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Heavy Metal Detection using AAS (Atomic Absorption Spectroscopy) in Leafy Vegetables: Part II

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ABSTRACT:

Earlier we have found that all plants are capable of absorbing and bioaccumulation of heavy metals in their own part in various concentrations, confirmed by using Atomic Absorption Spectroscopy (AAS). Rate of absorption and percentage of bioaccumulation varies among the species. In earlier study we have selected *Corchorus olitorious, Cassea tora, Raphanus Sativus, Marsilea quadrifolia and Amaranthus viridis* (Prabhas *et al*., 2018). This study is the follow up experiment of Part I and targeting some other leafy vegetable species, on the basis of their consumption and popularity among the people of central India. In this experiment we have selected *Moringa oleifera*, *Ipomea batatas*, *Brassica oleracea* var. *botrytis*, *Colocasia esculenta*, *Murraya koenigii*, *Bahunia verigeta.* Plant species were grown in the agriculture field up to optimum growth. Leaf and edible parts were collected from the plants and air dried. Dry matter were used for acid digestion after that quantity of heavy metals can be analyzed using Atomic Absorption Spectroscopy (AAS). Total six metal ions including Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Iron (Fe) and Copper (Cu) was targeted in this study. Lowest concentration of Pb was found in *M. Oleifera* (0.041 mg/L) and highest in *I. batatas* and *B. Oleracea var. botrytis*, Cr was low in *M. Oleifera* and higher in *I. batatas*, Zn was low in *B. Oleracea var. botrytis* and higher in *C. Esculenta*, Fe was lowest in *M. Oleifera* and high in *B. Verigeta* and Cu was low in *B. Oleracea var. botrytis* and higher in *I. batatas*. More or less all these metal ions are necessary for growth of plant. On the other hand bioaccumulation of these metals may also cause hazardous effect on human health after consumption as food source or medicinal source.

Keywords: Edible, leafy vegetable, bioaccumulation, consumption, Atomic absorption Spectroscopy, high, low, health.

INTRODUCTION:

Vegetables, crops and other food resources are cultivated in various types of soil under varied environmental conditions. Surrounding edifices always shows significant affect on the growth of all kind of living organism including plant, animals, microorganisms and human also. Likewise industrial waste released in water sources, dumping of domestic waste in soil and water resources, use of pesticides and fertilizers in agriculture field etc. Movement of

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heavy metal from their source of origin to agriculture field may occur with flood water, river, rain water and underground water also. Numerous reasons are present that can alter the concentration of heavy metals in soil, agriculture field and land where the plants are cultivated regularly. Waste containing heavy metals are directly absorbed by the plants to optimize their growth. In general terminology we call them Mineral ions in place of heavy metal ions. These metal ions are used by living organism for maintaining their cellular and metabolic activities as well. Many enzymes and metabolic transport depends upon presence and concentration of metal ions. But, we should always care about that the concentration of availability and utilization of each kind of mineral ions. They are categorized as micro and macro element on the basis of their requirement for growth of the plant. Higher the requirement they are included in the list of macro-elements (mg or gram) on other hand if they are required in small quantity in micro gram, they are listed in micro-elements.

Major objective of the study is to reveal bioaccumulation of heavy metal in plant occurs due to presence of high concentration of heavy metal ions in soil, where the edible plants are grown for human's utilization. Availability of high percentage of heavy metal in soil enforces more absorption by plant. More absorption and optimum requirement leads into the result of Bioaccumulation. Availability of heavy metal ions in soil can be natural or artificial/anthropogenic. Most probably plants are unaffected with their high concentration inside their parts but higher animals like humans are susceptible to high amount of heavy metals. Consumption and utilization of plants and their parts as food resources by humans and other higher animals is a matter of discussion. Various diseases and hazardous effect on human body are associated with altered concentration of heavy metal ions (Table 1.).

Agricultural productivity and all other plant products are major source of diet in India and all other Asian countries. Hence, it is necessary that food resources should be healthy and free from contamination. Moreover quality check of all dietary components is mandatory with changing climate and environmental conditions at regular time interval. This is a matter associated with food safety and our health. Altered concentration of heavy metal ions than expected is like an invisible healthy enemy that cannot be easily detected without standard and scientific approaches. Supply of healthy and clean food resources are mandatory for preventing health issues and enhancing immunological machinery inside them. Unavailability of technology and economic limitation is also a barrier for quality test of various food products. Clean and natural environment for the cultivation of plant is a major challenge nowadays. Food article must be clean from outside and inside too.

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Table 1: Effect of high concentration of heavy metal ions in human body

S. No.	Heavy Metal	Importance and Role of heavy metal in human body	Various diseases and health risk caused by higher concentration of heavy metal ions
1.	**Lead (Pb)**	Toxic to both human and plant	Liver damage, kidney damage, gastrointestinal disparity, mental retardation in children, retardation in growth, damage to sensory organs, paralysis and nervous disorder etc .
2.	**Cadmium (Cd)**	Non-biodegradable, a major source of soil pollution.	Lung diseases, blood pressure, damage to bone marrow, cancer, osteomalacia, renal dysfunction, emphysemia, gastrogenital disorders etc.
3.	**Chromium (Cr)**	Maintenance and regulation of Glucose metabolism in human body	Nervous breakdown, Glucosuria, DNA damages and alteration in DNA replication, altered protein synthesis, fatigue and irritability etc.
4.	**Zinc (Zn)**	Structural component of various enzyme and hormones	Altered bone formation, immunological imbalance, anaemia, reduced cell growth, damage to nervous system etc.
5.	**Iron (Fe)**	Structural component of haemoglobin, important function in transport channel, component of myoglobin, helps in function of various enzymes as co-factor.	Causes anaemia, cardiac disease, liver damage, various genetic disease, hemochromatosis etc.
6.	**Copper (Cu)**	Structural component of various enzyme i.e. ferroxidases, tyrosinase and some antioxidant enzymes too.	Resulting into anaemia, liver dysfunction, Wilson's disease etc.

Ref: Prabhas *et al*. (2018)

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METHODOLOGY:

Sample collection: Total six plant species of edible varieties are grown simultaneously *i.e. Moringa oleifera*, *Ipomea batatas*, *Brassica oleracea* var. *botrytis*, *Colocasia esculenta*, *Murraya koenigii*, *Bahunia verigeta*. Edible parts were collected from the plants. Samples were collected and dried well. All the selected plant species are well known for their consumption and popularity as dietary component in India and most part of Asia as well. Plants were grown in black soil, which is rich and mineral and heavy metal ions. All fresh samples were collected and weighed for 1000 gm and dried for further analysis.

Table 2: List of plant species from where test samples were collected

Botanical Name	Indian/Hindi Name	Common Name	Family	Edible Part
Moringa oleifera	Sahijan, Munaga Bhaji	Moringa or Drum Stick	Moringaceae	Leaf and Fruit
Murraya koenigii	Kari patta or Meetha neem	Curry leaf plant	Rutaceae	Leaf
Bahunia verigeta	Sonpatti	Senna	Fabaceae	Leaf
Ipomoea batatas	Shakarkandi P	Sweet Potato	Convolvulaceae	Fruit
Brassica oleracea var. *botrytis*	Phool Gobhi B	Cauliflower	Brassicaceae	Leaf and Flower

Spectrophotometric analysis

After deep washing using double distilled water, all samples were air dried. It was recorded that the moisture content of each sample was approximately 70 to 90 %, recorded after 168 hrs. Percent moisture content varies between the samples taken for the analysis. After removal of moisture samples were grinded to obtain fine powder using electronic grinder. Powdered samples were treated for acid digestion resulting into extraction of heavy metal through supernatant. Acid mixture was prepared from stock solution of Nitric acid (HNO_3) - 70%, Sulphuric acid (H_2SO_4) - 65% and perchloric acid ($HCLO_4$). All three acid stock solutions were taken in the ratio of 5:1:1 for final preparation of acid mixture solution. Each dried sample was treated with acid mixture solution and incubated till a clear transparent solution is obtained, approximately for 10 to 24 hrs at 80°C. Supernatant was filtered and separated from solid residues of the digested samples using Whatman's filter paper 1 at room temperature. Each sample was analyzed thrice (for obtaining mean value). Quantitative estimation of six heavy metals i.e. including Lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn), Iron (Fe) and Copper (Cu) (Table 1) done in Atomic Absorption Spectroscopy (AAS).

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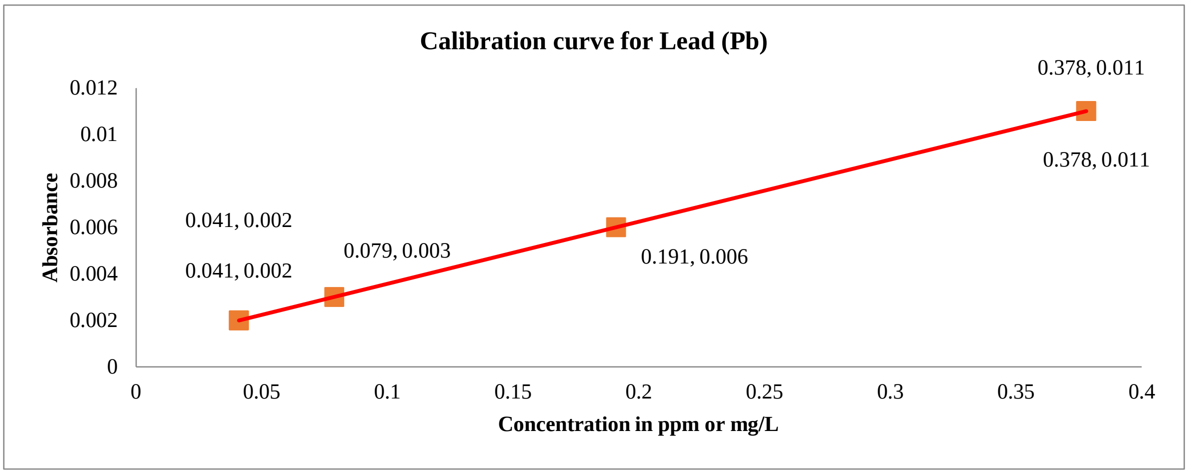
In Flame Atomic Absorption Spectroscopy different wavelengths (nm) were used for detection of each heavy metal ion. Lead was detected in the wavelength of 283.31 nm followed by Zinc (213.86 nm), Cadmium (257.25 nm), Chromium (357.87 nm), Copper (324.75 nm) and Iron (248.33 nm).

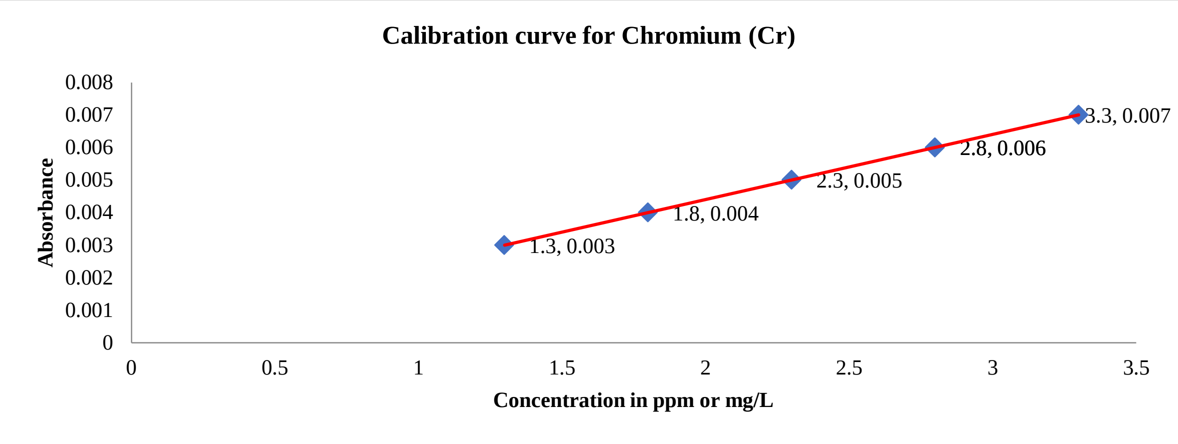
RESULT:

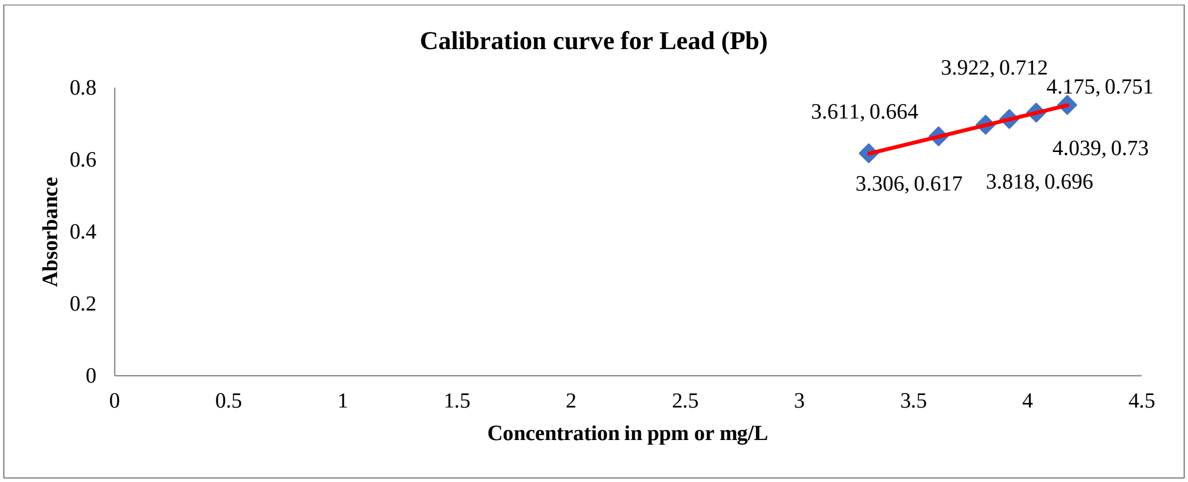
Spectrophotometric analysis (AAS) has shown significant result for the targeted heavy metals. Concentration was recorded in unit of ppm or mg/L. Quantitative analysis of various heavy metals reveals that varies among the selected plant species in mg/L. *M. oleifera* was containing the least amount of lead as 0.041 ppm (mg/L) and highest concentration of Pb was recorded in *I. batatas* and *B. oleracea var. botrytis*. Chromium was recorded as low as 1.300 ppm (mg/L) in *Moringa oleifera* and it was high in *I. batatas*. Lowest concentration of Zinc was recorded as 3.306 ppm (mg/L) in *B. oleracea var. botrytis* and highest concentration was found in *C. esculenta*. *B. verigeta* was rich in Iron as 8.639 ppm (mg/L) and it was low in *B. oleracea var. botrytis*. *B. oleracea var. botrytis* was containing low concentration of copper while on the other hand it was bioaccumulated in *M. oleifera*. Cadmium concentration was absent in all samples. As we can observed that in the table 3, concentration of copper (Cu) is quite high as compared to recommended value. All the samples were rich in iron content. Zinc (Zn) concentration is quite low in all the samples. Chromium (Cr) is slightly high in *I. batatas*, otherwise it is under the recommended value. Lead concentration was under the limit in first three samples i.e. *M. oleifera, M. koenigi* and *B. verigeta* and slightly higher in remaining three samples of *C. esculanta, I.batatas* and *B. oleracea var. botrytis*.

Botanical Name	**Pb**	**Cd**	**Cr**	**Zn**	**Fe**	**Cu**
Permissible value of heavy metal in vegetables (recommended)	**0.3 mg/kg or 0.1 mg/L**	**0.2 mg/kg or 0.06 mg/L**	**5.0 mg/kg or 3.0 mg/L**	**60 mg/Kg or 15.0 mg/L**	**450 mg/kg Or 6.0 mg/L**	**30 mg/kg Or 0.1 mg/L**
Moringa oleifera	0.041	Not detected	1.300	3.611	7.071	2.589
Murraya koenigii	0.041	Not detected	1.800	3.922	8.200	3.917
Bahunia verigeta	0.079	Not detected	2.800	3.818	8.639	3.620
Colocasia esculenta	0.191	Not detected	2.800	4.175	7.205	3.236

Ipomoea batatas	0.378	Not detected	3.300	4.039	7.967	3.998
Brassica oleracea var. botrytis	0.378	Not detected	2.300	3.306	6.336	2.420







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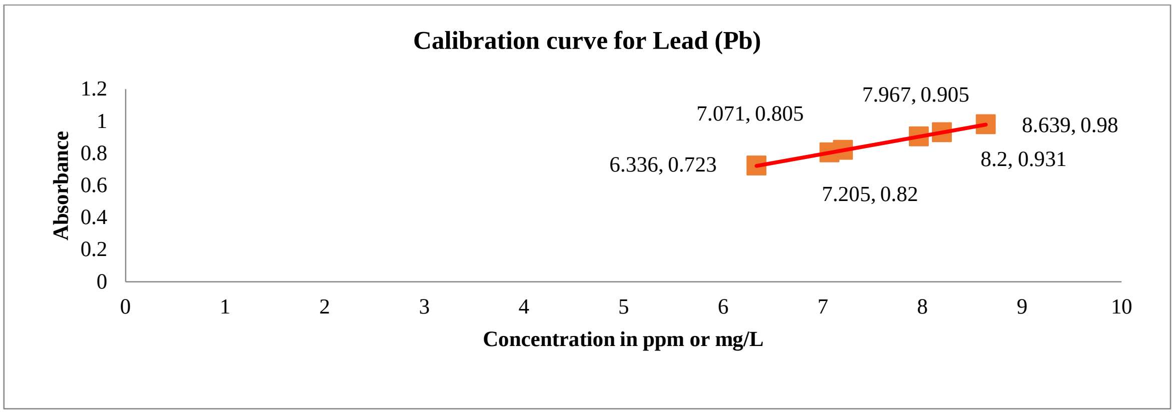

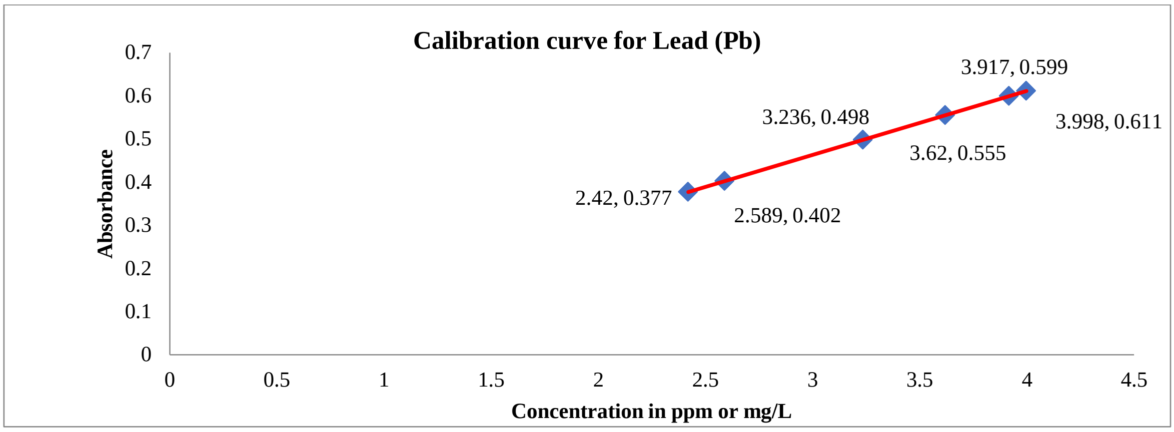

CONCLUSION AND DISCUSSION:

Mineral toxicity is the terminology used for altered and hazardous concentration of mineral ions for living organism. Hence, a specific concentration of mineral and metal ions is required by living organism, which can vary from one organism to other. But it is mandatory to identify the optimum or recommended value of concentration of various minerals and metal ion in dietary component of living organisms. Especially humans are highly susceptible to very high or low concentration of every essential and non essential mineral/ion. As we discussed earlier that plants and their products are major source of food for human beings. Hence, bioaccumulation of heavy metal ions in plants is the route of transport of heavy metal ions from environment to human body. Certainly they are needed for growth but up to the certain level. High concentration as well as deficiency both is dreadful for human health. Quantification of heavy metal ions accumulated in our dietary resources is an important aspect.

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