Plants genotoxicity as pollution bioindicator in Jordan using comet assay

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Received: 14 September 2017 / Revised: 23 November 2017 / Accepted: 5 December 2017 / Published online: 13 December 2017
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Abstract This study aimed to assess genotoxicity in wild plants grown in Jordan as a pollution bioindicator. Comet assay was used to evaluate the level of DNA damage in plants collected from different areas in Jordan. Significant differences in plant DNA damage index and frequency were observed among sites of collection. Results show that plants collected from Aqaba back road and Ghour Assaal had significantly higher damage values. In contrast, plants collected from Wadi Rum, Al Naqab Heights, Swaimih/Deadsea and Alshoneh Aljanobyeh showed low levels of DNA damage. A similar trend was observed for lipid peroxidation rates. Furthermore, heavy metal analysis showed that plants collected from Aqaba back road and Aqaba airport had the highest Al, Cr, Fe, Cu, Zn, Cd and Pb contents. A significant correlation was observed between DNA Damage Index, DNA Damage Frequency, lipid peroxidation rate, soil Cu, Cd and Pb biomarkers, indicating that heavy metals pollution is a major source for genotoxicity in these plant species. Finally, our results approved the feasibility of using plants and Comet assay system as a diagnostic tool for pollution in any environment adversely affected by different pollution sources.

Keywords Comet assay · DNA damage · Heavy metals · LPO · Pollution · Bioindicators

Introduction

Pollution is one of the serious problems affecting life on Earth. Living organisms are incessantly affected by pollutants, which subsequently alter their behavior and abundance. Anthropogenic activities such as mining, traffic and industry are the major sources of toxic substances (organic pollutants, heavy metals) (Jarup 2003). Heavy metal pollution of soil due to extreme urbanization and industrialization has become a crucial problem in many countries worldwide (Mireles et al. 2012). The accumulation of heavy metals in soil is affected by many environmental factors such as soil parent materials, chemical and physical properties of soil, and by human activities. Recently, it has been shown that heavy metal levels have increased significantly in soil and plants due to traffic pollution. Toxic levels of heavy metal affect different processes in plants. The most important effect is production of high amounts of reactive oxygen species (ROS) that damage pigments, cell membranes and nucleic acids (Beyersmann and Hartwig 2008).

The expansion of the industrial sector in Jordan has exposed many areas to environmental pollution. These pollutants are toxic to living organisms and their effect can be detected at the phenotypic and distributional level. Most of the environmental pollutants have genotoxic effects. Genotoxicity is defined as a destructive effect on living cell's genetic material. Genotoxic compounds have major health and environmental effects over long periods of exposure. These compounds affect a natural population's genetic structure, either directly by damaging the DNA (direct mutagenic effect) or by affecting a population's reproduction potential (population genetic effects) (Klapacz et al. 2016; Theodorakis et al. 2001).

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Using plants in environmental monitoring has many advantages over other systems: plants are inexpensive, sensitive and easy to handle. Different plant systems including *Vicia faba* and *Allium cepa* have been used as bioindicators to assess environmental pollution. Plants are the main food source for various organisms and, as a result, they transfer pollutants to higher trophic levels (Madejón et al. 2013; Notten et al. 2005). Different studies used plants as a tool in ecological risk assessment and solid wastes effect (Kovalchuk and Kovalchuk 2008).

Various biological assays including chromosomes aberration and cytogenetic tests have been used to detect the presence of pollutants worldwide. Recently, molecular markers have been used as a more accurate technique for monitoring DNA damage. The major advantages of measuring the effect of genotoxic compounds using DNA biomarkers are the sensitivity and accuracy of these applications.

Comet assay (also called single cell gel electrophoresis) is considered one of the most important and commonly used bioassays in the risk assessment of industrial chemicals, agrochemicals and pharmaceuticals. Comet assay detects different types of DNA damage including single and double-strand DNA breaks, apurinic/apyrimidinic sites, oxidation and fragmentation of bases and other specific lesions including 8OHdG (DeMarini 2013). It is a simple, sensitive and rapid technique, only requiring a small number of cells (Di Bucchianico et al. 2017; Tice et al. 2000).

In a comparative study of DNA damage in leaves of *Cassia occidentalis* grown on weathered fly ash or soil, higher levels of DNA damage was detected by comet assay in leaf tissues of *Cassia occidentalis* growing on fly ash than *C. occidentalis* grown on soil (Love et al. 2009). Sriussadaporn et al. (2003) conducted a study to compare the level of DNA damage in different plant species grown in roadside versus non-roadside environments using comet assay. They found that roadside grown plants showed higher levels of DNA damage than non-roadside grown plants.

One study also used comet assay to detect DNA damage in various agronomic crops (including alfalfa, lentil, maize, potato, and wheat) caused by the genotoxic agent ethyl methanesulphonate (EMS). Results showed that the increase in EMS concentration (from 2 to 10 mM) resulted in a significant increase in DNA damage level of all studied crops (Gichner et al. 2003). Furthermore, comet assay was used to assess the genotoxicity of organochlorine pesticide, endosulfan to White Clover. It has been shown that endosulfan significantly affect DNA stability of White Clover (Liu et al. 2009).

This study was conducted in various sites in Jordan that differed in pollution intensity. Eight plants species were

used as bioindicators to assess the toxicity status in different regions in Jordan.

Materials and methods

Study area and sampling

Plants were collected from different sites in Jordan (Fig. 1a) during the summer of 2015 and 2016. The majority of samples were collected from point sources of pollution such as major roads, industrial factories, and agricultural fields with heavy fertilization. However, some samples were collected from areas with relatively less pollution (reserves, desert). Aerial parts of widely distributed (*Citrullus colocynthis* (Cucurbitaceae), *Anabasis setifera* (Amaranthaceae), *Prosopis juliflora* (Leguminosae), *Prosopis farcta* (Leguminosae), *Suaeda vermiculata* (Amaranthaceae), *Ziziphus spina-christi* (Rhamnaceae), *Capparis spinosa* (Capparaceae), *Salsola vermiculata* (Amaranthaceae)) plant species were collected. These plant species were selected for this study due to their high abundance in the collection sites.

Plants of similar age and size were selected. Only plants free from visible signs of disease were collected. Twenty plants from each species/site were randomly harvested and stored in polyethylene bags at 4 °C for further analysis. Shoots were thoroughly washed in distilled water to remove irrelevant material before tissue analysis. Few leaves from each sample were used for comet assay, and the remaining tissues were used for Al, Cu, Cr, Fe, Zn, Cd and Pb analysis.

Soil samples were collected from rhizospheres of selected plants species grown in the study area. Samples were stored in sealed polythene bags at room temperature. Fifteen gram of dried soil was sieved (2 mm size sieve) and ground for further chemical analysis.

Comet assay

Five hundred mg of plants leaf samples were rinsed in distilled water twice and then dried with a paper towel. Leaves were placed in a glass petri plate containing 750 µL cold Tris-MgCl₂ (pH = 7.5). Leaves were gently sliced using a fresh razor blade to release nuclei into the buffer under dim orange light.

Slide preparation and the comet assay were done according to the manufacture procedure (Oxisselect comet assay kit, Cell Biolabs, Inc, San Diego, USA). Then, nuclei suspension was centrifuged at 2000g for 90 s, followed by mixing 20 µL of the suspension with 80 µL low melting point agarose (at 40 °C) to obtain final concentration of 1% (v/v). 100 µL of the mixture was immediately spread onto

Fig. 1 Plants and soil sampling sites in Jordan (a). Representative comet assay images of the different DNA damage classes used to calculate DNA damage index (b)

each well on the comet slides. Slides were kept on ice under dark conditions until the agarose and nuclear suspension mixture solidified. Then, slides were transferred to a pre-chilled lysis buffer for 60 min at 4 °C in the dark. Next, the lysis buffer was replaced with pre-chilled alkaline solution and slides were incubated under dark conditions for another 30 min at 4 °C.

For gel electrophoresis, slides were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (300 mM NaOH, 1 mM EDTA,

pH > 13). Subsequently, the electrophoresis was carried out at 1 V/cm with constant 300 mA for 40 min at 4 °C. Following electrophoresis, slides were neutralized by washing three times (5 min each) in the neutralization buffer (400 mM Tris–HCl, pH 7.5).

After drying, slides were stained with 70 μL/well Vista green DNA dye and incubated at room temperature for 10 min before viewing under the fluorescent microscope (EVOS[®]) on 100× magnification with an excitation filter of 470 nm and a barrier filter of 525 nm. A total of 200

randomly chosen cells were scored per individual plant. DNA damage index was calculated by grouping cells into five classes according to tail length (0: no tail, 25%: short tail, 50%: medium tail, 75%: long tail and 100% very long tail length) (Fig. 1b), then the average of all cells for each plant species from each site was calculated. Damage frequency (%) was calculated by dividing the number of cells that grouped as 25, 50, 75 or 100% over the total number of cells examined for each plant species from each site. The mean DNA damage index or frequency for each site was calculated by taking the average of the DNA damage index or frequency of all tested plant species from that site.

Lipid peroxidation assay

Lipid peroxidation was assayed as malondialdehyde content in leaves of different plant species according to Zhao et al. (2006). One gram of fresh leaves tissues was ground in 10 mL of 0.1% thiobarbituric acid (TCA) and centrifuged at 10,000g for 15 min. Then, 1 mL of the supernatant was added to 4 mL of 20% TCA solution and heated for 25 min at 97 °C using heat block followed by cooling on ice bath. The solution was centrifuged for 10 min at 12,000g. Finally, the absorbance was measured at 532 and 600 nm using UV–Vis Spectrophotometer (GENESYS 10S). MDA content was calculated using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Heavy metals analysis

All the collected plant species were washed with distilled water to clear them of detritus, and dried at 104 °C for 24 h. The ash content was determined by heating the sample at 520 °C for 5 h. Produced ash was digested in 10 mL of nitric acid followed by evaporation on hot plate. 2 mL of distilled water was added to the digested residue, filtered and then the overall volume was made to 20 mL with ultra pure water. For soil samples, 1 g from each collected soil sample was digested with 8 mL of 50% hydrochloric acid on a hot plate. After digestion, 1 g of boric acid was added to neutralize the sample. Then, samples were filtered and diluted to 50 mL using deionized distilled water. Heavy metals including iron (Fe), lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), nickel (Ni) and chromium (Cr) were analyzed using atomic absorption spectrophotometer. The instrument setting and operational conditions were done according to the manufacturer's recommendations.

Statistical analysis

Four slides were evaluated per plant species per site, and the whole experiment was repeated two times. Data

presented are mean $\pm$ SE. Data was analyzed using SPSS 16.0. One-way analysis of variance (ANOVA) was employed to examine any difference between species and sites. Means were separated using Tukey's test at $P \leq 0.05$.

Results

Comet assay

Comet assay results are expressed as damage index and damage frequency for different plant species collected from eight sites in Jordan (Fig. 2a, b). No significant differences in damage index and damage frequency were observed between plant species in each site. In contrast, a significant difference in damage index and damage frequency were observed between plant species collected from various sites ($P < 0.05$). Similar plant species showed different DNA damage index and frequency values when collected from different sites. Aqaba back road and Ghour Assaal sites had significantly higher damage values than other sites.

The highest DNA damage index was observed in plants collected from Ghour Assaal and Aqaba back road sites (ranging from 69 to 77 and 60 to 69, respectively). Similarly, these plants showed the highest DNA damage frequency (ranging from 70 to 86% and 73 to 93%, respectively). Plants collected from Aqaba airport showed intermediate DNA damage index and frequency values (55–63 and 46–69%, respectively). Finally, plants collected from Wadi Rum, Al Mujib dam/Deadsea, Swaimeh/Deadsea, Al Naqab Heights and Alshoneh Aljanobyeh sites showed low levels of DNA damage as expressed in DNA damage index and frequency, with the minimum in plants collected from Al Naqab Heights (Fig. 2).

Lipid peroxidation

Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation inside the cell and used widely as indicator of free radicals production and tissue damage. An increase in lipid peroxidation measured by malondialdehyde content was observed in plants collected from Ghour Assaal and Aqaba back road sites (ranging from 3.62 to 4.49 and 3.52 to $3.97 \mu\text{mol g FW}^{-1}$, respectively) ($P < 0.01$). Also, plants collected from Aqaba airport area showed relatively high lipid peroxidation levels (ranging from 2.57 to $3.43 \mu\text{mol g FW}^{-1}$). On the other hand, plants collected from Wadi Rum, Al Mujib dam/Deadsea, Swaimeh/Deadsea and Alshoneh Aljanobyeh showed moderate lipid peroxidation levels. In contrast, plants collected from Al

Fig. 2 DNA damage index (a) and damage frequency (b) of different plant species collected from eight sites in Jordan. Error bars represent standard error (n = 4). Means with different letters are considered significantly different according to Tukey's test at $P \leq 0.05$

Naqab Heights showed minimal lipid peroxidation levels ($0.27\text{--}0.34 \mu\text{mol g FW}^{-1}$) (Fig. 3).

Heavy metals analysis

Tables 1 and 2 demonstrate the heavy metal levels in the plant and soil samples collected from different areas in Jordan during 2015–2016. Analysis of variance showed a significant difference in all tested heavy metal levels among sampling sites ($P < 0.05$). Plants collected from Wadi rum, Al Mujib Dam, Swaimeh, Al Naqab heights and

Alshoneh Aljanobyeh showed low levels of Al. In contrast, plants collected from Aqaba back road and Aqaba airport showed the highest Al levels. The maximum Al content was observed in *Anabasis setifera* collected from Aqaba back road (226 mg/g) (Table 1).

The maximum level of chromium (5.4 mg/g) was observed in *Citrullus colocynthis* collected from Aqaba back road area and this area showed the highest level of chromium among the tested areas. For iron, plants collected from Aqaba back road and Aqaba airport showed the highest levels compared to plants collected from Swaimeh,

Fig. 3 Lipid peroxidation rate expressed as MDA content of different plant species collected from eight sites in Jordan. Error bars represent standard error (n = 4). Means with different letters are considered significantly different according to Tukey's test at $P \leq 0.05$

Al Naqab heights and Alshoneh Aljanobyeh which showed low levels (Table 1). The highest Cu value as an average of the collected plant species from one area was observed at Aqaba back road area (16.43 mg/g). All other studied sites showed relatively less Cu content than 50% of plants collected from Aqaba back road area. Similarly, plants collected from Aqaba back road showed the highest Zn levels (with an average of 139.93 mg/g) (Table 1).

As shown in Table 1, the average Cd content was the highest in plants collected from Aqaba back road and Ghour Assaal areas (1.40 and 1.31 mg/g, respectively). In contrast, plants grown in Al Naqab Heights and Wadi Rum showed the lowest Cd levels (0.20 and 0.26 mg/g respectively). Plants collected from Aqaba back road, Ghour Assaal and Aqaba Airport areas showed high levels of lead. The highest Pb level (1.88 mg/g) was observed in *Salsola vermiculata* plants collected from Ghour Assaal area. In contrast, the lowest Pb level (0.21 mg/g) was observed in *Capparis spinosa* plants collected from Wadi Rum area (Table 1).

The soil samples collected from Aqaba back road and Alshoneh Aljanobyeh showed Cr content (0.11 and 0.08 μg/g, respectively) (Table 2). All other sites show Cr content lower than 0.05 μg/g. The highest Cu level (1.13 μg/g) was observed in soil samples collected from Ghour Assaal area followed by Aqaba back road (0.92 μg/g). In contrast, soil samples collected from Wadi Rum showed very low levels of Cu (0.2 μg/g). Soil samples collected from Aqaba back road showed the highest Zn level (9.2 μg/g), while Zn levels in Wadi Rum sample was the lowest (0.26 μg/g). For Cd, soil samples collected from

Aqaba back road and Aqaba Airport areas showed high levels (0.48 and 0.45 μg/g, respectively). Finally, the highest Pb level was observed in soil sample collected from Ghour Assaal area (7.8 μg/g) followed by samples collected from Aqaba back road, Aqaba airport, Alshoneh Aljanobyeh and Swaimeh (4.87, 3.73, 3.42 and 3.01 μg/g, respectively) (Table 2).

Discussion

The present study found that high levels of DNA damage were present in all plant species collected from Aqaba back road and Ghour Assaal, followed by Aqaba airport area. The lowest DNA damage levels were observed in plant species collected from Al Naqab heights. Plants are sessile organisms, exposed to several environmental factors and endogenous processes that cause DNA damage and genotoxic stress. Stress conditions could be due to biotic (such as those caused by animals, insects and pathogen) or abiotic factors (temperature, water, salt, light or pollution). Damaging plant DNA may result in different physiological consequences including reduction in protein synthesis, alteration in photosynthesis and cell membrane damage, all of which can negatively affect plant growth and development (Britt 1999). Samples were collected from Aqaba back road, Aqaba airport, Ghour Assaal and Alshoneh Aljanobyeh which are located next to main roads and/or industrial activities. In contrast, Wadi Rum, Al Mujib Dam, Swaimeh and Al Naqab Heights areas are distant from heavy traffic and industrial areas. It has been shown that

Table 1 Heavy metals content (mg/g dry weight) in plants species collected from 8 different sites in Jordan

Sampling Site	Species	Al	Cr	Fe	Cu	Zn	Cd	Pb
Aqaba/Back Road	<i>Citrullus colocynthis</i>	194	5.40	435	14.44	76	1.47	1.28
	<i>Anabasis setifera</i>	226	4.27	282	17.09	131	1.25	1.64
	<i>Suaeda vermiculata</i>	114	4.53	279	18.00	197	1.49	1.85
	<i>Capparis spinosa</i>	142	3.95	308	16.20	155	1.38	1.57
	AVG	168.99	4.54	325.81	16.43	139.93	1.40	1.58
Ghour Assaal	<i>Citrullus colocynthis</i>	135	1.95	293	9.24	96	1.28	1.58
	<i>Salsola vermiculata</i>	128	1.60	259	7.75	73	1.50	1.88
	<i>Anabasis setifera</i>	118	2.07	313	8.73	64	1.08	1.71
	<i>Suaeda vermiculata</i>	100	1.80	233	9.63	89	1.40	1.23
	AVG	120	1.86	274	8.84	81	1.31	1.60
Wadi Rum	<i>Anabasis setifera</i>	57	0.49	145	6.31	67	0.21	0.67
	<i>Citrullus colocynthis</i>	44	0.87	94	6.09	59	0.24	0.55
	<i>Capparis spinosa</i>	31	0.61	88	5.14	68	0.33	0.21
	AVG	44.11	0.66	109.03	5.85	64.91	0.26	0.47
Aqaba Airport	<i>Citrullus colocynthis</i>	209	1.95	597	9.75	89	0.91	1.60
	<i>Anabasis setifera</i>	176	1.16	261	10.48	66	0.79	1.27
	<i>Suaeda vermiculata</i>	169	1.67	232	8.10	166	0.81	0.95
	AVG	184.76	1.59	363.17	9.45	107.15	0.84	1.27
AlMujib Dam Deadsea	<i>Salsola vermiculata</i>	22	0.44	46	4.60	39	0.62	0.68
	<i>Capparis spinosa</i>	25	0.90	47	3.79	44	0.66	0.85
	<i>Anabasis setifera</i>	55	1.13	56	8.46	33	0.68	0.93
	AVG	34.04	0.82	49.60	5.62	38.55	0.65	0.82
Swaimeh/DeadSea	<i>Suaeda vermiculata</i>	70	1.50	79	3.02	46	0.35	0.68
	<i>Capparis spinosa</i>	62	1.08	58	2.67	53	0.31	0.53
	<i>Ziziphus spina-christi</i>	54	1.27	48	3.87	42	0.27	0.59
	AVG	61.95	1.28	61.63	3.19	47.00	0.31	0.60
Al Naqab Heights	<i>Capparis spinosa</i>	38	0.47	24	2.43	44	0.21	0.64
	<i>Anabasis setifera</i>	26	0.29	34	1.87	31	0.19	0.44
	AVG	32.00	0.38	29.00	2.15	37.50	0.20	0.54
Alshoneh AlJanobyeh	<i>Ziziphus spina-christi</i>	34	3.39	64	7.66	53	0.44	0.70
	<i>Prosopis juliflora</i>	31	2.1	51	6.21	47	0.57	0.52
	<i>Prosopis farcta</i>	41	1.47	59	4.85	38	0.51	0.66
	AVG	35.20	2.32	58.16	6.24	45.96	0.51	0.63
Tukey's ($P \leq 0.05$)		12.18	0.83	15.47	1.62	8.28	0.18	0.26

Table 2 Heavy metals content ($\mu\text{g/g}$) in soil samples collected from 8 different sites in Jordan

	Cr	Cu	Zn	Cd	Pb
$\mu\text{g/g}$					
AlMujib Dam Deadsea	< 0.05	0.73	1.15	0.21	1.57
Ghour Assaal	< 0.05	1.13	6.28	0.36	7.80
Aqaba Airport	< 0.05	0.84	3.46	0.45	3.73
Wadi Rum	< 0.05	0.20	0.26	0.13	0.88
Al Naqab Heights	< 0.05	0.43	0.88	0.11	1.06
Swaimeh/DeadSea	< 0.05	0.77	3.41	0.20	3.01
Aqaba/Back Road	0.11	0.92	9.20	0.48	4.87
Alshoneh AlJanobyeh	0.08	0.74	3.57	0.29	3.42
Tukey's ($P \leq 0.05$)	0.02	0.11	0.37	0.05	0.41

Table 3 Pearson correlation coefficient (r) of Damage Index, Damage Frequency, LPO, soil Cr, Cu, Zn, Cd and Pb

	Damage freq	LPO	Soil Cr	Soil Cu	Soil Zn	Soil Cd	Soil Pb
Damage index	0.92**	0.90**	0.22	0.87*	0.74*	0.87**	0.84*
Damage freq		0.93**	0.35	0.80*	0.85*	0.86**	0.86*
LPO			0.33	0.71	0.84*	0.87*	0.81*
Soil Cr				0.26	0.57	0.52	0.26
Soil Cu					0.48	0.36	0.58
Soil Zn						0.43	0.31
Soil Cd							0.33

**, *Significant at 0.01 level $P < 0.01$ and at 0.05 level $P < 0.05$, respectively

high levels of heavy metals were observed in soil and plant samples collected from main roads in different countries (Xiong et al. 2017; Chen et al. 2010; Yassoglou et al. 1987).

Although high levels of DNA damage were observed in some plant species grown on areas like Aqaba back road, Ghour Assaal and Aqaba airport area, they were similar (phenotypically) to plants with low levels of DNA damage grown on other sites. This indicates that these plants may have the potential to repair damaged DNA easily and quickly without a significant effect on plant morphology. Plants have different DNA repair mechanisms following DNA damage (Al Khateeb and Schroeder 2009; Britt 2002). DNA repair pathways protect organisms from genetic damage through removal of damaged DNA fragments or by helping organisms to tolerate the damage (Singh et al. 2010). Different DNA repair mechanisms have been characterized in microorganisms, mammalian and plant model organisms. In general, plants have been shown to possess most of the DNA repair pathways originally discovered in other eukaryotes (including humans) (Britt 2002). Different DNA repair mechanisms are involved in repair and tolerance of the damage. Photoreactivation is one of the major DNA repair pathways most commonly used by plants. Excision repair pathway, which includes base excision repair (BER) and nucleotide excision repair (NER) are also used by plants to ensure genome stability. Mismatch repair is a specialized repair mechanism for the removal of incorrectly paired nucleotides. Other DNA repair mechanisms are also involved in DNA double-strand break repair such as Homologous recombination (HR) and Non-homologous end joining (NHEJ). It has been shown previously that these repair mechanisms are crucial in plants for the maintenance of genetic stability (Manova and Gruszka 2015).

The increase in plants DNA damage level can be associated with the high contaminants found in the studied areas. It has been shown that heavy metals have detrimental effects on all organisms and the surrounding environment (Jomova and Valko 2011). Inorganic elements may directly harm plants through their effect on the plants

physiological and biochemical processes. These effects may subsequently reduce the plant's growth. Inorganic elements may also indirectly harm plant's DNA by interfering with cellular redox regulation mechanisms or by generating ROS that induce oxidative stress (Beyersmann and Hartwig 2008; Jomova and Valko 2011). In this study, high levels of Cr, Cu, Zn, Cd and Pb were observed in Aqaba back road, Ghour Assaal and Aqaba airport areas. It has been shown previously by many studies that growing plants on soil contaminated with heavy metals resulted in DNA damage (Siddiqui 2015; Gichner et al. 2005).

A recent study compared the level of DNA damage caused by cadmium chloride and copper chloride using comet assay in *Pisum sativum* roots and leaves. The study demonstrated that root cells are more sensitive than leaves and that leaf cells showed the DNA damage phenotype only under higher heavy metals treatments (Hattab et al. 2009). Our study used leaf samples for comet assay and results showed great variation among sites even though leaves are less sensitive than roots as indicated in the previous study (Hattab et al. 2009). This indicates that those sites showing high levels of DNA damage were indeed polluted with genotoxic agents. Also, organic pollutants such as Polycyclic aromatic hydrocarbons (which are usually found in high amounts around highways) affect genome stability. These Polycyclic aromatic hydrocarbons bind covalently to DNA and form adducts (DNA binding products) or may result in oxidative stress to cells that induce mutations (Castorena-Torres et al. 2008; Pratt et al. 2011). A significant positive correlation (Table 3) was observed between DNA Damage Index, DNA Damage Frequency, lipid peroxidation rate, soil Cu, Zn, Cd and Pb content. This indicates that these soil heavy metals could be the source of pollution in the study area.

In conclusion, the data obtained in this study reveals the feasibility of using Comet assay as a diagnostic tool for genotoxicity induced by environmental genotoxic factors in wild grown plants. These findings could be used as a quick and accurate preliminary quantitative assessment of pollution level in areas of potential pollution risk problems worldwide.

Acknowledgements Thank you to Janelle Lazaro for providing helpful comments that improved this manuscript. This study was funded by the Deanship of Scientific Research, Yarmouk University, Jordan (Project # 8/2017).

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