Chemical Analysis of Uncultivated *Cichorium intubus*

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Summary: Uncultivated medicinal plants are useful for assessing the nutrients bioavailability in terrestrial soils ecology. Wild *Cichorium intubus* growing in Cholistan desert environment was selected to study variation in the concentration of nutrient ions like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cl^-, HCO_3^-, PO_4^{3-}, and SO_4^{2-}. A positive nutrient-to-nutrient correlation ($p < 0.01$) was observed for the certain nutrient pairs. The results of the current study were compared with the levels observed in adjoining soil. All nutrient levels except Na^+ and Ca^{2+} were found higher in plant than the adjoining soil. This base line data generated will be helpful for 'Hakims' in the preparation of 'Unani System of Medicines'.

Introduction

Life and diseases go together; where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through their uses as foods, fibers and shelter, but also plants have been used to control and ease diseases, therefore the use of plants as medicines is an ancient and reliable practice [1-5].

Besides water, plants contain inorganic nutrients from the soil for their metabolic activities. Some of the heavy metals are essential in trace amounts and play important role in plant and animal nutrition, enzymatic reactions and metabolic processes [6-8]. The sensitivity of plants and animals differs strongly for deficiency and excess levels of a particular metal. The availability of heavy metals depends upon soil properties such as pH, electrical conductivity, lime content, organic matter and texture. Therefore, their correlation studies are of utmost importance [9-14].

In deserts, the medicinal plants are traditionally used for the treatment of several diseases. Ailments and medicinal plants vary in the world, hence their nature; frequency and methods of administration can change in relation to geography, time and knowledge. Natural herbal treatments are also appealing to all the tiers of society simply, because they are far cheaper than the synthetics [15].

In the present study, *Cichorium intubus* is selected for the determination of nutrients from the plant and adjacent soil. The purpose of this research is to study variation in the concentration of nutrient ions found in plant and local soil habitat and to assess desert growing plant's nutritive values.

Pharmacological importance

Cichorium intubus also known as chicory and Kasni has been in use as a pot herb from an early period. The plant is found in grassy and uncultivated areas of Eurasia up to the borders of Afghanistan, Balochistan, Punjab, Sindh and Andhra Pradesh and is cultivated in Bihar, Punjab, Himachal Pradesh, Assam, Maharashtra, Gujarat, Tamil Nadu, Orissa, Andhra Pradesh and Kerala for preparation of herbal medicines [2, 15].

Subcontinent's Hakims used this plant as a resolvent and cooling medicine and prescribed in bilious complaints. Plant roots dried, roasted and reduced to powder is very extensively used in Europe as a substitute for coffee and for adulterating that article. The roots contain tannin phlobaphenes and reducing sugars. The seeds have carminative and cordial properties and are useful as a brain tonic and for headache, asthma and bilious vomiting. It is used in hepatic enlargement, fever, vomiting and abdominal pain and has cholagogue and anti-inflammatory properties [16, 17].

The crude extract of the plant has been shown to inhibit free-radical-mediated DNA damage in lab

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animals [18-20]. An alcoholic extract of the plant is found to be effective against chlorpromazine hepatic damage in albinos and also exhibit resorptive activity. The alcoholic aqueous extract shows cholagogue activity, which may be attributed to polyphenols. The alcoholic extract of the seeds has been reported to cause bradycardia in normal and hypodynamic hearts and elicit fall in the blood pressure [21-26].

Results and Discussion

Cichorium intubus (Table-1) have high potassium intake, i.e. 37.58 ppm to 42.82 ppm, parallel that for low sodium intake i.e. 6.56 ppm to 10.37 ppm. While the levels of sodium and potassium in adjoining soil are 17.04 ± 5.62 ppm and 3.99±1.76 ppm respectively. The statistical linear correlation study at $p < 0.01$ (Table-2 and 3) for soil and plant shows strong positive correlation (r = 0.817, 0.743) for Na^+-K^+ pairs respectively. Sodium and potassium are the electrolytes and sodium is the major component of the cation of extra cellular fluid while potassium is the cation of intracellular fluid. 139 meq / liter of sodium and 5 meq / liter of potassium is present in blood plasma of human beings. Both sodium and potassium controls the osmotic pressure including water retention. High concentration of sodium leads to hypertension while the high concentration of potassium leads to the dilation of arteries and normalize the blood pressure. Excessive amount of potassium leads to the failure of heart [27, 28].

The amount of calcium varies from 24.29 ppm to 56.32 ppm parallel to high level in the adjoining soil i.e. 32.35 ppm to 96.15 ppm. In case of soil (Table-2), calcium has strong positive correlation with magnesium, phosphate and bicarbonate where as in plant negative correlation (Table-3), is observed. Calcium is absorbed as divalent (Ca^{+2}). Most soils contain enough calcium for adequate plant growth, but acidic soils, where high rainfall occurs; the soils are deficient of calcium. The calcium is used in the synthesis of new cell walls. A protein found in Cytosol, to form calcium-calmodulin complex might be involved in regulation of many metabolic processes. 5 meq / liter of calcium is present in blood plasma of humans [7, 9, 12].

Kasni plant (Table-1 and 3) has high concentration of magnesium i.e. 43.28 ppm to 67.23 ppm than the calcium and shows strong positive correlation with sulphate (r = 0.929) in plant and with iron and aluminium in soil (Table 2). It is interesting to note that plant keeps high magnesium concentration even when the peripheral soil has low value

Table 1: Levels of nutritive elements found in *Cichorium intubus* and adjoining soil

Sample Code	Soil / Plant	pH	Moisture contents %	Organic contents %	Soluble salts %	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Fe^{3+} (ppm)	Al^{3+} (ppm)	Cl^- (ppm)	HCO_3^- (ppm)	PO_4^{3-} (ppm)	SO_4^{2-} (ppm)
A	Soil	7.72	16.25	3.24	2.45	10.56	2.34	96.15	33.62	287.75	220.75	55.96	12.31	0.4[illegible]	436.43
	Plant	-	-	-	-	6.56	37.58	24.29	43.28	838.35	-	689.13	44.37	0.57	304.86
B	Soil	7.71	3.42	3.57	4.47	20.23	3.79	32.35	28.85	288.95	440.31	78.45	8.95	0.25	326.92
	Plant	-	-	-	-	10.37	40.58	24.78	67.23	838.27	-	213.98	36.76	0.26	413.67
C	Soil	7.66	16.27	2.46	6.78	20.34	5.85	80.18	51.37	430.46	515.62	71.95	10.86	0.36	254.84
	Plant	-	-	-	-	9.24	42.82	56.32	48.56	720.64	-	217.96	29.89	0.18	365.52
*Soil	Range	7.66-7.72	3.42-16.27	2.46-3.57	2.45-6.78	10.56-20.34	2.34-5.85	32.35-96.15	28.85-51.37	287.75-430.46	220.75-515.62	55.96-78.45	8.95-12.31	0.25-0.45	254.84-436.43
	Average	7.70	11.98	3.09	4.57	17.04	3.99	69.56	37.95	335.72	392.23	68.79	10.71	0.35	339.40
	SD	±0.03	±7.41	±0.57	±2.17	±5.62	±1.76	±33.20	±11.87	±82.05	±153.20	±11.57	±1.68	±0.10	±91.44
*Plant	Range	-	-	-	-	6.56-10.37	37.58-42.82	24.29-56.32	43.28-67.23	720.64-838.35	-	213.98-689.13	29.89-44.37	0.18-0.87	304.86-413.67
	Average	-	-	-	-	8.72	40.33	35.13	53.02	799.0	-	373.69	37.01	0.44	361.35
	SD	-	-	-	-	±1.96	±2.63	±18.35	±12.58	±67.94	-	±273.18	±7.24	±0.37	±54.52

* Range, average and SD for Levels of nutritive elements found in Cichorium intubus and adjoining soil

Table 2: Linear correlation coefficient matrix for selected nutritive parameters studied in soil adjacent to *Cichorium intubus* (n = 3).

	pH	Moisture contents	Organic contents	Soluble salts	Sodium	Potassium	Calcium	Magnesium	Iron	Aluminium	Chloride	Bicarbonate	Phosphate
Moisture contents	-0.360												
Organic contents	0.901	-0.730											
Soluble salts	-0.946	0.040	-0.712										
Sodium	-0.636	-0.490	-0.237	0.851									
Potassium	-0.964	0.101	-0.754	0.998	0.817								
Calcium	-0.124	0.970	-0.543	-0.203	-0.687	-0.142							
Magnesium	-0.936	0.665	-0.996	0.773	0.325	0.810	0.464						
Iron	-0.989	0.495	-0.955	0.888	0.515	0.915	0.270	0.978					
Aluminium	-0.800	-0.271	-0.460	0.951	0.972	0.930	-0.495	0.539	0.703				
Chloride	-0.385	-0.722	0.055	0.662	0.957	0.615	-0.868	0.037	0.244	0.861			
Bicarbonate	0.077	0.902	-0.364	-0.395	-0.818	-0.338	0.980	0.278	0.072	-0.659	-0.950		
Phosphate	0.098	0.893	-0.344	-0.414	-0.830	-0.358	0.975	0.257	0.050	-0.675	-0.956	1.000	
Sulphate	0.884	0.117	0.593	-0.988	-0.923	-0.976	0.354	-0.664	-0.805	-0.988	-0.771	0.534	0.552

Values>0.3 or < -0.3 are significant at P< 0.01

Table 3: Linear correlation coefficient matrix for selected nutritive parameters studied in *Cichorium intubus* ($n = 3$).

	Sodium	Potassium	Calcium	Magnesium	Iron	Chloride	Bicarbonate	Phosphate
Potassium	0.743							
Calcium	0.242	0.829						
Magnesium	0.856	0.291	-0.294					
Iron	-0.229	-0.822	-1.000	0.307				
Chloride	-0.959	-0.902	-0.505	-0.676	0.494			
Bicarbonate	-0.706	-0.999	-0.858	-0.239	0.851	0.877		
Phosphate	-0.921	-0.945	-0.600	-0.588	0.589	0.994	0.926	
Sulphate	0.987	0.624	0.080	0.929	-0.067	-0.900	-0.581	-0.845

Values>0.3 or < -0.3 are significant at $P < 0.01$

i.e. 28.85 ppm to 51.37 ppm. Magnesium is absorbed as divalent Mg^{+2}. Magnesium is almost never limiting to the plant growth in soils. In plant cells, magnesium has a specific role in the activation of enzymes involved in respiration, photosynthesis and synthesis of DNA and RNA. Magnesium is also a part of the porphyrin component of the chlorophyll. It combines with ATP and allows it to function [8, 13].

Kasni has high concentration of iron i.e. 720.64 ppm to 838.35 ppm than the adjoining soil i.e. 287.75 ppm to 430.46 ppm. In soil, iron shows strong positive correlation with chloride and aluminium where as in plant with bicarbonate ($r = 0.851$). Iron plays an important role as a component of enzymes concerned with the transfer of electron (redox reaction), such as cytochromes. It undergoes alternative oxidation and reduction between Fe^{+2} and Fe^{+3} states as it acts as electron carrier. Iron deficiency causes the anemia disease, which may be cured by the use of this plant [7, 12, 13].

Concentration of chloride studied in Kasni is higher i.e. 213.98 ppm to 689.13 ppm than concentration of bicarbonates i.e. 29.89 ppm to 44.37 ppm. In Cholistan soil (Table-2) chloride shows negative correlation with bicarbonate, phosphate and sulphate where as in plant (Table-3) shows strong positive correlation with bicarbonate and phosphate. Chloride is essential in water balance, osmotic pressure regulation as well as acid base equilibrium and most of it may be required for cell division in leaves and roots [15].

Table-1 shows that concentration of Sulphate (361.35 ± 54.52) is greater than phosphate (0.437 ± 0.37) in Chicory plant. In soil, sulphate has strong positive correlation with bicarbonate ($r = 0.534$) and phosphate ($r = 0.552$) where as in plant the following pattern is observed: > calcium > potassium > magnesium > sodium. Sulpher in plants is absorbed as divalent SO_4^{-2}. Sulphate ions are present in proteins especially in amino acids cystein and methionine that are building blocks of proteins. It is an essential component of vitamins, biotin and co-enzyme. Phosphorus, as phosphate, is an integral component of a number of important compounds present in plant cells. It act as limiting element in soils and absorbed primarily as monovalent phosphate anion-H_2PO_4. The ability of these two forms of ions is controlled by soil pH i.e. monovalent is favored by pH below 7 and divalent form by pH above 7. Phosphorus remains as free phosphate or bound to organic compounds as esters in plants [29-33].

Experimental

During this study, 3 samples of *Cichorium intubus* were collected from each three different areas of Bahawalpur division, located in Cholistan desert (Table-4). The samples were then analysed for different cations and anions like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Cl^-, HCO_3^-, PO_4^{3-}, and SO_4^{2-}, using different laboratory methods [28, 29].

Chemical Reagents

All the reagents and chemicals used were of analytical reagent grade from Merck and Fluka. All the solution of standards and samples were prepared in freshly prepared deionised water using E.mil glasswares. Mitler PM 40 electric balance, Milwaukee pH meter and Corning – 460 flame photometer were used.

Plant Sample Preparation

The plants were cleaned visually to remove the dust particles and dried at 150 °C to a constant

Table 4: Sampling Locations.

Sample code	Sampling Areas.
A	Plant and soil samples collected from Bahawalpur city i.e. Aziz abad colony.
B	Plant and soil samples collected from Khanqah Sharif, Ganwar Shah road Bahawalpur.
C	Plant and soil samples collected Faisal colony, Bahawalpur.

weight. The dried plants were grinded to fine powder and then used for dry ashing. The pre-cleaned silica crucible was heated at 600 °C to a constant weight. The powdered plant material in the crucible was heated in a muffle furnace at 600 °C. The crucible containing plant ash was cooled at room temperature and moistened with deionised water to keep it overnight. The undissolved particles were filtered and make up the volume to 1000 ml. This solution was used as sample solution [28, 29].

Soil Sample Preparation

20 grams of dried soil was taken in a 500 ml beaker, stirred it with 100 ml deionised water for 30 minutes and filtered through 'Whattman 42' filter paper. The volume of the filtrate was made up to 1000 ml and is used for quantitative estimation of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Cl^-, HCO_3^-, PO_4^{3-}, and SO_4^{2-}[28, 29].

Methods

Sodium and potassium were determined by flame photometer model 'Corning – 40'. Calcium and magnesium were determined by complexometric titration [12, 28]. Iron was determined by oxidation-reduction titration using diphenylamine as internal indicator [15, 29]. Phosphates were determined by celorimetric method using 'ammonium dihydrogen phosphate' as standard solution and 'molybodate' as complexing agent [15, 28]. Sulphates were estimated gravimetrically [28, 29]. Bicarbonates were determined by titrimetric method and chlorides were determined by argentometric titration [15].

Statistical analysis

The data was statistically analysed by using SPSS 12 and STATSICA (StatSoft 1999) softwares on P-IV system. The concentrations of elements in soil and plant samples were correlated by linear correlation coefficient matrix (pearson).

Conclusions

The inorganic trace elements are active in very low concentration, so in therapeutic activity of medicinal plants the trace elements play major role. The trace minerals help in digestion, aid in replacing electrolytes and defend cells against toxic reaction and poisoning. It is concluded that the levels of nutritive elements like K^+, Mg^{2+}, Fe^{3+}, Cl^-, HCO_3^{-1}, PO_4^{3-}, and SO_4^{2-} are found higher in the *Cichorium intubus* than the soil in which the plant is being growing and the levels Na^+ and Ca^{+2} are found lesser in the plant even when the adjoining soil has their higher concentration. This data will help in establishing baseline levels on the use of the plant as medicine.

References

1. M. Arshad and A.R. Rao, Plant Genetic Resources of Cholistan Desert and their utilization. Arid Zone Research Supplement, Xinjiang Institute of Biology, Pedology and Desert Research, Urumqi China, (1995).
2. Atta-ur-Rahman, S.M.Hakim and Viqar-u-ddin, Pakistan Encyclopedia Planta Medica, Hamdard Foundation Pakistan and H. E. J. Research Institute of Chemistry-Karachi, Pakistan, (1986).
3. A.Q.S. Bukhari and M. Mirza, *National Health.*, 3, 26 (1985).
4. I. Ahmad, M. Arshad and H. Bibi, *Journal of Biological Sciences*, 1, 768 (2001).
5. H.M. Said, *Medicinal Herbal* Hamdard Foundation Karachi-Pakistan (1996).
6. D.C. Adrian, Trace Elements in the Terrestrial Environment. Springer, New York, (1986).
7. E.J. Hewitt and T.A. Smith, Plant Mineral Nutrition, The Eng. Univ. Press Ltd. London, (1974).
8. E.J. Underwood, Trace Elements in Human and Animal Nutrition, Academic Press, New York, (1977).
9. K.A. Pendias and H. Pendias, Trace Elements in Soils and Plants. CRC Press, Inc. Florida, (1986).
10. H.W. Martin, T.R. Young, D.I. Kaplan, L. Simon, D.C. Adriano, *Plant-Soil*, **182**, 199 (1996).
11. P. Brather and P. Schramel, Trace element analytical in medicine and biology. Walter de

Gruyter, Berlin, (1980).

12. V.Harold, Practical Clinical Biochemistry. 4th. Ed. Vazirani for Anrold Heineman Pvt. Ltd., p. 526. (1970).
13. J.B. Herry and B.E. Statland, Chemical Pathology and Clinical Chemistry, W. B. Saunders Company, Philadelphia, (1979).
14. Ochai and El–Chiro, *Bioinorganic Chemistry*. Boston, Allyn, (1936).
15. S. Yasmin, Determination of heavy and essential metal in Cichorium intubus (Kasni) grown in Cholistan desert. M. Sc. thesis, Department of Chemistry, Islamia University Bahawalpur, Pakistan, (2002).
16. M.M. Bhandari, Flora of Indian Desert. Scientific Publishers, Jodhpur, India, (1978).
17. J. Park and J. Yun, *Lett. Appl. Microbiol.*, **33**, 183 (2001).
18. D.P. Belesky, J.M. Fedders, K.E. Turner, J.H. Ruckle, *Agronomy Journal*, **91**, 450 (1999).
19. K.E. Turner, D.P. Belesky and J.M. Fedders, *Agronomy Journal*, **91**, 445 (1999).
20. Goel and Bhattacharyya, *Jour. Econ. Taxon. Bot.*, **2**, 157 (1981).
21. J.H. Kim, Y.J. Mun, W.H. Woo, K.S. Jeon, N.H. An, J.S. Park, *Int. Immunopharmacol.* **2**, 733 (2002).
22. L. Simon, J. Prokisch, B. Kovács, Z. Győri, Proceedings of 7th International Trace Element Symposium, Budapest, Hungary, 83 (1996).
23. S.R. Baquar, Medicinal and Poisonous Plants of Pakistan, Printas-Karachi, Pakistan, (1989).
24. L. Simon, Z. Pomázi, J. Fischinger, B. Kovács, J. Prokisch, Proceedings of 6th International Trace Element Symposium, Budapest, Hungary, 339 (1994).
25. L. Simon, B. Kovács, J. Prokisch, Z. Győri, Proceedings of the scientific conference: Soil environment degradation and improving, Agricultural University of Wroclaw, (1995).
26. L. Simon, J. Prokisch, B. Kovács, 3rd International Conference on the Biogeochemistry of Trace Elements, Paris, France, (1995).
27. Recommended Dietary Allowance (RDA), 19th Ed. National Academy of Sciences, Washington DC, (1965).
28. F. Chouhan, M.H.S. Wattoo, S.A. Tirmizi, *The Nucleus*, **39**, 195 (2002).
29. S.A. Tirmizi, F.H. Wattoo, M.H.S. Wattoo, S. Kanwal and J. Iqbal, *Jour. Chem. Soc. Pak.*, 27, 186 (2005).
30. Y.J. Cho, J. Sinha, J.P. Park, J.W. Yun, *Enzyme-Microb.-Technol*, **28**, 439 (2001).
31. Douglas and N. Z. Poll, *Jour. Exp. Agric.* **14**, 223 (1986).
32. Sama, *Indian Jour. Med. Res.* **64**, 738 (1976).
33. L. Simon, H.W. Martin, D.C. Adriano, *Water, Air, Soil Pollut.* **91**, 351 (1996).