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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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ABSTRACT

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 geberifolius* 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.

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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 (Rodríguez et al., 1997). There are various higher plant models as *Vicia faba*, *Allium cepa*, *Zea mays*, *Tradescantia paludosa*, *Nicotiana tabacum*, *Crepis capillaris*, 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 sophisticated and expensive instruments (Ceretti et al., 2015). The use of plants as sentinels, that is, as organisms capable to react to 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 Ixtacchuatl, 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 genotoxic 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 (Cerdeja et al., 1993). Koppen and Verschaevé (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 leaves, flowers in disc form in yellow inflorescences.

St. Luis, MO, USA: low and normal melting point agarose, EDTA, Tris, and ethidium bromide; Triton X-100 and phosphate saline buffer pH 7.4 (PBS) were purchased from Dubelcco's PBS (1 L per packet sterilized); sodium hydroxide (NaOH), sodium chloride (NaCl) and Dimethyl sulfoxide (DMSO, CAS number 67-68-5) were purchased from J.T. Baker (México). All other reagents used were of analytical grade.

2.2. Characteristics of the study sites

This study was realized in a rural place, a natural reserve named Iztacihuatl-Popocatepetl, specifically in the high-altitude station Altzomoni (ALTZ, Lat 19.12° N, Long 99.65° W, Alt 4 000 m) and in an urban site, the Centro de Ciencias de la Atmósfera (CCA) of Universidad Nacional Autónoma de México (Lat. 19.33° N, Long 99.65° W, Alt 2 260 m). Both sites are very different with respect to the presence of environmental pollution. Native plants that are widely distributed in these places, can be used as biomonitors to study air pollution and its effect on DNA damage.

2.3. Preparation of plant material

Taraxacum officinale seeds were obtained from a mountainous natural protected area in Santo Tomás, Ajusco, Tlalpan, México City. Selected seeds of this species were germinated in controlled conditions of light, temperature and humidity. When the plants reached 10–15 cm, 24 of them were exposed to both environments and to control conditions. On the other hand 30 very healthy plants of *R. gerberifolius* were collected from Izta-Popo National Park and placed, without damaging leaves and roots, in flowerpots containing sterile soil. Subsequently, they were maintained in a growth camera with controlled temperature, humidity and light, during six weeks.

2.4. Plant exposure

One third of the plants were placed in ALTZ, another third in CCA and the last third (the control) was maintained in a growth camera. After exposure for 0, 2, 4 and 6 weeks, two plants of each treatment and of the control, were collected and transferred to the laboratory. In order to remove the particles attached to the leaves of each treatment they were rinsed in distilled water and carefully dried with paper towel; one half of them was dried at 40 °C for its posterior chemical analysis. The other half was prepared for the comet assay, cuts of 1 cm² were maintained in cold phosphate saline buffer pH 7.4 (PBS) until the isolation of nuclei (≤ 15 min).

2.5. Comet assay

2.5.1. Isolation of nuclei from leaves

The nuclei were isolated mechanically, under dim or yellow light. Leaves cuts of 1.0 cm² were placed in a 60 mm glass Petri dish. Onto each 350 μ L of cold PBS, pH 7.4 was spread. Using a cold scalpel penknife the cuts were sliced perpendicular to central venation into the buffer. The Petri dish was kept on ice so that the nuclei can be collected by precipitation in the buffer. 50 μ L of nuclei suspension was added to micro vials with 50 μ L of molten low melting point agarose (LMPA), gently mixed by repeated pipetting using a cut tip. 80 μ L of the mixture were placed onto a cover slip (24 \times 50 mm), and immediately a microscope slide with frosted ends, previously covered by one side with 1.0% normal melting point agarose (NMPA), was carefully overlaid avoiding the formation of air bubbles. To solidify, the slide was placed on a cold surface for a minimum of 5 min, after which the cover slip was removed and a final layer of 80 μ L molten 0.5% LMPA was added to the slide that again was kept on the cold surface for 5 min.

2.5.2. Lyses

The cover slips were removed and all the slides immersed in staining jars with cold (4 °C) lyses solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, 10 N NaOH, 10% DMSO and 1% Triton X-100 in deionized water) pH = 10 for at least 1 h in darkness.

2.5.3. Unwinding and electrophoresis

All the slides were drained vertically. The bottom of the slides were dried to remove the lyses solution and placed in an electrophoresis chamber and immersed in electrophoresis buffer (10 N NaOH, 200 mM EDTA, pH > 13). The nuclei were incubated for 20 min to allow the DNA unwinding. Subsequently, the electrophoresis was conducted at 0.74 V/cm (25 V, 300 mA) for 20 min into a refrigerator at 4 °C in darkness. After electrophoresis, the slides were neutralized 3X with 0.4 M Tris buffer, and stained or incubated for 15 min in ethanol and left overnight to dry, after stored in slide boxes until its register.

2.5.4. Staining and comet scoring

The slides were stained with 50 μ L 0.2 mg/mL ethidium bromide and covered with a cover slip. Slides with stained nuclei were placed in a wet camera between two paper towel layers soaked in 0.45 μ M Millipore filtered water to avoid the contamination by bacteria and dye evaporation. Slides were protected from light with an aluminum foil to avoid the decay of dyeing. The comets were scored within three or four days. At least 50 cells for each slide and three slides by treatment were analyzed in an epifluorescence Axiostar Plus Carl Zeiss microscope with an exciting filter of 515–560 nm and a barrier filter of 590 nm. The computerized image analysis system Comet Assay IV (Perceptive Instruments)

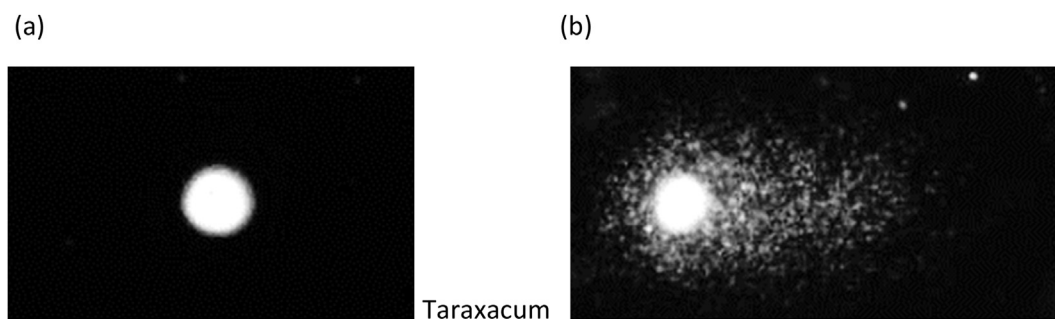


Fig. 3. (a) Damaged nucleus (comet) and (b) damaged nucleus (comet) of *Taraxacum officinale*.

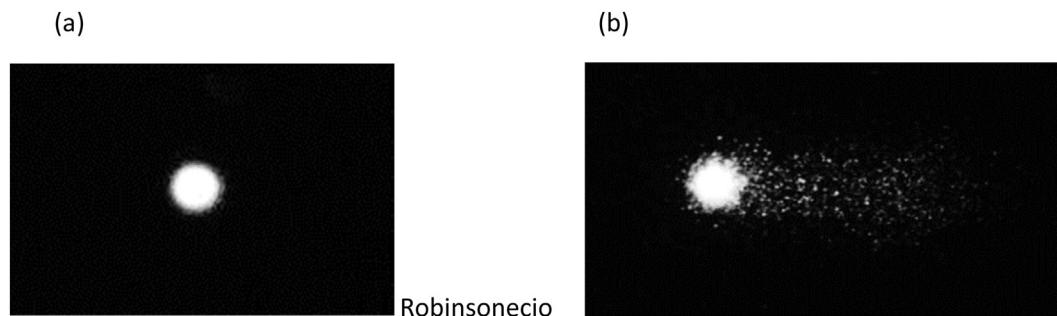


Fig. 4. (a) Normal nucleus and (b) damaged nucleus (comet) of *Robinsonecio gerberifolius*.

was used to measure the DNA damage by means of the tail moment. Only the nuclei images whose structure and brightness intensity was very good (Fig. 3a and b and 4a,b) were analyzed. All the slides were coded before scoring as to avoid bias.

2.6. Spectroscopic analysis

Physical analysis of pollution with a cavity ring down spectrometer (CRDS), allows to characterize the composition of terrestrial atmosphere, as well as to determine gaseous emissions. This method uses the absorption in the infrared spectra of the corresponding molecules.

The presence of CO₂, CH₄ and CO during the exposure periods in both seasons was registered with a cavity ring down spectrometer.

2.7. Chemical analysis

The dehydrated leaves of *Traxacum officinale* samples exposed during six weeks (total exposure time) were analyzed with an inductively coupled plasma mass spectrometer PQ3 (Thermo Elemental) to determine the following heavy metals: Mn, Fe, Cu, Zn, Mo, V, Cr, Co, Cd, Sb and Pb.

The samples were prepared in a digester, UltraWave model (Milestone). 0.2 g of sample was weighed and 5 mL of ultra-high grade HNO₃ were added. Samples were left in pre-digestion overnight under the extraction hood.

All measurements were carried out using an inductively coupled plasma mass spectrometer PQ3 (Thermo Elemental) in the Laboratory ICP-MS at the Instituto de Geofísica, Universidad Nacional Autónoma de México. The instrument was optimized prior to the analysis with a certified aqueous solution manufactured by High Purity Standards (ICP-MS-B), which contains a wide range of masses (Li, Co, In, Ba, Bi, Ce and U of 1 µg/L). The calibration curve was generated with eight points (0, 0.1, 0.5, 1, 10, 50, 100 and 250 µg/L) from a multi-element stock solution of 100 µg/mL of High Purity Standards (QCS-26).

The instrumental drift was corrected with the internal Indian standard. Detection limits were calculated by the following equation:

$$L.D. = \frac{3(SD \text{ int BCO})}{(\text{conc STD})}$$

$$(\text{int STD} - \text{aver int BCO})$$

Where:

SD int BCO – Standard deviation of the intensity of the target

conc STD – Concentration of the standard solution

int STD – Intensity of the standard solution

aver int BCO – Average target intensity

2.8. Statistics

Analysis of variance was used to test for differences among the exposed samples within each experimental series. When there was a significant F-value, between each exposed group and its corresponding negative control, a test for significance using Newman-Keuls multiple comparison was applied.

3. Results

Fig. 5 shows the data ± standard error of at least 300 values of tail moment, registered in three different slides and in two different experiments for *T. officinale*'s evaluation of genotoxicity, corresponding to 2015 dry and rainy seasons. In this figure it is also observed that in the second week there are significant differences with respect to control, in the dry season in CCA and in the rainy season in ALTZ, meanwhile in the fourth week only in the last case. In the sixth week the data were significant with respect to control, for the dry and rainy seasons at CCA, obtaining in the later the greater value, the same as for ALTZ.

Fig. 6 shows the results of DNA damage obtained from *R. gerberifolius*, corresponding to 2015 dry and rainy seasons, significant differences were observed when compared with the control. In the second week only in the dry season of CCA there was a significant difference, meanwhile in the fourth week the differences were significant only in dry and rainy seasons in ALTZ. During the sixth week, the tail moment was significantly different in the rainy season of CCA and in the dry and rainy seasons of ALTZ.

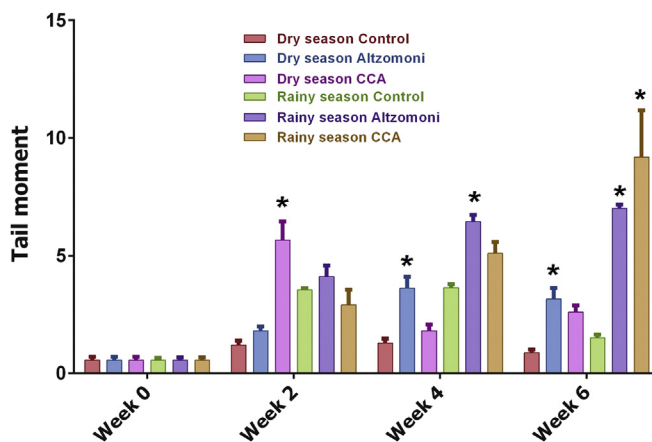


Fig. 5. Tail moment mean ± standard error in *T. officinale* exposed during 2, 4 and 6 weeks in dry and rainy seasons in Altzomoni (ALTZ) and Centro de Ciencias de la Atmósfera (CCA). * indicates a significant difference against the exposure time and seasons ANOVA two ways, post hoc Newman-Keuls $p < 0.05$.

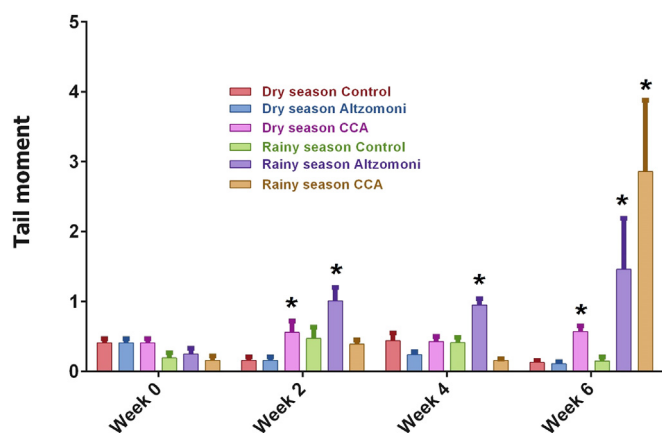


Fig. 6. Tail moment mean \pm standard error in *R. gerberifolius* exposed during 2, 4 and 6 weeks in dry and rainy seasons in Altzomoni (ALTZ) and Centro de Ciencias de la Atmósfera (CCA). * indicates a significant difference against the exposure time and seasons ANOVA two ways, post hoc Newman-Keuls $p < 0.05$.

In general, in the rainy season there was more DNA damage in both exposure sites, ALTZ and CCA, when compared to the control. Also, it can be appreciated an increase of genotoxicity with the time of exposure, and the higher effect was observed in the samples exposed during six weeks.

In Table 1 is observed the data of pollutants monitored by University Network of Atmospheric Observatories (RUOA), in the CCA and in the Altzomoni atmospheric station for 2, 4 and 6 weeks, corresponding to plants exposure, during 2015 dry and rainy seasons. It can be observed that for CO_2 , CH_4 and CO the data are similar in both sites in the two seasons and in the different weeks of exposure, except for CO that is lower in ALTZ. While for NO , NO_2 and NO_x in all the seasons in ALTZ is less than in the CCA, except for both seasons in ALTZ in the week six. In the case of O_3 , the highest values occur in the fourth and sixth weeks in ALTZ in the dry season. While for SO_2 , the highest values are observed in the CCA in the rainy season. $\text{PM}_{2.5}$, was only detected in CCA in both dry and rainy seasons. PM_{10} , was observed in both seasons in CCA and ALTZ, except in the latter in the second week that only was observed in rain season. UVA and UVB, are only observed in the CCA.

Heavy metal quantification with inductively coupled plasma mass spectrometer in leaves of the *Taraxacum officinale*, after 6 weeks of exposure during 2015 dry and rainy seasons in CCA and in ALTZ monitoring station are showed in Table 2 in which the data of the following detected metals are shown: Mn, Fe, Cu, Zn, Mo, V, Cr, Co, Cd, Sb and Pb. In almost all cases the determined values of heavy metals in both season and sampling sites, are higher than the control, except for lead, which was only higher in the rainy season. In this data it can be observed a tendency to the accumulation of almost all the heavy metals in the samples exposed during rainy season in relation with the dry season.

4. Discussion and conclusions

The evaluation of the genotoxic risk of the atmosphere is not easy because it is a complex mix (Piraino et al., 2006). This study propose an interesting strategy based on the statement of two wild plants as bio monitors or sentinel organisms to evaluate the genotoxic potential of the environment of two places located, one of them in a rural area (Altzomoni) and the other in an urban place (Centro de Ciencias de la Atmósfera) of the Mexico Valley, in the 2015 dry and rainy seasons.

Due to the important role that plants play in the trophic chain and to their sessile nature, they can be used as stable sensors in the ecosystems, being a good tool for obtaining information about lipophilic chemicals and heavy metals dangers that can induce genotoxic damage to different live organisms (Gichner et al., 2000; Ventura et al., 2013). Specifically, the leaves are an excellent tissue for the bio monitoring of genotoxic agents *in situ* during long time (Gichner et al., 1999, 2000; Ventura et al., 2013).

In relation to the genotoxic damage observed in this study, the first important observation is the different sensitivity to pollution effects between both plants, as *R. gerberifolius* showed greater sensitivity than *T. officinale*.

DNA damage in both plants can be related to the exposure time in dry and rainy seasons and agree with increased estimates of pollutants detected in CCA and ALTZ by inductively coupled plasma mass spectrometry. These high levels of pollution can be associated with the accumulation of these compounds in plant structures, which may generate a dysfunction in their cells (Gichner et al., 2004).

Table 1

Pollutants monitored by Cavity Ring-Down Spectrometer (CRDS) during 2015 dry and rainy seasons in Centro de Ciencias de la Atmósfera (CCA) and Altzomoni (ALTZ).

Pollutant	Week	0		2		4		6	
		CCA	ALTZ	CCA	ALTZ	CCA	ALTZ	CCA	ALTZ
^a CO_2	Dry	420.66	395.40	415.53	395.10	419.02	394.95	418.31	394.40
	Rainy	409.95	391.03	411.32	389.90	413.85	400.81	424.23	395.29
^a CH_4	Dry	1.94	1.84	1.93	1.83	1.99	1.84	1.10	1.85
	Rainy	1.99	1.83	2.02	1.86	2.02	1.85	0.86	1.87
^b CO	Dry	0.73	0.16	0.63	0.13	0.76	0.17	0.62	0.17
	Rainy	0.65	0.08	0.64	0.10	0.54	0.11	0.86	0.11
^b NO	Dry	12.00	—	13.04	—	9.44	—	12.62	—
	Rainy	13.13	0.16	12.49	0.16	15.36	0.66	23.01	0.61
^b NO_2	Dry	21.21	—	21.75	—	22.44	2.62	24.54	1.71
	Rainy	25.66	0.78	22.75	1.75	27.78	1.41	29.90	1.89
^b NO_x	Dry	36.17	—	34.79	—	32.38	3.28	37.14	49.32
	Rainy	39.74	1.03	34.73	1.86	43.11	1.61	52.90	39.41
^c O_3	Dry	32.98	—	27.37	—	36.45	56.23	33.73	49.32
	Rainy	28.03	33.36	20.92	37.91	26.81	41.69	19.43	39.41
^c SO_2	Dry	3.17	—	2.12	—	2.09	0.73	2.74	2.34
	Rainy	2.88	2.23	4.90	1.62	4.96	2.25	7.50	2.59

— there were not registered data.

^a Data are described in ppm.

^b Data are described in ppb.

^c Data are described in $\mu\text{g}/\text{m}^3$.

Table 2

Concentration of metals in leafs of *Taraxacum* exposed for 6 weeks to CCA and ALTZ atmospheres during 2015 dry and rainy seasons.

	D. L. (µg/L)	Control	CCA		ALTZ	
			Dry	Rainy	Dry	Rainy
Mn	0.061	19.33	49.59	51.19	112.77	529.73
Fe	0.25	29.12	81.15	102.92	93.51	202.89
Cu	0.03	5.78	5.22	81.15	5.65	13.99
Zn	0.18	18.13	13.81	16.84	55.43	0.06
Mo	0.01	1.15	0.69	0.44	1.83	3.10
V	0.01	78.73	315.13	560.52	274.09	495.32
Cr	0.01	139.10	302.59	314.47	152.06	526.94
Co	0.002	39.35	123.46	109.77	192.72	247.65
Cd	0	39.60	73.07	56.15	133.66	518.34
Sb	0.01	3.99	22.08	28.82	5.35	176.89
Pb	0.02	554.51	533.18	508.09	75.15	808.46

D.T. Detection Limit.

Data obtained in our observations, allows to relate a higher DNA damage in the exposed leaves in the CCA station during the rainy season, with an increase of several gases detected as CH₄, NO, NO₂, NO_x and SO₂. Additionally, a greater induced genotoxicity can be due to a physiological response of plants to a relative humidity increase, given that this condition favors stomatic opening to provide cell transpiration and thus favoring the intake of pollutant gases. Interaction of pollutant gases with UV radiation in the environment has been associated with DNA damage as single and double breaks effect in alkali labile sites, base oxidation, reactions DNA-DNA and cross-links DNA-protein (Ventura et al., 2013). Studies show that the exposure to pollutant gases as O₃ and CO₂ generate tail moments increased in relation to control (Tai et al., 2010). In the present study CO₂ and O₃, has high values in the dry and rainy seasons in both sites, and could be responsible for the increase in the genotoxicity detected in both species. Restivo et al. (2002) and Sriussadaporn et al. (2003), demonstrated increased DNA damage associated with the intensification of pollutant gases in plants exposed in busy roads.

On the other hand, the levels of pollutants monitored in ALTZ station were lower than those found in CCA because ALTZ is a protected zone with little vehicle transit and low emission of pollutant gases.

The high data of DNA damage observed in plants coming from ALTZ can be explained considering an increment of heavy metals during the six-week period of exposure, probably due to the continuous exhalations of the Popocatepetl volcano (that is near to Alzomoni station). This will have to be corroborated in a further investigation.

Heavy metals can bio accumulate and they can associate to organic molecules through non-covalent unions, they can inhibit protein activity or damage its structure, and generate reactive oxygen species (ROS). Damage induced by OH radicals can conduce to carcinogenesis (Navarro-Aviño et al., 2007). Lead, widely increased in the sixth week of the rainy season in ALTZ, may join phosphate groups of the DNA chain (Silva et al., 2017), forming clastogenic compounds (Leonard, 1988); lead is also related to disturbs in DNA synthesis, mutations and chromosomal aberrations (Rojas et al., 1999).

Cadmium, visibly increased in both sites during the dry and rainy seasons, possibly interacts with nucleic acids over phosphate groups (Jacobson and Turner, 1980; Koizumi and Waalkes, 1990), inducing DNA breaks, chromosomal aberrations and sister chromatid exchanges (Gómez-Arroyo et al., 1989; Mourón et al., 2004). Chromium, increased in ALTZ during the rainy season, is involved in the defense response against antioxidants, in the detoxification process and in the regulation of transcription genes (Karmous et al.,

2014).

Different studies regarding lead and cadmium have shown its carcinogenic effects (Tchounwou et al., 2012; Valverde et al., 2001). In the present study, many heavy metals quantified in the leaves of *Taraxacum officinale* were higher in the rainy season than in the dry season in both sites, ALTZ and CCA.

Genotoxic stress in plants is a critical factor and damages productivity and genome stability; the most frequent injuries in DNA bases are oxidation, alkylation, deamination, apurinic or apirimidic sites and single strand breaks (Tuteja et al., 2009). These alterations activate transduction signals for cell cycle arrest and regulation of genes repair expression, this nuclei damage, generates oxidative stress and can result in programmed cell death (Balestrazzi et al., 2013).

Various studies using higher plants as bio monitors have been developed in different countries (Carvalho-Oliveira et al., 2017; Cesa et al., 2014; De Paula et al., 2016; Illi et al., 2017; Loppi, 2014; Ord et al., 2016; Schultz et al., 2015). Nevertheless, the evaluation of air quality through DNA damage in plant cells in Mexico is practically non existing; several studies describe different genetic damages from environmental pollution over different plant species in other countries (Amato-Lourenco et al., 2017; Raj, 2016). For this reason, the results obtained in the present study may be very useful and highlight the importance of *Taraxacum officinale* and *Robinsonia gerberifolia* as excellent bio monitors of genetic damage induced by environmental pollutants, as several gases and heavy metals. It can be concluded that both organisms, and many other wild plants, can be used for this purpose.

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References

- Amato-Lourenco, L.F., Lobo, D.J., Guimarães, E.T., Moreira, T.C., Carvalho-Oliveira, R., Saiki, M., Saldiva, P.H., Mauad, T., 2017. Biomonitoring of genotoxic effects and elemental accumulation derived from air pollution in community urban gardens. *Sci. Total. Environ.* 575, 1438–1444.
- Balestrazzi, A., Confalonieri, M., Macovei, A., Dona, M., Carbonera, D., 2013. Genotoxic Stress, DNA Repair and Crop Productivity. Springer Science Business Media, New York, pp. 153–169.
- Carvalho-Oliveira, R., Amato-Lorenço, L.F., Moreira, T.C.L., Rocha Silva, D.R., Vieira, B.D., Mauad, T., Saiki, M., Nascimento Saldiva, P.H., 2017. Effectiveness of traffic-related elements in tree bark and pollen abortion rates for assessing air pollution exposure on respiratory mortality rates. *Environ. Int.* 99, 161–169.
- Cerda, H.V., Hofsten, B., Johanson, K., 1993. Identification of irradiated food by microelectrophoresis of DNA from single cells. In: Leonardi, M., Raffi, J.J., Belliardo, J.J. (Eds.), *Proceedings of the Workshop on Recent Advances on Detection of Irradiated Food*, Ancona Italy, 1991. Commission of the European Communities, Bruselas, Belgium, pp. 401–405.
- Ceretti, E., Zani, C., Zerbini, I., Viola, G., Moretti, M., Villarini, M., Dominici, L., Monarca, S., Feretti, D., 2015. Monitoring of volatile and non-volatile urban air genotoxins using bacteria, human cells and plants. *Chemosphere* 1120, 221–229.
- Cesa, M., Nimis, P.L., Buora, C., Lorenzonetto, A., Pozzobon, A., Raris, M., Rosa, M., Salvador, M., 2014. Moss bags as sentinels for human safety in mercury-polluted ground waters. *Environ. Sci. Pollut. Res.* 21, 6714–6722.
- Collins, A.R., 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol. Biotechnol.* 26, 249–261.
- De Paula, P.H.M., Mareus, L.M., Araripe, D.R., Duyck, C.B., Saint-Pierre, D.S., Gioda, A., 2015. Biomonitoring of metals for air pollution assessment using a hemi-epiphyte herb (*Struthanthus flexicaulis*). *Chemosphere* 138 (429), 437.
- Gichner, T., Plewa, M.J., 1998. Induction of somatic DNA damage as measured by single cell gel electrophoresis point mutation in leaves of tobacco plants. *Mutat.*

- Res. 401, 143–152.
- Gichner, T., Ptáček, O., Stavreva, J., Plewa, M.J., 1999. Comparison of DNA damage in plants as measured by single cell gel electrophoresis and somatic leaf mutations induced by monofunctional alkylating agents. *Environ. Mol. Mutagen* 33, 279–286.
- Gichner, T., Ptáček, O., Stavreva, D.A., Wagner, E.D., Plewa, M., 2000. A comparison of DNA repair using the comet assay in tobacco seedlings base on data of the comet assay and two recombination assays. *Mutat. Res.* 470, 1–9.
- Gichner, T., 2003. Differential genotoxicity of ethyl methane sulphonate, N-ethyl-N-nitrosourea and maleic hydrazide in tobacco seedlings base on data of the comet assay and two recombination assays. *Mutat. Res.* 538, 171–179.
- Gichner, T., Patkova, Z., Szakova, J., Demnerova, K., 2004. Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombination in tobacco leaves. *Mutat. Res.* 59, 49–57.
- Gichner, T., Znidar, I., Wagner, E., Plewa, M., 2009. The use of higher plants in the comet assay. In: Dhawan, A., Anderson, D. (Eds.), *Issues in Toxicology n° 5 the Comet Assay in Toxicology*. Royal Society of Chemistry, London, pp. 98–119.
- Gómez-Arroyo, S., Abarca-Hernández, J.C., Cortés-Eslava, J., Villalobos-Pietrini, R., 1989. Sister chromatid exchanges induced by cadmium in *Vicia faba*. *Rev. Int. Contam. Ambient.* 5, 71–82.
- Grant, W.F., 1994. The present status of higher bioassays for the detection of environmental mutagens. *Mutat. Res.* 310, 175–185.
- Illí, J.C., Vancetta, T., Alves, D.D., Migliavacca, Osório, D.M., Bianchin, L., Müller de Quevedo, D., Juchem, F., 2017. Integrated assessment of air pollution by heavy metals and source apportionment using ryegrass (*Lolium multiflorum* Lam.) in southern Brazil. *Environ. Sci. Pollut. Res.* 24, 2790–2803.
- Jacobson, K.B., Turner, J.E., 1980. The interaction of cadmium and certain other metal ions with proteins and nucleic acids. *Toxicology* 16, 79–95.
- Jha, A.N., 2008. Ecotoxicological applications and significance of the comet assay. *Mutagenesis* 23, 207–221.
- Karmous, I., Chaoui, A., Jaouani, K., Sheehan, D., El Fejarni, E., Scoccianti, V., Crinelli, R., 2014. Role of ubiquitin- proteasome pathway and some peptidases during seed germination and copper stress in bean cotyledons. *Plant Physiol. Biochem.* 76, 77–85.
- Koizumi, T., Waalkes, M.P., 1990. Effects of zinc on the binding of cadmium to DNA: assessment with testicular interstitial cell and calf thymus DNAs. *Toxicol. Vitro* 4, 51–55.
- Koppen, G., Verschaeye, L., 1996. The alkaline comet test on plant cells: a new genotoxicity test for DNA strand breaks in *Vicia faba* root cells. *Mutat. Res.* 360, 193–200.
- Leonard, A., 1988. Mechanisms in metal genotoxicity: the significance of *in vitro* approaches. *Mutat. Res.* 198, 321–326.
- Ligocki, M., Tarasewicz, Z., Zygmunt, A., Anisko, M., 2011. The common dandelion (*Taraxacum officinale*) as an indicator of anthropogenic toxic metal pollution of environment. *Acta Sci. Pl. Zootech.* 10, 73–82.
- Loomis, D., Grosse, Y., Laihy-Secretan, B., El Ghissassi, F., Bouvard, V., Brenbahim-Tallaa, L., Guha, N., Baan, R., Matocock, H., Straif, K., 2013. On behalf of the international agency of research cancer monograph working group IARC, Lyon, France. The carcinogenicity of outdoor air pollution. *Lancet Oncol.* 14, 1262–1263.
- Loppi, S., 2014. Lichens as sentinels for air pollution at remote alpine áreas (Italy). *Environ. Sci. Pollut. Res.* 21, 6714–6722.
- Mourón, S.A., Grillo, C.A., Dulout, F.B., Golijow, C.D., 2004. A comparative investigation of DNA strand breaks, sister chromatid exchanges and K-ras gene mutations induced by cadmium salts in cultures human cells. *Mutat. Res.* 568, 221–231.
- Navarette, M.H., Carrera, P., de Miguel, M., de la Torre, C., 1997. A fast comet assay variant for solid tissue cells. The assessment of DNA damage in higher plants. *Mutat. Res.* 398, 271–277.
- Navarro Aviño, J.P., Aguilar Alonso, I., López-Moya, J.R., 2007. Aspectos bioquímicos y genéticos de la tolerancia y acumulación de metales pesados en plantas. *Ecosistemas* 16, 10–25.
- Ord, J., Butler, H.J., McAinsh, M.R., Martin, F.L., 2016. Spectrochemical analysis of sycamore (*Acer pseudoplatanus*) leaves for environmental health monitoring. *Analyst* 141, 2896–2903.
- Owens, E.O., Patel, M.M., Kirrane, E., Long, T.C., Brown, J., Cote, I., Ross, M.A., Dutton, S.J., 2017. Frame work for assessing causality of air pollution related health effects for reviews of the National Ambient Air Quality Standards. *Reg. Toxicol. Pharmacol.* (in press).
- Petrova, S., Yurukova, L., Velcheva, I., 2013. *Taraxacum officinale* as a biomonitor of metals and toxic elements (Plovdiv, Bulgaria). *Bulg. J. Agric. Sci.* 19, 241–247.
- Piraino, F., Aina, R., Palin, L., Prato, N., Sgorbati, S., Santagostino, A., Citterio, S., 2006. Air biomonitoring: assessment of air pollution genotoxicity in the Province of Novara (North Italy) by using *Trifolium repens* L. and molecular markers. *Sci. Total. Environ.* 372, 350–359.
- Poli, P., Buschini, A., Restivo, F.M., Ficarelli, A., Cassoni, F., Ferrero, I., Rossi, C., 1999. Comet assay application in environmental monitoring: DNA damage in human leukocytes and plant cells in comparison with bacterial and yeast tests. *Mutagenesis* 14, 547–556.
- Pourrut, B., Pinelli, E., Celiz, M.V., Silvestre, J., Douay, F., 2015. Recommendations for increasing alkaline comet assay reliability in plants. *Mutagenesis* 30, 37–43.
- Pruski, J.F., 2012. Compositae of Central America-I. The tussilaginoide genus *Robinsonia* (*Senecionaceae*), microcharacters, generic delimitation, and exclusion of senecioid *Senecio cuchumatensis*. *Phytoneuron* 38, 1–8.
- Raj, P.K., 2016. Impacts of particulate matter pollution on plants: implications for environmental biomonitoring. *Ecotoxicol. Environ. Saf.* 129, 120–136.
- Restivo, F.M., Laccone, M.C., Buschini, A., Rossi, C., Poli, P., 2002. Indoor and outdoor genotoxic load detected by the comet assay in leaves of *Nicotiana tabacum* cultivars Bel B and Bel W3. *Mutagenesis* 17, 127–134.
- Rodrigues, G.S., Ma, T.H., Pimentel, D., Weinstein, L.H., 1997. *Tradescantia* bioassays as monitoring systems for environmental mutagenesis: a review. *CRC Crit. Rev. Plant Sci.* 16, 325–359.
- Rojas, E., Herrera, L.A., Poirier, L.A., Ostrosky-Wegman, P., 1999. Are metals dietary carcinogens? *Mutat. Res.* 443, 157–181.
- Ruston, A.C., 1921. The plant as an index of smoke pollution. *Ann. Appl. Biol.* 7, 390–403.
- Rzedowski, G.C., 1997. Compositae. Tribu tageteae. In: Rzedowski, G.C., Rzedowski, J. (Eds.), *Flora del Bajío y de Regiones Adyacentes. Fascículo 113*. Instituto de Ecología-Centro Regional del Bajío. Consejo Nacional de Ciencia y Tecnología y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro, Michoacán, México, 1–166.
- SEMARNAT, 2012. Informe de la Situación del Medio Ambiente en México. Compendio de Estadísticas Ambientales. Indicadores Clave y de Desempeño Ambiental, México, D.F.
- Schultz, S.T., Kruschel, C., Bakran-Petricioli, T., Petricioli, D., 2015. Error, power and blind sentinels: the statistics of seagrass monitoring. *PLoS One* 10, e0138378.
- Silva, S., Silva, P., Oliveira, H., Galvão, I., Matos, M., Pinto-Carnide, O., Santos, C., 2017. Pb low doses induced genotoxicity in *Lettuca sativa* plants. *Plant. Physiol. Biochem.* 112, 109–116.
- Sriussadaporn, C., Yamamoto, K., Fukushima, K., Simazaki, D., 2003. Comparison of DNA damage detected by plant comet assay in roadside and non-roadside environments. *Mutat. Res.* 541, 31–44.
- Stahl Jr, R.G., 1997. Can mammalian and non-mammalian sentinel species data be used to evaluate the human health implications of environmental contaminants? *Hum. Ecol. Risk Assess.* 3, 329–335.
- Tai, H.H., Percy, K.E., Karnosky, D.F., 2010. DNA damage in *Populus tremuloides* clones exposed to elevated O₃. *Environ. Pollut.* 158, 969–976.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. *Mol. Clin. Environ. Toxicol.* 101, 133–164. Series Experimentia Supplementum.
- Tuteja, N., Ahmad, P., Panda, B.B., Tuteja, R., 2009. Genotoxic stress in plants: shedding light on DNA damage repair and DNA repair helicases. *Mutat. Res.* 681, 134–149.
- Valverde, M., Trejo, C., Rojas, E., 2001. Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA-metal interaction? *Mutagenesis* 16, 265–270.
- Ventura, L., Giovannini, A., Savio, M., Doná, M., Macovei, A., Buttafava, A., Carbonera, D., Balestrazzi, A., 2013. Single cell gel electrophoresis (comet) assay with plants: research on DNA repair and ecogenotoxicity testing. *Chemosphere* 92, 1–9.
- Wang, W., 1991. Literature review on plants for toxicity testing. *Water Air Soil Pollut.* 59, 381–400.
- Wang, W.C., Freemark, K., 1995. The use of plants for environmental monitoring and assessment. *Ecotoxicol. Environ. Saf.* 30, 289–301.
- Weinstein, L.H., Laurence, J.A., Mandi, R.H., Walti, K., 1990. Use of native and cultivated plants as bioindicators and biomonitors of pollution damage. In: Wang, W., Gonsuch, J.W., Lower, W.R. (Eds.), *Plants for Toxicity Assessment*, ASTM STP 1091. American Society for Testing and Materials, Philadelphia, pp. 117–126.