Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **5(2),** 17-22, February **(2015)** *Res. J. Chem. Sci.*

Cytogenotoxicity evaluation of LOKPODJI and AGBOKOU sites of pollution using Allium Cepa assay

Arthur Romuald CAKPO[1], Etienne SAGBO[1], Daouda MAMA[2], et Mohamed M. SOUMANOU[3]
[1]Laboratoire de Chimie Inorganique et de l'Environnement (LaCIE), Faculté des Sciences et Techniques (FAST), Université d'Abomey -Calavi BP : 4521 Cotonou, Bénin,
[2]Laboratoire d'Hydrologie Appliquée (LHA), Université d'Abomey- Calavi, Bénin
[3]Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), Ecole polytechnique d'Abomey- Calavi (EPAC), Université d'Abomey-Calavi, Bénin

Available online at: www.isca.in, www.isca.me
Received 4 th January 2015, revised 14 th 2015, accepted 18 th February 2015

Abstract

The cytogenotoxic effects of LOKPODJI and AGBOKOU sites from Porto-Novo lagoon (0%, 25%, 50%, 75%, 100%) were evaluated using root tip cells of Allium cepa. In this study, root length and chromosomal aberration assays were used to determine the 96 h effective concentration (96h, EC $_{50}$), roots growth inhibition, mitotic index and chromosome aberration rate. According to the results obtained, water of AGBOKOU site was 2 times more toxic than those of LOKPODJI site and one has different significant between the average lengths of the roots of onions exposed in the various concentrations of the two sites ($P < 0.05$). This indicated that the root growth inhibition was concentrations dependent. The mitotic index (MI) decreases with increasing concentrations of water on AGBOKOU site on the other hand on the site of LOKPODJI, it decreases up to 50% and increases from 75% to 100%. Water of the two sites induced chromosomal aberrations in root tip cells of Allium cepa with chromosomal malformations such as vagrant chromosome, fragment, bridges and sticky chromosomes being most frequently observed. The common aberrations observed when the concentrations are weak are the fragments and bridges chromosomes. Genotoxicity test carried out on the chromosomes of onions roots tips made it possible to measure the genotoxic effects of water on two studied sites.

Keywords: Cytogenotoxicity, allium cepa, chromosomal aberrations, root growth inhibition, Benin.

Introduction

The African countries are confronted nowadays with innumerable difficulties which impede their development.The world economic crisis and the economic resection which was followed from there came to complicate the situation of these countries which pain with booster their economies.

Population, poverty and pollution are the three major problems of developing countries[1]. Our country the Benin one does not escape this assertion. These problems are accentuated fact dangerously the industrial great strides which make the African countries. The research of the improvement of the living conditions of the population obliged the governments to accept the installation of industries in our cities. The particular characteristic of these industries and their principal defect is the production of waste which directly or is indirectly poured out in the rivers and lagoons.

These industrial promoters who settle briskly a little everywhere in Africa, without being worried do not respect any regulation in force in the countries or they unload. They do not apply any provisions of fight anti pollution envisaged by the laws which govern the host countries pretesting that industrialization is the key of the development.

Industrial effluents are a main source of direct and often continuous input of pollutants/toxicants into aquatic ecosystems with long-term implications on ecosystem functioning[2,3,4,5]. Pollution decreases the quality of life in various aspects then affects the health and life span[1]. Moreover the direct effects on health, the danger of the toxic and mutagen pollutants is that they cause afflictions such as cancer, atherosclerosis, the cardiovascular diseases and premature ageing[1]. Several studies undertaken on waste water coming from the industrial or urban discharges showed the existence of the genotoxic activities[1,4,6,7]. A growing interest in genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicants in the air, water and soil[8]. The tests of genotoxicity are generally used to evaluate the potential genotoxic in the environment and on the level of the samples of effluents industrial[1,6,9].

In Africa generally and in all under African Western area, the phenomenon of agricultural, industrial and environmental pollution is a plague which grow worse day by day becoming disastrous extent. In Lagos, Nigeria, there are many industries that discharge their effluents into the different water bodies around the metropolis[10]. This industrial pollution is especially caused by textiles and paint industries which are major industries in Lagos and discharge large amount of effluent

among all the industries in the metropolis. There are very few reports on genotoxicity of industrial effluents in Nigeria[10]. Most reports concentrated on the genotoxicity evaluation of leachates from solid industrial/domestic wastes and landfills[11-13].

In the large cities of Benin, such as Cotonou, Porto-Novo and Parakou; several industries pour out their discharges in the lagoons and various rivers causing an environmental pollution of great scale. Domestic waste water and those of stream coming from all the city, especially in rainy season are also drained towards the rivers by canalization; what worsens the phenomenon of pollution.

Since always, the tests of toxicity carried out on experimental animals. They were very expensive and required a long period for their realization. Thus various other alternatives of search for determination of toxicity were implemented among which the test of toxicity based the roots of the plants which are often used in the biological tests[10]. Indeed the end of the roots is generally the first part, most exposed to the chemical contaminations in water, the ground and in nature. Observation of the root type system therefore constitutes a rapid and sensitive method for environmental monitoring[14].

Environmental pollution and the cytotoxicity can be evaluated by the in vivo onion roots tip cell test[15]. The results similar to this test are obtained with the in vitro animal cytotoxicity tests[16,17,18]. The studies undertaken by these various researchers revealed that the Allium is useful for the detection of potentially genotoxic substance in water screening programmes[9,19,20]. The test of onions was used to evaluate the toxicity and the genotoxicity of several industrial effluents[1,6,7,19].

In this study, Allium cepa root-tip assay was used to evaluate the cytogenotoxicity effects on the sites of LOKPODJI and AGBOKOU in Porto-Novo lagoon where activities of sand lagoon extraction are respectively undertaken by machines, and are poured out waste water then various chemical wastes resulting from the preparation of the soaps and the cosmetic products.

Material and Methods

Test organism: The onions (3 – 3.5 cm diameter) used for this experiment were procured from OUANDO market, Porto-Novo, Benin

Test agents: LOKPODJI and AGBOKOU water samples were used for this study. Water samples were collected directly from the two sites and they were collected in plastic container and stored in the refrigerator at 4°C pending use[21]. Before each test was carried out, the water samples were equilibrated to room temperature (26 ± 2°C) and diluted with dechlorinated tap water to produce the series of dilutions investigated.

Assay procedure: The assay was carried out using a plastic tube (diameter, 2.6 cm; length 8 cm) in a rack. Dechlorinated tap water was use as control (0%) and for dilutions of water samples. The yellow shallows and dry bottom plate inside the root primordial of Allium cepa were carefully removed prior to the test.

Root growth inhibition test: The growth inhibition assay was carried out at the Inorganic Chemistry laboratory and Environment (LaCIE), Faculty of Science and Techniques (FAST), University of Abomey - Calavi and was performed as a 96 h semi- static exposure test[22]. Allium cepa was exposed for 96 h to different dilutions of the water samples as follows: 0 %, 25 %, 50 %, 75%, 100 % for the two sites.

Each concentration was set-up in 5 replicates. The various dilutions used for the test of cytotoxicity were replaced every 24 hours with fresh diluted solutions. At the 4 days end of exposure, the length of the root bundle was measured for the five onions and two longest roots in each bulb were measured. Growth inhibition of the roots was estimated by EC_{50} (the effective concentration of a chemical producing 50% of the total effect).

Genotoxicity assay: The test of genotoxicity was carried out with water samples of the two sites. Five onions were put in culture during 48h in each concentration of 0%; 25%; 50%; 75% and 100% obtained by dilution of the water samples of the sites with dechlorinated tap water[22].

As for the growth inhibition test, the test solutions were changed after 24h and at 48h, one root tip (10 mm) from each bulb was cut for cytological study[10]. The root tips were fixed by a mixture of absolute ethanol and icy acetic acid in the proportions (3:1) during 12h. After fixing, the roots are then transferred in a test tube containing the mixture of ethanol and hydrochloric acid for their softening. The softened roots are rinsed with water then transferred in a small volume from dye, in a test tube which is heated with the flame of alcohol lamp. The dye used was prepared starting from one gram (1g) of acetic carmine (1 to 2%) mixed with 55ml of distilled water and 45ml of icy acetic acid; whole is heated during 5mm with the flame of a bottle with gas and the Bunsen burner. The dye is cooled then filtered on whatman paper; to the filtrate one adds a ferric chloride drop.

One takes 1 to 2 mm of the coloured roots tips which one deposits in a drop of fresh dye on the blade then crushed by a plate. For that it is necessary to press gently on the plate to flatten the final segment of the root in order to form a monocellular stratum by slightly moving the plate while pressing to induce cells dissociation. The excess of the dye on the blades is eliminated using a whatman paper, then to set under the microscope for the observation. It is important to wear gloves during handling.

Microscopic examination: All slides were coded and the samples of roots tips were examined under the microscope. The

mitotic index (MI) was determined by the examination of 500 cells per concentration (100 cells per slide). Characterization of mitosis and chromosomal aberrations were scored in 100 cells per slide.

Statistical analysis: The effective concentration EC_{50} and the regression equation were determined from a root length according to the percentage of control against the sample concentrations, by using a Microsoft Excel computer program. Pearson correlation of was used to check the relation between the root length and water samples concentrations. Analysis of variance (ANOVA) and Student Newman Keul's (SNK) tests were used to check the significant differences in the mean root lengths of Allium cepa exposed to different concentrations of LOKPODJI and AGBOKOU water samples. The tests were carried out at 5% significant rate. The analysis was performed with the software SPSS 20.0 (Statistical Package for the Social Sciences).

Results and Discussion

Root growth inhibition: The results of the macroscopic parameters (root length) used in testing for general toxicity (root growth inhibition) of onions exposed to various water concentrations of LOKPODJI and AGBOKOU sites are presented in figures 1, 2 and table-1.

The estimated EC_{50} (concentration of the chemical producing 50% of the total effect) of onions exposed to LOKPODJI (site 1) and AGBOKOU (site 2) was 80% and 40% respectively (figures-1 and 2).

No growth was observed on the level of onions exposed to AGBOKOU site. Generally a delay of growth was observed at onions put in culture in the water samples of the two sites having high concentrations (table-1). Further analysis based on the correlation of Pearson revealed that the root growth inhibition or delay of growth was significantly concentrations dependent ($P < 0.01$; $R^2 = 0.870$ for site 1 and $R^2 = 0.589$ for site 2) (figures-1 and 2). High growth level of the roots was observed in bulbs exposed to low concentrations of either LOKPODJI (site 1) or AGBOKOU (site-2) water samples (table-1).

Statistical analysis based on analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the mean root lengths of Allium cepa exposed to different concentrations of LOKPODJI (site 1) and AGBOKOU (site 2) water samples. Further analysis using Student Newman Keul's (SNK) test revealed that the root length of the control group onions (0% of two sites) was significantly different ($P < 0.05$) from the root length of onions exposed to all other concentrations but this difference is accentuated on the level of site 2. No significant difference ($P > 0.05$) was observed in root growth of onions exposed to 0% and 25% of LOKPODJI water samples (site 1) then on the level of the concentrations 25% and 50% of AGBOKOU water samples (site 2) (table-1).

Table-1
Mean (± SD) root length of Allium cepa exposed to different concentrations of LOKPODJI (site 1) and AGBOKOU (site 2) sites

LOKPODJI (site 1)		AGBOKOU (site 2)
Concentration (%)	**Mean root length (cm)**	**Mean root length (cm)**
Control (0)	9.50± 1.79	9.45± 3.48
25	9.18± 1.56	3.42± 0.78
50	8.65± 1.19	3.74± 0.55
75	5.00± 1.39	3.16± 0.96
100	2.50± 3.15	2.85± 1.18

SD: Standard deviation

Microscopic effects: The microscopic results of Allium cepa roots exposed to LOKPODJI (site 1) and AGBOKOU (site 2) water samples are summarized in tables 2 and 3 respectively.

Except in the Allium cepa exposed to 75% of LOKPODJI water samples, there was a decrease in the mitotic index (MI) with increasing concentration of the waste waters. Let us note that it was impossible to obtain 500 mitoses for the chromosomes selected in all the water concentrations of the two sites as one obtained in the control (0%) (table-2 and 3). Analysis of chromosomes showed that the water samples of two sites induced chromosomal aberrations significantly when compared to the control (0%). No aberration was recorded in the chromosome of Allium cepa exposed to the control (0%). Viscous or stickiness and bridges fragment were observed in chromosomes of onions exposed to different concentrations of two sites. Vagrant chromosomes were observed in concentrations 25% and 100% on the water samples of the two sites. Abnormal and multipolar anaphases were also observed in onions exposed to 25% on the two sites. The chromosomes attached in metaphase appeared for the concentrations 50% and 100% on the water samples of site 1 then 75% and 100% for the water samples of site 2. Binuclei cells were observed in the chromosome of onions exposed to 75% and 100% on the water samples of site 1 then 100% of the water samples of site 2 (table-2 and 3).

Discussion: The results obtained at the time of the macroscopic study based on growth inhibition of Allium cepa roots indicated clearly that the site of AGBOKOU (site 2) (96h EC_{50} = 40%) was 2 times more toxic than the site of LOKPODJI (site 1) (96h EC_{50} = 80%) (figures-1 and 2). At 100% concentration of LOKPODJI (site 1) and AGBOKOU (site 2) water samples there was respectively 26.32% and 30.16% root growth relative to control. ANOVA showed that there was significant difference in the mean root length of Allium cepa exposed to different concentrations of higher growth rate observed in onion bulbs exposed to low concentration of either LOKPODJI (site-1) ($R^2 = 0.870$) or AGBOKOU (site 2) ($R^2 = 0.589$) than those exposed to high concentrations (figures-1 and 2).

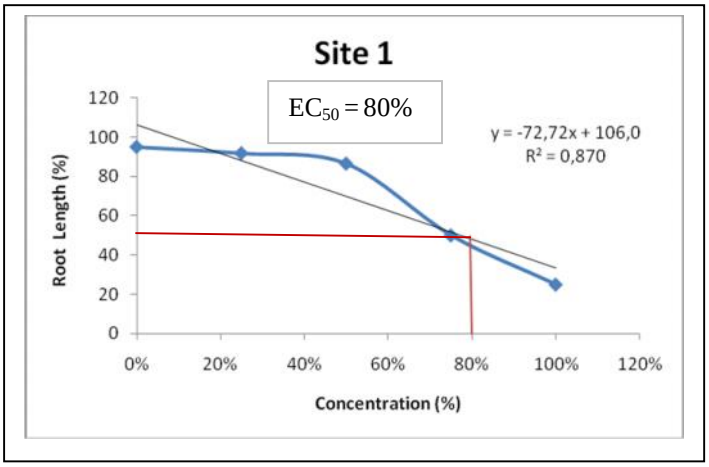

Figure-1
Growth inhibition of Allium cepa roots exposed to LOKPODJI (site 1) water samples

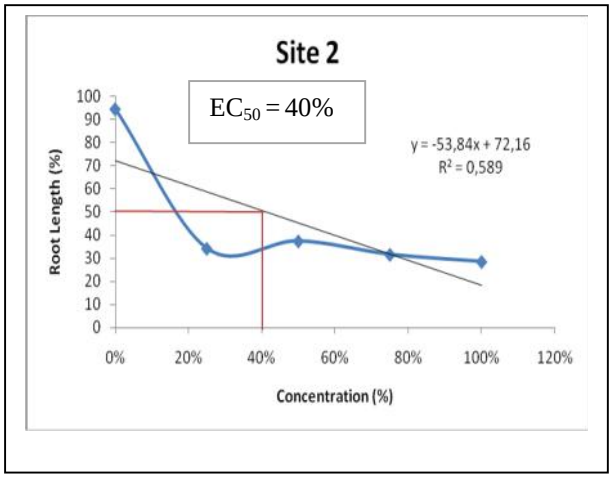

Figure-2
Growth inhibition of Allium cepa roots exposed to AGBOKOU (site 2) water samples

Table-2
Effects of treatments with different concentrations of LOKPODJI water samples

Concentration (%)	mitotic Index	Number of cell	Number of dividing cell	chromosome aberrations						
				Stickiness	Vagrant	Bridges fragment	Binuclei	Multipolar anaphase	Attached	% Aberration
Control (0)	8.00	500	40[$P_{15}M_{13}A_5T_7$]	0	0	0	0	0	0	0.00± 0.00
25	8.78	376	33[$P_{20}M_2A_{10}T_1$]	3	1	1	0	1	0	1.59± 0.12
50	8.13	246	20[$P_{15}M_2A_2T_1$]	2	0	1	0	0	1	1.63± 0.15
75	8.69	115	10[$P_5M_2A_1T_2$]	1	0	0	1	0	0	1.73± 0.22
100	9.40	234	22[$P_{19}M_1A_1T_1$]	1	2	2	0	0	0	2.14± 0.51

Mitotic index was calculated as : (number of dividing cells / number of cell) x 100

Table-3
Effects of treatments with different concentrations of AGBOKOU water samples

Concentration (%)	mitotic Index	Number of cell	Number of dividing cell	Chromosome aberrations						
				Stickiness	Vagrant	Bridges fragment	Binuclei	Multipolar anaphase	Attached	% Aberration
Control (0)	8.00	500	40[$P_{15}M_{13}A_5T_7$]	0	0	0	0	0	0	0.00±0.00
25	9.33	375	35[$P_{14}M_6A_8T_7$]	1	1	0	0	1	0	0.80± 0.05
50	9.27	356	33[$P_{28}M_2A_2T_1$]	1	0	2	0	0	0	0.84± 0.08
75	9.22	412	38[$P_{31}M_2A_3T_2$]	0	0	3	0	0	1	0.97± 0.17
100	8.93	291	26[$P_6M_5A_4T_{11}$]	0	1	1	0	0	1	1.03± 0.21

Mitotic index was calculated as : (number of dividing cells / number of cell) x 100

Estimate of 96h EC_{50} showed a great similarity in dose – effect[10,11,21]. The cytogenetic effects obtained on the level of onions root cells during their mitotic division were mentioned in tables 1 and 2 respectively for each site 1 and 2. The mitotic index (MI) except the control (0%) decreased with increasing concentrations of AGBOKOU water samples. Similar results were obtained when they carried out the test of cytogenotoxicity based on onions on the effluent of the textile factory (Nichemtex) of Lagos in Nigeria[10]. Same results were obtained after treating Allium cepa root cells with leachates from solid industrial wastes and on oil field waste water[11,21].
Then finally for their study on the sodium metabisulphite[23]. Inhibition of mitotic activities is often used for the tracing cytotoxic substances[10].

The highest index mitotic obtained during this study is that of the onions roots exposed to 100% of LOKPODJI (Site 1) water samples. This study showed that water of the two sites induced chromosomal aberrations in root tip cells of Allium cepa with bridges and fragments and sticky or viscous chromosomes, vagrant chromosome being most frequently observed. All these deformations indicate the presence of the cytogenotoxic substances in water of the two sites. Vagrant chromosomes have been described as a weak C mitotic effects indicating risk of

aneuploidy while sticky chromosomes indicate a highly toxic[10]. Irreversible effect probably leading to cell death[24,25]. There is a hypothesis that stickiness of chromosomes may cause incomplete separation of daughter chromosomes as a result of cross- linkage chromoproteins[26].

The number of aberrant mitotic cells caused by all water concentrations of the two sites was different from that obtained of the control then note that no chromosomal aberration was observed in the Allium cepa exposed to the control (0%). The variation of the number of chromosomal aberrations observed in this study was not dose dependent, except the case of bridges chromosomes where the number of chromosomal deformations reduced when the concentrations of water samples on the two sites increased what is in disagreement with study which reported that aberrant rate goes up with the concentrations[27].

But in agreement with result on genotoxicity of oil field waste water in Nigeria[21]. A plausible explanation for this phenomenon is that, with increasing concentration and consequently, increasing toxicity, there was an inhibitory effect on cell division[21].

The proportion of the cellular aberrations of the onions roots exposed to LOKPODJI water samples (site 1) is higher than cellular aberrations of the onions roots exposed to AGBOKOU water samples (site 2) (table-2 and 3) although according to EC_{50}, AGBOKOU site was more toxic than LOKPODJI site.

Generally the chromosomal aberration in the root tip of onions indicates their genotoxic effects. The Allium test has been used in the screening of other types of industrial waste and waste water. The genotoxicity screening of leachates from solid industrial wastes was indicated and genotoxicity effect was signalized of oil field waste water[11,21]. One can also quote the study, which evaluated the suitability of the Allium test in the screening of waste water for genotoxicity[28]. Then the one of study which evaluated the genotoxicity of waste water samples from sewage and industrial effluent using Allium cepa root anaphase aberration[1]. The efficiency of algal biofilters in bioremediation of toxic industrial effluents using Allium cepa genotoxicity bioassay have them also reported[6]. The impact of genotoxicity waste water on the environment and the importance to human health are difficult to predict because waste water are complex mixtures of chemical substances[21]. Complementary chemical analysis must be made to determine the chemical components which accumulate in a way persistent and dangerous in waste water and which pollutes the biota with their potentialities to harm human health dangerously. This study made it possible to show that genotoxic potential of LOKPODJI site (site 1) and of AGBOKOU site (site 2) can be detected with the test of chromosomal aberration in the root tip of Allium cepa.

Conclusion

It would be beneficial to apply Allium cepa chromosomal assay as a tool for monitoring the genotoxic effects of industrial effluent and waste waters thereby providing information on the need for environmental managers to further subject treated industrial effluent to Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) before they are finally discharged. This will enable proper chemical analysis of industrial effluent in order to identify the constituent that is really genotoxic and its prompt removal from the effluent before discharge[10].

References

1. Grover I.S. and Kaur S., Genotoxicity of wastewater samples from sewage and industrial effluent detected by the Allium root anaphase aberration and micronucleus assays, *Mutat. Res.*, **426**, 183-188 **(1999)**

2. Odeigah C. and Osanyipeju O., Genotoxic effects of two industrial effluent and ethylmethane sulfonite in clarias will lazera, *Food chem. Tox.*, **33**, 501-505 **(1995)**

3. Chan Y.K., Wong C.K., Hsieh D.P.H., Ng S.P., Lau T.K. and Wong P.K., Application of a toxicity identification evaluation for a sample of effluent discharged from has dyeing factory in Hong Kong, *Environ. Tox.*, **18**, 312-316 **(2003)**

4. Lah B., Gorjane G., Nekrep F.V. and Marinsek- Logar R., Comet assay of waste water genotoxicity using yeast cells, *Bull. Environ. Contam.* Tox., **72**, 607-616 **(2004)**

5. Smolders R., Bervoets L. and Blust R., In situ and laboratory bioassays to evaluate the impact of effluent discharges one receiving aquatic ecosystems, *Environ. Pol*., **132(2),** 231-243 **(2004)**

6. Abdel-Migid H.M., Azad Y. and Ibrahim W.M., Use of plant genotoxicity bioassay for the evaluation of efficiency of algal biofilters in bioremediation of toxic industrial effluent, *Ecotox. Environ. Safety*, **66**, 57-64 **(2007)**

7. Junior H.M., da-Silva J., Arenzon A., Portela C.S., de-Sa-Ferreira I.C. and Henniques J.A.P., Evaluation of genotoxicity and toxicity of water and sediment samples from a Brazilian stream influenced by tannery industries, *Chemosphere*, **67, 1211-1217 (2007)**

8. Grisolia C.K. and Cordeiro C.M.T., Variability in micronucleus induction with different mutagens applied to several species of fish, *Gen. Mol. Biol*., **23(1**), 235 – 239 **(2000)**

9. Cotelle S., Masfaraud J. and Ferard J., Assessment of the genotoxicity of contamined soil with the Allium Nicia : micronucleis and the Tradescantia – micronucleus assays, *Muta. Res*, **426**, 167-171 **(1999)**

10. Samuel O., Osuala F. and Odeigah P.G., Cytogenotoxicity evaluation of two indutrial effluent

using Allium cepa assay, *African Journal of Environnemental Science and Technology*, **4(1)**, 21-27 **(2010)**

11. Odeigah P.G., Ijimakinwa J., Lawal B. and Oyeniyi R., Genotoxicity screening of leachates from solid industrial wastes evaluated with the Allium test, *Atla*, **25**, 311-321 **(1997b)**

12. Bakare A.A., Mosuro A.A. and Osibanjo O., Cytotoxic effects of landfill leachate on allium cepa, *L. Biosci. Res. Com*., **11(1)**, 1-13 **(1999)**

13. Bakare A.A., Mosuro A.A, Osibanjo O., Effect of simulated leachate on chromosomes and mitosis in roots of Allium cepa (L), *J. Environ. Biol*, **21(3)**, 263-271 **(2000)**

14. Majer B.J., Grummt T., Uhi M. and Knasmuller S., Use of plant bioassays for the detection of genotoxins in the aquatic environment, *Acta Hydrochim. Hydrobiol*, **33(1)**, 45-55 **(2005)**

15. El-Shahaby A.O., Abdel-Migid H.M., Soliman M.I. and Mashaly I.A., Genotoxicity screening of industrial wastewater using the allium cepa chromosome aberration assays, *Pakistan J Biol. Sci*., **6**, 23-28 **(2003)**

16. Chauhan L.K.S., Saxena P.N. and Gupta S.K., Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of allium cepa, *Environ. Exp. Bot*., **42**, 181-189 **(1999)**

17. Vicentini V.E.P., Comparoto M.L., Teixeira R.O. and Mantovani M.S., Averrhoa carambola L, Syzygium cumini (L) Skeels and Cissus sicoydes L: Medicinal herbal tea effects on vegetal and animal test systems, *Acta Scientiarum*, **23**, 593-598 **(2001)**

18. Teixeira R.O., Comparoto M.L., Mantovani M.S. and Vicentini V.E.P., Assessment of two medecinal plants Psidium guajava L and Achillea millefolium L, in vitro and in vivo assays, *Gen. Mol. Biol.*, **26**, 551-555 **(2003)**

19. Rank J. and Nielson M.H., Genotoxicity testing of wastewater sludge using the Allium cepa anaphase telophase chromosome aberration assays, *Mutat. Res*., **418**, 113-119 **(1998)**

20. Moraes D. and Jordao B., Evaluation of the genotoxic potential of municipal waste water discharged into the Paraguay river during periods of food and drought, *Environ. Tox*., **16**, 113-116 **(2001)**

21. Odeigah P.G.C., Nurudeen O. and Amund O.O., Genotoxicity of oil field wastewater in Nigeria, *Hereditas*, **126**, 161-167 **(1997a)**

22. Rank J., The method of Allium anaphase-telophase chromosome aberration assays, *Ekologija*, **1**, 38-42 **(2003)**

23. Rencuzogullari E., Kayraldiz A., Ila H.B., Cakmak T. and Topaktas M., The cytogenetic effects of sodium metabisulfite, a food preservative in root tip cells of Allium cepa L, *Turk. J. Biol*, **25**, 361-370 **(2001)**

24. Fiskesjo G., The allium test as the standard in environmental monitoring, *Hereditas*, **102**, 99-112 **(1985)**

25. Fiskesjo G., The allium test: An alternative in environmental studies, The Relative toxicity of metal ions, *Mutat. Res*., **197**, 243–260 **(1988)**

26. Kong M.S. and Ma T.H., Genotoxicity of contaminated soil and shallow well water detected by plant bioassays, *Mutat. Res*., **426(2**), 221-230 **(1999)**

27. Qian X.W., Mutagenic effects of chromium trioxyde on root tips of Vicia faba, *J. Zhejiang Univ. Sci*., **5(12)**, 1570-1576 **(2004)**

28. Rank J. and Nielson M.H., Evaluation of the Allium anaphase : Telophase test in the relation to genotoxicity screening of industrial wastewater, *Mutat. Res*, **312**, 17-24 **(1994)**