International Journal of Innovation Sciences and Research
Vol.5, No, 10, pp. 878-884, October 2016

REVIEW ARTICLE

USE OF LICHENS AS BIO-INDICATORS IN ASSESSING THE LEVEL OF AIR POLLUTION IN THE REGION OF BIZERTE

***Karima Tabbabi and Tijani Karmous**

Laboratory of Natural Substances, University of Carthage, Faculty of Sciences of Bizerte, Zarzouna 7021, Bizerte, Tunisia

Accepted 16th September, 2016; Published Online 31st October, 2016

ABSTRACT

Since 1950, various plants with a strong ability to bioaccumulate have been used for continuous monitoring of the land contamination or water areas by the main categories of pollutants. These plants, containing certain chelating components, have the property of accumulation in large amounts of certain halogens, such as chlorine and fluorine. Heavy metals, like lead and cesium, are also retained. These pollution bioaccumulators can detect pollution difficult to be demonstrated by direct analysis of the water, soil or air.In the case of atmospheric contamination, the lichens are the most suitable bioindicators. They constitute powerful bioaccumulators because of their ability to collect contaminants in the air as they entirely depend on their mineral nutrition from the extraction of nutrients from the atmosphere. Lichens have been used as biological indicators of contamination by toxic or radioactive metals. The purpose of this work is to determine the level of pollution in the Bizerte region by atomic absorption spectrometry and by emission spectrometry with plasma torch.

Key words: Lichen, Atmospheric Pollutants, Heavy Metals, Atomic Absorption Spectrometry, Emission Spectrometry, Plasma Torch.

INTRODUCTION

The lichen constituents have recently gained great interest. They have been widely discussed by many researchers for several reasons. In fact, some of them provide highly-demanded fragrances (Moxham, 1981; Richardson, 1975) and very effective antibiotic drugs (Smith, 1980; Hanssen and Schadler, 1985), while those used in dyeing (Carbonnier, 1990; Hale, 1983; Henderson, 1984) are widely applied in various domains. In addition, all lichens are pollution indicators (Yuan Bao, 2016; Olena Yemets *et al*., 2015; Togwell A. Jackson, 2015; Autoren, 2014), which sometimes prevent them from surviving. For this reason, we try in this work to exploit the lichen behavior opposite to certain pollutants. We also study the characterization and analyze the heavy metals accumulated by some lichens collected from Bizerte region (Tunisia). To our knowledge, no research work, assessing the level of pollution in a Tunisian region, used the lichens as bio-accumulators. In this work, we first study the morphology of the samples collected to determine each lichen family and species because previous studies have shown that the accumulation of pollutants in a frond varies according to the species and the environmental conditions. This morphological determination requires the use of color indicators and microscopic examinations of the various fronds to be analyzed. Then, we move to the analytical part where several techniques are used to study the pollutants attached to the harvested thallus. Among these methods, we can mention the two following ones: the AAS and the emission spectrometry with plasma torch.

MATERIALS AND METHODS

Material

The analyzed lichen samples and various parts of each collected lichen are mentioned in Table 1. They all come from the Bizerte region (Zarzouna, Menzel Jemil, Djebel Ichkeul The fishery Sejnane El Alia, etc.). Indeed, they are fixed on different supports: rocks, soils (limestone, etc.), shrubs (rosemary rétamé, acacia) and trees (eucalyptus, olive, carob, fig, etc.).Lichens from the industrial suburb of Tunis will also be studied to determine the level of pollution in Bizerte. They are taken from sites near plants probably emitting some pollutants in the atmosphere, such as the factory of the Tunisian Company of Lubricants SOTULUB; cement Bizerte and the Brickyard Menzel Jemil… Other collection sites are characterized by agricultural or forestry utility.

Morphological analysis methods

Defining the lichen species recourse requires a review of the thallus by usual optical instruments and also the use of color reagents. We should note that each lichen is made up of a thallus representing the plant itself whose outer surface forms the cortex. On the latter, the producing organs (apothecia and soralies) are fixed.

Microscopic analysis

Many morphological characteristics are observed using a binocular microscope: the shape of the compartments, the form apothecia, etc…

****Corresponding author: Karima Tabbabi,***
Laboratory of Natural Substances, University of Carthage, Faculty of Sciences of Bizerte, Zarzouna 7021, Bizerte, Tunisia.

But, to determine the lichen species, the apothecium and the cortex must be microscopically examined. Thin sections of these parts are also noticed. Through this analysis, we tried to find especially the number of spores per ascus, shape, color and size.

Sample preparation and equipment

Cuts of apothecia and thallus, made using a razor blade, were placed between two microscopically-observed glass slides to study the various lichen bodies. This necessitated the use of an Olympus microscope which allowed magnifications up to 1000 x.

Coloring tests

Coloring tests were carried out to determine the lichen species. Each species had one or more lichen substances; some of which gave color reactions with potassium (K), hypochlorite (C) and para-phenylenediamine (PD).

Coloring tests

The appropriate reagent for each test was applied using a micropipette on the thallus of lichen to be analyzed morphologically. Color changes were observed after the application–of–the reagent because some tests have instant reactions whose effect disappeared quickly. If the execution of a test is followed by an appearance of coloration), this test is positive and noted K^+, C^+, PD^+ depending on the reagent used; otherwise it is noted K^-, C^-, PD^-.
The used reagents are:

Test C: an aqueous calcium hypochlorite solution Ca $(OCl)_2$, or sodium hypochlorite NaOCl (bleach).

K test: an aqueous solution of potassium hydroxide KOH to about 10 %.

PD test: an ethanoic solution of 1 to 5% of paraphenylenediamine.

Assay methods of heavy metals in lichens and analysis methods

Analysis methods

The most appropriate methods for the polluting substances identification are atomic absorption spectrometry (AAS) and emission inductively coupled with plasma atomic emission spectroscopy. The former is used to examine more than 70 elements (metals and nonmetals) in concentrations of ppm (mg / ml) or even ppb (1 µg / L).

Accuracy, speed and detection limits can make the plasma torch by issuing one of the best elemental analysis techniques available today. (Rouessac, 1994; Atkins, 1986). However, the latter, based on the observation of the lines emitted by past atoms in an excited state, usually ionized under the effect of very high temperatures (8000-9000 K). All items, except those which are made up of light atoms, can thus be examined. Besides, atomic emission spectrometers can dose several items in the sample at once.

Treating the analyzed sample by atomic absorption and emission by plasma torch

The sample was dried in an oven at 40 ° C and then grounded to obtain a fine powder. The sample solution annealing (0.5g for the plasma torch and 0.25 g for atomic absorption) was provided by an acid attack with a mixture of acids having the following proportions: (20ml HF, 50 ml $HClO_4$, 2 ml HNO_3). After complete dissolution, the solution was evaporated to dryness at 100 ° C and the residue obtained was taken up in 10 ml of concentrated HCl. We added, to the obtained chlorohydrique solution, 5 ml of LiCl (150 g / L) made up with distilled water to 50 ml (for the plasma torch) and 100 ml (for the atomic absorption). Then, numbers provided by the plasma torch are read. For Ag, As, Sn and Sb, mineralization was performed by mixing 10 ml of concentrated HCl and 5 ml of concentrated HNO3, followed by heating for 3 hours at 100 ° C in a water bath.

The multi-element analyses were carried out using:

- Plasma torch ICP- CB, ARC manufacturer, equipped with phased array and sequential cassettes.
- Atomic absorption spectrometry Perkin Elmer 306

RESULTS AND DISCUSSION

The aims of our experimental work done on the lichens of the Bizerte region are to determine their morphology and identify the pollutants accumulated in their thallus.

Morphological identification of the studied lichens

Table 2 summarizes the results of the lichens morphological identification. In this study, we tried, during harvest, to collect lichen species found in each of the chosen areas to compare the accumulation of each species capacity according to the environmental conditions. Thus, the morphological identification showed that the following three lichens: *Xanthoria parietina, Parmelia caperata* and *Diploschistes gypsaceus* were present in all sampling sites. Only these three species will be the objects of studying the pollutants accumulation. The species *Xanthoria parietina* is very dominant in the Mediterranean climate. This lichen is very abundant in areas located on the coast of Tunisia (Bizerte, Cap Bon, Sahel...) as it can grow inside the country (Kef, Makthar ...) (Karmous *et al*., 2001). It is a foliose lichen-shaped rosette, growing on the most varied substrates: tree branches, tiles, stones, etc… Indeed, foliose lichens, adhering more or less closely to their support, form cells in several lobes of variable thickness. They can be torn out easily with a knife after a period of rain. The shape of the thallus *Xanthoria parietina* is almost circular (diameter up to 50 cm) with a bit square limits. The orientation takes on the color of the lichen. In fact, sunshine promotes green orange, while the grayish green is the color of lichens growing in the shade.

Table 1. Samples of collected lichens submitted for analysis

Lichen reference	Support	Place of sampling and vocation	Observations and characteristics
L_1	Tile	Zarzouna, near the oil refinery STIR and society of SOTULUB lubricants highway	Foliose lichen , orange yellow , red apothecia
L_2	Acacia		Greenish lichen , foliose , soralies at the lobe tips
L_3	Stone		Lichen whitish appearance very mealy , black apothecia
L_4	Tile		Foliose lichen , yellow-orange , red apothecia
L_5	Rock		White lichen , very floury appearance , black apothecia
L_6	Olive tree	Bizerte, near Bizerte cement and dump	Orange lichen green frond leaf attached to the support by rhizines
L_7	Ground		white lichen highly adherent to the substrate, no apothecia
L_8	Stone		white lichen highly adherent to the substrate, no apothecia
L_9	Acacia		Lichen shaped rosette with broad rounded lobes devices and low cut
L_{10}	Eucalyptus	Menzel Jemil brick and textile industry	Thallus foliose , greenish yellow, lobes between 5 and 13 mm in diameter
L_{11}	Red tile		Lichen greenish yellow, brown apothecia few, bottom whitish
L_{12}	Carob tree		Lichen greenish yellow, brown apothecia few, bottom whitish
L_{13}	Ground		Lichen crustacean white , round black apothecia
L_{14}	Tile		Lichen crustacean whitish in remote Apothecia
L_{15}	Eucalyptus	El Alia farmland	Foliose lichen , yellowish to dark red apothecia present in the central part of the thallus
L_{16}	Olive tree		Foliose lichen in the form of large lobed rosette and soredia
L_{17}	Limestone		Lichen crustacean pleated surface , fitted with black apothecia
L_{18}	Eucalyptus	Djebel Ichkeul national park	Greenish yellow lichen , almost circular , red apothecia present in the central part
L_{19}	Limestone		Lichen white pleated surface irregularly shaped black apothecia
L_{20}	Oak		Greenish lichen , foliose , very few adherent to the substrate, thallus provided soredia
L_{21}	rosemary		
L_{22}	Olive tree		Greenish yellow lichen , almost circular , red apothecia present only in the central part
L_{23}	Limestone	Sejnane forest region	Lichen crustacean white and gray Apothecia
L_{24}	Eucalyptus		Whitish green lichen shaped peripheral rounded lobed leaves , very concave Apothecia
L_{25}	Ground		Thallus in yellowish leaves , many apothecia red, little adherent to the substrate
L_{26}	Cork oak		
L_{27}	Stone		Thallus greyish white Apothecia not pressed in the frond with black discs
L_{28}	Eucalyptus	Ben Arous industrial site	Foliose lichen yellow orange little adherent to the substrate, no apothecia
L_{29}	Eucalyptus		Greenish lichen-like scalloped perimeter lobed leaves , very concave Apothecia

Tale 2. Results of morphological identification of the collected lichens

Lichen references	Identified species	Main features
L_1, L_4, L_6, L_{11}, L_{12}, L_{15}, L_{18}, L_{22}, L_{25}, L_{26}, L_{28}	Xanthoria parietina	- Foliose lichen, greenish yellow pushing on a variety of substrates (rock, soil, tree trunks, etc.) - Red Apothecia present in the central part of 1 to 2 mm in diameter - Spore count ranges from 10 to 14 - Color reactions K^+ red, C^-
L_2, L_9, L_{10}, L_{16}, L_{20}, L_{21}, L_{29}	Parmelia caperata	- Foliose lichen , wide enough , shaped rosette - Lobes rounded and broad peripheral pushing, especially on the trunks and branches of trees possessing soralies at the lobe tips - Concave apothecia - Color reactions k^+ yellow, orange PD^+
L_3, L_5, L_7, L_8, L_{13}, L_{14}, L_{17}, L_{23}, L_{17}	Diploschistes gypsaceus	- Lichen crustacean, forming a whitish crust on some rocks or bark. - Chalky, wrinkled surface -Black apothecium -Lichen saxicole and calcicolous - Multicellular spores - Color reactions C^+ red

Table 3. multi-element analysis * (plasma torch, atomic absorption) of lichen samples from Zarzouna (STIR and SOTULUB)

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_1	Xanthoria parietina	50	41	53	16	204	≤1	9	2	1	8	≤1	5	35	≤1	17
L_2	Parmelia caperata	53	85	11	23	217	≤1	13	3	1	7	≤1	5	41	≤1	19
L_3	Diploschistes gypsaceus	430	186	308	60	179	7	50	3	2	7	≤1	12	48	2	21
L_4	Xanthoria parietina	61	51	97	16	211	≤1	16	2	2	5	≤1	6	37	≤1	18
L_5	Diploschistes gypsaceus	270	135	262	16	182	9	65	5	1	4	≤1	15	49	3	23
Average grade		173	99.6	146	26	199	3.2	30.6	3	1.4	6.2	≤1	8.6	42	1	20
Minimum content		50	41	11	16	179	1	9	2	1	4	≤1	5	35	≤1	17
Maximum content		430	186	308	60	217	9	65	5	2	8	≤1	15	49	3	23

* : values in ppm

Table 4: multi-element analysis * (plasma torch, atomic absorption) of lichen samples from Bizerte (Cement + landfill)

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_6	*Xanthoria parietina*	1467	1100	600	8	185	1	12	17	3	15	3	38	86	1	15
L_7	*Diploschistes gypsaceus*	5320	300	1800	78	167	6	51	85	2	16	2	56	97	1	13
L_8	*Diploschistes gypsaceus*	6700	2200	1500	93	162	9	43	72	2	14	2	55	95	2	12
L_9	*Parmelia caperata*	900	870	750	16	192	2	20	30	2	17	4	29	74	≤1	14
Average grade		3597	1118	1163	49	177	4.5	31.5	51	2.3	16	2.8	45	88	1.3	14
Minimum content		900	300	600	8	162	1	12	17	2	14	2	29	74	1	12
Maximum content		6700	2200	1800	93	192	9	51	85	3	17	4	56	97	2	15

* : values in ppm

Table 5. multi-element analysis * (plasma torch, atomic absorption) of lichen samples from Menzel Jemil (Brick + Textile)

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_{10}	*Parmelia caperata*	660	490	150	29	76	2	9	3	2	8	1	19	3	18	2
L_{11}	*Xanthoria parietina*	750	360	200	18	52	2	6	4	1	6	1	23	5	17	3
L_{13}	*Diploschistes gypsaceus*	2250	3330	350	57	43	5	55	19	2	7	3	47	5	21	2
L_{14}	*Diploschistes gypsaceus*	2100	2530	400	53	39	6	43	22	3	5	2	51	6	19	2
Average grade		1440	1678	275	39	52.5	3.75	28.3	12	2	6.5	1.8	35	4.8	19	2.3
Minimum content		660	360	150	18	39	2	6	3	1	5	1	19	3	17	2
Maximum content		2250	3330	400	57	76	6	55	22	3	8	3	51	6	21	3

* : values in ppm

Table 6. multi-element analysis * (plasma torch, atomic absorption) of lichen samples from El alia

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_{15}	*Xanthoria parietina*	210	80	65	5	24	3	45	9	2	≤1	≤1	2	≤1	≤1	37
L_{16}	*Parmelia caperata*	180	50	40	5	18	5	63	8	2	≤1	≤1	2	≤1	≤1	23
L_{17}	*Diploschistes gypsaceus*	450	30	410	80	31	2	81	11	3	≤1	≤1	4	≤1	≤1	51
Average grade		280	120	172	30	24.3	3.33	63	9.3	2.3	≤1	≤1	2.7	≤1	≤1	37
Minimum content		180	50	40	5	18	2	45	8	2	≤1	≤1	2	≤1	≤1	23
Maximum content		450	230	410	80	31	5	81	11	3	≤1	≤1	4	≤1	≤1	51

* : values in ppm

Table 7: multi-element analysis * (plasma torch, atomic absorption) of lichen samples from El alia

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_{18}	*Xanthoria parietina*	80	60	30	2	2.2	≤1	4	2	1	2	≤1	3	≤1	≤1	≤1
L_{219}	*Diploschistes gypsaceus*	286	203	45	26	2.9	2	3	8	2	3	≤1	11	≤1	≤1	≤1
L_{20}	*Parmelia caperata*	70	50	25	5	3	≤1	5	2	1	1	≤1	5	≤1	≤1	≤1
L_{22}	*Xanthoria parietina*	50	90	20	3	3.7	≤1	3	1	1	2	≤1	4	≤1	≤1	≤1
Average grade		122	100.8	30	9	3	2	11.3	3.3	1.3	2	≤1	5.8	≤1	≤1	≤1
Minimum content		50	50	20	2	3	2	3	1	1	1	≤1	3	≤1	≤1	≤1
Maximum content		286	203	45	26	3	2	33	8	2	3	≤1	11	≤1	≤1	≤1

* : values in ppm

Table 8. multi-element analysis * (plasma torch, atomic absorption) of lichen samples collected from Sejnane (Forest)

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_{23}	*Diploschistes gypsaceus*	306	180	27	23	≤1	1	11	6	2	1	≤1	5	≤1	≤1	≤1
L_{24}	*Parmelia caperata*	39	48	19	6	≤1	≤1	3	1	1	1	≤1	3	≤1	≤1	≤1
L_{25}	*Xanthoria parietina*	27	22	11	4	≤1	≤1	2	2	1	2	≤1	2	≤1	2	≤1
L_{26}	*Xanthoria parietina*	68	57	17	1	≤1	≤1	3	1	2	1	≤1	1	≤1	≤1	≤1
L_{27}	*Diploschistes gypsaceus*	480	250	28	29	≤1	2	15	5	3	3	≤1	11	≤1	3	≤1
Average grade		184	11.4	20.4	13	≤1	1.5	6.8	3	1.8	1.6	≤1	4.4	≤1	2.5	≤1
Minimum content		27	22	11	1	≤1	≤1	2	1	1	1	≤1	1	≤1	2	≤1
Maximum content		480	250	28	29	≤1	2	15	6	3	3	≤1	11	≤1	3	≤1

* : values in ppm

Table 9: multi-element analysis * (plasma torch, atomic absorption) lichen samples from Ben Arous

Ref	Species	Al	Fe	Ca	Mn	Pb	Sn	Zn	Cu	Be	Ni	As	Cr	Cd	Co	Hg
L_{28}	*Xanthoria parietina*	4300	3200	500	600	650	410	650	620	31	270	58	350	570	320	315
L_{29}	*Parmelia caperata*	3700	3600	450	750	770	330	770	830	43	280	75	420	380	410	285
Average grade		4000	3400	475	675	710	370	710	725	37	575	67	385	475	365	300
Minimum content		3700	3200	450	600	650	330	650	620	31	270	58	350	380	320	285
Maximum content		4300	3600	500	750	770	410	770	830	43	280	75	420	570	410	315

The thallus of this species comprises apothecial, 1 to 2 mm, and the number of spores varies from 10 to 14. The *Xanthoria parietina* is characterized by its high parietin, giving color reactions K^+ red and its bio- accumulative capacity of pollutants (F, Pb...) without being damaged. The second gave rise to extensive use of this species in the study of the environment quality. According to the morphological results, we were interested in a second species of lichen widespread in highly humid areas (Littoral, North...), such as *Diploschistes gypsaceus*, particularly saxicole and corticolous.

This lichen, which is difficult to harvest, forms a crust on some rocks or bark. Its color varies from white to gray white. Indeed, a blade wide short knife is the best instrument to detach from the holder. The apothecia of chalky Lichen with pleated and very uneven surface have black discs surrounded by a thick rim thallin. This species gives a red color reaction C^+ because of its richness in lecanoric acid. The third identified species is *Parmelia caperata*. It represents foliose lichen with rather large diameter (lobe 13 to 15 mm). Its colour is greenish yellow. It grows especially on trunks, branches and old trees, but rarely on rocks and soils. Lichen calcifuges push in areas where rainfall is abundant. Its apothecia are concave on board thallin soredia (Figure 3). The thallus of this species is adherent to the substrate. Lichen *Parmelia caperata* is photophile and very sensitive to pollution. It is rich not only in caperatic acid (fatty acid), but also in atranoric acid, usnic acid and protocetraric acid, which explains the results of the color tests: K^+ yellow for the thallus and PD^+ orange for surface of the medulla. All of the three species are morphologically identified. Each of the selected sampling areas were analyzed to determine and characterize the pollutants accumulated in each of the species and to assess the level of air pollution of the studied site.

Identification of the pollutants accumulated in lichens of Bizerte region

Three different species, *Xanthoria parietina, Parmelia caperata* and *Diploschistes gypsaceus*, were chosen to study the air quality in the region. This choice is dictated by the fact that all species do not behave in the same way opposite to pollutants. In addition, these three species occured in each sampling site, which shows the effect of location and climate. They also had large areas of contact with the atmosphere. By analyzing lichens collected in the Bizerte region using atomic absorption spectrometric methods and emitting plasma torch, we obtained the results presented in Tables 3, 4, 5, 6, 7 and 8. These techniques reveal the elements accumulated in the different lichens: aluminum, lead, tin, zinc, copper, beryllium, nickel, arsenic, chromium, cadmium, cobalt and mercury. The determination of contaminants in the studied lichens allowed us to measure the level of pollution in the region and informed us about these pollutants possible emission sources. It is worth-noting that the identification analyses were carried out in duplicate. In fact, an average of two determinations was calculated for each analysis to further confirm our results.

Pollutants identified in lichens of Zarzouna (Table 3)

Analysis of Zarzouna lichens showed the presence of iron, aluminum and calcium. According to the contents of the mentioned elements, lichens can be classified into two groups. The first includes the *Diploschistes gypsaceus*. It represents a crustacean species having high levels of Fe (135-186 ppm), Al (270-430 ppm) and Ca (262-308 ppm). However, the second group, containing the leafy lichens *Xanthoria parietina* and *Parmelia caperata*, is characterized by much lower amounts of Fe (41-85 ppm), Al (50-61 ppm) and Ca (11-97 ppm). These results reveal that the lichen morphology enhanced the pollutants absorption capacity. Thus, *Diploschistes* lichens, which were very adherent to the support and difficult to remove, were always taken together with strips of rock or stone in general substrate. This explains the high percentages of Fe, Al and Ca detected in *Diploschistes.* For Against, the presence of Fe, Al and Ca in leafy lichens can be attributed to the formation of solid particles deposit on the analyzed fronds. Significant concentrations of lead were observed in three species of lichens from Zarzouna; the strongest ones (of 204-217 ppm) were identified in leafy lichens *Xanthoria parietina* and *Parmelia caperata*. One factor behind this finding is the peculiarity of having leaf-like frond with major surfaces in contact with the atmosphere.

The main source of lead emissions in the atmosphere remains the traffic, i.e. the automotive exhaust gas of motor gasoline (Scerbo *et al*., 2002). This source of energy, which is the regular or premium gasoline, contains tetraethyl lead. The latter is an additive whose role is to increase the gasoline octane in order to improve engine performance. It has not been used in Tunisia since 2008. We can mention other sources emitting this toxic pollutant, such as the burning of petroleum and coal products (104). It is argued that the presence of lead in Zarzouna lichens is probably related to the traffic and the industrial activities, like oil refining and recycling of lubricating oils, in this region.

The studied lichens also revealed significant amounts of Zn (9-65 ppm) Cd (3-49 ppm) and mercury (17 to 23 ppm). Zinc is ubiquitous in nature. The intensity of traffic explains its significant content observed in lichens (69). Cadmium always accompanies Pb for several reasons. First, it is also present in fossil fuels. Second, Pb tetraethyl is prepared from Cd diethyl. Thus, gasolines, diesel and fuel are potential sources that may emit Cd (Scerbo *et al*., 2002). Mercury, observed at doses ranging from 17 to 2 » ppm, could come from the combustion of fuel by the factories in Zarzouna to produce energy (Seaward, 1992). Actually, lichens of Zarzouna contain relatively low concentrations and sometimes negligible Cu, Be, Ni, Ar, Sn and Co. Certainly, they constitute airborne contaminants that can be absorbed by the plant to be analyzed. Regarding the level of pollution in Zarzouna site (Bizerte), the contents of pollutants Pb , Zn, Hg and Cd in lichens, indicate that the atmosphere of this area has a level of air pollution by far lower than that reported in the literature and on certain industrial areas in Italy (104) (105). Using the lichen *Xanthoria parietina*, the study of the air quality in the Italian province "Livorno" recorded 1130-79190 ppm for Pb, while the average contents of Cd and Zn respectively reached 160 and 46000 ppm. The main causes resulting in such high level of pollution are the chemical industry, metallurgy, power plants and traffic (Scerbo *et al*., 1999).

Identified pollutants in lichens Bizerte (Table 4)

The samples of the collected lichen in Bizerte (close to the cement and dump) exhibited high contents of Al (900-6700 ppm) Fe (300-2200 ppm) and Ca (600-1800 ppm). These elements are associated with minerals, such as limestone and clay used as raw materials to prepare cement and hydraulic lime. The latter are manufactured by the nearby factory of the sampling. It is believed that these minerals are atmospheric contaminants that have been deposited as dust on lichens frond lying around the factory. Obviously, the *Diploschistes* showed an affinity of such minerals, much greater than that of leaf-like. The relatively high concentrations of Pb (162-192 ppm) and Hg (1to 15 ppm) of the three species of the analyzed lichens were normally expected due to use of a fossil fuel by the cement and the fumes resulting from the incineration of waste near a dump of samplings and the relatively dense automobile traffic on the site. The results for the Bizerte lichens highlight Zn (1 to 51 ppm) and Cd (74-97 ppm). Data provided in the literature support the hypothesis that these two elements always accompany the lead from car exhausts (Scerbo *et al*., 2002).

The Ni (14 to 17 ppm), Sn (1-9 ppm) and Cr (29-56 ppm) showed significantly higher levels in lichens of Bizerte than those of Zarzouna due to the presence of the sampling point nearby garbage dump. Other elements, such as Be, Sn and As, reveal the same trends as those found in Zarzouna lichen. Obviously, the city of Bizerte has a very low level of pollution compared to other Mediterranean and European regions. The area of Pisa in Italy, with a very large industry (Scerbo *et al.*, 2002), has 3440 pm Pb , 30150 ppm Zn and 2235 ppm Cr. Some cities , such is the case of Athens where the situation is so dire that we had to regulate traffic since the 1980s, alternate with license plates corresponding odd and even days. (Ramade, 2002). To determine nationally the level of pollution in Bizerte, we studied the lichens collected at Ben Arous which is an important industrial site in the southern suburbs of Tunis.

Identified pollutants in lichens of Menzel Jemil (Table 5)

The *Diploschistes* lichens from Menzel Jemil are very rich in Al (2100 to 2250 ppm) and Fe (2530 to 333A ppm). The amounts of these elements in the *Xanthoria* samples are small. They (expressed as ppm) are respectively equal to 750 ppm and 360 ppm. Indeed, the brick is involved in such contamination, as clay and sand which represent the components of manufacturing refractory articles. This industrial plant releases dusts rich with Fe and Al. The same plant could be the origin of the average rates of Pb (39-76 ppm) and Hg (2 to 3 ppm) detected in the collected samples. The use of fuel oil fat was behind the presence of these toxic pollutants (Scerbo *et al.*, 1999). Note that the Co doses (17 to 21 ppm) , Cr (19-51 ppm) and Cu (3-22 ppm), observed in samples of Menzel Jemil, are more important than in other sites. This can be explained by the existence of a polluting textile industry employing dyes and synthetic mordant. It is worth-noting that this activity uses the burning remains of fabrics produced by many textile manufacturing plants located in the periphery of the site.

Identification of Ben Arous lichen Pollutants

First, we note the scarcity of lichens in Ben Arous. The *Diploschistes* which are crustaceans growing in such a microclimate were not found. These findings prove that the pollution has reached such intensity that the existence of some lichen species is threatened. Concentrations of three digits for most contaminants (table 9), found in lichens of Ben Arous, were in argument with the disappearance of *Diploschistes* in this site. Furthermore, Aluminum (3700-4300 ppm) and calcium (450 to 500 ppm) may be atmospheric contaminants emitted by an industrial activities related to the cement and lime manufacturing. It has been reported that the cement Jebel Jeloud is not far from the sampling site. Iron (3200-3600 ppm) probably comes from the discharge of steel industries and metal processing installed in Megrine. Mercury (285-315 ppm) is produced by the Rades thermal plant that uses the fuel in huge quantities to produce energy. It can be emitted from the electronic components industries, pesticides, fungicides and Steel… (Scerbo *et al.*, 2002). The fuel combustion and the very heavy traffic in the harvest area generate the emission of significant concentrations of Pb (650-770 ppm). The arsenic (58-75 ppm), in which coal combustion is the principal source, can be emitted by paintings and non-ferrous alloys industries already installed in the site, such as paints, non-ferrous alloys. However, plating industries, dyeing, painting and steel production (19) explain the atmospheric contamination by nickel (270-280 ppm) and chromium (350-420 ppm). Cadmium (380-570 ppm) has the same sources as lead. But, the presence of zinc (650-770 ppm) is attributed to various industrial activities (rubber, pesticides, fertilizers, paints...) and urban (waste incineration, road traffic...). (Scerbo *et al.*, 1999). These pollution levels, although modest if compared with those of other capitals of the Mediterranean, do not prevent to take precautions in order to avoid worsening the ecological situation. Among the proposed solutions, we can mention the use of non-leaded petrol and catalytic exhausts, filters, which may stop the emission of solid dust by some factories... The situation of air pollution in Bizerte did not lead to currently imposing draconian measures to reduce certain pollutants emissions into the atmosphere. The urban development of the region calls for increased vigilance in this area read.

Conclusion

In this study, we exploited the lichens ability to capture and accumulate contaminants in the atmosphere to specify the level of pollution in the Bizerte region. The objective of this work was attained in the sense that we firstly determined each of the contaminants content in the analyzed lichens. Secondly, we were able to locate the pollution level of each studied site. The emitters of most pollutants were also identified. From the experimental results, we deduce that:

- Zarzouna and Bizerte provide the highest levels of lead. This is explained by the presence of a petroleum refining industry and recycling lubricants. Indeed, the road traffic and the burning of fossil fuels in factories can also result in the emission of lead, zinc and cadmium.
- Bizerte cement and Menzel Jemil brick are responsible for the presence of particles containing aluminum, calcium and iron. The minerals of these elements are behind the development of ceramic products and cement.
- Waste incineration is considered as an issuing source of nickel, tin and cadmium.
- The use of fertilizers in agriculture in el Alia promotes the issuance of the element mercury into the atmosphere.
- Lichens *Diploschistes* crustaceans are bio-accumulators whose capacity exceeds that of leafy *Xanthoria* and *Parmelia.*

We proved that the level of pollution in Bizerte is very low compared to that of Tunis or those of other Mediterranean countries. This is explained by the weakness of the industrial fabric and the average size of the city.

Acknowledgement

We thank everyone who helped us complete this work

REFERENCES

Atkins P.W. 1986. “Physical chemistry” Oxford university. Press 3rd Ed.

Autoren: Shukla, Vertika, D.K., Upreti, Bajpai, Rajesh, 2014. “Selection of biomonitoring species “, Lichens to Biomonitor the Environment, pages 47-60

Carbonnier, J. 1990. « Le devenir des plantes utiles :Un exemple de l’intérêt des lichens dans la lutte contre la sécheresse ». *Bull Soc. Industrielle de Mulhouse 4, pages.51-58.*

Garty, J., Ammann, K.1987. “the amounts of Ni, Cr , Zn Pb, Cu, Fe and Mn in some lichens growing in Switzerland”. Env. Exp. Botany 27; 127-138.

Hale, M.E. 1983. “The biology of lichens. 3rd Ed, *Edward Arnold*, *London 138.*

Hanssen, H.P., Schadler, M. 1985. “Pflanzen in der Tradionellen chinesischen Medizin”. *Dtsch Apoth. Ztg 125, pages.1239-1243.Paris.*

Henderson, A. 1984. “Some memorabilia on the industrial manufacture of lichen dyestuffs, Cudbears and orchil II”. *Bull. Br. Lichen Soc*. 55, *pages.19-21.*

Karmous, T., Ayed, N. “Orchil lichens of Tunisia” 2001. Dyes in history and archeology 16/17; 119-128.

Moxham T.H. 1981. “Lichens in perfume industry”. *Drogo Rep*. *2, pages31-39.*

Olena Yemets, Yngvar Gauslaa, Knut Asbjørn Solhaug. August 2015. “Monitoring with lichens – Conductivity methods assess salt and heavy metal damage more efficiently than chlorophyll fluorescence “, *Ecological Indicators, Volume 55, Pages 59-64*

Ramade, F. 2002. “Dictionnaire encyclopédique de l’écologie et des sciences de l’environnement”. DUNOD 2ème édition, p88.

Richardson D.H.S. 1975. “The Vanashing lichens”. David and Charle, *North Pomfret V*, *pages.95-97.*

RouessacF., Rouessac A. « Analyse chimique : méthodes et techniques instrumentales modernes »Masson. Paris, 2ème édition (1994).

Scerbo, R., Possenti, L., Lampugnani, L., Ristori, T., Borale, R., Barghigiani, C. 2002. “Lichen *(Xanthoria parietina)* biomonitoring of trace element contamintion and air quality assessment in Livorno province (Tuscany, Italy)”. Sci Total Environ. 286; 27-40.

Scerbo, R., Possenti, L., Lampugnani, L., Ristori, T., Borale, R., barghigiani, C. 1999. “Lichen *(Xanthoria parietina)* biomonitoring of trace element contamintion and air quality assessment in Livorno province (Tuscany, Italy)”. Sci Total Environ. 241; 91-106.

Seaward, M.R.D.1992. “large-scale air pollution monitoring using lichens “1992. Geojournal 28; 403-411.

Smith, A.L. 1980. “Lichens”. *Cambridge University Press,* London.

Togwell A. Jackson. August 2015. “Weathering, secondary mineral genesis, and soil formation caused by lichens and mosses growing on granitic gneiss in a boreal forest environment “, *Geoderma, Volumes 251–252, Pages 78-91*

Yuan Bao, Yiwen Ju, Boping Li, Yimin Sun, “Migration of trace elements from basalt substrate to co-located vegetation (lichens and mosses) at the Wudalianchi volcanos, Northeast China”, *Journal of Asian Earth Sciences*, *Volume 118, 15 March 2016, Pages 95–100*

****