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Ability of metal trace elements accumulation by Lichens, *Xanthoria parietina* and *Ramalina farinacea*, in Megres area (Setif, Algeria)

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Abstract: *The accumulating ability of the atmospheric Metal Trace Elements (MTE) of two lichenic species thalli; Xanthoria parietina and Ramalina farinacea were evaluated in the region of Megres. The recorded concentrations of MTE (Fe, Cu, Mn, Cd, and Pb) were determined by atomic absorption spectrophotometry (AASF). The ability to accumulate MTE in X. parietina thalli is considerably greater than that of the fruticulous lichen R. farinacea in all stations studied. The general pattern of the elements accumulated in the thalli of the two species in decreasing order of their concentrations was Fe> Mn> Pb> Cu> Cd. The Fe values are very high in X. parietina thalli with an average of 35237.5 ± 3394.2 mg/kg* dry wt. *In contrast, the Pb concentrations are high, especially in the southern station of the Megres region. The results showed that X. parietina is a hyper-accumulating species of MTE, compared to R. farinacea. This work highlights the ecological importance of this species as a stable and resistant pioneer in this fragile region*.

Keywords: Air pollution; Biomonitoring; *Ramalina farinacea*; *Xanthoria parietina*; Megres; Algeria

Introduction

Air pollution is a complex mixture of several toxic compounds to the environment and human health [1-3], in especially heavy metals mainly of anthropogenic origin [4-5]. Environmental monitoring is an important part of the risk assessment process; however, air quality assessment methods remain a challenge, since environmental degradation is influenced by several ecological factors [6].

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Bio monitoring of atmospheric contaminants by the use of lichens is an important approach for its use simplicity as bio-accumulators [7]. The use of lichens in the bio indication, because of their physiology and morphology, gives excellent results for bioaccumulation [8-11]. Currently, several species of lichens have been appreciated as air bio monitors [12-17].

Lichens are among the most effective bio monitors in the assessment of toxic pollutants such as heavy metals; they absorb most atmospheric substances [18]. They are able to accumulate minerals well above their needs [19-22]. These organisms are highly resistant to the adverse effects of air pollution [23].

Xanthoria parietina is a tolerant species colonizing highly polluted sites, as well as urban and industrial areas [24], while *Ramalina farinacea* is a forest species colonizing natural sites [25]. These two species are used as heavy metal indicators frequently, while occupying different sites [26-28].

The aim of this study is to compare the accumulation capacity and to quantify the air pollution by MTE in the region of Megres (Setif, Algeria), using two different lichenic thalli, *X. parietina* with foliaceous thallus and *R. farinacea* with fruticulous thallus.

Materials and methods

Sample collection

The lichens *X. parietina* and *R. farinacea* (Figure 1), widespread in the study area and widely used in similar studies, have been selected as bio accumulators. Samples were taken from four sites in Megres Mountains, in the Setif region. The stations are distributed according to the four orientations of the mountain (North, South, East and West) (Figure 2).

Figure 1. Lichens used in the study

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Samples were collected avoiding the usage of tools or containers, which may contaminate them. Each sampled site consists of an area of a maximum 100 m, located near traffic roads and secondary roads. The Megres region is part of a series of mountain ranges. It is located between 36° 33' North and 5° 35' East, with a peak of 1737 m above sea level. The study area covers 1500 hectares. This region is characterized by a semi-arid continental climate with hot summers and cold winters. Precipitation is in the form of rain, and snow in winter. The average annual temperature is 10°C.

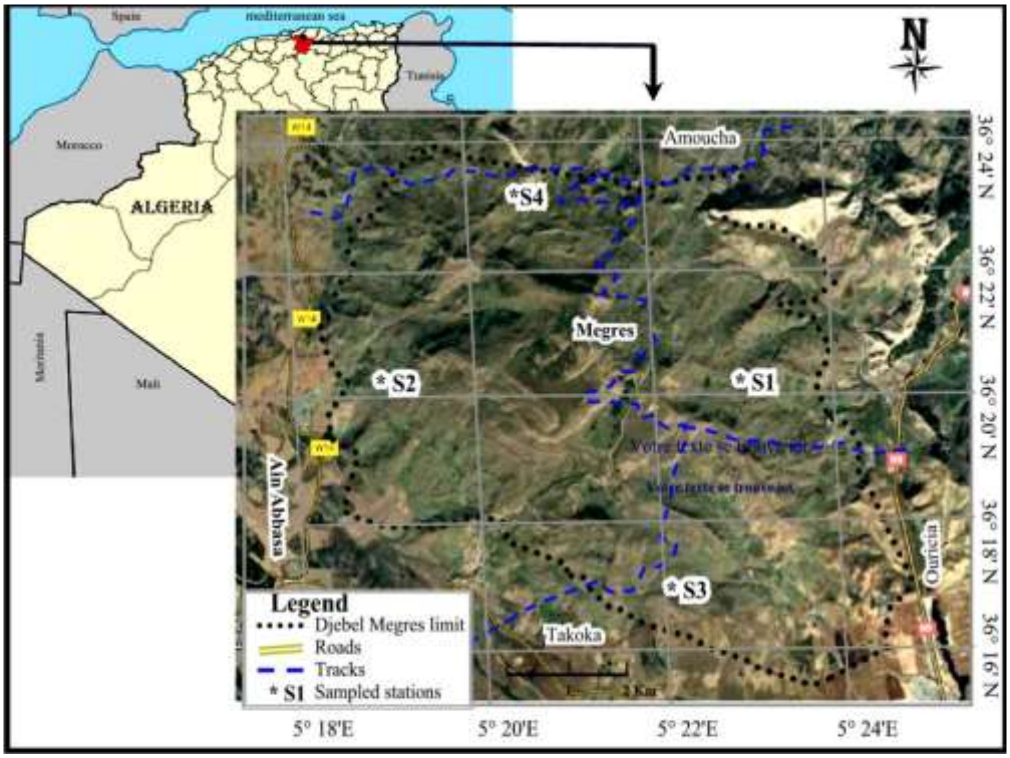

Figure 2. Location of the study area (Megres region)

Samples treatment

Lichen samples were digested in the laboratory in aseptic conditions, using a mixture of $HNO_3/HF/H_2O$ in Teflon containers [29-30]. A solution of our samples is prepared using the grinded solid material to which 10 ml of 40% hydrofluoric acid (HF) and 3 ml of 70% perchloric acid ($HClO_4$) were added. Evaporation takes place on a hotplate at 160°C. After a quasi-total evaporation, 1 ml of 65% nitric acid (HNO_3) and 10 ml of distilled water were added. The samples are then left for 30 min at 4°C in the refrigerator. The dissolution of the residue is performed by placing the samples on a hotplate at 60°C for 1h. The resulting mixture is transferred to a 100 ml flask for filtration, adjusting the volume with distilled water.

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Analytical methods for MTE concentration measurements in lichens

The concentrations of the following elements: Fe, Cd, Cu, Mn, and Pb were determined by Atomic Absorption Spectro-photometry with Flame (AASF). There are no established standards of trace elements concentration in lichens. To interpret the results of each element studied we used as a standard reference, values of the European Commission BCR information CRM-482 [16, 31] (Table 1).

Table 1. Certified CRM-482 values of trace elements (mg/kg) using AASF

MTE	Symbols	Certified values	Uncertainty
Cadmium	Cd	0.56	0.02
Copper	Cu	7.03	19
Iron	Fe	804	160
Lead	Pb	40.90	1.40
Manganese	Mn	33	0.50

Statistical analysis

Data were first subjected to Principal Components Analysis (PCA) to examine the relationship among the trace elements and the bioaccumulation by lichens, and the relation between the presence of these elements and the pollution. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan Distance Matrix to look for hierarchical associations among the elements and the locations. Statistical analyses were carried out using Statistica 10 software.

Results

The determination of trace metals, in the thalli of *X. parietina* and *R. farinacea*, by atomic absorption spectrophotometry (AAS), shows that the accumulation of MTE, Fe, Cu, Mn, Cd, and Pb, in the two species is very different (Table 2).

The results reveal the presence of a significant variation in metal concentrations between the stations studied and between the two species *X. parietina* and *R. farinacea*. The Iron accumulation in *X. parietina* thalli is very high in all the stations studied, with an average of 35237.5 ± 3394.2 mg/kg dry wt, far exceeding the certified standard. The results show that this lichenic species is hyper-accumulator of iron. The rate of Iron accumulation is lower in the thalli of *R. farinacea*, but exceeds the certified standard (Figure 3).

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Table 2. Concentrations of Metal Trace Elements in lichenic thalli (mg/kg dry wt)

Lichens	Station	Pb	Cu	Fe	Mn	Cd
X. parietina	*Est*	103	62	36000	400	12
	Ouest	125	52	34650	400	11
	Sud	200	61	31050	350	12
	Nord	106	66	39250	350	12
	Average	133.5	60.25	35237.5	375	11.75
	SD	45.39	5.91	3394.20	28.87	0.50
	RSD	34.00	9.81	9.63	7.70	4.26
R. farinacea	*Est*	41	18	193	120	2
	Ouest	56	13	210	131	4
	Sud	33	10	245	157	2
	Nord	41	12	225	147	3
	Average	42.75	13.25	218.25	138.75	2.75
	SD	9.60	3.40	22.11	16.46	0.96
	RSD	22.47	25.69	10.13	11.86	34.82
Certified values		**40.9**	**7.03**	**804**	**33**	**0.56**

In addition, a strong accumulation of iron should be noted in all the stations studied, in particular in the North for *X. parietina* and in the South for *R. farinacea*; these two stations are located near a Djebel Anini iron mine and the various quarries, in particular that of Adjel.

Concerning the other four MTE, their concentrations in the thalli of the two species follow the same path. The concentration is higher in the thalli of *Xanthoria parietina* than in *Ramalina farinacea* thalli (Figure 4).

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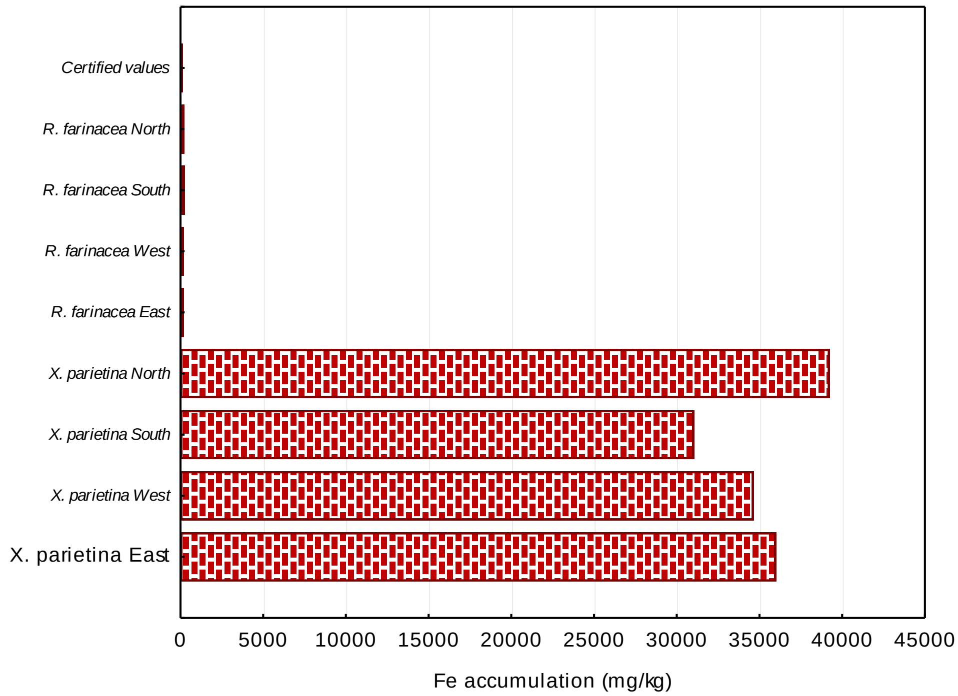

Figure 3. Iron accumulation in the *X. parietina* and *R. farinacea* thalli

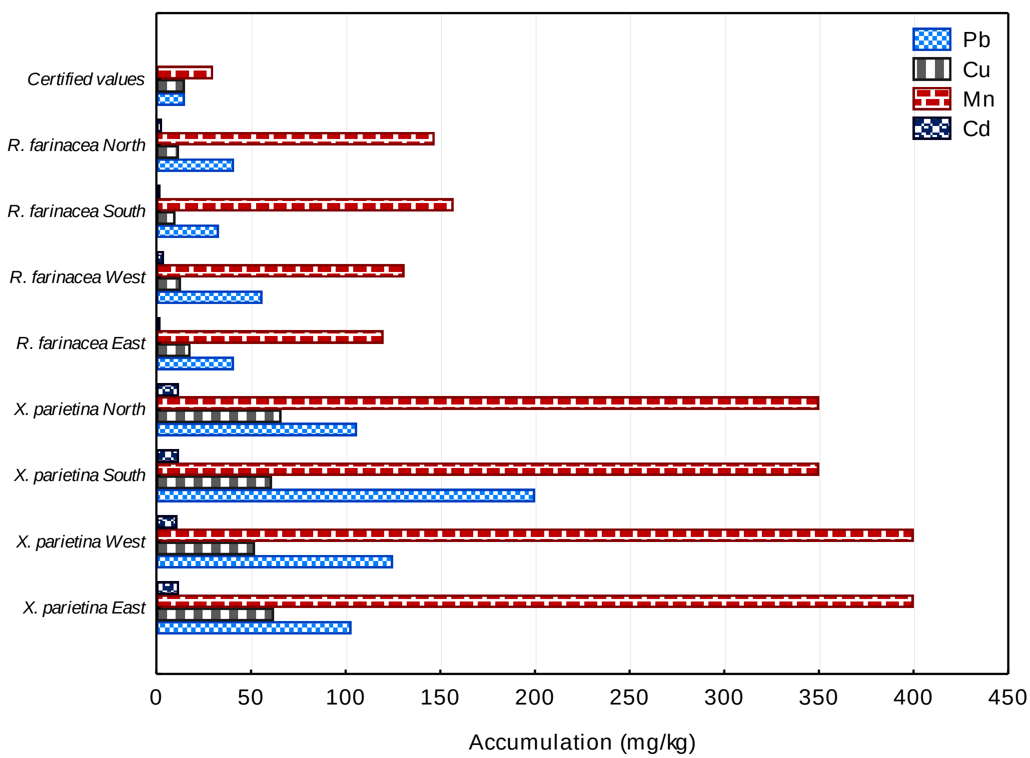

Figure 4. MTE accumulation in *X. parietina* and *R. farinacea* thalli

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The level of lead accumulated in the *X. parietina* thalli, presents values exceeding the certified standard, with an average of 133.5 ± 45.39 mg/kg dry wt. The thalli sampled from the southern station of Megres has the highest rate of accumulated lead (200 mg/kg dry wt), while the eastern station has the lowest rate. On the other hand, the accumulation of lead in the thalli of *R. farinacea* is low, with an average of 42.75 ± 9.6 mg/kg dry wt, although the lead content exceeds the certified standard.

The determination of copper in *X. parietina* thalli shows a strong accumulation with an average rate of 64.25 ± 5.91 mg/kg dry wt, exceeding the certified limits. The accumulation in the thalli of *R. farinacea* is low. The high Cu rate in the North and East in the thalli of *X. parietina* is probably due to the proximity of these stations to agricultural areas. The accumulated manganese in thalli of *X. parietina*, in all study sites, shows values exceeding the certified standard; on the other hand, its accumulation in the thalli of *R. farinacea* is lower. Levels of Cadmium in thalli of *X. parietina* are very close and higher than the certified standard, while the concentrations in the thalli of *R. farinacea* are low, but exceeding the certified standard in all stations.

All elements quantified in two lichen species far exceed the standard values. The estimation of the MTE concentrations in the different samples, allowed us to establish the order of the concentrations in the species: Fe > Mn > Pb > Cu > Cd. This MTE order is confirmed by the UPGMA (Figure 5). This analysis separates the trace elements into two very distinct groups. The first is represented by Fe, with very high levels accumulated in the thalli. The second group brings together the elements with high concentrations in the thalli.

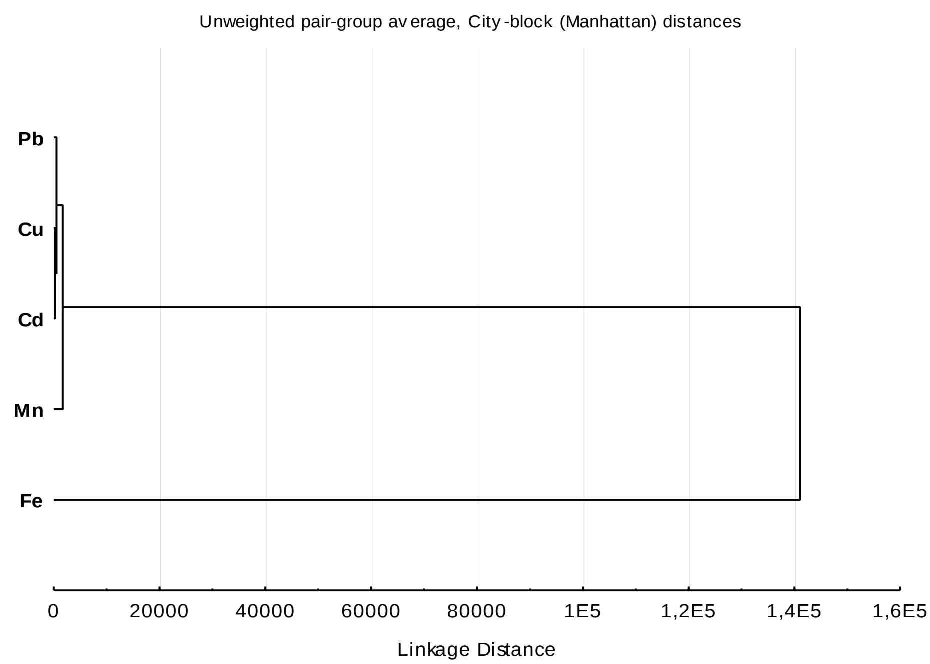

Figure 5. Cluster dendrogram of the elements in *X. parietina* and *R. farinacea*

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The rate of accumulation in MTE frond in different sampling sites, show a very high variability (Figure 6). The variation of the Fe rate, in *X. parietina* thalli is to be noticed, especially in the southern station. This variability is followed by the concentrations of the Mn element and Pb. The elements Cu and Cd show very little variability.

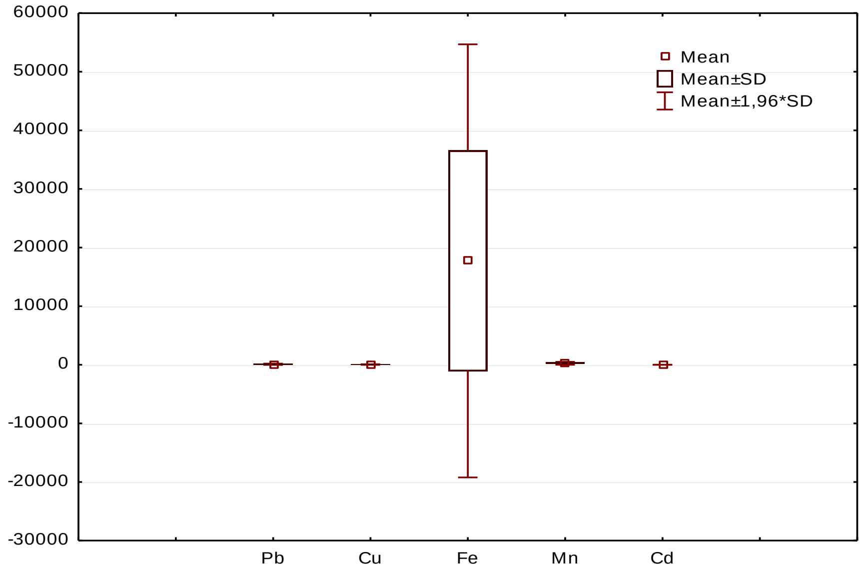

Figure 6. Variability of MTE concentrations in *X. parietina* and *R. farinacea* thalli

The results of trace element concentrations were subjected to a principal component analysis (PCA). The three-dimensional spatial projection of the stations, based on the first three axes from the PCA has been applied to find significant differences between the lichen species studied (Figure 7). This analysis did not allow a good characterization of the stations into homogeneous groups, because the separation is not clear. However, the two lichen species stand out and form two well-separated clusters.

The species *X. parietina* is characterized by high level of metal trace elements, thus indicating that this species is a super accumulator of MTE. On the other hand, *R. farinacea* accumulates very little MTE, although the accumulated rates exceed the standard values.

A second statistical analysis, based on the Unweighted Pair Group Method with Average (UPGMA) (Figure 8), shows the clear separation of the two species studied, based on the accumulation of MTE. Considered bio-accumulating, *X. parietina* isolates itself with high rates of MTE, whereas *R. farinacea* which is less accumulating than the first species forms the second group. In general, *R. farinacea* thalli accumulate the MTE in the same way, in the four sampled sites, whereas *X. parietina* shows differences in the accumulation to the different stations.

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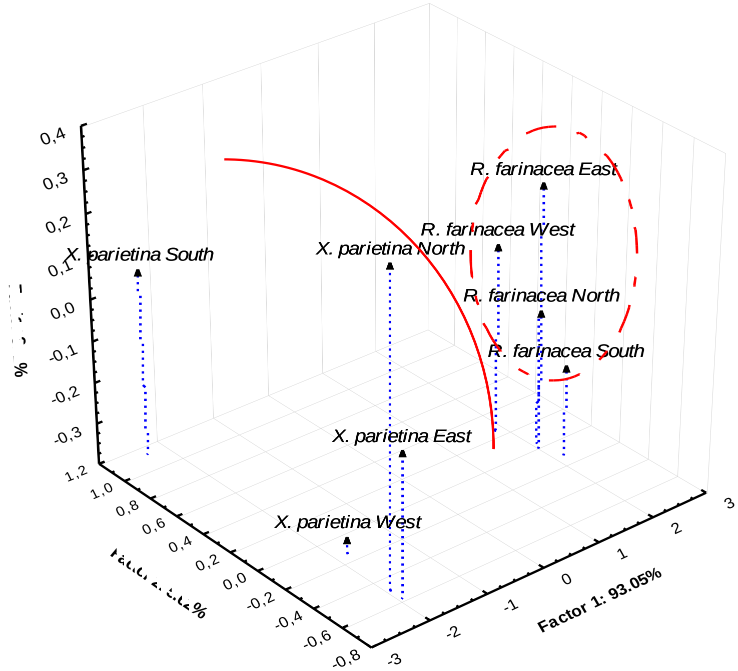

Figure 7. Spatial projection of stations based on the first three axes from the PCA

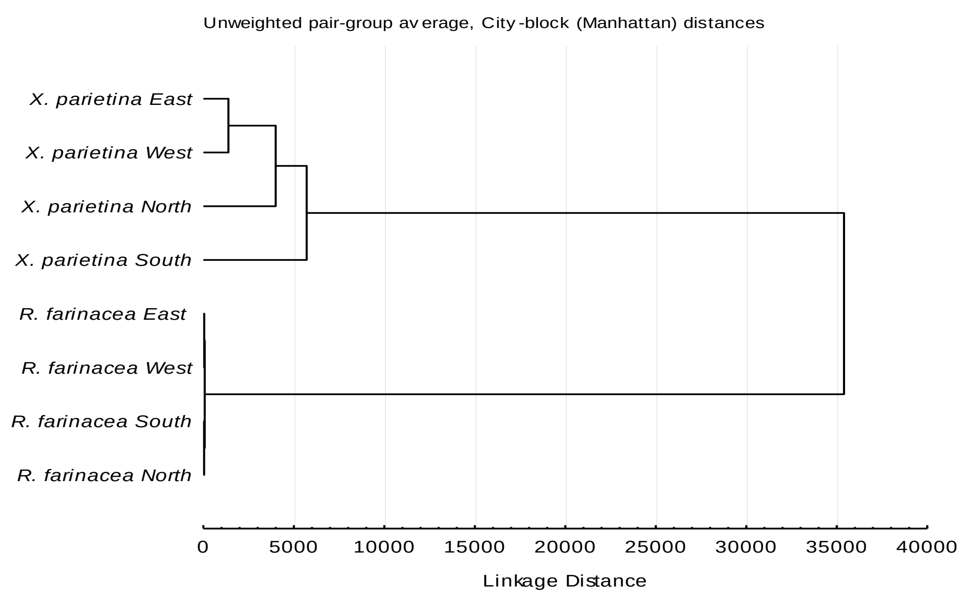

Figure 8. UPGMA based on the concentration of heavy metals

The southern site of *X. parietina* shows the presence of high rates of MTE, and separates from the rest of the stations. It is the most polluted station, followed by the North, East and West stations. *X. parietina* sites can be classified as follows: South> North> East> west.

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In order to determine the different levels of pollution in the study region (Megres mountains), a distribution of the sampled sites was carried out based on the concentrations of MTE in the thalli of the two species *X. parietina* and *R. farinacea* (Figure 9). A clear separation of the two species is noticed. *X. parietina* shows high concentrations of MTE, with a high rate in the southern part of Megres, while *R. farinacea*, with lower concentrations, the four stations are grouped in the low concentration of MTE.

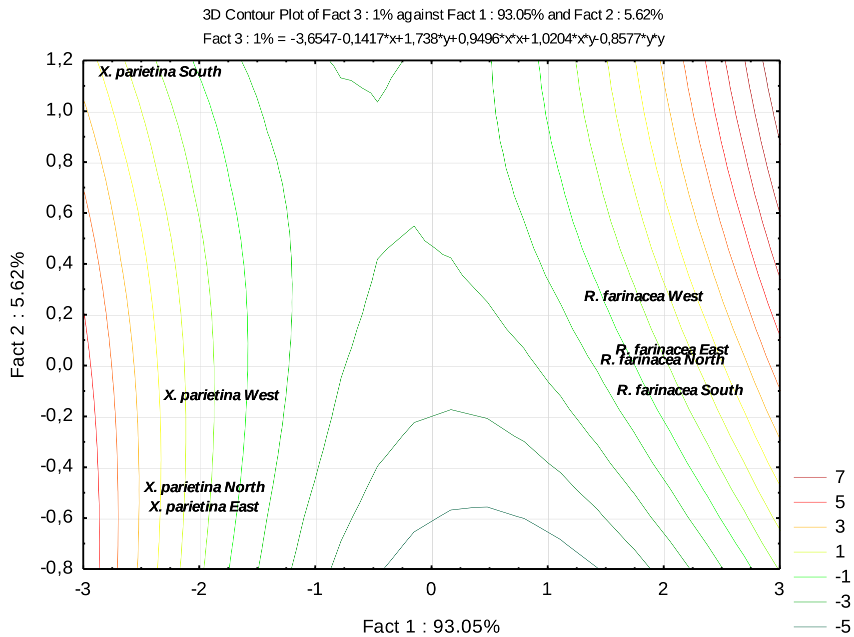

Figure 9. Distribution of sites sampled, based on the accumulation of MTE in Megres region

The highest values of accumulation are those of Fe in *X. parietina* thalli (35237.5 ± 3394.2 mg/kg dry wt) and of Mn (375 ± 28.87 mg/kg dry wt). The highest Fe level is noted in the North station (39.250 mg/kg dry wt); this station is close to an iron mine, while the Mn rate was recorded in both East and West stations (400 mg/kg dry wt). In contrast, *R. farinacea* thalli accumulate fewer MTE, although the rates exceed certified standard. The lowest levels of Cd are recorded in the thalli of *R. farinacea* with an average of 2.75 ± 0.96 mg/kg dry wt.

Discussion

The concentrations of the MTE in the thalli of lichens examined showed quantitative differences between the two species studied, *X. parietina* and *R. farinacea*. Lichen foliose *X. parietina* accumulates greater amounts of MTE against the lichen fruticose *R. farinacea*. This difference in accumulation is probably due to the ecology and type of phorophyte [8, 32]; *R. farinacea* is a saxicolous species, while *X. parietina* is a cortical species.

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A study in Turkey has shown that foliaceous lichens accumulate more metal than fruticulous lichens [33]. The same observations have been reported for the species *X. parietina* and *Evernia prunastri* [34, 35]; generally, when comparing two morphologically similar species, the differences are minimal [8, 36]. The study by Bergamaschi et al. [37] in the north of Italy, on four lichen species showed that the concentrations of accumulation of MTE were all significantly different. The morphology of the lichen is a key factor in the accumulation of MTE [38].

The southern station of Megres reveals a high rate of MTE; it is the most polluted station by contribution to the sites studied. The transport of airborne particles from urban and industrial areas of the city of Setif, 20 km away, contributes significantly to this pollution. In addition, this station is very close to high traffic roads, and to high industrial emissions [5, 15, 22, 24, 39-41].

High lead concentrations, exceeding standard values, are accumulated in *X. parietina* thalli. The analyzed samples are collected near the roads of the region, in particular in the southern part, at the intersection of the four road axes (National road 9, National road 9B, National road 75 and departmental road). The high rates of this element have been positively correlated with road traffic. The exhaust emissions of vehicles have an important role [42-46]. A study carried out in China indicating a close relationship between the content of heavy metals, in particular Pb and the volume of traffic on the roads [47].

The determination of copper concentrations shows a strong accumulation in the North and the East in the thalli of *X. parietina*. This accumulation is probably due to the intensive agricultural operations that surround the study region, especially in the north, which affects air quality using pesticides and the chemical spraying [22, 48-50].

The high levels of Cu and Mn accumulation north of Megres are linked not only to agricultural activities, but also to emissions from the large open-air landfill in the commune of Amoucha. This type of discharge generates toxic heavy metals associated with airborne particles [51-55]. This study is consistent with the results from other work, showing the positive relationship between the contamination of air with strong metals (Cu and Mn) and their bioaccumulation in lichens located near solid waste landfills [56-57].

Extremely high values of Fe were measured in all stations studied, particularly in North in the *X. parietina* thalli and in the south in *R. farinacea* thalli. These high rates are probably due to the proximity of these two stations to the Djebel Anini iron extraction mine and the various quarries, in particular that of Adjel which contribute significantly to air pollution [58-59].

The same observations were carried out in the Taihang region of China, higher iron concentrations were observed near stations with mining activities [39]; this high accumulation of Iron in the thalli can also be of biogenic origin, which confirms the capacity of hyper-accumulator of heavy metals by *X. parietina* [60-62].

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Conclusion

The dosing of metal trace elements, by atomic absorption spectrophotometry (AAS) in the *X. parietina* and *R. farinacea* thalli shows an accumulation of metals. The two lichenic species studied are bio-accumulators of MTE, which are excellent bio-indicators for the estimation of the metal air pollution; however, *X. parietina* has a higher accumulation than *R. farinacea*. The results show that *X. parietina* is a hyper-accumulator of MTE, whereas the *R. farinacea* species is weakly accumulator. Therefore, the species *X. parietina* shows some tolerance for elements studied (Pb, Cu, Cd, Fe, and Mn).

Analysis of MTE concentrations in lichen thalli of the Megres region shows that the two species have the same accumulation pattern, whose elements can be classified in descending order according to the following order Fe> Mn> Pb > Cu> Cd. The results clearly underline that the Megres region, a natural and pristine ecosystem, is under the direct impact of metallic contaminants of local origin (iron mine, quarries and road traffic) and the intensive agricultural operations that surround the study region.

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