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POTENTIAL HEALTH RISK ASSESSMENT ASSOCIATED WITH HEAVY METAL ACCUMULATION IN NATIVE *URTICA DIOICA*

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Abstract. Metals concentrations of Cr, Ni, Co, Cu, Zn, Cd, and Pb in nettle (*Urtica dioica*) and soil samples, collected in April 2019 from several villages around Targoviste City, were determined. The samples were collected in the springtime (April 2019) when it is considered that the plants have reached the maturity and because inhabitants pick nettles for their purposes or sell them in markets to other consumers as well. This study revealed that concentrations of Cr, Ni, Co, Cu, Zn, Cd, and Pb in soil samples collected in April 2019 were higher than the alert level established by Romanian legislation. In the same time, Cd and Pb contents were higher than maximum admitted levels (established by Romanian Order no. 975/1998) and the daily intake values for Cr, Co, Cu, Cd, and Pb were higher than reference doses (recommended by USEPA) in all analyzed samples. The obtained results can extend understanding of the human risks of heavy metals in soil and fresh leaves and stem of nettle regarding the consumption and medical purposes. Also, this study will provide insights into pollution levels, concerning human health risk.

Key words: native plant, heavy metal, ICP-MS, daily intake, health risk index.

1. INTRODUCTION

Urtica dioica (traditionally called nettle or stinging nettle) is an herbaceous perennial flowering plant from the family *Urticaceae*, well known for its efficiency in the treatment of allergic-like symptoms [1]. *U. dioica* grows wild in regions with high humidity and temperate climate [2]. It is a perennial plant that has 1–2 m tall, mainly in summer, with soft, green leaves with long triangular teeth that are 3–15 cm long [3]. The leaves contain sterols such as sitosterol, glycoproteins, acids (*i.e.*, salicylic, malic, carbonic, and formic), flavonoids (*e.g.*, rutin, kaempferol, quercetin, etc.), essential minerals (calcium, potassium), amines (histamine, etc.), tannins, etc. On the other hand, the roots of nettle contain polysaccharides, lectins, sterols, and their glucosides (*i.e.*, 3-beta-sitosterol, sitosterol-3-d-glucoside, etc.), lignans, fatty acids, etc. [4]. One of the main organic compounds is the scopoletin, a coumarin that is found in both leaves and roots of *U. dioica* [5]. The presence of chemical substances such as histamine, organic acids, acetylcholine, leukotrienes, 5-hydroxytryptamine, and other irritants, causes a painful sting or paresthesia and the development of an erythematous macule, and itching or numbness for a period lasting from minutes to days [4]. For a long time, this plant has been used in the medical field as a diuretic and as a therapy for joint diseases, as well as for human food [2].

Currently, in medicine, *U. dioica* is used internally as a depurative, for acne, diarrhea, diabetes, to improve circulation and low blood pressure and external as a remedy for hair loss, against seborrhea and dandruff of the scalp [3, 6]. Mittman reported that the freeze-dried nettles are helpful for symptoms in allergic rhinitis and the juice of the nettle is prescribed as an antidote to the rash [7]. Also, it was reported that fresh leaves of *U. dioica* present anti-tumor activity in animal studies [4] and the extract significantly induces apoptosis in breast cancer cells [8]. Most of the elements, mainly heavy metals, in perennial plants are naturally occurring. They are acquired due to the bioaccumulation and translocation process from the soil and to the effects of the geological setting, including climatic factors [9–12]. However, the heavy metals content in wild perennial plants including *U. dioica* also depends on the contaminating effects of industry, human settlements, agricultural activities, traffic, and tailing dumps [13–15]. As previously mentioned, nettle is widely utilized for medical purposes and as food in the human diet as well. Linked to mineral requirements of the humans, mainly in spring, the fresh leaves of *U. dioica* represent a significant micromineral intake, thus determining the minimal nutrient intake that prevents the development of different illnesses like anemia, cardiovascular diseases, diabetes, allergic rhinitis, etc. Besides essential elements (*e.g.*, Fe, Ca, Mg, Na, K, Cl), these plants can bring an important intake of toxic metals (*i.e.*, Cr, Ni, Co, Cu, Zn, Cd, and Pb) in human diets. Heavy metals can induce modifications in other macro or micronutrients that could affect the absorption of the essential elements or even can influence the biochemical or physiological parameters employed in the assessment of human health status [16–19]. Besides the status of the heavy metal in fresh leaves of *U. dioica*, it can be detected by the requirements based on epidemiological studies of mineral nutrients status carried out in healthy populations with different nutrient intake profiles [20–23].

The case-control studies demonstrate a direct relationship between the consumption of fresh plants and beneficial effects for human health, provided that these plants grow in ecological conditions [4, 20, 24]. In this respect, and based on previous studies of the authors [11, 12, 16–20], the aim of this research can be considered on the one hand as a continuation of the investigation process of heavy metals content in wild plants due to the pollution and on the other hand as a toxic metal intakes calculation from fresh leaves and stem of native *U. dioica,* which can influence the human health. The daily intake of metals (DIM) and health risk index (HRI) were calculated for the selected vegetables. Consumption and medical use of contaminated native *Urtica dioica* by the rural population around Targoviste City, Romania, may become a potential pathway for human exposure to heavy metal pollution from industrial plants. It is well known that some heavy metals such as copper and zinc are essential nutrients for humans but become toxic at higher concentrations. Also, the human intake of high dose of Zn and Cu may lead to disrupt protein metabolism and cause different diseases such as the ones cardiovascular, arteriosclerosis, gastrointestinal, immunity disorder, and even neurodegenerative illness [23]. On the other hand, the consumption and use of fresh leaf and stem nettle in medical purposes that contain cadmium, chromium, nickel, cobalt, and lead is related to the emergence of tumoral diseases, and this should be studied carefully by the Romanian authorities.

2. MATERIALS AND METHODS

In the spring of the 2019, precisely in April (*e.g.*, when it is considered that the plants have reached the maturity provided by the *Romanian Pharmacopoeia* [25], regarding the chemical composition of organic compounds and essential nutrients), 10 nettle samples (*U. dioica*, samples were coded Ud_1–Ud_{10}) and 10 soil samples (coded S_1–S_{10}) were collected from several villages around Targoviste City (Fig. 1).

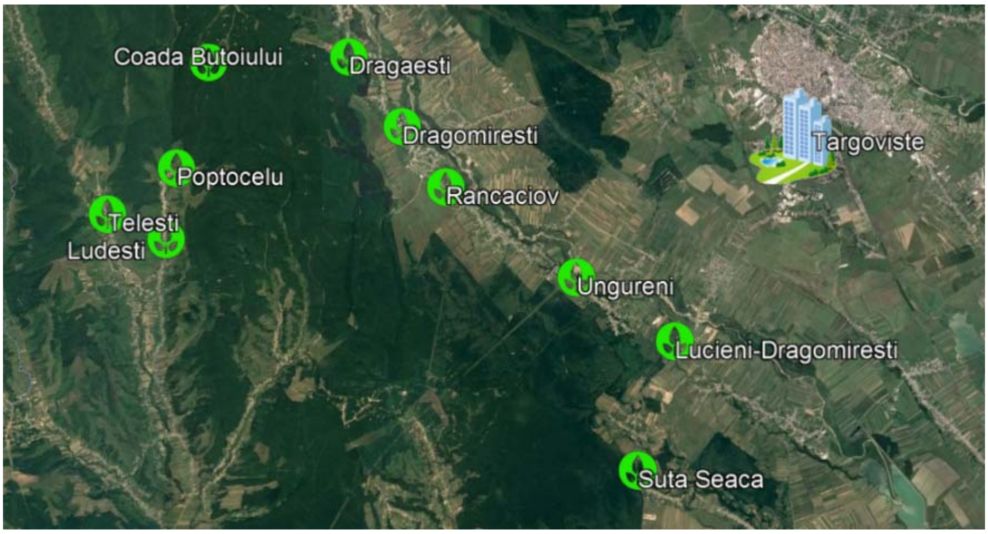

Fig. 1 – The map of collection points for soil and *Urtica dioica* samples.

This rural area was chosen to be investigated for heavy metals accumulation in native plants; it is considered a polluted area, because it is situated in the southwest of industrial plants of Targoviste City (Romania). In this matter, the samples were harvested mainly from humidity areas, out of rural settlements, along of Dambovita River, knowing that due the microclimatic and pedoclimatic factors, the heavy metals contamination of soil and implicit native plants is much more common. It is also known that the inhabitants of the area pick nettles and use them for their own purposes or sell them in markets to other consumers.

The plant and soil samples were collected according to Plant Sampling [26] and Soil Sampling Guidelines [27]. In each sampling point, up to six subsamples (n = 6) were collected and were mixed into a homogeneous representative sample. These samples were analyzed in terms of heavy metals concentration by ICP-MS. The nettle samples were rinsed with deionized water and then separated into leaf (Ln_1–Ln_{10}) and stem (Sn_1–Sn_{10}). The native plant and soil samples were dried in the oven with forced airflow circulation at 40 °C for 72 hours, milled and sifted to obtain a homogeneous representative sample.

For soil, 500 mg samples were digested with aqua regia (*i.e.*, a mixture of 3 mL nitric acid – HNO_3 65%, Merck – and 9 mL hydrochloric acid, HCl 37%, Merck) at 110 °C for 6 hours [28, 29]. For plant tissues, 300 mg samples were digested with 6 mL concentrated nitric acid and 1 mL hydrogen peroxide (H_2O_2 30%, Merck) [17, 30]. The mineralization process was performed using the TopWave microwave digestion system (Analytik Jena, Germany) according to data included in Tables 1 and 2.

Table 1

The parameters for the mineralization process of the soil samples

Parameter	Step 1
Temperature [°C]	175
Pressure [bar]	40
Power [%]	90
Ramp [min]	1
Time [min]	10

Table 2

The parameters for the mineralization process of the plant samples

Parameter	Step 1	Step 2
Temperature [°C]	170	200
Pressure [bar]	40	40
Power [%]	80	90
Ramp [min]	5	1
Time [min]	10	15

After the mineralization process was completed, the samples were cooled at room temperature for 30 minutes. Finally, the clear mineralized solutions were filtered by 0.45 mm filter membrane and diluted to 50 mL with deionized distilled water [9, 17, 29, 30].

The concentration of Cr, Ni, Co, Cu, Zn, Cd, and Pb in samples (*i.e.*, plants and soils) was determined by inductively coupled plasma-mass spectrometry using an ICP-MS system (iCAP Qc, Thermo Fisher Scientific, Waltham – Massachusetts, USA). This mass spectrometer is equipped with flow nebulizers, cyclonic spray chambers and wide bore injectors (which ensure the highest plasma robustness), Peltier cooling system (to ensure the highest stability to sample injection), an ergonomically designed quadrupole, and innovative RAPID (Right Angle Positive Ion Deflection) lens technology for separation of ions and neutrals. All quantitative measurements were performed in triplicates using the standard mode (STD). Several well-known isobaric interferences were automatic corrected [9, 30–35]. The ICP-MS quantification was performed by a standard curve procedure. Metals calibration curves showed good linearity over the concentration range (0.1 to 10.0 mg/L) and R^2 correlation coefficients ranged between 0.995 and 0.999. The analytical curves for investigated metals were prepared using a stock standard solution (Merck). Standard reference materials *i.e.* NIST SRM 2710a Montana Soil and 1515 Apple leaves, respectively were used to verify the accuracy and traceability of both methods. Accuracy and precision in the ranges of 94–107% and 1–8% (*i.e.*, soil) while 96–103% and 1–6% (*i.e.*, plant), respectively were considered satisfactory.

The transfer factor (TF), well known as accumulation factor, represents the ratio between the heavy metal concentration in plant C_{plant} (*i.e.*, average values of content in leaf and stem) and the heavy metal concentration in the soil C_{soil} [16, 17]:

$$\mathrm{TF} = \frac{C_{plant}}{C_{soil}}. \tag{1}$$

The daily intake of metals (DIM) was assessed to estimate the average daily loading of metal into the body system of the specified bodyweight of a consumer [30, 36, 37]. The daily intake of metal in this study was calculated based on the formula (2):

$$\mathrm{DIM} = \frac{C_{plant} \times I}{BM}, \tag{2}$$

where: I = average intake (ingest) rate of an adult (I = 0.345 kg); BM = the average body mass of an adult (BM = 65 kg).

The health risk index (HRI) by consumption of contaminated vegetables was calculated as the ratio between the DIM and oral reference dose RfD values [37].

$$\mathrm{HRI} = \frac{\mathrm{DIM}}{\mathrm{RfD}}, \tag{3}$$

where RfD represents the average reference dose (*i.e.*, $RfD_{Cr(VI)} = 0.003$; $RfD_{Cr(III)} = 1.000$; $RfD_{Ni} = 0.020$; $RfD_{Co} = 0.002$; $RfD_{Cu} = 0.040$; $RfD_{Zn} = 0.300$; $RfD_{Cd} = 0.001$; $RfD_{Pb} = 0.0035$) [38–46].

3. RESULTS AND DISCUSSION

The average of metals content (*i.e.*, Cr, Ni, Co, Cu, Zn, Cd, and Pb) in the soil samples, determined by ICP-MS, is reported in Table 3. Among the detected heavy metals, the average of Cu concentration was the highest in eight soil samples (*i.e.*, S_1–S_3, S_5, S_6, S_8–S_{10}) followed by Cr in six soil samples (*i.e.*, S_2–S_5, S_9, S_{10}) and Cd in four soil sample (*i.e.*, S_2, S_3, S_5, and S_{10}), while the Zn concentration was the lowest comparative with the AL (alert level) values stipulated by the Romanian Order no. 756/1997 – Regulations on environmental pollution assessment [47]. Compared with the alert level specified in by Romanian regulation, the concentrations of Ni and Pb in soils did not exceed these thresholds, except sample S_{10} in the case of Pb.

Table 3

The average of metals content [mg/kg d.w.] of soil samples (*n* = 6) determined by ICP-MS, comparative with values established by Romanian Order no. 756/1997 [47]

	Cr	Ni	Co	Cu	Zn	Cd	Pb
S_1	248.62	57.39	82.47	**322.11**	184.35	3.51	47.32
S_2	**416.21**	94.60	**152.77**	**595.72**	323.28	**6.51**	72.98
S_3	**442.01**	61.95	89.38	**397.87**	176.08	**6.19**	132.29
S_4	**350.78**	37.27	36.04	201.65	86.11	2.75	27.37
S_5	**402.27**	88.69	**153.78**	**457.19**	254.59	**5.69**	133.61
S_6	228.38	51.07	64.16	**302.74**	156.36	4.43	70.99
S_7	152.31	40.42	44.41	198.55	177.25	2.08	24.17
S_8	250.10	51.81	59.43	**378.39**	175.51	4.18	70.95
S_9	**320.50**	71.83	**107.70**	**294.05**	78.94	2.12	29.36
S_{10}	**390.46**	49.29	49.87	**746.05**	408.56	**9.40**	**265.36**
RSD[1] [%]	5.00–8.32	2.40–6.11	1.75–7.02	4.45–6.98	5.02–9.01	1.00–5.02	3.12–7.78
NV[2]	30.00	20.00	15.00	20.00	100.00	1.00	20.00
AL[3]	300.00	200.00	100.00	250.00	700.00	5.00	250.00
IL[4]	600.00	500.00	250.00	500.00	1500.00	10.00	1000.00

[1] RSD – *Relative Standard Deviation*; [2] NV – *Normal Value*; [3] AL – *Alert Level*; [4] IL – *Intervention Level* – according to Romanian Order no. 756/1997. Values written with bold font represent values higher than alert levels.

The metals content (*i.e.*, Cr, Ni, Co, Cu, Zn, Cd, and Pb) in leaf and stem samples, determined by ICP-MS, is reported in Table 4, comparative with the Romanian Order no. 975/1998 – on the approval of the hygienic-sanitary norms for food, especially for vegetables with leaf. Chromium, especially in the case of a high intake of Cr(VI) from the soil *via* native plants, may be accountable for gastrointestinal bleeding and severe kidney problems related to necrosis to humans. Chromium in soil samples was record values between 152.31 mg/kg d.w. (S_7) and 442.01 mg/kg d.w. (S_3), with an average value of 320.16 mg/kg d.w. (Table 3). The average of Cr concentration exceeded the NV in all investigated soil samples and even overtaking the AL for six soil samples, with the highest value in sample S_3 (*i.e.*, 442.01 mg/kg d.w.). Regarding the Cr content in nettle samples, it is not possible to make a comparison with the values allowed in the Romanian legislation because they are not stipulated (Table 4). To the best of our knowledge, high human intake of Ni caused health problems, mainly contact or systemic dermatitis and more dangerously, skin tumors. In this regard, the nickel content values in soil samples (Table 3) were ranged between 37.27 mg/kg d.w. (S_4) and 94.60 mg/kg d.w. (S_2), with an average value of 60.42 mg/kg d.w. The Ni content in soil samples exceeded their respective NV but is lower than AL according to Order 975/1998. For Ni, it is not possible to make a comparison with the national legislation of the obtained concentrations for nettle leaves and stems because no limit thresholds have been determined for this metal in plants.

Table 4

The average of metals content [mg/kg d.w.] of leaf and stem samples (*n* = 6) determined by ICP-MS, comparative with values established by Romanian Order no. 975/1998 [70]

	Cr	Ni	Co	Cu	Zn	Cd	Pb
Ln_1	1.83	0.78	0.83	18.07	37.66	0.17	**1.50**
Ln_2	0.70	2.76	3.29	14.10	14.84	**0.24**	**3.25**
Ln_3	1.11	2.75	0.42	20.73	36.14	0.01	**2.32**
Ln_4	0.72	1.34	1.61	40.51	24.99	0.11	**0.87**
Ln_5	0.76	0.40	0.53	19.25	19.87	0.08	**2.19**
Ln_6	1.01	0.89	0.82	30.84	44.46	0.10	**4.66**
Ln_7	0.77	0.62	0.49	26.62	36.46	**0.81**	**3.98**
Ln_8	0.19	0.66	0.95	40.26	38.90	0.05	0.22
Ln_9	3.45	3.72	6.00	20.01	40.57	**0.29**	**5.05**
Ln_{10}	1.87	2.16	2.37	19.24	44.22	**1.22**	**12.77**
Sn_1	2.04	3.09	0.52	13.64	49.61	**1.11**	**2.50**
Sn_2	2.13	3.12	0.53	13.68	49.59	**1.12**	**2.52**
Sn_3	2.10	3.08	0.52	13.62	49.19	**1.11**	**2.51**
Sn_4	1.86	2.88	0.49	13.20	45.90	**1.10**	**2.46**
Sn_5	1.41	1.77	0.11	6.54	10.02	**0.79**	**1.52**
Sn_6	1.54	2.40	0.45	9.61	35.79	**0.93**	**2.06**
Sn_7	0.59	1.68	0.37	7.70	11.72	**0.62**	**1.68**
Sn_8	2.02	3.04	0.52	13.59	49.41	**1.12**	**2.51**
Sn_9	1.77	5.24	0.50	11.91	37.06	**1.00**	**2.27**
Sn_{10}	1.63	7.54	0.64	4.35	25.90	**0.58**	**1.34**
RSD[1] [%]	1.00–7.06	1.25–6.94	2.05–5.18	3.19–7.55	2.48–8.01	1.09–6.08	1.85–6.94
MAL_{VL}[2]	uv[3]	uv[3]	uv[3]	uv[3]	uv[3]	0.20	0.50

[1] RSD – *Relative Standard Deviation*; [2] MAL_{VL} – *maximum allow limits for vegetables with leafs according to Romanian Order no. 975/1998*; [3] uv – *unregulated value.* Values written with bold font represent values higher than maximum allow limits.

Cobalt is a microelement, being a component of vitamin B_{12}, which has an essential role in the formation of red blood cells, red blood cells. It is known for its vasodilating properties and is also used to treat palpitations, anxiety, high blood pressure, coronary heart disease, arthritis, spasms, and vascular blockages [48]. No daily doses have been set, but usually, the need for the human body is reduced, on the order of a maximum of 8 mg per day [49]. On the other hand, the International Agency for Research on Cancer (IARC) stipulated that cobalt metal with tungsten carbide is *probably carcinogenic to humans* (IARC Group 2A Agent), whereas cobalt metal without tungsten carbide is *possibly carcinogenic to humans* (IARC Group 2B Agent) [50]. In this respect, the Co concentration must be monitored closely. Due to their interference with Ni and Fe, most often Co is omitted to be investigated. This is the reason that it was considered that the determination of the Co content in the analyzed samples can bring an additional novelty of this study. According to the result presented in Table 3, the cobalt concentration was ranged between 36.04 mg/kg d.w. (S_4) and 153.78 mg/kg d.w. (S_5), with an average value of 84.00 mg/kg d.w. The Co contents in soil samples were below their respective alert thresholds, excepting for S_2, S_5 and S_9 samples. For Co, it is not possible to compare with the values stipulated by national legislation because no limit thresholds have been determined for this metal in plants.

Copper is responsible for structural and catalytic properties of multiple enzymes necessary for normal body functions (*e.g.*, bone strength, red and white cell maturation, iron transport and brain development, especially at children's) [51]. Anemia, Menkes disease, hypopigmentation of the hair and skin, hypotonia, impaired growth, increased incidence of infections, altered immunity, and bone abnormalities (osteoporosis, fractures, etc.) are the main manifestations described by the copper deficiency. Currently, copper deficiency is associated with cardiovascular mortality due to the low copper intake and/or low serum copper levels. On the other hand, the high copper content in the human body is widely linked with "cuprous fever" a severe disease. In addition, the human intake of a high content of Cu may cause different diseases such as the ones cardiovascular, gastrointestinal, and even neurodegenerative illness. The recorded mean values for copper (Table 3) were between 198.55 mg/kg d.w. (S_7) and 746.05 mg/kg d.w. (S_{10}), with an average value of 389.43 mg/kg d.w. According to Romanian legislation, it can be observed that Cu contents were highly elevated than NV and AL, while two samples (S_2 and S_{10}) exceed even their intervention threshold (IL, 500.00 mg/kg d.w.), the determined values being 746.05 mg/kg d.w. and 746.05 mg/kg d.w., respectively. Generally, the highest concentrations of Cu in plant samples occurred in leaf, while the lower concentrations of Cu were obtained in stem (Table 4). For Cu, it is not possible to compare with the values stipulated by national legislation because no limit thresholds have been determined for this metal in plants. However, it can be concluded that Cu contents in both leaf and stem have been higher than the concentration of other analyzed heavy metals, with values comparable to those of zinc (Table 5).

Table 5

The Health Risk Index induced by *Urtica dioica* consumption

	Cr	Ni	Co	Cu	Zn	Cd	Pb
Ud1	**3.981**	0.597	**2.083**	**2.446**	0.898	**3.950**	**3.527**
Ud2	**2.911**	0.907	**5.894**	**2.143**	0.663	**4.197**	**5.087**
Ud3	**3.302**	0.900	**1.450**	**2.650**	0.878	**3.456**	**4.258**
Ud4	**2.654**	0.651	**3.240**	**4.144**	0.729	**3.734**	**2.936**
Ud5	**2.232**	0.335	0.987	**1.990**	0.307	**2.685**	**3.271**
Ud6	**2.623**	0.508	**1.960**	**3.121**	0.825	**3.178**	**5.925**
Ud7	**1.399**	0.355	**1.327**	**2.648**	0.496	**4.413**	**4.990**
Ud8	**2.273**	0.571	**2.268**	**4.154**	0.908	**3.610**	**2.407**
Ud9	**5.369**	**1.382**	**10.029**	**2.463**	0.799	**3.981**	**6.454**
Ud10	**3.600**	**1.497**	**4.644**	**1.820**	0.721	**5.555**	**12.440**

* HRI values written with bold font are higher than 1.

Zinc is an essential element being a catalytic component of over 300 enzymes and has an important role in the structure of proteins and membranes [52, 53], as well. For humans, Zn is responsible for growing, normal development, DNA synthesis, immunity, and sensorial functions [54–56]. The zinc deficiency is observed by growth retardation [57], delayed skeletal maturation [58], lower cell-mediated immunity [59], decreased resistance to infections linked by immunity as well, anorexia [60], behavioral problems [61, 62], skin lesions [63], and alopecia [64], Alzheimer's disease [65] etc. Recently [66], it was concluded that zinc deficiency should be very similar to that of iron deficiency and both elements can be powerful indicators in the assessment of human health at the global level. Also, the human intake of high levels of Zn leads to disrupt protein metabolism and cause arteriosclerosis. Regarding Zn content, it was determined values that ranged between 78.94 mg/kg d.w. (S_9) and 408.56 mg/kg d.w. (S_{10}), with an average of 202.10 mg/kg d.w. Concentrations of Zn in the studied areas showed soil Zn concentration ranges that were above NV values from Romanian Order, but lower than ones of AL. Zn content in plant samples showed concentrations similar in terms of magnitude order (Table 4), the highest value of Zn in leaf being in Ln_6 (44.46 mg/kg d.w.) while in stem the elevated Zn content was in Sn_1 (49.61 mg/kg d.w.).

Regardless of concentration, cadmium is primarily responsible for kidney disorders, especially injuries, and has negative health effects on the cardiovascular, musculoskeletal, brain, and lung systems [67]. Cadmium was record values between 2.08 mg/kg d.w. (S_7) and 9.40 mg/kg d.w. (S_3), with an average value of 4.69 mg/kg d.w. The study revealed that Cd concentration in the soil was above the NV (1.00 mg/kg d.w.) provided in Romanian legislation (Table 3) and four values exceed the AL (*i.e.*, 5.00 mg/kg d.w.). In addition, for plants, it was observed (Table 4) that stem of nettles accumulated high content of Cd, in all analyzed samples exceeding the maximum allowed limit provided by Romanian Order no. 975/1998 (limit of 0.20 mg/kg d.w.). In four leaf samples (Ln_2, Ln_7, Ln_9 and Ln_{10})

are obtained concentration in Cd that exceed the MAL values, according to data shown in Table 4. High Pb concentration in nettle is a known causative of elevated blood pressure, renal and tumor infection, improper hemoglobin synthesis and reproductive system [68, 69].

For Pb content were determined values between 24.17 mg/kg d.w. (S_4) and 265.36 mg/kg d.w. (S_2), with an average value of 87.44 mg/kg d.w. Pb was the main heavy metal that posed potential risks to human health (Table 4 and Table 5), from nettles consumption and the risks must be considered in the future. High values of Pb are found both in leaf and stem of nettles, which is explained by a good transfer of Pb from the soil in the vegetative part of plant [51].

The heavy metals that exceeded the limits according to the Romanian threshold in nettles and soil samples were different. The excess of the heavy metal limit is more concerning for soil than native plants, excepting Pb and Cd. Even if the Romanian regulation does not set limits for all analyzed metals, it can be easily observed that from all subsamples, only Ln_8 have Cd and Pb content are under the MAL_{VL} [70]. All stem subsamples exceed these levels, probably because the nutritive elements – as well as, heavy metals – are transferred from soil to leaf by the stem.

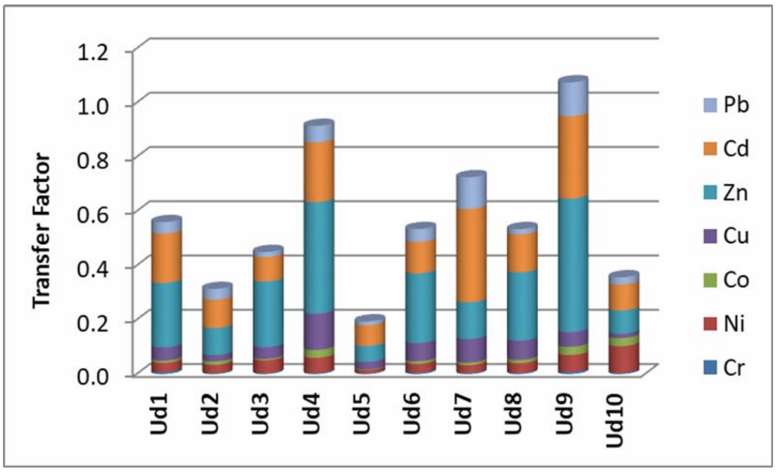

Fig. 2 – The transfer factor calculated for *Urtica Dioica* samples.

For each Ud_{1-10} sample was established the average content (stem and leaf) for all elements, in order to calculate the transfer factor (*e.g.*, based on equation (1)). The obtained results are presented in Fig. 2. The transfer factor is a parameter used in the evaluation of the phytoremediation capacity of plants. If the transfer factor value is higher than 1, then the plant is considered hyperaccumulator of one or more heavy metal [16, 17]. In this study, for all analyzed samples, were obtained values lower than 1 (for all metals). The capacity to transfer metals from soil to plant tissues depends on the pH and humidity of the soil [11, 12, 18].

Based on the same values reported in Table 4 and using the equation (2), the daily intake metal (DIM) was calculated. This parameter represents the quantity of intake metal (or other contaminants) if an adult, with the body mass of 65 kg, consume 345 g/day of *Urtica dioica*. The obtained results are presented in Fig. 3.

Since exist many different ways in which people (children or adult) can be exposed to contaminants, World Health Organization (WHO) was established values for provisional tolerable weekly intake (PTWI) which represents the cumulative exposure from all sources [71]. Copper and zinc are essential elements for humans, but they become toxic at higher concentrations (Fig. 3), and they cause different diseases such as the ones cardiovascular, arteriosclerosis, gastrointestinal, immunity disorder, and even neurodegenerative illness.

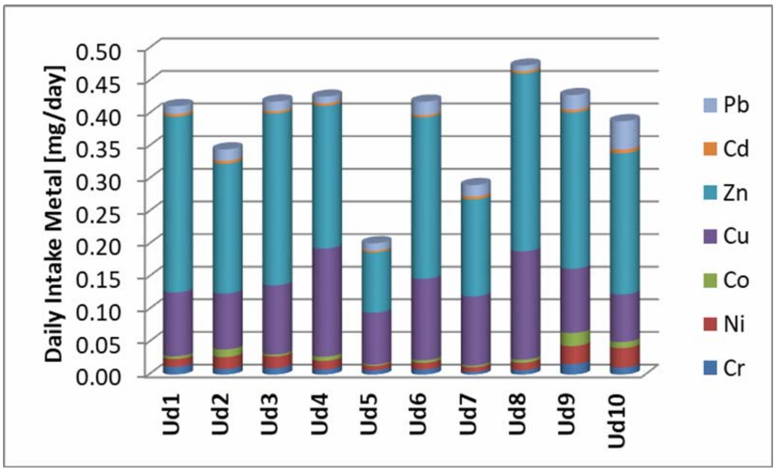

Fig. 3 – The amount of daily intake metal [mg/day], calculated for *Urtica Dioica* samples.

But the DIM factor is not enough to evaluate the plant toxicity; for this reason, the Health Risk Index (HRI) is used. U.S. Environmental Protection Agency – Integrated Risk Information System (IRIS) was established the oral reference dose (RfD) for each metal as the maximum quantity which does not generate adverse effects [39–45]. Based on the obtained results for DIM and RfD values, the HRI values were calculated using equation (3). The obtained values are reported in Table 5. Thus, exposed humans were likely to experience detrimental health effects from consuming leaf nettles. If the HRI value is less than 1, then the exposed population was safe, but in this case, all samples exceed the value 1 for all metals excepting Ni and Zn. However, the RfD values refer to the total amount ingested in a day, not just in one foodstuff.

4. CONCLUSIONS

To the best of our knowledge, heavy metal bioaccumulation in fresh *U. dioica* used by the rural population from Romania had not been studied, and the human health risks of nettle to the population who use the leaves as a decoction in medical purposes are unclear, as well. Copper and zinc are essential nutrients for humans, but they become toxic at higher concentrations, causing different diseases such as the ones cardiovascular, arteriosclerosis, gastrointestinal, immunity disorder, and even neurodegenerative illness. On the other hand, consumption of fresh leaf and stem nettle containing cadmium, chromium, nickel, cobalt, and lead is related to the emergence of cancer in humans.

The present study revealed that concentrations of Cr, Ni, Co, Cu, Zn, Cd, and Pb in soil samples collected in April 2019 were significantly high than the alert level (established by Romanian Order no. 756/1997). This fact has led to metals content in vegetables (*i.e.* Cd and Pb) higher than maximum admitted level (established by Romanian Order no. 975/1998) and daily intake values (*i.e.*, for Cr, Co, Cu, Cd, and Pb) higher than reference oral doses (recommended by USEPA) in all analyzed samples. This study represents the base for further monitoring campaigns because the health risk by ingestion of metals through consumption of contaminated vegetables is not negligible.

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