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Research Article

ANALYSIS OF HEAVY METALS CONTENTS AND THEIR EFFECTS ON HUMAN HEALTH BIOACCUMULATED IN SEED OIL OF PLANT *MOMORDICA CHARANTIA* OF ARID ZONE

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ABSTRACT

A study was conducted on accumulation of heavy metals in seed oil of *Momordica charantia* (bitter gourd) from arid resources of Rajasthan, India. Heavy metals are natural components of the Earth's crust. These can neither be degraded nor destroyed. These enter our bodies via food, drinking water and air as trace elements, some heavy metals are essential to maintain the metabolism of the human body. However, when present in higher concentrations can lead to poisoning. The plant *Momordica charantia* is selected for study is a medicinal plant. The seeds, the leaves, and the vines of the bitter gourd have also been extensively used in a variety of herbal medicines and infusions. Fatty acid analysis was done. Gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric and ferulic acids were identified in the seed oil. Among these, vanillic acid was predominant. The content of metals and their species (chemical forms) in edible seed oils depend on several factors. The metals can be incorporated into the oil from the soil or be introduced during the production process. An atomic absorption spectroscopic technique has been used for the determination of metals (Cd, Zn, Fe, Ni and Pb) in seed oils of Momordica charantia after open vessel digestion. The concentrations of metals Cd (0.09 *103µg/L), Fe (104.35*103µg/L), Cu (3.53*103 µg/L) and Ni (8.94*103 µg/L) were observed. The presence of trace metals such as Cu, Fe, Cd, Pb, and Ni is known to have adverse effects on the plants and human health.

Key Words: *Solanum Malongena, Atomic Absorption Spectroscopy, Mineral Metal Analysis, Physico-Chemical Properties*

INTRODUCTION

Heavy metals are natural components of the earth's crust and these can enter the water and food cycles through a variety of chemical and geochemical processes. Advancement in technology and modern civilization inducts a wide range of pollutants into the atmosphere through various anthropogenic activities such as industry, mining, transportation, etc.

Despite the fact that it is almost impossible to visualize a soil without trace of levels of heavy metals and most of the heavy metals are essential elements for living organisms, but their excess amounts are generally harmful to plants, animals and human health (Azevedo *et al*,. 2005; Jarup *et al*,. 2003). Currently, contamination of soil in cultivated fields with toxic heavy metals such as cadmium, copper, lead, iron, nickel and zinc has emerged as a new threat to agriculture (Singh et al,. 2007). Cadmium is an unnecessary element for both plants and animals and has toxic effects when its concentration has exceeded a limit. Generally, it makes negative effect on their metabolism by influencing the activity of cellular enzymes (Yang *et al.*, 1986). Copper is widely prevalent and was considered as an essential element for all living organisms including plants (Underwood , 1977) and Shi *et al*,.1993)) .Copper is one of the common heavy metals in industrial discharge of aeronautic, metal and metallurgy, and refinery industries shows toxic effects on plants and animals. Copper causes injury at cellular level by the formation of free radicals. Nickel is naturally present in soil and water, usually in trace amount. Several plants nutritionally require nickel for various metabolic activities (Welch *et al*,. 1981)). Nickel is known to cause cancer. Lead accumulation results first in reduced functioning of kidney, liver and brain cells and later in complete breakdown of the tissues. Iron typically damages cells in the heart and liver which can cause cancer, coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome and long-term organ damage.

The plant Momordica charantia commonly known as the bitter gourd or Chinese gourd belongs to the Cucurbitaceae family. Bitter gourd is a fast growing warm seasonal climbing annual, native to South Asia. It is nutritious gourds with medicinal properties. The fruit of this plant has a very bitter taste. Medicinal use is chiefly made of the fruit, as it is considered the safest and most easily cured part of the plant, however, the seeds, the leaves, and the vines of the bitter gourd have also been extensively used in a variety of herbal medicines and infusions. It is widely cultivated in tropical climates for use as a vegetable and a medicine. Fruit contains ascorbic acid. Leaves and flowers contain alkaloids named momordicine. Plant contains a bitter glucoside, resin, aromatic oils, a substance very similar to that of saponin and mucilage. The fruit is green and contains white seeds, when young and after maturity, turns to orange-yellow with red seeds. Leaves and fruits of the plant are a source of nutritional substances such as iron, calcium, phosphorous, vitamin C, protein, riboflavin, carotene, thiamine and some pharmacologically active substances.

A compound known as 'charantin' present in the bitter gourd is used in the treatment of diabetes to lower blood sugar levels. Fruit of the bitter gourd when consumed can lead to a significant lowering of the sugar levels in the blood and urine of a person. Turkish herbalist uses the fruit of the bitter gourd to treat all types of ulcers affecting the body. Herbal remedies made from the bitter gourd are also a favourite of herbalist in the West Indies, they use it as a cure all or super herb, treating cases of worms, problems related to urinary stones, and in the treatment of fever. The purgative action of the juice of the fruit is also extensively used of in the West Indies. The herbal remedy is also prescribed for treating colic and abdominal gas in different people. Herbal oil derived from the seed oil is used on wounds for topical relief and long term remedial benefits. Other important pharmacological activities of bitter gourd are its antibacterial activity, antiviral activity (including HIV infection), anthelmintic activity, abortifacients and antifertility activity, immunomodulatory activity, analgesic and anti-inflammatory activity, hypotensive and anti prothrombin activity, hypocholesterolemic and anti-oxidant potential, antipsorisis activity, anticancer activity and anti-ulcer activity. Antiviral activity observed in vitro has been attributed to a 30kD protein called MAP30, which has been isolated from bitter gourd seeds (Gupta *et al,*. 2006)). This protein has been reported to inhibit HIV viral integrate and cause irreversible relaxation of super coiled viral nucleic acids (Wang et al,.(2000)). These changes render viruses unable to integrate into host cell genomes. Rates of T lymphocyte infection with HIV-1 and reduced rates of viral replication in infected cells have also been reported in vitro (Lee-Huang *et al.*, 1995)).

The use of bitter gourd in decolourisation and removal of textile dyes and phenolic compounds from effluents are other potential applications In contrast to most of the commonly available seed oils, the seed oil of M. charantia contains a large amount of eleosteric acid .The presence of eleosteric acid in substantial amounts improves the drying rates of thin films of fatty oils when exposed to air. Seed oil of M. charantia had been identified as a good material for making wrinkle varnish.

The aim of the study is to analyze contents of heavy metals accumulated in seed oil of Bitter gourd plant. For this purpose, the concentrations of Pb, Zn, Cd, Fe, and Ni were measured for the estimation of heavy metal pollution.

MATERIALS AND METHODS

***Sampling*:** The seeds of bitter gourd plants were collected from arid region of Rajasthan, India. Seeds were dried in air. Extraction of oil from seeds was done by solvent extraction method. The clean and dried seed samples were crushed in mortar and oil was extracted from the crushed seeds by extraction with petroleum ether (60-800C) in a Soxhlet apparatus for 6 hrs. The solvent is removed under reduced pressure. The obtained oil was stored in cool place (refrigerator) until further investigation.

Reagents: All reagents were of analytical reagent grade. Double deionized water was used for all dilutions. HNO_3, H_2SO_4, H_2O_2, HF, $HClO_4$ and HCl were of superior quality. All the plastic and glassware were cleaned by soaking in dilute HNO_3 and were rinsed with distilled water prior to use. The working standard solutions of heavy metals used for calibration were prepared by diluting a stock solution of 1000 µg/L (Pb, Cd, Zn, Fe, and Ni).

Mineral metal analysis: One of the methods for determination of the total contents and speciation analysis of heavy metals of their environmental concentrations is atomic absorption spectroscopy (Cabrera et al,. (1994) and Kerber et al,. (2002)).This method is simple and very selective. In this paper we present determination of heavy metals in seed oil of bitter gourd plant by atomic absorption spectroscopy method.

Preparation of standard for metal: In spectrophotometric measurements we are concerned with solution having very small concentration of the metal to be determined. It follows that the standard solution which will be required for analysis must also contain very small concentration of the relevant metal. Standards are prepared by dissolving 1gm of metal cadmium, nickel, iron lead and zinc dissolve in minimum quantity of aquaregia (1:3) HCL and HNO_3, made up to 1 litre in volumetric flask by adding deionized water. This is a stock solution which contains about 1000µg/L of required metal and then the working standard solution is prepared by suitable dilution of stock solution. The calibration curves for metal ions were drawn by taking working standard of 0-40 µg/L as required for the calculations.

Digestion of seed oil: For the seed oil samples analysis, seed oil was digested in 100 ml Pyrex glass beaker. For this we took 1g of seed oil added 10 mL Concentrate Nitric acid. Kept first for cold digestion for 24 hours and then heat at 50^oC for 4hours. The solution was finally boiled with 1:5 mixtures of concentrate acids HCl and HNO_3 in order to digest all organic matter (Tuzen (2003)) and then filtered after cooling. Finally volume of the extract was made up to 25 mL using double distilled water (Lark et al,.(2002)).From the calibration curves for these standard metal ions, concentration of metals in seed oil sample was calculated.

Fatty acid analysis: The fatty acids composition of bitter gourd plant oil was determined in two steps. In first step hydrolysis of oil was done and mixed fatty acids were obtained, and in second step this mixture of fatty acids was further derivatised to their methyl esters. The formation of methyl esters was confirmed by thin layer chromatography (TLC). The methyl esters so obtained were analyzed by HPLC (Browne et al,.(2000)). The physico-chemical properties such as Saponification value, acid value, iodine value and peroxide value of the Momordica charantia seed oils were determined, using the method described by AOCS (Adelaja, 2006 and Nukhet *et al*,. 2001).

RESULTS AND DISCUSSION

Fatty acid analysis results: The chemical compositions of fatty acids of bitter gourd plant seed oil and methyl esters from Soxhelet extraction observed were - Caprilic acid (C:8-0) Capric acid (C:10-0) Lauric acid (C:12-0) Myristic acid (C:14-0) Palmitic acid (C:16-0) Stearic acid (C:18-0) Oleic acid (C:18-1) Linoleic acid (C:18-2) Punicic acid (ctc,9,11,13–18:3) α-Eleosteric acid (ctt,9,11,13–18:3) Catalpic acid (ttc, 9,11,13–18:3) β-Eleosteric acid (ttt, 9,11,13–18:3).

Physico-chemical properties: The Physico-chemical properties of seed oils were obtained using the method described by AOCS are given in table1.

Mineral metal analysis results: The reason for the accumulation of heavy metals in plants is that they can relatively easily take up by food crops and especially by vegetables. Also it may be due to the foliar absorption of atmospheric deposits on plant. Different plant species accumulate different metals depending on environmental conditions (Linde et al,. (2001) and Dos Santos et al,.(2006)), metal species, plant available and forms of heavy metals. Studies have shown that uptake and accumulation of metals by different plant species depend on several factors, and various researchers have studied them (Demirbas et al,.(2001) and Gast et al,.(1988)).

Apparatus: An Atomic Absorption Spectrophotometer was used in this study for analysis of heavy metals. All the measurement for metals (Cd, Ni, Fe, Pb and Zn) was carried out in an air/acetylene flame. The instrumental parameters and operating conditions are given in table2.

Table1: Physico-chemical properties of the bitter gourd plant seed oils.

Properties	
Moisture content of fresh seeds (% w/w)	53 ± 13
Kernel to seed dry basis (% w/w)	60 ± 4.7
Specific gravity	0.92 ± 0.01
Refractive index at 25 °C	1.4895 ± 0.0050
Colour	Reddish brown
Acid value (mg/g)	2.73 ± 0.876
Saponification value (mg/g)	190.70 ± 1.82
Iodine value (cg/g)	115.96 ± 3.46

Table 2: AAS data for elements

S.No.	**Element**	**Wave length of main resonance line λ (nm)**	**Type of Flame***	**Absorbance [μg /L1%]**
1	Cd	228.8	AA	25
2	Fe	243.3	AA	100
3	Ni	232.0	AA	100
4	Pb	283.3	AA	500
5	Zn	213.9	AA	15

**AA – Air Acetylene mixture*

Table 3: Concentration of heavy metals detected in seed oil of Bitter Gourd plant

Metal	**Clock Interval (in seconds)**	**Standard Deviation**	**Related Standard Deviation%**	**Concentration in mg/L**
Cu	27.44	1.918	15.66	3.53
Pb	1.970	4.053	20.21	Not Traceable
Fe	5.779	5.198	29.66	104.35
Ni	6.223	0.9740	716.7	8.94
Cd	25.97	21.24	54.03	0.09

In AAS the absorbance is linearly related to concentration. The instrument used in this experiment is designed for flame absorption studies. It utilizes an integrated aspirator burner with electric ignition, the burner being fed with air and acetylene, and the sample being directly aspirated into the flame. For absorption studies, the hollow cathode is selected for the element under analysis, and is operated at about 50% of its current rating. The grating monochromator is employed between the flame and the detector. The wavelength of radiation allowed to pass onto the detector is chosen by selecting the element to be studied which automatically adjusts the monochromator system. Five adjustments were essentially made to set the monochromator at peak emission.

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Signal strength adjustment is accomplished by adjusting the flame position vertically with respect to the AA optical path, by controlling the fuel and oxidizer flow rates to the burner, and by electronic adjustments on the control panel.
Concentrations of heavy metals in the analyzed samples are given in Table3.

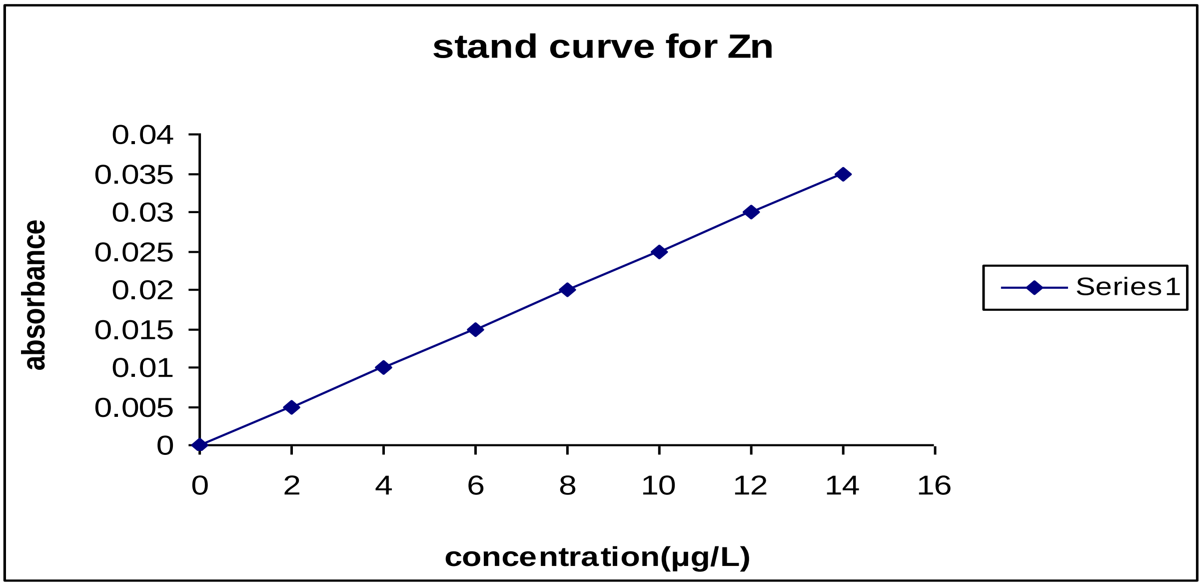

Figure 1: Calibration curve for **Zn**

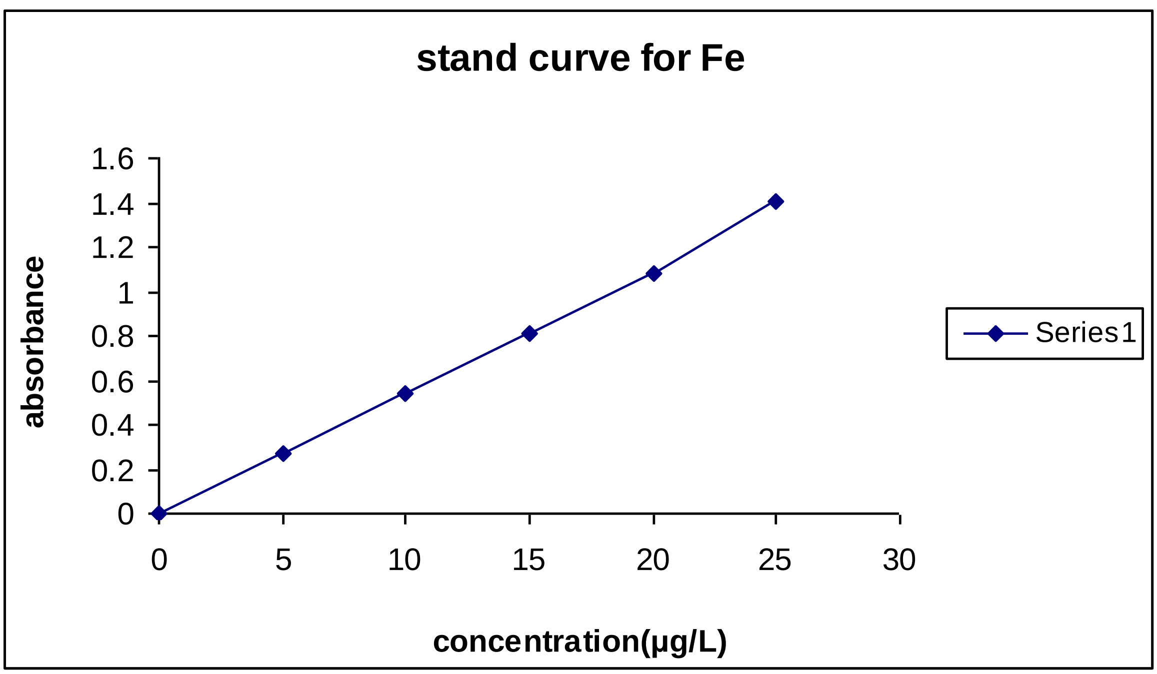

Figure 2: Calibration curve for **Fe**

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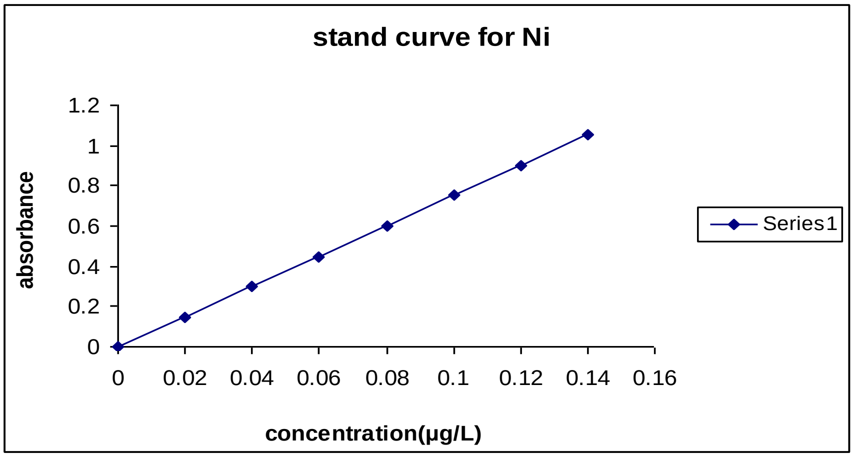

Figure 3: Calibration curve for **Ni**

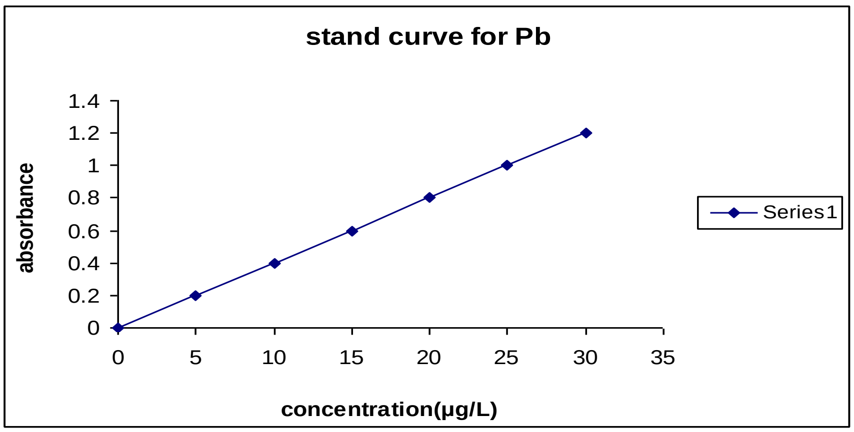

Figure 4: Calibration curve for **Pb**

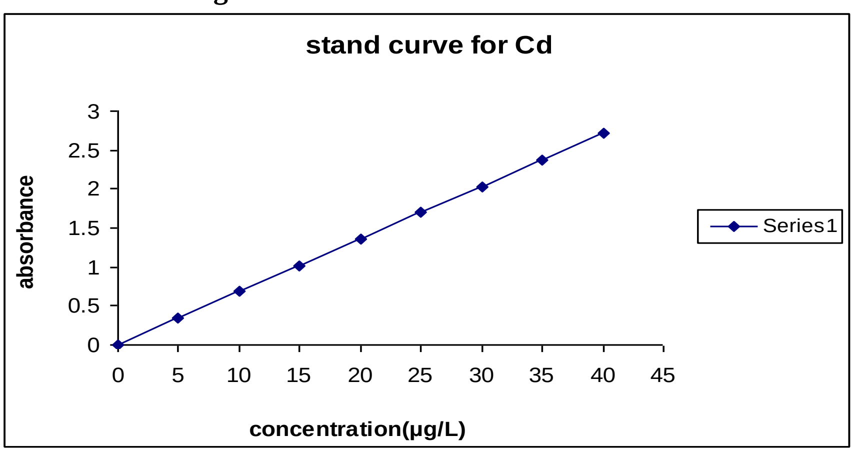

Figure 5: Calibration curve for **Cd**

***Calibration curves for metals*:** A calibration curve used in atomic absorption measurements is plotted by aspirating samples of solutions containing known concentrations of metal into the flame, measuring the absorbance of each solution and then constructing a graph in which measured absorbance is plotted against the concentration of solution.

Using the calibration curve, it is a simple matter to evaluate the concentration of relevant metal in test solution from the measured absorbance.

Calibration curve for metals are given as follow

***Concentration of heavy metals detected in seed oil of egg plant*:** Heavy metals concentration analyzed in seed oil sample is given in table3. All the metal concentrations were determined on dry weight basis.

Most of the laboratory research on biosorption of heavy metals indicated that no single mechanism is responsible for metal uptake. In general, two mechanisms are known to occur, viz. 'adsorption', which refers to binding of materials on to the surface and 'absorption', which implies penetration of metals into the inner matrix. Either one of these or both of the mechanisms might be involved in the transportation of metals into the plant body. Accumulation of these heavy metals in vegetables could be attributed to the use of industrial waste water for their cultivation. From the results, it is found that concentration of Cd, Pb is considerably high.

From the results it is found that the average content of Ni was 8.94 ppm the toxic level ranges from 7 to 100 ppm. The detected level of Cu was ranged from 3.54 ppm. This indicates the effect of anthropogenic activities and heavy traffic activities by which the Cu metal may accumulate in the soil. The high content of Fe due to physical conditions like salinity, temperature, pH of the medium, and presence of other metals influence the process of uptake and accumulation of Fe in medicinal plant. The concentration of metals (Ni, Cu and Fe) is more than toxic level. Hence there is need to have some safety measure to overcome this environmental metal toxicity before using plant for nutritional and medicinal purpose.

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