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Biomonitoring of heavy metals by lichens in Setif Area (East of Algeria)

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In the present work, lichen *Xanthoria parietina* is used as an accumulator of four heavy metals in the Setif region. The sampling of biological material is performed using a natural sensor, represented by a lichen species corticolous, *X. parietina*, from 16 stations located in proximities of 04 highways (RN 5, NR 9, RN 28 and RN 74). Samples were taken and four heavy metals (Cr, Zn, Cu and Ni) were selected for the analysis. The metal contents recorded in the various stations are very variable. The (Cr. Zn and Ni) are high in all the stations relative to normal levels; these stations are characterized by high traffic and / or an intense industrial activity. For the Cu contents, recorded for all stations, are low. These results show a tolerance of *X. parietina* the metal stress and therefore its efficiency to be used in biomonitoring programs of air quality.

Keywords: Heavy metals, Biomonitoring, Bioaccumulation, *Xanthoria parietina*, Setif, Algeria.

INTRODUCTION

Lichens are organisms that react to atmospheric pollution. These organizations are very popular in studies of Biomonitoring, bioaccumulation and measures concentrations of substances in organisms, resulting from pollution (Clement et al., 1995; Bargagli, 1998; Wyttenbach et al., 1990; Van Dobben et al., 2001; Bargagli et al., 2002; Bergamaschi et al., 2007; Maizi et al., 2010). They are very sensitive to emissions of gases, especially those containing heavy metals (Garty, 1993; Rao et al., 1997; Sarret et al., 1998). Lichens are among the bioindicators most commonly used (Ferry et al., 1973; Markert, 1993; Bargagli, 1998; Nash, 1988; Dzubaj et al., 2008).
The mineral nutrition of lichens depends on wet and dry deposition from the atmosphere; they are considered appropriate tools for monitoring the relative levels of air pollution; in fact, they are able to accumulate and store many airborne substances in the environment (Nieboer and Richardson, 1981; Baffi et al., 2002). The anthropogenic input of heavy metals in the atmosphere has increased over the last sixty years (Patterson and Settle, 1987; Nriagu and Pacyna, 1988). This contamination of the atmosphere is due to industrial production and vehicular traffic (leaded petrol combustion). These metals are responsible for a dangerous and irreversible pollution and can have very harmful effects on human health.
Lichens are the most dominant organizations in the evaluation of air quality, by their slow growth are ideal for long-term studies of air pollution (Stamenkovic and Cvijan, 2003). Lichen *Xanthoria parietina* is common in urban areas and one of species the most commonly used in biomonitoring programs (Nimis et al., 2000, 2001). The value of lichens as bioaccumulators is largely attributed to their surface/volume. *X. parietina* has a large area of contact with air pollutants, so it is able to accumulate large amounts of heavy metals Sarret et al., 1998; Bargagli, 1998; Scerbo et al., 1999, 2000; Nayaka et al., 2003; Cuny et al., 2004). Similarly the lack of roots, waxy cuticle and stomata, facilitates the penetration of contaminants inside the thallus (Puckett et al., 1973; Sloof, 1995; Reis et al., 1999). This study was undertaken to evaluate the metal

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contamination levels in the thalli of *X. parietina*, collected in the communes of Setif region (Algeria).

MATERIALS AND METHODS

Sample Collection

The foliose lichen *Xanthoria parietina*, widespread in the study area, and widely used in similar studies in several countries, has been selected as bioaccumulators. The samples were collected at 16 stations spread in Setif Province (Figure 1). Samples are collected, in avoiding the use of tools or containers may contaminate the sample, (steel tool or stainless steel containers whose walls contain pigments based on trace elements, for example PVC).

Each station consists of areas of maximum 100 m^2, located near high traffic roads (Table 1). The Setif province is characterized by a semi-arid continental climate, with hot, dry summers and severe winters. Rainfall is inadequate and irregular both in time and space. The geographical coordinates of each sampled stations were noted using a GPS.

Treatment of samples

Lichen samples were digested in a clean room at the laboratory, using a mixture $HNO_3/HF/H_2O$ in Teflon vessels (Rusu, 2002; CERTU, 2004). The Solutionization of the metal elements and performed on the milled samples, from 10 ml of hydrofluoric acid (HF) at 40% and 3 ml of perchloric acid ($HClO_4$) at 70%. Evaporation takes place to dryness on a hotplate at 160°C. The residue is collected with 1 ml of nitric acid (HNO_3) at 65%. Then we added 10 ml of distilled water, and allowed to stand for 30 min to cold. The dissolution of the residue is performed by placing the samples 1h on a hotplate at 60°C. The obtained mixture is transferred to a 100 ml flask by filtration, adjusting with distilled water to the mark.

Analytical procedures for lichens concentrations

The concentrations of the following elements Cr, Cu, Ni, Cd were determined by Atomic Absorption Spectrophotometry with Flame (AASF). There are no established standards of trace elements concentration in lichens (Bettinelli et al., 1996). To interpret the results of each element studied, we use as standard reference values, the unit's concentration ranges (Quevauviller et al., 1996) (table 2).

Statistical analysis

Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the trace elements and the bioaccumulation by lichens, and the Relations between the presence of these elements and the vehicle circulations. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the elements and stations; to test for significant differences among means of samples, the ANOVA is used. The statistical analyses were carried out using STATISTICA 10 software.

RESULTS AND DISCUSSION

The analysis of metal elements accumulated in the thalli of *Xanthoria parietina* by AAS gave the contents of metal elements that vary from one station to another (Table 3). The element displaying the highest average concentration in *X. parietina* sample was Zn (91.70 ± 40.62), while Cu was least abundant. The analyzed metal concentrations were distributed in the following order: Zn > Cr > Ni > Cu.

The Zn presents the highest variation (97.14 ± 45.27), followed by Cr (89.44 ± 34.99) then Ni (39.52 ± 19.15), by against Cu has a very low variability. The higher are the Zn while Cu is less abundant. The concentrations of the metals analyzed are distributed in the following order: Zn > Cr > Ni > Cu. The analyses of the concentrations of the five metals in the 16 samples showed a subdivision into two clusters (figure 2).

The rate of Cr accumulated in the *X. patietina* thallus, show values exceeding the certified standard, which is 4.12 mg / kg (reference standard). There are three class groups of accumulation of this metal. Very high concentrations relate the stations, El-Eulma, Guergour, Tizi N'bechar, Setif, Aïn Oulmene, El Ouricia and Zanadia. The stations of Beni-Ouartilene, Salah-Bey, Amoucha, Guellal and Ouled Sabor, show high rates, while the lowest rate was observed in the municipalities of Ain-Arnat, Bougâa, Beni-Chebana and Mezloug.

The accumulation of Cu is very high in the Ain-Arnat station, and exceeds the certified standard (7.03 mg/kg), while the rest of the stations have low levels. The Ni accumulated in *X. parietina* thalli, present in all stations, values exceeding the certified standard, which is 2.47 mg/kg. The Zn rate accumulated in the *X. parietina*, shows values below the standard certified (100.6 mg/kg), except for stations of El-Ouricia and Ain-Arnat which slightly exceed the standard, against the stations of Setif, Aïn-Oulmene, Bougâa well above the certified standard. The concentration of heavy metals in the lichen has a significant difference, and analysis of these metals exhibit a high variability between the plants studied (Figure 3).

The three-dimensional spatial projection of stations, based on the first three axes resulting from the PCA (Figure 4) shows the separation of populations of Ain Arnet and Bougâa, while the remaining populations are less separated and the distinction between them is not clear.

The UPGMA confirms the separation of stations into many groups (Figure 5). This separation, based on the (Unweighted Pair-Group Average) and Euclidean distance, is due to the concentration of heavy metals in lichen thalli. This separation reflects heterogeneity in the metal accumulation in *X. paretina*.

The first group is represented by the stations of Ain Arnat and Bougaa, the second group split into two groups; one notices the separation of Setif and Ain Oulemene. The rest of the stations form an amalgam representing the same groups as those of the CPA except for the station of El-Eulma.

The analysis shows the presence of a quantitative diversity, which can be explained by environmental hazards, demographic and especially those related to the use of vehicles that accentuate the accumulation of these metals in lichen thalli. The two statistical analyzes (PCA and UPGMA) have failed to separate our stations in distinct homogeneous groups. Our goal is to find the relationships between stations, located on the same road, and concentrations of metallic elements. For that we applied a third statistical analysis (ANOVA). This technique allowed us the comparison between the average concentrations of heavy metals and roads (table 4).

The comparison of average metal concentrations, of different sites with the certified standard, shows that the values fluctuate with remarkable peaks for chromium and nickel, and a slight increase for the zinc in the RN 74. These results are superior to certified values, while copper levels remain below the standards set.

The ANOVA did not reveal significant difference inter-stations for the accumulation of metals. However, a highly significant difference was observed in the stations (highway) for the Chrome ($p = 0.0006 > 0.001$). For copper and nickel, there's a very significant difference between the axes studied (p = 0.028

and $p = 0.018 > 0.01$). By cons there is no significant difference between the average grades of the samples analyzed regardless of the study area for zinc ($p=0.7 > 0.05$) (tableau 5). The use of test comparing averages (LSD - Fischer) allowed us to differentiate between the elements and roads (Table 6).

The schematic representation of these results allowed us to better identify the relationships between groups. Regarding the Zn, the test LSD-file shows no significant difference between the stations and the certified standard. This test shows a significant difference between the stations and the standard for Ni; and a non-significant difference between the stations (Figure 6).

Concerning Cr one notices the separation of stations and standard; by against the axes have significant differences. We note that there are no significant differences between the stations and the standard for copper Cu. For the classification of traffic routes according to metallic element accumulation, the Score method was applied (Table 7). The RN 5 is ranked first with a score of 4, meaning that this axis is the most contaminated by heavy metals. While RN9, RN 28 and RN 74 with a score of 3, are less polluted than the axis of the RN 5.

DISCUSSION

The accumulation of Cr and Ni in the thallus of *Xanthoria patietina* in the study sites has values exceeding the certified standards. This high concentration is due to the intense movement of vehicles in these axes, which contributes to air emissions and industrial activities (Scerbo et al., 1999, 2000; Brunialti and Frati, 2007; Doğrul-Demiray et al., 2012; Delmas-Gadras, 2000). The atmospheric deposition of chromium, copper, nickel and zinc, in Hungary, on the foams are associated with different sources (chemical, steel mills, coal combustion, and transportation and energy production) (Otvos et al., 2003; Figueiredo et al., 2007).

The concentration of zinc in lichens of the stations of El Ouricia and Ain Arnet (urban area) slightly exceeds the standard, by against in Setif (Downtown), Aïn Oulmene and Bougaa (urban area) stations, the concentrations are well above the certified standard. This trend can be explained by the difference in the intensity of car traffic between the two sites; in addition, the towns of Setif, Aïn Oulmene and Bougaa are near of industrial activities.

Concerning the roads traversing the study area, two groups are distinguished. The first group is represented by the RN5, characterized by high pollution by heavy metals. This axis is characterized by very important road traffic. The second group, formed by the RN 28, RN 74 and RN 9, the pollution is moderate. This heavy metal concentration is explained by the intensity of car traffic (Sarmoum et al., 2014).

The traffic and road infrastructure are an important source of heavy metals released into the environment, the main pollutants emitted, the lead, zinc and cadmium are mainly present in the exhaust gas and the brake linings. Zinc is also present in tires, lubricants and particularly in the guard rails (Deletraz, 2000). The Brakes represents a significant source of copper (Pagotto, 1999). Human activities are also capable of emitting various substances, mainly in the large urban centers (industry, energy production...), the mining areas, extraction and high agricultural productivity sectors (Agnan et al., 2013).

CONCLUSION

Concentrations of four heavy metals (Cr. Zn. Cu and Ni) were determined in the thalli of *Xanthoria parietina*, harvested from 16 stations in the Setif region. This study showed that X. parietina, is a species accumulating heavy metals (pollutants) at a variable degree of tolerance.

The concentration of (Cr. Zn and Ni) is high in all the stations relative to the normal content; for Cu, the contents registered in all the stations are low. On the basis of the rate of accumulation of heavy metals by lichen, we have defined two groups of roads according to the degree of pollution: RN 5 is a highly contaminated axis, it represented by urban sites in Setif region. While the RN 28, RN 74 and RN 9, are moderate pollution stations. This pollution is mainly due to traffic and road infrastructure.

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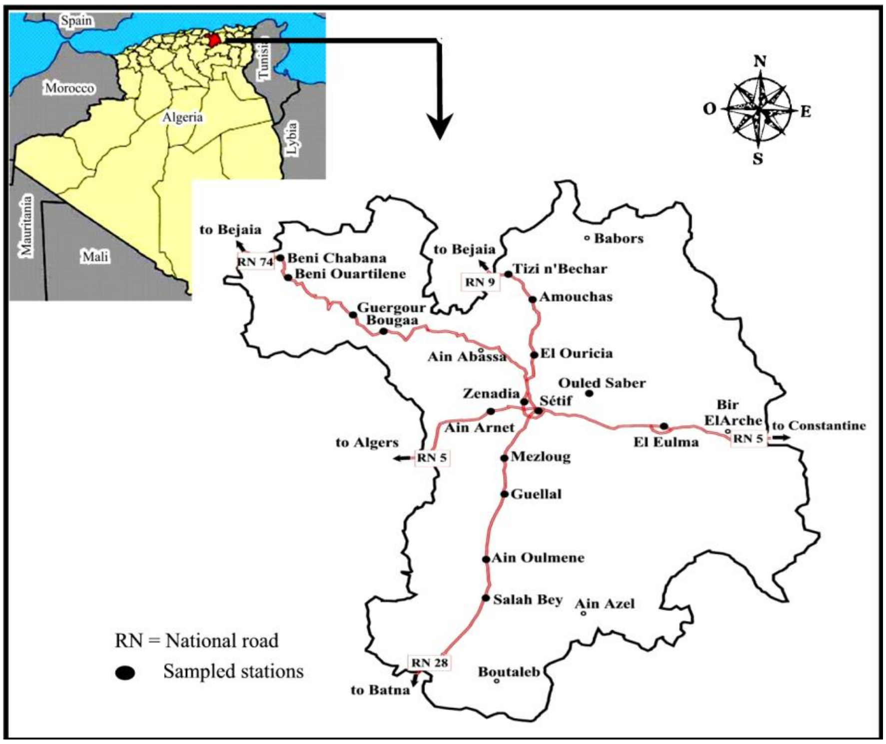
Figures

Figure 1: Location of the sampling stations
Figure 2: Dendrogram of the elements, based on the data of table 3
Figure 3: Concentration variability in heavy metals in *Xanthoria parietina*
Figure 4: Spatial projection of stations, based on the first three axes, from the ACP
Figure 5: UPGMA, based on the concentration of heavy metals
Figure 6: Relationship between groups and the standards certified



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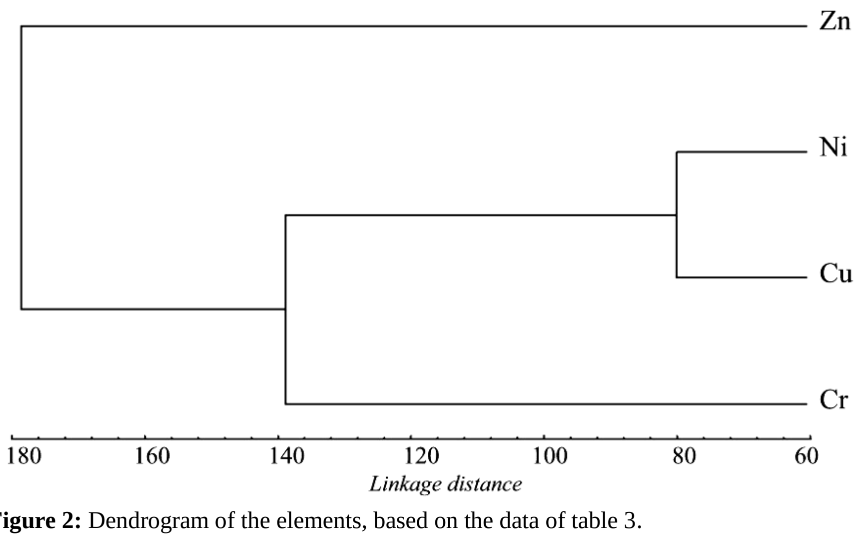

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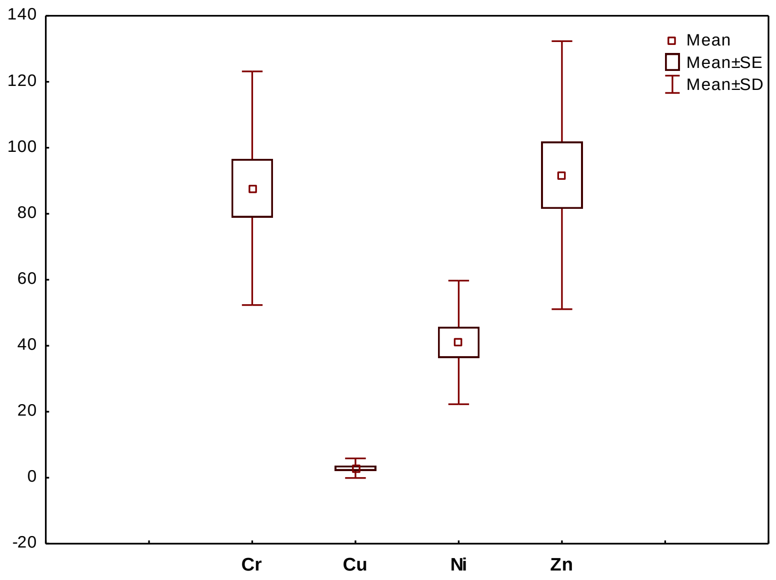

Figure 3: Concentration variability in heavy metals in *Xanthoria parietina*

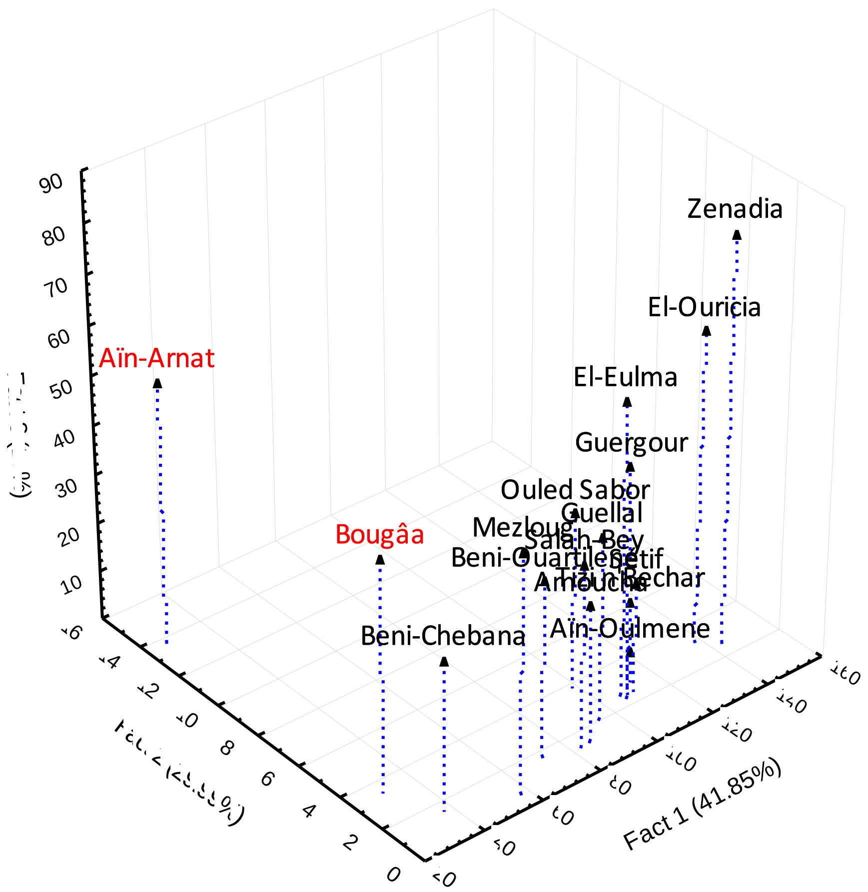

Figure 4: Spatial projection of stations, based on the first three axes, from the ACP

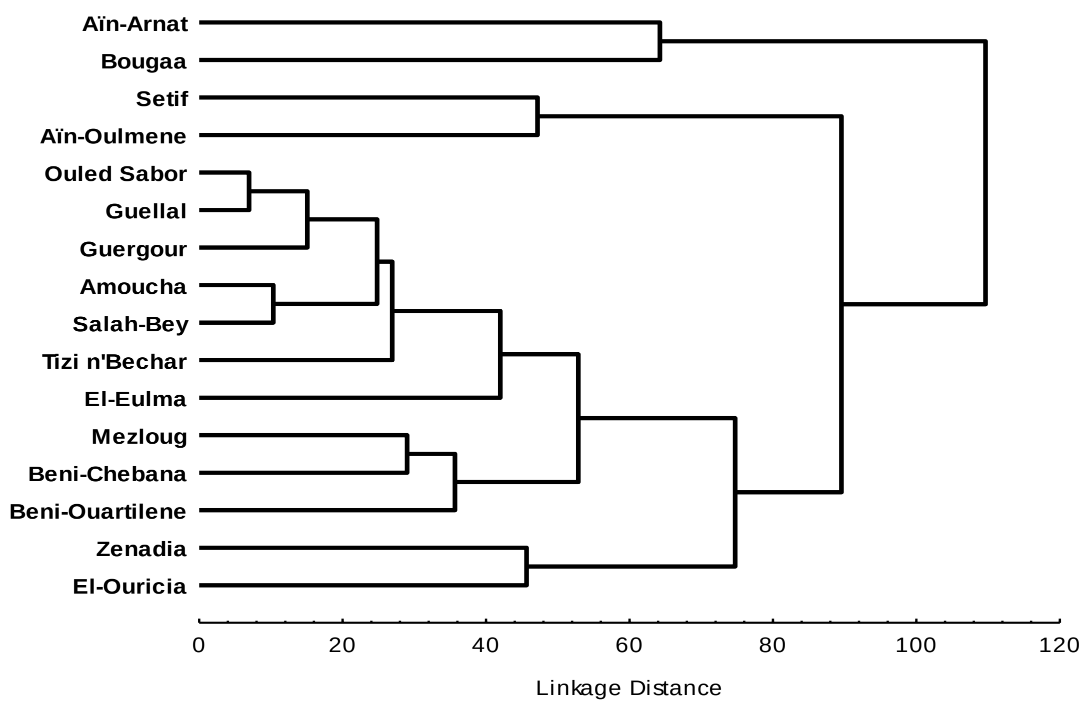

Figure 5: UPGMA, based on the concentration of heavy metals

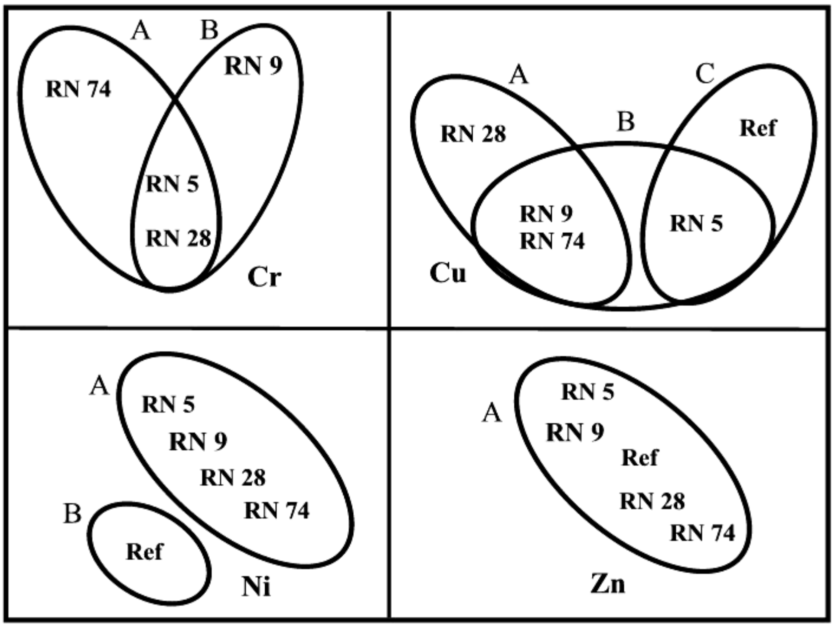

Figure 6: Relationship between groups and the standards certified

Tables

Table 1: Geographic coordinates of the stations sampled
Table 2: Certified values according to (AASF) of trace elements
Table 3: Concentrations of trace elements (mg/kg) in *Xanthoria parietina*
Table 4: Average concentrations of heavy metals
Table 5: Variance analysis of heavy metal
Table 6: the Difference between the elements traces and roads (LSD – Fischer)
Table 7: Classification of areas according to scoring method

Table 1: Geographic coordinates of the stations sampled

Localities	Altitude (m)	Geographic coordinates	National road (RN)
Ain Arnat	1018	36° 11′ N ; 05° 19′ E	RN5
Setif	1100	36° 09′ N ; 05° 26′ E	
Ouled Saber	1075	36° 09′ 46″ N ;05° 33′ 43″ E	
El Eulma	950	36° 09′ 23″ N ; 05° 41′ 06″ E	
Tizi N'Bechar	895	36° 25′ 52″ N ; 05° 21′ 36″ E	RN 9
Amoucha	800	36° 23′ 17″ N ; 05° 24′ 39″ E	
El Ouricia	1138	36° 17′ 01″ N ; 05° 24′ 34″ E	
Zenadia	1150	36° 09′ N ; 05° 26′ E	
Mezloug	915	36° 06′ 28″ N ; 05° 20′ 13″ E	RN 28
Guellal	905	36° 02′ 42″ N ; 05° 19′ 41″ E	
Ain Oulmene	928	35° 54′ N ; 05° 17′ E	
Saleh bey	974	35° 51′ 15″ N ; 05° 17′ 30″ E	
Beni Ourtilane	900	36° 26′ N ; 04° 54′ E	RN 74
Beni Chebana	750	36° 27′ 54″ N ; 04° 52′ 34″ E	
Guergour	647	36° 19′ N ; 05° 04′ E	
Bougâa	840	36° 19′ 57″ N ; 05° 05′ 19″ E	

Table 2: Certified values according to (AASF) of trace elements

Elements	Wavelength (nm)	Detection Limit (mg/kg)	Certified values (mg/kg)
Cr	357,9	0,06	4,12
Cu	324,7	0,03	7,03
Ni	232	0,1	2,47
Zn	213,9	0,01	100,6

Table 3: Concentrations of trace elements (mg/kg) in *Xanthoria parietina*

Road*	Station	Cr	Cu	Ni	Zn
RN 5	Aïn-Arnat	26.4	13.6	53.57	121.69
	Setif	109.82	2.35	22.46	124.75
	Ouled Sabor	98.2	3.7	35.85	77.03
	El Eulma	105.59	2.44	59.75	38.98
RN 9	Zanadia	143.08	2.48	82.62	63.35
	El Ouricia	137.8	3.05	63.46	104.44
	Amoucha	85.53	1.25	28.02	59.7
	Tizi N'Bechar	107.71	2.34	19.37	62.91
RN 74	Bougaa	29.57	3.32	47.18	184.7
	Guergour	106.12	2.22	47.39	74.97
	Beni-Chebana	36.96	1.46	30.7	83.77
	Beni-Ouartilene	71.8	1.58	35.85	99.5
RN 28	Mezloug	57.02	0.6	48.83	73.33
	Guellal	93.95	1.93	37.5	72.09
	Aïn-Oulmene	111.93	2.8	6.18	168.97
	Salah-Bey	82.36	1.22	37.5	57.08
	Average	87.74	2.90	41.01	91.70
	SD	35.40	2.97	18.73	40.62
	Min.	26.40	0.60	6.18	38.98
	Max.	143.08	13.60	82.62	184.70
	Certified standard**	4.12	7.03	2.47	100.6

Table 4: Average concentrations of heavy metals

Road	Elements	Average	Median	Sum.	Min.	Max.	SD	Variance
RN 5	Cr	85.00	101.90	340.01	26.40	109.82	39.36	1549.39
	Cu	5.52	3.07	22.09	2.35	13.60	5.42	29.38
	Ni	42.91	44.71	171.63	22.46	59.75	16.98	288.42
	Zn	90.61	99.36	362.45	38.98	124.75	40.75	1660.53
RN 9	Cr	118.53	122.76	474.12	85.53	143.08	26.96	726.70
	Cu	2.28	2.41	9.12	1.25	3.05	0.75	0.57
	Ni	48.37	45.74	193.47	19.37	82.62	29.75	885.30
	Zn	72.60	63.13	290.4	59.70	104.44	21.29	453.22
RN 28	Cr	86.32	88.16	345.26	57.02	111.93	23.01	529.42
	Cu	1.64	1.58	6.55	0.60	2.80	0.95	0.90
	Ni	32.50	37.50	130.01	6.18	48.83	18.34	336.47
	Zn	92.87	72.71	371.47	57.08	168.97	51.27	2628.58
RN 74	Cr	61.11	54.38	244.45	29.57	106.12	35.20	1239.39
	Cu	2.15	1.90	8.58	1.46	3.32	0.85	0.72
	Ni	40.28	41.52	161.12	30.70	47.39	8.36	69.85
	Zn	110.74	91.64	442.94	74.97	184.70	50.34	2534.43

Table 5: Variance analysis of heavy metal

		SS	MS	F	p
Cr	highway	29049.7	7262.4	8.9772	658.10^{-6}
	Error	12134.7	809.0		
	Total	41184.4			
Cu	highway	92.0	23.0	3.6428	28897.10^{-6}
	Error	94.7	6.3		
	Total	186.7			
Ni	highway	5280.4	1320.1	4.1774	18053.10^{-6}
	Error	4740.1	316.0		
	Total	10020.6			
Zn	highway	3163.5	790.9	0.5434	706445.10^{-6}
	Error	21830.3	1455.4		
	Total	24993.9			

Table 6: the Difference between the elements traces and roads (LSD – Fischer)

	Cr			Cu			Ni		Zn
highway	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**A**
1	****	****			****	****	****		****
2		****		****	****		****		****
3	****	****		****			****		****
4	****			****	****		****		****
5			****			****		****	****

Table 7: Classification of areas according to scoring method

Area	Average				Score
	Cr	Cu	Ni	Zn	
RN 5	AB 85.00	BC 5.52	A 42.91	A 90.61	1 + 1 + 1 + 1 = 04
RN 9	B 118.53	AB 2.28	A 48.37	A 72.60	1 + 0 + 1 + 1 = 03
RN 28	AB 86.32	A 2.46	A 32.50	A 92.87	1 + 0 + 1 + 1 = 03
RN 74	A 61.11	AB 2.15	A 40.28	A 110.74	1+ 0 + 1 + 1 = 03
Standard values	C 4.12	C 7.03	B 2.47	A 100.45	
Max	AB 85.00	C 7.02	A 48.37	A 110.74	

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