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NETTLE PLANTS FINGERPRINT BASED ON XRF ANALYSIS

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Abstract

Stinging nettle (Urtica dioica L.) is a perennial crop well adapted to a varied range of environmental conditions. The cultivation of nettle could help meet the high demand of nutritious plants, for food, nutraceuticals and as a substitute for artificial fibers for different industries. Nettles are well known as hyperaccumulators of heavy metals, growing as weed, worldwide, which makes them suitable for the present study. The aim of this study is to present the possibility of using X-ray fluorescence (XRF) spectrometry as a valuable tool to create fingerprints to evaluate the environmental pollution of a specific area using soil and nettle plants samples, as well as verifying the quality of plants utilized as potential food sources. Nettle plants and soil samples were randomly collected from different areas of Romania, dried under controlled environment, then prepared for XRF Analysis. All samples were analyzed in triplicate using Hitachi X-MET8000 XRF analyzer and the results were statistically evaluated using statistical programs. The results show the possibility to obtain valuable fingerprints based on the elemental composition correlated to the geographical origins of plants.

Key words: *Urtica dioica, heavy metals, food safety, mathematical models.*

INTRODUCTION

The distribution of mineral elements in the ecological environment is irregular, having major changes in the structure and content of mineral elements in water, soil and air (Franke et al., 2005).

The X-ray Fluorescence (XRF) technique presents a strong reliability in the identification of chemical elements existing in soil and plant samples, regardless of the instrumental conditions and the type of analyzed plant sample (roots, stems or leaves) (Panebianco et al., 2023).

The characterization of trace elements in natural and environmental samples are frequently made by using conventional methods, i.e., atomic spectroscopic techniques, including ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy), ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) (Panebianco et al., 2023).

The term analytical fingerprint refers to scientific techniques that are used as a traceability tool to identify the source or origin of the products of interest (Watzel, 2023).

The stinging nettle (*Urtica dioica* L., *Urticaceae*), a widespread weed plant - prominent in desert areas, with unpleasant hairs on stems and leaves, is defined by significant economic potentials, being used in human nutrition and medicine since Antiquity. (Rafajlovska et al., 2013, Kavalali, 2003). Due to the existence of some precious compounds, such as proteins, macro and micro elements, tannins, flavonoids and fatty acids, phenolic components, chlorophylls the nettle plants and /or parts are used in several ways (Rafajlovska et al., 2013). Nettle leaves show high levels of vitamins (A and C) and minerals (iron, calcium, manganese and potassium) (Bordean, 2012).

The "fingerprint" of micro-chemical differences suggests a reference for the traceability of the origin of products/plants due to the elemental structure which varies between nettles originated from different places. The detailed characterization of the multi-mineral elements of the plant materials suggests a possible link for the research of the traceability of other valuable products of geographical indication (Xue et al., 2022).

Chemical fingerprints of plants, measured in general concentrations, contribute to research in a number of environmental, biomonitoring and biological analysis sectors (Djingova et al., 2004). Founded in 1990 by O'Byres and Wackernagel, the ecological footprint has the special meaning of combining data from several sectors, expressing them in the form of an equation with four variables (Odagiu et al., 2012).

Heavy metals represent a massive category of genotoxic environmental contaminants that present a serious danger to the environment and human health.

The mineral nutrient content in plant foods is influenced by the plants' capacity to absorb and integrate specific minerals from their growing environment. The term "concrete mineral nutrients" implies particular and well-defined minerals that the plants assimilate based on the conditions of the growing climate.

The essential source of mineral nutrients for plants is the soil, which contains important nutrients for plant growth, such as nitrogen, potassium, phosphorus, magnesium, calcium, sulfur, iron, zinc, copper, manganese, boron, molybdenum, chlorine, nickel (Kirkby, 2013).

The aim of this study is to present the possibility of using X-ray fluorescence (XRF) spectrometry as a valuable tool to create fingerprints to evaluate the environmental pollution of a specific area using soil and nettle plants samples, as well as verifying the quality of plants utilized as potential food sources.

MATERIALS AND METHODS

Collection and preparation of samples

The nettle plant and soil samples were collected during autumn 2003, from Romania: Turcinesti - Gorj county, Vadu-Dobrii - Hunedoara, Bazos, Recas - and Timisoara, Timis county, as presented in Figure 1.

The location L3 is close to the Dendrological Park, located in a grand secular oak forest, which received the status of a protected area for the protection of the biodiversity of the Geno fond and Eco fund since 1994 (Wasicsek A., 2020).

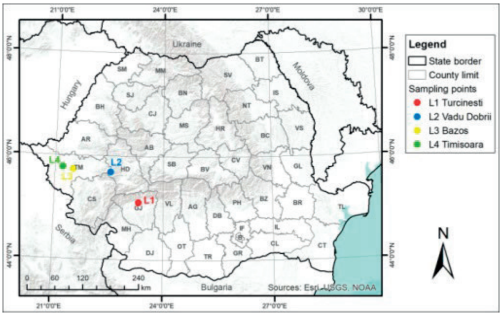

Figure 1. Collection areas of soil and nettle sample

The 5 random selected nettle plants together with the soil samples were collected from each mentioned location (L1-L4). All sub-samples were mixed to create the laboratory soil samples and laboratory nettle samples. The soil samples were sieved through a 2 mm sieve to remove non-soil material, including plant fragments, then homogenized using a grinder, dried under controlled environment and stored in tight sealed containers.

The plant samples were carefully washed with tap water to remove dust and soil particles, followed by rinsing with distilled water to ensure that any residual minerals from tap water were eliminated. This was done to reduce the risk of contamination and maintain the integrity of the samples. The soil was carefully shaken from the roots. Roots were separated from plant stems and leaves. All plant parts were air-dried and homogenized by grinding in a laboratory mill designed for processing food samples.

Samples analysis

The mineral content of soil and plant samples was analyzed using XRF Hitachi XMET8000 portable spectrometer, which can be used to measure a wide range of atomic elements, from sodium (Z11) to uranium (Z92), while providing elementary detection limits from small parts per million (ppm) by weight high percentage (% weight).The XRF method was selected because XRF (X-ray fluorescence spectrometry) is an essential evaluation method of soil and plant samples at medium air pressure, frequently used for qualitative and quantitative analyses (Yonehara et al., 2010). XRF spectrometry is beneficial because it provides the concentrations and presence of chemical elements quickly, with high accuracy (Panebianco et al., 2023).

Statistical analysis

PAST and MVSP programs were used for statistical analysis and mathematical modeling.

Cluster analysis is a method that has the role to identify groups (clusters) or patterns in a set of data on the basis of their similarities or differences. Correlation is a statistical tool used to assess the degree of association of two quantitative variables measured in each member of a group (Aggarwal & Ranganathan, 2016).

Generalized linear models (GLM) is a more flexible form of linear regressions (Guo, 2022), providing a common approach to a broad range of response modelling problems (Faraway, 2010).

RESULTS AND DISCUSSIONS

XRF technique is of great help to obtain fast and reliable informations regarding the elemental composition of the investigated samples. The results are presented in Table 1.

Table 1. Mineral composition of nettle plants and soil samples

Element/S	L1S	L1P	L2S	L2P	L3S	L3P	L4S	L4P
K	18067	67624.33	15521	53359.33	14469	66529.33	16448	45271.67
Ca	7642	54660.67	6923	36390.67	5725	55785.67	47340	46191.67
Fe	27183	1437.67	74907	1361.67	39425	2282	38983	715
Mn	900	206.5	2609	335	491	198.67	973	130
Ti	4225	234.5	8360	133.67	5084	230.67	4699	63
Cu	17	29	56	39	24	20.67	51	1
Mo	0	8	5	6.5	0	7	3	6.33
Zn	72	65	247	74.67	105	61.33	207	23.67
Ni	26	23	94	19	52	19	61	2
Co	43	0	0	0	0	0	40	0
Ba	556	162	657	128	344	122	621	0
Zr	295	10.5	335	6	251	7	335	0
Sr	151	161.33	100	39	153	128.33	192	57.5
Rb	119	9.33	197	91.33	126	6.33	123	3.5
Cr	47	15	141	18	78	12	87	0
Sn	45	42	48	0	39	35	40	0
Ta	32	19	47	20.67	31	18	28	17.5
Th	13	0	16	5	14	0	15	0
Sb	0	19	0	12	0	19.5	0	18.5
Hg	8	5	9	5.5	6	6	11	7
Sc	0	315.5	0	259	0	259.67	161	237.33
Pb	32	0	57	4	31	3	53	0
Cd	0	17	0	0	8	0	0	0

Legend: L1P- location 1 plant, L2P-location 2 plant, L3P-location 3 plant, L4P-location 4 plant, L1S-location 1 soil, L2S-location 2 soil, L3S-location 3 soil, L4S-location 4 soil.

As we can observe all soil samples are described by high content of iron, manganese, titanium and barium, while plant samples are defined by high content of potassium and calcium. The analyzed soil from location 2, has the highest content of rubidium, tantalum, lead and thorium compared to the other three analyzed soil samples, which recorded lower values.

The results show that L1 soil samples present high content of Cobalt, while L2 soil samples are characterized by high quantities of Fe, Cu, Mg. Vanadium was approximately twice as abundant in the soil sample from location 2 compared to other analyzed soils. Additionally, location 1 demonstrated the highest molybdenum content in nettle plants, and the mean strontium content was greater in plants from location 1 compared to others (Table 1).

The L3 soil samples elemental analysis result proves that Bazos (the protected area) is an unpolluted zone. The nettle plants collected from L3 present the highest content of potassium and calcium (Table 1).

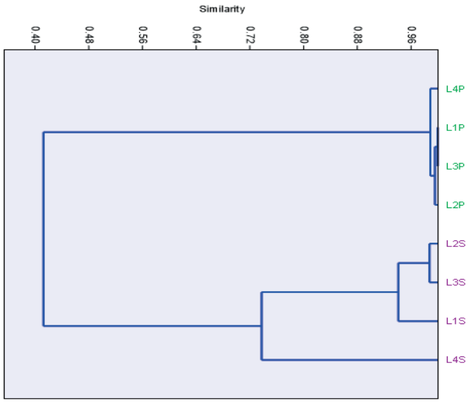

Figure 2. Graphical representation of cluster analyzes

Legend: L1P- location 1 plant, L2P-location 2 plant, L3P-location 3 plant, L4P-location 4 plant, L1S-location 1 soil, L2S-location 2 soil, L3S-location 3 soil, L4S-location 4 soil

Location 4 soil samples are described by the highest content of Ca, Mo, Sb showing a potential contamination with Sc.

The results of this study show that the nettle plants collected from the four different locations in Romania can be considered a source of valuable bio-elements. Nettle plants collected from different locations can be considered valuable sources of bio-elements due to their rich nutritional content, medicinal properties (Devkota et al, 2022), adaptability, sustainability (being used as an indicator of high soil fertility), and versatility in consumption (Schreiner, 1959 cited by FEIS, 2023).

The cluster analysis (Figure 2) was performed based on paired group algorithm and correlation as similarity measure. As we can observe the data group belonging to the nettle plants (LP 1-4) is more homogenous based on the mineral content compared to the cluster corresponding to the soil minerals data (LS 1-4).

Clustering soil and plant samples based on mineral concentrations provides a structured approach to understanding complex datasets in various scientific and agricultural contexts, like: *Pattern Recognition* (Butler et al., 2020), *Environmental Assessment* (Stein et al., 2017), *Precision Agriculture* (Gavioli et al., 2019), *Research and Hypothesis Testing* (Ahmad et al., 2022), *Verification of Food Safety* (Kuang et al., 2022) etc.

The fingerprint specific to each location is presented in Figures 3-6. The X-axis represents different minerals being analyzed in the mentioned samples, while y-axis represents the percentage composition of each respective mineral in the sample. The percentage indicates the proportion of each mineral relative to the total composition of minerals in the sample. The selected type of graph commonly used in elemental analysis, provides a visual represent-tation (fingerprint) of the relative abundance of different minerals, aiding in the interpretation of the sample's overall mineral composition.

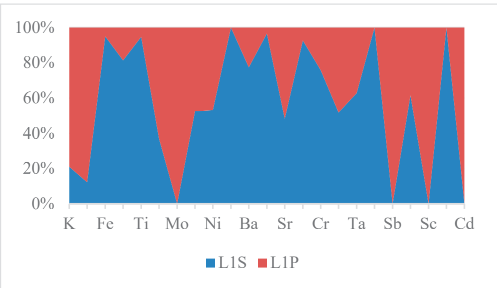

Figure 3. Mineral content fingerprint of the studied soil and plant samples collected from location 1 (Turcinesti - Gorj County)
Legend: L1P- location 1 plant, L1S-location 1 soil

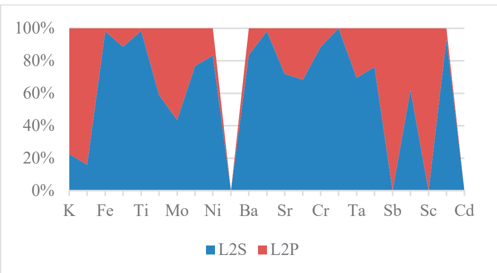

Figure 4. Mineral content fingerprint of the studied soil and plant samples collected from location 2 (Vadu-Dobrii - Hunedoara County)
Legend: L2P-location 2 plant, L2S-location 2 soil

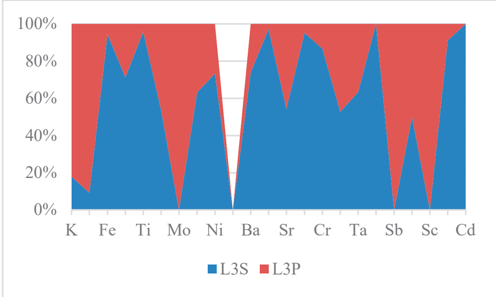

Figure 5. Mineral content fingerprint of the studied soil and plant samples collected from location 3 Bazos, Timis county
Legend: L3P-location 3 plant, L3S-location 3 soil

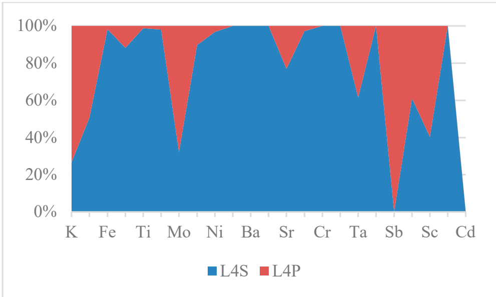

Figure 6. Mineral content fingerprint of the studied soil and plant samples collected from location 4 Timisoara, Timis County,
Legend: L4P-location 4 plant, L4S-location 4 soil

The General linear models (GLM) presented in Figures 7-10 describe the relation between soil and nettle plants for each samples collecting area from the perspective of multiple regression analysis, predicting the variation of "a dependent variable in terms of a linear combination (weighted sum) of several reference functions" (GLM-BrainVoyager v23.0).

For creating the GLM mathematical models, all variables and parameters were used. On the *x-axes* are distributed the data values corresponding to the to the minerals detected in soil samples and on *y-axis* are represented the data values corresponding to the minerals detected in plant samples.

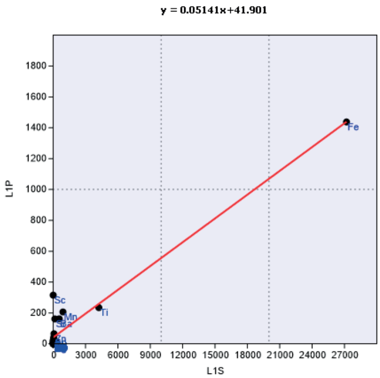

Figure 7. General linear model specific for location 1
Legend: L1P- location 1 plant, L1S-location 1 soil,
p (slope = 0): 7.548E-63

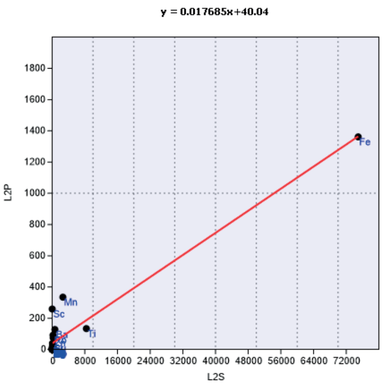

Figure 8. General linear model specific for location 2
Legend: L2P-location 2 plant, L2S-location 2 soil
p (slope = 0): 2.015E-52

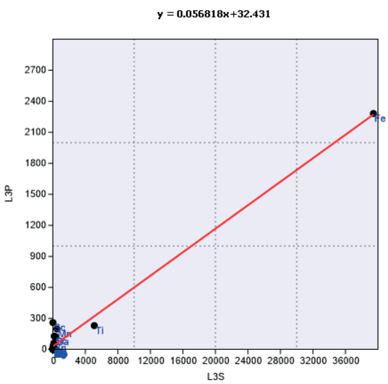

Figure 9. General linear model specific for location 3
Legend: L3P-location 3 plant, L3S-location 3 soil
p (slope = 0): 0

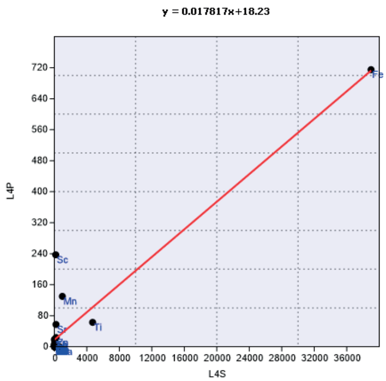

Figure 10. General linear model specific for location 4
Legend: L4P-location 4 plant, L4S-location 4 soil.
p (slope = 0): 0

The low p-value provides support for the presence of a statistically significant relationship between the predictor variable and the response variable in the presented GLM.

CONCLUSIONS

The X-ray fluorescence technique is rapid and offers multiple opportunities for elemental analysis, to identify the mineral uptake by plants or to detect the variations of pollution of various areas. As we can observe (figures 4-7), based on the mineral fingerprint of each location studied samples (soil and nettle plants) we can identify the plants desired to be used as food or medicine, or we can monitories the contamination level of soil and plants. Based on these observations we can suggest that the fingerprints created based on the XRF studies can generate maps of pollutants or desired minerals and to monitories the response of biocenosis to the environmental changes. The mathematical models based on spatial correlation are valuable tools to motorize both: cause and effect.

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