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# Comparative Analysis of Bioindicator and Genotoxicity Indicator Capacity of Lichens Exposed to Air Pollution

Sümer Aras, Demet Cansaran-Duman, Çiğdem Vardar and Esin Başaran
*Ankara University, Science Faculty,*
*Department of Biology, Biotechnology Section, Ankara*
*Turkey*

## 1. Introduction

Air pollution is very harmful on both the environment and on living organisms at high concentrations. Furthermore polycyclic aromatic compounds (PACs), heavy metals and halogenated aliphatic hydrocarbons have been shown to be genotoxic to the living organisms (Grant, 1998). Polycyclic aromatic hydrocarbons (PAHs) are capable of forming covalent interaction with nucleophilic centres of DNA (Piraino et al., 2006). They also cause base pair substitutions, frameshift mutations, deletions, S-phase arrest, strand breakage and a variety of chromosomal alterations. (Singer and Grunberger, 1983; Dipple, 1985; Baird et al., 2005). Further studies have also pointed out that long-term exposure to air pollution can cause cancer (Cohen and Pope, 1995; Sawicki, 1977; Jerrett et al., 2005).

Unfortunately, current physical and chemical methods for estimating air genotoxicity provide insufficient information to accurately quantify the risk to biota (Piraino et al., 2006).

In contrast to physical and chemical methods, biological methods allow the direct assessment of the genotoxic potential of air pollutants. Plants have been widely used as bioindicators in many studies up to now. Lichens can also be considered sensitive and efficient indicators of genotoxicity. They in particular have been widely used as trace element atmospheric biomonitors as they are widespread and capable of absorbing elements directly from the atmosphere and accumulating them in their tissues (Aras et al., 2010).

This chapter reports the results of biomonitoring experiments aimed at assessing the genotoxic potential of air pollutants. We tried to detect DNA damage using molecular marker technonolgy. Development of this technology has provided new tools for the detection of genetic alteration by looking directly at the level of DNA sequence and structure. Various types of molecular markers are available. In particular the PCR (Polymerase Chain Reaction) based molecular markers are useful for DNA analysis in complex genomes. With the PCR reaction almost any type of mutational event can be screened e.g.: point mutation, small insertion, deletion and rearrangement (Conte et al., 1998).

The experimental data generated in our studies could be used to develop a thematic map of air genotoxicity with the aim of defining the air quality due to the presence of genotoxic stressors.

## 2. Air pollution

According to the Environmental Protection Agency, air pollution is a mixture of solid particles and gases in the air. Car emissions, chemicals from factories, dust, pollen and mold spores may be suspended as particles in the air (Forman and Alexander, 1998). Some air pollutants are poisonous and inhaling them can increase the human health problems. Air pollution represents a serious threat to both the environment and living organisms. Millions tons of toxic pollutants are released into the air each year. Following activities are the major reasons of such kind of pollution; mobiles (cars, buses, trucks, etc.) and industrial sources (factories, refineries, power plants, etc.) (Wolterbeek, 2002).

Presumably heavy metal accumulation is the most important component of air pollution and also the major part of the environmental pollution. Heavy metals can easily mobilize, disperse and to some extent produce toxic effects, which in turn can lead to growth inhibition and decline in crop yield (Wolterbeek, 2002). Toxic heavy metals in air, soil, and water are global problems that are a growing threat to the environment. There are hundreds of sources of heavy metal pollution, including the coal, natural gas, paper, glassware and industries.

Environmental pollution is a serious problem in many parts of the world. The major impacts of air pollution can be stated as health problems with exposed human populations, forest decline, loss of agricultural productivity, contamination of ecosystems, etc. Those problems has been a cause of increasing public concern throughout the world. Concern about atmospheric pollutants and contaminants underlies the efforts to establish control programmes in many countries. The necesarry quantitative information on chemical element air pollution is generally obtained by modelling of the dispersion (source oriented approach, making use of a priori known information on emission sources) or by field measurements of the immission (receptor oriented approach) (Wolterbeek, 2002; Garty et. al., 2002). Monitoring air pollution is a complex process because of the high number of potentially dangerous substances, the difficulty of estimating their synergistic or antagonistic effects, the large spatial and temporal variation of pollution phenomena, the high cost of recording instruments, and hence the low sampling density of a purely instrumental approach. For these reasons it is hard to establish a region-wide monitoring system to reveal environmental risk assessment levels. Increasing awareness of the potential hazards of large scale contamination of ecosystems by pollutants has highlighted the need for continuous monitoring of the levels of contaminants in the environment.

## 3. Bioindication and biomonitoring

In a general sense, biomonitoring may be defined as the use of bioorganisms (biomonitors) to obtain quantitative information on certain characteristics of the biosphere (Wolterbeek, 2002; Garty et al., 2002). In general bioindicators are organisms that can be used for the identification and qualitative determination of human generated environmental factors, while biomonitors are organisms used for the quantitative determination of contaminants (Conti and Cecchetti, 2001). The use of cosmopolite organisms to assess pollution has developed notably during the last few decades. Such organisms assume environmental contaminants and may be used as indicators of the bioavailability of a given contaminant over time, allowing, in certain cases, comparison between contamination levels in geographically differenet areas (Conti and Cecchetti, 2001).

The use of living organisms as indicators for environmental stability has long been widely recognised. Higher plants, animals, alga, fungi, bacteria and lichen have been employed as bioindicators and biomonitors in air, soil and water pollution surveys over the past few decades (Garty, 1999; Galun et. al.,1987; Castello et al., 1990; Bargagli, 1998; Conti and Cecchetti, 2001; Wolterbeek, 2002). The fundamental aim of biomonitoring can be explained as supplying data for an effective ecological control system. In particular, biomonitoring should act as an early warning system by providing information about the sensitivity of living organisms.

## 4. Use of lichens as indicators and monitors of air pollutant effects

Lichens are prominent examples of symbiotic organisms, in which alga and fungi form an intimate biological union (Nash, 1996). They are slow-growing associations of fungi (mycobionts) and green alga or cyanobacteria (photobionts).

Lichens were recognised as potential indicators of air pollution as early as the 1860's Britain and Europe, since then lichens have played prominent roles in air pollution studies throughout the world because of their sensitivity to different gaseous pollutants, particularly sulphurdioxide (Marquez, 2008). They have also been found to act as acummulators of trace and radioactive elements.

Lichens are made up of a few distinct characters morphologically. The most obvious structure is the lichen thallus. The form of the thallus is a result of the fungal species involved in the simbiont. The thallus is the main body of the lichen. The top surface is called cortex which is normally a layer of tightly packed hyphae. There is an algal layer below this where the photobiont lives. Unlike higher plants, lichens have no roots or a well developed cuticle and they strongly depend on deposited material from the atmosphere to obtain their mineral nutrients. On the other hand the lichen surface, structure and roughness facilitate the interception and retention of particles (Marquez, 2008). These features of lichens, combined with their extraordinary capability to grow at a large geographical range and to accumulate mineral elements far above their need, rank them among the best bioindicators of air pollution (Cansaran-Duman et al., 2009).

Lichens in particular have been widely used as trace element atmospheric biomonitors as they are widespread and capable of absorbing elements directly from the atmosphere and accumulating them in their tissues. As a result of these proporties of lichens, several papers have been published on heavy metal monitoring of lichens in different geographic areas, even Antarctic regions (Garty et al., 1977; Ölmez et al., 1985; Bargagli, 1989; Bermudez et al., 2009; Villarini et al., 2009).

Lichen biomonitoring is often used as receptor based method in air quality studies. It can be useful in risk assessment for human health and it can be a powerful tool for administrators inolved in environmental planning.

## 5. Genotoxic effects of air pollution and genotoxicity assessment strategies

Mixed environmental toxicants are also known to affect the genetic structure of natural populations. In other words, in living organisms heterogenous air pollutants are considered as a major source of DNA damage. They act either through the direct action of the toxicant at the DNA level (direct mutagenic effect) or via toxicant-mediated mortality and/or curtailment of reproduction (population genetic effects). Furthermore polycyclic aromatic

compounds (PACs), heavy metals and halogenated aliphatic hydrocarbons, have been shown to be genotoxic to the living organisms (Grant, 1998). Polycyclic aromatic hydrocarbons (PAHs) are capable of covalent interaction with nucleophilic centres of DNA (Piraino et al., 2006). They also cause base pair substitutions, frameshift mutations, deletions, S-phase arrest, strand breakage and a variety of chromosomal alterations. (Singer and Grunberger, 1983; Dipple, 1985; Baird et al., 2005)

### 5.1 Comparative analysis of air pollution genotoxicity by molecular markers (RAPD and AFLP) and bioindicator capacity in the exposed samples of *Pseudevernia furfuracea* province of Kayseri

We conducted studies in our laboratory in this context and the studies concluded that lichens could be utilized as sensitive and efficient indicators of genotoxicity in addition to their bioindicator capacity. Different studies which were applied to evaluate the genotoxic potential of the atmospheric environment by lichens were analysed comparatively. We aim to detect DNA damage using Randomly Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP), very sensitive molecular tools for the detection of DNA fragmentation and chromosomal mutations (Citterio et al., 2002).

One of our genotoxicity work reported the results of biomonitoring experiments, aimed to assess the genotoxic potential of air pollutants throughout the Kayseri Province (central Anatolia). For this investigation *Pseudevernia furfuracae* L. Zopf lichen species was chosen as a suitable bioindicator since its sensitivity to organic and inorganic compounds were well documented. Heavy metal accumulation was analysed by using Atomic Absorption Spectrometer (AAS) and effects of environmental pollution on DNA was investigated by RAPD and AFLP analysis in lichen species. *P. furfuracea* collected from Çat Forests located around the province of Sivas (central Anatolia), and then exposed at 12 polluted sites in the province of Kayseri. Lichen samples were transplanted to the different pollution sources in province of Kayseri for two time periods (dry and wet seasons) of the year (Fig 1) (Aras et al., 2010). Economic development in the province is facilitated by the close proximity of a major road network, with arterial roads extending from east to west and north to south. This network supports heavy traffic, a major source of air pollution to the region. Also land use in the province is diverse: apart from agriculture there are both large and small industrial districts located around the city. Interestingly, both industrial districts are characterised by different activities (mechanical, chemical, textile, food). There are definite boundaries which distinguishes urban and suburban sites in the city of Kayseri (central Anatolia). Pollution sites were urban roadsides, urban sites, urban park sites, industrial sites, rural areas and shanty areas (Table 1). Urban sites were chosen at least 10 m away from a main road, and the samples from urban roadsides were selected from sites close to the city centre, along busy main roads. Urban roadside samples were chosen between 0 and 5 m, usually not more then 2 m away from the busy road. Urban park sites were chosen from two large parks of Kayseri. Industrial sites were chosen from the industrial parts of the city. Shanty areas were selected from two shanty zones around the city and rural samples were chosen from south of the Kayseri which were more than 10 km away from any source of pollution (Aras et al., 2010).

The experimental data generated in this study provided a thematic map of air genotoxicity for the province of Kayseri with the aim of defining the air quality due to the presence of genotoxic stressors. The study area was characterized by the presence of numerous industrial activities, such as steel works, glassworks and metallurgical, mechanical, chemical

and other industries. These were potential sources of heavy metals and other mixed pollutants in the environment (Aras et al., 2010).

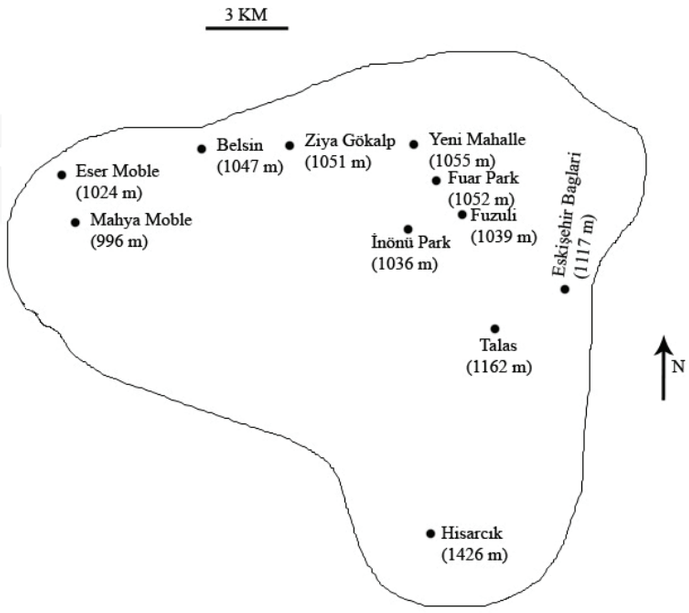



Fig. 1. Map of the study area (Aras et al., 2010)

| **Control** |
|---|
| Hisarcık |
| **Industry** |
| Eser Moble |
| Mahya Moble |
| **Urban Park** |
| Fuar Park |
| İnonu Park |
| **Shanty** |
| Ziya Gökalp |
| Eşkişehir Bağları |
| **Urban** |
| Yenimahalle |
| Belsin |
| **Urban roadsite** |
| Fuzili |
| Talas street |

Table 1. Study area of samples

The AAS data were evaluated via pollution scales, and specimens were analysed using RAPD and AFLP-PCR to detect the probable pollution effects and changes on DNA molecules. Polymorphism was calculated in relation to the appearance of new bands and disappearance of normal bands considering the control's band patterns.

Seven primers used in the study yielded new band appearance and disappearance in the exposed samples compared with the control. For example, the primer TubeA01 (5´CAGGCCCTTC3´) yielded eight clear, reproducible bands in the control which only one of them was disappeared in the exposed samples, Urban Park II, Urban I, II and Urban roadside II in wet season. Same primer yielded seven bands in control for the dry season and more band appearances and disappearences were noticed. Three primers TubeA02 (5´TGCCGAGCTG3´), TubeA03 (5´AGTCAGCCAC3´), TubeA04 (5´AATCGGGCTG3´) yielded higher polymorhism values compared to the other primers for the wet season in which the TubeA04 was displayed the highest polymorphism. TubeA02 (5´TGCCGAGCTG3´) displayed only one band disappearance in all the samples in dry season. TubeA12 (5´TCGGCGATAG3´) also showed maximum three band disappearance out of 14 in dry season (Aras et al., 2010). The results of RAPD analysis displayed an interesting distinction in band patterns of wet and dry seasons. According to our results, the differences among the band patterns between dry and wet seasons were remarkable. Many studies have documented the differences in cell wall permeability due to factors like precipitation and subsequent better performance of lichens as a bioaccumulator during wet period. In the RAPD analysis, different band patterns were obtained for the samples collected during dry and wet season but the polymorphism ratios of the primers were not informative to make any suggestion about the harmful effect of the genotoxic agents in a certain season. But apparently DNA polymerization during PCR reactions were affected in a way and different band patterns were obtained (Aras et al., 2010).

With the aim of verifying the effect of environmental pollutants on the genetic material of the lichen samples, AFLP analysis was performed from the same *P. furfuracea* lichen samples exposed to polluted sites in the province of Kayseri. The primers used in the are the combination of E22-M3, E32-M7, E32-M3, E22-M6 and E32-M6. The AFLP profiles showed substantial differences between unexposed and exposed lichen samples, with apparent band changes in the number of amplified DNA fragments at different locations.

The nineteen primers used displayed significant differences between the control and polluted samples collected from various parts of Kayseri. The highest number of band appearance and disappearence was determined at the samples collected from Urban road site-I for wet season and Shanty-II for the dry season with all of the five primer pairs used. But the other areas also displayed high band appearance and disappearance compared to the original control samples. Polymorphisms obtained, were due to loss and/or gain of amplified bands observed in exposed samples compared with the control (Atienzar et. al., 1999). Meanwhile, 5 pairs of primer combination gave a total of 231 bands in wet period, 147 bands in dry period. Different polymorphic bands were detected at each location and periods for different primer combinations. Average value of polymorphism (P) obtained from the amplification of the primers used in the research was P (%) = 45.02 for wet period and 64.62 for dry period. In all cases, polymorphism was due to the loss and/or the gain of amplified bands in the exposed samples compared with the control.

In addition, genomic template stability ratios (GTS) were calculated. GTS implies qualitative measure reflecting changes in RAPD and AFLP profiles. Changes in RAPD and AFLP profiles were expressed as reductions in GTS (a qualitative measure reflecting the obvious

changes of the number and intensity of DNA bands in DNA patterns generated by toxicant exposed) in relation to profiles obtained from control samples.
In RAPD analysis, we obtained 96 polymorphic bands in wet season (Aras et al., 2010). In AFLP analysis we obtained 104 polymorphic bands in the same season. 48 polymorphic bands were observed by RAPD analysis in dry season. But 95 polymorphic bands were observed by AFLP analysis in the same season. Similarly GTS results were compared in two different seasons per method. In RAPD analysis the highest GTS value (75%) was obtained from Park-I samples, in AFLP analysis the highest GTS value was obtained from Rural-II (85.8%) samples in wet season. In dry season, Urban-II (87.5%) and Urban roadsite-I (87.5%) samples showed the highest GTS values by RAPD analysis and Rural-II (88.8%) samples showed the highest GTS values by AFLP analysis.
According to results, AFLP profile changes provide all sensitive markers to detect genotoxicity in lichens. Thus, the AFLP method has been successfully used as a sensitive means of detecting DNA damage and shows potential as a reliable and reproducible assay for genotoxicity. Furthermore, DNA affects in conjunction with other biomarkers from higher levels of biological organization would to be a powerful ecotoxicological tool. Our studies revealed that results of AFLP analyes were paralled with atomic absorbtion results.
We also studied on some methods to establish a correlation between RAPD, AFLP and AAS results. We tried to construct a special mathematical model between the RAPD results and AAS values. Excel correlation function was used in order to design this model but we have not been able to construct a model showing the direct correlation between the band patterns and AAS data yet.

### 5.2 Comparative analysis of air pollution genotoxicity by molecular markers (RAPD) and bioindicator capacity of the exposed samples of *Pseudevernia furfuracea* province of Ankara

In this study, we aimed to describe the heavy metal contents of exposed *P. furfuracea* lichen samples to various polluted areas in the province of Ankara (central Anatolia). Lichen samples collected from the Yenice Forest (province of Karabük) and transplanted in bags to different sites in Ankara and exposed to pollution (Fig 2). Samples were exposed for three and six months and then the concentrations of six trace elements (Cd, Cu, Mn, Ni, Pb and Zn) in *P. furfuracea* were determined. Heavy metal concentrations of *P. furfuracea* were determined by inductively coupled plasma-mass spectrometry (ICP-MS) technique.
Ankara, the capital of Turkey, is located in central Anatolia, and it is the second most crowded city in the country. Ankara has gained a bad reputation for the black smog that hung over the city during the winter. The primary source of the pollution was the usage of coal, as a main fuel for residential and industrial heating during winter times. Since the 1980s, however, Turkey has made investments for the development of an extensive network of natural gas pipelines that serve all the major cities and most towns. Consequently, natural gas has replaced coal as the primary source of fuel in most the populated centers, and urban air has become cleaner than the past.
The districts chosen for the study are given in Fig 2. Kızılay is the downtown of the city. Sıhhıye is the closest district to Kızılay. Ulus is the downtown of the old town of the city and Dışkapı is close to Ulus. All these districts are very crowded and polluted areas especially in terms of vehicular traffic. Tandogan, Emek, Yenimahalle and Etlik are the major residential areas where natural gas is the main energy source used for heating. Mamak is a shanty area

where coal burning is still used for heating purposes. Eryaman is a suburban site which is 30 km far from downtown and natural gas is the only energy source used.

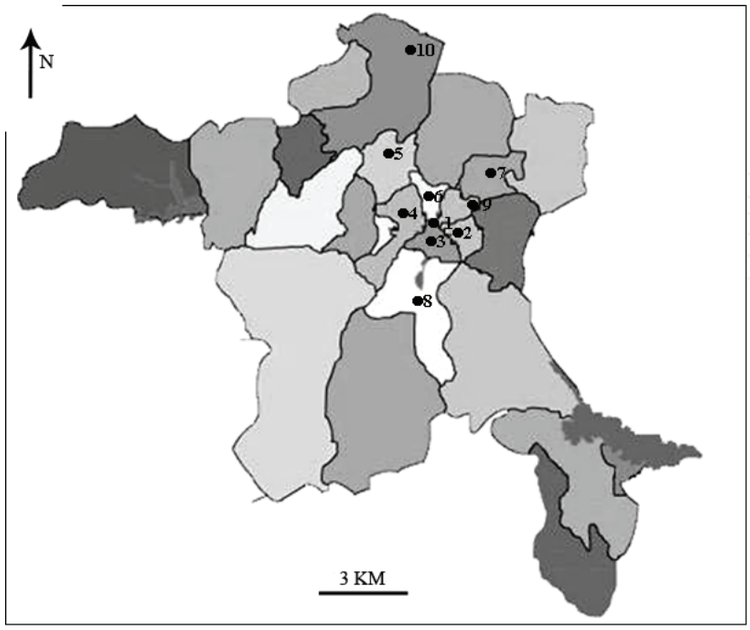



Fig. 2. Ankara
Site 1= Kızılay, Site 2= Sıhhıye, Site 3= Ulus, Site 4= Tandogan, Site 5= Emek, Site 6= Yenimahalle, Site 7= Etlik, Site 8= Mamak, Site 9= Dışkapı, Site 10= Eryaman

| | |
|---|---|
| **Control** | Karabuk, Yenice, North of Yalnızca plateau, GPS:41°15′ N, 32°34′ E, 1200m |
| **Site 1** | Eryaman-5th street, in front of Ugur taxi |
| **Site 2** | Dışkapı- in front of Yıldırım Beyazıt Hospital |
| **Site 3** | Mamak- in front of Muhabere Okul Komutanlığı |
| **Site 4** | Kızılay- in front of Turkish Air Force |
| **Site 5** | Sıhhıye- in front of Refik Saydam National Public Health Agency |
| **Site 6** | Ulus- TBMM Garden |
| **Site 7** | Etlik Kasalar-in front of The Ministry of Healt of Turkey Etlik İhtisas Hospital |
| **Site 8** | Yenimahalle- İvedik street, in front of Yayla taxi station |
| **Site 9** | Emek-8. street, opposite of Karacaoğlan restraurant |
| **Site 10** | Tandogan-in front of Ankara University |

Table 2. The localities of the lichen samples used in Ankara

Comparisons of the Pb concentrations of the samples from exposed sites with the control yielded very significant variations. Kızılay and Sıhhıye with the highest human activities, together with high vehicular density congestion, showed the highest Pb with the values of, 47.00 $gg^{-1}$ in three months and 52.80 $gg^{-1}$ in six months which were significantly higher than the control site, 22.70 $gg^{-1}$. It can be concluded that Pb concentration is the highest in Kızılay district because it is the central part of the city where human activities and density of traffic are very intense.
The mean Cd concentration in Eryaman, Emek and Tandogan are slightly higher than the control site. The other districts; Dışkapı, Mamak, Kızılay and Sıhhıye showed significantly

higher Cd concentrations than control site in six months. The concentrations of Cd in three and six months were significantly higher in Dışkapı, Mamak, Kızılay and Sıhhıye than the control site, probably indicating the contaminants from motor vehicles, dust raised by metal industry and other human activities. The most important sources of Cd pollution were regarded as fossil fuels used by the vehicles, metal business, plastics, house tools construction and sewer. Increases in trace element concentration confirm that vehicular traffic plays a prominent role on air pollution of Ankara.

In addition, DNA alterations in the exposed lichen samples were aimed to be described by RAPD analysis. Out of 17 decamer oligonucleotide primers tested, six of them showed clear and reproducible bands. In RAPD analyses some of the primers displayed significant differences between the control and polluted samples exposed to various parts of the city of Ankara.

The number of band appearance and disappearance was the highest in the samples collected from Mamak district for three months and Dışkapı for the six months for all the six primers used. The size of the disappearing bands ranged from 220 bp to 1600 bp. But the other areas also displayed high band appearance and disappearance compared with the original control sample.

For example, the primer OPOO3 (5´CTGTTGCTAC3´) yielded 11 clear, reproducible bands in the control which only one of them was disappeared in the exposed samples in Emek, Mamak, Dışkapı, Kızılay and Sıhhıye in three months. Same primer yielded more band appearances and disappearances in six months (Mamak, Dışkapı, Kızılay and Sıhhıye). One of the other primers used in the study OPB16 (5´TTTGCCCGGA3´), also showed maximum three band disappearance (Mamak) out of 12 in six months. As could be seen from results the sample exposed in Mamak yielded 15 polymorphic bands and the sample from Dışkapı showed 14 polymorphic bands. Sıhhıye and Kızılay districts followed them with 13 and 11 polymorphic bands, respectively in three months. In six months Kızılay district samples showed the highest polymorphism with 15 band variation. The results prove that these districts are the most populated and polluted areas in Ankara (Cansaran-Duman et al., under review).

The highest DNA band variation was recorded in the samples exposed for six months in Dışkapı. *P. furfuracea* samples transplanted to Mamak and Dışkapı showed higher DNA variation than the other sites after three and six months. This result might be correlated with the elevated Pb accumulation as a result of very dense vehicular traffic.

Results indicated that both accumulating trace elements and DNA variations by RAPD analysis were the highest in samples exposed for six months.

### 5.2.1 Comparison of chemical content and RAPD profiles in the exposed samples of *P. furfuracea* province of Ankara

The results for Zn, Cd and Pb elements from Dışkapı and Mamak samples were found high, as expected. In these districts coal burning in stoves is common which stimulates Zn and Cd accumulation in that region and also the areas are hollow where circulation and reverse inversion do not exist. All these factors are the main reasons for air pollution in these districts and explain the elevated levels of heavy metals recorded for areas. Likewise, the results of RAPD analysis yielded the highest band variations in the samples from these districts.

According to the results of chemical analysis, the sample exposed in Eryaman which is 30 km away from downtown (Kızılay) yielded the lowest metal values which are close to the values obtained from the control sample. In Eryaman accumulation in the sample is not high

as the central natural gas system is used commonly for heating. Likewise, RAPD profiles of Eryaman sample displayed the most similar band pattern with the control among all the samples. According to our observations obtained from chemical analysis, Mamak and Dışkapı were the most polluted areas because of usage of stoves for heating, Kızılay and Ulus were polluted because of the traffic.

We also recorded a slight increase in metal contents of the samples from three months to six months. Likewise DNA band variation intensities were increased and in accord the GTS values were decreased in the samples exposed for 6 months. As a result, the present study confirms lichens as efficient metal accumulators and their appropriate use in biomonitoring studies. The concentrations of six elements detected in *P. furfuracea*, after exposure in bags in the urban area of Ankara, compared with the element content in control site gave a clear indication of urban air contamination by trace elements. The correlation between Cd, Cu, Mn, Ni, Pb and Zn confirm that vehicular traffic plays a prominent role in Ankara's air pollution.

### 5.3 Comparison of the results obtained from two different provinces (Ankara-Kayseri, Turkey)

The experiments were carried out in Ankara and Kayseri using the bag technique with the same type of lichens. Both cities were in central Anatolia region and their climate had similar aspects. Same types of lichens were transplanted using the bag technique in especially shanty areas and the parts with traffic of both cities.

When the RAPD results were compared, it was observed that there were numerous band changes for the lichen species especially near the highways like Kızılay, Sıhhiye, Ulus in Ankara and Kayseri.

Band changes were observed clearly for the lichen species in regions with high consumption of coal as a fuel; shanty areas in Kayseri and Mamak, Dışkapı provinces of Ankara. RAPD results were compared with the atomic absorption spectrometer data. It was identified that results for Mg, Zn and Mn showed high values in Sıhhiye, Mamak, shanty areas and near the highways in Kayseri but the results were not correlated with mathematical expressions and models.

### 5.4 Comparative analysis of heavy metal accumulation in the samples of *Pseudevernia furfuracea, Evernia prunastri, Usnea hirta, Hypogymnia physodes* province of Iron-steel Factory in Karabük and examples to genotoxicity

#### 5.4.1 Accumulation of heavy metals in *Pseudevernia furfuracea*

The study was initiated with the objective to provide baseline information on metal accumulation in lichen species growing in and around the Iron-steel Factory in Karabük, Turkey. *P. furfuracea* lichen specimens were collected from every 5 km starting from around the Iron-steel Factory located in the central area of Karabük province (Anatolia), up to Yenice Forest (Table 3). The locations of the districts were also given in the map (Fig 3). Zn, Cu, Mn, Fe, Pb, Ni, Cd, Cr concentration were analyzed in the samples collected from polluted and unpolluted areas. In the study *P. furfuracea* sample from Yenice Forest was used as a control. The reason for the choise of Yenice Forest was the abundance of species diversity, and therefore sample collection might cause a very low impact on natural population density. The forest is among the 100 forested areas that must be urgently taken under protection according to World Wildlife Fund (WWF) researches. The present investigation has involved the collection of ten *P. furfuracea* samples growing on *Pinus* sp. from 10 sites in and around Karabük Iron-steel Factory area, Karabük, Turkey. The metal concentration of *P.*

*furfuracea* samples collected from Yenice Research Forest (Yenice-Karabük) and Karabük Iron-steel Factory were analyzed by atomic absorbsion spectrometry. The thalli of *P. furfuracea* were used to determine the levels of eight metals (Zn, Cu, Mn, Fe, Pb, Ni, Cd, Cr).

| **Locality No** | **Date of collection** | **GPS co-ordinates** | **Locality name** | **Altitude** (m) |
|---|---|---|---|---|
| 1 | 15.11.2005 | 44°62′ N, 45°73′ E | Karabük, Yenice, Kuzdağ district | 1125 |
| 2 | 15.11.2005 | 41°15′ N, 32°35′ E | Karabük, Yenice, Kabaklı kaya | 1140 |
| 3 | 15.11.2005 | 41°13′ N, 32°28′ E | Karabük, Yenice, vicinity of Hamzakıran district | 1140 |
| 4 | 15.11.2005 | 41°14′ N, 32°35′ E | Karabük, Yenice, Dikilitaş | 1125 |
| 5 | 15.11.2005 | 41°12′ N, 32°25′ E | Karabük, Yenice, vicinity of Kuzdere, Hamdioğlu district | 1400 |
| 6 | 15.11.2005 | 41°15′ N, 32°34′ E | Karabük, Yenice, North of Yalnızca plateau | 1200 |
| 7 | 15.11.2005 | 41°11′ N, 32°27′ E | Karabük, Yenice, Acısu Center | 1375 |
| 8 | 15.11.2005 | 41°14′ N, 32°33′ E | Karabük, Yenice, Kazancıoğlu district | 1750 |
| 9 | 15.11.2005 | 41°12′ N, 32°29′ E | Karabük, Yenice, Hacıömerler district | 1380 |
| 10 | 15.11.2005 | 41°12′ N, 32°29′ E | Karabük, Yenice, Kızılgöz kayası | 1385 |
| 11* | 15.11.2005 | 41°10′ N, 32°24′ E | Karabük, Yenice, vicinity of Cami district | 1100 |

* 11.= control sample

Table 3. The localities of the lichen samples used in Karabük

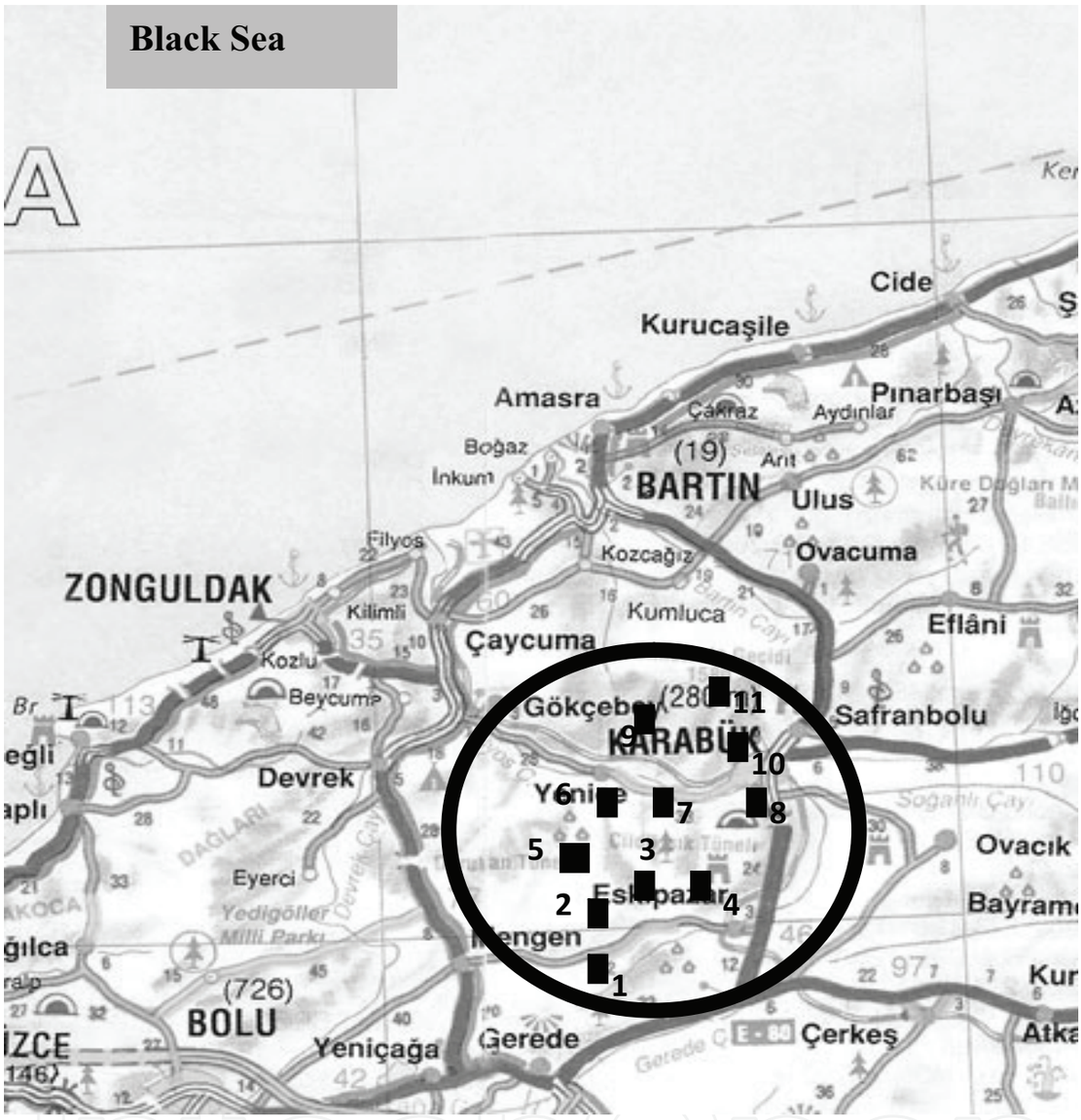



Fig. 3. Map of the Karabük

The 5th and 8th stations which were located close to Iron-steel Factories and major motor vehicle traffic, manifested the highest level of Mn (Table 3). At site 8, combustion of coal and other kind of fuels rather than natural gas seem to be the reason of air pollution. Site 10 with the highest human activities, together with high vehicular density congestion, showed the highest Pb levels with the value of 9.750 mg/kg which is significantly higher than that of the control site (4.00 mg/kg) (Table 4). The most important sources of Cr pollution are indicated as industrial activities like refining works and Iron-steel Factories (Cansaran-Duman et al., 2009).

Results of the study displayed significant variations among the concentration of these elements between stations. As expected, the pollution sources such as Iron-steel Factory, roads and railroads, industry, heavy traffic and waste treatment plants have major impact on the heavy metal accumulation in *P. furfuracea* and in accordance to their location samples

8 and 10 displayed high element accumulation. Surprisingly, although Yenice Forest is under protection, results of our study showed that the region is becoming polluted by the influence of many pollution sources in the area. The present study also confirms the efficient metal accumulation capacity of lichens.

| Site | Zn | | Cu | | Mn | | Fe | |
|---|---|---|---|---|---|---|---|---|
| | N | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| 1 | 5 | 26.280±0.158 | 5 | 3.090±0.111 | 5 | 45.496±3.197 | 5 | 2379.000±44.193 |
| 2 | 5 | 25.680±0.316 | 5 | 2.050±0.100 | 5 | 32.500±0.174 | 5 | 1273.030±17.408 |
| 3 | 5 | 24.410±0.095 | 5 | 1.940±0.032 | 5 | 41.730±2.103 | 5 | 356.460±7.906 |
| 4 | 5 | 30.162±3.455 | 5 | 2.770±0.047 | 5 | 56.290±0.142 | 5 | 965.250±15.811 |
| 5 | 5 | 25.610±0.063 | 5 | 3.350±0.190 | 5 | 119.860±0.380 | 5 | 766.350±15.812 |
| 6 | 5 | 28.078±0.040 | 5 | 3.300±0.016 | 5 | 92.950±0.016 | 5 | 199.030±3.162 |
| 7 | 5 | 40.628±0.561 | 5 | 2.990±0.551 | 5 | 71.890±1.059 | 5 | 1558.960±46.833 |
| 8 | 5 | 32.360±0.158 | 5 | 4.450±0.047 | 5 | 112.970±1.091 | 5 | 3016.000±13.835 |
| 9 | 5 | 31.362±0.119 | 5 | 3.550±0.016 | 5 | 31.720±2.087 | 5 | 1560.132±6.665 |
| 10 | 5 | 53.802±0.340 | 5 | 4.560±0.047 | 5 | 54.080±0.190 | 5 | 1185.600±39.845 |
| 11* | 5 | 28.640±0.174 | 5 | 1.810±0.010 | 5 | 42.250±0.965 | 5 | 918.452±7.471 |
| ANOVA | | | | | | | | |
| F ratio | | 0.065 | | 6.750 | | 0.051 | | 0.081 |

| Site | Pb | | Ni | | Cr | | Cd | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| 1 | 5 | 7.200±0.158 | 5 | 5.170±0.079 | 5 | 4.540±0.047 | 5 | 0.725±0.002 |
| 2 | 5 | 4.900±0.158 | 5 | 2.090±0.063 | 5 | 2.950±0.032 | 5 | 0.690±0.008 |
| 3 | 5 | 5.100±0.152 | 5 | 1.490±0.063 | 5 | 2.730±0.031 | 5 | 0.490±0.019 |
| 4 | 5 | 6.000±0.073 | 5 | 2.350±0.158 | 5 | 3.242±0.024 | 5 | 0.706±0.007 |
| 5 | 5 | 4.130±0.095 | 5 | 1.974±0.052 | 5 | 2.940±0.016 | 5 | 0.618±0.005 |
| 6 | 5 | 3.000±0.079 | 5 | 1.450±0.079 | 5 | 2.730±0.007 | 5 | 0.632±0.004 |
| 7 | 5 | 3.150±0.080 | 5 | 1.240±0.159 | 5 | 2.620±0.032 | 5 | 0.668±0.002 |
| 8 | 5 | 4.700±0.160 | 5 | 2.490±0.063 | 5 | 4.100±0.063 | 5 | 0.671±0.003 |
| 9 | 5 | 4.600±0.158 | 5 | 1.880±0.063 | 5 | 3.440±0.063 | 5 | 0.720±0.006 |
| 10 | 5 | 9.750±0.128 | 5 | 4.190±0.079 | 5 | 3.390±0.079 | 5 | 0.770±0.007 |
| 11* | 5 | 4.000±0.035 | 5 | 2.100±0.100 | 5 | 2.280±0.007 | 5 | 0.630±0.007 |
| ANOVA | | | | | | | | |
| F ratio | | 2.717 | | 3.199 | | 5.712 | | 1.651 |

Table 4. Average Zn, Cu, Mn, Fe, Pb, Ni, Cr and Cd concentrations in *P. furfuracea* (μgg$^{-1}$) in the Karabük city, with Standart deviations (SD)

### 5.4.2 Accumulation of heavy metals in *E. prunastri*

Heavy metal concentrations of *E. prunastri* samples taken from polluted sites and control group are summarized in Table 5. All stations were statistically analyzed to determine their relationships with respect to each heavy metal. SPSS 11.5 analysis was used to show the

relationships of the stations and some results were shown with tables (Table 5) (Cansaran-Duman et al., 2011).

The highest levels of Manganase (Mn) in the *E. prunastri* were found in sites 8 (82.7 µg/g), 5 (77.0 µg/g) and 6 (73.7 µg/g). In order to compare the ability of the lichen species to accumulate some heavy metals, they were compared with the element concentrations in the baseline material. For example, the highest levels of Mn in the *E. prunastri* were found in the site 8 (82.7 µg/g) (control is 28.8 µg/g) (Table 5) (Cansaran-Duman et al., 2011).

| Site | Zn | | Cu | | Mn | | Fe | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| 1 | 5 | 46.698±0.058 | 5 | 3.673±0.007 | 5 | 34.425±5.665 | 5 | 943.032±5.238 |
| 2 | 5 | 43.359±0.772 | 5 | 3.002±0.025 | 5 | 44.185±0.641 | 5 | 443.061±0.417 |
| 3 | 5 | 24.139±0.826 | 5 | 1.903±0.190 | 5 | 32.201±0.706 | 5 | 1023.900±3.069 |
| 4 | 5 | 16.532±0.058 | 5 | 1.549±0.007 | 5 | 30.428±2.646 | 5 | 540.792±6.833 |
| 5 | 5 | 21.895±0.261 | 5 | 1.724±0.082 | 5 | 77.026±1.209 | 5 | 419.541±11.863 |
| 6 | 5 | 17.891±0.029 | 5 | 1.558±0.021 | 5 | 73.773±2.687 | 5 | 775.832±8.405 |
| 7 | 5 | 16.215±0.161 | 5 | 2.022±0.019 | 5 | 57.955±1.699 | 5 | 1289.000±11.765 |
| 8 | 5 | 19.055±0.008 | 5 | 2.693±0.014 | 5 | 82.773±0.685 | 5 | 2187.200±71.983 |
| 9 | 5 | 17.860±0.555 | 5 | 1.601±0.009 | 5 | 26.333±1.409 | 5 | 786.969±0.638 |
| 10 | 5 | 15.526±0.316 | 5 | 1.551±0.015 | 5 | 54.663±2.812 | 5 | 827.505±32.554 |
| 11* | 5 | 12.543±0.332 | 5 | 1.469±0.032 | 5 | 28.830±0.172 | 5 | 460.228±0.302 |
| ANOVA | | | | | | | | |
| F ratio | | 0.026 | | 0.156 | | 0.254 | | 0.046 |

| Site | Pb | | Ni | | Cr | | Cd | |
|---|---|---|---|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| 1 | 5 | 5.171±0.236 | 5 | 7.819±0.201 | 5 | 2.719±0.017 | 5 | 0.620±0.001 |
| 2 | 5 | 0.316±0.005 | 5 | 2.665±0.010 | 5 | 2.718±0.029 | 5 | 0.644±0.002 |
| 3 | 5 | 1.037±0.033 | 5 | 4.627±0.082 | 5 | 3.364±0.011 | 5 | 0.604±0.004 |
| 4 | 5 | 0.958±0.092 | 5 | 1.862±0.073 | 5 | 1.801±0.007 | 5 | 0.682±0.001 |
| 5 | 5 | 1.011±0.097 | 5 | 1.970±0.071 | 5 | 1.821±0.017 | 5 | 0.624±0.006 |
| 6 | 5 | 0.995±0.143 | 5 | 0.950±0.137 | 5 | 2.329±0.013 | 5 | 0.505±0.003 |
| 7 | 5 | 3.087±0.886 | 5 | 1.830±0.485 | 5 | 5.752±0.012 | 5 | 0.630±0.003 |
| 8 | 5 | 1.606±0.473 | 5 | 2.290±0.445 | 5 | 4.650±0.091 | 5 | 0.696±0.003 |
| 9 | 5 | 2.863±0.578 | 5 | 2.426±0.375 | 5 | 2.851±0.096 | 5 | 0.560±0.018 |
| 10 | 5 | 1.030±0.264 | 5 | 2.312±0.209 | 5 | 2.395±0.011 | 5 | 0.609±0.003 |
| 11* | 5 | 1.315±0.292 | 5 | 0.594±0.037 | 5 | 1.694±0.029 | 5 | 0.306±0.006 |
| ANOVA | | | | | | | | |
| F ratio | | 1.466 | | 0.318 | | 0.023 | | 0.081 |

Table 5. Average Zn, Cu, Mn, Fe, Pb, Ni, Cr and Cd concentrations in *E. prunastri* (µgg$^{-1}$) in the Karabük city, with Standart deviations (SD)

Comparisons of the Pb concentrations of *E. prunastri* specimens from polluted sites with the control yielded very significant variations. Sites 1, 7 and especially sites 9 with the highest

human activities, together with high vehicular density congestion, showed the highest Pb with the values of, 5.17 g/g which were significantly higher than the control site (1.31 g/g). It could be concluded that Pb concentration was highest in site 1 as it is the central part of the city where human activities and density of traffic are very intense. Similar kinds of observations were made by Cansaran-Duman *et al.* (2009) while studying *Pseudevernia furfuracea* thalli as an indicator of air pollution in the same province (Karabük) in Turkey. All sites, especially site 8 (0.69 µg/g), showed significantly higher Cd concentrations than the sample from control site (0.30 µg/g) (Cansaran-Duman et al., 2011).
In the same study the samples were also evaluated to detect DNA damage in thallus, caused by environmental pollutants. The region surveyed in the study suffers from substantial historical and current air contamination principally due to the presence of the steel and iron industry, which have been active since 1925 until now. The use of biological responses to contaminant exposure by lichen species has become a useful tool in environmental quality evaluation and risk assesment (Cansaran-Duman et al., 2011).

### 5.4.3 Chemical content in *Hypogymnia physodes* and *Usnea hirta*

Lichen *Hypogymnia physodes* and *Usnea hirta* samples were collected in 2005 from 10 stations around Iron-steel Factory in Karabük, Turkey (Fig. 3). *H. physodes* and *U. hirta* samples from Yenice Forest were used as a control. The aim was to evaluate the bioaccumulation ability and to determine the environmental impact of an Iron-steel Factory in Karabük (Cansaran-Duman, 2011). The analytical results were compared statistically by using Statistical Package for the Social Sciences (SPSS). As expected, the study area (Yenice Forest, Karabük) was chosen as control site (site no 11) (Table 2) showed significantly lower impact in comparison to other site (site no 1-10). Compared with the two lichen species, *H. physodes* was the species with the highest accumulation capacity while *U. hirta* had the lowest one. These criteria attested the best suitability for *H. physodes,* followed by *U. hirta* (Cansaran-Duman, 2011).
Around the Karabük Iron-steel Factory, the highest levels of Zinc (Zn) in the *H. physodes* were found in site 4 (33.1 $\mu g g^{-1}$), site 8 (30.2 $\mu g g^{-1}$) and site 5 (30.1 $\mu g g^{-1}$), respectively. Sites 6, 7, 10 and 1 were determined close to each other value in the *H. physodes* species (Fig4). Also, the highest levels of Zinc (Zn) in the *U. hirta* were found in sites (1, 5 and 7) (21.1, 21.4 and 21.2 $\mu g g^{-1}$, respectively). In addition to these sites, Zn concentration in sites 3(19.0 $\mu g g^{-1}$) and 9 (20.6 $\mu g g^{-1}$) was high value in *U. hirta.* Zn concentration in the lichen samples was linearly related to the vehicle traffic, railway and activity of industrial units. The highest levels of Manganase (Mn) in the *H. physodes* were found in sites 2 (195.8 $\mu g g^{-1}$), and 4 (202.7 $\mu g g^{-1}$), respectively. The highest levels of Mn in the *U. hirta* were found sites 3 (195.9 $\mu g g^{-1}$) and 4 (150.3 $\mu g g^{-1}$) with a control value of 19.3 $\mu g g^{-1}$. We considered that both of samples were higher for Mn concentration in site no 4. The reason for this, motor vehicles are known to be a source of Mn in urban areas (Monaci *et al.*, 2000) and could explain the reason of elevated Mn concentrations in site 4 (Cansaran-Duman, 2011).
Comparisons of the Pb concentrations of the *H. physodes* and *U. hirta* species from polluted sites with the control yielded very significant variations, especially *U. hirta* species. Sites 3, 5, 8 and especially sites 1, 10 (Fig 3) with the highest human activities, together with high vehicular density congestion, showed the highest Pb with the values of, 8.78 $g g^{-1}$ in *U. hirta* species which were significantly higher than the control site, 1.32 $g g^{-1}$ (Fig. 4). It could be concluded that Pb concentration was highest in sites 1 and 10 because they are the central

part of the city where human activities and density of traffic are very intense (Cansaran-Duman, 2011).

Although the highest levels of chromium (Cr) in the *H. physodes* was found in site 7 (3.86 $\mu gg^{-1}$), and 8 (4.56 $\mu gg^{-1}$), respectively, these sites slightly higher than the controls. The chromium (Cr) concent in sites 5 (6.75 $\mu gg^{-1}$), 6 (4.18 $\mu gg^{-1}$), and 7 (3.15 $\mu gg^{-1}$) were significantly higher than control site (1.96 $\mu gg^{-1}$) in *U. hirta*. The most important sources of Cr pollution are indicated as industrial activities like refining works and Iron-steel Factories (Cansaran-Duman, 2011).

Copper (Cu) contents in the *H. physodes* samples ranged from 2.44 to 3.94 $\mu gg^{-1}$. Cu content in site 7 (3.11 $\mu gg^{-1}$) in *U. hirta* was significantly higher than control site (1.59 $\mu gg^{-1}$). These two species showed high Cu concentration. Nickel (Ni) concentrations in site 3 was found as 10.81 $\mu gg^{-1}$ *H. physodes* and 8.66 $\mu gg^{-1}$ in site 9 of *U. hirta* (Cansaran-Duman, 2011).

The mean Cd concentration in sites 1 (0.85 $\mu gg^{-1}$), 8 (0.84 $\mu gg^{-1}$) and 10 (0.87 $\mu gg^{-1}$) are slightly higher than the control site (0.73 $\mu gg^{-1}$) in *H. physodes*. All sites, especially site no 4 (0.61 $\mu gg^{-1}$), showed significantly higher Cd concentrations than control site (0.17 $\mu gg^{-1}$) in *U. hirta*. The concentrations of Cd in *U. hirta* were significantly higher in all sites than from the control site, probably indicating the contaminants from motor vehicles, dust raised by metal business and other human activities. The most important sources of Cd pollution were regarded as fossil fuels used by the vehicles, metal business, plastics, house tools construction and sewer (Markert, 1992). Markert (1992) was recorded the Cd levels in between 0.01 and 0.3 $gg^{-1}$ for unpolluted natural environments and also reported that all of the study sites have been polluted except rural sites.

### 5.4.4 Comparison *H. physodes, U. hirta, P. furfuraceae* heavy metal accumulation

The presence of heavy metals in *P. furfuracea* was already reported in Cansaran-Duman *et al*. 2009 and to allow the comparison with *U. hirta* and *H. physodes* species in the present review the results are expressed. It was compared *H. physodes*, *U. hirta* with *P. furfuracea*, there was no different in Zn concentration. Especially, sites 7 and 10 in *P. furfuracea* were significantly higher than *U. hirta* and *H. physodes* (Fig. 4).

In the site 10, Pb concentration of *P. furfuracea* was highest than *U. hirta* and *H. physodes*. Thus, *H. physodes* and *U. hirta* the highest concentration of Pb which can be related to a selective cation uptake as was informed previously by Cansaran-Duman et al. 2009. The authors attributed this finding to a greater affinity between Pb cations and the lichen cell wall exchange sites that are probably strongly attached to binding sites (Fig 4).

*H. physodes* in the levels of Fe metal accumulated in site 8 were similar to those obtained by Cansaran-Duman et al. 2009 in *P. furfuracea* species from same site of Iron-steel Factory in Karabük. Highest Fe concentration was found in *P. furfuracea* to site no 1, while Fe concentration was lower in *U. hirta* and *H. physodes* (Fig 4).

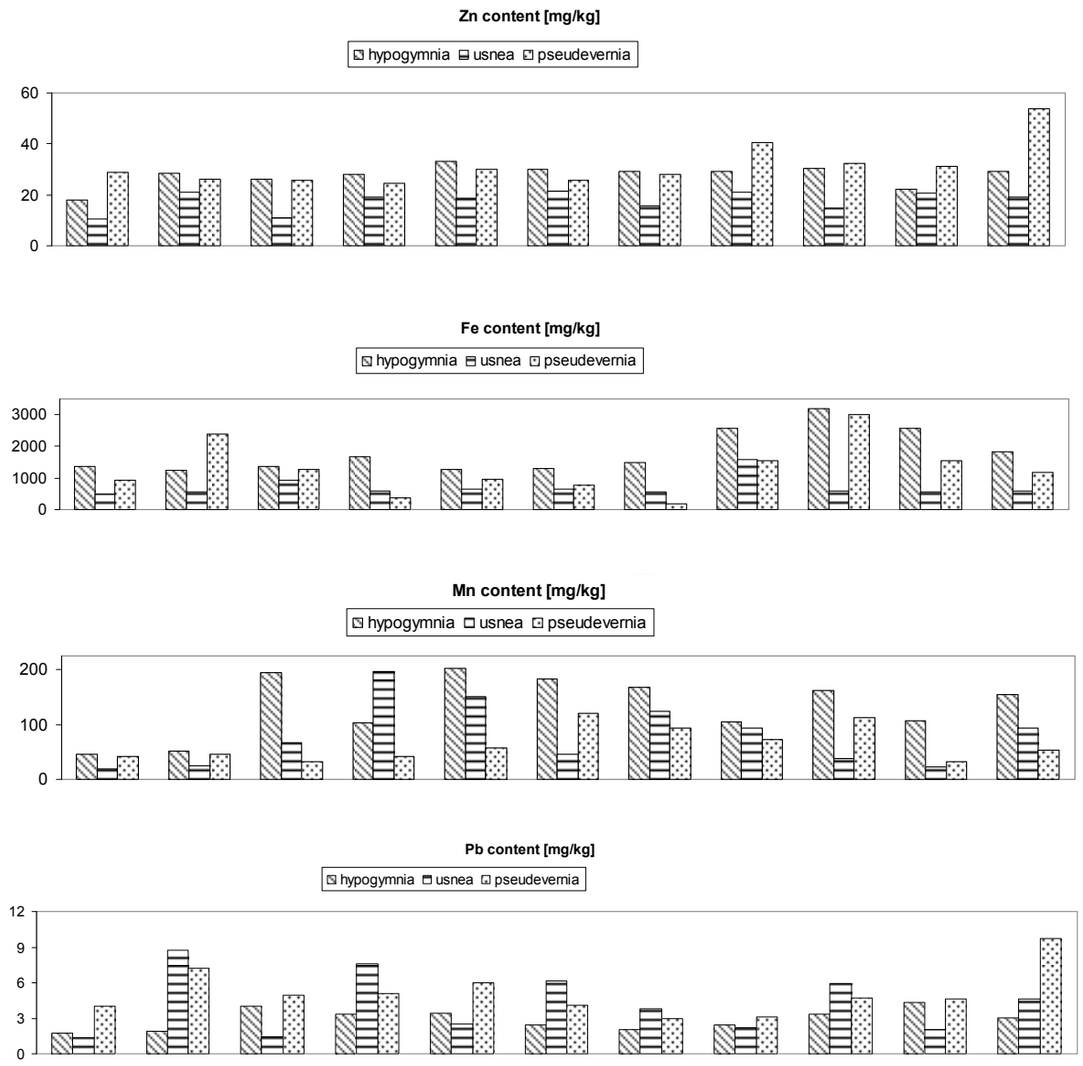
The order of magnitude of accumulation on Mn at 2, 4 and 10 sites were *H. physodes* > *U. hirta* > *P. furfuracea*. Although *U. hirta* showed the highest levels of Mn in site 3, *H. physodes* was higher accumulated than *P. furfuracea* and *U. hirta* sites 5 and 9 (Fig 4). Mn could be tracer of both eolic dust particles as well as vehicular traffic, since this element has recently been used as a substitute for Pb in additives (Ardeleanu *et al*., 1999).

Our findings are consistent with data reported by Cansaran-Duman et al. (2009), who have monitored *P. furfuracea* species in the same district. In concordance with findings made by Cansaran-Duman et al. (2009) on *P. furfuracea*, this study demonstrated the importance of

heavy metals accumulation on the choose of lichen species. Heavy metals were the highest concentration around the Iron-steel Factory in Karabük, which is in accordance with previous data (Cansaran-Duman et al., 2009).

Generally, previous studies that used *P. furfuracea* as a passive biomonitor in a Iron-steel Factory in the province of Karabük showed concentrations higher than *U. hirta* species found in the present study. *P. furfuracea* and *H. physodes* were close quarters to heavy metal accumulation in Iron-steel Factory. According to our observations in result of this study, *H. physodes* higher than the values to *P. furfuracea* (Cansaran-Duman et al., 2009).

The presence of heavy metals in *P. furfuracea* was already reported in Cansaran-Duman et al. 2009. Moreover, results of this study are comparison with *U. hirta* and *H. physodes* species. It was observed that the accumulation of metals in *P. furfuracea* was similar to the one observed in *H. physodes*, with significantly higher values of all elements in samples exposed in the Karabük. Moreover, in previous study *P. furfuracea* and in the current study *H. physodes* were proved to be almost similar bioaccumulator than *U. hirta* species.



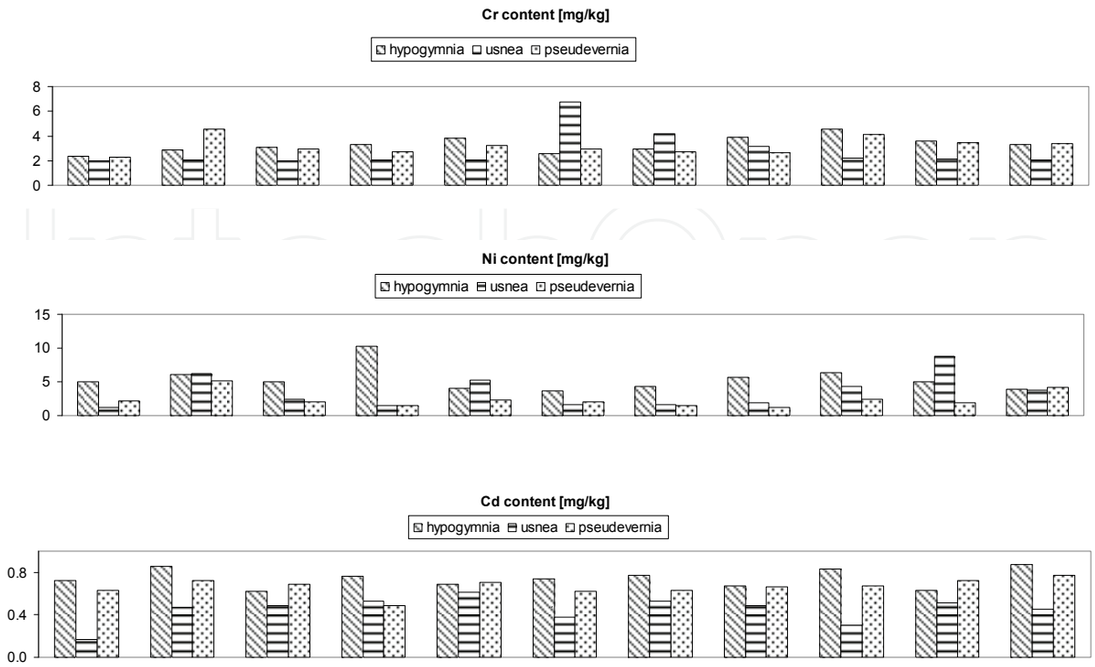



Fig. 4. Comparison *H. physodes, U. hirta, P. furfuraceae* heavy metal accumulation

### 5.4.5 RAPD profiles of *P. furfuraceae*

In this study the genotoxic effects of various environmental pollutants were tested with the samples collected from their natural habitats from the Karabük region. 10 of the primers yielded clear and reproducible bands in RAPD analysis. Among the primers used, TubeA03 (AGTCAGCCAC) showed the highest polymorphism while TubeA02 (TGCCGAGCTG) and TubeA04 (AATCGGGCTG) showed monomorphic band patterns.

Additionally, genomic template stability ratios (GTS) were calculated. GTS related to the level of DNA damage, the efficiency of DNA repair and replication, Atienzar et al., (1999) could explain the appearance and disappearance of bands. The lowest values were obtained in sample numbers 10, 9 and 8. Generally in samples 8, 9 and 10, the lowest GTS values were obtained, which might imply the sensitivity of lichens to genotoxic stressors near the Iron-steel Factory. Previous studies have also indicated that mutations, chromosomal rearrangements and other DNA lesions could be the reason for the variation in RAPD band patterns. It was demonstrated that changes in RAPD profiles induced by pollution could be regarded as modifications in genomic template stability. On the other hand mutations could be displayed as an appearance of new bands in RAPD assay if the same locus hosts mutations in a sufficient number of cells which might require at least 10% of the mutations (Atienzar et al., 2000).

In a another study, a controlled experiment with one type of heavy metal treatment was also conducted in our laboratory in order to show the effects of a genotoxic agent under *in vitro* environment (Aras et al., 2010). In the study a clean sample of *P. furfuracea* collected from Yenice Forest was exposed to various doses of Pb in different time intervals. Although the polymorphism percentages were not calculated in the study obvious band changes were visualized especially after 24 and 48h. of Pb treatments. Results of the study displayed that

even only one kind of stressor (Pb) might induce DNA changes in *P. furfuracea* samples under *in vitro* conditions (Aras et al., 2010). Thus, it might become easier to explain the level of polymorphisms recorded, in the studies in samples exposed to polluted environment.

### 5.4.6 RAPD profiles of *E. prunastri*

Genotoxic contamination was monitored by RAPD analyses with *Evernia prunastri* lichen samples exposed to differently polluted sites in Karabük (Table 3). A clear genotoxic influence is demonstrated in *E. prunastri* exposed naturally to railways, motorways and the Iron-steel Factory in Karabük. DNA damage assessed by RAPD analyses in the *E. prunastri* province of the Iron-steel Factory in Karabük, Turkey. Results of this study was exhibited similar results *P. furfuraceae* lichen species in Karabük (Cansaran-Duman et al., 2011).

### 5.4.7 Comparison of *P. furfuraceae* and *E. prunastri* RAPD fingerprint results

The high values of DNA damage were obtained by both lichen species (*E. prunastri* and *P. furfuracea)* exposed naturally to various polluted sites around the Iron-steel Factory in Karabük. Cansaran-Duman et al. (2011) have recorded high deterioration of DNA integrity in *E. prunastri* exposed at the same site in 2005, while data presented here showed moderate DNA damage in *P. furfuracea* exposed at the same location. But direct comparison of results may be difficult due to differences in the biology of the employed species, different season and duration of exposure and because of constant alternations in quality and quantity of air pollution. However, the level of DNA damage measured in *P. furfuracea* was lower in *E. prunastri* in the same region. However, these measurements cannot provide detailed data on a variety of specific chemicals or their interactions responsible for genotoxic impact during the investigated period, and are therefore used here only as an indication of polluted status (Stambuk et al., 2009). This may be due to adaptive mechanisms developed in lichen species continuously inhabiting polluted environments. Nevertheless, further studies which focuse especially on the influence of other ecological factors on DNA damage are needed.

## 6. Conclusion

The results of all these studies pointed the advantages of biomonitoring with lichens over instrumental monitoring. Lichens accumulate most of the elements of the periodic table, are usable at low expense, do not depend on electricity for their operation, do not need treatment and are easy to hide, thus discouraging vandalism. In contrast to physical and chemical methods, biological methods allow the direct assessment of the genotoxic potential of air stressors. Thus biological data can be used to estimate environmental and the potential impact on other organisms, including humans.

Ultimately, usage of lichens may allow the ecotoxicological examination of the link between molecular alternations and measurable adverse effects at higher levels of biological organizations. The techniques also might provide an early warning system with a higher sensitivity than the conventional techniques.

## 7. Acknowledgements

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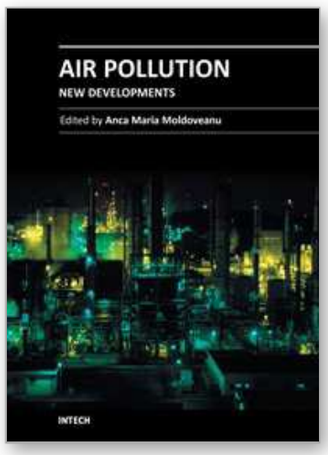


**Air Pollution - New Developments**

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Today, an important issue is environmental pollution, especially air pollution. Due to pollutants present in air, human health as well as animal health and vegetation may suffer. The book can be divided in two parts. The first half presents how the environmental modifications induced by air pollution can have an impact on human health by inducing modifications in different organs and systems and leading to human pathology. This part also presents how environmental modifications induced by air pollution can influence human health during pregnancy. The second half of the book presents the influence of environmental pollution on animal health and vegetation and how this impact can be assessed (the use of the micronucleus tests on TRADESCANTIA to evaluate the genotoxic effects of air pollution, the use of transplanted lichen PSEUDEVERNIA FURFURACEA for biomonitoring the presence of heavy metals, the monitoring of epiphytic lichen biodiversity to detect environmental quality and air pollution, etc). The book is recommended to professionals interested in health and environmental issues.



**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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