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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (17): 821-827, 2010
ISSN 1028-8880

Influence of Humic Acid Derived from Composted Wastes of Nigeria Origin on Oxidative and Antioxidant Status of African Mud Catfish (*Clarias gariepinus*)

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Abstract: The utilization of organic fertilizer, a product of composted organic wastes, is being advocated in Nigeria as a solid waste management alternative. The application of such products near surface water could increase organic matter load of the aquatic environment, thus increasing the humic substance concentration that influence metal speciation and bioavailability in water. In this study, Humic Acid (HA), a major humic substance fraction, derived from composted organic wastes of Nigeria origin was evaluated for effects on the oxidative and antioxidant status of African Mud Catfish (*Clarias gariepinus*) exposed to different HA (100, 250, 500 and 1000 mg L^{-1}) concentrations in static water culture. Lipid peroxidation was estimated via malondialdehyde (MDA) using thiobarbituric acid assay while oxidative stress was assessed spectrophotometrically, via superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) using standard enzymatic assay techniques. Results showed that treatments increased MDA by 20 to 70% but decreased SOD, CAT and GSH by 10 to 42.56%, 43.62 to 64.09% and 9.84 to 67.68%, respectively. Negative coefficient (r) was obtained for CAT ($r = - 0.491$; $p > 0.10$), GSH ($r = - 0.551$; $p > 0.10$) versus HA concentration but correlation was positive for MDA ($r = 0.998$; $p = 0.012$) and the latter. Study revealed humic acid-mediated oxidative stress and lipid oxidation in the fish. The adverse impact was a function of humic acid concentration and an assessment of heavy metal-humic acid mixture effect on the oxidative and antioxidant status of fresh water fish is recommended.

Key words: Bioassessment, biochemical analysis, catfish, composted waste, humic acids

INTRODUCTION

Pollution of the aquatic environment is a global problem but is heightened in the developing countries including Nigeria, where wastes are willfully disposed into water bodies such as freshwater ecosystem. Chemical pollutants that find entry into fresh water are mainly industrial in source. However, there are natural compounds, though not classified as toxicants, on entry into the water influence a wide variety of chemical reactions affecting the behavior of environmental pollutants. A typical example is organic matter, with emphasis on the humic substance component. Humic substances (essentially humic acid and fulvic acids) are refractory organic substances consisting of complex aromatic polymers and oxygenated functional groups that chelate with dissolved pollutants in water and enhance their assimilation or uptake by organisms (fauna and flora) (Stevenson, 1994).

Of recent in Nigeria, composting of organic solid waste materials is seen as an economically feasible waste management option and the resulting solid and/or liquid fertilizers are utilized in organic farming and horticultural purposes for urban greening in order to boost food security, combat global warming and consequent climate change (Adekunle, 2009, 2010). These organic fertilizers, similar to peat, lignite, soil organic matter, sediment, inherently contain humic acid. On application of the fertilizers to the soil, processes such as stormwater, irrigation and rainfall leaching, could transfer some humic substance fractions to the surrounding water bodies, thereby, adding to the existing dissolved humic matter in the receiving waters (Matthews *et al*., 1995; Paul and Jayakumar, 2010).

Nigerian agriculture is mainly rain fed, especially in the southern part of the country. There are two main cropping seasons in the country based on early and late rainfall seasons. To facilitate the continual cultivation in

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order to exploit the dry season and to increase farmer income potential, government initiated the small scale low cost farmer managed irrigation scheme to develop Fadama lands (flood plains). The National Fadama Development Programme (NFDP) was to assist the qualifying states of the Federation through the World Bank supported Agricultural Development Projects (ADP) to among others, finance constructing fadama infrastructures, organizing fadama farmers for irrigation management (Afolabi, 2010). Fadama farming in the southern part of the country is prevalent along river banks and other wetland areas and the crops mainly grown are vegetables. The utilization of organic fertilizer in this farming practice could increase organic matter load of the aquatic environment, thus increasing the humic substance concentration that influence metal speciation and bioavailability in water. It is therefore necessary to assess the safety of aquatic organisms exposed to compost-derived humic substances.

Bio-analytical risk assessment procedures involve the use of biomarkers such as indices of oxidative stress and lipid peroxidation (Cheng *et al*., 1999; Osman *et al*., 2009), which include malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT). Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene ($-CH_2-$) groups that possess reactive hydrogens. Elevated concentration of MDA indicates lipid peroxidation (Knight *et al*., 2003).

Superoxide dismutases are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids, being important antioxidant defense in nearly all cells exposed to oxygen. Simply put, SOD outcompetes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. Hence, high levels of free radicals can cause damage to SOD (Alscher *et al*., 2002; Rajaraman *et al*., 2008).

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. Glutathione has multiple functions: It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms. Through direct conjugation, it detoxifies many xenobiotics (foreign compounds) and carcinogens, both organic and inorganic (Johansen *et al*., 2005). Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide oxygen. Catalase has one of the highest turnover numbers of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Rajaraman *et al*., 2008).

When pollutants such as xenobiotic molecules enter the body, they undergo redox cycling and generate Reactive Oxygen Species (ROS). The body will produce more antioxidant enzymes such as SOD and CAT to get rid of the undesired ROS. This response is also called induction of antioxidant enzymes. When the generation of ROS overwhelms the antioxidant system, damages to lipids, proteins and DNA occur and this condition is referred to as oxidative stress. Under normal conditions, an elevation in oxidative stress rapidly induces various antioxidant defenses (particularly antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase) that quickly reduce the stress. These have the potential to help establish pathogenic stages of and risk for disease (Johansen *et al*., 2005; Rajaraman *et al*., 2008; Klaunig *et al*., 2010).

There are no readily available data on the toxicological effect(s) of HA isolated from composted organic wastes of Nigeria origin to aquatic organisms such as catfish (*Clarias gariepinus*), a commonly consumed fresh water organism in Nigeria, which is a significant dietary protein source. The present study, therefore, examined the influence of humic acid derived from composted organic wastes of Nigeria origin on the oxidative and antioxidative status of *C. gariepinus* in static water culture, using biomarkers SOD, CAT, MDA and GSH.

MATERIALS AND METHODS

This research project was conducted from June 2008 to January 2009.

Exposure of fish to compost derived humic acid: Source separated municipal solid wastes generated from Abeokuta city, Nigeria, consisting largely of vegetable matter, crop and food residues were composted for 75 days via in-vessel technique after the procedures of

(Adekunle, 2009, 2010). Humic acid was extracted from the cured product using alkaline method, purified and characterized using the method of (Adekunle and Onianwa, 2001; Adekunle *et al*., 2007a) with slight modification. Prognostic markers of oxidant and antioxidant status: malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) activities and glutathione concentration (GSH) of *C. garipinus* exposed to different concentrations of HA (0, 100, 250, 500 and 1000 mg L^{-1}) in controlled water culture for 45 days, were determined using standard clinical techniques.

Twenty five juvenile fishes of average weight and length 43.2±0.5 g and 18.6±0.8 cm, respectively, fed with 2 mm copen fish feed, were utilized for the experiment at 5 per aquarium of 45 L capacity. Temperature, electrical conductivity, nitrate, phosphate, dissolved oxygen, total hardness and alkalinity of the process water were also analyzed using standard procedures reported in Adekunle *et al*. (2007b, c).

Blood sample collection: At the end of the exposure period, the fishes were removed from water and directly placed in clove oil to make them inactive. Blood samples were then taken from the caudal vein by heparinized plastic syringe, transferred to heparinized tubes and centrifuged at 4, 000 rpm for 8 min and the resulting plasma was stored by refrigeration at -18°C.

Biochemical analysis: Prognostic markers of oxidant and antioxidant status: malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) activities and glutathione concentration (GSH) of *C. garipinus* were measured spectrophotometrically, using standard clinical techniques via Randox kit supplied by Randox Laboratories Ltd. UK. Lipid peroxidation was estimated via MDA using thiobarbituric acid assay while oxidative stress was assessed via SOD, GSH and CAT using standard enzymatic assay techniques (Buege and Aust, 1978; Clairborne, 1986; Das *et al*., 2000).

Assessment of lipid oxidation activity: The extent of lipid peroxidation was estimated in terms of Thiobarbituric Reactive Species (TBA) using malondialdehyde (MDA) as by the method of (Buege and Aust, 1978). Exactly 1 ml of plasma was added to 2.0 mL of the TCA-TBA-HCl reagent consisting of 15% (w/v) TCA, 0.375% (w/v) TBA and 0.25 M HCl. The content was boiled for 15 min, cooled and centrifuged at 4000 rpm for 8 min. The absorbance was then read at 535 nm.

Assessment of superoxide dismutase activity: This assay was carried out according to the procedure of Das *et al*. (2000) in which 1.4 mL aliquot of the reaction mixture comprising 1.1 mL of 50 mM phosphate buffer (pH = 7.4), 0.075 mL of 20 mM L-methionine, 0.4 mL of 1% (v/v) Triton X-100, 0.075 mL of 10 mM hydroxylamine and 0.1 mL of 50 mM EDTA. This aliquot (1.4 mL) was added to 0.1 mL of the plasma and incubated at 30°C for 5 min. This was followed by the addition of 80 μL of 50 μM riboflavin and the tubes were exposed for minutes to 200 watts Philip lamps. After the exposure time, 1 mL of Greiss reagent (mixture of equal volume of 1% sulphamlamide in 5% phosphoric acid) was added and the absorbance of the colour formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrate formation under the assay condition.

Assessment of catalase activity: Catalase activity was assayed using the method described by Clairborne (1986). This method is based on the ability of catalase to oxidize hydrogen peroxide. Catalase activity was therefore measured spectrophotometrically by observing the decrease in light absorption at 240 nm during decomposition of H_2O_2 by the enzyme. To this effect, exactly 40 μL of the plasma was added to 1 mL of 5 mM phosphate buffer pH 7.0. Then 1.0 mL of 30% H_2O_2 was added to the buffered serum to initiate the reaction. The change in the absorbance for 1 min at 10 sec interval was recorded at 240 nm. One unit activity is defined as the amount of enzyme catalyzing the formation of water and oxygen from hydrogen peroxide under assay conditions. Catalase activity (unit/ mg protein) = (Change in absorbance per minute x 100 x D.F)/ 43.6 x 2, where 43.6 is the molar absorptivity index for H_2O_2 at 240 nm and D.F is the dilution factor.

Assessment of glutathione: Glutathione was assayed using the method described by (Boyne and Ellman, 1972). Exactly 0.02 mL of plasma was thoroughly mixed with 9 mL of 50% acetone -water (1:1) mixture and 1 mL of 0.1 M phosphate buffer pH 8.0. The mixture (3 mL) was read at 420 nm as blank. Another 3 mL of the mixture was added to 0.02 mL of 0.01 M DTNB (5, 5'-dithio-bisnitrobenzoic acid). This was mixed thoroughly and left for 2 min for colour to develop. The absorbance was read at 420 nm within 20 min of preparation.

Statistical analysis: Data were subjected to descriptive statistics, one-way analysis of variance and Pearson

Correlations, using SPSS 16.0 for Windows®, to calculate mean±standard deviation, establish significant variations and relationships, respectively.

RESULTS AND DISCUSSION

Results

Water quality and humic acid characteristics: The quality of the process water: 27°C for temperature, 6.4 for pH, 20 mg L^{-1} for nitrate, 154 g L^{-1} for hardness, 80 mg L^{-1} for alkalinity. The chemical characteristics of the humic acid used in this study revealed functional groups whose absorption bands were at regions 3320 to 3470 cm^{-1} for H-bonded OH, N-H; 2471 - 2320 cm^{-1} for C-N multiple bond; 1721 to 1631 cm^{-1} for carbonyl (C=O) of carboxylic acid, ketone, aldehyde and quinine; 2897 cm^{-1} for C-H of hydrocarbon skeleton; 1591 cm^{-1} for C-H of substituted benzene and 1102 cm^{-1} for C-O bonds. Quantitatively, the humic acid total acidity (3,300 cmol kg^{-1}) consisted of 86.67% COOH (2,860 cmol kg^{-1}) and 13.33% OH (440 cmol kg^{-1}) and the E_4/E_6 ratio was obtained as 4.08.

Malondialdehyde concentration: Malondialdehyde (MDA) concentrations, presented in Fig. 1, showed that when compared to the control group, the MDA levels on exposure to 100 and 250 mg L^{-1} of HA in water, were found to be statistically insignificant at $p = 0.05$. Increased concentration of HA (500 and 1000 mg L^{-1}) gave significant variation from the control value at $p<0.05$. Relative to the control, MDA increased by 20% for 100 and 250 mgHA L^{-1}, 40 and 70% for 500 and 1000 mgHA L^{-1}, respectively. Correlation showed positive relationship between MDA level and HA concentration ($r = 0.998$; $p = 0.012$). This implies increasing MDA level with HA concentration in water.

Superoxide dismutase activity: The mean level of dismutase activity (Fig. 2) in the control was 0.21±0.08 unit mg^{-1} protein. The level (0.12±0.01 to 0.19±0.04 unit mg^{-1} protein) found in the test groups, were less when compared to the control. The highest depletion of SOD, relative to the control was obtained at 100 mgHA L^{-1} while the least was at 500 mgHA L^{-1} (42.56% for 100, 14.29% for 250, 10% for 500 and 28.57% for 1000). Hence, correlations gave positive, though not significant ($r = 0.16$; $p>0.10$) relationship for SOD and HA concentration, suggesting that the depletion of SOD by HA relatively reduced at raised concentration of the latter.

Glutathione concentration and catalase activity: The mean glutathione level (1.93±0.61 unit mg^{-1} protein) in the control fish group was high when compared to the mean values (0.59±0.12 to 1.74± 0.15 unit mg^{-1} protein) in the test group (Fig. 3). Significant variation at $p<0.05$ was only found between the control value and those obtained at 250, 500 and 1000 mg HA L^{-1}. The decrease, relative to the control, ranged from 43.20 to 78.52%. Correlation gave negative coefficient (-0.551; $p>0.10$), suggesting decreasing GSH concentration with increasing HA concentration in water. The mean catalase activity (2.98±1.24 unit/mg protein) in the control fish group was significantly ($p<0.05$) high when compared to the mean values (0.64±0.13 to 1.68± 0.33 unit mg^{-1} protein) obtained in the exposed population (Fig. 4). Reductions, relative to the control, were obtained as 9.84, 67.68, 50.26 and 55.44%

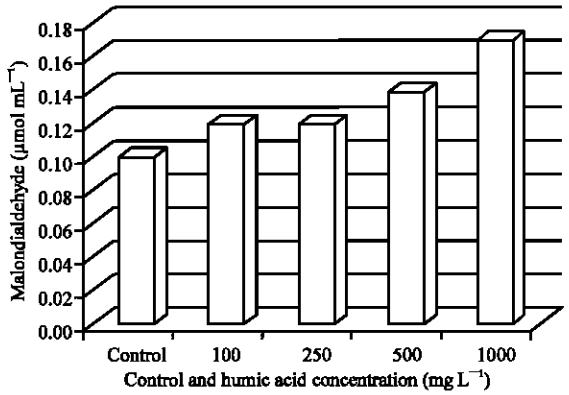

Fig. 1: Variation in malondialdehyde (MDA) concentration in *C. graiepinus* on exposure to varying HA concentrations in water

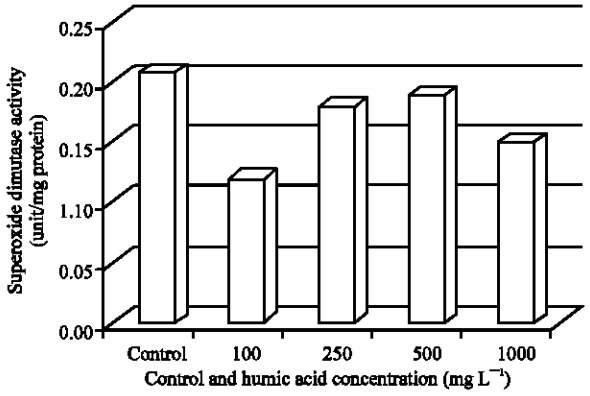

Fig. 2: Variation in superoxide dismutase activity in *C. gariepinus* on exposure to varying HA concentrations

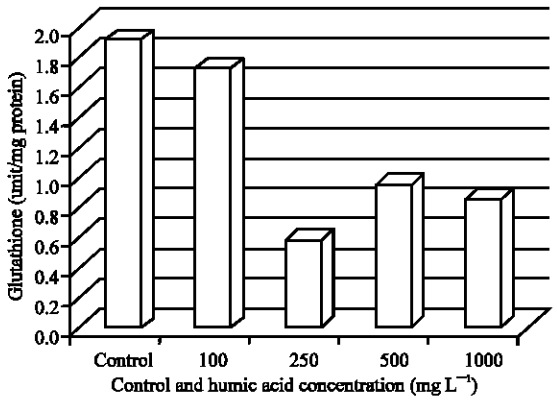

Fig. 3: Variation in glutathione concentration in *C.gariepinus* on exposure to varying HA concentrations in water

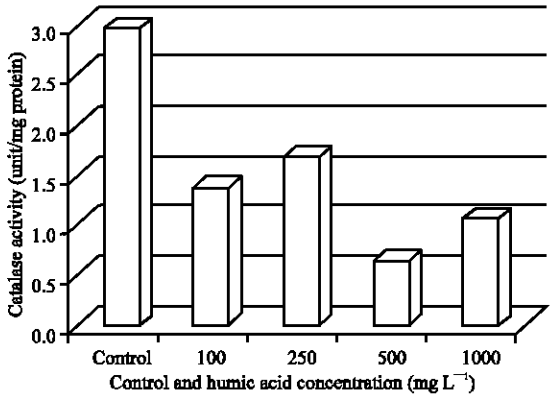

Fig. 4: Variation in catalase activity in *C.gariepinus* on exposure to varying HA concentrations in water

for 100, 250, 500 and 1000 mgHA L^{-1}, respectively. Correlation gave negative coefficient (-0.491; $p>0.10$), suggesting decreasing CAT activity with increasing HA concentration in water.

Inter-biomarker ratio: Results showed that the biomarker ratios for the control groups differed from test population. The inter-biomarker ratios were lower in the exposed groups when compared with the control with the exception of CAT/GSH, at 250 mgHA L^{-1}. For instance, the CAT/GSH ratio was 1.544 for control and 0.667 to 2.847 for the exposed fish. CAT/SOD was 14.90 for the control but ranged from 3.368 to 11.500 for the test group. Again, CAT/MDA was 29.800 for the control and varied from 4.571 to 14.000 in the test population. The concentration of GSH relative to SOD in the control was 9.190 but varied from 0.145 to 5.733 in the test group. The ratio of GSH/MDA was 19.320 in the control but was found in test population to vary from 4.917 to 14.500. Finally, SOD/MDA ratio in the control was 2.100 but ranged from 0.882 to 1.500 in the exposed fish samples.

DISCUSSION

The quality of process water used was suitable for aquaculture (Adekunle *et al*., 2007b). The increased concentration of malondialdehyde, depletion of superoxide dismutase, catalase activity and glutathione concentrations in the test fish population, relative to the control group, on exposure to HA, as was found in this study, were predictive of lipid peroxidation and oxidative stress. This is an indication of imbalance in the generation and removal of oxygen radical species in the test fish. The changes in the biomarker ratios in the test group relative to the control indicate alteration in the fish physiological constitution.

The content of oxo-functional group in the humic acid used in this study is similar to the reports of Adekunle and Onianwa (2001) and Adekunle *et al*. (2007a) as well as with report elsewhere that humic substances have a relatively high content of oxygen rich functional groups, presumably of the semiquinone type, which are more prominent in humic acids than the other components (Balarezo *et al*., 2002). Cooper *et al*. (1989), reported that absorption of light by humic substances can initiate a number of photochemical processes, producing peroxy radicals and hydroxyl radicals as well as hydrated electrons, hydrogen peroxide, singlet oxygen and superoxide. These chemical species can promote redox reactions, which explains the depletion of SOD, CAT and GSH. In addition, the oxidizing species could have enhanced the level of MDA in the exposed fish population in this study. Cheng *et al*. (1999) reported that HA initiated oxidative stress on red blood cells, resulting in their dysfunction and Ho *et al*. (2003) reported that humic acid promoted lipid peroxidation.

The result on relative reduction of SOD depletion with increased HA concentration was attributed to the possibility of humic acid reducing the availability of pollutants at elevated concentration. Humic substances can function as surfactants with the ability to bind both hydrophobic and hydrophilic materials. This function in combination with their colloidal properties, makes HAs effective agents that control the bioavailability of both organic and inorganic contaminants (Balarezo *et al*., 2002). Humic substances are major controlling materials for metal speciation, pollutant binding and nutrient availability (Samamidon *et al*., 1991). It is possible that at increased concentration, HA played a protective role of detoxification of pollutants from the fish body via chelation, which is consistent with the report of

Osman *et al.* (2009), who treated fish (*O. niloticus*) exposed to Cd with 15, 30 and 50 mg L^{-1} of humic acid in the pond water and reported that 15 mg HA L^{-1} had no significant effect on the Cd exposed fish while treatment with 30 and 50 mg L^{-1} humic acid significantly reversed the effects of the cadmium toxicity.

The reduced E_4/E_6 ratio (<5.0), obtained in this study, indicate condensed molecular fragment, indicating the presence of benzenoid structures. Carboxyl (COOH) and hyroxyl (OH) groups attached to benzene (aromatic) hydrocarbons are considered to be stronger chelating agents (Stevenson, 1994). By increased aromaticity, humic substances in aquatic ecosystems have been shown to be important in influencing the bioavailability, toxicity and fate of organic xenobiotics (Stevenson, 1994; Matthews *et al*., 1995). Carboxylic group predominated in the humic acid from this study, unlike the predominance of hydroxyl obtained in the reports of Adekunle *et al.* (2007a) for soil humic acid. The dominance of COOH functional group in the compost derived humic acid relative to OH group suggest the possibility of chelation potential with any metallic ion present in the water.

Farombi *et al.* (2007) recorded alteration in the antioxidant enzymes, glutathione system and induction of lipid peroxidation in *C. gariepinus* from Nigeria Ogun river, which contained Zn, Cu, Pb, As, Cd that accumulated in the liver, kidney, gills and heart; pointing to the heavy metals as causative factors. Most water samples collected from pond, hand-dug wells, boreholes, fresh water, utilized for aquaculture in Nigeria have been reported to contain elevated heavy metal concentrations (Farombi *et al*., 2007; Adekunle *et al*., 2007b, c). The water used in this study was from a borehole (to reflect the common practice) and contained Pb at up to 5 mg L^{-1}. The identified COOH and OH groups in the humic acid molecular structure, if attached to benzene structure, could make the compost derived humic acid play a significant role in the migration and redistribution of potentially toxic metal ions from the water into the fish body, resulting in lipid peroxidation and oxidative stress.

Consequently, the results obtained from this study were attributed to two possibilities: (i) enhancement of pollutant- induced oxidative stress and lipid peroxidation by humic acid or (ii) solely humic acid-mediated lipid peroxidation and oxidative stress. Based on the results from this study, the application of excess composted organics in farms located close to surface water bodies poses risk to aquatic life. This is of importance in terms of repeated applications which has the potential to increase organic matter load of the aquatic system, especially in standing waters.

CONCLUSIONS AND RECOMMENDATION

Results revealed that humic acid isolated from composted organic wastes of Nigeria origin at concentration range 100 to 1000 mg L^{-1} induced lipid peroxidation and oxidative stress. The adverse impact was found to be concentration dependent. Assessment of heavy metal-humic acid mixture effect, at an environmentally realistic range for dissolved organic matter in water (<100 mg L^{-1}), is recommended.

ACKNOWLEDGMENT

The authors acknowledge Prof. G.N.O Ezeri of the Department of Fisheries and Aquaculture Management, College of Environmental Resources Management, University of Agriculture, Abeokuta for his valuable advice on fish sample procurement and feeding protocol.

REFERENCES

Adekunle, I.M. and P.C. Onianwa, 2001. Functional group characteristics of humic and fulvic acids extracted from some agricultural wastes. J. Sci., 35: 15-19.

Adekunle, I.M., T.A. Arowolo, N.P. Ndahi, B. Bello and D.A. Owolabi, 2007a. Chemical characteristics of humic acids in relation to lead, copper and cadmium levels in contaminated soils of southwest Nigeria. Annals Environ. Sci., 1: 23-34.

Adekunle, I.M., T.A. Arowolo, I.T. Omoniyi and O.T. Olubambi, 2007b. Risk assessment of nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) exposed to cassava effluent. Chem. Ecol., 23: 283-292.

Adekunle, I.M., M.T. Adetunji, A.M. Gbadebo and O.P. Banjoko, 2007c. Toxicological assessment of groundwater quality in a typical rural settlement of south west Nigeria. Int. J. Environ. Res. Public Health, 4: 307-318.

Adekunle, I.M., 2009. Temperature effect on water extractability of cadmium, copper, lead and zinc from composted organic solid wastes of South-West Nigeria. Int. J. Environ. Res. Public Health, 6: 2397-2407.

Adekunle, I.M., 2010. Evaluating environmental impact of Nigerian composted wastes using laboratory extraction test. Environ. Eng. Manage. J., 9: 721-729.

Afolabi, J.A., 2010. Fadama farming as an environment friendly and viable enterprise in Ondo State, Nigeria. J. Hum. Ecol., 30: 93-97.

Alscher, R.G., N. Erturk and L.S. Heath, 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot., 53: 1331-1341.

Balarezo, A.L., V.N. Jones, H. Yu and H. Hwang, 2002. Influence of humic acid on 1-aminopyrene ecotoxicity during solar photolysis process. Int. J. Mol. Sci., 3: 1133-1144.

Boyne, A.F. and G.L. Ellman, 1972. A methodology for analysis of tissue sulfhydryl components. Anal. Biochem., 46: 639-653.

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

Cheng, M.L., H.Y. Ho., D.T.Y. Chiu and F.J. Lu, 1999. Humic acid-mediated oxidative damages to human a possible mechanism leading to anemia in Blackfoot disease. radical biology. Free Radic. Biol. Med., 27: 470-477.

Clairborne, A., 1986. Catalase Activity. In: Handbooks for Methods for Oxygen Radical Research, Greenwald, R.A. (Ed.). CRC Press Inc., Boca Raton, FL, pp: 283-284.

Cooper, W.J., R.G. Zika, R.G. Petasne and A.M. Fischer, 1989. Sunlight-Induced Photochemistry of Humic Substances in Natural Waters: Major Reactive Species. In: Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants, Suffet, I.H. and P. MacCarthy (Eds.). American Chemical Society, Washington, D.C., pp: 333-362.

Das, K., L. Samanta and G.B.N. Chainy, 2000. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys., 37: 201-204.

Farombi, E.O., O.A. Adelowo and Y.R. Ajimoko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. Int. J. Environ. Res. Public Health, 4: 158-165.

Ho, K.J., T.S. Liu and F.J. Huang, 2003. Humic acid mediates iron release from ferritin and promotes lipid peroxidation *in vitro*: A possible mechanism for humic acid-induced cytotoxicity. Arch. Toxicology, 77: 100-109.

Johansen, J.S., A.K. Harris, D.J. Rhychly and A. Ergul, 2005. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovascular Diabetol., 4: 5-9.

Klaunig, J.E., L.M. Kamendulis and B.A. Hocevar, 2010. Oxidative stress and oxidative damage in carcinogenesis. Toxicol. Pathol., 38: 96-109.

Knight, T.R., M.W. Farris, A. farhood and H. Jaeschke, 2003. Role of lipid peroxidation as a mechanism of liver injury after acetaminophen overdose in mice. Toxicol. Sci., 76: 229-236.

Matthews, J.C., J.E. Ekuta, K.D. Drake, C.F. Watson and W.H. Benson, 1995. Nature and extent of the interactions of humic acids with a water treatment algicide and a fungicide. Chemosphere, 30: 1565-1572.

Osman, H.A.M., T.B. Ibrahim, A.T. Ali and H.I.M. Derwa, 2009. Field application of humic acid against the effect of cadmium pollution on cultured tilapia oreochromis niloticus. World Applied Sci. J., 6: 1569-1575.

Paul, V.I. and P. Jayakumar, 2010. A comparative analytical study on the cadmium and humic acid contents of two Lentic water bodies in Tamil, India. Iran J. Environ. Health. Sci. Eng., 7: 137-144.

Rajaraman, P., A. Hutchinson, N. Rothman, P.M. Black and H.A. Fine *et al*., 2008. Oxidative response gene polymorphisms and risk of adult brain tumors. Neuro-Oncology, 10: 709-715.

Samamidon, V., I. Papadoyannis and G. Vasilikiotis, 1991. Mobilization of heavy metals from river sediments of Northern Greece by humic substances. Environ. Sci. Health, 26: 1055-1068.

Stevenson, F.H., 1994. Humus Chemistry: Genesis, Composition, Reactions. 2nd Edn., Wiley, New York, pp: 378-486.