



Biomonitoring of heavy metals by lichen *Xanthoria parietina* in Bejaia area (East Algeria)

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Abstract

The metallic air pollution in the Bejaia region (northern Algeria) were examined, using the lichen *Xanthoria parietina* as a bio-monitoring organism. The biological material from 42 stations were sampled and analyzed by flame atomic absorption spectrophotometry (AASF). In order to determine the relationship between the potential sources of contaminants in the study area and the degree of deposition of metals in air, five heavy metals (Cd, Cu, Pb, Mn, and Ag) were analyzed. The results show that the metal contents recorded in the various sampled stations are fluctuating and lead to the following decreasing series: Pb > Mn > Cu > Ag > Cd. The air quality in the town of Amizour is considerably poor compared to the rest of the studied stations. The Pb and Mn levels are very high in all stations with an average of $(134.62 \pm 148.53 \text{ mg/kg})$ and $(290.88 \pm 175.13 \text{ mg/kg})$ respectively. The highest concentrations of heavy metals were observed around the municipalities of Bejaia, Akbou and Tichy. The current situation suggests an obvious need to mitigate atmospheric pollution by MTE and to control the emissions of toxic metals, in particular Pb from industrial sources and road traffic in large cities.

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Introduction

The environmental contamination by toxic chemicals from human activities is a serious public health problem worldwide (Gu, 2018; Kittner *et al.*, 2018; Weinmayr *et al.*, 2018; Kresovich *et al.*, 2019). These chemical compounds are widely distributed in the environment, mainly due to emissions from industrial activities, mining, domestic, and agricultural activities, but above all by intensive automobile traffic (Lodenus *et al.*, 2010; Pandey *et al.*, 2014; Gonzalez-Castanedo *et al.*, 2014; Parzych *et al.*, 2016; Chen *et al.*, 2016). Therefore, the use of bio indicators in diagnostic studies of these dangerous elements in the long term is very advantageous, since the cost is low and can be used on a large scale with high efficiency (Stamenkovic *et al.*, 2013; Antonucci *et al.*, 2016; Belguidoum *et al.*, 2020).

Lichens have a high capacity for absorption and accumulation of atmospheric pollutants, in particular heavy metals, because of their biological characteristics, which allow the absorption of many contaminants directly from the atmosphere by the surface of the thallus (Conti *et al.*, 2011; Augusto *et al.*, 2015; Loppi and Paoli, 2015; Bargagli, 2016; Will-Wolf *et al.*, 2017). These characteristics of lichens, combined with their extraordinary ability to grow in large geographic areas, allow them to be classified as a reliable biomonitor and ideal for assessing air pollution (Garty, 2001; Antonucci *et al.*, 2016).

Frequently, the lichens are used in bio-monitoring studies of metallic air pollutants (Bermejo-Orduna *et al.*, 2014, Nascimbene *et al.*, 2014, Agnan *et al.*, 2015). In many studies, lichens are of great help in determining of heavy metals concentrations released by traffic and accumulated by thalli (Douibi *et al.*, 2015; Gauslaa *et al.*, 2016; Adjiri *et al.*, 2018; Demkova *et al.*, 2017, 2019).

The *Xanthoria parietina* species has a large contact surface with atmospheric pollutants (surface / volume). It is very resistant to air pollution, so it is able to bioaccumulate large amounts of heavy metals in polluted areas (Cuny *et al.*, 2004; Demiray *et al.*,

2012; Adjiri *et al.*, 2018; Parviainen *et al.*, 2019; Vitali *et al.*, 2019).

Generally, atmospheric metal contamination attracts greater attention in urban areas than in rural areas that are less affected by human activities (Wan *et al.*, 2016). The wilaya of Bejaia is located north of the country on the Mediterranean coast. It is an Algerian province served by an important road network and has an airport and a port (port of international trade and hydrocarbons), which occupies an important place in the country by its volume of activity (Belkhiri and Djemili, 2016).

The objective of this study is to assess the amount of heavy metals accumulated in the thalli of the lichen *Xanthoria parietina*, in order to estimate the air quality in the various municipalities of the province of Bejaia.

Materials and methods

Study area

The study area covers an area of 3268 km². It is distinguished by a mountainous relief with a steep gradient, at relatively high altitudes and very marked slopes (80% of the territory have a slope greater than 25%). The wilaya of Bejaia is a coastal and mountainous province; it covers an area of 3268 km². The region's climate is Mediterranean, with mild and rainy winters in the coastal strip but cold and snowy on the mountains. Average annual precipitation can expect 1100 mm. The precipitation regime there is generally favorable for the development of vegetation. The average annual temperatures range from 16.7°C to 18.6°C.

Sample Collection

The foliaceous lichen *Xanthoria parietina*, widespread in the studied areas and widely used in similar studies. Samples were collected in 42 locations spread throughout Bejaia province (North of Algeria) (Fig. 1).

Each sampled site consists of a maximum area of 100 m², located near roads, as much as possible, with

heavy traffic and secondary roads. The samples were collected avoiding the use of tools or containers likely to contaminate them. After collection, the samples were transported to the laboratory for analysis. The

lichen samples were dehydrated at 40°C for 48h, cleaned to remove all types of traces of foreign matter (dust, leaves, soil, wood chips) and then ground with an agate mortar.

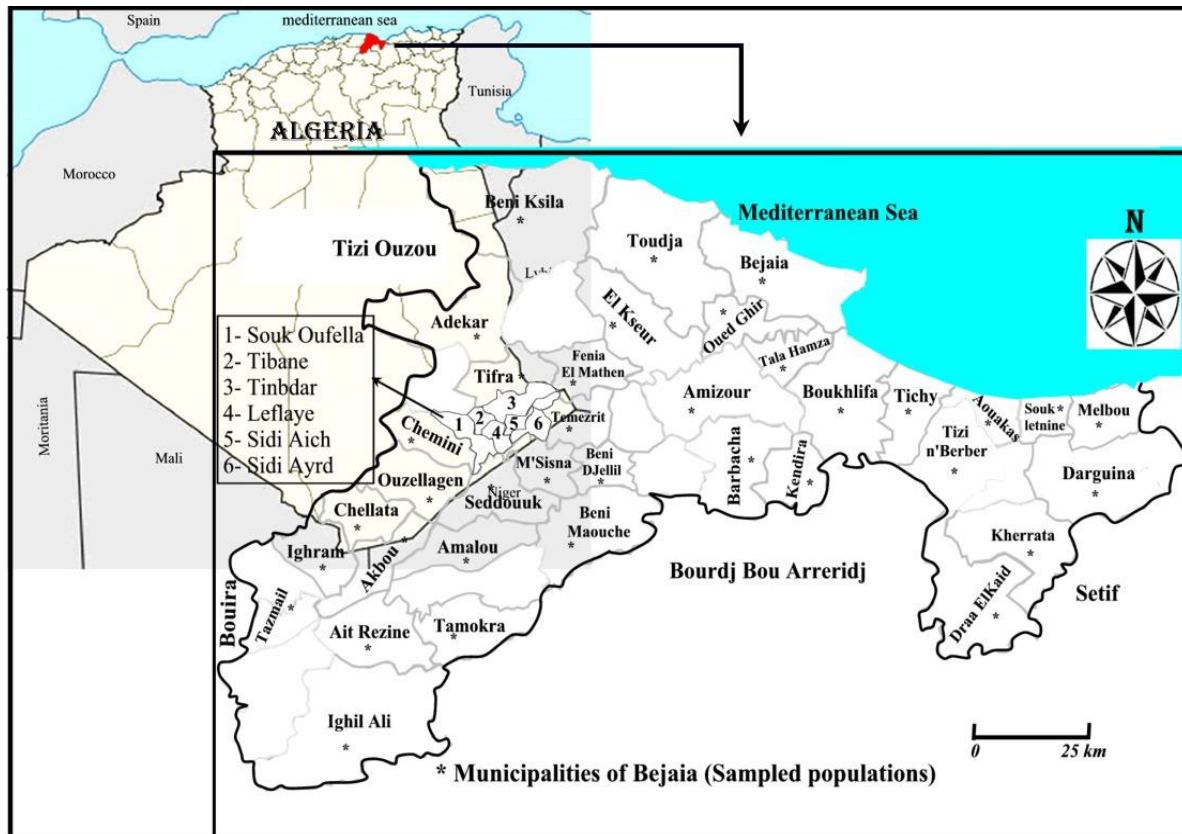


Fig. 1. Sampled populations of *Xanthoria parietina*.

Samples treatment

Lichen samples were digested in the laboratory in aseptic conditions, using a mixture of $\text{HNO}_3/\text{HF}/\text{H}_2\text{O}$ in Teflon containers (Hébrard-Labit and Meffray 2004; Adjiri *et al.* 2018).

A solution of our samples is prepared using the ground solid materials 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.

Analytical methods for MTE concentration measurements in lichens

The concentrations of the following elements Pb, Mn, Ag, Cu and Cd, were determined by Atomic Absorption Spectro-photometry with Flame (AASF). There are no established standards of trace elements concentration in lichens (Hébrard-Labit and Meffray, 2004). To interpret the results of each element studied we used as a standard reference, the values of the European Commission BCR information CRM-482 (Table 1).

Statistical analysis

Data were 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 analyzes were performed using the software STATISTICA 10.

Results

The concentrations of the elements (Ag, Cd, Cu, Mn and Pb) accumulated in the thalli of *Xanthoria parietina* from 42 municipalities in the Provence of Bejaia were analyzed using flame atomic absorption spectrophotometry (SAAF) (Table 2). The

concentrations of MTE accumulated by *X. parietina* are variable and exceed standard values.

The accumulation of Pb in the thalli of *X. parietina* is very high in all stations studied, with an average of 291 ± 175 mg/kg, far exceeding the certified standard, in particular in the town of Akbou. This high rate in this commune is probably due to the proximity of a large vehicle market and to the dust emissions emitted by four aggregate quarries, south of the city of Akbou, which persistently contribute to the atmospheric pollution.

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

MTE	Symbols	Certified values	uncertainty
Lead	Pb	40.9	1.4
Manganese	Mn	33	0.5
Copper	Cu	7.03	0.19
Cadmium	Cd	0.56	0.02
Silver	Ag	2.9*	--

* Minimum value observed (Daillant, 2003).

The concentrations of Mn and Cu in *X. parietina* thalli, with an average of 252.19 ± 134.30 mg/kg for Mn and 134.62 ± 148.53 mg/kg for Cu, reveal values exceeding the certified standards. The thalli collected from the Amizour station have the highest rates with 735 mg / kg and 894 mg / kg for Mn and Cu, respectively. Whereas Tamokra and M'Sisna stations have the lowest rates of these elements.

Cadmium contents are low and very close, but still exceeding the certified standard in all stations. The highest concentration of Cd was recorded in the municipality of Bejaia (8.21 mg/kg).

This contamination is largely explained by automobile traffic and anthropogenic activities, which represent an important source of Cadmium and lead in this urban environment.

A strong accumulation of Ag should be noted in the thalli of *X. parietina* with an average of 8.03 ± 5.94 mg/kg. The highest rate is observed in the commune of Ighram (33.35 mg/kg), near sites devoted to

arboriculture, especially olive and fig trees. Statistical analysis of the results shows that the HTAs are separated into two groups. The group with a high concentration in the thalli is formed by the elements Mn and Pb. The second group gathers the elements (Cu, Ag and Cd) (Fig. 2). This analysis allowed us to classify these elements according to the following relation: $Pb > Mn > Cu > Ag > Cd$.

The element Pb has high concentrations in *X. parietina* thalli with an average of 290.88 ± 175.13 mg/kg, while the Cd is the least abundant in the thalli ($3.06 \pm 2, 22$ mg/kg). The Pb shows the greatest variation in the samples, followed by the Mn (252.19 ± 134.30 mg/kg). The levels of variation in Ag and Cd concentration are relatively small (Fig. 3).

The three-dimensional spatial projection of the stations, based on the first three axes from the PCA, shows a grouping of the point cloud with the individualization of certain populations, So five populations are isolated, Amizour, Bedjaia, Tichy, Akbou and Ighrem (Fig. 4).

Table 2. Accumulation of MTE in *X. parietina* thalli (mg/kg).

Stations	Cd	Cu	Pb	Mn	Ag	Stations	Cd	Cu	Pb	Mn	Ag
Bejaia	8.21	232	597	591	9.36	Tizi n'Berber	4.01	99	115	189	6,69
Akbou	7.76	81	752	444	6.25	Boudjelil	1.96	81	219	342	5,59
Amizour	5.32	894	576	735	3.15	Tamokra	0.50	48	98	125	6,37
Adekar	5.98	351	365	234	21.25	Tibane	0.40	54	81	138	12,2
Sedouk	5.43	69	253	258	5.86	Ighil Ali	2.75	196	611	275	15,6
Kherrata	4.15	108	419	282	7.25	Toudja	4.50	202	464	358	12,4
Timezrit	3.69	87	287	378	6.36	Thala Hamza	0.60	48	115	234	7,26
Chemini	5.35	345	345	199	24.19	Chellata	1.95	52	139	155	4,93
Ousellaguen	3.27	78	458	255	3.96	Souk Oufella	6.96	235	98	190	2,77
Aokas	2.89	66	502	228	9.78	Tifra	0.50	73	117	182	3,37
El Kseur	2.75	99	303	195	6.37	Sidi Ayob	2.31	77	169	185	5,59
Sidi Aich	6.25	126	425	270	7.56	Boukhalifa	1.68	122	143	168	6,63
Darguina	2.70	87	258	177	6.36	M'Sisna	0.21	45	164	145	5,79
Souk El Tenine	6.54	69	526	252	5.39	Malbou	1.50	110	266	209	8,99
Tazmalt	2.20	60	235	243	6.99	Ben Ksala	0.30	85	151	127	7,14
Barbacha	4.53	75	398	195	7.14	Tinebdar	2.16	105	203	170	3,58
Tichy	3.05	99	629	648	12.18	Amalou	0.50	57	168	149	5,79
Drâa El Kaid	2.50	51	293	183	3.46	Kendira	0.50	98	183	157	3,47
Oued Ghir	2.75	126	267	228	6.37	Leflaye	0.60	77	140	177	6,13
Ait Rizine	4.52	60	237	318	5.89	El Mathen	0.40	84	134	163	3,99
Ighrem	3.64	441	190	243	33.35	Beni Maouche	0.90	102	124	198	4,67
		Moyenne					3,1	134,6	291	252	8,03
		SD					2,2	148,5	175	134	5,94
		RSD					72,7	110,3	60,2	53,3	74
		Min.					0,2	45	81	125	2,8
		Max.					8,2	894	752	735	33,4
		Valeur Standard					0.6	7.1	40.9	33	2.9

These populations represent the most polluted sites in the Bejaia region. The concentrations of MTE found in the thalli of the Amizour population are high, in particular for Cu and Mn. These high rates are probably due in large part to significant agricultural activities of the region, especially tree crops (olive and fig), to industrial activities and uncontrolled local mining activities (the Amizour mine at Cu, Pb and Zn), and the excessive increase in frequent and severe road traffic in the region. In contrast, the Tamokra population thalli accumulate low MTE concentrations, with the lowest Mn concentration compared to the different stations studied (125 mg/kg).

The use of the UPGMA shows the heterogeneity of the accumulation of metals in the 42 populations of *X.*

parietina and confirms the separation of the sites studied into two very distinct groups (Fig. 5).

The first group is represented by the Amizour station with the highest accumulation of MTE in the thalli, especially for Cu and Mn. This station has a high degree of pollution. While the second group splits into two branches. The Bejaia, Tichy and Akbou stations represent the first branch, with a very high accumulation of Pb, between 597 and 752 mg/kg. The remaining sites form the second branch, which seems to be the least polluted. However, their accumulation rates are much higher than standard values.

Generally the concentrations of Pb, Mn and Cu are high in the stations studied, which shows a strong impact of the sources of air pollution, especially in the

stations which are crossed by the national roads with heavy traffic such as Amizour and Bejaia. The lowest rates of MTE concentrations were observed in the

commune of Tamokra, it is a station with low population density, without industrial activity and low road traffic.

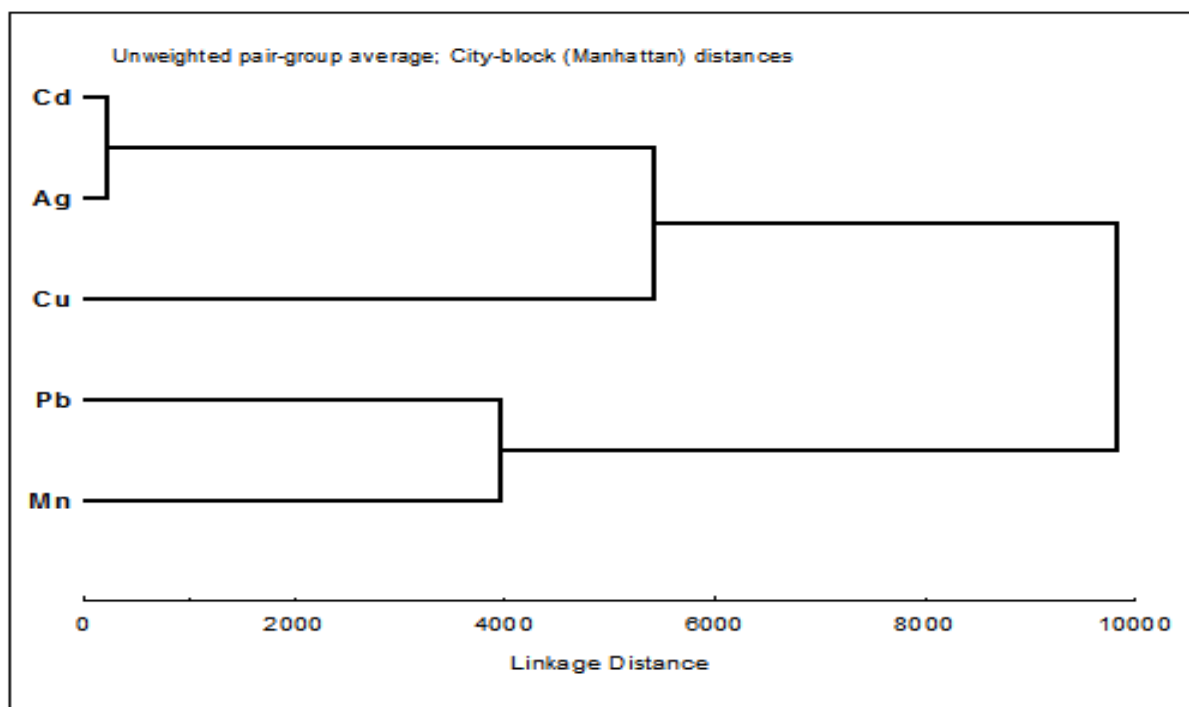


Fig. 2. Relationships among MTE accumulations in *X. parietina* of Bejaia Region.

Discussion

The concentration of MTE accumulated by *Xanthoria parietina* thalli in the wilaya of Bejaia, for the five metals, shows the following decreasing series: Pb > Mn > Cu > Ag > Cd. These results differ from those of the literature, whose Pb is always higher than Mn (Balabanova *et al.*, 2012; Nimis *et al.*, 2000).

The assessment of metal accumulation levels in the municipalities of the province of Bejaia showed extremely high values in the thalli of *X. parietina*.

The Amizour station is the most affected compared to the stations studied. En general, the high concentrations of these elements are closely linked to local uncontrolled mining activities, especially with regard to lead and copper extracted from the Amizour mine. Similar findings were made by some authors, (Boamponsem *et al.*, 2010; Li *et al.*, 2014; Mwaanga *et al.*, 2016; Liu *et al.*, 2016; Demkova *et al.*, 2019). Thus, emissions from road traffic and frequent severe in the region contribute significantly to the increase

in these items in the atmosphere (Liu *et al.*, 2016).

The high concentrations of Pb, Cu, and Mn observed can be linked to the vicinity of sampling points in industrial activity zones, including the metallurgical and chemical industries (González-Castanedo *et al.*, 2014; Pollard *et al.*, 2015), and agricultural activities (Pascaud *et al.*, 2014; Nguyen *et al.*, 2016; Ratier *et al.*, 2018).

The highest accumulation of Pb is recorded in thalli of *X. parietina* of Akbou town; this station is located near a large vehicle market; thus, this accumulation is directly or indirectly associated with emissions from diesel fueled vehicles (Liu *et al.*, 2016; Ahmad *et al.*, 2018; Kazimirova *et al.*, 2016; Coufalík *et al.*, 2019). Monaci *et al.*, (2000) in Italy made similar observations. In fact, it is confirmed that the deposit of Pb is mainly related to vehicle emissions (Tokahoglu and Kartal, 2006; Wang *et al.*, 2019). In addition, the commune of Akbou reveals high levels of Mn, Cd and Cu. These high rates are probably due to

the excessive use emissions of explosives for extracting sand, and to the dust which emanates from four quarries of aggregates, on the mount of Piton in

the south of the city of Akbou and which contribute in a way persistent to air pollution (Peng *et al.*, 2016; Paoli *et al.*, 2017).

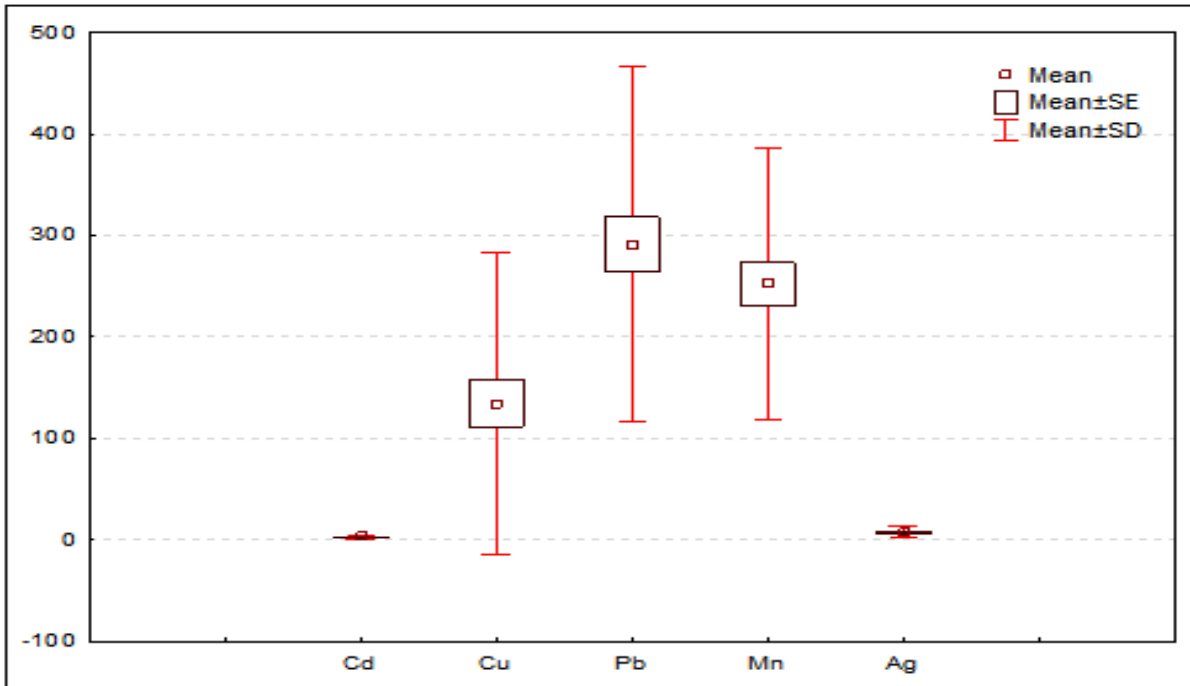


Fig. 3. Variability of MTE concentrations in thallus *X. parietina*.

Extremely high values of Cd and Pb have been observed in the municipality of Bejaia. These high rates are due to automobile traffic and anthropogenic activities, which represent an important source of

Cadmium and lead in this urban environment (Alkama *et al.*, 2009; Benaissa *et al.*, 2016; Wan *et al.*, 2016; Lin *et al.*, 2017; Ben Aissa *et al.*, 2018; Hamma and Petrișor, 2018).

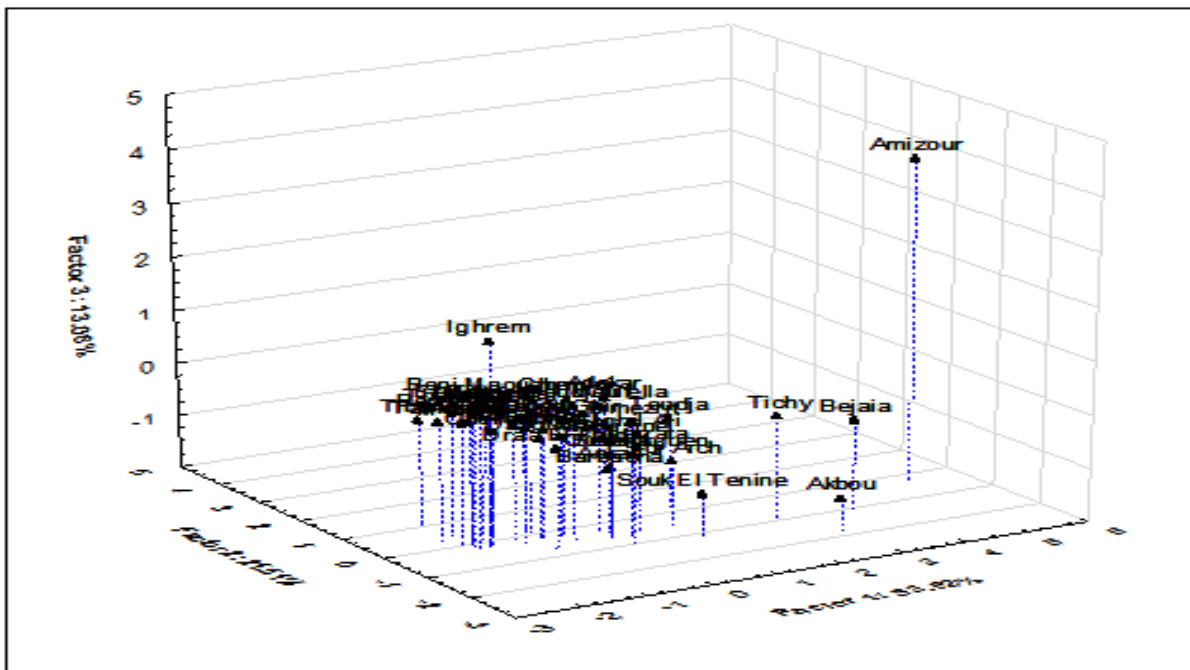


Fig. 4. Spatial projection of the populations, based on the first three axes.

The same results, high concentrations of Cd and Pb in urban areas, were found by Aghaei and Khademi (2016) in Iran, in France (Dron *et al.*, 2016), in Algeria (Adjiri *et al.*, 2018), in Spain (Parviainen *et al.*, 2019) and in Bosnia and Herzegovina (Ramić *et*

al., 2019). The sea is also considered as a persistent source of MTE emission in the municipality of Bejaia, by the processes of the exchange of these elements by the biogeochemical cycles between the sea and the atmosphere (Bagnato *et al.*, 2013; Zhang *et al.*, 2019).

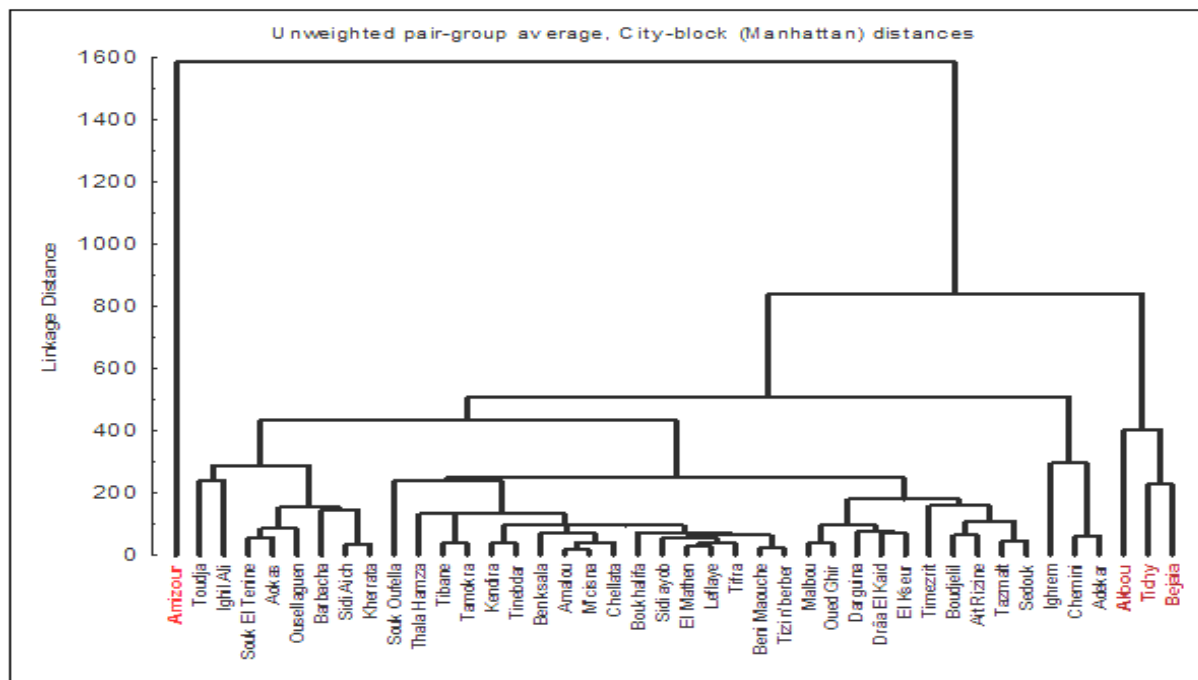


Fig. 5. UPGMA based on the concentration of heavy metals in *X. parietina* thalli.

Repeated ballasting or degassing, which are carried out in violation of regulations near the coast, can also be important sources of Cd, Pb, Mn and Cu in the air (Cram *et al.*, 2006; Piazzolla *et al.*, 2015; Ahmed *et al.*, 2017). A small accumulation of Ag in the sites studied should be noted, with values between (2.77 and 33.35 mg / kg). However, the concentration of Ag in the commune of Ighram was high, due to the intensive use of pesticides, herbicides and fertilizers in arboriculture (Pelletier and Campbell, 2004; Onakpa *et al.*, 2018), and from extraction and processing of mining products (Austruy, 2012), and especially the use of the photographic industry and film development (Daillant, 2003).

Conclusion

The concentrations of five elements (Pb, Cd, Cu, Mn and Ag) were controlled using a lichenic organism *Xanthoria parietina*, collected in 42 municipalities of Bejaia region. MTE analysis revealed significant air pollution in most of the municipalities studied.

The results indicate a good agreement between the concentrations of metals in lichens and the anthropic impacts, which are notably represented by mining, agricultural activities, chemical industries, and the combustion processes linked to vehicle emissions. The results showed that the highest contaminant levels were recorded for Pb, Mn and Cu, and particularly affect the municipalities of Bejaia, Tichy and Akbou. As well as the environmental change in the municipality of Amizour, the closest to the Pb mine.

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