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Biomonitoring of air pollutants by using lichens (*Evernia prunastri*) in areas between Kenitra and Mohammedia cities in Morocco

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Abstract: El Rhzaoui G., Divakar, P.K., Crespo, A. & Tahiri, H. *Biomonitoring of air pollutants by using lichens (Evernia prunastri) in areas between Kenitra and Mohammedia cities in Morocco. Lazaroa 36: 21-30 (2015).*

In this study, *Evernia prunastri*, a lichen growing in its natural habitat in Morocco was analysed for the concentration of five heavy metals (Fe, Pb, Zn, Cu and Cr) from eleven sites between Kenitra and Mohammedia cities. The control site was Dar Essalam, an isolated area with low traffic density and dense vegetation. In the investigated areas, the concentration of heavy metals was correlated with vehicular traffic, industrial activity and urbanization. The total metal concentration was highest in Sidi Yahya, followed by Mohammedia and Bouznika. The coefficient of variation was higher for Pb and lower for Cu, Zn and Fe. The concentrations of most heavy metals in the thalli differed significantly between sites ($p<0.01$). Principal component analysis (PCA) revealed a significant correlation between heavy metal accumulation and atmospheric purity index. This study demonstrated also that the factors most strongly affecting the lichen flora were traffic density, the petroleum industry and paper factories in these areas. Overall, these results suggest that the index of atmospheric purity and assessment of heavy metals in lichen thalli are good indicators of the air quality at the studied sites.

Keywords: Biomonitoring, *Evernia prunastri*, heavy metals, atomic absorption spectrometry (AAS), index of atmospheric purity.

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En este trabajo se presentan los resultados de un estudio realizado en *Evernia prunastri*, un liquen frecuente en hábitats naturales de Marruecos. Se analizó la concentración de cinco metales pesados (Fe, Pb, Zn, Cu y Cr) en líquenes de once localidades diferentes, entre Kenitra y Mohammedia. La localidad de control es Dar Essalam, una zona aislada con baja densidad de tráfico y con vegetación densa. En las áreas investigadas, la concentración de metales pesados se correlacionó con el tráfico de vehículos, la actividad industrial y la densidad de población. La concentración total de metal fue más alta en Sidi Yahya, seguida de Mohammedia y Bouznika. El coeficiente de variación fue mayor para Pb e inferior para Cu, Zn y Fe. Las concentraciones de la mayoría de los metales pesados en los talos difirieron significativamente entre poblaciones ($p<0,01$). El análisis de componentes principales (PCA) reveló una correlación significativa entre la acumulación de metales pesados y el índice de pureza atmosférica. Este estudio demostró también que los factores que afectan con mayor fuerza a la flora de líquenes fueron la densidad del tráfico, la industria del petróleo y las fábricas de papel. En general, estos resultados sugieren que el índice de pureza atmosférica y la evaluación de los metales pesados en los talos de liquen son buenos indicadores de la calidad del aire en las poblaciones estudiadas.

Palabras clave: Biomonitoreo, *Evernia prunastri*, metales pesados, espectrometría de absorción atómica (AAS), índice de pureza atmosférica.

INTRODUCTION

Lichens are valuable as bioindicators of environmental pollution, climate change and ecological continuity (Hawksworth & *al.*, 2005; Nash, 2008; Bjerke, 2011; Leavitt & St. Clair, 2011). Several aspects of lichen biology are useful as indicators for evaluating air quality

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(Calvelo & *al*., 2009) and can determine local air quality patterns on the basis of their sensitivity (Hawksworth & Rose, 1970; Cuny, 2012; Evju & Bruteig, 2013). Epiphytic lichens are very sensitive to air pollution. Alterations in air quality directly affect lichen diversity and are therefore generally considered to be good indicators of air quality (Seaward, 1993; Van Doven & Ter-Braak, 1999; Nimis & *al*., 2002; Crespo & *al*., 2004; Adamo & *al*., 2007; Balabanova & *al*., 2012; Cuny, 2012; Evju & Bruteig, 2013)

Lichens are the biotic samples most frequently used as biomonitors because of their low cost and easy sampling, which allow extensive areas to be monitored (Cansaran-Duman & *al*., 2011). Additionally, lichens have certain characteristics, such as their perennial nature, the absence of roots or other special organs for the uptake of nutrients, and the lack of cuticle (enables them to absorb metals directly from the atmosphere), that make them ideal biomonitoring organisms. Their great capacity for accumulating air pollutants (e.g. heavy metals) is another feature that makes them highly suitable organisms for biomonitoring studies (reviewed in Garty, 2001; Nimis & *al*., 2002; Nash, 2008 and Giordano & *al*., 2011).

The diversity of epiphytic lichens is commonly used as a sensitivity indicator of the biological effects of air pollutants, and mapping lichen diversity has become routine in several countries since it is quick, inexpensive and provides results on which predictions for human health can be based (Nimis & *al*., 2002; Hauck & *al*., 2013). In addition to floristic changes, lichens have been widely used to monitor the deposition patterns of heavy metals. A comparison of the elemental composition of the thalli from isolated sites can reveal different levels of accumulation of certain elements, thereby providing clues to the sources of pollution (Calvelo & *al*., 2009). They integrally reflect the environmental influence and are one of the best tools for assessing heavy metal contamination (Van Doven & Ter Braak 1999; Shukla & Upreti 2007, 2008; Bajpai & *al*., 2004, 2010; Rani & *al*., 2011). There is usually a close correlation between the pattern of lichen species distribution and the trace element content of the surrounding air (Shukla & Upreti, 2007).

Valuable information can be obtained by analysing and comparing the floristic composition at the evaluated sites. While qualitative information can be gathered simply by listing the presence or absence of species, quantitative information can be amassed by calculating indices, such as the Index of Atmospheric Purity (IAP, Calvelo & *al*., 2009). The IAP identifies how lichen distribution, frequency and cover change over space and/or time with respect to pollution gradients (Gries, 1996). The method is used in many countries to measure air quality (Deruelle & García Schaeffer, 1983; Giralt & *al*., 1989; Pirinstos & *al*., 1993; Loppi & *al*., 1996; McCarthy & *al*., 2009).

A few studies have compared the data concerning element accumulation in lichen thalli with the IAP (reviewed in Garty, 2001; McCarthy & *al*., 2009). For example, the correlation between concentration of accumulated elements in species used as biomonitors and IAP values were analysed by Herzig (1993). The author found a negative correlation between IAP and Al, Cl, Cu, Fe, Li, Pb and Zn, and a negative correlation between IAP and Cd, Cr, Cu, Hg, Ni, Pb and Zn in *Flavoparmelia caperata*. Similarly, the elemental composition of *Hypogymnia physodes* in Slovenia were analysed and compared the results with the IAP Jeran & *al*. (2002), also finding a negative correlation between IAP and As, Cr and Zn. However, there have so far been no studies published that assess air quality in Morocco using biological agents.

E. prunastri is a common lichen species that is widespread in the region. For this study, *E. prunastri* was chosen as a suitable bioindicator since its sensitivity to organic and inorganic compounds is well documented (Conti & *al*., 2004; Cansaran-Duman & *al*., 2011; Balabanova & *al*., 2012; Lackovicová & *al*., 2013; Sujetoviene & Silumpaite, 2013).The aim of this work was to assess air quality by 1) determining the concentration of heavy metals in the thalli of *Evernia prunastri*, and 2) measuring the correlation between the IAP and heavy metal concentrations. To address these objectives, many samples of *E. prunastri* were collected from different sites in Morocco. The levels of six heavy metals were measured and analysed. The heavy metal contents of the collected samples were determined by atomic absorption spectroscopy (AAS).

MATERIALS AND METHODS

STUDY AREA

The studied regions depicted in Figure 1 and Table 1 are located on the Atlantic coast between Kenitra and Mohammedia, Morocco, and enjoy a Mediterranean climate with an oceanic influence. They are highly susceptible to interference from the Atlantic Ocean during the rainy season. The summer, on the other hand, is dry and sunny, but this does not preclude the appearance of banks of mist and dew, which are both quite common during this period. However, rainfall is very variable between the north and the south. The total mean annual rainfall is 60 mm in Kenitra, 560 mm in Rabat and 400 mm in Casablanca. In contrast, temperatures are rather homogeneous, with the Atlantic currents moderating the climate to give mild winters and summers. The average temperature in January is 12-13°C; while in summer it is around 23°C.

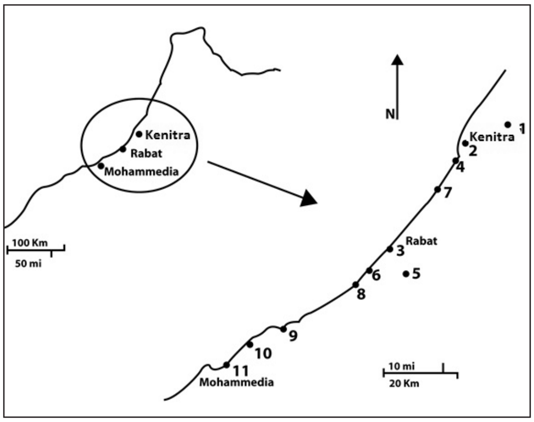

Figure 1. — Map of collection sites along the coastline between Kenitra and Mohammedia, Morocco.

Table 1
Localities of the lichen samples used in the study

Localities	Phorophyte	Pollution level
1. SidiYayha	*Quercus suber*	Open forest, high pollution
2. Kenitra	*Quercus suber*	Dense forest (Mamora), moderate pollution
3. Rabat	*Quercus suber*	Open area, moderate pollution
4. Mehdia	*Quercus suber*	Dense forest, low polution
5. Dar Essalam	*Quercus suber*	Dense forest, No pollution
6. Harhoura	*Quercus suber*	Dense forest, low pollution
7. Plage des Nations	*Quercus suber*	Dense forest, low polltion
8. Plage Sidi Abed	*Quercus suber*	Dense forest, low pollution
9. Bouznika	*Quercus suber*	Dense forest, moderate pollution.
10. Forêt Mansouriya	*Pinus sp.*	Dense forest, moderate pollution
11. Mohammedia	*Quercus suber*	Dense forest, high pollution.

Estimation of heavy metal concentrations

The concentrations of six heavy metals, Cr, Ni, Pb, Zn, Mn and Fe, in the thalli of *E. prunastri* collected from 11 sites were analysed (Table 1). In the laboratory, the thalli were carefully removed from the bark using a snapper blade and were put in the oven to dry at 120°C. The dried samples of *E. prunastri* were then ground to a powder and separated into 0.5 g amounts for further metal analysis. Lichen samples were chemically analysed after extraction with a mixture of HCL and HNO_3 (3:1) and heating to 80°C. Digestion was completed with the addition of a few drops of perchloric acid. This digest was filtered through Whatman filter paper, and the filtrate diluted to the desired volume with DDW. The total metal concentrations in the solutions were determined by atomic absorption spectrometry (AAS) using a PerkinElmer 2380 spectrometer in a flame correcting for Ca, Si and Al with HACH DR 30 (Rader & Grimaldi, 1961).

Index of atmospheric purity

The index of atmospheric purity (IAP) (LeBlanc & De Sloover, 1970; Garty, 2001; Calvelo & *al*., 2009; McCarthy & *al*., 2009) makes it possible to map the air quality of a determined area. The method evaluates the level of atmospheric pollution, based on the number (n), coverage frequency (f) and tolerance of the lichens in the study area. We used the formula:

$$IAP = \frac{1}{10} \sum_{1}^{n} Q \times f$$

where n=number of epiphytic species, Q=resistance factor (ecological index) of each species, and f=coverage frequency score of each species.

RESULTS AND DISCUSSION

Assessment of heavy metals with AAS

Heavy metal concentrations of *E. prunastri* samples taken from the studied sites, including the control site, are summarised in Table 2. Similarly, IAP values are presented in Table 3. The concentrations of Zn, Fe and Cu varied the least between the eleven sites.

In the case of zinc, the highest concentration was found in Sidi Yahya (1366 µg/g), followed by Mohammadia (108 µg/g) and Harhoura (103 µg/g). The contamination could have been due to charcoal, fuel use or the wear of vehicle tyres. The lowest concentration was found in the Sidi Abed area (25µg/g). The heavy metal content in *E. prunastri* collected in Karabük Turkey were measured by Cansaran-Duman & *al*. (2011), and the highest concentration of Zn 46.6 µg/g were detected in Kabakli kaya site. In the industrial site of central Italy the Zn concentration in this species was even much lower (7.0 µg/g; Conti & *al*., 2004). It is worth to underline that the values detected in these studies were relatively much lower than we found in the present investigation.

The level of lead (Pb) was also highest in Sidi Yahya (45.75 µg/g), followed by Bouznika, with a concentration of 27.89 µg/g and the Forêt de Mansouriya site (27.6 µg/g). Highly toxic Pb is released as a result of various industrial processes and is also associated with the vehicular traffic. Mohammedia is an industrial area whose chemical and parachemical industries are probably the source of the air contamination in Bouznika and at the Forêt de Mansouriya site. In previous studies Pb concentration was also detected in this species in other countries. Such as in urban area in central Lithuania the values were measured between 5-7 µg/g (Sujetoviene & Silumpaite, 2013) and in central Italy 3-7µg (Conti & *al*., 2004). Similarly, in Karabük, Turkey the value was measured between 1.31-5.17 µg /g (Cansaran-Duman & *al*., 2011).

Table 2
Heavy metal concentrations in *Evernia prunastri* thalli collected from different sites between Kenitra and Mohammedia. Concentrations are given in µg/g with variance (mean ± standard error) and coefficient of variation percent

Localities	Zn	Pb	Fe	Cu	Cr
Sidi Yahya	1366 ± 0.62	45.75 ± 0.77	15000 ± 443.84	13.86 ± 0.01	1774 ± 0.96
Kenitra	85 ± 2.99	5.7 ± 0.17	1990 ± 3.10	32.5 ± 0.31	570.33 ± 8.50
Rabat	95 ± 0.79	2.85 ± 0.06	2640 ± 4.54	66.66 ± 0.72	59.98 ± 0.73
Mehdia	69 ± 0.48	4.27 ± 0.05	2700 ± 99.83	31.96 ± 0.94	404.2 ± 1.947
Dar Essalam (control site)	67 ± 0.48	4.03 ± 0.016	1360 ± 2.07	7.34 ± 0.18	17.99 ± 0.82
Harhoura	103 ± 0.62	4.85 ± 0.08	2050 ± 10.80	42.65 ± 0.71	716.63 ± 1.09
Plage des Nations	109 ± 0.89	5.62 ± 0.18	2150 ± 3.60	17.64 ± 0.01	478.74 ±1.73
Plage Sidi Abed	25 ± 0.65	4.32 ± 0.21	1120 ± 4.28	13.07 ± 0.12	868.99 ± 1.79
Bouznika	92 ± 0.40	27.89 ± 0.36	3150 ± 3.49	38,31 ± 0.24	683.99 ± 1.79
Forêt Mansouriya	77 ± 0.48	27.6 ± 0.19	2450 ± 58.495	30.05 ± 0.48	675.61 ± 0.81
Mohammedia (plage Mimosa)	108 ± 28.52	4.6 ± 0.12	1550 ± 5.28	31.89 ± 0.06	80.14 ± 0.66
Coefficient of variation	1.85	28.17	1.40	0.52	2.16

The highest concentration of iron was registered, once again, in Sidi Yahya's samples (15.000 µg/g), while the lowest concentration was found at the Plage Sidi Abed site (1120 µg/g). The highest concentration of copper was recorded in Rabat (66.66 µg/g), followed by Harhoura (42.65 µg/g), the lowest concentration being found at the control site of Dar Essalam (7.34 µg/g). Compared the accumulated Cu content in *E. prunastri* to other studies, in this investigation Cu concentration was relatively high (7.34-66.66 µg/g; CANSARAN-DUMAN & *al*., 2011) with the content detected in Karabük, Turkey: 1.54-3.67 µg/g; and 4-7 µg/g in central Lithuania (SUJETOVIENE & SILUMPAITE, 2013); and 2–6 µg/g in central Italy (CONTI & *al*., 2004). The highest concentration of chromium was also found in Sidi Yahya (1174 µg/g), followed by Plage Sidi Abed (868.99 µg/g). In sites of other countries the recorded value was relatively very less such as the highest value of 5.75 µg/g was determined in Karabük, Turkey (CANSARAN-DUMAN & *al*., 2011). The most important sources of Fe, Cr and Cu pollution could be indicated as industrial activities like refining works and heavy traffic activity. There are installations of the paper and refining industry in Sidi Yahya, which could be the source of these contaminations in this area.

Evernia prunastri has been considered as a good bio-accumulator of metals (CERCASOV & *al*., 2002; CONTI & *al*., 2004). In our study, the highest concentrations of heavy metals in *E. prunastri* thalli were found in Sidi Yayha, followed by Mohammedia site. The high concentration of most of the heavy metals could be explained by air pollution arising from industrial activities and heavy vehicular traffic throughou the year.

The coefficient of variation for element concentration in *E. prunastri* was generally greater for Pb and Cr than for Cu, Fe and Zn, reflecting the nature of the elements entrapped by lichen. GARTY & *al*. (1977) suggested that copper is dispersed in small particles, while the higher coefficients of variation for Pb and Cr reflect a low rate of dispersion. In this study it is important to stress that metal accumulation in *E. prunastri* is exceptionally high in comparison with that found in *Phaeophyscia hispidula* in India by SHUKLA &UPRETI (2007), in which accumulated lead and iron had concentrations in the range of 231-425.9 µg/g and of 4.5-10.92 µg/g, respectively. It has been also estimated the range of concentrations of Fe and Pb in *Pseudevernia furfuracea* from Switzerland to be in the range of 1.56-1.097 µg/g and 39-177 µg/g, respectively (GARTY & AMMAN, 1987).

INDEX OF ATMOSPHERIC PURITY

Forty-four species of lichen were found along the coast between Kenitra and Mohammedia. The most common were *E. prunastri*, *Diploicia canescens*, *Physcia adscendens* and *Xanthoria parietina* growing on eutrophytic bark. At least five phorophytes have been analysed (CRESPO & *al*., 1977). In order to recover coefficient, we considered species with + as r=0 (BRAUN-BLANQUET, 1964), since r=0 does not give importance to less common species. With r=0 we obtained a simple representation of the different sites and a good individualization of zones, although the IAP was low because many species were found to be r = + (Table 3).

Table 3
IAP values of the sampled localities

Localities	IAP
1. Sidi Yayha	2
2. Kenitra	4.2
3. Rabat	15.6
4. Mehdia	17.64
5. Dar Essalam (control site)	24
6. Harhoura	4.88
7. Plage des Nations	13.84
8. Plage Sidi Abed	4.48
9. Bouznika	9
10. Forêt Mansouriya	6.92
11. Mohammedia (plage Mimosa)	7.2

COMPARISON OF THE IAP AND THE CONCENTRATION OF HEAVY METALS

The IAPs obtained were compared with the concentrations of heavy metals (Zn, Pb, Fe, Cu and Cr) measured in the specimens of *E. prunastri* from the 11 sites sampled (Table 1). To perform a multivariate analysis, an observation matrix based on 44 individuals, five elements (Zn, Pb, Fe, Cu, and Cr) and the IAP index (quantitative variable) and more qualitatively variable species was created. The measurements of the five elements in *E. prunastri* and the IAP from 11 localities were used to construct a correlation matrix (Table 4). Most pairs of variables were positively correlated except those featuring IAP, which was negatively correlated with all the elements.

In the PCA analysis (Table 5) the first two principal components explained 94.57% of the total variation, and all variables were positively correlated with the component. The second principal component explained 17.78% of the total variation. Quantitative variables are depicted in Figure 2. The principal component analysis separated the IAP from the contaminant elements, placing Cr, Fe and Zn in one group, leaving Cu and Pb in an isolated positions. Based on the values of heavy metal concentrations and the IAP values, the PCA analysis separated the localities into two groups and observation 1 (Sidi Yayha) that can be considered as an outlier (Figure 3). The analysis confirmed Sidi Yayha as the most contaminated area in having Pb, Zn, Cr and Fe with very low values of IAP. This may be attributed to the large paper industry works located at this site, since they are probably responsible for the emission of all the heavy metals emitted into air, especially Zn, Cr and Fe. The second cluster groups Harhoura, Sidi Abed, Bouznika, Mansouriya and Mohammedia. This group is characterized by an IAP between 4.88 and 9 with a high level of Cu, due to the emissions from the petrol industry that is concentrated in Mohammedia. Harhoura and Sidi Abed had low IAPs due to the traffic density and emissions of the cement industry and the metallurgic activities in Temara. The third cluster includes sites with high PCA values, between

Table 4
Matrix of correlation

Variables	localities	Zn	Pb	Fe	Cu	Cr	IAP
Localities	1	-0.479	-0.032	-0.487	0.101	-0.452	-0.095
Zn	-0.479	1	0.764	0.990	-0.286	0.992	-0.385
Pb	-0.032	0.764	1	0.810	-0.123	0.788	-0.441
Fe	-0.487	0.990	0.810	1	-0.213	0.986	-0.368
Cu	0.101	-0.286	-0.123	-0.213	1	-0.318	-0.024
Cr	-0.452	0.992	0.788	0.986	-0.318	1	-0.440
IAP	-0.095	-0.385	-0.441	-0.368	-0.024	-0.440	1

13.84 and 24. These sites are near forests, such as the Plage des Nations locality, Mehdia, which is a nature reserve, and the Dar Essalam site, which is dense forest far from heavy traffic and anthropogenic activities. The low concentration of most heavy metals in the lichens from Dar Essalam reflects very low traffic activity, its proximity to a very dense forest, and its distance from industrial activities.

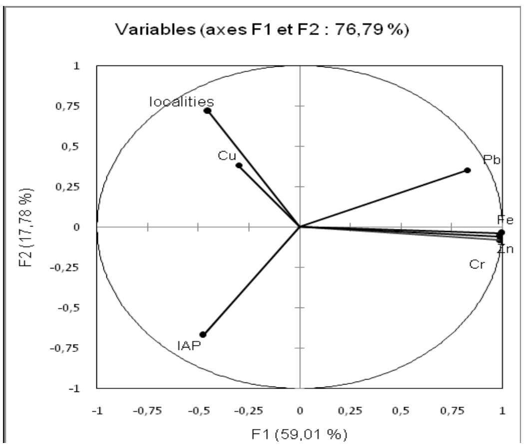

Figure 2. — First factorial plane of PCA: quantitative variables.

During the last 30 years, many studies have championed the possibility of using lichens as biomonitors of air quality on the basis of their sensitivity to various environmental factors, which can provoke changes in some of their components and/or specific parameters (for review, see (for review, see CONTI & CECCHETTI, 2001). As expected, our study with the AAS analysis and IAP index showed that the concentration of absorbed heavy metal element increased from Rabat to Sidi Yayha.

Table 5
Percent variance explained and coordinates of active variables for the first components from the principal component analysis. Percent variance explained (variance explained for each axis/total variance) *100 IAP: Index of atmospheric purity.

	F1	F2
Percent variance explained	59.009	17.777
Zn	23.417	0.523
Pb	16.611	10.069
Fe	23.470	0.276
Cu	2.194	11.674
Cr	23.877	0.116
IAP	5.506	36.062

While we found a positive correlation of most of the elements in IAP analysis, we unable to correlate our data with gaseous air pollutants, such as nitrogen compounds and sulphur dioxide. Gaseous air pollutants as another factor cannot be discarded. Further, in a coastal site, effects of differences in salt brought inland by spray and winds from the sea may well also influence the observed lichen distribution patterns.

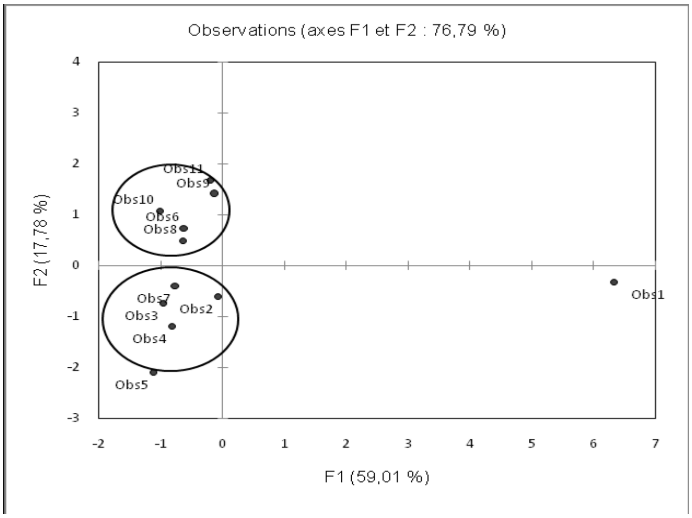

Figure 3. — First factorial plane of PCA: collection. Obs represents observation and Numbers refer to localities of Table 1.

CONCLUSION

We have identified three sources of contamination. The first was the paper industry, which is responsible for the emission of zinc, chrome and iron, and negatively affects air quality. Further studies should investigate contamination of the soil and that in other areas, to make an assessment of nearby sites. The second source of contamination was the combination of the dense traffic and the petroleum industry in Mohammedia, affecting the entire area of Mohammedia-Mansouriya-Bouznika. The final source was the cement industry in Temara, which affects the air quality of Harhoura and Sidi Abed. We unable to analyze gaseous air pollutants (e.g. nitrogen compounds and sulphur dioxide) and these could be other sources of contamination in the studied areas. Moreover, effects of differences in salt brought inland by spray, winds from the sea and dust eutrophication may well also influence the observed lichen distribution patterns in coastal areas.

The analysis of heavy metals provides information about the air quality in the study area and can also identify possible sources of contamination. However, this kind of study requires expensive equipment. In contrast to heavy metal analysis, IAP is a simple, non-destructive method that can be used when an analysis needs to be done over a large scale and over a long period of time in order to investigate how the contamination varies. It is an inexpensive method but one that does require specialised knowledge to identify lichenous flora.

Although the two methods are complementary, environmental assessment could initially be done with IAP analysis to identify critical areas, which could then be investigated further by the other method in order to obtain reliable results.

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