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Safety Evaluation of Osun River Water Containing Heavy Metals and Volatile Organic Compounds (VOCs) in Rats

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Summary: This study evaluated the pH, heavy metals and volatile organic compounds (VOCs) in Osun river water. It also evaluated its safety in rats. Heavy metals were determined by atomic absorption spectrophotometry (AAS) while VOCs were determined by gas chromatography coupled with flame ionization detector (GC-FID). Male and female rats were exposed to Osun river water for three weeks and then sacrificed. The abundance of heavy metals in Osun river followed the trend Pb > Cd > Zn > Fe > Cr > Cu while VOCs followed the trend benzene < ethylbenzene < toluene < xylene. The concentrations of Pb, Cd and benzene were higher than the permissible limits of Standards Organization of Nigeria (SON) and World Health Organization (WHO) respectively. Rats exposed to Osun river water for three weeks had increased WBC, thiobarbituric acid reactive substances (TBARS), serum proteins and serum aminotransferases. There were also significant decreases ($P < 0.05$) in HCT, PLT, liver aminotransferases and liver glutathione compared to the control. These results show that the pollutants in Osun river water are capable of inducing hematological imbalance and liver cell injury. The toxicity induced in blood was sex-dependent affecting female rats more than male rats.

Keywords*:* Osun river, Haematology, Biochemical parameters, Heavy metals, Volatile Organic Compounds.

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INTRODUCTION

Water is essential for the continuity of life and surface water such as river is the cheapest source of water supply for drinking, laundry, recreational, agricultural and religious activities. Meeting the water quality for these purposes poses great concerns because of volumes of wastes received by river (Olajire and Imeokpara, 2001; Elleta, 2012). Wastes generated from industries, dumpsites and agricultural activities are washed into the river from uncontrolled management while some people also defecate into the river (Eletta, 2012). These wastes which are sources of heavy metals, poly aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and many more are detrimental to humans and aquatic lives abound in river. Some of these pollutants are confirmed carcinogens and can induce oxidative stress which causes damage to DNA, protein and other cellular constituents (Rechenmacher et al., 2010). Illnesses such as diarrhea, cholera, dysentery, typhoid, poliomyelitis, morbidity, mortality and hematological disorders have been attributed to poor quality of water (Wahab et al., 2012). Cancers, respiratory diseases, organ failures, mental retardation of the intellect, gastrointestinal disorders, tremor, ataxia, paralysis, vomiting and convulsion, depression, irritation and pneumonia are some of the diseases that have been traced to these pollutants (Nkolika and Benedict, 2009; Rechenmacher et al., 2010; Eletta, 2012).

Studies conducted by Olajire and Imeokparia (2000; 2001) on heavy metal concentrations and physico-chemical properties of Osun river reported high pollution with Pb, Cd, Ni, Cr, Zn, cyanide ion and ammonia which were mainly from farming, industrial activities and domestic discharges into this river. Equally, Wahab et al. (2012) reported high occurrence of pathogenic organisms in the river which could be as a result of unregulated activities such as defecating into the river. Due to the significance of this river as an international cultural heritage as well as national monument and its usefulness for both recreational and religious activities, there is need to assess its quality for the aforementioned applicable activities. However, no study has reported the concentrations of VOCs which have deleterious effects on both human and aquatic lives in the river. Though, studies have assessed the concentrations of heavy metals in Osun river, the most recent was in 2001 by Olajire and Imeokpara. Since wastes are continuously discharged into this river, there is every tendency that the concentrations of heavy metals might have changed, thus the need to determine the concentrations of heavy metals. Also, no study has evaluated the toxicological implications of drinking Osun river water on health. Therefore, this study determined the concentrations of VOCs and heavy metals in Osun river and evaluated their effects on hematological and biochemical parameters in wistar rats.

MATERIALS AND METHODS

Study location: Osun river is housed by Osun groove within which there is Osun shrine. Osun worshipers, traditionalists and tourists from all walks of life and different countries gather to celebrate the Osun festival annually. It is one of the rivers ascribed to mythology and there is belief that it cures worshippers of various diseases when taken. It is located on latitude of 6°33'35"N and longitude of 4°03'47"E.

Sampling and analyses: Water samples were collected between November, 2014 and January, 2015 at 5m away from the shrine. Part of it was preserved with concentrated hydrochloric acid (HCl) and stored at room temperature for the determination of heavy metals while another part was used for the determination of VOCs. pH was determined in-situ with Jenway 3505 pH meter (USA).

Heavy metals analysis: Water samples were digested according to the method of Sallah et al., (2011). 50 cm^3 of each sample was treated with $5cm^3$ of conc. HNO_3 and heated on a hot plate with gradual addition of conc. HNO_3 as necessary until the solution boiled. It was then evaporated to about $20cm^3$; $5cm^3$ of conc. HNO_3 was finally added, covered, and allowed to cool, and then filtered. The filtrate was poured into a $50cm^3$ standard volumetric flask and made up to the mark with distilled-deionized water. Portion of the solution was used for metal analysis with atomic absorption spectrophotometer (AAS) S series 711047v1.22. Triplicate digestions and analyses were run and average values reported in the results.

VOCs analysis: VOCs in Osun river were determined using gas chromatography coupled with flame ionization detector (GC-FID Hewlett-Packard Model, 501, USA). 10 ml of water samples was emptied into pre-cleaned 20 ml vial. The vial with content was placed in the vial holder of headspace sampler for GC analysis programming. Vial temperature for headspace sampler was set at 40 °C, looping at 100 °C with the transfer line temperature of 100 °C. Vial was shaken for 1 min equilibrating for 0.15 min and pressurized for 1 min. An HP 6890 CP-Sil 5CBcolumn (25 m x 0.32 μm i.d x 0.12 μm film thickness) was used with the initial oven temperature set at 35°C for 2 min., increasing at a rate of 5°C/min. to 80°C and then holding for 10 min. Detector and injector temperatures were maintained at 300°C and 150°C respectively. The target VOC species were identified by their individual retention time. Dechlorinated-distilled water was used as blank and was analyzed just as the Osun river sample. Standards of VOC mixtures containing all investigated constituents were prepared and calibration curves gave significant correlation coefficients (r^2) between 0.9996 and 0.9998. Triplicate analyses were done.

- **Animal groupings and water administration:** 20 male and female albino wistar rats weighing between 141± 29 g were bought from animal breeding unit of Department of Biochemistry, College of Medicine, University of Ibadan. The animals were randomly divided into four groups having five animals each: CM; control males, EM; experimental males, CF; control female, EF; experimental female. They were acclimatized at 25±6°C with day and night cycle of 12 h each for two weeks on commercial feed and tap water ad libitum. The rats in EM and EF were thereafter exposed to Osun river water *ad libitum* for another three weeks. Groups CM and CF received dechlorinated tap water during this period. Good hygiene was maintained by cleaning the cages of faeces and spilled-off food every day. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals (NIH, 1985) in accordance with the principles of Good Laboratory Procedure (GLP) (WHO, 1998).
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Collection of blood and preparation of serum and tissue homogenates: The procedure described by Yakubu et al., (2005) was used in the preparation of serum and tissue homogenates.

Haematological Indices Analysis: White Blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and platelet (PLT) were all determined according to the manufacturer's procedure using automated haematological analyzer X model KX-2IW.

Biochemical Assays: Biochemical parameters were determined using standard methods for protein (Gornall et al., 1949) and alanine aminotranstranfers (ALT) and aspartate aminotransferase (AST) (Reitman and Frankel, 1957). Reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were assayed as detailed below:

Reduced glutathione (GSH) was determined by the method of Ellman (1959). Briefly, 0.5 ml of the liver supernatants was precipitated with 2 ml of 5% TCA and then centrifuged at 604 x g for 20 minutes. A known volume (1 ml) of the resulting supernatant was mixed with 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0). Glutathione was used as standard. The absorbance was read at 412 nm and the concentration determined from a prepared standard.

MDA in liver supernatants was determined colorimetrically by evaluating thiobarbituric acid reactive substances (TBARS) using the procedure described by Buege and Aust (1978). MDA reacts with thiobarbituric acid (TBA) forming a 1:2 adduct (MDA-TBA_2) which produces a complex aromatic structure that strongly absorbs at 532 nm. Briefly, 0.1 ml of the liver supernatants was added to 2 ml of TBA-TCA-HCl reagent in the ratio of 1:1:1 (0.3% TBA, 0.25 N HCl and 15% trichloroacetic acid, TCA). The mixture was then placed in boiling water bath for 15 minutes, cooled and centrifuged at 1073 x g for 30 minutes. The absorbance of the clear supernatant was read at 535 nm against distilled water blank. The values were expressed as nmol MDA/mg protein using the following expression:

$$\text{MDA} = \frac{\text{Absorbance x sample volume x dilution factor}}{1.56 \text{ x } 10^5 \text{ x total volume x mg protein/ml}}$$

STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation of three replicates. They were subjected to one-way ANOVA followed by Duncan Multiple Test. SPSS 15 version was used for the statistical analysis. Significant differences were tested at $p < 0.05$.

RESULTS

pH, heavy metals and VOCs: Table 1 presents pH, concentrations of heavy metals and VOCs in Osun river water with their standards. The values obtained for pH of Osun river water showed that it was slightly acidic. The trend of abundance of these metals followed Pb > Cd > Zn > Fe > Cr > Cu. The concentrations of Pb and Cd were higher than acceptable limits of Standards Organization of Nigeria (SON) while the concentrations of others were lower. These concentrations were higher than what were obtained by Olajire and Imeokparia, (2000). The abundance of VOCs in Osun river followed the trend benzene < ethylbenzene < toluene < xylene. Except benzene whose concentration was higher than World Health Organization (WHO) limit for drinking water, others were below.

Haematological parameters: Table 2 presents the hematological indices of male and female rats exposed to Osun river water and control. There were significant ($p < 0.05$) elevations in WBC and significant ($p < 0.05$) reductions in HCT and PLT in all rats exposed to Osun river water compared to the control. These increases and decreases were sex-dependent with higher elevations in WBC recorded for female rats (88.1%) than male rats (33.58%), higher reductions in HCT of female rats (23.65%) than male rats (12.5%) and PLT of female rats (43.48%) than male rats (19.95%). No significant ($p > 0.05$) changes were obtained in RBC, Hb, MCV, MCH and MCHC.

Biochemical and toxicological parameters: Table 3 presents biochemical parameters of male and female rats exposed to Osun river water and control. There were significant increases ($p < 0.05$) in the levels of liver TBARS, serum total protein and activities of AST and ALT in serum of rats exposed to Osun river water. Significant reductions ($p < 0.05$) were obtained in the levels of liver GSH and activities of AST and ALT in

Table 1: pH, heavy metals and VOCs concentrations in Osun river water

Pollutants	Measured concentration	Standard value
Fe (mg/l)	0.07 ± 0.0016	0.3*
Cd (mg/l)	0.15 ± 0.0011	0.003*
Zn (mg/l)	0.14 ± 0.0002	3.0*
Cu (mg/l)	0.0012 ± 0.0001	1.0*
Cr (mg/l)	0.004 ± 0.0001	0.05*
Pb (mg/l)	1.69 ± 0.0040	0.01*
pH	6.02 ± 0.02	6.5-7.5*
Benzene (ppb)	11.42 ± 2.03	10**
Toluene (ppb)	72.98 ± 13.06	700**
Ethylbenzene (ppb)	66.33 ± 5.92	300**
Xylene (ppb)	237.68 ± 24.07	500**

Data are mean ± standard deviation of three values. Fe – Iron, Cd – Cadmium, Zn – Zinc, Cu – Copper, Cr – Chromium, Pb – Lead, * Standard Organization of Nigeria values, **World Health Organization drinking water guidelines, 2008.

Table 2: Haematological indices of rats exposed to Osun river water and control

Parameter	CM	EM	CF	EF
WBC (10^3/µl)	15.87±2.36	21.20±0.60[a]	12.77±1.03	24.02±2.07[b]
RBC (10^6/ µl)	8.60± 0.67	7.05±0.04	8.47±0.43	6.32±0.27
Hb (g/dl)	13.53± 0.31	12.93±0.42	14.93±0.72	11.43±0.21
HCT (%)	48.23±0.99	42.2±1.80[a]	44.70±3.06	34.13±2.64[b]
MCV (fl)	58.77±1.40	56.73±0.60	59.97±2.21	59.80±0.70
MCH (pg)	17.4±0.36	16.73±0.11	18.70±0.96	17.10±0.36
MCHC (g/dl)	29.63±0.51	29.57±0.15	30.17±0.55	29.04±0.98
PLT (µl)	932.33±13.05	746.33±15.82[a]	1292.67±108.58	730.67±95.52[b]

Data are mean ± standard deviation of five values. CM – control male rat; CF – control female rats; EM – experimental male rats; EF – experimental female rats; a significantly different at p < 0.05 from Corresponding CM across the row; b significantly different at p < 0.05 from corresponding CF; WBC - white blood cell; RBC - red blood cell; Hb - hemoglobin; HCT - hematocrit; MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration; PLT – platelet Count.

Table 3: Biochemical parameters in rats exposed to Osun river water and control

Parameter	CM	EM	CF	EF
Liver TBARS (nmol MDA/mg protein)	1.27±0.21	4.32± 0.24[a]	0.97± 0.02	3.32± 0.43[b]
Serum TP (g/L)	0.11±0.01	0.16± 0.02	0.17± 0.04	0.32± 0.02[b]
Liver GSH (mmol/dl)	66.27±4.02	45.62± 2.43[a]	43.73 ± 2.24	27.06± 5.27[b]
Liver AST (nmol min^{-1} mg^{-1} protein)	27.23±2.16	14.20±0.53[a]	45.35±1.42	38.57±7.77[b]
Serum AST (nmol min^{-1} mg^{-1} protein)	48.22±5.26	70.86±2.70[a]	26.64±4.04	39.05±3.39[b]
Liver ALT (nmol min^{-1} mg^{-1} protein)	73.09±3.90	33.94±4.56[a]	72.44±15.48	31.92±2.82[b]
Serum ALT (nmol min^{-1} mg^{-1} protein)	35.92±7.57	70.88±10.59[a]	29.73±3.57	56.40±10.36[b]

Data are mean ± standard deviation of five values. CM – control male; EM – experimental male; CF – control female; EF – experimental female; a significantly different at $p < 0.05$ from corresponding CM across the row; b significantly different at $p < 0.05$ from Corresponding CF across the row; TBARS - thiobarbituric acid reactive substances; MDA – malondialdehyde; TP – total protein; GSH – glutathione; AST – aspartate aminotransferase ALT – alanine aminotransferase.

the liver of male and female rats exposed to Osun river water compared to control. There were higher elevations in the levels of serum total protein of male rats than female while no sex-depedence was obtained in TBARS levels. There were higher reductions in the levels of GSH in liver of female rats than males. There were higher elevations of AST activity in serum of female rats than male rats while higher elevations of ALT activity in serum in male rats than female rats were obtained.

DISCUSSION

Toxicological evaluation of wastes washed into the river in rats are necessary due to their deleterious effects. This is so because humans, aquatic life and other living organisms are constantly exposed to them. Therefore, assessment of the effects of contaminated water on the liver and blood of rats is of great importance for a possible prediction of such effects on humans. Human exposure to contaminated water, at high levels, may result in damage to several tissues, leading to death at excessively high levels (Pari and Amali, 2005; Rechenmacher et al., 2010). Given the priority Osun river is accorded because of its mythology, this study assessed the possible health effects of drinking its water.

Heavy metals have been reported to adversely affect health due to their toxicity and bioaccumulative tendencies (Kori-Siakpere and Ikomi, 2011). Exposure to these heavy metals has been attributed to oxidative stress and which has been shown to produce a variety of alterations in tissues which underlie the etiology of many diseases (Jadhav et al., 2007). The high concentrations of heavy metals such as Cd and Pb in Osun river water indicate that it is highly polluted beyond the allowable limits of SON. This could be as a result of washed off torrents from dumpsites, local automobile repair workshops, welders and farming activities around this river (Olajire and Imeokpara, 2000, 2001). VOCs in water constitute health risks even at low concentrations because of their carcinogenicity and mutagenicity (Nikolaou et al., 2002). Therefore, the high concentrations of these compounds especially benzene whose concentrations exceeded permissible limit of WHO in Osun river water could significantly affect health when the river water is consumed untreated. Their presence in the river could have been from pharmaceutical waste materials, dumpsites, spent engine oil and paint wastes washed into the river (Azeez et al., 2013).

Useful information is obtained about diseases and their pathologies when hematologic variables are measured (Saadat et al., 2004; Mansour et al., 2007). Elevations in white blood cell (WBC) count are attributable to acute infections (Jee et al., 2005). This increase could be from counteraction of WBC to fight off deleterious effects of toxic pollutants (Elkind et al., 2001; Jassim and Hassan, 2011). The Osun river water mediated increase in WBC suggests that rats could be reacting to the presence of toxic agents in the water or even infectious agents since the contaminated water might contain microorganisms. Such ability of Osun river to cause increase in WBC might not be unconnected to the presence and concentrations of heavy metals and VOCs in it. These pollutants especially benzene is known to cause leukemia which is the proliferation of white blood cells, thereby increasing their number (Uboh et al., 2008b, Olajire and Azeez, 2012). WBCs are also elevated in response to inflammation by toxic agents. Our results show that exposed female rats were more susceptible to immune system disturbances than exposed male rats. This was also observed by Uboh et al., (2008b) that increase in WBC was sex-dependent. RBC, Hb, HCT, MCV, MCH and MCHC are red blood cell indices and their reductions could result in anemia. Decreases in RBC, Hb and HCT are indicative of anemia, hemorrhage, disturbance in heme biosynthesis and cancer (Eyoung et al., 2004; Mansour, 2007; Kori-Siakpere and Ikomi, 2011). It is not immediately clear why significant reductions were observed for HCT alone. However, increase in blood volume may lead to decrease in the number of cells per unit volume, which is the HCT. This reduction might be from the exposure of rats to Osun river water determined to contain pollutants such as benzene, ethylbenzene, xylenes and Pb which have been reported to destroy red blood cells (Uboh et al., 2008b; Olajire and Azeez, 2012). This observation was also sex-dependent. Decreases in PLT indicate blood clotting problems (Yilmazet al., 2004). The significant reductions in exposed rats may have been the result of response to the presence of pollutants in Osun river water. Pollutants such as xylene have been linked with the reductions of PLT (Olajire and Azeez, 2012a). Our results show that exposed female rats were more susceptible to PLT reduction than exposed male rats.

The measurement of TBARS which are products of lipid peroxidation is a convenient method to monitor oxidative damage induced by pollutants (Pari and Amali, 2005). Heavy metals and VOCs have been reported to induce free radicals which cause lipid peroxidation (El-Gendy et al., 2010; Olajire and Azeez, 2012). The significant increase in the TBARS in rats treated with Osun river water is indicative of the carcinogenic and mutagenic effects of the pollutants in Osun river because the polyunsaturated membrane lipids succumb easily to deleterious actions of reactive oxygen species (ROS). Increased ROS can be generated from the metabolism of toxicants. The presence of toxicants in the water could have resulted in an increase in ROS production, which has overwhelmed the natural antioxidant system, resulting in oxidative stress. This oxidative stress could have led to oxidative attack on membrane lipids leading to the observed increase in the amount of TBARS. These findings are consistent with reports by Salau et al. (2016) that a toxic agent; diethylnitrosamine caused an increase in concentration of lipid peroxidation products.

GSH plays an important role in preventing the oxidation of cellular macromolecules especially in the lung. It keeps up the cellular levels of active forms of vitamins C and E in blood (Yilmaz et al., 2004; Pari and Amali, 2005). The observed decrease in liver glutathione is indicative of oxidative stress which further corroborates the observed increase in lipid peroxidation. Increased lipid peroxidation and decreased GSH, a natural cellular antioxidant have been observed previously in rats treated with aflatoxin B1 and diethylnitrosamine; two potent carcinogenic agents (Ajiboye et al., 2014; Salau et al., 2016).

Activities of transferases increase in the serum when there is damage to vital organs in the body especially the liver. This can be used as a tool to assess the toxicity of pollutants. ALT and AST are markers of liver cell injury, with ALT being a more sensitive biomarker for the liver than AST (Pramyothin et al., 2006). Increase in the activities of ALT and AST in serum with corresponding decrease in liver observed in this study show that the enzymes have probably leaked from the liver to the extracellular fluid. This may be due to the ealier observed disruption of the ordered bilayer of the liver cell membrane caused by peroxidation of the polyunsaturated phospholipids of the plasma membrane induced by the pollutants in the water. Since AST and ALT are cytosolic and mitochondrial enzymes, their leakage is indicative of the compromise of plasma membrane integrity and possibly mitochondrial membrane. Heavy metals such as Cd and Pb have been well documented to increase the activities of these enzymes in serum (Edewor et al., 2007; Uboh et al., 2008a). The decrease in the activities of these enzymes in the liver would adversely affect carbohydrate and amino acid metabolism and subsequently energy production.

The amount of serum total protein is an index of the synthetic ability of the liver since the plasma proteins; majorly albumin and globulin are synthesized in the liver. It is also a reflection of the balance between protein anabolism and catabolism (Yakubu and Musa, 2012). Increased serum protein has been observed in cases of liver cirrhosis (Mayne, 2005). The increased serum protein in the present study could have resulted from the increased amount of AST and ALT in the blood or imbalance in protein synthesis and degradation. The synthetic ability of the liver might also have been increased as a response to the presence of toxic agents in the water which the rats were exposed to. Increased serum protein was also reported in previous studies where rats were treated with diethylnitrosamine, extracts of *Crateva adansonii*, aqueous leaf extract of *Ficus exasperata* and aqueous root bark extracts of *Anogeissus leiocapus* and *Terminalia avicenniodes* (Salau, 2013; Salau et al., 2012; Akanji et al., 2013)

CONCLUSION

This study has reported the concentrations of pollutants such as heavy metals and volatile organic compounds (VOCs), some of which were higher than the allowable limits of SON and WHO. These pollutants have led to liver cell injury as indicated by alterations in some hematological parameters, increased liver lipid peroxidation, decreased liver GSH, decreased liver cellular enzymes, increased serum protein and increased serum enzyme activities. This means that Osun river water is not safe for drinking despite the religious beliefs and mythology ascribed to it.

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