# Study of Lichens of the Moroccan Atlantic Coast Safi - Essaouira: Bioindication of Climate Disturbances and Nitrogen Pollution

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**Abstract:** ***In order to explore an ecological correlation between lichens and global air pollution on the Moroccan Atlantic coast Safi - Essaouira, we used a qualitative approach based on the spatial distribution of lichenic flora, mainly saxicolous, which reflects special environmental pollution conditions. Used as good indicators of air quality, lichens can also reflect climate trends. The biological spectrum of studied lichens, characterized by the low presence of fruticose lichens (9%) and foliose lichens (17%) and the dominance of crustose lichens (74%), indicates the arid climate of the region. A comparison between ancient and current lichenic flora revealed the disappearance of Ramalina implexa, lichen with a tropical affinity, which probably had to disappear from this study area, reflecting the loss of a local tropical microclimate within an increasingly arid regional mesoclimate. This observation has been confirmed by the study of temperature and precipitation variations in the region since 1966 to 2020. The identification of 36 species of saxicolous lichens and their categorization by the eutrophication index revealed a gradient of nitrogen pollution that tends to shift the composition of the lichen community from a structure dominated by oligotrophic species to one dominated by eutrophic species. This trend revealed the southern phytogeographic limit of the species Coscinocladium gaditanum.***

**Keywords:** Bioindication - Ramalina implexa - Coscinocladium gaditanum - Biological spectrum - Nitrogen pollution - Climate change

## 1. Introduction

Air pollution constitutes the most pressing environmental health risk facing our global population, 92% of the world's population are estimated to breathe toxic air quality (WHO, 2016). Due to its geographical location and its socio - economic development, Morocco is one of the most vulnerable countries to atmospheric disturbances. The cost of air pollution is estimated at 1.05% of the moroccan GDP (Croitoru et al.2017). Lichens are known as indicators of various types of pollution (Nimis et *al.*, 2002). The potential of a lichen as an indicator depends on the species and the pollutants (Nash, 2008), different studies have explored the relationships between lichens and pollutants (Bates, 2002).

The use of lichens is one of the biological assessment approaches to environmental change. Lichens are good examples for assessing air pollution (Garrec and van Haluwyn, 2002). Used for a long time as good indicators of the air quality, they are also precious tools to highlight the change of atmospheric conditions (Temperature, precipitation etc.). Happe David (2016) reported that epiphytic lichens are particularly important sentinel species to monitor periodic temperature changes: Some species described as very rare in northern Europe are more commonly observed, in recent years, in these areas. Changes in epiphytic lichen flora in the Netherlands indicate a significant change towards species preferring warm conditions (Van Herk et *al.*, 2002). Similar observations have been reported in Germany (De Bruyn et *al.*, 2009, Stapper et *al.*, 2011).

Bioindication is an ecological approach that seeks to evaluate possible air disturbances from the presence/absence of key species in a specific lichenic survey. The first catalog of Moroccan lichens published by Werner and Gattefossé (1931) includes 542 species. A major scientific study of the Moroccan lichenic flora was conducted by Werner between 1931 and 1976. Egea (1996), in his catalog, gave an update on the studies of Moroccan lichen flora between 1879 and 1990, and gives a commented list of lichens in our country. In 2013, a synthesis on the lichens of the Rabat herbarium (RAB) was published by Ajaj et *al.* (2013).

The nutrition of lichens is strongly dependent on the atmosphere, which makes them exposed to atmospheric pollutants (in particular SO2, NOx) to which they respond at the cellular, morphological and community levels; this allows to use them as indicators of the air quality. The combustion of fossil fuels (NO and NO2), the industry (NOx) and the agriculture (NH3) contribute to the majority of nitrogen emissions into the atmosphere. Deposits of these pollutants and secondary pollutants derived therefrom (eg NH4+, NO3 - and HNO3) have a negative effect on vegetation. The rate of this pollution is related to the size of the population (amount of fossil fuel burned per person) and the intensity of agriculture. Human activities have altered the global nitrogen cycle to a greater degree. In California, critical nitrogen loads are exceeded for many years, resulting in significant changes in lichen indicator groups (U. S. Forest Service, 2018). A number of studies have assessed the effects of ammonia on epiphytic lichens (van Herk 1999; Wolseley*et al.*2004, 2006; Leith et al.2005). In addition to ammonia, emissions of nitrogen oxides from traffic contribute to atmospheric nitrogen and several studies have shown that lichens respond to nitrogen from this source (Davies *et al.*2007, Lisowska, 2011, Seed *et al.*2013).

The present study aims to study the lichenic flora of the Atlantic coastal fringe from the commune of Cap Cantin to the province of Essaouira over a length of 160 km, using different approaches, historical and bioindication, to highlight possible changes in certain climatic parameters, and to qualitatively evaluate the evolution of the composition of the saxicolous lichen community as a result of nitrogen pollution. This is the first initiative of its kind in this region of study.

## 2. Material and Methods

The Moroccan Atlantic coast Safi - Essaouira includes two urbanized areas (the city of Safi and the city of Essaouira) and less urbanized areas. Since the 1960s, it is home to a major industrial phosphate processing plant located south of the Safi city, and accused of being one of the contributors to the deterioration of the air quality in the city. The region is characterized by a semi - arid to arid climate, rains fall regularly in autumn and winter. The average annual rainfall is estimated at 280 mm. Temperatures are moderate with an average annual value of 18 ° C. The relative humidity of the air is on average between 70% and 80%. The winds are particularly strong in the coastal zone. They are predominantly northwest and are frequent by the end of the afternoon.

The approach adopted in this study is to consult the old publications on the Moroccan lichenic flora relating to the coastal region of Safi - Essaouira and to compare them with the current local flora. The goal is to highlight any changes in this flora indicating ecological disturbances.

Seven sites were selected assuming an increasing air pollution gradient between non - urban and urban areas (Fig.1). Earlier work developed by Essilmi & al (2017) and completed in 2020 in this study has established a list of the majority of lichen species of this region, but two species of lichens have been identified in previous studies (Werner R. G, before 1975), but in past ecological contexts different from those present and aroused the curiosity of our work: they are Ramalina implexa and Coscinocladium gaditanum. After comparison between old and recent inventories we noted the disappearance of Ramalina implexa and the geographic expansion towards the south of Coscinocladium gaditanum.

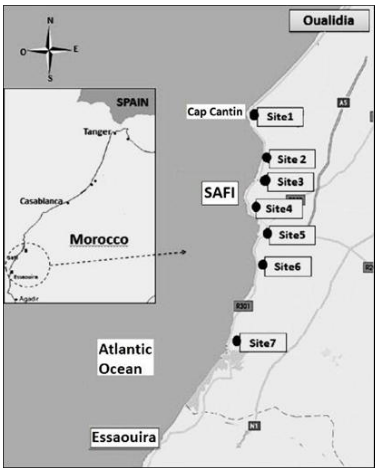



**Figure 1:** Location of the Safi - Essaouira coastline and the seven studied sites

***Bioclimatic indicators***

To verify a possible impact of climate change on lichens in the region, the analysis of bioclimatic indicators was also included. The Emberger and De Martonne indices were calculated using monthly temperature and precipitation data.

The Emberger pluviothermic quotient is determined by the combination of the 3 main climate factors, it is defined by the formula:

$$Q = \frac{1000 \cdot P}{((M+m)/2) \cdot (M-m)} = \frac{2000 \cdot P}{M^2 - m^2}.$$

Q: The Emberger pluviothermic quotient
M: average of maxima (maximum daily temperatures) of the hottest month, in kelvins
M: average of the minimum (minimum daily temperatures) of the coldest month, in kelvins P: total annual precipitation, in millimeters

The aridity Martonne index is defined by the formula:

$$I_a = \frac{P}{t+10}.$$

I: is the De Martonne index in mm / ° C,
T: is the average annual temperature in ° C P: total annual precipitation, in millimeters.

The interpretation of I values is:
If I < 10, the climate is arid
If 10 < I <20, the climate is semi - arid
If 20 < I < 50, the climate is cold, temperate or tropical If I > 50, the climate is equatorial or mountainous

***Bio - indication of atmospheric NOx levels***

The saxicolous lichens studied have different tolerances to nitrogen. . Nimis & Martellos proposed an eutrophication index: this value expresses the degree of nitrogen pollution. There are five values:
1= no eutrophication
2= very weak eutrophication 3= weak eutrophication
4= rather high eutrophication 5= very high eutrophication

## 3. Results

### 1) Bionindication of a climatic disturbance

Before 1975, Werner R. G reported the presence of Ramalina implexa in Cap Cantin near Safi; Ramalina implexa is a fruticose saxicolous or corticolous species, filiform and entangled, yellowish green in color; its branches have longitudinal folds with hooked ends, apothecia are very rare. It is P+ orange and K+ yellow then brown (Figure 2).

Current lichen surveys in Cap Cantin (Site 1) have revealed the absence of Ramalina implexa. This may be due to a disturbance of its bioclimatic space caused by air pollution or a disruption of climatic factors. But Cap Cantin is a less polluted rural locality because it is less urbanized, non - industrialized and where the presence of certain lichens like Ramalina lacera, Ramalina pollinaria, Roccela phycopsis and Telochistes villosus indicates good air quality. In his phytogeographic synthesis of North Africa lichenic flora, Werner R. G (1955) described Ramalina implexa as a tropical species that probably returned to North Africa to settle in Cap Cantin.

**Figure 2:** Ramalina implexa, (saxicolous form). (Photo: French Association of Lichenology, 2020)

To correlate the disappearance of Ramalina implexa with a climatic disturbance, we used the climatic data (precipitation and temperature), provided by the National Department of Meteorology (DMN) which covered the period from 1966 to 2020 for the meteorological station of Safi. The calculations allow following the evolution of:

- The Emberger pluviothermic quotient (Q) (Fig.3)
- The aridity Martonne index (I) (Fig.4)
- The evolution of annual precipitation (Fig.5).

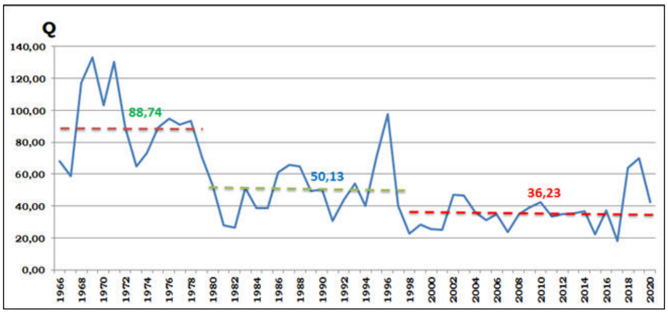



**Figure 3:** The pluviothermic Quotient evolution in the Safi province since 1966 to 2020

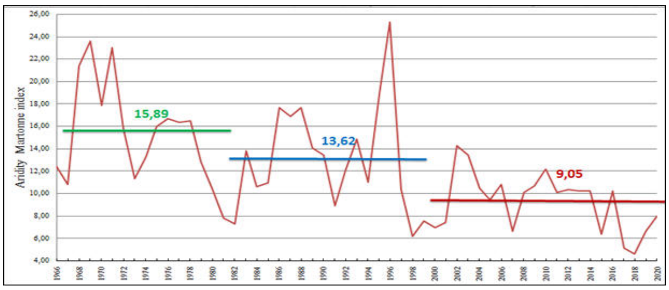



**Figure 4:** The aridity Martonne index evolution in the Safi province since 1966 to 2020

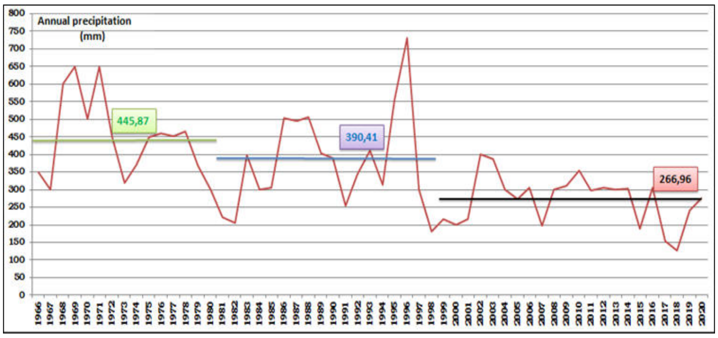



**Figure 5:** Evolution of annual precipitations in the province of Safi since 1966 to 2020

1 - Bioindication of atmospheric NOx levels

According to the values of the eutrophication index, we have classified the saxicolous lichens harvested in three categories: O = oligotroph (eutrophication index =1, 2 and 3), M = mesotroph (eutrophication index =4), and E = eutroph (eutrophication index =5) (table 1).

**Table 1:** Inventory of saxicole lichens harvested and identified in each Site ("+" means species present). O = oligotroph, M = mesotroph and E = eutroph.

| | | Sites | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
| *Caloplaca citrina* | E | + | + | + | + | + | + | + |
| *Diploschistes scruposus* | E | + | + | | | | | + |
| *Lecania atrynoide* | *E* | + | | | | | + | + |
| *Lecania erysibe* | *E* | + | + | + | + | + | + | + |
| *Myriolecis bandolensis* | *E* | + | + | | | + | | |
| *Opegrapha rupestris* | *E* | + | | | | | | + |
| *Physcia adscendens* | *E* | + | + | | | | + | + |
| *Verrucaria nigrescens* | *E* | + | + | + | | + | + | + |
| *Xanthoria calcicola* | *E* | + | + | + | | + | + | + |
| *Xanthoria parietina* | *E* | + | + | + | | + | + | + |
| *Caloplaca aurantia* | *M* | + | + | + | | + | + | + |
| *Caloplaca crenulatella* | *M* | + | | + | | + | + | + |
| *Coscinocladium gaditanum* | *M* | + | + | + | | + | + | + |
| *Diploicia canescens* | *M* | + | | | | | + | + |
| *Diplotomma alboatrum* | *M* | + | + | + | | | + | + |
| *Lecania spadicea* | *M* | + | + | + | | | + | + |
| *Myriolecis albescens* | *M* | + | + | + | | | + | + |
| *Myriolecis dispersa* | *M* | + | + | + | | | + | + |
| *Ramalina pollinaria* | *M* | + | + | | | | | + |
| *Toninia aromatic* | *M* | + | | | | | | + |
| *Verrucaria viridula* | *M* | + | + | + | | + | + | + |
| *Acrocordia conoidea* | *O* | + | + | | | | + | + |
| *Alyxoria subelevata* | *O* | + | + | | | | | + |
| *Alyxoria variiformis* | *O* | + | + | | | | | |
| *Arthonia calcarea* | *O* | + | + | | | | + | + |
| *Aspicilia calcarea* | *O* | + | + | + | | + | + | + |
| *Bagliettoa calciseda* | *O* | + | + | | | | + | + |
| *Buellia dispersa* | *O* | + | | | | | | + |
| *Caloplaca chalybaea* | *O* | + | + | + | | | | + |
| *Caloplaca erythrocarpa* | *O* | + | + | + | | | + | + |
| *Caloplaca lactea* | *O* | + | + | + | | + | + | + |
| *Caloplaca subochracea* | *O* | + | + | | | | | + |
| *Dirina massiliensis* | *O* | + | + | | | | + | + |
| *Myriolecis crenulata* | *O* | + | + | + | | | + | + |
| *Parabagliettoa cyanea* | *O* | + | + | | | | + | + |
| *Rocella phycopsis.* | *O* | + | + | | | | + | + |

Comparing the proportions of oligotrophic, mesotrophic and eutrophic species from one site S1 to S7, there is a change in the composition of the saxicolous lichen community that reveals a nitrogen gradient (Figure 6, table 1). The closer we get to downtown Safi (site S3) and the industrial area (site S4), the more the community of saxicolous lichen is dominated by eutrophs. Sites containing higher proportions of eutrophic and less oligotrophic species therefore receive more nitrogen (sites S3, S4, and S5) than sites containing many oligotrophs and few eutrophs (sites S1, S2, S6 and S7).

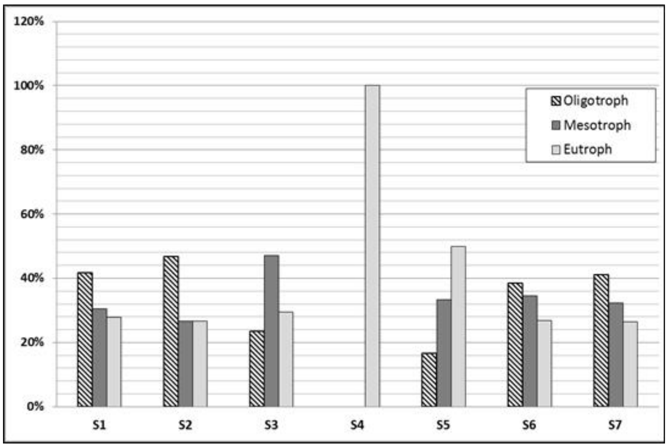



**Figure 6:** Percentage of oligotrophic, mesotrophic and eutrophic lichens in the seven sites

## 4. Discussion

### 1) Bioindication of aridity by biological spectrum

To compare the lichen biological spectrum with the ecological conditions of the biotope, Renaut et *al.* (1968) reported in a study carried out in Morocco that a spectrum showing 50% of crustose corresponds to a pluviothermic quotient Q = 80; a spectrum with 70% of crustose would correspond to Q=60; Finally a spectrum with 80% of crustose would indicate a pluviothermic quotient less than 60. The study of the biological spectrum of lichens in this study area showed that crustose lichens reach an average frequency of 74% (Figure7). This value reflects the current bioclimatic semi - arid to arid character of the city.

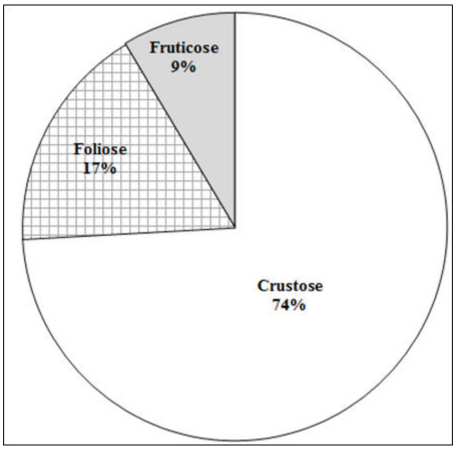



**Figure 7:** Biological spectrum of harvested lichens

### 2) Historical Emberger pluviothermic quotient Q

The evolution of the regional pluviothermic quotient between 1966 and 2020 (Figure 3) is characterized by three distinct periods: the first from 1966 to 1980 with an average of 88, 74; the second from 1980 to 1998 with an average of 50, 13 and the third from 1998 to 2020 with an average of 36.23. The year 2017 recorded a very low regional pluviothermic quotient Q = 18.08. The value of Q over the last 20 years is on average 36, 23. This value is reflected by the dominance of crustose lichens which represent 74% of the biological spectrum. The decrease of the pluviothermic quotient between 1966 and 2020 can be explained by an increase in the aridity of the region.

### 3) Historical variation of the aridity Martonne index

The mean aridity index calculated over the period 1966 - 1980, the period 1981 - 1997 and the period 1998 - 20120 are respectively 15.89, 13.62 and 9.05 (Figure 4). These results show the climate trend of this region towards more aridity. Studies have confirmed that over the past 30 years, aridity has been shifting from southern to northern Morocco Driouech (2010) and Sebbar et *al.* (2012). The lichenic biological spectrum with low presence of fruticose lichens (9%), foliose lichens (17%), and dominance of crustose lichens (74%) indicate and confirm the current arid climate of the region.

### 4) Bioindication of paleomicro climate

Ramalina implexa is a hygrophilous species requiring open habitat and a humid climate in very foggy sites (Nimis). Its absence in Cap Cantin is due to a disturbance of its bioclimatic space caused by a disturbance of climatic factors. Knippertz et al. (2003) have noted a decrease in rainfall in the Mediterranean region since the mid - 1970s. Sebbar et *al.* (2013) confirmed that since the 1970s, Morocco has experienced difficult weather conditions by the persistence and the severity of the drought; the annual rainfall totals in Morocco have undergone significant downward trends. In Safi region, the annual rainfall trend between 1966 and 2020 is characterized by three distinct periods (Figure 5): the first from 1966 to 1980 with an average of 445.87 mm/year, the second from 1981 to 1997 with an average of 390.41 mm/year and the third with an average of 266.96 mm/year. With the exception of 1996, which was a record year along the Atlantic coast up to Agadir (Driouech, 2010), there is a reduction in rainfall potential in the region. Similar results have been reported in other regions of Morocco (Driouech, 2006, Sebbar et *al.*, 2011). The increase in rainfall in the region from 1966 to 1980 is due to tropical inputs (sebbar 2012). Werner R. G (1901 - 1977) describes Ramalina implexa as a tropical species that probably went back to North Africa to settle in Cap Cantin. Before 1975, the regional pluviothermic quotient averaged Q=88.74. This value reflects climatic conditions favoring the development of fruticose lichens such as Ramalina implexa. (Renaut et *al.*, 1968).

**Table 2:** Evolution of the bioclimatic zone of the region from 1966 to 2020

| Periods | 1966 - 1980 | 1981 - 1997 | 1998 - 2020 |
|---|---|---|---|
| Q2 average | 88, 74 | 50, 13 | 32, 86 |
| m average (°C) | 7, 79 | 6, 77 | 7, 31 |
| bioclimatic zone | Sub - humid with warm winter | Semi - arid with temperate winter | Arid with à warm winter |

According to the value of Q and m, the Emberger climagram of the region allows to characterize the evolution of the climate from 1966 to 2020: There is a clear transition from a warm sub - humid climate to a warm arid climate. (Figure 8, Table 2).

Before 1975, the presence of Ramalina implexa indicates a tropical local microclimate at Cap Cantin within a warm sub - humid regional mesoclimate (m = 7.79 °C and Q= 88.74), however this regional climate over time knew a tendency towards the aridification whose consequence is a loss of the tropical climatic character in Cap Cantin. This finding is confirmed by Driouech (2010) and Sebbar & al. (2012) who reported that over the last 30 years, aridity has changed from southern to northern Morocco. This evolution must be correlated with a decrease in precipitation and more severe drought frequencies, which is confirmed by global warming (Sebbar). The disappearance of Ramalina implexa from Cap Cantin is also correlated with its rarity in the Mediterranean and European region: In 1989 Ramalina implexa was reported in the Red List of Macrolichens in the European Community (Sérusiaux, 1989), In 2003 Claude Roux reported this species as a macrolichens to protect in France and in 2016 it was reported as a rare species in Italy (Nimis).

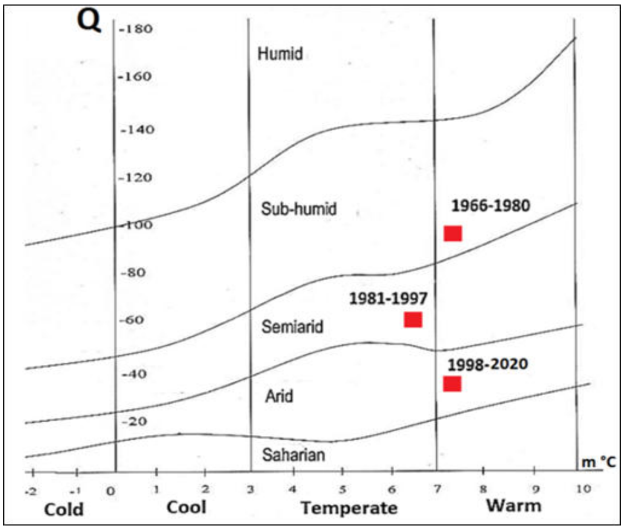



**Figure 8:** Projection of the study area in the Emberger climagramm.

**5) Bioindication of nitrogen pollution**

Before the studies of the Crespo et *al*. (2004), the lichen Coscinocladium gaditanum was classified as Buellia lisbonensis. In 1976, Werner reported that Buellia lisbonensis is known in Morocco between Rabat and Cap Cantin (north of Safi) on maritime rocks and coastal limestones or agglomerated sands. It seems, therefore, that the southern phytogeographic limit of this mediterranean species is 36 km north of Safi. The update of its phytogeographical distribution was published in 2004 by Crespo and confirms this limit north of Safi. Our field surveys show that Coscinocladium gaditanum is present 160 km south of Cap Cantin.

Coscinocladium gaditanum is reported by Nimis (2021) as a nitrophilous species and it appears that the increase in the air nitrogen content has favored the proliferation of mesotrophic and eutrophic species in the Safi city at the expense of the oligotrophic species. This increase of the air nitrogen can be discussed on two levels:

***Socioeconomic level:***

During the last 15 years, Morocco has experienced strong economic growth, the population has increased by 1.25% per year on average (Haut Commissariat au Plan.2015); while the urban population, mainly concentrated in coastal areas, grew even more rapidly (2.1% per year) (In 2014 the population of the Safi city reached 347, 925 inhabitants). Coastal cities, such as Safi, host most of the country's economic activities, such as energy and industry, and have experienced rapid growth in road traffic (United Nations Economic Commission for Europe 2014). These activities have resulted in a rapid increase in local air pollutant emissions. In 2014, the Moroccan Ministry of the Environment published the annual average NO2 recorded at Safi, which increased from 28 µg/m3 to 53.62 µg/m**3** between 2007 and 2011, exceeding the annual standard of 50µg/m**3** in 2010 and 2011; This annual increase in NOx concentrations is correlated with its national increase in recent years (World Bank Open Data.2018) (Figure 9).

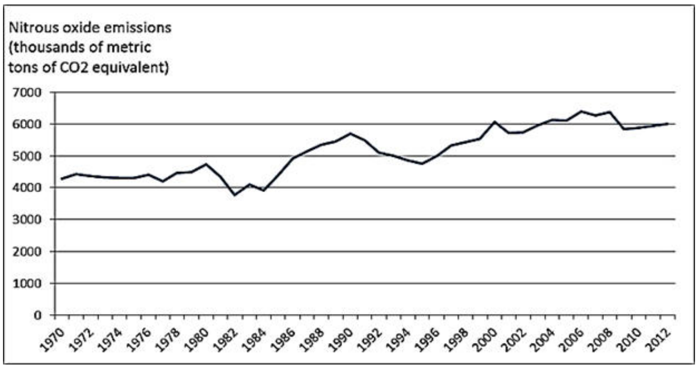



**Figure 9:** Evolution of nitrous oxide emissions in Morocco from 1970 to 2012

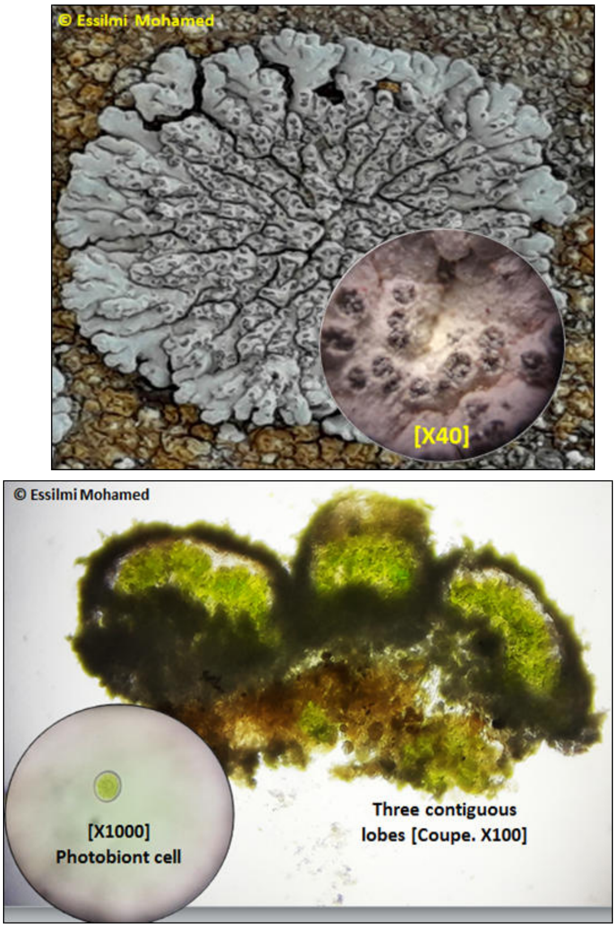
***Ecological level:***

Lachat et al. (2011) report that eutrophication promotes the differential development of some lichen species resistant to the effects of pollution. It has been shown that the composition of lichen communities near highways is evolving towards species associations with more nitrophyte lichens (Madl, P.2009).

In general, increased nitrogen deposition affects the composition of plant diversity and can lead to the dominance of nitrophyte species (Frati et *al*.2007, Riddell et *al*.2011). One of the mechanisms leading to the extreme sensitivity of oligotrophic lichens to an excessive supply of nitrogen compounds could be the binding of these compounds to the cell walls until concentrations become toxic by causing an electrolyte leak, as it has been demonstrated in Evernia prunastri by Munzi et al. (2009). The NOx gases are known to negatively affect the physiological status of oligotrophic lichens: Once in solution in lichen, NOx are powerful catalysts for membrane lipid peroxidation, which results in membrane damages (Das *et al*.2011, Paoli *et al*.2014, Lucadamo et *al*.2015).

**Update of the southern limit of Coscinocladium gaditanum**

Coscinocladium gaditanum is a crustose lichen forming rosettes with 1cm to 3cm in diameter, whitish gray to white, with a bluish tinge in the central part. Its reproduction is asexual and ensured by blackish soralia more or less in cups (Figure 10). The Chemical reagents have no action (K - , C - , KC - , P -).




(b)

**Figure 10:** Microscopic observation of Coscinocladium gaditanum (a) Morphology of the thallus; (b) Section of the thallus. (Photos Mohamed Essilmi - 01/08/2019)

Coscinocladium gaditanum was reported by Werner (1976) and Crespo (2004) as a species whose air distribution is limited to 36 km north of Safi; its geographical expansion towards the south must be due to an increase of the nitrogen rate of the air in the studied zone which favored the proliferation of the mesotrophic and eutrophic lichens in the city of Safi at the expense of the oligotrophic species, but at the same time This change has facilitated the expansion of Coscinocladium gaditanum.

Mostly known in Portugal and Spain, Coscinocladium gaditanum is generally a saxicolous species but can also be found on the mortar receiving bird droppings and on the fence walls of agricultural fields where the nitrogen supply is well marked. Currently, C. gaditanum is present until the province of Essaouira (figure 11).

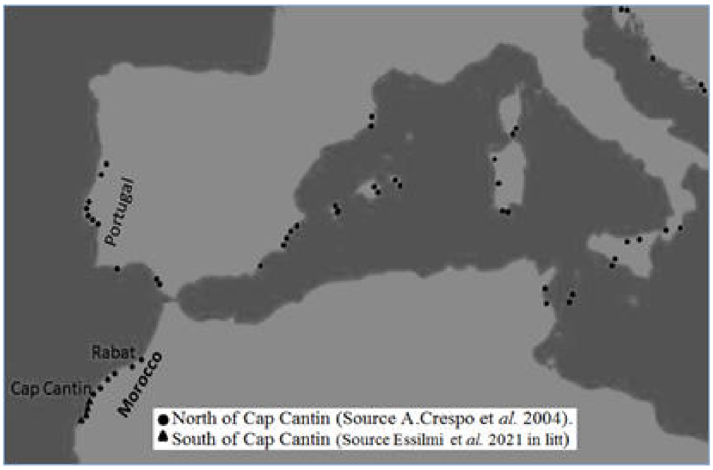



**Figure 11:** The distribution of Coscinocladium gaditanum

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## 5. Conclusion

The bioindication study using the eutrophication index allows concluding that in the industrial zone of Safi (site S4) we have a strong eutrophication due to nitrogen pollution as evidenced by the proliferation of eutrophic lichens and the disappearance of the mesotrophic and oligotrophic lichens. The further away from this area, the lower is the rate of nitrogen pollution. The nitrogen pollution gradient in the region therefore tends to modify the structure of the saxicolous lichen community from a structure dominated by oligotrophic species, in less polluted sites, to a structure dominated by eutrophic species in polluted sites. The Atlantic Coast Safi - Essaouira seems to be correlated with the road traffic, the degree of urbanization and the economic activity of the region.

The study of the lichenic biological spectrum has shown that the tendency of the region's climate towards aridity favors the development of crustose lichens whose rate exceeds 70% of the lichenic cover. The study of the temperature and precipitation variations of the region since 1966 to 2020 has allowed highlighting that the climate of the region over time knew a tendency toward aridification resulting in a loss of a tropical microclimate in Cap Cantin.

Several studies suggest the relationship between ecological disturbances and the geographical distribution of organisms of various taxa. These disturbances facilitate the expansion of lichens with a high tolerance of habitat and described as generalists or "Winners" as the case of Coscinocladium gaditanum; while lichens with restricted habitat profiles lose their range and described as specialists or "losers" such as Ramalina implexa,.

## 6. Acknowledgments

We warmly thank the members of the French association of lichenology (AFL) for their contribution to the identification of certain species during the determination session at the laboratory of the forest ecology station of Fontainebleau (France, 2016). We also thank Claude Roux for the contribution to the the determination of corticolous species (December 2019)

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