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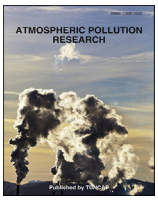

In situ biomonitoring of air quality in rural and urban environments of Mexico Valley through genotoxicity evaluated in wild plants

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Air pollution is one the main causes of DNA damage in living organisms. Continuous exposure to the complex mixture of gases of polluted atmospheres affects health in many ways. Sentinel organisms are good biological models to assess the genotoxic damage caused by various chemicals such as atmospheric pollutants.

In this study the plant species *Taraxacum officinale* and *Robinsonecio gerberifolius* were exposed during 2015, in the dry and rainy seasons, for 0, 2, 4 and 6 weeks to two different atmospheres of Mexico Valley, one rural in Altzomoni atmospheric observatory (ALTZ) and other urban in the atmospheric observatory of Centro de Ciencias de la Atmósfera (CCA), located in Universidad Nacional Autónoma de México (UNAM).

Leaves of exposed plants were processed to analyze genotoxic damage by single-cell gel electrophoresis. To found any relation, the presence of pollutants in the atmosphere of both sites was analyzed with a Cavity Ring-Down Spectrometer (CRDS) and in the leaves the presence of heavy metals with an inductively coupled plasma mass spectrometer.

Single-cell gel electrophoresis results showed higher damage in the leaves exposed to higher pollution in the UNAM atmospheric station in comparison to the ALTZ and controls, which was maintained in growth chambers under controlled conditions. Significant differences between rainy and dry seasons were found. Chemical analysis showed a significant increase in various heavy metals, especially in rainy season in both exposure sites. Increased DNA damage observed in both plant species at CCA station could be caused by accumulation trough six weeks.

1. Introduction

Air pollution is one of the most serious environmental problems in the world. Anthropogenic activities release to the environment millions of tons of contaminants as a complex mixture that includes inorganic gases, volatile organic compounds, heavy metals, and several biologic agents; different studies present evidence of deleterious effects of this mixture (Ceretti et al., 2015; Owens et al., 2017). The International Agency for Research on Cancer (IARC) classified atmospheric pollution as carcinogenic for humans (Group 1) and there are enough evidence that the exposure to it is a cause of lung cancer (Loomis et al., 2013).

Since 1990 Mexico City is considered one of the more contaminated places in Latin America. The chemical composition of its

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atmosphere has changed with time due to the emissions of industries like petroleum and petrochemical, metallurgy, iron and steel, automotive, paper, glass, food and drinks, amongst others, as well as of natural sources. The orography of the Mexico valley favors the accumulation of harmful compounds for human health, as well as the high population density and considerable vehicular traffic (SEMARNAT, 2012). Native plants that are widely distributed in these places, can be used as biomonitors to study air pollution and its effect on DNA damage.

Parallel to physical analysis of pollution, biomonitoring allows determining biologic effect of air pollution (Piraino et al., 2006).

Unfortunately, studies about the risk of continued exposure to atmospheric contaminants to health are scarce in México, and this aspect receives little importance, in particular with plant models. Notwithstanding that since the 20th century, higher plants were used to evaluate the presence and damage induced by several physical and chemical environmental agents (Ruston, 1921). Plants can give information about toxic compounds accumulated in them (Weinstein et al., 1990). Different studies show that plants are sensible organisms to atmospheric pollutants as sulfur dioxide, nitrogen oxide, ozone, formaldehydes, ammonia, and other complex mixes as cigarette smoke, or diesel combustion (Rodrígues et al., 1997). There are various higher plant models as *Vicia faba*, *Allium cepa*, *Zea mays*, *Tradescantia paludosa*, *Nicotiana tabacum*, *Crepis capilaris*, and *Hordeum vulgare* (Gichner et al., 2009; Grant, 1994; Ventura et al., 2013). Biomonitoring *in situ* by using higher plants as models can be useful to identify the effect of air pollutants in specific areas without the use of sophisticate and expensive instruments (Ceretti et al., 2015). The use of plants as sentinels, that is, as organisms capable to react at any environmental pollutant before it impacts over humans, allows to identify toxicological effects and its damage (Stahl, 1997).

Higher plants, as sessile organisms, are continually exposed to environmental pollution and particularly to chemical stressors. Therefore, higher plants have been used for the evaluation of genotoxic effects of environmental chemicals and for the biomonitoring of terrestrial ecosystems (Wang, 1991; Wang and Freemark, 1995).

In our study the selected plants: *Taraxacum officinale* and *Robinsonecio gerberifolius* show important characteristics to use them as biomonitors of air quality because they are widely distributed in Mexico Valley. *T. officinale* (Fig. 1) is an herbaceous evergreen common species, with yellow flowers in inflorescences composed of 80–250 flowers, seeds with white pappus of 5–8 cm. It is of broad distribution, from 1 200 to 4 000 m of altitude (Rzedowski, 1997), in different temperatures and meteorological conditions, as well as in sites with higher indices of contamination, near to roads or industrial zones (Ligocki et al., 2011), it is easy to identify and the sampling is simple and economical (Petrova et al., 2013). *R. gerberifolius* (Fig. 2) is a native plant of recent identification distributed in alpine and sub-alpine regions of México Valley as Popocatepetl and Ixtaccihuatl, place of our study. It is an herbaceous plant of 15–33 cm usually with 1–3 scapus, large and etiolated leaves, flowers in disc form in yellow inflorescences present between July and December (Pruski, 2012).

During the last decade the single-cell gel electrophoresis or comet assay has been one of the most used techniques for the detection of genotovic damage caused by environmental pollutants, due to its simplicity, sensitivity, versatility, fast and economical (Collins, 2004; Jha, 2008).

Among the assays used to evaluate environmental pollution, the comet assay, in its neutral version, was used for the first time with plant tissues 20 years ago (Cerda et al., 1993). Koppen and Verschaeve (1996) developed the alkaline version on broad bean (*Vicia faba*) roots a few years later. However, the absence of free

Fig. 1. *Taraxacum officinale* (dandelion), shows open flowers and seed heads.

cells in plants and the presence of a cell wall, which is a resistant barrier to cell lysis (Poli et al., 1999), cause technical issues for performing the comet assay on plant tissues. To overcome these problems, a simple and efficient mechanical extraction to isolate cell nuclei was developed by Navarette et al. (1997). This technique was improved by Gichner and Plewa (1998) and Poli et al. (1999). Most researchers actually employ the same nucleus isolation buffer and both, unwinding and electrophoresis conditions, based on protocols developed by Gichner et al. (1999, 2004) and Gichner (2003). Recently researchers have made efforts to improve the comet assay performance to increase its reliability and reproducibility (Pourrut et al., 2015).

The aim of this study was to evaluate the DNA damage induced by air pollutants in the leaves of *Taraxacum officinale* and *Robinsonecio gerberifolius*, exposed to two different atmospheres in Mexico Valley, one rural and other urban during 2015 dry and rainy seasons, through the comet assay and relationed it with spectroscopic and chemical analyses of any pollutants presents in both sites.

2. Material and methods

2.1. Chemicals

The following chemicals were purchased from Sigma-Aldrich,

Fig. 2. *Robinsonecio gerberifolius*, shows large and etiolated leafs, flowers in disc form in yellow inflorescences.

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