

HEAVY METAL CONTENTS OF *BRASSICA OLERACEA* AS BIOINDICATORS DETERMINED BY XRF AND AAS ANALYTICAL METHODS

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Abstract. Energy Dispersive X-Ray Fluorescence, Flame Atomic Absorption Spectrometry and Graphite Furnace Atomic Absorption Spectrometry, have been used to determine the content of Mn, Fe, Ni, Cu, Zn, Cd and Pb in different tissues of *Brassica oleracea* used as bioindicator. The results were compared with the maximum admitted limits recommended by FAO/WHO as well as with the those of Markert's "Reference plants" (Water, Air Soil Poll. **64**, 533-538, 1992). Excepting Cd and Pb whose content was 2 to 6 and respectively 2 to 7 time higher then the FAO/WHO recommendations, the content of the other elements never overpassed FAO/WHO thresholds. Both Principal Component Analysis and Factor Analysis as well as Cluster Analysis showed the presence of a main cluster consisting of Mn, Fe, Ni, Cd and Pb, attesting a certain degree of industrial contamination.

1. INTRODUCTION

Heavy metals, such as Ni, Cu, Zn, Cd or Pb, whose presence in soil could be attributed to the industrial activity of neighbouring plants or to an excessive use of phosphate fertilizer, usually relatively rich in Cd [1] are among the best bioindicators. Their content could be determined with high accuracy and precisions by using more atomic and nuclear analytical methods whose performances are well established. Among them, Atomic Absorption Spectrometry in two variants, Flame Atomic Absorption Spectrometry (FAAS) and Grafit Furnace Atomic Absorption Spectrometry (GFAAS) [3]; Atomic Emission Spectrometry (AES) in two variants, Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma with Mass Spectrometer (ICP-MS) [5] or Wave Dispersive (WDXRF) and Energy Dispersive X-Ray Fluorescence (EDXRF) [6–9] were inten-

sively used in the past decades.

Vegetables, fruits, leaves of plants, mosses and lichens, mushrooms, etc., which are at the base of any trophic chains and have a high absorption power of chemical elements from soil and atmosphere are among the best vegetal bioindicators [7, 8, 10]. In this regard, the leafy vegetables distinguish themselves by a considerable capacity to absorb and retain different heavy metals [11]. Moreover, in interpreting large amount of data, multivariate statistic method of analysis such as Principal Component Analysis (PCA) and Factor Analysis (FA) [12] showed to be helpful in grouping different pollutants according to their biological or chemical activity.

In view of this, EDXRF, FAAS and GFAAS were used to determine the contents of seven heavy metals, *i.e.* Cu, Fe, Mn, Ni, Zn, Cd and Pb in 36 samples of *Brassica oleracea* samples collected from the Petresti (Dambovită County) agricultural area. For a better interpretation of the experimental data, the results were compared with the FAO/WHO recommendations [13] as well as with the corresponding content of the Reference Plant (RP) as defined by Markert [14]. The results thus obtained will be further presented and interpreted.

2. MATERIALS AND METHODS

2.1. SAMPLING AND SAMPLE PREPARATION

Brassica oleracea plants were collected at six different locations of Petresti agricultural area, Dâmbovița County. Geographical location was performed using a GPS. From each collecting point, six different exemplars were gathered which further were sub-divided in outer, intermediate and spine leaves, core and stem. The resulting 36 samples were washed and oven dried at 40 °C. After drying, the samples were subjected to a grinding process to be crushed and kept in sterile polypropylene vials. The method for the preparation of samples for EDXRF experiments consisted of weighing a quantity of milled material (in powder form), amount of 3-5 g of each sample placed in plastic containers provided with Mylar windows of 6 microns, containers optimally adapted to the geometry of EDXRF spectrometer.

For the subsequent AAS measurements, about 0.2 g of each sample was digested with 8 ml of HNO₃(65 %) and 10 ml H₂O₂(30 %) in a microwave oven. Digestion was carried out at 1200 °C, at a pressure of 75 MPa for 20 minutes. After that, the solutions were filtered and placed in a glass flask containing a volume of 50 ml of deionized distilled water. The accuracy was checked by preparing in the same conditions the Apple leaves SRM-NIST 1515 standard material [15].

Table 1

The contents, expressed (in mg/kg), of K, Ca, Mn, Cu, Fe, Zn, Cd and Pb in the SRM-NIST 1515 sample as determined by EDXRF, FAAS and GFAAS (only Cd and Pb).

Element	Content		
	SRM-NIST 1515 (certified value)	ED-XRF experimental	AAS experimental
K	1.61±0.02	1.92±0.46	1.78±0.19
Ca	1.53±0.02	1.64±0.43	1.60±0.22
Mn	54±3	57.4±6.7	53.2±4.3
Cu	5.64±0.24	5.85±0.52	5.23±0.48
Fe	83±53	91.3±7.4	87.2±6.7
Zn	12.5±0.3	14.2±0.6	13.0±0.8
Cd	0.01±0.00	not detected	0.01±0.00
Pb	0.47±0.03	not detected	0.51±0.04

2.2. ANALYTICAL METHODS

The heavy metals contents in *Brassica oleracea* were determined by means of an ElvaX Energy Dispersive X-ray spectrometer as well as by the Avanta GBC Flame Atomic Absorption spectrometer and GBC Avanta Ultra Z Graphite Furnance Atomic Absorption spectrometer.

The experimental data were analysed by means of StatSoftTM Statistica 11.

3. RESULTS AND DISCUSSION

The accuracy of measurements was checked by measuring in the same conditions as in the case of *Brassica oleracea* samples the content of K, Ca, Mn, Cu, Fe, Zn, Cd and Pb in the SRM-NIST1515 Apple Leaves calibration samples. The results as obtained by EDXRF, FAAS and GFAAS and reproduced in Table 1 showed a good agreement between measured and corresponding certified values of the NIST sample.

In these conditions it was possible to determine the content of the same elements in different kinds of *Brassica oleracea* tissue such as outer, spine and inner leaves, core as well as strains (Table 2). As it could be remarked, the average contents of considered elements, excepting Cd, are relative close to the RP, showing a relative unpolluted environment. Moreover, the average Mn content varied between one fifth and one third of the RP one. On contrary, the high content of Cd, which overpass eight to twenty six time the corresponding RP, prove the existence of a pollution source (see Fig. 1a). This could be either an industrial unit either, as mentioned above, the use of some phosphate fertilizer whose Cd content was not checked.

To get more information about a possible origin of the Cd, a PCA based on

Table 2

The average content of heavy metals (in mg/kg) of different tissues of *Brassica oleracea* as determined by EDXRF, FAAS and GFAAS (only Cd and Pb). For comparison, the content of the same elements as sated by FAO/WHO [13] and by proposed by [14] is reproduced too. The average values refer to six different locations.

Element	Tissue					FAO/WHO	RP
	Outer leaves	Inner leaves	Spine leaves	Core	Stem		
Mn	87 ±5	62±3	50±3	64±4	36±2	-	200
Fe	225±13	191±11	161±10	165±10	161±10	-	150
Ni	2.5±0.1	2.3±0.1	1.8±0.1	2.3±0.1	1.7±0.1	-	1.5
Cu	9.4±0.4	12.9±0.6	14.2±0.6	8.7 ±0.4	10.3 ±0.4	73.3	10
Zn	25±1	41±1	30 ±1	62±1	12±1	99.4	50
Cd	1.1±0.2	0.9±0.1	0.6±0.1	0.7±0.1	0.5±0.1	0.2	0.05
Pb	2.0±0.1	1.0±0.1	1.0±0.1	0.7±0.1	1±0.1	0.3	1.0

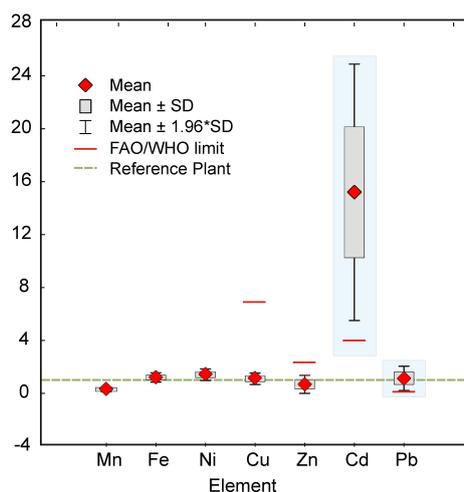


Fig. 1 – A box-and-whiskers diagram illustrating the content of investigated heavy elements in *Brassica oleracea* tissues normalized to Reference Plant [14]. It should be remarked the Cd increased content with respect to FAO/WHO [13] threshold values.

correlation was performed on both raw and normalized to RP data. Final result represented by a Factor 2 vs. Factor 1 bi-plot showed at last two cluster, one well defined consisting of Mn, Fe, Ni, Cd and Pb, and other consisting of Cu and Zn, less closed but significantly distanced by the first one (see Fig. 1b). O similar construction was obtained by using the cluster analysis. In this case, a tree diagram constructed by considering the Pearson's correlation as well as the Ward's amalgamation (joining)

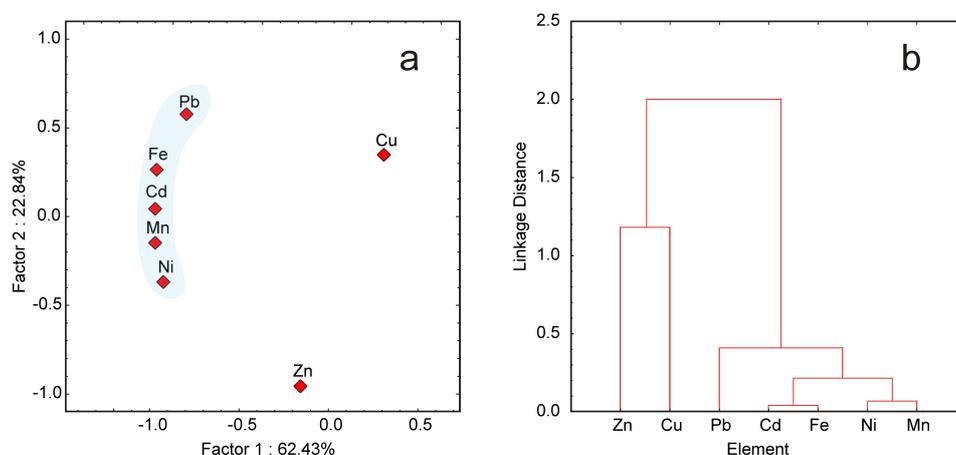


Fig. 2 – The results of PCA illustrated by a Factor 2 vs. Factor biplot (2a) as well as of the CA tree-diagram (2b) regarding the heavy metal content of *Brassica oleracea* tissue. Both diagrams illustrate, in different manner, the presence of the same two clusters.

rule showed with clarity the existence of the same two clusters.

Accordingly, the increased content of Cd as well as the fact that Cd forms a cluster with Mn, Fe, Ni and Pb suggests rather a moderate industrial contamination. At the same time it should be remarked that the Cd content, significantly higher than the RP, when compared with the FAO/WHO recommendations overpass them two to six times the maximum values (Table 2). Cd, as Pb is a non-essential trace element for living organisms, but it is considered toxic even at sub mg/kg contents, especially in the case of children [16]. For this reason, its presence should be carefully monitored.

4. CONCLUDING REMARKS

Three different analytical methods were used to determine the content of seven heavy metals, *i.e.* Mn, Fe, Ni, Cu, Zn, Cd and Pb in cabbage (*Brassica oleracea* L.) collected from the Petresti village, Arges County. Reference Plant as well as the FAO/WHO recommended values were used to quantify the degree of local pollution. Moreover, Principal Component, Factor as well as Correlation Analyses were used to evidence and correlations between the content of investigated elements.

While the content of Mn, Fe, Ni, Cu and Zn were lower or comparable with those of the Reference Plant, the Pb and especially Cd overpassed both Reference Plant contents and FAO/WHO recommended thresholds, this finding suggesting the existence of a certain degree of anthropogenic pollution, whose source, by taking

into account the Cd toxicity, should be removed and the land rehabilitated.

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