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Air-quality biomonitoring: Assessment of genotoxicity of air pollution in the Province of Kayseri (Central Anatolia) by use of the lichen *Pseudevernia furfuracea* (L.) Zopf and amplified fragment-length polymorphism markers

Çiğdem Vardar[a], Esin Basaran[a], Demet Cansaran-Duman[b], Sümer Aras[a,*]

[a] *Biotechnology Section, Department of Biology, Faculty of Science, University of Ankara, Tandogan, Ankara 06100, Turkey*
[b] *Ankara University, Biotechnology Institute, Tandogan, Ankara 06100, Turkey*

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ABSTRACT

Mixed air pollutants are considered a major cause of DNA damage in living organisms. In this study, samples of the lichen *Pseudevernia furfuracea* (L.) Zopf were used as bioindicators to assess the genotoxicity of air pollutants in the province of Central Anatolia, Kayseri. The study area is characterized by the presence of numerous industrial activities, such as steel works, glassworks, and ship-building, metallurgical, mechanical and chemical industries. In the study, two biomonitoring experiments were performed during the dry and wet seasons of 2005. *P. furfuracea* lichen samples were exposed to various pollutants at 12 monitoring sites, distributed throughout the different parts of the province, and each experiment lasted for a period of four weeks. Genotoxic effects of environmental pollutants were evaluated with amplified fragment-length polymorphism (AFLP) molecular markers. The results indicate that the mixture of pollutants might have contributed to the changes in the band patterns obtained by AFLP analysis, reflecting the presence of DNA damage. The average value of polymorphism obtained from the amplification of the primers used was 45.0% for the wet period and 64.6% for the dry period.

Genomic template stability (GTS) ratios revealed that the highest values belong to the *P. furfuracea* samples from Rural Site I and Rural Site II (97.9%, 99.3% respectively for the dry season), the lowest values were from Shanty II and Urban Road Site I (85.8%, 85.2%, respectively for the wet season). The present results indicate that the licxhen species *P. furfuracea*, which is known for its bioindicator–biomonitor capacity, also has a high capacity as indicator of genotoxicity. AFLP markers are cheap, reliable and, therefore, an important tool for studying genotoxicity in lichen species.

1. Introduction

Air pollution represents a serious threat to both the environment and to human health. Millions of tons of toxic pollutants such as ozone, particular matter, carbon monoxide, nitrogen oxides, sulfur dioxide and lead are released into the air each year. Automobiles (cars, buses, trucks, etc.) and industrial sources (factories, refineries, power plants, etc.) are the major contributors to such kind of contamination. Furthermore, polycyclic aromatic compounds (PACs), heavy metals, and halogenated aliphatic hydrocarbons, have been shown to be genotoxic to living organisms [1]. Polycyclic aromatic hydrocarbons (PAHs) are capable of covalent interactions with nucleophilic centers in DNA [2]. They also cause base-pair substitutions, frameshift mutations, deletions, strand breakage, a variety of chromosomal alterations, and *S*-phase arrest [3–5]. Further studies have pointed out that in humans long-term exposure to air pollution is one of the factors involved in the development of cancer [6–8].

It is known that lichens generally compete with plants for access to sunlight, water and other needs. They can survive in extreme environments such as deserts, and on frozen soil of the Arctic regions [9]. A major ecophysiological advantage of lichens is that they are poikilohydric (*poikilo*- variable, *hydric*- relating to water): although they have little control over the status of their hydration, they can tolerate irregular and extended periods of severe desiccation [10].

Unfortunately, current physical and chemical methods for estimating genotoxicity in ambient air provide insufficient information to accurately quantify the risk for biota [2]. In contrast to physical and chemical methods, biological methods allow direct assessment of the genotoxic potential of air pollutants (sulfur dioxide, nitrogen oxide, carbon monoxide, etc.). Plants have been widely used

* Corresponding author. Tel.: +90 312 2126720/1088; fax: +90 312 2232395.
E-mail address: aras@science.ankara.edu.tr (S. Aras).

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as bioindicators in many studies up to now [11,12]. Lichens in particular have been extensively used as biomonitors of atmospheric trace elements, as they are widespread and capable of absorbing elements directly from the air and accumulating them in their tissues [13–19]. The development of molecular marker technology has provided new tools for the detection of genetic variation. In particular, PCR-based molecular markers are useful for DNA analysis in complex genomes. With the PCR reaction almost any type of mutational event can be scored, *e.g.*, point mutation, small insertion and deletion, rearrangement [20]. *Tradescantia virginiana* [21], *Allium cepa* [1,22], *Vicia faba* [23], *Solanum tuberosum* [24], *Cucumis sativus* [25], and *Abelmoschus esculantus* (okra) [26] are only some of the plant species that have been used successfully in biomonitoring studies to assess genotoxicity in ambient air. Lichens can also be considered as sensitive and efficient indicators of genotoxicity. Lichen thallus does not possess roots or waxy cuticles and depends mainly on an atmospheric input of mineral nutrients. These features of lichens, combined with their extraordinary capability to grow over a large geographical range and to accumulate mineral elements far above their own needs, rank them among the best bioindicators of air pollution. In recent years, genotoxicity studies with lichen species have demonstrated the possible ecotoxicological impact of atmospheric contamination by providing valuable information on environmental pollution and improving the process of risk assessment through analyses with molecular markers [19,27–30].

This paper shows the results of studies aimed at assessing the genotoxic potential of air pollutants throughout the Kayseri Province. Kayseri is located in central Anatolia, and it is among the most crowded cities in Turkey. The low-quality coal used to heat houses, buildings and industrial sites is generally an important source of air pollution in Kayseri. In recent years, rapid unplanned urbanization, improperly locating industrial facilities, a lack of good regulations to control industrial emissions, and an increasing number of motor vehicles have caused high levels of air pollution in Kayseri.

Heavy metals, ozone, particular matter, carbon monoxide, nitrogen oxides and sulfur dioxide, which constitute a mixture of pollutants, have been a major concern for human and environmental health in recent years. In this regard, lichens can be used effectively in biomonitoring studies of air pollution. Biomonitoring with lichens provides advantages over instrumental analyses in that lichens accumulate most of the elements of the periodic table and they are cost-effective, do not depend on electricity for their operation and do not need any kind of treatment. Biological methods, in contrast to physical and chemical methods, allow the direct assessment of the genotoxic potential of air contaminants. Thus biological data can be used to estimate the environmental impact of pollutants on living organisms. Lichens are considered sensitive and efficient indicators of genotoxicity [31]. For this investigation *Pseudevernia furfuracae* L. Zopf was chosen as a suitable bioindicator since its sensitivity to organic and inorganic compounds has been well documented. We tried to detect probable DNA-damage induction by use of the amplified fragment-length polymorphism (AFLP) method, a very sensitive molecular tool for the detection of DNA alterations.

2. Materials and methods

2.1. Study area

The lichen samples were collected from Çat forest (Sivas) from 1582 m above sea level, far from large urban and industrial settlements, and transplanted to 12 polluted sites in Kayseri. The study was conducted throughout the different polluted sites of Kayseri

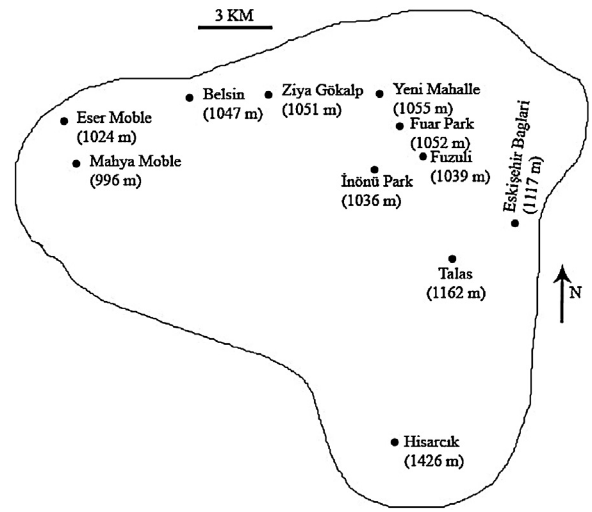

Fig. 1. Map of the study area.

province. The province itself is situated in the middle of the Central Anatolia region. According to measurements from these stations, the study area has a Mediterranean climate characterized by dry summers and high temperatures. In Kayseri, the annual rainfall is 368.4 mm and the mean annual temperature is 10.6 °C. The urban area of Kayseri is affected by contamination from SO_2 and particle matter (PM) in the atmosphere. However, the SO_2 and PM values are still below the limit values set by the EC and WHO [32]. There are definite boundaries that distinguish urban and suburban sites in the city of Kayseri. The samples collected from Çat forest were exposed to air at different sites, selected on the basis of traffic and industrial activities by use of bag technique, which was used evaluation of sampling technique for air pollution, during the wet and dry periods of the year 2005. Industrial districts to which the lichen samples were transplanted were characterized by different activities (mechanical, chemical, textile, food). Urban sites, urban park sites, a main road and urban roadsides were close to the city center. Urban roadside samples were among the areas between 0 and 5 m, usually not more than 2 m away from the busy road. Urban park sites were chosen from two large inner city parks of Kayseri. *P. furfuracae* samples were also transplanted to two shanty zones around the city and to two rural areas in the south of the Kayseri province, more than 10 km away from the city center (Fig. 1, Table 1).

2.2. Lichen sampling and bag preparation

Pseudovernia furfuracea samples were collected from the bark of pine trees from Çat forest (Sivas province) (39°24.665′ N, 35°51.369′ E, Turkey), located far from large urban and industrial settlements. In the laboratory, *P. furfuracea* samples were cleaned from soil particles and treated with seven consecutive washings with distilled water. Spherical bags of 3–4 cm in diameter were assembled with nylon mesh (10 cm × 10 cm wide, with 1-mm^2 meshes) and closed with nylon wire. About 400–450 mg *P. furfuracea* thalli were placed in each prepared bag.

The *P. furfuracea* samples were transplanted to 12 polluted sites in Kayseri (Table 1). The control samples from Çat forest were transported to the lab immediately on iceblocks and ground to powder with mortar and pestle in liquid nitrogen and then stored at −70 °C until use.

2.3. Sample collection and chemical content of *P. furfuracea*

In order to evaluate in the element accumulation in lichen samples at two different time periods, the dry (June–September 2005)