



# Lichen diversity and bioaccumulation of heavy metals in northern Tunisia: a study to evaluate environmental pollution

Mohamed Mendili<sup>1</sup> · Tayssir Ben Jrad<sup>1</sup> · Ayda Khadhri<sup>1</sup>

Received: 18 June 2023 / Accepted: 6 August 2023 / Published online: 29 September 2023  
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## Abstract

This study focused on the diversity of lichens present in selected regions of northern Tunisia and assessed their resistance to atmospheric pollution. Twenty-five species of lichen were identified, divided into 13 families and 17 different genera. The results show that the *Cladoniaceae* and *Parmeliaceae* families predominate. Foliose and complex lichens are the most abundant. The main objective of this study was to examine, via atomic absorption spectrometry, the bioaccumulation of heavy metals (lead, zinc, copper, and cadmium) in the lichens *Cladonia rangiformis*, *Flavoparmelia caperata*, *Parmotrema perlatum*, and *Evernia prunastri*, which were selected due to the presence of similar lichens at the sites. The results showed high accumulations of lead, copper, and zinc at all stations (Nefza, Babouche, Oued Zen) characterized by intense road traffic and/or industrial activity, while cadmium levels were low at all stations. *Flavoparmelia caperata* proved to be a species tolerant of metal stress, making it a promising candidate for air quality biomonitoring programs aimed at assessing air pollution (17.53 mg/g DW of Pb and 89.8 mg/g DW of Zn in Nefza, and 2.36 mg/g DW of Cd and 10.13 mg/g DW of Cu in Oued Zen). These results highlight the importance of lichens as biological indicators for assessing pollution, the need to carefully monitor heavy metal concentrations in urban environments, and to provide future projects with funding for their potential transplantation to polluted.

**Keywords** Air pollution · Atmospheric · Bioaccumulation · Biomonitoring · Environment · Heavy metals · Lichen

## Introduction

Due to growing urbanization, industrial activity, rising traffic emissions, and a lack of urban planning, air pollution is one of the most significant environmental problems in many cities in the world (Benítez et al. 2019; Shahi Khalaf Ansar et al. 2022). The urban region is also acknowledged as the primary source of air pollution (Azimi-Yancheshmeh et al. 2021); it is responsible for around 78% of the region's major air pollutants and carbon emissions (Abas et al. 2022). The overabundance of heavy metals has become one of the major causes of environmental pollution. Also, the use of organic contaminants such as poly- and perfluoroalkyl substances

poses serious environmental problems for soil and water (Kavusi et al. 2023). This pollution negatively affects the ability of the ecosystem to support life (Syed Salleh and Abas 2023). Previous research has demonstrated that levels of potentially toxic elements (PTEs) in soil, water, and air are above environmental quality standards (Fry et al. 2021; Abas et al. 2022). The most prevalent elements in total suspended particle matter are primarily Cu, Co, V, Ni, Fe, and Zn (Abas et al. 2022).

Air quality can only be saved by detecting and measuring air pollution. Although it is possible to assess air quality using measuring devices, their high cost will never allow complete coverage of the territory (Zahra et al. 2014). In addition to physical–chemical methods, there is another simple and less costly alternative for studying environmental conditions. This is biomonitoring, or biological monitoring, which uses animal or plant species to report environmental conditions according to their sensitivity (Asgari Lajayer et al. 2019). Lichens are good examples; they can be used for assessing air quality and to directly assess the environmental impacts of pollutants (Déruelle and Richard 1983). They

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Responsible Editor: Giulia Guerriero.

✉ Ayda Khadhri  
khadriayda@yahoo.fr

<sup>1</sup> Faculty of Sciences, Plant, Soil, Environment Interactions Laboratory, The University of Tunis El Manar, Campus Academia, 2092 Tunis, Tunisia

are a suitable alternative for studying how various contaminants affect the environment. Their types, variety, existence or absence, and species are the results of the integration of these pollutants over time (Lawal et al. 2023). Lichens are symbiotic associations of a fungus (mycobiont) and an algal or cyanobacterial partner and/or cyanobacteria (photobiont) (Sharma and Mohammad 2020). They are lichenized fungi (Grimm et al. 2021). Lichens do not have roots—they receive most, if not all, of their nutrients from the atmosphere as dry or wet deposits (Anderson et al. 2022). Furthermore, lichens grow in all climates and are very resistant (Boustie and Grube 2005). About 20,000 species have been described (Grimm et al. 2021). Most lichens are sensitive to changes in their environment and are generally sensitive to atmospheric pollution (Anderson et al. 2022). They have been widely used as indicators of changes in the environment as well as for air quality monitoring (Seed et al. 2013; Chahloul et al. 2022, 2023; Lawal et al. 2023). Studies and research on Tunisian lichen flora remain negligible to this day, although Tunisia offers a great diversity of lichens. Most of the work has focused on the secondary metabolites of lichens and their biological proprieties (Khadhri et al. 2019; Mendili et al. 2019, 2021a, 2021b, 2022a, 2022b). However, studies that have touched on the atmospheric pollution of lichens remain rare (Chahloul et al. 2022, 2023).

The impact of environmental pollution on our planet has become a major concern. The principal goal of this paper is to examine the use of lichens as an indicator and accumulator of environmental contaminants, with a focus on the accumulation of some metal contaminants in Northern Tunisia. In addition, this study aimed to evaluate the collection sites available and to select the lichen that accumulates the most pollutants in the cleanest areas, to contribute to their transplantation in polluted areas in future projects.

## Materials and methods

### Chemicals and equipment

The chemicals and equipment used in the experimental work are listed below.

**Chemicals:** potassium hydroxide (KOH), sodium hypochlorite (NaOCl), 1-phenylenediamine, and hydrochloric acid.

**Equipment:** silica crucible, oven (Protherm Furnaces) for calcination, and an atomic absorption spectrometer (AANA-LYST 400).

### Characteristics of the study areas

The study areas are all located in the humid bioclimatic zone, characterized by a high rainfall of 1000 mm. The Bizerte Governorate, located in the north of Tunisia, is characterized by a mixture of coastal, urban, and industrial environments, making it a region prone to pollutant emissions from a variety of human activities (Table 1).

The Beja Governorate, also located in northern Tunisia, is characterized by its rural landscape and agricultural activities, making it an environment potentially exposed to agricultural pollutants (Table 1).

The Jandouba Governorate, located in the north of Tunisia, has an environment characterized by forested and humid zones. There is a limited presence of industrial activities but a certain influence of road traffic and forest fires on air quality in this governorate (Table 1).

The distribution maps of sulfur dioxide (SO<sub>2</sub>), ozone (O<sub>3</sub>), nitrogen dioxide (NO<sub>2</sub>), carbon monoxide (CO), and the aerosol index in the governorates of Bizerte, Beja, and Jandouba in 2019 shown in Fig. 1 provide the maximum values for each pollutant in the three zones. For all pollutants, the maximum values are obtained in the Bizerte area. Therefore, we can conclude that the most polluted of the three zones is the Bizerte zone. It has the highest concentrations of SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, CO, and aerosols. We used data collected from the Google Earth Engine (GEE) platform and the Sentinel 5P satellite.

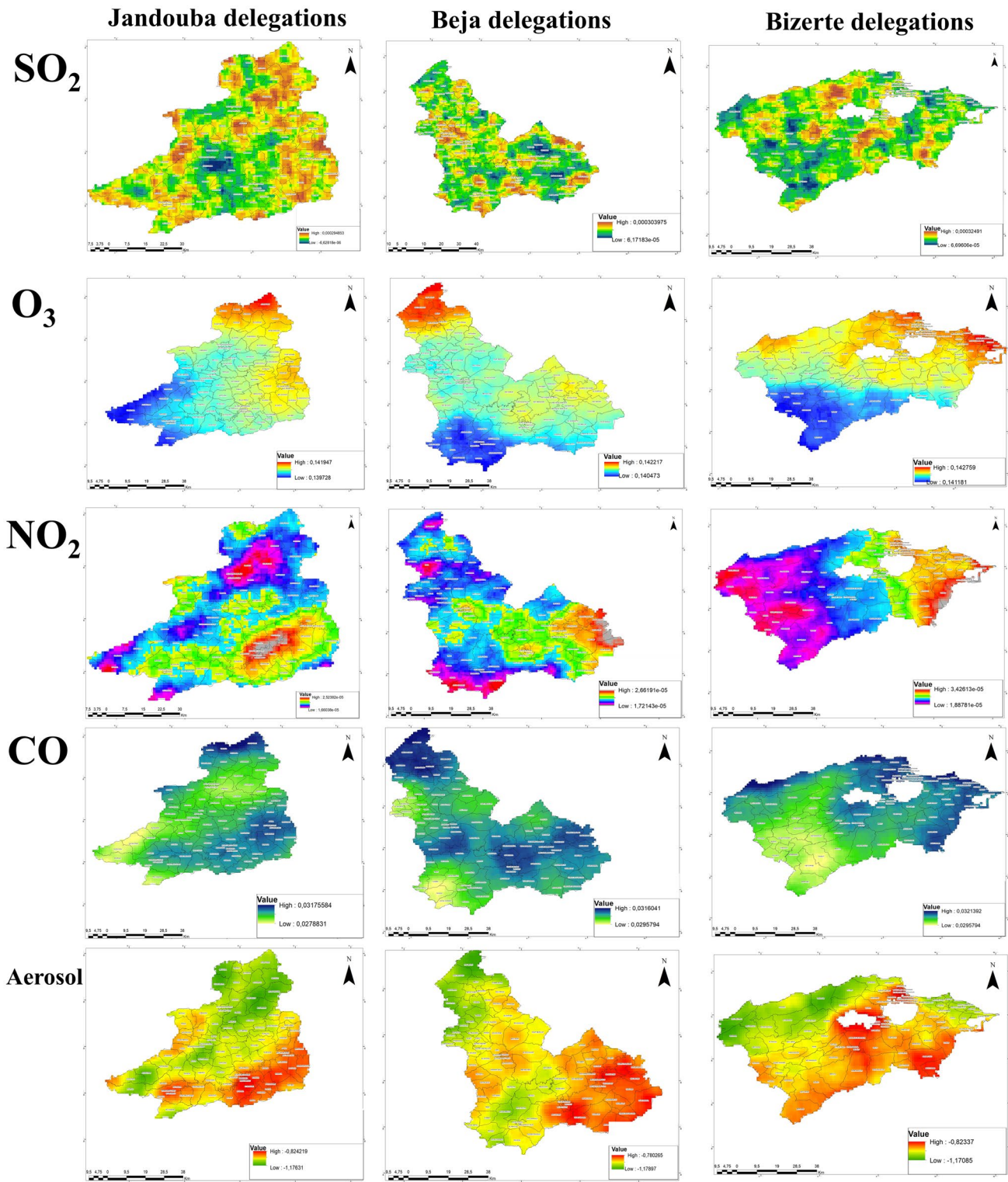
### Lichen sampling

Lichen samples were collected in February, March, and April 2019 from the Bizerte [Ichkeul National Park: IC (37° 07' 43" N: 9° 39' 50" E) and Bazina: B.Z (36° 57' 36" N: 9° 17' 45" E)], Beja [Nefza: NF (37° 02' 11" N:

**Table 1** Characteristics of the stations sampled

Governorate	Delegation	Industrial activities	Anthropogenic action	Urbanization	Car traffic
Bizerte	Bazina	+	++	++	++
	Ichkeul	++	++	++	++
Beja	Nefza	+	++	++	++
Jandouba	Babouche	0	+	+	++
	Oued Zen	0	+	+	++

0 absent, + medium, ++ high



**Fig. 1** Distribution maps of SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, CO, and the aerosol index in the governorates of Bizerte, Beja, and Jandouba in 2019 obtained using the Google Earth Engine platform and the Sentinel 5P satellite

9° 05' 12" E)], and Jandouba [Babouche: B.B (36° 48' 08" N, 8° 40' 36" E) and Oued Zen: O.Z (36° 24' 0" N, 9° 30' 0" E)] regions of Tunisia (Fig. 2). Voucher specimens have been deposited in the lichenological herbarium of the Department of Biology, Faculty of Sciences of Tunisia.

### Lichen identification

On the one hand, the identification of lichens was carried out by macroscopic observations to determine the general morphological characteristics of different species of lichens, such as the type of thallus (leprose, crustose, squamous, foliose, fruticose, composite, or gelatinous), the color of the thallus (yellow, orange, green, blue-green, brown), the orientation of the tips, and their habitat. The samples were analyzed in the area. On the other hand, the identification of lichen was carried out by performing chemical tests with certain reagents which give specific colorations when in contact with the thallus (Van Haluwyn and Asta 2013; Van Haluwyn et al. 2014; Asta et al. 2016).

The main reagents used were as follows:

- K test: 10% KOH solution in water
- C test: NaOCl, an aqueous calcium hypochlorite solution
- KC test: K and C solutions applied one after the other
- P test: 1% 1-phenylenediamine in a 10% aqueous sodium thiosulfate solution.

For the addition of each reagent, a + followed by the observed color is noted if the reaction is positive, and a – is indicated if no color has appeared (a negative reaction).

### The state of the air quality in the areas

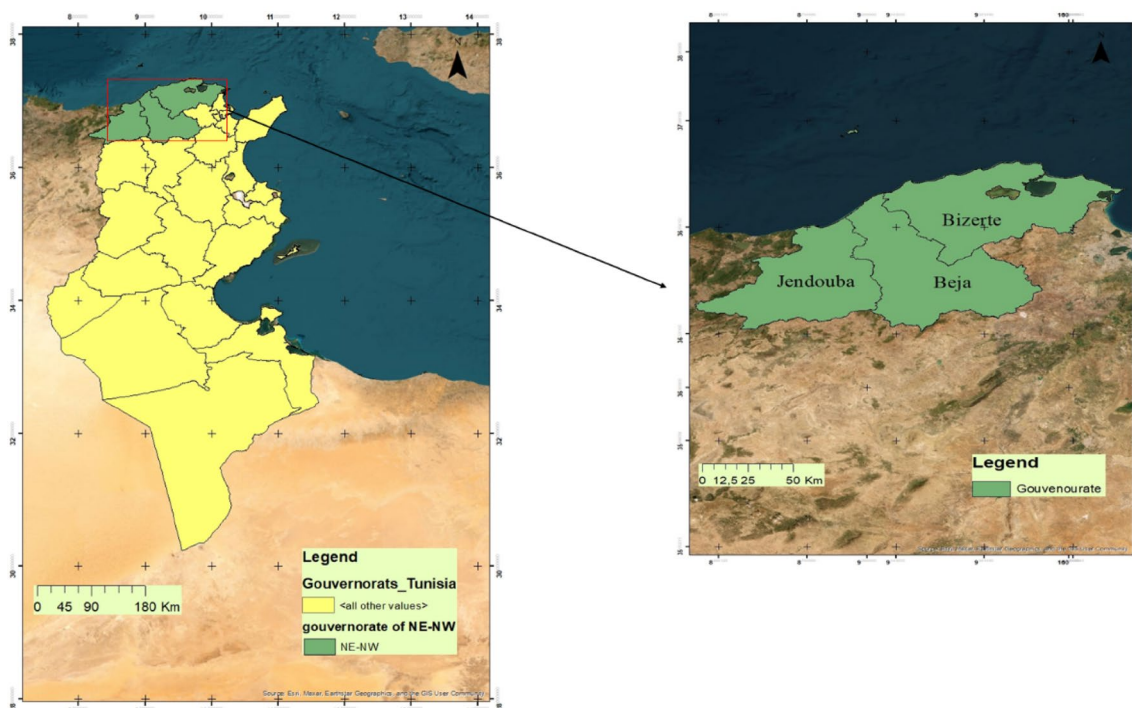
The Van Haluwyn and Lerond (1986) method was used to compare air quality in the areas studied; this formed the basis for classifying the lichens according to their degree of resistance to pollution.

### Mineralization

The amounts of the four trace elements Zn (zinc), Pb (lead), Cu (copper), and Cd (cadmium) were determined by following the methods reported by Adjiri et al. (2018). In addition, three replicates were used for each treatment.

### Preparing samples for mineral analysis

The samples were stored in a refrigerator at 4 °C and dried at 70–80 °C until a constant mass was obtained. The samples were then ground to a powder using a grinder and stored in pillboxes. The determination of the elements required the sample to be placed in solution. The organic matter was destroyed in a dry process.



**Fig. 2** Location maps of the prospecting stations in the north of Tunisia (ArcGIS 10.8)



## Calcination

The first step in calcination was to weigh 0.5 g of lichen powder into a silica crucible. The crucible was placed in an oven (Protherm Furnaces) and the temperature was gradually raised to 450 °C and maintained at that for 4–6 h, depending on the nature of the sample. It was then allowed to cool. Next, the ash was moistened with 3 mL of distilled water and 1 mL of concentrated HCl was added. Finally, evaporation was performed on a hot plate.

## Atomic absorption spectrometry assay

For the determination of the four trace elements (Pb, Zn, Cu, and Cd), we used the atomic absorption spectrometry technique. The instrument used was an AANALYST 400 atomic absorption spectrometer.

## Statistical analysis

The data were analyzed using statistical software. A two-way permutation ANOVA was performed to test for differences between species and sites, using Duncan's test for post hoc

comparisons. Statistical analysis was performed using SPSS software (SPSS, 20.0). The data for the pair chart and heat map were generated using Python Pandas Dataframe and Google Colab.

Calculations and graphical representations were made using Microsoft Excel and Google Colab. The safety intervals of the means were calculated at the 95% level.

## Results

### Lichen identification

Lichen identification is challenging in Tunisia due to limited knowledge about the lichen flora. As a result, thallus reactions and macroscopic observations were carried out to identify the lichens. Indeed, the collection stations (Bazina, Ichkeul National Park, Nefza, Babouche, and Oued Zen) show substantial lichen diversity.

Our work involved chemical tests with various reagents for which contact with the thallus results in a range of colors (Table 2). Therefore, they are distributed as shown in Table 3.

**Table 2** Results of chemical reactions with lichen thalli

Lichens	K test	C test	KC test	P test
<i>Anaptychia ciliaris</i>	–	–	–	–
<i>Anaptychia runcinata</i>	–	–	–	–
<i>Cladonia cervicornis</i>	–	–	–	+ Red
<i>Cladonia chlorophaea</i> agg.	–	–	–	+ Red
<i>Cladonia foliacea</i>	–	–	+ Yellow	+ Red
<i>Cladonia fimbriata</i>	–	–	–	+ Red
<i>Cladonia furcata</i>	–	–	–	+ Red
<i>Cladonia rangiformis</i>	+ Yellow	–	–	–
<i>Cladonia symphicarpi</i>	+ Yellow to brown-red	–	–	+ Orange
<i>Collema flaccidum</i>	–	–	–	–
<i>Diploschistes ocellatus</i>	+ Yellow then red	–	–	+ Orange
<i>Evernia prunastri</i>	+ Yellow	–	–	–
<i>Flavoparmelia caperata</i>	+ Yellow	–	–	+ Yellow-range to red
<i>Lepraria membranacea</i>	+ Yellow to orange	+ Yellow	–	+ Orange
<i>Lobaria pulmonaria</i>	+ Yellow to orange	–	± Yellow	+ Orange
<i>Ochrolechia parella</i>	–	–	+ Yellow	–
<i>Parmotrema perlatum</i>	–	+ Yellow-orange	+ Orange	+ Orange
<i>Parmotrema hypoleucinum</i>	–	+ Yellow	–	+ Yellow-orange
<i>Peltigera horizontalis</i>	–	–	–	–
<i>Ramalina farinacea</i>	+ Red	–	–	+ Orange
<i>Ramalina fraxinea</i>	–	–	–	–
<i>Rocella phycopsis</i>	+	+ Red	+ Yellow	–
<i>Squamarina cartilaginea</i>	–	+	+ Yellow	–
<i>Xanthoparmelia stenophylla</i>	+ Yellow then red	–	–	–
<i>Xanthoria parietina</i>	+ Red-purple	–	–	–

**Table 3** Localities of species recorded in the north of Tunisia

Lichens	Locality				
	B.Z	I.C	N.F	B.B	O.Z
<i>Anaptychia ciliaris</i>	–	–	–	–	+
<i>Anaptychia runcinata</i>	–	–	–	+	–
<i>Cladonia cervicornis</i>	+	–	–	–	–
<i>Cladonia chlorophaea</i> agg.	–	–	+	–	–
<i>Cladonia foliacea</i>	+	–	+	–	–
<i>Cladonia fimbriata</i>	–	+	–	+	–
<i>Cladonia furcata</i>	+	–	+	–	–
<i>Cladonia rangiformis</i>	+	+	–	+	+
<i>Cladonia symphicarpi</i>	–	–	–	–	+
<i>Collema flaccidum</i>	+	–	–	–	–
<i>Diploschistes ocellatus</i>	+	–	–	–	–
<i>Evernia prunastri</i>	–	–	+	+	+
<i>Flavoparmelia caperata</i>	–	–	+	+	+
<i>Lepraria membranacea</i>	–	–	–	+	–
<i>Lobaria pulmonaria</i>	–	–	–	+	+
<i>Ochrolechia parella</i>	–	–	–	+	–
<i>Parmotrema perlatum</i>	–	–	+	+	+
<i>Parmotrema hypoleucinum</i>	–	–	–	–	+
<i>Peltigera horizontalis</i>	–	–	–	+	–
<i>Ramalina farinacea</i>	–	–	–	+	+
<i>Ramalina fraxinea</i>	–	–	–	–	+
<i>Roccella phycopsis</i>	–	+	–	–	–
<i>Squamarina cartilaginea</i>	+	–	–	–	–
<i>Xanthoparmelia stenophylla</i>	–	–	+	–	–
<i>Xanthoria parietina</i>	+	–	–	+	–

B.Z Bazina, I.C Ichkel, N.F Nefza, B.B Babouche, O.Z Oed Zen

– Absent; + present

Depending on their habitats, the lichens collected are corticolous, muscicolous, saxicolous, and terricolous lichens. The morphological characteristics of the lichens collected enabled us to distinguish seven types of thallus: complex, crustose, foliose, fruticose, gelatinous, leprose, and squamulose (Table 4).

The first results show a high biodiversity of lichens, with 25 species. Of these lichens, 32% are foliose, 24% are complex, and 20% are fruticose, while leprose constitute only 4% (Fig. 3).

In Fig. 4, we can see that terricolous and corticolous lichens are the most dominant lichens in the three governorates in which lichens were sampled.

The systematic classification of all the identified lichen species is shown in Table 5. The results obtained reveal 25 species of lichenized fungi, which are divided into 13 families and 17 genera (Fig. 5). These species are important bioindicators of atmospheric SO<sub>2</sub> content and heavy metal accumulation.

## Sulfur dioxide and air pollution

Lichen species are distributed according to specific ecological conditions (Abas and Din 2020). Some species only grow on acidic substrates (rocks, bark, etc.). Others will only grow on a neutral or basic substrate. Some require very high light conditions and others do not, depending on the humidity, dryness, temperature, and even the degree of air pollution (Van Herk 2001). As shown in Table 6, some lichens have been found to be bioindicators of the approximate atmospheric SO<sub>2</sub> content (in µg/m<sup>3</sup> of air) (Tiévant 2001).

## The state of the air quality

The most widely used method for assessing the air quality of a given ecosystem is that of Van Haluwyn and Lerond (1986), which is based on classifying lichens according to their degree of resistance to pollution. This method was used to compare air quality in the areas studied. As shown in Table 7, the species most sensitive to pollution (*Anaptychia ciliaris*, *Parmotrema perlatum*, and *Ramalina fraxinea*) are found in Nefza, Oed Zen, and Babouche. However, the predominance of tolerant lichens in Bazina and Ichkel suggests that some parts of these areas may be exposed to high levels of pollutants (Table 7).

According to the distribution maps of SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, CO, and the aerosol index, the maximum values for all pollutants are obtained in the Bizerte area. Therefore, we can conclude that the most polluted of the three governorates is the Bizerte zone. It has the highest concentrations of SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, CO, and aerosols (Fig. 1).

The areas situated in the governorate of Bizerte have a low diversity of species. They are characterized by important industrial activities and a high density of road traffic. However, Beja and Jandouba are characterized by limited industrial activity.

For this reason, the selection of lichens is based on the presence of similar lichens at the less polluted sites.

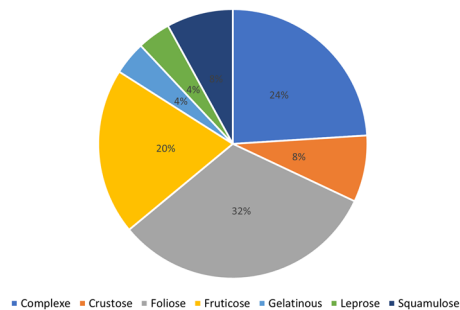
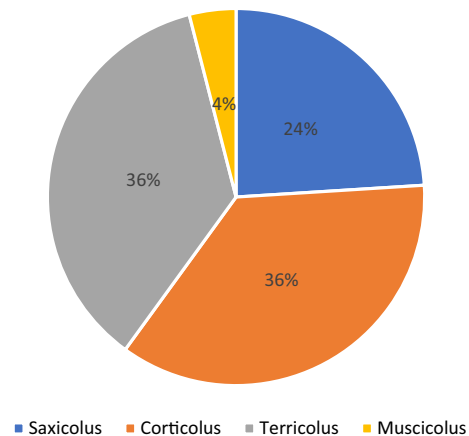
## Heavy metal contents

Our study focuses on the accumulation of zinc, lead, copper, and cadmium by the selected lichens (*Cladonia rangiformis*, *Flavoparmelia caperata*, *Parmotrema perlatum*, and *Evernia prunastri*) collected from the selected sites in Babouche, Oued Zen, and Nefza. The selection of lichens is based on the presence of similar lichens at the sites.

Duncan's test reveals non-significant differences between these four lichens in the levels of the four bioaccumulated PTEs at the same site.

**Table 4** Morphological characteristics of the lichens

Family	Species	Thallus		Ecology
		Type	Color	
<i>Cladoniaceae</i>	<i>Cladonia cervicornis</i>	Complex	Brown	Terricolous
	<i>Cladonia chlorophae</i> agg.	Complex	Gray-green	Corticolous
	<i>Cladonia foliacea</i>	Squamulose	Green-yellow	Terricolous
	<i>Cladonia fimbriata</i>	Complex	Green-yellow	Corticolous
	<i>Cladonia furcata</i>	Complex	Gray-green to brown	Terricolous
	<i>Cladonia rangiformis</i>	Complex	Gray-green	Terricolous
	<i>Cladonia symphicarpia</i>	Complex	Green	Terricolous
<i>Collema</i>	<i>Collema flaccidum</i>	Gelatinous	Black	Muscicolous
<i>Lobariaceae</i>	<i>Lobaria pulmonaria</i>	Foliose	Gray-green when dry, dark green when wet	Corticolous
<i>Ochrolechiaceae</i>	<i>Ochrolechia parella</i>	Crustose	Creamy white	Saxicolous
<i>Parmeliaceae</i>	<i>Flavoparmelia caperata</i>	Foliose	Greeny	Corticolous
	<i>Evernia prunastri</i>	Fruticose	Green, yellow to green, gray	Corticolous
	<i>Parmotrema perlatum</i>	Foliose	Blue-gray	Terricolous
	<i>Xanthoparmelia stenophylla</i>	Foliose	Gray-green	Terricolous
	<i>Parmotrema hypoleucinum</i>	Foliose	Light gray-green	Corticolous
	<i>Peltigeraceae</i>	<i>Peltigera horizontalis</i>	Foliose	Black
<i>Physiaceae</i>	<i>Anaptychia ciliaris</i>	Fruticose	Dark gray to gray-brown	Corticolous
	<i>Anaptychia runcinata</i>	Foliose	Brown	Saxicolous
<i>Ramalinaceae</i>	<i>Ramalina farinacea</i>	Fruticose	Gray to green-yellow	Corticolous
	<i>Ramalina fraxinea</i>	Fruticose	Olive green	Corticolous
<i>Roccellaceae</i>	<i>Roccella phycopsis</i>	Fruticose	Ashy gray	Saxicolous
<i>Stereocaulaceae</i>	<i>Lepraria membranacea</i>	Leprose	Light yellow	Saxicolous
<i>Squamarinaceae</i>	<i>Squamarina artilaginea</i>	Squamulose	Greenish white	Saxicolous
<i>Teloschistaceae</i>	<i>Xanthoria parietina</i>	Foliose	Yellow-orange	Corticolous
<i>Thelotrema</i>	<i>Diploschistes ocellatus</i>	Crustose	Grayish white	Saxicolous

**Fig. 3** Numeric assignment of recorded specimens according to thallus type**Fig. 4** Numeric assignment of recorded specimens by substrate type










## Zinc content

Figure 6 shows that the Zn levels vary between the sites and that the species collected at Nefza have the highest Zn levels of all the species examined. The concentration of Zn shows significant differences between Babouche and Nefza; on the other hand, the Oued Zen site does not show any significant difference from those two sites in

the Zn concentration ( $p > 0.05$ ). The levels are lower at the Babouche and Oued Zen sites. The highest zinc contents are on the order of 39.8 mg/g DW (in *Flavoparmelia caperata* obtained from Nefza) and 32.26 mg/g DW (in *Flavoparmelia caperata* collected from Oued Zen).



**Table 5** Classification of collected lichens (all lichens were collected in February, March, and April 2019)

Classification	Pictures	Locations	Species: <i>Cladonia furcata</i> subsp. subrangiformis		
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia cervicornis</i>	 <i>Cladonia cervicornis</i>	Bazina			
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia chlorophae</i> agg	 <i>Cladonia chlorophae</i> agg	Nefza	Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia rangiformis</i>	 <i>Cladonia rangiformis</i>	Babouche
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia symphicarpia</i>	 <i>Cladonia foliacea</i>	Nefza	Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia symphicarpia</i>	 <i>Cladonia symphicarpia</i>	Oued Zen
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia fimbriata</i>	 <i>Cladonia fimbriata</i>	Babouche	Division : Ascomycota Class : Lecanoromycetideae Order : Peltigerales Family : Collemataceae Genus : Collema Species : <i>Collema flaccidum</i>	 <i>Collema flaccidum</i>	Bazina
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Cladoniaceae Genus : Cladonia Species : <i>Cladonia furcata</i> subsp. subrangiformis	 <i>Cladonia furcata</i> subsp. subrangiformis	Nefza	Division : Ascomycota Class : Lecanoromycetideae Order : Peltigerales Family : Lobariaceae Genus : Lobaria Species : <i>Lobaria pulmonaria</i>	 <i>Lobaria pulmonaria</i>	Babouche

## Lead content

Pb concentration shows significant differences ( $p < 0.05$ ) between Nefza and the other two sites (Babouche and Oued

Zen) ( $p > 0.05$ ). The results in Fig. 7 show that higher levels of lead were observed at the Nefza site compared to the other sites. The lead content varies from 24.6 mg/g DW in *Cladonia rangiformis* to 10.35 mg/g DW in *Parmelia perlata* (Fig. 7).



**Table 5** (continued)




















<p>Division : Ascomycota Class : Lecanoromycetidae Order : Pertusariales Family : Ochrolechiaceae Genus : Ochrolechia Species : <i>Ochrolechia parella</i></p>	 <p><i>Ochrolechia parella</i></p>	<p>Babouche</p>	<p>Species: <i>Xanthoparmelia stenophylla</i></p>		
<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Flavoparmelia Species : <i>Flavoparmelia caperata</i></p>	 <p><i>Flavoparmelia caperata</i></p>	<p>Nefza</p>	<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Parmotrema Species : <i>Parmotrema hypoleucinum</i></p>	 <p><i>Parmotrema hypoleucinum</i></p>	<p>Oued Zen</p>
<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Flavoparmelia Species : <i>Flavoparmelia caperata</i></p>	 <p><i>Flavoparmelia caperata</i></p>	<p>Nefza</p>	<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Peltigeraceae Genus : Peltigera Species : <i>Peltigera horizontalis</i></p>	 <p><i>Peltigera horizontalis</i></p>	<p>Babouche</p>
<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Evernia Species : <i>Evernia prunastri</i></p>	 <p><i>Evernia prunastri</i></p>	<p>Babouche</p>	<p>Division : Ascomycota Class : Lecanoromycete Order : Caliciales Family : Physciaceae Genus : Anaptychia Species : <i>Anaptychia ciliaris</i></p>	 <p><i>Anaptychia ciliaris</i></p>	<p>Oued Zen</p>
<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Parmelia Species : <i>Parmelia perlatum</i></p>	 <p><i>Parmelia perlatum</i></p>	<p>Nefza</p>	<p>Division : Ascomycota Class : Lecanoromycete Order : Caliciales Family : Physciaceae Genus : Anaptychia Species : <i>Anaptychia runcinata</i></p>	 <p><i>Anaptychia runcinata</i></p>	<p>Babouche</p>
<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Parmeliaceae Genus : Xanthoparmelia Species : <i>Xanthoparmelia stenophylla</i></p>	 <p><i>Xanthoparmelia stenophylla</i></p>	<p>Nefza</p>	<p>Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Ramalinaeae Genus : Ramalina Species : <i>Ramalina farinacea</i></p>	 <p><i>Ramalina farinacea</i></p>	<p>Babouche</p>



Table 5 (continued)

Division : Ascomycota Class : Lecanoromycete Order : Caliciales Family : Physiaceae Genus : Anaptychia Species : <i>Anaptychia ciliaris</i>	 <p style="text-align: center;"><i>Anaptychia ciliaris</i></p>	Oued Zen	Division : Ascomycota Class : Lecanoromycetideae Order : Lecanorales Family : Stereocaulaceae Genus : Lepraria Species : <i>Lepraria membranacea</i>	 <p style="text-align: center;"><i>Lepraria membranacea</i></p>	Babouche
Division : Ascomycota Class : Lecanoromycete Order : Caliciales Family : Physiaceae Genus : Anaptychia Species : <i>Anaptychia runcinata</i>	 <p style="text-align: center;"><i>Anaptychia runcinata</i></p>	Babouche	Division : Ascomycota Class : Lecanoromycetideae Order : Lecanorales Family : Stereocaulaceae Genus : <i>Squamarina</i> Species : <i>Squamarina cartilaginea</i>	 <p style="text-align: center;"><i>Squamarina cartilaginea</i></p>	Bazina
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Ramalinaceae Genus : Ramalina Species : <i>Ramalina farinacea</i>	 <p style="text-align: center;"><i>Ramalina farinacea</i></p>	Babouche	Division : Ascomycota Class : Lecanoromycetideae Order : Teloschistales Family : Teloschistaceae Genus : Xanthoria Species : <i>Xanthoria parietina</i>	 <p style="text-align: center;"><i>Xanthoria parietina</i></p>	Bazina
Division : Ascomycota Class : Lecanoromycete Order : Lecanorales Family : Ramalinaceae Genus : Ramalina Species : <i>Ramalina fraxinea</i>	 <p style="text-align: center;"><i>Ramalina fraxinea</i></p>	Oued Zen			
Division : Ascomycota Class : Arthoniomycetideae Order : Arthoniales Family : Roccellaceae Genus : Roccella Species : <i>Roccella phycopsis</i>	 <p style="text-align: center;"><i>Roccella phycopsis</i></p>	Ichkeul			

For Oued Zen, the highest content was recorded in *Flavoparmelia caperata* (12.1 mg/g DW); for Babouche, the highest content (11.85 mg/g DW) was recorded in *Parmotrema perlatum*.

### Copper content

The analysis reported in Fig. 8 shows that the species at the sites in Oued Zen and Babouche contain the highest levels of copper. The Cu concentrations at the three sites are not significantly different ( $p > 0.05$ ). For the Oued Zen and Babouche sites, *Flavoparmelia caperata* shows the highest content (11.5 mg/g DW), followed by *Parmotrema perlatum* (8.9 mg/g DW). For the site in Nefza, the highest content is about 6.8 mg/g DW, in *Flavoparmelia caperata*.

### Cadmium content

The results obtained when determining cadmium are shown in Fig. 9. The Cd contents at Nefza are significantly different ( $p < 0.05$ ) to those at the other two sites (Babouche and Oued Zen) ( $p > 0.05$ ). The Cd contents are very similar and generally low (ranging from 0 to 2.36 mg/g DW). Figure 9 shows that the species harvested in Babouche have rather high cadmium contents: on the order of 2.36 mg/g DW for *Flavoparmelia caperata*, 0.86 mg/g DW for *Evernia prunastri*, and 0.65 mg/g DW for *Parmotrema perlatum*. At the Nefza site, the cadmium content is only about 0.16 mg/g DW in *Evernia prunastri*.

### Correlation analysis

The correlation heat map (Fig. 10) shows the pairwise correlations between Zn (zinc), Pb (lead), Cu (copper), and Cd (cadmium). We find that Zn shows moderately positive correlations with Pb and Cu, with  $r = 0.61$  and  $r = 0.27$ . This suggests that higher values of Zn tend to be associated with higher values of Pb and Cu (Table 8).

Cadmium shows negative correlations with Zn and Pb ( $r = -0.48$  and  $r = -0.33$ , respectively). This means that there may not be a strong linear relationship between Cd and the other elements (Table 8).

The pair chart shown in Fig. 11 allows us to visualize the relationships between all the heavy metals at once. It confirms the observations made based on the correlation heat map.

The diagonal of the pairwise diagram displays histograms showing the distributions of the heavy metals.

## Discussion

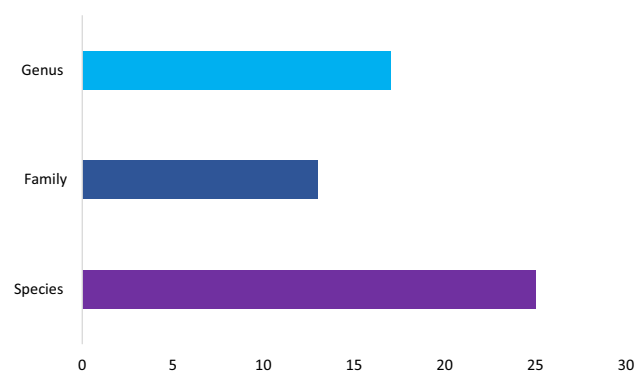
Due to both natural occurrence and human activity, heavy metal contamination is a significant environmental issue on a global scale. The release of harmful heavy metals into ecosystems has grown considerably because of anthropogenic processes such as mining, industrial waste disposal, urbanization, and agricultural practices (Asgari Lajayer et al. 2019). In addition, air, water, and soil pollution have considerable consequences for ecosystems and the loss of biodiversity (Shahi Khalaf Ansar et al. 2022).

This work aimed to provide a better understanding of the lichens living in the selected regions and to determine their resistance to environmental pollution. The lichens were collected from different types of substrates (soil, moss, tree trunks, rocks, etc.).

The results enabled us to identify 25 lichens. They were divided into 13 families and 17 genera. The results show the dominance of the *Cladoniaceae* and *Parmeliaceae* families in terms of the number of species. Analysis of the physiognomic types of the lichens identified showed a dominance of foliose and complex thalli, followed by fruticose, then squamulose, and finally leprous and gelatinous thalli, which were the least represented.

The lichens collected during our studies were distributed according to the ecological conditions, such as the climatic conditions and substrate characteristics. Indeed, most of the species collected were corticolous and foliose. These results agree with those of Déruelle and Richard (1983), who showed that the species most resistant to atmospheric pollution were of the foliose type (Chahloul et al. 2022).

Studies of Tunisia's diversity of lichens are still relatively rare. Indeed, there are not many studies of lichens in Tunisia. However, we can cite the work of El Mokni et al. (2010, 2011).



**Fig. 5** Numeric distribution of the recorded lichen specimens in different taxa (families, genera, and species)

**Table 6** Eventual presence of lichens as a function of the atmospheric SO<sub>2</sub> content (µg/m<sup>3</sup> of air)

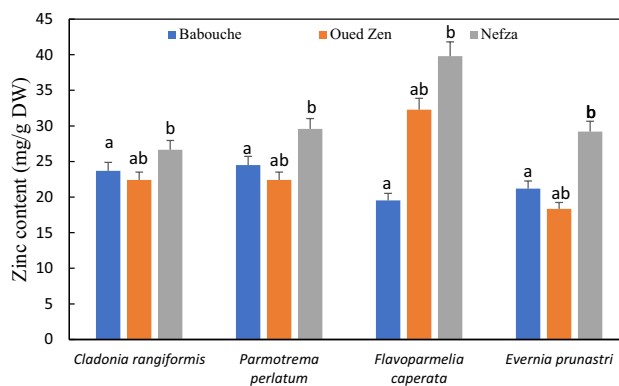
Lichens	Estimated atmospheric SO <sub>2</sub> content (in µg/m <sup>3</sup> of air)
<i>Anaptychia ciliaris</i> (L.) Körb. ex A.Massal	[SO <sub>2</sub> ] < 50 approx.
<i>Evernia prunastri</i>	[SO <sub>2</sub> ] < 60 approx.
<i>Lobaria pulmonaria</i> (L.) Hoffm	[SO <sub>2</sub> ] < 30 approx.
<i>Parmotrema perlatum</i>	[SO <sub>2</sub> ] < 50 approx.
<i>Parmotrema hypoleucinum</i> (Huds.) M. Choisy	[SO <sub>2</sub> ] < 35 approx.
<i>Ramalina farinacea</i> (L.) Ach	[SO <sub>2</sub> ] < 60 approx.
<i>Ramalina fraxinea</i> (L.) Ach	[SO <sub>2</sub> ] < 30 approx.
<i>Xanthoria parietina</i> (L.) Th. Fr	[SO <sub>2</sub> ] < 70 approx.

Estimated atmospheric SO<sub>2</sub> content (in µg/m<sup>3</sup> of air) (Fiore-Donno 1997; Gaveriaux 1999; Sérusiaux et al. 2004)

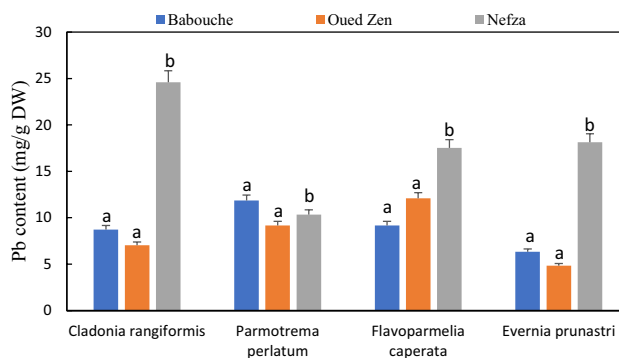
The Van Haluwyn and Lerond (1986) method for assessing the air quality of a given ecosystem showed that the areas belonging to the Bizerte Governorate are the most polluted. This is explained by satellite data. We can conclude that the most polluted of the three governorates is the Bizerte area. It has the highest concentrations of SO<sub>2</sub>, O<sub>3</sub>, NO<sub>2</sub>, CO, and aerosols. Soleimany et al. (2021) found strong correlations between satellite data and in-situ measurements. For example, they showed strong correlations between the aerosol index and in situ PM2.5 and PM10 concentrations.

As a developing country, Tunisia, particularly in the northern governorates (Bizerte), has experienced a gradual increase in air pollutant emissions. This agrees with Azimi-Yancheshmeh et al. (2021), who indicate that Iran (a developing country) faces high levels of PM2.5.

In this context, the selection of lichens was based on the presence of similar lichens at the least polluted sites. Lichens provide a means of determining the degree of atmospheric pollution without the need for often-complicated chemical methods. In this way, lichen bioindicators can be used to assess air quality. The analysis of trace metal elements (Pb,



**Fig. 6** Zn contents (mg/g DW) in the four lichen species at three different sites



**Fig. 7** Pb contents (mg/g DW) in the four lichen species at the three sites

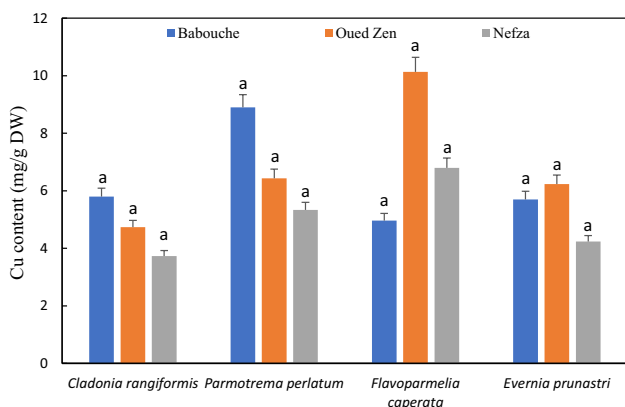
Cd, Zn, and Cu) in the lichens studied show that the accumulation of these metals depended on the site and the lichen species considered. Based on the results for heavy metal levels, we found that the number and coverage rate of lichen flora are related to the degree of pollution. This enabled us to determine that *Flavoparmelia caperata* is the most resistant to atmospheric pollution and accumulates more heavy metals.

**Table 7** Air quality assessed using the method of Van Haluwyn and Lerond (1986) in the areas studied

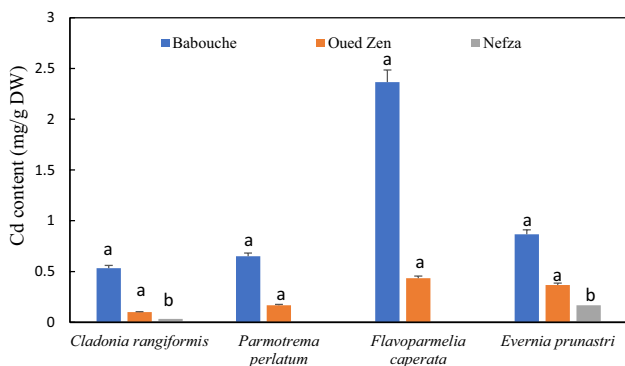
	Pollution	Locality				
		B.Z	I.C	N.F	B.B	O.Z
A	Extreme	–	*	–	–	–
B	Very high	–	–	–	–	–
C	High	–	–	–	–	–
D	Fair	–	–	–	–	–
E	Medium	*	–	*	*	*
F	Low	–	–	*	*	*
G	Very low	–	–	*	*	*

– Absent  
\* Present





**Fig. 8** Cu content (mg/g DW) in the four lichen species at the three sites



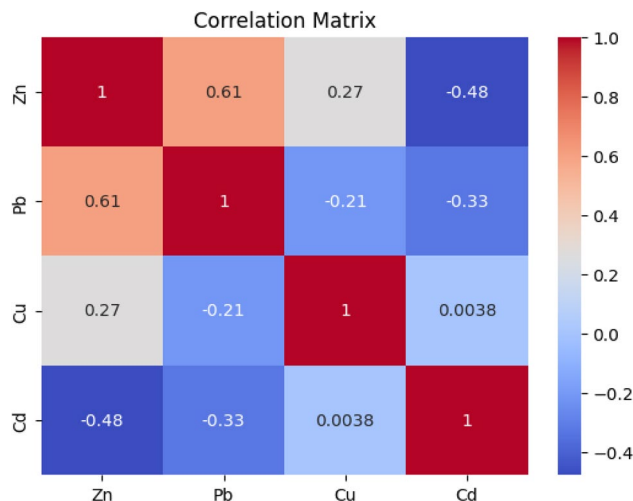
**Fig. 9** Cd content (mg/g DW) in the four lichen species at the three sites

Lichens can accumulate heavy metals because of their anatomical characteristics (the absence of a cuticle) and their physiological characteristics: continuous activity, the absence of stomata, and regulatory mechanisms (Van Haluwyn and Garrec 2002). The high resistance of lichens to environmental extremes is due to their main ecophysiological and metabolic characteristics (Lorenz et al. 2023).

In our work, *Falavoparmelia caperata* collected in Babouche was found to have accumulated the highest levels of the most toxic metal, cadmium.

We note that lead and zinc accumulation was high in urban and suburban sites near roads with heavy traffic (in Nefza). The high zinc levels recorded at the Nefza stations are explained by their locations, which are close to road activities.

As a result of our analysis, we found higher levels of lead and cadmium in some areas, particularly in Nefza. These elevated levels can be attributed to several



**Fig. 10** Correlation matrix of PTEs

**Table 8** Pearson’s correlation coefficients between PTEs

	Zn	Pb	Cu	Cd
Zn	1	<b>0.607*</b>	0.266	-0.476
Pb	<b>0.607*</b>	1	-0.212	-0.327
Cu	0.266	-0.212	1	0.004
Cd	-0.476	-0.327	0.004	1

Bold =  $p < 0.05$

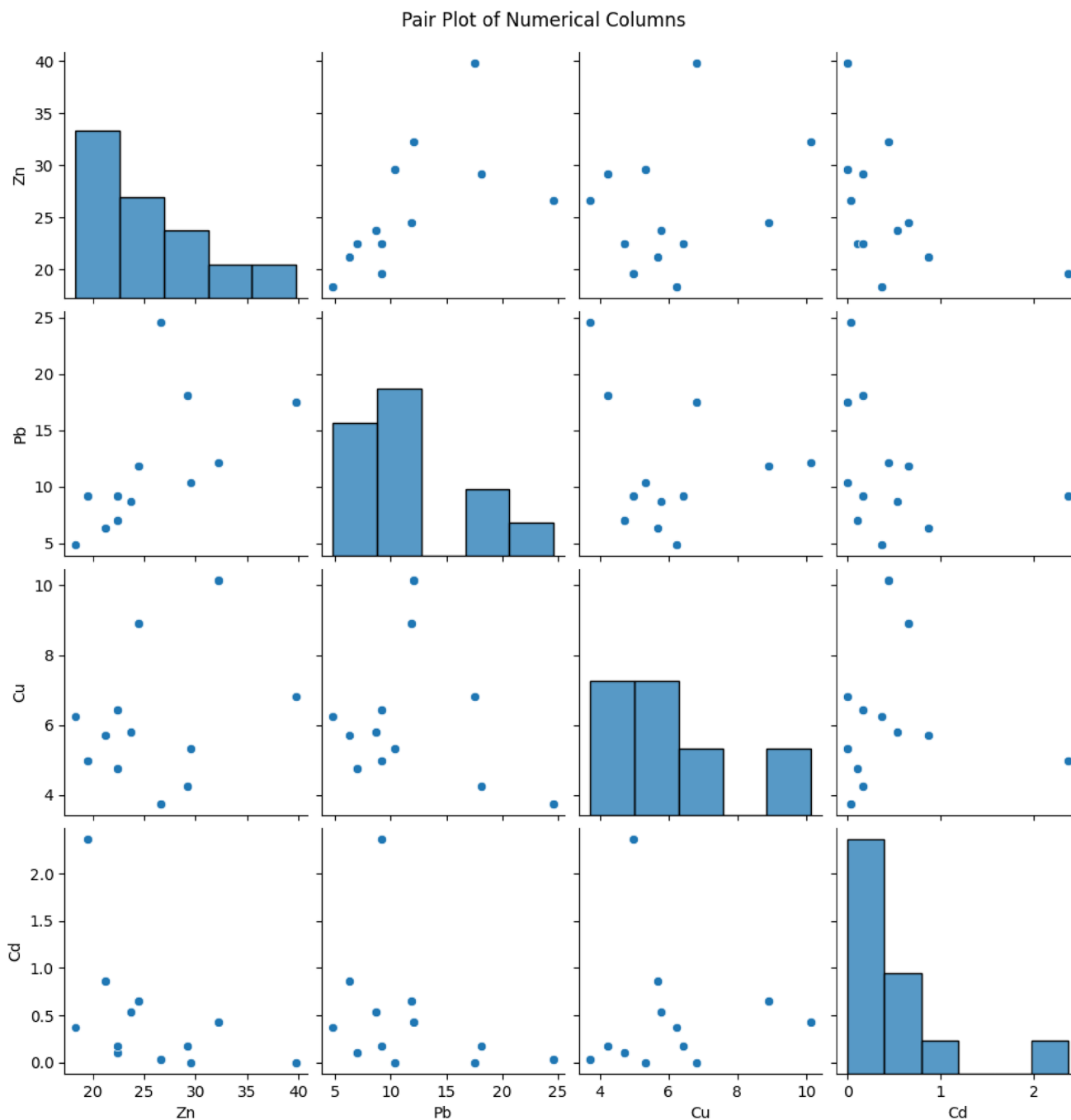
\*Correlation is significant at the 0.05 level (two-tailed)

environmental factors, including increased road traffic and the presence of intensive agricultural activities in the area, which can lead to an intensified release of these heavy metals into the atmosphere.

This hypothesis is based on studies that have shown that lichens located in proximity to roads generally have higher concentrations of lead due to vehicle emissions (Benítez et al. 2019; Sujetoviene and Sliumpaite 2013).

In addition, the correlations between the elements highlight potential links between certain sources of pollution. The positive correlations between zinc, lead, and copper suggest common sources of pollution, while the negative correlation with cadmium indicates a distinct source for this metal. Overall, our findings conform to those previously reported by Chahloul et al. (2023), indicating that a significant correlation between the elements in lichens was observed.

Based on the rate of accumulation of heavy metals by lichens, we can classify the selected stations into two categories according to the degree of pollution. (1) A heavily polluted station, Nefza: this is the urban station. Intense



**Fig. 11** Pair chart for PTEs

road traffic and industrial activity are characteristics. (2) Moderately polluted stations: Babouche and Oued Zen. Since urbanization is a consequence of the explosion in industrialization and road traffic, and these activities emit enormous quantities of pollutants into the atmosphere, posing numerous environmental problems, such as air pollution. Llop et al. (2012) indicated that lichens are sensitive to pollution caused by road traffic. These activities lead to a decline in lichens.

## Conclusion

In conclusion, this study assessed the potential of lichens as indicators of air pollution and accumulators of heavy metals in northern Tunisia. The results showed a high biodiversity of lichens in the region, with both pollution-sensitive and pollution-tolerant species. The state of the air quality suggests that Bizerte areas are the most polluted, due to intensive industrial and road activities. Among the

lichens studied, *Flavoparmelia caperata* was found to be the most resistant to environmental pollution and to accumulate the highest levels of heavy metals, particularly lead, zinc, and copper. The high levels of these metals in some lichens indicate significant environmental contamination by these pollutants. These results confirm the value of lichens as biological indicators and accumulators of metal contaminants. Further study of more sites and lichens would be enable a more comprehensive assessment of the air pollution in northern Tunisia and their transplantation in polluted areas in future projects.

**Data availability** The research data associated with this article is available.

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