

Full Length Research Paper

Acute toxicity of potassium permanganate to fingerlings of the African catfish, *Clarias gariepinus* (Burchell, 1822)

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Laboratory static bioassays were conducted to determine the 96-h LC₅₀ and the lethal levels of concentrations of the aquaculture therapeutant, potassium permanganate (KMnO₄) on fingerlings (mean weight, 6.24 ± 0.15 g and mean length, 4.25 ± 0.07 cm) of the African catfish, *Clarias gariepinus*. A total number of one hundred and eighty (180) fingerlings of mixed sex and brood stock were used for the toxicity bioassay. The 96-h median lethal concentration (LC₅₀) value obtained for treatment of the fingerlings of the catfish *C. gariepinus* with potassium permanganate was 3.02 mg/L KMnO₄ with lower and upper 95% confidence limits of 0.40 and 3.73 mg/L KMnO₄, respectively. There was very strong and positive correlation between the variables ($r = 0.96$) with 93% (r^2) association. Toxicity curve revealed 11.20 mg/L KMnO₄ at 8.20 h as the threshold value for the 96 h assay of potassium permanganate on fingerlings of *C. gariepinus*. The 96 h LT₅₀ for 10, 8 and 6 mg/L KMnO₄ to the test fish were also determined to be 10.40, 11.30 and 17.80 h, respectively. Varying behavioural patterns were observed in the fish, which included erratic swimming, loss of reflexes, total loss of equilibrium, paleness of skin and gasping for air. The value of water quality parameters monitored during the exposure period did not differ significantly ($P > 0.05$) within the various concentrations of the therapeutant (KMnO₄) as well as with the control. However, pH and total alkalinity tended to increase with increasing concentration of the toxicant.

Key words: Potassium permanganate, acute toxicity, LC₅₀, LT₅₀, behaviour, *Clarias gariepinus*, Nigeria.

INTRODUCTION

Potassium permanganate (KMnO₄) is one of the widely used inorganic chemicals worldwide and much information is available on its chemistry, manufacture and various uses (Duncan, 1978). It has been used as a therapeutant and prophylactic for fish diseases since 1918 when the first controlled test of its efficacy against myxobacteriosis was performed by Davis (1922). The therapeutic use of KMnO₄ was extended to control invertebrate parasites of fish by Hess (1930) and methods of its application were developed by Kingsbury and Embury (1932). The chemical became popular for the treating of diseased trout because it could be used in soft waters where other chemicals, such as copper sulphate, were too toxic to use. When channel catfish *Ictalurus punctatus* culture became established in the United States, KMnO₄ was found to be effective for

control of some warm-water bacterial, parasitic and fungal diseases (Wellborn, 1985).

Currently, in aquaculture worldwide, KMnO₄ has been used as a water/bath treatment for protozoan parasites in commercial and ornamental fish culture; however, it is not approved by U. S. Environmental Protection Agency (EPA) or the Food and Drug Administration (FDA), for therapeutic use in aquaculture. Regulatory action on the use of KMnO₄ has been deferred pending the outcome of ongoing research. Straus and Griffin (2002) described the four legal options for using aquatic chemotherapeutics in the United States as follows: 1) the FDA has approved the use of compound as a therapeutant; 2) the therapeutant is the subject of an Investigational New Animal Drug (INAD) exemption; 3) the therapeutant has been determined by the FDA to be of low regulatory priority; or

4) the therapeutant is not of low regulatory priority but regulatory action has been deferred pending the outcome of research. Currently only formalin (Formalin-F, Paracide-F, and Parasite-S), oxytetracycline (Terramycin), sulfadimethoxine and ormetropin (Romet 30), and sulfamerazine (no longer manufactured) are FDA-approved therapeutants; though each approval is for specific uses (Greenlees, 1997).

Users of aquaculture chemicals have been frequently advised to use chemicals sparingly and only when needed to avoid stressing the treated fish and possibly introducing greater harm than benefit (Wellborn, 1985; Tucker and Robinson, 1990).

A limited amount of information is available about the toxicity of KMnO_4 . The acute toxicity of KMnO_4 to various fish species has been studied (Marking and Bills, 1975; Tucker, 1987) including several studies investigating striped bass (Wellborn, 1969; Hughes, 1971; Bills et al., 1993; Reardon and Harrell, 1994) and the hybrid striped bass (Straus, 2004); however, there are no data for most African fish species. Most commonly, KMnO_4 is used in freshwater systems at 2 mg/L to control ectoparasites, bacteria and fungi. Effective concentrations are determined by the KMnO_4 demand of the water being treated (Marecaux, 2006).

The African catfish, *Clarias gariepinus* (Burchell, 1822), was selected as the test organism in this study for its great aquaculture and commercial value in Nigeria and elsewhere in the developing world. *C. gariepinus* is a benthopelagic (bottom feeder), omnivorous feeder that occasionally feeds at the surface. Their diets include insects, crabs, plankton, snails and fish but also have been seen to consume young birds, rotten flesh, plants and fruits (Teugels, 1986). *C. gariepinus* also referred to as mudfish, is very hardy and tasty. They are able to tolerate adverse aquatic conditions where other cultivable fish species cannot survive (Olatunde, 1983). It is widely cultivated and used as experimental fish (Musa and Omoregie, 1999).

Experiments were conducted on fingerlings, based on the fact that fingerlings are more sensitive to environmental changes than older and more mature fish. The specific objective of the research was to investigate the acute toxicity of KMnO_4 to fingerlings of *C. gariepinus*.

MATERIALS AND METHODS

Fish samples

Live fingerlings specimens of the African catfish, *C. gariepinus* of mixed sex and broodstock (mean weight, 6.24 ± 0.15 g and mean length, 4.25 ± 0.07 cm) were procured from a local fish farm at Sapele, Delta State, Nigeria. They were transported to the laboratory at the Department of Zoology, Delta State University, Abraka and kept in plastic aquaria supplied with clean water. All fish were maintained in the laboratory for a minimum period of two weeks during which they were fed with commercial fish pellets (Copens 2 mm), mixed with ground shrimps and half of the water was changed daily.

Bioassay technique

Bioassay followed the standard semi-static procedure with the observation of all necessary traditions and precautions (Sprague, 1973; Ward and Parrish, 1982; APHA, 1998). Appropriate modifications were made where necessary. In essence, the process involved a range determination step during which the experimental fish were exposed to a wide range of concentrations of the chemotherapeutants, potassium permanganate (KMnO_4) relying on preliminary trials and/or historical data. After establishing the ranges of concentrations to be tested, necessary dilutions from the appropriate stock solution were made for the experimental concentrations for acute tests based on the logarithmic bisection of the intervals. In all cases, the diluted water was aerated to saturation *ab initio* and all the tests were replicated with the appropriate controls.

The containers used consisted of plastic aquaria of 40 litres capacity. The upper part of each aquarium was covered with a lid made of fine polyethylene gauze screen of 1 mm mesh size. Each experimental set up and control (each aquarium) was stock with 10 fish specimen in each of the concentrations tested. The acute toxicity bioassay lasted 4 days (96 h).

Stock solution of potassium permanganate (KMnO_4) was prepared from 1 g standard AnalaR grade granules in 1 litre of distilled/deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared. The acute concentrations used were 10, 8, 6, 4, and 2 mg/L KMnO_4 . Fish specimens were starved for 24 h, weighed and then introduced into the test solution/water (10 specimens in each aquarium). In order to monitor the depletion of dissolved oxygen, the effects of evaporation and ammonia concentration during experimentation and reduce stress, 50% of the toxicant water added after removing the exact volume from the exposure set up daily. During the period of experimentation, some water quality parameters were monitored daily using the method described by APHA (1998). The water quality parameters monitored include: temperature, hydrogen ion concentration (pH), dissolved oxygen, free carbon IV oxide and total alkalinity. Mortality was also recorded.

Water quality parameters

The water quality parameters of the experimental set up with KMnO_4 toxicant and control bioassay were monitored by the procedures according to APHA (1998) as follows.

Temperature

The temperature ($^{\circ}\text{C}$) was measured by dipping a dry bulb thermometer to about 10 cm below the water surface in each aquarium and allowed to equilibrate for 5 min before the reading was taken.

Hydrogen ion concentration (pH)

Ten (10) ml of water sample was drawn from the aquaria and transferred into a beaker; and the pH reading was taken with a Jenway model pH meter.

Dissolved oxygen

250 ml of water sample was collected into a 250 ml reagent bottle, avoiding air bubbles during collection. 2 ml of wrinkler A solution followed by 2 ml of wrinkler B solution were added with different measuring cylinders to the water sample in the reagent bottle. The bottle was then stoppered and inverted vigorously a few times to

Table 1. Water quality parameters for acute toxicity bioassay of potassium permanganate on fingerlings of the African catfish, *Clarias gariepinus* over a period of 96 h.

Parameter	Concentration of potassium permanganate (mg/L)					
	0.0	2.0	4.0	6.0	8.0	10.0
Temperature (°C)	24.50±0.01	24.40±0.02	24.40±0.02	24.40±0.02	24.00±0.02	24.00±0.03
pH	7.09±0.01	7.13±0.11	7.18±0.20	7.34±0.12	7.38±0.11	7.82±0.21
Dissolved oxygen (mg/L)	7.46±0.12	7.54±0.20	7.50±0.11	7.21±0.21	7.12±0.08	7.01±0.18
Free carbon (IV) oxide (mg/L)	4.23±0.12	4.24±0.21	4.21±0.12	4.16±0.02	4.10±0.14	4.01±0.04
Total alkalinity (mg/L)	76.84±0.09	78.21±0.06	79.32±0.12	80.04±0.23	82.47±1.02	86.73±1.32

Values are mean±SE.

ensure thorough mixing of the reagents with the water sample. The flocculent was allowed to settle to about 1/3 of the volume of the bottle after which the stopper was removed and 2 ml of concentrated tetraoxosulphate (VI) acid (H₂SO₄) was carefully added. The bottle was then stoppered gently to avoid entry of air bubbles and vigorously inverted until the precipitate dissolved leaving a yellow colour of free iodine. 200 ml of the water sample from the reagent bottle was then transferred into a conical flask and 2 ml of fresh starch solution was added to the sample. The resulting blue-black coloured sample was then titrated with 0.25 N sodium thiosulphate.

Free carbon (IV) oxide

100 ml of water sample was drawn into a 250 ml conical flask to which 10 drops of phenolphthalein indicators was added. If sample turned pink 0.0 mg/L was recorded; otherwise it was titrated with N/44 NaOH solution to achieve a weak pink endpoint.

Free Carbon (IV) Oxide (mg/L) = 10 x vol of N/44 NaOH used.

Total alkalinity

Determination of total alkalinity involves phenolphthalein and methyl orange alkalinities thus.

Phenolphthalein alkalinity

In this, 100 ml of water sample was drawn into a volumetric flask to which four drops of phenolphthalein indicators was added. If the sample remains clear 0.0 mg/L was recorded, otherwise it was titrated with 0.02N H₂SO₄ from a burette until it became clear.

Phenolphthalein alkalinity = 10 x vol of 0.02N H₂SO₄ used in mg/L CaCO₃.

Methyl orange alkalinity

This involves the measuring of 100 ml of water sample into a volumetric flask and adding 3 drops of methyl orange indicator. The sample was then titrated with 0.02 N H₂SO₄ till the greenish yellow colour turns pink orange.

Methyl orange alkalinity = 10 x vol of 0.02N H₂SO₄ used in mg/L CaCO₃.

Total alkalinity (mg/L) = Phenolphthalein alkalinity + methyl orange alkalinity

Visual examination

Visual examination was conducted during the acute toxicity assay, on the behavioural pattern of the test fish, which included the erratic movement, leaping, gasping for air and instability among other things.

Data analysis

The bioassay results were analysed using the probit method to estimate the LC50, according to the procedure of Finney (1971). The percentage mortality were calculated and transformed into probit kill using the probit table. The probit kill was plotted against logarithm concentration after which a straight-line equation was used to find the regression line between the points plotted on the graph sheet.

RESULTS

Water quality parameters

The values of the water quality parameters monitored during the exposure period did not differ within the various concentrations of the therapeutant as well as with the control. However, pH and total alkalinity tended to increase with increasing concentrations of potassium permanganate (KMnO₄), respectively. The result of the mean water quality parameters measured during the 96 h exposure period is as presented in Table 1.

Behavioural patterns

The behavioural patterns observed in *C. gariepinus* juveniles, the acute toxicity bioassay included loss of stability such as erratic swimming, total loss of equilibrium, agitated swimming, loss of reflexes and gasping for air.

Acute toxicity

The acute toxicity result obtained (Tables 2 and 3) from the exposure of fingerlings of the *C. gariepinus* to various acute concentrations of potassium permanganate (KMnO₄)

Table 2. Mean[#] cumulative mortalities of fingerlings of the African catfish *Clarias gariepinus* exposed to various acute concentrations of potassium permanganate during the 96 h of exposure.

Concentration of KMnO ₄ (mg/L)	No of specimen	Mortalities/ h							
		6	12	24	36	48	60	72	96
10	10	2.67	7.67	10.0	10.0	10.0	10.0	10.0	10.0
8	10	1.33	6.67	9.33	10.0	10.0	10.0	10.0	10.0
6	10	1.00	4.00	7.00	8.33	8.33	9.67	10.0	10.0
4	10	0.00	2.33	3.00	3.67	4.33	4.33	4.33	4.33
2	10	0.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00
0 (Control)	10	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33

[#]Obtained from triplicate exposure.

Table 3. Log. Concentration; Total Mortality; Percentage Mortality; Corrected Mortality and Probit Kill of fingerlings of the African Catfish *Clarias gariepinus* exposed to various acute concentrations of Potassium permanganate during the 96 hours of exposure.

KMnO ₄ (mg/L)	Log. Conc.	Total mortality	% mortality	#Corrected mortality	Probit Kill
10	1.0000	10.00	100.00	100.00	8.72
8	0.9031	10.00	100.00	100.00	8.72
6	0.7782	10.00	100.00	100.00	8.72
4	0.6021	04.33	43.30	41.37	4.78
2	0.3010	02.00	20.00	17.23	4.06
0 (Control)	-	00.33	3.30	0.00	-

Abbott's Formula for Corrected Mortality.

Corrected Mortality (%) = $\{(M_O - M_C) / (100 - M_C)\} \times 100$ where M_O = Observed treatment mortality in % and M_C = Control Mortality in %.

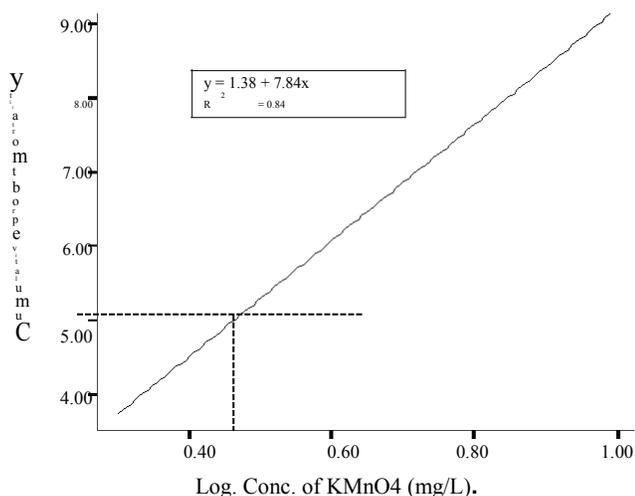


Figure 1. Log. Conc. against Probit Mortality of fingerlings of *Clarias gariepinus* exposed to various acute concentrations of KMnO₄ (mg/L) during 96 h exposure period. LC₅₀ = 3.02 mg/L KMNO₄; R = 0.96; R² = 0.93; Upper and lower confidence limit = 3.73 and 0.4 mg/L KMNO₄.

during the 96 h of exposure revealed 100% mortalities in 10, 8, and 6 mg/L KMnO₄ after 12, 24, 36 and 72 h, respectively. 43.33, 20.0 and 3.3% mortalities were

recorded in 4, 2 and 0 mg/L KMnO₄ after 96 h, respectively. To eliminate the factor(s) that led to the mortality in 0 mg/L KMnO₄, Abbott's formula for corrected mortality was used and thus revealed 100, 100, 100, 41.37, 17.23 and 0% for 10, 8, 6, 4, 2 and 0 mg/L KMnO₄, respectively.

Median lethal concentration

A plot of Log concentration against Probit Kill (Figure 1) showed that the 96 h LC₅₀ of KMnO₄ to the test fish *C. gariepinus* was 3.02 mg/L. This implies that the accumulation of KMnO₄ above 3.02 mg/L for 96 h is lethal for the fingerlings of *C. gariepinus*. The upper and lower 95% confidence limits were 3.73 and 0.40 mg/L, respectively. There was very strong and positive correlation between Log concentration of KMnO₄ and probit mortality as shown by the value of regression analysis ($r = 0.96$). This also showed that 93% of the association is dependent on the variables (Log concentration and probit mortality).

Median lethal time

A plot of time against Mortality (Figure 2) showed that the

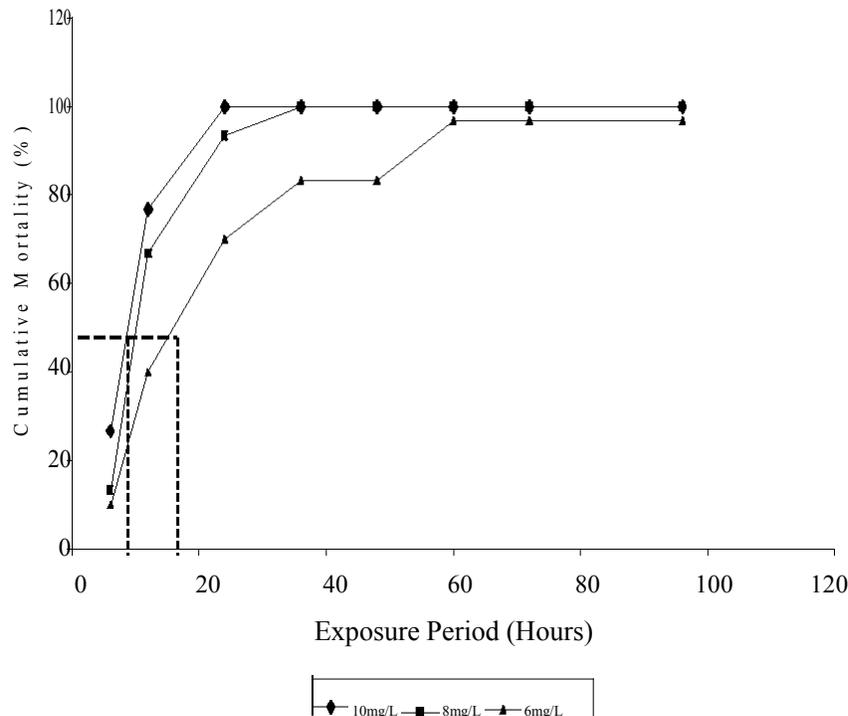


Figure 2. Linear Relationship between Cumulative Mean Mortality and exposure period of fingerlings of *Clarias gariepinus* exposed to various acute concentrations of KMnO_4 during the 96 h of exposure period. LT 50 for 10 mg/L KMnO_4 is 10.40 h; LT 50 for 8 mg/L KMnO_4 is 11.30 h; LT 50 for 6 mg/L KMnO_4 is 17.80 h.

96 h LT_{50} of 10, 8 and 6 mg/L KMnO_4 to the fingerlings of *C. gariepinus* were 10.40, 11.30 and 17.80 h, respectively for the fingerlings of *C. gariepinus* during the 96 h exposure period.

Toxicity curve

A plot of concentration against Median Lethal Time (Figure 3) revealed 11.20 mg/L KMnO_4 as lethal threshold value. Thus implies that any value from 11.20 mg/L KMnO_4 is lethal for the fingerlings of *C. gariepinus* for the 96 h of exposure.

DISCUSSION

All the tested fish were able to survive during acclimatization because amongst the culturable species of fish, *C. gariepinus* is known to be one of the hardest, capable of tolerating long periods of adverse conditions, particularly low concentrations of dissolved oxygen. Grasping of air observed in this study revealed that the toxicant (KMnO_4) interferes with the respiratory mechanisms of the fish especially in water of higher pH by forming tiny particles of manganese oxide salts, which clog fish gills. The observed paleness of the skin, on the other hand

may be related to the corrosive effect of potassium permanganate. The result obtained herein indicated that increasing concentrations of potassium permanganate led to increased mortality of fingerlings of *C. gariepinus* with a 96-h LC_{50} value of 3.02 mg/L. The lethal effect of exposure to KMnO_4 appears to be unrelated to metallic manganese toxicity, but rather the strong oxidative action of the permanganate (MnO_4^-) ion (Griffin et al., 2002). It has been suggested that water of high pH can cause the disruption of MnO_4^- (Noga, 1996), which in turn increases the rate of mortality as observed in this study.

The acute toxicity of KMnO_4 to channel catfish is greater at lower temperatures, at higher pH and in harder water (Marking and Bills, 1975). Tucker (1987) reported that toxicity to fingerling channel catfish was closely related to the chemical oxygen demand (COD) of the culture water. The 96 h LC_{50} increased from 4.5 to 17.6 mg/L as the COD increased from 21 to 118 mg/L. Straus (2004) demonstrated that the acute toxicity of KMnO_4 to hybrid striped bass juveniles is lower in waters of lower total alkalinity and total hardness. Marking and Bills (1975) also reported that the acute toxicity of KMnO_4 to rainbow trout *Oncorhynchus mykiss* and channel catfish *L. punctatus* was greatest in very hard waters, and at 96 h these toxicity differences were also statistically significant. In the above study, (Marking and Bills 1975), it was demonstrated that the toxicity of KMnO_4 to ten species of

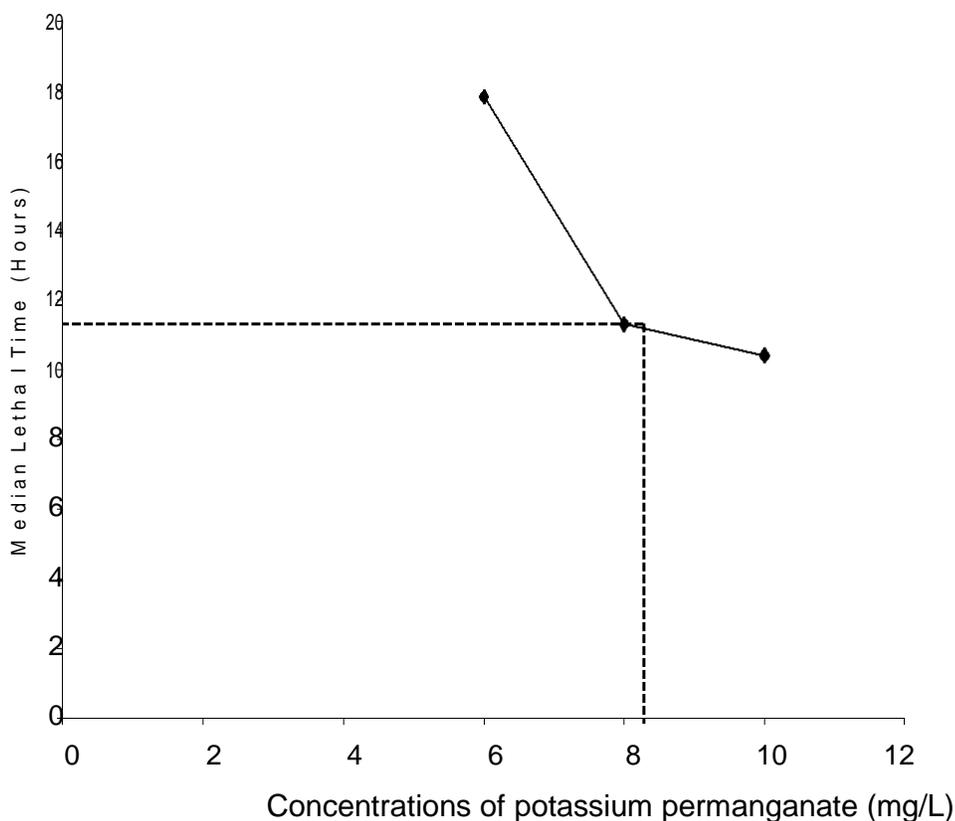


Figure 3. Linear Relationship between various Concentrations and Median Lethal Time of fingerlings of *Clarias gariepinus* exposed to various acute concentrations of KMnO_4 during 96 h exposure period. The threshold value is 11.20 mg/L KMnO_4 at 8.20 h for the fingerlings of *Clarias gariepinus* for the 96 h of exposure.

fish changed little after 24 h. Tucker (1987) found that the 15 min Potassium Permanganate Demand (PPD) was highly correlated with the toxicity of KMnO_4 to juvenile channel catfish that were exposed in various pond waters.

Several other authors have reported similar findings such as Hughes (1971) who reported a 96 h LC_{50} for potassium permanganate in 1 month old striped bass fingerlings to be 4.0 mg/L; Golow and Godzi (1996) also reported 96 h LC_{50} for potassium permanganate on *Oreochromis niloticus* to be 6.1 mg/L. Similarly, Dureza (1988) showed that the 96 h LC_{50} for potassium permanganate on fry and fingerlings of *Tilapia nilotica* were 2.9 and 3.3 mg/L, respectively. These researchers' findings are in the range with the findings reported in this study. However, the little variation observed may be probably due to the ability of different species of fish to tolerate different levels of a given toxicant. Variation in the size of the experimental fish could also be responsible for the difference in the values of LC_{50} .

Recently, results from a study (Mareceaux, 2006) suggested that KMnO_4 at concentrations of 0.5 and 1.0 mg/L may be safe for use in water containing sailfin mollies *Poecilia latipinna* in water of salinities of 2, 15 and

30 g/L. Mareceaux, 2006; however stated that KMnO_4 should not be used at concentrations of 3.0 mg/L in 2, 15 and 30 g/L salinity water on the sailfin molly until further research was conducted and that the toxicity of potassium permanganate increased in the higher salinity groups (15 and 30 g/L) compared to the low salinity group (2 g/L).

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