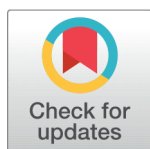


Determination of essential and non-essential elements in *Xylanthemum macropodum* of Balochistan, Pakistan

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ABSTRACT

Background: Medicinal plants have been used to treat various diseases for several years throughout the world. *Xylanthemum macropodum* is a medicinal plant with a vast application as a home remedy in Balochistan.

Objective: The current study was conducted to determine the levels of essential and non-essential elements in *Xylanthemum macropodum* collected from Quetta (Balochistan, Pakistan).

Methods: Analysis was conducted for eleven elements by using atomic absorption spectrophotometer and flame photometer.

Results: The essential and non-essential elements that were detected in *Xylanthemum macropodum* are K, Na, Ca, Fe, Cu, Co, Ni, Mn, Cr, Cd and Pb, respectively. The result of the concentration of determined elements are in the order of K> Na> Ca> Fe> Cu> Co> Ni> Mn> Cd> Cr> Pb in *Xylanthemum macropodum* 3000> 1600> 790.25> 92.36> 85.31> 49.24> 40.94> 20.94> 6.655> 1.61> 1.18 µg/g respectively.

Conclusions: Mn, Na and Pb were found within the permissible limit given by World Health Organization, while Ca, Cu, Fe, Ni, Cd and Cr were beyond the permissible limits. However, there is no permissible limit for K and Co.

Keywords AAS, flame photometer, heavy metals, *Xylanthemum macropodum*

INTRODUCTION

The uncontrolled and rapid industrialization and urbanization of developing countries have elevated the contamination levels in the ecosystem. Various sources can participate in the contamination of the environment such as; industries, solid waste dumpsites, automobile exhaust, municipal waste management and agricultural activities. Circulation and migration of toxic metals in the environment include various processes like; volcanic eruption, sedimentary, rock decay, forest fires and evaporation of oceans.¹ Despite their therapeutic effects, medicinal plants may also have adverse effects on human health, such

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as hypertension, depression, headache, skin eruption, intestinal ulcer, anaemia, alopecia, hypoglycemia, and abdominal pain due to the presence of heavy metals. Therefore, it is essential to monitor the amount of such elements when used in medication.²

Since the beginning of human civilization, medicinal plants are used by local people to cure various diseases like; fever, hepatitis, typhoid, asthma, bronchitis, cough, diarrhoea, urinary tract infections, constipation, ulcers, pain, scrofula, kidney troubles, nervous system, immune system and heart diseases.³ Plants play an essential role in transferring elements from the biotic to the abiotic environment. They absorb metals by roots from growth media (water, soil and air) and accumulate them in various parts. Plants' ability to accumulate heavy metals depends upon various levels of organization: cell, tissue and organ.⁴

From the past decade's humankind have shown interest in the power and potential of medicinal plants.⁵ Several countries such as; Pakistan (Herbal Medicines), India (Ayurvedic Medicine), China (Traditional Chinese Medicine), Japan (Kampo Medicine), Sri Lanka, Africa, South Asia, America, France, Arabs, Ireland, England, and Germany; use medicinal plants for the cure of various illness since from ancient era.⁶ In the world, 2,50,000 plant species are discovered, of which 80,000 species are known for medicinal properties. As described by World Health Organization (WHO), 80% of the human's population depends upon medicinal plants for their primary health care, and 40% of pharmacological industries synthesize drugs from medicinal plants.⁷

Pakistan shares rich flora of 5700 plants due to its unique climate, nine major ecological zones, many topographical regions and agricultural soil. Among these, 2000 plants are medicinally important. About 80% of the local people of Pakistan rely upon herbal medicines, while 90% of medicinal plants are imported.⁸ However, the trade digits of medicinal plants are low than neighbouring countries such as China and India. Therefore, there is a dire need for Pakistan to develop more pharmacological industries to utilize herbal plants. Less developed areas of Pakistan such as Balochistan, Gilgit Baltistan and FATA are well-known for their biodiversity. And, the local government of these areas should participate in the development of pharmacological industries, and the therapeutic knowledge of local people must be utilized.⁹

Due to limited reports about the levels of essential and non-essential elements in *Xylanthemum macropodum*, we sought to investigate the concentrations of some of these elements in *Xylanthemum macropodum* collected from Quetta (Balochistan, Pakistan).

MATERIALS AND METHODS

Chemicals and reagents

All the standards and reagents were of analytical grade. The mixture of concentrated Nitric Acid (HNO₃), Sulphuric Acid (H₂SO₄) and Per Chloric Acid (HClO₄) was used for digestion purpose. Merck KgaA, Damnsstadt, manufactured the reagents used. For calibration purposes, 1000 ppm stock solution (Merck) of various metals (Ca, Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, Pb) were used, and distilled water was utilized dilution of samples and

standards.

Instrumentation

Flame atomic absorption spectroscopy (FAAS) model S4 AA (Thermo) was used for the analysis of Cu, Cd, Co, Pb, Fe, Ni·Mn (Atomize in the presence of Acetylene gas) and Ca, Cr (Atomize in the presence of Acetylene and Nitrous gases). FAAS is an analytical technique used for the quantification of 70 elements. Atomic Absorption Spectroscopy is the most suitable technique as it is highly sensitive, low detection limit, low cost and easy to operate.¹⁰ AAS works on a principle of absorption of electromagnetic radiation (EMR) by the vaporized atom of the specific element. For the analysis purpose, the solution was aspirated into flame, then the vaporized atoms absorbed at a specific wavelength and the degree of absorbed radiation was measured.

Flame photometer model PFP7 (Jenway) was used for the analysis of K and Na. Flame atomic emission spectroscopy (FAES) is a quantitative analytical technique used to determine alkali and alkaline earth metals.¹¹ It is the most suitable and low-cost technique for the analysis of sodium and potassium. It works on the principle of emission of radiation by vaporized excited atom. For the analysis purpose, the sample is aspirated into flame then the vaporized atoms absorb thermal energy and become excited. The excited atoms emit radiation of a specific wavelength, and the degree of emitted radiation is measured.

Sample collection and sample preparation

The selected medicinal plant *Xylanthemum macropodum* (Figure 1) was collected from Hanna valley, North of Quetta, Balochistan (Figure 2). The collected plant sample was brought to the laboratory of the Department of Chemistry, University of Balochistan in Quetta and then, it was washed with ultra-pure deionized water and dried carefully without exposure to direct sunlight. Finally, the dried plant sample was ground in an electric grinder to obtain fine powder for further analysis.



Figure 1 *Xylanthemum macropodum* plant.



Figure 2 Map of Balochistan.

Acid digestion

One gram of powdered *Xylanthemum macropodum* plant sample was weighed and taken in a 50 ml Erlenmeyer flask, and the mixture of three acids (HNO_3 , H_2SO_4 , HClO_4) was used in the ratio of 10:2:1 for digestion. The prepared mixture of the plant was heated on an electric hot plate at 80-85 °C in a fuming hood. The sample was heated until complete digestion is accomplished, indicated by the occurrence of white fumes. The sample was allowed to cool down at room temperature and then was transferred to a 50 mL volumetric flask and was diluted by distilled water up to the mark. Then, the diluted sample was filtered by Whatman No. 42 filter paper and further used for elemental analysis.¹²

Blank and standards

A 0.5 M blank of HNO_3 was prepared for the dilution of standards of various concentrations. By utilizing 1000 ppm, stock solution of various metals five standards of various concentration of each metal was prepared for calibration of the instrument. After calibration of the instrument, the sample was aspirated into flame to analyse various metals, and the obtained data was interpreted.

RESULTS AND DISCUSSION

Medicinal plants may be contaminated with heavy metals due to various environmental processes that occur during the plant's growth. It is necessary to determine essential and non-essential metals in plants to protect humans from the toxic effects of non-essential metals and determine the nutritional value of medicinal plants.³ In the present study, the con-

centration of eleven metals was analyzed utilizing FAAS and FAES in *Xylanthemum macropodum*. The concentrations order of the eleven metals (K, Na, Ca, Fe, Cu, Co, Ni, Mn, Cd, Cr, Pb) in *Xylanthemum macropodum* were 3000, 1600, 790.25, 92.36, 85.31, 49.24, 40.94, 20.94, 6.655, 1.61, 1.18 $\mu\text{g/g}$ respectively (Table 1). Consequently, the comparison of the concentration of these metals is accomplished by plotting a graph shown in. The concentration of eleven metals in *Xylanthemum macropodum* is compared with the maximum permissible limits (MPL) of metals in medicinal plants set by the World Health Organization (Table 2). Their impacts on human health are also discussed.

Table 1 The concentration of elements in *Xylanthemum macropodum* ($\mu\text{g/g}$).

Ca	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	K	Na
790.25	6.655	1.61	49.24	85.31	92.36	1.18	20.94	40.94	3000	1600

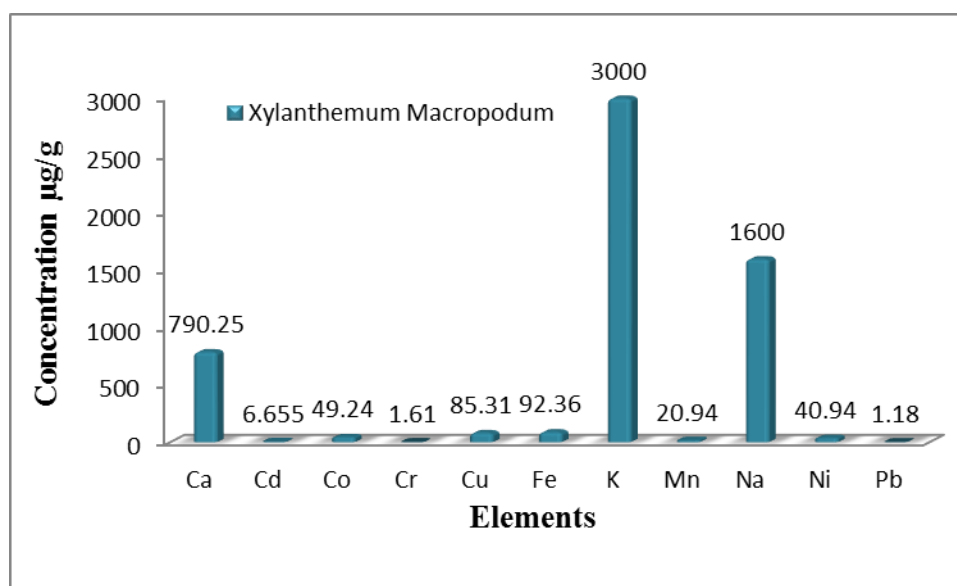


Figure 3 Comparison of concentration of various metals in *Xylanthemum macropodum*.

Determination of essential metals

Calcium (Ca)

Calcium is one of the most essential and abundant elements in the human body. It plays various roles in the human body, such as building strong bones & teeth, absorption of vitamin B, preventing osteoporosis, releasing hormones, activating enzymes, and contraction of muscles. Its deficiency results in weakness of bones and abnormality in heartbeats which may lead to a heart attack. Recommended Dietary Allowance (RDA) for Ca is 500-1000 mg/day for children and 800 mg/day for adults. MPL of Ca for the medicinal plant is 614

ppm.¹³ The concentration of Ca in *Xylanthemum macropodum* is 790.25 $\mu\text{g/g}$, and it is concluded that the concentration of calcium was higher than the permissible limit.

Chromium (Cr)

Cr is the 7th most abundant element. Chromium is a trace element in the human body. It has essential functions in carbohydrate metabolism, cholesterol synthesis from acetate in the liver, stimulation of fatty acid, and improved sugar metabolism. Cr deficiency causes an increase in haematological parameters. RDA for Cr is 35 mg/day for men and 25 mg/day for female.¹⁴ MPL of Cr for edible plants is 0.02 ppm ((Food and Agricultural Organization) FAO/WHO, 1984), and for the medicinal plant is 1.5 mg/kg (WHO). The concentration of Cr in *Xylanthemum macropodum* is 1.61 $\mu\text{g/g}$, and it was concluded that chromium was in a higher concentration than the permissible limit.

Cobalt (Co)

Co is a naturally occurring metal in the earth crust.⁵ Cobalt is an essential trace element for the human body and is an essential component of vitamin B₁₂, a high intake of cobalt results in heart diseases. At the same time, its deficiency results in anaemia and hypothyroidism. The RDA for Co is 5-40 $\mu\text{g/day}$. No MPL are determined for Cobalt in medicinal plants.¹³ The concentration of Co in *Xylanthemum macropodum* was 49.24 $\mu\text{g/g}$.

Copper (Cu)

Cu is the third most abundant (150mg) trace element found in the human body. High intake of Cu has adverse effects on human health like; liver toxicity, kidney damage, nausea, anaemia and cancer (Group D carcinogenic).⁸ At the same time, its deficiency results in cardiovascular diseases. RDA for Cu is 2-5 mg/day. MPL of Cu for edible plants is 3.00 ppm (FAO/WHO26, 1984) and for the medicinal plant is 10 mg/kg (WHO).¹⁴ The concentration of Cu in *Xylanthemum macropodum* is 85.31 $\mu\text{g/g}$, and it was concluded that copper was in a higher concentration than the permissible limit.

Iron (Fe)

Fe is an essential element for all living organisms and the second most abundant element on earth crust. Iron is the most abundant metal (3-4 g) and is considered an essential element for the human body. In humans, it is the main component of several enzymes, haemoglobin and myoglobin. In comparison, Iron deficiency results in anaemia. RDA for Fe is 8 mg/day for males and 18 mg/day for females. MPL of Fe for edible plants is 20 ppm (FAO/WHO, 1984), and for the medicinal plant is 15 mg/kg (WHO).¹³ Therefore, the con-

centration of Fe in *Xylanthemum macropodum* is 92.36 $\mu\text{g/g}$, and it was concluded that iron was in a higher concentration than the permissible limit.

Table 2 Recommended dietary allowance and Maximum permissible limit of medicinal and edible plants.¹⁵

Elements	RDA (mg/day)	WHO's MPL	
		Medicinal plants (mg/kg)	Edible plants (ppm)
Calcium	500-1000 (child) 800 (adults)	614 ppm	—
Cadmium	—	0.3	0.2-0.81
Chromium	35 (males) 25 (females)	1.5	0.02
Cobalt	5-40 $\mu\text{g/day}$	—	—
Copper	2-5	10	3
Iron	8 (males) 18 (females)	15	20
Lead	—	10	0.43
Manganese	2.3 (males) 1.8 (females)	200	2
Nickel	80-130 $\mu\text{g/day}$	1.5	1.63
Potassium	2300	—	—
Sodium	1-3.8	51340	—

Manganese (Mn)

On the earth crust, Mn is the 12th most abundant element (average concentration 0.1%). It is a trace element (12 mg) found in the human body and it is one of the essential nutrients for human health. High intake of Mn can result in neurological disorders. While its deficiency results in dermatitis, deafness, changes in hair color, hypercholesterolemia, infertility, impaired glucose tolerance and skeletal abnormalities. RDA for Mn is 2.3 mg/day for men and 1.8 mg/day for women. MPL of Mn for edible plants is 2.00 ppm (FAO, 1984) and for the medicinal plant is 200 mg/kg (WHO).¹³ The concentration of Mn in *Xylanthemum macropodum* was 20.94 $\mu\text{g/g}$, and it was concluded that manganese was within the range of MPL.

Nickel (Ni)

Nickel is a transition metal that is naturally found in the environment. It is essential in trace amounts while high Ni intake has adverse effects on human health such as; sickness and dizziness, respiratory diseases, asthma and chronic bronchitis, lung cancer, larynx cancer, nose cancer, prostate cancer, birth defects, and birth defects, allergic reactions and heart disorders.¹⁵ RDA for Ni is 80-130 $\mu\text{g/day}$. MPL of Ni for edible plants is 1.63 ppm (FAO/WHO, 1984), and for the medicinal plant is 1.5 mg/kg (WHO).¹³ Therefore, the concentration of Ni in *Xylanthemum macropodum* was 40.94 $\mu\text{g/g}$, and it was concluded that nickel was in a higher concentration than the permissible limit.

Potassium (K)

Potassium is one of the richest element in living organisms. It plays a vital role in the maintenance of cell function such as nerve and muscle cells. Potassium is beneficial for reducing blood pressure, improve glucose control and insulin resistance. The RDA for K is 2300 mg/day. The concentration of K in *Xylanthemum macropodum* was 3000 $\mu\text{g/g}$.⁹

Sodium (Na)

Sodium is one of the essential and richest elements in the human body. It is essential for the maintenance of physiology and optimal growth of the human body. A high concentration of Na results in hypertension. At the same time, its deficiency causes fatigue, mode change, hair loss, muscle cramps, dehydration and hypotension. RDA for Na is 1-3.8 g/day. MPL of Na for the medicinal plant is 51340 ppm.⁷ The concentration of Na in *Xylanthemum macropodum* was 1600 $\mu\text{g/g}$, and it was concluded that sodium was within the range of MPL.

Determination of non-essential metals

Cadmium (Cd)

Cadmium is the 7th toxic heavy element on earth (ATSDR), and it has adverse effects on human health. In humans, it causes high blood pressure damages the kidney and liver.¹⁶ The MPL of Cd for edible plants is 0.2 to 0.81 ppm (WHO22) and for the medicinal plant is 0.3 mg/kg (WHO).¹² The concentration of Cd in *Xylanthemum macropodum* was 6.655 $\mu\text{g/g}$, and it was concluded that Cd was in a higher concentration than the permissible limit.

Lead (Pb)

Lead is a highly toxic metal. Industrial processes, storage batteries, food and smoking, gasoline and house paint, drinking water and domestic sources are causes of lead contamination in the environment. There is no biological role of lead in the human body. However, lead harms human health, and high concentration can damage the kidney, brain, reproductive system, nervous system and cause high blood pressure.¹⁷ The MPL of Pb for edible plants is 0.43 ppm (FAO/WHO, 1984), and for the medicinal plant is 10 mg/kg (WHO).¹³ The concentration of Pb in *Xylanthemum macropodum* was 1.18 $\mu\text{g/g}$, and it was concluded that lead was within the range of MPL.

CONCLUSIONS

The present study shows that the concentration of detected metals in *Xylanthemum macropodium* was within the range of acceptable value fixed by FAO/WHO. The concentration of both essential and non-essential elements was determined. The concentration of calcium, iron, potassium and sodium were found higher among the assessed metals. These metals are beneficial for human health. However, other elements were within the range of permissible limit by FAO/WHO. Thus, the plant *Xylanthemum macropodium* has non-toxic effects on human health and safe to use as a drug. Nonetheless, the plant must be monitored before using it as medicine because several environmental factors can cause contamination of medicinal plants.

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DECLARATIONS

Authors' contributions

AK, NK, AT, SB and HT contributed to the practical part of this work. MB wrote the original draft. SK supervised the project. All authors reviewed and approved this paper before publication.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approvals

This work was done and approved by the Department of Chemistry, University of Balochistan, Quetta in November 2020.

Data availability

The data associated with this work can be requested from the corresponding author.

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