 ELEMENTAL ANALYSIS OF INDIGOFERA GERARDIANA WALL BY ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

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ABSTRACT

Background: Increase in frequency of environmental pollution particularly soil contaminated with heavy metals direct their uptake in human food through plant parts. Tissue precipitation of macro-nutrients and micro-nutrients during utilization of produces adversely affects one’s health. The current study is directed to assess and quantify the presence of metallic elements in whole plant of Indigofera gerardiana.

Methodology: The fresh specimen of Indigofera gerardiana Wall was collected from upper Dir. The sample of the plant was dried under shadow, cut into small pieces and grinded to coarse powder. Powder was then used to analyse for mac-ro nutrients by flame photometry and for micronutrients by atomic absorption spectrophotometer.

Results: The window of acceptability for metallic ion is being exceeded by Fe, Ni, Cr & Co in certain parts of plants but normal in others. Mostly, the micro-nutrients detected in leaves were beyond normal limits.

Conclusions: Our study showed the presence of substantial concentrations of micro- and macro-nutrients in samples of root, leaves and stem.

Keywords: Indigofera gerardiana, Medicinal plants, Micronutrients, Macro nutrients, AAS (Atomic Absorption spectrophotometer)

INTRODUCTION

Flora of Pakistan is rich in medicinal plants, which are therapeutically used. More than 1,000 species pos-sess medicinal properties which are used by local communities to cure different diseases. These plants are commonly used due to their affordability and less side effects. World Health Organization (WHO 2002) esti-mates that in developing countries about 80 % of the total population uses phytotherapy for their primary health care, and hence it is mandatory to check these plants for their safety, efficacy and potency. The Indigofera gerardiana is generally known as Ghorega, which is a species of Family Fabaceae (Leguminosae) and sub-family Faboideae. It is widely distributed in northern areas.

The whole plant extract is mostly used in hepatitis and whooping cough. It is also used as an anti-spasmodic, tonic and hypoglycaemic agents; the flowers, leaves and small shoots have a cooling and demulcent effect. They are used in treatment of leprosy and cancerous infection. Its leaves are used to cure abscesses. The alcoholic extract of the dried shoots is reported to have an anti-inflammatory activity; the root bark if chewed, helps to relieve the abdominal pain; it is also reported that its bark, leaves and roots have an anti-bacterial activity. The current study is directed to check the presence of metallic elements and also quantify it, in different parts of indigofera.
gerardiana.

## MATERIAL AND METHODS

### Collection of Plants

The fresh specimen of Indigofera gerardiana was collected from upper Dir, Khyber Pakhtunkhwa, Pakistan during the month of August as a whole plant. The sample plant was identified by a plant taxonomist.

### Chemical Reagents

Reagents used in elemental analysis were of analytic grade including nitric acid, perchloric acid, potassium iodide, sulphuric acid, oxalic acid, ascorbic acid, and standard test metals Ca, Na, K, Zn, Cu, Cr, Co, Cd, Fe, Pb, Ni and Mn (Sigma Aldrich).

### Method

Standard protocol was followed to arrange the samples (10). 1.00 ± 0.05 gram test sample powder was put in a flask to which 10 ml of conc. HNO₃ was added and was kept for 12 hours at room temperature, then 4 ml of HClO₄ was added. The solution was concentrated at 60°C on a hot plate until 1 ml of clear solution was left. After cooling, the deionized double distilled water was added followed by filtration through Whatman (# 42) filter paper. Finally, 100 ml solution was made with deionized water as the stock solution. The test sample was analyzed for the analysis of micro-nutrients using Flame Atomic Absorption Spectrophotometer (polarized Zeeman Hitachi 2000) and Flame Photometer (Jenway PFP7, UK) was used for the determination of macronutrients. Then the stock solution of standard test metals, containing 1000 ppm of each metal, was used. Afterwards, the diluted stock solution was prepared for the construction of standard calibration curve. All chemicals and reagents used in the study were of analytical grade.

## RESULTS

### Elemental analysis results

High level of Ca concentration was found in all parts of the plant. However for Na and K the values were within the permissible limits. Na concentration was 348.84, 376.26 and 589.99 ppm in stem, root and leaf respectively. K concentration was 3822.08, 4903.81 and 8199.91 ppm in root, stem and leaf respectively. The results for the micronutrient contents of the materials are listed in Table 1.

High concentration of Fe was found in all parts of the plant. However in Ni the concentration is high in leaf, i.e. 2.199 and within limit in stem and root, i.e. 1.59 and 1.28 respectively. Significant amount of Co was also observed. Cu values are within the permissible limit.

### Table 1: Micronutrient of the plant

<table>
<thead>
<tr>
<th>Metal</th>
<th>Leaves (ppm)</th>
<th>Stem (ppm)</th>
<th>Root (ppm)</th>
<th>Permissible Limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>589.99</td>
<td>348.84</td>
<td>376.26</td>
<td>2100-51340 (11)</td>
</tr>
<tr>
<td>K</td>
<td>8199.91</td>
<td>4903.81</td>
<td>3822.08</td>
<td>6380-36,600 (11)</td>
</tr>
<tr>
<td>Ca</td>
<td>32999.67</td>
<td>17342.76</td>
<td>22774.08</td>
<td>44-614 (11)</td>
</tr>
</tbody>
</table>

### Table 2: Macronutrients

<table>
<thead>
<tr>
<th>Metal</th>
<th>Leaves (ppm)</th>
<th>Stem (ppm)</th>
<th>Root (ppm)</th>
<th>Permissible Limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>1435.98</td>
<td>332.90</td>
<td>604.00</td>
<td>36-241 (11)</td>
</tr>
<tr>
<td>Ni</td>
<td>2.199</td>
<td>1.59</td>
<td>1.28</td>
<td>1.5 (12)</td>
</tr>
<tr>
<td>Cu</td>
<td>6.499</td>
<td>7.57</td>
<td>4.45</td>
<td>10 (12)</td>
</tr>
<tr>
<td>Cr</td>
<td>3.299</td>
<td>1.096</td>
<td>0.495</td>
<td>1.5 (12)</td>
</tr>
<tr>
<td>Pb</td>
<td>1.299</td>
<td>6.578</td>
<td>Nil</td>
<td>10 (2)</td>
</tr>
<tr>
<td>Mn</td>
<td>45.49</td>
<td>8.47</td>
<td>14.258</td>
<td>200 (12)</td>
</tr>
<tr>
<td>Co</td>
<td>1.599</td>
<td>1.196</td>
<td>0.9901</td>
<td>0.2 (12)</td>
</tr>
<tr>
<td>Cd</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>0.3 (2)</td>
</tr>
</tbody>
</table>
possible limits i.e. 6.499, 7.57 and 4.45 in leaf, stem and root respectively. Similarly, Pb and Mn values are also within the permissible limits. However, Cr levels were found high in leaf only whereas in stem and root they are within the range. The results for the micronutrient contents of the materials are listed in Table 2.

**DISCUSSION**

**Micronutrients**

Iron is the most abundant essential element in body tissues. Animals, plants and microorganism require optimal Fe levels for their body maintenance. According to WHO, approximately 48% of world’s pregnant women and 46% of the children suffer from anaemia. Iron deficiency affects immune response in different ways and causes alteration of brain functions i.e. irreversible. All plant parts possess significant concentrations of iron. The plant under study is not supposed to be a good natural source of iron because it exceeds the permissible limits as described in Table 2.

Earth’s crusts are natural source of Nickel. Its deficiency is not that much common as it is abundantly found in almost all types of food. Abnormalities associated with Nickel intoxication are skin allergies, variable degrees of cardiovascular and kidney system poisoning, lung fibrosis and activation of neoplastic transformation. Liver disorders may also be associated with its deficiency. Pancreas is its main source and has an important role in the synthesis of insulin. Permissible limit for Ni in plants is 1.5 ppm. Our data suggests that concentration of Nickel was exceeded permissible limit except in plant roots as shown in Table 2.

Copper is a crucial element for both plants and animals. It is important for different human metabolic systems. It is required for regulation of various bio-logical systems i.e. energy production, oxidation-reduction reaction, connective tissue formation, neurotransmitter synthesis and iron metabolism. Industry and sewage sludge, pesticides and fertilizers are the most abundant sources of copper in soils. Melting and cutting of copper may generate fumes, dust and inhaled exposure to which is hazardous to health. In-toxication of Cu may cause liver and kidney damage. Samples of all parts of the plant do not exceed the permissible limit (10 ppm).

Chromium is crucially required for the metabolism of proteins, carbohydrates and lipids, biosynthesis of cholesterol and fatty acids, and also facilitates the action of insulin. For the very reason, supplements having Cr are used to improve weight loss. Chromium exposure is related with Cr toxicity and symptoms are skin rashes, respiratory problems, liver and kidney damage, weakened immune systems, stomach upset, ulcers, mutation of genetic material, lung cancer and ultimately death. Permissible limit of Cr in plants is 1.5 ppm. Leaves of plants exceed the normal permissible limits i.e. 3.29 ppm, while stem and root have Cr in normal range i.e. 1.096 and 0.495 ppm, respectively.

Lead (Pb) is highly poisonous metal and affects almost every organ of the body. Long term exposure to lead may alter function of nervous system. In pregnant women increase level of lead may cause miscarriage and in male reduces fertility. Excessive level of lead may be associated with delaying the puberty in girls. A permissible limit in plants is 10ppm. The leaves and stem of Indigofera gerardiana have lead in normal permissible limits i.e. 1.29 and 6.5 ppm respectively, while absent in roots.

One of other essential metallic element for growing plant and animal is Manganese. Parkinsonism is associated with intoxication of Mn, which is usually progressive and irreversible, showing some extent of permanent destruction of neurologic structures. All parts of plant have normal levels of Mn i.e. not exceeding permissible limits as shown in Table 2. Permissible limit of Mn in plants is 200 ppm.

Cobalt is essential element for all animals and is key constituent of cobolamine (vit B12). Its deficiency in sheep may lead to “Bush sickness”. Excessive level of cobalt in body may lead to cardiomyopathy and contact dermatitis. Normal intake of cobalt is 0.2mg/kg. Permissible limits for cobalt is 0.2 ppm. All parts of plant possesses excessive level of cobalt which exceed the permissible limits i.e. 1.59, 1.16 and 0.99 in leaves, stem and roots respectively.

**Macronutrients**

Calcium is an essential macronutrient and in combination with phosphorus, is a structural component of teeth, bones and soft tissues. It also provides thermodynamic stability to proteins by binding to the human surface of growth hormones. Ca is necessary for the regulation and monitoring of nerve functions and muscle, blood vessel dilation and contraction and glandular secretions.

Bone fractures are associated with low level of Ca. Its deficiency may also cause abnormality in heart beat that may lead to heart failure and spasm of skel-etal muscle.

All parts of the plant possess Ca levels that exceed
normal limits as mentioned in table 1. According to “Food and Nutrition Board”, 1000 mg/day Ca is required to our body. Thus, for dietary Ca it may not be a good source.

One of the most critically important macronutrient for human body is sodium, and table salt (NaCl) is its main dietary source. It plays a vital role in the main-tenance of normal physiology. Optimal ingestion of Na is mandatory for growth, and is the most abundantly found cation in the extracellular fluid; its fluctuation alters overall balance of fluid and distribution.13.

Na deficiency may reflect changes in mood, fatigue, muscle cramps, hair loss, dehydration and hypotension.15. Significant sodium concentration was ob-served in all plant parts and not exceeding permissible limit i.e. 589.9, 348.8, 376.2 ppm in leaves, stem and root respectively. Daily intake of 1-3.8 mg/day of Na is recommended.17. Hypertension is associated with high levels of Na.

Potassium is an important macronutrient in humans. It is crucial because it regulate the action potentials and also take part in intercellular signaling in electrified active cells. Irrespective of cell excitability, K channels regulate different functions including transduction of signals, insulin secretion, membrane poten-tial regulation, cell volume and immune response, regulation of vascular tone, and hormone release.11. For adult men the average range of daily potassium intake is 3100 mg/day and 2300 mg/day for adult women.12. Our study explores that significant level of K is there in all plant parts, which did not exceed the permissible limit.

CONCLUSION

Current study showed that all parts of the plant contain significant concentrations of macro- and micro-nutrients. However, the levels of some metallic elements e.g. Fe, Ni, Cr, Mn, Cu and Pb, do exceeded permissible limits. Long term health complications are associated with excessive level of these ions, and even could be lethal. It is, therefore, compulsory to per-form proper safety evaluation of therapeutically used medicinal plant i.e. determination of heavy metal content prior to formulation into finished medicines.

REFERENCES


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