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Review

May the Diversity of Epiphytic Lichens Be Used in Environmental Forensics?

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Abstract: Epiphytic (tree inhabiting) lichens, well-known biomonitors of atmospheric pollution, have a great potential for being used in environmental forensics. Monitoring changes in biodiversity is a useful method for evaluating the quality of an ecosystem. Lichen species occurring within an area show measurable responses to environmental changes, and lichen biodiversity counts can be taken as reliable estimates of environmental quality, with high values corresponding to unpolluted or low polluted conditions and low values to polluted ones. Lichen diversity studies may be very useful in the framework of environmental forensics, since they may highlight the biological effects of pollutants and constitute the base for epidemiological studies. It is thus of paramount importance that great care is taken in the interpretation of the results, especially in the context of a rapidly changing environment and facing global change scenarios. For this reason, it seems advisable to produce several zonal maps, each based on different species groups, and each interpreted in a different way. This exercise could also be a valid support in the framework of a sensitivity analysis, to support or reject the primary results. In addition, a clear and formal expression of the overall uncertainty of the outputs is absolutely necessary.

Keywords: air pollution; biodiversity; bioindicators; biomonitoring; environment; uncertainty

1. Introduction

Environmental forensics relates to the understanding of the extent, duration, and responsibility for environmental contamination in a legal context. More in detail, environmental forensics deals with scientific investigations that address environment contamination, its sources, and the historical reconstruction of its release into the environment. Issues involved in environmental forensics most commonly include the distinction between polluted and unpolluted areas, as well as the identification and quantification of the contributions from various sources to contaminated sites [1].

Forensic botany, curiously, usually remains confined almost exclusively to criminal investigations, i.e., to the understanding of where and when a crime was committed and who committed the crime [2]. Forensic botany is now well accepted by the legal system and has been used also in the framework of war crimes [3]. Likewise, forensic mycology is also popular in criminal investigations, and there are reports of studies using lichens [4]. Besides their valuable use in criminal investigations, epiphytic (tree inhabiting) lichens, well-known biomonitors of atmospheric pollution, have a great potential for being used in environmental forensics.

2. Biological Monitoring

The use of living organisms for the evaluation of environmental quality is now widely recommended to support and integrate the data derived from physico-chemical measurements [5]. Biomonitoring techniques are based on the fact that modifications in environmental parameters cause changes in the biota: Assessment of such changes provides useful information on the state of health of the environment [6]. When a natural habitat is disturbed by human activities, biological communities accurately reflect ongoing changes. Biomonitoring may thus be used to evaluate the state of the environment through the evaluation of changes in the biodiversity of sensitive components of the ecosystems [5].

The use of biomonitors is necessarily complementary to conventional monitoring by instrumental devices, but it should be remembered that it is the only way to evaluate the biological impact of pollutants [7]. In addition, the biological consequences of pollution events may be much more longer lasting than the events themselves or, alternatively, long-lasting low pollution events, which are not likely to be identified by devices, may cause biological consequences.

When assessing air pollution by chemico-physical analyses, two approaches are possible: Source-oriented dispersion modeling, based on the combination of emission loads and meteorological data, and receptor-oriented measurement of atmospheric pollutants. Owing to economic constraints, the former is attracting a wider interest, since field measurements require expensive instruments and maintenance. For this reason, monitoring devices are usually deployed at a few polluted sites or in the proximity of specific sources. Biomonitoring is a valid alternative for greatly increasing the area that can be surveyed, or the density of sampling sites, or both. Thus, the use of biomonitors in field studies has the great advantage of permitting environmental monitoring without the widespread establishment and maintenance of sophisticated and costly equipment.

3. Lichen Biomonitoring

Lichens are notoriously among the most valuable biomonitors of atmospheric pollution [8]. Lichens are peculiar living organisms since they are mycobionts (fungi) living in symbiosis with one or even two photobionts, usually a green alga, but sometimes also a cyanobacterium, thus representing a unique association of two or sometimes three different kingdoms. They are perennial, slow growing, and maintain a fairly uniform morphology in time. They are also highly dependent on the atmosphere for nutrients and do not shed plant parts as readily as vascular plants. Additionally, the lack of a waxy cuticle and stomata, typical of higher plants, allows many contaminants to be absorbed over their whole surface [9].

Biomonitoring with lichens is based on the detection of changes in the community composition (biodiversity), in trace element content and in the physiological status, providing useful evidence for spatial and temporal trends in ambient pollution burdens [7]. Using lichens as biomonitors of air quality may become very useful in urban and industrialized areas, where the high density of different emitting sources makes monitoring of air pollution with conventional physico-chemical methods an extremely difficult task due to the variety of pollutants [10]. In addition, there is evidence that lichen biomonitoring may be used as a proxy for the effects of air pollution on human health [11].

4. Lichen Diversity

Monitoring changes in biodiversity provides a useful method for evaluating the status (quality) of an ecosystem [12]. Lichen species occurring within an area show measurable responses to environmental changes and lichen biodiversity counts can be taken as reliable estimates of environmental quality, with high values corresponding to unpolluted or low polluted conditions and low values to polluted ones [7].

The mapping of the lichen biodiversity has become routine in several countries [13] since it gives an indication of the quality of the air based on the biological impact of air pollution. In addition, although this bioindicator requires a time lag for tracking changes in air quality, which must be sufficient to allow for community changes in species number and composition, there is evidence that lichens respond quickly to changing concentrations of air pollutants [14]. Monitoring the lichen biodiversity is cost effective and provides results on which predictions for human health can be based [11].

5. Measuring the Diversity of Epiphytic Lichens

The standard method for measuring the diversity of epiphytic lichens [15] is based on the sampling of the lichen species present within a grid of 15 × 50 cm divided into five quadrats of 10 × 10 cm. The grid has to be placed systematically on the bole of standard trees at the four main cardinal exposures, at a height of 1 m from the ground. For each species, a frequency value in the range 1–20 is obtained as the number of quadrats in which the species is present. The lichen diversity value (LDV) of each tree is calculated as the sum of frequencies of all species present.

6. Interpretation and Presentation of the Results

The interpretation of the results is based on the deviation of the lichen diversity from conditions in undisturbed ecosystems. The LDV measured in unpolluted areas is taken as the maximum potential diversity, and the LDV values are consequently scaled and divided into classes of alteration from such a value [16,17].

The outcome of biomonitoring studies based on the lichen diversity is usually a zonal map depicting different parts of the study area that are classified into different air quality zones. Several studies confirmed that lichen diversity values are correlated with acidic gaseous pollutants such as SO_2 and NO_x [18,19].

7. Lichen Diversity and Environmental Forensics

Application of the lichen diversity in biomonitoring studies of air pollution is very useful since the area under investigation may be classified into air quality zones according to the epiphytic lichen communities. The results of lichen diversity studies may be seen as based on receptor modeling integrated spatially. As a consequence, the resulting map can be interpreted as a map of the biological effects of air pollutants and can have a great value in depicting the situation of biological consequences in a given area. In other words, areas with few lichens shall be regarded as risk areas.

However, to be relevant in the framework of environmental forensics, the outcomes of lichen diversity surveys must be relevant in the source evaluation. This is the most crucial point of any monitoring study based on receptor modeling, irrespective of whether it is biological or physico-chemical. A clear link to source identification based on environmental concentrations of pollutants is not simple and is not always feasible.

As an example, coal-based thermal power plants are well-known emitters of great amounts of SO_2 and NO_x to the atmosphere, and hence lichen diversity studies are well suited for monitoring the impact of such installations. In the literature there are many examples on this topic, also as follow up studies after technical improvement to the discharge chimneys. Rao and LeBlanc [20] showed a decreased lichen diversity around isolated factories emitting primarily SO_2. Nash [21] found that lichen species diversity is markedly reduced near an isolated zinc factory where both SO_2 and zinc were released. Nimis et al. [22] and Castello et al. [23] found highly reduced lichen diversity around two coal fired power plants in Liguria. Showman [24] reported that epiphytic lichens are suitable bioindicators around a coal-fired power generating plant in Ohio (USA), but noted that even though large quantities of SO_2 were released by the power plant, the effect on lichens was not as severe as that in many urban areas. After some years, the same author reported that air quality improvement near the same coal-fired power plant led to great lichen recolonization [25].

However, when the power plants are located in a complex environment, where many other pollution sources are present, e.g., vehicular traffic, domestic heating, other industrial plants, and harbors, the link with the source may not be clear [26]. The situation in urban and industrial areas is very complex. Smoke is emitted from a number of low-level sources, and usually contains a mixture of phytotoxic compounds. These variables create a very complex system and meaningful conclusions are indeed difficult to obtain. In addition, if more than one source affects the results, it is very unlikely that lichen diversity counts may help allocate between sources [26].

A characteristic of lichen diversity biomonitoring (which is a weak point in forensic cases, but has advantages and disadvantages) is that the results are largely nonspecific and cannot be attributed with certainty to a single pollutant, as in the case of, e.g., ozone monitoring using sensitive tobacco plants [27]. So, the results of such studies have often to be considered as explorative over more or less large areas to pinpoint to risk areas, i.e., areas where there is a risk of biological consequences from air pollutants. However, in case of before–after studies, under the assumption of unchanging conditions other than the work under study, establishing a network for the study of the lichen diversity may be very helpful in detecting negative changes in the surrounding environment. Will-Wolf [28] investigated the lichen communities before and after the starting up of a coal-fired power plant in Wisconsin (USA) to determine if SO_2 air pollution from the plant had a negative effect and found that no lichen species were lost due to SO_2, but communities in higher-SO_2 areas exhibited more changes in species frequencies than did communities in lower-SO_2 areas. An elegant example of a long-term lichen biomonitoring program may be found in Paoli et al. [29], which used the diversity of epiphytic lichens and the accumulation of selected trace elements in the lichen *Flavoparmelia caperata* to assess the impact of a landfill in central Italy across 14 years of waste management. The results indicated an increased deposition of some elements (i.e., Cd, Cr, Fe, and Ni) and a decrease of the lichen diversity around the landfill. The authors recommended the use of lichens for monitoring air pollution arising from dumping activities, since it provides essential ecological information for the industrial regulatory process.

8. Possible Pitfalls in Interpreting the Results of Lichen Diversity Monitoring

There is enough evidence that lichen biomonitoring is a reliable tool for the evaluation of biological effects caused by air pollutants released from geothermal power stations [30], and the diversity of epiphytic lichens has successfully been used for the assessment of air quality in geothermal areas [31–36]. Studies indicated the highly toxic gaseous pollutant hydrogen sulfide (H_2S), which is emitted at relatively high concentrations, as the main cause of lichen decline around geothermal power plants.

Loppi and Nascimbene [37] clearly demonstrated that not only H_2S but also NH_3 released from geothermal power plants shapes the epiphytic lichen vegetation, making the interpretation of lichen diversity counts in terms of air pollution by H_2S difficult. In fact, relatively high diversity values, unrelated with the levels of air pollution by H_2S, were found around geothermal installations as a consequence of the presence of nitrophytic lichen species, determined by NH_3. However, in another nearby geothermal area not affected by NH_3 release, the interpretation of lichen diversity results was correct and not biased by the presence of nitrophytic species. Based on these findings, the authors recommended that in the presence of NH_3, nitrophytic species are not included in the calculation of lichen diversity values.

9. Present Challenges in Using Lichen Diversity in Air Pollution Monitoring

Over the last decades, there has been a huge drop in SO_2 atmospheric concentrations as a result of policies specifically dedicated to the abatement of the release of this pollutant [38]. Conversely, the release of nitrogen compounds, especially reduced ones linked to agricultural activities, has greatly enhanced [39]. The sensitivity of lichens to nitrogen pollution is well established [40,41], and NH_3 is widely recognized as a key factor driving the composition of lichen assemblages [42]. An increase in nitrophytic lichen species and a decrease in acidophytic species was noted in areas of the Netherlands with high concentrations of atmospheric NH_3, and this circumstance was particularly evident on trees with an acid bark; since on these substrates, nitrophytes were previously scarce or absent [43]. More recently, following decreasing atmospheric NH_3 concentrations, a decrease in nitrophytic lichen species has been reported [44]. According to Van Herk [42], the effects of ammonia on nitrophytes are not primarily caused by the increased availability of nitrogen, determined by ammonium, but rather by the rise in bark pH, caused by the adsorption of NH_3. On the contrary, acidophytes are sensitive both to a rise of the pH caused by ammonia and to an increase in the ammonium content of the bark [42,45]. In fact, NH_4^+ is nitrified into NO_3^-, and it add considerably to acidification [45]. However, in areas with a warm and dry climate, such as the Mediterranean basin, detection of the effects of nitrogen may be hampered by the dominating effect of dust [46]. In fact, in the Mediterranean area, dust impregnation, light, and dry conditions also raise bark pH, allowing nitrophytic species to develop on normally acid-barked trees [47,48]. In such cases, monitoring the diversity of epiphytic lichens on pine trees may be very useful [49].

Based on the positive reaction of nitrophytic species to increased atmospheric nitrogen, several authors suggested that epiphytic lichen communities should be distinguished into two main functional groups: Nitrophytic species, which are expected to dominate at medium–high nitrogen concentrations, and oligotrophic species, which are associated with low levels of nutrients [50,51]. Under conditions of high nitrogen deposition, the relationship of air pollutants causing a decrease in epiphytic lichens becomes much more complex [44,52]. However, as bioindication is largely a matter of data interpretation [16], this does not mean that the effects of individual pollutants could not be detected, but it means that great care must be taken in the interpretation of the results.

In this context, when monitoring the diversity of epiphytic lichens, the interpretation of the results could consist of several maps (Figure 1), each based on different groups of species, and each interpreted in a different way [52,53]. According to these authors, the exclusion of nitrophytic species (objectively selected using an online database) from the calculation of the index of lichen diversity led to more realistic results; conversely, the use of only nitrophytic species allowed the mapping of the eutrophication in the area, which resulted in lichens heavily affected by agricultural activities. Mapping using only strictly nitrophytic species showed "hot spots" where ammonia emission from animal husbandry plays an important role.

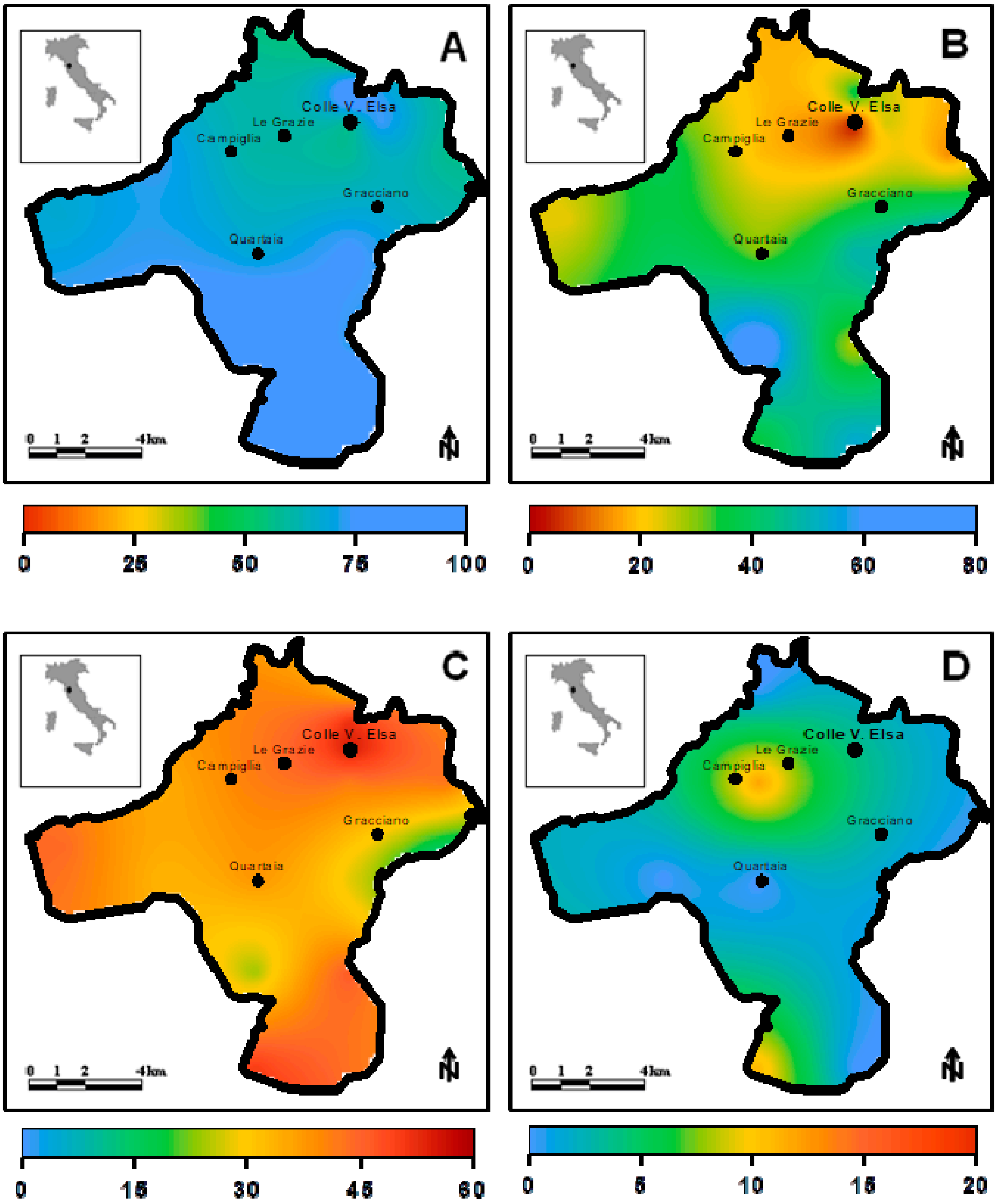

Figure 1. (**A**) Maps of the biodiversity of all epiphytic lichens, (**B**) biodiversity excluding nitrophytic species, (**C**) biodiversity of nitrophytic species, and (**D**) biodiversity of strictly nitrophytic species. Note that high values in Figure 1A,B correspond to low pollution, while high values in Figure 1C,D correspond to high pollution (after [52]).

10. Monitoring Lichen Diversity under a Climate-Change Scenario

It is well known that climate change is greatly influencing ecosystems, causing many different ecological responses. In the Mediterranean area, climate change is foreseen to induce a wide range of consequences, mostly related to the exacerbation of already existing problems such as land desertification, water scarcity, and food production [54]. Higher temperatures, lower water availability, and increased salinity that relate to climate change have adverse effects on plant photosynthesis and productivity [55,56].

Lichens are responding to global warming [57] and some long-term monitoring programs proved their sensitivity, mainly through a community approach [58], while only few studies investigated ecophysiological responses to climate change [59,60]. There is evidence that lichens can profitably be used as indicators of climate change [61–63].

Loppi et al. [64] investigated the epiphytic lichen vegetation of central Italy in agricultural and non-agricultural areas along an altitudinal transect characterized by different climatic conditions. The results showed that lichen communities are influenced more by climate than by agricultural practices or at least that climatic parameters can mask any effects of agriculture. Similar results were achieved by Matos et al. [65], which showed that lichen diversity metrics mainly reflect changes in climate rather than in nitrogen pollution level.

11. Proxies for Lichen Diversity

Lichen diversity studies require a great taxonomic skill for correct species identification [66]. However, this usually implies a great financial load for these studies. Aiming at constraining the costs of wide-scale lichen diversity studies has led to the search for valid proxies of global biodiversity, through the use of rapid biodiversity assessment methods. Giordani et al. [67] showed that simplified lists based on morphospecies may give a good correlation with the total biodiversity only when used by operators with a high taxonomic knowledge, though without reducing the time needed in the field. Nevertheless, the lichen monitoring method currently in use in the USA [68] makes use only of the macrolichens and gives a visual estimate of the abundance at the site. However, a direct comparison of the European and the USA methods showed that both approaches give highly similar interpretations [65].

12. Toward a Worldwide Lichen Diversity Index

The search for a worldwide valid biological index for monitoring the effects of air pollution on ecosystems has led to the comparison of the European and USA methods of lichen biomonitoring in terms of taxonomic diversity, functional diversity, and community composition shifts [65]. The results were encouraging and showed that despite the use of very different protocols and different absolute values, both methods give similar interpretations and overall highlight the same degree of change.

13. The Spatial Scale

Biodiversity assessments are strongly dependent on the spatial scale of observation, with natural spatial variation or the spacing of sampling units that may greatly influence the results [69]. According to Wolterbeek et al. [70], in order to have biomonitoring results of good quality, one key point is that the signal at the wider scale of the study (survey variance) is greater than the noise at the local scale of the site (site variance).

Lichen biomonitoring surveys usually focus on between-site variability using a high sampling density at this scale and do not sufficiently consider possible within-site variability, probably because this would imply sampling more trees, and thus increase the cost of the survey [71]. Giordani et al. [72] compared four sites in Italy, along gradients of atmospheric pollution and climate, to test the partitioning of the variance components of lichen diversity across spatial scales (from trunks to landscapes). Despite environmental heterogeneity, these authors observed a comparable spatial variance, but residuals often overcame between-plot variability, leading to biased estimation of atmospheric pollution effects.

Ferretti et al. [71] investigated the reliability of different sampling densities for estimating and mapping lichen diversity in biomonitoring studies, and found that the relative error in mean lichen diversity value and the error associated with the interpolation of lichen diversity values increased as the sampling density decreased. However, the relationship was not linear and even a considerable reduction (up to 50%) in the sampling effort may lead to small error increases in the mean estimates (<6%) and mapping (<18%). In addition, changing the spatial location of the sampling sites causes changes in the output maps generated, and only a random selection of sites or a random selection of the origin of a systematic sampling may prevent from this bias. The authors concluded that any evaluation of the sampling density cannot be taken without deciding an a priori acceptable level of error of the investigation that can only be estimated considering the objectives of the study, its spatial scale, local conditions, and available resources.

14. Uncertainty of Lichen Diversity Biomonitoring

Brunialti et al. [73] investigated the intercomparability of lichen diversity biomonitoring results obtained by different, but well-experienced, field crews faced with the same problem, at the same time, under the same field conditions, and following the same standard operating procedures. The results showed that surveys run by different teams may be poorly comparable and suggested that comparison of the results of different lichen surveys may lead to incorrect evaluation if there is no formal expression of the acceptable uncertainty.

A key feature for using biological indicators to monitor the state of the environment is that they must clearly reflect a change in the environment, and a common criticism is that they do not account for uncertainty [74]. Uncertainty is unavoidable in environmental studies, and may either be inherent to natural or anthropogenic processes or be introduced during the process of measuring the variable(s) of interest. As a consequence, applied ecologists and environmental biologists need to be effective at informing environmental managers and policy-makers by using approaches that explicitly take into account the uncertainty [75].

Lichen diversity studies require a balance point between scientific reliability and economic constraints, which finds its clearer expression when deciding, based on an a priori established economic budget, whether to focus on statistical robustness at the sampling site scale or to give priority to the spatial variability of the data. In the assessment of atmospheric pollution using lichen diversity counts, coping with uncertainty is mandatory in cases of environmental forensics, when understanding whether an area is polluted or not, or tracing a given pollution source is of paramount importance.

In the framework of environmental forensic studies, a lichen biomonitoring survey may be planned to depict different air quality zones, discriminating, if possible, the contribution of specific sources, and constituting the base for epidemiological studies, usually based on a case–control design, to highlight any relationship with human health. It is clear that in such cases there is the need for a very high technical quality and good reliability of the resulting lichen maps. In addition, a clear expression of the uncertainty affecting the maps is mandatory, since people living inside different zones of the lichen-derived maps are assigned to the different zones for all epidemiological calculations. Thus, any possible uncertainty in clearly defining the border between zones may have great consequences on the attribution of people to a given air quality zone, with great implications for the whole epidemiological analysis.

15. Sensitivity Analysis

Sensitivity analysis play a crucial role in assessing the robustness of the findings or conclusions of any study. It is a critical way to assess the impact, effect, or influence of key assumptions or variations, such as different methods of analysis, definitions of outcomes, protocol deviations, missing data, and outliers on the overall conclusions of a study [76]. Sensitivity analysis is especially used by epidemiologists, who need to carefully evaluate the causality of an association between, e.g., a prescription drug and a health outcome. Despite their importance, epidemiologic studies have often been criticized for the incompleteness of their information on potential confounders that can influence the results. The basic concept of sensitivity analysis is to make informed assumptions about potential residual confounding and quantify its effect on the relative risk estimate of, e.g., the drug–outcome association [77].

The same concepts may apply to any lichen diversity biomonitoring study: The general question is about the overall confidence of the results and possible changes in the outcome on changing some parameters, e.g., varying the cutoff levels in defining air quality zones, or changing the methods of data analysis, or determining the influence that minor protocol deviations may have on the conclusions. The above questions can be addressed by performing a sensitivity analysis for testing the effect of these "changes" on the observed results.

Although not directly cited, checking the results by redoing the calculations excluding nitrophytic species, if there is a suspicion that nitrogen pollution may play an influence, is a kind of sensitivity analysis: If the influence of nitrophytic species is minimal, the results will remain stable. Another possibility is changing the way the cutoffs between the air quality zones are defined and checking if the results are still consistent. Including and excluding from the analysis those sites that experienced small deviations from the standard protocol and comparing the results is another possible way of running a sensitivity analysis of a lichen diversity survey.

If, after performing a sensitivity analysis, the findings are consistent with those from the primary analysis and would lead to similar conclusions, the results and the conclusions may be regarded as robust, and there is a clear indication that the investigated factors of change had little or no influence at all on the primary conclusions.

16. Lichen Biomonitoring and Environmental Justice

Environmental justice deals with the preferential exposure to environmental risk factors across sociodemographic groups that may lead to inequalities in health and most often put disadvantaged groups at higher risk for environmental health effects [78]. Establishing links between socioeconomic deprivation and the mixtures of air pollutants is thus fundamental. However, the complexity of air pollutant mixtures makes it very difficult to characterize environmental inequalities across territories in relation to atmospheric pollution [79]. In this context, the use of lichen biomonitoring can be very useful. Occelli et al. [80], in a study of environmental justice in northern France, showed that the most disadvantaged populations live in a strongly contaminated environment, as depicted by the accumulation of trace elements by lichens. In northern Italy, Contardo et al. [81] found a close correlation between socioeconomic deprivation and air pollution evaluated through the use of lichens as bioaccumulators of heavy metals and clearly showed that the most disadvantaged population is clustered in the most polluted areas. Lanier et al. [82] used the lichen diversity to assess the level of eutrophication as part of a composite biological pollution index in an environmental justice assessment study in northern France.

17. Conclusions

The lichen diversity is a powerful tool for monitoring the biological effects of air pollution. It can be of great help in depicting air quality zones of a given study area and in the framework of long-term monitoring programs, especially in the case of before–after studies.

Indeed, a crude interpretation of overall lichen diversity counts may be at risk of some pitfall, especially when more environmental drivers play a contrasting role on lichen communities. It is thus of paramount importance that great care is taken in the interpretation of the results, especially in the context of a rapidly changing environment and facing global change scenarios. Climate change and nitrogen pollution seem to be the main current drivers of the lichen diversity worldwide. For this reason, is seems advisable to produce several zonal maps, each based on different species groups, and each interpreted in a different way. This exercise could also be a valid support in the framework of a sensitivity analysis, to support or reject the results.

Lichen diversity studies may be very useful in the framework of environmental forensics: They may highlight biological effects of pollutants and constitute the base for epidemiological studies. However, besides a smart interpretation and a sensitivity analysis of the results, to be effective, a clear and formal expression of the overall uncertainty of the outputs is absolutely necessary.

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