Studies Concerning Heavy Metals Accumulation of *Carduus nutans L.* and *Taraxacum officinale* as Potential Soil Bioindicator Species

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This study assessed the level of heavy metal uptake by two weeds such as Carduus nutans L., and Taraxacum officinale which are growing naturally, without specific requirements, alongside the roads, disturbed sites, on strongly polluted places such as smelters, railway tracks, coal mines, and other industrial plants from Romania, in order to identify the hyper-accumulator species that could be used for future phytoremediation purpose. Concentrations of elements including Pb, Cr, Cd, Cu, Zn and Ni were analyzed in the soil and leaf samples collected from two historical polluted sites: North Năvodari Camp and former Fertilizer Chemical Plants (USAS) Navodari. Trace metals concentrations (i.e. Cd and Cr) in leaf samples were determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The other metal concentrations including Zn, Ni, Pb and Cu were performed by Flame Atomic Absorption Spectrometry (FAAS). The bioaccumulation factors have been calculated as well. This investigation has shown that Taraxacum Officinale L. and Cardus Nutans L. accumulate high levels of Pb (BF > 1 depending the sampling period) as well as Cu, from soil to leaves, and from this reason it is expected that these weeds to be used with success as bioindicator plants for metal contaminated soil.

Keywords: biaccumulation factor, heavy metal bioindicator, Carduus nutans L., Taraxacum officinale

In the last century, the idea of using plants to clean the polluted environment was developed [1-5]. It is well known that the monitoring of environment against anthropogenic pollution is a real problem of the world. This monitoring includes the assessing of the degree of metal accumulation in plants as well. For this purpose, the best are organisms commonly found in a variety of habitats, less sensitive or insensitive to intoxication, with a wide geographic range of distribution. Examples of such species may be: mosses [6-8], lichens [8,9], mushrooms [10-12], perennial medicinal plants [13], common dandelion [14-17], and other noxious weeds [18-22] as well. Most of these species are already considered hyper-accumulator of different elements including heavy metals (e.g. Cd, Pb, As, Cr, Ni, Mn). Thus, in the present, a promising alternative to remove the hazardous heavy metals from contaminated sites is the possibility to use different metal-hyper-accumulators. Obviously, this useful technique is called phytoremediation. Long times, in Romania, toxic weeds, such as musk thistle or *Carduus nutans L.*, and dandelion or *Taraxacum officinale*, were considered useless. Currently, these weeds are appreciated due their therapeutically properties in traditional medicine. Thus, it can emphasize the hepatoprotective properties of musk thistle and dandelion dry extracts. As medicinal plant, *Taraxacum officinale*, is very useful in the treatment of different diseases such as intestinal disorders, hypo gastritis, obesity, gout, rheumatism, atherosclerosis, varicose veins and so on.

The musk thistle (*Carduus nutans L.*) is native to southern Europe and is an invasive biennial and aggressive species [23, 24] that can form extremely dense stands along of the roadsides, or on disturbed sites, and grain fields. Musk thistle does not have specific climatic requirements, and from this reason grows in Romania, primarily in Navodari area due to the alkaline soil with a high sand content, nutrient deficient and low water content. Also, the common dandelion (*Taraxacum officinale)* grows wild as a ubiquitous weed [25] of road-sides, gardens, on strongly polluted such as smelters, railway tracks, coal mines, and other industrial plants. It is well known that this plant has so many benefits due to the higher amount of beta-carotene (which the liver converts to vitamin A), pectin and vitamin C, as well as a high content of vitamin K (which helps the liver function properly being an important vitamin to increase vitality and the longevity of life) and vitamin E (a powerful antioxidant which reduces the risk of heart attack and it protects LDL cholesterol from oxidation) [26].

Several studies have investigated the accumulation of heavy metals in musk thistle [14,15], dandelion [27-29] and *Populus Nigra L.* [30] samples collected at different polluted sites but have not demonstrated how much of the metals from soil or atmosphere absorb these weeds. It is well known that the amount of heavy metals in soil and weeds can vary seasonally due to the environmental factors which play an important role to uptake the elements by these plants. The possible chemical forms of heavy metals in a natural system are hydrated metal ions, metal

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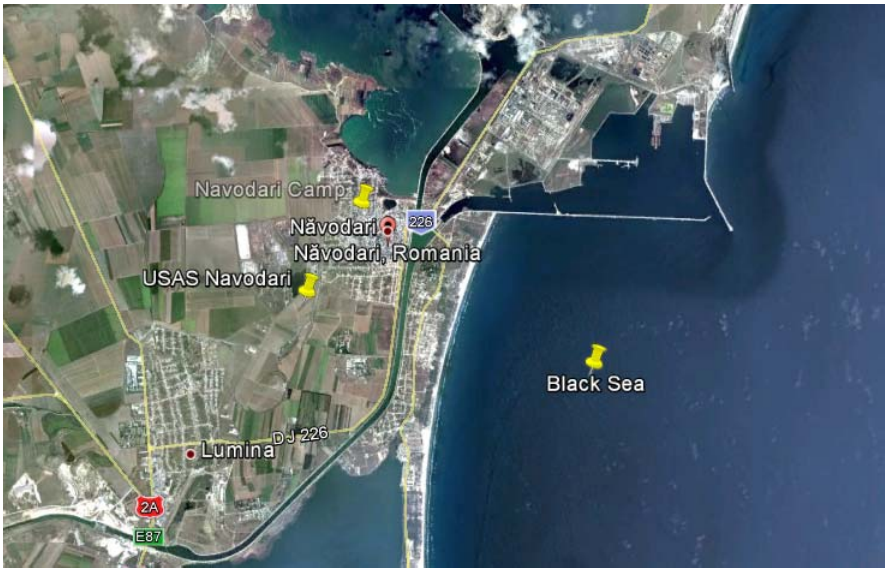

Fig.1. Navodari studied area

precipitates, soluble metal complexes, metal ions adsorbed to organic matter and bacterial residues and metal ions adsorbed to the surface and interstices of minerals. In analytical procedures, the concentrations of trace metals in weeds require processing a large numbers of samples, to accurately characterize their abundance and to reach reliable conclusions. Generally, in these types of studies, knowledge on the total trace element content is sufficient, without the necessity of speciation. In order to determinate the heavy metals amount from different plants, some widely analytical techniques such as GFAAS (Graphite Furnace Atomic Absorption Spectrometry) and FAAS (Flame Atomic Absorption Spectrometry) were used.

The aim of this paper is to present, for the first time, comparative data about heavy metals accumulation of the wild plants species (i.e. *Carduus nutans L.*, and *Taraxacum officinale*) which grow in contaminated sites in Navodari city and around, in order to identify the hyper-accumulator species that could be used for future phytoremediation and phytoextraction purposes. Also, this study attempts to determine the magnitude of environmental contamination of Navodari sites with several heavy metals, including Cd, Cr, Cu, Ni, Pb, and Zn, being well-known the historical pollution of studied area.

Site characterization

Navodari area (i.e. **site 1** located at 44^{0}19'27.13''N and 28^{0}36'23.58''E, elevation 31 m, around of Navodari Summer Camp; **site 2** located at 44^{0}17'04.50''N and 28^{0}34'46.44''E, elevation 30 m, around of the former Chemical Fertilizer Plant - USAS) was chosen because it was observed an increasing number of population, due to the development of a petrochemical plant, Cape Midia Shipyard and the touristic development on Black Sea Coast. The largest summer camp for children from Romania is in Navodari as well. In this respect, in summer, from June to the end of August, the traffic density in this area was around at least 2000 vehicles a day.

The main activities of the Navodari city, but also the main sources of pollution in this region, are a petrochemical plant, Cape Midia Shipyard, and former Chemical Fertilizer Plant transformed in waste landfill. Treatment plant of oil refinery discharged wastewaters into the Black Sea, in the northern of Navodari. Chemical Fertilizer Plant was closed, but around the sole remained sections was huge amounts of toxic waste (some radioactive), serious sources of environmental pollution. Moreover, it was discovered that the pipe that leave from the former settling basins of the plant and stops in the see, still flows water with phosphogypsum. In 2008, the National Commission for Control of Nuclear Activities (CNCAN) concluded that the phosphogypsum deposits which are located inside the ex - Chemical Fertilizer Plant, have a radioactive content which exceeds the normal range. In addition to the phosphogypsum waste (2.5 million tones in settling basins), on the plant there still exists about 5 tons of sulfuric acid sludge deposited on the bottom collector basins, almost 100 tons of pyrite ash, and over 120 tons of vanadium catalysts, in contact stoves. These sources are toxic for peoples and potentially polluted sources for environment. In the last ten years a new corporation intended to open a big waste landfill but has not obtained all the support of the Romanian authorities. However, these anthropogenic activities, auto traffic and its effects on environment can be showed by weeds, such as *Carduus nutans L.*, and *Taraxacum officinale* which can respond at the pollution degree of the region in the changing climatic conditions. The dominant soil types from Navodari area were formed and evolved mostly on loess, sand and are represented by calcic and calcarocalcic chernozem.

The sites experiences a unique climate affected by continental and maritime especially climates due to the Black Sea. The summers are dry and hot and the winters are cold. Precipitation in the form of heavy snowfall is a common feature of winter. Rain is generally common in spring or at the beginning of autumn. According to the data published by National Meteorological Administration of Romanian Ministry of Environment and Forests (ANM, 2011) the annual average temperature lies around 13.8^{o}C [31], annual average maximum temperature around 16.7^{o}C and the annual average minimum temperature were around 8.5^{o}C. The annual precipitation in 2011 was 340 mm [32].

Experimental part

Chemicals and standard materials

The used chemicals included nitric acid (65% Merck), hydrochloric acid (37% Aldrich), hydrogen peroxide (30% Aldrich), sulphuric acid (96% Aldrich) and potassium chloride (Aldrich). Distilled deionized water had a resistivity bigger than 17.5 MΩcm. Certified standard reference materials NIST SRM 1515 (Apple leaves) for leaves and NIST SRM 2709, 2710 and 2711 for soil were used. The solutions used for calibration of GFAAS and FAAS were prepared from standard solution (Merck) of the studied elements.

Plant and soil sampling

The study was carried out in the period of April, 2011 to September, 2011. Sampling points were located by GPS (Global Position System). Samples of *Carduus nutans L.*, and *Taraxacum officinale* were collected from two historical polluted sites (fig. 1) from Navodari area, Romania (i.e. Navodari Camp and USAS Navodari). Samples were collected from 15 points per sampling site, around of summer Camp and USAS (minimum of 50 g fresh weight leaves without stalks). Both weeds form both selected sites were chosen by respect some criteria, such as: the distance between weeds and Camp, respectively USAS was 20-30 m, approximately along an expected gradient of anthropogenic pollution; the youngest leaves on top of the long shoots were not used; the leaves with obvious imperfections have been removed as well. In order to estimate the soil contamination from the both sites under study, fifteen soil samples from each site were collected from the root aria of each weed at 20 cm depth by using a disposable plastic scalpel.

Sample preparation and analytical procedures

The leaves of musk thistle were dark green, coarsely bipinnately lobed, with a smooth, waxy surface and sharp yellow-brown to whitish spines at the tips of the lobes. Also, the leaves of dandelion were 10–25 cm long, simple and basal, entire or lobed, which forming a rosette above the central root. All samples were washed with distilled water. Leaf samples were dried in oven at 70°C for 24 h.

Approximately 0.2 g of dried powdered leaf samples, were weighed and then introduced in the digestion vessels (DAP-60K) of Berghof MWS-2 microwave digestion system. 8 mL of HNO_3 (65% Merck) and 10 mL H_2O_2 (30% Merck) were used for digestion. The digestion was carried out at 220°C, at a pressure of 75 MPa for 20 min (add 15 min. for cooling of the system). The clear solutions were filtered and were brought in 50 mL volumetric bottle with distilled deionized water.

The total metal content of the solid samples were performed by Atomic Absorption Spectrometry (AAS) technique [33, 34]. The GBC Avanta AAS with flame and GBC Avanta Ultra Z equipped with graphite furnace spectrometers and autosampler, which provided a good sensitivity, were used.

Thus, trace metals concentrations (i.e. Cd and Cr) in leaf mineralized solutions were analyzed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The other metal concentrations including Zn, Ni, Pb and Cu were performed by Flame Atomic Absorption Spectrometry (FAAS). Determination of elemental concentrations in the leaf samples were performed using the method of calibration curve according to the absorber concentration. Several solutions of different known concentrations were prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve. All samples concentrations were reported as mg/kg dry weight of material. For the quality assurance, the results were checked by carrying out a triplicate analysis according to certified standard reference materials NIST SRM 1515 (Apple leaves). The relative standard deviation was routinely between 0.001 and 3.5%.

The soil samples were dried at 105°C for 48 h. The dried material was triturated in a porcelain mortar to a dusty form; ensuring uniformity of chemical composition throughout the mass of the sample, then was passed through a 100 μm sieve and stored for analysis. Therefore, a quantity of approximately 0.5 g from each sample was introduced in the digestion vessels (DAP-60K) of Berghof MWS-2 microwave digestion system and then, in according to 3051 EPA standard, each soil sample was treated with 12 mL HNO_3 (65% Merck), 6 mL HCl (37% Aldrich), 4 mL HF (40% analytical solution) and 1 mL H_2SO_4 (96% Aldrich). The vessels were heated in a microwave oven with the following temperature program: rising to 145°C in 10 min and maintaining for 5 min, rising to 180°C in 5 min and maintaining for 10 min, cooling to 100°C in 1 min and maintaining for 10 min. Finally, the samples were cooled 30 min at room temperature. Final solutions were filtered and were brought to 50 mL volumetric bottle with distilled deionized water. Heavy metals concentrations in the final solutions of soil were analyzed by Flame Atomic Absorption Spectrometry (FAAS). To check the analytical precision, randomly chosen samples (about 20% of the total numbers) were measured in triplicate according to International Standard Reference Material: NIST SRM 2709, 2710 and 2711 for soil. Average recoveries (n=5) were 83, 73, 76, 85, 91 and 99% for Zn, Cd, Cr, Cu, Pb and Ni, respectively.

Measurements of pH values of soil were carried out in deionized water ratio of 1:2 according to the NF ISO 10390/2005 procedure. 10 g of soil samples were mixed with 50 mL 0.1 N KCl solution, for 30 min, under stirring. After one hour, the mixture was filtered and the *p*H determination was done with Consort P501 pH-meter at room temperature (20.5°C). The conductivity measurement in saturated solution of soil extract was done by HACH CO150 instrument.

Results and discussions

Two noxious weeds such as *Carduus nutans L.,* and *Taraxacum officinale* were selected to evaluate the accumulation of several heavy metals including Cu, Zn, Pb, Ni, Cr, and Cd from soil in leaves from two metal-contaminated sites from Navodari area, Romania (fig. 1). Sampling of heavy metal accumulation in these plants in **site 1** (i.e. Northern Navodari Camp) and **site 2** (i.e. former Chemical Plant, USAS) was carried out in the period April – September, 2011.

The concentrations of Cu, Zn, Pb, Ni, Cr, and Cd in leaves samples and soils varied significantly on the studied sites according with the obtained data from tables 1-4. Thus, the mean concentrations of Cu in leaves of musk thistle collected from **site 1** (table 1) were considerably higher comparative with those samples collected from **site 2** (table 2) and this is surprising considering the fact that this is a Summer Camp for children (**site 1**). The high value of copper was found in dandelion leaves samples collected from both sites as well (tables 3 and 4). It is well-known that in the Northern of Dobrudja Plateau are copper in calcic deposits and this metal is used in non-ferrous metallurgy. This can explain the higher amounts of copper in both weed samples and soil as well. High concentrations of Pb in musk thistle and dandelion leaf samples, as well as in soil collected from **sites 1** and **2** can be explained by the elimination of the leaded gasoline years ago, a high number of coal-fired heating installations in the city, as well as by petrochemical plant's activities. Certainly high values of lead observed in leaf samples of musk thistle collected in June and July, 2011 from both sites (tables 1 and 2) perhaps, are due to intensify the touristic traffic to the Black Sea. From table 3 it can be seen that in the period when the summer Navodari Camp (i.e. April to May, 2011) has entered into renovation, high concentrations of lead were obtained in dandelion samples such as 12.4650 mg/kg d.w in April and 13.7874 mg/kg d.w in May, respectively.

Obviously, zinc concentrations were higher in leaves of both weeds samples for both studied sites especially in

Table 1
HEAVY METAL CONCENTRATIONS IN LEAF OF *CARDUUS NUTANS L.* AND SOIL SAMPLES COLLECTED FROM NAVODARI SUMMER CAMP

Month	Heavy metals concentration [mg/kg d.w]											
	Cu*		Ni*		Pb*		Zn*		Cr**		Cd**	
	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil
April	8.0355	32.8911	0.2424	12.0827	2.5824	12.2790	15.2640	328.9650	0.0076	0.2797	0.0320	1.5020
May	7.7523	24.3629	0.2114	8.6555	2.1800	17.5702	16.9141	169.2350	0.0026	0.2293	0.0107	0.9797
June	14.1299	41.6082	0.3223	11.4127	25.6321	21.4449	25.0372	256.8925	0.0002	0.2270	0.0010	1.0961
July	15.0963	24.4651	0.2875	12.8988	10.9508	46.4177	31.0074	190.3116	0.0001	0.1021	0.0005	0.3958
August	12.1898	51.2110	0.3370	12.4739	3.5699	18.8579	18.2897	244.0714	0.0247	0.1803	0.0032	0.8902
September	11.3895	28.2410	1.0522	8.9282	5.0314	37.4220	21.1321	138.2003	0.0185	0.3934	0.0534	0.5200
Mean	11.4322	33.7966	0.4088	11.0753	3.8069	24.8505	21.2741	221.2793	0.0090	0.2353	0.0168	0.8973
RDS	0.5-2.5	1.1-2.3	0.0-0.8	0.1-0.5	0.05-1.3	0.2-1.75	0.1-2.5	1.75-4.3	0.01-0.1	0.02-0.15	0.01-0.15	0.09-0.2

*Flame Atomic Absorption Spectrometry (FAAS)

**Graphite Furnace Atomic Absorption Spectrometry (GFAAS) technique applied for leaf samples

Table 2
HEAVY METAL CONCENTRATIONS IN LEAF OF *CARDUUS NUTANS L.* AND SOIL SAMPLES COLLECTED FROM USAS NAVODARI

Month	Heavy metals concentration [mg/kg d.w]											
	Cu*		Ni*		Pb*		Zn*		Cr**		Cd**	
	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil
April	5.3315	45.8816	0.2364	11.2218	4.2230	15.0819	14.2667	196.7451	0.0006	0.3243	0.0023	0.7990
May	5.1790	24.2792	0.2110	8.3947	2.1982	30.1709	12.6597	136.8753	0.0002	0.4417	0.0005	0.5658
June	13.4471	28.0802	0.2144	16.3340	23.4448	19.7634	25.0624	234.2660	0.0018	0.3661	0.0073	0.9284
July	15.2060	16.0796	0.2570	10.5777	13.4713	37.2859	27.1995	183.9150	0.0011	0.2550	0.0045	0.8374
August	3.4887	29.4584	1.2987	10.3976	6.9721	17.4974	27.9162	216.4829	0.0046	0.2758	0.0151	0.7918
September	1.8832	19.8495	0.6921	5.8801	6.8779	27.3623	23.0181	158.5705	0.0164	0.3011	0.0177	0.3677
Mean	7.4226	32.8656	0.4849	8.5510	5.5505	21.2221	21.6871	177.6578	0.0041	0.3127	0.0079	0.5834
RDS	0.75-3.58	1.5-2.5	0.05-0.24	0.1-1.1	0.1-1.07	0.86-2.5	0.3-3.9	1.2-4.86	0.02-0.13	0.08-0.15	0.1-0.25	0.1-0.23

*Flame Atomic Absorption Spectrometry (FAAS)

**Graphite Furnace Atomic Absorption Spectrometry (GFAAS) technique applied for leaf samples

Table 3
HEAVY METAL AMOUNTS IN LEAF OF *TARAXACUM OFFICINALE* AND SOIL SAMPLES COLLECTED FROM NAVODARI CAMP

Month	Heavy metals concentration [mg/kg d.w]											
	Cu*		Ni*		Pb*		Zn*		Cr**		Cd**	
	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil
April	7.3229	32.8911	0.1080	12.0827	12.4650	12.2790	20.4745	328.9650	0.0159	0.2797	0.0675	1.5020
May	7.3861	24.3629	0.1894	8.6555	13.7874	17.5702	17.9917	169.2350	0.0189	0.2293	0.0786	0.9797
June	12.7232	41.6082	0.3125	11.4127	3.7277	21.4449	35.4911	256.8925	0.0020	0.2270	0.0084	1.0961
July	13.8382	24.4651	0.2607	12.8988	8.2829	46.4177	25.4302	190.3116	0.0004	0.1021	0.0017	0.3958
August	7.5043	51.2110	0.8120	12.4739	8.8159	18.8579	26.8884	244.0714	0.0210	0.1803	0.0089	0.8902
September	5.7024	28.2410	0.3212	8.9282	6.0965	37.4220	21.3376	138.2003	0.0180	0.3934	0.0228	0.5200
Mean	9.0795	33.7966	0.3340	11.0753	9.2808	24.8505	24.6023	221.2793	0.0127	0.2353	0.0313	0.8973
RDS	0.6-4.0	1.1-4.3	0.1-0.4	0.1-0.5	0.1-1.2	0.1-1.5	0.06-2.9	1.75-4.5	0.008-0.15	0.02-0.15	0.01-0.2	0.09-0.25

*Flame Atomic Absorption Spectrometry (FAAS)

**Graphite Furnace Atomic Absorption Spectrometry (GFAAS) technique applied for leaf samples

summer according with data presented in tables 1-4. The elements concentration in the Northern of Navodari Camp area was significantly higher than those from the USAS Navodari. These results show that elements such as Cu and Zn were more associated to crustal elements, though the effects of anthropogenic source could not be totally excluded. The highest concentrations of Zn were obtained at **site 1** while the lowest concentrations were recorded in **site 2** (tables 1-4). Certificated reference material NIST SRM 1515 Apples leaves that contains 0.013 ± 0.002 mg Cd/kg, 5.65 ± 0.24 mg Cu/kg, 0.91 ± 0.12 and 12.5 ± 0.3 mg Zn/kg was used to check the quality of analyzed leaves plants data.

The level of metals in weeds can vary seasonally and to interpret the results presented in tables 1-4, it has to discuss about the climate factors in the region where the analyzed sites are situated. Besides the concentration of metals in the soil, environmental factors play a critical role to metal uptake by the plant as well. Therefore the Dobrudja Plateau is windy, especially in autumn, the winds direction being from North to East. The extremes mean annual speeds registered in 2011 were 5.16 m/s in July and the mean annual temperature was of 13.8^{0}C as well. This region is characterized by a low pluvial regimen with the annual mean less than 350 mm. It is known that the rain plays an important role in the plants life. In this context, the metals accumulation, increased in the dry period from May to the end of August 2011. The short episodes of precipitations, with high intensity, appeared at the summer end, stopped for a while, the metal's bioaccumulation, especially in leaves, in both sites. The plants leaves were chosen because their preparation is less laborious and give a higher certainty of accuracy. Also, the leaves are easier to wash from external dirt, dry faster, and are easier to grind to obtain homogeneous material. In most cases, a higher concentration of elements (Cr, Cu, Cd, Zn, Ni, and Pb) was found in the dry matter of leaves and this is advantageous for the accuracy and precision of the measurements. The

Month	Heavy metals concentration [mg/kg d.w]											
	Cu*		Ni*		Pb*		Zn*		Cr**		Cd**	
	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil	Leaf	Soil
April	9.1385	45.8816	0.2062	11.2218	1.1178	15.0819	25.8671	196.7451	0.0006	0.3243	0.0024	0.7990
May	8.0559	24.2792	0.4957	8.3947	2.2742	30.1709	20.4495	136.8753	0.0002	0.4417	0.0005	0.5658
June	11.8596	28.0802	0.2965	16.3340	8.3491	19.7634	15.1803	234.2260	0.0012	0.3661	0.0049	0.9284
July	13.6744	16.0796	0.1893	10.5777	9.5931	37.2859	24.6140	183.9150	0.0013	0.2550	0.0053	0.8374
August	6.5746	29.4584	0.1499	10.3976	5.7408	17.4974	20.8747	216.4829	0.0149	0.2758	0.0045	0.7918
September	12.3126	19.8495	0.8173	5.8801	7.0493	27.3623	29.8610	158.5705	0.0270	0.3011	0.0337	0.3677
Mean	10.2693	32.8656	0.3592	8.5510	4.0836	21.2221	22.8078	177.6578	0.0075	0.3127	0.0086	0.5834
RDS	0.4-3.0	1.5-2.5	0.1-0.5	0.1-1.1	0.09-1.4	0.8-2.75	0.17-2.1	1.55-4.8	0.01-0.1	0.05-0.15	0.02-0.25	0.1-0.2

*Flame Atomic Absorption Spectrometry (FAAS)

**Graphite Furnace Atomic Absorption Spectrometry (GFAAS) technique applied for leaf samples

Table 4
HEAVY METAL AMOUNTS IN LEAF OF *TARAXACUM OFFICINALE* AND SOIL SAMPLES COLLECTED FROM USAS NAVODARI

Metals	TF – Site 1						TF – Site 2					
	1	2	3	4	5	6	1	2	3	4	5	6
Cu	0.24	0.31	0.33	0.61	0.24	0.40	0.15	0.21	0.48	0.95	0.12	0.10
Ni	0.02	0.02	0.02	0.02	0.03	0.12	0.02	0.02	0.01	0.02	0.12	0.12
Pb	0.21	0.12	**1.20**	0.24	0.19	0.13	0.28	0.07	**1.19**	0.36	0.40	0.25
Zn	0.05	0.10	0.10	0.16	0.08	0.15	0.07	0.09	0.11	0.15	0.13	0.15
Cr	0.03	0.01	0.00	0.00	0.13	0.05	0.00	0.00	0.00	0.00	0.02	0.05
Cd	0.02	0.01	0.00	0.00	0.00	0.10	0.00	0.00	0.01	0.01	0.02	0.05

1-May; 2-June; 3-July; 4-August; 5-September; 6- October

Table 5
BIOACCUMULATION FACTOR OF HEAVY METALS FROM SOIL TO LEAVES OF *CARDUS NUTANS L.* COLLECTED FROM SITES **1** AND **2**

pH of all soil samples taken from the study sites were in the alkaline domain. There were no significant differences in the values determined for the pH in both sites (minimum 7.79±0.01 and maximum 7.99±0.01). Also, no significant variation of the electrical conductivity has been recorded (minimum 12.71 mS/cm and maximum 13.51 mS/cm). Musk thistle and dandelion grow, as all weeds, in well-drained soil with pH between 6.0 and 8.9. Therefore, the *p*H values of studied soil samples, which ranged between 7.79 and 7.99, are suitable for grow and life cycle of musk and dandelion. It is true that these species are most abundant in fertile soils but can also be found in nutrient-poor soils including soil collected from **site 2.**

The bioaccumulation factor (BF) from soil to leaves, expressed as the ratio of metal concentration in leaf divided by the concentration of metal in soil, may be an indicator of the studied weeds accumulation behaviour. In this study are estimated and compared the values of the bioaccumulation factor (BF) of Cd, Zn, Ni, Cu, Pb and Cr (tables 5 and 6) from soil to leaf considering two wild species. Obviously, only with BF it is not possible to establish if the studied weeds may be considerate as hyperaccumulator species for certain metals. However these data can be used in further studies when they will be associated with other data resulted by calculus of translocation factor (TF) and potential risk pollution factor (PRF).

Bioaccumulation factor is calculated with relation:

$BF = C_p/C_s$

where: C_p is the concentration of metal in leaf sample (mg/kg) and C_s is the concentration of metal in soil sample (mg/kg).

If the BF > 1 then the plants can be accumulators; BF = 1 has no influence and if the BF < 1 then the plant can be an excluder.

The bioaccumulation factors (BF) of Cd, Cu, Ni, Pb, Cr and Zn for two plant species are given in figures 1 and 2.

The BF for leaves of Cu and Zn - essential microelements for the plants grow – registered values less than 1.0, and the same variation trend. BF values > 1 for Pb in the leaves of plant species show that only this element was highly absorbed from soil into leaves. Pb is known to be the metallic element with the highest deposition speed and the highest overtaking of the maximum admissible concentration in all the environment components, especially in the soils with big concentration of phosphorus. It is the element that provokes disorders in the micro-organisms metabolism, affecting the respiratory process and the cells multiplication. High concentration of this element has been registered in the leaves of *Taraxacum Officinale L.* in April (BF =1.02 – **site 1**) and in the leaves of *Cardus Nutans L.* in June (BF =1.20 – **site 1** respectively BF = 1.19 – **site 2**). These data confirm that both weeds including musk thistle and dandelion can be used as potential accumulator species mainly for Pb, and for Cu as well. Implicitly these plants can be potential bioindicator for these metals (tables 5 and 6) depending on the climatic conditions and the characteristic geochemical background of the sites. The metal-accumulators properties of studied weeds depend as well, on the soil physico-chemical parameters, especially *p*H and redox potential of the soil from Navodari area. The BF values obtained for Pb and Cu (figs. 2 and 3) can indicate a positive relationship between the content of these metals in leaves of studied plants. The

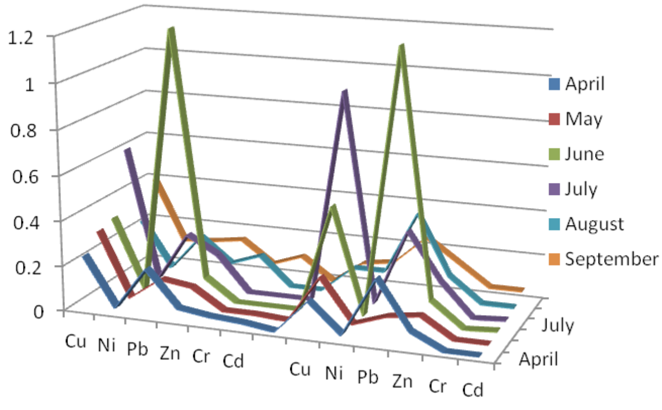

Fig. 2. The transfer factors in soil to musk thistle of Cd, Cu, Ni, Pb, Cr and Zn.

Metals	TF – Site 1						TF – Site					
	1	2	3	4	5	6	1	2	3	4	5	6
Cu	0.22	0.30	0.30	0.57	0.15	0.20	0.19	0.33	0.42	0.85	0.22	0.62
Ni	0.01	0.02	0.02	0.02	0.06	0.4	0.01	0.06	0.02	0.02	0.01	0.04
Pb	**1.02**	0.78	0.17	0.18	0.46	0.16	0.07	0.08	0.42	0.25	0.33	0.26
Zn	0.06	0.11	0.14	0.13	0.11	0.15	0.13	0.15	0.06	0.13	0.10	0.19
Cr	0.06	0.08	0.01	0.01	0.11	0.05	0.01	0.00	0.00	0.00	0.05	0.08
Cd	0.05	0.08	0.01	0.00	0.01	0.04	0.00	0.00	0.01	0.01	0.01	0.09

1-May; 2-June; 3-July; 4-August; 5-September; 6- October

Table 6
BIOACCUMULATION FACTOR OF HEAVY METALS FROM SOIL TO LEAVES OF *TARAXACUM OFFICINALE L.* COLLECTED FROM SITES 1 AND 2

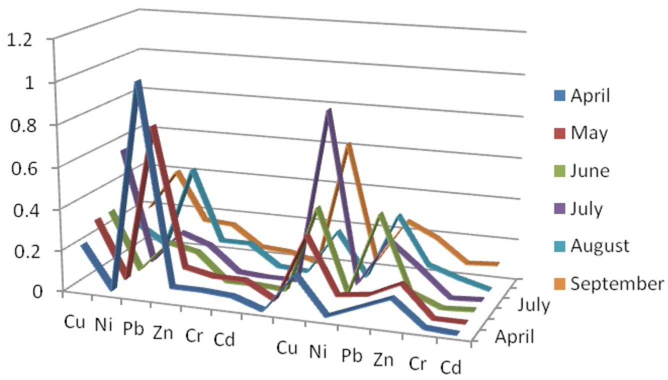

Fig. 3. The transfer factors in soil to dandelion of Cd, Cu, Ni, Pb, Cr and Zn.

accumulation of copper in leaves of dandelion as examples is higher than accumulation of lead in the same period (e.g. for Cu: BF = 0.57 in June – **site 1** and BF = 0.85 in June or BF = 0.62 in September, 2011 – **site 2** comparative with the BF values of lead: BF = 0.18 – **site 1** and BF ranged between 0.25 and 0.26 – **site 2**). These BF values showed a slow migration of lead from soil in dandelion leaves comparative with copper migration and this suggests a better suitability of the leaves to monitor the metal-contamination of the soils from both sites.

It is well-known that to grow and complete the life cycle these weeds have acquired from soil important macronutrients including N, P, K, S, Ca, and Mg as well as some essential micronutrients such as Zn, Ni, Cu and other metals. These plants have evolved highly specific mechanisms to take up, translocate, and store these nutrients. It can conclude that these weeds do not only accumulate high levels of essential micronutrients, but can also absorb significant amounts of nonessential metals, such as Cd, Pb or Cr. The mechanism of Cd accumulation has not been elucidated, but it is well-established that several plants can accumulate Cd in the constituent parts when the Cd concentration in soil increases [35]. In this respect, some studies [36, 37] about tolerant mechanism of Cd tolerant plants have been reported. Sometimes is possible that the uptake of Cd in leaves of different plants is, via a system involved in the transport of another essential divalent micronutrient, possibly Zn^{2+}. Cadmium is a chemical analogue of the latter, and plants may not be able to differentiate between the Cd^{2+} and Zn^{2+}. In our case, for both weeds it can be seen (tables 5 and 6) that Zn and Cd as well are very less accumulated from soil to leaves. Also, chromium is a toxic metal for plants; this study shows that musk thistle and dandelion do no accumulate this metal. It can be concluded that these species are not accumulators for Zn, Cr and Cd, and it is possible to be extruders for these metals.

Conclusions

This investigation relates to the study of concentration of heavy metal accumulation in leaves of two weeds (i.e. *Cardus Nutans L.* and *Taraxacum Officinale L.)*, as potential bioindicators of metal-contamination of soil. These wild species are growing naturally, without specific requirements, in Navodari area, Romania. It was chosen this region because is characterized by a historical pollution due to the industrial activity (petrochemical plant, Shipping Midia-Navodari, and former USAS Navodari), high traffic, especially in touristic period, and domestic pollution as well. In this respect, sampling of leaves and soil was achieved in the period April, 2011 to September, 2011 from two representative sites such as: **site 1** – Summer Camp of students from the Northern of Navodari city and **site 2** – around of former Fertilizer Chemical Plant, USAS, Navodari. This study has shown that *Taraxacum Officinale L.* and *Cardus Nutans L.* accumulate high levels of Pb (BF > 1 depending on the sampling period) as well as of Cu, from soil to leaves and from this reason is expected that these weeds to be used with success as bioindicator plants for actual pollution degree of the environment with heavy metals. Therefore, these weeds might be useful for the phytoremediation of soil contaminated with Cu and Pb. For other metal including Zn, Cd, Cr and Ni it was observed a less accumulation in leaf samples from soil of both sites. Obviously the climatic factors, botanical structure and mineral composition of analyzed soil were responsible for variation in elemental content of metals including Cu, Ni, Pb, Zn, Cr, and Cd. The further approaches will be focused on root to shoot translocation of heavy metals, especially Pb and Cu, because already exist a primary results, as well as on the joint effect of these metals absorption in roots and shoots of musk thistle and dandelion.

By increasing the studies concerning the remarkable capacity of plants to accumulate and tolerate metals it will continue to provide a wealth of information to further enhance the understanding of the low-cost phytoremediation process.

References

1.LASAT M.M., Journal of Hazardous Substance Research, 2, nr. 5, 2000, p.1.

2.BROOKS R.R., Plant Soil 48, 1977, p. 541.

3.CHANEY R.L., Li Y-M, ANGLE J.S., BAKER A.J.M., REEVES R.D., BROWN S.L., HOMER F.A., MALIK M., CHIN M., Improving metal-hyperaccumulators wild plants to develop commercial phytoextraction systems: Approaches and progress. In Phytoremediation of Contaminated Soil and Water, eds. N. Terry and G.S. Ba uelos, CRC Press, Boca Raton, FL., 1999.

4.KABATA-PENDIAS A., PENDIAS H. Trace elements in the soil and plants. CRC Press, Boca Raton, FL., 1989.

5.RASKIN I., Nanda Kumar P.B.A., Dushenkov S., Salt D.E., Curr Opin Biotechnol, 5, 1994, p.285-290

6.STATE G., POPESCU I.V., RADULESCU C., MACRIS C., STIHI C., et al. Bull. Environ. Contam. Toxocol., 82 nr. 3, 2012, p. 580.

7.POPESCU I.V., FRONTASYEVA M., STIHI C., CIMPOCA GH.V., RADULESCU C., STATE G., GHEBOIANU A., OROS C., CILICOV O., BANCUTA I., DULAMA I., Romanian Reports on Physics, 63, 2011, p.1205.
8.POPESCU, I.V., FRONTASYEVA, STIHI, C., CIMPOCA, GH.V., RADULESCU C., GHEBOIANU, A., OROS, C., VLAICU, GH., PETRE, C., BANCUTA, I., DULAMA I.D., Romanian Journal of Physics, 55, nr. 7-8, 2010, p. 821.
9.STATE, G., POPESCU, ION V., GHEBOIANU, A., RĂDULESCU, CRISTIANA, DULAMA, I., BANCUTA, I., STIRBESCU, R., Romanian Journal of Physics, **56, nr.** 1-2, 2011, p. 233.
10.STIHI, C., RADULESCU C., BUSUIOC, G., POPESCU, I.V., GHEBOIANU, A., ENE A., Romanian Journal of Physics**,** 56, nr. 1-2, 2011, p. 257.
11.RADULESCU C., STIHI, C., BUSUIOC, G., GHEBOIANU, A.I., POPESCU, I.V., Bull. Environ. Contam. Toxocol., 84, nr. 5, 2010, p. 641.
12.RADULESCU C., STIHI, C., BUSUIOC, G., GHEBOIANU, A.I., POPESCU, I.V., CIMPOCA, GH. V., Romanian Biotechnological Letters, 15, nr. 4, 2010, p. 5444.
13.RADULESCU C., STIHI, C., POPESCU, I.V., IONITA, I., DULAMA, I., CHILIAN A., BANCUTA, O.R., CHELARESCU, E.D., LET D., Romanian Reports in Physics, 65, nr. 1, 2013.
14.KROLAK E., Polish Journal of Environmental Studies, **12**, nr. 6, 2003, p. 713.
15.CZARNOWSKA K., MILEWSKA A., Polish Journal of Environmental Studies, **9**, nr. 2, 2000, p.125.
16.FINZAR N., TLUSTOS P., LESTAN D., Plant Soil Environ., 53, nr. 5, 2007, p. 225.
17.LIGOCKI M., TARASEWICZ Z., ZYGMUNT A., ANISKO M., Acta Sci Pol., Zootechnica, 10, nr. 4, 2011, p.73.
18.MIRIK M., STEDDOM K., MICHELS jr. G.J., Rangeland Ecol Manag., 59, 2006, p. 44.
19.HAMRICK J.L., LEE J.M., American Journal of Botany, 74, nr. 3, 1987, p. 451.
20.BAKER A.J.M., BROOKS R.R., Biorecovery, 1, 1989, p. 81.
21.NOURI J., KHORASANI N., LORESTANI B., KARAMI M., HASSANI A.H., YOUSEFI N., Environ Earth Sci, 59, 2009, p. 315.
22.CUI Y-J., ZHU Y-G., ZHAI R-H., CHEN D.-C., HUANG Y.-Z., QIU Y., LIANG J.-Z., Environment International, 30, 2004, p.785.
23.HULL A.C. JR, EVANS J.O., Journal of Range Management, 26, nr 5, 1973, p. 383.
24.DESROCHERS A.M., BAIN J.F., WARWICK S.I., Canadian Journal of Plant Science, 68, 1988, p.1053.
25.***USDA, The PLANTS Database. National Plant Data Center, Natural Resources Conservation Service, United States Department of Agriculture. Baton Rouge, LA, 2010. http://plants.usda.gov
26.WICHTL M, ANTON R, Plantes therapeutiques, TEC & DOC Paris, 2003, p. 596.
27.DJINGOVA R, KULEFF I., Monitring of heavy metals by Taraxacum officinale, In Markert B, Ed. Plants as Biomonitors: Indicators for Heavy Metals in Terrestrial Environment, VCH, New York, 1993, p. 435.
28.KABATA-PENDIAS A., DUDKA S., Environ.Geochem. Health, 13, 1991, p. 108.
29.HUSAIN, I., KHAN L., Int. J. Pharamacognosy & Phytochem. Res., 2, 2010, p.15.
30.BĂRBULESCU, A., BARBEŞ, L., Mathematical Models and Methods in Applied Sciences, 2012, p. 151.
31.MAFTEI, C., BĂRBULESCU, A., Lecture Notes in Engineering and Computer Sciences, WCE 2008, vol. II, 2008, p.1082.
32.MAFTEI, C., BĂRBULESCU, A., MAUSAM, 63, nr. 4, 2012, p. 553.
33.RADULESCU C., STIHI C., Metode analitice complementare pentru determinarea concentratiei de metale grele, Ed. Bibliotheca, Târgovişte, 2011.
34.POPESCU I.V., RĂDULESCU C., STIHI C., CIMPOCA GH.V., DULAMA I.D., Tehnici analitice utilizate in studiul poluarii mediului, Ed. Bibliotheca, Târgovişte, 2011.
35.SUN Y., ZHOU Q., DIAO C., Bioresource Technology, 99, 2008, p. 110.
36.ZHOU Q.X., Journal of Basic Science and Engineering, 2, 1994, p. 81.
37.ZHAO F.J., HAMON R.E., LOMBI E., MCLAUGHLIN M.J., Journal of Experimental Botany, 53, 2002, p. 535

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