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Nutrient Analysis of Rhizospheric and Non-Rhizospheric Soil of *Saussurea obvallata* (DC.) Edgew. (Brahma Kamal) from Kedarnath, Uttarakhand, India

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Abstract
Saussurea obvallata (DC.) Edgew. (Brahma Kamal) is an endangered medicinal herb of the high altitude Himalayan region with immense cultural significance. This study is on the analysis of micro and macro nutrients in the rhizospheric and non-rhizospheric soils from *S. obvallata*. The amount and availability of nutrients absorbed through the plant roots is an important factor affecting growth and development. The macro-elements and micro-elements are essential for healthy plant growth. For the present study, soil samples were collected from four sites where this species was known to be present as well as from area where the plant was introduced in recent times. The results indicated that soil nutrients, including Zn, Cu, Fe, Mn ranged from 0.22 – 2.02, 0.56 – 1.27, 0.00 – 19.23, and 0.00 – 2.02 ppm, respectively. The amount of available carbon, potassium, and phosphorus ranged from 0.64 – 0.99 %, 99.83 – 301.35, and 8.93 – 26.90 Kg/hectare, respectively. This investigation gives baseline information about the nutrients in rhizospheric and non-rhizospheric soil of *S. obvallata*. Such data are expected to be helpful for developing conservation and restoration strategies for this endangered plant.

Keywords- Brahma Kamal, Kedarnath Valley, Micro-Nutrients, Macro-Nutrients, Non-Rhizospheric, Rhizospheric Soil.

1. Introduction

A better understanding of the edaphic and climatic conditions along with the biology of an endangered plant are helpful in developing a successful conservation programme (Steinbrecher et al., 2015). Healthy soil and nutrient availability is critical for the plant establishment, growth, and reproduction. Soil represents the organic and inorganic material on the surface of the earth that provides the basic medium for plant growth. It is important to understand the nutrient status of the soil in which any plant species is growing (rhizospheric and non-rhizospheric soil). *Saussurea obvallata* (DC.) Edgew. (Brahma Kamal) is the state flower of Uttarakhand, India. It is a hermaphrodite herb and distributed between 3,000-4,800 m above mean sea level (amsl). The plant holds religious and medicinal value (Pant and Semwal, 2013; Semwal et al., 2014). The recent Conservation Assessment and Management

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Plan (CAMP) had categorized *S. obvallata* as an endangered species (Sastry and Chatterjee, 2000).

2. Material and Methods

2.1 Sample Collection

Soil samples were collected from a depth of 15 cm from four different sites (rhizospheric soil samples were taken from close vicinity of root system of *S. obvallata*, and for non-rhizospheric samples, soil was collected from area some distance away of the root zone of *S. obvallata*) from Kedarnath valley in September, 2015. The samples were packed in plastic bags and immediately brought to the laboratory. The samples were air dried at room temperature, passed through a 2 mm sieve, and stored at 4 ºC until further processing. The sample collection sites are shown in Table 1 and Figure 1, respectively.

Table 1. Sample collection sites in Kedarnath valley

S.No.	Collection Sites	Altitude (m amsl)	Latitude	Longitude
A	Madhu Ganga (MG)	4325	30˚44’577”	79˚03’290”
B	Maha Panth (MP)	4450	30˚44’982”	79˚04’992”
C	Cheer Ganga (CG)	4265	30˚43’801”	79˚03’216”
D	Hathi Parwat (HP)	4335	30˚40’739”	79˚05’203”

Figure 1. Natural populations of Brahma Kamal in Kedarnath valley; (A) Madhu Ganga, (B) Maha Panth, (C) Cheer Ganga, (D) Hathi Parvat

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2.2 Soil Analysis

The analysis of soil samples was carried out at the District Soil Testing Laboratory, Dehradun, Government of Uttarakhand for physical (pH, moisture content) and chemical (organic carbon, phosphorus, potassium, zinc, manganese, iron, and copper) parameters using standard methods developed by the Department of Agriculture & Cooperation, Ministry of Agriculture, Government of India (Soil testing in India, 2011) and other published protocols. The estimation of micronutrients in the soil extract was performed with the help of Atomic Absorption Spectrophotometer (EC Electronics Corporation of India Limited; AAS Element AS AAS4141). All estimations were conducted in triplicate.

2.3 Soil pH and Moisture Content

10 g of dried soil samples were suspended in 20 mL of distilled water. The suspension was stirred thoroughly and then allowed to stand for 30 min. The pH was recorded with a pH meter (Cyber Scan 500). For moisture content, 500 mg of soil samples were taken and kept at 110 ^{0}C until constant weight. The loss in weight was considered to be due to the moisture contained in the soil samples, and the calculations were done accordingly.

2.4 Organic Carbon (OC)

The quantity of organic carbon in the soil was estimated by the method of Jackson, (1967). One g fine ground soil samples were taken in 500 mL conical flask, to which 5 mL of 1 N Potassium Dichromate and 10 mL Conc. H_2SO_4 were added and then the mixture was shaken for 30 min. After this 100 mL of distilled water, 5 mL Orthophosphoric Acid, and 1 mL of diphenylamine indicator were added. The solution was titrated against standard 0.5 N Ferrous Ammonium Sulfate (FAS), till the colour changes from blue to green. Blank titration was carried out without soil.

2.5 Available Phosphorus (P)

Available phosphorus was estimated by the method described by Olsen et al. (1954) using the bicarbonate extraction procedure. In this procedure, 42 g of sodium bicarbonate was dissolved in 1000 mL of distilled water and pH adjusted to 8.3. Soil samples (2.50 g) were weighed into a 150 mL flask, and 50 mL of Olsen's reagent (0.5 M $NaHCO_3$) and 1 g of activated carbon were added. The flask was kept for 30 min on a shaker and then the contents were passed through Whatman filter paper (No. 40). Filtrate (5 mL) was taken and mixed with 5 mL of ammonium molybdate reagent for neutralizing the solution and the volume was made up to 20 mL by adding distilled water. Colour was found to develop when 1 mL of diluted $SnCl_2$ (Stannous Chloride) from the stock solution (10 g $SnCl_2$ in 25 mL of con. HCl) was added and the mixture was shaken thoroughly. The blue colour was read at 660 nm after 10 min using a spectrophotometer with appropriate blank.

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2.6 Available Potassium (K)

The flame photometeric method (Jackson, 1958) was employed to estimate potassium present in the soil. 5 g of air dried sample was taken in 150 mL flask and 25 mL of ammonium acetate (1 M) was added to the flask. The contents were shaken for 5 min on a shaker and filtered through Whatman filter paper No. 1. The filtrate was collected in a fresh beaker. 5 mL of this filtrate was diluted with 25 mL of distilled water and the reading was recorded by using a flame photometer.

2.7 Micronutrient Analysis

Commonly studied micronutrients include Zn, Cu, Fe, Mn, B, and Mo. Different extractants were developed for assessing plant available nutrients (elements) in the soil. Pentetic Acid/Diethylenetriaminepentaacetic Acid (DTPA) is widely used chelating agent employed for simultaneous extraction of elements, like Zn, Cu, Fe, and Mn. DTPA extracting solution prepared by DTPA 0.005M (1.98 g), 0.01 M $CaCl_2.2H_2O$ (1.47 g) and 0.1 M TEA (13.3 mL) extractant. The pH was adjusted to 7.3 by using 1 M HCl before making the volume with distilled water to 1000 mL. The estimation of elements in the extract was done with the help of Atomic Absorption Spectrophotometer (AAS). In this experiment, 10 g of soil samples were taken and mixed with 20 mL of DTPA extracting solution, mixed well for 2 h and filtered with Whatman no. 40 filter paper. Filtered extracts were stored in new bottles and allowed to stand before testing.

3. Results and Discussion

3.1 Nutrient Analysis of Soil Samples Collected from Kedarnath

Soil is a living entity and vital for the sustenance of life on planet earth. It is a provider of nutrients, anchoring support and moisture for the growth and survival of plants. Therefore, soil moisture content is an important parameter for the health of the plants. The average soil moisture content of the examined four different samples was recorded to be between 36.93 ± 3.17 % to 52.73 ± 2.30 %; results indicate that the soil moisture content in the rhizospheric soil was invariably higher than found in the non-rhizospheric soil samples (Table 2). Soil pH values indicate it to be slightly acidic to mildly alkaline; nutrient availability and the activity of many micro-organisms are affected by the soil pH. It also influences the type of crops that can be grown, and overall plant growth. The pH of all samples was found to range between 6.50 ± 0.04 to 7.41 ± 0.04. By and large the pH of rhizospheric soil was neutral to mildly alkaline, and that of the non-rhizospheric soil was slightly acidic to neutral. The per cent carbon values were found to be between 0.64 ± 0.09 to 0.99 ± 0.01, while the available phosphorus and available potassium were found to be between 8.93 ± 0.02 to 26.90 ± 0.09 Kg/hectare and 99.83±0.76 to 301.35±1.29 Kg/hectare, respectively. Atomic Absorption Spectroscopy was used for the estimation of Zn, Cu, Fe and Mn. The observed values for various elements were in parts per million (ppm): Zinc (0.22 ± 0.00) to (2.02 ± 0.03), Mn (0.00 ± 0.00) to (8.74 ± 0.11), Fe (0.00 ± 0.00) to (19.23 ± 0.04) and Cu (0.56 ± 0.00) to (1.27

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± 0.00). For elemental estimations using AAS, hollow cathode lamps, specific to various elements were used.

Table 2. Physico-chemical characteristics of rhizospheric and non-rhizospheric soil collected from Kedarnath valley

Sample ID	Sample Name	Moisture Content (%)	pH	Organic Carbon (%)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Zinc (ppm)	Manganese (ppm)	Iron (ppm)	Copper (ppm)
1	N.S. MG	40.47 ± 3.78	7.28 ± 0.03	0.64 ± 0.09	26.90 ± 0.09	120.75 ± 0.67	1.10 ± 0.10	1.11 ± 0.01	11.61 ± 0.11	0.75 ± 0.01
2	R.S. MG	48.67 ± 1.15	7.41 ± 0.04	0.92 ± 0.03	8.93 ± 0.02	171.33 ± 1.52	1.27 ± 0.00	5.82 ± 0.00	19.23 ± 0.04	0.85 ± 0.00
3	N.S. CG	39.47 ± 1.52	6.83 ± 0.03	0.99 ± 0.01	22.29 ± 0.25	165.54 ± 1.25	0.49 ± 0.00	0.25 ± 0.18	0.00 ± 0.00	0.56 ± 0.00
4	R.S. CG	49.07 ± 7.76	7.31 ± 0.02	0.75 ± 0.03	17. 55 ± 0.66	232.47 ± 2.16	1.35 ± 0.10	8.74 ± 0.11	3.25 ± 0.00	1.27 ± 0.00
5	N.S. HP	39.13 ± 2.88	6.93 ± 0.03	0.81 ± 0.02	13.44 ± 0.07	179.44 ± 1.07	0.22 ± 0.00	2.30 ± 0.00	3.83 ± 0.00	0.56 ± 0.00
6	R.S. HP	52.73 ± 2.30	6.53 ± 0.06	0.79 ± 0.03	22.08 ± 0.50	301.35 ± 1.29	0.41 ± 0.01	0.00 ± 0.00	0.81 ± 0.00	0.60 ± 0.00
7	N.S. MP	36.93 ± 3.17	6.50 ± 0.04	0.82 ± 0.02	13.40 ± 0.03	99.83 ± 0.76	1.52 ± 0.01	4.70 ± 0.00	8.60 ± 0.00	0.85 ± 0.00
8	R.S. MP	46.00 ± 7.00	7.09 ± 0.02	0.80 ± 0.04	22.2 ± 0.42	181.36 ± 2.36	2.02 ± 0,03	3.40 ± 0.00	0.00 ± 0.00	0.96 ± 0.71

N.S. stands for non-rhizospheric soil and R.S. stands for rhizospheric soil; MG, CG, HP, and MP refer to various sites as shown in Table 1.

According to Maurya et al. (2014), altitude is one of the most important topographical factors which can influence the fertility status of soil. They reported the values for pH: 5.80; OC: 2.07 %; P: 98.87 Kg/ha; K: 1860.98 Kg/ha for soil samples collected from Almora district at 4000-4500 feet amsl. These values are higher than the observed values in the present study. Shukla et al. (2015) also reported an investigation of soil structure and nutrient analysis of soils collected from 13 districts of Uttarakhand and according to this report the reported range for Zn was (0.11 to 17.18), Mn (0.03 to 64.42), Fe (0.47 to 106.14), and Cu (0.11 to 4.82) in ppm from Rudraprayag district (current study area) and the recorded values for Kedarnath sites are close to these values. Thus the micro-nutrient mapping done for the soil nutrients by Shukla et al. (2015) is in agreement with the current study.

4. Conclusion

The results of this study provide baseline information for initiating a conservation program for this endangered, culturally and medicinally important plant, *S. obvallata*, and may also help to understand the nutrient dynamics at high altitudes. Further research is required to understand the correlation between soil nutrient availability, plant population distribution with nutrient cycling in areas that support *S. obvallata.*

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