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A novel screening method to identify air pollution by genotoxic compounds☆

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ABSTRACT

Genotoxic compounds, as common contaminants of the air environment, are of interest in air pollution monitoring. There are several methods to determine the level of these contaminants in different localities, many of which may be difficult to access with the use of conventional active and passive samplers. In the present study, the needles *Pinus mugo* Turra and *Picea abies* were used to monitor sampling localities in Austria, Slovakia, and the Czech Republic. Needles were extracted and chemical analysis and the genotoxicity bioassay SOS chromotest were used to obtain complex information about the chemical mixture of pollutants present and their genotoxic effects. The SOS chromotest method was optimized by using a CPRG chromogenic substrate to reduce the false positive genotoxic effect of needle extracts. *Pinus mugo* Turra and *Picea abies* were identified as suitable passive sampling matrices for long-term air monitoring using the same plants sampled at the same time. The presented study brings an innovative method for the fast screening and identification of localities loaded by genotoxic active air contaminants.

1. Introduction

Persistent organic pollutants (POPs) resulting from anthropic activities (stationary and mobile sources) cause a significant deterioration of air quality (Holoubek et al., 2007a,b). Some of these compounds are known, or suspected, to be human carcinogens (Claxton and Woodall, 2007).

Conventionally, POPs are monitored using high-volume air samplers. However, these are expensive and their use in remote localities is complicated (Levy et al., 2007). Alternatively, spatial and temporal POPs concentrations can be monitored via passive samplers. This is cheaper than the conventional approach (Harner et al., 2003; Jaward et al., 2004; Klánová et al., 2009), yet, as in the case of high-volume samplers, the installation of passive or active samplers can be very challenging or not even possible in some localities. For remote sampling and poorly accessible environments and localities, the use of vegetation can be a useful sampling alternative. The ability of vegetation to accumulate organic compounds was described in details and demonstrated in several studies, these involving needles (Chropeňová et al., 2016; Piccardo et al., 2005), lichens (Augusto et al., 2007; Fuga et al., 2008), and moss (Lead et al., 1996; Lim et al., 2006).

Conifer needles, which were used in the present study, are covered by an epicuticular wax layer, which accumulates lipophilic compounds (Eriksson et al., 1989; Hellstrom et al., 2004; Piccardo et al., 2005). Air pollutants in the vapor phase can diffuse into the waxy layer, while particle-associated compounds are deposited on the needle surface (Jensen et al., 1992). Conifers accumulate air pollutants over years and their amounts increase with the age of the needles (Kylin and Sjödin, 2003).

Many toxic and harmful substances which are responsible for adverse health effects may be difficult to identify by chemical analysis due to their low concentration in the environment. Moreover, the toxic effects of some chemicals can be observed only after their activation by other pollutants in a mixture, where various interactions can produce synergistic, antagonistic, or additive effects (Donnely et al., 1990). The chemical analysis of air samples alone does not provide enough information about the health impacts of a wide variety of air pollutants.

There is a sufficiency of chemical studies focused on quantification of the levels of air pollutants in needles (Di Guardo et al., 2003; Eriksson et al., 1989; Iozza et al., 2009; Kylin and Sjodin, 2003; Piccardo et al., 2005) or comparison between the profile

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and concentration of POPs in needles and those in active and passive air samplers (Holt et al., 2016; Klanova et al., 2009; Levy et al., 2007). There are many studies which have used bioassays to evaluate the genotoxic effects of air pollution. The first type, *in situ biomonitoring*, describes a method in which mature plants (grown in standardized conditions in a greenhouse) are exposed to air pollution for a short time, and after that, the genotoxicity test is used (Amato-Lourenco et al., 2017; Pereira et al., 2014). In the second approach, air samples are collected by active (Hi-Vol, Low-Vol) or passive (polyurethane foam (PUF), semipermeable membrane device (SMPD), or XAD-2 resin (XAD-PAS)) air samplers. Usually, samples are extracted (for example with dichloromethane) and crude extracts are used for biological experiments (Aammi et al., 2017; Binkova et al., 2003; Brits et al., 2004; Cerna et al., 2000; Cupr et al., 2006; Skarek et al., 2007; Škarek et al., 2007a). However, each of the mentioned chemical approaches or bioassays brings certain limitations described below.

Conventional high-volume air sampling is associated with high cost and sampling campaigns are usually restricted to a small number of sites and very limited time periods; consequently, information on the spatial and temporal trends of POPs concentrations in the atmosphere is still sparse (Klánová et al., 2006). In contrast, passive air sampling (PUF, XAD, SPMD etc.) as a cheap and versatile alternative to conventional active air sampling can provide information about long-term contamination of a particular site; however, such contamination can only be exposed over a period of several weeks or months and the sampling campaign preparation procedure is sophisticated (precleaning of the sampling medium, necessity of sampling medium protective chambers, protection against robbery and damage, etc.) (Ockenden et al., 1998; Pozo et al., 2006). By contrast, conifers (pine or spruce), which are endemic and evergreen, can accumulate POPs for several years and can be used for long-term monitoring over a broad spatial area or at remote and poorly accessible locations (Chropeňová et al., 2016). It is relatively easy to establish the age of needles; thus, the same tree branch containing one-to four-year-old needles (or older, depending on the species) can be sampled at the same time. Then, needles are separated according to their age and analyzed in a one-run analysis which allows the evaluation of temporal trends in air pollution (Baráková et al., 2017; Kylin and Sjodin, 2003; Piccardo et al., 2005).

Studies which use only bioassays as indicators of air pollution are limited by not being able to determine the exact composition of the air pollution mixture or the possible sources of the pollution. For this, other complementary analyses are required (Piraino et al., 2006). *In situ biomonitoring* (already mentioned above) can give current information about air pollution by genotoxic active compounds or information only about short-term air pollution (depending on the time of mature plant exposure); however, this method does not provide spatial or temporal information about air pollution (Amato-Lourenco et al., 2017; Isidori et al., 2003; Pereira et al., 2014).

The combination of chemical analysis and biological assay can give an overall picture of the concentration of biologically active pollutants which can interact with the human body, and subsequently, this obtained information can be used to assess human health risks. To evaluate the toxicity and genotoxicity levels of atmospheric pollution, several bioassays can be used, such as the Ames test using the bacterial strain *Salmonella typhimurium* (Cerna et al., 2000), the Microtox and SOS chromotest (Aammi et al., 2017; Škarek et al., 2007b), micronuclei assay using *Tradescantia pallida* (Vivian Sposito et al., 2017), the Pollen Abortion Test (Fleck et al., 2016; Greguskova and Micieta, 2013), and bioassays using human cell lines (Sevastyanova et al., 2007). All these approaches use methods of active or passive sampling to determine the chemical composition of the investigated air pollution and the biological activity of these the respective samples.

Unlike these methods, the innovative approach presented here allows the identification of genotoxic compounds absorbed onto conifer needles (i.e. needles from plants growing directly in the locality of interest); that is, such needles are used as passive air samplers. Among other advantages, the presented method allows (i) the monitoring of remote areas; (ii) long-term monitoring using the same tree branch with needles of a different age; (iii) rapidly-acquired screening information about the genotoxicity of the air pollution mixture, which can be used for later complex analysis focusing in particular on concrete groups of contaminants; and (iv) the cheap and fast identification of localities loaded by genotoxic active contaminants.

In this study, experimentally obtained data on the bacterial mutagenic potencies of air pollutants accumulated in pine and spruce needles were investigated and compared with the levels of POPs determined using the same extract from needles. *Pinus mugo* Turra and *Picea abies* were used as model species in the present study as they are the more common coniferous species in Europe. *Pinus mugo* Turra is widely distributed in high mountain ecosystems and *Picea abies*, a species of spruce, is widely planted across Northern, Central, and Eastern Europe. As both species differ by their structure and properties, there are also differences in POPs uptake (Di Guardo et al., 2003); however, determining these differences was not the aim of the present study as samples of *Picea abies* and *Pinus mugo* Turra were taken from different localities. Mountains are very important environments for studying levels of pollutants as these are transported to mountainous zones by air mass movements. The enhanced deposition of harmful substances in these habitats is related to the higher levels of precipitation (Chropeňová et al., 2016). This study explores the use of two coniferous species - spruce and pine – as passive air samplers and their possible employment in the evaluation of the genotoxic effects of air pollution.

2. Materials and methods

2.1. Sampling sites

In this study, two species of coniferous trees – *Picea abies* (Project Monairnet) and *Pinus mugo* Turra (Project Needlenet) – were used. Needles were sampled between late September and October 2012 for the main Monairnet campaign (Šnábl, 2010) and between June and July 2014 for the main Needlenet campaign (Chropeňová et al., 2016). Nine sampling sites in South Moravia, the Czech Republic and in alpine habitats in northern Austria were chosen for the Monairnet project and twelve sampling sites in the mountain alpine habitats of the Western Carpathians in the Malá Fatra, Velká Fatra and Tatra Mts for the Needlenet project. The GPS coordinates of the sampling sites and their description are shown in Supplementary Information (SI), Table S1.

2.2. Sampling design and procedure - Monairnet

Three to five branches with different orientations were cut from the top 7th whirl of two dominant, vital adult spruce trees. Six-month-old twigs of the current year were collected, pooled, and transferred to the laboratory in airtight pre-cleaned 3L glass jars. After sampling, the jars were immediately sealed and stored under dry ice for transport to the laboratory. The de-needling of shoots in the laboratory was achieved by their immersion in liquid nitrogen. After about five minutes needles could be removed easily by stirring. The defoliated twigs were removed with tweezers and needles were skimmed off with a filter and placed in similar but