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# Trace metal analysis of the leaves of *Azima tetracantha* lam

**Abirami H[1*], Ilamathy P[2]**
[1-2] Associate Professor, Department of Biotechnology, Cauvery College for Women (Autonomous), Tiruchirappalli, Tamil Nadu, India

**Abstract**
Heavy metals, also known as trace metalss are naturally occurring elements which are required in trace concentrations for the development and life processes of an organism. The various applications of heavy metals in industrial, agricultural, domestic, medical and technological applications have led to their wide distribution in the environment. This has raised concerns over their potential effects on human health and environment. The toxicity of the heavy metals depends on the concentration, route of exposure, duration of exposure as well as the age, gender and genetic makeup of the individual who is getting exposed. *Azima tetracantha* Lam. is a medicinal plant commonly found in India which has many medicinal and therapeutic values. The various parts of the plant have been administered to treating pyrexia, dropsy, dyspepsia, chronic diarrhea, diabetes and arthritis. As the plant is meant for consumption, it is needed to assess the concentration of trace metals present or accumulated in the plant before exploiting the medicinal values of the plant and its parts. This article aims for the same and reveals the concentration of trace elements Iron, Copper, Nickel, Cadmium, Chromium, Lead and Zinc which are found to be the predominantly occurring heavy metals in the polluted environment.

**Keywords:** heavy metals, toxicity, pollution, trace metals, *azima tetracantha* lam.

## 1. Introduction
India is a land of biodiversity. It is known for its diverse resources including flora and fauna. During the pandemic situation, medicinal plants and herbs are gaining much interest to explore the possibilities of developing therapeutic drugs and vaccines from the phytochemical compounds or bioactive compounds present in them. India has a history of developing therapeutic drugs and therapeutic compounds from various medicinal plants and herbs. The phytochemical compounds present in the medicinal plants either act as potent chemotherapeutic compounds or they may also act as lead molecules for drug designing and Molecular modelling.

The interest in our country for these plants is much greater because of the possibility of exportation. From plant nutrition studies, it is known that plants require a certain amount of trace elements (also referred to as heavy metals) that they respond differently to an enhanced or lowered trace element supply, and that, in some cases, agricultural products may be contaminated with toxic heavy metals (Krug, 1986) [1].

Several investigators have performed several studies on the residual levels of toxic metals in medicinal herbs (Schilcher, 1982; Ali, 1983; Peters and Schilcher, 1986; Schilcher *et al*., 1987) [2, 3, 4, 5]. Most studies on residual levels of toxic metals in medicinal herbs have focused on lead, cadmium, and mercury (Schilcher, 1986; Schilcher *et al*., 1987) [5].

The accumulation of heavy metals in some desert plants may open a new perspective for application of these species as 'accumulators' of heavy metals to clean-up contaminated soils in arid environments.

The medicinal plants such as Withinia coagulas, Sarcococca saligna, Cronopus didymus, Senecio chrysanthamoides, Aerva javanica, Vinca major, Salvadora sps (yellow), Impatiens walleriana, Pteris vittata, Calotropis procera, Eichhornia crassipes, Pinus walliachiana were subjected for the trace metal analysis including Magnesium, Potassium, Chromium, Copper, Nickel, Iron, Arsenic, Cobalt, Lead and Cadmium. It was concluded that lead was present in the highest amount among all the plants. Among all the plants, Pinus walliachiana contained the highest amount of lead 450.60 ppb. Other metals were also present but their concentration was less as compared to lead (Naeem *et al*., 2009) [6].

A.baccifera and A.tetracantha showed the toxic elements Pb, Cd, Hg and As at BDL (Below Detectable Limit) (DL 0.1 mg $kg^{-1}$) and in M.madaraspatana, toxic elements Cd and Pb were found but at very low concentrations whereas Hg and as were found at Below Detectable Level (Shanthy *et al*., 2013) [7]. The concentration of heavy metals such as Cobalt (Co), Nickel (Ni), Chromium (Cr), Copper (Cu), Iron (Fe) and Zinc (Zn) and non-essential heavy metals Cadmium (Cd), Lead (Pb) were analyzed in Aloe vera leaves and Tamarix aphylla by using Flame atomic absorption spectrophotometer which showed that Tamarix aphylla was found to be resistant to heavy metals uptake as compared to Aloe vera (Iqbal *et al*., 2013) [8].

Chemical profiling of nine heavy metals (Mn, Cr, Pb, Fe, Cd, Co, Zn, Ni and Hg) was undertaken in stem and leaf samples of ten medicinal plants (Acacia nilotica, Bacopa monnieri, Commiphora wightii, Ficus religiosa, Glycyrrhiza glabra, Hemidesmus indicus, Salvadora oleoides, Terminalia bellirica, Terminalia chebula and Withania somnifera) collected from environmentally diverse regions of Haryana and Rajasthan states in North-Western India. Concentration of all heavy metals, except Cr, was within permissible limits in the tested stem and leaf samples. Leaf samples had consistently more Cr compared to respective stem samples with highest concentration in leaf samples of Bacopa monnieri (13.19 ± 0.0480 ppm) and stem

samples of Withania somnifera (4.93 ± 0.0185 ppm). This amount was beyond the permissible limit of 2.0 ppm defined by WHO for raw herbal material and suggested the cultivation of medicinal plants and other dietary herbs should be curtailed near environmentally polluted especially industrial areas for avoidance of health hazards (Kulhari *et al*., 2013)[9].

Considering the above, the present study was carried out to find out the concentrations of Fe, Cu, Zn, Pb, Cd, Cr and Ni in the leaves of Azima tetracantha.

## 2. Materials and Methods

### 2.1. Collection of Plant Material

Fresh Azima tetracantha leaves were collected during January 2020, in and around Keelapaluvur, Ariyalur District, and Tamilnadu, India. The plant has been identified by RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's College campus, Tiruchirappalli, Tamil Nadu, India and a voucher specimen no. RHT. 63874 have been deposited in the herbarium.

### 2.2. Experimental Protocol and Procedure

Fresh Azima tetracantha leaves were carefully removed and washed with running tap water and then with double distilled water. The cleaned leaves were shade dried and were ground with mortar and pestle. After drying, 1 g of leaves samples was treated with aqua-regia mixture in Teflon bomb and was incubated at 140°C for 2-3 days. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. Then the extraction was analyzed for trace metals (Fe, Cu, Zn, Pb, Cd, Cr and Ni) analysis. The concentrations of the studied metals in the solutions were determined by the 797 VA Computrace voltametry, Metrohm. To avoid contamination, the devices were rinsed with acidified water (10% $HNO_3$). All the equipments and containers were soaked in 10% $HNO_3$ for 24 h and rinsed thoroughly in de-ionized water before use. Below detectable limit (BDL) of the instrument was also determined.

## 3. Results and Discussion

The plant leaves exhibited the concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn as 0.05, below detectable level (BDL), BDL, 0.18, 0.42, BDL, 0.06 and 0.38 mg $kg^{-1}$ respectively (**Table 1**). Normally in the raw plant, the toxic metals (Cu, Pb and Cd) concentrations would be high due to its open exposure, land absorptions but the leaves of Azima tetracantha revealed the higher concentration of Fe as 0.42 mg $kg^{-1}$ followed by Zn in the concentration of 0.38 mg $kg^{-1}$.

Atmosphere and soil are continuously being polluted with chemicals and heavy metals due to dynamic development of industries and motorization along with extensive use of pesticides and fertilizers. In turn, these pollutants and heavy metals are getting deposited in the plants. The trace metals Cd, Cr, Cu, Fe, Ni, Pb and Zn were found in the leaves of Azima tetracantha but the concentrations were recorded within the permissible limits and the levels are 0.05, below detectable level (BDL), BDL, 0.18, 0.42, BDL, 0.06 and 0.38 mg $kg^{-1}$ respectively.

Cadmium (Cd) is another hazardous heavy metal which can cause significant reduction in plant yield at concentrations ranging from 5–30 mg/kg. Recently, it is gaining more attention due to wide occurrence in water, soil, milk, dietary products, medicinal plants and herbal products. The major sources leading to accumulation of cadmium in soil and plants are phosphate fertilizers, non-ferrous smelters, lead and zinc mines, sewage sludge application and combustion of fossil fuels. At the same time, the leaves of Azima tetracantha revealed the presence of Cd within the acceptable range that is 0.5 mg $kg^{-1}$.

Chromium (Cr), regarded as one of the most toxic pollutants in the world is released by tanneries, steel industries, and sewage sludge applications along with alloys in motor vehicles. Its concentrations between 5–30 mg/kg are considered critical for plants as it causes heavy reduction in plant growth and yield. The permissible limit for Cr in raw herbal materials is 2.0 ppm and that for finished products is 0.02 mg/day (WHO, 2007) [10]. However the leaves of Azima tetracantha have Cr concentrations below detectable limit (BDL).

The concentration of copper was recorded as 0.18 mg $kg^{-1}$ in the leaf sample of Azima tetracantha. Critical concentration for copper in plants is in between 20-100 mg/kg. Maximum permissible limit (MPL) for Copper (Cu) recommended by World Health Organization (WHO) is 10 mg $kg^{-1}$ while the daily dietary intake (DDI) is 2- 3 mg $kg^{-1}$ (Gupta, 1975) [11]. High levels of copper may cause metal fumes fever with flue like symptoms, hair and skin discoloration, dermatitis, irritation of the nasal mucosa and nausea (Shad *et al*., 2008) [12].

Iron (Fe) is an important trace element and iron protein mixtures play a vital role in metabolism in all living organisms. However, Fe overdose has been one of the leading reasons of death caused by toxicological agents in children younger than 6 years of age. Individuals demonstrate signs of gastro-intestinal toxicity after ingestion of more than 20 mg/kg iron while moderate intoxication occurs when ingestion of elemental Fe exceeds 40 mg/kg and ingestions exceeding 60 mg/kg can cause severe toxicity and may be lethal also (Spanierman, 2011) [13]. But the concentration of iron was found to be 0.42 mg $kg^{-1}$ in the leaves of Azima tetracantha. On the other hand, Fe was present beyond the acceptable range 1000 µg/day in Ocimum canum, Clausena anisata and Rauwolfia vomitoria (Annan *et al*., 2010) [14].

Nickel (Ni) is a trace element required for a variety of biological processes. Ni is directly coordinated by proteins (Zhang *et al*., 2009) [15]. Nickel was recognized as an allergen of the year in 2008 by the American Contact Dermatitis Society and its minimal risk level was set to be 0.2 µg/$m^3$ for inhalation during 15 – 364 days, however, no limit has been set for food stuffs (Bhat *et al*., 2010) [16]. In the present investigation, the leaves of Azima tetracantha consist of Ni in below detectable level.

Lead (Pb) is highly hazardous for plants, animals and microorganisms. Continuous consumption of fertilizers, fuel combustion and sewage sludge are the major reasons leading to escalation in Pb pollution. The permissible limit of Pb is10 ppm defined by WHO (2007) [10]. However the concentration of Pb in the leaf samples was found to be 0.06 mg $kg^{-1}$.

Zinc (Zn) is an essential component of thousands of proteins in plants, although it is toxic in excess quantities. In the present investigation, Zn concentration was found to be 0.38 mg $kg^{-1}$ in Azima tetracantha leaves. The level of Pb, Cd, Cu and Zn in various fruits and vegetables sold in Egyptian markets were evaluated and their concentrations were found to be significantly higher but was noted below the acceptable range in strawberries, cucumber, date and spinach (Radwan and Salama, 2006) [17]. On the other hand, level of four most hazardous heavy metals Pb, Cd, Cu and Cr were found beyond the permitted limits in Glycyrrhiza

glabra, Onosma bracteatum, Viola odorata, Foeniculum vulgare, Cuminum cyminum, Coriandrum sativum and Zingiber officinalis collected from southern, eastern, and western zones of Karachi, Pakistan (Hina *et al*., 2011) [18].

**Table 1:** Trace Metals Analysis of the Leaves of *Azima tetracantha* Lam.

| Sampling Site Name | Sample Name | Cd* | Cr* | Cu* | Fe* | Ni* | Pb* | Zn* |
|---|---|---|---|---|---|---|---|---|
| Keelapaluvur, Ariyalur (DT), Tamil Nadu | *Azima tetracantha* | 0.05 | BDL | 0.18 | 0.42 | BDL | 0.06 | 0.38 |

BDL- below Detectable Limit *Concentration expressed in mg/kg on Fresh Weight Basis

## 5. Conclusions

As the leaves of the plant are meant for consumption as therapeutic agents, the leaves were analyzed for trace metal levels. The plant leaves exhibited the concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn as 0.05, below detectable level (BDL), BDL, 0.18, 0.42, BDL, 0.06 and 0.38 mg $kg^{-1}$ respectively. The leaves of the plant *Azima tetracantha* revealed the higher concentration of Fe as 0.42 mg $kg^{-1}$ followed by Zn in the concentration of 0.38 mg $kg^{-1}$.

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