Comparative Bioaccumulation of Heavy Metals in Two Bioindicator Lichen Species: Implications For Air Quality Biomonitoring

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ABSTRACT

Atmospheric deposition of pollutants, such as heavy metals, has detrimental effects on human health and disrupts the balance of terrestrial ecosystems. Assessing metallic element pollution in the city of Djelfa can be achieved through lichen bioaccumulation, which constitutes an excellent tool for air quality biomonitoring. This study analyzes atmospheric metallic contamination in this region using two species of crustose epiphytic lichens, Xanthoria parientina (Xpa) and Physia aiplioide (Pai). The results reveal significant multi-source pollution, associated with road traffic, industrial activities, and incineration, with a marked presence of 16 heavy metals, including iron (Fe), bromine (Br), cobalt (Co), rubidium (Rb), barium (Ba), and arsenic (As). The comparative approach highlights the key role of the species in bioaccumulation: Xpa emerges as a more effective and sensitive bioindicator than Pai for monitoring metallic deposition, due to its higher accumulation capacity. The combined use of both species would thus allow for a more comprehensive and nuanced picture of air quality, distinguishing chronic contamination from acute pollution episodes.

Key words : Bioaccumulation, Biomonitoring, Heavy metals, Djelfa, Lichens, Pollution

INTRODUCTION

The presence of heavy metals in the environment represents a critical challenge for the integrity of ecosystems and human health, due to their ability to accumulate along trophic chains (Abas, 2021). Monitoring this diffuse pollution, which is often complex and costly using conventional physicochemical methods, requires the development of integrative and sensitive tools. In this context, biomonitoring, which uses living organisms as indicators of environmental stress, stands out as a powerful and complementary approach (Counouy, 2022; Agnan, 2021; Vanhaluwayn et al., 2013; Dalquier, 2022). Among the most remarkable bioindicators, lichens occupy a prominent place (Vanhaluwayn et al., 2013; Lucking, 2012; Stephenson, 2010; Counouy, 2022). Due to their particular physiology (absence of a root system and cuticle, nutrition dependent on atmospheric deposits), lichens passively accumulate elements

present in their environment in proportion to the deposits. (Gilbert, 1998 ; Giordani, 2007 ;Nash, 2008 ; Lucking, 2012 ; Agnan et al., 2017 ; Loppi, 2019; Nelsen et al., 2019; Nimis & Martellos, 2020).

This high bioaccumulation capacity, particularly with respect to heavy metals (lead, cadmium, zinc, nickel, mercury, etc.), makes them exceptional natural sensors (Kirschbaum and Wirth, 1997; Vanhaluwayn et al., 2013; Giordani, 2019; Agnan, 2021; Counouy, 2022). Lichen biomonitoring exploits this property to assess air quality, map pollutant deposition, identify emission sources, and monitor the temporal evolution of contamination on scales ranging from local to regional (Vanhaluwayn et al., 2013 ; Nelsen et al., 2019 ; Ghennam, 2021).

This study covers six of the densest forest stations in the wilaya of Djelfa. In this steppe ecosystem, where lichen biodiversity remains very poorly documented despite its potential ecological role, we specifically evaluate two foliose epiphytic lichens, Xanthoria parietina and Physcia aipolia, as bioindicators and bioaccumulators.

The use of lichens as bioaccumulators and bioindicators has yielded significant results in recent years in different regions of the world, particularly in humid and sub-humid climates, as evidenced by various studies carried out (Yahia & Marniche, 2019 ; Berrayah et al., 2016 ; Omar et al., 2015 ; Ouali alami et al., 2014 ; Adjiri et al., 2018)

The main objective of this study is to analyze the mechanisms of bioaccumulation of metallic elements by lichens, by examining the factors that influence this process, such as lichen species and the properties of the metals. It also aims to assess the usefulness of these bioindicators for environmental quality monitoring, particularly through a statistical analysis of bioaccumulation data.

MATERIALS AND METHODS

Study area

With an area of 32,256.35 km², the municipality of Djelfa is organized into 36 communes. Its relief presents notable altitudinal variations, peaking at 1613 meters and descending to 150 meters. The territory has a population of 1,508,535 inhabitants (DPSB, 2018). Its climate, generally arid to lower semi-arid, is characterized by marked seasonal contrasts: cold winters marked by frost and snow, and hot, arid summers, with local variations from one commune to another (CF, 2015).

Experimental Design

The chosen study area was structured into 6 stations, each comprising three points approximately 0.5 to 1 km apart. At each point, 10 trees were selected, corresponding to a total of 30 trees per station. These stations were distributed across all the communes of the wilaya, covering both forests and wooded areas, with targeted sampling on the dominant species, Aleppo Pine (CF, 2015), see Figure 1.

The lichens were collected between December 2022 and March 2023. Collection was carried out using a knife or manually, taking care to avoid any contamination. After collection, the specimens were sorted, packaged in envelopes, and then identified at the laboratory of the DIFAR department (Dipartimento di Farmacia) at the University of Genoa, Italy. Identification was performed by microscopic examination of the morphological characteristics of the thallus and spores (size and color), based on taxonomic identification keys: the website (Italic 8.0) and other works by lichenologists (Clauzade and Roux,1985 ; Ozenda and Clauzade ,1970 ; Kirschbaum et Wirth ,1997 ; Emmanuel et al.,2004 ; Van Haluwyn and Asta, 2009 ; Maizi et al., 2010 ; Rebbas et al., 2011 ; Ait hammou et al., 2011 ;Roux ,2011 ;Hassani et Djeddi,2013; Ghennam, 2017 ; Amrani et al, 2018 ; Boumakhleb, 2021) .
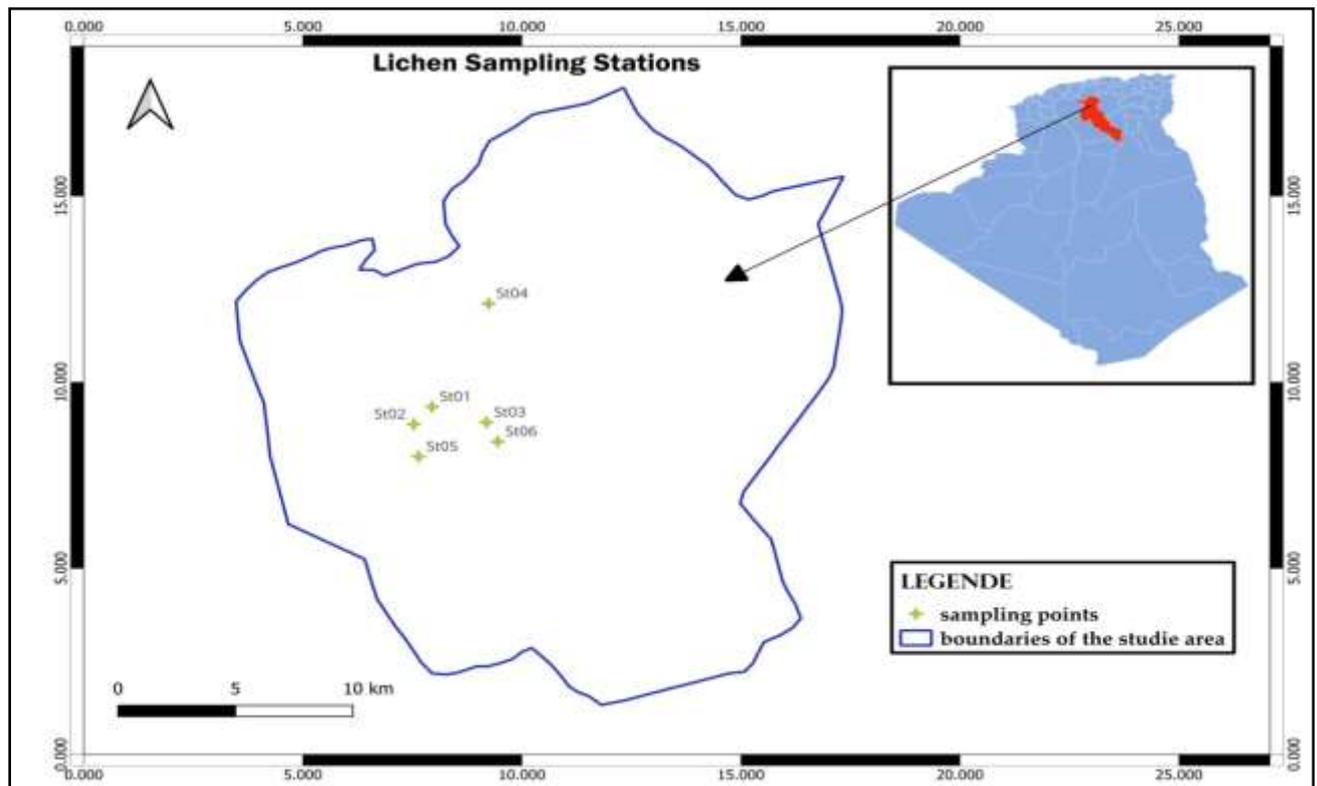
Figure 1 : The sampling points in the study area

STATISTICAL ANALYSIS
All statistical analysis were performed using R software (CRAN, 2024).

CHOICE OF SPECIES
Two foliose lichen species, *Xanthoria parietina* and *Physcia aipolioides* (Figure 2), were selected for this study. This choice was based on several essential criteria. Their foliose morphology facilitates sampling due to limited adherence to the substrate. These species are also cosmopolitan, presenting a wide distribution in the study area and are commonly used in similar biomonitoring studies of trace metal elements (TME). In particular, *Xanthoria parietina* has been the subject of numerous studies worldwide: in Italy (Scerbo et al., 1999; Nimis et al., 2000), in Algeria (Rahali, 2003; Maatoug et al., 2010; Sarmoum et al., 2014), and in Morocco (Ouali et al., 2014).

Other foliose species have also been used in studies in Italy (Loppi et al., 2004; Sorbo et al., 2008), Portugal (Reis et al., 1999; Costa et al., 2002), China (Zhang et al., 2002), and Ghana (Nyarko et al., 2006). Finally, these two species are recognized for their good metal accumulation capacities.

| *Physcia aipolioides* | *Xanthoria parietina* |

Figure 2: illustration of the studied species

LABORATORY ANALYSIS PROTOCOL

PREPARATION OF LICHENS FOR HEAVY METAL ASSAY

The preserved lichens are washed three times with bi-distilled water and dried in an oven at 65°C until completely dry. They are then ground and sieved to obtain a fine, homogeneous powder.

NEUTRON ACTIVATION ANALYSIS PROCEDURE

The samples are weighed using an analytical balance (~1 mg) with a mass not exceeding 100 mg (60-100 mg). Two replicates were prepared for each type, which were subsequently packaged in high-purity aluminum envelopes. In this part, we introduced standards with the same matrix as the studied samples, namely: CRM-GSV4 and CRM-Lichen336. These standards must accompany the samples to be analyzed in all phases of the analytical work. The method uses reference standards, and each sample is duplicated to improve precision and accuracy.

The samples and standards were placed in an irradiation capsule and then irradiated with a thermal neutron flux. This work was carried out using the neutron activation analysis technique implemented around the Es-salam reactor (Nuclear Research Centre of Birine, Algeria).

After a cooling period, these samples were measured using a high-resolution gamma spectrometer, and the concentration of each element is given in ppm

GAMMA SPECTROMETRY

Spectrum acquisition was carried out using a computer-controlled gamma spectrometer, employing a high-purity germanium (HPGe) detector biased at +4000 V. The determination of medium and long half-life elements required two measurement campaigns. The spectra were then processed using Genie 2K and InterSpectre software.

SAMPLE PREPARATION

For each sample, three test portions, with a mass between 100 and 130 mg, were wrapped in high-purity aluminum foil. The same preparation was applied to two IAEA certified reference materials (CRMs), namely CRM-GSD-12 and CRM-GSD-04, in order to validate the analytical results.

IRRADIATION PARAMETERS

The samples and standards thus prepared were irradiated for four hours in the Es-Salaam research reactor.

GAMMA SPECTRUM ACQUISITION

Gamma spectrum acquisition was carried out using a CANBERRA spectrometer equipped with a high-purity germanium detector (HPGe, relative efficiency of 35%), controlled by Genie 2k software (version 3.2). Measurements were performed over a duration of 7,200 seconds (2 hours) for each sample and standard, at four distinct cooling intervals, ranging from four days to two weeks after irradiation (Avino et al., 2006, 2007).

RESULTATS AND DISCUSSION

STATISTICAL SUMMARY OF METALLIC ELEMENTS IN THE TWO SPECIES

The two tables present descriptive statistics of the concentrations of 16 heavy metals in two species, Xpa and Pai. The analysis reveals significant environmental contamination, but with clearly different levels of contamination.

In Xpa (Table 1), the concentrations are markedly higher, dominated by iron (mean of 13,830 ppm), barium, and zinc. Certain elements (Sb, Np, Yb) are present only in trace amounts. The high dispersion of values for iron, zinc, and barium indicates a heterogeneous distribution of these metals.

In Pai (Table 2), the concentrations are generally lower: iron peaks at an average of 3,655 ppm, nearly four times less than in Xpa. Variability is moderate for most elements, suggesting a more homogeneous environment. Of note: ytterbium is completely absent, while neptunium and europium are present only in trace, stable amounts.

Based on this summary, the superiority of Xpa as a bioindicator of metal pollution is evident. The particularly high concentrations of iron, barium, and zinc recorded in this species indicate a strong anthropogenic influence, linked to industrial discharges (iron and steel industry, metallurgy) and urban emissions (Pacyna and Pacyna, 2001; Mbengue, 2016). The notable presence of barium likely originates from road traffic, particularly from the wear of braking systems and fuel combustion (Mbengue, 2016), while the significant iron levels suggest a contribution from dust from construction sites, building materials, and disturbed soils (Douibi, 2020). Zinc, meanwhile, can be associated with both tire abrasion and the leaching of certain wastes (Douibi, 2020). These results show the capacity of the lichen Xpa to accumulate these elements, confirming its value for monitoring atmospheric fallout of anthropogenic

origin (Nimis et al., 2000; Dron et al., 2016), unlike the lichen Pai, which reflects a less impacted environment.

Table 1 : Statistical summary of the species *Xanthoria Parietina*

Metal	Mean	Standard deviation	Median	Minimum	Maximum
As	5.642	0.937	5.790	4.210	6.750
Ba	219.707	28.521	226.275	167.020	243.670
Br	48.496	7.458	50.710	33.765	53.860
Ce	38.723	9.407	42.150	24.395	50.030
Co	7.952	0.831	8.250	6.315	8.490
Cr	1.476	0.737	1.705	0.130	2.205
Cs	3.947	1.328	4.350	2.200	5.320
Eu	1.811	0.844	1.830	0.785	3.020
Fe	13829.675	2051.392	14943.590	9980.740	15094.120
Hg	2.338	0.544	2.450	1.380	2.840
La	22.470	4.964	22.320	16.090	31.110
Rb	60.725	11.448	63.940	41.740	75.340
Sb	0.454	0.080	0.445	0.345	0.580
Np	0.150	0.144	0.145	0.000	0.310
Yb	0.100	0.245	0.000	0.000	0.600
Zn	65.064	20.487	73.895	29.315	83.840

Table 2 : Statistical summary of the species *Physia Aiplioide*

Metal	Mean	Standard deviation	Median	Minimum	Maximum
As	2.900	0.477	2.990	2.28	3.42
Ba	125.683	20.884	123.250	99.63	159.32
Br	17.728	1.474	18.235	15.43	19.00
Ce	29.615	4.977	32.435	22.91	33.72
Co	2.978	0.613	2.905	2.31	3.89
Cr	1.560	0.823	1.805	0.07	2.43
Cs	2.742	1.092	3.225	1.33	3.92
Eu	0.687	0.178	0.680	0.45	0.92
Fe	3654.975	208.885	3678.470	3387.42	3905.12
Hg	1.715	0.298	1.830	1.20	2.03
La	19.338	6.207	23.010	11.04	23.67
Rb	32.113	7.683	31.965	24.49	45.98
Sb	0.398	0.203	0.400	0.18	0.63
Np	0.335	0.237	0.285	0.12	0.67
Yb	0.000	0.000	0.000	0.00	0.00
Zn	51.193	15.489	57.320	31.76	65.12

DISTRIBUTION OF HEAVY METAL CONCENTRATIONS IN LICHEN SPECIES

This graph compares, on a logarithmic scale, the distribution of metallic elements in the two species (Pai in blue, Xpa in orange) (Figure 3). Overall, Xpa shows higher bioaccumulation than Pai for most elements, with a particularly marked difference for Fe, Br, Co, Rb, Ba, and As, indicating an anthropogenic origin, mainly related to road traffic and combustion processes (Kuik and Wolterbeek, 1995; Cuny et al., 2001). Conversely, metals such as Cr and Sb show similar distributions between the two species, originating from road traffic and local industrial activities (Francovà et al., 2017), suggesting a negligible interspecific

difference. Certain trace elements (Np, Yb) show very low values, close to the detection limits, which makes their interpretation difficult. Despite notable variability between stations for several metals, the general trend confirms that Xpa accumulates atmospheric deposition more efficiently than Pai, especially for the dominant metals (Parzych et al., 2016).
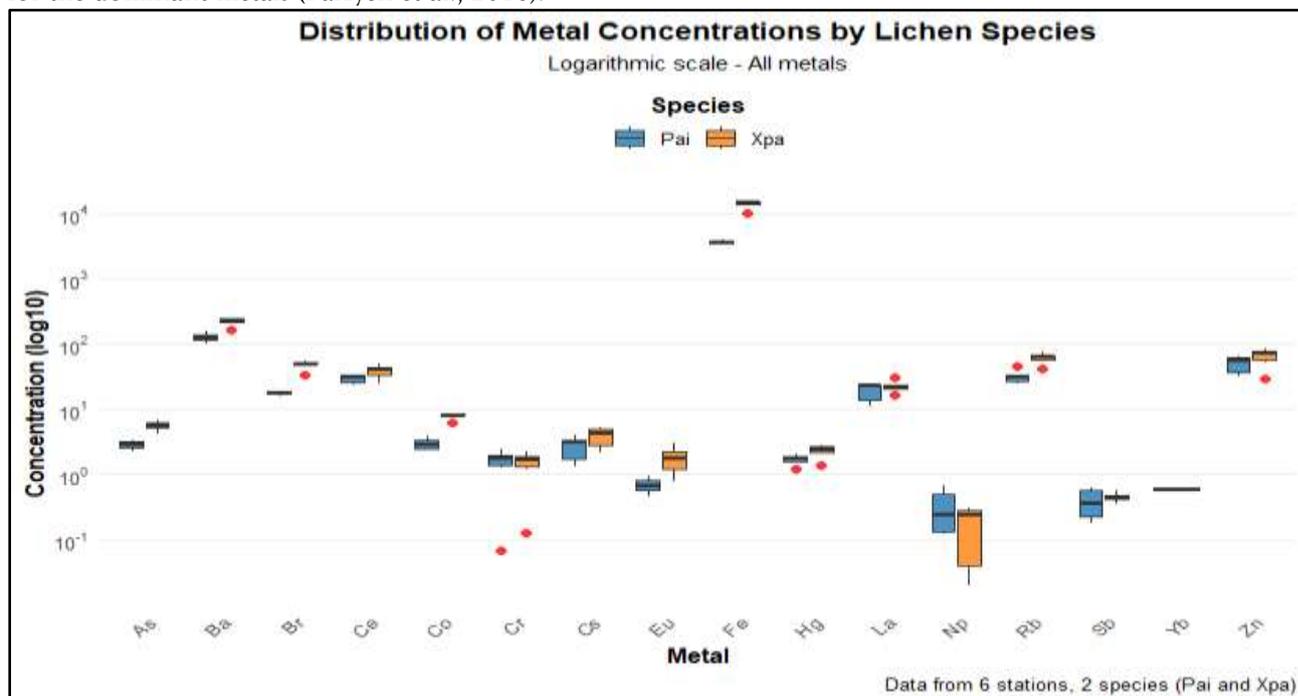

Figure 3: distribution of heavy metals by the two lichen species

CLASSIFICATION DENDROGRAM AND PCA: POSITIONING OF STATIONS BY SPECIES ACCORDING TO THEIR METALLIC PROFILES

The analysis of the dendrogram (Figure 4a) reveals a spatial organization of sites based on their metallic signatures. The first cluster, closely associating St4, St5, and St6, then St1 somewhat more distantly, suggests the existence of a relatively homogeneous zone where trace element inputs are likely governed by common sources or similar deposition mechanisms. Conversely, the clear distinction of the cluster formed by St2 and St3, which only joins the main group at a high level of dissimilarity, reflects a singular metallic composition. This singularity argues for a particular local influence, whether anthropogenic (point source discharge) or natural (specificity of the substrate). Finally, the distinct positioning of the two species within the same station constitutes a key finding: it demonstrates that metal accumulation is also governed by biological factors (Nieboer et al., 1976). This interspecific divergence between Pai and Xpa underscores the importance of an active biological filter whose response varies depending on the species considered (Aslan et al., 2011; Francovà et al., 2017).

The PCA confirms and complements this structuring. The first axis (PC1), which alone captures 58.6% of the total variance (Figure 4b), opposes the samples along a major gradient of metal concentrations and allows for a coherent grouping of stations with similar signatures. The second axis (PC2), with 19.7%, refines this discrimination by isolating certain stations with a more atypical profile, notably one of them positioned very low on this axis, suggesting the influence of a specific local source or a particular metal assemblage. All these results highlight a marked spatial differentiation between stations, with some sites distinguished by more singular pollution signatures.
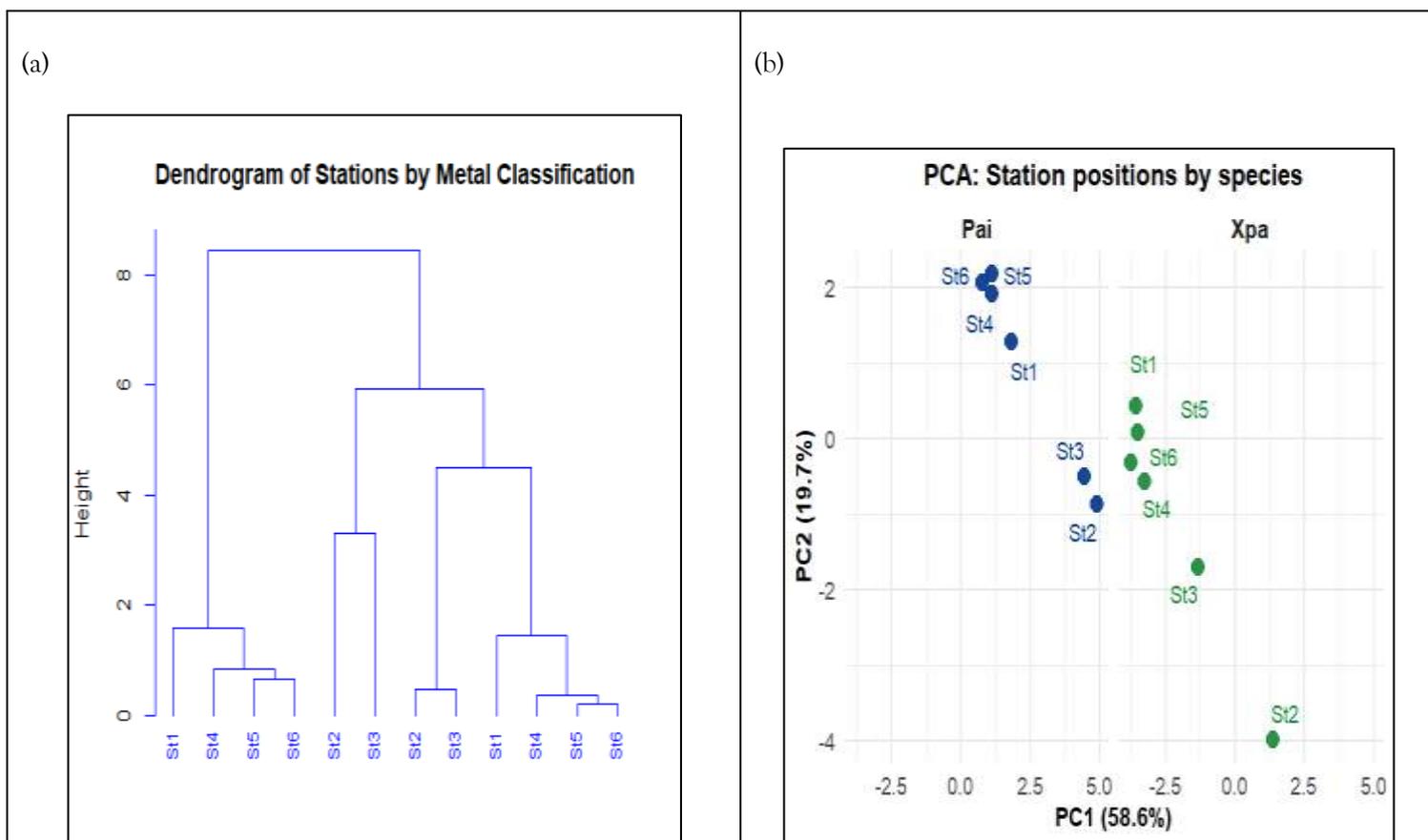
(a)
(b)

Figure 4 : Classification of stations by metallic profiles (a) and PCA of projection species (b)

INTERSPECIFIC COMPARISON OF HEAVY METAL BIOACCUMULATION BY (PAI AND XPA)

Statistical analysis using the Wilcoxon test (Table 3) highlights notable differences in bioaccumulation between the two lichens studied, Pai and Xpa. The latter exhibits higher median concentrations for twelve of the sixteen metals analyzed. The most striking disparities concern Fe, with a median almost four times higher in Xpa, followed by Br and Co, whose levels are approximately 2.8 times higher. As, Ba, and Rb are also 1.8 to 2 times more concentrated in Xpa. Although the differences for eleven metals are significant before correction, the application of the FDR correction makes these results more nuanced and calls for caution. In contrast, elements such as La, Sb, Cr, and Yb show no significant difference between the two species. These results suggest that Xpa constitutes a more effective bioindicator for monitoring atmospheric metal fallout (Sarmoum et al., 2014; Francová et al., 2017). These findings confirm that Xpa is a more sensitive bioindicator than Pai for monitoring atmospheric metal deposition, particularly for the most abundant elements or those with the highest enrichment factors (Aslan et al., 2011; Dron et al., 2016).

Table 3 : Result of wilcoxon test

Metal	Median_Pai	Median_Xpa	Median_logRatio	Direction	P_value	FDR
As	2.990	5.790	0.56521218	Xpa>Pai	0.03603169	0.05240973
Ba	123.250	226.275	0.56247132	Xpa>Pai	0.03603169	0.05240973
Br	18.235	50.710	1.00646336	Xpa>Pai	0.03603169	0.05240973
Ce	32.435	42.150	0.24911502	Xpa>Pai	0.03603169	0.05240973
Co	2.905	8.250	0.79281500	Xpa>Pai	0.03603169	0.05240973
Cs	3.225	4.350	0.29284134	Xpa>Pai	0.03603169	0.05240973
Eu	0.680	1.830	0.44965448	Xpa>Pai	0.03603169	0.05240973
Fe	3678.470	14943.590	1.36264471	Xpa>Pai	0.03603169	0.05240973
Hg	1.830	2.450	0.22607406	Xpa>Pai	0.03603169	0.05240973
Rb	31.965	63.940	0.66963756	Xpa>Pai	0.03603169	0.05240973

Np	0.285	0.145	-0.12392979	Pai>Xpa	0.03603169	0.05240973
Zn	57.320	73.895	0.16382531	Xpa>Pai	0.05917207	0.07889609
La	23.010	22.320	0.14944268	Xpa>Pai	0.40167817	0.45906076
Sb	0.400	0.445	0.03730924	Xpa>Pai	0.40167817	0.45906076
Cr	1.805	1.705	0.02381927	Xpa>Pai	0.83393541	0.88953111
Yb	0.000	0.000	0.00000000	Pai>Xpa	1.00000000	1.00000000

With:

P-value: statistical significance threshold for the Wilcoxon test.

FDR (False Discovery Rate): false discovery rate, applied to correct the error risk associated with multiple tests.

INTERSPECIFIC DISSIMILARITY OF METALLIC PROFILES AND COMPARISON OF BIOACCUMULATION CAPACITIES BETWEEN XPA AND PAI

(Figure 5c) shows that the contamination gap between the two lichens varies according to the station. At St3, the dissimilarity is higher (0.100) than the average (0.087), indicating a clear difference between their chemical signatures at this site. This is explained by a local pollution source or a particular composition of atmospheric fallout. At the five other stations, the values are more stable (between 0.075 and 0.085), suggesting a moderate and reproducible interspecific difference under common conditions.

Furthermore, Figure (5d) confirms that Xpa accumulates far more metals than Pai, with an average concentration nearly four times higher (14,309 ppm vs. 3,944 ppm). This difference validates the trend observed metal by metal in Table 3 and reinforces the idea that Xpa is a more efficient bioaccumulator (Cuny et al., 2009).

Even if the results by metal became marginal after statistical correction, these global analyses clearly confirm the trend: Xpa is not only more efficient at accumulating metals, but also more sensitive to local variations (Sarmoum et al, 2014; Dron et al, 2016), as at St3. It therefore appears to be a more suitable bioindicator for detailed monitoring of metal deposits. Although combining the two species helps to better differentiate natural variations from localized pollution peaks, this disparity reflects physiological and morphological differences between them in their ability to absorb and retain metallic elements (Branquinho et al., 2008; Aslan et al., 2011; Francovà et al., 2017).
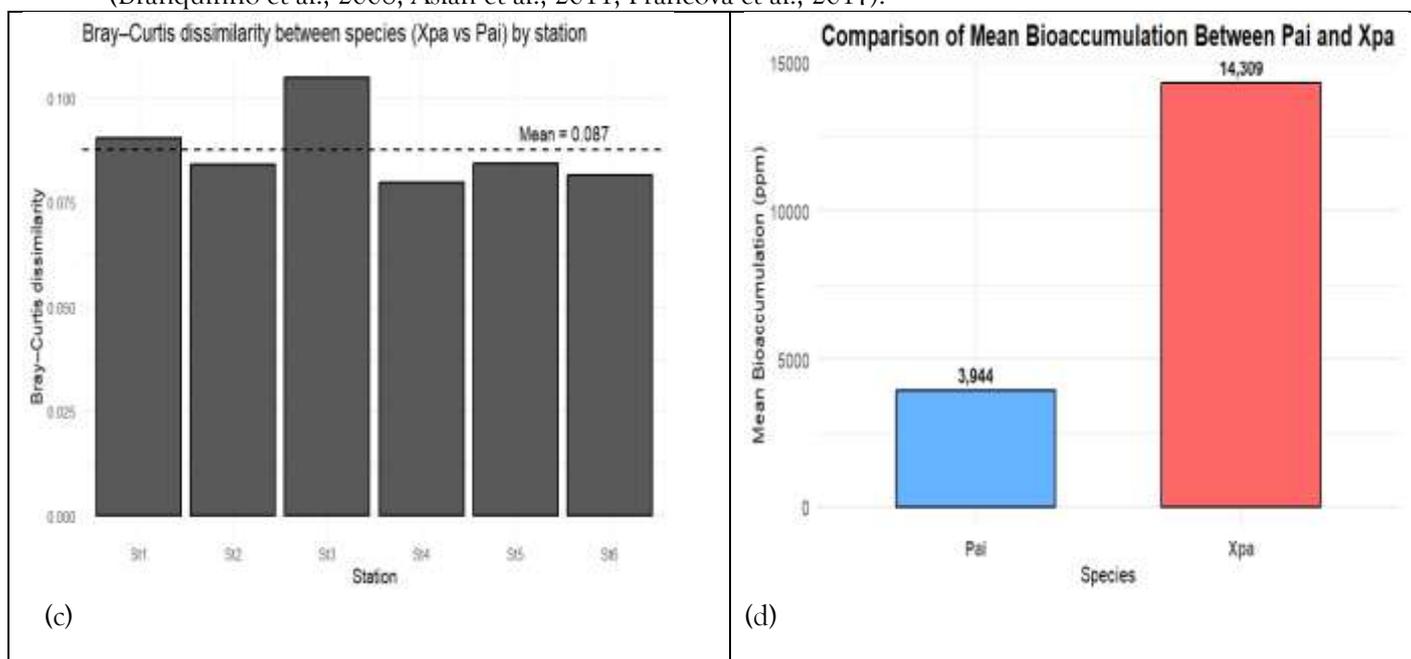
Figure 5 : Bray-Curtis dissimilarity between species by station (c); Comparison of mean bioaccumulation between Pai and Xpa (d).

CONCLUSION

This study highlights significant multi-source contamination of the city of Djelfa by trace metal elements, combining high anthropogenic pollution from road traffic, industrial activities, and incineration (Fe, Br, Co, Rb, Ba). The choice of lichen species in assessing atmospheric metallic fallout proved to be very important. Xpa stands out as a particularly effective bioaccumulator, exhibiting iron levels nearly four times higher than Pai and marked sensitivity to local anomalies, particularly at station St3. For its part,

Pai provides a more stable and homogeneous estimate of the ambient background. The spatial analysis reveals a coherent organization of stations, with a homogeneous group (St1, St4, St5, St6) and a distinct cluster (St2-St3) with particular metallic profiles, reflecting specific local influences. The differences observed between the two species at the same site confirm that metal accumulation results from both environmental exposure and specific biological characteristics. These observations encourage the adoption of a strategy combining Xpa and Pai, making it possible to accurately differentiate regional background noise from localized pollution episodes, thereby improving air quality monitoring.

ACKNOWLEDGMENTS

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