



Bioaccumulation and sources identification of atmospheric metal trace elements using lichens along a rural–urban pollution gradient in the Safi-Essaouira coastal area

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ABSTRACT

Atmospheric contamination by metallic trace elements emitted by various human activities constitutes an important threat to human and environmental health. This study aims to determine metal accumulation and the sources of air metallic pollution in the Safi urban-industrial area using lichens as biomonitors. Ten trace elements (As, Cd, Co, Cr, Cu, Ni, Pb, Ti, V and Zn) concentrations and $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ isotopic ratios were analyzed by ICP-MS in four lichen species: *Xanthoria Parietina*, *Ramalina Lacera*, *Xanthoria Calicicola* and *Ramalina Pollinaria*. The results showed significant differences among study sites for most elements with higher concentrations in the industrial, urban and peri-urban sites compared to the reference site chosen as a natural rural area far from any human activities. Significant differences were found between saxicolous and corticolous species especially for Cd, Cu and Zn. The values of Zn/Cu, Zn/Pb and Pb isotope ratios measured in lichens revealed that vehicular traffic and industrial emissions are the main sources of atmospheric Pb contamination. Other anthropic activities (waste incineration, artisanal pottery...) might be the source of other trace metal elements accumulated by lichens. Airborne contaminants in Safi appear to be exported from their sources by air mass movements driven by the regional wind profile.

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Introduction

Atmospheric contamination by metal trace elements (MTE) has become a serious concern worldwide owing to their toxic health effects [1,2]. Several studies suggested that industrial activities, vehicular exhaust and road dust emissions are the main sources contributing to air contamination by MTE in many large cities [3–5].

Airborne metallic micropollutants occur in dissolved or particulate chemical forms that are highly bioassimilable by organisms [6]. The quantification of these toxic elements in atmospheric particles by chemical techniques is highly onerous, and therefore other

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bioassessment approaches have been used to indirectly assess the content of MTE in the air from urban and industrial areas [7–10]. Several organisms, such as lichens [11] and mosses [12], have been used as bioindicators in air pollution monitoring. Lichens are considered among the most efficient biological monitors of air pollution owing to their sensitivity to various metallic pollutants and their ability to accumulate and integrate high content of atmospheric MTE over time [13–15]. Several studies have widely used lichens as low-cost biomonitors of air pollution, since the pollutants concentrations accumulated in their thalli can be directly correlated with those present in the environment [14,16]. Furthermore, the determination of Pb isotopic composition of lichens has proven to be a valuable tool for tracking the air pollution sources and its circulation between different environmental matrices [17–19]. Different studies have used Pb isotopic ratios to identify and quantify Pb anthropogenic emissions sources [20–23]. Elemental ratios between two MTEs (e.g. Zn/Cu, Zn/Pb) are also used as indicators of anthropic and lithogenic sources contribution [23–25].

The aim of this study is to assess metal accumulation and identify the sources of air metallic emissions along a pollution gradient in the Safi urban-industrial area using lichens as biomonitors. The accumulated concentrations of As, Cd, Co, Cr, Cu, Ni, Pb, Ti, V and Zn were analyzed using four lichen species, two corticolous (*Xanthoria parietina* and *Ramalina lacera*) and two saxicolous (*Xanthoria calcicola* and *Ramalina pollinaria*). Finally, we determine the Pb isotope composition, Zn/Cu and Zn/Pb elemental ratios of lichens to identify and track the potential emissions sources of air pollution in the study area.

Materials and methods

Study area

Eight sampling sites were selected along an increasing air pollution gradient from rural to sub-urban and urban in Safi-Essaouira coastal area. Located in western Morocco (32°17' N, 9°14' W), this Atlantic coastal zone extends over 200 km from the rural commune of Oualidia (Site 1, 60 km north of Safi; considered as an uncontaminated reference site), to the vicinity of Essaouira city (site 8) (Figure 1). Table 1 presents a description of the 8 selected sites.

Lichen samples collection

Thalli of two saxicolous lichens, *Xanthoria calcicola* and *Ramalina pollinaria*, growing on the calcareous rocks and two epiphytic lichens, *Xanthoria parietina* and *Ramalina lacera*, growing on the tree trunk of *Acacia dealbata* at 1.5 m, were collected randomly in March 2017 in each sampling site. Four replicates samples were performed for each lichen species. Lichens were carefully sorted, packaged and labelled. Each sample was placed in a small paper bag and marked with the appropriate indications (date, station, type of substrate ...); the bags corresponding to the same station are gathered in a large plastic bag clearly labelled.

Sample preparation and metal analysis

The determination of MTE content in lichens has been carried out according to the standard lichens analysis method: NF X43-904 (AFNOR, 2013). The lichens samples were first

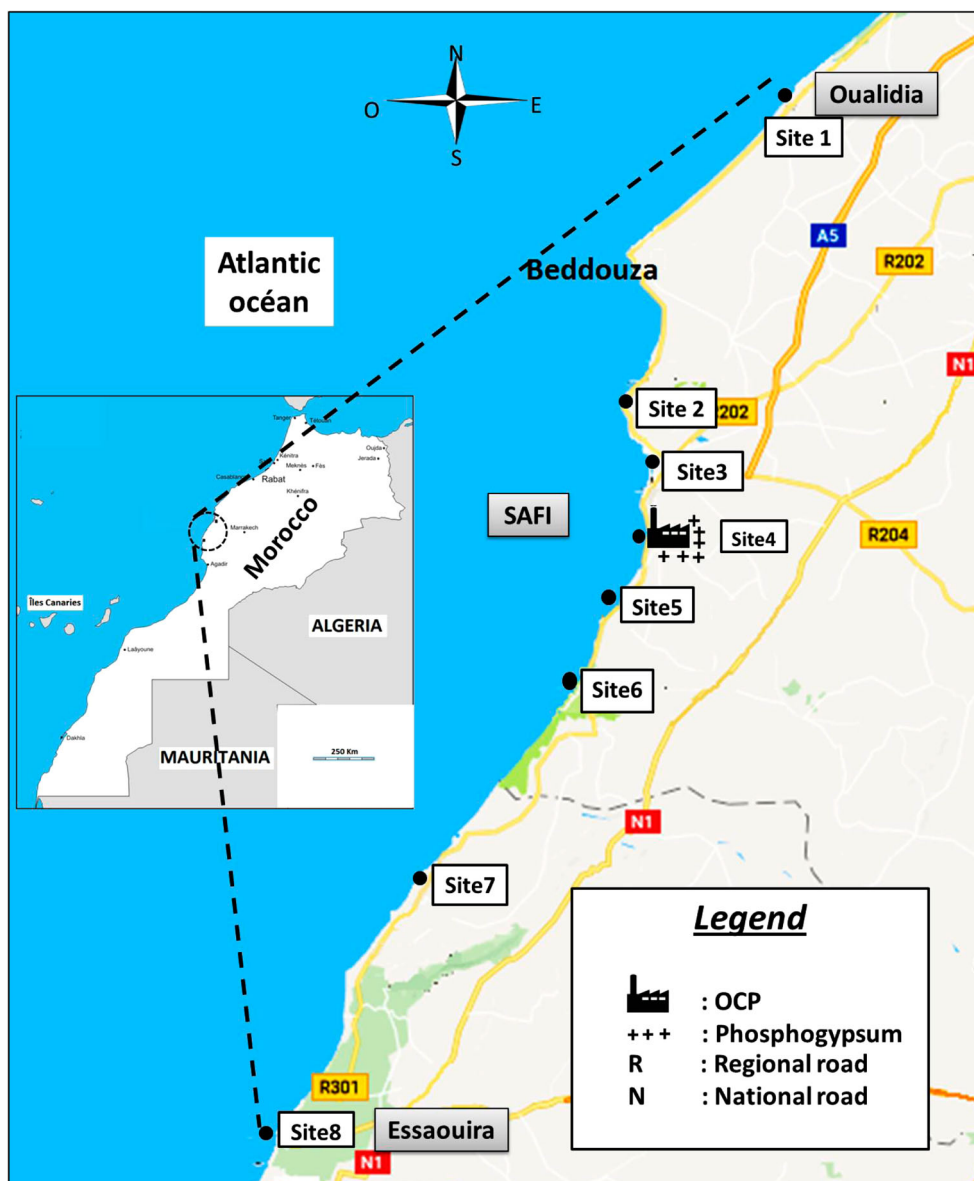


Figure 1. Location of the study area and the eight sampling sites.

dried in an oven at 105°C for 24 h, 2 g of each dried sample was powdered using an agate mortar and then calcined at 550°C for 5 h. After calcination, each sample undergoes oxidation with 5 mL of a H_2O_2 at 30% w/w (110 vol.) and then a hot acid attack with a mixture of 50% HCl (37% PA-ACS-ISO) and 50% HNO_3 (65% PA-ISO). The samples were analyzed with a ThermoFisher iCAP RQ ICP-MS spectrometer (International Equipment Trading, USA) using the Qtegra software. Four replicates were performed for each sample and 10 metallic trace elements were analyzed: As, Cd, Co, Cr, Cu, Ni, Pb, Ti, V and Zn. Lead isotopes ^{206}Pb , ^{207}Pb and ^{208}Pb were also assayed by ICP-MS to identify Pb anthropogenic sources of atmospheric pollution. The analytical quality of the MTE measurements was

Table 1. characteristics of the sample sites.

Sites	Description	Distance to the industrial zone of Safi in Km
S1	The site of Qualidia (Village Lakouassem) is a rural site far from any urban or industrial pollution, it was chosen as a control site.	60
S2	The northern part of Safi city, no industrial activity and a very low population density.	20
S3	It is the centre of the city with a high population density whose activities (urban traffic; domestic activities; ..) pollute the air and whose impact on air quality is added to that of industrial origin.	8 à 10
S4	It is the industrial zone where air quality is strongly influenced by the activities of the phosphate processing complex (OCP) and other industrial and urban activities.	0
S5	In 2017, the construction of a coal plant has already been launched to be operational in 2019.	12
S6	It is the village of Souiria, it is a little urbanised site with a summer tourist vocation.	33
S7	It is the village of Bhibeh, little urbanised, It is a beach with modest tourist vocation.	58
S8	The city of Essaouira, a small non-industrial city with a tourist vocation, the main source of air pollution is urban traffic and industrial processing activities (agri-food, textile, parachechemical etc.).	120

verified by using a SPEX Certiprep CLMS-SETN multi-element standard solution (Thermo-Fisher) (ISO/IEC 17025:2005).

Statistical analysis

The analysis of MTE was done in four replicates per sampling site. The results are expressed in mean \pm standard error (SE). To assess the significant differences between sampling sites, One-way Analysis of Variance (ANOVA) and Tukey tests were performed using IBM SPSS statistics 20.0 software (IBM corp. 2020). Multivariate analysis was made to identify correlations between the individuals (sampling sites as Observations) and variables (lichens species and MTE concentrations) using Principal Component Analysis (PCA) with the software R version 3.4.3 [26]. To determine the sources of MTE atmospheric contamination, we also used Zn/Pb and Zn/Cu ratios. Our reference is the value of these two ratios in the upper continental crust (UCC). Anthropogenic inputs of Zn, Cu and Pb cause the value of this ratio to shift below or above its geological value.

Results

Spatial variation of MTE contents

The ANOVA analysis showed significant ($p < 0.05$) effect of sampling site on lichens MTE bioaccumulation. Figure 2 shows the variation in the MTE for *Xantoria parietina* and supplementary Figures 1, 2 and 3 present it for the other lichen species analyzed in this work. In all species, the MTE concentrations were significantly increased with increasing urbanisation. The highest levels of bioaccumulated MTE were recorded in peri-urban and urban sites, especially those located in industrial area of Safi (S3 and S4). In contrast, the MTEs concentrations were significantly lowest in the rural sites (S1 and S7) far from any urban activity.

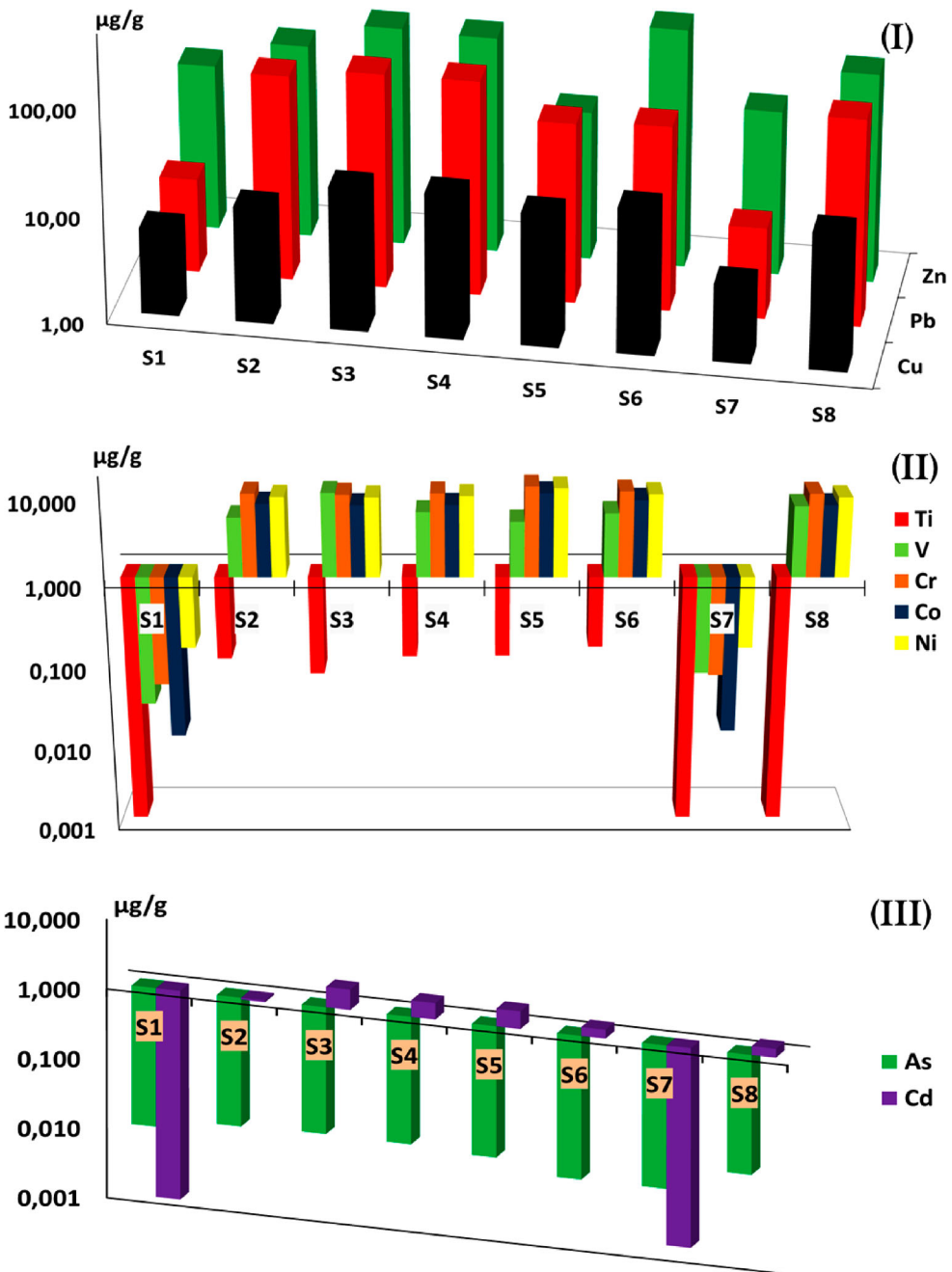


Figure 2. MTE contents in *Xanthoria parietina*. Each value represents the mean \pm SD of four replicates. The concentration means are significantly different at $p = 0.05$. Graphs (I), (II) and (III) are in semi-log scale.

Three profiles of MTE lichen bioaccumulation can be distinguished: MTE with concentrations less than $2 \mu\text{g/g}$ (As and Cd), MTE with concentrations between 2 and $20 \mu\text{g/g}$ (Ti, V, Cr, Co, Ni) and MTE with concentrations exceed $20 \mu\text{g/g}$ (Cu, Pb and Zn). MTE with

concentrations below 2 µg/g (As and Cd) show non-significant variations between the rural site S1, urban and peri-urban sites, but MTE with concentrations greater than 15 µg/g show significant variations between rural sites S1 and S7 (low levels), urban and peri-urban sites (very high levels).

The sampling site seems to have an impact on the bioaccumulation potential of the studied lichens. Using our database of bioaccumulation levels in the studied samples and to visualise possible correlations between the 8 sites of this study, the PCA analysis by the HCPC function (Hierarchical Principal Component Clustering) [from the FactoMineR package] [26] has differentiated sites where lichen samples have similar bioaccumulation values (Figure 3):

- The sites S1 and S7 (Cluster 1).
- The sites S2, S5 and S6 (Cluster 2).
- The site S8 (Cluster 3).
- The sites S3 and S4 (Cluster 4).

Effect of lichen species on MTE accumulated levels

The foliose lichens (*Xanthoria calcicola* and *Xanthoria parietina*) accumulate more Co, Cd, Cu, Pb and Zn than the fruticose lichens (*Ramalina pollinaria* and *Ramalina Lacera*), however, no significant difference was observed for Pb (Figure 4 Supp).

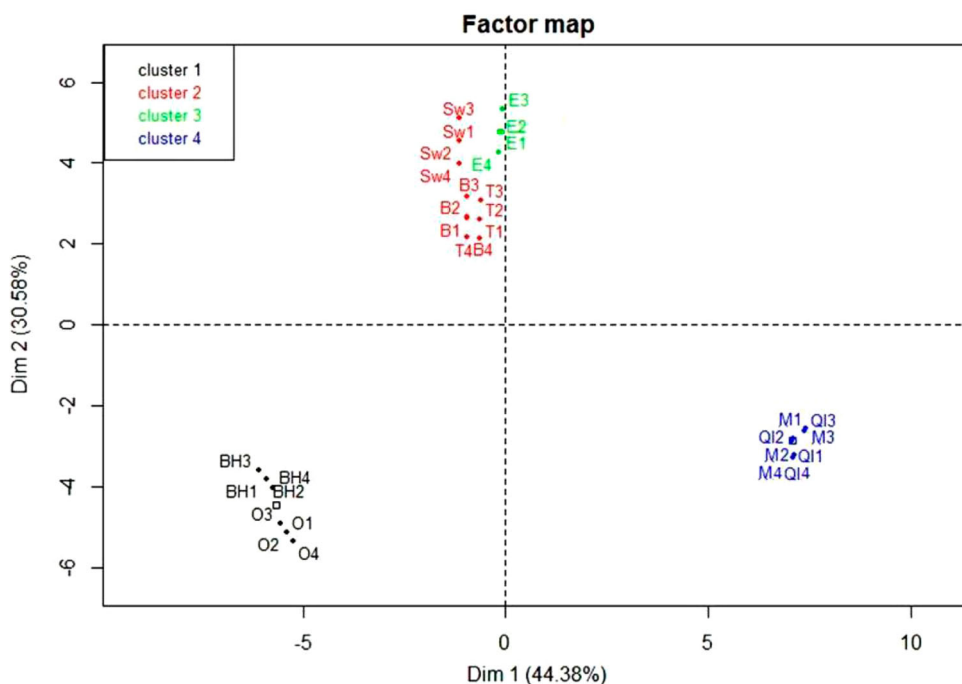


Figure 3. Factorial map of the studied sites (Performed with the HCPC function (Hierarchical Principal Component Clustering) [from the FactoMineR package] [26]). O: Site S1(Oualidia village) B: Site S2 (Borj Nador) M: Site S3 (Massira) QI: Site S4 (Industriel Zone) T: Site S5 (Thermique de Safi) Sw: Site S6 (Swiria Qdima) BH: Site S7 (Bhibeh village) E: Site S8 (Essaouira city).

The genus *Ramalina* accumulates more Nickel (*Ramalina Lacera* 8.33 µg/g (± 0.057), *Ramalina pollinaria* 9.58 µg/g (± 0.004)) than the genus *Xanthoria* (*Xanthoria calcicola* 6.73 µg/g (± 0.12), *Xanthoria parietina* 7.21 µg/g (± 0.19)). PCA analysis (by R. software [26]), of bioaccumulated MTE levels in lichens (Figure 5 Supp), confirmed this observation and revealed two groups:

- A group that represents the phenomenon of bioaccumulation in *Xanthoria* genus;
- The second group represents the phenomenon of bioaccumulation in *Ramalina* genus.

The regional annual precipitation (Prec) and the mean annual temperature (Temp) seem to not influence the bioaccumulation of MTE in these lichens. The genus *Ramalina* is more influenced than *Xanthoria* genus by the distance between the industrial zone and sampling sites (DistPoll).

Field exploration confirms this difference in sensitivity to air pollution between the two genus: in the urban sites S3 and S4, the genus *Ramalina* as well as all fruticose lichens disappear [11].

Effect of the support

Results show that corticolous form of *Ramalina* accumulated higher Cd levels than the saxicolous form at all sites (Figure 4). The same remark for the *Xanthoria* genus with the exception of the control site S1 and the rural site S7 where the saxicolous form accumulated more Cd than the corticolous form. The ANOVA test shows a p -value = 0.002 for *Ramalina* for all sites; however, the difference was not significant for *Xanthoria* in sites S2, S3, S4, S5, S6 and S8.

Lichens living on limestone (saxicolous) accumulate more Cu and Zn than the forms living on the tree (corticolous) except for *Ramalina* in the case of copper where the corticolous form and the saxicolous form present very close maximum mean contents. In this case, the ANOVA test performed on the means of Zn and Cu bioaccumulated in the

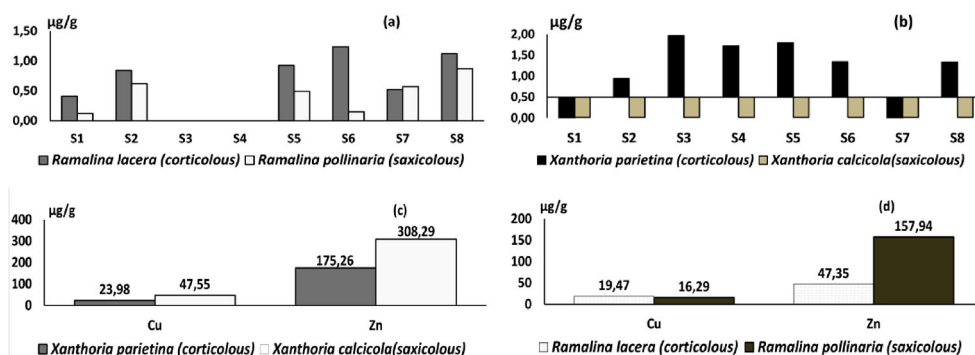


Figure 4. Maximum Cd levels in the genus *Ramalina* (a) and in the genus *Xanthoria* (b). (c) and (d): Comparison of maximum Cu and Zn levels according to the support. The concentrations are significantly different at $p = 0.05$. The scale of the graph is arithmetic. (Corticolous: living on tree) (Saxicolous: living on rock).

different sites showed a p -value lower than 0.05, which expresses a significant difference between the saxicolous and corticolous.

Isotopic signature: $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$

The $^{206}\text{Pb}/^{207}\text{Pb}$ ratio varies in the studied lichens between 1.15 (± 0.0021) and 1.23 (± 0.0375), while the $^{208}\text{Pb}/^{207}\text{Pb}$ ratio varies between 1.75 (± 0.001) and 2.01 (± 0.013).

In *Xanthoria calcicola*, the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ isotope ratios are maximum at the S2 site and minimum at S1 and S7; for *Xanthoria parietina* the maximum is at the S4 site while the minimum is at S7. In the case of the *Ramalina Lacera*, the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ isotope ratios are maximal at site S6 and minimal at S7. In *Ramalina pollinaria* the $^{208}\text{Pb}/^{207}\text{Pb}$ ratio shows a maximum at the S6 site and a minimum at S7. $^{206}\text{Pb}/^{207}\text{Pb}$ ratio is almost stable along the study transect (Figure 6 Supp).

PCA analysis, of the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ ratio values in lichens (HCPC by R. software [26]) confirmed this observation and revealed three groups of isotope ratios values (Figure 7 Supp):

- Very low values of isotopic ratios ratios $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ especially in corticolous forms (*Xanthoria parietina* and *Ramalina Lacera*) at rural sites (S1 and S7) (Cluster 1 on Figure 7 supp).
- Very high values of isotopic ratios ratios $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ especially in the genus *Xanthoria* at urban sites (S3 and S4) (Cluster 3 on Figure 7 supp).
- Intermediate values of isotopic ratios ratios $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ for both genus especially in the peri-urban site S5 (Cluster 2 on Figure 7 supp).

Table 2 presents a comparison between the isotopic signature ($^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$) of the studied lichens and other measurements. The value of the $^{206}\text{Pb}/^{207}\text{Pb}$ isotopic ratio of the studied lichens is greater than that of leaded gasoline (marketed in Morocco before 2009), on the other hand, this value is very close to that of road dust, road traffic brake and tire wear [27]. We observe the reverse for the value of the $^{208}\text{Pb}/^{207}\text{Pb}$ isotope ratio, this allows us to hypothesise that the source of lead bioaccumulated by lichens in Safi is not related to the gasoline used in road traffic. Indeed these lichens were collected 8 years after the end of the use of leaded gasoline in Morocco.

Use of elemental ratios

The calculation of an average value of Zn/Cu and Zn/Pb ratios in the studied lichens is summarised in the Table 1 Supp. Figure 5 compare these ratios to elemental ratios referenced in the literature [34–38].

➤ Zn/Pb ratio

Any increase in the Pb content decreases the value of Zn/Pb ratio below the natural geochemical background value ($\text{Zn/Pb}_{\text{UCC}} = 4.18$ [39]) reflecting various anthropogenic sources generating this element. This is the case for Fuel combustion activities (Zn/Pb

Table 2. Comparison between the isotopic signature ($^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$) of the studied lichens and other measurements.

	$^{206}\text{Pb}/^{207}\text{Pb}$	$^{208}\text{Pb}/^{207}\text{Pb}$	Location	Date	Reference
Lichens	1.1538–1.2308	1.7498–2.0128	Safi	2021	In literature
	1.082–1.147	2.337–2.370	Johannesburg	2006	[28]
	1.146–1.186	2.423–2.460	Agadir	2011	[4]
	1.1465–1.1640	—	France	2018	[19]
Aérosols	1.141 ± 0.001	2.418 ± 0.001	Maroc (Nador)	1999	[29]
Gasoline with lead	1.116 and 1.125	2.404	Agadir	1997	[30]
	1.076–1.081 and	2.348–2.360.	Agadir	2011	[4]
Road dust	1.1163–1.1417	2.117–2.1446	London	2017	[27]
Brake dust	1.2200	1.9598			
Tire wear	1.1502	2.1075			
Phosphogypse	1.84 ± 0.2	2.399 ± 0.2	Safi	2006	[31]
Phosphate of Morocco	2.08 ± 0.3	2.37 ± 0.3			
Aerosols	1.12–1.14	2.02–2.15	Tangier	2004	[32]
Pb industry	1.13–1.17	2.05–2.15	France	2015	[22]
natural mineral matter	1.19	2.47	—	1993	[33]
upper crust exposed to weathering					
Saharan dust	1.23	2.49	—	2003	[34]

= 0.7) [39]. The Zn/Pb ratio decreases in the site S2, S3, S5, S6 and S8 with values lower than 2 indicating anthropogenic activities generating Pb (waste incineration, fuel combustion). On the other hand, at the control site S1 and at the site S7, the level of Pb is low, which increases the value of the Zn/Pb ratio (Figure 5).

➤ Zn/Cu ratio

The value of the natural geochemical background is $\text{Zn}/\text{Cu}_{\text{UCC}} = 2.87$ [39]. The Zn/Cu ratio in urban and peri-urban sites is greater than 3 indicating anthropogenic activities that generate Zn (waste incineration, fuel combustion). Sites S3 and S5 revealed a Zn/Cu values greater than 10: Four times greater than the geochemical background value (Figure 5).

Discussion

- The atmospheric MTE levels bioaccumulated by the four lichen species *Xanthoria calicicola*, *Xanthoria parietina*, *Ramalina Lacera* and *Ramalina pollinaria* show variations depending on the species and the space. Lead and zinc showed maximum levels of 126.90 and 308.28 $\mu\text{g/g}$ respectively. As, Ti, Cd and Co have mean levels that do not exceed 2.05 $\mu\text{g/g}$. V, Cr and Ni have mean contents which oscillate between 5 and 13 $\mu\text{g/g}$ while copper reaches a mean content of 47.55 $\mu\text{g/g}$. The maximum mean levels of bioaccumulated MTE were very different depending on the sampling site. The approach by hierarchical classification on principal components (Hierarchical Clustering on Principal Components) [26] revealed three types of sites (Figure 3):
- Type 1: Safi city centre and the industrial zone (S3 and S4) where the disappearance of *Ramalina* genus indicates poor air quality and a high level of MTE contamination.
- Type 2: Borj nador site, thermal station, Souiria and Essaouira (S2, S5, S6 and S8). These are the areas where the MTE contamination is medium.

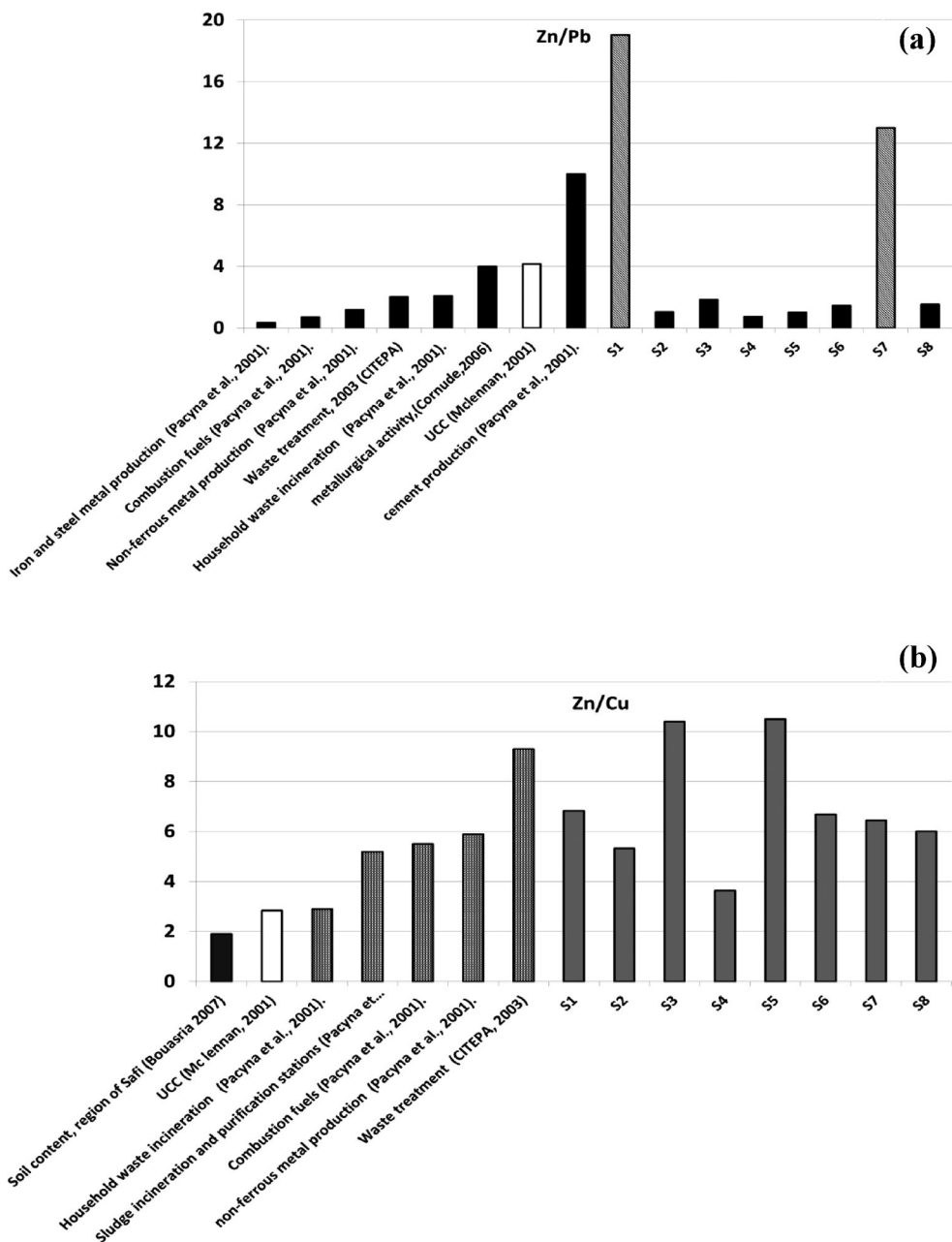


Figure 5. Zn/Pb ratio (a) and Zn/Cu ratio (b) of different emissions sources compared to the studied sites (S1 to S8).

- Type 3: Oualidia and Bhibeh villages (S1 and S7), these are rural areas with a low level of contamination and good air quality.

The disappearance of the genus *Ramalina* from the S3 and S4 sites reflects a significant effect of the sampling site on its ability to bioaccumulate MTE, this effect is reflected in

the genus *Xanthoria* by high concentrations of MTE. The extra figure 8 summarises this result in the studied species for lead.

- Since the ban on leaded gasoline, many studies have reported an increase in the values of the $^{206}\text{Pb}/^{207}\text{Pb}$ ratio in the studied samples [40,41]. In Morocco, the marketing of unleaded gasoline began in 2009, so the lichens collected in Agadir in 2005 (Table 2) [4] still had a legacy of bioaccumulated Pb before 2009. On the other hand, the lichens collected in Safi in 2017 must live under ecological conditions where human activities used gasoline without Pb. In the studied lichens, the decrease of the $^{208}\text{Pb}/^{207}\text{Pb}$ isotope ratio and the slight increase in the value of the $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio can be linked to the ban on the use of leaded gasoline. The Pb bioaccumulated is the result of an overlap of anthropogenic sources as transport, waste incineration, industry and geochemical origin (Figure 6).
- The Zn/Pb ratio in the studied sites is close to 2 while Zn/Cu is greater than 5. These two values indicate contamination of the studied lichens by emissions from waste incineration activities and potentially wear and tear on vehicle tires and brakes. This remark can be explained by the industrial character of the city. Safi is considered the pottery capital of Morocco and there are 42 workshops equipped with 27 gas kilns and 72 traditional kilns (Provincial delegation of handicrafts in Safi city, 2020). The traditional kilns use energy from the incineration of wood branches, plastic waste and tires, these materials generate gaseous emissions and ash ((Figure 9 Supp). In the United States in 1990, analyses confirmed that Zn is released into the air from waste incineration and tire wear [42]. In urban areas, Zn, Pb and Cu are most released from tire and brake wear [43].

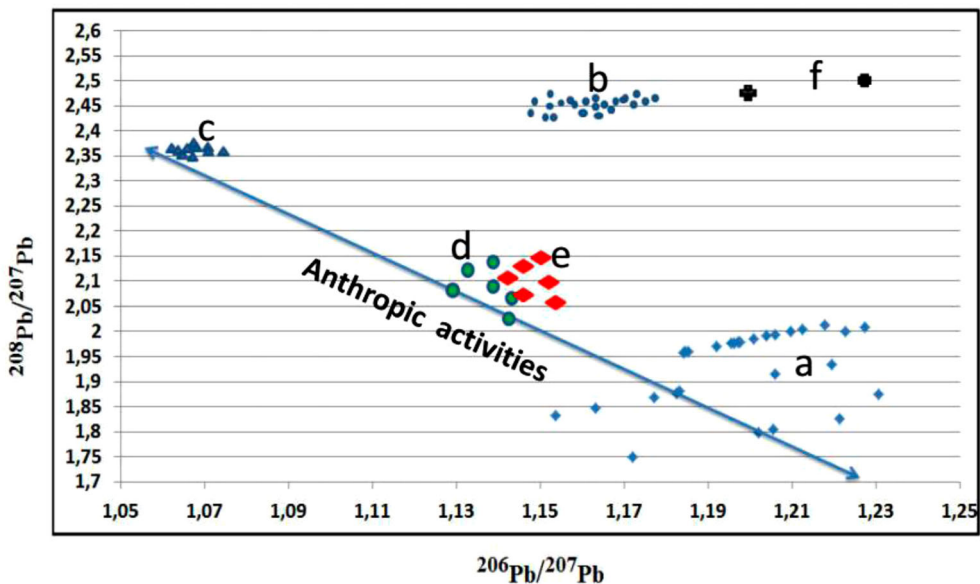


Figure 6. Diagram of Isotopic signature $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ of the lichens collected at Safi in 2017 (a) compared with lichens collected at Agadir city in 2011 (b) [4], leaded gasoline sampled in Morocco in 2005 (c) [4], Aerosols of Tangier city (d) [32], Pb industry in France (e) [27] and natural mineral matter (f) [33,34].

- The 'Office Chérifien des Phosphates (OCP)' is an important industrial complex of phosphate processing installed in the city of Safi since 1960s. The solid chemical discharges of this industry include phosphogypsum (PG) stored near the plants, its erosion releases highly polluting substances, namely: sulphates, fluorosilicates, hydrogen fluorides, phosphorus, cadmium, 226Ra [44] and MTE [45]. Atmospheric agents can transport these pollutants towards the neighbouring areas [46]. In the studied urban and peri-urban sites (S2, S3, S4, S5 and S6), lichens show maximum levels of bioaccumulated Pb, Zn and Cu. The erosion of PG embankments and wind transport seems to promote the enrichment of the air at Safi by these MTE. Numerous studies from around the world have confirmed that MTE such as Cd, Cu, Pb and Zn can be dispersed downwind from their sources [47,48]. Samples of the lichen *Hypogymnia physodes* were collected between 2002 and 2017 within a radius of approximately 150 km of an oil sands extraction site in Canada. Increased levels of bioaccumulated MTE (aluminium, nickel, strontium, vanadium) in these lichens were observed, these MTE were transported from the oil sands extraction site and dispersed by wind [49].

At the S4 site, air quality measurements showed extremely high fine particle pollution around the OCP plant in Safi city. The 'SWISSAID' research team [50] measured, in February and March 2019, between 150 and 430 $\mu\text{g}/\text{m}^3$ of fine particles $\text{PM}_{2.5}$. This is an exceedance of 6–16 times the daily value (25 $\mu\text{g}/\text{m}^3$) recommended by the World Health Organization.

The majority of $\text{PM}_{2.5}$ at site S4 come from phosphogypsum backfills, their composition in MTE should be very similar to the phosphogypsum [45]; their dispersal by the wind and their landing on the lichens would therefore be one of the causes of enrichment of lichens by MTE. Indeed, the data relating to the wind recorded in Safi by the meteorological services highlight daily, weekly and monthly variations in the speed and direction of the wind, which mix the air masses and allow the dispersion of pollutants over long distances.

Conclusion

The bioaccumulation approach and ICP/MS analysis were used to assess the levels of MTE bioaccumulated by four lichen species. Two MTE had maximum levels: lead and zinc, with 126.90 and 308.28 $\mu\text{g}/\text{g}$ respectively. Arsenic is the least present with contents sometimes null and the mean does not exceed 0.02 $\mu\text{g}/\text{g}$. The other MTE (V, Cr, Mn, Cu, Ti, Co, Ni) have mean contents that oscillate between 0.01 and 20 $\mu\text{g}/\text{g}$.

The coastal Safi-Essaouira area is characterised by a rural–urban gradient of MTE pollution. The maximum mean levels of MTE bioaccumulated by the studied lichens were very high in the urban sites S3 and S4. These levels decrease in the peri-urban sites (S2 and S5) and in low urbanised sites (S6 and S8), and they were very low in rural sites S1 and S7. This conclusion confirms the results of our previous research using lichen bioindication approaches.

The lead isotopic signature ($^{206}\text{Pb}/^{207}\text{Pb}$, $^{208}\text{Pb}/^{207}\text{Pb}$) of studied lichens indicates that the source of bioaccumulated lead is not related to gasoline used in road traffic but to other human activities. The Zn/Cu, Zn/Pb ratios indicate that there is an input of MTE from multiple anthropogenic activities which overlap with the geochemical inputs. In

urban and peri-urban areas of Safi, the phosphate industry, waste incineration, the artisanal activity of pottery and the particles emitted by the wear of tires, brakes and car exhausts are potential sources of enrichment of lichens by MTE. The movements of the air masses facilitate the dispersion of aerosols carrying MTE from the generating sources to be deposited on the lichens.

In Safi-Essaouira coastal area, the accumulation of MTE by lichens presents a highly significant correlation with the proximity of the urban and industrial area of Safi city. This accumulation also depends on ecological conditions such as wind dominance, lichen species, lichens biological spectrum and the nature of the lichen support.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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