential amino acids has been demonstrated previously (Dwivedi et al. 2012). The effects of As toxicity on protein concentrations were significant for potato ($P < 0.001$) and non-significant for lettuce except for Mix3. An induction in net protein concentrations of lettuce was observed in solely As stressed plants, while both induction and reduction occurred at binary and tri mixture treatments (Fig. 4.4). Tomato showed a reduction under all treatment levels and the effects increment with increasing concentration of As. Similarly, in potato cultivars induction in protein concentrations were observed for different treatment levels except As3, AsCd2 and Mix3. The effects were significant for potato ($P < 0.001$) and less significant for lettuce ($P < 0.05$) except for Mix3 ($P < 0.01$). The significant effects on potato may be due to the interaction of As with N at

root exudates. According to previous literature, As is responsible for the production of reactive oxygen species that effect protein synthesis (Gunes et al. 2007).

P is an essential macro- nutrient and the basic components of energy transfer systems (Marschner, 1995) like Adenosine diphosphates (ADPs) and Adenosine triphosphates (ATPs), but exposure to metal contaminated soil the plant P concentrations showed different effects. The results indicated that P concentrations were significantly ($P < 0.001$) affected by As stress, however the effects were not consistent for different plant species and contamination levels. In potato, decrements were observed in P concentrations in all treatment levels, except AsPb1 and AsPb2. The net P concentrations in lettuce were incremented at low concentration, while a significant decrement was observed at high concentration. Parsons et al. (2008) also reported a decrement in P concentrations (50%) in the plants grown in As contaminated soil as compared to control. Since As and P have some similarities in their chemical properties, As replace P effecting their transport to aerial tissues of plants and ATP synthesis (Wang et al. 2010; Zhao et al. 2009). Similarly, other studies revealed that P transporters are the main route for As accumulation in plants (Meharg and Macnair, 1992), therefore soil P may suppressed As bioaccumulation (Khan et al. 2014; Lihong and Guilan, 2009).

K is an essential macro-nutrients required for plant growth. The results indicated that the K concentrations in potato, tomato and lettuce reduced significantly. Unlike N and P the K concentrations showed antagonistic effects to As stress as single metal, binary mixture and tri mixture at different contamination level and for different plant species (Fig. 4.4). Maximum reduction (47%) in K concentration was observed at Mix3 in potato. Among different plant species, potato showed the highest reduction for all treatment level (Fig. 4.4). As toxicity significantly ($p < 0.01$) affected the K concentrations in plants, while a reduction (60%) in total K

concentrations in corn seedlings were also observed by Parsons et al. (2008). This decrement in K may be due to plaque formation on root system that inhibit root to shoot translocation of K. Rosas-Castor et al. (2014) reported that plant use for As intoxication observed significant changes in nutritional components like Ca, Mg and K.

Heavy metals have been observed to severely reduce the vitamin C concentrations in plants (Iyengar and Nair, 2000). The results indicated that As toxicological effects on vitamin C concentrations varied from specie to specie and both synergistic and antagonistic effects were observed in this study (Fig. 4.4). Since an induction was occurred in lettuce and tomato vitamin C concentrations under different treatment levels of As, the maximum increments (64% at AsPb3 and 49% at Mix3) were observed for lettuce and tomato, respectively. In lettuce the maximum effects were observed by tri mixture followed by binary mixture with Cd and Pb, respectively. Similarly, in tomato cultivar the binary mixture with Pb showed maximum induction at all three levels followed by binary mixture with Cd and tri mix. Potato showed decrements in vitamin C concentrations with maximum reduction (54%) at AsCd3 and AsPb1. The decrements in vitamin C concentrations may be due to the inverse correlation of vitamin with heavy metals as reported previously (Widowati, 2012), however, this is not true for all plant species and heavy metals as different environmental condition and soil physiochemical characteristic have strong influence on heavy metals interaction (Khan et al. 2015b).

The results of macro- and micro- nutrient concentrations indicated that As toxicity severely affect the nutritional balance of lettuce, potato and tomato that may directly and indirectly affect the consumers health. Since, the toxicity of As is strongly influenced by the nutritional status of the consuming population, exposure duration and dose (Markley and

Herbert, 2009) therefore, the frequency of health and nutritional effects vary from person to person along different region of the world.

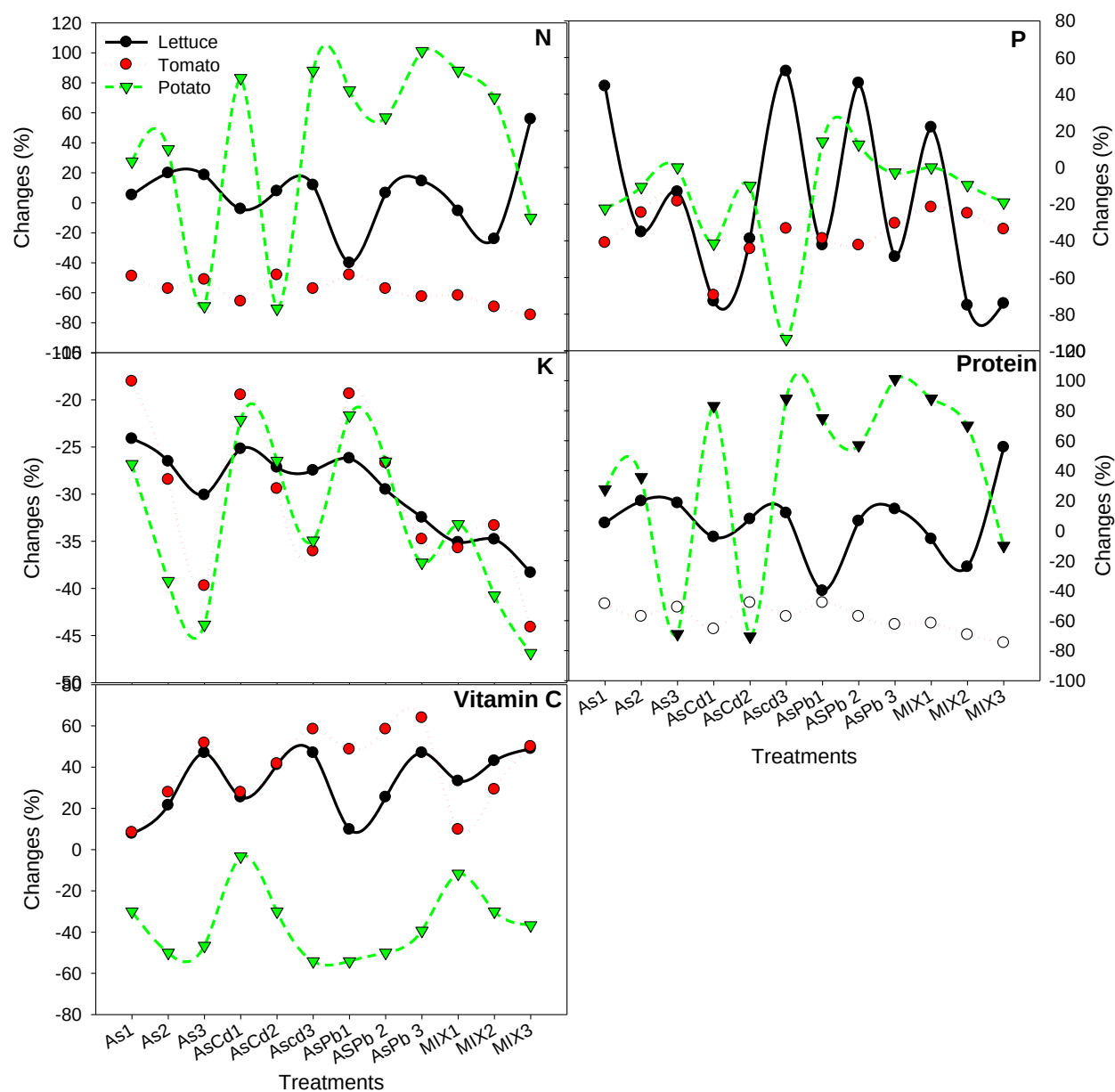


Fig. 4.4. Changes (%) in macro- nutrient concentrations of vegetables under different treatment levels of As

4.2.4. Effects on daily intake of nutrients

Food plants are vital source of macro- and micro- nutrients, however their contamination with As and other toxic substances severely affect their nutritional balance resulting in malnutrition and severe health hazards in the consuming population. The DIM values of macro- (g d^{-1}) and micro- (mg d^{-1}) nutrients showed significant variations for different treatment levels and age group of the people (Table Anx.2.1-2.3). Among micro- nutrients, the highest intake was observed for Mg for both children and adults and lettuce provided more nutrients than potato. Like micro- nutrients, the macro- nutrients also showed variation in daily dietary intake through consumption of As contaminated vegetables (Table Anx. 2.2-2.3).

The dietary intake ($\text{mg kg}^{-1}\text{d}^{-1}$) of Fe showed great variation at different treatment levels and for different age groups. Among selected vegetables, lettuce showed the highest intake followed by tomato and potato. In potato, a progressive decrease was observed with increasing the contamination level, while lettuce showed an increase with increasing As level. The dietary intake of other micro- nutrients such as Ca, Mg and Mn also affected by As toxicity. Ca dietary intake was higher for contaminated vegetables as compared to the control, while both increases and decreases in dietary intake were observed for Mg and Mn depending upon the type of vegetables and contamination level (Table Anx. 2.4). Among macro- nutrients there was a significant variation ($P<0.01$) in estimated intake of N between the vegetable grown on the control and As contaminated soils (Table Anx. 2.5). The effects were more significant ($p<0.01$) for potato and tomato than lettuce ($p<0.05$) at different treatment levels and age groups. Similarly, the concentrations of other macro- nutrients such as P, K, protein and vitamin C showed uneven responses to different treatment levels (Table Anx. 2.5). The cumulative estimated intake of micro- and macro- nutrients from consumption of metal contaminated

vegetables showed the highest intake for Mg followed by Ca and N for children (Table 4.4). However, protein and P showed the lowest estimated intakes for both adults and adolescents (Table 4.4), while protein and P had less contribution to estimated dietary intake. These nutrients showed significant ($P < 0.01$) variation for different treatment levels and age groups. The results indicated that As contamination have substantial effects on nutritional balance of children followed by adults and adolescent. These findings indicated that young children and those with old age are more at risk because of As toxicity. Previously, it is revealed that the variation in dietary intake of essential nutrients may be due to the presence and/or absence of some toxic and essential nutrients in the body (Lonnerdal and Hernell, 1994). Excessive medication (Martinez et al. 1985) and presence of essential nutrients such as carbohydrates (Seaborn and Stoecker, 1989), vitamins (Seaborn and Stoecker, 1990) and amino acids (Dowling et al. 1990) may severely affect the dietary intake of essential and non essential metals.

Table 4.4. Cumulative DIM of heavy metals and macro- and micro- nutrients of As contaminated vegetables

	Cd	Pb	Fe	Mn	Ca	Mg	N	P	K	Protein	Vitamin C
Children											
Control	2.58E-03	1.77E-03	1.01E-01	5.22E-02	3.98E-01	8.79E-01	2.46E-03	1.99E-03	3.21E-03	1.40E-03	1.04E-02
As1	2.45E-03	1.80E-03	1.30E-01	3.81E-02	5.43E-01	9.63E-01	2.06E-03	1.78E-03	2.45E-03	1.17E-03	9.70E-03
As2	3.01E-03	9.31E-04	1.51E-01	3.60E-02	4.42E-01	1.04E+00	2.11E-03	1.53E-03	2.17E-03	1.20E-03	1.01E-02
As3	2.61E-03	6.86E-04	1.37E-01	3.25E-02	5.33E-01	1.11E+00	1.58E-03	1.77E-03	1.97E-03	9.02E-04	1.17E-02
AsCd1	2.97E-03	1.98E-03	1.57E-01	4.33E-02	6.50E-01	1.29E+00	2.11E-03	7.72E-04	2.49E-03	1.20E-03	1.20E-02
AsCd2	2.35E-03	1.30E-03	1.34E-01	4.65E-02	4.53E-01	1.14E+00	1.54E-03	1.36E-03	2.32E-03	8.76E-04	1.18E-02
AsCd3	2.84E-03	1.30E-03	1.55E-01	4.67E-02	5.50E-01	9.77E-01	2.35E-03	1.41E-03	2.15E-03	1.34E-03	1.18E-02
AsPb1	2.76E-03	1.34E-03	1.36E-01	4.91E-02	5.04E-01	9.37E-01	2.02E-03	1.55E-03	2.49E-03	1.15E-03	1.07E-02
AsPb2	2.94E-03	1.83E-03	1.39E-01	4.76E-02	5.45E-01	1.14E+00	2.14E-03	2.01E-03	2.32E-03	1.22E-03	1.16E-02
AsPb3	2.31E-03	1.75E-03	1.43E-01	4.83E-02	5.22E-01	9.49E-01	2.37E-03	1.46E-03	2.08E-03	1.35E-03	1.26E-02
MIX1	2.36E-03	2.00E-03	1.33E-01	4.95E-02	4.19E-01	8.98E-01	2.17E-03	1.95E-03	2.10E-03	1.24E-03	1.09E-02
MIX2	3.74E-03	1.95E-03	1.63E-01	3.66E-02	4.95E-01	9.88E-01	1.85E-03	1.31E-03	2.02E-03	1.05E-03	1.13E-02

MIX3	2.86E-03	1.28E-03	7.77E-02	3.15E-02	2.80E-01	8.32E-01	1.89E-03	1.18E-03	1.81E-03	1.08E-03	1.21E-02
Adolescent											
Control	1.01E-03	6.92E-04	3.93E-02	2.04E-02	1.55E-01	3.43E-01	9.60E-04	7.75E-04	1.25E-03	5.47E-04	4.04E-03
As1	9.56E-04	7.03E-04	5.07E-02	1.49E-02	2.12E-01	3.76E-01	8.05E-04	6.95E-04	9.57E-04	4.59E-04	3.79E-03
As2	1.17E-03	3.64E-04	5.88E-02	1.40E-02	1.73E-01	4.04E-01	8.23E-04	5.98E-04	8.47E-04	4.69E-04	3.93E-03
As3	1.02E-03	2.68E-04	5.37E-02	1.27E-02	2.08E-01	4.35E-01	6.18E-04	6.92E-04	7.70E-04	3.52E-04	4.59E-03
AsCd1	1.16E-03	7.74E-04	6.12E-02	1.69E-02	2.54E-01	5.03E-01	8.23E-04	3.01E-04	9.72E-04	4.69E-04	4.67E-03
AsCd2	9.16E-04	5.06E-04	5.24E-02	1.82E-02	1.77E-01	4.44E-01	6.00E-04	5.33E-04	9.08E-04	3.42E-04	4.62E-03
AsCd3	1.11E-03	5.07E-04	6.05E-02	1.82E-02	2.15E-01	3.82E-01	9.17E-04	5.50E-04	8.40E-04	5.23E-04	4.60E-03
AsPb1	1.08E-03	5.25E-04	5.30E-02	1.92E-02	1.97E-01	3.66E-01	7.91E-04	6.05E-04	9.71E-04	4.51E-04	4.17E-03
AsPb2	1.15E-03	7.15E-04	5.41E-02	1.86E-02	2.13E-01	4.44E-01	8.34E-04	7.86E-04	9.08E-04	4.75E-04	4.52E-03
AsPb3	9.01E-04	6.83E-04	5.59E-02	1.89E-02	2.04E-01	3.71E-01	9.27E-04	5.71E-04	8.12E-04	5.29E-04	4.93E-03
MIX1	9.20E-04	7.83E-04	5.21E-02	1.93E-02	1.64E-01	3.51E-01	8.48E-04	7.60E-04	8.20E-04	4.84E-04	4.26E-03
MIX2	1.46E-03	7.63E-04	6.35E-02	1.43E-02	1.93E-01	3.86E-01	7.23E-04	5.11E-04	7.90E-04	4.12E-04	4.40E-03
MIX3	1.12E-03	4.98E-04	3.03E-02	1.23E-02	1.09E-01	3.25E-01	7.37E-04	4.63E-04	7.07E-04	4.20E-04	4.72E-03

Adults											
Control	1.26E-03	8.67E-04	4.92E-02	2.56E-02	1.94E-01	4.30E-01	1.20E-03	9.71E-04	1.57E-03	6.85E-04	5.06E-03
As1	1.20E-03	8.80E-04	6.35E-02	1.87E-02	2.66E-01	4.71E-01	1.01E-03	8.70E-04	1.20E-03	5.75E-04	4.74E-03
As2	1.47E-03	4.55E-04	7.36E-02	1.76E-02	2.16E-01	5.06E-01	1.03E-03	7.48E-04	1.06E-03	5.88E-04	4.92E-03
As3	1.28E-03	3.36E-04	6.72E-02	1.59E-02	2.61E-01	5.45E-01	7.74E-04	8.67E-04	9.64E-04	4.41E-04	5.75E-03
AsCd1	1.45E-03	9.70E-04	7.66E-02	2.12E-02	3.18E-01	6.30E-01	1.03E-03	3.77E-04	1.22E-03	5.88E-04	5.85E-03
AsCd2	1.15E-03	6.34E-04	6.57E-02	2.28E-02	2.21E-01	5.56E-01	7.52E-04	6.68E-04	1.14E-03	4.29E-04	5.79E-03
AsCd3	1.39E-03	6.35E-04	7.57E-02	2.28E-02	2.69E-01	4.78E-01	1.15E-03	6.88E-04	1.05E-03	6.54E-04	5.76E-03
AsPb1	1.35E-03	6.57E-04	6.64E-02	2.40E-02	2.46E-01	4.58E-01	9.90E-04	7.58E-04	1.22E-03	5.65E-04	5.23E-03
AsPb2	1.44E-03	8.95E-04	6.78E-02	2.33E-02	2.67E-01	5.56E-01	1.04E-03	9.84E-04	1.14E-03	5.95E-04	5.66E-03
AsPb3	1.13E-03	8.55E-04	7.00E-02	2.36E-02	2.55E-01	4.64E-01	1.16E-03	7.15E-04	1.02E-03	6.62E-04	6.17E-03
MIX1	1.15E-03	9.80E-04	6.52E-02	2.42E-02	2.05E-01	4.39E-01	1.06E-03	9.52E-04	1.03E-03	6.06E-04	5.34E-03
MIX2	1.83E-03	9.56E-04	7.95E-02	1.79E-02	2.42E-01	4.83E-01	9.05E-04	6.40E-04	9.89E-04	5.16E-04	5.51E-03
MIX3	1.40E-03	6.24E-04	3.80E-02	1.54E-02	1.37E-01	4.07E-01	9.23E-04	5.80E-04	8.86E-04	5.26E-04	5.92E-03

Results indicated that consumption of As contaminated vegetable may contribute >50% of RDA for Mn for different plant species, with highest intake by adolescent (Table Anx. 2.6). Similarly, Fe and Mn showed great variations in RDA for different plant species, age groups and gender. There was a significant difference between the dietary intakes of Mg, these changes were modest in term of Mg RDA. For children, a single servant would provide and RDA of 17, 6, 8 and 8 (potato), 12, 18, 20, 21 (tomato) and 16, 23, 23 and 14% (lettuce) for control, Mix1, Mix2 and Mix3, respectively. The changes in Mg RDA for adults and adolescents by consumption of control and contaminated soil grown potato showed almost similar effects. Unlike Fe and Mn, the RDA provided by Ca was <3% for different treatment levels and age groups except for Mix2 (lettuce). Although Ca had minimal contribution to RDA and a significant ($P<0.01$) difference was observed among different treatment levels and plant species (Table Anx. 2.6).

The contribution to %RDA of macro- nutrients was <15% for all the macronutrients (Table 4.5 and Anx. 2.7). P showed substantial contribution to %RDA for both the control and As contaminated vegetables. Although other macro- nutrients showed less contribution to %RDA, still there was a significant variation ($P<0.01$) among different treatment levels and age groups. The %RDA of N for potato grown As contaminated soil was almost double than the control, while lettuce showed variable effects under different treatment levels. Similarly, tomato showed a reduction in N contribution to %RDA. The contributions to %RDA of vitamin C and K were significantly ($P<0.01$) decreased in plants grown in contaminated soil (Table 4.5 and Anx. 2.7).

The cumulative %RDA also varied significantly for different treatment levels (Table 4.5). Substantial variation in % RDA of macro- and micro- nutrients was observed for both male and female of different age groups with significant changes for children. Mn provided the highest RDA for female and children followed by Fe. Nutritional assessment study of different age

groups and gender revealed that macro- and micro- nutrients had more contributions to RDA for female than male for Mn, Mg, N and protein, while for the rest of the nutrients, RDA was similar for both male and female. Similarly, for different age groups the order of contributions to RDA were adult>adolescent>children for Fe, K and vitamin C, adolescent>children>adult for Mn, adult>children>adolescent for Ca and P, while children>adolescent>adults for Mg, N and protein, respectively (Table 4.5). Generally, the RDA was highest for Mn, medium for P, K, Mg and Fe and lowest for Zn, Ca, N, protein and vitamin C for different plant species and age groups.

Table 4.5. Cumulative RDA (%) of macro- and micro- nutrients provided by dietary intake of As contaminated vegetables

	Fe		Mn		Ca	Mg		N		P	K	Protein		Vitamin C
	M	F	M	F	M-F	M	F	M	F	M-F	M-F	M	F	M-F
Children														
Control	30.3	30.3	87.1	91.3	1.04	15.0	15.4	1.36	1.35	6.74	4.45	0.12	0.12	0.58
As1	39.1	39.1	63.6	66.7	1.41	16.4	16.9	1.14	1.13	6.04	3.40	0.10	0.10	0.54
As2	45.3	45.3	59.9	62.9	1.15	17.7	18.2	1.17	1.16	5.19	3.01	0.11	0.11	0.56
As3	41.4	41.4	54.1	56.8	1.39	19.0	19.6	0.88	0.87	6.01	2.74	0.08	0.08	0.65
AsCd1	47.2	47.2	72.1	75.6	1.69	22.0	22.6	1.17	1.16	2.62	3.46	0.11	0.11	0.66
AsCd2	40.4	40.4	77.5	81.3	1.18	19.4	19.9	0.85	0.84	4.63	3.23	0.08	0.08	0.66
AsCd3	46.6	46.6	77.8	81.6	1.43	16.7	17.2	1.300	1.29	4.78	2.99	0.12	0.12	0.65
AsPb1	40.9	40.9	81.9	85.9	1.31	16.0	16.5	1.12	1.11	5.26	3.45	0.10	0.10	0.59
AsPb2	41.7	41.7	79.3	83.2	1.42	19.4	19.9	1.18	1.17	6.82	3.23	0.11	0.11	0.64
AsPb3	43.1	43.1	80.5	84.5	1.36	16.2	16.7	1.31	1.30	4.96	2.89	0.12	0.12	0.70

MIX1	40.2	40.2	82.4	86.5	1.09	15.3	15.8	1.20	1.19	6.61	2.92	0.11	0.11	0.61
MIX2	49.0	49.0	60.9	63.9	1.29	16.9	17.3	1.02	1.01	4.44	2.81	0.09	0.09	0.63
MIX3	23.4	23.4	52.6	55.1	0.73	14.2	14.6	1.04	1.03	4.02	2.52	0.10	0.09	0.67
Adolescent														
Control	34.0	24.9	88.3	121.4	0.95	11.8	12.4	0.97	1.30	5.90	4.97	0.09	0.12	0.64
As1	43.9	32.2	64.4	88.6	1.29	13.0	13.6	0.81	1.09	5.29	3.79	0.07	0.10	0.60
As2	50.9	37.3	60.8	83.5	1.05	13.9	14.6	0.83	1.11	4.55	3.36	0.08	0.10	0.62
As3	46.4	34.1	54.9	75.5	1.27	15.0	15.7	0.62	0.84	5.27	3.05	0.06	0.08	0.73
AsCd1	52.9	38.8	73.1	101	1.55	17.3	18.1	0.83	1.11	2.30	3.86	0.08	0.10	0.74
AsCd2	45.4	33.3	78.6	108	1.08	15.3	16.0	0.61	0.81	4.06	3.60	0.06	0.07	0.73
AsCd3	52.3	38.4	78.9	109	1.31	13.2	13.8	0.92	1.24	4.19	3.33	0.08	0.11	0.73
AsPb1	45.9	33.7	83.0	114	1.200	12.6	13.2	0.80	1.07	4.61	3.85	0.07	0.10	0.66
AsPb2	46.8	34.3	80.4	1116	1.30	15.3	16.0	0.84	1.13	5.98	3.60	0.08	0.10	0.72
AsPb3	48.4	35.5	81.7	112	1.24	12.8	13.4	0.94	1.25	4.35	3.22	0.09	0.11	0.78
MIX1	45.1	33.0	83.6	115	1.00	12.1	12.7	0.86	1.15	5.79	3.25	0.08	0.10	0.68
MIX2	54.9	40.3	61.8	84.9	1.18	13.3	13.9	0.73	0.98	3.89	3.13	0.07	0.09	0.70
MIX3	26.3	19.3	53.3	73.3	0.67	11.2	11.7	0.74	1.00	3.52	2.81	0.07	0.09	0.75
Adults														
Control	46.7	20.8	84.4	107.9	1.23	10.5	13.6	0.93	1.17	10.5	4.97	0.08	0.11	0.64
As1	60.3	26.8	61.6	78.8	1.68	11.5	14.9	0.78	0.98	9.44	3.79	0.07	0.09	0.60
As2	69.9	31.1	58.1	74.3	1.37	12.3	16.0	0.80	1.01	8.13	3.36	0.07	0.09	0.62
As3	63.9	28.4	52.5	67.1	1.65	13.3	17.3	0.60	0.76	9.41	3.05	0.05	0.07	0.73
AsCd1	72.8	32.3	69.9	89.4	2.01	15.3	19.9	0.80	1.01	4.10	3.86	0.07	0.09	0.74
AsCd2	62.4	27.7	75.2	96.1	1.40	13.5	17.6	0.58	0.73	7.25	3.60	0.05	0.07	0.73
AsCd3	71.9	32.0	75.5	96.5	1.70	11.6	15.1	0.89	1.12	7.47	3.33	0.08	0.10	0.73
AsPb1	63.1	28.0	79.4	101.5	1.56	11.2	14.5	0.77	0.97	8.23	3.85	0.07	0.09	0.66

AsPb2	64.4	28.6	76.9	98.3	1.69	13.5	17.6	0.81	1.02	10.7	3.60	0.07	0.09	0.72
AsPb3	66.5	29.6	78.1	99.8	1.62	11.3	14.7	0.90	1.13	7.76	3.22	0.08	0.10	0.78
MIX1	62.0	27.5	79.9	102.1	1.30	10.7	13.9	0.82	1.04	10.3	3.25	0.08	0.09	0.68
MIX2	75.6	33.6	59.1	75.5	1.53	11.8	15.3	0.70	0.88	6.94	3.13	0.06	0.08	0.70
MIX3	36.1	16.1	51.0	65.1	0.87	9.91	12.9	0.71	0.90	6.29	2.81	0.07	0.08	0.75

M; Male, F; Female

4.3 As, Cd and Pb effects on heavy metals

4.3.1. As effects on heavy metals

Arsenic (As) uptake by vegetables is strongly correlated with its concentration in the respective soil (Huang et al. 2006). Food crops have the capability to accumulate toxic metals in the edible parts (Mapanda et al. 2007). The mean concentration of Cu, Cr, Ni and Zn show significant variation at different treatment levels (Fig. 4.5). Lettuce Cu concentration slightly increases with increase concentration of As and tomato concentration decreases by 19% in As3, while in potato a significant decrease ($p < 0.01$) was observed except for As1 which increased by 5%. Similarly, binary mixture of As and Cd have no significant effects on lettuce Cu concentration while in potato (25%) and tomato (48%) a significant decrease at AsCd2 (Fig.4.5) was observed. Among different contamination levels the highest decrease in Cu concentration was observed at binary mixture of As and Pb for lettuce followed by potato and tomato, while the tri mixture have less effect on Cu concentration except for tri mixture (Mix3) in tomato and lettuce. Lettuce is hyper accumulator for Cr as compare to potato. Cr concentration in potato observed a significant increase with increase concentration of As and its binary and tri mixture, however the increase in Cr concentration was not consistent for all the treatments. Results show that the binary and tri mixture effects were more significant than individual element

concentration (Fig. 4.5). Similarly, lettuce and tomato show a progressive decrease in Cr concentration with increasing As concentration, almost similar results were observed under tri mixture treatment for lettuce, however, lettuce cultivated in binary mixture of As-Cd and As-Pb observed an increase in Cr concentration but the increase is not significant except for AsPb3 ($P < 0.05$). Contrastingly under binary and tri mixture Cr concentration was significantly increased in tomato ($P < 0.001$).

Ni is a toxic environmental pollutant that may cause serious health problems when increase their threshold limits (Oliver et al. 1997). Ni concentrations in potato, tomato and lettuce were significantly reduced with increased doses of As, while in tomato the concentration of Ni increased with increasing As stress, while in tomato the concentration of Ni increased with increasing As stress. Similarly, under binary mixtures a reduction in lettuce and potato and an induction in tomato Ni concentration was observed with few exceptions where an induction in lettuce and potato occurred. In tri mixture treatments Ni bioaccumulation in all three plant species reduced with increasing concentration of metal mixtures, while in lettuce the effects of binary mixture was less significant and that of tri mixture was more significant ($P < 0.01$) than potato and tomato (Fig. 4.5).

Zn is a well known environmental pollutant causes serious hazards to plant, animals and human beings (ASTDR, 2011; Trivedi and Axe, 2000). Zn, under high pH, exhibit amphoteric characteristics and form complex molecules with other elements (Zak and Deja, 2015). Results of Zn concentrations under As stress show significant increase, with few exceptions, in both lettuce and potato plants, while its concentration decreased in tomato. In lettuce a slight decrease in Zn concentration was observed in tri mixture treatment (Mix3), while potato showed significant decrease at Mix3 and Mix2, the rest of the treatments showed high concentration than

control. However, tomato under stress of As and its binary and tri mixture showed a progressive decrease with increase level of contaminants in the soil (Fig. 4.5). In a field study conducted by Shi et al. (2013), their findings show that with increase As concentration the Zn concentration in edible plant parts also increase. Similarly, Beccaloni et al. (2013) observed almost similar pattern of increase in Zn contents with increase As concentration in different leafy and non leafy vegetables. The differences in bioaccumulation pattern of heavy metals by the three plant species may be due to change in the physiological characteristics of these plants, as different plant species have differential responses to same element and within the same plant specie the bioaccumulation varies for different elements and even for the same element at different concentrations.

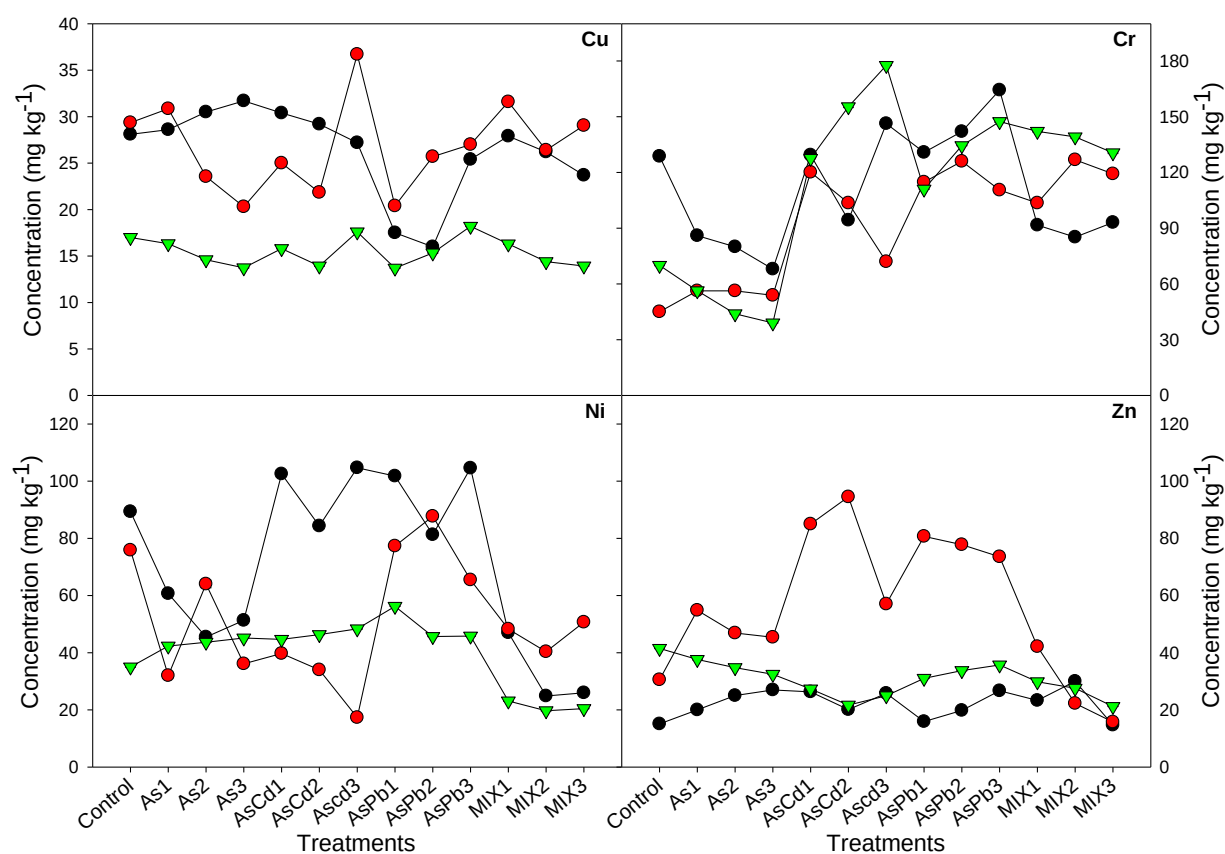


Fig. 4.5. Heavy metals concentration of As contaminated vegetables

4.3.2. Cd and Pb effects on heavy metals uptake

Food crop cultivated on contaminated soil may not only uptake essential minerals but also toxic elements like heavy metals (Xu et al. 2013a). Food plants are good nutritional dietary source containing macro- nutrients and several trace minerals (Kawashima and Soares, 2003) and provide defensive mechanism against oxidative stress and diseases (Llorach et al. 2008).

Cr concentrations showed great variation at different treatments of Cd and Pb (Fig. 4.6). In potato elevated concentrations of Cr were observed with increase dose of Cd and Pb showing positive correlation with both heavy metals. In tomato the concentration of Cr decreased with increasing concentration of Cd and increased with Pb at contamination level Pb₁ and Pb₃, while decreased at level Pb₂. Similarly, increase in Cr concentrations was observed under combined treatments. The differences in Cr concentrations between control and combined treatments was significant at $P < 0.01$. The bioaccumulation of heavy metals is strongly influenced by other metals, resulting in both antagonistic and synergistic effects (An et al. 2004). The Cu concentration in potato was significantly reduced by Cd and Pb at contamination level Cd₁, Cd₃, Pb₁ and Pb₃. The results showed that Cd and Pb has antagonistic relationship with Cu, as the concentration of these metals increase in soil the bioaccumulation of Cu decreases (Fig. 4.6). These results are in agreement with the findings of An et al. (2004).

Ni concentration varies greatly at different treatment levels. Ni exhibit both synergistic and antagonistic association with Cd and Pb depend on type of plant species, as in potato Ni concentration increased under Cd and Pb stress, while in tomato the effects were synergistic for Cd and antagonistic for Pb, similarly the binary mixture of Cd-Pb exhibit synergistic effects in lettuce. This behavior of Ni showing both synergistic and antagonistic effects to same level of Cd

and Pb at different plant species may be due to the difference in the mechanisms involve in metal uptake by plant (Garty 2001). When compare with different contamination levels the potato Zn concentrations was observed a progressive increase with increase concentrations of Cd and Pb in the soil except for Cd3 and Pb3 which showed no significant changes in Zn, while in tomato the Zn contents decreased with increase concentration of Cd and Pb, except for Cd3 (44 mg kg⁻¹) and Pb1 (46 mg kg⁻¹) where the concentrations increase as compare to control (42 mg kg⁻¹) environment. The progressive increase in Zn concentrations with increase in soil Cd content may be due to the mechanism involve in Cd and Zn transport to plants, different studies revealed that the mechanism of transport of both the elements are similar (Liu et al. 2003; Kabata-Pendias and Pendias, 1984). Similarly, in lettuce cultivar the Zn concentrations increased with increase dose of Pb. Binary mixture of Cd-Pb have synergistic effects on Zn contents of potato and lettuce and antagonistic effects on tomato. Furthermore, the Zn concentration in binary mixture treatments were in order of CdPb1>CdPb2>CdPb3, CdPb1>CdPb2>CdPb3 and CdPb1>CdPb3>CdPb2 for potato, tomato and lettuce, respectively (Fig. 4.6).

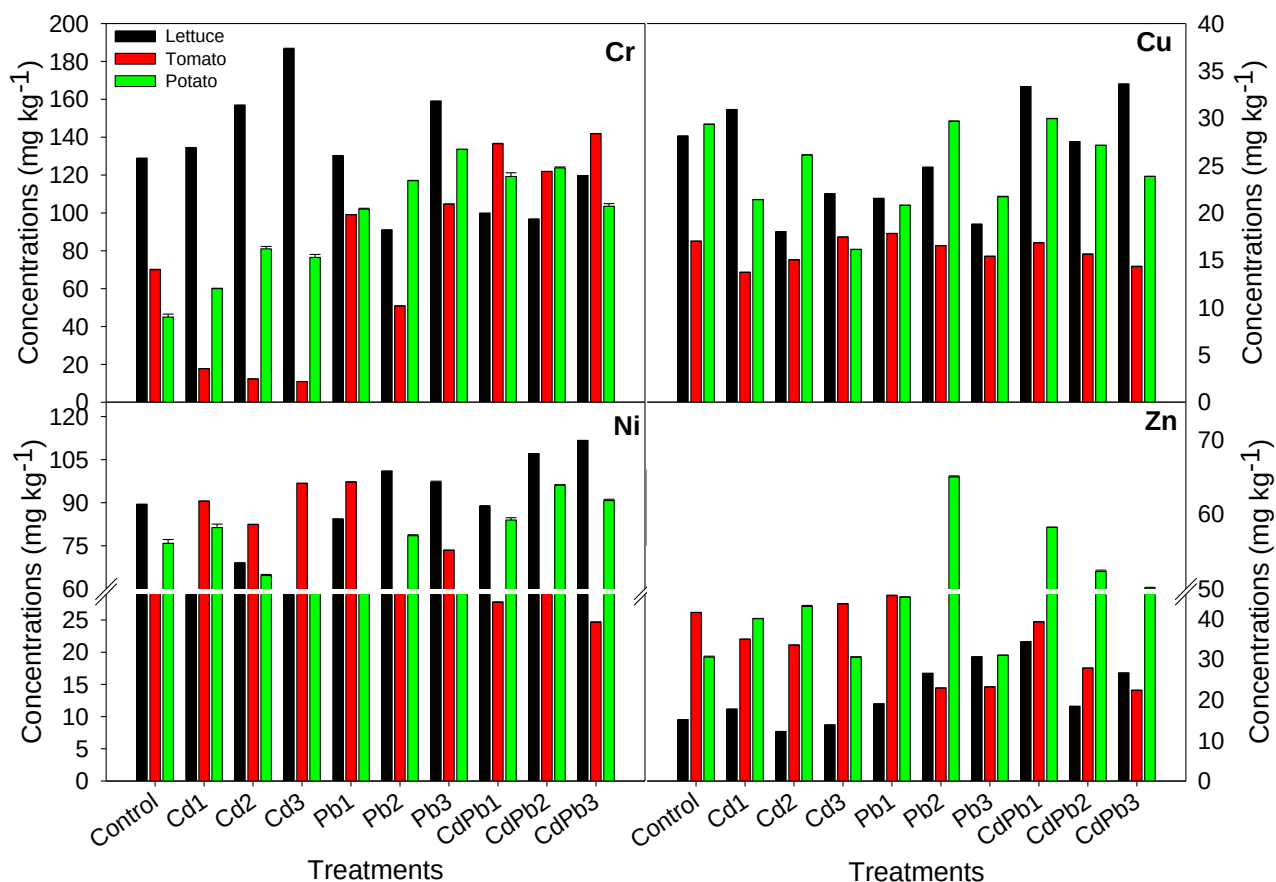


Fig. 4.6. Heavy metals concentration of Cd and Pb contaminated vegetables

4.3.3. Daily Intake of metals

The recommended daily dietary intake values have been suggested for several trace and essential elements. These values are used to determine whether a particular element is essential or toxic. The essentiality and toxicity of these elements have been discussed previously (Goldhaber, 2003). The DIM values (mg d⁻¹), individual and cumulative, of Cr were significantly higher for contaminated soil than control, with few exceptions (Table Anx.3.1-3.4). The DIM values through consumption of As contaminated vegetables were significantly higher for potato than tomato and lettuce ($P < 0.01$). The cumulative intake showed that AsPb3 and tri mixtures

provided maximum intake of Cr for both adults and children. Cd and Pb contaminated vegetables showed almost similar results for Cr intake. Similarly, the DIM ($\text{mg kg}^{-1} \text{d}^{-1}$) values of Cr through consumption of As contaminated vegetables indicated that among different vegetables tomato provide maximum intake followed by lettuce and potato (Table Anx. 3.5), while among different treatment levels the contribution to dietary intake was highest for AsCd3 (tomato). The results of Cd and Pb contaminated vegetables were almost similar that of As contaminated vegetables, however in Cd and Pb contaminated vegetables the maximum intake was observed for lettuce (Table Anx. 3.6). Among different age groups the significant intake was observed for children for vegetables cultivated in As, Cd and Pb contaminated soil (Table 4.6-4.7 and Anx. 3.5-3.6). Vegetables grown on contaminated soil showed a progressive decrease in Cu dietary intake (mg d^{-1}) with increase concentration of As, Cd and Pb, however, this decrease is not consistent for all vegetables and age groups (Table Anx. 3.1-3.4). Similarly, the cumulative intake was highest for treatment levels AsCd3 and CdPb1 respectively (Table Anx. 3.2 and 3.4). The dietary intake ($\text{mg kg}^{-1} \text{d}^{-1}$) values of Cu was highest for potato followed by lettuce and tomato in As contaminated vegetables while followed the order of lettuce>potato>tomato in Cd and Pb contaminated vegetables (Table Anx. 3.5-3.6). Among different age groups the cumulative intake was highest for children followed by adults and adolescents. Ni dietary intake (mg d^{-1} and $\text{mg kg}^{-1} \text{d}^{-1}$) showed variable effects under different contamination levels and age groups. The binary mixture of AsPb and CdPb observed an increase while in rest of the treatments the Ni concentrations were decreased (Table 4.6-4.7).

Table 4.6. Cumulative Daily dietary intake of metals (mg kg⁻¹ d⁻¹) of As contaminated vegetables

	Children				Adolescent				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Control	6.41E-02	1.96E-02	5.26E-02	2.29E-02	2.50E-02	7.65E-03	2.06E-02	8.95E-03	3.13E-02	9.58E-03	2.57E-02	1.12E-02
As1	5.22E-02	1.99E-02	3.55E-02	2.96E-02	2.04E-02	7.78E-03	1.39E-02	1.16E-02	2.55E-02	9.75E-03	1.74E-02	1.45E-02
As2	4.74E-02	1.81E-02	4.03E-02	2.80E-02	1.85E-02	7.05E-03	1.57E-02	1.10E-02	2.32E-02	8.83E-03	1.97E-02	1.37E-02
As3	4.23E-02	1.73E-02	3.49E-02	2.76E-02	1.65E-02	6.75E-03	1.36E-02	1.08E-02	2.07E-02	8.45E-03	1.70E-02	1.35E-02
AsCd1	9.91E-02	1.87E-02	4.92E-02	3.65E-02	3.87E-02	7.31E-03	1.92E-02	1.42E-02	4.85E-02	9.16E-03	2.40E-02	1.78E-02
AsCd2	9.29E-02	1.71E-02	4.33E-02	3.58E-02	3.63E-02	6.67E-03	1.69E-02	1.40E-02	4.54E-02	8.36E-03	2.12E-02	1.75E-02
AsCd3	1.04E-01	2.14E-02	4.48E-02	2.83E-02	4.07E-02	8.37E-03	1.75E-02	1.11E-02	5.09E-02	1.05E-02	2.19E-02	1.39E-02
AsPb1	9.37E-02	1.36E-02	6.19E-02	3.36E-02	3.66E-02	5.30E-03	2.42E-02	1.31E-02	4.59E-02	6.64E-03	3.03E-02	1.64E-02
AsPb2	1.06E-01	1.50E-02	5.64E-02	3.45E-02	4.13E-02	5.86E-03	2.20E-02	1.35E-02	5.18E-02	7.34E-03	2.76E-02	1.69E-02
AsPb3	1.11E-01	1.86E-02	5.68E-02	3.58E-02	4.34E-02	7.25E-03	2.22E-02	1.40E-02	5.43E-02	9.08E-03	2.78E-02	1.75E-02
MIX1	8.87E-02	1.99E-02	3.11E-02	2.50E-02	3.46E-02	7.79E-03	1.22E-02	9.78E-03	4.34E-02	9.75E-03	1.52E-02	1.23E-02
MIX2	9.23E-02	1.76E-02	2.23E-02	2.10E-02	3.61E-02	6.88E-03	8.72E-03	8.20E-03	4.52E-02	8.62E-03	1.09E-02	1.03E-02
MIX3	9.01E-02	1.75E-02	2.55E-02	1.36E-02	3.52E-02	6.85E-03	9.97E-03	5.30E-03	4.41E-02	8.58E-03	1.25E-02	6.64E-03

Table 4.7. Cumulative Daily dietary intakes ($\text{mg kg}^{-1} \text{d}^{-1}$) of heavy metals of Cd and Pb contaminated vegetables.

	Children				Adolescent				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Control	6.39E-02	1.96E-02	5.26E-02	2.29E-02	2.50E-02	7.65E-03	2.06E-02	8.95E-03	3.13E-02	9.55E-03	2.58E-02	1.12E-02
Cd1	5.58E-02	1.74E-02	5.39E-02	2.44E-02	2.18E-02	6.78E-03	2.11E-02	9.52E-03	2.73E-02	8.48E-03	2.64E-02	1.19E-02
Cd2	6.59E-02	1.56E-02	5.68E-02	2.33E-02	2.57E-02	6.07E-03	2.22E-02	9.09E-03	3.22E-02	7.62E-03	2.78E-02	1.14E-02
Cd3	7.20E-02	1.46E-02	4.49E-02	2.31E-02	2.82E-02	5.71E-03	1.75E-02	9.05E-03	3.52E-02	7.15E-03	2.20E-02	1.13E-02
Pb1	8.72E-02	1.58E-02	5.55E-02	2.89E-02	3.40E-02	6.17E-03	2.17E-02	1.13E-02	4.26E-02	7.74E-03	2.71E-02	1.41E-02
Pb2	6.80E-02	1.87E-02	5.67E-02	3.01E-02	2.66E-02	7.29E-03	2.21E-02	1.17E-02	3.33E-02	9.12E-03	2.77E-02	1.47E-02
Pb3	1.04E-01	1.47E-02	5.45E-02	2.23E-02	4.08E-02	5.74E-03	2.13E-02	8.71E-03	5.11E-02	7.19E-03	2.67E-02	1.09E-02
CdPb1	9.36E-02	2.11E-02	5.27E-02	3.46E-02	3.65E-02	8.24E-03	2.06E-02	1.35E-02	4.57E-02	1.03E-02	2.58E-02	1.69E-02
CdPb2	9.00E-02	1.85E-02	6.15E-02	2.59E-02	3.51E-02	7.22E-03	2.40E-02	1.01E-02	4.40E-02	9.04E-03	3.01E-02	1.27E-02
CdPb3	9.60E-02	1.89E-02	5.97E-02	2.60E-02	3.75E-02	7.39E-03	2.33E-02	1.02E-02	4.69E-02	9.24E-03	2.92E-02	1.27E-02

Unlike Ni, Zn concentration increased except for tri mixture treatments in potato, while increased in lettuce and tomato for both adults and children. The exposure to metals toxicity through consumption of contaminated vegetables was highest for children than adults these results are in agreements with previous study of Beccaaloni et al. (2013), they reported that among different age groups infants were the most sensitive risk group exposed to metals toxicity. A number of studies have been conducted to evaluated Cd interaction with other elements, Cd has the capability to interfere with other elements like Cu and Zn and affect their metabolism (Peraza et al. 1998).

The NAS, (2001) has not recommended RDA and upper intake limit values for Cr therefore RDA was not calculated for this element. While for Ni there is no RDA value available, however NAS (2001) has suggested upper intake level (UL) for Children (0.37 mg d^{-1}), adolescents (1.0 mg d^{-1}) and adults (1.0 mg d^{-1}), therefore the RDA values were calculated on the basis of UL. In the present study lettuce was the main source of Ni contribution to RDA for both As and Cd-Pb contaminated vegetables (Table Anx. 3.7-3.8). Similarly, the cumulative RDA was more significant for children than adults (Table 4.8-4.9). The individual plants cultivated on control and contaminated soil showed the highest RDA of >50% for Cu for different age groups and treatment levels. Cu contribution to RDA through consumption of As contaminated vegetables were in order of potato>lettuce>tomato for different age groups (Table Anx. 3.7-3.8). Similarly, Cd and Pb contaminated soil contributed to Cu RDA with an average of 88, 73 and 72 % for children, adolescent and adults, respectively (Table 4.9). The Cu RDA is similar for both male and female.

Zn have <30% contribution to RDA for individual plant species and <20% for cumulative intake of the three vegetables cultivated in As contaminated soil, while, Zn have <10%

contribution to RDA for children and <20% for adults and adolescents with few exceptions eliciting great variation in RDA for different age groups and gender under different contamination levels. Zn contribution to cumulative RDA by consumption of contaminated vegetables was significant for As contaminated vegetables (Table Anx. 3.7 and 4.8) and less significant for Cd and Pb (Table Anx. 3.8 and 4.9). The maximum contribution to RDA by Zn was observed for treatment level AsCd1 for children. Similarly, among different age groups the contribution to Zn RDA was more significant for children than adults, while in adults female contributed more than male (Table 4.8 and 4.9).

Table 4.8. Cumulative RDA of different trace metals through consumption of As contaminated vegetables

	Children			Adolescent				Adults			
	Cu	Ni	Zn	Cu	Ni	Zn	Zn	Cu	Ni	Zn	Zn
	M+F	M+F	M+F	M+F	M+F	M	F	M+F	M+F	M	F
Control	99.87	355.7	10.75	81.77	195.7	7.740	9.470	80.86	195.7	7.740	10.65
As1	101.7	239.8	13.87	83.23	132.0	10.00	12.22	82.31	132.0	10.00	13.74
As2	92.09	272.1	13.16	75.40	149.7	9.480	11.59	74.56	149.7	9.480	13.03
As3	88.18	235.5	12.93	72.19	129.6	9.320	11.39	71.39	129.6	9.320	12.81
AsCd1	95.51	332.1	17.10	78.20	182.7	12.32	15.06	77.33	182.7	12.32	16.94
AsCd2	87.17	292.6	16.81	71.37	161.0	12.11	14.80	70.58	161.0	12.11	16.65
AsCd3	109.3	302.6	13.29	89.51	166.5	9.580	11.70	88.52	166.5	9.580	13.17
AsPb1	69.22	418.1	15.74	56.67	230.1	11.34	13.86	56.04	230.1	11.34	15.59
AsPb2	76.51	381.4	16.20	62.64	209.9	11.67	14.26	61.94	209.9	11.67	16.05
AsPb3	94.71	383.6	16.77	77.54	211.0	12.08	14.77	76.68	211.0	12.08	16.61
MIX1	101.7	210.4	11.75	83.25	115.7	8.470	10.35	82.33	115.7	8.470	11.64
MIX2	89.88	150.9	9.850	73.59	83.01	7.100	8.670	72.77	83.01	7.100	9.760
MIX3	89.45	172.51	6.37	73.24	94.92	4.590	5.610	72.42	94.92	4.590	6.310

M+F: Male + Female: the values is samr for both genders

Table 4.9. Cumulative RDA of different trace metals through consumption of Cd and Pb contaminated vegetables

	Children			Adolescent				Adult			
	Cu	Ni	Zn	Cu	Ni	Zn	Zn	Cu	Ni	Zn	Zn
	M+F	M+F	M+F	M+F	M+F	M	F	M+F	M+F	M	F
Control	99.27	355.7	3.030	81.77	195.7	7.730	9.470	80.87	195.7	7.73	10.63
Cd1	88.00	364.1	3.230	72.50	200.4	8.230	10.07	71.70	200.4	8.230	11.33
Cd2	78.87	383.9	3.100	64.97	211.2	7.900	9.630	64.23	211.2	7.900	10.87
Cd3	74.07	303.3	3.070	61.00	166.9	7.800	9.530	60.37	166.9	7.800	10.77
Pb1	80.17	374.9	3.830	66.00	206.3	9.800	11.97	65.33	206.3	9.800	13.43
Pb2	94.70	382.9	4.000	78.00	210.7	10.17	12.43	77.13	210.7	10.17	13.97
Pb3	74.53	368.4	2.930	61.37	202.7	7.570	9.200	60.73	202.7	7.570	10.37
CdPb1	106.8	356.1	4.600	87.97	195.9	11.70	14.33	86.97	195.9	11.70	16.13
CdPb2	93.67	415.5	3.430	77.17	228.6	8.770	10.73	76.30	228.6	8.770	12.03
CdPb3	95.70	403.4	3.430	78.83	222.0	8.830	10.77	77.97	222.0	8.830	12.13

CHAPTER 5

Conclusions and Recommendations

5.1 Conclusions

Vegetables are the main dietary source having major nutritional contribution. Among food plants potato, tomato and lettuce are the major vegetables that consumed all over the world. These vegetables are rich source of macro- and micro- nutrients and contamination of these vegetables with heavy metals may severely affect human health. Heavy metal effects on human health are direct and indirect, the direct impacts involve major diseases caused by consumption of heavy metals contaminated vegetables, these effects include both cancer and non-cancer health risks, while, among the indirect effects reduction in nutritional values of contaminated vegetables is the most important effect. The results of the study indicated that the concentration of heavy metals were much higher in contaminated vegetables than control. In the background study the concentration of heavy metals were well above their permissible limits set by different countries and organization. These heavy metals have significantly affected the nutritional parameters of selected vegetables. The results indicated that heavy metal concentrations in soil are inversely related to plant nutritional values.

The first study indicated that the cultivation of vegetables on metals contaminated soil can significantly affect the nutritional composition of these vital food sources which may lead to malnutrition. Different vegetables showed great variation in heavy metal concentrations, which might be due to the difference in uptake and translocation mechanisms. The effects of Cd and Pb on nutritional composition of selected vegetables showed great variation with changing concentrations of these metals. Cd and Pb showed both synergistic and antagonistic effects on

macro- and micro- nutrients uptake. Similarly, the contribution to %RDA also varied for different elements. N, P, K, Ca, Fe, Mn and protein showed a decrease in %RDA, while Mg and vitamin C showed an increase in %RDA. From the findings of this study, it is suggested that consumption of metals contaminated food should be avoided and different remediation strategies should be adopted to reclaim the contaminated soil for safe cultivation. It is further suggested that the obtained data must be carefully interpreted because the average body weight of individual and daily vegetables consumption may vary from region to region and may be over and/or underestimated.

The second study was focused on As contamination and nutritional effects. The bioaccumulation of As in the edible tissues of potato, tomato and lettuce were significantly higher in contaminated soil than control. From the present study it is concluded that As contamination, with and without Cd and Pb, has deleterious effects on plant nutritional composition. Concentrations of some nutrients (Ca, Fe, Mg and vitamin C) increased in the edible tissues, while other (Mn, N, P, K and protein) decreased. The effects of As on macro- and micro- nutrients concentrations changed significantly with increasing the concentration and changing the metal mixtures. The daily dietary intake of As contaminated vegetables showed great variation depend upon type of vegetables, contamination level, metal mixture, age group and gender. Generally, children were more affected than adults. The contribution to RDA also varied for different macro- and micro- nutrients. From the findings of this study, we concluded that As and its binary and tri mixture had significant effects on macro- and micro- nutrient concentrations in vegetables and their consumption may severely affect the body nutrient balance resulting in malnutrition and health effects. It is strongly recommended to avoid the consumption of metal(s) contaminated vegetables. The data may be over or under estimated as the daily

dietary intake of vegetables, food habits, individual's body weight and standard of living varies greatly across the globe, therefore, great care must be given during data interpretation.

The third study reveal that heavy metals like As, Cd and Pb and their binary and tri mixture concentrations in soil have substantial effects on mobility and bioavailability of other trace metals, further more the bioavailability of trace metals under heavy metals stress is also affected by plant species. Cu, Cr, Ni and Zn observed significant changes under different contamination levels. The three metals and their binary and tri mixtures have both synergistic and antagonistic effects on trace metals bioavailability depend upon type of plant species, metal mixture and concentration. The daily dietary intake of Cr, Ni and Zn were higher for contaminated vegetables than control with few exceptions, while Cu observed a decrease in contaminated soil than control. Similarly, the contribution to RDA also varies for different elements depend upon type of vegetable, contaminants and their concentration. The highest contribution to RDA was observed for Ni followed by Cu and Zn, while no RDA was calculated for Cr. The results showed that As, Cd and Pb are significant environmental pollutants that have substantial effects on other metals bioavailability.

From the present study it can be concluded that heavy metals have significant negative impacts on elemental concentration of contaminated vegetables. Consumption of these contaminated vegetables has severe negative impacts on human nutrition. Continuous use of metals contaminated vegetables may result in malnutrition and associated health risks.

5.2 Recommendations

Based on the above mentioned information it is strongly recommended to avoid the consumption of metal(s) contaminated vegetables. Furthermore, it is suggested to adopt different

remediation strategies to minimize the possible health effects. The data presented may be under or over estimated because the dietary intake of vegetables, individual body weight, food habits and standard of living varies greatly from region to region, therefore, the data presented must be carefully interpreted. Further and more intensive studies are suggested to be carried out to assess the effects of different heavy metals on other macro- and micro- nutrients in both field and control environment experiments.

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Annexure-1

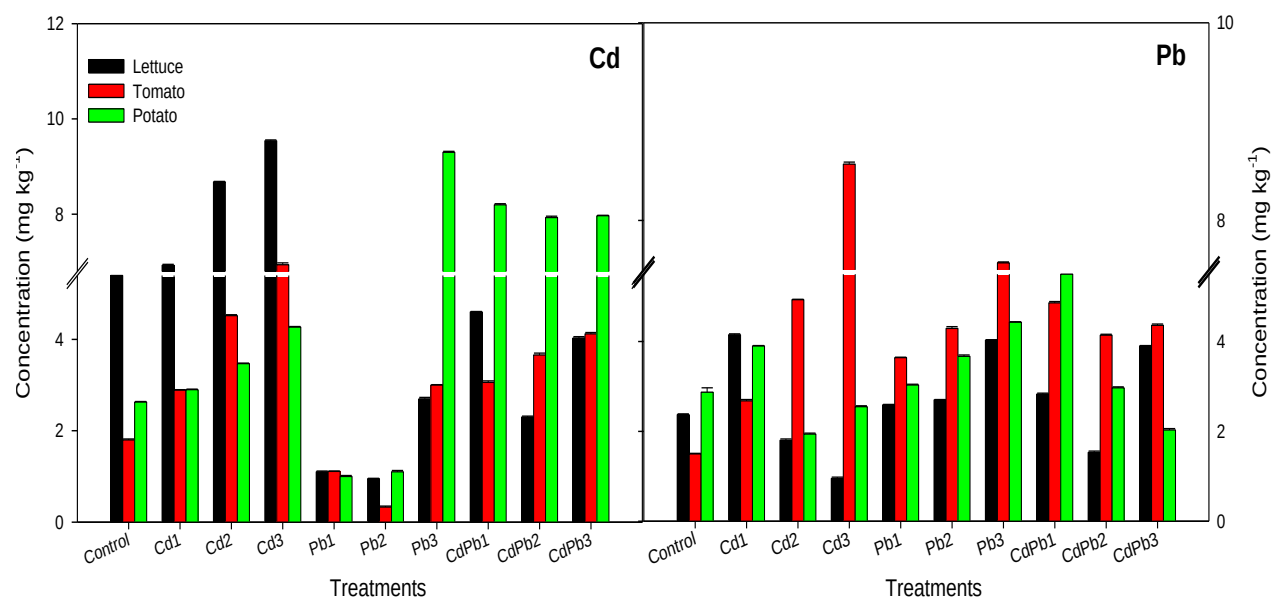


Fig. Anx. 1.1 Elemental analysis of vegetables cultivated on Cd and Pb contaminated soils. The error bars indicate standard deviation.

Table Anx. 1.1

Daily dietary Intake of Cd, Pb and micro- nutrients (mg d⁻¹) through consumption of vegetables

	Adults						Children					
	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg
Potato												
Control	7.7E-02	8.4E-02	1.5E+00	1.5E+00	1.1E+01	3.7E+01	5.2E-02	5.7E-02	1.0E+00	9.9E-01	7.3E+00	2.5E+01
Cd1	8.5E-02	1.1E-01	2.2E+00	1.4E+00	2.2E+01	4.8E+01	5.7E-02	7.7E-02	1.4E+00	9.3E-01	1.5E+01	3.2E+01
Cd2	1.0E-01	5.7E-02	1.2E+00	1.7E+00	1.3E+01	3.2E+01	6.8E-02	3.8E-02	7.8E-01	1.1E+00	9.0E+00	2.1E+01
Cd3	1.3E-01	7.5E-02	1.4E+00	2.2E+00	1.0E+01	2.9E+01	8.4E-02	5.0E-02	9.4E-01	1.5E+00	6.8E+00	1.9E+01
Pb1	2.9E-02	8.9E-02	2.8E+00	1.2E+00	7.5E+00	2.4E+01	2.0E-02	6.0E-02	1.9E+00	8.0E-01	5.0E+00	1.6E+01
Pb2	3.2E-02	1.1E-01	1.2E+00	2.2E+00	1.8E+01	3.4E+01	2.2E-02	7.2E-02	7.9E-01	1.4E+00	1.2E+01	2.3E+01
Pb3	2.7E-01	1.3E-01	1.3E+00	2.8E+00	2.7E+01	4.8E+01	1.8E-01	8.7E-02	8.5E-01	1.9E+00	1.8E+01	3.2E+01
CdPb1	2.4E-01	1.6E-01	2.0E+00	6.7E-01	2.1E+01	3.5E+01	1.6E-01	1.1E-01	1.4E+00	4.5E-01	1.4E+01	2.3E+01
CdPb2	2.3E-01	8.7E-02	1.6E+00	7.8E-01	1.3E+01	2.7E+01	1.6E-01	5.9E-02	1.1E+00	5.2E-01	8.5E+00	1.8E+01
CdPb3	2.3E-01	6.0E-02	1.0E+00	1.1E+00	5.1E+00	2.5E+01	1.6E-01	4.0E-02	7.0E-01	7.2E-01	3.5E+00	1.7E+01
Tomato												
Control	5.3E-02	4.4E-02	2.0E+00	1.7E+00	6.7E+00	2.6E+01	3.5E-02	3.0E-02	1.4E+00	1.2E+00	4.5E+00	1.7E+01
Cd1	8.5E-02	7.9E-02	1.0E+00	9.7E-01	5.3E+00	3.5E+01	5.7E-02	5.3E-02	6.9E-01	6.5E-01	3.6E+00	2.4E+01
Cd2	1.3E-01	1.4E-01	2.1E+00	2.3E+00	4.6E+00	3.2E+01	8.9E-02	9.7E-02	1.4E+00	1.5E+00	3.1E+00	2.2E+01
Cd3	2.0E-01	2.5E-01	1.9E+00	8.9E-01	5.5E+00	3.7E+01	1.4E-01	1.7E-01	1.3E+00	6.0E-01	3.7E+00	2.5E+01

Pb1	3.3E-02	1.1E-01	2.5E+00	1.3E+00	6.2E+00	3.5E+01	2.2E-02	7.2E-02	1.7E+00	8.9E-01	4.2E+00	2.3E+01
Pb2	9.7E-03	1.3E-01	2.0E+00	1.4E+00	4.2E+00	4.3E+01	6.5E-03	8.5E-02	1.3E+00	9.2E-01	2.8E+00	2.9E+01
Pb3	8.8E-02	2.2E-01	1.4E+00	1.9E+00	8.2E+00	4.9E+01	5.9E-02	1.5E-01	9.3E-01	1.3E+00	5.5E+00	3.3E+01
CdPb1	9.0E-02	1.4E-01	1.6E+00	1.1E+00	6.0E+00	3.2E+01	6.0E-02	9.6E-02	1.1E+00	7.4E-01	4.1E+00	2.1E+01
CdPb2	1.1E-01	1.2E-01	1.2E+00	1.3E+00	5.1E+00	3.9E+01	7.2E-02	8.2E-02	8.2E-01	9.0E-01	3.4E+00	2.6E+01
CdPb3	1.2E-01	1.3E-01	1.4E+00	1.4E+00	7.0E+00	4.2E+01	8.1E-02	8.6E-02	9.5E-01	9.6E-01	4.7E+00	2.8E+01
Lettuce												
Control	1.6E-01	7.0E-02	7.7E+00	2.6E+00	2.7E+01	3.5E+01	1.1E-01	4.7E-02	5.2E+00	1.8E+00	1.8E+01	2.4E+01
Cd1	2.0E-01	1.2E-01	5.9E+00	1.8E+00	1.3E+01	3.1E+01	1.4E-01	8.2E-02	3.9E+00	1.2E+00	8.8E+00	2.1E+01
Cd2	2.5E-01	5.3E-02	6.7E+00	1.3E+00	2.3E+01	4.6E+01	1.7E-01	3.5E-02	4.5E+00	8.7E-01	1.6E+01	3.1E+01
Cd3	2.8E-01	2.8E-02	9.2E+00	1.1E+00	3.5E+01	6.6E+01	1.9E-01	1.9E-02	6.2E+00	7.1E-01	2.4E+01	4.5E+01
Pb1	3.2E-02	7.6E-02	1.2E+01	2.6E+00	2.7E+01	6.1E+01	2.2E-02	5.1E-02	8.1E+00	1.8E+00	1.8E+01	4.1E+01
Pb2	2.8E-02	7.9E-02	9.5E+00	1.2E+00	2.3E+01	3.8E+01	1.9E-02	5.3E-02	6.4E+00	8.1E-01	1.6E+01	2.6E+01
Pb3	7.9E-02	1.2E-01	6.5E+00	1.1E+00	1.3E+01	3.3E+01	5.3E-02	7.9E-02	4.3E+00	7.1E-01	8.6E+00	2.2E+01
CdPb1	1.3E-01	8.3E-02	5.8E+00	1.7E+00	1.1E+01	1.3E+01	9.1E-02	5.6E-02	3.9E+00	1.2E+00	7.3E+00	8.8E+00
CdPb2	6.7E-02	4.5E-02	6.1E+00	2.0E+00	1.1E+01	2.6E+01	4.5E-02	3.0E-02	4.1E+00	1.4E+00	7.5E+00	1.7E+01
CdPb3	1.2E-01	1.1E-01	5.5E+00	1.4E+00	6.3E+00	2.4E+01	7.9E-02	7.7E-02	3.7E+00	9.1E-01	4.2E+00	1.6E+01

Table Anx. 1.2

Daily dietary Intake of macro- nutrients (mg d⁻¹) through consumption of vegetables

	Adults					Children				
	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C
Potato										
Control	6.26E-02	7.44E-02	1.50E-01	3.57E-02	4.40E-01	4.21E-02	5.00E-02	1.00E-01	2.40E-02	2.96E-01
Cd1	5.65E-02	6.01E-02	1.00E-01	3.22E-02	3.15E-01	3.80E-02	4.04E-02	7.00E-02	2.16E-02	2.12E-01
Cd2	8.42E-02	5.95E-02	9.00E-02	4.80E-02	1.76E-01	5.66E-02	4.00E-02	6.00E-02	3.23E-02	1.18E-01
Cd3	1.35E-01	5.96E-02	9.00E-02	7.72E-02	3.30E-01	9.11E-02	4.01E-02	6.00E-02	5.19E-02	2.22E-01
Pb1	1.27E-01	6.94E-02	1.20E-01	7.25E-02	3.30E-01	8.56E-02	4.67E-02	8.00E-02	4.88E-02	2.22E-01
Pb2	1.22E-01	6.48E-02	1.00E-01	6.96E-02	2.57E-01	8.21E-02	4.36E-02	7.00E-02	4.68E-02	1.73E-01
Pb3	9.24E-02	6.39E-02	9.00E-02	5.27E-02	2.38E-01	6.21E-02	4.30E-02	6.00E-02	3.54E-02	1.60E-01
CdPb1	1.44E-01	7.08E-02	1.10E-01	8.19E-02	1.54E-01	9.66E-02	4.76E-02	8.00E-02	5.51E-02	1.04E-01
CdPb2	1.24E-01	6.19E-02	1.00E-01	7.04E-02	1.58E-01	8.31E-02	4.16E-02	7.00E-02	4.74E-02	1.06E-01
CdPb3	1.12E-01	4.24E-02	9.00E-02	6.38E-02	1.69E-01	7.52E-02	2.85E-02	6.00E-02	4.29E-02	1.13E-01
Tomato										
Control	1.34E-01	8.40E-02	9.74E-02	7.66E-02	5.28E-01	9.04E-02	5.65E-02	6.55E-02	5.15E-02	3.55E-01
Cd1	4.01E-02	5.74E-02	7.43E-02	2.29E-02	7.48E-01	2.70E-02	3.86E-02	5.00E-02	1.54E-02	5.03E-01
Cd2	1.22E-01	5.64E-02	5.90E-02	6.96E-02	8.65E-01	8.21E-02	3.79E-02	3.97E-02	4.68E-02	5.82E-01
Cd3	8.89E-02	3.00E-02	5.23E-02	5.07E-02	9.31E-01	5.98E-02	2.02E-02	3.52E-02	3.41E-02	6.26E-01
Pb1	4.75E-02	4.40E-02	7.63E-02	2.71E-02	1.03E+00	3.20E-02	2.96E-02	5.13E-02	1.82E-02	6.90E-01
Pb2	3.84E-02	3.39E-02	6.49E-02	2.19E-02	7.92E-01	2.58E-02	2.28E-02	4.36E-02	1.47E-02	5.32E-01
Pb3	7.32E-02	4.50E-02	5.50E-02	4.17E-02	6.45E-01	4.92E-02	3.02E-02	3.70E-02	2.81E-02	4.34E-01
CdPb1	4.72E-02	4.10E-02	7.76E-02	2.69E-02	6.16E-01	3.17E-02	2.76E-02	5.22E-02	1.81E-02	4.14E-01
CdPb2	4.41E-02	3.88E-02	8.01E-02	2.52E-02	8.36E-01	2.97E-02	2.61E-02	5.39E-02	1.69E-02	5.62E-01

CdPb3	3.90E-02	3.17E-02	5.91E-02	2.22E-02	6.60E-01	2.62E-02	2.13E-02	3.98E-02	1.49E-02	4.44E-01
Lettuce										
Control	7.70E-02	6.31E-02	1.10E-01	4.39E-02	1.87E-01	5.18E-02	4.24E-02	7.40E-02	2.95E-02	1.26E-01
Cd1	8.42E-02	4.80E-02	8.20E-02	4.80E-02	2.38E-01	5.66E-02	3.23E-02	5.51E-02	3.23E-02	1.60E-01
Cd2	9.03E-02	4.57E-02	8.24E-02	5.15E-02	2.86E-01	6.07E-02	3.07E-02	5.54E-02	3.46E-02	1.92E-01
Cd3	3.98E-02	1.86E-02	7.46E-02	2.27E-02	3.19E-01	2.68E-02	1.25E-02	5.02E-02	1.53E-02	2.14E-01
Pb1	4.21E-02	4.04E-02	8.01E-02	2.40E-02	2.75E-01	2.83E-02	2.72E-02	5.39E-02	1.61E-02	1.85E-01
Pb2	7.90E-02	4.09E-02	7.83E-02	4.50E-02	2.86E-01	5.31E-02	2.75E-02	5.27E-02	3.03E-02	1.92E-01
Pb3	1.13E-01	3.71E-02	7.66E-02	6.44E-02	2.57E-01	7.59E-02	2.49E-02	5.15E-02	4.33E-02	1.73E-01
CdPb1	7.80E-02	4.45E-02	7.96E-02	4.45E-02	2.05E-01	5.25E-02	2.99E-02	5.35E-02	2.99E-02	1.38E-01
CdPb2	8.31E-02	2.21E-02	7.63E-02	4.74E-02	2.20E-01	5.59E-02	1.49E-02	5.13E-02	3.19E-02	1.48E-01
CdPb3	8.93E-02	5.24E-02	7.37E-02	5.09E-02	2.13E-01	6.00E-02	3.53E-02	4.95E-02	3.42E-02	1.43E-01

Table Anx. 1.3

Cumulative Daily dietary Intake of Cd, Pb and macro- and micro- nutrients (mg d⁻¹) for all three vegetables

	Cd	Pb	Fe	Mn	Ca	Mg	N	P	K	protein	Vitamin
Adults											
Control	9.60E-02	6.60E-02	3.70E+00	1.90E+00	1.50E+01	3.30E+01	9.13E-02	7.38E-02	1.19E-01	5.21E-02	3.85E-01
Cd1	1.20E-01	1.00E-01	3.00E+00	1.40E+00	1.40E+01	3.80E+01	6.02E-02	5.52E-02	8.59E-02	3.43E-02	4.34E-01
Cd2	1.60E-01	8.50E-02	3.30E+00	1.80E+00	1.40E+01	3.70E+01	9.88E-02	5.39E-02	7.77E-02	5.63E-02	4.42E-01
Cd3	2.00E-01	1.20E-01	4.10E+00	1.40E+00	1.70E+01	4.40E+01	8.81E-02	3.61E-02	7.17E-02	5.02E-02	5.27E-01
Pb1	3.10E-02	9.10E-02	5.80E+00	1.70E+00	1.30E+01	4.00E+01	7.23E-02	5.13E-02	9.07E-02	4.12E-02	5.44E-01
Pb2	2.30E-02	1.00E-01	4.20E+00	1.60E+00	1.50E+01	3.80E+01	7.99E-02	4.65E-02	8.24E-02	4.55E-02	4.45E-01
Pb3	1.50E-01	1.60E-01	3.00E+00	1.90E+00	1.60E+01	4.30E+01	9.28E-02	4.86E-02	7.44E-02	5.29E-02	3.80E-01
CdPb1	1.60E-01	1.30E-01	3.10E+00	1.20E+00	1.30E+01	2.70E+01	8.96E-02	5.21E-02	9.06E-02	5.11E-02	3.25E-01
CdPb2	1.40E-01	8.40E-02	3.00E+00	1.40E+00	9.60E+00	3.10E+01	8.36E-02	4.09E-02	8.50E-02	4.77E-02	4.04E-01
CdPb3	1.60E-01	1.00E-01	2.70E+00	1.30E+00	6.10E+00	3.10E+01	8.01E-02	4.22E-02	7.48E-02	4.56E-02	3.47E-01
Children											
Control	6.50E-02	4.40E-02	2.50E+00	1.30E+00	9.90E+00	21.97137	6.14E-02	4.96E-02	8.01E-02	3.50E-02	2.59E-01
Cd1	8.40E-02	7.10E-02	2.00E+00	9.40E-01	9.10E+00	25.75695	4.05E-02	3.71E-02	5.77E-02	2.31E-02	2.92E-01
Cd2	1.10E-01	5.70E-02	2.20E+00	1.20E+00	9.30E+00	24.76858	6.65E-02	3.62E-02	5.22E-02	3.79E-02	2.97E-01
Cd3	1.40E-01	7.90E-02	2.80E+00	9.20E-01	1.10E+01	29.54161	5.92E-02	2.43E-02	4.82E-02	3.38E-02	3.54E-01

Pb1	2.10E-02	6.10E-02	3.90E+00	1.20E+00	9.00E+00	26.92431	4.86E-02	3.45E-02	6.10E-02	2.77E-02	3.66E-01
Pb2	1.60E-02	7.00E-02	2.80E+00	1.10E+00	1.00E+01	25.8683	5.37E-02	3.13E-02	5.54E-02	3.06E-02	2.99E-01
Pb3	9.90E-02	1.10E-01	2.00E+00	1.30E+00	1.10E+01	29.15148	6.24E-02	3.27E-02	5.00E-02	3.56E-02	2.56E-01
CdPb1	1.00E-01	8.70E-02	2.10E+00	7.80E-01	8.60E+00	17.89097	6.03E-02	3.50E-02	6.09E-02	3.44E-02	2.19E-01
CdPb2	9.10E-02	5.70E-02	2.00E+00	9.30E-01	6.50E+00	20.72736	5.62E-02	2.75E-02	5.72E-02	3.21E-02	2.72E-01
CdPb3	1.10E-01	6.80E-02	1.80E+00	8.60E-01	4.10E+00	20.5203	5.38E-02	2.84E-02	5.03E-02	3.07E-02	2.33E-01

Table Anx. 1.4

Daily dietary Intake of Cd, Pb and micro- nutrients on individual body weight basis (mg kg⁻¹ d⁻¹) for single plant specie

	Children						Adolescent						Adults					
	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg
Potato																		
Control	2.07E-03	2.26E-03	4.10E-02	3.97E-02	2.92E-01	9.84E-01	8.10E-04	8.84E-04	1.60E-02	1.55E-02	1.14E-01	3.85E-01	1.01E-03	1.11E-03	2.01E-02	1.94E-02	1.43E-01	4.82E-01
Cd1	2.29E-03	3.08E-03	5.78E-02	3.71E-02	6.03E-01	1.30E+00	8.94E-04	1.20E-03	2.26E-02	1.45E-02	2.36E-01	5.07E-01	1.12E-03	1.50E-03	2.83E-02	1.81E-02	2.95E-01	6.35E-01
Cd2	2.74E-03	1.53E-03	3.12E-02	4.57E-02	3.62E-01	8.54E-01	1.07E-03	5.98E-04	1.22E-02	1.78E-02	1.41E-01	3.34E-01	1.34E-03	7.49E-04	1.53E-02	2.23E-02	1.77E-01	4.18E-01
Cd3	3.37E-03	2.01E-03	3.77E-02	5.84E-02	2.70E-01	7.74E-01	1.32E-03	7.86E-04	1.47E-02	2.28E-02	1.06E-01	3.02E-01	1.65E-03	9.84E-04	1.85E-02	2.86E-02	1.32E-01	3.79E-01
Pb1	7.89E-04	2.39E-03	7.63E-02	3.21E-02	2.01E-01	6.48E-01	3.08E-04	9.34E-04	2.98E-02	1.25E-02	7.86E-02	2.53E-01	3.86E-04	1.17E-03	3.73E-02	1.57E-02	9.84E-02	3.17E-01
Pb2	8.68E-04	2.89E-03	3.18E-02	5.80E-02	4.89E-01	9.24E-01	3.39E-04	1.13E-03	1.24E-02	2.26E-02	1.91E-01	3.61E-01	4.24E-04	1.42E-03	1.55E-02	2.84E-02	2.39E-01	4.52E-01
Pb3	7.34E-03	3.49E-03	3.41E-02	7.48E-02	7.30E-01	1.29E+00	2.87E-03	1.36E-03	1.33E-02	2.92E-02	2.85E-01	5.03E-01	3.59E-03	1.71E-03	1.67E-02	3.66E-02	3.57E-01	6.30E-01
CdPb1	6.47E-03	4.34E-03	5.41E-02	1.81E-02	5.74E-01	9.33E-01	2.53E-03	1.69E-03	2.11E-02	7.07E-03	2.24E-01	3.65E-01	3.16E-03	2.12E-03	2.65E-02	8.86E-03	2.81E-01	4.57E-01
CdPb2	6.26E-03	2.34E-03	4.28E-02	2.09E-02	3.42E-01	7.40E-01	2.44E-03	9.15E-04	1.67E-02	8.17E-03	1.34E-01	2.89E-01	3.06E-03	1.15E-03	2.10E-02	1.02E-02	1.67E-01	3.62E-01
CdPb3	6.29E-03	1.60E-03	2.80E-02	2.86E-02	1.38E-01	6.66E-01	2.46E-03	6.25E-04	1.09E-02	1.12E-02	5.39E-02	2.60E-01	3.08E-03	7.83E-04	1.37E-02	1.40E-02	6.75E-02	3.26E-01
Tomato																		
Control	1.42E-03	1.18E-03	5.44E-02	4.68E-02	1.81E-01	6.98E-01	5.55E-04	4.62E-04	2.13E-02	1.83E-02	7.09E-02	2.73E-01	6.95E-04	5.79E-04	2.66E-02	2.29E-02	8.87E-02	3.41E-01
Cd1	2.28E-03	2.11E-03	2.76E-02	2.62E-02	1.42E-01	9.45E-01	8.90E-04	8.26E-04	1.08E-02	1.02E-02	5.56E-02	3.69E-01	1.12E-03	1.03E-03	1.35E-02	1.28E-02	6.96E-02	4.62E-01
Cd2	3.57E-03	3.89E-03	5.52E-02	6.18E-02	1.25E-01	8.73E-01	1.39E-03	1.52E-03	2.16E-02	2.41E-02	4.87E-02	3.41E-01	1.74E-03	1.90E-03	2.70E-02	3.02E-02	6.10E-02	4.27E-01
Cd3	5.47E-03	6.76E-03	5.02E-02	2.39E-02	1.49E-01	9.85E-01	2.14E-03	2.64E-03	1.96E-02	9.33E-03	5.83E-02	3.85E-01	2.68E-03	3.31E-03	2.46E-02	1.17E-02	7.30E-02	4.82E-01
Pb1	8.76E-04	2.87E-03	6.75E-02	3.58E-02	1.67E-01	9.32E-01	3.42E-04	1.12E-03	2.64E-02	1.40E-02	6.53E-02	3.64E-01	4.28E-04	1.40E-03	3.30E-02	1.75E-02	8.18E-02	4.56E-01
Pb2	2.60E-04	3.38E-03	5.28E-02	3.70E-02	1.13E-01	1.15E+00	1.02E-04	1.32E-03	2.06E-02	1.44E-02	4.40E-02	4.51E-01	1.27E-04	1.66E-03	2.58E-02	1.81E-02	5.50E-02	5.65E-01
Pb3	2.37E-03	5.97E-03	3.72E-02	5.15E-02	2.21E-01	1.31E+00	9.24E-04	2.33E-03	1.45E-02	2.01E-02	8.63E-02	5.13E-01	1.16E-03	2.92E-03	1.82E-02	2.52E-02	1.08E-01	6.43E-01
CdPb1	2.41E-03	3.83E-03	4.32E-02	2.97E-02	1.62E-01	8.60E-01	9.43E-04	1.50E-03	1.69E-02	1.16E-02	6.35E-02	3.36E-01	1.18E-03	1.88E-03	2.12E-02	1.45E-02	7.95E-02	4.21E-01
CdPb2	2.89E-03	3.27E-03	3.29E-02	3.60E-02	1.36E-01	1.05E+00	1.13E-03	1.28E-03	1.29E-02	1.41E-02	5.32E-02	4.11E-01	1.41E-03	1.60E-03	1.61E-02	1.76E-02	6.66E-02	5.15E-01
CdPb3	3.25E-03	3.44E-03	3.82E-02	3.84E-02	1.88E-01	1.14E+00	1.27E-03	1.34E-03	1.49E-02	1.50E-02	7.33E-02	4.45E-01	1.59E-03	1.68E-03	1.87E-02	1.88E-02	9.18E-02	5.58E-01
Lettuce																		
Control	4.26E-03	1.87E-03	2.06E-01	7.02E-02	7.19E-01	9.54E-01	1.66E-03	7.30E-04	8.06E-02	2.74E-02	2.81E-01	3.73E-01	2.08E-03	9.14E-04	1.01E-01	3.43E-02	3.52E-01	4.67E-01

Cd1	5.47E-03	3.27E-03	1.58E-01	4.94E-02	3.50E-01	8.47E-01	2.14E-03	1.28E-03	6.16E-02	1.93E-02	1.37E-01	3.31E-01	2.67E-03	1.60E-03	7.72E-02	2.42E-02	1.71E-01	4.14E-01
Cd2	6.85E-03	1.42E-03	1.81E-01	3.49E-02	6.26E-01	1.25E+00	2.67E-03	5.55E-04	7.09E-02	1.36E-02	2.45E-01	4.87E-01	3.35E-03	6.95E-04	8.87E-02	1.71E-02	3.06E-01	6.09E-01
Cd3	7.53E-03	7.49E-04	2.47E-01	2.82E-02	9.48E-01	1.79E+00	2.94E-03	2.93E-04	9.64E-02	1.10E-02	3.70E-01	6.98E-01	3.68E-03	3.67E-04	1.21E-01	1.38E-02	4.64E-01	8.74E-01
Pb1	8.68E-04	2.04E-03	3.24E-01	7.02E-02	7.13E-01	1.65E+00	3.39E-04	7.98E-04	1.27E-01	2.74E-02	2.79E-01	6.45E-01	4.24E-04	9.99E-04	1.59E-01	3.43E-02	3.49E-01	8.08E-01
Pb2	7.49E-04	2.13E-03	2.56E-01	3.26E-02	6.22E-01	1.03E+00	2.93E-04	8.32E-04	1.00E-01	1.27E-02	2.43E-01	4.01E-01	3.67E-04	1.04E-03	1.25E-01	1.59E-02	3.04E-01	5.02E-01
Pb3	2.13E-03	3.18E-03	1.74E-01	2.86E-02	3.44E-01	8.96E-01	8.32E-04	1.24E-03	6.78E-02	1.12E-02	1.34E-01	3.50E-01	1.04E-03	1.55E-03	8.49E-02	1.40E-02	1.68E-01	4.39E-01
CdPb1	3.63E-03	2.23E-03	1.56E-01	4.63E-02	2.90E-01	3.54E-01	1.42E-03	8.72E-04	6.10E-02	1.81E-02	1.13E-01	1.38E-01	1.77E-03	1.09E-03	7.64E-02	2.27E-02	1.42E-01	1.73E-01
CdPb2	1.81E-03	1.21E-03	1.63E-01	5.51E-02	3.00E-01	6.95E-01	7.09E-04	4.71E-04	6.38E-02	2.15E-02	1.17E-01	2.71E-01	8.87E-04	5.90E-04	7.99E-02	2.70E-02	1.47E-01	3.40E-01
CdPb3	3.18E-03	3.08E-03	1.48E-01	3.65E-02	1.69E-01	6.57E-01	1.24E-03	1.20E-03	5.78E-02	1.43E-02	6.59E-02	2.57E-01	1.55E-03	1.50E-03	7.23E-02	1.79E-02	8.26E-02	3.21E-01

Table Anx. 1.5

Daily dietary Intake of macro- nutrients on body weight basis ($\text{g kg}^{-1} \text{d}^{-1}$) for single plant specie

	Children					Adolescent					Adults				
	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C
Potato															
Control	1.68E-03	2.00E-03	4.04E-03	9.60E-04	1.18E-02	6.58E-04	7.82E-04	1.58E-03	3.75E-04	4.62E-03	8.24E-04	9.79E-04	1.98E-03	4.70E-04	5.79E-03
Cd1	1.52E-03	1.62E-03	2.73E-03	8.66E-04	8.48E-03	5.93E-04	6.32E-04	1.06E-03	3.38E-04	3.31E-03	7.43E-04	7.91E-04	1.33E-03	4.23E-04	4.15E-03
Cd2	2.26E-03	1.60E-03	2.46E-03	1.29E-03	4.73E-03	8.84E-04	6.25E-04	9.63E-04	5.04E-04	1.85E-03	1.11E-03	7.83E-04	1.21E-03	6.31E-04	2.32E-03
Cd3	3.64E-03	1.60E-03	2.37E-03	2.08E-03	8.87E-03	1.42E-03	6.26E-04	9.26E-04	8.11E-04	3.47E-03	1.78E-03	7.84E-04	1.16E-03	1.02E-03	4.34E-03
Pb1	3.42E-03	1.87E-03	3.11E-03	1.95E-03	8.87E-03	1.34E-03	7.29E-04	1.22E-03	7.62E-04	3.47E-03	1.67E-03	9.13E-04	1.52E-03	9.55E-04	4.34E-03
Pb2	3.29E-03	1.74E-03	2.79E-03	1.87E-03	6.90E-03	1.28E-03	6.81E-04	1.09E-03	7.32E-04	2.70E-03	1.61E-03	8.52E-04	1.37E-03	9.16E-04	3.38E-03
Pb3	2.48E-03	1.72E-03	2.46E-03	1.42E-03	6.41E-03	9.71E-04	6.71E-04	9.62E-04	5.53E-04	2.50E-03	1.22E-03	8.41E-04	1.21E-03	6.93E-04	3.14E-03
CdPb1	3.87E-03	1.90E-03	3.08E-03	2.20E-03	4.14E-03	1.51E-03	7.44E-04	1.20E-03	8.61E-04	1.62E-03	1.89E-03	9.32E-04	1.51E-03	1.08E-03	2.03E-03
CdPb2	3.32E-03	1.66E-03	2.65E-03	1.89E-03	4.24E-03	1.30E-03	6.50E-04	1.04E-03	7.40E-04	1.66E-03	1.63E-03	8.14E-04	1.30E-03	9.27E-04	2.07E-03
CdPb3	3.01E-03	1.14E-03	2.46E-03	1.72E-03	4.54E-03	1.18E-03	4.46E-04	9.62E-04	6.70E-04	1.77E-03	1.47E-03	5.58E-04	1.20E-03	8.39E-04	2.22E-03
Tomato															
Control	3.62E-03	2.26E-03	2.62E-03	2.06E-03	1.42E-02	1.41E-03	8.82E-04	1.02E-03	8.05E-04	5.55E-03	1.77E-03	1.10E-03	1.28E-03	1.01E-03	6.95E-03
Cd1	1.08E-03	1.54E-03	2.00E-03	6.15E-04	2.01E-02	4.21E-04	6.03E-04	7.81E-04	2.40E-04	7.86E-03	5.28E-04	7.55E-04	9.78E-04	3.01E-04	9.84E-03
Cd2	3.28E-03	1.52E-03	1.59E-03	1.87E-03	2.33E-02	1.28E-03	5.93E-04	6.20E-04	7.31E-04	9.09E-03	1.61E-03	7.42E-04	7.77E-04	9.15E-04	1.14E-02
Cd3	2.39E-03	8.08E-04	1.41E-03	1.36E-03	2.50E-02	9.34E-04	3.16E-04	5.50E-04	5.33E-04	9.78E-03	1.17E-03	3.95E-04	6.88E-04	6.67E-04	1.23E-02
Pb1	1.28E-03	1.18E-03	2.05E-03	7.29E-04	2.76E-02	5.00E-04	4.63E-04	8.01E-04	2.85E-04	1.08E-02	6.26E-04	5.80E-04	1.00E-03	3.57E-04	1.35E-02
Pb2	1.03E-03	9.12E-04	1.74E-03	5.89E-04	2.13E-02	4.04E-04	3.56E-04	6.82E-04	2.30E-04	8.32E-03	5.06E-04	4.46E-04	8.54E-04	2.88E-04	1.04E-02
Pb3	1.97E-03	1.21E-03	1.48E-03	1.12E-03	1.74E-02	7.69E-04	4.73E-04	5.77E-04	4.38E-04	6.78E-03	9.63E-04	5.92E-04	7.23E-04	5.49E-04	8.49E-03
CdPb1	1.27E-03	1.10E-03	2.09E-03	7.24E-04	1.66E-02	4.96E-04	4.31E-04	8.15E-04	2.83E-04	6.47E-03	6.21E-04	5.40E-04	1.02E-03	3.54E-04	8.10E-03
CdPb2	1.19E-03	1.04E-03	2.16E-03	6.77E-04	2.25E-02	4.64E-04	4.08E-04	8.42E-04	2.64E-04	8.78E-03	5.81E-04	5.10E-04	1.05E-03	3.31E-04	1.10E-02
CdPb3	1.05E-03	8.54E-04	1.59E-03	5.98E-04	1.77E-02	4.10E-04	3.33E-04	6.21E-04	2.34E-04	6.93E-03	5.13E-04	4.18E-04	7.78E-04	2.93E-04	8.68E-03
Lettuce															
Control	2.07E-03	1.70E-03	2.96E-03	1.18E-03	5.03E-03	8.09E-04	6.63E-04	1.16E-03	4.61E-04	1.96E-03	1.01E-03	8.30E-04	1.45E-03	5.77E-04	2.46E-03
Cd1	2.26E-03	1.29E-03	2.20E-03	1.29E-03	6.41E-03	8.84E-04	5.04E-04	8.61E-04	5.04E-04	2.50E-03	1.11E-03	6.31E-04	1.08E-03	6.31E-04	3.14E-03

Cd2	2.43E-03	1.23E-03	2.22E-03	1.38E-03	7.69E-03	9.49E-04	4.80E-04	8.66E-04	5.41E-04	3.00E-03	1.19E-03	6.02E-04	1.08E-03	6.77E-04	3.76E-03
Cd3	1.07E-03	5.01E-04	2.01E-03	6.11E-04	8.58E-03	4.18E-04	1.96E-04	7.84E-04	2.39E-04	3.35E-03	5.24E-04	2.45E-04	9.82E-04	2.99E-04	4.20E-03
Pb1	1.13E-03	1.09E-03	2.16E-03	6.45E-04	7.40E-03	4.42E-04	4.25E-04	8.42E-04	2.52E-04	2.89E-03	5.54E-04	5.32E-04	1.05E-03	3.16E-04	3.62E-03
Pb2	2.13E-03	1.10E-03	2.11E-03	1.21E-03	7.69E-03	8.30E-04	4.29E-04	8.23E-04	4.73E-04	3.00E-03	1.04E-03	5.38E-04	1.03E-03	5.93E-04	3.76E-03
Pb3	3.04E-03	9.97E-04	2.06E-03	1.73E-03	6.90E-03	1.19E-03	3.89E-04	8.05E-04	6.76E-04	2.70E-03	1.49E-03	4.88E-04	1.01E-03	8.47E-04	3.38E-03
CdPb1	2.10E-03	1.20E-03	2.14E-03	1.20E-03	5.52E-03	8.20E-04	4.67E-04	8.36E-04	4.67E-04	2.16E-03	1.03E-03	5.85E-04	1.05E-03	5.85E-04	2.70E-03
CdPb2	2.24E-03	5.95E-04	2.05E-03	1.27E-03	5.92E-03	8.74E-04	2.33E-04	8.01E-04	4.98E-04	2.31E-03	1.09E-03	2.91E-04	1.00E-03	6.24E-04	2.89E-03
CdPb3	2.40E-03	1.41E-03	1.98E-03	1.37E-03	5.72E-03	9.38E-04	5.51E-04	7.74E-04	5.35E-04	2.23E-03	1.17E-03	6.90E-04	9.69E-04	6.70E-04	2.80E-03

Table Anx. 1.6

RDA (%) of micro- nutrients for individual plant specie

	Children				Adolescent				Adults									
	Fe	Mn	Ca	Mg	Fe	Fe	Mn	Mn	Ca	Mg	Mg	Fe	Fe	Mn	Mn	Ca	Mg	Mg
	M+F	M+F	M+F	M+F	M	F	M	F	M+F	M	F	M	F	M	F	M+F	M	F
Potato																		
Control	12.4	66.2	1.2	32.4	13.9	10.2	67.1	92.3	0.7	13.3	13.9	19.1	8.5	64.2	82.0	0.7	13.3	13.9
Cd1	17.4	61.8	2.5	42.7	19.5	14.3	62.6	86.1	1.4	17.5	18.3	26.9	11.9	59.9	76.6	1.4	17.5	18.3
Cd2	9.4	76.1	1.5	28.1	10.5	7.7	77.2	106.1	0.9	11.5	12.0	14.5	6.4	73.8	94.3	0.9	11.5	12.0
Cd3	11.4	97.3	1.1	25.5	12.8	9.4	98.6	135.6	0.6	10.4	10.9	17.5	7.8	94.4	120.6	0.6	10.4	10.9
Pb1	23.0	53.5	0.8	21.3	25.8	18.9	54.3	74.6	0.5	8.7	9.1	35.4	15.7	51.9	66.3	0.5	8.7	9.1
Pb2	9.6	96.6	2.0	30.4	10.7	7.9	98.0	134.7	1.2	12.4	13.0	14.8	6.6	93.7	119.7	1.2	12.4	13.0
Pb3	10.3	124.6	3.0	42.4	11.5	8.5	126.4	173.8	1.7	17.3	18.1	15.8	7.0	120.9	154.4	1.7	17.3	18.1
CdPb1	16.3	30.2	2.4	30.7	18.3	13.4	30.6	42.1	1.4	12.6	13.1	25.1	11.2	29.3	37.4	1.4	12.6	13.1
CdPb2	12.9	34.8	1.4	24.3	14.5	10.6	35.3	48.6	0.8	10.0	10.4	19.9	8.8	33.8	43.2	0.8	10.0	10.4
CdPb3	8.4	47.7	0.6	21.9	9.5	6.9	48.4	66.5	0.3	9.0	9.4	13.0	5.8	46.3	59.1	0.3	9.0	9.4
Tomato																		
Control	16.4	78.0	0.8	23.0	18.4	13.5	79.0	108.7	0.4	9.4	9.8	25.3	11.2	75.6	96.6	0.4	9.4	9.8
Cd1	8.3	43.6	0.6	31.1	9.3	6.8	44.3	60.8	0.3	12.7	13.3	12.8	5.7	42.3	54.1	0.3	12.7	13.3
Cd2	16.6	103.0	0.5	28.7	18.7	13.7	104.5	143.6	0.3	11.8	12.3	25.7	11.4	99.9	127.7	0.3	11.8	12.3
Cd3	15.1	39.8	0.6	32.4	17.0	12.4	40.3	55.5	0.4	13.3	13.9	23.3	10.4	38.6	49.3	0.4	13.3	13.9
Pb1	20.3	59.6	0.7	30.6	22.8	16.7	60.4	83.1	0.4	12.5	13.1	31.4	13.9	57.8	73.9	0.4	12.5	13.1
Pb2	15.9	61.6	0.5	38.0	17.8	13.1	62.5	85.9	0.3	15.6	16.3	24.5	10.9	59.8	76.4	0.3	15.6	16.3
Pb3	11.2	85.9	0.9	43.2	12.6	9.2	87.1	119.7	0.5	17.7	18.5	17.3	7.7	83.3	106.4	0.5	17.7	18.5

CdPb1	13.0	49.5	0.7	28.3	14.6	10.7	50.2	69.0	0.4	11.6	12.1	20.1	8.9	48.0	61.3	0.4	11.6	12.1
CdPb2	9.9	59.9	0.6	34.6	11.1	8.2	60.8	83.6	0.3	14.2	14.8	15.3	6.8	58.1	74.3	0.3	14.2	14.8
CdPb3	11.5	64.0	0.8	37.5	12.9	9.5	64.9	89.3	0.4	15.4	16.1	17.7	7.9	62.1	79.3	0.4	15.4	16.1
Lettuce																		
Control	62.1	117.0	3.0	31.4	69.7	51.1	118.6	163.1	1.7	12.9	13.4	95.9	42.6	113.5	145.0	1.7	12.9	13.4
Cd1	47.5	82.3	1.5	27.9	53.3	39.1	83.4	114.7	0.8	11.4	11.9	73.3	32.6	79.8	102.0	0.8	11.4	11.9
Cd2	54.6	58.2	2.6	41.0	61.3	45.0	59.0	81.1	1.5	16.8	17.5	84.3	37.5	56.4	72.1	1.5	16.8	17.5
Cd3	74.3	47.1	4.0	58.7	83.4	61.2	47.7	65.6	2.3	24.1	25.1	114.7	51.0	45.7	58.3	2.3	24.1	25.1
Pb1	97.7	117.0	3.0	54.3	109.6	80.4	118.6	163.0	1.7	22.2	23.3	150.7	67.0	113.4	144.9	1.7	22.2	23.3
Pb2	77.1	54.3	2.6	33.7	86.5	63.4	55.1	75.7	1.5	13.8	14.4	118.9	52.9	52.7	67.3	1.5	13.8	14.4
Pb3	52.3	47.6	1.4	29.5	58.7	43.0	48.3	66.3	0.8	12.1	12.6	80.6	35.8	46.2	59.0	0.8	12.1	12.6
CdPb1	47.0	77.2	1.2	11.6	52.8	38.7	78.3	107.6	0.7	4.8	5.0	72.6	32.3	74.9	95.7	0.7	4.8	5.0
CdPb2	49.2	91.9	1.2	22.8	55.2	40.5	93.1	128.1	0.7	9.4	9.8	75.9	33.7	89.1	113.8	0.7	9.4	9.8
CdPb3	44.5	60.8	0.7	21.6	50.0	36.7	61.7	84.8	0.4	8.8	9.2	68.7	30.5	59.0	75.4	0.4	8.8	9.2

Table Anx. 1.7

RDA (%) of macro- nutrients for individual plant specie

	Children							Adolescent							Adults						
	N		P	K	Protein		Vitamin	N		P	K	Protein		Vitamin	N		P	K	Protein		Vitamin
							C							C							C
	M	F	M-F	M-F	M	F	M-F	M	F	M-F	M-F	M	F	M-F	M	F	M-F	M-F	M	F	M-F
Potato																					
Control	0.93	0.92	6.79	5.61	0.08	0.08	0.66	0.66	0.89	5.95	6.26	0.06	0.08	0.73	0.64	0.8	10.63	6.26	0.06	0.07	0.73
Cd1	0.84	0.83	5.49	3.79	0.08	0.08	0.47	0.6	0.8	4.81	4.22	0.05	0.07	0.53	0.58	0.72	8.59	4.22	0.05	0.07	0.53
Cd2	1.25	1.24	5.43	3.42	0.11	0.11	0.26	0.89	1.2	4.76	3.82	0.08	0.11	0.29	0.86	1.08	8.5	3.82	0.08	0.1	0.29
Cd3	2.02	2	5.44	3.29	0.18	0.18	0.49	1.44	1.92	4.77	3.67	0.13	0.18	0.55	1.38	1.74	8.51	3.67	0.13	0.16	0.55
Pb1	1.89	1.88	6.33	4.32	0.17	0.17	0.49	1.35	1.81	5.55	4.82	0.12	0.16	0.55	1.3	1.63	9.91	4.82	0.12	0.15	0.55
Pb2	1.82	1.8	5.91	3.88	0.17	0.16	0.38	1.29	1.73	5.18	4.33	0.12	0.16	0.43	1.25	1.57	9.25	4.33	0.11	0.14	0.43
Pb3	1.37	1.36	5.83	3.42	0.13	0.12	0.36	0.98	1.31	5.11	3.82	0.09	0.12	0.4	0.94	1.19	9.13	3.82	0.09	0.11	0.4
CdPb1	2.14	2.12	6.46	4.28	0.19	0.19	0.23	1.52	2.04	5.67	4.77	0.14	0.19	0.26	1.46	1.84	10.12	4.77	0.13	0.17	0.26
CdPb2	1.84	1.82	5.65	3.68	0.17	0.17	0.24	1.31	1.76	4.95	4.11	0.12	0.16	0.26	1.26	1.59	8.84	4.11	0.11	0.14	0.26
CdPb3	1.66	1.65	3.87	3.42	0.15	0.15	0.25	1.19	1.59	3.39	3.81	0.11	0.14	0.28	1.14	1.44	6.06	3.81	0.1	0.13	0.28
Tomato																					
Control	2	1.98	7.66	3.64	0.18	0.18	0.79	1.42	1.91	6.72	4.06	0.13	0.17	0.88	1.37	1.73	11.99	4.06	0.12	0.16	0.88
Cd1	0.6	0.59	5.24	2.78	0.05	0.05	1.12	0.42	0.57	4.59	3.1	0.04	0.05	1.25	0.41	0.51	8.2	3.1	0.04	0.05	1.25
Cd2	1.82	1.8	5.15	2.21	0.17	0.16	1.29	1.29	1.73	4.51	2.46	0.12	0.16	1.44	1.24	1.57	8.06	2.46	0.11	0.14	1.44
Cd3	1.32	1.31	2.74	1.95	0.12	0.12	1.39	0.94	1.26	2.4	2.18	0.09	0.12	1.55	0.91	1.14	4.29	2.18	0.08	0.1	1.55
Pb1	0.71	0.7	4.02	2.85	0.06	0.06	1.53	0.5	0.68	3.52	3.18	0.05	0.06	1.71	0.48	0.61	6.29	3.18	0.04	0.06	1.71
Pb2	0.57	0.57	3.1	2.42	0.05	0.05	1.18	0.41	0.55	2.71	2.7	0.04	0.05	1.32	0.39	0.49	4.85	2.7	0.04	0.05	1.32
Pb3	1.09	1.08	4.11	2.05	0.1	0.1	0.96	0.78	1.04	3.6	2.29	0.07	0.09	1.08	0.75	0.94	6.43	2.29	0.07	0.09	1.08
CdPb1	0.7	0.7	3.74	2.9	0.06	0.06	0.92	0.5	0.67	3.28	3.23	0.05	0.06	1.03	0.48	0.61	5.86	3.23	0.04	0.06	1.03
CdPb2	0.66	0.65	3.54	2.99	0.06	0.06	1.25	0.47	0.63	3.1	3.34	0.04	0.06	1.39	0.45	0.57	5.54	3.34	0.04	0.05	1.39
CdPb3	0.58	0.58	2.9	2.21	0.05	0.05	0.99	0.41	0.55	2.54	2.46	0.04	0.05	1.1	0.4	0.5	4.53	2.46	0.04	0.05	1.1

Lettuce																					
Control	1.15	1.14	5.76	4.11	0.1	0.1	0.28	0.82	1.09	5.05	4.58	0.07	0.1	0.31	0.78	0.99	9.01	4.58	0.07	0.09	0.31
Cd1	1.25	1.24	4.38	3.06	0.11	0.11	0.36	0.89	1.2	3.84	3.42	0.08	0.11	0.4	0.86	1.08	6.86	3.42	0.08	0.1	0.4
Cd2	1.34	1.33	4.17	3.08	0.12	0.12	0.43	0.96	1.28	3.66	3.43	0.09	0.12	0.48	0.92	1.16	6.53	3.43	0.08	0.11	0.48
Cd3	0.59	0.59	1.7	2.79	0.05	0.05	0.48	0.42	0.57	1.49	3.11	0.04	0.05	0.53	0.41	0.51	2.66	3.11	0.04	0.05	0.53
Pb1	0.63	0.62	3.69	2.99	0.06	0.06	0.41	0.45	0.6	3.24	3.34	0.04	0.05	0.46	0.43	0.54	5.78	3.34	0.04	0.05	0.46
Pb2	1.18	1.17	3.73	2.93	0.11	0.11	0.43	0.84	1.12	3.27	3.26	0.08	0.1	0.48	0.81	1.01	5.84	3.26	0.07	0.09	0.48
Pb3	1.68	1.66	3.38	2.86	0.15	0.15	0.38	1.2	1.6	2.96	3.19	0.11	0.15	0.43	1.15	1.45	5.29	3.19	0.1	0.13	0.43
CdPb1	1.16	1.15	4.06	2.97	0.11	0.1	0.31	0.83	1.11	3.56	3.32	0.08	0.1	0.34	0.8	1	6.35	3.32	0.07	0.09	0.34
CdPb2	1.24	1.23	2.02	2.85	0.11	0.11	0.33	0.88	1.18	1.77	3.18	0.08	0.11	0.37	0.85	1.07	3.16	3.18	0.08	0.1	0.37
CdPb3	1.33	1.32	4.79	2.75	0.12	0.12	0.32	0.95	1.27	4.19	3.07	0.09	0.12	0.35	0.91	1.15	7.49	3.07	0.08	0.1	0.35

Annexure-2

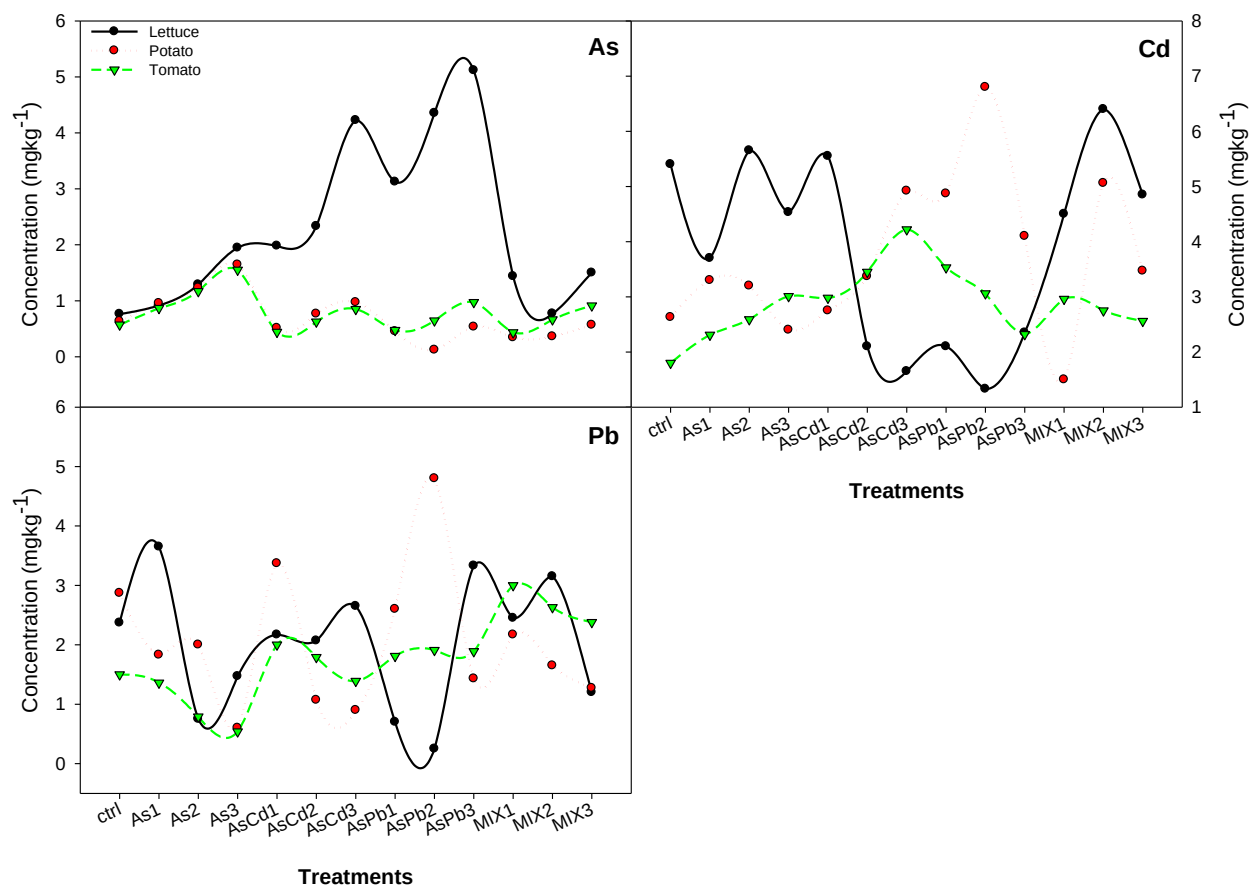


Fig Anx. 2.1. Effects of As on Cd and Pb concentrations in vegetables cultivated in metals contaminated soil

Table Anx. 2.1. Daily dietary Intake of heavy metals and micro- nutrients (mg d⁻¹) through consumption of vegetables

	Adults						Children					
	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg
Potato												
Control	7.7E-02	8.4E-02	1.5E+00	1.5E+00	1.1E+01	3.7E+01	5.2E-02	5.7E-02	1.0E+00	9.9E-01	7.30E+00	2.46E+01
As1	9.7E-02	5.4E-02	1.5E+00	1.5E+00	2.6E+01	3.8E+01	6.5E-02	3.6E-02	1.0E+00	1.0E+00	1.74E+01	2.53E+01
As2	9.4E-02	5.9E-02	1.7E+00	1.7E+00	1.2E+01	3.7E+01	6.3E-02	3.9E-02	1.1E+00	1.1E+00	8.38E+00	2.46E+01
As3	7.0E-02	1.8E-02	1.2E+00	1.5E+00	2.1E+01	3.9E+01	4.7E-02	1.2E-02	8.3E-01	9.9E-01	1.43E+01	2.61E+01
AsCd1	8.1E-02	9.9E-02	1.0E+00	1.3E+00	2.8E+01	3.9E+01	5.4E-02	6.6E-02	7.0E-01	8.7E-01	1.92E+01	2.59E+01
AsCd2	9.9E-02	3.1E-02	1.0E+00	2.2E+00	2.3E+01	3.5E+01	6.6E-02	2.1E-02	7.0E-01	1.5E+00	1.53E+01	2.32E+01
AsCd3	1.4E-01	2.6E-02	5.5E-01	1.7E+00	2.8E+01	2.7E+01	9.7E-02	1.8E-02	3.7E-01	1.1E+00	1.90E+01	1.82E+01
AsPb1	1.4E-01	7.6E-02	6.3E-01	2.6E+00	2.2E+01	3.2E+01	9.6E-02	5.1E-02	4.2E-01	1.7E+00	1.50E+01	2.13E+01
AsPb2	2.0E-01	1.4E-01	1.2E+00	2.5E+00	1.8E+01	4.1E+01	1.3E-01	9.5E-02	7.8E-01	1.7E+00	1.22E+01	2.74E+01
AsPb3	1.2E-01	4.2E-02	1.6E+00	2.4E+00	2.2E+01	2.7E+01	8.1E-02	2.8E-02	1.1E+00	1.6E+00	1.45E+01	1.79E+01
MIX1	4.4E-02	6.4E-02	1.0E+00	1.1E+00	7.3E+00	1.3E+01	3.0E-02	4.3E-02	6.8E-01	7.3E-01	4.90E+00	8.74E+00
MIX2	1.5E-01	4.8E-02	7.5E-01	5.3E-01	5.4E+00	1.7E+01	1.0E-01	3.3E-02	5.0E-01	3.6E-01	3.65E+00	1.12E+01
MIX3	1.0E-01	3.7E-02	8.8E-01	4.0E-01	5.3E+00	1.7E+01	6.8E-02	2.5E-02	5.9E-01	2.7E-01	3.55E+00	1.17E+01
Tomato												
Control	5.3E-02	4.4E-02	2.0E+00	1.7E+00	6.7E+00	2.6E+01	3.5E-02	3.0E-02	1.4E+00	1.2E+00	4.54E+00	1.74E+01
As1	6.8E-02	4.0E-02	2.2E+00	1.7E+00	5.9E+00	2.9E+01	4.6E-02	2.7E-02	1.5E+00	1.1E+00	3.94E+00	1.95E+01
As2	7.6E-02	2.3E-02	2.4E+00	1.5E+00	5.4E+00	3.1E+01	5.1E-02	1.6E-02	1.6E+00	9.8E-01	3.65E+00	2.11E+01
As3	8.8E-02	1.6E-02	2.5E+00	1.3E+00	4.7E+00	3.5E+01	5.9E-02	1.1E-02	1.7E+00	9.0E-01	3.16E+00	2.36E+01
AsCd1	8.7E-02	5.9E-02	1.8E+00	2.0E+00	5.3E+00	3.4E+01	5.9E-02	3.9E-02	1.2E+00	1.3E+00	3.55E+00	2.28E+01
AsCd2	1.0E-01	5.2E-02	1.5E+00	1.9E+00	6.3E+00	3.1E+01	6.8E-02	3.5E-02	1.0E+00	1.3E+00	4.26E+00	2.11E+01
AsCd3	1.2E-01	4.1E-02	1.4E+00	1.8E+00	5.7E+00	3.0E+01	8.3E-02	2.7E-02	9.7E-01	1.2E+00	3.85E+00	2.00E+01

AsPb1	1.0E-01	5.3E-02	1.5E+00	1.4E+00	6.3E+00	3.1E+01	7.0E-02	3.6E-02	1.0E+00	9.4E-01	4.26E+00	2.11E+01
AsPb2	9.0E-02	5.6E-02	1.7E+00	1.3E+00	6.7E+00	3.2E+01	6.0E-02	3.8E-02	1.1E+00	9.0E-01	4.52E+00	2.17E+01
AsPb3	6.8E-02	5.5E-02	1.8E+00	1.2E+00	5.9E+00	3.6E+01	4.6E-02	3.7E-02	1.2E+00	8.1E-01	3.93E+00	2.40E+01
MIX1	8.7E-02	8.8E-02	1.7E+00	2.0E+00	5.9E+00	3.8E+01	5.8E-02	5.9E-02	1.1E+00	1.3E+00	3.97E+00	2.56E+01
MIX2	8.1E-02	7.7E-02	1.4E+00	1.4E+00	7.0E+00	4.3E+01	5.4E-02	5.2E-02	9.6E-01	9.2E-01	4.68E+00	2.86E+01
MIX3	7.5E-02	7.0E-02	1.3E+00	1.1E+00	7.3E+00	4.6E+01	5.0E-02	4.7E-02	8.6E-01	7.6E-01	4.89E+00	3.08E+01
Lettuce												
Control	1.6E-01	7.0E-02	7.7E+00	2.6E+00	2.7E+01	3.5E+01	1.1E-01	4.7E-02	5.2E+00	1.8E+00	1.80E+01	2.39E+01
As1	1.1E-01	1.1E-01	1.1E+01	1.1E+00	2.9E+01	4.1E+01	7.3E-02	7.2E-02	7.2E+00	7.2E-01	1.94E+01	2.74E+01
As2	1.7E-01	2.2E-02	1.3E+01	8.8E-01	3.1E+01	4.7E+01	1.1E-01	1.5E-02	8.6E+00	5.9E-01	2.11E+01	3.19E+01
As3	1.3E-01	4.3E-02	1.2E+01	8.1E-01	3.3E+01	5.0E+01	8.9E-02	2.9E-02	7.8E+00	5.5E-01	2.24E+01	3.39E+01
AsCd1	1.6E-01	6.4E-02	1.5E+01	1.6E+00	3.9E+01	7.1E+01	1.1E-01	4.3E-02	9.9E+00	1.0E+00	2.60E+01	4.78E+01
AsCd2	6.2E-02	6.1E-02	1.2E+01	1.1E+00	2.1E+01	6.1E+01	4.1E-02	4.1E-02	8.3E+00	7.6E-01	1.44E+01	4.09E+01
AsCd3	4.8E-02	7.8E-02	1.5E+01	1.7E+00	2.7E+01	5.2E+01	3.3E-02	5.2E-02	1.0E+01	1.1E+00	1.83E+01	3.50E+01
AsPb1	6.2E-02	2.1E-02	1.3E+01	1.5E+00	2.8E+01	4.1E+01	4.1E-02	1.4E-02	8.7E+00	1.0E+00	1.85E+01	2.79E+01
AsPb2	3.9E-02	7.3E-03	1.3E+01	1.4E+00	3.6E+01	5.4E+01	2.6E-02	4.9E-03	8.5E+00	9.6E-01	2.41E+01	3.61E+01
AsPb3	6.9E-02	9.8E-02	1.3E+01	1.7E+00	3.1E+01	4.4E+01	4.6E-02	6.6E-02	8.4E+00	1.2E+00	2.07E+01	2.94E+01
MIX1	1.3E-01	7.2E-02	1.2E+01	2.5E+00	3.4E+01	4.9E+01	8.9E-02	4.8E-02	8.2E+00	1.7E+00	2.26E+01	3.30E+01
MIX2	1.9E-01	9.2E-02	1.6E+01	2.2E+00	4.3E+01	5.1E+01	1.3E-01	6.2E-02	1.1E+01	1.5E+00	2.88E+01	3.43E+01
MIX3	1.42E-01	3.52E-02	6.50E+00	1.98E+00	1.87E+01	2.96E+01	9.56E-02	2.37E-02	4.37E+00	1.33E+00	1.25E+01	1.99E+01

Table Anx. 2.2. Daily dietary Intake of macro- nutrients (mg d⁻¹) through consumption of vegetables

	Adults					Children				
	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C
Potato										
Control	0.06	0.07	0.15	0.04	0.44	0.04	0.05	0.10	0.02	0.30
As1	0.08	0.06	0.11	0.05	0.31	0.05	0.04	0.07	0.03	0.21
As2	0.09	0.07	0.09	0.05	0.22	0.06	0.04	0.06	0.03	0.15
As3	0.02	0.07	0.08	0.01	0.23	0.01	0.05	0.06	0.01	0.16
AsCd1	0.11	0.04	0.12	0.07	0.43	0.08	0.03	0.08	0.04	0.29
AsCd2	0.02	0.07	0.11	0.01	0.31	0.01	0.05	0.07	0.01	0.21
AsCd3	0.12	0.00	0.10	0.07	0.20	0.08	0.00	0.07	0.05	0.14
AsPb1	0.11	0.09	0.12	0.06	0.20	0.07	0.06	0.08	0.04	0.14
AsPb2	0.10	0.08	0.11	0.06	0.22	0.07	0.06	0.07	0.04	0.15
AsPb3	0.13	0.07	0.09	0.07	0.27	0.08	0.05	0.06	0.05	0.18
MIX1	0.12	0.07	0.10	0.07	0.39	0.08	0.05	0.07	0.05	0.26
MIX2	0.11	0.07	0.09	0.06	0.31	0.07	0.05	0.06	0.04	0.21
MIX3	0.06	0.06	0.08	0.03	0.28	0.04	0.04	0.05	0.02	0.19
Tomato										
Control	0.13	0.08	0.10	0.08	0.53	0.09	0.06	0.07	0.05	0.35
As1	0.07	0.05	0.08	0.04	0.57	0.05	0.03	0.05	0.03	0.38
As2	0.06	0.06	0.07	0.03	0.67	0.04	0.04	0.05	0.02	0.45
As3	0.07	0.07	0.06	0.04	0.80	0.04	0.05	0.04	0.03	0.54
AsCd1	0.05	0.03	0.08	0.03	0.67	0.03	0.02	0.05	0.02	0.45

AsCd2	0.07	0.05	0.07	0.04	0.75	0.05	0.03	0.05	0.03	0.50
AsCd3	0.06	0.06	0.06	0.03	0.84	0.04	0.04	0.04	0.02	0.56
AsPb1	0.07	0.05	0.08	0.04	0.78	0.05	0.03	0.05	0.03	0.53
AsPb2	0.06	0.05	0.07	0.03	0.84	0.04	0.03	0.05	0.02	0.56
AsPb3	0.05	0.06	0.06	0.03	0.87	0.03	0.04	0.04	0.02	0.58
MIX1	0.05	0.07	0.06	0.03	0.58	0.03	0.04	0.04	0.02	0.39
MIX2	0.04	0.06	0.06	0.02	0.68	0.03	0.04	0.04	0.02	0.46
MIX3	0.03	0.06	0.05	0.02	0.79	0.02	0.04	0.04	0.01	0.53
Lettuce										
Control	0.08	0.06	0.11	0.04	0.19	0.05	0.04	0.07	0.03	0.13
As1	0.08	0.09	0.08	0.05	0.20	0.05	0.06	0.06	0.03	0.14
As2	0.09	0.04	0.08	0.05	0.23	0.06	0.03	0.05	0.04	0.15
As3	0.09	0.05	0.08	0.05	0.27	0.06	0.04	0.05	0.04	0.18
AsCd1	0.07	0.02	0.08	0.04	0.23	0.05	0.01	0.06	0.03	0.16
AsCd2	0.08	0.04	0.08	0.05	0.26	0.06	0.03	0.05	0.03	0.18
AsCd3	0.09	0.10	0.08	0.05	0.27	0.06	0.06	0.05	0.03	0.18
AsPb1	0.05	0.04	0.08	0.03	0.21	0.03	0.02	0.05	0.02	0.14
AsPb2	0.08	0.09	0.08	0.05	0.23	0.06	0.06	0.05	0.03	0.16
AsPb3	0.09	0.03	0.07	0.05	0.27	0.06	0.02	0.05	0.03	0.18
MIX1	0.07	0.08	0.07	0.04	0.25	0.05	0.05	0.05	0.03	0.17
MIX2	0.06	0.02	0.07	0.03	0.27	0.04	0.01	0.05	0.02	0.18
MIX3	0.12	0.02	0.07	0.07	0.28	0.08	0.01	0.05	0.05	0.19

Table Anx. 2.3; Cumulative Daily dietary Intake of heavy metals and macro- (g d⁻¹) and micro-nutrients (mg d⁻¹)

	Cd	Pb	Fe	Mn	Ca	Mg	N	P	K	Protein	Vitamin C
Adults											
Control	0.10	0.07	3.74	1.94	14.8	32.7	0.09	0.07	0.12	0.05	0.38
As1	0.09	0.07	4.83	1.42	20.2	35.8	0.08	0.07	0.09	0.04	0.36
As2	0.11	0.03	5.60	1.34	16.4	38.5	0.08	0.06	0.08	0.04	0.37
As3	0.10	0.03	5.11	1.21	19.8	41.4	0.06	0.07	0.07	0.03	0.44
AsCd1	0.11	0.07	5.82	1.61	24.2	47.8	0.08	0.03	0.09	0.04	0.44
AsCd2	0.09	0.05	4.99	1.73	16.8	42.3	0.06	0.05	0.09	0.03	0.44
AsCd3	0.11	0.05	5.75	1.74	20.4	36.3	0.09	0.05	0.08	0.05	0.44
AsPb1	0.10	0.05	5.05	1.83	18.7	34.8	0.08	0.06	0.09	0.04	0.40
AsPb2	0.11	0.07	5.15	1.77	20.3	42.3	0.08	0.07	0.09	0.05	0.43
AsPb3	0.09	0.07	5.32	1.80	19.4	35.3	0.09	0.05	0.08	0.05	0.47
MIX1	0.09	0.07	4.96	1.84	15.6	33.4	0.08	0.07	0.08	0.05	0.41
MIX2	0.14	0.07	6.04	1.36	18.4	36.7	0.07	0.05	0.08	0.04	0.42
MIX3	0.11	0.05	2.89	1.17	10.4	30.9	0.07	0.04	0.07	0.04	0.45
Children											
Control	0.06	0.04	2.51	1.31	9.94	21.9	0.06	0.05	0.08	0.04	0.26
As1	0.06	0.04	3.25	0.95	13.6	24.1	0.05	0.04	0.06	0.03	0.24
As2	0.08	0.02	3.76	0.90	11.0	25.9	0.05	0.04	0.05	0.03	0.25
As3	0.07	0.02	3.43	0.81	13.3	27.9	0.04	0.04	0.05	0.02	0.29
AsCd1	0.07	0.05	3.91	1.08	16.3	32.2	0.05	0.02	0.06	0.03	0.30
AsCd2	0.06	0.03	3.36	1.16	11.3	28.4	0.04	0.03	0.06	0.02	0.30

AsCd3	0.07	0.03	3.87	1.17	13.7	24.4	0.06	0.04	0.05	0.03	0.29
AsPb1	0.07	0.03	3.39	1.23	12.6	23.4	0.05	0.04	0.06	0.03	0.27
AsPb2	0.07	0.05	3.46	1.19	13.6	28.4	0.05	0.05	0.06	0.03	0.29
AsPb3	0.06	0.04	3.58	1.21	13.0	23.7	0.06	0.04	0.05	0.03	0.32
MIX1	0.06	0.05	3.33	1.24	10.5	22.5	0.05	0.05	0.05	0.03	0.27
MIX2	0.09	0.05	4.06	0.91	12.4	24.7	0.05	0.03	0.05	0.03	0.28
MIX3	0.07	0.03	1.94	0.79	6.99	20.8	0.05	0.03	0.05	0.03	0.30

Table Anx. 2.4. Daily dietary Intake of heavy metals and micro- nutrients on individual body weight basis (mg kg⁻¹ d⁻¹) for single plant specie

	Children						Adolescent						Adults					
	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg	Cd	Pb	Fe	Mn	Ca	Mg
Potato																		
Control	2.07E-03	2.26E-03	4.10E-02	3.97E-02	2.92E-01	9.84E-01	8.10E-04	8.84E-04	1.60E-02	1.55E-02	1.14E-01	3.85E-01	1.01E-03	1.11E-03	2.01E-02	1.94E-02	1.43E-01	4.82E-01
As1	2.60E-03	1.44E-03	4.02E-02	4.01E-02	6.95E-01	1.01E+00	1.02E-03	5.64E-04	1.57E-02	1.57E-02	2.72E-01	3.95E-01	1.27E-03	7.06E-04	1.97E-02	1.96E-02	3.40E-01	4.95E-01
As2	2.52E-03	1.58E-03	4.46E-02	4.53E-02	3.35E-01	9.85E-01	9.86E-04	6.16E-04	1.74E-02	1.77E-02	1.31E-01	3.85E-01	1.23E-03	7.72E-04	2.18E-02	2.21E-02	1.64E-01	4.82E-01
As3	1.89E-03	4.73E-04	3.32E-02	3.95E-02	5.74E-01	1.04E+00	7.40E-04	1.85E-04	1.30E-02	1.54E-02	2.24E-01	4.08E-01	9.26E-04	2.32E-04	1.62E-02	1.93E-02	2.81E-01	5.11E-01
AsCd1	2.17E-03	2.66E-03	2.80E-02	3.49E-02	7.66E-01	1.04E+00	8.47E-04	1.04E-03	1.09E-02	1.36E-02	2.99E-01	4.05E-01	1.06E-03	1.30E-03	1.37E-02	1.71E-02	3.75E-01	5.07E-01
AsCd2	2.66E-03	8.44E-04	2.79E-02	5.83E-02	6.11E-01	9.28E-01	1.04E-03	3.30E-04	1.09E-02	2.28E-02	2.39E-01	3.63E-01	1.30E-03	4.13E-04	1.36E-02	2.85E-02	2.99E-01	4.54E-01
AsCd3	3.88E-03	7.10E-04	1.48E-02	4.57E-02	7.61E-01	7.29E-01	1.52E-03	2.77E-04	5.76E-03	1.78E-02	2.97E-01	2.85E-01	1.90E-03	3.47E-04	7.22E-03	2.23E-02	3.72E-01	3.57E-01
AsPb1	3.84E-03	2.05E-03	1.70E-02	6.89E-02	5.99E-01	8.53E-01	1.50E-03	8.01E-04	6.62E-03	2.69E-02	2.34E-01	3.33E-01	1.88E-03	1.00E-03	8.30E-03	3.37E-02	2.93E-01	4.17E-01
AsPb2	5.36E-03	3.79E-03	3.12E-02	6.82E-02	4.89E-01	1.10E+00	2.10E-03	1.48E-03	1.22E-02	2.67E-02	1.91E-01	4.29E-01	2.62E-03	1.85E-03	1.53E-02	3.34E-02	2.39E-01	5.37E-01
AsPb3	3.23E-03	1.13E-03	4.35E-02	6.55E-02	5.80E-01	7.14E-01	1.26E-03	4.41E-04	1.70E-02	2.56E-02	2.26E-01	2.79E-01	1.58E-03	5.52E-04	2.13E-02	3.21E-02	2.84E-01	3.49E-01
MIX1	1.18E-03	1.71E-03	2.71E-02	2.91E-02	1.96E-01	3.49E-01	4.62E-04	6.69E-04	1.06E-02	1.14E-02	7.65E-02	1.36E-01	5.79E-04	8.37E-04	1.33E-02	1.42E-02	9.58E-02	1.71E-01
MIX2	3.99E-03	1.30E-03	2.02E-02	1.43E-02	1.46E-01	4.48E-01	1.56E-03	5.08E-04	7.89E-03	5.58E-03	5.70E-02	1.75E-01	1.95E-03	6.37E-04	9.88E-03	6.98E-03	7.14E-02	2.19E-01
MIX3	2.74E-03	1.00E-03	2.37E-02	1.06E-02	1.42E-01	4.69E-01	1.07E-03	3.91E-04	9.24E-03	4.16E-03	5.55E-02	1.83E-01	1.34E-03	4.90E-04	1.16E-02	5.21E-03	6.95E-02	2.29E-01
Tomato																		
Control	1.42E-03	1.18E-03	5.44E-02	4.68E-02	1.81E-01	6.98E-01	5.55E-04	4.62E-04	2.13E-02	1.83E-02	7.09E-02	2.73E-01	6.95E-04	5.79E-04	2.66E-02	2.29E-02	8.87E-02	3.41E-01
As1	1.82E-03	1.07E-03	5.98E-02	4.55E-02	1.58E-01	7.81E-01	7.12E-04	4.19E-04	2.34E-02	1.78E-02	6.16E-02	3.05E-01	8.91E-04	5.25E-04	2.93E-02	2.23E-02	7.72E-02	3.82E-01
As2	2.04E-03	6.23E-04	6.42E-02	3.90E-02	1.46E-01	8.44E-01	7.98E-04	2.43E-04	2.51E-02	1.52E-02	5.70E-02	3.30E-01	9.99E-04	3.05E-04	3.14E-02	1.91E-02	7.14E-02	4.13E-01
As3	2.37E-03	4.26E-04	6.66E-02	3.60E-02	1.26E-01	9.43E-01	9.27E-04	1.66E-04	2.60E-02	1.41E-02	4.93E-02	3.68E-01	1.16E-03	2.08E-04	3.26E-02	1.76E-02	6.17E-02	4.61E-01
AsCd1	2.35E-03	1.58E-03	4.72E-02	5.31E-02	1.42E-01	9.11E-01	9.18E-04	6.16E-04	1.85E-02	2.08E-02	5.55E-02	3.56E-01	1.15E-03	7.72E-04	2.31E-02	2.60E-02	6.95E-02	4.46E-01
AsCd2	2.72E-03	1.41E-03	4.09E-02	5.09E-02	1.70E-01	8.44E-01	1.06E-03	5.52E-04	1.60E-02	1.99E-02	6.66E-02	3.30E-01	1.33E-03	6.91E-04	2.00E-02	2.49E-02	8.33E-02	4.13E-01
AsCd3	3.33E-03	1.10E-03	3.86E-02	4.91E-02	1.54E-01	8.01E-01	1.30E-03	4.28E-04	1.51E-02	1.92E-02	6.01E-02	3.13E-01	1.63E-03	5.36E-04	1.89E-02	2.40E-02	7.52E-02	3.92E-01

AsPb1	2.78E-03	1.43E-03	4.10E-02	3.76E-02	1.70E-01	8.44E-01	1.09E-03	5.58E-04	1.60E-02	1.47E-02	6.66E-02	3.30E-01	1.36E-03	6.98E-04	2.01E-02	1.84E-02	8.33E-02	4.13E-01
AsPb2	2.41E-03	1.51E-03	4.52E-02	3.61E-02	1.81E-01	8.67E-01	9.43E-04	5.89E-04	1.77E-02	1.41E-02	7.07E-02	3.39E-01	1.18E-03	7.37E-04	2.21E-02	1.76E-02	8.85E-02	4.24E-01
AsPb3	1.83E-03	1.49E-03	4.94E-02	3.26E-02	1.57E-01	9.58E-01	7.15E-04	5.82E-04	1.93E-02	1.27E-02	6.15E-02	3.74E-01	8.95E-04	7.29E-04	2.42E-02	1.59E-02	7.70E-02	4.69E-01
MIX1	2.33E-03	2.37E-03	4.46E-02	5.30E-02	1.59E-01	1.03E+00	9.12E-04	9.24E-04	1.74E-02	2.07E-02	6.21E-02	4.01E-01	1.14E-03	1.16E-03	2.18E-02	2.59E-02	7.77E-02	5.02E-01
MIX2	2.17E-03	2.07E-03	3.83E-02	3.69E-02	1.87E-01	1.14E+00	8.47E-04	8.10E-04	1.50E-02	1.44E-02	7.32E-02	4.47E-01	1.06E-03	1.01E-03	1.88E-02	1.81E-02	9.16E-02	5.59E-01
MIX3	2.02E-03	1.88E-03	3.44E-02	3.06E-02	1.96E-01	1.23E+00	7.89E-04	7.33E-04	1.35E-02	1.19E-02	7.64E-02	4.81E-01	9.88E-04	9.18E-04	1.69E-02	1.49E-02	9.57E-02	6.02E-01
Lettuce																		
Control	4.26E-03	1.87E-03	2.06E-01	7.02E-02	7.19E-01	9.54E-01	1.66E-03	7.30E-04	8.06E-02	2.74E-02	2.81E-01	3.73E-01	2.08E-03	9.14E-04	1.01E-01	3.43E-02	3.52E-01	4.67E-01
As1	2.92E-03	2.88E-03	2.89E-01	2.88E-02	7.76E-01	1.10E+00	1.14E-03	1.12E-03	1.13E-01	1.12E-02	3.03E-01	4.28E-01	1.43E-03	1.41E-03	1.42E-01	1.41E-02	3.80E-01	5.36E-01
As2	4.46E-03	5.92E-04	3.43E-01	2.36E-02	8.44E-01	1.28E+00	1.74E-03	2.31E-04	1.34E-01	9.20E-03	3.30E-01	4.99E-01	2.18E-03	2.89E-04	1.68E-01	1.15E-02	4.13E-01	6.25E-01
As3	3.57E-03	1.16E-03	3.12E-01	2.18E-02	8.98E-01	1.35E+00	1.40E-03	4.53E-04	1.22E-01	8.54E-03	3.51E-01	5.29E-01	1.75E-03	5.67E-04	1.53E-01	1.07E-02	4.39E-01	6.63E-01
AsCd1	4.38E-03	1.71E-03	3.94E-01	4.17E-02	1.04E+00	1.91E+00	1.71E-03	6.69E-04	1.54E-01	1.63E-02	4.07E-01	7.47E-01	2.14E-03	8.37E-04	1.93E-01	2.04E-02	5.09E-01	9.36E-01
AsCd2	1.66E-03	1.63E-03	3.34E-01	3.04E-02	5.76E-01	1.64E+00	6.47E-04	6.38E-04	1.30E-01	1.19E-02	2.25E-01	6.39E-01	8.10E-04	7.99E-04	1.63E-01	1.49E-02	2.82E-01	8.01E-01
AsCd3	1.30E-03	2.09E-03	4.11E-01	4.53E-02	7.34E-01	1.40E+00	5.08E-04	8.17E-04	1.61E-01	1.77E-02	2.87E-01	5.48E-01	6.37E-04	1.02E-03	2.01E-01	2.21E-02	3.59E-01	6.86E-01
AsPb1	1.66E-03	5.52E-04	3.49E-01	4.09E-02	7.41E-01	1.11E+00	6.47E-04	2.16E-04	1.36E-01	1.60E-02	2.90E-01	4.35E-01	8.10E-04	2.70E-04	1.71E-01	2.00E-02	3.63E-01	5.45E-01
AsPb2	1.05E-03	1.97E-04	3.39E-01	3.85E-02	9.65E-01	1.45E+00	4.10E-04	7.70E-05	1.32E-01	1.50E-02	3.77E-01	5.65E-01	5.13E-04	9.65E-05	1.66E-01	1.88E-02	4.72E-01	7.07E-01
AsPb3	1.85E-03	2.63E-03	3.37E-01	4.68E-02	8.28E-01	1.17E+00	7.24E-04	1.03E-03	1.31E-01	1.83E-02	3.24E-01	4.59E-01	9.07E-04	1.28E-03	1.65E-01	2.29E-02	4.05E-01	5.74E-01
MIX1	3.55E-03	1.93E-03	3.28E-01	6.63E-02	9.02E-01	1.32E+00	1.39E-03	7.55E-04	1.28E-01	2.59E-02	3.52E-01	5.15E-01	1.74E-03	9.45E-04	1.61E-01	3.24E-02	4.41E-01	6.46E-01
MIX2	5.05E-03	2.48E-03	4.29E-01	5.85E-02	1.15E+00	1.37E+00	1.97E-03	9.71E-04	1.68E-01	2.28E-02	4.50E-01	5.36E-01	2.47E-03	1.22E-03	2.10E-01	2.86E-02	5.63E-01	6.71E-01
MIX3	3.83E-03	9.47E-04	1.75E-01	5.34E-02	5.02E-01	7.97E-01	1.49E-03	3.70E-04	6.83E-02	2.09E-02	1.96E-01	3.11E-01	1.87E-03	4.63E-04	8.56E-02	2.61E-02	2.45E-01	3.90E-01

Table Anx. 2.5. Daily dietary Intake of macro- nutrients on individual body weight basis ($\text{g kg}^{-1} \text{d}^{-1}$) for single plant specie

	Children					Adolescent					Adults				
	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C	N	P	K	Protein	Vitamin C
Potato															
Control	1.68E-03	2.00E-03	4.04E-03	9.60E-04	1.18E-02	6.58E-04	7.82E-04	1.58E-03	3.75E-04	4.62E-03	8.24E-04	9.79E-04	1.98E-03	4.70E-04	5.79E-03
As1	2.15E-03	1.56E-03	2.96E-03	1.23E-03	8.28E-03	8.41E-04	6.08E-04	1.15E-03	4.79E-04	3.24E-03	1.05E-03	7.61E-04	1.45E-03	6.00E-04	4.05E-03
As2	2.29E-03	1.79E-03	2.45E-03	1.31E-03	5.92E-03	8.95E-04	7.00E-04	9.59E-04	5.10E-04	2.31E-03	1.12E-03	8.76E-04	1.20E-03	6.39E-04	2.89E-03
As3	5.25E-04	2.01E-03	2.27E-03	2.99E-04	6.31E-03	2.05E-04	7.83E-04	8.86E-04	1.17E-04	2.47E-03	2.57E-04	9.81E-04	1.11E-03	1.46E-04	3.09E-03
AsCd1	3.09E-03	1.17E-03	3.14E-03	1.76E-03	1.14E-02	1.21E-03	4.58E-04	1.23E-03	6.88E-04	4.47E-03	1.51E-03	5.73E-04	1.54E-03	8.62E-04	5.59E-03
AsCd2	4.97E-04	1.80E-03	2.97E-03	2.83E-04	8.28E-03	1.94E-04	7.04E-04	1.16E-03	1.11E-04	3.24E-03	2.43E-04	8.82E-04	1.45E-03	1.39E-04	4.05E-03
AsCd3	3.17E-03	1.31E-04	2.63E-03	1.81E-03	5.42E-03	1.24E-03	5.10E-05	1.03E-03	7.07E-04	2.12E-03	1.55E-03	6.38E-05	1.29E-03	8.85E-04	2.65E-03
AsPb1	2.95E-03	2.29E-03	3.16E-03	1.68E-03	5.42E-03	1.15E-03	8.94E-04	1.24E-03	6.58E-04	2.12E-03	1.45E-03	1.12E-03	1.55E-03	8.24E-04	2.65E-03
AsPb2	2.65E-03	2.26E-03	2.97E-03	1.51E-03	5.92E-03	1.04E-03	8.81E-04	1.16E-03	5.90E-04	2.31E-03	1.30E-03	1.10E-03	1.45E-03	7.39E-04	2.89E-03
AsPb3	3.40E-03	1.95E-03	2.53E-03	1.94E-03	7.18E-03	1.33E-03	7.61E-04	9.90E-04	7.56E-04	2.80E-03	1.66E-03	9.53E-04	1.24E-03	9.47E-04	3.51E-03
MIX1	3.17E-03	2.01E-03	2.70E-03	1.81E-03	1.05E-02	1.24E-03	7.84E-04	1.05E-03	7.07E-04	4.08E-03	1.55E-03	9.81E-04	1.32E-03	8.85E-04	5.11E-03
MIX2	2.87E-03	1.81E-03	2.39E-03	1.64E-03	8.28E-03	1.12E-03	7.08E-04	9.35E-04	6.39E-04	3.24E-03	1.40E-03	8.86E-04	1.17E-03	8.01E-04	4.05E-03
MIX3	1.52E-03	1.62E-03	2.15E-03	8.66E-04	7.49E-03	5.93E-04	6.34E-04	8.38E-04	3.38E-04	2.93E-03	7.43E-04	7.93E-04	1.05E-03	4.23E-04	3.67E-03
Tomato															
Control	3.62E-03	2.26E-03	2.62E-03	2.06E-03	1.42E-02	1.41E-03	8.82E-04	1.02E-03	8.05E-04	5.55E-03	1.77E-03	1.10E-03	1.28E-03	1.01E-03	6.95E-03
As1	1.85E-03	1.33E-03	2.15E-03	1.05E-03	1.54E-02	7.23E-04	5.20E-04	8.38E-04	4.12E-04	6.01E-03	9.05E-04	6.51E-04	1.05E-03	5.16E-04	7.52E-03
As2	1.55E-03	1.70E-03	1.87E-03	8.81E-04	1.81E-02	6.04E-04	6.64E-04	7.32E-04	3.44E-04	7.09E-03	7.56E-04	8.31E-04	9.16E-04	4.31E-04	8.87E-03
As3	1.77E-03	1.84E-03	1.58E-03	1.01E-03	2.15E-02	6.90E-04	7.19E-04	6.17E-04	3.93E-04	8.41E-03	8.64E-04	9.00E-04	7.72E-04	4.93E-04	1.05E-02
AsCd1	1.24E-03	6.84E-04	2.11E-03	7.08E-04	1.81E-02	4.85E-04	2.67E-04	8.24E-04	2.77E-04	7.09E-03	6.08E-04	3.35E-04	1.03E-03	3.46E-04	8.87E-03
AsCd2	1.88E-03	1.25E-03	1.85E-03	1.07E-03	2.01E-02	7.33E-04	4.90E-04	7.22E-04	4.18E-04	7.86E-03	9.18E-04	6.14E-04	9.04E-04	5.23E-04	9.84E-03
AsCd3	1.55E-03	1.50E-03	1.67E-03	8.81E-04	2.25E-02	6.04E-04	5.87E-04	6.54E-04	3.44E-04	8.78E-03	7.56E-04	7.36E-04	8.19E-04	4.31E-04	1.10E-02

AsPb1	1.88E-03	1.38E-03	2.11E-03	1.07E-03	2.11E-02	7.33E-04	5.39E-04	8.25E-04	4.18E-04	8.24E-03	9.18E-04	6.75E-04	1.03E-03	5.23E-04	1.03E-02
AsPb2	1.55E-03	1.30E-03	1.92E-03	8.81E-04	2.25E-02	6.04E-04	5.08E-04	7.50E-04	3.44E-04	8.78E-03	7.56E-04	6.36E-04	9.39E-04	4.31E-04	1.10E-02
AsPb3	1.35E-03	1.57E-03	1.71E-03	7.71E-04	2.33E-02	5.28E-04	6.13E-04	6.67E-04	3.01E-04	9.09E-03	6.62E-04	7.67E-04	8.35E-04	3.77E-04	1.14E-02
MIX1	1.38E-03	1.77E-03	1.68E-03	7.87E-04	1.56E-02	5.39E-04	6.90E-04	6.58E-04	3.07E-04	6.09E-03	6.75E-04	8.64E-04	8.23E-04	3.85E-04	7.62E-03
MIX2	1.10E-03	1.69E-03	1.75E-03	6.29E-04	1.83E-02	4.31E-04	6.61E-04	6.82E-04	2.46E-04	7.16E-03	5.40E-04	8.27E-04	8.54E-04	3.08E-04	8.97E-03
MIX3	9.11E-04	1.50E-03	1.46E-03	5.19E-04	2.13E-02	3.56E-04	5.84E-04	5.72E-04	2.03E-04	8.32E-03	4.46E-04	7.32E-04	7.16E-04	2.54E-04	1.04E-02
Lettuce															
Control	2.07E-03	1.70E-03	2.96E-03	1.18E-03	5.03E-03	8.09E-04	6.63E-04	1.16E-03	4.61E-04	1.96E-03	1.01E-03	8.30E-04	1.45E-03	5.77E-04	2.46E-03
As1	2.18E-03	2.45E-03	2.24E-03	1.24E-03	5.42E-03	8.52E-04	9.56E-04	8.77E-04	4.86E-04	2.12E-03	1.07E-03	1.20E-03	1.10E-03	6.08E-04	2.65E-03
As2	2.48E-03	1.10E-03	2.17E-03	1.42E-03	6.11E-03	9.71E-04	4.29E-04	8.49E-04	5.53E-04	2.39E-03	1.22E-03	5.37E-04	1.06E-03	6.93E-04	2.99E-03
As3	2.46E-03	1.47E-03	2.07E-03	1.40E-03	7.40E-03	9.60E-04	5.74E-04	8.08E-04	5.47E-04	2.89E-03	1.20E-03	7.19E-04	1.01E-03	6.85E-04	3.62E-03
AsCd1	1.99E-03	4.58E-04	2.21E-03	1.13E-03	6.31E-03	7.76E-04	1.79E-04	8.64E-04	4.43E-04	2.47E-03	9.72E-04	2.24E-04	1.08E-03	5.54E-04	3.09E-03
AsCd2	2.24E-03	1.04E-03	2.15E-03	1.27E-03	7.10E-03	8.74E-04	4.05E-04	8.41E-04	4.98E-04	2.77E-03	1.09E-03	5.07E-04	1.05E-03	6.24E-04	3.47E-03
AsCd3	2.32E-03	2.59E-03	2.14E-03	1.32E-03	7.40E-03	9.06E-04	1.01E-03	8.38E-04	5.16E-04	2.89E-03	1.13E-03	1.27E-03	1.05E-03	6.47E-04	3.62E-03
AsPb1	1.24E-03	9.77E-04	2.18E-03	7.08E-04	5.52E-03	4.85E-04	3.82E-04	8.53E-04	2.77E-04	2.16E-03	6.08E-04	4.78E-04	1.07E-03	3.46E-04	2.70E-03
AsPb2	2.21E-03	2.48E-03	2.08E-03	1.26E-03	6.31E-03	8.63E-04	9.68E-04	8.14E-04	4.92E-04	2.47E-03	1.08E-03	1.21E-03	1.02E-03	6.16E-04	3.09E-03
AsPb3	2.37E-03	8.69E-04	2.00E-03	1.35E-03	7.40E-03	9.27E-04	3.39E-04	7.80E-04	5.29E-04	2.89E-03	1.16E-03	4.25E-04	9.77E-04	6.62E-04	3.62E-03
MIX1	1.96E-03	2.07E-03	1.92E-03	1.12E-03	6.70E-03	7.66E-04	8.08E-04	7.50E-04	4.36E-04	2.62E-03	9.59E-04	1.01E-03	9.39E-04	5.47E-04	3.28E-03
MIX2	1.57E-03	4.20E-04	1.93E-03	8.97E-04	7.20E-03	6.15E-04	1.64E-04	7.53E-04	3.50E-04	2.81E-03	7.70E-04	2.05E-04	9.43E-04	4.39E-04	3.52E-03
MIX3	3.23E-03	4.37E-04	1.82E-03	1.84E-03	7.49E-03	1.26E-03	1.71E-04	7.12E-04	7.19E-04	2.93E-03	1.58E-03	2.14E-04	8.92E-04	9.01E-04	3.67E-03

Table Anx. 2.6; RDA (%) of micro- nutrients for individual plant specie

	Children						Adolescent						Adults							
	Fe	Mn	Mn	Ca	Mg	Mg	Fe	Fe	Mn	Mn	Ca	Mg	Mg	Fe	Fe	Mn	Mn	Ca	Mg	Mg
	M,F	M	F	M,F	M	F	M	F	M	F	M,F	M	F	M	F	M	F	M,F	M	F
	Potato																			
Control	12.36	66.19	69.43	0.76	16.81	17.28	13.86	10.17	67.11	92.28	0.70	13.26	13.86	19.06	8.47	64.20	82.03	0.90	11.73	15.25
As1	12.11	66.85	70.12	1.81	17.27	17.75	13.58	9.96	67.78	93.20	1.66	13.62	14.24	18.68	8.30	64.83	82.84	2.16	12.05	15.66
As2	13.42	75.46	79.16	0.87	16.82	17.30	15.06	11.05	76.51	105.20	0.80	13.27	13.87	20.71	9.21	73.19	93.51	1.04	11.74	15.26
As3	10.00	65.87	69.09	1.49	17.83	18.33	11.22	8.23	66.78	91.82	1.37	14.07	14.70	15.43	6.86	63.88	81.62	1.78	12.44	16.18
AsCd1	8.43	58.24	61.09	2.00	17.71	18.21	9.46	6.94	59.05	81.19	1.83	13.97	14.61	13.01	5.78	56.48	72.17	2.38	12.36	16.07
AsCd2	8.40	97.19	101.95	1.59	15.85	16.30	9.42	6.91	98.55	135.50	1.46	12.50	13.07	12.96	5.76	94.26	120.44	1.89	11.06	14.38
AsCd3	4.44	76.12	79.85	1.98	12.45	12.80	4.99	3.66	77.18	106.12	1.81	9.82	10.27	6.86	3.05	73.82	94.33	2.36	8.69	11.29
AsPb1	5.11	114.90	120.53	1.56	14.56	14.97	5.73	4.20	116.50	160.19	1.43	11.49	12.01	7.88	3.50	111.44	142.39	1.86	10.16	13.21
AsPb2	9.40	113.72	119.29	1.27	18.74	19.26	10.54	7.73	115.30	158.54	1.17	14.78	15.45	14.50	6.44	110.29	140.92	1.52	13.07	17.00
AsPb3	13.12	109.25	114.60	1.51	12.19	12.54	14.72	10.79	110.77	152.31	1.38	9.62	10.06	20.23	8.99	105.95	135.38	1.80	8.51	11.06
MIX1	8.17	48.54	50.91	0.51	5.97	6.14	9.17	6.73	49.21	67.67	0.47	4.71	4.92	12.61	5.60	47.07	60.15	0.61	4.16	5.41
MIX2	6.08	23.80	24.96	0.38	7.65	7.87	6.83	5.01	24.13	33.17	0.35	6.04	6.31	9.38	4.17	23.08	29.49	0.45	5.34	6.94
MIX3	7.13	17.75	18.62	0.37	8.01	8.23	8.00	5.87	18.00	24.74	0.34	6.32	6.60	11.00	4.89	17.21	21.99	0.44	5.59	7.26

Tomato																				
Control	16.39	77.96	81.78	0.47	11.91	12.25	18.40	13.49	79.04	108.69	0.43	9.40	9.83	25.29	11.24	75.61	96.61	0.56	8.31	10.81
As1	18.02	75.82	79.53	0.41	13.34	13.71	20.22	14.83	76.87	105.70	0.38	10.52	11.00	27.80	12.35	73.53	93.95	0.49	9.31	12.10
As2	19.35	65.04	68.22	0.38	14.41	14.82	21.71	15.92	65.94	90.67	0.35	11.37	11.89	29.85	13.27	63.07	80.60	0.45	10.06	13.07
As3	20.06	60.08	63.02	0.33	16.10	16.55	22.51	16.51	60.92	83.76	0.30	12.70	13.27	30.95	13.76	58.27	74.45	0.39	11.23	14.60
AsCd1	14.23	88.57	92.91	0.37	15.56	16.00	15.97	11.71	89.80	123.48	0.34	12.27	12.83	21.96	9.76	85.90	109.76	0.44	10.86	14.11
AsCd2	12.31	84.81	88.96	0.44	14.41	14.82	13.82	10.13	85.99	118.24	0.41	11.37	11.89	19.00	8.44	82.25	105.10	0.53	10.06	13.07
AsCd3	11.64	81.90	85.91	0.40	13.68	14.06	13.06	9.57	83.04	114.18	0.37	10.79	11.28	17.95	7.98	79.43	101.50	0.48	9.55	12.41
AsPb1	12.36	62.62	65.68	0.44	14.41	14.82	13.86	10.17	63.49	87.30	0.41	11.37	11.89	19.06	8.47	60.73	77.60	0.53	10.06	13.07
AsPb2	13.62	60.12	63.06	0.47	14.80	15.22	15.28	11.21	60.96	83.82	0.43	11.68	12.21	21.02	9.34	58.31	74.50	0.56	10.33	13.43
AsPb3	14.89	54.30	56.95	0.41	16.37	16.83	16.71	12.25	55.05	75.70	0.38	12.91	13.50	22.97	10.21	52.66	67.29	0.49	11.42	14.85
MIX1	13.44	88.31	92.63	0.41	17.51	18.00	15.08	11.06	89.54	123.11	0.38	13.81	14.44	20.74	9.22	85.64	109.43	0.49	12.22	15.88
MIX2	11.55	61.53	64.54	0.49	19.53	20.08	12.96	9.50	62.38	85.78	0.45	15.41	16.11	17.82	7.92	59.67	76.25	0.58	13.63	17.72
MIX3	10.38	50.92	53.41	0.51	21.01	21.60	11.64	8.54	51.63	70.99	0.47	16.58	17.33	16.01	7.12	49.38	63.10	0.61	14.66	19.06
Lettuce																				
Control	62.13	117.01	122.73	1.87	16.30	16.76	69.71	51.12	118.63	163.12	1.71	12.86	13.44	95.86	42.60	113.48	145.00	2.23	11.37	14.79
As1	87.20	47.99	50.33	2.02	18.71	19.24	97.84	71.75	48.65	66.90	1.85	14.76	15.43	134.53	59.79	46.54	59.47	2.41	13.06	16.97
As2	103.23	39.26	41.18	2.20	21.80	22.41	115.83	84.95	39.80	54.73	2.01	17.20	17.98	159.27	70.79	38.07	48.65	2.62	15.21	19.78
As3	94.09	36.42	38.20	2.34	23.14	23.78	105.57	77.42	36.92	50.77	2.14	18.25	19.08	145.16	64.52	35.32	45.13	2.78	16.14	20.99

AsCd1	118.80	69.51	72.91	2.71	32.67	33.59	133.30	97.75	70.47	96.90	2.48	25.77	26.94	183.28	81.46	67.41	86.13	3.23	22.80	29.63
AsCd2	100.60	50.62	53.09	1.50	27.95	28.74	112.88	82.78	51.32	70.56	1.37	22.05	23.05	155.20	68.98	49.09	62.72	1.78	19.51	25.36
AsCd3	123.79	75.46	79.16	1.91	23.94	24.61	138.89	101.86	76.51	105.20	1.75	18.88	19.74	190.98	84.88	73.19	93.51	2.27	16.70	21.71
AsPb1	105.21	68.17	71.50	1.93	19.03	19.56	118.05	86.57	69.11	95.03	1.77	15.01	15.69	162.31	72.14	66.11	84.47	2.30	13.28	17.26
AsPb2	102.16	64.13	67.27	2.51	24.69	25.38	114.63	84.07	65.02	89.41	2.30	19.47	20.36	157.62	70.05	62.20	79.47	2.99	17.23	22.39
AsPb3	101.38	78.04	81.86	2.16	20.05	20.62	113.75	83.42	79.12	108.80	1.97	15.82	16.54	156.41	69.52	75.68	96.71	2.57	13.99	18.19
MIX1	98.86	110.43	115.84	2.35	22.54	23.17	110.93	81.35	111.97	153.96	2.15	17.78	18.58	152.53	67.79	107.10	136.85	2.80	15.73	20.44
MIX2	129.30	97.44	102.21	3.00	23.42	24.08	145.08	106.39	98.80	135.85	2.75	18.47	19.31	199.48	88.66	94.50	120.75	3.57	16.34	21.24
MIX3	52.70	88.98	93.33	1.31	13.61	13.99	59.13	43.36	90.21	124.05	1.20	10.73	11.22	81.30	36.14	86.29	110.26	1.55	9.49	12.34

Table Anx. 2.7; RDA % of macro- nutrients for individual plant specie

	Children							Adolescent							Adults						
	N		P	K	Protein		Vitamin C	N		P	K	Protein		Vitamin C	N		P	K	Protein		Vitamin C
	M	F	M-F	M-F	M	F	M-F	M	F	M-F	M-F	M	F	M-F	M	F	M-F	M-F	M	F	M-F
Potato																					
Control	0.93	0.92	6.79	5.61	0.08	0.08	0.66	0.66	0.89	5.95	6.26	0.06	0.08	0.73	0.64	0.8	10.63	6.26	0.06	0.07	0.73
As1	1.19	1.18	5.28	4.11	0.11	0.11	0.46	0.85	1.14	4.63	4.58	0.08	0.1	0.51	0.82	1.03	8.26	4.58	0.07	0.09	0.51
As2	1.27	1.26	6.08	3.41	0.12	0.11	0.33	0.9	1.21	5.33	3.8	0.08	0.11	0.37	0.87	1.09	9.51	3.8	0.08	0.1	0.37
As3	0.29	0.29	6.81	3.15	0.03	0.03	0.35	0.21	0.28	5.97	3.51	0.02	0.03	0.39	0.2	0.25	10.65	3.51	0.02	0.02	0.39
AsCd1	1.71	1.7	3.98	4.37	0.16	0.15	0.64	1.22	1.63	3.49	4.87	0.11	0.15	0.71	1.17	1.48	6.23	4.87	0.11	0.13	0.71
AsCd2	0.27	0.27	6.12	4.12	0.03	0.02	0.46	0.2	0.26	5.36	4.6	0.02	0.02	0.51	0.19	0.24	9.58	4.6	0.02	0.02	0.51
AsCd3	1.76	1.74	0.44	3.65	0.16	0.16	0.3	1.25	1.68	0.39	4.07	0.11	0.15	0.34	1.2	1.52	0.69	4.07	0.11	0.14	0.34
AsPb1	1.63	1.62	7.76	4.4	0.15	0.15	0.3	1.16	1.56	6.8	4.9	0.11	0.14	0.34	1.12	1.41	12.15	4.9	0.1	0.13	0.34
AsPb2	1.47	1.45	7.65	4.12	0.13	0.13	0.33	1.04	1.4	6.71	4.6	0.1	0.13	0.37	1	1.26	11.98	4.6	0.09	0.12	0.37
AsPb3	1.88	1.86	6.61	3.52	0.17	0.17	0.4	1.34	1.79	5.8	3.92	0.12	0.16	0.44	1.29	1.62	10.35	3.92	0.12	0.15	0.44
MIX1	1.76	1.74	6.81	3.75	0.16	0.16	0.58	1.25	1.68	5.97	4.18	0.11	0.15	0.65	1.2	1.52	10.65	4.18	0.11	0.14	0.65

MIX2	1.59	1.57	6.15	3.32	0.14	0.14	0.46	1.13	1.52	5.39	3.71	0.1	0.14	0.51	1.09	1.37	9.62	3.71	0.1	0.13	0.51
MIX3	0.84	0.83	5.5	2.98	0.08	0.08	0.42	0.6	0.8	4.82	3.32	0.05	0.07	0.46	0.58	0.72	8.61	3.32	0.05	0.07	0.46
Tomato																					
Control	2	1.98	7.66	3.64	0.18	0.18	0.79	1.42	1.91	6.72	4.06	0.13	0.17	0.88	1.37	1.73	11.99	4.06	0.12	0.16	0.88
As1	1.02	1.01	4.52	2.98	0.09	0.09	0.85	0.73	0.98	3.96	3.32	0.07	0.09	0.95	0.7	0.88	7.07	3.32	0.06	0.08	0.95
As2	0.86	0.85	5.77	2.6	0.08	0.08	1.01	0.61	0.82	5.05	2.9	0.06	0.07	1.12	0.59	0.74	9.03	2.9	0.05	0.07	1.12
As3	0.98	0.97	6.24	2.19	0.09	0.09	1.2	0.7	0.93	5.47	2.44	0.06	0.09	1.33	0.67	0.84	9.77	2.44	0.06	0.08	1.33
AsCd1	0.69	0.68	2.32	2.93	0.06	0.06	1.01	0.49	0.66	2.04	3.27	0.04	0.06	1.12	0.47	0.59	3.63	3.27	0.04	0.05	1.12
AsCd2	1.04	1.03	4.26	2.57	0.09	0.09	1.12	0.74	0.99	3.73	2.86	0.07	0.09	1.25	0.71	0.9	6.66	2.86	0.06	0.08	1.25
AsCd3	0.86	0.85	5.1	2.33	0.08	0.08	1.25	0.61	0.82	4.47	2.59	0.06	0.07	1.39	0.59	0.74	7.99	2.59	0.05	0.07	1.39
AsPb1	1.04	1.03	4.69	2.93	0.09	0.09	1.17	0.74	0.99	4.11	3.27	0.07	0.09	1.31	0.71	0.9	7.33	3.27	0.06	0.08	1.31
AsPb2	0.86	0.85	4.41	2.67	0.08	0.08	1.25	0.61	0.82	3.87	2.97	0.06	0.07	1.39	0.59	0.74	6.91	2.97	0.05	0.07	1.39
AsPb3	0.75	0.74	5.32	2.37	0.07	0.07	1.29	0.53	0.71	4.66	2.65	0.05	0.07	1.44	0.51	0.65	8.33	2.65	0.05	0.06	1.44
MIX1	0.76	0.76	5.99	2.34	0.07	0.07	0.87	0.54	0.73	5.25	2.61	0.05	0.07	0.97	0.52	0.66	9.38	2.61	0.05	0.06	0.97
MIX2	0.61	0.61	5.74	2.42	0.06	0.06	1.02	0.43	0.58	5.03	2.7	0.04	0.05	1.14	0.42	0.53	8.98	2.7	0.04	0.05	1.14
MIX3	0.5	0.5	5.08	2.03	0.05	0.05	1.18	0.36	0.48	4.45	2.27	0.03	0.04	1.32	0.35	0.43	7.94	2.27	0.03	0.04	1.32
Lettuce																					
Control	1.15	1.14	5.76	4.11	0.1	0.1	0.28	0.82	1.09	5.05	4.58	0.07	0.1	0.31	0.78	0.99	9.01	4.58	0.07	0.09	0.31

As1	1.21	1.2	8.31	3.12	0.11	0.11	0.3	0.86	1.15	7.28	3.48	0.08	0.11	0.34	0.83	1.04	13	3.48	0.08	0.09	0.34
As2	1.37	1.36	3.73	3.02	0.13	0.12	0.34	0.98	1.31	3.27	3.37	0.09	0.12	0.38	0.94	1.19	5.83	3.37	0.09	0.11	0.38
As3	1.36	1.35	4.99	2.87	0.12	0.12	0.41	0.97	1.3	4.37	3.2	0.09	0.12	0.46	0.93	1.17	7.81	3.2	0.08	0.11	0.46
AsCd1	1.1	1.09	1.56	3.07	0.1	0.1	0.35	0.78	1.05	1.36	3.43	0.07	0.1	0.39	0.75	0.95	2.43	3.43	0.07	0.09	0.39
AsCd2	1.24	1.23	3.52	2.99	0.11	0.11	0.39	0.88	1.18	3.08	3.34	0.08	0.11	0.44	0.85	1.07	5.51	3.34	0.08	0.1	0.44
AsCd3	1.28	1.27	8.78	2.98	0.12	0.12	0.41	0.91	1.22	7.7	3.32	0.08	0.11	0.46	0.88	1.11	13.74	3.32	0.08	0.1	0.46
AsPb1	0.69	0.68	3.32	3.03	0.06	0.06	0.31	0.49	0.66	2.91	3.38	0.04	0.06	0.34	0.47	0.59	5.19	3.38	0.04	0.05	0.34
AsPb2	1.22	1.21	8.41	2.9	0.11	0.11	0.35	0.87	1.17	7.37	3.23	0.08	0.11	0.39	0.84	1.05	13.16	3.23	0.08	0.1	0.39
AsPb3	1.31	1.3	2.95	2.77	0.12	0.12	0.41	0.94	1.25	2.58	3.09	0.09	0.11	0.46	0.9	1.13	4.62	3.09	0.08	0.1	0.46
MIX1	1.08	1.07	7.02	2.67	0.1	0.1	0.37	0.77	1.04	6.15	2.97	0.07	0.09	0.42	0.74	0.94	10.98	2.97	0.07	0.09	0.42
MIX2	0.87	0.86	1.42	2.68	0.08	0.08	0.4	0.62	0.83	1.25	2.99	0.06	0.08	0.45	0.6	0.75	2.23	2.99	0.05	0.07	0.45
MIX3	1.79	1.77	1.48	2.53	0.16	0.16	0.42	1.27	1.71	1.3	2.82	0.12	0.16	0.46	1.22	1.54	2.32	2.82	0.11	0.14	0.46

Annexure-3

Table Anx.3.1. Daily intake (mg d⁻¹) of metals through consumption of As contaminated vegetables

	Children				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Potato								
Control	0.89	0.58	1.50	0.60	1.32	0.86	2.22	0.90
As1	1.11	0.61	0.63	1.08	1.65	0.90	0.94	1.61
As2	1.11	0.46	1.26	0.92	1.65	0.69	1.88	1.38
As3	1.06	0.40	0.71	0.90	1.58	0.60	1.06	1.33
AsCd1	2.37	0.49	0.78	1.68	3.52	0.73	1.17	2.49
AsCd2	2.04	0.43	0.67	1.86	3.04	0.64	1.00	2.77
AsCd3	1.42	0.72	0.34	1.12	2.11	1.08	0.51	1.67
AsPb1	2.26	0.40	1.52	1.59	3.37	0.60	2.27	2.37
AsPb2	2.48	0.51	1.73	1.53	3.69	0.75	2.57	2.28
AsPb3	2.18	0.53	1.29	1.45	3.24	0.79	1.92	2.16
MIX1	2.04	0.62	0.95	0.83	3.04	0.93	1.42	1.24
MIX2	2.50	0.52	0.80	0.44	3.72	0.77	1.18	0.65
MIX3	2.35	0.57	1.00	0.31	3.50	0.85	1.49	0.46
Tomato								
Control	1.38	0.34	0.69	0.82	2.05	0.50	1.03	1.22
As1	1.11	0.32	0.83	0.74	1.65	0.48	1.24	1.10
As2	0.87	0.29	0.86	0.69	1.29	0.43	1.28	1.02
As3	0.77	0.27	0.89	0.64	1.14	0.40	1.32	0.95
AsCd1	2.51	0.31	0.88	0.54	3.74	0.46	1.31	0.80
AsCd2	3.06	0.27	0.91	0.43	4.56	0.41	1.36	0.64
AsCd3	3.50	0.35	0.95	0.49	5.21	0.52	1.42	0.73
AsPb1	2.19	0.27	1.11	0.61	3.26	0.40	1.65	0.91
AsPb2	2.65	0.30	0.90	0.67	3.94	0.45	1.34	0.99
AsPb3	2.91	0.36	0.90	0.70	4.32	0.53	1.34	1.05
MIX1	2.80	0.32	0.46	0.59	4.17	0.48	0.68	0.87
MIX2	2.74	0.28	0.39	0.54	4.08	0.42	0.58	0.81
MIX3	2.57	0.27	0.40	0.42	3.83	0.41	0.60	0.62
Lettuce								
Control	2.54	0.55	1.76	0.30	3.77	0.82	2.62	0.44
As1	1.70	0.56	1.20	0.39	2.52	0.84	1.78	0.59
As2	1.58	0.60	0.90	0.49	2.35	0.89	1.33	0.73
As3	1.34	0.63	1.01	0.53	1.99	0.93	1.50	0.79
AsCd1	2.55	0.60	2.02	0.52	3.79	0.89	3.01	0.77
AsCd2	1.86	0.58	1.66	0.40	2.77	0.86	2.47	0.59

AsCd3	2.88	0.54	2.06	0.51	4.29	0.80	3.07	0.76
AsPb1	2.58	0.35	2.01	0.31	3.84	0.51	2.99	0.47
AsPb2	2.80	0.32	1.60	0.39	4.16	0.47	2.38	0.58
AsPb3	3.24	0.50	2.06	0.53	4.82	0.74	3.07	0.78
MIX1	1.81	0.55	0.93	0.46	2.69	0.82	1.38	0.68
MIX2	1.68	0.52	0.49	0.59	2.50	0.77	0.73	0.88
MIX3	1.83	0.47	0.51	0.29	2.73	0.70	0.76	0.43

Table Anx.3.2. Cumulative Daily intake (mg d^{-1}) of metals through consumption of As contaminated vegetables

	Children				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Control	1.60	0.49	1.32	0.57	2.38	0.73	1.96	0.85
As1	1.31	0.50	0.89	0.74	1.94	0.74	1.32	1.10
As2	1.18	0.45	1.01	0.70	1.76	0.67	1.50	1.04
As3	1.06	0.43	0.87	0.69	1.57	0.64	1.30	1.02
AsCd1	2.48	0.47	1.23	0.91	3.68	0.70	1.83	1.36
AsCd2	2.32	0.43	1.08	0.90	3.45	0.64	1.61	1.33
AsCd3	2.60	0.54	1.12	0.71	3.87	0.80	1.66	1.05
AsPb1	2.34	0.34	1.55	0.84	3.49	0.50	2.30	1.25
AsPb2	2.65	0.37	1.41	0.86	3.93	0.56	2.10	1.28
AsPb3	2.77	0.46	1.42	0.89	4.13	0.69	2.11	1.33
MIX1	2.22	0.50	0.78	0.63	3.30	0.74	1.16	0.93
MIX2	2.31	0.44	0.56	0.52	3.43	0.65	0.83	0.78
MIX3	2.25	0.44	0.64	0.34	3.35	0.65	0.95	0.50

Table Anx.3.3. Daily dietary Intake of metals (mg d⁻¹) through consumption of Cd and Pb vegetables

	Children				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Potato								
Control	0.89	0.58	1.50	0.60	1.30	0.86	2.20	0.90
Cd1	1.20	0.42	1.60	0.79	1.80	0.63	2.40	1.20
Cd2	1.60	0.51	1.30	0.85	2.40	0.77	1.90	1.30
Cd3	1.50	0.32	0.74	0.60	2.20	0.47	1.10	0.89
Pb1	2.00	0.41	0.58	0.89	3.00	0.61	0.87	1.30
Pb2	2.30	0.59	1.50	1.30	3.40	0.87	2.30	1.90
Pb3	2.60	0.43	0.72	0.61	3.90	0.64	1.10	0.91
CdPb1	2.40	0.59	1.70	1.10	3.50	0.88	2.50	1.70
CdPb2	2.40	0.54	1.90	1.00	3.60	0.80	2.80	1.50
CdPb3	2.00	0.47	1.80	0.99	3.00	0.70	2.70	1.50
Tomato								
Control	1.40	0.34	0.69	0.82	2.10	0.50	1.00	1.20
Cd1	0.35	0.27	1.80	0.69	0.52	0.40	2.70	1.00
Cd2	0.24	0.30	1.60	0.66	0.36	0.44	2.40	0.98
Cd3	0.22	0.34	1.90	0.86	0.32	0.51	2.80	1.30
Pb1	2.00	0.35	1.90	0.90	2.90	0.52	2.90	1.30
Pb2	1.00	0.33	0.71	0.45	1.50	0.49	1.10	0.67
Pb3	2.10	0.30	1.40	0.46	3.10	0.45	2.20	0.68
CdPb1	2.70	0.33	0.55	0.77	4.00	0.49	0.81	1.10
CdPb2	2.40	0.31	0.61	0.55	3.60	0.46	0.91	0.82
CdPb3	2.80	0.28	0.49	0.44	4.20	0.42	0.72	0.65
Lettuce								
Control	2.50	0.55	1.80	0.30	3.80	0.82	2.60	0.44
Cd1	2.60	0.61	0.65	0.35	3.90	0.91	0.97	0.52
Cd2	3.10	0.35	1.40	0.24	4.60	0.53	2.00	0.36
Cd3	3.70	0.43	0.72	0.27	5.50	0.65	1.10	0.40

Pb1	2.60	0.42	1.70	0.37	3.80	0.63	2.50	0.56
Pb2	1.80	0.49	2.00	0.52	2.70	0.73	3.00	0.78
Pb3	3.10	0.37	1.90	0.60	4.70	0.55	2.90	0.90
CdPb1	2.00	0.66	1.70	0.68	2.90	0.98	2.60	1.00
CdPb2	1.90	0.54	2.10	0.36	2.80	0.81	3.10	0.54
CdPb3	2.40	0.66	2.20	0.52	3.50	0.99	3.30	0.78

Table Anx.3.4. Cumulative Daily dietary Intake of metals (mg d⁻¹) through consumption of Cd and Pb contaminated vegetables

	Children				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Control	1.60	0.49	1.30	0.57	2.40	0.73	2.00	0.85
Cd1	1.40	0.43	1.30	0.61	2.10	0.65	2.00	0.91
Cd2	1.60	0.39	1.40	0.58	2.40	0.58	2.10	0.87
Cd3	1.80	0.37	1.10	0.58	2.70	0.54	1.70	0.86
Pb1	2.20	0.40	1.40	0.72	3.20	0.59	2.10	1.10
Pb2	1.70	0.47	1.40	0.75	2.50	0.69	2.10	1.10
Pb3	2.60	0.37	1.40	0.56	3.90	0.55	2.00	0.83
CdPb1	2.30	0.53	1.30	0.87	3.50	0.78	2.00	1.30
CdPb2	2.20	0.46	1.50	0.65	3.30	0.69	2.30	0.96
CdPb3	2.40	0.47	1.50	0.65	3.60	0.70	2.20	0.97

Table Anx.3.5. Daily dietary Intake of metals (mg kg⁻¹ d⁻¹) through consumption of As contaminated vegetables

	Children				Adolescent				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
Potato												
Control	3.55E-02	2.32E-02	5.98E-02	2.41E-02	1.39E-02	9.04E-03	2.34E-02	9.41E-03	1.74E-02	1.13E-02	2.93E-02	1.18E-02
As1	4.44E-02	2.43E-02	2.52E-02	4.33E-02	1.73E-02	9.51E-03	9.86E-03	1.69E-02	2.17E-02	1.19E-02	1.23E-02	2.12E-02
As2	4.44E-02	1.86E-02	5.05E-02	3.70E-02	1.73E-02	7.26E-03	1.97E-02	1.45E-02	2.17E-02	9.09E-03	2.47E-02	1.81E-02
As3	4.25E-02	1.60E-02	2.85E-02	3.58E-02	1.66E-02	6.25E-03	1.11E-02	1.40E-02	2.08E-02	7.83E-03	1.39E-02	1.75E-02
AsCd1	9.47E-02	1.97E-02	3.13E-02	6.70E-02	3.70E-02	7.70E-03	1.22E-02	2.62E-02	4.63E-02	9.65E-03	1.53E-02	3.28E-02
AsCd2	8.16E-02	1.72E-02	2.68E-02	7.45E-02	3.19E-02	6.73E-03	1.05E-02	2.91E-02	3.99E-02	8.43E-03	1.31E-02	3.65E-02
AsCd3	5.68E-02	2.89E-02	1.37E-02	4.50E-02	2.22E-02	1.13E-02	5.34E-03	1.76E-02	2.78E-02	1.42E-02	6.69E-03	2.20E-02
AsPb1	9.05E-02	1.61E-02	6.10E-02	6.37E-02	3.54E-02	6.29E-03	2.38E-02	2.49E-02	4.43E-02	7.87E-03	2.98E-02	3.11E-02
AsPb2	9.94E-02	2.03E-02	6.92E-02	6.14E-02	3.88E-02	7.92E-03	2.70E-02	2.40E-02	4.86E-02	9.92E-03	3.39E-02	3.00E-02
AsPb3	8.72E-02	2.13E-02	5.16E-02	5.80E-02	3.40E-02	8.32E-03	2.02E-02	2.27E-02	4.26E-02	1.04E-02	2.53E-02	2.84E-02
MIX1	8.16E-02	2.49E-02	3.81E-02	3.32E-02	3.19E-02	9.74E-03	1.49E-02	1.30E-02	3.99E-02	1.22E-02	1.86E-02	1.63E-02
MIX2	1.00E-01	2.08E-02	3.18E-02	1.76E-02	3.91E-02	8.13E-03	1.24E-02	6.86E-03	4.89E-02	1.02E-02	1.56E-02	8.59E-03
MIX3	9.41E-02	2.29E-02	4.00E-02	1.24E-02	3.67E-02	8.95E-03	1.56E-02	4.85E-03	4.60E-02	1.12E-02	1.96E-02	6.08E-03
Tomato												
Control	5.52E-02	1.34E-02	2.76E-02	3.27E-02	2.16E-02	5.24E-03	1.08E-02	1.28E-02	2.70E-02	6.56E-03	1.35E-02	1.60E-02
As1	4.44E-02	1.29E-02	3.34E-02	2.97E-02	1.74E-02	5.03E-03	1.30E-02	1.16E-02	2.17E-02	6.30E-03	1.63E-02	1.45E-02
As2	3.46E-02	1.15E-02	3.44E-02	2.74E-02	1.35E-02	4.50E-03	1.35E-02	1.07E-02	1.69E-02	5.63E-03	1.69E-02	1.34E-02
As3	3.08E-02	1.08E-02	3.56E-02	2.56E-02	1.20E-02	4.23E-03	1.39E-02	9.99E-03	1.50E-02	5.30E-03	1.74E-02	1.25E-02
AsCd1	1.01E-01	1.25E-02	3.52E-02	2.16E-02	3.93E-02	4.87E-03	1.38E-02	8.42E-03	4.92E-02	6.10E-03	1.72E-02	1.05E-02
AsCd2	1.23E-01	1.10E-02	3.65E-02	1.71E-02	4.79E-02	4.29E-03	1.43E-02	6.68E-03	6.00E-02	5.37E-03	1.79E-02	8.36E-03
AsCd3	1.40E-01	1.39E-02	3.81E-02	1.97E-02	5.47E-02	5.42E-03	1.49E-02	7.68E-03	6.85E-02	6.79E-03	1.86E-02	9.62E-03
AsPb1	8.76E-02	1.08E-02	4.44E-02	2.45E-02	3.42E-02	4.22E-03	1.73E-02	9.56E-03	4.28E-02	5.29E-03	2.17E-02	1.20E-02
AsPb2	1.06E-01	1.21E-02	3.60E-02	2.66E-02	4.14E-02	4.72E-03	1.41E-02	1.04E-02	5.19E-02	5.92E-03	1.76E-02	1.30E-02
AsPb3	1.16E-01	1.44E-02	3.62E-02	2.82E-02	4.54E-02	5.61E-03	1.41E-02	1.10E-02	5.68E-02	7.02E-03	1.77E-02	1.38E-02
MIX1	1.12E-01	1.29E-02	1.82E-02	2.35E-02	4.38E-02	5.02E-03	7.13E-03	9.19E-03	5.48E-02	6.29E-03	8.92E-03	1.15E-02

MIX2	1.10E-01	1.14E-02	1.55E-02	2.18E-02	4.29E-02	4.44E-03	6.07E-03	8.50E-03	5.37E-02	5.56E-03	7.60E-03	1.06E-02
MIX3	1.03E-01	1.10E-02	1.61E-02	1.67E-02	4.02E-02	4.29E-03	6.29E-03	6.53E-03	5.04E-02	5.37E-03	7.88E-03	8.18E-03
Lettuce												
Control	1.01E-01	2.22E-02	7.05E-02	1.19E-02	3.96E-02	8.66E-03	2.75E-02	4.65E-03	4.96E-02	1.08E-02	3.45E-02	5.83E-03
As1	6.78E-02	2.26E-02	4.79E-02	1.58E-02	2.65E-02	8.81E-03	1.87E-02	6.16E-03	3.32E-02	1.10E-02	2.34E-02	7.72E-03
As2	6.31E-02	2.41E-02	3.59E-02	1.97E-02	2.47E-02	9.40E-03	1.40E-02	7.70E-03	3.09E-02	1.18E-02	1.75E-02	9.65E-03
As3	5.36E-02	2.50E-02	4.05E-02	2.13E-02	2.10E-02	9.77E-03	1.58E-02	8.32E-03	2.62E-02	1.22E-02	1.98E-02	1.04E-02
AsCd1	1.02E-01	2.40E-02	8.09E-02	2.07E-02	3.98E-02	9.37E-03	3.16E-02	8.10E-03	4.99E-02	1.17E-02	3.96E-02	1.01E-02
AsCd2	7.44E-02	2.30E-02	6.65E-02	1.59E-02	2.91E-02	9.00E-03	2.60E-02	6.19E-03	3.64E-02	1.13E-02	3.25E-02	7.76E-03
AsCd3	1.15E-01	2.15E-02	8.26E-02	2.04E-02	4.51E-02	8.38E-03	3.23E-02	7.95E-03	5.64E-02	1.05E-02	4.04E-02	9.96E-03
AsPb1	1.03E-01	1.38E-02	8.03E-02	1.25E-02	4.03E-02	5.39E-03	3.14E-02	4.90E-03	5.05E-02	6.75E-03	3.93E-02	6.14E-03
AsPb2	1.12E-01	1.26E-02	6.41E-02	1.56E-02	4.38E-02	4.93E-03	2.50E-02	6.10E-03	5.48E-02	6.17E-03	3.14E-02	7.64E-03
AsPb3	1.30E-01	2.00E-02	8.25E-02	2.11E-02	5.06E-02	7.83E-03	3.22E-02	8.23E-03	6.34E-02	9.80E-03	4.04E-02	1.03E-02
MIX1	7.23E-02	2.20E-02	3.71E-02	1.84E-02	2.82E-02	8.60E-03	1.45E-02	7.18E-03	3.54E-02	1.08E-02	1.81E-02	8.99E-03
MIX2	6.72E-02	2.07E-02	1.96E-02	2.37E-02	2.63E-02	8.07E-03	7.66E-03	9.24E-03	3.29E-02	1.01E-02	9.60E-03	1.16E-02
MIX3	7.34E-02	1.87E-02	2.05E-02	1.16E-02	2.87E-02	7.30E-03	8.01E-03	4.53E-03	3.59E-02	9.14E-03	1.00E-02	5.67E-03

Table Anx.3.6. Daily dietary Intake of metals on individual body weight basis (mg kg⁻¹ d⁻¹) for single plant species through consumption of Cd and Pb contaminated vegetables

	Children				Adolescent				Adults			
	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn	Cr	Cu	Ni	Zn
	Potato											
Control	3.55E-02	2.32E-02	5.98E-02	2.41E-02	1.39E-02	9.04E-03	2.34E-02	9.41E-03	1.74E-02	1.13E-02	2.93E-02	1.18E-02
Cd1	4.73E-02	1.69E-02	6.42E-02	3.16E-02	1.85E-02	6.59E-03	2.51E-02	1.23E-02	2.32E-02	8.26E-03	3.14E-02	1.54E-02
Cd2	6.39E-02	2.06E-02	5.10E-02	3.39E-02	2.50E-02	8.04E-03	1.99E-02	1.32E-02	3.13E-02	1.01E-02	2.50E-02	1.66E-02
Cd3	6.03E-02	1.27E-02	2.96E-02	2.41E-02	2.36E-02	4.97E-03	1.15E-02	9.40E-03	2.95E-02	6.22E-03	1.45E-02	1.18E-02
Pb1	8.05E-02	1.64E-02	2.33E-02	3.57E-02	3.14E-02	6.41E-03	9.12E-03	1.40E-02	3.94E-02	8.03E-03	1.14E-02	1.75E-02
Pb2	9.23E-02	2.34E-02	6.19E-02	5.13E-02	3.61E-02	9.14E-03	2.42E-02	2.00E-02	4.51E-02	1.14E-02	3.03E-02	2.51E-02
Pb3	1.05E-01	1.71E-02	2.89E-02	2.45E-02	4.11E-02	6.69E-03	1.13E-02	9.55E-03	5.15E-02	8.37E-03	1.41E-02	1.20E-02
CdPb1	9.41E-02	2.36E-02	6.63E-02	4.59E-02	3.67E-02	9.23E-03	2.59E-02	1.79E-02	4.60E-02	1.16E-02	3.24E-02	2.25E-02
CdPb2	9.76E-02	2.14E-02	7.57E-02	4.13E-02	3.81E-02	8.36E-03	2.96E-02	1.61E-02	4.77E-02	1.05E-02	3.70E-02	2.02E-02
CdPb3	8.16E-02	1.88E-02	7.16E-02	3.95E-02	3.19E-02	7.35E-03	2.80E-02	1.54E-02	3.99E-02	9.20E-03	3.50E-02	1.93E-02
	Tomato											
Control	5.52E-02	1.34E-02	2.76E-02	3.27E-02	2.16E-02	5.24E-03	1.08E-02	1.28E-02	2.70E-02	6.56E-03	1.35E-02	1.60E-02
Cd1	1.40E-02	1.08E-02	7.14E-02	2.76E-02	5.46E-03	4.22E-03	2.79E-02	1.08E-02	6.84E-03	5.29E-03	3.49E-02	1.35E-02
Cd2	9.70E-03	1.19E-02	6.50E-02	2.64E-02	3.79E-03	4.63E-03	2.54E-02	1.03E-02	4.75E-03	5.80E-03	3.18E-02	1.29E-02
Cd3	8.65E-03	1.37E-02	7.63E-02	3.44E-02	3.38E-03	5.37E-03	2.98E-02	1.35E-02	4.23E-03	6.73E-03	3.73E-02	1.69E-02
Pb1	7.81E-02	1.41E-02	7.67E-02	3.60E-02	3.05E-02	5.49E-03	3.00E-02	1.41E-02	3.82E-02	6.88E-03	3.75E-02	1.76E-02
Pb2	4.01E-02	1.30E-02	2.84E-02	1.81E-02	1.57E-02	5.10E-03	1.11E-02	7.06E-03	1.96E-02	6.38E-03	1.39E-02	8.84E-03
Pb3	8.26E-02	1.21E-02	5.80E-02	1.83E-02	3.23E-02	4.75E-03	2.26E-02	7.14E-03	4.04E-02	5.94E-03	2.84E-02	8.94E-03
CdPb1	1.08E-01	1.33E-02	2.19E-02	3.09E-02	4.21E-02	5.19E-03	8.56E-03	1.21E-02	5.27E-02	6.49E-03	1.07E-02	1.51E-02
CdPb2	9.61E-02	1.23E-02	2.44E-02	2.19E-02	3.75E-02	4.82E-03	9.51E-03	8.57E-03	4.70E-02	6.03E-03	1.19E-02	1.07E-02
CdPb3	1.12E-01	1.13E-02	1.95E-02	1.76E-02	4.37E-02	4.42E-03	7.60E-03	6.88E-03	5.47E-02	5.53E-03	9.52E-03	8.62E-03

Lettuce												
Control	1.01E-01	2.22E-02	7.05E-02	1.19E-02	3.96E-02	8.66E-03	2.75E-02	4.65E-03	4.96E-02	1.08E-02	3.45E-02	5.83E-03
Cd1	1.06E-01	2.44E-02	2.61E-02	1.40E-02	4.14E-02	9.52E-03	1.02E-02	5.45E-03	5.18E-02	1.19E-02	1.28E-02	6.83E-03
Cd2	1.24E-01	1.42E-02	5.44E-02	9.62E-03	4.83E-02	5.55E-03	2.13E-02	3.76E-03	6.05E-02	6.95E-03	2.66E-02	4.71E-03
Cd3	1.47E-01	1.74E-02	2.88E-02	1.09E-02	5.75E-02	6.78E-03	1.13E-02	4.25E-03	7.20E-02	8.49E-03	1.41E-02	5.32E-03
Pb1	1.03E-01	1.70E-02	6.64E-02	1.50E-02	4.01E-02	6.62E-03	2.59E-02	5.85E-03	5.02E-02	8.30E-03	3.25E-02	7.33E-03
Pb2	7.17E-02	1.96E-02	7.97E-02	2.09E-02	2.80E-02	7.64E-03	3.11E-02	8.17E-03	3.51E-02	9.57E-03	3.90E-02	1.02E-02
Pb3	1.25E-01	1.48E-02	7.67E-02	2.41E-02	4.90E-02	5.79E-03	2.99E-02	9.43E-03	6.14E-02	7.25E-03	3.75E-02	1.18E-02
CdPb1	7.86E-02	2.63E-02	6.99E-02	2.71E-02	3.07E-02	1.03E-02	2.73E-02	1.06E-02	3.85E-02	1.28E-02	3.42E-02	1.32E-02
CdPb2	7.63E-02	2.17E-02	8.44E-02	1.45E-02	2.98E-02	8.47E-03	3.30E-02	5.67E-03	3.73E-02	1.06E-02	4.13E-02	7.10E-03
CdPb3	9.44E-02	2.65E-02	8.81E-02	2.10E-02	3.69E-02	1.04E-02	3.44E-02	8.20E-03	4.62E-02	1.30E-02	4.31E-02	1.03E-02

Table: Anx.3.7. RDA (%) of different trace metals through consumption of As contaminated vegetables

	Children			Adolescent				Adults			
	Cu	Ni	Zn	Cu	Ni	Zn	Zn	Cu	Ni	Zn	Zn
	M+F	M+F	M+F	M+F	M+F	M	F	M+F	M+F	M	F
Potato											
Control	118.12	404.37	11.30	96.71	222.49	8.14	9.95	95.63	222.49	8.14	11.20
As1	124.16	170.55	20.29	101.65	93.84	14.62	17.87	100.52	93.84	14.62	20.11
As2	94.78	341.10	17.35	77.60	187.68	12.50	15.28	76.73	187.68	12.50	17.19
As3	81.70	192.56	16.80	66.89	105.95	12.10	14.79	66.14	105.95	12.10	16.64
AsCd1	100.61	211.75	31.45	82.37	116.51	22.66	27.70	81.46	116.51	22.66	31.16
AsCd2	87.94	181.21	34.96	71.99	99.71	25.19	30.79	71.19	99.71	25.19	34.64
AsCd3	147.70	92.36	21.10	120.92	50.82	15.20	18.58	119.58	50.82	15.20	20.91
AsPb1	82.10	412.15	29.86	67.22	226.77	21.51	26.29	66.47	226.77	21.51	29.58
AsPb2	103.43	467.58	28.78	84.68	257.27	20.74	25.35	83.74	257.27	20.74	28.52
AsPb3	108.66	348.94	27.22	88.96	191.99	19.61	23.97	87.98	191.99	19.61	26.97
MIX1	127.17	257.27	15.59	104.12	141.55	11.23	13.73	102.96	141.55	11.23	15.44
MIX2	106.25	215.05	8.24	86.99	118.33	5.94	7.26	86.02	118.33	5.94	8.16
MIX3	116.91	270.06	5.83	95.72	148.59	4.20	5.13	94.65	148.59	4.20	5.77
Tomato											
Control	68.42	186.54	15.35	56.01	102.64	11.06	13.52	55.39	102.64	11.06	15.21
As1	65.72	225.61	13.92	53.81	124.13	10.03	12.26	53.21	124.13	10.03	13.79
As2	58.76	232.75	12.86	48.11	128.06	9.27	11.33	47.57	128.06	9.27	12.75
As3	55.26	240.53	12.00	45.24	132.34	8.65	10.57	44.74	132.34	8.65	11.89
AsCd1	63.59	238.08	10.11	52.06	130.99	7.29	8.91	51.48	130.99	7.29	10.02
AsCd2	56.06	246.93	8.02	45.90	135.86	5.78	7.06	45.39	135.86	5.78	7.94
AsCd3	70.83	257.59	9.22	57.99	141.73	6.65	8.12	57.35	141.73	6.65	9.14
AsPb1	55.14	299.69	11.48	45.14	164.89	8.27	10.11	44.64	164.89	8.27	11.37
AsPb2	61.70	243.41	12.48	50.51	133.93	8.99	10.99	49.95	133.93	8.99	12.36
AsPb3	73.25	244.26	13.21	59.97	134.40	9.52	11.63	59.30	134.40	9.52	13.09
MIX1	65.60	123.28	11.04	53.71	67.83	7.95	9.72	53.11	67.83	7.95	10.93
MIX2	57.95	105.00	10.21	47.45	57.77	7.36	8.99	46.92	57.77	7.36	10.11
MIX3	56.06	108.89	7.84	45.90	59.91	5.65	6.91	45.39	59.91	5.65	7.77
Lettuce											
Control	113.09	476.10	5.59	92.59	261.96	4.03	4.92	91.56	261.96	4.03	5.54
As1	115.10	323.35	7.40	94.24	177.91	5.33	6.52	93.19	177.91	5.33	7.33
As2	122.75	242.34	9.25	100.50	133.34	6.66	8.15	99.38	133.34	6.66	9.16
As3	127.58	273.42	9.99	104.45	150.44	7.20	8.80	103.29	150.44	7.20	9.90
AsCd1	122.34	546.46	9.73	100.17	300.67	7.01	8.57	99.05	300.67	7.01	9.64

AsCd2	117.52	449.56	7.44	96.21	247.36	5.36	6.55	95.14	247.36	5.36	7.37
AsCd3	109.47	557.86	9.55	89.62	306.94	6.88	8.41	88.63	306.94	6.88	9.46
AsPb1	70.43	542.57	5.88	57.66	298.53	4.24	5.18	57.02	298.53	4.24	5.83
AsPb2	64.39	433.20	7.33	52.72	238.35	5.28	6.45	52.13	238.35	5.28	7.26
AsPb3	102.22	557.44	9.88	83.69	306.71	7.12	8.70	82.76	306.71	7.12	9.79
MIX1	112.28	250.50	8.62	91.93	137.83	6.21	7.59	90.91	137.83	6.21	8.54
MIX2	105.44	132.55	11.10	86.33	72.93	8.00	9.78	85.37	72.93	8.00	11.00
MIX3	95.38	138.57	5.44	78.09	76.25	3.92	4.79	77.22	76.25	3.92	5.39

Table Anx.3.8. RDA (%) for individual plant specie through consumption of Cd and Pb contaminated vegetables

	Children			Adolescent				Adult			
	Cu	Ni	Zn	Cu	Ni	Zn	Zn	Cu	Ni	Zn	Zn
	M+F	M+F	M+F	M+F	M+F	M	F	M+F	M+F	M	F
Potato											
Control	117.40	404.37	3.20	96.70	222.49	8.10	10.00	95.60	222.49	8.10	11.20
Cd1	85.60	433.47	4.20	70.50	238.50	10.70	13.00	69.70	238.50	10.70	14.70
Cd2	104.40	344.67	4.50	86.00	189.64	11.50	14.00	85.00	189.64	11.50	15.80
Cd3	64.50	199.70	3.20	53.10	109.88	8.10	9.90	52.60	109.88	8.10	11.20
Pb1	83.20	157.76	4.70	68.50	86.80	12.10	14.80	67.80	86.80	12.10	16.60
Pb2	118.70	418.54	6.80	97.80	230.29	17.30	21.20	96.70	230.29	17.30	23.80
Pb3	86.80	195.44	3.20	71.50	107.53	8.30	10.10	70.70	107.53	8.30	11.40
CdPb1	119.80	447.70	6.10	98.70	246.33	15.50	19.00	97.60	246.33	15.50	21.40
CdPb2	108.50	511.65	5.50	89.40	281.52	14.00	17.10	88.40	281.52	14.00	19.20
CdPb3	95.40	483.67	5.20	78.60	266.12	13.40	16.30	77.70	266.12	13.40	18.40
Tomato											
Control	68.00	186.54	4.30	56.00	102.64	11.10	13.50	55.40	102.64	11.10	15.20
Cd1	54.80	482.50	3.70	45.20	265.48	9.30	11.40	44.70	265.48	9.30	12.80
Cd2	60.20	439.12	3.50	49.60	241.61	8.90	10.90	49.00	241.61	8.90	12.30
Cd3	69.70	515.49	4.60	57.40	283.63	11.60	14.20	56.80	283.63	11.60	16.00
Pb1	71.30	518.16	4.80	58.70	285.10	12.20	14.90	58.10	285.10	12.20	16.70
Pb2	66.20	191.82	2.40	54.50	105.54	6.10	7.50	53.90	105.54	6.10	8.40
Pb3	61.60	391.63	2.40	50.70	215.48	6.20	7.50	50.20	215.48	6.20	8.50
CdPb1	67.30	148.01	4.10	55.50	81.44	10.50	12.80	54.80	81.44	10.50	14.40
CdPb2	62.50	164.53	2.90	51.50	90.53	7.40	9.10	50.90	90.53	7.40	10.20
CdPb3	57.30	131.48	2.30	47.20	72.34	6.00	7.30	46.70	72.34	6.00	8.20
Lettuce											
Control	112.40	476.10	1.60	92.60	261.96	4.00	4.90	91.60	261.96	4.00	5.50
Cd1	123.60	176.41	1.80	101.80	97.07	4.70	5.80	100.70	97.07	4.70	6.50

Cd2	72.00	367.75	1.30	59.30	202.34	3.30	4.00	58.70	202.34	3.30	4.50
Cd3	88.00	194.70	1.40	72.50	107.12	3.70	4.50	71.70	107.12	3.70	5.10
Pb1	86.00	448.82	2.00	70.80	246.95	5.10	6.20	70.10	246.95	5.10	7.00
Pb2	99.20	538.30	2.80	81.70	296.18	7.10	8.60	80.80	296.18	7.10	9.70
Pb3	75.20	518.05	3.20	61.90	285.04	8.20	10.00	61.30	285.04	8.20	11.20
CdPb1	133.20	472.59	3.60	109.70	260.02	9.10	11.20	108.50	260.02	9.10	12.60
CdPb2	110.00	570.28	1.90	90.60	313.78	4.90	6.00	89.60	313.78	4.90	6.70
CdPb3	134.40	595.17	2.80	110.70	327.47	7.10	8.70	109.50	327.47	7.10	9.80

