HEAVY METAL DETECTION USING AAS (ATOMIC ABSORPTION SPECTROPHOTOMETER) IN LEAFY VEGETABLES: PART 1

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ABSTRACT

The major aim of this study is to target standing position of bioaccumulation of various heavy metals in edible greens, consumed by the people as major dietary resource in Asian territories, especially in India. This may reveal much important information regarding their fitness for consumption and associated health problems. Five different species of edible greens has been selected for heavy metal detection. Such as, *Corchorus olitorious, Cassea tora, Raphanus Sativus, Marsilea quadrifolia and Amaranthus viridis*. Samples were collected freshly from agriculture field. Air-drying method was used and followed by extraction of heavy metals by acid digestion method. Flame absorption method by Atomic Absorption Spectrophotometer (AAS) has been used to detect amount of heavy metals in selected samples. Heavy metal has been selected on the basis of their role in human health and availability in agricultural field. Six metal ions i.e. Cadmium (Cd), Lead (Pb), Copper (Cu), Zinc (Zn), Iron (Fe) and Chromium (Cr) has been quantified. Amount of different metal ions detected in various samples of green are iron (Fe) 6.336-8.988 mg/L, zinc (Zn) 3.170- 4.208 mg/L, Copper (Cu) (2.069-4.025 mg/L), Chromium (Cr) (1.321-3.837), Lead (Pb) 0.041-0.416 and Cadmium (Cd) was not detected in any of the selected sample of greens. *R. sativus, M. Quadrifolia* and *A. Viridis* were found to comprise lead above permissible level. Bioaccumulation of other metal ions was under permissible level. But some of them are found in much smaller amount, hence more consumption is recommended for fulfilling requirement of proper body growth and metabolic functioning of cell.

Keywords: Greens, Bioaccumulation, Atomic Absorption Spectrophotometer, Heavy metal

I INTRODUCTION

Large scale use of chemical fertilizers and loss of soil fertility is a major issue to produce healthy food resources along with high nutritional values. However, use of chemical fertilizers and various kinds of pesticides are responsible for contaminating food produce in agriculture field. Increase in the amount of metal ions absorbed by the plant above permissible value may give rise to various problems associated with human health. Rise in amount of various heavy metals inside plant body is termed as bioaccumulation. Detection of amount of heavy metals in agriculture produces may provide important information regarding suitability for consumption as dietary component.

Greens are one of the major sources of dietary components in Asian territory especially in India, China, Srilanka, Bangladesh etc. In Chhattisgarh - a state of tribal people (India), the life and economy of the local people are intimately connected with the agriculture produce. Leafy vegetables play a major role in fulfilling the nutritional requirement of local people in urban and remote parts of the Chhattisgarh. Green leafy vegetables are assumed to be very healthy and easily available source to obtain vitamins, iron, mineral, carbohydrates, amino acids, proteins etc. Almost all green leafy vegetables are also known for therapeutics value. Green are also chief source of secondary metabolites which provide us cure from many diseases, local forest product which are collected and sell them to earn their livelihood the present study based on identification of heavy metals which are found in the edible plant leaves. Primarily availability of greens depends upon their seasonal dependency, but some of them are available throughout the year, species which are studied in this study. Some plant species known for edible fruit, root, stem and seeds along with edible leaf are also considered in this project to maximize the approach of the research study. Greens are very popular among people in fresh form but some of them are also used as powder (dried from).

Now the issue is that the nutritional value of edible greens are still same as found earlier, when they were cultivated without using chemical fertilizers and other synthetic substances. Moreover loss of soil fertility, changing climate and rise in pollution level is really a serious issue, which raises a question with the nutritional value of edible produces. Potential in green leafy vegetable as good edible source is exhibited by the presence of various types of metal ions. Those metal ions participate in many metabolic activities inside living cells essential for cell growth via., proper functioning of cell organelles, various types of transportation, hormone production, protein synthesis and other physiochemical processes. Metal ions are essential for growth, more or less in all types of living cells but at some sort of extent or in very small amount. After and above required degree of necessity, metal ions are termed as heavy metal, responsible to cause toxicity in cells of living plant and animals. Those heavy metals include Copper (Cu), Lead (Pb), Zinc (Zn), Cadmium (Cd), Chromium (Cr) etc. These metal ions are also responsible to raise environmental pollution. These metal ions are generated in very high concentration through industrial effluent, petroleum products, pesticides, refineries and various types of

chemical fertilizers. Metal ions are absorbed by the plants through soil and enter into the human body after consumption as food article. High amount of consumption and accumulation in living organism is termed as Bioaccumulation. This study especially focuses on Ethanobotanical uses of edible plant species.

Table 1: Essential and non-essential role of heavy metals in living cells i.e.

Metal Type	**Importance**	**Disorder caused by bioaccumulation in human being**
Iron (Fe)	Major component of haemoglobin and myoglobin,	Anemia, liver disease, may provoke diabetes, and cardiac failure. Genetic disease - hemochromatosis
Copper (Cu)	Major component of enzyme ferroxidase, regulate iron transport and storage, structural element in tyrosinase, various type of oxidases and also in antioxidant enzymes.	Anemia, liver malfunction Genetic disorder – Wilson's disease
Zinc (Zn)	Structural constituent of many enzymes i.e. alcohol and dehydrogenase, carbonic anhydrase, RNA's DNA polymerase etc., Cofactor for gene regulatory proteins, Important component of Follicle stimulating hormone ,luteinizing hormone, DNA's structure & functioning and antioxidant superoxide	Nervous system, skin, intestine, cell growth, cell division, immune cell disorder, ageing, anaemia and reduced bone formation etc.
Chromium (Cr)	Regulation of Glucose Metabolism	DNA damages and alteration of Replication and Transcription process Hyperglycemia, Glucosuria, damage of nervous system, fatigue, irritability
Cadmium (Cd)	Xenobiotic in nature: A commonly occurring pollutant in nature	Renal dysfunction, lung disorder, skeletal and bone defects, blood pressure, kidney disease, bronchitis, gatrogenital disorder, bone marrow, cancer, Proteinuria,

		Glucosuria, Osteomalacia, Aminoaciduria, Emphysemia.
Lead (Pb)	Extensively toxic for both plant as well as human body	Mental retardation in children, slow growth, encephalopathy, congenital paralysis, sensor neural deafness, nervous system, liver, kidney and gastrointestinal damage.

II MATERIAL AND METHOD

Collection and Sampling

Variety of plants may differ in secretion and concentration of heavy metals, which lick quantity crucial for regulation of entire endocrine systems or biological activities. Proper scientific approach toward identification and quantification of metal ion in food resource could be beneficial to recognize healthiness of dietary habit in our daily life. More than 50 green leafy vegetables were listed based on an ethno-botanical filed study in various districts of Chhattisgarh state including tribal and urban region. Some of them are cultivated and some species of greens are wild varieties. Here we are selecting more commonly available species of greens, easily available in market area and popular among local people as dietary source. This means that the selection of plant species is based on cultivation, consumption level and availability. The selected plant species are *Corchorus olitorius, Cassea tora,Raphanus sativus,Marsilea quadrifolia* and *Amaranthus viridis*, all are cultivated and collected from the agriculture field.

AAS Analysis

All edible leaf samples were collected and weigh up for obtaining 1000 gm of each sample followed by washing with double distilled water. Each sample was taken thrice to obtain the mean value for validating the expected result. Washing was followed by air-drying method for next 96 hrs in dry state and also placed in hot air oven at 70°C for 1 hr with the help of silica crucible. Dried samples were grounded to obtained fine powder form of leafy vegetable samples. Extraction of heavy metal is done through acid digestion method. Acid solution was prepared by the mixture of Sulphuric acid (H_2SO_4) - 65%, Perchloric acid ($HClO_4$) – 65%, and HNO_3 (70%) at the ratio of 1:1:5. 1 gm of each powdered sample was treated with the prepared acid solution at the temperature of 80°C until transparent solution is obtained. Transparent solution was incubated for next 1hr for cooling under

room temperature. Thereafter solution was filtered with whatman filter paper No. 1 and suspension is prepared upto 100 ml using double distilled water.

Each sample was estimated for six metal ions i.e. Cd, Pb, Cu, Zn, Fe and Cr via. Atomic Absorption Spectrophotometer (AAS) (Company name).

III RESULT AND DISCUSSION

Some of the metal ions are essential for living cells for proper functioning and rest of them are responsible to cause toxicity. But the quantity in terms of concentration is a major factor, which derives their specific role inside living cell of each metal ion. Determination of quantity of metal ion in food material can reveal their effect on human health. Cadmium was not detected in any of the sample analyzed by AAS method. Cadmium was not detected by AAS in all the samples. Amount of different metal ions detected in various samples of green are iron (Fe) 6.336-8.988 mg/L, zinc (Zn) 3.170- 4.208 mg/L, Copper (Cu) (2.069-4.025 mg/L), Chromium (Cr) (1.300-3.800), Lead (Pb) 0.041-0.416 and Cadmium (Cd) was not detected in any of the selected sample of greens. Amount of lead detected in *R. sativus, M. Quadrifolia* and *A. Viridis* were found to comprise lead above permissible level. Many researchers have been revealed adverse effect of lead on human health. Metals like Iron and Zinc are essential for proper functioning of metabolic activities of cell in human body. Although greens are rich in Iron and Zinc content but regular intake is necessary for proper functioning of metabolic activities because requirement is much higher than the amount detected in samples by AAS. Many more conclusions may be prepared on the basis of result obtained through this study. But still more effort is required for the extension of this type of study to spot various affect of changing technique and methodology in the field of agriculture.

IV CONCLUSION

This research study concludes that a massive effort is required in this field of study. There are many metal ions are present in our surrounding atmosphere and are absorbed by edible plants through water and soil. This is important to quantify them in terms of Nutraceutical values as well as their dreadful effect on human health. Moreover huge population of plant is available and utilized as source of dietary component all over the world. This is really a major challenge to fulfil requirement of food for such a large population of world without sacrificing health benefits of food products. Many more other studies also reveal that food values are gradually decreasing with changing climate and environmental conditions. Absorption and accumulation of metal ions by the plants depends upon various factors such as-

a. Plant physiology
b. Metabolic requirement of the plant cell
c. Adaptability
d. Genetic variability
e. Nutritional requirement etc.

Therefore, this is essential to quantify heavy metals in each species separately. This could be a long journey but this kind of study may bring important information regarding quality food production. Our team will try to extend this study by selecting some other different edible species of plant. We also expect that research will appreciate this kind of work and they will contribute in this study.

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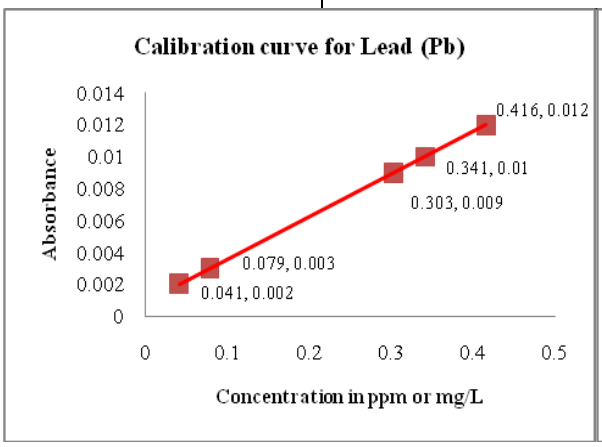
Table 2: Showing heavy metal detection in ppm by AAS method mg/L or ppm or mg/kg

Botanical Name	Indian/Hindi Name	Common Name	Family	Pb (ppm)	Zn (ppm)	Cd (ppm)	Cr (ppm)	Cu (ppm)	Fe (ppm)
Corchorus olitorius	Chech bhaji	Jute	Tiliaceae	0.041	3.358	Not detected	1.300	2.319	7.698
Cassea tora	Charota Bhaji	Faoetid Cassia, Tora, Sickle senna, Wild senna, sickle pod & coffee pod etc.	Fabaceae	0.079	4.143		2.300	4.025	8.988
Raphanus sativus	Mooli	Winter radish & White radish	Brassicaceae	0.303	4.000		3.300	2.717	8.442
Marsilea quadrifolia	Teenpaniya Bhaji/ Chunchuni a bhaji	Four leaf clover, European water clover	Masileaceae	0.416	3.170		2.300	2.069	8.263
Amaranthus viridis	Kheda Bhaji, Jadi bhaji	Callaloo Kallaloo & Bhaaji	Amarantahcae	0.341	4.208		3.800	3.034	8.451
Permissible value of heavy metal in Vegetables recommended by WHO				**0.3 mg/kg**	**60 mg/kg**	**0.2 mg/kg**	**5.0 mg/kg**	**30 mg/kg**	**450 mg/kg**

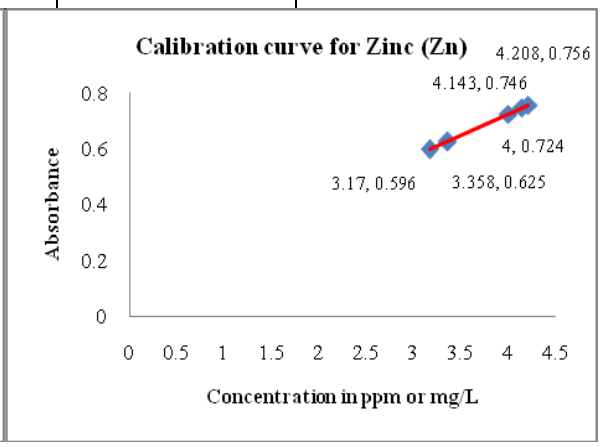
Table Showing: Instrument's parameter for different metal ion detection in plant sample

Measure Method by AAS: Flame Absorption

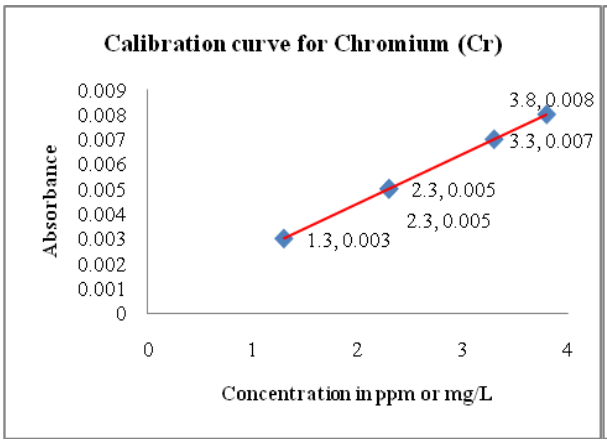
Elements	Wavelength (nm)
Lead (Pb)	283.31
Zinc (Zn)	213.86
Cadmium (Cd)	257.25
Chromium (Cr)	357.87
Copper (Cu)	324.75
Iron (Fe)	248.33



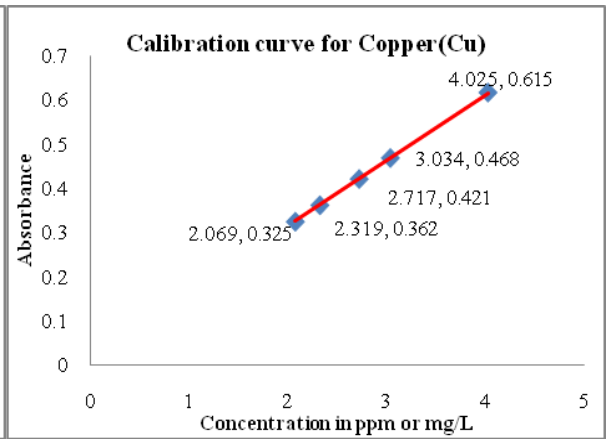
Graph 1: Detection of Lead by AAS



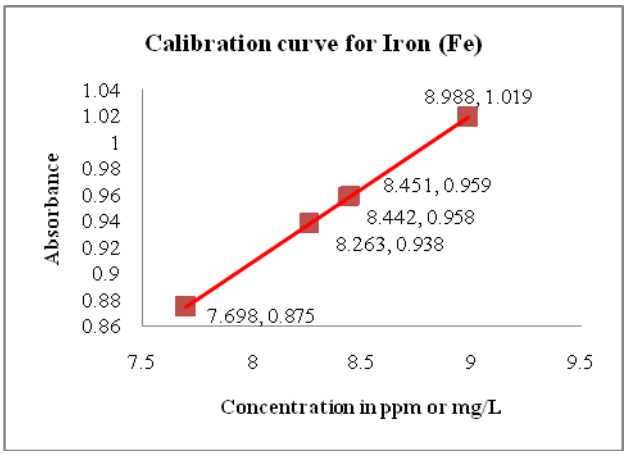
Graph 2: Detection of Zinc by AAS



Graph 3: Detection of Chromium by AAS



Graph 4: Detection of Copper by AAS



Graph 5: Detection of Iron by AAS

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