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Research Article

DETECTION OF HEAVY METAL CONTENTS IN THE SEED OIL OF SOLANUM MALONGENA (EGG PLANT) OF ARID ZONE

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ABSTRACT
Determination of heavy metals in edible seed oil is of importance, as heavy metals are useful micronutrients for plants, humans and animals but become toxic for them when their concentration exceeds a limit. The study of heavy metal contamination in plants, serve to indicate the metal concentration status of the site, where plants have grown. This is an important tool for phytoremediation. A study was conducted on accumulation of heavy metals in seed oil of solanum malongena from arid resources of Rajasthan, India. Mrystic, palmitic, stearic, arachidic, myristoleic, palmitoleic, oleic, linoleic and gadoleic fatty acids were observed in the seed oil sample of solanum malongena. An atomic absorption spectroscopic method has been used for the determination of concentration of metals (Cd, Zn, Fe, Ni and Pb) in seed oil sample of solanum malongena after open vessel digestion. The concentrations of metals Cd (39.32 μg/L), Fe (17.53 μg/L), Pb (20.05 μg/L), Zn (12.24 μg/L) and Ni (0.139 μg/L) were observed. The presences of these heavy metals such as (Cd, Zn, Fe, Ni and Pb) in higher concentration are known to have adverse effect on growth of plants, animals and human health.

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

INTRODUCTION
Heavy metals are naturally present in the environment. Their occurrence, however, has gradually been increasing with the increase of industrialization. Agricultural soils, as an essential part of the environment, are no exception of this phenomenon. Cadmium (Cd), lead (Pb), and Zinc (Zn) are among the most abundant heavy metals in the agricultural soils (Förstner, 1995). Nickel and Zinc, when present in low concentrations, are important micronutrients, while in high concentrations, these two metals become toxic to plants (Lester G (1997)). Zn has ability to occupy low symmetry site in enzyme (Olivares, Uauy 1996), Ikeda and Murakami (1995) and Corn (1993)) and cause disturbance in enzymatic function. As far as non-essential elements are concerned, 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. Cadmium and its compounds are also toxic to humans. They produce acute and chronic symptoms varying in intensity from irritation to extensive metabolic disturbances. The levels of toxic metals (Cd and Pb) were determined in seed oil sample of egg plant. Although Cadmium and lead have unknown role as nutrients, but plants readily accumulate them in their system (Mido, Satake 2003).
Iron typically damages cells in the heart and liver which can cause cancer, coma, metabolic acidosis, liver failure, circulatory shock and long-term organ damage.
The ability of plants to accumulate heavy metals is used in the process of phytoremediation where the green plants are employed to cleanness of contaminated soils (Vangronsveld *et al.*, 2000).
Phytoremediation refers to the natural ability of certain plants called hyper accumulators to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air. Metals, pesticides, solvents, explosives etc are Contaminants. Phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology, as opposed to mechanical cleanup methods such as soil excavation or pumping polluted groundwater. Heavy metals like Cd, Co, Cu, Ni, Zn, and Cr are phytotoxic either at all concentrations or above certain threshold levels. Toxic metals are biologically magnified through the food chain. They infect the environment by affecting soil properties its fertility,

biomass and crop yields and ultimately human health. It is a big issue of accumulation of heavy metals in soils as a result of industrial effluents and atmospheric emissions like paper mill, fertilizers, glasses and Mining wastes (Baker and Brooks (1994) and Baker *et al.,* 1989). Certain plants absorb these toxic metals and help to clean up them from soils these plants are termed hyper accumulators. These plants have been shown to be resistant to heavy metals and are capable of accumulating them into their roots and leaves and transporting these soil pollutants to high concentrations. Phytoremediation of toxic metals from the contaminated soil basically involves the extraction or inactivation of these metals in soils. Phytoremediation is one new approach that offers more ecological benefits and a cost efficient alternative. One major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on plant growth, tolerance to toxicity, and bioaccumulation capacity (Angle *et al.,* 2001) and Chaney 1983).

Egg plant, *Solanum melongena* is a common and popular vegetable crop grown in the subtropics and tropics. It is called brinjal in India. Egg plant is a perennial but grown commercially as an annual crop. The name egg plant derives from the shape of the fruit of some varieties, which are white and shaped very similarly to chicken eggs **(**Chadha 1993). Egg plant is a common vegetable on our diet. Egg plant is a bushy plant and grows to a height of 60 to 120 centimeters. The plant is erect, compact, and well branched. It has a rather fibrous or lignified root system. The leaves are large, simple, lobed and alternate on the stems. The flowers are large, violet- or white-colored, and solitary, or in clusters of two or more. The stems, leaves, and calyx of some cultivars are spined. The fruit is a pendant, fleshy berry (Tsao and Lo 2006) and Doijode (2001). The shape of fruit varies from ovoid, oblong, obovoid, or long cylindrical; the color of fruit varies from (shiny) purple, white, green, yellowish, or striped. The seeds are borne on the fleshy placentae filling the locular cavity completely. Its composition per 100 g of edible portion is Calories 24.0Kcal, Moisture content 92.7%, Carbohydrates 4.0%, Protein 1.4 g, Fat 0.3 g and vitamins 130g. Bitterness in egg plant is due to the presence of glycol alkaloids which are of wide occurrence in plants of Solanaceae family. It may contain certain medicinal properties because medicinal uses of egg plant have been reported. For example, white egg plant is good for diabetic patients. It can cure toothache if fried egg plant fruit oil is taken. It has also been recommended as an excellent remedy for those suffering from liver complaints and asthma (Alcodia and Cabanting 1996).

The aim of the present work is to investigate contents of heavy metals accumulated in seed oil of egg plant. For this purpose, the concentrations of Pb, Zn, Cd, Fe, and Ni were measured for the estimation of heavy metal pollution. The high concentration of heavy metals in soils is reflected by higher concentrations of metals in plants, and consequently in animal and human bodies. The ability of some plants to absorb and accumulate heavy metals makes them useful as indicators of environmental pollution.

MATERIALS AND METHODS

Sampling: The seeds of egg 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 (60-80^{0}C) in a Soxhlet apparatus for 6hr. 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 and Concepts 2002).This method is simple and very selective. In this

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paper we present determination of heavy metals in seed oil of egg 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 aqua regia (1:3) HCL and HNO3, made up to 1liter in volumetric flask by adding deionized water. This is a stock solution which contain about 1000µg/L of required metal and then the working standard solution are 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 require 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 take 1g of seed oil add10 ml Concentrate Nitric acid .Keep it 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 and then filtered after cooling. Finally volume of the extract was made up to 25 ml using double distilled water.

Fatty acid analysis: The fatty acids composition of egg 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 were 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 & Armstrong 2000). The physico-chemical properties such as Saponification value, acid value, iodine value and peroxide value of the Solanam melongena seed oils were determined, using the method describes by AOCS (Adelaja 2006) and Nukhet *et al.*, 2001).

RESULTS AND DISCUSSION

Fatty acid analysis results: The chemical compositions of fatty acids of egg plant seed oil and methyl esters from Soxhelet extraction observed were - Mrystic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), myristoleic (14:1), palmitoleic (16:1), oleic (9c-18:1), linoleic (9c, 12c-18:2) and gadoleic (20:1). Unsaturated fatty acid as myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic (9c, 12c, 15c-18:3) acid and gadoleic (9c-20:1).

Physico-chemical properties:

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

Table 1: Physico-chemical properties of the egg plant seed oils.

Oil properties	
Acid value (mg KOH/g oil)	0.361
Saponification value (mg KOH/g oil)	160.2
Iodine value (g I/l00 g oil)	146.8
Peroxide value (mmol peroxide/kg oil)	19.0

Mineral metal analysis: 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

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depending on environmental conditions, 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, 2001; 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.

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*

In AAS the absorbance is linearly related to concentration. So for the determination of concentration of metals in plant sample by AAS the sample volume, ramp and hold time for digestion were kept optimized before analysis to obtain maximum absorbance and minimum background.

The use of HNO3/ HCL mixture in digestion of seed oil sample allow the determination of total content of heavy metals analyzed in seed oil sample of egg plant.

For the analysis of metal take the hollow cathode lamp of related metal in the operating position, adjust the current, select the appropriate resonance line and adjust the operating conditions to give a fuel lean air-acetylene flame.

Starting with the least concentrated solution, then aspirate successively the standard solution of metals in to the flame and finally the test solution, in each case absorbance is recorded.

Concentrations of heavy metals in the analyzed samples are given in Table 3.

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.

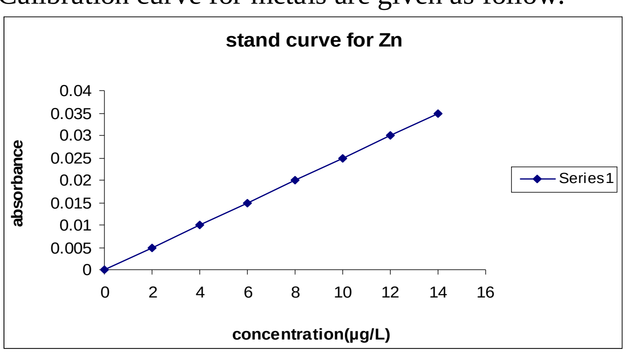

Figure1: Calibration curve for Zn

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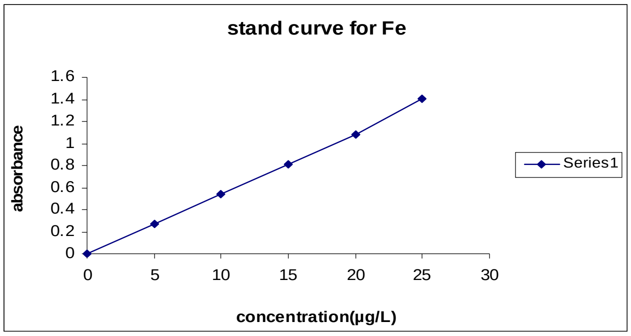

Figure 2: Calibration curve for Fe

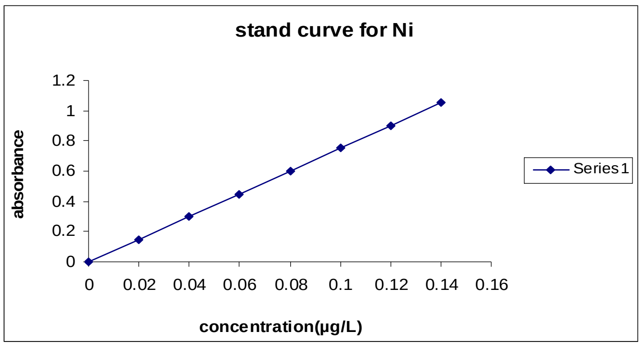

Figure 3: Calibration curve for Ni

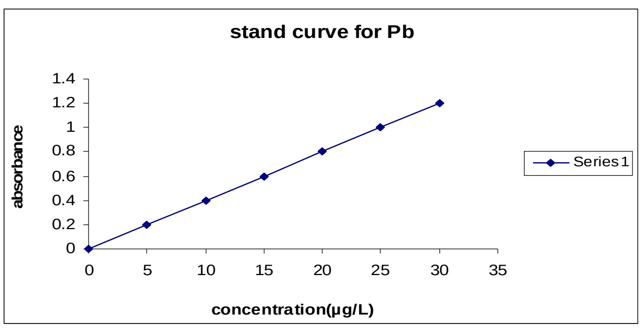

Figure 4: Calibration curve for Pb

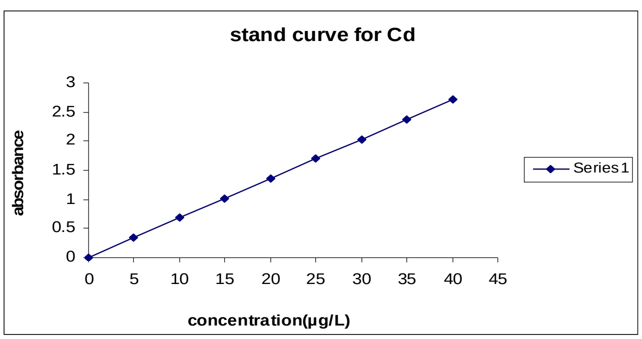

Figure 5: Calibration curve for Cd

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

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

Metal	Clock Interval (in seconds)	Standard Deviation	Related Standard Deviation%	Concentration in µg/L
Zn	27.44	1.918	15.66	12.24
Pb	1.970	4.053	20.21	20.05
Fe	5.779	5.198	29.66	17.53
Ni	6.223	0.9740	716.7	0.1359
Cd	25.97	21.24	54.03	39.32

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 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. Results of the study proves that egg plant accumulate heavy metals in seed oil. The concentration of metal accumulated in seed oil of plant depending on environmental condition, metal species, form of heavy metals, collecting site of sample and distance from the source of pollution. From the results it is found that the concentration of cadmium and lead is too high. The reason for the accumulation of cadmium is that cadmium is relatively easily taken up by food crop. The concentration of lead in sample is depending upon traffic volume, high concentration of lead show high traffic volume around the site where plants have grown. Thus, the plant can be recommended as bioindicators for determination of pollution levels of the environment.
Research results also show that plant has significant potential to use for remediation of heavy metals from soil.

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