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# Studies of toxic metals (Pb, Cd, Cu, Zn, Fe, Ni) content in green leafy vegetables locally available in Mysuru city, India

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## Abstract

*The importance of this study was to calculate the toxicity levels of toxic metals (Pb, Cd, Cu, Zn, Fe, Ni) found in leafy vegetable (Spinach, Coriander, Mint, Amaranthus, Curry leaves, Methi) grown in urban area of Mysuru city. In order to ascertain green leafy vegetable for human consumption major toxic metals were evaluated and standard methods were used for determining of toxic metals in leafy vegetable by Atomic Absorption spectrometry (AAS). Toxic metals in vegetables, food, water is one of the main routes through which these elements enter the human body; however toxic metals can cause disease. Concentration for each toxic metal in the samples was calculated and compared with the permissible levels set by the FSSAI, Food & Agriculture Organization and World Health Organization. The toxic metal intake in the human diet was estimated and to calculate the risk of human health. In our research work the presence of toxic metals in leafy vegetable in the order Fe > Mn > Zn > Cu > Pb > Ni > Cd. Based on these research, we conclude that the hazard for human consumption.*

**Keywords:** Leafy vegetable, Contamination, Toxic metals, Daily intake.

## Introduction

Water pollution of the natural environment by toxic metals is a universal problem because these toxic metals are indestructible and most of them toxic effect on living organisms. Toxic meals frequently reported in literature with regards to potential hazards and occurrences[1]. Leafy vegetables are common diet taken by people throughout the world, being rich sources of antioxidants, essential nutrient and metabolites by acting as buffering agent for acidic substance produced during the digestion process. These food stuffs are important components of human diet hence, toxic metal contamination of leafy vegetable cannot be underestimated. Toxic metal contamination of the food items is one of the most important aspects of food quality assurance[2-4]. These vegetable are valuable sources of vitamins A & C, Iron, Calcium and Dietary fibre. In recent years their consumption is increasing gradually, particularly among the urban community. A toxic metal ranks high among the chief contaminants of the vegetable[5,6]. Toxic metals are easily accumulated in the edible parts of leafy vegetable as compared to grain or fruit crops[7,8]. Vegetable constitute an important part of the human diet since they contain both trace and toxic element over a wide range of concentration. Metal accumulation in leafy vegetable may pose a direct threat to human health[9]. Leafy vegetable taking up the toxic metals by absorbing them polluted water, contaminated soil as well as from deposits on different parts of the leafy vegetable exposed to the water and air from polluted environment[10,11]. Concentration of traces toxic metals in leafy vegetable is essential for good health and growth of human beings but the toxic metal should be within permissible limits of FSSAI/WHO. Concentration higher or lower than the recommended permissible limits may causes various diseases like tumours, heart disease, psychic condition that damage the memory of human from the above views, it was decided to investigate the concentration of selected toxic metals (Fe, Pb, Cd, Cu, Zn, Ni) in leafy vegetable[12,13]. Intake of vegetable is an important path of toxic metals to human being. Regular monitoring of these metals in leafy vegetable and in other food material is essential for preventing excessive build-up of the metals in food chain[14]. The aim of this study was to find the concentration of toxic metals in leafy vegetable in Mysuru city, Karnataka, India and to calculate their contribution of the metals in the daily intake.

## Materials and methods

The area which we had been studied Mysuru is having around 11 lakh populations and state of Karnataka. It lies between 12°8' and 11°7' latitude and 77°8' longitude and general elevation is little more than 1801 ft above sea level. The climate of the city is moderated throughout the year with temperature during summer ranging from 32°C to 40°C. Winter season is from October to February and rainy season is from April to October. The main source of water in Mysuru city is mainly from the Cauvery river water and ground water for domestic, industrial and irrigation purpose. Growing cities of Karnataka Mysuru is one of the clean city in India due to presence of industrial resources and a well-developed communication network At present situation industrialization has become main cause of city's growth. Because of the diversity in industrial and scape growth of Mysuru with haphazard distribution. The industrial areas are distributed all over the city and its surroundings with lack of order and regulation in industrial location. Number of small and medium scale industries exist in and around the Mysuru city. Engineering chemical, pharmaceutical food brewery, textile, steel and metal smelting. most of all medium scale industries we found in and around Mysuru city.

**Sampling and sample preparation:** From January 2014 to June 2014 Six numbers of leafy vegetable Name Spinach (Spinaciaoleracea), Corriander (Coriandrumsativum), Mint (Labiateae), Amaranthus (Amaranthaceae), curry leaves (Rutaceae), Methi (Fabaceae) were randomly collected. They were considered as representation of leafy vegetable species commonly consumed in the area under study. Collected samples were taken in polythene cover, brought to the department on the same day. All sample was washed with tap water and distilled water to remove any possible dust, contaminants, such as mud , pesticides, the edible parts were cut in small pieces and then oven dried at 75–80°C to attain constant weight[15,16]. The dried vegetable samples were grind, and sample was kept in cool condition up to the complete analysis.

**Sample digestion:** All standard chemical were NIST traceable and Milli-Q water was used for standard solution preparation. All the glassware's were cleaned, soaked in 5% HNO3 for > 44 hrs then rinsed with double Distilled and Milli-Q water and oven dried. All leafy vegetable 0.25 to 0.45 g of dried sample was digested with 6ml of 65% ultra pure Nitric Acid (Merck Chemical), 2ml $H_2O_2$ and 2 ml Mill-Q water in a microwave digestion system (The Anton Paar microwave digester was used for digestion of leafy vegetable samples at 200°C with hold time of 15 min by using[17]. The digested samples were then transferred to volumetric flask and total volume was made up to 50ml with Milli-Q water. All analyses were processed in triplicate. Toxic metals (Pb, Cd, Cu, Zn, Ni) were determined in this standard solution by Atomic Absorption spectrometry (AAS) (iCE 3000 series Thermofisher Scientific). Accuracy of the method was verified by analysing the standard (NIIST) traceable reference material calculated Estimated Daily intake of trace metals (EDEM) through vegetable was dependent on toxic metal concentration in leafy vegetable, daily leafy vegetable consumption as well as body weight (BW) which was calculated with the formula given below:

$$EDEM = \frac{DIM}{BW}$$

DIM=Daily intake of metals ions = Leafy vegetable consumption daily x mean concentration of metals ions in leafy vegetable, BW= body weight.

Where, daily consumption of leafy vegetable rate for adult resident was an approximately of 170g and the body weight of an adult resident was set to 60kg.

**Calculation of health risk:** In our study, the leafy vegetable consumption by people was calculated based on the target hazards quotients (THQs) and health risk. This method was used for estimating of risk by using THQ was provided in the U.S. EPA Region III risk-based concentration table[18] and is based on the equation given below:

$$THQ = \frac{EFxEDXFIxMC}{RfDxBWxAT} x10^{-3}$$

EF = exposure frequency (365 days/year), ED = exposure duration (70 years), FI = food ingestion (g/person/day), MC = the metal ion concentration in leafy vegetable (mg/kg, on fresh weight basis), RfD = oral reference dose (mg/kg/day), BW = average body weight (Adult 60 kg), AT = average time for non-carcinogens (365 days/year x number of exposure years, assuming 60 years in this study). The oral reference doses were based on 0.5, 0.02, 0.04, 0.3, 0.0004, 0.0005 mg/kg/day for Iron, Nickel, Copper, Zinc, Lead, Cadmium respectively[14].

**Table-1:** Target hazard Quotients (THQ).

| Sample | Fe | Mn | Cu | Zn | Pb | Ni | Cd |
|---|---|---|---|---|---|---|---|
| Curry leaves | 0.536 | 0.7 | 0.15 | 0.097 | 2.17 | 0.71 | 0.11 |
| Coriander | 2.36 | 0.6 | 0.22 | 0.144 | 1.12 | 0.31 | 0.12 |
| Methi | 2.01 | 0.33 | 0.16 | 0.15 | 0.9 | 0.16 | - |
| Amaranthus | 3.41 | 0.45 | 0.23 | 0.19 | 1.06 | 0.31 | 0.5 |
| Pundina | 3.6 | 0.4 | 0.42 | 0.17 | 2.3 | 0.3 | 0.25 |
| Spinach | 2.05 | 1.94 | 1.6 | 0.137 | 1.11 | 0.46 | 0.12 |

**Table-2:** Daily intake of metal ions from vegetables.

| Sample | Fe | Mn | Cu | Zn | Pb | Ni | Cd |
|---|---|---|---|---|---|---|---|
| Curry Leaves | 290 | 36.7 | 6.01 | 40.2 | 9.36 | 17 | 0.07 |
| Corriander | 850 | 101 | 10.7 | 337 | 4.83 | 6.75 | 0.06 |
| Methi | 677 | 55.4 | 8.83 | 43.7 | 4.34 | 4.13 | 0 |
| Amaranthus | 627 | 66.8 | 12.6 | 65.2 | 4.76 | 6.34 | 0.22 |
| Pundina | 966 | 63.7 | 17.8 | 55 | 9.81 | 6.07 | 0.12 |
| Spinach | 782 | 122 | 15.5 | 66.7 | 5.7 | 6.06 | 0.2 |

## Results and discussion

The concentrations of toxic metals (mg/kg fw) in the leafy vegetable are shown in Figure-1-6. Among leafy vegetable variety and sample locations, the concentrations of toxic metals ions are differing greatly. The mean concentration of toxic metals ions in leafy vegetable samples were in the decreasing order as: Fe > Mn > Zn > Cu > Ni > Pb > Cd. The concentration of toxic metals ions in different leafy samples in the study area was compared with the standards for vegetable by the guideline values are decided based on contaminants, toxins and many substances regarding their occurrence in food and their significance for human and animal health[19].

The obtained results of the recent study showed that the concentration of Fe in the leafy vegetable is much high than other vegetable. However, spinach, coriander, pudina maintained highest Iron concentration (521.4, 531.6, 566.5 mg/kg) content. Our results were much higher than recorded by Zahir et al. who analysed different samples of vegetable and reported a high concentration (7.9 – 24.8 mg/kg) of Fe in Pakistan[20]. In another study Fe (17.0 – 35.6 mg/kg) in some raw food stuffs grown in waste water industrial areas.

Manganese content showed a higher value in leafy vegetable as compared to those in the other investigated vegetable spinach and pudina has the highest Mn concentration (117.2 and 42.4 mg/kg). Whereas manganese concentration were in the safe limit permitted for food. Chauhan et al. recommended 2–9 mg per day for an adult[21].

Concentration of copper in the leafy vegetable (spinach, amaranthus, methi and coraiander amounted to 10.8, 8.41, 5.54 and 7.14 mg/kg). These are ntoc4eable high significance level in spinach and gradually decreased in curry leaves. It was found that toxic metal accumulated more in leafy vegetable because these leaves considered as entry points of toxic metals from air. Demirezen et al reported that levels of Cu (22.19 – 76.50mg/kg) were higher in the leafy species than the non-leafy vegetable species from Turkey[22]. Sharma et al. reported concentration of Cu (2.25 – 5.42 mg/kg) in vegetable grown in waste water areas of Varanasi, India to be within the safe limit[23]. Our study revealed that leafy vegetable accumulated Cu higher than the permissible levels (10.0 mg/kg,)[24]. The concentration was high in spinach leaves 10.8 mg/kg.

The obtained results declared that Zn values to be higher in all leafy vegetable. The amount of Zn in the leafy sample (Amaranthus, Curry leaves, Pudina, Spinach, Methi, 41.4, 49.3, 36.6, 33.3 mg/kg). Generally the present study demonstrated the concentration of Zn to be within the set limits of International Standards (5.00 mg/kg. The results showed a relative increase of Zinc content in most leafy vegetable more than reported by Singh et al and Itanna e .al. reported that the Zn concentration (3.56 – 4.59 mg/kg) was within the recommended International Standards[25,26].

The concentration of Pb in leafy vegetable collected from different areas have highest value of 7.47, 7.28, 4.76 mg/kg respectively. Increased of Pb levels in leafy vegetable in areas 5 and 6 was attributed to industrial and heavily traffic in this area which leads to accumulation of Pb emitted from cars exhaustions. Munchuveti et al. reported the Pb concentration (17.5-25 mg/kg) in vegetable grown industrial areas[27]. Geetha Tiwari et al. reported the levels of Pb (6.77 mg/kg) in vegetable irrigated with mixtures of waste water and sewage from Zimbabwe to be higher than WHO safe limit (2mg/kg)[28].

The concentration of Cd in leafy vegetable (Amaranthus and Pudina) collected from different areas have highest value of 0.53 and 0.31 mg/kg respectively. In our study, concentration of Cd was noted to be above the critical level of 0.1 mg/kg as reported by WHO and thus might be a great thereat for the human consumers[24].

The concentration of Ni in leafy vegetable (curry leaves, Amaranthus, Pudina, Spinach) collected from different area have highest value (16.4, 7.39, 6.48 and 5.61 mg/kg). Our study revealed that leafy vegetable accumulated Ni higher than permissible level 1.5 mg/kg[24].

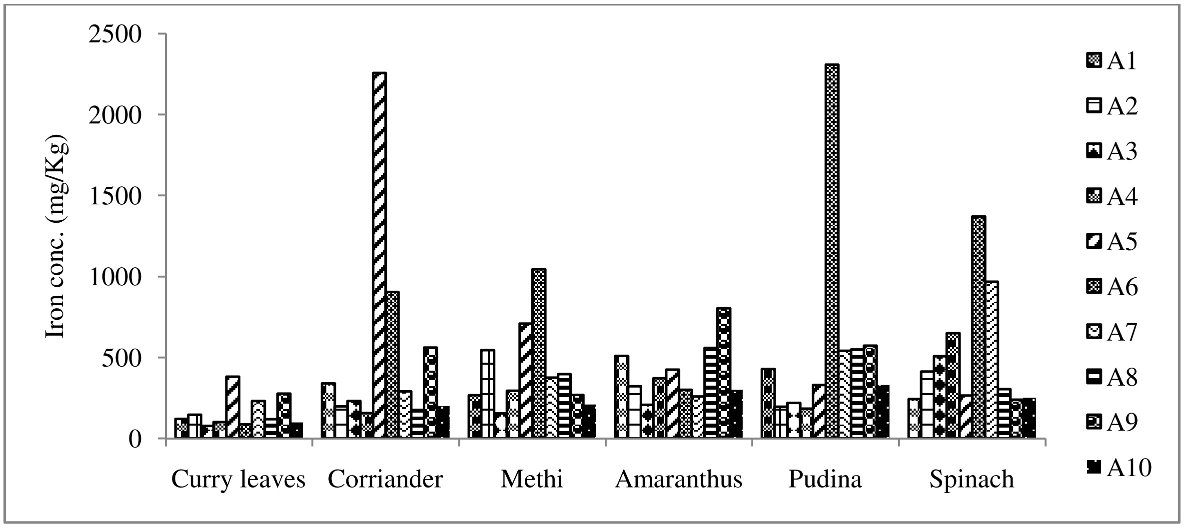


**Figure-1:** Concentrations of Iron (mg/kg fw) in the leafy vegetable.

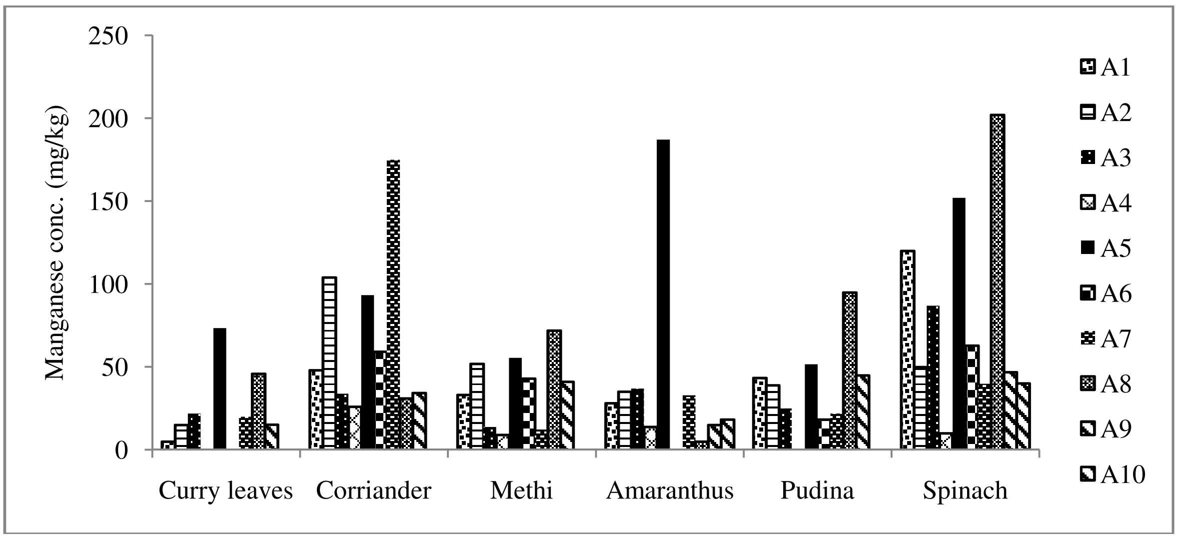


**Figure-2:** Concentrations of Manganese (mg/kg fw) in the leafy vegetable.

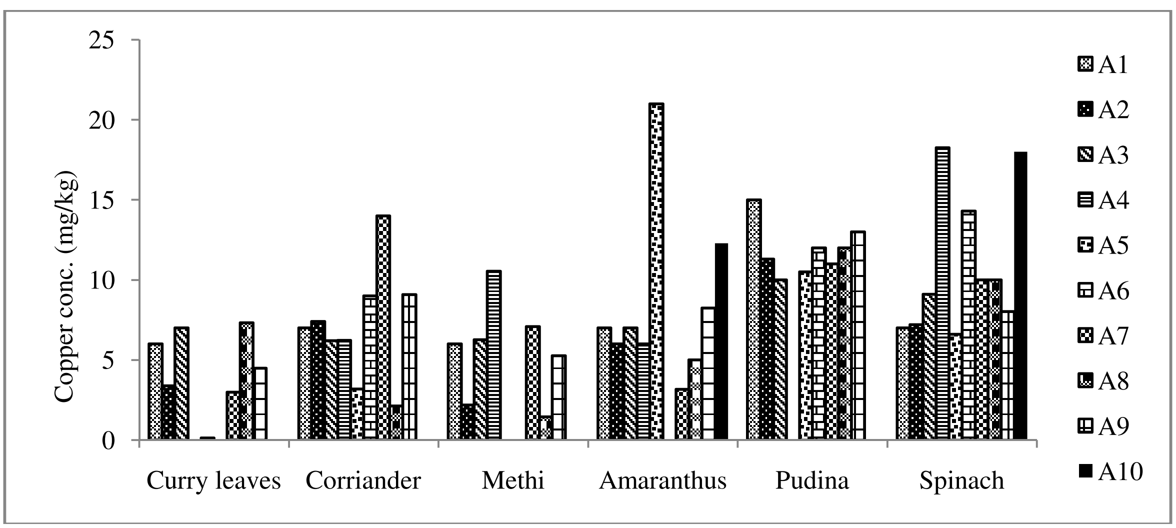


**Figure-3:** Concentrations of Copper (mg/kg fw) in the leafy vegetable.

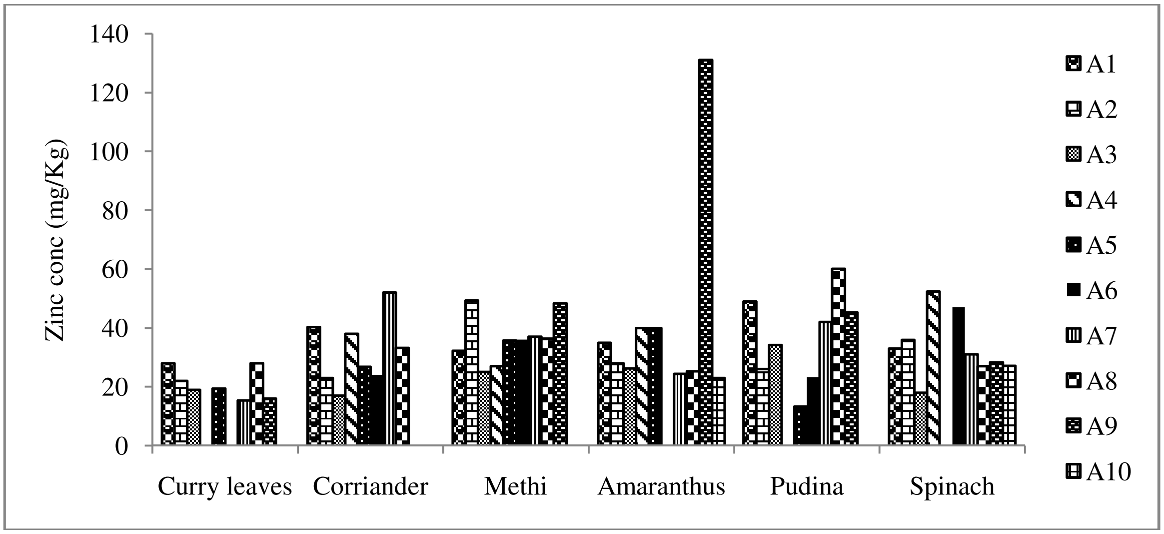


**Figure-4:** Concentrations of Zinc (mg/kg fw) in the leafy vegetable.

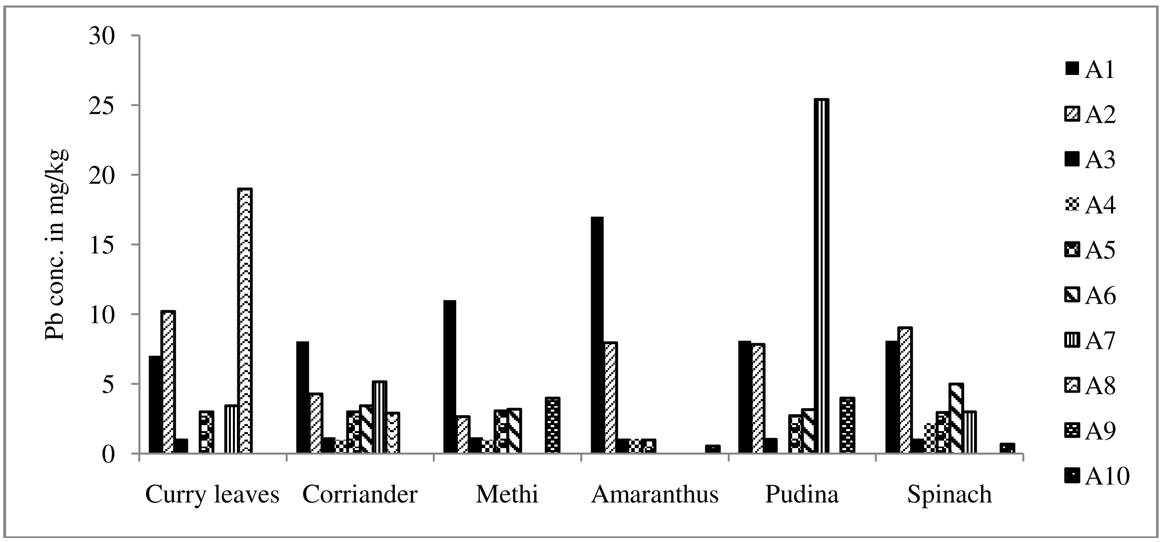


**Figure-5:** Concentrations of Pb (mg/kg fw) in the leafy vegetable.

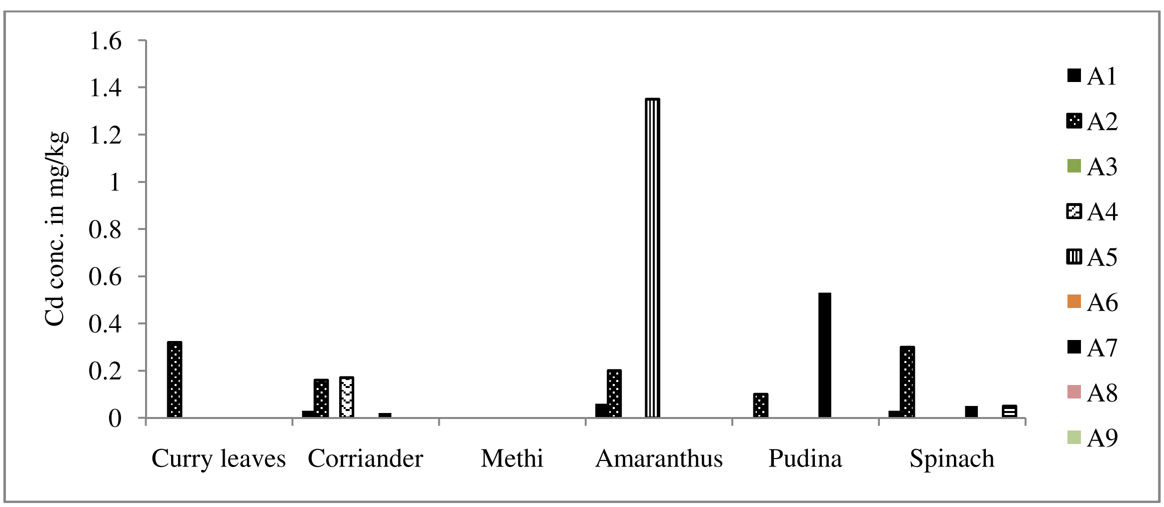


**Figure-6:** Concentrations of Cd (mg/kg fw) in the leafy vegetable.

**Health risk assessment:** The target hazard quotient (THQ) was developed by US EPA as on quantitative way to evaluate potential health risks associated with long term exposure to chemical pollutants in food stuffs and the THQ is measured concentration and the oral reference dose. The accumulation of toxic metals in the leafy vegetable may be the impact on the human health of local people in nearby areas. Hence, the metal ions polluted vegetable may be a affected to the people in the study area. The concentration of Cu, Mn and Pb in most leafy vegetable crossed the standards of food safety (Table-1). Estimation was done by daily intake of metal ions (EDI) Target Hazard Quotients (THQ) was used to assessment of the risk of human health related with toxic metal contamination of selected leafy vegetable. The THQ of each toxic metals ions through consumption of leafy vegetable in the study areas increases in the order. Fe > Mn > Pb > Ni > Cu > Zn > Cd (Table-2). The intake of a single metal ions indicated that through consumption of leafy vegetable was not much effect on potential health hazard except Cu, Pb and Mn Health of human risks from contamination to leafy vegetable are therefore of some concern.

## Conclusion

Through the above results it is clear that the importance of some minerals which has benefits for human body like Fe in leafy vegetable as a natural and rich source of this mineral.

In our study, it is found that it should be regular monitoring of toxic metals ions in leafy vegetable should be performed in order to prevent excessive build-up of these toxic metals in the human food chain. Appropriate precaution should also be taken at the time of transportation and marketing of leafy vegetable.

The magnitude of toxic metals detected in different kinds of leafy vegetable was arranged as Fe > Mn > Zn > Cu > Pb > Ni > Cd. The leafy vegetable contained the highest values of most toxic metals especially those collected from area 5, 6, and 7 due to heavy industrial activities and heavy traffic in those areas. Furthermore the concentration of most studies metals are above the standard permissible levels thus might be in concern for human consumers.

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