Heavy metal analysis in *Ziziphus nummularia* leaf stem and fruit parts

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Abstract: The present study was aimed to investigate the heavy metal of the leaves, Bark and fruit of *Ziziphus nummularia* belonging to family Rhamnaceae. This medicinal plants from local area Dehradun India and discusses a possible correlation between their curative effects and their trace elements content. Further, a possible accumulation of adverse heavy metals could be excluded (Lead, cadmium, arsenic and mercury) were determined using inductively coupled plasma (ICP)-mass spectrometry (ICP-MS), ICP-sector field-mass spectrometry (ICP-sf-MS) techniques. The results of the present study showed no heavy metal accumulation in the plants. Pb, Cd, As and Hg were found only in trace concentrations significantly below the global limits. This justifies its use in the traditional medicine for the treatment of different diseases such as ulcer, asthma, dysentery and fever.

1.0 Introduction

Heavy metals are metals and metalloids, which have atomic weights between 63.5 and 200.6 g/mol, and density greater than 4.5 g/cm3. Heavy metals are conservative in nature, meaning they persist in the environment for longer periods. According to Pekey (2006), heavy metals are deemed serious pollutants because of toxicity, persistence and non-biodegradability in the environment. Heavy metals such as mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), selenium (Se), copper (Cu), zinc (Zn), chromium (Cr) and vanadium (V) are considered potentially hazardous (Lata & Rohindra, 2002) to the human health and the environment. "Heavy metals" are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Lide 1992). There are 35 metals that concern us because of occupational or residential exposure; 23 of these are the heavy elements or "heavy metals": antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc (Glanze 1996). Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning). Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are not uncommon and repeated long-term contact with some metals or (their compounds) may even cause cancer (International Occupational Safety and Health Information Centre 1999).

WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1989, 1998). Medicinal herbs are easily contaminated during growth, development and processing. After collection and transformation into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions. Plants are able to take up and accumulate certain environmental contaminants such as heavy metals. When the plants are ingested by man, these contaminants are transferred along the food chain. Due to the poorly regulated medicinal plant trade in India, many opportunities exist for heavy metal contamination of medicinal plants namely contaminated harvest sites as well as poor drying, processing, storage, transport and manufacturing conditions. With the unregulated medicinal plant trade in many developing countries, several opportunities for contamination exist. According to WHO (2003) potentially harmful contaminants in medicinal plants may come from Environments where the

medicinal plants are grown and conditions where they are collected, Conditions under which they are dried and processed, Transport and storage conditions; and/or, Manufacturing processes during the final stage of preparation. Despite the popularity of traditional medicines, scientific research on safety and efficacy is limited. However documented fatalities and severe illness due to lead poisoning are increasingly recognized to be associated with traditional medicinal use. Contamination by heavy metals is among the main problem related to phytotherapy. Contamination with heavy metals generally originates from polluted irrigation water, particulate air material, polluted soils and in appropriate storage conditions. Despite this, analytical methodology to determine heavy metals in medicinal plants has not received the same research effort as has been dedicated to the evaluation of phytotherapeutic properties.

In the present day scenario, the importance of herbal drugs is increasing due to their lesser side effects and acceptability to the majority of the population of third world countries. Thus, there is an urgent need to establish the identity, purity and quality assurance of herbal products in order to have full efficacy and safety.

In the proposed work, measurable amounts of heavy metals- Cd, Hg, As and Pb was detected in *Z. nummularia* different plant parts (Leaves, stem & Fruits) and compared with the permissible limits of these heavy metals in herbal drugs as stated by WHO and AYUSH for the quality control of herbal drugs. The permissible limits for these heavy metals are 10ppm, 1ppm, 0.3ppm and 3ppm for lead, mercury, cadmium and arsenic respectively. This will allow reliable determination of heavy metal content in *Ziziphus nummularia* quality control.

Table 1 **National limit for heavy metals in medicinal plants/herbal products Country wise**

Country	**As (Arsenic)**	**Cd (Cadmium)**	**Cr (Chromium)**	**Cu (Copper)**	**Hg (Mercury)**	**Pb (Lead)**
India	3 ppm	0.3 ppm	-	-	1.ppm	10 ppm
Canada	5 ppm	0.3 ppm	2 ppm	-	0.2 ppm	10 ppm
China	2 ppm	1 ppm	-	-	0.5 ppm	10 ppm
Malaysia	5 mg/kg	-	-	-	0.5 mg/kg	10mg/kg
Singapore	5 ppm	-	-	150 ppm	0.5 ppm	20 ppm
Thailand	4 ppm	0.3 ppm	-	-	-	10 ppm
Europe	15 mg/kg	7 mg/kg	-	-	1.6 mg/kg	25 mg/kg
Philippines	0.3 ppm	0.3 ppm	-	-	0.5 ppm	10 ppm
Tanzania	5 ppm	0.3 ppm	-	-	0.5 ppm	10 ppm
W.H.O	1mg/kg	0.3mg/kg	-	-	-	10mg/kg

2.0 Materials and Method

2.1 Plant material

Fresh parts (Leaves, Bark and fruit) of *Ziziphus nummularia* was collected from the Local area of Dehradun, Uttarakhand India. Identification and confirmation were done by Department of Botany, Forest research Institute (FRI), Dehradun India. Where voucher specimens were deposited. The above fresh material were dried under shade, powdered and pass through 40 mesh sieve and stored in closed container for further use.

2.2 ICP-MS Method:

Prepare the standard solution [Lead, cadmium, Arsenic and Mercury] or as per the requirement containing the element of interest by the following method.

2.2.1 Preparation of multiple standard solutions:

Reference standard solution (*100 ppm*): Standard solution of *100 ppm* supplied by Merck.

Stock standard solution (*100 ppb*): From reference standard solution prepare stock standard solution containing *1000 ppb* by diluting 0.5 ml of reference standard solution to 50 ml with Milli-Q water.

Working standard solutions: From stock standard solution prepare working standard solution containing *100 ppb, 50 ppb, 25 ppb, 10ppb, 5 ppb* and *1 ppb* of standard with Milli-Q water.

2.2.2 Preparation of working standard solution:

100 ppb- pipette out the 5 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

50 ppb- pipette out the 2.5 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

25 ppb- pipette out the 1.25 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

10 ppb- pipette out the 0.5 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

5 ppb- pipette out the 0.25 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

1 ppb- pipettes out the 0.05 ml of stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

2.2.3 Preparation of Mercury standard solution:

Reference standard solution (*1000 ppm*): Standard solution of *1000 ppm* supplied by Merck.

Stock standard solution (*10 ppm*): From reference standard solution, prepare stock standard solution containing *10 ppm* by diluting 1 ml of reference standard solution to 100 ml with Milli-Q water.

Stock standard solution (*100 ppb*): Dilute 1.0 ml of primary stock solution containing 10 ppm to 100 ml with Milli-Q water.

Working standard solutions: From the secondary stock standard solution prepare working standard solution containing *10 ppb, 7.5 ppb, 5 ppb, 2.5 ppb,* and *1 ppb* of Mercury with Milli-Q water.

2.2.4 Preparation of working standard solution:

10 ppb- pipette out the 5 ml of secondary stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

7.5 ppb- pipette out the 3.75 ml of secondary stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

5 ppb- pipette out the 2.5 ml of secondary stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

2.5 ppb- pipette out the 1.25 ml of secondary stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

1 ppb- pipette out the 0.5 ml of secondary stock standard solution to 50 ml volumetric flask and diluted with Milli-Q water and make up the volume to 50 ml with same solvent.

2.2.5 Standard blank preparation: Milli-Q water used as standard blank.

2.2.6 Sample preparation: Weigh accurately about 0.5 gm of finely powdered sample into the Teflon vessel of microwave digestion system and add 7 ml of Nitric acid (supra pure / highly pure). Swirl gently to complete the wetting. Close and keep the vessel inside the microwave digestion system. Digest the sample at 170° C for 25 minutes. After the digestion, cool the vessels and open it. Filter the sample and make up to 50 ml with Milli-Q water.

2.2.7 Sample blank solution:

Prepare the blank sample solution without sample using 7 ml of nitric acid and follow the same as mentioned under sample preparation.

2.3 Analysis:

2.3.1 Stabilization: Stabilize the instrument as per the standard operating procedure of the instrument and check the performance as per SOP.

Aspirate the tuning solution and record the count / intensity should be above the acceptable range as per the limits laid down in the SOP. Choose the element to be analyzed in the instrument software as per SOP.

2.3.2 Standard Solution: Aspirate the standard blank solution followed by working standard solution into the ICP-MS system and checks the performance of the instrument by the count / intensity. Create the standard calibration curve for each element and ensure that the R^2 values of each curve are above 0.99.

2.3.3 Sample solution: Aspirate the sample blank and followed by sample solution and record the count / intensity.

2.3.4 Calculation:

The concentration of each element can be calculated by feeding the sample dilution in the **instrument soft ware** or using below formula.

$$\text{Content of element } (ppb) = \frac{C_1 - C2}{\text{Weight of sample (gm)}} \times \text{Total volume of sample in ml}$$

$$\text{Content of element } (ppm) = \frac{C_1 - C2}{\text{Weight of sample (gm)}} \frac{1}{1000} \times \text{Total volume of sample in ml}$$

C_1 – concentration of element in sample solution in ppb
C_2 – concentration of element in sample blank solution in ppb

Alternatively the concentration of each element can be calculated manually using the following formula.

$$\text{Content of element } (ppb) = \frac{C_1 - C2}{C_3 - C_4} \frac{S}{W} \times V$$

$$\text{Content of element } (ppm) = \frac{C_1 - C2}{C_3 - C_4} \frac{S}{W} \times V$$

C_1 - Counts/intensity in sample solution
C_2 - Counts/intensity in sample blank solution
C_3 - Counts/intensity in standard solution
C_4 - Counts/intensity in standard blank solution
S – Standard concentration in ppb
T – Total sample volume made in ml
W – Weight of sample in g

3.0 Result

From the study, Lead (Pb) was below the detection limit. Cadmium was also within the permissible limits, Arsenic (As) and Mercury (Hg) was also below the detection limit. Thus we have observed that in the plant parts of Ziziphus nummularia the heavy metals (pb, Cd, As, Hg) were below the detection limit [Tables 2] shown in histogram fig: 1.

Table: 2 Heavy metal analysis of *Ziziphus nummularia* Leaves, Stem and Fruits

Plant Name	Parts used	*Lead (pb)*	*Cadmium (Cd)*	*Arsenic (As)*	*Mercury (Hg)*
Ziziphus nummularia	Leaves	3.48	0.10	0.51	0.01
	stem	1.16	0.03	0.31	0.03
	fruit	2.98	0.11	0.84	0.01

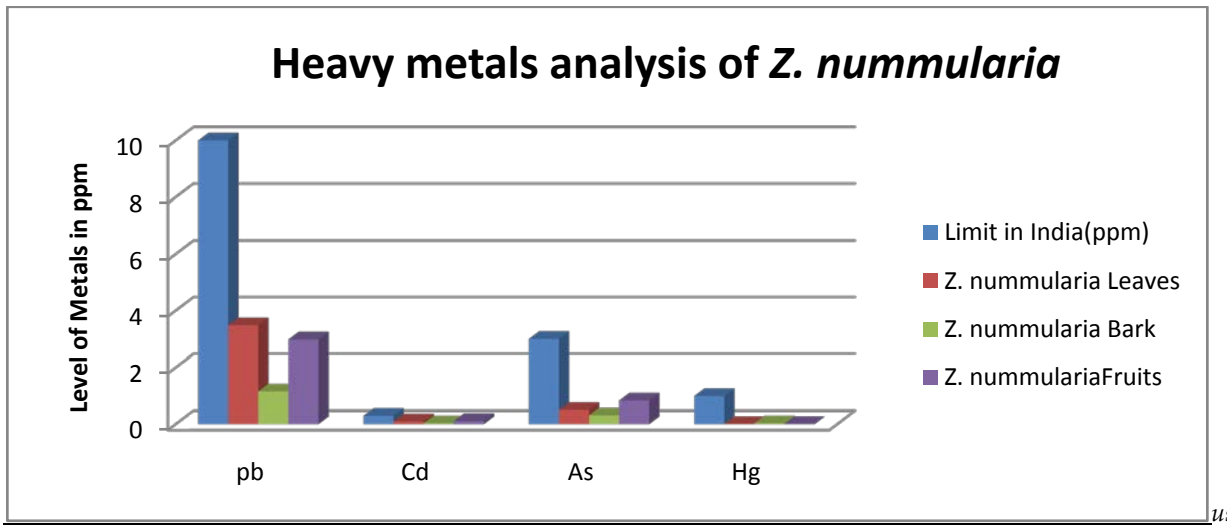

Fig: 1 Heavy metals analysis of *Z. nummularia* Leaves, Stem and Fruits

4.4 Discussion

A comprehensive analysis of published data indicates that heavy metals such as arsenic cadmium, chromium, lead, and mercury, occur naturally. In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang S, Shi X, 2001). Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis (Chang LW et al 1996, Beyersmann D & Hartwig A 2008). Several studies have demonstrated that reactive oxygen species (ROS) production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic (Yedjou CG & Tchounwou PB 2006, Yedjou CG & Tchounwou PB 2007, Tchounwou PB et al 2004), cadmium [Tchounwou PB et al 2001], chromium (Patlolla A et al 2009, Patlolla A et al 2009), lead [Yedjou GC & Tchounwou PB 2008, Tchounwou PB et al 2004], and mercury (Sutton DJ & Tchounwou PB 2007, Sutton D et al 2002). Because of their high degree of toxicity, these five elements rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. According to the United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as either "known" or "probable" human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals. Recent reports have pointed out that these toxic elements may interfere metabolically with nutritionally essential metals such as iron, calcium, copper, and zinc (López Alonso M et al 2004, Abdulla M & Chmielnicka J 1990). They are important constituents of several key enzymes and play important roles in various oxidation-reduction reactions (WHO/FAO/IAEA 1990). However, each metal is known to have unique features and physic-chemical properties that confer to its specific toxicological mechanisms of action. Medicinal plants have been cited as a potential source of heavy metal toxicity to both man and animals.(Arruti A et al 2010) The most common heavy metals implicated in human toxicity include lead, mercury, arsenic, and cadmium, although aluminum and cobalt may also cause toxicity. Therefore, the world health organization recommends that medicinal plants, which form the raw materials for most herbal remedies, should be checked for the presence of heavy metals. From the study, the levels of these metals were detected in *Ziziphus nummularia*. The plant parts collected from local area of Dehradun and then authenticated from Forest Research Institute, Dehradun. This work provides an analysis of the environmental occurrence and exposure of arsenic, cadmium, lead, and mercury from the *Ziziphus nummularia* species.

4.5 Conclusion

Our study has shown that *Ziziphus nummularia* which are traditionally used as medicinal plants, growing in Dehradun India locations, have low levels or within the permissible limit of heavy metals. Thus, medicinal plants for the formulation of herbal remedies should be harvested from pollution-free natural habitat. Our findings further indicate that the medicinal plants, used for local or pharmaceutical purposes, should be collected from areas not contaminated with heavy metals. It is, therefore, advised that the metal content in medicinal plants be checked for levels of heavy metals before their use for local and pharmaceutical purposes.

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