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Determination of trace elements in various parts of *Eichhornia crassipes*

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Abstract

Eichhornia crassipes, commonly known as water hyacinth, can absorb large quantities of minerals from the surrounding water column. Experimental research focusing on this plant's chemical constituents as well as medicinal use are scarce. Insight into the trace element concentration of this plant can perhaps be harnessed to postulate the rationale behind its use in traditional medicine. A microwave assisted digestion method was employed for digesting the segregated parts of the dried plant. Elemental analysis was carried out using inductively coupled plasma optical emission spectrometry and Inductively coupled plasma mass spectrometry. The concentration values of Cu, Fe, Mn, Zn, Co, Ni, Se, I in *E. crassipes* are reported. A broad difference in the concentration range of the plant was observed. The concentrations of elements were found in the order of Mn > Fe > Zn > Cu > Ni > Co > I in *E. crassipes*.

Keywords: Trace elements, ICP-MS, ICP-OES, elemental determination

1. Introduction

Eichhornia crassipes, commonly known as water hyacinth, is an aquatic floating macrophyte, native to the Amazon basin. The plant is hardy and very difficult to eradicate because it can survive extremely harsh conditions. It can absorb into its tissues large quantities of minerals from the water column. Plant and plant derived products form an important alternative medicine category in health care that are often used for treatment of several disorders. According to folk medicine, Water hyacinth has been used for treatment of several ailments. In Java, green parts, inflorescences and flowers spikes are cooked and eaten. There are reports of its use as a stytic in Chhattisgarh, India. The fresh juice of this weed is used to treat fresh wounds as the tribes believe that it stops further spread of infection and relieves stress associated with the injury. Along with vinegar, it has been reported to use in treatment of septic wounds^[1]. *E. crassipes* has also been used to ease swelling, burning, haemorrhage, and goiters. In India and Nigeria, the oil from flowers has been used for skin care as well as for treatment of skin diseases. In Assam, the flowers and roots have been used in treatment of stomach ache and pneumonia. In Bangladesh, the plant has been used in treating hepatic disorders^[3]. In recent years, there are reports of *E. Crassipes* being used as an insecticide, antioxidant, antitumor, antiinflammatory and as an antimicrobial agent^[4-7]

Trace elements constitute less than 0.01% of the weight of the human body; however, despite their relative scarcity, they play an important role in human growth and development. Deficiency or excess of trace elements are associated with several metabolic disorders. An investigation focusing to understand the relationship between trace elements and human health has led to the conquest of diseases such as pernicious anaemia and recently, Keshan disease.

The purpose of this study is to determine the distribution of some trace elements in different parts of the *E. crassipes* (Water Hyacinth). It is expected that the metal distribution within the plant species would be a representation of the concentration of the water in which it is found. Plants have the ability to accumulate non-essential and essential trace elements and this ability could be exploited to not only remove pollutant metals from the environment, but also to investigate its potential for specific pharmaceutical or cosmeceutical studies like diarrhoea, skin burns, goiter etc^[8,9]. Although, only elemental analysis data, is insufficient to decode the mechanisms involved in the use of *E. Crassipes* in folk medicine, and a more detailed study needs to be undertaken with respect to its secondary metabolite profiling. Nevertheless, insight into the trace element concentration of this plant can perhaps be harnessed to postulate the rationale behind its use in traditional medicine.

2. Materials and Methods

2.1 Collection of Plant material

Eichhornia crassipes (Water Hyacinth) was collected in the month on March 2018 from Pashan Lake, Pune, India.

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The Leaves, petioles and roots of the plant were segregated and set to dry under shade for 20 days until constant weight of the dry mass was obtained. The dried samples were then ground to fine powder.

2.2 Sample Preparation and digestion

The samples were separately weighed, following which they were subjected to microwave digestion (Mars Express Vessel) according to the parameters given in Table 1.

For digestion, 0.5 g of sample was taken into a digestion vessel to which 3ml of Nitric acid (HNO₃ 69% Suprapure grade), 7 ml of Hydrochloric acid (HCL 30% Suprapure grade) was added. Blank solutions were prepared similarly, omitting the sample. Digested samples were transferred to falcon tubes and diluted with acidified water to a volume of 50 mL. The resulting solutions were subjected to ICP-OES and ICP-MS analysis.

2.2.1 Iodine sample preparation

For Iodine determination, 0.5g of the sample was digested in basic medium by taking 1 mL tetra methyl ammonium hydroxide (TMAH) to which 5 ml of water was added. The solution was subjected to centrifugation, after which it was kept in oven for 2 hrs at 80 °C The final volume was made up to 50ml water.

2.3 Standard Preparation

Stock solutions containing 1000 mgL⁻¹ of Perkin Elmer Pure was used to prepare different custom-grade multielement standard solutions for Co, I, Se, Ni, Cu, Mn, Zn, Fe by diluting with 50 mL acidified water. In-house certified reference materials (CRMs) for biological materials, developed using the "ISO Guide 80," were used for comparison purposes (10). Calibration curves for each element were constructed in triplicate using six different concentration. An internal-standard stock solution of 100 mg/L Lutetium (Lu) was prepared from single-element stock solutions.

Suprapure grade nitric acid and hydrochloric acid was used for preparing stock solutions of 100ppm and 10ppm for ICP-OES and ICP-MS respectively.

2.4 ICP-OES and ICP-MS Analysis

All samples and blank solutions were subjected for elemental determination by technique of Inductively Coupled Plasma Optical Emission Spectrometry ICPOES (Thermo Fisher Scientific, Bremen Germany, i CAP 6300) and Inductively Coupled Plasma Mass Spectrometry ICPMS (Agilent 7700 series).

3. Result and Discussion

3.1 Choice of isotopes and correction of interferences

After careful evaluation of all possible isotopes, their abundances, potential interferences (meaningful in the analysis of plants) and the corrections introduced, isotopes selected for quantitative estimation of the respective elements were; ⁵⁹Co, ¹²⁷I, ⁶⁰Ni, ⁶⁶Cu. Pure single element standard solutions of the interfering elements were used to establish the actual interferences on all isotopes under investigation.

The corrections were calculated according to the following equation [11]

$$S_{\text{cor}} = S_{\text{meas}} - (S_{\text{inter}} A)$$

Where S_{cor} is the corrected signal of the analyte; S_{meas} is the measured signal; S_{inter} is the signal of the interfering element and A is the % of formation of the respective interfering species determined in the solution of the interfering element at the working conditions specified in Table 2 and Table 3. S_{inter} is measured in each sample and the isotope abundance is accounted for.

3.2 Element concentration

The concentration values of Co, I, Se, Ni, Cu, Mn, Zn, Fe in various parts like roots, leaves and petiole as expressed in ug/ml are given in Table 4. The total range of concentration of elements was found as low as 0.94 mg/g for I and as high as 5659 mg/g for Mn. Roots of the plant are in direct contact with the surrounding water and as a result they form the principle part of element uptake. Concentration of all measured elements was found to highest in roots. From the roots the elements are transported to the petiole. The concentration of Ni, Cu, Mn, was found to be highest in Roots>Petiol> Leave. Whereas the concentration of Zn and Fe was found to be highest in Roots followed by leaves and finally petiole. This finding suggests alteration in transport mechanism or storage process involved with Zn and Fe. More detailed studies involving 2D-element mapping are necessary to evaluate the transport and storage mechanism of these elements in *E. Crassipes*. Concentraion of Co was high in roots but in petiole and leaves it was almost similar. An interesting finding was the occurrence of measurable quantities of Iodine in all the parts of the plant despite its non marine source.

3.3 Recovery

Concentration of Co, Ni, Cu was also determined in the digested plant samples of leaves, roots and petiole after spiking with an standard element solution containing 2 ppm concentration. Spiked concentration for Co, Ni, Cu was found in order of Roots>Petiole>Leaves. Concentration of Cu was too high in the unspiked root samples itself, hence it was not measurable in the spiked root samples. After spiking concentration of 2 ppm the recovery obtained was as follows: in leaves recovery of Co and Ni was 83.5% and 82%, in roots it was 89.5% and 84.5% and in Petiole it was 98% and 82.5%. The recovery was not obtained in Mn, Zn and Fe because concentration of these elements was already very high in unspiked samples.

3.4 Limits of detection and quantification

The calibration curves for all the analytes were built on six different concentrations and LOD and LOQ values were determined.

The limits of detection (LOD) were calculated by taking three times the standard deviation of the 10 individually prepared blank solutions.

The limits of quantification (LOQ), were calculated by taking ten times the standard deviation of the 10 individually prepared blank solutions [7] LOD and LOQ values for the measured elements are given in Table 5.

3.5 Linearity

The correlation coefficients were calculated for the calibration curves for all the seven trace elements on six standard replicates for six different concentrations. Concentration used for determining linearity was between 1.0 - 5.0 ppm. R² values for the individual elements are shown in Table 5. Good

correlation was obtained for all the measured elements. Observed R^2 values were in the range of 0.9976 to 1.

4. Conclusion

E. Crassipes is a hyperaccumulator. Evaluation of its elemental profile gives an idea of the surrounding water that it grows in. Minor and trace element analysis of the plant was performed with the objective of deciphering evidence based rationale behind the use of *E crassipes* in treatment of various ailments like antimicrobial, antiinflammatory, antioxidant, etc., which will present the basis for its potential use in modern treatment. Though there are many works citing the use of this plant in bioremediation and energy production, the plant has been exploited only to a certain extent in terms of its phytochemical uses. *E. Crasipes* can be considered a potential source of nutrition and mineral supplements. However, the mineral composition of the plant obtained here was based on chemical analysis only. Biological evaluation using human and animal feeding studies would be required to establish the nutritional value of this plant as a source of minerals. Although we are fully aware that it has been widely acknowledged that this plant accumulates heavy metals from contaminated water sources, if the utility of the plant in Pharmacaeuticals/Cosmeceuticas is established, it would be worthwhile to cultivate this rapidly reproducing plant in neutral water bodies for this purpose. A more detailed scientific protocol for evaluation of *E. crassipes* with respect to its phytochemical composition and pharmacological/cosmeceutical activity evaluation along with secondary metabolite profiling, is under process by the authors. With more such studies, a plant that has been considered a threat to the environment and economy, could be constructively harvested.

Table 1: Operating conditions for the microwave digestion system.

Step	Power	Temperature	Ramp time	Hold Time
1	1500W	170 °C	15 min	15min

Table 2: Instrumental analytical conditions for ICP-OES of element analysis

Parameter	Settings
Pump rate	50
RF Power	1150W
Plasma View	Axial
Auxillary gas flow	0.5 L min ⁻¹
Nebuliser gas (Argon)	0.5 L min ⁻¹
Stabilisation time	10min
Plasma gas	12 L/min

Table 3: Instrumental analytical conditions for ICP-MS of element analysis (Agilent 7700 Series)

Parameter	Settings
RF Power	1550W
RF Matching	1.00 Volts
Sample depth	8 mm
Carrier gas (Argon)	0.99 L min ⁻¹
Scanning time	2 min
Pump speed	0.5rpm
Plasma gas flow	0.5 L/min ⁻¹
Auxillary gas flow	0.5 L/min ⁻¹
Nebuliser gas flow	0.5 L/min ⁻¹
Sweeps/ reading	100
Scan mode	No gas mode
Run time	3 min

Table 4: Unspiked Trace Element concentration in *Eichhornia crassipes*

Element	Roots (mg g ⁻¹)	Petiole (mg g ⁻¹)	Leaves (mg g ⁻¹)	Total (mg g ⁻¹)
⁵⁹ Co*	4.06±0.04	0.13±0.02	0.14±0.02	4.33± 0.02
¹²⁷ I*	0.37±0.01	0.21±0.01	0.36±0.01	0.94±0.01
⁶⁰ Ni*	7.42±0.1	1.1±0.01	0.97±0.01	9.54± 0.01
⁶⁶ Cu*	19.1±0.1	7.5±0.02	1.38±0.05	28.04 ±0.1
Mn**	4450±0.4	696±0.8	513±0.15	5659 ±0.16
Zn**	24.5±0.5	4.3±0.2	11.3±0.15	40.26±0.07
Fe**	3138±1.2	89.6±0.4	189±0.17	3417 ± 0.64

* elements measured by ICP MS, ** elements measured by ICPOES

Table 5: Parameters of calibration obtained external calibration, correlation coefficient (R^2), limit of detection (LOD) and limit of quantification (LOQ) by using ICP-OES and ICP-MS

Elements	Linear Equation	R ²	LOD (µg g ⁻¹)	LOQ (µg g ⁻¹)
Co	y = 24783x - 15714	0.9986	0.001866	0.05598
I	y = 271156x + 1E+07	0.9299	0.001954	0.05862
Ni	y = .4x - 5023.8	0.9976	0.02623	0.786
Cu	y = 31295x + 117.87	1	0.000257	0.000855
Mn	y = 94308x + 1164	0.9998	0.000049	0.000164
Zn	y = 19802x + 3.3093	1	0.000055	0.000185
Fe	y = 20247x + 0.7623	1	0.000226	0.000753

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