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Behavioural Responses in Mudskipper (*Periopthalmus Papilio*) Exposed to Sodium Bromide under Laboratory Conditions

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Abstract

One hundred and eighty (180) mudskipper (*Periophthalmus papilio*) of equal size (mean length 18.74 ± 2.64cm and mean weight 156.68 ± 1.81g) were exposed to different concentrations (0.0mg/l – control, 5.0, 10.0, 15.0, 20.0 and 25.0mg/l) of sodium bromide a biocide, to determine the Behavioural responses of opercula beat frequency (OBF), tail beat frequency (TBF), 96 hour lethal concentration (96h.LC_{50} and median lethal time (MLT_{50}). The result obtained indicated that *P. papilio* showed initial stress responses such as increased opercula ventilator rate, erratic swimming and gasping for air. The pattern of response of TBF to time and concentrations of the biocide was irregular, whereas that of OBF tended to decrease with increase in time, but increased with increase in concentrations. Also cumulative mortality values increased with exposure time and concentrations.

Introduction

Aquatic systems are the final recipient of both natural and artificial inputs of pollutants into the environment. Releases of toxic substances such as chemicals have been responsible for the reduction in the populations of aquatic organisms across the globe. Even sub-lethal concentrations of toxicants may prove to be equally devastating to fish populations [1, 2]. The upsurge in human activities is one of the main factors leading to the increasing level of contaminants in aquatic environments. Coastal pollution has been increasing significantly over the recent years and further expanding environmental problems in many developing countries. The discharge of waters from cites, public places and industries have resulted in pollution of aquatic ecosystems [3, 4].

Fishes are exposed to pollutants through water and in their feeding patterns. They enter their bodies through the skin, gills and even via the mother in uteri [5]. One of the most insidious characteristics of pollutants is that because they are lipid – soluble they tend to accumulate in animal body tissues. This problem is further compounded by the process of biomagnifications in which chemical concentrations increase at higher tropic levels [6]. In amphibians contaminant concentration are often many time greater in body tissue than those in the surrounding waters [7]. Chemicals from house hold and industrial sources have been recognized as one of the major pollutants in the aquatic bodies [8]. One of such chemicals whose utilization cut across home and industries is biocide.

The use of biocides is indispensable in modern agricultural technology to control weeds or pests for the production of more food and management of public health. Biocides such as sodium bromide are routinely employed to control the growth and development of fouling organisms. In water cooling systems the working life of a biocide depends on environmental factors, the amount added, the physical and chemical fate of the compound. Unfortunately, there exists residual quantities of biocides and their degradation/ transformation present in the effluents of these systems, are sometimes discharged into rivers, creeks, estuaries and lagoons [9].

Benli, et al. defined Behavioural toxicity as a change, which is induced by stress that exceeds the homeostasis [10]. It is believed that Behavioural changes in fish are the most sensitive measures of neurotoxicity and this may have motivated the large number of studies on animals [11]. Results from several investigations involving the use of several fish species support the concept that toxicant – induced stress on organisms can be quantified by methods other than mortality [12, 13, 14]. Hence, changes in fish behavior can be used as a sensitive indicator of acute and sub lethal toxicant exposure. Fish altered behaviors can be grouped as follows: a) Locomotors activity e.g. opercula beat frequency (OBF), tail beat frequency (TBF), (b) avoidance, (c) inhibition of feeding, (d) schooling, (d) migratory behavior (e) spawning and (f) homing. Some fish behaviors (e.g. locomotors and avoidance) are extremely sensitive to pollutant chemicals, whereas others (e.g. aggression, feeding and spawning) seem to be refractory [15].

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Mudskipper *Periophthalmus Papilio* is an amphibious fish that is highly active during low tides and spend most of its time out of water in mangrove habitats. According to Akinrotimi et al. [16] they form a high density on tidal mud flats in the coastal areas of Niger Delta. This species is widely distributed and notably present in many reverie communities in Niger Delta, Nigeria, thus, it is essential to evaluate the toxicity of biocides in the fish, as many research activities in coastal environment have been focusing on other species. Hence, the present investigation is aimed to assess the physiological responses of the mudskipper *P. Papilio* exposed to the biocide, sodium bromide in order to understand the biocide mode of action, stress responses and organ dysfunction in experimental fish.

Materials and Methods

Experimental Location

The experiment was carried out at the brackish water research station of African Regional Aquaculture Centre, Buguma, and Rivers State, Nigeria.

Source of Experimental Fish

One hundred and eighty (180) adult, mudskipper *P.Papilio* of equal size (mean length 18.74±2.64 cm and mean weight 156.68±1.81 g) was collected from the mangrove swamps in Buguma creek at low tide using locally made fishing trap. They were then transferred in six 50 liter plastic tanks to the laboratory for acclimation process.

Acclimation and Feeding Of Fish

The experimental fish was acclimated in four 150L capacity circular plastic tanks containing 150L de-chlorinated water for and mud at the bottom, 7 days to experimental conditions at room temperature Netted materials with central slits was tied to the tops of the tanks to prevent escape of fish. Water renewal was done every two days. The fish were feed with freshly killed crabs, cut into small pieces.

Procurement of Test Solution

A commonly used biocide sodium bromide (NaBr) was purchased off shelf, from "Analytical" chemical shop, Garrison, Port Harcourt, Rivers State, Nigeria. Sodium bromide is an inorganic compound with the formula NaBr. It is a high-melting white, crystalline solid that resembles sodium chloride. It is a widely used source of bromide ion and has many applications.

Preparation of Test Solution

The solution of the chemical in water was prepared by serial dilution using the dilution formula described by Begum [17].

Exposure of Fish to Biocide Sodium Bromide

10 Mudskippers each were introduced individually into 18, tanks of dimension 1.5m x 1m x 0.5m containing various concentrations of biocide: 0.00 (control), 5.00, 10.00, 15.00, 20.00 and 25.00 mg/L^{-1}. Each treatment(s) and control was replicated three times and the experimental duration lasted for a period of 96 hours. The solution for each concentration was renewed daily, with freshly prepared solution of biocide. The tanks were covered with netted materials and supported with heavy objects to prevent the mudskipper from escaping. Opercula beat frequency (OBF), Tail beat frequency (TBF) and mortality was monitored at fixed intervals of 12, 24, 36, 48, 60, 72, 84 and 96 hours. Dead fish were counted at 12h intervals and revived from aquaria. A fish was considered dead when it showed no visible movement (e.g. gill movements) and touching its caudal peduncle did not produce any reaction.

Evaluation of Water Quality Parameters

Temperature measurements were made with a mercury-filled Celsius thermometer. PH was done with pH meter. Electrical conductivity and nitrate of both effluent and the water samples were determine using Horiba water checker (Horita U-10 models). While phosphate and ammonia was measured using a test kit with a calorimetric chant produced by SUNPV, Biochem, Beijin China and the salinity was evaluated using hand held refractometer (model HRN-2N, Atago, Products, Japan). This was done by dropping two drops of water and effluents on refractive glass and then viewed from the back side, the reading were then recorded. Biological water samples for Oxygen Demand (BOD) were incubated for 5 days in the dark at room temperature. Then the Winkler's method (APHA [18]) was then used.

Statistical Analysis

Data obtained from the experiments were subjected to ANOVA using Statistical Package for the Social Sciences, (SPSS) version 10 and differences among means were separated by Duncan Multiple Range test at 0.05%. The dependent variables in the trials (OBF, TBF, mortality, blood parameters and biochemical indices) were compared based on the concentration of the toxicants. An analysis of the lethal concentration (LC50) values for the 24, 48, 72 and 96 hours and the median lethal time for the various concentrations of herbicide was done with Probity Analysis. Safe concentration of the herbicide at the various time intervals were obtained by multiplying the lethal concentration by a factor 0.1 [19].

Results

Water Quality Parameters

The result of the physico-chemical properties of the exposure tanks in chronic toxicity studies are presented in table 1. The water quality variables in the aquaria of *P. Papilio* exposed to sodium bromide for 96 hours indicated a significant reduction ($p < 0.05$) in the values of dissolved oxygen. Also, significant increases with increasing concentration of the chemical were however recorded in the values of ammonia, electrical conductivity. Chloride, sulphide and total hardness of water. While other parameters such as temperature, pH and salinity were within the same range comparable to the control at all concentrations of the chemical.

General Behavioural Changes

As fish came into contact with the toxicant, abnormal behavior was observed. The abnormal behavior exhibited was a Neural Paralytic Syndrome (NPS). Strong restlessness and

Table 1: Physico - Chemical Parameters of Water in Experimental Tanks of P. Papilio exposed to Sodium Bromide for 96 hours

	Concentrations of Sodium Bromide (MlL^{-1})					
Parameters	**0.0**	**5.0**	**10.0**	**15.0**	**20.0**	**25.0**
Temperature (°C)	27.37 ± 4.21^{a}	27.68± 4.21^{a}	27.49 ± 4.11^{a}	27.74± 4.12^{a}	28.02±1.18^{a}	27.61± 1.18^{a}
Ph	6.38± 1.67^{a}	6.36± 1.24^{a}	6.33± 1.18^{a}	6.36± 1.24^{a}	6.39± 1.34^{a}	6.37± 1.17^{a}
Ammonia (mgL^{-1})	0.39± 0.01^{a}	1.01± 0.038^{b}	0.49 ± 0.04^{a}	1.03± 0.01^{b}	1.06± 0.01^{b}	1.12± 0.18^{b}
Dissolved Oxygen (mgL^{-1})	6.78± 1.12^{c}	5.81± 0.81^{b}	6.13± 0.91^{b}	3.88± 1.16^{a}	3.24± 1.34^{a}	3.02± 1.018^{a}
Nitrite (mgL^{-1})	00.04± 0.01^{a}	0.02± 0.01^{ab}	0.009± 0.02^{ab}	0.036± 0.01^{c}	0.04± 0.02^{a}	0.05± 0.03^{a}
Sulphate (mgL^{-1})	0.02± 0.01^{a}	0.04± 0.01^{a}	0.02± 0.01^{a}	0.05± 0.01^{a}	0.06± 0.02^{a}	0.07± 0.01^{a}
Salinity (0/00)	10.88± 1.68^{a}	10.87± 1.24^{a}	10.81±1.02^{a}	10.81± 1.02^{a}	10.89± 1.12^{a}	10.88± 1.11^{a}
Electrical Conductivity mmho/cm)	386.31± 10.41^{a}	398.61± 11.21^{a}	383.61± 12.61^{a}	42871± 13.81^{b}	348.61± 14.21^{a}	487.66± 15.61^{c}
Chloride (mgL^{-1})	7.81± 1.89^{a}	8.14± 2.18^{a}	8.64 ± 1.67^{a}	14.68± 2.84^{ab}	20.61± 241^{d}	24.68± 3.18^{c}
Sulphide (mgL^{-1})	0.34± 0.01^{a}	0.41± 0.02^{a}	6.38± 0.02^{a}	0.53± 0.12^{b}	0.68± 0.08^{c}	0.78± 1.18^{d}
Total Hardness (mgL^{-1})	**19.74± 1.21^{a}**	**24.68± 1.18^{b}**	**23.68± 2.41^{ab}**	**28.61± 3.14^{ab}**	**26.71± 1.81^{db}**	**32.68±1.18^{c}**

Mean within the same superscripts in the row are not significant different (*P < 0.05*)

excitation was reflected by an increased reaction to exogenous stimuli, loss of movement co-ordination began as well as loss of orientation in water. Twisting of abdomen and weakening of jerks (areflexia) was also observed. Jumps above the water surface especially those that received the highest concentration of the toxicant, also rapid opercula movement and incessant gulping of air was obvious. There were no obvious changes in fish behavior in the lower concentrations (5.0, 10.0 and 15.0 mg/L). These abnormalities displayed by fish increased with increasing concentration of sodium Bromide water but decreased with time/period of exposure.

Behavior Responses (Opercula Beat Frequency and Tail Beat Frequency) and Mortality of *P.Papilio* exposed to Sodium Bromide for 96hours

The pattern of response of OBF/min and TBF min to time of exposure and concentration of the toxicant were similar, it increased with time and concentration and latter decreased,

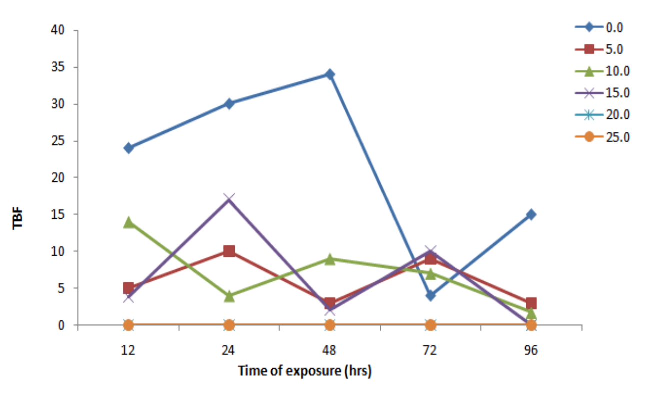

Figure 1: Tail beat frequency (TBF) in *P.papillio* exposed to various concentrations of sodium bromide for 96 hours

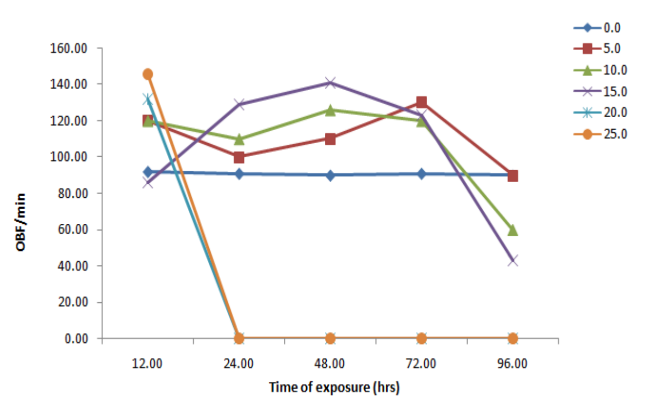

Figure 2: Tail beat frequency (TBF) in *P.papillio* exposed to various concentrations of sodium bromide for 96 hours

especially at the highest concentration and time (Figures 1 and 2). The range of variation of OBF/Min and TBF/min was similar, both were narrow. The cumulative mortality of the fish increased with time and was significantly different for the various time intervals and concentration.

Analysis of Variance (ANOVA) showed that various concentration extraction of biocide produced significant differences in the values of OBF and TBF (*P > 0.001*) (Table 2 and 3). The time of exposure produced significant changes both in the OBF (*P > 0.0001*) and TBF (*P > 0.001*). Interaction between the exposure time and concentration of the toxicant produced marked changes in the OBF and TBF (*P > 0.0001*). ANOVA showed that the time of exposure (*P > 0.001*) interactions between exposure time and toxicant concentration (*P > 0.0001*) had no significant impact on the cumulative mortality of the fingerlings (Table 4). Mortality increased with time and concentration of the toxicant (Figure. 3). No morality was recorded in the control group.

Table 2: Changes in the tail of opercula beat frequency and cumulative mortality (%) of *P. Papilio* exposed to various concentration of Sodium Bromide for 96 hours (Mean ± SD)

Variable	Time of exposure (hrs)					
	24	48	72	96		
TBF/min	15.49 ± 4.41^{ab}	7.87± 4.41^{ab}	21.12± 5 .22^{b}	4.49± 5.40^{b}		
OBF/min	113.10± 7 .60^{a}	115.23± 7.23^{a}	10.79± 8 99^{a}	65.15± 9 .15^{b}		
Cum. Mortality	37.227± 31 .21^{d}	56.11± 36 .16^{c}	63.89± 32 .39^{b}	73.33± 29 .31^{a}		
Concentration of Sodium bromide (Mg/L)						
	0	5	10	15	20	25
TBF/min	15.73 ± 4.41^{a}	5.79± 4.41 ^{a}	16.46± 4 .41^{b}	9.00± 4.63^{b}	7.04 ± 5.92^{a}	0.00 ± 0.00
OBF/min	96.71± 7 .60^{a}	123.27± 8.00^{a}	108.58± 8 00$^{a\text{-}c}$	100.93± 8 .00$^{a\text{-}c}$	73.42± 10 .19^{c}	124.72± 16 .99^{ab}
Cum. Mortality		22.67± 12 .80^{d}	24.00± 24 .43^{d}	32.67± 19 .81^{a}	50.67± 37 .70^{b}	77.33± 36 .15^{d}

Means with the same superscripts in the row are not significantly different *(P > 0.05)*

Table 3: Mean squares from ANOVA of OBF/min., TBF/min. and cumulative mortality of *P.Papilio* exposed to sodium bromide (mean±SD)

Source of variation	Dependent variable	df	Mean square
	TBF	4	0.017^{ns}
	OBF	4	532.06***
Time	MORT	4	2880.46**
	TBF	6	267.72^{ns}
	OBF	6	2757.12**
Conc.	MORT	6	1951.03***
	TBF	24	419.78^{ns}
	OBF	24	1335.49^{ns}
Time ´ Conc.	MORT	24	481.17**
	TBF	49	292.03
	OBF	49	876.02
Error	MORT	49	150.14

TBF-tail beat frequency; OBF-opercula beat frequency, and mort. - Cumulative mortality (%), ns-non-significant, F-testsignificant level:*-0.5, **-0.01, ***-0.001.

Table 4: Lethal concentration and associated 95% confidence limits of *P.Papilio* exposure to Sodium bromide

Time (hrs)	Lethal Concentration (mean +C.L.)	Safe Concentration	Probit model estimation equation
24	LC50- 20.81 (19.58-22.48)	2.08	
24	LC90- 26.44 (24.45-31.47)	2.64	
48	LC50- 18.50 (16.67-19.40)	1.85	y=-5.18+0.29´
48	LC90- 22.54 (20.88-26.09)	2.25	
72	LC50- 17.11 (16.30-17.84)	1.71	y=-5.30+0.31´
72	LC90- 21.44 (20.59-23.31)	2.14	
96	LC50-15.88 (14.99-16.64)	1.59	y=-5.06+5.06´
96	LC90- 19.91 (18.97-21.42)	1.2	

Where y=dependent variable, x= undependable variable

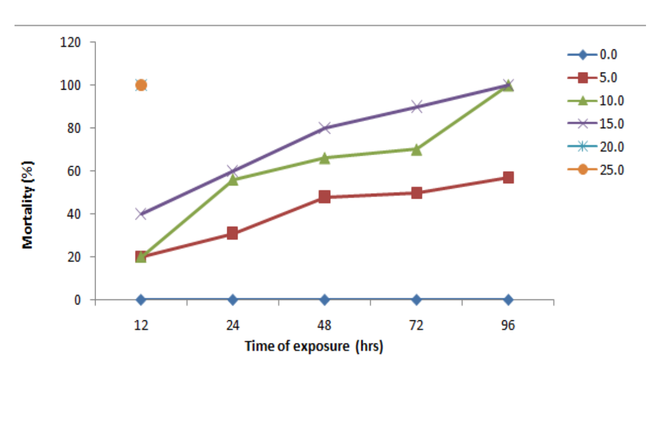

Figure 3: Mortality of *P.papillio* exposed to various concentrations of sodium bromide for 96 hours

Lethal Concentration (LC) of Sodium bromide to *P. pappilio* for 96 hours

The lethal effects of bromide on *P. Papilio* exposed to LC5, LC50 and associated 95% confidence limits of the toxicant concentration as shown in table 4, indicated that the range of the values between 24hrs LC50 and 96hr LC50 was narrow. A safe concentration at LC50 of bromide to *P.Papilio* was very high (2.08 mg/l for 24 hours and 1.20 mg/l for 96 hours). The toxicity factor (T.F) of the various concentrations increased with time of exposure from 24 to 96 hours as indicated by the observed decreased in LC50 value. At 24 hours LC50 T.F recorded was 1.0 while 96 hours LC50 was 1.26.

Discussion

Four major phases have been identified in the responses of fish to toxicants; the contact phase (brief period of excitability), exertion (visible avoidance characterized by fast swimming, leaping, and an attempt to jump out of the toxicant), loss of equilibrium and lethal (death) phase, when opercula movement and response to tactile stimuli cease completely [19]. Rao [20] reported the same Behavioural responses in *C. gariepinus* and other fish species exposed to acute concentrations of toxicants. The abnormal response such as hyperactivity increased ventilator rate, dash and erratic swimming, increased surfacing and other abnormal behaviors may have increased the energy demand for metabolism beyond normal, leading to fatigue and stress [21].

The stressful behavior responses of the exposed *P.papilio* suggest that they suffered respiratory impairment due to the effect of the biocide on the gills and general metabolism. Oxygen exchange across the gills might have been impaired as evidenced by the disruption of the structure of the gills and increased mucus production by mucus cells in exposed fish. These observations are in line with those of previous workers who assessed the acute effects of Cazmirm chloride and sodium bromide [22]. They attributed these behaviours to nervous disorder elicited by the toxicants.

Contamination of aquatic environment by biocide poses a series threat to aquatic life forms. The 24^{hr} LC50 value of sodium bromide to *P. Papilio* was found to be 2.64mg/l, indicating that sodium bromide is toxic to fish. The toxicity of sodium bromide varies depending on the fish species [23].This result is in line with that of Anees [24], in Channa punctatus exposed to insecticide in the laboratory. The toxicity of bromide in fish is traceable to a combination of three factors, a sensitive central nervous system, slow hydrolytic detoxification and direct absorption via the gills into the blood stream [25]. Furthermore biocide may also secondarily induce on osmotic imbalance that contributes to their toxicity [26]. Due to neurotoxin effect, sodium bromide might have affected the synthesis process regulation in nerve cells [27].

During chronic exposure of *P. papillo* to high doses of sodium bromide, the fish shows changes in its behavioural attitude, drastic variation in opercula beat rates, loss of its equilibrium, sluggish and settlement at the bottom. These ultimately led to respiratory distress paralysis and mortality especially at higher concentrations. The dead mudskipper was covered with a layer of mucous on the surface of the body. The hypersensitivity of the fish to biocide toxicity may be due to specie specific differences in biocide metabolism, however high sensitivity of the piscine nervous system to toxicants might be the possible reason [28].

The response pattern of tail beat frequency of exposed fish with respect to time and concentration of the biocide in this study agrees with the findings of Viran *et al.* [29] who studied the acute effects of deltamethrin on guppies (*Poecilia reticulate*). But it differed from the findings of Hoentella *et al.*, [30] in rainbow trout exposed to cadmium salts. In these studies the values of TBF appeared to increase with time and concentration of the toxicant. The mode of action of biocide on the exposed fish may include impairment of nerve impulse transmission as was the case in fishes exposed to monocotophos, an organic phosphate insecticide [31].The generally low levels and less variable values of the TBF recorded in this study may suggest that the fish was less sensitive to the biocide when compared to the response of *C. gariepinus* exposed to cypermerthrin (pyrethroid) possibly due to its mode of action and size of fish [32]. Haphazard response and cessation of TBF before OBF, and subsequent death may indicate the trend in available metabolic energy. This appears to be the usual trend in the responses of *C. gariepinus* to acute concentrations of toxicants [33].

OBF appeared to be a more responsive parameter in measuring behavioural toxicity in comparison to TBF in *C. gariepinus* exposed to Roundup. The trend in OBF in this study and several others is that it is usually raised, peaks and then falls with time for the various concentrations of the toxicants tested [34]. However, variations observed in this study could be accounted for by differences in the fish species, life stage and mode of action of the toxicant. Obomanu *et al.* [35] observed that increased opercula rate may result from decreased efficiency in oxygen uptake, transport or increased metabolic rate. To cope with stress caused by exposure to the herbicide, the fish may have increased its TBF and OBF concurrently, but the latter particularly to increase the rate of water flow over the gills to enhance oxygen uptake from the water [36].

In this study, *P. pappillis* exposed to acute concentrations of Sodium Bromide exhibited an increased in pigmentation of the skin. According to Anderson [37] a layer of mucus (glycoproteins, preteoglycans and proteins) forms the interface between the skin of the fish and the external environment in addition to scales in scaly fish. The layer is continuously replenished by mucus secreting cells and the rate can increase in response to infection, chemicals or physical irritants. In this study, exposed fish had a greater amount of mucus covering their bodies and gills than that in the control. Mucus production is response to an irritant like Sodium Bromide is one of the strategies of fish to protect itself against damage, but this has grave implications particularly for the gills as this impairs oxygen uptake. Increased mucus as a result of increased activity of mucus cells was reported in *C. gariepinus* on exposured to petroleum products [34]. And Cirrhinus mrigala O exposed to cypermethrin.

Conclusion

This study proved that exposure to sodium bromide did not affect the water quality parameters except the dissolved oxygen which reduced, also chloride and electrical conductivity increased with increasing concentration of the chemical. In the toxicity LC50 (96hr) value of sodium bromide for *P. papilio* was found to be 1.59 indicating that sodium bromide is moderately toxic to the fish. There was a rapid response after the fish had contact with the biocide, as indicated by imbalance or disturbances in the studied biomarkers.

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