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MOLECULAR RAPID ASSAY ON PLANT CELLS: A TEST FOR DNA DAMAGE CAUSED BY POLLUTION FROM AL-DAURA THERMAL POWER STATION ON *CITRUS AURANTIUM* L. PLANTS

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ABSTRACT

Genotoxicity testing is generally used for hazard recognition with relevance to DNA damage that can be caused by many chemicals or pollutants. In this study, alkaline version of comet assay was used to investigate the DNA damage in the Iraqi sour orange (*Citrus aurantium* L.) leave and root cells responding to the pollutants from Al-Daura Thermal Power Station in Baghdad-Iraq such as Sulfur Dioxide (SO_2), Oxides of Nitrogen (NOx), Hydrogen Sulfide (H_2S), Carbon Monoxide (CO), Ozone (O_3), Heavy metals like Nickel (Ni), Cadmium (Cd) and Copper (Cu) and other pollutants. Three selected sites with gradual distances from the station were chosen for sampling according to downwind for site. In addition, one of unpolluted site upwind from the station was chosen for comparison as control site. The samples were collected for two seasons; season one was in December 2014 and season two was in March 2015. The comet parameters (tail length, % DNA in the tail and tail moment) of *Citrus aurantium* L. cells in all sites are significantly higher when compared with those of control site. The plants in site 2 were the most plants affected by pollutants according to Comet assay parameters. Comparison between the results of Comet parameter of *Citrus aurantium* cells for the two seasons show that there was slight significant increase in tail moment (mean ± SE) in season 2 other than that of season 1. Concluded that the high DNA damage in *Citrus aurantium* L. leave and root cells detected in this study is of great concern and highlighted the increase effect of pollutants resulted from Al-Daura thermal power station in Baghdad / Iraq.

KEYWORDS: Genotoxicity, Comet assay, Thermal power station, Pollution, Heavy metals, *Citrus aurantium* L.

INTRODUCTION

Genotoxic and mutagenic products causes an environmental pollution affecting the exposed organism animals, and in this case man is included, plants and microorganisms.[1] There are many of pollution sources that release such pollutants to the surrounding environment such as industrial activities, vehicles, oil refineries and thermal power stations.[2,3] Thermal Power stations are the main source of the generation of electricity for any developing country and are the most popular power station existing because of its high production and it is dependence on fossil fuel or their derivatives as a major fuel. So, it is classified as a high pollution project.[4] The pollutants that are produce from thermal power stations can be divided to gaseous pollutants, fluid pollutants as well as heat pollution. The gaseous emissions from combustion installations within thermal power stations which they are use large amounts of fossil fuels or other raw material, leads to the generation of emissions to air, soil and water which are considered to be one of the main environment issues. The main important emissions to air of the combustion of fossil fuels to air are NO_x, SO_2, Particulate Matter (PM), unburned hydrocarbons and other Volatile Organic Compounds (VOCs), heavy metals and greenhouse gases such as CO_2.[5,6]

Since this wide range of genotoxic substances is present into the environment, it could influence human health, animal and plant, making the monitoring process even more problematic. Therefore, the analysis of pollution by using biosensors (living organisms which are able to assess the quality of air, soil or water) is definitely essential. A number of verious biosensors were successfully used in the past including bacteria, animal and plants.[7] Plants are used as biomonitor of pollution because they are eukaryotes and like animals, are able to process complex pollutant molecules, resistant to environmental stresses, easy to grow, do not contaminate easily, and in many cases more sensitive than other available systems making them perfect for risk assessment of probable environmental mutagens, allow assays of a range of environmental conditions and for outdoor monitoring. The available data show that plant bioassays are influential tests for detection of genotoxic contamination in the environment and the establishment of controlling systems.[8]

Many genotoxicity assays have been developed to identify DNA damage. The Comet assay or the single cell gel electrophoresis (SCGE) assay is a gel electrophoresis-based method that

can be used to measure DNA damage in individual eukaryotic cells.[9] For its sensitivity and simplicity and as it is the only technique that directly measures DNA damage in individual cells make it an invaluable tool and has gained rapidly importance for clinical applications, human bio-monitoring, genotoxicity, genetic ecotoxicology and carcinogenesis.[10,11]

MATERIALS AND METHODS

Collection of Samples

The plant that selected to be tested for genotoxicity within this study was Iraqi Sour orange (*Citrus aurantium* L.) tree. The chosen plant under study is an exemplary and generic tree in Iraq. It grows in everywhere fields, even with the high rate of pollution in the weather. The plant was found within all three suspected polluted sites and for control site with large number and can be easily specified. Roots and leaves are used to measuring the effects of heavy metals pollutants at these sites.

Three selected locations with gradual distances from Al-Daura thermal power station within (0.5 Km, 1 Km, and 2 Km, respectively) were chosen for sampling according to the downwind for site, which are confirmed as polluted locations by Iraqi Ministry of Environment.[12,13] In addition, one of unpolluted site was chosen for comparison as a control site in Baghdad around 4 Km upwind from Al-Daura thermal power station. All samples were collected for two seasons; season one was in December 2014 and season two was in March 2015. 48 samples (24 roots, 24 leaves) as 2 roots with 2 leaves were collected from each one of *Citrus aurantium* L. trees for each season.

Comet Assay Method

Preparations of solutions and stains for the comet assay were prepared according to Dhawan et al. (2003).

Nuclei Preparation and Treatment for the Comet Assay

Nuclei isolation was conducted according to Gichner (2003) and Gichner et al. (2004). An individual leaf or root of *Citrus aurantium* was excised and placed on ice in 60 mm petri dish containing 300 µl of cold 400 mM Tris-HCl buffer, pH 7.5. Using a razor blade, the leave or root was gently sliced into a "fringe" to release nuclei into the buffer. The Petri dish is kept tilted in the ice so that the isolated nuclei will collected in the buffer.

Preparation of Microscope Slides for Comet Assay

Microscope slides for comet assay were prepared according to Gichner (2003) and Gichner et al. (2004). Previously cleaned slides were dipped into melted 1% normal melting agarose (NMA) and left to be solidified, a second layer of 100 µl mixture of 1% of low melting agarose (LMA) and freshly prepared nuclei suspension (a ratio of 1:1) was placed on previously coated slides with NMA, covered with cover slip, placed on an ice until solidified, then the cover slip was removed and the slides dipped again but with 1% of low melting agarose to get a third layer, placed on an ice until solidified and remove the cover slip to be ready for the DNA unwinding step.

Unwinding of DNA

For toxicity study, the Alkaline version of the Comet technique with DNA unwinding and electrophoresis at pH>13 was done. So, the slides were sat in the alkaline buffer for 15 minutes before electrophoresis to allow for unwinding of the DNA.

Electrophoresis Optimization for the Comet Assay for *Citrus aurantium* L. Plants

The electrophoresis conditions used in this study were optimized as they proved to provide minimum level of DNA damage in control cells and linear response for the induction of comets after pollutants exposure from thermal power station. Optimization of the Comet assay indicates to get the most suitable time for DNA unwinding and electrophoresis. It needs to ensure the least DNA migration in control plant samples, and at the same time extreme sensitivity of treated suspected polluted samples. All operations were conducted under dark field. This method of isolating the nuclei was to provide low values of DNA damage in control cells. Electrophoresis was done on 24 V, 250 milliamperes for 30 min at 4 °C and adjusts the current to 250 mA by raising or lowering the buffer level.

DNA Staining

Ethidium bromide as a flourochromes was used in the comet assay for DNA staining.

Examination of Comet Cells

The step followed the staining is the examination of comet cells under fluorescence microscope using a software that linked to a digital camera mounted on the microscope, automatically analyses individual Comet images.

The mean values for the parameters of DNA damage extent are the % of tail DNA, tail length and tail moment (TM) were obtained which were used in the analysis of the results.

Statistical analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors (Site and Season) in study parameters. Least significant difference-LSD test was used to significant compare between means in this study.

RESULTS AND DISCUSSION

The samples were taken for two seasons; Season one was in December 2014 with mean air temperature 13.9 °C, total monthly rainfall 3.9 mm, relative humidity 65 %, mean wind speed 2.4 m/s and prevailing wind North West. Whereas season two was in March 2015 with mean air temperature 17.9 °C, total monthly rainfall 26.1 mm, relative humidity 43 %, mean wind speed 3.0 m/s and prevailing wind North.

Genetically Assessment of DNA Damage Using the Comet Assay

The comet assay is selected as a toxicological monitor to evaluate the genotoxic effects of Al-Daura thermal power station pollution on leaves and roots of *Citrus aurantium* L. trees. The association between DNA damage and pollutants could be evaluated through comparison of Comet parameters (tail length, % DNA in the tail and tail moment), among trees in all study sites and control site for two seasons.

Statistical differences in all three parameters of comet assay are observed among the three sites and control site in the two seasons:

Tail Length (Px)

As Tail length refers to the distance from the head to the end of the tail[18], the results of the tail length parameter are listed in the table 1 for the leaves and table 2 for the roots of *Citrus aurantium* L. cells.

The results showed that there were no significant differences in the tail length of leave and root cells between the two seasons, whereas there were significant differences among all sites within the season. The mean of tail length (Mean ± SE) of site 2 (30.535 ± 0.49 and 27.655 ± 0.40 for leaves and roots, respectively) was higher than those of all other sites. There was significant difference for interaction value of sites and seasons and it was higher in site 2 of season 2, whereas the lowest value for leaves and roots for control site was in season 1.

Table 1: Effect of site and season in the tail length (px) (mean ± SE) of *Citrus aurantium* cell of leaves

Site	Season		Mean ± SE
	1	2	
Control	5.42 ± 0.31	5.63 ± 0.33	5.525 ± 0.21 D
1 (0.5 Km distance)	24.51 ± 0.45	24.98 ± 0.63	24.745 ± 0.36 B
2 (1 Km distance)	30.30 ± 0.75	30.77 ± 0.79	30.535 ± 0.49 A
3 (2 Km distance)	8.44 ± 0.35	8.47 ± 0.25	8.455 ± 0.23 C
Mean ± SE	17.16 ± 3.17 A	17.46 ± 3.21 A	---
LSD value of Site: 1.127 * , Season: 0.79 NS , Interaction value for Site × Season: 1.682 * * (P<0.05), NS: Non-significant			

Table 2: Effect of site and season in the tail length (px) (mean ± SE) of *Citrus aurantium* cell of roots

Site	Season		Mean
	1	2	
Control	4.98 ± 0.10	5.38 ± 0.13	5.18 ± 0.12 D
1 (0.5 Km distance)	22.3 ± 0.48	22.82 ± 0.52	22.56 ± 0.34 B
2 (1 Km distance)	27.52 ± 0.51	27.79 ± 0.65	27.655 ± 0.40 A
3 (2 Km distance)	7.35 ± 0.31	7.47 ± 0.24	7.41 ± 0.21 C
Mean ± SE	15.53 ± 2.89 A	15.86 ± 16.0 A	----
LSD value of Site: 0.871 *, Season: 0.616 NS, Interaction value for Site × Season: 1.323 * * (P<0.05), NS: Non-significant			

The Percentage of DNA in the Tail

The percentage of tail DNA also called percentage of migrated DNA,[18] which is based on the fluorescence intensity is illustrated in tables 3 and 4 for leave and root cells of *Citrus aurantium* L.

The present study found that there was no significant difference in the % DNA in the tail for the cells of *Citrus aurantium* leaves and roots between the two seasons. Also, there were significant differences in the % DNA in the tail among all sites, where site 2 recoded the higher value (they were 14.49 ± 0.40 and 13.11± 0.15 for leaves and roots, respectively) between all sites.

The higher interaction value for leaves and roots with significant difference was found in season 2 at site 2, whereas the lowest value found in season 1 at control site.

Table 3: Effect of site and season in % DNA in the tail (mean ± SE) of *Citrus aurantium* cell of leaves

Site	Season		Mean ± SE
	1	2	
Control	3.17 ± 0.04	3.29 ± 0.07	3.23 ± 0.06 D
1 (0.5 Km distance)	10.57 ± 0.58	10.97 ± 0.62	10.77 ± 0.17 B
2 (1 Km distance)	14.36 ± 0.65	14.62 ± 0.60	14.49 ± 0.40 A
3 (2 Km distance)	5.26 ± 0.08	5.35 ± 0.06	5.305 ± 0.08 C
Mean ± SE	8.34 ± 1.33 A	8.55 ± 1.34 A	----
LSD value of Site: 0.722 * , Season: 0.511 NS , Interaction value for Site × Season: 1.101 * * (P<0.05), NS: Non-significant			

Table 4: Effect of site and season in % DNA in the tail (mean ± SE) of *Citrus aurantium* roots cell

Site	Season		Mean ± SE
	1	2	
Control	3.09 ± 0.09	3.23 ± 0.06	3.16 ± 0.06 D
1 (0.5 Km distance)	10.38 ± 0.27	10.82 ± 0.16	10.6 ± 0.17 B
2 (1 Km distance)	12.99 ± 0.19	13.23 ± 0.21	13.11 ± 0.15 A
3 (2 Km distance)	5.12 ± 0.07	5.17 ± 0.14	5.145 ± 0.06 C
Mean ± SE	7.895 ± 1.19 A	8.112 ± 1.24 A	---
LSD value of Site: 0.331 * , Season: 0.234 * , Interaction value for Site × Season: 0.501 * * (P<0.05), NS: Non-significant			

Tail Moment

The parameter of tail moment refer to appropriate index of induced DNA damage in considering both the migration of genetic material as well as the relative amount of DNA in the tail.[19]

The results in tables 5 and 6 listed the values of tail moment of leaves and roots cells of *Citrus aurantium* and show that there were increases with significant differences in the values of tail moment of leaves and roots in season 2 more than that in season 1. In the same time, there were significant differences in the values of tail moment among all sites. Where, the values of tail moment for leaves and roots cells in site 2 was much higher than that of all other sites.

The higher interaction values with significant differences were for leaves and roots in season 2 at site 2, while the lowest interaction values were in season 1 at control sites.

Table 5: Effect of site and season in the tail moment (mean ± SE) of *Citrus aurantium* leave cells.

Site	Season		Mean ± SE
	1	2	
Control	0.197 ± 0.01	0.214 ± 0.02	0.205 ± 0.01 C
1 (0.5 Km distance)	2.65 ± 0.09	2.80 ± 0.12	2.725 ± 0.17 B
2 (1 Km distance)	4.43 ± 0.15	4.60 ± 0.26	4.515 ± 0.21 A
3 (2 Km distance)	0.474 ± 0.01	0.490 ± 0.05	0.482 ± 0.03 C
Mean ± SE	1.937 ± 0.51 B	2.026 ± 0.56 A	---
LSD value of Site: 0.350 *, Season: 0.247 *, Interaction value for Site × Season: 0.535 * * (P<0.05), NS: Non-significant			

Table6: Effect of site and season in the tail moment (mean ± SE) of *Citrus aurantium* root cells.

Site	Season		Mean ± SE
	1	2	
Control	0.179 ± 0.009	0.207 ± 0.02	0.193 ± 0.01 D
1 (0.5 Km distance)	2.36 ± 0.11	2.53 ± 0.09	2.445 ± 0.19 B
2 (1 Km distance)	3.64 ± 0.07	3.79 ± 0.13	3.715 ± 0.07 A
3 (2 Km distance)	0.407 ± 0.01	0.424 ± 0.02	0.415 ± 0.027 C
Mean ± SE	1.646 ± 0.44 B	1.737 ± 0.49 A	---
LSD value of Site: 0.148 *, Season: 0.104 * , Interaction value for Site × Season: 0.226 * * (P<0.05), NS: Non-significant			

According to the results obtained here, all the comet parameters of *Citrus aurantium* L. cells in all sites are significantly higher when compared with those in control site. The increase of the DNA damage in these sites resulted from the growing of these plants in polluted atmosphere. Thus, the plants in site 2 were the most plants affected by pollutants than others according to Comet assay parameters, which is explained what had been stated by Akoteyon, (2012) that the release of pollutants into the environment is not limited to areas adjacent to the point sources, such as industrial facilities.

Comparison between the results of Comet parameters of *Citrus aurantium* cells for the two seasons show that there was slight significant increase in tail moment in season 2 (2.026 ± 0.56 and 1.737 ± 0.49 for leaves and roots, respectively) other than that of season 1 (1.937 ± 0.51 and 1.646 ± 0.44 for leaves and roots, respectively) which was due to the increase of heavy metals in plant tissues of season 2. The amounts of rainfall throughout seasons and the increase of ambient temperature influences have important effects on the enhancement of citrus trees response to uptake of metals. In subtropical climates, citrus plants appear higher

stem water potential when it is warmer weather with more rain; in contrast, a reduction in stem water potential is found when it is colder weather with less rain, in which plants may get fewer levels of some trace elements from the soil solution.[21]

The results in this study are agree with several studies which have indicated that the plants growing on contaminated soils have higher concentrations of heavy metals than those grown on unpolluted soil and the chronic exposure to heavy metals leads to chronic toxicity, causing an accumulation of the toxic metals in the plant tissues and induced genotoxicity by the interaction of these metals with DNA, either directly or indirectly through the induction of oxidative stress.[15,22,23]

Tom´aˇs et al. (2008) applied the alkaline protocol of SCGE for detecting the extent of induced DNA damage in the roots of potato plants (*Solanum tuberosum* L.) that were treated for 1 and 2 weeks with 2.5 to 12.5 µM $Cd^{2+,}$ a slight but significant ($P < 0.05$) increase in the % tail DNA was observed at 7.5 µM Cd^{2+} and above. Similarly, 2 week continuous treatment with concentrations from 5 to 12.5 µM Cd^{2+} resulted in an increase of % tail DNA from 18.0±2.1% to 49.4±3.5% , compared to the control 7.3±0.6% . In all cases, the increase of % DNA in the tail was associated with strongly distorted and yellowish leaves and with an inhibition of root growth.

Pourrut et al. (2011) mentioned that treatment of the whole plant roots of *Vicia faba* L. that was grown hydroponically under controlled conditions with Pb^{2+} induced significant increase in DNA damage correlated with increasing concentrations of lead (1–20 µM). The % DNA in tail significantly increased ($P < 0.01$) with dose-dependent manner to 64 ± 2.1% for lead treatments when compared with negative control (23± 1.2%).

Many studies used plant species as a tool of environmental pollution monitor in situ and a source of information for the genotoxic impact of dangerous agents ,where Sriussadaporn et al. (2003) used *Ginkgo biloba* L.; *Epipremn umaureum* and *Vinca rosea* plant species (all were roadside plants that exposed to CO, SO2, O3 and other pollutants), which were all tolerant to environmental stresses. As a result, they noted high levels of DNA damage in leaves, indicating that these plants were challenged with over stress, above the resistance limit.

Whereas, the results in this study disagree with Chakraborty and Mukherjee (2011) when they applied the comet assay protocol on Vetiver plant (*Vetiveria zizanioide* L.) that exposed to fly ash and other pollutants of thermal power station and founded no DNA damage observed by Comet parameters in the root nuclei and only limited amounts of the heavy metals were pass from roots to above ground parts demonstrating long-term survival ability in the plant on the polluted soil. Contrariwise, it meet other results in the same study when another plant such as Allium cepa show a highly sensitive to fly ash and highly levels of DNA damage were revealed by using SCGE.

REFERENCES

1. Vishal Vasistha. Effects of pollutants produced by thermal power plant on environment: A Review. Int. J. Mech. Eng. & Rob. Res, 2014; 3(2): 202-207.
2. Dan'azumi, S. and Bichi, M. H. Industrial Pollution and Implication on Source of Water Supply in Kano, Nigeria, International Journal of Engineering & Technology IJET-IJENS, 2010; 10(1): 2227-2712.
3. Narimisa, M.R. and Basri, N.E.A. A case study for environmental impact assessment of oil refinery in Iran with emphasis of Environmental and Social Action Plan (ESAP). 2nd International Conference on Environmental Science and Technology - IPCBEE. IACSIT Press, Singapore, 2011; 6: 334 -337.
4. Kumar, S.; Katoria, D. and Sehgal, D. Environment impact assessment of thermal power plant for sustainable development. International Journal of Environmental Engineering and Management, 2013; 4(6): 567-572.
5. Kwon, W.; Kim, D. and Kim, Y. Characterization of Heavy Oil Fly Ash Generated from a Power Plant. The AZo Journal of Materials Online (Azojomo), 2005; 6(2): 260-263.
6. Singh, R.; Singh, R.K.; Gupta, N. C. and Guha, B.K. Assessment of heavy metals in fly ash and groundwater – a case study of NTPC Badarpur thermal power plant Delhi, India. Poll Res, 2010; 29(4): 685-689. India.
7. Kovalchuk, I and Kovalchuk, O. Transgenic plants as sensors of environmental pollution genotoxicity. Sensors, 2008; 8:1539-1558.
8. Maluszynska, J. and Juchimiuk, J. (2005). Plant genotoxicity: a molecular cytogenetic approach in plant bioassays. Plant Genotoxicity.
9. Olive, P.L. And Banáth, J.P. The comet assay: a method to measure DNA damage in individual cells. Nat Protoc, 2006; 1(1): 23-29.

10. Choucroun, P.; Gillet, D.; Dorange, G.; Sawicki, B. and Dewitte, J. Comet assay and early apoptosis. Mut. Res., 2001; 478: 89–96.
11. Ventura, L.; Giovannini, A.; Savio, M.; Donà, M. and Macovei, A. et al, Single cell gel electrophoresis (Comet) assay with plants: Research on DNA repair and ecogenotoxicity testing. Chemosphere., 2013; 92: 1–9.
12. Sahieb. B. H.; Ghalib. M. S.; Hasin, S. K. and Ishaq, A. The effect of residual heavy Metals originated from electric power station on internal and surrounded soils of polluted site. (Study report). Ministry of Environment/ Directory of Environmental Protection and Enhancement in the Middle Region/ Directory of Baghdad Environment / Branch of Agriculture Pesticides. 2013. Iraq. (Unpublished report).
13. Lafta, A.; Johi, H.; Kazar, A. and Abood, S. A study on the Impact of Al-Daura power station emissions on neighboring areas. (Study report). Ministry of Environment/ Directory of Environmental Protection and Enhancement in the Middle Region/ Directory of Baghdad Environment / Branch of Monitoring air quality and noise. 2014. Iraq. (Unpublished report).
14. Dhawan, A.; Bajpayee, M.; Pandey, A. and Parmar, D. Protocol for the Single Cell Gel Electrophoreses/Comet Assay for Rapid Genotoxicity Assessment, Developmental Toxicology Division Industrial Toxicology Research Centre, 2003. India.
15. Gichner, T. Differential genotoxicity of ethyl methanesulphonate, Nethyl-N-nitrosourea and maleic hydrazide in tobacco seedlings based on data of the Comet assay and two recombination assays. Mutat. Res., 2003; 538: 171–179.
16. Gichner, T.; Patkov´a, Z.; Sz´akov´a, J. and Demnerov´a, K. Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombination in tobacco leaves. Mutat. Res., 2004; 559: 49–57.
17. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc.: 2012; Cary. N.C. USA.
18. Cotelle, S. and Fe`rard, J. F. Comet assay in genetic ecotoxicology. Environmental and Molecular Mutagenesis, 1999; 34: 246–255.
19. Olive, P. L.; Banath, J. P. and Durand, R. E. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the Comet assay. Radiat Res., 1990; 122: 86 –94.
20. Akoteyon, I. S. Evaluation of groundwater quality using contamination index in parts of alimosho, Lagos- Nigeria. Journal of American Academic & Scholarly Research, 2012; 4 (4): 13-19.

21. Ribeiro, R. V. and Eduardo, C. M. Some aspects of citrus ecophysiology in subtropical climates: re-visiting photosynthesis under natural conditions. Braz. J. Plant Physiol., 2007; 19(4): 393-411.
22. Komatina, M. "Medical Geology, Effects of Geological Environmental on Human Health-Development in Earth and Environment Sciences-2". 2004; Oxford, UK, 482p.
23. Kabata-Pendias, A.. Trace elements in soils and plants, 4th ed. London, New York: Taylor &Francis: 2011.
24. Tom´aˇs, G.; Zdeˇnka P.; Jiˇrina S.; Irena ˇZ. and Anita, M. DNA damage in potato plants induced by cadmium, ethyl methanesulphonate and γ-rays. Environmental and Experimental Botany., 2008; 62: 113–119.
25. Pourrut, B.; Jean, S.; Silvestre, J. and Pinelli, E. Lead-induced DNA damage in *Vicia faba* root cells: Potential involvement of oxidative stress. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 2011; 726(2): 123-128.
26. Sriussadaporn, C.; Yamamoto, K.; Fukushi, K. and Simazaki, D. Comparison of DNA damage detected by plant comet assay in roadside and non-roadside environments. Mutat. Res., 2003; 541: 31–44.
27. Chakraborty, R. and Mukherjee, A. Technical note: vetiver can grow on coal fly ash without DNA damage. Int. J. Phytoremediation, 2011; 13: 206–214.