 

# Determination of Heavy Metals Bioaccumulation in Two Green Leafy Vegetables by Atomic Absorption Spectroscopy

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**Abstract**
Two edible green leafy vegetables viz., Amaranthus (*Amaranthus sp.*) and Dill (*Anethum graveolens*) leaves collected randomly from three locations in Kolar district like Bethamangala, Bangarpet and K.G.F. are critically examined for heavy metal pollutants like Lead, Copper, Cadmium, Chromium and Zinc using Atomic Absorption Spectrophotometer. Results revealed that, Amaranthus and Dill leaves contain lead, copper, cadmium, chromium and zinc in all the sampling stations. Amaranthus leaves recorded significantly high lead concentration (41.57 ppm to 48.89 ppm) crossed permissible limits in all the stations. Copper concentration varied considerably in Bethamangala (4.26 ppm), Bangarpet (12.22 ppm) and KGF (21.59 ppm). Cadmium concentration at Bethamangala (2.06 ppm), Bangarpet (2.78 ppm) and KGF (2.96 ppm) recorded above permissible level. Chromium was detected in all the stations and values are not exceeding the permissible limit. Zinc concentration is higher in K.G.F (62.01 ppm) and in other two stations it is below the safe limit. Dill leaves showed high lead content (22.51 ppm-32.20 ppm) in three stations crossed safe value limit recommended by FAO/WHO. Copper concentration in Bethamangala (3.29 ppm), Bangarpet (12.22 ppm) and KGF (18.52 ppm) are in permissible limit. Cadmium content in Bethamangala (0.92 ppm) and Bangarpet (1.44 ppm) are below detection level and in KGF (2.32 ppm) exceeding the permissible limit. Chromium was detected in all three stations and values are below detection level. Zinc concentration is high in K.G.F (63.04 ppm) and in remaining two stations it is below the safe limit.
**Keywords:** Heavy metals, leafy vegetables, amaranthus, dill, spectrophotometry.

## 1. Introduction

Green leafy vegetables were extensively used in the preparation of soups across India. Intake of vegetables is an important path of heavy metal toxicity to human beings and based on persistent nature and cumulative behaviour as well as the probability of potential toxicity effects of heavy metals as a result of consumption of leafy vegetables. It is known fact that, majority of population suffers from malnutrition and therefore, nutrient deficient syndromes are visible in human beings. Variety of leafy vegetables used in balanced diet (116g/day) as they are rich in minerals and vitamins. Implication associated with heavy metal contamination is of great concern, particularly in agricultural production. Heavy metals are responsible significantly for health risk to humans (Gupta & Gupta, 1998). Dietary exposure to heavy metals like cadmium, lead, zinc and copper has observed as a risk factor to human health through vegetables consumption (Kachenko & Singh, 2006). Furthermore, consumption of heavy metals contaminated food can significantly reduce essential nutrients in the body responsible for decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Arora *et al*., 2008). Leafy vegetables are very common in daily intake of diet by all most all populations throughout the world due to their richness in vitamins, minerals, fibers and anti-oxidative effects. However, leafy vegetables such as amaranth and cabbage are said to be good in absorbing heavy metals from soil (Lokeshwari & Chandrappa, 2006; Eslami *et al*., 2007). Leafy vegetables grown in heavy metals contaminated soils accumulate high amount of metals than those cultivated in uncontaminated soils as they absorb the metals through roots (Muhammad Farooq *et al*., 2008). Vegetables accumulate heavy metals in both their edible and non-edible parts. Absorption capacity of heavy metals depends upon the nature of vegetables and some of them have a greater potential to accumulate higher concentrations of heavy metals than others (Akan *et al*., 2009).

Heavy metals accumulation in vegetables may be due to the deposition of metals on aerial parts by polluted air or from contaminated soil through the crop roots and incorporated them into the edible part of plant tissues or by up taking of water from the contaminated soil (Haiyan & Stuanes, 2003; Nwajei, 2009). Recent reports indicated that, heavy metals take driver's seat among the chief contaminants of leafy vegetables. Heavy metals are non-biodegradable and thermo stable, thus readily accumulate to toxic levels (Sharma *et al*., 2007). Though, metals are indispensable part of our environment and play positive role in various biological processes such as signalling, homeostasis and enzyme catalysis, higher concentration of metals tend to toxic effects since they are prone to bio-accumulation and bio-magnification along the food chain. Industrialization and urbanization as well as anthropogenic activities are main source for heavy metal contamination that undoubtedly affected lakes and tanks (Ramesh & Yogananda Murthy, 2012). Considering the significance of heavy metals and consumption of vegetables, present investigation was carried out to determine heavy metals levels in selected green leafy vegetables that are consumed regularly. These vegetables serve as food sources and thus offer rapid and ideal means of providing adequate vitamins, mineral salts, trace essential elements and fibre as suggested by Ihekeronye & Ngoddy (1995).

## 2. Material and Methods

### *2.1. Collection of Leaf Samples*

Two green Leafy vegetables viz., Amaranthus (*Amaranthus sp.*) and Dill (*Anethum graveolens*) samples were collected randomly from three stations such as Bethamangala, Bangarpet and K.G.F. in Kolar district. Control leafy vegetables were obtained from normal irrigation practicing areas.

### *2.2. Preparation of Leaf Samples*

Leafy vegetables were washed thoroughly with tap water followed by distilled water to remove adsorbed elements. Samples were cut into small pieces, air dried for 48 hrs and kept in hot air oven at 100 °C ± 1 °C for 4 hrs. Dried samples were grounded to fine powder and then pass through a 1 mm sift. 0.5 g of sample is taken in reference vessels, add 4 ml of $HNO_3$ and 0.2 ml of $H_2O_2$ and carousel was positioned into microwave unit. The system was pre-programmed for 1 min. of microwave digestion at 250W power and another 5 min. at 500W power and left to automatic ventilation for 10 min. Digested solution was cooled, filtered using Whatman filter paper No. 40 and made up to 100 ml with distilled water and stored in plastic bottles for analysis.

### *2.3. Sample Analysis*

Leaf samples were analysed for heavy metals viz., Lead, Copper, Cadmium, Chromium and Zinc concentrations. A serial dilution method was used to prepare the standard samples and metals concentration in each sample was analysed using Atomic Absorption Spectrophotometer (Varian AA 525) equipped with a digital readout system at Azyme Biosciences Private Limited, Jayanagar, Bangalore-560069. Data obtained were analyzed using Microsoft Excel and results were expressed in mean.

## 3. Results and Discussion

Heavy metal contents obtained from two green leafy vegetables viz., Amaranthus and Dill leaf samples are tabulated in Table 1 and 2 respectively.

Lead (Pb) is a toxic element harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. Wong *et al*., (1996) reported that, Chinese cabbage picks up Pb more readily compared to other heavy metals such as Cd, Cu, Ni, and Zn. Present results revealed that, Pb concentration is found highest in both the vegetables in all sampling stations including control. In Amaranthus leaves, Pb concentration was highest in K.G.F (48.89 ppm) followed by Bangarpet (41.57 ppm) and Bethamangala (41.57 ppm) compared to control (8.2 ppm), whereas Dill leaves recorded highest Pb content in K.G.F. (32.20 ppm), Bangarpet (26.35 ppm) and Bethamangala (22.51 ppm) with control (9.0 ppm). Pb contents of the vegetables in this study are higher compared to FAO/WHO (2001) safe limit. Study showed that, in leaf samples, Pb contents are exceeding the permissible limit. Thus, Pb level in leafy vegetables seems alarming for consumption. High level of lead in some plants may probably be attributed to pollutants in irrigation water, farm soil, small scale industries, due to pollution from traffic etc. (Qui *et al*., 2000). Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human consumption (Muhammad Farooq *et al*., 2008). Adu *et al*., (2012) reported that, Pb level in lettuce leaves (0.01 mg/kg) is lower compared to the present results. Lead concentration in leafy vegetables was much higher than other vegetables (Rahlenbeck *et al*., 1999). Uptake of lead in plants is regulated by pH, particle size and Cat-ion exchange capacity of soil as well as by root exudation and other physico-chemical parameters (Lokeshwari & Chandrappa, 2006). Present results are in conformity with Anita Singh *et al*., (2010) and Abida Begum & Harikrishna (2010) as they have observed the presence of lead in leafy vegetables examined. Introduction of lead into food chain may affect human health and thus, studies concerning lead accumulation in vegetables have increasing importance (Coutate, 1992).

Copper (Cu) is an essential micronutrient functions as biocatalyst required for body pigmentation. It maintains a healthy central nervous system and prevents anemia in the body (Akinyele & Osibanjo, 1982). Most plants contain Cu inadequate for normal growth that is usually ensured by artificial or organic fertilizers (Itanna, 2002). Among all heavy metals, Cu is the most abundant element, recorded highest concentration of 21.59 ppm in amaranthus and 18.52 ppm in dill leaves in K.G.F. and least concentration of 4.26 ppm in amaranthus and 3.29 ppm in dill leaves in Bethamangala. Elbagermi *et al*., (2012) reported Cu content in carrot, cucumber and spinach 5.00, 5.75 and 5.32 mg/kg respectively. Cu contents in this study are within the permissible limit (30.00 ppm) of FAO/WHO (2001) in vegetables.

 

Cadmium (Cd) content in *Amaranthus* leaves is significantly high at K.G.F (2.96 ppm) followed by Bangarpet (2.78 ppm) and in Bethamangala (2.06 ppm) and the values are more than safe limit (2.0 ppm) of FAO/WHO (2001). Dill leaves recorded high Cd content in K.G.F (2.32 ppm) more than safe limit and in Bangarpet (1.44 ppm) and Bethamangala (0.92 ppm) the values are in safe limit FAO/WHO (2001). Cd is non-essential in foods and natural waters and it accumulates principally in the kidney and liver (Divrikli *et al*., 2006). Various sources of environmental contamination have been reported for its presence in food and various values have been reported for leafy vegetables that include 0.090 mg/kg for fluted pumpkin by Sobukola *et al*., (2010), 0.049 mg/kg for lettuce by Muhammad Farooq *et al*., (2008). Cd concentration is significantly high in both Amaranthus and Dill leaves and regular monitoring is required over a long period as the vegetables are transported from different sources.

Chromium (Cr) concentration in *Amaranthus* and *Dill* leaves samples in all the sampling points recorded well within the FAO/WHO (2001) safe limit (20.0 ppm). Cr concentration is below detection level (BDL) at K.G.F (<0.24 ppm) followed by Bangarpet (<0.14 ppm), Bethamangala (<0.09 ppm) and in Dill leaves at K.G.F (<0.30 ppm) followed by Bangarpet (<0.22 ppm), Bethamangala (<0.12 ppm). Exposure to Chromium may occur through breathing air, drinking water or eating food containing Cr or even through skin contact. In human beings and animals, it is considered to be an essential metal for carbohydrates and lipid metabolism within a certain range of concentrations (up to 200µg/day). However exceeding normal concentrations leads to accumulation and toxicity that can result in hepatitis, gastritis, ulcers and lung cancer (Garcia *et al*., 2001).

Zinc (Zn) concentration in Amaranthus leaves in all the stations varied considerably and except at K.G.F (62.01 ppm), in other stations it does not exceed the FAO/WHO (2001) safe limits (60 ppm). Availability of Zn ranged from 26.33 ppm to 62.01 ppm compared to control (24.60 ppm). In Dill leaves, Zn concentration exceeded the permissible limits at K.G.F (63.04 ppm), whereas in Bethamangala (24.31 ppm), Bangarpet (42.16 ppm) and control (21.66 ppm) showed values far below the FAO/WHO (2001) safe limit (Table 1 & 2). Zinc is the least toxic and an essential mineral element that is naturally present in some foods, added to others and available as a dietary supplement. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of enzymes and plays a role in functioning of immune system in human diet, protein synthesis and wound healing. Zinc also supports normal growth and development. Zn deficiency in the diet may be highly detrimental to human health than too much Zn in the diet, but high concentration of Zn in vegetables may cause vomiting, renal damage, cramps etc. (ATSDR, 1994). Regular consumption of these two leafy vegetables may assist in preventing the adverse effect of zinc deficiency that results in retarded growth and delayed sexual maturation because of its role in nucleic acid metabolism and protein synthesis (Barminas *et al*., 1998). Sobukola *et al*., (2010) have reported that, Zn level of 0.011, 0.070 and 0.050 mg/kg in bitter leaf, water leaf and cabbage respectively. Sridhara Chary *et al*., (2008) have also found varied Zn concentration in leafy vegetables of waste water irrigated areas of Hyderabad, Andhra Pradesh. Abida Begum & Harikrishna (2010) observed similar trends in Zn concentration of leafy vegetables in periurban areas of Bangalore. Zn is present in appreciable amounts in leafy vegetables and appears to have higher uptake from continued sewage irrigated land in Bellandur, Bangalore urban district (Lokeshwari & Chandrappa, 2006).

## 4. Conclusions

From the results it is confirmed that, green leafy vegetables collected from three different stations contained substantial amounts of heavy metals. Lead and Cadmium metals concentrations are exceeding the FAO/WHO (2001) safe limits. Copper concentrations found to be within the safe limits and Chromium concentrations are below safe limit. Zinc concentration is exceeding the FAO/WHO (2001) safe limit in K.G.F and in other stations it is below the safe limit. This is an important result as human health is directly affected by consumption of vegetables. Monitoring of heavy metals in vegetables needs to be continued; because these are the main sources of food for human beings in many parts of the world and are considered as bio indicators of environmental pollution.

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| Localities | Pb | Cu | Cd | Cr | Zn |
|---|---|---|---|---|---|
| Bethamangala | 41.57 | 4.26 | 2.06 | BDL <0.09 | 26.33 |
| Bangarpet | 43.35 | 12.22 | 2.78 | BDL <0.14 | 39.04 |
| K.G.F. | 48.89 | 21.59 | 2.96 | BDL <0.24 | 62.01 |
| Control | 8.2 | 12.20 | 0.8 | BDL <0.05 | 24.60 |
| FAO/WHO Safe limit (2001) | 5.0 | 30.0 | 2.0 | 20.0 | 60.0 |

Table: 1. Heavy metal concentration (ppm) in Amaranthus leaves

| Localities | Pb | Cu | Cd | Cr | Zn |
|---|---|---|---|---|---|
| Bethamangala | 22.51 | 3.29 | 0.92 | BDL <0.12 | 24.31 |
| Bangarpet | 26.35 | 12.22 | 1.44 | BDL <0.22 | 42.16 |
| K.G.F. | 32.20 | 18.52 | 2.32 | BDL <0.30 | 63.04 |
| Control | 9.0 | 8.20 | 0.68 | BDL <0.06 | 21.66 |
| FAO/WHO Safe limit (2001) | 5.0 | 30.0 | 2.0 | 20.0 | 60.0 |

Table: 2. Heavy metal concentration (ppm) in Dill leaves