

IN VIVO EFFECTS OF ZINC OXIDE-*Chromolaena odorata* NANOPARTICLE TREATED OYUN RIVER WATERArise R.O.^{1,3*}, Alabi A.B.², Maccarthy O.S.¹, Olurombi T.M.¹, Olufemi O.¹, Farohunbi O.S.¹ & Anthony T.O.^{4,5}¹ Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.² Department of Physics, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria³ Department of Biological Sciences, Landmark University, Omu-Aran, Nigeria.⁴ Department of Physics, Osun State University, Osogbo, Nigeria.⁵ Deceased**Abstract**

This study evaluated the purifying effect of the zinc oxide-*Chromolaena odorata* (ZnO-CO) nanoparticle on water samples collected from Oyun River water. Physicochemical and bacteriological characteristics of untreated and treated Oyun river water were determined and their effects on hematological parameters, liver and kidney of rats were also investigated. Thirty-two albino rats (with mean weight $\pm$ SD of 132.00 ± 7.50 g) were randomized into four groups (A-D). Rats in groups A, B, C and D were maintained on distilled water, untreated Oyun river water (UW), ZnO-CO-treated Oyun river water (ZW), and alum-treated Oyun river water (AW), respectively for 30 days. Thermo-tolerant and enteric bacteria were undetectable in UW samples after treatment with the ZnO-CO nanoparticle. WBC, RBC and platelet counts of rats maintained on UW, ZW, and AW were significantly different from those in the control. Liver aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) activities of rats maintained on ZW were increased significantly ($p < 0.05$) when compared to the control. Serum globulin and creatinine concentrations of rats maintained on ZW also increased significantly when compared with the control. The results of this study suggest that Oyun river water is polluted, and its consumption may produce deleterious effects in humans. Treatment of Oyun river water with the synthesized ZnO-CO nanoparticle is more effective than alum in purifying the polluted water. However, ZW also impacted negatively on the liver and renal functions of rats. Hence, ZnO-CO nanoparticle treated water may not be safe as drinking water.

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1. Introduction

Water is essential for the sustenance of life. Although water is abundant on earth, the availability of fresh water is limited and its quality is constantly affected adversely by natural and anthropogenic influences (WWAP, 2017). Climate change, pollution, population growth and developmental activities are major factors that contribute to an insufficient supply of safe and accessible water. Preservation of fresh water quality is important for food production, and for domestic and recreational water uses, and more importantly, when used as a drinking-water supply (WHO, 2018). The quality of water can be compromised by the presence of infectious agents, toxic chemicals and radiological hazards. Hence, effective management and monitoring of fresh water bodies are vital to dealing with the global issue of water inadequacy.

Rivers are the most important freshwater resource for man. Indiscriminate disposal of industrial waste, refuse and sewage affect the physicochemical characteristics and microbiological quality of river waters, thereby leading to pollution (Koshy & Nayar, 1999). Pollution of water bodies is a serious and growing problem. Increasing quantities of industrial, commercial, and agricultural waste discharged into the surface water bodies, often in excess of the capacity of these ecosystems to breakdown the pollutants, have led to various deleterious effects on aquatic organisms (Gerardi & Zimmerman, 2005; Adewoye, 2010). Industrial wastes compromise the capacity of surface water to inactivate and destroy pathogens by altering the pH and providing excessive nutrients for microorganisms, threatening human and animal health and safety (Adekunle & Eniola, 2008). At least 2 billion people worldwide, use a drinking water source that is contaminated by faeces. Transmission of diseases such as cholera, dysentery, diarrhea, typhoid, polio, and

hepatitis A, has been linked to contaminated water (WHO, 2014).

Water treatment methods including sedimentation, filtration, chemical and membrane technologies are expensive and could generate secondary toxic pollutants into the ecosystem (Padmanabhan *et al.*, 2006; Gaya & Abdullah, 2008). In addition, these treatment methods alone may not guarantee a safe water supply that is completely free of pathogens. Disinfection of drinking water involves the use of physical and chemical disinfectants such as chlorine, chloramine, ozone and UV treatment. Each of these conventional water disinfection processes usually generates by-products; which can react with naturally occurring organic matter in the water (WHO, 2000). Chlorine is the most widely used disinfectant, but it is reported to generate various chlorine by-products (CBPs) which are potential carcinogens (Gallard & Gunten, 2002).

Heterogeneous photocatalysis is being considered as a promising and innovative alternative to conventional water purification techniques (Baruah & Dutta, 2009). It produces highly reactive transitory species for degradation of refractive complex organic molecules, which mineralizes disinfectant by-products as well as distorts microbial cell walls, thereby immobilizing them. Application of nanotechnology in water purification processes will likely remove the finest contaminant from polluted water due to the large surface area to volume ratios of the nanostructures (Hornyak *et al.*, 2008). Hence, this study evaluated the purifying effect of the zinc oxide-*Chromolaena odorata* (ZnO-CO) nanoparticle on Oyun river water. Additionally, the effect of the ZnO-CO nanoparticle treated Oyun river water on selected hematological parameters and indices, as well as liver, and kidney function and histology of the experimental rats, were assessed.

2. Material and Methods

2.1 Study Area

This study was conducted on Oyun river water, collected along the Kwara State Polytechnic stretch, Oyun, Ilorin, Kwara state. The Oyun river is one of the major rivers in the Kwara state, Nigeria. This river is a fresh water, non-tidal type river which flows northwards passing through Ijagbo, Ajase-ipo, Idofian, University of Ilorin Bridge, Oyun, finally converging with the Asa River at the Sobi barracks area to form the Awon River.

2.2 Materials

2.2.1 Water sample collection

Grab samples were collected from the Oyun river, along the Kwara State Polytechnic stretch, Oyun, Kwara State, following the standard sampling guidelines and methods (WHO, 1997). Water samples were collected in duplicate into pre-sterilized bottles, kept in ice-boxes and transported immediately to the laboratory for physicochemical and bacteriological analyses.

2.3 Chemicals and Assay Kits

Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Acid phosphatase (ACP), Alanine aminotransferase (ALT), Albumin, Total Protein, Globulin, Bilirubin, Urea and Creatinine (Randox Laboratories Ltd. Co-Atrim, UK) and aluminium potassium sulphate ($KAl(SO_4)_2 \cdot 12H_2O$) (Drury Industries, Ogun State). The ZnO-CO nanoparticle, synthesized from Zinc nitrate and *Chromolaena odorata* was obtained from the Department of Physics, University of Ilorin, Ilorin, Nigeria. All reagents used were of analytical grade.

2.4 Experimental animals and treatment groups

Thirty-two (32) healthy male and female albino rats (with mean weight $\pm$ SD of $132.00 \pm 7.50g$) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Ethical approval was obtained from the University of Ilorin Ethical Review Committee (Ref No. UIL/UERC/LSC/03/47KC147). The rats were kept in well-ventilated conditions (Temperature: 28-30°C), and fed *ad libitum* with commercial feed (Premier Feed Mills Ltd, Lagos, Nigeria) and distilled water for seven days during acclimatization after which they were randomly assigned to one of four groups (n= eight rats per group)

Group A - maintained on distilled water (control group)

Group B - maintained on untreated water (UW)

Group C - maintained on water treated with the ZnO-CO nanoparticle (ZW)

Group D - maintained on water treated with alum (AW)

Water was orally administered to the rats under the four treatment regimens for 30 days. Weights of the rats were recorded prior to and after 30 days of water administration. At the end of the experiment, the rats were sacrificed by anaesthetizing in a jar containing cotton wool soaked in diethyl ether. The jugular veins were cut and 5 ml of the blood was collected into centrifuge tubes, allowed to stand for 10 minutes at room temperature (clotting) and centrifuge for $685 \times g$ for 10 minutes. The serum was aspirated with Pasteur pipettes into dry, sample bottles and used within hours of preparation for the biochemical assays. The liver and kidney were also carefully removed, cleaned of fatty materials and placed in cold 0.25 M sucrose solution to maintain the integrity of the tissue. The organs were later blotted with tissue paper, weighed separately and homogenized in ice-cold 0.25 M sucrose solution using mortar and pestle. The homogenates were appropriately diluted

(1:5 w/v) with sucrose after which they were centrifuged at $33.5 \times g$ for 15 minutes to obtain the supernatants which were then collected into sample bottles and frozen at -20°C until required for various biochemical assay.

2.5 Methods

2.5.1 Synthesis of ZnO-*Chromolaena odorata* nanoparticles

A weight of 25 g of zinc nitrate was added to 250 ml boiled solution of Akintola (*Chromolaena odorata*) leaf extract. The mixture was then boiled until it reduced to a deep yellow coloured paste which was collected in a ceramic crucible and heated in an air-heated furnace at 400°C for 2 hours. A light yellow coloured powder was obtained, which was carefully collected and mashed into finer particles for characterization. The marched ZnO-*Chromolaena odorata* nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM). The XRD was used to inquire into the crystalline nature of the nanomaterial in addition to the ordering and regularity of ions that form lattice in the sample (Seeck & Bridget, 2015). The morphology of the nanoparticles was investigated via SEM; an acceptable and conventional microscopy method (Zhou et al., 2007). The average grain size of zinc oxide nanoparticles using *Chromolaena odorata* extract as reducing agent was $1.3699 \times 10^{-8}\text{m}$.

2.5.2 Treatment of water samples

ZnO-CO nanoparticles weighing 1.2 g was added to 15 L of Oyun river water in a clean container. The mixture was continuously stirred in sunlight for 3 hours for maximum photo degradation of pollutants. Similarly, 0.6 g of alum was added to 15 L of Oyun river water. Both treated Oyun river water samples were covered with aluminum foil and left in a dark room for 24 hours. After 24 hours, samples were taken from untreated and treated Oyun river water for

physicochemical and bacteriological analyses. Subsequently, both the treated and untreated Oyun river water were fed to rats for a period of 30 days. The rate of water intake of rats maintained on distilled water, UW, ZW and AW for 30 days period was measured. The weight of rats before and after administration of distilled water, UW, ZW and AW water samples was recorded.

2.5.3 Physiochemical Analysis

Water samples were analysed for the physicochemical parameters including pH, electrical conductivity, dissolved oxygen (DO), total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total hardness, nitrate, and sulphate, following standard methods (APHA, 1985; Ademoroti, 1996). Metal levels were determined using atomic absorption spectrophotometer (AAS).

2.5.4 Bacteriological Analysis

Bacteriological analysis of the water samples was carried out using the standard plate count (SPC) to enumerate total heterotrophic bacteria (THC), and the multiple tube test fermentation technique to enumerate total coliforms (TC), and total thermotolerant coliforms (TTC) (Fawole & Oso, 2004; WHO, 2006). Total coliforms and total thermotolerant coliforms were detected and quantified using Eosin methylene blue (EMB) agar and incubated at 37°C and 44.5°C , respectively (Ochei & Kolhatkar, 2004; Tang & Stratton, 2006).

2.5.5 Determination of Hematological Parameters

Hematological parameters of the blood samples collected were determined using standard clinical methods described by Khalaf-Allah (1999). Blood samples were collected into sample bottles containing EDTA to prevent blood clotting. Red Blood Cell (RBC), White Blood Cell (WBC) and Hemoglobin (Hb) were determined using Wintrobe's micro hematocrit,

improved Neubauer haematocytometer and Sahli's methods. Differential WBC counts were taken using the Wright's stain.

2.5.6 Biochemical Parameters

Under ether anesthetic condition, the jugular veins around the neck region was slightly displaced to prevent the mixing of interstitial fluid with blood, and then cut with a sharp sterile blade. The rats were made to bleed into EDTA sample bottles, left undisturbed at room temperature for 20 minutes. Using a Uniscope Laboratory centrifuge (model SM800B, Surgifried Medicals, England), the blood containing bottles were centrifuged at $33.5 \times g$ for 15 minutes. The sera were then aspirated using a Pasteur pipette into clean, dry, sample bottles. Previously used standard methods were used to determine the various biochemical parameters- ALT and AST activities (Reitman & Frankel, 1957); ALP and ACP activities (Wright *et al.*, 1972a & 1972b); protein concentration (Gornall *et al.*, 1949); serum albumin level (Doumas *et al.*, 1971); serum globulin level (Tietz, 1995); serum bilirubin level (Evelyn & Mallock, 1938); serum urea level (Veniamin & Vakirtzi, 1970); serum creatinine level (Bartels *et al.*, 1972)

2.5.7 Histopathology of Liver and Kidney

The liver and kidney of the sacrificed rats were removed and cleaned with blotting paper and weighed, fixed in 10% (v/v) formaldehyde, dehydrated through ascending grades of ethanol (70%, 90% and 95% v/v), cleaned in xylene and embedded in paraffin wax (melting point 56°C). Tissue sections were prepared according to the procedure described by Drury and Wallington (1967) and stained with haematoxylin/eosin (H and E). The histology slides were read with a light microscope (OLYMPUS, Model CX21FSI, Philippines).

2.6 Statistical Analysis

Results were expressed as the mean of four determinations $\pm$ SD. The data was statistically analyzed using the Duncan Multiple Range Test complemented with the Student's t-test. Values were considered statistically different at $p < 0.05$. All statistical analyses were performed using the SPSS software.

3 Results

3.1 Physicochemical Parameters

The physicochemical properties of untreated and treated Oyun river water, and the WHO and EPA standards for drinking water are presented in Table 1. All the physicochemical parameters evaluated were within the WHO and EPA recommended limits for drinking water. However, COD and TSS were above the values set by the EPA for surface water (EPA, 2001). COD level of 1504 mg/l for UW considerably reduced to 256 mg/L and 64 mg/L, after it was treated with ZnO-CO nanoparticle and alum, respectively. Similarly, TSS levels of UW (600 mg/L), ZW (570 mg/l) and AW (580 mg/L) were above the EPA recommended limit of 50 mg/L for surface water.

3.2 Bacteriological Parameters

Table 2 shows the bacteriological parameters of untreated (UW), ZnO-*Chromolaena odorata* nanoparticle treated (ZnO-CO), and alum treated Oyun river water (AW). The total coliform count of UW was 1100 MPN/100mL, which was significantly reduced to 150 MPN/100mL and 35 MPN/100mL, when treated with ZnO-CO nanoparticles and alum, respectively. Similarly, the total bacterial count of 880×10^3 CFU/mL obtained for UW decreased to 230×10^3 CFU/mL and 580×10^3 CFU/mL after being treated with ZnO-CO nanoparticles and alum, respectively. The thermo-tolerant (faecal coliform) bacteria count for UW was 14×10^3 CFU/mL, while the enteric

bacteria count was 21×10^3 CFU/mL. The results obtained show that thermotolerant and enteric bacteria were undetectable in Oyun river water samples after treatment with ZnO-CO nanoparticles and alum.

3.3 Rate of Water Intake

Table 3 shows the rate of water intake of rats maintained on distilled water, UW, ZW and AW during the experimental period of 30 days. There was gradual decrease in the consumption of UW, ZW and AW, by the rats when compared to the rats maintained on distilled water (control).

Table 1: Physicochemical parameters of untreated (control), ZnO-*Chromolaena odorata* nanoparticle-treated and alum treated Oyun river water

Parameter	Untreated	ZnO-nanoparticle treated	Alum treated	WHO Recommended Limit (2017)	EPA Recommended Limit (2001)
pH	7.70	7.90	8.20	6.50-8.50	6.50-9.50
EC ($\mu\text{S}/\text{cm}$)	300	320	300	-	2500
Turbidity (NTU ^o)	4.47	2.60	2.63	5.00	5.00 (USEPA 2018)
NO ₃ ⁻ (mg/l)	38.82	43.14	23.72	50.00	50.00
SO ₄ ²⁻ (mg/l)	22.94	23.77	24.47	250	250
DO (mg/l)	6.20	5.50	6.40	-	? 5.00
COD (mg/l)	1504	256	64	-	40.00
BOD (mg/l)	2.00	0.00	2.40	-	5.00
TDS (mg/l)	150	150	150	? 600	500 (USEPA 2018)
TSS (mg/l)	600	570	580	-	50
Total hardness (mg/l)	138	124	124	500	-
Pb (ppm)	-	0.01	-	0.01	0.01
Cu (ppm)	0.03	0.02	-	2.00	2.00
Zn (ppm)	-	0.17	-	5.00	5.00

Table 2: Bacteriological parameters of untreated, ZnO-*Chromolaena odorata*-nanoparticle treated and alum treated Oyun river water

Sample	Total Coliform count (MPN/100ml)	Total Bacteria count $\times 10^3$ (CFU/ml)	Thermotolerant Bacteria count $\times 10^3$ (CFU/ml)	Enteric Bacteria count $\times 10^3$ (CFU/ml)
Untreated	1100	880	14	21
ZnO-nanoparticle treated	150	230	-	-
Alum treated	35	580	-	-

Table 3: Rate of water intake of rats administered with untreated and treated Oyun river water

	Day 1-6 (cl)	Day 7-12 (cl)	Day 13-18 (cl)	Day 19-24 (cl)	Day 25-30 (cl)
Control	83.30	84.50	83.21	85.00	84.70
Untreated Oyun River Water	79.90	74.65	73.35	68.44	62.60
ZnO Nanoparticle Treated River Water	80.23	79.49	78.76	78.90	75.82
Alum Treated River Water	80.05	72.67	76.00	73.35	71.55

3.4 Effect of administration of untreated and treated Oyun river water on the weight of rats

Table 4 shows the weights of rats in the control and treatment (3 groups) prior to exposure and at the end of exposure trial of 30 days. There was no significant difference in the weights of the rats before and after administration of AW. However, the weights of rats before and after administration of UW decreased significantly. Similar result was obtained for the group of rats administered ZW, in which the weights of rats before and after administration reduced significantly.

on UW, ZW, and AW were not significantly different from the control. Similarly, there was no significant difference between the kidney-body weight ratios of rats administered with UW and AW when compared with that of the control. However, there was significant increase in kidney-body weight ratio of rats administered with ZW when compared with the control.

3.6 Hematological parameters

Table 6 shows the effect of the administration of UW, ZW and AW on hematological parameters of the experimental rats.

Table 4. Mean weight ($\pm$ Standard Deviation) of rats (n=4) before and after administration of untreated and treated Oyun water samples

Groups	Weight Before (g)	Weight After (g)
Control	124.25 $\pm$ 3.30 ^a	133.75 $\pm$ 9.46 ^a
Untreated Oyun River Water	134.00 $\pm$ 7.39 ^{ab}	130.75 $\pm$ 11.44 ^a
ZnO Nanoparticle Treated River Water	141.50 $\pm$ 9.33 ^b	135.75 $\pm$ 7.41 ^a
Alum Treated River Water	128.25 $\pm$ 4.03 ^a	126.50 $\pm$ 8.58 ^a

*p < 0.05.

Table 5. Effect of consumption of untreated and treated Oyun river water on organ-body weight ratios of rats (n=4)

Parameters	Liver	Kidney
Control	2.75 $\pm$ 0.16 ^a	0.52 $\pm$ 0.04 ^a
Untreated Oyun River Water	2.77 $\pm$ 0.07 ^a	0.55 $\pm$ 0.02 ^a
ZnO Nanoparticle Treated River Water	2.74 $\pm$ 0.10 ^a	0.66 $\pm$ 0.01 ^b
Alum Treated River Water	2.80 $\pm$ 0.27 ^a	0.55 $\pm$ 0.04 ^a

*p < 0.05.

3.5 Organ-body weight ratio

The effect of administration of UW, ZW, and AW on organ-body weight ratio of rats is presented in Table 5. The liver-body weight ratio of the rats maintained

There was no significant difference between mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte count (LYM) and mean platelet volume (MPV) of rats administered untreated, ZW, AW and

3.7 Liver function

the control. In contrast, white blood cell (WBC) count, platelet (PLT) count, platelet distribution width (PDW), and red blood cell (RBC) count of rats maintained on UW, ZW, and AW, were significantly different from the control. There was no significant difference in hematocrit count (HCT) of rats maintained on ZW and AW relative to the control. However, HCT counts of rats maintained on UW differed significantly relative to the control. Hemoglobin (HGB) counts of rats administered UW and AW, were significantly reduced when compared with the control. In contrast, there was no significant difference in HGB counts of rats maintained on ZW and the control. Red cell distribution width coefficient of variation (RDW-CV) percentage of rats maintained on ZW and AW were not significantly different from the control. In contrast, RDW-CV of rats administered UW decreased significantly when compared with the control. Red cell Distribution Width Standard Deviation (RDW-SD) of rats maintained on AW was significantly increased relative to the control. In contrast, there were no significant difference in RDW-SD of rats maintained on UW, ZW and the control.

The effect of administration of UW, ZW and AW on parameters that assessed liver function is presented on Table 7. There was no significant difference between total serum protein concentrations of rats administered with UW and ZW relative to the control. However, total serum protein concentration of rats maintained on AW decreased significantly when compared with the control. Serum globulin concentrations of rats maintained on UW, ZW, and AW, differed significantly from the control. Similarly, total serum bilirubin concentrations of rats maintained on UW, ZW, and AW decreased significantly relative to the control. There was no significant difference between direct serum bilirubin concentrations of rats administered with AW and the control. In contrast, serum direct bilirubin concentration of rats maintained on UW and ZW differed significantly from the control. Serum albumin concentration of rats maintained on ZW was not significantly different from the control. However, serum albumin concentrations of rats maintained on UW and AW significantly increased when compared with the control.

Table 6: Effect of administration of untreated and treated Oyun river water on Hematological parameters of the rats (n=4)

Parameters	Control	Oyun River Water	ZnO Nanoparticle + River Water	Alum + River Water
WBC ($\times 10^3 / \mu\text{L}$)	10.10 $\pm$ 0.87 ^a	6.63 $\pm$ 0.51 ^a	13.70 $\pm$ 1.02 ^a	12.55 $\pm$ 0.52 ^a
RBC ($\times 10^6 / \mu\text{L}$)	7.46 $\pm$ 0.61 ^a	6.43 $\pm$ 0.50 ^a	7.38 $\pm$ 0.53 ^{bc}	6.66 $\pm$ 0.25 ^{bc}
HGB (g/dL)	10.67 $\pm$ 0.81 ^a	9.05 $\pm$ 0.73 ^a	10.68 $\pm$ 0.19 ^a	9.63 $\pm$ 0.75 ^{bc}
HCT (%)	40.78 $\pm$ 2.49 ^a	32.08 $\pm$ 3.11 ^a	40.35 $\pm$ 2.43 ^a	39.03 $\pm$ 2.58 ^a
MCV (fL)	56.95 $\pm$ 4.85 ^a	55.85 $\pm$ 4.19 ^a	55.63 $\pm$ 2.89 ^a	55.40 $\pm$ 4.61 ^a
MCH (pg)	14.90 $\pm$ 0.84 ^a	14.80 $\pm$ 0.69 ^a	15.10 $\pm$ 1.06 ^a	14.03 $\pm$ 1.21 ^a
MCHC (g/dL)	26.18 $\pm$ 0.86 ^a	26.55 $\pm$ 1.23 ^a	27.15 $\pm$ 0.45 ^a	26.10 $\pm$ 1.45 ^a
PLT ($\times 10^3 / \mu\text{L}$)	259.50 $\pm$ 11.70 ^a	173.50 $\pm$ 6.76 ^a	96.50 $\pm$ 1.73 ^a	65.5 $\pm$ 2.52 ^a
LYM (%)	91.40 $\pm$ 5.67 ^a	96.83 $\pm$ 1.64 ^a	96.45 $\pm$ 0.87 ^a	95.20 $\pm$ 3.00 ^a
RDW-SD (fL)	31.08 $\pm$ 1.31 ^a	29.33 $\pm$ 0.17 ^a	30.90 $\pm$ 2.57 ^a	34.28 $\pm$ 2.92 ^a
RDW-CV (%)	15.18 $\pm$ 1.05 ^a	13.15 $\pm$ 1.14 ^a	15.15 $\pm$ 1.00 ^a	15.90 $\pm$ 0.24 ^a
PDW (fL)	9.90 $\pm$ 0.67 ^{bc}	9.35 $\pm$ 0.40 ^a	9.13 $\pm$ 0.71 ^a	9.05 $\pm$ 0.35 ^a
MPV (fL)	7.10 $\pm$ 0.56 ^a	7.15 $\pm$ 0.47 ^a	7.07 $\pm$ 0.45 ^a	6.95 $\pm$ 0.21 ^a

*p<0.05.

3.8 Kidney function

Table 8 shows the effect of administration of UW, ZW and AW parameters related to the kidney. Serum creatinine concentrations of rats maintained on UW significantly increased relative to the control.

from the control. In contrast, there was no significant difference in AST activity in the kidney of rats maintained on UW, ZW, AW and the control. Similarly, liver AST activity of rats maintained on UW was not significantly different from the control. However, there were significant increases in the liver AST activity of rats maintained on with ZW and AW when compared to the control.

Table 7: Effect of the administration of untreated and treated Oyun river water on liver function of rats (n=4)

Parameters	Distilled Water	Oyun River Water	ZnO Nanoparticle + River Water	Alum + River Water
Serum total protein (g/dL)	8.63 ± 0.23 ^a	8.94 ± 0.11 ^a	8.93 ± 0.16 ^a	7.70 ± 0.34 ^b
Serum globulin (g/dL)	3.37 ± 0.35 ^a	2.66 ± 0.45 ^b	4.14 ± 0.74 ^c	1.65 ± 0.15 ^c
Serum albumin (g/dL)	5.26 ± 0.28 ^a	6.28 ± 0.46 ^b	4.79 ± 0.23 ^a	6.05 ± 0.35 ^b
Serum direct bilirubin (mg/dL)	11.21 ± 1.32 ^a	14.15 ± 0.87 ^b	8.50 ± 0.41 ^c	12.01 ± 1.17 ^a
Serum total bilirubin (mg/dL)	18.94 ± 1.26 ^a	16.99 ± 1.31 ^b	15.58 ± 0.73 ^b	16.33 ± 1.06 ^b

Data are means ± Standard Deviations. *p<0.05.

Table 8: Effect of the administration of untreated and treated Oyun river water on kidney function parameters of rats (n=4)

Parameters	Distilled Water	Oyun River Water	ZnO Nanoparticle + River Water	Alum + River Water
Serum Creatinine (mg/dL)	13.64 ± 0.30 ^a	20.75 ± 0.96 ^b	23.00 ± 0.82 ^c	23.84 ± 0.67 ^c
Serum Urea (mg/dL)	117.67 ± 6.16 ^a	100.04 ± 5.28 ^b	92.08 ± 6.59 ^b	115.29 ± 1.19 ^a

Data are means ± Standard Deviations. *p<0.05.

There was no significant difference in serum urea concentrations of rats maintained on AW and the control. On the contrary, serum urea concentrations of rats maintained on UW and ZW were significantly different from the control.

3.9 Enzymatic parameters

3.9.1 Aspartate aminotransferase (AST)
Figure 1 shows the activity of AST in the serum, liver and kidney of rats administered with UW, ZW and AW. Serum AST activity of rats maintained on UW, ZW and AW differed significantly

3.9.2 Alkaline phosphatase (ALP)

Activity of ALP in the serum, liver and kidney of rats maintained on UW, ZW, and AW is presented in Figure 2. Serum ALP activity of rats maintained on UW, ZW and AW were not significantly different from the control. In contrast, ALP activity in the kidney in rats administered with UW, ZW and AW significantly differed from the control. Liver ALP activity of rats maintained on UW and AW differed significantly from the control. However, there was no significant difference in the liver ALP activities of rats maintained on ZW and the control.

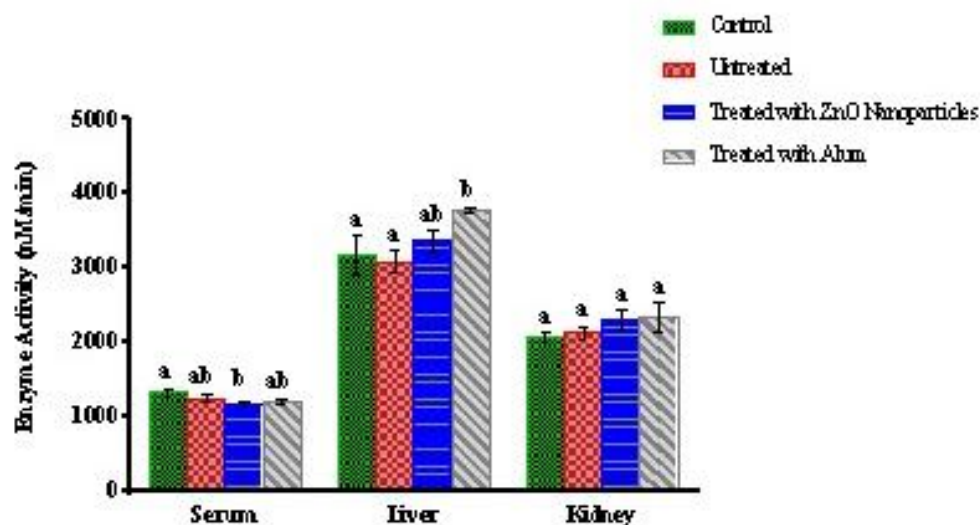


Figure 1: Activity of AST in the serum, liver and kidney of rats administered with untreated and treated Oyon river water

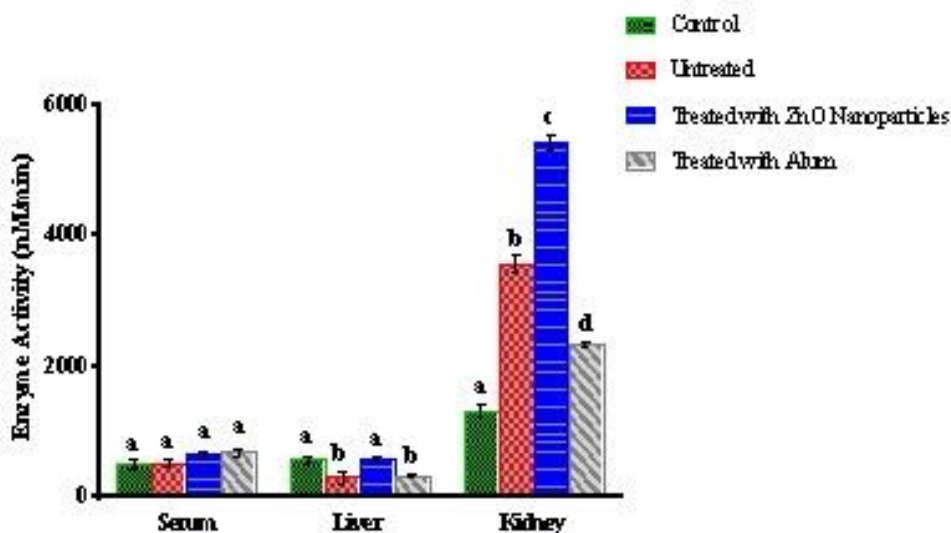


Figure 2: Activity of ALP in the serum, liver and kidney of rats administered with untreated and treated Oyon river water

3.9.3 Alanine aminotransferase (ALT)

Activity of ALT in the serum, liver and kidney of rats maintained on UW, ZW, and AW is presented in Figure 3. Serum ALT activity of rats maintained on UW, ZW, and AW differed significantly from the

control. Similarly, there was a significant difference in liver ALT activity of rats maintained on UW, ZW, and AW when compared with the control. The activity of ALT in the kidney of rats maintained on UW, ZW and AW were not significantly different from the control.

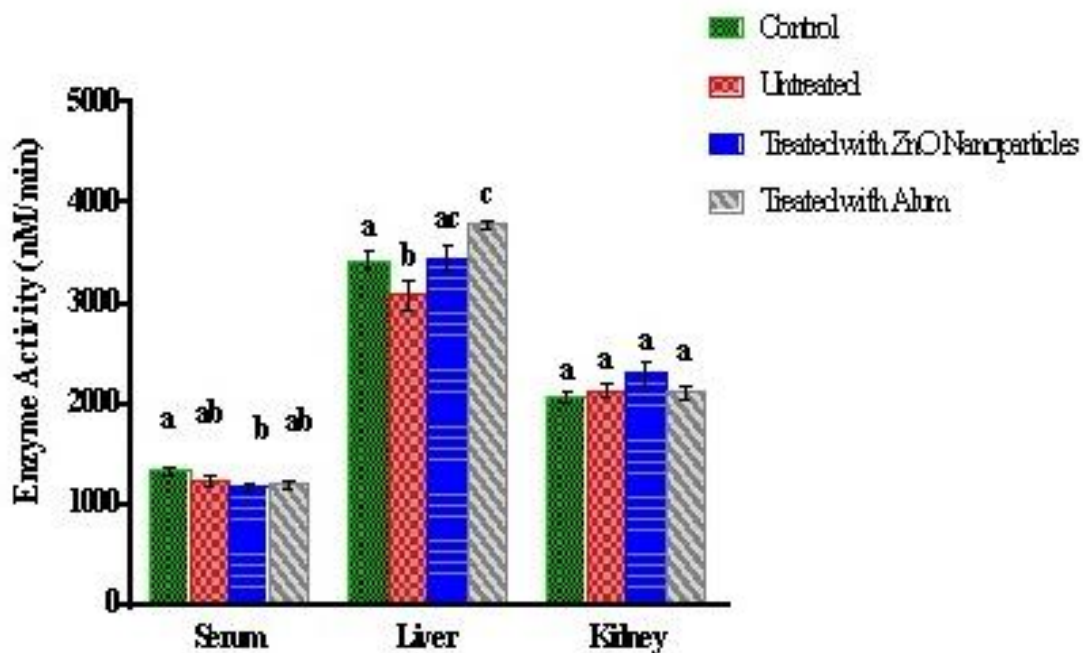


Figure 3: Activity of ALT in the serum, liver and kidney of rats administered untreated and treated Oyun river water

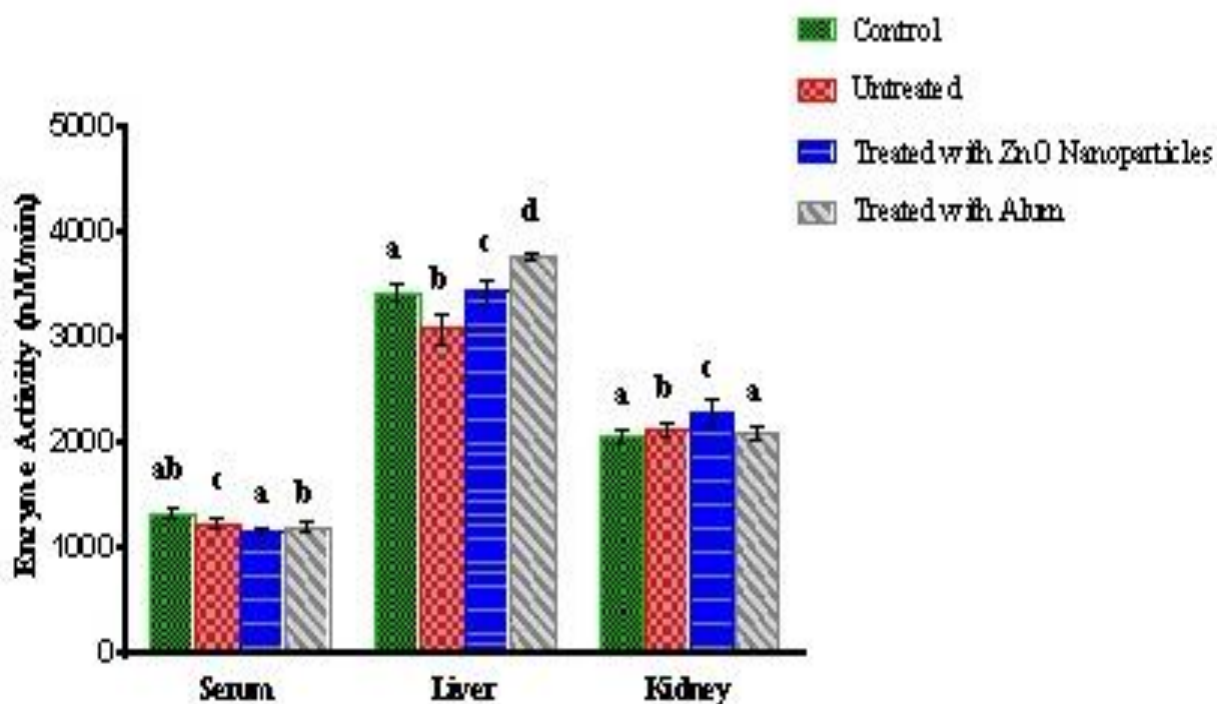


Figure 4: Activity of ACP in the serum, liver and kidney of rats administered with untreated and treated Oyun river water

3.9.4 Acid phosphatase (ACP)

Figure 4 shows the activity of ACP in the serum, liver and kidney of rats administered UW, ZW, and AW. Serum ACP activity of rats maintained on UW, ZW, and AW differed significantly from the control. Similarly, the Liver ACP activity of rats maintained on UW, ZW, and AW differed significantly relative to the control. There was no significant difference in kidney ACP activity of rats maintained on AW and the control. In contrast ACP activity in the kidneys of rats maintained on UW and ZW differed significantly when compared with the control.

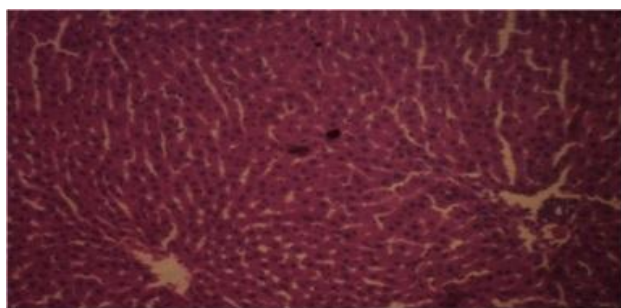


Plate 1. Photomicrograph of the cross section of liver of a rat maintained on distilled water (x400; H and E)

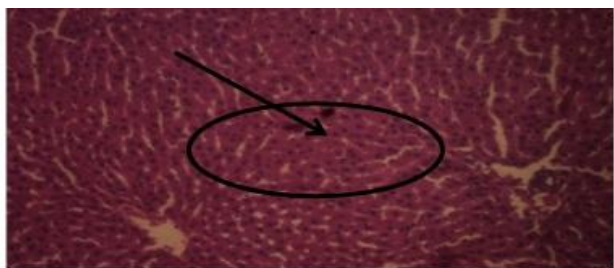


Plate 2. Photomicrograph of the cross section of liver of a rat maintained on untreated Oyun river water (x400; H and E) showing irregular hexagonal plates of hepatocytes with central vein

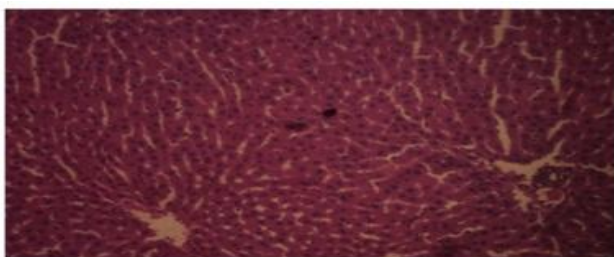


Plate 3. Photomicrograph of the cross section of liver of a rat maintained on Oyun river water treated with Zinc-oxide nanoparticle (x400; H and E) showing distortion of hepatic tissue with interstitial congestion

3.10 Histological parameter

Histological results of the liver and kidney of rats maintained on distilled water, UW, ZW, and AW are depicted in Plate 1–8. The liver and kidney of rats maintained on distilled water exhibited no distortion, adhesion or inflammation as shown in Plate 1 and 5, respectively. Plate 2 and 4 revealed irregular hexagonal plates of hepatocytes with central vein in the liver of the rats maintained on UW, and AW respectively. Plate 3 shows mild distortion of hepatic tissue with interstitial congestion on the cross-section of the rats maintained on ZW. The cortex and medulla of the kidneys maintained on UW exhibited normal histological features as shown in Plate 6. In contrast, Plate 8 revealed mild distortion of nephrons with congested and vascular dilation of the cross-section of the kidney of rats maintained on AW. Plate 7 shows the cross-section of the kidney of rats maintained on ZW which exhibited normal nephrons but with congestion.

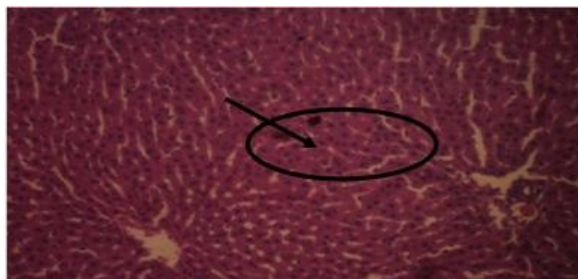


Plate 4. Photomicrograph of the cross section of liver of a rat maintained on Oyun river water treated with Alum (x400; H and E) showing irregular polyhedral plates of hepatocytes with central vein

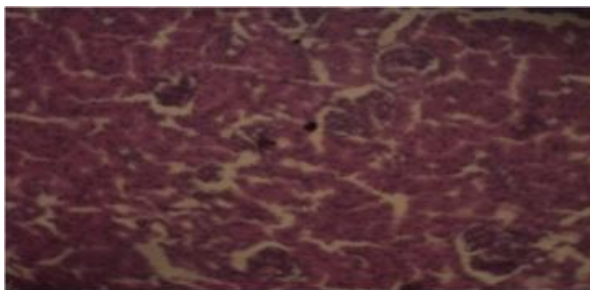


Plate 5: Photomicrograph of the cross section of kidney of a rat maintained on distilled water (x400; H and E)

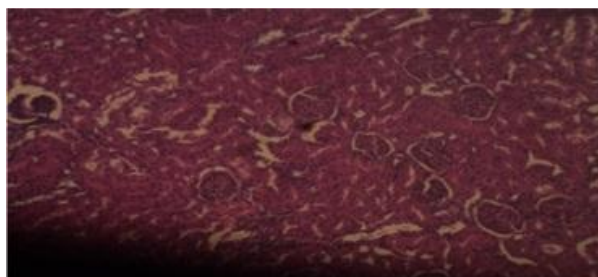


Plate 6. Photomicrograph of the cortex and medulla of kidney of a rat, maintained on untreated Oyun river water (x400; H and E). The cortex shows numerous glomeruli with normal histological features

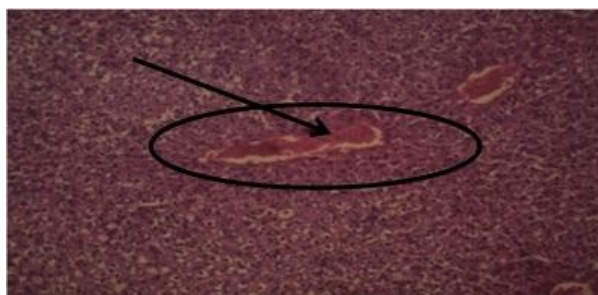


Plate 7. Photomicrograph of the cross section of kidney of a rat maintained on Oyun river water treated with Zinc-oxide nanoparticle water (x400; H and E) showing normal nephron and glomeruli but with congestion

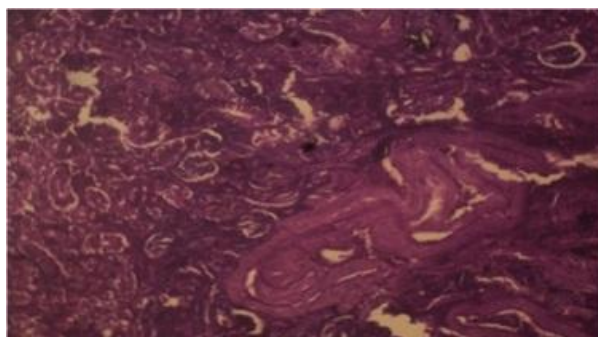


Plate 8. Photomicrograph of kidney of the cross section of a rat maintained on Oyun river water treated with Alum (x400; H and E) showing mild distortion and abnormal nephron and glomeruli with congested and vascular dilation

4. Discussion

The nanocrystalline structure of the zinc oxide was successfully synthesized by heating at a temperature of 400°C for 2 hours. The mean size of the particle was estimated to be approximately $1.3699 \times 10^{-8}\text{m}$ (13.7nm); this affirmed that, the synthesized structures are in the nanoscale range. The sizes of the particles were estimated using X-ray diffractometer analysis. The surface

morphology of the nanoparticles samples were characterized using the Scanning Electron Microscope (SEM). The morphology of the nanoparticles was attained via a self-assembling process orchestrated via the interplay of size and molecular interactions which, in turn, is determined by the distribution of the nanomaterials in the matrix (Obasuyi et al., 2017).

Physicochemical analysis of water is essential for the assessment of water quality and purity. The extent of waste discharge onto surface water can be estimated by measuring parameters such as DO, BOD, COD, TSS and TDS. Although their measurements have no direct health implications to humans, analysis of these parameters are an important indicator of overall water quality and performance efficiency of treatment plants. In this study, COD and TSS levels UW exceeded the EPA recommended limit for surface water (EPA, 2001). These results indicate the extent of water pollution in Oyun river water as a result of runoff from the waste deposited along the bank of the river, direct discharge of waste into the river or as a result of agricultural activities that take place around the river. COD and TSS levels were still above the recommended limits after treatment. This suggests that ZnO-CO nanoparticle is not sufficient for the removal of waste materials discharged into the Oyun river.

High total coliform counts observed in UW are characteristic of water bodies that have been contaminated by organic pollutants such as industrial waste or decaying organic material (Olayemi, 1994). Treatment of Oyun river water with ZnO-CO nanoparticles significantly reduced total coliform counts, although observed value exceeded the limit recommended by the WHO (0/100 ml of total coliforms in drinking water) (WHO, 2017). Although the total bacteria counts of UW significantly reduced after treatment with ZnO-CO nanoparticles, its

level was above the USEPA acceptable value for the heterotrophic plate count in municipal drinking water (USEPA, 2012). These observations imply that ZW was inadequate for total clearance of coliform and heterotrophic bacteria contaminants. Thermotolerant bacteria count, which is an index of faecal contamination, was undetected in the ZW sample. ZnO nanoparticles have been reported to have very good antibacterial activity on a broad spectrum of bacteria. This may have been due to the photocatalytic generation of H_2O_2 responsible for antimicrobial action of ZnO (Senthilkumar & Sivakumar, 2014). Similarly, ZnO-CO nanoparticles successfully removed the enteric bacteria from untreated Oyun river water. These results indicate high level of faecal contamination of the Oyun river, which was successfully eradicated by treatment with ZnO-CO nanoparticles (Santo Domingo *et al.*, 2007).

Any factor influencing water intake of the rats will also affect their rate of feed consumption (Leeson & Summers, 2009). Changes in weights of the rats maintained on distilled water, treated and untreated Oyun river water reflect the patterns of their rates of water intake during the experiment. Significant losses in weights of rats maintained on UW and ZW nanoparticle treated Oyun river water, suggest unacceptability of the water by the rats, which might be due to taste or odour of the water.

Organ-body weight ratio can serve as an indicator to assess the effect of a compound on specific organs of exposed experimental animals, as significant differences in organ weight between treated and untreated animals may occur in the absence of any morphological changes (Bailey *et al.*, 2004). The kidneys might be the target organ for potential effect of ZnO-CO nanoparticles, as there

was significant increase in organ-body weight ratio of rats maintained on ZW (Yan, *et al.*, 2012).

The significant decrease in RBC, HGB, HCT and RDW-CV observed in the rats maintained on UW sample suggests anemia, which might be as a result of impaired or decreased production of erythrocytes (Seelev *et al.*, 1998). Similar results were observed in rats maintained on AW, although HCT counts in this group was not significantly different from the control. The observed significant increase in RDW-SD of rats maintained on AW might be an indication of normocytic heterogeneous anemia (Bessman *et al.*, 1983). WBC counts of rats maintained on UW decreased significantly, probably due to infections from pathogens present in the water sample that resulted in increased utilization and destruction, or impaired production of white blood cells, or both (Nicholson *et al.*, 1995). However, in rats maintained on ZW and AW, WBC counts were observed to increase significantly, which might be due to infections, particularly those caused by viral pathogenic organisms present in the water sample (Chabot-Richards & George, 2014). The significant reduction in PLT counts observed in rats maintained on UW, ZW, AW, may be an indication of platelet production impairment or increased platelet destruction induced by infections or exposure to other constituents in the water (Ali & Auerbach, 2017). PDW indicates volume variability in platelet size (Vagdatli *et al.*, 2010), which increases during platelet activation, reflecting platelet anisocytosis. Thus, the significant decrease in PDW of rats maintained on untreated, ZW, and AW suggests that it is probably due to decreased production or increased destruction of platelets.

A significant increase in AST and ALT activity observed in the liver of rats maintained on ZW, and AW may be attributed to the induction of expression of the enzyme in the liver (Pappas, 1989). Reduction in liver ALT activity of rats maintained on UW may be due to

inhibition or inactivation of the enzyme by the constituents of untreated Oyun river water. Increased kidney ALP activity of rats maintained on UW, ZW, and AW may be due to increased synthesis of the enzyme by induction (Kaplan & Righetti, 1970). However, the significant decrease in liver ALP activity of rats maintained on UW and AW suggests inhibition of the enzyme by the constituents of the water (Kristensen, 1994; Arise *et al.*, 2011). Elevated ACP activity in the kidney of rats maintained on UW and ZW may be attributed to enzyme induction since there was no significant increase in serum ACP activity (Collins & Lewis, 1971). Similarly, the observed increase in ACP activity in the liver of rats maintained on ZW, and AW suggests increased synthesis or activation of the enzyme. Inhibition or inactivation of liver ACP by the constituents of the polluted Oyun river water could be responsible for the significant reduction in activity observed in rats maintained on UW (Akanji *et al.*, 1993; Arise *et al.*, 2015a & b).

Alterations in total serum protein are mainly caused by a change in the concentration of one or more of the specific proteins in the plasma or a change in the volume of plasma water. These alterations usually reflect physiological or pathological processes (Killingsworth, 1979). The significant decrease in total serum proteins observed in rats maintained on AW may be attributed to reduced synthesis of plasma proteins as a result of liver damage or increased loss of plasma proteins due to kidney injury (Constable *et al.*, 2017). The observed reduction in serum globulin levels of rats maintained on UW and AW may be due to acute dehydration or decreased production of the proteins (Eckersall, 2008). However, an elevated level of serum globulin in rats maintained on ZW may be as a result of infection or inflammation (Eckersall, 2008). Plasma albumin levels could be used to assess the synthetic function of the liver, as well as the severity of hepatic injury (Yakubu *et al.*, 2003). Significant increases in serum albumin levels observed in rats

maintained on UW and AW probably resulted from acute dehydration. This observation is supported by considerable decrease in the rate of water intake that occurred in this treatment group. Increased plasma albumin levels are usually seen during acute dehydration since the rate of synthesis of albumin and intravascular-extravascular equilibration usually occurs fairly rapidly to stabilize relative osmotic pressures (Johnson, 2006). Bilirubin is produced from the catabolism of haem within the reticuloendothelial system, where it is released as an unconjugated form and converted to conjugated forms by the liver (Mauro *et al.*, 2006). An elevated level of conjugated bilirubin in rats maintained on UW suggests liver damage, which might be a result of infection (Friedman *et al.*, 2003).

The significant increase in serum creatinine concentrations of rats maintained on UW, ZW, and AW may indicate impaired kidney function or dehydration (Panda, 1989; Lamb *et al.*, 2006), probably resulting from infections, or reduced blood flow to the kidneys.

Decrease in the serum urea concentration is of less clinical diagnostic significance than the elevated serum urea concentration (Lum & Leal-Khoury, 1989). Reduction in serum urea levels of rats maintained on UW, and ZW may be due to decreased urea production as a result of acute liver failure.

Histopathological lesions may arise from infectious diseases and parasites, provoking necrotic and degenerative alterations to which the organism responds with an inflammatory or defensive reaction. The liver and kidney of rats maintained on distilled water showed normal and non-obsolete histology, unlike those maintained on untreated and treated Oyun river water with varying distortions of cellular architecture of the nephrons and the hepatocytes.

6. Conclusion

The results from this study revealed that Oyun river water is contaminated, and that its consumption produced deleterious effects on the organs of rats. Treatment of Oyun river water with synthesized ZnO-CO is more effective than alum in purifying the polluted water. However, ZW impacted negatively on the liver and renal functions of rats. Hence, ZnO-*Chromolaena odorata*-nanoparticle treated water may not be safe for drinking but may be used for other non-consumptive uses.

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