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HEAVY METAL LEVELS IN VEGETABLES WITH GROWTH STAGE AND PLANT SPECIES VARIATIONS

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Abstract

Field experiment was conducted to compare and investigate the concentration levels of heavy metals in leafy vegetables with growth stage and plant species variations on an experimental field near the net house of Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh during November 2008 to January 2009. Seeds of spinach (*Spinacia oleracea*), red amaranth (*Amaranthus tricolor*) and amaranth (*Amaranthus oleraseus*) were sown on 14 November 2008. Plant and soil samples were collected at different growth stages, such as at 20, 30, 40, and 50 days after sowing (DAS). The concentrations of lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), and chromium (Cr) in plant increased with the age of the plant, but the increase was not linear. The rate of increase of concentration of these metals at 20 to 30 DAS was found lower than that at 30 to 40 DAS, except Cr. Heavy metal content gradually increased at the early growing stage and fall during later stages of growth. The significant differences ($P < 0.01$) were observed between the mean metal concentrations in the three vegetables species. The Pb and Co concentrations in amaranth were found higher compared to those found in spinach and red amaranth. Spinach exhibited higher levels of Cd and Cr than those of other vegetables. However, the three vegetables did not differ significantly in its Ni concentration. The order of heavy metal level in different vegetables was Cd<Co<Pb<Ni<Cr. In vegetable species in respect of heavy metal concentration Cd, Ni, and Cr was highest in spinach and amaranth showed highest concentration in Pb and Co. The highest correlation between soil-plant was found for Cd, while the lowest for Ni. Metal concentrations in the vegetables studied were found lower than the maximum allowable level in India but the concentrations of Cd and Cr were higher than the allowable levels set by the World Health Organization (WHO).

Key words: Vegetables, heavy metal, concentration, growth stage, plant species

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1. Introduction

Soil pollution by heavy metals is great concern to public health (Goyer, 1996). The source of heavy metal in plant is the environment in which they grow and their growth medium (soil) from which heavy metals are taken up by roots or foliage of plants (Okonkwo *et al.*, 2005). Plants grown in polluted environment can accumulate heavy metals at high concentration causing serious risk to human health when consumed. Moreover, heavy metals are toxic because they tend to bioaccumulate in plants and animals, bioconcentrate in the food chain and attack specific organs in the body ((Akinola *et al.*, 2006; Chatterjee and Chatterjee, 2000).

Vegetables constitute an important part of the human diet since there contain carbohydrates, proteins, as well as vitamins, minerals and heavy metals. Heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh vegetables (Bigdeli and Seilsepour, 2008). A number of elements, such as lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), chromium (Cr), Copper (Cu) and Selenium (Se) (IV) can be harmful to plants and humans even at quite low concentrations (Bowen, 1979). Soil pollution is caused by misuse of the soil, such as poor agricultural practices, disposal of industrial and urban wastes, etc. (Buchaver, 1973). Soil is also polluted through application of chemical fertilizers (like phosphate and Zn fertilizers), and herbicides (Demi˙rezen and Aksoy, 2004). Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality, crop growth (Ma *et al*., 1994; Msaky and Calvert, 1990; Fergusson, 1990) and environmental health.

Plant species have a variety of capacities in removing and accumulating heavy metals. So there are reports indicating that some plant species may accumulate specific heavy metals (Markert, 1993). The uptake of metals from the soil depends on different factors, such as their soluble content in it, soil pH, plant species, fertilizers, and soil type (Lubben and Sauerberck, 1991). Vegetables, especially leafy vegetables, accumulate higher amounts of heavy metals (Sharma and Kansal, 1986). Roots and leaves of herbaceous plants retain higher concentration of heavy metal than stems and fruits (Yargholi and Azimi, 2008). There are limited studies on heavy metal content at different growth stages of vegetables, the most studies focused on the status of metal content in edible parts of vegetables. And an investigation of the literature also shows a scarcity of data on comparison of metal content at different leafy vegetable species in Bangladesh. Therefore, the present study was undertaken (i) to compare and investigate the concentration levels of heavy metals (Pb, Cd, Ni, Co, and Cr) at different growth stages of the commonly grown leafy vegetables; (ii) to find out a growth stage, which stage is less content of heavy metals; and iii) to quantify the concentrations of heavy metal content by different vegetable species.

2. Materials and Method

2.1. Experimental field

Experimental plots were set up on an experimental field near the net house of Soil Science Division, Joydebpur, Gazipur, Bangladesh during November 2008 to January 2009. The experimental field was top-dressed by foreign soil near about three years before. So, it is not represent the characteristics of Gazipur soils. Experimental plots consisted of three replicate rectangular areas (5 m × 2 m) for each leafy vegetable and each replicate was separated by 1 ft buffer area which was left as barren. Seeds of spinach (*Spinacia oleracea*), red amaranth (*Amaranthus tricolor*) and amaranth (*Amaranthus oleraseus*) were sown on 14 November 2008. Fertilizers NPK were applied @ 75, 25, and 60 kg/ha from the source of Urea, triple super phosphate (TSP), and muriate of potash (MoP), respectively. Before fertilizer application and seeding initial soil sample (top soils, 0-15 cm) was collected. Initial soil and fertilizers sample were analyzed in the laboratory.

2.2. Plant and soil sample collection

Plant and soil samples were collected at four growth stages of vegetables at 20, 30, 40, and 50 days after sowing. The samples were collected carefully using hand trowel to dig the soil around the plant and the plants were pulled out carefully, ensuring that no part of the root was lost. Plant samples were kept in separate polythene bags and properly labeled. Soil samples were collected at a depth of 0-15 cm from the same point of collecting plant samples. The samples were kept in polythene bags and labeled properly. The plant and soil samples were analyzed in the laboratory.

2.3. Preparation and preservation

The vegetable samples were washed in fresh running water to eliminate dust, dirt, possible parasites or their eggs and then again washed with deionized water. The clean vegetable samples were air-dried and placed in an electric oven at 65 °C for 72–96 h depending on the sample size. The dried vegetables samples were homogenized by grinding using a ceramic coated grinder used for metal analysis. All soil samples were spread on plastic trays and allowed to dry at ambient temperature for 8 days. The dried samples of soils were ground with a ceramic coated grinder and sieved through a nylon sieve. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis.

2.4. Digestion and determination

One gram of dry matter was weighed into 50-ml beaker, followed by the addition of 10 ml mixture of analytical grade acids HNO_3: $HClO_4$ in the ratio 5:1. The digestion was performed at a temperature of about 190 ºC for 1.5 h. After cooling, the solution was made up to a final volume (30 ml) with distilled water in a volumetric flask. The metal (Pb, Cd, Ni, Co, and Cr) concentrations were determined by atomic absorption spectrometry using a VARIAN model AA2407 Atomic Absorption Spectrophotometer (AAS). Analysis of each sample was carried out three times to obtain representative results and the data reported in µg/g (on a dry matter basis). Statistical differences were performed by Tukey's multiple comparisons test by using Excel Statistics version 4.0 (Esumi Co. Ltd., Tokyo, Japan).

3. Results and Discussion

In initial soil, organic matter concentration was 0.49% and soil pH (H_2O) was 7.3. The concentrations [µg/g of (dry weight (DW)] of nutrients and heavy metals in initial soil were 260 N, 54 P, 3121 K, 478 S, 4.04 Pb, 0.69 Cd, 14.4 Ni, 11.9 Co, and 21.9 Cr. The heavy metal content in initial soil was quite high. The source of soil contamination by heavy metal may be as a result of topdressd-soil, which was collected from Kodda, Joydebpur, Gazipur, a highly industrial effluents polluted area. There is no doubt that heavy metals are also present in soil naturally and non-degradable (Adeyeye, 2005 and Nriagu, 1990). Moreover, the soil may be contaminated with heavy metals as a result of the application of chemical fertilizers. The concentrations (µg/g) of heavy metals in applied fertilizers urea – triple super phosphate (TSP) – muriate of potash (MoP) were 0.0–64.5–0.47 Pb, 0.0–1.01–0.21 Cd, 0.0–28.6–0.60 Ni, 0.0–56.8–2.01 Co, 0.0–41.4–1.60 Cr, respectively. The results are in good agreement with the data obtained by Zhou *et al.* (2000). They stated that the main sources of soil pollution by heavy metals are phosphate fertilizers. For example, Cd is found predominantly in phosphatic fertilizers, resulting from the presence of Cd as an impurity in all phosphate rocks.

Heavy metals concentrations in plant and soil at different growth stages of vegetables are shown in Figures 1–2. The variation of heavy metals concentrations in soil at different growth stages was non-significant. The concentrations of plant Pb, Cd, Ni, Co, and Cr increased with the age of the plant up to 40 DAS with the exception of Cr but the increasing trend was not linear. Chromium concentrations in spinach increased up to 30 DAS and after that, the concentration decreased up to the final harvest at 50 DAS. The metal concentrations of the third harvested (40 DAS) plant samples were higher than those of others with the exception of Pb concentration in spinach and Cd in amaranth. The pattern of metal concentration was found different and irregular among the plant species. Only Ni concentration showed the similar trend in the three plant species. Spinach had a higher level of Cd but red amaranth had much lower. Lead concentration was higher in amaranth where as Cd concentration was higher in spinach. Cobalt concentration showed the similar trend like Pb. The rate of increasing or slope of metal concentrations was found different at different growth stages. The rate of increasing at 20 DAS to 30 DAS was comparatively low than that at 30 DAS to 40 DAS, except Cr. Cadmium concentrations in spinach, red amaranth and amaranth were increased by 66–182%, 286–534%, 81–132%, respectively for the same number of days as Pb. Similarly Ni, Co, and Cr concentrations in the three vegetables showed increases (in percentage) of metal content, with the exception of Cr concentration in spinach (49–32%, decreases).

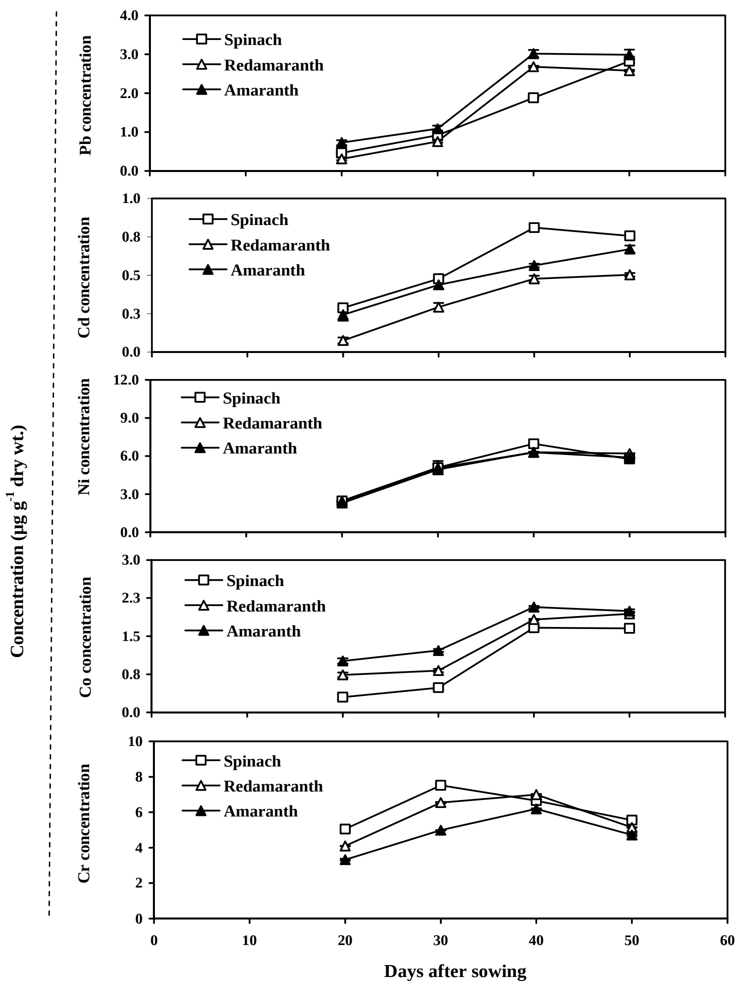

Fig. 1. Pattern of heavy metal concentrations in plant at different growth stages of vegetables

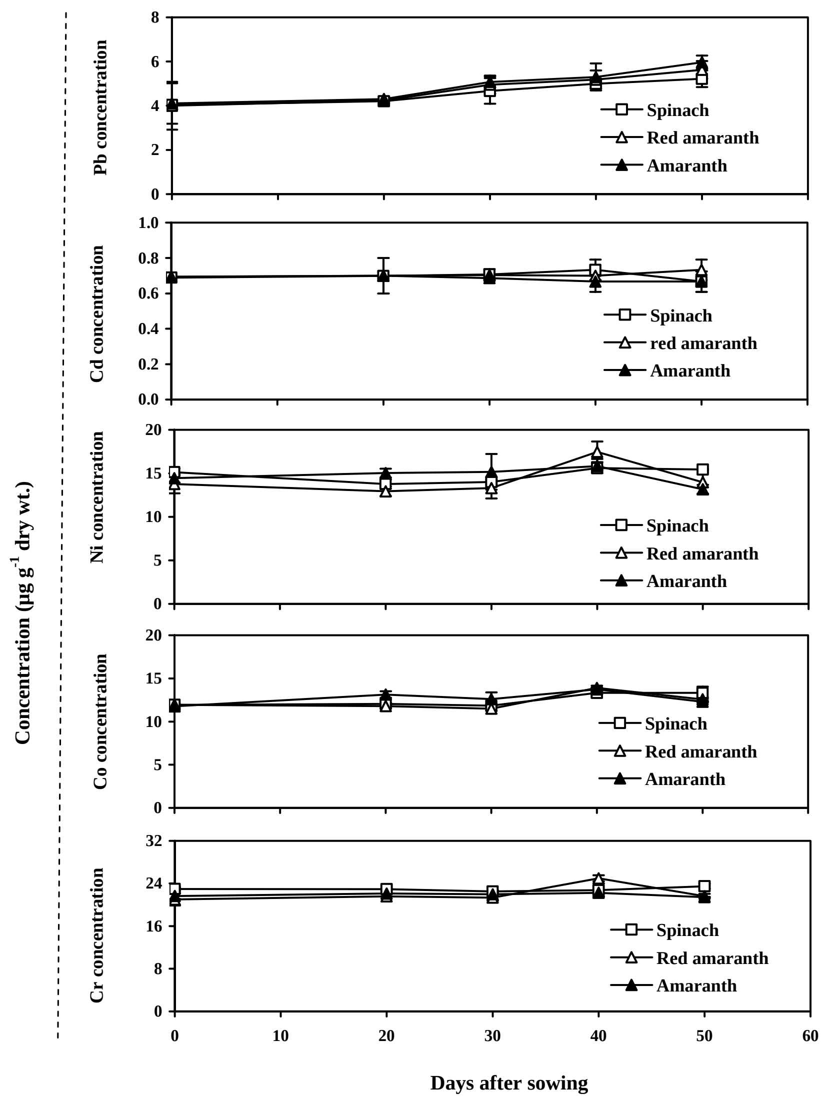

Fig. 2. Pattern of heavy metal concentrations in soil at different growth stages of vegetables

In this experiment, metal content gradually increased at the early growing stages and fall during later stages of growth. The present results are shown to be in good agreement with the result obtained by Oliveira *et al.* (1994); and Lutts *et al.* (2004). Mensah *et al.* (2008) reported that the Pb concentrations in lettuce increased consistently during the period of growth with time. Plant metal content varies with time and stage of maturity (Sauerbeck, 1991). However, the magnitude of time dependence of plant metal concentration variations differed among crops and metals according to the study.

The order of level of heavy metals in vegetables was Cd<Co<Pb<Ni<Cr (Table 1–2). Heavy metal content by plants can be affected by several factors including metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of plants, and plant age. It is generally accepted that the metal concentration in soil is the dominant factor (Adriano, 1986). Heavy metal availability can also be directly affected by plant itself (Zhang *et al.*, 1991). In the present study, it was observed that the concentration of Pb in plant was higher than that of Cd. In general, vegetables studied from different areas had a concentration of Pb higher than that of Cd (Demi˙rezen and Aksoy, 2006). The maximum concentration of Cd, Ni, and Cr were recorded in spinach, but the highest Pb and Co contents were noted in amaranth.

Table 1. Mean Pb, Cd, Ni, Co, and Cr concentration (±, standard deviation) of leafy vegetables.

Vegetable	Heavy metal (µg/g)				
	Pb	Cd	Ni	Co	Cr
Spinach	1.52±0.02a	0.583±0.01c	5.07±0.14	1.03±0.03a	6.20±0.03c
Red amaranth	1.58±0.01a	0.337±0.01a	4.93±0.11	1.33±0.03b	5.70±0.01b
Amaranth	1.95±0.05b	0.478±0.02b	4.92±0.07	1.57±0.03c	4.81±0.01a

Values in a column followed by a common letter are not significantly different at $P < 0.01$.

Table 2. Mean Pb, Cd, Ni, Co and Cr concentration (±, standard deviation) of soils where the vegetables grown.

Vegetable	Heavy metal (µg/g)				
	Pb	Cd	Ni	Co	Cr
Spinach	4.77±0.23	0.70±0.01	14.7±0.17	12.6±0.19	22.9±0.34
Red amaranth	5.00±0.20	0.71±0.03	14.4±0.61	12.4±0.14	22.4±0.07
Amaranth	5.16±0.15	0.68±0.02	14.8±0.47	12.9±0.30	21.9±0.27

The result indicated that there was significant difference ($P < 0.01$) in mean heavy metal content in the three vegetable species. The result showed significantly higher level of Pb concentration in amaranth compared to spinach and red amaranth. Spinach exhibited significantly higher levels of Cd and Cr than the other vegetables. The difference in level of heavy metal contamination between spinach and amaranth species was due to their morpho-physiological differences in terms of heavy metal content, exclusion, accumulation, foliage deposition and retention efficiency (Carlton-Smith and Davis, 1983). Cadmium concentration mostly appeared in leafy vegetables and was in order of spinach>amaranth>red amaranth. The level of Cd in the samples analyzed in this study was ranged from 0.34 to 0.58 µg/g of Dw. The Cd concentration of vegetables in this study was in agreement with the findings of Yusuf *et al.*, (2003) who found Cd concentration in leafy vegetables to be 0.09 (µg/g of DW). Singh and Kumar (2006) reported that Pb and Cd content in spinach ranged between 1.7 and 7.0 and 2.0 and 7.1 µg/g DW, respectively. The three vegetables did not differ significantly in Ni content. Cobalt showed similar trend as of Pb. The Cr concentration in vegetables from this study were higher than those recorded by Nagajyoti *et al.* (2008) who found Cr concentration in five leafy vegetables ranging between 0.89 to 1.08 (µg/g of DW). Haiyan and Stuanes (2003) reported that the Cr content in the vegetables grown in controlled area ranged from 0.4 to 2.7 (µg/g of DW).

Table 3. Correlations between heavy metal content[§] in soils and in vegetables (§=µg/g, *=p < 0.05, **=p < 0.01, ns=non significant)

Vegetable	Heavy metal				
	Pb	Cd	Ni	Co	Cr
Spinach	0.944ns	0.965*	0.679ns	0.999**	0.955*
Red amaranth	0.930ns	0.957*	0.670ns	0.772ns	0.948ns
Amaranth	0.819ns	0.988*	0.911ns	0.693ns	0.700ns

Table 4. Transfer factor (±, standard deviation) of Pb, Cd, Ni, Co and Cr for the soils to vegetables species.

Vegetable	Heavy metal				
	Pb	Cd	Ni	Co	Cr
Spinach	0.320±0.016	0.831±0.015	0.345±0.008	0.081±0.002	0.271±0.005
Red amaranth	0.317±0.014	0.475±0.011	0.343±0.021	0.107±0.003	0.255±0.001
Amaranth	0.379±0.006	0.703±0.018	0.332±0.015	0.122±0.002	0.219±0.003

The variation of mean concentrations of the metals in soils at different growth stages of three vegetables plots was non-significant. The Pb, Cd, and Ni concentrations in soils from this study were found lower than those obtained by Demi˙rezen and Aksoy (2006), who found the concentrations (µg/g) of Pb, Cd, and Ni to be 95, 1.83 and 29, respectively, in the soils of non-contaminated rural area. Nickel and Cr showed higher values in soils compared to Pb and Cd contents. The Cr concentration in soils from this study was much lower than those obtained by Haiyan and Stuanes (2003) who found Cr concentration to be approximately 108 (µg/g DW). The order of heavy metal level in soil was Cd<Pb<Co<Ni<Cr. Heavy metal concentrations values are higher in soil samples compared to vegetables samples. Demi˙rezen and Aksoy (2006) also reported that the level of heavy metals in vegetables were generally lower than the soil samples. Such results might be attributed due to root activity, which seems to act as a barrier for translocation of metals (Davies and White, 1981; Yusuf *et al.*, 2003). Heavy metal absorption is governed by soil characteristics, such as pH and organic matter content. High levels of heavy metals in soils do not always indicate similar high concentrations in plants. The extent of accumulation and toxic level depend on the plant and heavy metal species (Alloway, 1996).

In order to ascertain probable relationship between Pb, Cd, Ni, Co, and Cr content of soils and vegetables, correlations were calculated. The results showed (Table 3) positive correlation between metal concentration in soils and vegetables, and the correlation varied widely. The highest correlation between soil-plant was found with Cd, while the lowest was with Ni. The relationship between soil and plant depend on available forms of metal ions in soil.

Transfer factor (TF) of different heavy metals from soil to vegetables was calculated as the ratio between the concentrations of heavy metals in vegetables and their respective concentration in soils (Table 4). The TF is one of the key components of human exposure to metals through the food chain. In all three vegetables an opposite trend in TF was observed (Cd>Ni>Pb>Cr>Co) as of metal concentration in vegetable. The mobility of metals from soil to plant is a function of the physical and chemical properties of the soil and of vegetable species, and is altered by innumerable environmental and human factors (Zurera *et al.*, 1987). The highest TF values 0.831, 0.475, and 0.703 were found for Cd in spinach, red amaranth and amaranth, respectively. This might be due to higher mobility of Cd with a natural occurrence in soil (Alam *et al.*, 2003), and the low retention of Cd (II) in the soil than other toxic cations (Lokeshwari and Chandrappa, 2006). The result also supports the findings that accumulation of Pb and Ni is comparatively less than that of Cd in plants (Olaniya *et al.*, 1998).

The accumulation concentration of Cd and Cr was higher and that of Pb and Ni was lower than the maximum allowable level set by WHO (Fig. 3). The allowable level of Pb, Cd, Ni, and Cr as set by the WHO were 2.0, 0.02, 10.0 and 1.30 µg/g, respectively (Lone *et al.*, 2003). However, metal concentration of vegetables in this study was lower than the maximum allowable level for Pb, Cd, Ni, and Cr (2.5, 1.5, 5.0, and 20 µg/g DW, respectively) as set in the Prevention of Food Adulteration Act (PFA), 1954, India (Lokeshwari and Chandrappa, 2006).

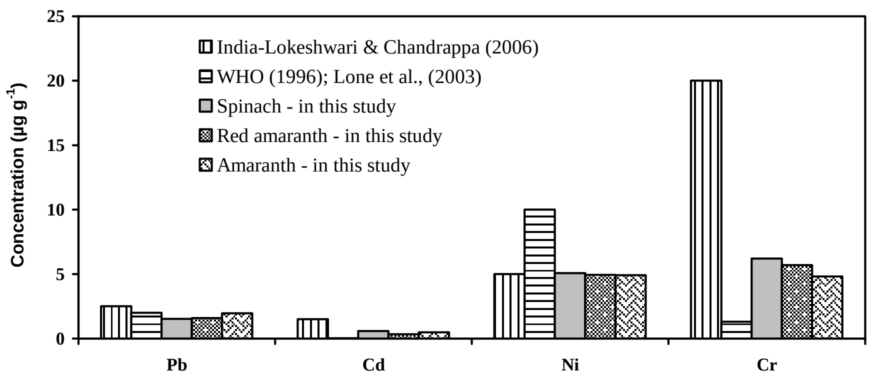

Fig. 3. Total concentrations of metal in vegetables and maximum allowable limits

4. Conclusion

Heavy metal content in different leafy vegetables varies significantly. The content varies with time of harvesting and stage of maturity of crops. The Cd and Cr contents in leafy vegetables in this study were detected higher while Pb and Ni were within the permissible limits as per the WHO standard but all the metals were within the maximum allowable level as per PFA, 1954, India. The magnitude of time dependence of plant metal concentration variations differed among crop species and metals. Further research is needed to obtain more specific information about the effect of age of the plants on accumulation and distribution of the heavy metal in the different plant parts, variations in uptake between different plant species, cropping history and fertilization.

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