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Research Article
Oxidative Stress and Neurological Impairment in *Chrysichthys nigrodigitatus* Following Subacute Exposure to Water Treatment Agent

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Abstract

Background and Objective: The water in Niger Delta, Nigeria has significant amounts of iron and manganese. To remove them, a precipitation procedure that involves potassium permanganate is required. In the end, the slush is empty into the aquatic environment without treatment, hence, we investigated the effects of the $KMnO_4$, a major water treatment agent on *Chrysichthys nigrodigitatus*, a dominant freshwater fish in Niger Delta Nigeria. **Materials and Methods:** Fifteen *Chrysichthys nigrodigitatus* were self-bred and subjected to one-tenth of the range of $KMnO_4$ concentrations used for water treatment in Nigeria (0.50, 1.00, 1.50 and 2.00) mg L^{-1} and a control. At the end of each experimental period, a fish is removed from each treatment and blood samples are taken from the caudal in anticoagulant-free centrifuge tubes and serum is obtained by centrifugation of blood at 3,000 rpm for 10 min. Similarly, on day 30th, immediately after collection of the blood, a fish from each treatment was dissected and the organs (brain, liver, gills and muscles) were excised, transferred to liquid nitrogen and kept at -25°C until the analysis. The activities of glutathione s-transferase (GST) and acetylcholinesterase (AChE) in these organs were measured spectrophotometrically. **Results:** The slight increase in the activity of GST and the decrease in the activity of AChE demonstrated the incapability of the vital organs to neutralize the $KMnO_4$ generated in the oxidative stress condition and neurological impairment. **Conclusion:** These studies have proven that $KMnO_4$ suppresses AChE activity and has a dramatic impact on the antioxidant enzyme in *C. nigrodigitatus*. Therefore, sludge from water plants should be kept harmless before discharging into the rivers.

Key words: Metals, potassium permanganate, *Chrysichthys nigrodigitatus*, glutathione-s transferase, acetylcholinesterase, Niger Delta, serum

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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

Organic chemicals, microbes and inorganic contaminants are the three primary types of pollutants found in groundwater. Because metals are not biodegradable, metal poisoning of groundwater from inorganic pollution poses a significant environmental health concern[1]. This means it will not decompose naturally and will thus linger in the water, posing a health danger to individuals who consume it.

According to studies[2], iron and manganese, which naturally occur in groundwater, are frequently removed from the water for aesthetic reasons because the substances can cause issues like turbid water, precipitation of the substances in distribution pipes, discoloured laundry and a different taste and odour of the water. Manganese and iron in groundwater can be hazardous to human health and possibly cause chronic disorders. They also state that the presence of manganese and iron in groundwater, which will eventually become drinking water, is a severe environmental concern that must be addressed and that the two chemicals must be removed. In the earth's crust, manganese is the sixth most frequent metal[1].

Manganese does not pose a health hazard until it reaches roughly 0.5 mg L^{-1}, according to an information sheet issued by scientists[3]. According to the same article, elevated manganese concentrations can cause nervous system damage over time, resulting in a situation that resembles Parkinson's disease. Infants and small children appear to absorb more manganese and excrete less than later age groups, resulting in them being more exposed to the material than others, perhaps affecting their nervous system development.

Potassium permanganate ($KMnO_4$) is a strong oxidant on acrylamide. A small amount of $KMnO_4$ was needed to reduce acrylamide concentrations to an acceptable level. Some reducing agents in water may influence the oxidation effectiveness of $KMnO_4$, hydrogen sulphide has a large influence and its presence at the molar ratio in water may greatly reduce the oxidation efficiency[4,5]. Permanganate pre-oxidation offers the benefits of cheap cost, ease of operation and maintenance over current specific procedures for treating water with high organic content, such as ozonation, activated carbon and so on.

The use of potassium permanganate peroxidation in the Philadelphia Suburban Water Company's Crum and Pickering facilities established its usefulness as a disinfectant[6] and it can improve the performance of chemical coagulation of water[6] treatment with $KMnO_4$ at dosage rates of between 0.5 and 1.5 mg L^{-1}, in combination with feeds of 30.8 mg L^{-1} alum and up to 10 mg L^{-1} powdered activated carbon, was used to regulate taste and smell at these sites. In addition, when raw water was treated with 0.75 mg L^{-1} potassium permanganate, the MPN was reduced by 95%[7]. A plant-scale treatment investigation was carried out at a water purification plant in Kansas City, Missouri. Doses of $KMnO_4$ were adjusted to match the water's real permanganate requirement. The 1.0 mg L^{-1} in December, 1.1 mg L^{-1} in January, 1.3 mg L^{-1} in February and 2.7 mg L^{-1} in March were the monthly averages[6]. The coliform content was decreased to less than detectable levels with a few outliers, faecal streptococci clearance varied from 48-96%. The elimination of actinomycetes ranged from 96-27%. Aschner *et al.*[8] investigated the use of alum coagulation to remove monomeric precursors. When the raw water had large amounts of monomeric precursors, they found that using $KMnO_4$ as a pre-treatment method before alum coagulation considerably reduced the quantity of chloroform in the treated water.

The efficiency and mechanism of $KMnO_4$ oxidation for the removal of acrylamide from water were examined by Jusoh *et al.*[9]. They discovered that potassium permanganate is a powerful acrylamide oxidant. To bring acrylamide concentrations down to a safe level, a little quantity of $KMnO_4$ was used. Some reducing substances in the water may affect $KMnO_4$'s ability to oxidise. Hydrogen sulphide has a significant impact and its presence in water at a molar ratio can significantly limit oxidation efficiency. The effect of iron (II) on acrylamide oxidation is also extremely obvious. The addition of humic and fulvic acids may impair the effectiveness of acrylamide oxidation somewhat. The oxidation of acrylamide by potassium permanganate appears to be unaffected by nitrate or manganese. García-Mendieta *et al.*[10] investigated the effects of several treatment strategies on algae and particle removal in direct filtration, as well as the effects of $KMnO_4$ on algae and particle removal. They demonstrated the effect of permanganate pretreatment followed by dual coagulant coagulation (ferric sulphate and cationic polymer). They discovered that $KMnO_4$ peroxidation and coagulation enhance the effective removal of algae and other particulate particles in direct filtration[11].

Since the 1990s, when a growing number of Pharmaceutical and Personal Care Products (PPCP) and Endocrine Disruptor Compound (EDC) residues were discovered in natural aquatic ecosystems, the wastewater and water treatment sectors have faced issues. These chemicals have been linked to a variety of changes in aquatic habitats, including reduced fertility and gender shifts in birds, fish and mammals. These modifications raise concerns that they may have a long-term health effect on people when consumed in large quantities of seafood. As a result, the goal of this research is to see what effect a range of $KMnO_4$

concentrations used for water treatment in the Niger Delta area of Nigeria has on neurological and oxidative stress enzymes in *C. nigrodigitatus*, a major fish in the Niger Delta ecological zone.

MATERIALS AND METHODS

Study materials: The *C. nigrodigitatus* used for this investigation were self-bred and the broodstock used to have rapid growth potential, more resistant to low dissolved oxygen levels and poor water quality. They were regularly inspected for their health, maturity and parasite and disease-free status. The project began on March 15th, 2021 and we'll wrap it up on May 15th, 2022,

Experimental design: To replicate the fish's natural environment, a greenhouse was built and cleaned every day. Five males and ten females of *C. nigrodigitatus* were collected from the wild and housed in clay ponds with the aid of a local fisherman. Secondary sex traits were employed in determining sex. It is well knowledge that males tend to be bigger and have wider heads than females. Males grow leaner, more muscular and darker in colour as the spawning season draws closer. When seen from the top, the heads of females were smaller than their bodies. Soft, bulging bellies appear as the spawning season nears.

In each of the breeding tanks (one female and two males were kept). The pituitary gland was injected into the females. After injection, spawning usually takes 4-6 hrs. Water-borne milt from males fertilizes an egg delivered by females. The fish need 18 hours of incubation time. The female releases eggs which was fertilized by the milt released by males in water. The incubation period for the fish was 18 hrs. Hatching occurs in 5 days at 20 to 22°C.

Fifteen 60-gallon hexagonal ponds were built with clayey soil, each measuring Hexagon: 27 1/4×24 1/8×29 ½. A total of ten fingerlings were placed in each of the final ponds, where they were fed three times daily on a finely powdered cake and rice bran. Aquaria were checked, cleaned and replenished every day. Stale water was pumped out of the system using manual pumping equipment. There were no deaths after the 12th week.

Clayey soil was used in constructing fifteen earthen ponds each with the dimension of 60 Gallon Hexagon: 27 1/4×24 1/8×29 ½. Ten fingerlings were transferred into each of the final ponds and were fed on a finely powdered cake and rice bran thrice daily for 12 weeks. Water in the aquaria was monitored daily, cleaned and refilled. A manual pumping machine was used to siphon stale water. At the end of the 12th week, no mortality was observed. After which, each of the final ponds with their replicates was exposed to the range of the concentrations of $KMnO_4$ used in water treatment (2.50, 5.00 and 7.50) mg L^{-1} and the control for 30 days. Throughout the experiments, control and experimental fish were also fed twice daily at approximately 3% of their body weight. Water and toxicant were completely replaced every 24 hrs and the earthen ponds were maintained at the highest possible hygiene.

The physicochemical parameters of the water were taken daily throughout the investigation period. The unused fish were returned to the main pond. At the end of each experimental period (2, 9, 16, 23 and 30) days, one fish is removed from each treatment (including the replicates), taken into the laboratory in a well-ventilated container and immediately anaesthetized with MS222 (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma).

The blood samples were taken from the caudal vein just behind the backbone of each fish as described[11]. This blood was collected in anticoagulant-free centrifuge tubes. Serum was obtained by centrifugation of blood at 3.000 rpm for 10 min. Serum samples were then stored at –80°C until the analysis.

Enzymatic assay: Acetylcholinesterase (AChE) activities were monitored indirectly using 10-acetyl-3,7-dihydroxy-phenoxamine, a sensitive fluorogenic probe for hydrogen peroxide. AChE was estimated by a colorimetric assay by Ni *et al.*[12] as given by de Moura Nunes Fernandes *et al.*[13] using acetylthiocholine. About 2 mL of the serum in phosphate buffer (pH 8) were reacted with 200 ll of acetylthiocholine iodide (15 mM) and 1000 ll of DTNB (3 mM) and incubated for 15 min at 30 C. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each lasting 13 sec. After that, 200 mL of AChE (0.3 U mL^{-1}) solution was added to the initial mixture to start the reaction and then the absorbance was determined. Enzymatic activities were expressed as units of activity (U) per mg of protein. Each unit of activity corresponded to 1 nmol of substrate hydrolyzed per minute. The GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Vontas *et al.*[14] a method as modified by name Hernandez *et al.*[15]. The DetectX® Glutathione S-Transferase Fluorescent activity kit was used to measure GST activity in the samples. The kit makes use of a non-fluorescent molecule that serves as a substrate for the GST enzyme, which binds covalently to glutathione to produce a highly fluorescent result. In the endpoint mode, a fluorescent product is produced by mixing the sample or standard with the provided detection reagent and GST, which is read at 460 nm in a

fluorescent plate reader with excitation at 390 nm. This kit evaluates GST activity quantitatively by comparing it to a GST standard curve. The standard curve was subjected to a four-parameter curve analysis.

Statistical evaluation: The one-way analysis of variance and the Student's t-test SPSS (14.0 version), SPSS Inc., Chicago, USA, was used to calculate the significance of differences between control and experimental means. Statistical significance was defined as p-values of 0.05 or less[16]. In this study, many bars and line charts were employed to depict the assessment endpoints.

RESULTS

Physicochemical parameters: The physicochemical properties of the test media after the sub-lethal exposure of *C. nigrodigitatus* to various concentrations of $KMnO_4$ and the control group were shown in Table 1, indicating that the parameters did not change substantially as the toxicant concentrations increased.

Enzymatic assessments: The acetylcholinesterase and glutathione-s transferase activities in the erythrocyte of *C. nigrodigitatus* exposed to different concentrations of $KMnO_4$ were shown in Fig. 1 and 2. The responses of the enzymes to the toxicant were dose and time-dependent. The enzyme activities in the control groups were relatively the same and the slight changes were not significant (p>0.05).

The AChE activities on day 2, at 0.5, 1, 1.5 and 2.0 mg L^{-1} of $KMnO_4$ were 30.01, 26.70, 23.50 and 20.45 µmol/(min% hematocrit). No significant (p>0.05) changes in AChE activity in all the treatments on day 2. The enzyme activities on days 9, 16, 23 and 30, at 0.50 mg L^{-1} of $KMnO_4$ were 28.45, 26.79, 23.20 and 23.11 min% hematocrit, at 1.0 mg L^{-1} of $KMnO_4$ were 24.40, 22.11, 20.20 and 20.10 min% hematocrit. At 1.50 $KMnO_4$ were 20.08, 16.56, 14.23 and 14.11 min% hematocrit and at 2.00 mg L^{-1} $KMnO_4$ were 18.34, 13.28, 12.10 and 10.30 min% hematocrit, respectively.

In the tissues of the fish, the inhibition of the enzyme activities was more in the brain, gills and muscles and least in the liver. Percentage inhibition at the end of experimentation revealed, brain (83%), gills (33%), muscles (25%) and the liver (58%) (Fig. 2). Comparing the inhibition of the activities in the control with the treatments revealed that AChE activities vary significantly (p<0.05), in the brain and the gills only.

The GST activities in the fish erythrocytes of *C. nigrodigitatus* show concentrations and time-dependent induction. The higher the concentration and the exposure duration the more the induction and it varies significantly as shown in Fig. 3. On day 2, the enzyme varies significantly (p<0.05) at 2.0 mg L^{-1} concentrations of the inducer. While, on days 9, 16, 23 and 30th, GST activities vary significantly (p<0.05) at 1.5, 1.0 and 2.0 mg L^{-1} of $KMnO_4$ intoxication.

GST activities in the control group were, liver (27.80), brain (19.20), gills (12.30) and muscles (15.10). The induction of this enzyme in these organs revealed that the liver had the highest percentage of induction (66%) and the gills had the least (13.90%).

In the liver, the enzyme activities vary significantly (p<0.05) at 1.0, 1.50 and 2.0 mg L^{-1} of treated fishes. While in the other organs, the variation was observed at 2.0 mg L^{-1} $KMnO_4$ treated fish only (Fig. 4).

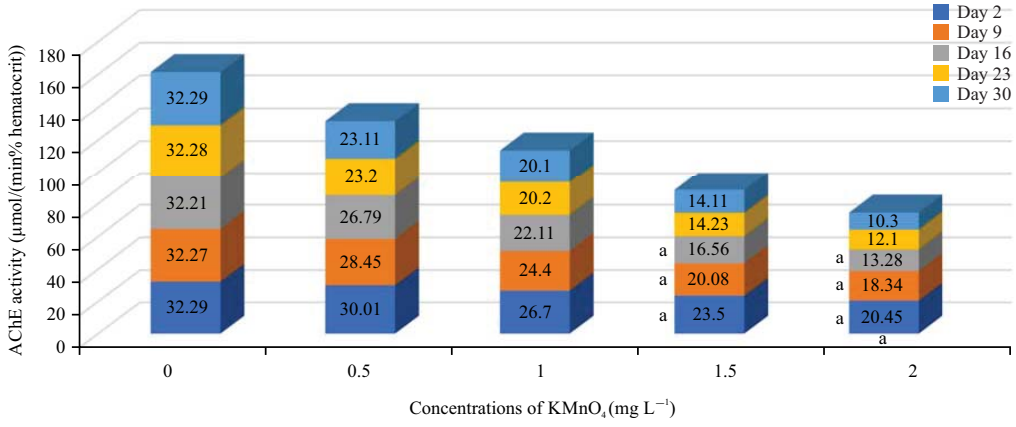

Fig. 1: AChE activity in the erythrocyte of *C. nigrodigitatus* exposed to sub-lethal concentrations of $KMnO_4$
Data presented as mean±SE, [a]: Significant differences between the control and the experimental groups (p<0.05)

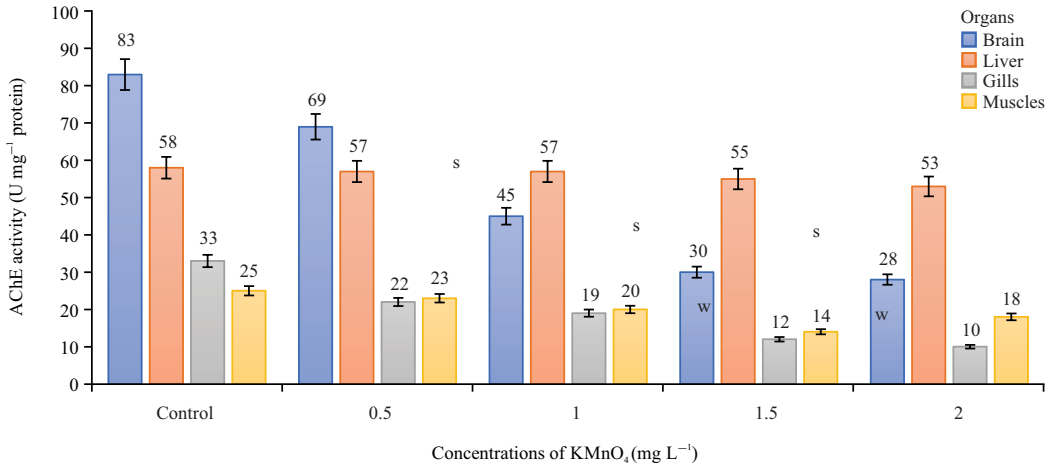

Fig. 2: AChE activity in the tissues of *C. nigrodigitatus* exposed to sub-lethal concentrations of $KMnO_4$ on day 30th
Data presented as mean±SE, [S, W]: Significant differences between the control and the experimental groups (p<0.05)

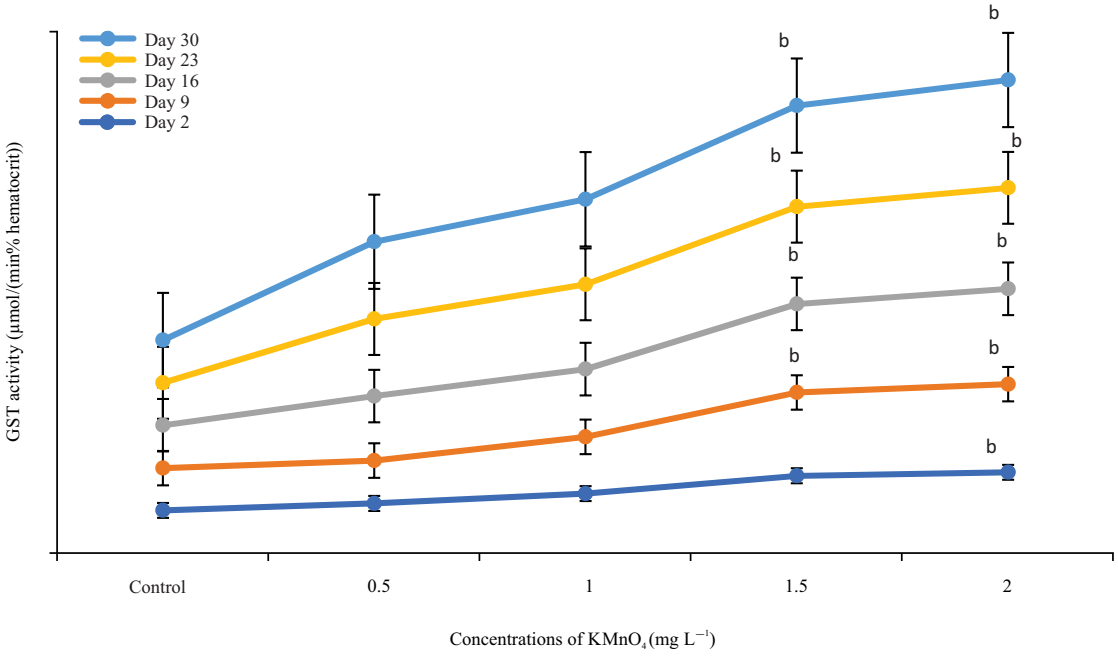

Fig. 3: GST activity in the erythrocyte of *C. nigrodigitatus* exposed to sub-lethal concentrations of $KMnO_4$
Data presented as mean±SE, [b]: Significant differences between the control and the experimental groups (p<0.05)

Table 1: Physiochemical parameters of the test media during sub-lethal exposure of *C. nigrodigitatus* to different concentrations (mg L^{-1}) of $KMnO_4$ after 30 days of exposure

	Mean±SE				
Parameters	Control	0.50	1.00	1.50	2.000
Ph	7.00±0.10^a	7.30±0.40^b	6.70±0.20^c	7.10±0.10^d	7.80±0.30^f
Temperature (°C)	26.30±0.50^s	26.12±0.11^x	26.05±0.04^y	25. 80±0.39^z	24.90±0.24^r
Alkalinity (mg L^{-1})	17.20±0.40^o	17.12±0.30^p	17.47±1.20^q	17.13±0.50^r	17.19±0.10^t
Total hardness (mg L^{-1})	31.30±1.12^a	31.33±3.19^b	30.50±1.70^c	31.70±1.10^d	31.30±0.50^f
Dissolve oxygen (mg L^{-1})	8.10±0.08^k	7.86±0.15^l	8.10±0.14^m	8.05±0.08^n	7.90±0.90^c

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

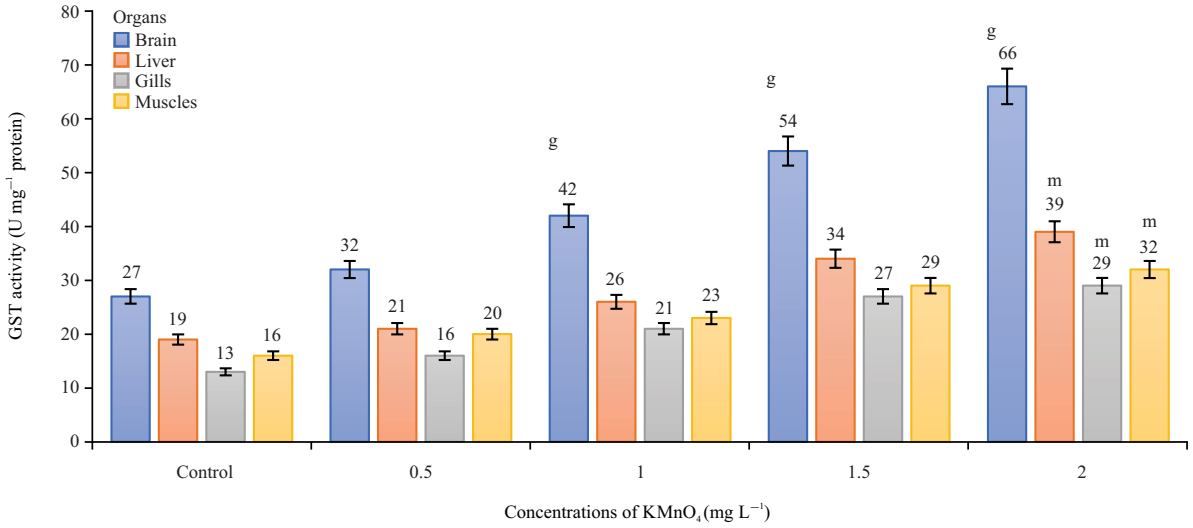

Fig. 4: GST activity in the tissues of *C. nigrodigitatus* exposed to sub-lethal concentrations of $KMnO_4$ on day 30th
Data presented as mean±SE, ^{g,m}: Significant differences between the control and the experimental groups ($p<0.05$)

DISCUSSION

Fish are a naturally occurring and non-exhaustible resource, it is possible to maintain good stock levels, but they require a healthy habitat. Unfortunately, both marine and freshwater fish populations are at risk of being exposed to potentially harmful compounds. Some of these dangerous substances include water treatments, nutrient pollution and the results of maritime activities such as oil spills and ballast water discharges[17]. In addition, Bruland and Lohan[18] mentioned that synthetic products, such as bisphenol and water treatment agents, among other compounds, seep from sewage water into aquatic environments and are associated with the observed changes in secondary sex characteristics of male and female fish. Additionally, there are additional substantial consequences that may be seen in fish and these effects can be detected. These include a decrease in the levels of reproductive hormones (such as estrogens and androgens), an inhibition of gonadal development, the presence of the proteins found in female eggs (vitellogenin, VTG) in the blood of male fish, gonadal histopathology and even intersex fish with "testes-ova"[19].

The physicochemical parameters of the test media during the sub-lethal exposure of *C. nigrodigitatus* to various concentrations of $KMnO_4$ as well as the control group revealed that the parameters did not change significantly ($p>0.05$) with an increase in toxicant concentrations and were within the regulatory limit set by various regulatory bodies in Nigeria[20].

Fish that were treated with $KMnO_4$ exhibited a significant decrease in their AChE activity, both in the red blood corpuscles and the organs. It was discovered that the erythrocyte AChE activity was more vulnerable to the harmful effects of $KMnO_4$ than the activities of other organs, except for the brain. In light of this, the acetylcholinesterase activity of erythrocytes may prove to be a more reliable signal for use in environmental monitoring. It is not difficult to get a sample of blood and the analysis of several effect characteristics is straightforward and quick. In the current investigation, none of the fish was killed in the process of collecting their blood samples. Additionally, there was no significant difference ($p>0.05$) in the levels of acetylcholinesterase activity in erythrocytes and brain tissue.

The brain inhibited AChE activity the most, followed by the blood, gills, muscles and liver. Excessive acetylcholine (ACh) buildup at synapses and neuromuscular junctions, leading to overstimulation of ACh receptors[21], might explain the highest AChE activity inhibition observed in the brain. The enzyme activity in all tissues was inhibited as the toxicant concentrations in the tissues increased. Similar observations were reported in the tissues of *Ctenopharyngodon idella* exposed to organophosphate pesticide[22-24]. As a result of the suppression of AchE activities, a cholinergic crisis develops, with increased glandular secretions, paralysis, miosis and muscular fasciculation, all of which can lead to death[25-27]. Except for the liver tissues, AChE activity differs substantially ($p<0.05$) between the control and different treatments in all of the tissues studied. The low amount of AChE activity disruption seen in the muscles might be related to the tissue's lower quantity of neural transmissions[28]. The activity of numerous enzymes in the mammalian liver always increased

dramatically as a result of toxins. These enzymes are engaged in a variety of xenobiotic detoxification and conjugation processes, as well as preventing peroxidative damage. Glutathione-s transferase is one of the most notable of these enzymes. The GST activities in *C. nigrodigitatus* fish erythrocytes demonstrated concentrations and time-dependent induction in this study. Only the liver had a larger induction % than the blood when compared to the organs, hence the erythrocyte of fish serves as an excellent measure for investigating toxicity.

Except for the maximum concentrations of 2.0 mg L^{-1} of $KMnO_4$, a similar trend was detected in other tissues (brain, gills and muscles), although it was not significant ($p>0.05$). The fact that the liver has the largest induction might be due to its role as the primary site of detoxification[29]. Furthermore, since it was in close touch with pollutants acquired from the environment, the liver is the greatest organ of metabolism and has the capacity for bioactivation, biotransformation and excretion of xenobiotics[27]. Similar observation was reported by Abdelazim *et al.*[30] on the liver of Oncorhynchus mykiss on exposure to the fish to various dosages of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,2-bis(p-chlorophenyl)-1,1-dichloroethane (p,p-DDE).

Potassium permanganate caused stress response in the tested fish and the toxicity of $KMnO_4$ to nontarget aquatic organisms has not been seriously considered because only a few standard toxicity test data on the toxicity of potassium permanganate to nontarget freshwater organisms are available. Furthermore, as the fish attempts to adjust to the altered environment, the community composition of the catfish's external biome will be altered, affecting energy transfer along the trophic levels. More information about the fate and duration of $KMnO_4$ residuals in potential effluent in aquatic habitats is critical for regulatory agencies.

CONCLUSION

These findings showed that $KMnO_4$ reduces AChE activity while also having a significant impact on GST activity in *C. nigrodigitatus*. It is imperative to know that $KMnO_4$ poisoning has similar effects on human health and can lead to major life-threatening consequences. As anthropogenic sources of $KMnO_4$ exposure are the most common, population sensitization is essential and water treatment agencies should follow the manufacturer's directions. Similarly, before being discharged into the rivers, sludge from water plants should be rendered harmless.

SIGNIFICANCE STATEMENT

This study demonstrated that $KMnO_4$ harms fish and the data generated from this experiment may be used in assessments of the potential effects of $KMnO_4$ on aquatic resources whether an accidental or intentional exposure. Similarly, the findings indicate that, due to its harsh oxidizing properties, $KMnO_4$ should not be applied to fish more frequently and that extreme caution should be exercised during application. As a result, now is the time for serious consideration of the environment, the aquatic life and, therefore, indirectly, our future.

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