NISEB JOURNAL Vol. 12, No. 2, April 30, 2012
Printed in Nigeria

1595-6938/2012 $5.00 + 0.00

http://www.nisebjournal.org

NISEB 2012046/12206

Biochemical effects and genotoxic evaluation of Warri Refinery and Petrochemical Company (WRPC) wastewater

D.I. Olorunfemi[1]*, L. Tudararo-Aherobo[2], O. Amadasun[1], and R Ofomata[1]

[1]Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria
[2]Department of Environmental Sciences, Federal University of Petroleum Resources, Effurun, Nigeria

(Received April 2, 2012; Accepted April 26, 2012)

ABSTRACT: Wastewater samples from Warri Refinery and Petrochemical Company (WRPC) were collected from the discharge point and 500m away from the point of discharge and analysed for physicochemical parameters using standard methods. Serially prepared effluent concentrations were subjected to aceto-orcein squash chromosome aberration *Allium cepa* assay for genotoxic evaluations. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were assessed on root-tips of *A. cepa* L. exposed to the wastewater samples. Physicochemical analysis showed that levels of lead, chromium, zinc, manganese, cadmium, iron and copper exceeded SON permissible limits and were higher at the discharge point than downstream. There was a rapid decrease in mitotic index and statistically significant ($p<0.05$) increase in chromosome aberrations in the root tip cells with increasing effluent concentration. SOD, CAT and GSH-Px enzyme activities decreased in the common onion root tip cells indicating high rate of genotoxicity. The findings reveal that the toxic chemicals in the wastewater are responsible for the observed genotoxic effects by oxidative damage on the onion root tip cells.

Keywords: Petrochemical wastewaters, *Allium cepa*, chromosomal aberrations, Ubeji community

Introduction

Refinery and petrochemical plants generate waste water characterised by chemicals including heavy metals and organic compounds which are assimilated by aquatic species, pass through the food chain, and bioaccumulate upon long-term exposure (Sang and Li, 2004). The wastes, which arise as a result of ineffective purification systems, contain environmental mutagens including heavy metals and could be an important risk factor for human health (Cerna *et al*., 1991, Bakare *et al*., 2007). Besides the direct health effects, they may be mutagenic or carcinogenic and could lead to several human afflictions like cancer and cardiovascular diseases (Hallenbeck, 1986).

Although the mechanism of metal carcinogenicity is largely unknown, several lines of experimental evidence suggest that a genotoxic effect may be involved (Snow, 1992, Bolognesi *et al*., 1999). It has been asserted that determination of the chemical composition and the genotoxic potential of wastewaters are crucial for environmental protection and public health (Durgo *et al*., 2009).

*Author to whom all correspondence should be addressed.
E-mail: udanfem@gmail.com; Tel: +2348023372455

Biological tests, especially short-period bioassays have been found detect a wide range of substances that can cause genetic damage and enable quantification of mutagenic hazard even when there are no sufficient data about identity and physico-chemical properties of compounds present in wastewater (Valko *et al*., 2006, Gana *et al*., 2008). In recent times there is a clamour for the preferential use of higher plants as sensors of environmental pollution because they are ethically more acceptable and aesthetically more appealing than animals (Kovalchuk and Kovalchuk, 2008). As a matter of fact, *Allium cepa* L. (2n=16) has been used to as genotoxic endpoints of refinery wastewaters to reflect DNA damage or biomarkers of exposure (Hoshina & Marin-Morales, 2009, Rodrigues *et al*., 2009). This assay is low cost and easy to use. The ability of plants to activate promutagens further adds to their suitability as test organisms (Plewa, 1978, Vig, 1978). Furthermore, there is a strong correlation between chromosomal aberrations induced by the same chemical in plant cells and in cultured mammalian cells as a result of similarity in their genetic compositions (Grant, 1978). The use of *A. cepa* bioassay for environmental mutagen monitoring has been endorsed by the Royal Swedish Academy of Sciences and the WHO (Fiskesjö, 1985). Similarly, the activities of SOD, CAT, and GSH-Px are recently used to monitor the development and extent of damage due to oxidative stress (Fatima and Ahmad, 2005, Olorunfemi and Lolodi, 2011).

Built in 1978, the Warri Refinery and Petrochemical Company (WRPC) is the first Nigerian government wholly owned and largest oil refinery in Nigeria. Originally built to process 100,000 barrels of crude oil per day, it was later de-bottlenecked to process 125,000 barrels per day of crude oil in 1987 (Nduka and Orisakwe, 2009). The most recent reports on WRPC wastewater assessment was done with physicochemical analysis which showed serious pollution burden on the potable water quality from the effect of the refinery effluent (Nduka & Orisakwe, 2009, Uzoekwe & Oghosanine, 2011). To the best of our knowledge, no investigations have been carried out for evaluating the genetic and oxidative effects of WRPC wastewaters. This study was aimed at elucidating and evaluating the genetic and oxidative effects induced by different concentrations WRPC wastewaters using *A. cepa* bioassay and the activities of antioxidant enzymes such as SOD, CAT and GSH-Px respectively.

Materials and Methods

Collection of Refinery Effluents: WRPC effluent samples were collected in February 2011 from point of discharge and 500m downstream in Ubeji community located in latitude 5° 34' 4.57"N and longitude 5° 42' 24.07" E. Ubeji is Delta State and has an average elevation of about 21 meters above sea level. The composite wastewater sampling method was employed in collecting the samples. Temperature, pH and DO were determined *in situ*. The samples were collected with clean plastic containers and kept in an ice chest. These were eventually stored in the refrigerator at 4°C and analysed within 24 h of collection.

Metal Analysis: Refinery effluent samples, together with control, were analysed for a number of standard physicochemical properties, including TDS, chlorides, nitrates, and phosphates, according to methods described by APHA (2005). Eleven metals namely lead, copper, cadmium, chromium, iron, zinc, aluminium, nickel, magnesium, mercury and manganese were analysed in the water samples according to standard analytical methods (USEPA, 1996; APHA, 2005) using an atomic absorption spectrometer (AAS) (PerkinElmer A Analyst 100). The metal standards were prepared to known concentrations, labelled, and kept inside plastic bottles that were pre-cleansed with concentrated nitric acid and distilled water.

***Allium cepa* Test:** Onion bulbs *(Allium cepa* L., 2n=16) of the purple variety of average size (15-22 mm diameter) were purchased locally in Benin City ($6^{0}15^{1}$ N and $5^{0}25^{1}$ E), Edo State, Nigeria. The bulbs were sundried for 8 weeks before use, to ensure that bulbs that were rotten and or mouldy were all discarded. Within 6 hours of the experimental set up, the outer scales of the bulbs and brownish bottom plates were carefully cut off, leaving the ring of root primordia untouched. The bulbs were set up to test for root growth toxicity and chromosomal aberration (cytogenetics).

Macroscopic Evaluation: To determine the root growth toxicity, 21 onion bulbs of good quality were selected were planted in group of 7 bulbs directly into dechlorinated tap water of good quality with pH 7.3 and relative hardness (Fiskesjö, 1985). The base of each of the bulbs was planted directly in 100 ml beakers containing tap water at room temperature in the dark for 96 hours with the tap water changed daily. After 96 hours, 5 bulbs with good growth were selected and the length of each root from each bulb measured (cm).

Microscopic Evaluation: For the evaluation of induction of chromosomal aberration, five onion bulbs were suspended in the tap water for 48 hours at the end of which root tips from these bulbs were cut and fixed in ethanol:glacial acetic acid (3:1, v/v) for 24 hours after which the root tips were hydrolyzed with 1N HCl at 65^{o}C for 3 minutes. They were rinsed in distilled water and two root tips squashed on a slide, stained with acetocarmine for 10 minutes and cover slips carefully lowered on the slides to exclude air bubble. The cover slips were sealed on the slides with clear fingernail polish as suggested by Grant (1982). This is to prevent drying out of the preparation by the heat of the microscope (Sharma, 1983). Six slides were prepared and were analyzed at ×1000 magnification for the induction of chromosomal aberrations. The mitotic index and the frequency of aberrant cells (%) were determined as in previous study (Olorunfemi *et al*., 2011).

Estimation of Peroxidation and Antioxidant Enzyme Activities: The onion bulbs were prepared for total protein and peroxidation determination and enzymatic assays as in previous studies (Olorunfemi and Lolodi, 2011). The outer scales of onion bulbs were carefully removed and the brownish bottoms were scraped away without destroying the root primordia. The peeled bulbs were placed in tap water during the cleansing procedure to protect the primordia from drying, were randomly placed on beakers (4.5 cm diameter, 5 cm length) filled with the test liquids such that the bases were constantly moistened and observed for 48 hours. The experiments were performed at room temperature (27 ± 2°C) and in the dark. The test solutions were replaced daily. The bulbs with the poorest growth were discarded in each group. On day two when mitotic activity is presumed optimal (Fiskesjö, 1985), the root tips were excised from each bulb and prepared for determination of total protein and peroxidation and enzymatic assays. The concentrations used were 0 (control), 5, 10, 20, 30, 40 and 5% of each of the WRPC effluents.

For the estimation of peroxidation, the level of lipid peroxidation products in samples was expressed as 2-thiobarbituric acid reactive materials, aldehydes, mainly malondialdehyde (MDA) and endoperoxides (Buege and Aust, 1978). 2-Thiobarbituric acid-reactive materials in samples were assayed according to the modified method of Heath and Packer (1968). Total protein estimated by a modified method of Lowry *et al*., (1951). SOD activity was determined by measuring its ability to inhibit the auto-oxidation of adrenaline in aqueous solution to adrenochrome in the presence of the superoxide anions (Misra and Fridovich, 1972). The amount of enzyme producing 50% inhibition is defined as one unit of the enzyme activity. Catalase determination is based on the method of Cohen *et al*., (1970). It hinges on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2) after the addition of the material containing the enzyme by reacting it with excess potassium tetraoxomanganate (VII), ($KMnO_4$) and then measuring the residual $KMnO_4$ spectrophotometrically at 480 nm. GSH-Px activity was measured using hydrogen peroxide as substrate (Carlberg and Mannervik, 1972). Potassium azide was added to inhibit CAT activity. Conversion of NADPH was monitored continuously spectrophotometrically at 340 nm for 3 min at 25°C.

Statistical Analysis: The results of the root inhibition and chromosome aberrations are presented as mean ± standard error for five onion bulbs per concentration and One-Way ANOVA was used for testing significance. Statistical significant differences between control and the different concentrations of the effluents were determined using Tukey post-hoc test. All statistical analyses were carried out using SPSS®14.0 statistical package.

Results and Discussion

The values obtained from the analysis of WRPC effluents collected from the discharge point and 500m downstream are presented in Table 1. The heavy metals: Cu, Fe, Zn, Mg, and Cr were comparatively higher in the effluent from the discharge point than downstream. Similarly, total dissolved solids, alkalinity, sulphates and nitrates were also higher in the effluent from the discharge point, however, the pH of both effluents were not significantly different from each other (8.01 and 7.79 respectively).

Table 1: Water quality parameters and chemical characteristics of refinery effluent samples from WRPC

Parameters	Discharge Point	500m from Discharge Point	SON (2007) Limit	FEPA (1991) Limit	USEPA (1999) Limit
Temperature	28.0	25.2	Ambient	30	NS
pH	8.01	7.79	6.5-8.5	6-9	6.5-8.5
BOD_{25} @ 20^oC	7.7	5.31	NS	50	NS
TDS	0.13	0.11	500	2000	500
Alkalinity	3.17	1.67	NS	NS	NS
Hardness	236.67	303.33	150	NS	NS
Sulphates	92.5	67	100	500	250
Nitrate	83.5	64	50	20	10
Nickel	ND	ND	0.02	<1	0.05
Copper	0.3	0.1	1.0	<1	0.009
Iron	7.1	1.3	0.3	20	0.30
Cadmium	0.1	0.1	0.003	<1	0.002
Mercury	ND	ND	NS	0.05	NS
Manganese	0.4	0.2	0.2	5	0.05
Zinc	9.3	5.5	0.3	<1	0.12
Aluminium	ND	ND	NS	NS	NS
Chromium	0.2	0.1	0.05	0.05	NS
Lead	0.1	0.1	0.01	<1	0.003
Magnesium	110.7	109.5	NS	200	NS

All values are means of 3 replicates for each treatment. Values are in mg/l except temperature (°C), pH with no unit.
TDS: Total dissolved solids
BOD: Biochemical oxygen demand
ND: Less than determined limit

The physicochemical properties of WRPC wastewater show that most of the parameters were within FEPA limits but exceeded USEPA limits. In particular, the concentrations of Zn, Cr and Pd the wastewaters exceeded FEPA limits. Although some metals like Zn, Mn, Ni and Cu may act as micronutrients, capable of enhancing plant growth at lower concentrations, they become toxic at higher concentrations posing health risk to the plants in the environment and consumers alike (Satarug *et al*., 2000; Eriyamremu *et al*., 2005; Muchuweti *et al*., 2006).

The onion bulbs grown in effluents 500m away from the discharge point had higher root growth in all concentrations (Fig. 1) than those grown in effluents from the discharge point. Highest root growth recorded was 3.95cm at 5% effluent concentration 500m downstream while the lowest was 1.96cm at 50% effluent concentration

at the discharge point. Compared to the onion bulbs grown in the control, the root tips of onion bulbs grown in the refinery effluents were shorter and characterised by root malformations.

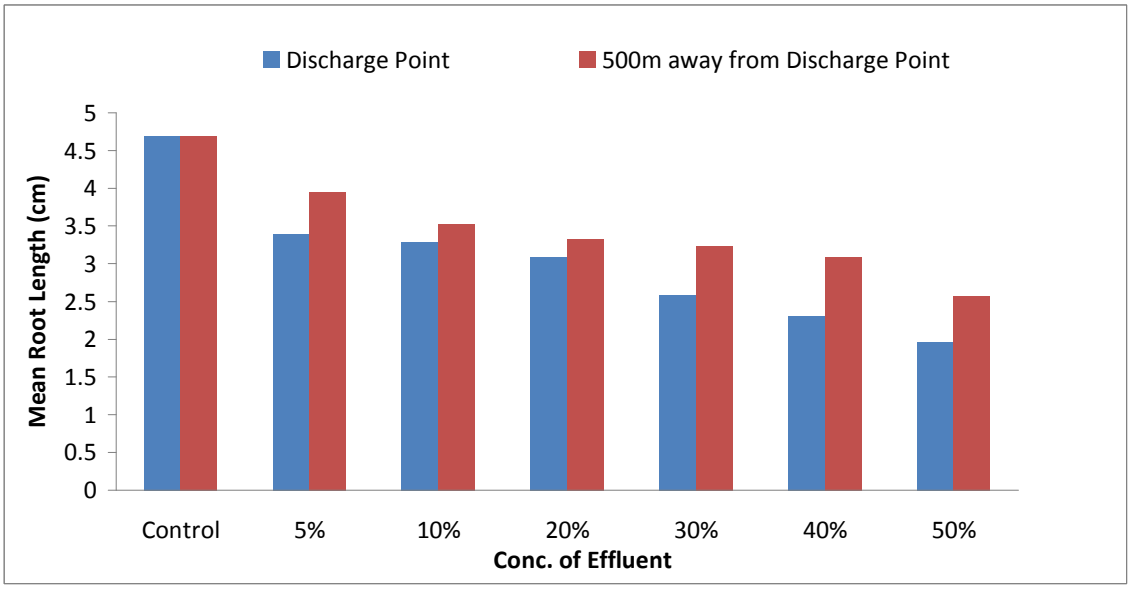

Fig. 1: Root growth of onion bulb cultivated in WRPC effluents at discharge point and 500m downstream at different concentrations.

Table 2: Effects of WRPC effluents on mitotic indexes and % aberrant cells in *A. cepa* root tips

Effluent concentration (%)	Mitotic Index (MI)±SE		% Aberrant cells±SE*	
	Discharge Point	500m Downstream	Discharge Point	500m Downstream
Control	65.2 ±0.70	65.2 ±0.70	0.0 ± 0.00	0.0 ± 0.00
5	59.8 ± 0.81	61.0 ± 0.81	5.0 ±0.52	4.5 ± 0.52
10	54.8 ± 0.78	55.6 ± 0.78	7.7 ± 0.55	6.8 ±0.55
20	53.6 ± 0.74	54.4 ± 0.74	8.9 ±0.45	7.7 ± 0.45
30	50.2 ± 0.70*	51.6 ± 0.70*	10.7 ± 0.53	9.3 ±0.53
40	48.6 ± 0.73*	49.8 ± 0.73*	12.3 ± 0.51	10.8 ± 0.51
50	46.0 ±0.71*	47.0 ± 0.71*	14.3 ±0.52	12.8 ± 0.52

Values are expressed as Mean±S.E.
*Means are statistically different from the controls at P<0.05

The types of chromosomal aberrations induced in the refinery wastewaters were sticky chromosomes, disoriented chromosomes, vagrants, bridges and micronuclei, the most frequent aberrations being sticky chromosomes. The mean values of the mitotic indexes (MI) and percentage aberrations obtained from WRPC effluents at the discharge point and 500m downstream at various concentrations are presented in Table 2. The results showed that, compared to the control, 30, 40 and 50% WRPC effluents at the point of discharge caused significantly lower MI while significantly higher % aberrant cells were recorded at all effluent concentrations. Similarly, the mitotic indexes of the effluent at the discharge point were lower than those downstream at each concentration. In the same vein, % aberrant cells of effluents at the discharge point were higher than those downstream and these were concentration dependent.

The results of this study have also established that the wastewaters caused the onset of genotoxicity. The effluents inhibited root growth indicating toxicity (Swierenga *et al.*, 1991). The presence of heavy metals in industrial wastewaters have been analysed in *A. cepa* genotoxicity test and found to induce significant chromosome aberrations (Nelson and Rank, 1998). Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Aberrations induced were stickiness, vagrants, breaks, bridges. Stickiness reflects high toxicity of a substance as well as irreversibility of the change (Turkoglu, 2007) while acentric fragments in anaphase is the result of chromosome or chromatids interruptions indicating interference with DNA while bridges probably occur by the interruption and joining chromosomes or chromatids or as a result of chromosome stickiness, or it may be ascribed to unequal translocation or inversion of chromosome segments (Turkoglu, 2007, Gömürgen, 2005). Bridges and fragments are clastogenic effects, both resulting from chromosomal and chromatid breaks (Kovalchuk *et al*. 1998). Vagrants arise as a result of irregular separation and dislocation of chromosomes; thereby constituting a risk of aneuploidy (Maluszynska and Juchimiuk, 2005).

The results of antioxidative enzyme responses in the WRPC wastewater at different concentrations are presented in Figs. 1 – 4. The activities of SOD, CAT and GSH-Px were all found to decrease in the wastewater both at the point of discharge and 500 m downstream and these were statistically significant ($P<0.05$).

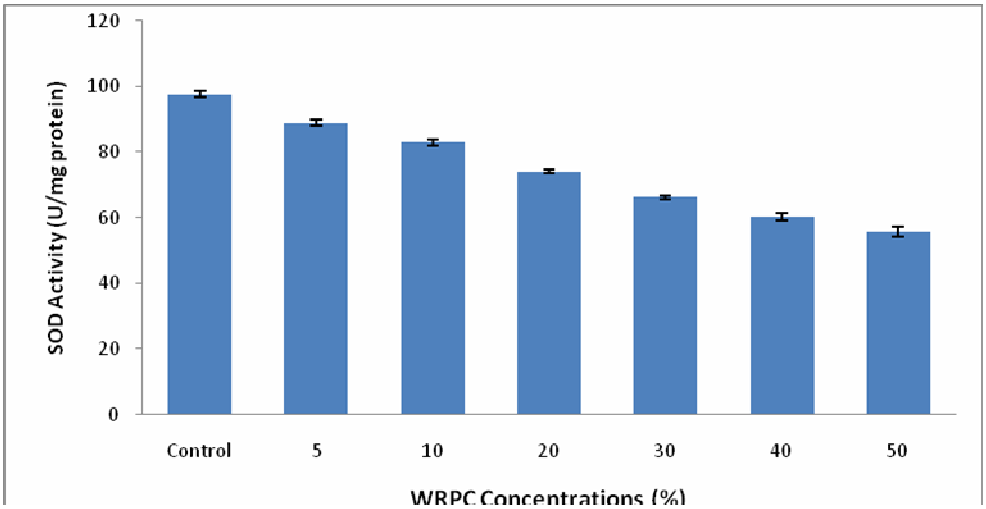

Fig. 2: Changes in SOD activity in *Allium* roots cultivated in WRPC effluents at different concentrations

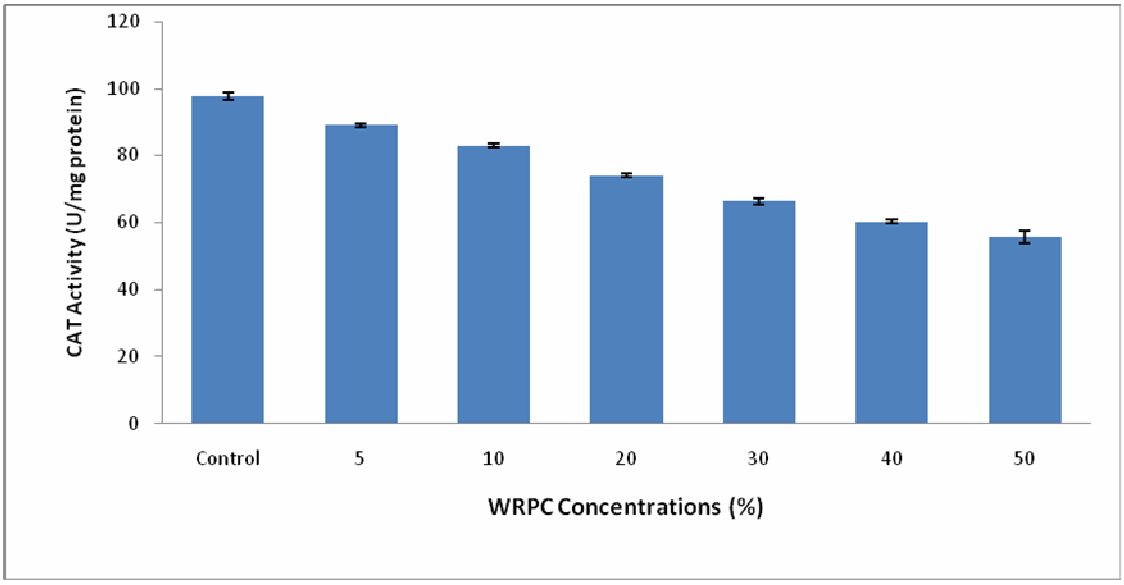

Fig. 3: Changes in Catalase activity in *Allium* roots grown WRPC effluents at different concentrations

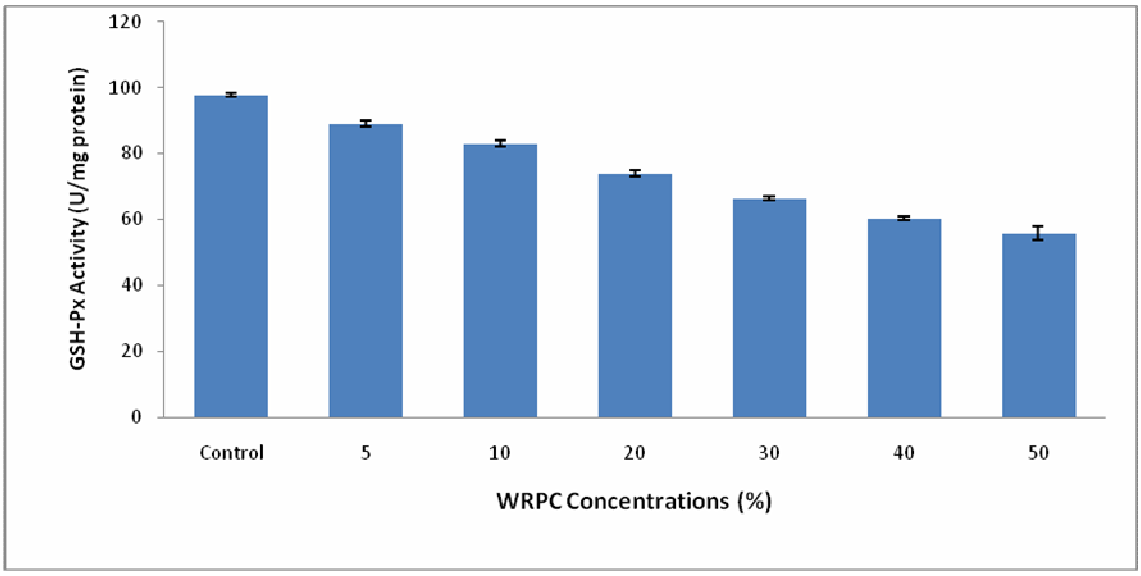

Fig. 4: Changes in GSH-Px activity in *Allium* roots grown WRPC effluents at different concentrations

The activities of SOD, CAT, and GSH-Px have been extensively used to monitor the development and extent of damage due to oxidative stress (Spronck and Kirkland, 2002, Fatima and Ahmad, 2005, Olorunfemi and Lolodi, 2011). The decreased activities of main antioxidant enzymes in this study are consistent with these reports. These have been attributed to high levels of and interactions of some heavy metals like Pb, Fe, Cd, Ni, Cr or Cu which

could lead to the formation of DNA strand breaks (Moriwaki *et al*., 2007). Such increases in oxidative stress have been repoted to cause DNA damage (Nagy *et al*., 2005).

In conclusion, the results obtained from the *Allium cepa* assay shows that it is a reliable tool for first-tier rapid environmental risk assessment monitoring. Decreased activities of SOD, CAT, and GSH-Px also show that WRPC wastewater induced oxidative stress and chromosomal aberrations in *A. cepa* model and needs to be properly treated. The relevant regulatory bodies should take urgent steps to safeguard the inhabitants of Ubeji community who are presently exposed to WRPC effluents which are indiscriminately discharged into the environment as exposure of the wastewater could lead to adverse health effects for exposed human and animal populations.

ACKNOWLEDGEMENTS: The authors are grateful to Geirid Fiskesjö for literature materials donated towards *Allium cepa* L. research in our Cytogenetics and Environmental Mutagenesis Research Laboratory. We also thank Miss Sandra Isibor for her assistance with wastewater sample collection.

References

American Public Health Association, APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association, Washington DC, 1220p.

Bakare, A.A., Pandey, A.K., Bajpayee, M., Bhargav, D., Chowdhuri. D.K., Singh, K.P., Murthy, R.C. and Dhawan, A. 2007. DNA damage induced in human peripheral blood lymphocytes by industrial solid waste and municipal sludge leachates, *Environmental and Molecular Mutagenesis, 48: 30-37*.

Bolognesi, C., Landini, E., Roggieri, P., Fabbri, R. and Viarengo, A. 1999. Genotoxicity biomarkers in the assessment of heavy metal effects in mussels: Experimental studies. *Environmental and Molecular Mutagenesis, 33: 287-292*.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. *Methods in Enzymology, 52: 302-310.*

Carlberg, I. and Mannervik, B. 1972. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *Journal of Biological Chemistry, 250: 5475–5480*.

Cerna, M., Hajek, V., Stejskalova, E., Dobia, L., Zudova, Z. and Rossner, P. 1991. Environmental genotoxicity monitoring using *Salmonella typhimurium* strains as indicator system, *Science of the Total Environment, 101: 139–147*.

Cohen, G., Dembiec, D. and Marcus, J. 1970. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry 34: 30-38*.

Durgo, K., Orešcanin, V., Luliç, S., Kopjar, N., Želježiç, D. and Coliç, J.F. 2009. The assessment of genotoxic effects of wastewater from a fertilizer factory. *Journal of Applied Toxicology, 29: 42–51*.

Eriyamremu, G.E., Asagba, S.O., Akpoborie, A. and Ojeaburu, S.I. 2005. Evaluation of lead and cadmium levels in some commonly consumed vegetables in the Niger-Delta oil area of Nigeria. *Bulletin of Environmental Contamination and Toxicology, 75: 278 – 283.*

Fatima, R.A., and Ahmad M., Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. *Science of the Total Environment, 346: 256-273.*

Fiskesjö, G. 1985. The Allium test as a standard in environmental monitoring. *Hereditas, 102: 99-112*.

Gana, J.M., Ordonez, R., Zampini, C., Hidalgo, M., Meoni, S. and Isla, M.I. 2008. Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina. *Journal of Hazardous Material, 155: 403-406.*

Gömürgen, A.N 2005. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tip of *Allium cepa* L. *Cytologia, 70: 119-128*.

Grant, W.F. 1978. Chromosome aberrations in plants as a monitoring system. *Environmental Health Perspectives, 27: 37-43*.

Grant, W.F. 1982. Chromosome aberration assays in *Allium*: A report of the U.S EPA Gene Tox. Program. *Mutation Research, 99: 273 -291*.

Hallenbeck, W.H. 1986. Human health effects of exposure to cadmium. *Experientia Supplement, 50: 131-137*.

Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stiochiometry of fatty acid peroxidation. *Archives in Biochemistry and Biophysics, 25: 189-198.*

Hoshina, M.M. and Marin-Morales, M.A. 2009. Micronucleus and chromosome aberrations induced in onion (*Allium cepa*) by a petroleum refinery effluent and by river water that receives this effluent. *Ecotoxicology and Environmental Safety, 72(8): 2090–2095*.

Kovalchuk, I. and Kovalchuk, O. 2008. Transgenic plants as sensors of environmental pollution genotoxicity. *Sensors, 8:1539-1558*.

Kovalchuk, O., Kovalchuk, I., Arkhipov, A., Telyuk, P., Hohn, B. and Kovalchuk, L. 1998. *Allium cepa* chromosome aberration test reliably measures genotoxicity of soils of inhabited areas of the Ukraine contaminated by the Chernobyl accident. *Mutation Research, 415: 47-57*.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin-Ciocalteu phenol reagent. *Journal of Biological Chemistry, 193: 265-275*.

Maluszynska, J. and Juchimiuk J. 2005. Plant genotoxicity: A molecular cytogenetic approach in plant bioassays. *Arh Hig Rada Toksikol, 56: 177-84.*

Misra, H. and Fridovish, I. 1972. The role of superoxide anion I. The autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry, 247: 3170*

Moriwaki, H., Osborne, M.R. and Phillips, D.H. 2007. Effects of mixing metal ions on oxidative DNA damage mediated by a Fenton-type reduction, *Toxicology In Vitro, 22: 36-44.*

Muchuweti, M., Birkett, J.W., Chinyanga, E., Zvauya, R., Scrimshaw, M.D. and Lester, J.N. 2006. Heavy metal content of vegetables irrigated with mixture of waste water and sewage sludge in Zimbabwe: implications for human health. *Agriculture Ecosystem and the Environment, 112: 41 – 48*.

Nagy, E., Johansson, C., Zeisig, M. and Moller, M. 2005. Oxidative stress and DNA damage caused by the urban air pollutant 3-NBA and its isomer 2-NBA in human lung cells analyzed with three independent methods", *Journal of Chromatography. B: Analytical Technologies in the Biomedical and Life Sciences, 827: 94–103.*

Nduka, J.K. and Orisakwe, O.E. 2009. Effect of effluents from Warri Refinery Petrochemical Company WRPC on water and soil qualities of "Contiguous host" and "Impacted on communities" of Delta State, Nigeria. *The Open Environmental Pollution & Toxicology Journal, 1: 11-17.*

Olorunfemi, D.I. and Lolodi, O. 2011. Effect of cassava processing effluents on antioxidant enzyme activities in *Allium cepa* L. *Biokemistri, 23(2): 49-61*.

Olorunfemi, D.I., Okoloko, G.E., Bakare, A.A. and Akinboro, A. 2011. Cytotoxic and genotoxic effects of cassava effluents using the *Allium cepa* test, *Research Journal of Mutagenesis, 1: 1-9*.

Plewa, M.J. 1978. Activation of chemicals into mutagens by green plants: A preliminary discussion. *Environmental Health Perspectives, 27: 45-50*.

Rank, J. and Nielsen, M.H. 1998. Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telophase chromosome aberration assays. *Mutation Research 418: 113 – 119.*

Rodrigues, F.P., Angeli, J.P.F., Mantovani, M.S., Guedes, C.L.G. and Jordão, B.Q. 2009. Genotoxic evaluation of an industrial effluent from an oil refinery using plant and animal bioassays. *Genetics and Molecular Biology, 32: 369-372*. (Assessed online).

Sang, N. and Li, G. 2004. Genotoxicity of municipal landfill leachate on root tips of *Vicia Faba*, *Mutation Research, 560: 159–165*.

Satarug, S., Haswell-Elkins, M.R. and Moore, M.R. 2000. Safe levels of cadmium intake to prevent renal toxicity of human subjects. *British Journal of Nutrition, 84: 791 – 802.*

Sharma, C.B.S.R. 1983. Plant meristems as monitors of genetic toxicity of environmental chemicals. *Current Science, 52: 1000-1002*.

Snow, E.T. 1992. Metal carcinogenesis: mechanistic implications. *Pharmacology and Therapeutics, 53: 31– 65.*

Spronck, J.C. and Kirkland, J.B. 2002. Niacin deficiency increases spontaneous and etoposide induced chromosomal instability in rat bone marrow cells *in vivo*, *Mutation Research, 508, 83-97.*

Swierenga, S.H., Heddle, J.A., Sigal, E.A., Gilman, J.P., Brillinger, R.L., Douglas, G.R. and Nestmann, E.R. 1991. Chromosome aberrations and sister-chromatid exchange in Chinese hamster ovary. *Mutation Research, 24: 301-322.*

Turkoglu S. 2007. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. *Mutatation Research, 626: 4-14.*

United States Environmental Protection Agency (USEPA) 1999. National recommended water quality criteria – Correction: EPA 822/Z-99-001, USEPA, Washington DC.

Uzoekwe, S. A. and Oghosanine, F.A. 2011. The effect of refinery and petrochemical effluent on water quality of Ubeji creek Warri, Southern Nigeria. *Ethiopian Journal of Environmental Studies and Management, 4(2): 107-116.*

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 106: 1–40.*

Vig, B.K. 1978. Somatic mosaicism in plants with special reference to somatic crossing over. *Environmental Health Perspectives, 27: 27-36*