

Research Article

Determination of heavy metals concentration in *Astragalus anisacanthus* and *Ebenus stellata* of Balochistan, Pakistan

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Abstract

Various elements along with heavy metals, present in different medicinal plants, play important roles in human's life. Assuming this, the concentrations of nine different elements including heavy metals were analyzed in selected medicinal plants i.e. *Astragalus anisacanthus* and *Ebenus stellata*. Wet digestion method was performed for the mineralization of plant samples employing three strong acids such as sulphuric acid (H₂SO₄), nitric acid (HNO₃) and per chloric acid (HClO₄) in ratio of 1:5:0.5mL respectively followed by filtration and analysis by flame atomic absorption spectroscopy. For Na and K, flame photometer was used and samples were diluted up to hundred folds for obtaining results within the range of the instrument. The increasing order of concentration of elements in *A. anisacanthus* is as: Cd<Pb<Co<Ni<Cu<Mn<Fe<K<Na, whereas in *E. stellata* was found as: Cd<Co<Ni<Cu<Mn<Pb<Fe<K<Na. The accumulation of heavy metals in plants was different in concentration according to the study. Furthermore, the concentrations were also within the range established by international organization. In future, the usefulness of this research work is that it will result in the synthesis of many new drugs as well as it will also be used for providing public protection regarding the adverse effects of heavy metals.

Keywords: Flame atomic absorption spectroscopy; Flame photometer; Flame emission spectroscopy; Heavy metals; Wet digestion

Introduction

Plants are mainly photosynthetic eukaryotic multicellular organisms which belong to kingdom plantae. They are comprised of conifers, flowering plants and gymnosperms [1]. The term "medicinal plants" refers to a large group of plants which are used in the

field of herbalism [2]. Elements which are not necessary for plant growth, but which can affect the production of crops, animals and human health are becoming increasingly important [3]. Some elements are known as micronutrients or trace elements which play

important role for plant's growth including Zn, Ni, Mn, etc. [4].

Determination of necessary elements present in plants and soil is essential if products are to be regulated at highly efficient production level [2]. For the human body, to maintain good health, metallic and non-metallic elements are needed. Subsequently, the elemental composition in edible plants and food is necessary for knowing their beneficial significance [5, 6-8]. There are 92 naturally occurring elements. Almost 30 metals and metalloids are fatal to human beings [9]. Heavy metals are member of specific subset of elements including transition metals, lanthanides and actinides [10]. When the heavy metal ions are absorbed through root system, they start to deposit on various part of plant resulting in the reduce growth of plants [11, 12]. Heavy metals are not toxic at low concentration, but when the concentration reaches to the threshold stage causes damage to human body [4].

Among heavy metals, Pb is one of the most abundantly found element on earth crust [13]. The environmental pollutants include the heavy metals except the natural calamities. The anthropogenic activities include the adverse effects of heavy metals on the environment [11]. Many plant species have been used for numerous infectious diseases ranging from minor infection to dangerous ailments like skin, asthma and horde of indication [14]. As stated by WHO report, about 70–80% of world population still primarily depends upon the non-conventional medicines, which are derived from herbal plants [5]. About 33 million people use medicinal plants in developing countries to treat various diseases [2]. The traditional medicine exercise is extensively employed in China, India, Japan, Sri Lanka and Thailand. The traditional tribal medicine in China is attributed by medicine consumption of about 40% [15].

Pakistan has specific climatic region and great biodiversity. About 6,000 types of higher plants are present. It has been reported that 600–700 species of which are used as medicinal plants [16]. The climate of Balochistan, the largest province of Pakistan regarding area, makes it rich in diverse species of medicinal plants [2]. To determine the whole concentration of metals, cannot give the complete information about their forms, but to find out heavy metals which are different from other metals because these are non-biodegradable toxic chemical species [17].

Atomic Absorption spectroscopy (AAS) is a helpful method for the analysis of trace elements, because of its high specificity, low detection limit, simple to use and easy sample preparation [18]. AAS is highly sensitive and concentration of different elements can be determined at lower level of concentrations [19]. AAS is the most suitable method and accepted generally for elemental analysis [18]. In spectroscopy, we study the interaction of electromagnetic radiation (EMR) with sample followed by measuring the attenuation of EMR intensity [20]. The quantity of light absorbed at a specific wavelength will rise as the number of atoms of the selected element increases in the light path and thus is proportional to the concentration of the element [21].

Ebenus stellata belongs to family Fabaceae and class Magnoliopsida and is locally known as “Sassai” [22]. The extracts of many plants belonging to Fabaceae family have shown toxicity and among them, *E. stellata* has been found as the least toxic leading to its usage as an anti-convulsant [23]. *E. stellata* is used in a group of medicine called as anti-convulsant (antiepileptic drugs or anti-seizure drugs) [24].

A. anisacanthus is a eukaryotic plant belongs to kingdom plantae; division, tracheophyte; genus, vedler (Astragalus)

and family, Papilionaceae [22]. Astragalus species contain a wide range of constituent including more than 40 saponins and several flavonoids including isoflavones and polysaccharides free from amino acids. *A. anisacanthus* is a neutral dietary source that is used for various adverse health conditions such as to treat common cold [22, 24]. Astragalus has been used as a traditional Chinese medicine for centuries. Its main use is to boost up the body immune system, to treat adverse health conditions like heart diseases [22, 24] and the folk people have employed these plants as medicine [22].

In this study, the concentrations of different heavy metals including Cd, Fe, Ni, Co, Cu, Pb and Mn present in *A. anisacanthus* and *E. stellata* was determined using AAS analytical technique. Whereas, concentrations of Na and K was assessed by employing flame photometric technique.

Materials and methods

Reagents and solutions

Different analytical grade standards (1000mgL^{-1}) of metals (Cd^{2+} , Fe^{2+} , Ni^{2+} , Co^{2+} , Cu^{2+} , Pb^{2+} and Mn^{2+}) were purchased from Merck, Marker for the analysis of heavy metals. Working standard solutions ($0.1\text{--}20\text{mgL}^{-1}$) of these metals were arranged by diluting the required aliquots from their stock solutions with the mixture of H_2SO_4 , HNO_3 and HClO_4 in ratio of 1:5:0.5 respectively. For the digestion of plants, a mixture of acids H_2SO_4 , HNO_3 and HClO_4 in ratio of 1:5:0.5 respectively was used. This mixture was arranged from the analytical grade commercially available stock solutions of these acids.

Instrument and glassware

Atomic absorption spectrophotometer (Thermo electron S4 AA) was used for the determination of heavy metals with hollow cathode lamps of different metals and a flame of air-acetylene. Whereas, Na and K were detected in aforementioned plants through flame emission spectrophotometer (Jenway

PFP7). Various glassware including round bottom flasks, conical flasks and beakers of pyrex glass were used. These glass wares were precleaned with surfactant, followed by soaking in HCl bath (10% v/v) for a week, and rinsing several times with deionized water.

Sample collection

Two medicinal plants were collected from Baberr Jungle situated in District Zhob, Balochistan in the month of August 2018 and recognition of the above-mentioned plants were done by a prominent taxonomist Dr. Rasool Bakhsh Tareen. Both plants were dried for almost two weeks in shadow or in absence of direct sun light. After the drying procedure, the medicinal plants were carefully grinded and prepared for further research work.

Sample preparation

The collected plants after drying were crushed into powder form via an electrical blender, which was used for further analysis through AAS and flame photometer. Digestion of the powdered plants was accomplished according to a previously reported protocol [25]. Accordingly, 0.25g powder of each plant was taken in a 50mL round bottom flask and was then added 6.5mL mixture of acids (5mL nitric acid, 1mL sulphuric acid and 0.5mL per chloric acid). These acids were taken from their commercially available stock solutions. Individually, each plant sample containing mixture of acids was heated on a hot plate (JENWAY 1000) at $80\text{--}85^\circ\text{C}$ until white fumes come out from the flask. These white fumes indicate that the digestion have been completed. Successively, few drops of distilled water was added to it, removed it from the hot plate and allowed it to cool for about 5–10 minutes. The digestion mixture was then transferred into 50mL volumetric flask and made the volume up to the mark i.e. 50mL by pouring distilled water in it followed by filtration using filter paper

Whatmann No.1 in the labelled plastic bottles. These prepared solutions after the wet digestion were studied for detection of elements using AAS and flame photometer. The dilutions of different concentrations were prepared from analytical grade stock standards of 1000ppm for the purpose of calibration. These dilutions were prepared immediately before running the samples. Deionized water was used throughout the investigation. FES of Jenway PFP7, the

technique was applied for the evaluation of sodium and potassium. For obtaining results within the range of flame photometer, the samples were diluted 100 folds with deionized water.

Result

After analysis, the obtained results (mgL^{-1}) for Cd, Pb, Co, Ni, Cu, Mn, Fe, K and Na are shown in (Table 1) and their comparison is given in (Figure 1).

Table 1. Concentration (mgL^{-1}) of various elements in selected medicinal plants

Plant specie	Cd	Pb	Co	Ni	Cu	Mn	Fe	K	Na
<i>A. anisacanthus</i>	0.0145	0.0727	0.0986	0.1064	0.2579	0.347	14.10	582	753
<i>E. stellata</i>	0.0162	0.726	0.0771	0.092	0.2044	0.629	14.098	514	698

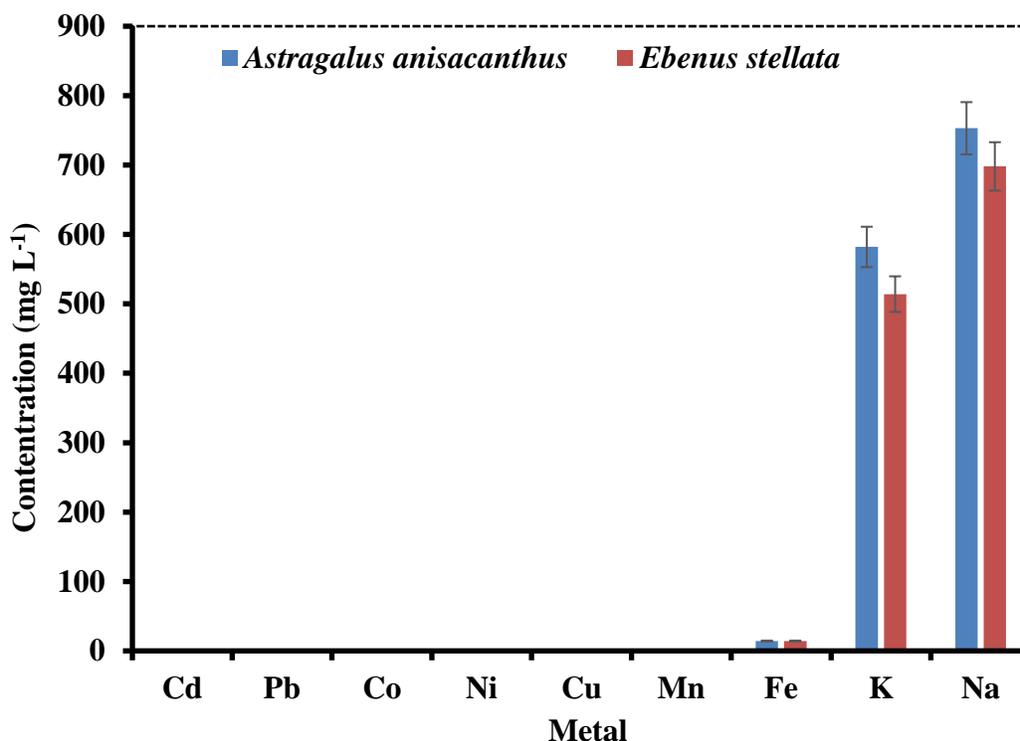


Figure 1. Comparison of the concentrations of various metals in *A. anisacanthus* and *E. stellata*

Discussion

AAS is used for the estimation of various heavy metals because of its accuracy. In addition, flame emission spectroscopy is a simple method for the determination of

sodium and potassium. For Na and K, samples were obtained by the dilution technique using deionized water considering the range of calibration curves of different elements. As AAS was connected with

computer device and it gives calculated concentration of various elements upon detection whereas flame photometer was not able to give the calculated concentrations so, the concentration of sodium and potassium were obtained through the regression equation.

Satisfactory results were obtained through the wet digestion method for the mineralization of plant materials. This method eradicates the surrounding of organic compounds around the minerals, only the minerals are left behind in an aqueous solution by using the strong oxidizing agents. This method is simple and faster than other methods of digestion like dry ashing and microwave digestion.

Plants need copper for their growth and development [26]. Cu plays an important role in metabolic process retarding the growth of higher plants [27]. The ailments like wounds inflammation, chest and arthritis are mainly healed via the presence of copper in plants [28, 29]. In addition, the existence and use of Pb makes it unique regarding to environmental toxicity that is why it is very important to study heavy metal like Pb [20].

Furthermore, the permissible limit of copper in plants is 10mg/kg which is used remedially 2–3mg/day for the consumption of humans [30]. The concentration of Cu in *A. anisacanthus* was 0.2579mgL⁻¹ whereas, in *E. stellata* 0.2022mg L⁻¹.

The mammals need cobalt in a very minor quantity. Cobalt can be used to treat anemic patients and also various cancers can be treated by the small amount of it [28, 29]. Cobalt is a major part of vitamin B-12 [31]. The tolerable limit of cobalt for human intake is 1.6–8µg/kg body weight per day [32]. The mean concentration of cobalt in *A. anisacanthus* was found as 0.0986mgL⁻¹ and in *E. Stellata* was 0.0771mgL⁻¹.

Nickel is vital for human body and also for plants in a very minute quantity as it is

required for the insulin production. However, its deficit leads to liver malfunction. In its high concentration, it is detrimental and causes many ailments which includes cardiac, loss of body weight and liver problems. According to WHO, the permissible limit of Ni is 1.5mg/kg in the plants and for dietary intake it is 1mg/day [30]. The mean concentration of Ni found in *A. anisacanthus* was 0.1064mgL⁻¹ and in *E. Stellata* was 0.0092mgL⁻¹.

To produce red blood cells in human body iron is an essential element. Anemia is caused by the low quantity of iron in the human body [33]. The human body tissues are damaged due to the high amount of iron [34]. According to WHO, the acceptable limit of iron in plants is 20mg/kg while 10 to 28mg/day for human consumption [30]. The mean concentrations of iron in *A. anisacanthus* and *E. stellata* were 14.10mgL⁻¹ and 14.098mgL⁻¹ respectively. Manganese play an important role in the growth of both plants and animals. It causes reproductive and skeletal problems due to its deficiency in mammals. In excessive amounts, it leads to several lung and brain complications [35]. According to WHO standards, the tolerable concentration of manganese for plants and animals are 200mg/kg and 11mg respectively [30].

The mean concentrations of manganese in *A. anisacanthus* and *E. stellata* were 0.347mgL⁻¹ and 0.629mgL⁻¹ respectively.

Even in the low concentration, cadmium is toxic and non-essential heavy metal for human health. The impediments in learning and hyperactivity in offspring is all due to cadmium [36]. The permissible limit according to WHO for cadmium is in therapeutic plants is 0.3mg/kg [30]. The average concentration of cadmium in *A. anisacanthus* and *E. stellata* were 0.0145mgL⁻¹ and 0.0162mgL⁻¹ respectively.

Lead is a non-essential heavy metal. It is stored in the bones and teeth which causes the bones to become stiff and hard and is the reason of weakens of the limbs and wrists. The deposition of lead in the soft tissues, renal, immune, reproductive and nervous systems of the body affects their functions. The safety limit of lead for human consumption is 1.5mgL^{-1} [33, 37]. While in medicinal plants, the permissible limit is 10mg/kg [30]. The average concentration of lead in *A. anisacanthus* and *E. stellata* were 0.0727mgL^{-1} and 0.726mgL^{-1} respectively. Sodium acts as a transporter for the substances which are involved in the respiration process such as amino acids, glucose and other metabolites. Mood swings, muscles cramps, dehydration and hair loss are some of the medical complications caused by the sodium deficiency. The recommended value of sodium for human intake is 13.8mg/day [38]. The average concentration of Na in *A. anisacanthus* and *E. stellata* were 753mgL^{-1} and 698mgL^{-1} respectively.

Potassium is involved in the regulation of hormones, secretion of insulin and response of immune system. Potassium works to lower the blood pressure. It is involved in the remedy of liver and kidney problems. The permissible limit of potassium for woman and man is 2300mg/day and 3100mg/day respectively [39]. The average concentration of K in *A. anisacanthus* and *E. stellata* were 519mgL^{-1} and 698mgL^{-1} respectively.

Conclusion

This study showed that the concentration of heavy metals in plants such as *A. anisacanthus* and *E. stellata* is below from their secure limits given by different international organizations like FAO and WHO. Nine elements including essential and non-essential elements were estimated in these plants among which the concentration of sodium and potassium were

found to be higher in both plants and concentration of heavy metals were within the permissible ranges. These plants are nontoxic to use as traditional medicines and therefore are not at danger. The accurate and precise determination of metals are very necessary. The results obtained in this investigation will be supportive for the manufacturers in the synthesis of new drugs with different combination of medicinal plants for curing various ailments.

Authors' contributions

Conceived and designed the experiments: I Kiazai, Samiullah, A Baqi & A Ghaffar, Performed the experiments: I Kiazai & A Baqi, Analyzed the data: Samiullah, N Khan & A Rehman, Contributed materials/analysis/ tools: A Rehman & Samiullah, Wrote the paper: I Kiazai, A Baqi & Samiullah.

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