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Determination of heavy metals and other indicators in waters, soils and medicinal plants from Ave valley, in Portugal, and its correlation to urban and industrial pollution.

Pinto D, Fernandes A, Fernandes R, Mendes I, Pereira S, Vinha A, Herdeiro T, Santos E, Machado M.

Centro de Investigação em Tecnologias da Saúde – CICS. IPSN-CESPU. 4760 Vila Nova de Famalicão, Portugal.

The aim of this study was to evaluate pollution indicators on Rio Ave and surrounding environment, namely water, soil and medicinal plants in order to identify areas where contamination could cause ecosystem degradation. Plants, soil, surface water and ground water samples were collected seasonally at five different sampling sites along the Ave valley. Conductivity, pH, ammonium, nitrite and nitrate contents were evaluated. Cadmium, copper, lead, and zinc were measured in the water, soil and plants by Atomic Absorption Spectrophotometry. Results showed an increase on conductivity in waters from the origin to the mouth of the river. Nitrite levels were also higher than expected, especially in areas of wide industrialization and agricultural practices, where fertilizers widely used. Moreover, heavy metals in plants presented higher values when compared to those found in soils revealing that increased levels of heavy metals on soils are reflected similarly in plants. Levels of the analyzed parameters were often close or above to those legally acceptable in water and soils.

Key words: pollution, heavy metals, Ave Valley, soil, water, medicinal plants.

1. Introduction

Among substances that may cause risk to human health there are nitrogen compounds at different oxidation states (ammoniac nitrogen, nitrite and nitrate) and dissolved heavy metals in river water. Water contamination by nitrogen compounds is increasing and becomes a global problem due to its large and diverse origin.[1-3] It is known that their consumption through drinking water is associated with two adverse health effects such as induction of methemoglobinemia, especially in children, and the formation of carcinogenic nitrosamines and nitrosamides. 4,5 In Portugal, the situation is getting worse because, since the 90's, it has been increasing significantly concentrations of ammoniacal nitrogen, nitrite and nitrate in river water.[6,7] In equilibrium with the nitrogen cycle, other parameters such as pH and conductivity are also commonly altered in polluted environments. pH is an important indicator of environmental quality because it can predict serious imbalances in the nitrogen cycle, with excessive production of nitrite in rivers or estuaries with pH under 6. Conductivity measurement is a parameter positively correlated with nitrite concentration allowing the prediction of waters nitrification.[7,8,9]

Urban growth, resource extraction and landfill waste are some of the processes that may cause negative impacts on soil and groundwater.[10] Pollution represents a real threat to ecosystems and to people who live or work in surrounding areas. Contaminants can achieve significant distances due to the high potential mobility of many compounds and to the interaction soil / groundwater by percolation effects.[10] Local contamination is generally associated with mining, industry, landfills. Diffuse pollution is associated with atmospheric deposition, agricultural practices and to inadequate recycling and treatment of waste and wastewater. The introduction of contaminants in soil can result in loss of some or several soil functions and even cause groundwater contamination. The occurrence of soil contaminants can cause multiple negative consequences in food chain, public health, ecosystems and natural resources.[11]

Heavy metals are highly reactive, bioaccumulative and extremely toxic elements above threshold concentrations. Lead (Pb) is the cause of multiple endocrine effects as those associated with renal dysfunction, male fertility, etc.[12] In excess, copper (Cu) leads to neurological disorders such as depression, irritability and nervousness, and to muscular pains. Zinc (Zn) is an essential element that intervenes in the metabolism of proteins and nucleic acids. However, in excess, promotes decreases in immune function and HDL levels.

Plants have been used to detect and monitor the deposition, accumulation and distribution of heavy metals.[13] In the last decades there has been an increase in the use of leaves of higher plants as biomonitors of heavy metal pollution of soils.[14]

2. Materials and Methods

2.1 Study area

The study area is located on Ave valley, throughout Rio Ave (94 km) (Fig. 1). Samples were collected in three climatic seasons during the year: Spring, Summer and Autumn and were obtained from five sites (S1–S5), (Fig.1).

2.3.4 Heavy metals/ Atomic absorption spectrophotometry

Concentration of heavy metals, namely, cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) was obtained after concentration, extraction and digestion with HNO₃/HClO₄ or HNO₃, followed by atomic absorption spectrophotometry (AAS) (Sensai Dual GBC Scientific Equipment).[5] The AAS incorporate an air/acetylene flame system and it was performed calibration curves with Pb, Cd, Cu and Zn standards.

Soils Samples were dried at 35-40°C and sieved with a 2mm HDPE sieve. In this paper, the obtained results concerning Cd, Cu, Pb and Zn, were based on its extraction by aqua regia.[17]

2.4 Statistics

We applied the Kolmogorov–Smirnov test to determine whether the data followed a normal distribution or not. For data with a normal distribution, analysis of variance (ANOVA) and Dunnett Post Hoc Test were applied for determination of significant differences between seasons and sites. Pearson coefficient test was applied to access the association between heavy metals levels in plants and soils. The level of significance was $p < 0.05$.

2.5 Results

Sampling of surface water, groundwater, soil and plants were accomplished according to figure 1 in 5 different sites through Ave valley along Rio Ave. Concerning to water samples, pH values did not show a trend of results according to the sampling point, but values gradually increases from spring to autumn. Conductivity shows a clear increase from the first site of sampling (33.3 S.cm⁻¹, S1) to the mouth of the river (1056 S.cm⁻¹, S5) ($p < 0.001$), and from spring to autumn ($p < 0.05$). Parameters related to nitrogen cycle presented maximum values in summer to nitrite (NO₂⁻)(0.37 mg. L⁻¹, S5). The nitrate (NO₃⁻) presented maximum values held in site 5 in the summer (21.3 mg. L⁻¹, S5). In groundwater (S2 and S3), maximum values were 15.1 mg. L⁻¹ and 13.7 mg. L⁻¹, respectively (Table 1).

Table 1. pH, conductivity, nitrate and nitrite evaluated from spring to autumn in five sampling sites (S1 to S5) of surface water, groundwater and soil.

Site	Season	pH			Conductivity (µS.cm ⁻¹)			Nitrate (mg.L ⁻¹)			Nitrite (mg.L ⁻¹)		
		SW	GW	S	SW	GW	S	SW	GW	S	SW	GW	S
S1	Spring	7,11	6,47	4,99	33,3	43,1	93,03	0,35	0,18	2,24	0,00	0,32	0,379
	Summer	7,23	6,38	5,16	36,0	34,40	62,90	0,26	0,13	0,31	0,33	0,34	0,371
	Outumn	7,29	7,25	3,87	39,7	47,10	11,34	0,00	0,00	0,00	0,00	0,00	1,009
S2	Summer	6,91	6,64	5,60	75,0	154,6	60,70	0,92	1,94	1,23	0,34	0,46	0,420
	Outumn	7,25	7,22	4,19	146,3	213,2	20,41	0,00	4,20	0,00	0,29	0,00	0,585
S3	Spring	6,29	7,06	4,82	116,3	182,4	55,93	1,01	1,72	4,49	0,43	0,31	2,227
	Summer	7,17	6,71	3,95	159,0	135,9	34,02	1,06	1,36	0,00	0,35	0,34	0,305
	Outumn	7,28	7,29	4,25	441,3	173,5	29,48	1,98	0,00	0,00	0,00	0,00	0,814
S4	Summer	7,27	-	4,23	170,2	-	97,53	1,54	-	19,32	0,35	-	3,115
	Outumn	7,45	-	3,90	718,9	-	65,77	0,00	-	7,71	0,14	-	0,196
S5	Spring	7,10	-	6,43	237,0	-	156,23	1,76	-	4,05	0,36	-	0,706
	Summer	7,13	-	6,27	302,0	-	94,73	2,68	-	2,07	0,37	-	0,671
	Outumn	7,30	-	5,30	1056,0	-	92,99	8,97	-	14,6	0,21	-	10,848

SW, surface water; GW, groundwater; S, soil.

The higher concentration on heavy metals was found for cadmium and lead on site 5 (3.54 ng.L⁻¹ and 9.53 ng.L⁻¹, respectively). Copper presented a maximum of 4.9 ng.L⁻¹ in site 4 and zinc 74.8 ng.L⁻¹ in site 3 (Table 2). As these metals accumulate in living organisms the use of this water must be controlled, especially if the destination is the consumption, irrigation of agricultural areas and like. Regarding to groundwater, maximum values of heavy metals, were found in site 3 (Cd-3; Cu-32, Zn-100 and Pb-18 ng.L⁻¹).

Table 2. Cadmium, copper, lead and zinc evaluated from spring to autumn in five sampling sites (S1 to S5) of surface water, groundwater and soil.

Site	Season	Cd (ng.L ⁻¹)			Cu (ng.L ⁻¹)			Zn (ng.L ⁻¹)			Pb (ng.L ⁻¹)		
		SW	GW	S	SW	GW	S	SW	GW	S	SW	GW	S
S1	Spring	3,28	1,12	1,20	0,00	0,00	9,03	0,00	13,0	103,80	0,00	0,00	19,15
	Summer	3,50	2,68	0,99	0,00	0,00	13,70	0,00	66,3	68,98	0,00	0,00	18,85
S2	Summer	2,62	1,90	1,33	0,00	14,8	16,58	22,80	48,8	139,20	0,12	0,00	23,63
	Autumn	2,62	1,62	1,93	0,00	31,2	17,43	20,10	71,0	165,88	0,16	1,20	36,83
S3	Spring	3,06	2,68	1,11	1,60	6,70	11,93	74,80	57,1	111,08	3,05	18,40	23,05
	Summer	3,00	2,74	0,94	2,20	31,6	7,03	28,50	93,4	110,28	0,68	0,00	22,38
	Autumn	2,50	3,12	1,16	1,40	18,0	10,53	9,40	99,9	103,38	1,13	2,000	19,93
S4	Summer	3,18	-	1,82	4,90	-	25,48	18,60	-	182,53	1,09	-	23,25
	Autumn	2,22	-	1,05	0,60	-	23,53	30,40	-	166,18	1,21	-	26,08
S5	Spring	2,70	-	1,39	2,80	-	68,68	32,80	-	251,25	0,55	-	43,35
	Summer	3,52	-	1,08	0,60	-	54,25	5,20	-	227,30	9,53	-	31,75
	Autumn	3,28	-	-	0,00	-	-	0,00	-	-	0,00	-	-

SW, surface water; GW, groundwater; S, soil.

Soil analysis showed pH values higher during the seasons of spring and summer, while the conductivity showed a decreasing trend from spring to autumn. For instance, in site 1 the conductivity in spring was 11 S.cm⁻¹, in summer 63 S.cm⁻¹ and in autumn 93 S.cm⁻¹ (Table 1). Nitrogen content in soil samples was evaluated by determining the concentration of nitrite (NO₂⁻) and nitrate (NO₃⁻). Soil sample collected in site 4 showed the maximum value of nitrite (4 mg.L⁻¹) in summer, while the maximum amount of nitrates was found in site 5, in spring (32.3 mg.L⁻¹) (Table 1). The amount of heavy metals on soils depends on pH. Generally the highest values were found in site 5, on river mouth (Cd-1.39, Cu-69, Zn-251 and Pb-43 mg.Kg⁻¹) (Table 2). Plants with the most frequent sampling through the different sites were peppermint and foxglove. Results for parameters such as pH, conductivity, nitrates and ammonium can be observed in table 3. In general, site 5 presented higher values of conductivity, nitrogen ammonia and nitrate, whose highest expression was found in the analysis of hyacinths harvested waterfront in summer (803 S.cm⁻¹, 21 mg.L⁻¹ and 1945 mg.L⁻¹, respectively).

Table 3. pH, conductivity, ammonium and nitrate evaluated in spring, summer and autumn in five sampling sites (S1 to S5) of 7 plant species, *Mentha piperita* (MP), *Digitalis purpurea* (DP), *Matricaria recutita* (MR), *Melissa officinalis* (MO), *Petroselinum Sativum* (PS), *Pimpinella anisum* (PA), *Eichhornia crassipes* (EC).

Site	Season	pH							Conductivity (μS.cm ⁻¹)						
		MP	DP	MR	MO	PS	PA	EC	MP	DP	MR	MO	PS	PA	EC
S1	Spring	5,8	5,8	-	-	-	-	-	213	305	-	-	-	-	-
	Summer	6,2	6,1	6,0	-	-	-	-	216	361	200	-	-	-	-
	Autumn	5,1	5,7	-	-	-	-	-	251	-	-	-	-	-	-
S2	Summer	5,7	-	-	-	-	-	-	120	-	-	-	-	-	-
	Autumn	5,8	-	-	-	-	-	-	348	-	-	-	-	-	-
S3	Spring	6,2	5,4	-	-	5,7	5,9	-	233	293	-	406	293	450	-
	Summer	5,7	5,9	-	5,8	-	-	-	152	268	-	-	-	-	-
	Autumn	5,6	5,6	-	-	-	5,7	-	361	-	-	-	-	494	-
S4	Summer	5,9	-	-	-	-	-	-	381	-	-	-	-	-	-
	Autumn	5,7	-	-	-	-	-	-	349	-	-	-	-	-	-
S5	Spring	6,3	5,8	6,1	-	5,7	-	-	384	261	465	-	605	-	-
	Summer	5,6	-	-	-	5,3	-	5,8	248	-	-	-	681	-	803
	Autumn	-	-	-	-	-	-	6,2	-	-	-	-	-	-	328

Site	Season	NH ₄ (mg.L ⁻¹)							Nitrate (mg.L ⁻¹)						
		DP	MR	MO	PS	PA	EC	MP	DP	MR	MO	PS	PA	EC	
S1	Spring	26,6	-	-	-	-	-	104	86	-	-	-	-	-	
	Summer	0,0	6,2	-	-	-	-	147	128	54	-	-	-	-	
	Autumn	4,8	-	-	-	-	-	213	128	-	-	-	-	-	
S2	Summer	-	-	-	-	-	-	91	-	-	-	-	-	-	
	Autumn	-	-	-	-	-	-	448	-	-	-	-	-	-	
S3	Spring	0,7	-	0,4	4,6	14,2	-	143	61	-	520	54	35	-	
	Summer	0,0	-	-	-	-	-	74	293	-	-	-	-	-	
	Autumn	1,4	-	-	-	11,2	-	175	275	-	-	-	239	-	
S4	Summer	-	-	-	-	-	-	120	-	-	-	-	-	-	
	Autumn	-	-	-	-	-	-	310	-	-	-	-	-	-	
S5	Spring	23,6	3,3	-	-	-	-	169	43	75	-	-	-	-	
	Summer	-	-	-	4,5	-	21,3	302	-	-	246	-	-	1945	
	Autumn	-	-	-	-	-	16,1	-	-	-	-	-	-	295	

Regarding heavy metals concentration in plants, results are summarized in table 4. Generally levels are quite similar among analyzed plants with the exception of *Petroselinum Sativum* that reveal higher values of Cu, and *Mentha piperita* that showed the lower levels on Zn.

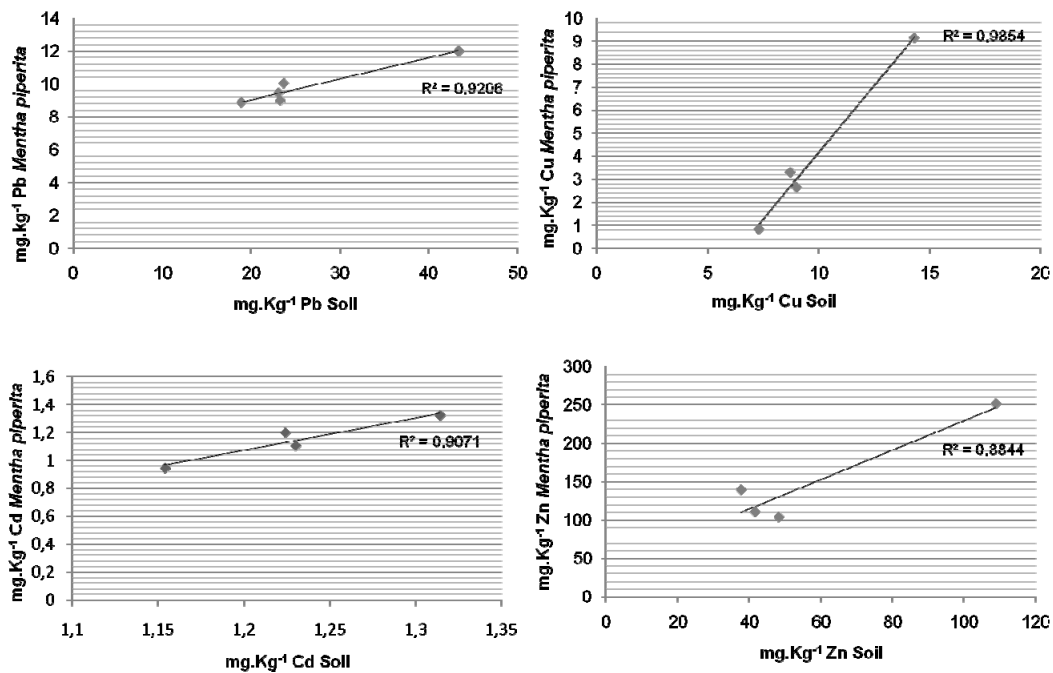
Table 4. Cadmium, copper, lead and zinc evaluated on spring, summer and autumn in five sampling sites (S1 to S5) of 7 plant species, *Mentha piperita* (MP), *Digitalis purpurea* (DP), *Matricaria recutita* (MR), *Melissa officinalis* (MO), *Petroselinum Sativum* (PS), *Pimpinella anisum* (PA).

Site	Season	Cd (ng.L ⁻¹)						Cu (ng.L ⁻¹)					
		MP	DP	MR	MO	PS	PA	MP	DP	MR	MO	PS	PA
S1	Spring	1,9	1,2	-	-	-	-	11	10	-	-	-	-
	Summer	1,3	1,4	1,6	-	-	-	7	13	24	-	-	-
S2	Spring	1,3	-	-	-	-	-	14	-	-	-	-	-
	Summer	1,2	1,2	-	1,4	1,3	1,6	9	8	-	9	29	10
S3	Spring	1,2	2,0	-	-	-	-	11	6	-	-	-	-
	Summer	1,2	-	-	-	-	-	11	-	-	-	-	-
S4	Spring	1,2	1,4	2,3	-	4,1	-	10	8	13	-	17	-
	Summer	1,2	-	-	-	-	-	-	-	-	-	-	-
S5	Spring	50	196	-	-	-	-	9	12	-	-	-	-
	Summer	65	473	225	-	-	-	11	11	8	-	-	-
S2	Spring	55	-	-	-	-	-	12	-	-	-	-	-
	Summer	64	228	-	216	165	86	12	12	-	11	14	16
S3	Spring	42	134	-	-	-	-	11	9	-	-	-	-
	Summer	42	134	-	-	-	-	11	9	-	-	-	-
S4	Spring	91	-	-	-	-	-	12	-	-	-	-	-
	Summer	109	90	106	-	215	-	10	9	11	-	11	-

Analyzing the association among heavy metals in plants and soils it was found a linear correlation between them, namely on peppermint (Figure 2) and foxglove (Figure 3).

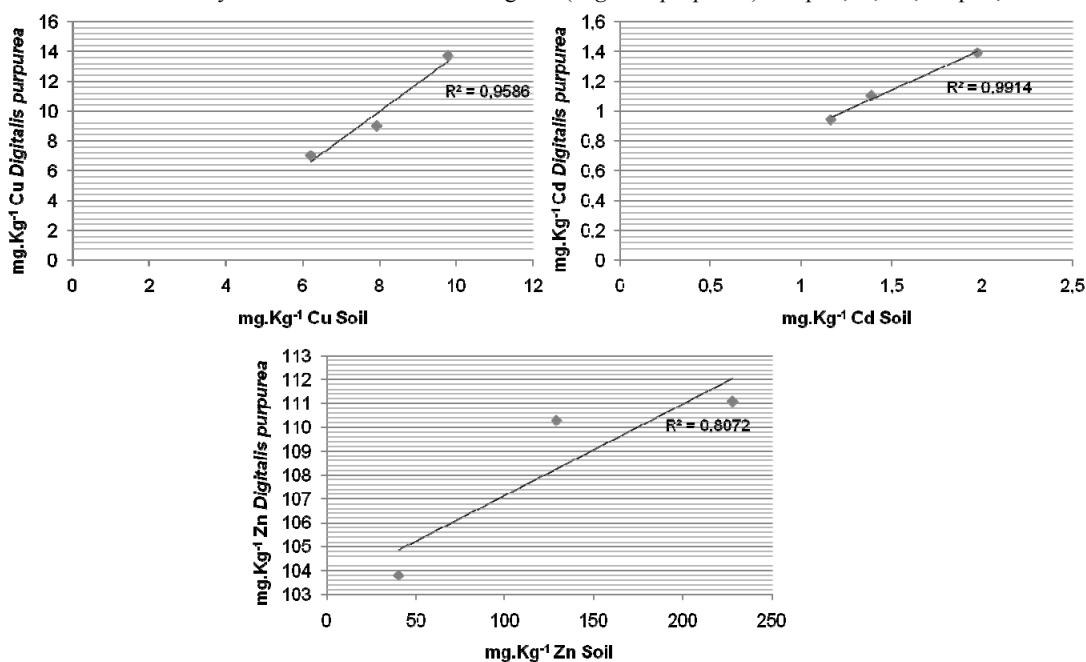
The relation between metal elements in soils and in peppermint revealed a significant association to all the heavy metals evaluated (Pb, Cu p<0,001; Cd p<0,05; Zn p<0,05).

Figure 2. Relation between heavy metal levels in soils and peppermint (*Mentha piperita*). Pb, Cu p<0,001; Cd p<0,05; Zn p<0,05.



The association between metal elements in soils and in foxglove also revealed to be significant in all evaluated heavy metals (Cd p<0,01; Cu, Zn p<0,05).

Figure 3. Relation between heavy metal levels in soils and foxglove (*Digitalis purpurea*). Cd $p < 0,01$; Cu, Zn $p < 0,05$.



It must be noted that analyzed parameters have not remained similar over time. The inconsistency of weather associated to the industrial pollution found in this region, led to values that do not exhibit a particular pattern of seasonal fluctuation.

Discussion

The level of pollution of Rio Ave is quiet evident. Considering the law which defines the conditions necessary for a drinking water, it was found that Rio Ave is far from a desired source of drinking water. In Rio Ave, there is an increase on conductivity up from site 1 to site 5, this trend is also evident throughout the seasons ($p < 0.05$). Indeed, at the mouth of the river there is a conductivity value equal to 1056 S.cm^{-1} in the autumn. This value was higher than the allowed in waters used to produce drinking water ($\approx 1000 \text{ S.cm}^{-1}$) and much higher than allowed for the quality of water for human consumption.[18]

Nitrite levels compared to what is acceptable (0.1 mg.L^{-1}), are also higher than expected. In site 3 was found the highest value on this parameter ($0,43 \text{ mg.L}^{-1}$) that is in accordance with the wide industrialization occurring in this area. All the groundwater analyzed, collected from private wells, showed a content of nitrites higher than the minimum values allowable for drinking water quality. That fact may also be due to agricultural practices and uncontrolled use of fertilizers.

Nitrate levels although being below the limit set for water consumption quality in (50 mg.L^{-1}) it showed an upward trend since site 1 to site 5, related to the increasing on industrial and domestic pollution.

Among all studied heavy metals it was verified that their levels are within the required parametric values for water consumption; however, it must be empathized that despite these, their capacity to accumulate in living organisms makes them important targets of health control. Curious is the fact that groundwater showed higher levels than surface water. This may be due to leaching of metals from soil, where they are in greater quantity. Indeed, Zn and Cd levels are higher than those recommended by law.[19] This is particularly evident in site 3 and 4, areas of heavy industrialization and highways nearby. On soils, limits set for heavy metal content depends on pH of the soil, therefore, in site 5 Cu, Zn and Pb values are higher since pH is also higher. Despite those values are close to the limit allowed by law (Cd-3, Cu-100, Zn-300 mg.Kg^{-1}) they are higher than alert values (Cd-0,6; Cu-30-35; Zn-150 mg.Kg^{-1}).[20,21]

On medicinal plants there are no legislation establishing limits concerning the analyzed parameters. However, these elements are important indicators of environmental pollution being important their study. Therefore, in the most industrialized sites of sampling (site 2, 3, 4) and at the mouth of the river (S5), nitrates, ammonia nitrogen and conductivity levels are generally higher for these plants. According to WHO recommendations, regarding heavy metals concentrations, the maximum levels allowed for Cd (0.3 mg.Kg^{-1}) and Pb (10 mg.Kg^{-1}) are generally exceeded. For Cu and Zn there is no legislation advising their limits.

Results showed that some of the element concentrations (Cd, Zn) in plants were higher than those in soil, and were greater than those in the water. It was also found that heavy metals levels in soil are correlated with those parameters in plants, revealing that increased levels of heavy metals on soils are reflected similarly in plants. This shows the ability of

the studied plants to extract and bioaccumulate heavy metals. Despite the interest that this feature represents on phytoremediation as a tool in pollution control, it represents a risk for public health, once they can be ingested as food or for medicinal purposes.[22-24]

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