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Impacts of sub-lethal potassium permanganate exposure on endocrine disruption and oxidative stress in *Clarias gariepinus*

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ABSTRACT

The water in Southern Nigeria is considered unsuitable for immediate consumption due to elevated concentrations of iron and manganese. To remove these metals, a precipitation process involving potassium permanganate (KMnO4) is employed. However, untreated sludge generated during this process becomes hazardous to aquatic environments. This study investigates the effects of KMnO4 on *Clarias gariepinus*, a prevalent fish species in the region. Fifteen self-bred specimens were exposed to concentrations equivalent to one-tenth of the levels used in water treatment, along with a control group. Fish were sampled at five intervals (days 2, 9, 16, 23, and 30) during the experiment. At each interval, blood samples were collected using anticoagulant-free centrifuge tubes and analyzed for abnormalities in hydrocortisone and superoxide dismutase (SOD) activity. On the 30th day, fish were dissected, and their organs were extracted and analyzed for SOD activity. Spectrophotometry was used to quantify the biomarkers. Results showed marginal upregulation of hydrocortisone secretion and disrupted SOD activity, indicating that critical organs and tissues were unable to mitigate the oxidative stress induced by KMnO4. These findings demonstrate that KMnO4 interferes with endocrine functions and significantly impacts the antioxidant defense system of fish. Consequently, sludge from water treatment facilities must be rendered safe before being discharged into aquatic ecosystems.

Keywords: *Clarias gariepinus*; Hydrocortisone; Metals; Potassium permanganate; Superoxide Dismutase

1. INTRODUCTION

Metals' indestructibility and contamination of groundwater constitute a major environmental health risk since the contaminants stay in the water for an extended

period, rendering it unsafe for human consumption (Ahmed et al., 2010). Some aesthetic problems that have prompted the extraction of natural iron and manganese from groundwater include turbidity, precipitation in distribution pipes, discoloured clothing, and changes in the water's flavour and odour. Contamination of groundwater with iron and manganese, according to Linde et al., (2005), can endanger human health and cause long-term health problems.

The presence of contaminants like manganese and iron in groundwater is a pressing environmental issue that demands quick resolution. This contamination could lead to the contamination of drinkable water. Manganese is the sixth most abundant element in the Earth's mantle, according to (Ahmed et al., 2010). In humans, manganese is not dangerous at concentrations below 0.5 mg/l, according to. An earlier study this year found that elevated manganese levels, when exposed to for an extended period of time, can induce Parkinson's disease symptoms.

Manganese may affect the development of the central nervous system in children less than two years old because they absorb more of it than adults but excrete less of it. Knocke, (1991) and Yahya et al., (1990) found that KMnO4 pre-oxidation effectively removed manganese and iron from groundwater, controlled odours and tastes (especially those caused by algae), and disinfected the water. Compared to ozonation and activated carbon processes, permanganate pre-oxidation has a number of benefits for treating water with high organic content, including low cost, simple operation, and minimal maintenance. The Crum and Pickering plants of Philadelphia Suburban Water Company were treated with KMNO4 to demonstrate its ability to decrease turbidity.

Studies have shown that potassium permanganate peroxidation can improve coagulation processes (Basiouny et al., 2008). Along with KMnO4 treatment at dose rates ranging from 0.5 to 1.5 mg/L, alum feeds of 30.8 mg/L and powdered activated carbon up to 10 mg/L were used to regulate the taste and odour in these locations. According to Shull, (1962), the levels of MPN in raw water were reduced by 95% after treatment with 0.75 mg/L of potassium permanganate. Performed a plant-scale treatment research at a water purification plant in Kansas City, Missouri. The amounts of KMnO4 were adjusted based on the actual permanganate demand of the water. The average for December was 1000 g/L, followed by January at 1.1%, February at 1.3%, March at 1.7%, and April at 2700 g/L.

The majority of faecal streptococci strains were eliminated, and the coliform concentration was brought down to levels that could not be detected. Actinomycete eradication rates were between 27% and 96%. The alum coagulation method was employed by Betty and Ty, (1985) to eliminate monomeric precursors. The researchers found that when the raw water contained high levels of monomeric precursors, the chloroform concentration of the treated water was dramatically reduced by pre-treating it with KMnO4 before alum coagulation. In order to determine how effective KMnO4 oxidation is for acrylamide extraction from water, conducted an investigation. To reduce acrylamide concentrations to an acceptable level, a modest quantity of KMnO4 was needed.

Significantly reducing the oxidation efficiency is the presence of hydroxide sulphide (H2S) in water at a molar ratio, which has a major effect on the efficiency of KMnO4 oxidation. The acrylamide oxidation of potassium permanganate appears to be unaffected by manganese and nitrate, and the presence of fulvic and humic acids may decrease its effectiveness. The efficacy of several treatment approaches for algae and KMnO4 removal in direct filtration was investigated by (Petrusevsici et al., 1996). Dual coagulation with ferric sulphate and cationic polymer followed permanganate pretreatment, which was found to be effective. Researchers discovered that KMnO4 peroxidation and coagulation were effective in removing algae and other particle materials through direct filtering (Basiouny et al., 2008).

This investigation was carried out in the Niger-Delta region of Nigeria from November 15, 2020, to May 15, 2022. The water treatment and wastewater sectors have been facing problems since the 1990s, when increasing amounts of PPCP and EDC residues were found in Nigeria's natural aquatic environments. Many changes in aquatic ecosystems, including those of birds, fish, and other species, have been attributed to toxic substances. Because of these changes, people are starting to wonder what effect eating seafood has on their health in the long run. Therefore, it is believed that this study's findings would provide insight into how the oxidative and neurological enzymes of the fish are affected by the amounts of KMnO4 used to purify water in the oil-rich Niger Delta region of Nigeria.

2. MATERIALS AND METHODS

The *C. gariepinus* employed in this study were self-bred, and the broodstock used had quick growth potential, increased resilience to low dissolved oxygen, and poor water quality. They were inspected regularly to assess their health and maturity, and freedom from parasite and disease infection. A greenhouse was constructed to depict the natural habitat of the fish and was cleaned daily to maintain

maximum hygiene. Fifteen adults of *C. gariepinus* (5 males and 10 females) were captured from the wild with the help of local fishermen and were kept in earthen ponds. Secondary sex characteristics were used in sex determination. Males are usually larger and have broader heads than females.

As spawning season approaches, males become leaner, develop even larger, muscular heads, and turn a dark bluish-to-black color. Females' heads are narrower than their bodies when viewed from the top. As the spawning season approaches, they develop soft, swollen bellies. One female and two male fish were placed in spawning tanks. The pituitary gland was inserted into the female. Typically, spawning takes place four to six hours following injection. When a male discharges his milt into water, it fertilizes the egg. The fish needed 18 hours to hatch. Under conditions of 28–31°C, hatching takes 4 days, whereas under conditions of 20–22°C, it takes 5 days. Fifteen 60-gallon hexagonal ponds measuring 27 1/4 x 24 1/8 x 29 ½ were built using clayey soil.

For twelve weeks, ten fingerlings were moved to each of the ponds and given a mixture of rice bran and finely powdered cake three times a day. The pond water was changed, cleaned, and monitored every day. No mortality by the conclusion of the 12th week. For 30 days, each of the ponds with their replicates, was subjected to water treatment solutions containing KMNO4 at concentrations of 2.5, 5, and 7.50 mg/l, as well as the control, All the fish, whether they were in the control or experimental group, were fed about 3% of their body weight twice a day. The clay ponds were kept in the utmost sanitary condition, and the water and toxicants were changed out entirely every 24 hours. Throughout the study, daily readings of the water's physicochemical properties were recorded. The fish that were not needed were put back into the main pond.

One fish from each treatment (including replications) is withdrawn following every session of experimentation (2, 9, 16, 23, and 30 days). The fish is then taken into the lab and given anesthesia with MS222 (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma) in a container with good ventilation. Following the protocol outlined by Congleton and LaVoie, (2001), blood samples were collected from the caudal vein, which is located directly behind the backbone. Blood samples were collected using anticoagulant-free centrifuge tubes and subjected to analysis to identify potential abnormalities of hydrocortisone and SOD. Likewise, immediately following the collection of blood on the 30th day, organs were removed from each treated fish and examined for superoxide dismutase (SOD) activity.

Enzymatic Assay

Serum extracts were analyzed utilizing The ELISA reagent comprises antibodies that are specific to cortisol. The kit contains numerous reagents and pre-coated wells that are required for the assay. Wells that had been coated with specific antibodies that bound to cortisol were supplemented with the sera. Thus, the antibodies on the plate can bind with the cortisol in the sample. By washing away unbound substances, any non-specific interactions were eliminated. Supplementary antibodies, which have been fluorophore-tagged, are introduced. Antibodies recognize and bind to any cortisol that has adhered to the plate. Upon the addition of a substrate solution, the enzyme induces a colour change.

The quantity of cortisol present in the sample is directly proportional to the magnitude of this change. A spectrophotometer was used to quantify the colour change, to ascertain the concentrations of cortisol in a blood sample. The interpretation of the results is predicated on a standard curve derived from established cortisol concentrations; this enables the estimation of the true cortisol concentration in the blood of the fish investigated. A solution containing epinephrine and buffer was utilized to generate superoxide radicals. Incorporating the serum into superoxide radicals. By adding an oxidase, an agent that stimulates the production of superoxide radicals, the reaction was initiated.

Using a spectrophotometer, the initial absorbance of the reaction mixture was determined at a specific wavelength of 480 nm. The rate at which absorbance decreases is proportional to the capacity of SOD to eliminate superoxide radicals. As the level of SOD activity increases, the rate of absorbance degradation also decreases. By comparing the rate of absorbance decrease of samples treated with various concentrations of SOD to that of a control, SOD activity in the blood sample of the fish was determined. The SOD activity was quantified in units per milliliter of sample or units per milligram of protein, with one unit of SOD activity corresponding to the quantity of enzyme required to impede the reaction by 50%.

Statistical Analysis

Student's t-test and one-way analysis of variance SPSS (14.0 version), SPSS Inc., Chicago, USA, was employed to calculate the significance of the differences between control and experimental means and within various treatments. P values of 0.05 or less were

considered statistically significant (Fisher, 1950). Multiple bars and line charts were used in this study for the pictorial representation of assessment endpoints.

3. RESULTS

Physicochemical Parameters

The physicochemical parameters of the test media and control group during sub-lethal exposure of *C.gariepinus* to KMnO4 at various doses are shown in (Table 1). The data revealed that the parameters remained relatively constant irrespective of the concentration of the toxicant and duration of treatments

Table 1 Physiochemical parameters of the test media during sub-lethal exposure of *C.gariepinus* to different concentrations (mg/l) of KMNO4 after 30 days exposure

Parameters	**Control** **Mean ± SE**	**0.50** **Mean ± SE**	**1.00** **Mean ± SE**	**1.50** **Mean ± SE**	**2.00** **Mean ± SE**
pH	7.00 ± 0.10 a	7.30 ± 0.40 b	6.70 ± 0.20 c	7.10 ± 0.10d	7.80 ± 0.30f
Temperature (OC)	26.30 ±0.50 s	26.12± 0.11 x	26.05± 0.04 y	25. 80± 0.39 z	24. 90± 0.24r
Alkalinity (mg/l)	17.20 ±0.40 o	17.12 ±0.30 p	17.47±1.20 q	17.13 ±0.50 r	17.19 ±0.10t
Total hardness (mg/l)	31.30 ±1.12 a	31.30 ±1.12 a	31.33 ±3.19 b	30.50 ±1.70 c	31.30 ±0.50f
Dissolve Oxygen (mg/l)	8.10 ±0.08 k	7.86 ±0.15 l	8.10 ±0.14 m	8.05 ±0.08 n	7.90 ±0.90c

Mean with the same superscript in the row are significantly different * ($p < 0.05$)

Enzymatic Assessments

In the cortisol fish, there was a gradual increase in the plasma cortisol level from day 2 (5.20) ug/dl to day 30 (5.30 + 0.010) ug/dl. In the treated fishes, the increment was obvious as compared to the control fishes. On day 2 in the treated fishes, three was a spontaneous increase in the cortisol level with the increase in the concentration of the toxicant on day 2, the concentration of 0.05mg/L the cortisol level was (6.1+ 0.010) ug/dl, and at 0.20ug/L concentration, the cortisol level was 7.20 + 1.03ug/l, and no significant ($p>0.05$) different between the control and various treatment on the same day. From day 19th to 30th, the increment in the cortisol level was proportional to the exposure concentration and was significant between the control and various treatments (Table 2).

Table 2 Responses of hydrocortisone (µg/dL) in the plasma of C.gariepinus exposed to Low dose of KMNO4 (mg /L)

Days	**Control** **Mean ± SE**	**1.00** **Mean ± SE**	**1.50** **Mean ± SE**	**2.00** **Mean ± SE**	**2.50** **Mean ± SE**
2	5.20 ± 0.01a	6.10 ± 0.01 a	6.30 ± 0.15a	6.90 ± 0.20a	7.20 ± 0.18a
9	5.26 ± 0.01a	8.30 ± 0.10b	9.10 ± 0.50b	9.90 ± 0.12b	9.90 ± 0.80b
16	5.60 ± 0.08a	9.10 ± 0.10b	9.30 ± 0.20c	11.20 ± 0.50c	11.30 ± 0.20c
23	5.90 ± 0.02a	9.90 ± 0.20b	11.10 ± 0.10c	12.10 ± 0.10c	13.10 ± 0.10c
30	5.90 ± 0.01a	10.10 ± 0.13b	12.20 ± 0.20c	14.30 ± 0.20c	15.30 ± 0.90c

* : a not significant ($p \leq 0.05$); b significant ($p \leq 0.05$), c highly significant ($p \leq 0.01$)

Induction of SOD activities in the erythrocytes of *C. gariepinus* is dependent on concentrations and time. As depicted in Figure 1, there is a clear correlation between the induction and changes in concentration and exposure time. Significant variations in the enzyme activities were only observed at 2.0 mg/l concentrations on day two ($p < 0.05$). However, SOD activities were significant ($p < 0.05$) at 1.5, 1.0, and 2.0 mg/l of KMNO4 intoxication on days nine, sixteen, twenty-three, and thirty.

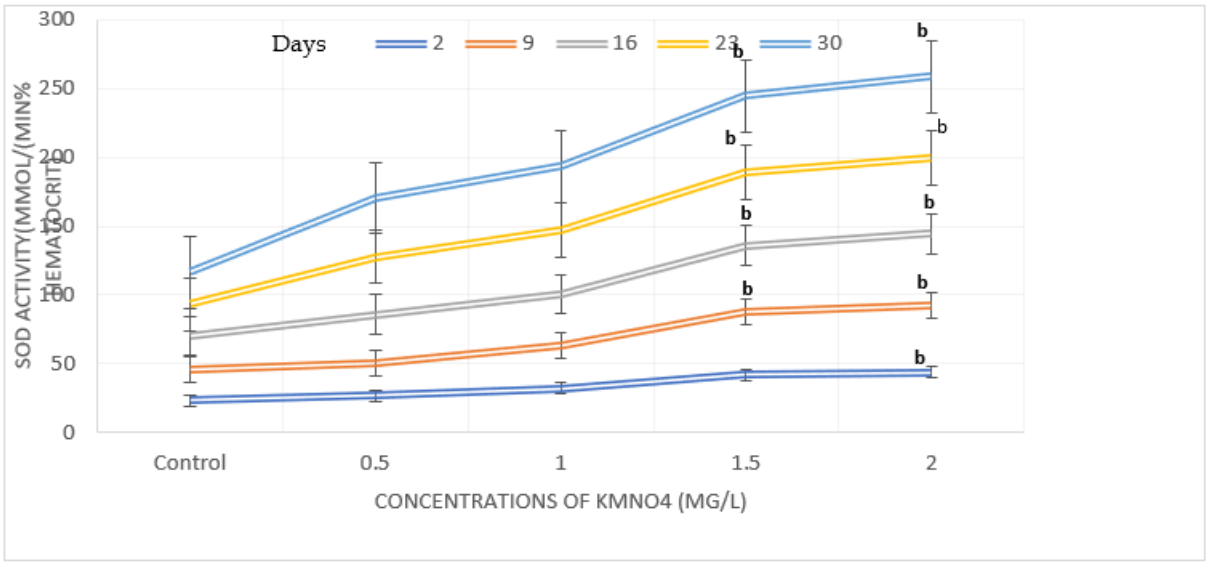

Figure 1 SOD Activity in the erythrocyte of *C. gariepinus* exposed to sub-lethal concentrations of KMNO4: Data presented as mean ± SE. A letter above the bars indicates significant differences between the control and the experimental groups ($p<0.05$).

The control group exhibited the following enzyme activities in the organs: liver (27.80), brain (19.20), gills (12.30), and muscles (15.10). The percentage of induction of this enzyme in each of these organs varied, with the gills exhibiting the lowest induction rate (13.90%) and the liver having the highest (72%). The enzyme activities in the liver of treated fish differ significantly ($p < 0.05$) at concentrations of 1.0, 1.50, and 2.0 mg/l. However, the observed variation in the other organs was limited to fish treated with 2.0mg/l KMNO4 (Figure 2).

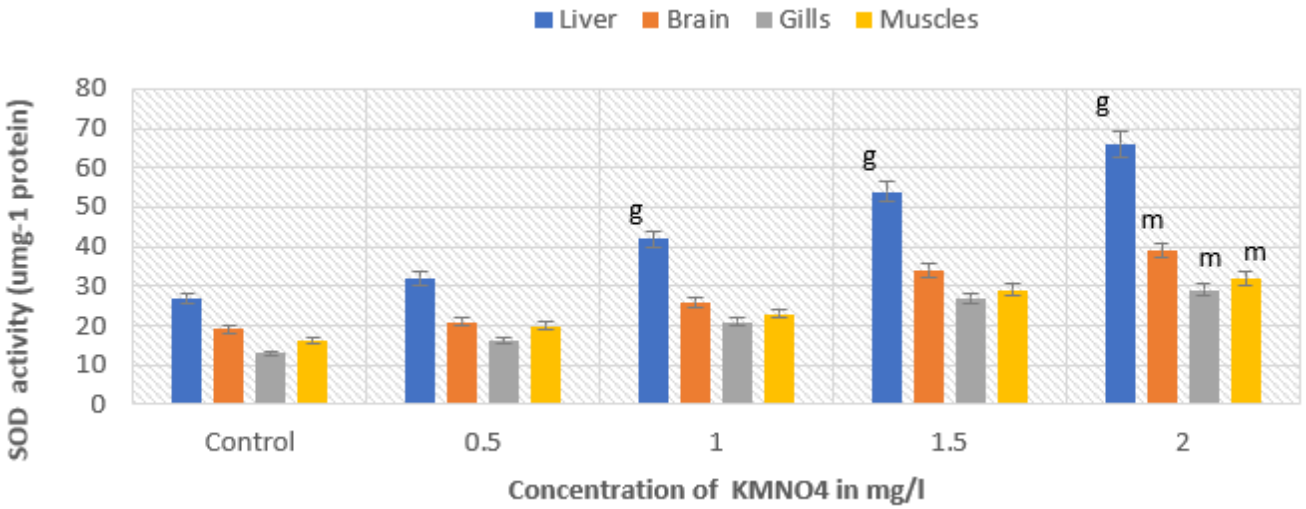

Figure 2 SOD Activity in the tissues of *C. gariepinus* exposed to sub-lethal concentrations of KMNO4 on day 30th. Data presented as mean ± SE. A letter above bars indicates significant differences between the control and the experimental groups ($p<0.05$)

4. DISCUSSION

Human activities, such as water treatment, and anthropogenic activities such as transportation, ballast water discharges, and oil spills, are the sources of contaminants that pose a hazard to marine and freshwater fish populations (OSPAR, 2000). According to sewage water contains synthetic substances like bisphenol and water treatment agents, among others, which have been found to alter the

secondary sex traits of fish. The inhibition of gonadal growth, the emergence of female egg proteins (vitellogenin; VTG) in male fish blood, changes in gonadal histopathology, and the occurrence of "testes-ova" in intersex fish are some of the additional significant effects that have been identified in fish (Jobling et al., 1994).

In this study, when *C. gariepinus* was exposed to sub-lethal doses of KMnO4, the physicochemical parameters of the test media were not affected. Even though the toxicant concentration and duration were increased, the parameters did not change significantly ($p > 0.05$), and they were within the limits that were established by Nigerian regulatory agencies (FMWR, 2007). The findings demonstrated that the KMnO4 had a significant impact on the amount of cortisol in erythrocytes. The endocrine response, which is initiated in reaction to environmental stressors such as pollutants is an integral component of the homeostatic physiological system that is activated in response to environmental stressors (Sinhorin et al., 2014).

The corticosteroid cortisol, which is the most abundant and metabolically active corticosteroid in fish blood, has surprisingly maintained a constant response throughout different periods of this investigation. Cortisol has a significant impact on the liver, gut, and gills in addition to the blood (Gagnon et al., 2006). The blood is not the only organ or tissue that cortisol impacts. These organs and tissues are a reflection of the two primary adaptive roles of cortisol that have been recognized up to this point: Osmoregulation and the regulation of energy balance. It has been stated by Hontela, (2005) that plasma cortisol is an outstanding indication of functional alterations in the HPI axis.

In reaction to nearly any kind of environmental stress, teleost fish will naturally secrete the steroid hormone cortisol from their internal tissues. This is an intrinsic response that they exhibit. According to Gagnon et al., (2006), exposure to metals and other toxins that suppress cortisol secretion may affect social interactions and processes that are dependent on cortisol. According to Ezemonye and Ikpesu, (2011), the stress indicator that is utilized in fish the most frequently is an increase in plasma cortisol levels. One possible interpretation of this behavior is that it is the fish's reaction to the discovery of a potentially harmful or toxic material in its environment.

In a study that was conducted by Scott et al., (2003), it was shown that the levels of cortisol in the plasma of rainbow trout increased when they were exposed to an alarm substance. This alarm substance was a chemical that was secreted by the epidermal epithelium. Cadmium was found to be able to suppress this increase in cortisol levels. According to the findings of a study that was carried out by Gagnon et al., (2006), it was shown that increased concentrations of copper have a direct toxic effect on adrenocortical cells, which inhibits the release of cortisol. On the other hand, in vitro data showed that exposure to ecologically relevant Cu concentrations for thirty days led to an increase in cortisol release in response to ACTH at low doses.

Given that corticoids are known to regulate electrolyte balance and gill ATPase activity, an elevated concentration of cortisol may be connected with either the creation of abnormal chloride and ATPase levels or the attempt to return them to normal levels (Fiess et al., 2007). This is because corticoids help regulate the levels of chloride and ATPase in the body. The spike in plasma cortisol levels that was observed during the treatments that were carried out in the current investigation could have been attributable to the secretion of cortisol from interrenal tissue as a response to stress, an aberrant plasma chloride level, or an attempt to return the values to normal. It appears that erythrocyte cortisol would be a more appropriate indicator to monitor in terms of the influence on the environment.

Because the marker is easy to assess and convenient. A significant rise in the activity of a large number of enzymes that are found in the liver of mammalian organisms is induced in the process of defending against peroxidative damage, these enzymes are involved in a variety of processes that are related to the detoxification and conjugation of xenobiotics. Among these enzymes, superoxide dismutase stands out as particularly important. In this study, the levels of SOD activities in the tissues of the fish (erythrocytes, liver, brain, gills, and muscles) spontaneously rise in response to changes in the concentration of KMnO4 and the duration of exposure. This is because superoxide dismutase reacts with harmful chemicals as well as sensitive and essential cellular targets, such as nitrogen oxide radicals.

Abhijit and Poopal, (2012) examined the plasma, gastrointestinal tract, and liver of *Catla Catla* that had been subjected to methyl parathion, and found that there was an increase in the activity of superoxide dismutase. Naz et al., (2017) reported an increase in the SOD activity of *Catla catla, Cirrhinus mrigala*, and *Labeo rohita*, the three varieties of carp that are endemic to India when they were exposed to a cocktail of pesticides. A higher response of SOD activity indicates an increased antioxidant capacity that is working to counterbalance the harmful effects of reactive oxygen species (ROS). The induction was highest in the liver This may be because the liver is the primary system responsible for detoxifying. Furthermore, the liver is the metabolic powerhouse, capable of bioactivation, biotransformation, and xenobiotic excretion (Danielson, 2002).

Furthermore, it is in direct touch with pollutants that are absorbed from the environment (Bernet et al., 1999). A comparable observation was documented by Petrivalsky et al., (1997) regarding the liver of *Oncorhynchus mykiss* under varying concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,2-bis(p-chlorophenyl)-1,1-dichloroethane. In line with prior research, Nwani et al., (2013) and Sinhorin et al., (2014) discovered that the outcomes of their investigations mirrored those of the aforementioned studies. Based on the elevation in SOD activity observed in this investigation, it is possible to infer that the exposure to KMnO4 resulted in an upsurge in ROS generation.

5. CONCLUSION

These findings show that KMnO4 disrupts the endocrine system and has a significant impact on SOD activity in fish. This research indicates that KMnO4 poisoning potentially may cause comparable detrimental impacts on human health and, in extreme cases, significantly endanger human life. Because the primary causes of KMnO4 exposure are anthropogenic, sensitization of the community is essential, and water remediation agencies ought to adhere to the guidelines provided by the manufacturer. Similar precautions should be taken to render sewage from water facilities non-hazardous before its release into rivers.

Author's Contribution

ITO: Develop the concept and involve in the samplings and data analysis

ICG: Liaised with the communities where samples were collected, analyzed and interpreted data, and take care of the correspondence.

Ethical approval & declaration

In this article, the animal regulations followed as per the ethical committee guidelines of Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria; the authors observed the impacts of sub-lethal potassium permanganate exposure on endocrine disruption and oxidative stress in *Clarias gariepinus*. The Animal ethical guidelines are followed in the study for species observation, identification & experimentation.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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