Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Sciences

ISSN 1819-3412
DOI: 10.3923/rjes.2018.14.20

Research Article
Quality Assessment of Waterside River, Ogbor Hill Aba 3: Effect of Three-Point Samples on Kidney Histo-architecture and Some Seric Bio-functional Indicators of Wistar Rats

[1]Anthony Cemaluk Chinedum Egbuonu, [2]Okechukwu Chibuike Atasie and [3]Sunday Oyewole Oyedemi

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria
[2]Department of Biochemistry, Abia State University, Uturu, Nigeria
[3]Department of Nature Conservation and Ethnobotany, Mangosuthu University of Technology, Jacobs, 4026 Durban, South Africa

Abstract

Background and Objective: Water as a universal solvent plays important metabolic roles hence its quality is critical to overall animal physiology. This study assessed the potential hydrogen, pH, temperature, chloride (Cl), phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) values of three-point samples from waterside river, Ogbor hill Aba (WRO) and the effect of the samples on rats' kidney histo-architecture and some seric bio-functional indicators. **Methodology:** Sample collection, quality analysis and animal study were carried out using standard protocols. All numeric data collected were analyzed by one way analysis of variance (ANOVA) using SPSS. **Results:** As compared among the samples, the respective pH was not different ($p>0.05$) but lower ($p<0.05$) than, while the respective temperature (either air or surface) was not different ($p>0.05$) but within the recommended standard value range. However, the chloride and sulphate compositions of the samples were lower ($p<0.05$) while the phosphate composition of the upstream and downstream was higher ($p<0.05$), than the recommended standard. The serum urea, creatinine and potassium concentration and the calculated potassium: Sodium ratio of rats exposed to the various WRO samples were higher ($p<0.05$), whereas that of sodium concentration was lower ($p<0.05$), than those of the corresponding control. Kidneys sections collected from rats in all the groups showed normal renal histo-architecture with normal Glomeruli, G. **Conclusion:** Thus, the determined quality indicators suggested inherent acidic property hence compromised quality of the WRO samples, whereas, the WRO samples probably affected the rats' serum functional indicators of the kidney without affecting their renal histo-architecture. The dose and duration effects of WRO samples were not explored hence further studies and urgent interim intervention measures are warranted and recommended as significant changes in organ histo-architecture are dose and duration-dependent.

Key words: Water quality, kidney, histo-architecture, duration-dependent, health-implications

Citation: Anthony Cemaluk Chinedum Egbuonu, Okechukwu Chibuike Atasie and Sunday Oyewole Oyedemi, 2018. Quality assessment of waterside river, Ogbor hill Aba 3: effect of three-point samples on kidney histo-architecture and some seric bio-functional indicators of Wistar rats. Res. J. Environ. Sci., 12: 14-20.

Corresponding Author: Anthony Cemaluk Chinedum Egbuonu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria Tel: +23480-3636-6565

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is a natural resource and as a universal solvent plays important metabolic and physiologic roles. Thus, water quality is critical to overall animal, including human physiology[1]. Some water characteristics including pH, chloride, phosphate, sulphate and temperature are the basis for determining water quality status[2,3]. Water source could be polluted by prevailing environmental factors and human activities including discharges from municipal wastewater or silage and effluent discharges from farms and factories[4]. Either banks of waterside river, Ogbor hill Aba traverse the rear of many factories, including breweries (soft drinks and beer) as well as soaps and detergents factories. Other prevailing activities that could predispose WRO to contamination and pollution include that as from abattoirs (hide and skin burning), auto mechanic workshops as well as human wastes (feces, urine) and general refuse disposal by the teaming inhabitants. Contamination of rivers due to the activities of the residents and industries was reported earlier[5].

From the foregoing, WRO may be polluted with attendant compromised quality. Waterside area is a densely populated community in Aba, Abia state, Southeast Nigeria. The inhabitants rely on riverside water for most of their daily water needs, especially in dry season. Water characteristics including pH, phosphate, chloride, sulphate and temperature indicated water quality status[6-8]. Hence, there is need for quality assessment of water sources especially including streams and rivers that are utilized by inhabitants for their overall water needs. There is also need to study the possible effects of such water sources using animal models. This study aimed at determining the pH, temperature, phosphate, sulphate and chloride values of three-point samples of waterside river, Ogbor hill Aba (WRO) and assessing the effect of the water samples on kidney histo-architecture and some seric bio-functional indicators of Wistar rats. The results of this study could provide deep insight into the quality status and possible renal health implications of consuming, WRO. Generally, the kidney performs important physiological functions including excretion, blood volume control, maintenance of ionic balance, pH regulation, red blood cell concentration regulation and vitamin D synthesis while creatinine, a breakdown product of creatine phosphate in muscle, urea, potassium and sodium concentration in the serum indicated renal health status.

MATERIALS AND METHODS

Sample collection: Three-point water samples were collected from the waterside river, Ogbor hill Aba thus: The first point, upstream, the second point, downstream and the third point, midstream. The water samples were collected using a properly labeled plastic containers and used without any treatment. The study was carried out between October, 2015 and July, 2017 at Biochemistry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Animal study

Animal procurement and exposure groups: Twenty adult male albino rats (*Rattus novergicus*) used in this study were procured from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatized for 2 weeks and then randomized (based on weight) to four experimentation groups with sample size of five rats.

Rats in the Group A (control) were sham-exposed to 0.5 mL of clean sachet water while rats in Groups B, C and D were, respectively exposed to 0.5 mL of WRO sample collected upstream, midstream and downstream. The exposure was per oral, using a gavage and daily for 4 weeks (28 days). Rats in all the groups were allowed free access to water and rat chow (commercial growers mesh feeds (Vital Feeds, Jos Nigeria).

Sacrifice, blood sample collection and preparation: After 4 weeks (28 days) exposure, the rats were fasted overnight and sacrificed the next day by cardiac puncture after cervical dislocation and the blood samples of the respective rats were collected individually into clean non-heparin tubes[9,10]. The respective blood sample thus collected was allowed to clot and then centrifuged at 3000 rpm for 10 min. The resultant serum sample was separated and stored in a refrigerator at 0°C and used the next day to determine the studied serum bio-indicators of kidney functions viz: Urea, creatinine, potassium and sodium ion concentration.

Ethical consideration: This study considered and adhered to the standard ethical use of experimental animals. Throughout the experimentation (acclimatization and exposure periods), all rats were housed at 25°C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat feed (Vital Feeds, Jos Nigeria) and tap water and generally received humane care in accordance with the guidelines of the National institute of Health, USA for ethical treatment of laboratory animals as

approved by the various (Departmental and College) ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

Determination of some water quality indicators (pH, temperature, phosphate, chloride and sulphate values) of WRO samples: The pH, temperature, phosphate, chloride and sulphate contents, respectively of the water samples was determined by methods described earlier[11]. In particular, the pH was determined with the aid of a glass electrode pH meter, pre-calibrated with carbonate buffer (at pH 4.70) and sulfate buffer (at pH 10.0) at 29°C. The temperature (air and surface) was determined at the site with mercury-in glass thermometer that was allowed standing on surrounding air or water surface for 5 min before taking the reading in degree centigrade. The bulb of the mercury-in-glass thermometer was washed in distilled water after taking each reading.

Phosphate concentration in the water samples was determined by adding 2.0 mL ammonium molybdate reagent and 0.2 mL stannous chloride reagent to 50.0 mL of the water sample and thoroughly mixed. The solution was incubated for 10 min. The absorbance of the samples was read using spectrophotometer at 690 nm and the phosphate concentration obtained by reading off absorbance from the standard curve of known concentration treated as the samples.

Sulphate concentration was determined based on the principle that sulphate ions in aqueous medium produce turbidity with barium chloride ($BaCl_2$). In brief, one hundred milliliters of the samples were taken and 20 mL of buffer added to it followed by addition of $BaCl_2$ crystals. The solution was stirred and the absorbance read at 460 nm with a spectrophotometer. Sulphate level was read off from a standard curve of known concentration treated the same way as the samples. Chloride concentration was determined based on the principle that in neutral or slightly alkaline medium, silver chloride precipitates on titration with silver nitrate. To one hundred milliliters of the sample in beaker, four drops of silver solution was added. Standard 0.014 M $AgNO_3$ solution was titrated until a precipitate was formed and the chloride concentration calculated according to instruction[11].

Determination of some seric bio-functional indicators of kidney (urea, creatinine, potassium and sodium concentration): Serum urea concentration was determined based on the principle that urea in serum when hydrolysed to ammonia in the presence of urease could be measured spectrophotometrically at 546 nm[12]. Serum creatinine concentration was determined by a colorimetric method based on the principle that creatinine on reacting with picric acid forms a coloured complex that was directly proportional to the creatinine concentration[12].

Serum potassium and sodium ion concentration were determined using flame photometer[13]. The samples were mixed with lithium nitrate and passed into the burner of a flame photometer equipped with filters to isolate the spectral lines of potassium and sodium. The intensity of light produced was compared to identically prepared standard and blank solution using propane and air flame for both potassium and sodium.

Examination of kidney histo-architecture: Kidneys sections were collected and fixed in 10% v/v phosphate buffered formalin for a minimum of 48 h. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70, 80, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned to 5 μm thick with a rotary microtome, floated in water bath and incubated at 60°C for 30 min. The 5 μm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70%). The sections were then stained with hematoxylin for 15 min. Blueing was achieved with ammonium chloride while differentiation was with 1% acidic alcohol before counter staining with eosin. Permanent mounts were made on degreased glass slides using a mountant DPX. The prepared slides were examined with a Motic™ compound light microscope using 4, 10 and 40× objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at 400× magnifications.

Statistical analysis: All numeric data collected were analyzed by one way analysis of variance (ANOVA) using the statistical package for Social Science (SPSS version 17, SPSS Inc., Chicago.IL.,USA). Results were presented as means±standard deviation (Mean±SD) at 95 % significance level ($p<0.05$).

RESULTS

The result showed that the pH of the respective samples was not significantly ($p>0.05$) different as compared among the samples but significantly ($p<0.05$) lower than the recommended standard pH range. As compared among the water samples, either the air or surface temperature was not significantly ($p>0.05$) different but was within the recommended standard range. The chloride and sulphate

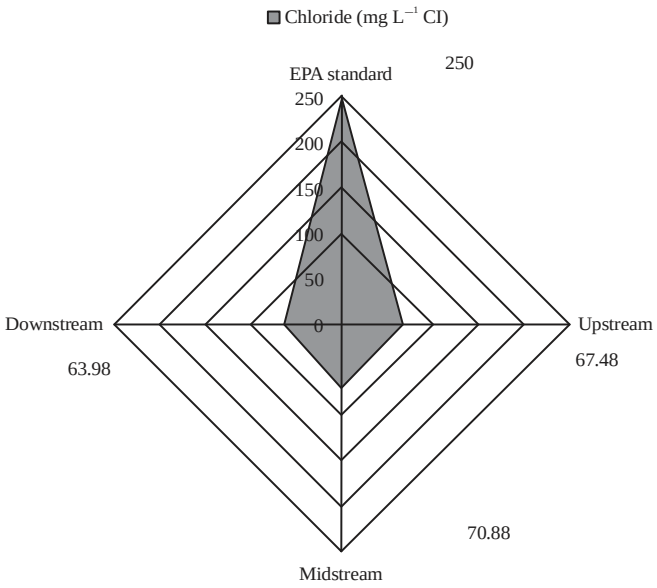

Fig. 1: Chloride composition of three-point (upstream, midstream and downstream) water samples from waterside river, Ogbor hill Aba, Abia State, Nigeria

Table 1: pH, temperature (air and surface), phosphate and sulphate values of three-point (upstream, midstream and downstream) water samples from waterside river, Ogbor hill Aba, Abia State, Nigeria

Parameters	EPA standard#	Upstream	Midstream	Downstream
pH	6.5-9.5	5.21±0.01	5.20±0.00	5.32±0.02
Air temperature(°C)	20-33	31.45±0.01	30.95±0.01	30.95±0.01
Surface temperature (°C)	20-33	29.55±0.01	29.75±0.01	29.51±0.01
Phosphate(mg L^{-1} P)	0.5-0.7	1.02±0.01	0.69±0.01	0.99±0.01
Sulphate (mg L^{-1} SO_4)	200-250	25.20±0.01	24.20±0.01	25.20±0.01

Value presented as Mean±SD of triplicate determinations. Significant difference at $p<0.05$. #: 4

Table 2: Effect of three-point (upstream, midstream and downstream) water samples from waterside river, Ogbor hill Aba, Abia State on serum kidney function parameters of normal male Wistar rats

Parameters	Control	Upstream	Midstream	Downstream
Potassium (mmol L^{-1})	3.70±0.12	4.60±0.17	4.02±0.15	4.52±0.28
Sodium (mmol L^{-1})	2.58±1.36	1.53±1.20	1.55±5.47	1.67±8.36
Potassium:Sodium	1.43±0.22	3.00±0.19	2.59±0.46	2.71±0.35
Urea (mg/100 mL)	3.90±2.00	4.82±2.94	5.50±1.58	6.00±2.12
Creatinine (mg/100 mL)	1.35±0.04	1.76±0.03	2.50±10.00	2.16±15.16

Value presented as Mean±SD of sample size, n = 5 rats. Significant difference at $p<0.05$

compositions of the water samples were markedly lower ($p<0.05$) while the phosphate composition for upstream and downstream were higher ($p<0.05$) than the recommended standard value (Table 1 and Fig. 1).

As depicted on Table 2, the effect of three-point water samples from WRO on serum kidney function parameters revealed that with the exception of sodium concentration that was lower ($p<0.05$), the serum urea, creatinine and potassium concentration of rats exposed to the various samples from WRO were higher ($p<0.05$) than that of the corresponding control. The calculated potassium: sodium ratio of the rats exposed to the various samples from WRO was higher ($p<0.05$) than that of the control with the highest value recorded in the rats exposed to the upstream sample followed by those in downstream and in midstream.

Kidney sections collected from rats in the normal group and in the other groups showed normal renal histo-architecture with normal Glomeruli, G (Fig. 2-5).

DISCUSSION

Some basic determinants of water quality including pH, temperature, phosphate, sulphate and chloride of three-point samples from waterside river, Ogbor hill Aba (WRO were

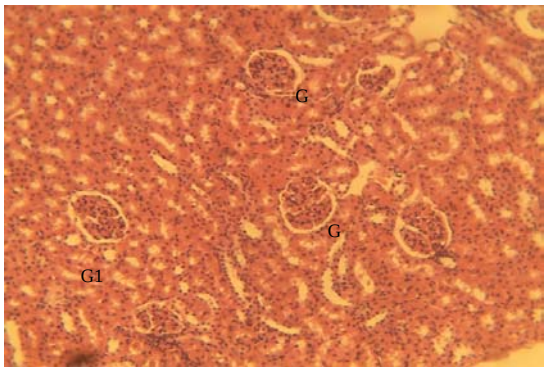

Fig. 2: Section of the kidney collected from the normal group showing the normal renal histo-architecture, Glomeruli (G) (400×)

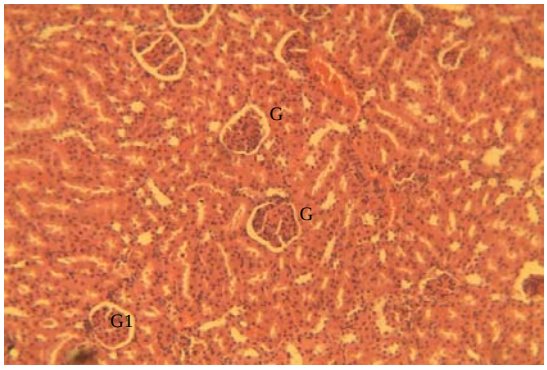

Fig. 3: Section of the kidney collected from the group that received upstream water showing the normal renal histo-architecture, Glomeruli (G) (400×)

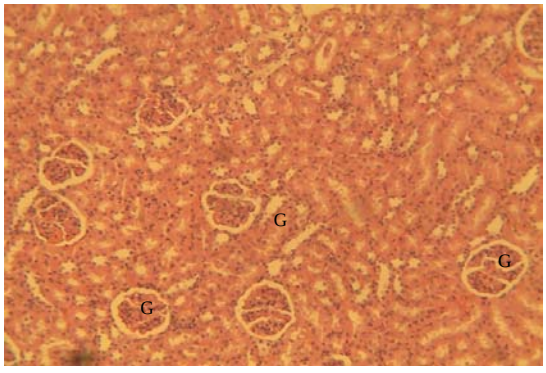

Fig. 4: Section of the kidney collected from the group that received midstream water showing the normal renal histo-architecture, Glomeruli (G) (400×)

determined and the effect on the kidney histo-architecture and some seric bio-functional indicators of Wistar rats was assessed by standard protocols. The results of the determined

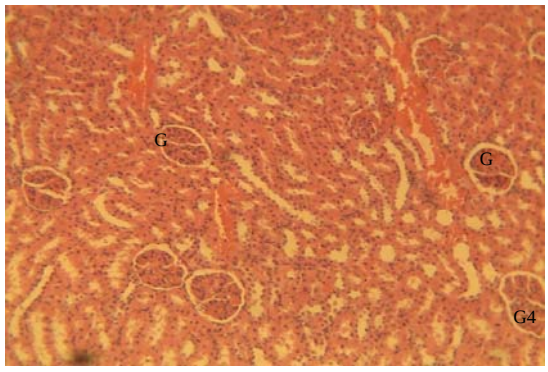

Fig. 5: Section of the kidney collected from the group that received downstream water showing the normal renal histo-architecture, Glomeruli (G) (400×)

water quality indicators (Table 1, 2 and Fig. 1) could imply inherent acidic property with possible organoleptic consequences, hence compromised quality of the water samples from WRO. This could impact negatively on the metabolic processes of the rats since water of good quality is important in diverse metabolic processes[14]. Compromised or low quality was attributed to low water to high organic water content[15]. This could imply possible high organic content in waterside River Ogbor hill Aba. The pH of the water samples in this study did not compare with the values obtained in earlier studies[15,16]. Also as obtained in this study, the temperature values were higher while the chloride values were lower than the average reported by Jamali and Amir[17]. The chloride value for the respective water samples was higher than the range 38.98-67.98 mg L^{-1} and the phosphate values were lower than, although that of the of the upstream compared with the lower range, 1.78-4.69 mg L^{-1} reported by Mohita *et al.*[18].

Water temperature in particular served as an indicator of compromised water quality since increased temperature could be as a consequence of enhanced light absorption by suspended particles[19]. The air temperature values of the WRO samples were comparatively higher than the surface temperature values of the respective water samples implying enhanced absorption perhaps by suspended particles in, hence compromised quality of, the samples. The lower ($p<0.05$) pH in this study compared to the standard range seemingly confirmed acidic status and apparently compromised quality, of the WRO samples[4,7]. However, the higher ($p<0.05$) phosphate composition in the upstream and downstream samples compared to the standard implicated sewage discharges while the lower ($p<0.05$) chloride and sulphate compositions of the WRO compared to the respective standard seemingly precluded organic wastes

particularly of animal origin as contributing to the apparent compromised quality of the samples and the attendant adverse effects, including on respiratory system[4,20].

Generally, the kidneys perform important physiological functions including excretion, blood volume control, maintenance of ionic balance, pH regulation, red blood cell concentration regulation and vitamin D synthesis, hence renal dysfunction could have significant adverse health implications. The serum urea, creatinine and potassium concentration and the calculated potassium:sodium ratio of rats exposed to the various WRO samples were higher ($p<0.05$), whereas that of sodium concentration was lower ($p<0.05$), than those of the corresponding control. The highest potassium:sodium ratio was recorded in the rats exposed to the upstream sample followed by those in downstream and the least in rats exposed to the midstream sample. This could be suggestive of impaired kidney functions, including excretory, resulting to enhanced accumulation of these metabolic products in the blood as observed in the serum and consequent dehydration[21]. However, the contribution of the ionic composition of the WRO samples which could influence the serum chemistry outcome was not explored in this study, hence warranted. High serum creatinine and urea and potassium and sodium ion concentration indicated renal failure[22]. In particular, the lower serum sodium concentration particularly in concert with higher serum potassium concentration as noted in this study following exposure to the various WRO samples compared with that of earlier report though following sub-chronic oral exposure of rats to esculetin (6, 7-Dihydroxy-coumarin) was associated with renal failure[23,24]. This may be so as the resultant higher calculated potassium:sodium ratio of the rats exposed to the samples as against the control could be in response to altered ionic balance, associated metabolic processes and bio-functions that are dependent on the critical physiological potassium:sodium ratio balance of 3:2 (or 1.5).

In this study, kidneys sections collected from rats in the normal group and in the other groups showed normal renal histo-architecture with normal Glomeruli, G. Histological changes are confirmatory indication of toxic assault on organs as organ damage occurs with or without serum chemistry changes[25]. The results generally seem surprising considering the enormity of on going activities in the area that could compromise the quality status of WRO. However, similar observation on other studied water quality indicators of the samples was noted in earlier report and attributable to inherent self purification potential of flowing rivers[26]. Furthermore, there is need to study the dose and duration effects of WRO samples as significant changes in organ histo-architecture are dose and duration-dependent.

CONCLUSION

Thus, the determined quality indicators suggested inherent acidic property hence compromised quality of the WRO samples whereas the WRO samples probably affected the rats' serum functional indicators of the kidney without affecting their renal histo-architecture. The dose and duration effects of WRO samples were not explored hence further studies and urgent interim intervention measures are warranted and recommended as significant changes in organ histo-architecture are dose- and duration-dependent.

SIGNIFICANCE STATEMENTS

This study discovers the possible acidic property hence compromised quality of samples from waterside river, Ogbor hill Aba which could alter the serum indicators of normal rats' kidney functions. This study will help the researcher to uncover the renal health implications of drinking water from waterside river, Ogbor hill Aba that many researchers have never explored. Thus, a new drinking water source for the inhabitants may be provided.

REFERENCES

1. Okonko, I.O., O.D. Adejoye, T.A. Ogunnusi, E.A. Fajobi and O.B. Shittu, 2008. Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. Afr. J. Biotechnol., 7: 617-621.
2. Adekunle, I.M., M.T. Adetunji, A.M. Gbadebo and O.B. Banjoko, 2007. Assessment of groundwater quality in a typical rural settlement in Southwest Nigeria. Int. J. Environ. Public Health, 4: 307-318.
3. Agbaire, P.O. and C.G. Obi, 2007. Seasonal variations of some physico-chemical properties of River Ethiope Water in Abraka, Nigeria. J. Applied Sci. Environ. Manage., 13: 55-57.
4. Bradley, C., C. Byrne, M. Craig, G. Free and T. Gallagher *et al*., 2015. Water Quality in Ireland 2010-2012. Environmental Protection Agency, Ireland, ISBN: 978-1-84095-602-3, pp: 52-86.
5. Raji, M.I.O., Y.K.E. Ibrahim, B.A. Tytler and J.O. Ehinmidu, 2015. Physicochemical characteristics of water samples collected from River Sokoto, Northwestern Nigeria. Atmos. Climate Sci., 5: 194-199.

6. Singh, J., D.K. Agrawal and S. Panwer, 2008. Seasonal variations in different physico-chemical characteristics of Yamuna river water quality in proposed Lakhwar hydropower project influence area. Int. J. Applied Environ. Sci., 3: 107-117.
7. Akubugwo, I.E., C.J. Ofoegbu and C.U. Ukwuoma, 2007. Physicochemical studies on uburu salt lake Ebonyi State-Nigeria. Pak. J. Biol. Sci., 10: 3170-3174.
8. Akan, J.C., F.I. Abdulraham, G.A. Dimari and V.O. Ogugbuaja, 2008. Physicochemical determinants of pollutants in wastewater and vegetable samples along Jakava wastewater chanel in Kano metropolis, Kano state Nigeria. Eur. J. Sci. Res., 9: 213-218.
9. Egbuonu, A.C.C. and G.A. Ekwuribe, 2017. Pulverized *Mangifera indica* (Mango) seed-kernel modulated serum lipid profile in monosodium glutamate-challenged rats. J. Applied Biotechnol., 5: 72-87.
10. Egbuonu, A.C.C. and S.O. Oriji, 2017. Pulverized *Mangifera indica* (mango) seed kernel mitigated monosodium glutamate-intoxicated rats' kidney histology and bio-functions. J. Nutr. Health Food Sci., 5: 1-7.
11. APHA., 1998. Standard Methods of Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC., pp: 348-350.
12. Egbuonu, A.C.C. and L.U.S. Ezeanyika, 2013. L-arginine exposure improves renal function markers of metabolic syndrome in female rats. Am. J. Biochem. Mol. Biol., 3: 50-60.
13. Dioka, C.E., O.E. Orisakwe, F.A.A. Adeniyi and S.C. Meludu, 2004. Liver and renal function tests in artisans occupationally exposed to lead in mechanic village in nnewi, nigeria. Int. J. Environ. Res. Public Health, 1: 21-25.
14. Smitha, P.G., K. Byrappa and S.N. Ramaswamy, 2007. Physico-chemical characteristics of water samples of Bantwal Taluk, South-Western Karnataka, India. J. Environ. Biol., 289: 591-595.
15. Kangabam, R.D., S.D. Bhoominathan, S. Kanagaraj and M. Govindaraju, 2017. Development of a Water Quality Index (WQI) for the Loktak Lake in India. Applied Water Sci., 7: 2907-2918.
16. Sharma, A.S.C., S. Gupta and N.R. Singh, 2013. Studies on the physico-chemical parameters in water of Keibul Lamjao National Park, Manipur, India. J. Environ. Biol., 34: 1019-1025.
17. Jamali, M.Y. and S. Amir, 2017. Study and evaluation of the physico chemical groundwater quality of an agricultural region around beni mellal city. Int. J. Dev. Res., 7: 11055-11064.
18. Mohita, K., S. Birjesh and P. Subrata, 2017. Impact of industrial effluents on water quality of Betwa River near Mandideep, Raisen. Int. J. Adv. Res. Biol. Sci., 4: 169-181.
19. Policht-Latawiec, A., A. Bogdal, W. Kanownik, T. Kowalik and K. Ostrowski, 2015. Variability of physicochemical properties of water of the transboundary Poprad river. J. Ecol. Eng., 16: 100-109.
20. Lenntech, 2008. Water treatment and air purification. Water Treatment, Lenntech, Rotterdmseweg, Netherlands, pp: 173-177.
21. Egbuonu, A.C.C., O. Obidoa, C.A. Ezeokonkwo, P.M. Ejikeme and L.U.S. Ezeanyika, 2010. Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats 1: Body weight changes, serum cholesterol, creatinine and sodium ion concentrations. Toxicol. Environ. Chem., 92: 1331-1337.
22. Hazilawati, H., S.M. Rosly, A.S. Tarmizi, K. Subramanian and J. Johaimi *et al.*, 2009. Comparison of blood urea nitrogen and serum creatinine in gentamicin-induced nephrotoxicity in rats and mice. Proceedings of the 21st Veterinary Association Malaysia Scientific Congress, August 7-9, 2009, Malaysia, pp: 346-349.
23. Egbuonu, A.C.C., A.E. Ogbu and L.U.S. Ezeanyika, 2015. Sub-chronic oral esculetin (6, 7-dihydroxy-coumarin) exposure in male Wistar rats: Effect on some serum functions and organ histology. Asian J. Biochem., 10: 67-77.
24. Al-Naama, L.M., J.A.A. Barry and S.S. Strak, 2007. Serum potassium, magnesium, sodium and chloride levels in elderly population. Med. J. Babylon, 4: 323-330.
25. Egbuonu, A.C.C., L.U.S. Ezeanyika, P.M. Ejikeme and O. Obidoa, 2010. Histomorphologic alterations in the liver of male wistar rats treated with l-arginine glutamate and monosodium glutamate. Res. J. Environ. Toxicol., 4: 205-213.
26. Atasie, O.C. and A.C.C. Egbuonu, 2017. Quality assessment of waterside River, Ogbor hill, Aba 1: Effect of three-point samples on some hematological parameters of Wistar rats. Int. J. Hydrol., Vol. 1, No. 3.