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Neurotoxicity, oxidative stress biomarkers and haematological responses in African catfish (*Clarias gariepinus*) exposed to polyvinyl chloride microparticles

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ABSTRACT

The aquatic environment is outrageously littered with resin pellets and particles of plastic origin which can jeopardise the health of aquatic organisms. The present study investigated the effect of polyvinyl chloride (PVC) microparticles on blood parameters, leucocytes, lipid peroxidation and antioxidant system (brain and gill) of *Clarias gariepinus*. *C. gariepinus* is a fresh water indicator species often used as model for ecotoxicological assay. Fish specimens were exposed to diets spiked with PVC microparticles (95.41 ± 4.23 μm) at the following concentrations; 0.50%, 1.50% and 3.0% and control diet for 45 days, followed by a depuration trial which lasted for 30 days. Blood and tissues (brain and gill) were sampled every 15 days interval for haematology, antioxidant enzymes and lipid peroxidation assay. The result obtained revealed that PVC orchestrated the marked alterations in haematological indices. Mean cell volume and mean cell haemoglobin values reduced significantly in all concentration treated groups and were time-dependent. Neutrophil counts decreased with a corresponding increase in PVC exposure time while lymphocytes and monocytes values showed no significant difference between the control and exposed fish groups. Glutathione peroxidase activity was altered substantially in the brain and gill of the exposed groups compared to the control. Superoxide dismutase activity was inhibited in the brain and gill of the exposed groups compared to the control, as well as the different exposed periods. Catalase activity reduced significantly in the brain of 0.5% PVC exposed groups, and also decreased in a time-dependent manner while its activity in the gill did not change significantly among the exposed groups relative to the control. Lipid peroxidation levels in the brain of PVC exposed groups increased significantly in a dose and time-dependent manner. However, PVC caused no significantly change in the gill lipid peroxidation level of the exposed fish, but elevated the lipid peroxidation levels as the exposure time increased. Acetylcholinesterase activity in the brain and gill of the exposed fish reduced substantially with increase in the exposure time. Variations in haematology, antioxidant enzymes, lipid peroxidation and acetylcholinesterase activities are indicative of oxidative stress and neurotoxicity in fish. *C. gariepinus* is an indispensable bioindicator to measure environmental impact of PVC microparticles.

1. Introduction

Plastic is a multiuse material that is widely extant in everyday life (Rao, 2019). Plastics are used in most different applications and this is as a result of its attributes such as malleability, low weight, durability and cost (Pellini et al., 2018). The increasing request for plastic items to support social and infrastructural development has intensely enhanced yearly plastic production from 1.5 million tonnes in the 1950s to 322 million tonnes in 2015 (Plastics Europe, 2016; Pellini et al., 2018). One of the consequences of the plastic revolution is the contamination it creates in the aquatic environment (Pellini et al., 2018). Rao (2019) stated that approximately 10% of the plastics produced worldwide ends up in the aquatic environment. Boucher and First (2017) claimed that an estimated 9.5 million tonnes of plastic waste is found in the ocean annually. Jovanović (2017) stated that plastic mass in the oceans relative to fish biomass is predicted to reach a ratio of 1 to 3 by 2025. The sources of plastics present in the aquatic systems differ, but include photodegradation of larger plastic waste items such as drink containers, synthetic fiber clothing, macro beads and cosmetic products (Foley et al., 2018). Occasional accidental loss of plastic resin/pellets into

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rivers and other watercourses can occur during these steps or during logistic stages (e.g., terrestrial/aquatic transport), and these plastics will eventually migrate to other water tributaries especially the coastal and marine waters (Ribeiro et al., 2017). There are no reliable estimates of the amount of plastic found in the aquatic environment, however, a substantial amount of plastic debris has been documented (Plastics Europe, 2016). The amount of plastic litter is so substantial that its footmark on the globe is now considered a pointer of the Anthropocene (Arias-Andres et al., 2018). The presence of plastics in the aquatic environment poses serious health risk issues to both flora, fauna and humans at large (Critchell and Hoogenboom, 2018; Scherer et al., 2018).

Polyvinyl Chloride (PVC) with the chemical formula $(C_2H_3Cl)n$ is a thermoplastic made of 57% chlorine and 43% carbon and is the second most used synthetic plastic polymer for the production of different forms of plastics worldwide after polyethylene (Wu et al., 2019) and this is due to its long term durability, weather ability, and electrical properties (Rao, 2019). PVC in different forms or state is used for insulation coatings, wire and cabling, synthetic leather making and flexible flooring (Espinosa et al., 2018). In recent times, PVC microplastic has been acknowledged to be one of foremost emerging contaminant of the aquatic environment (Jovanovic et al., 2018). PVC materials in various forms and sizes have been traced and documented to be found in water sediments, column and surface of different water bodies (Foley et al., 2018). Trace of PVC microplastics were found in Southern Ocean (Isobe et al., 2017), Rhine river (Mani et al., 2014) and marine sediments (Jahnke et al., 2017). Li et al. (2018) stated that PVC and its leachates have been detected in drinking waters and fresh water systems. PVC has been reported to be the most toxic (carcinogenic) and hazardous plastic (Plastics Europe, 2017; Gomiero et al., 2019), and contains leachates such as phthalates, bisphenol A, lead, cadmium and dioxins, which are highly toxic to both humans and aquatic lives (Wu et al., 2019). PVC has also been reported to promote endocrine disruption in organisms (Chen and Hollert, 2019). Espinosa et al. (2017) noted significant increase in serum enzyme (Aspartate amino transferase), globulin and albumin in *S. auratus* exposed to diet spiked with PVC microplastic after 30 days duration trial. PVC microplastic exposure induced oxidative stress and impaired some immune parameters (globulin and respiratory burst activity) in *S. auratus* (Espinosa et al., 2018). The authors also reported significant reduction of phagocytes in the fish. Wright et al. (2013) reported depleted lipid reserves and inflammatory response in marine worms (*A. marina*). Apoptotic accumulation has also been reported in the embryo of Zebra fish (*Danio rerio*) exposed to PVC microplastic (Gokce et al., 2018). Gomiero et al. (2018) stated that *Hediste diversicolor* suffered oxidative stress and genotoxicity when exposed to PVC microplastic spiked sediment.

Plastics are ingested by aquatic organisms accidentally or intentionally (Jovanović, 2017; Abbasi et al., 2018), due to their appealing coloration, buoyancy and semblance to food (De Sa et al., 2018). High prevalence of this scenario may likely occur in benthic dwellers, omnivorous species and predatory fishes (De Sa et al., 2018; Chan et al., 2019). High content of microplastics were found in fish (De Sa et al., 2018) and Clam (Ribeiro et al., 2017). Uptake of plastics of different size ranges by fish species have been reported (Güven et al., 2017; Rezania et al., 2018). Considering their different sizes and state, plastics can travel faster via biological membranes into cells of aquatic organism and bioaccumulate in them thus can cause biological impacts from sub-cellular to ecosystem level (Espinosa et al., 2018; Li et al., 2018). Aftermath effect of plastic uptake by aquatic organisms is X-rayed or manifested behavioural and physiological levels (Barboza et al., 2018).

Clarias gariepinus belonging to the family Claridae, is an important commercial species and highly relished in Nigeria and other parts of West Africa for its flesh quality and nutritional value. *C. gariepinus* is an omnivorous species and feast on any food material aside plants and fish (Iheanacho et al., 2019). *C. gariepinus* possess certain unique characteristics which include fast growth rate, better feed conversion rate, high resistance to poor water quality condition, easy adaptability in captivity and high survivorship (Odo et al., 2017; Iheanacho et al., 2019). This fish species inhabits almost all inland fresh waters including flooding plains and swamps (Okoro et al., 2019) and may likely ingest plastic resins and pellets. *C. gariepinus* is hardy and can survive tough situations, hence is often used as model fish for ecotoxicological studies (Odo et al., 2017). Nigeria is one of countries of the world highly dependent on plastic materials for diverse production of finished plastic products. Currently, there are no stringent measures put in place to check plastic pollution in the country. Substantial quantities of these plastic wastes end up in the aquatic environment while some are intentionally discharged into rivers and streams without restrictions. Information regarding the effect of microplastics on this fish species is lacking. To the best of our knowledge, this is the first study to assess the effect of PVC microplastic on *C. gariepinus*. The aim of this study (in vitro assay) is to examine the effect of PVC micro particles on *C. gariepinus* (an important and highly relished fish in the Sub-Saharan Africa) and evaluate the possible ecotoxicological risk of this pollutant in the species. The gill organ is the first line of contact of ingested food and non-food materials, while the brain is critical for examining the ecotoxicological effect of toxicant in aquatic models (Pala and Serdar, 2018). The impact of PVC micro particles in *C. gariepinus* was assessed via a battery of biomarkers of oxidative stress (superoxide dismutase, catalase, glutathione peroxidase), neurotoxicity (acetylcholinesterase) and lipid peroxidation.

2. Materials and methods

2.1. Biological model and rearing condition

In nature, African catfish *Clarias gariepinus* inhabits almost all zones of fresh water bodies including rivers, lakes, flooded plains, large and shallow streams (Idodo-Umeh, 2003). Moreover, *C. gariepinus* is a bottom omnivorous feeder, feeding on almost anything they come in contact with (Ogueji et al., 2019). *C. gariepinus* is a widely accepted model fish species, hence informs the choice of fish species used for this experiment. Total of 180 African catfish juveniles *Clarias gariepinus* were procured from the Department of Fisheries and Aquaculture farm, Alex Ekwueme Federal University Ndufu Alike Ikwo. Fish specimens were transferred to the departmental wet laboratory in 150 l capacity plastic container. On arrival at the laboratory, the fish were first quarantined in a holding fish tank prior to the acclimation period. Fish specimens were stocked in a 200 l aquarium tank and acclimated for two weeks prior to the commencement of the experiment. During the acclimatization period, fish were fed with commercial fish feed (3mmCoppens, Netherlands) at 3% body biomass twice (8:00 h–18:00 h) daily.

2.2. Polyvinyl chloride and particle size determination

Technical grade of polyvinyl chloride (PVC) polymer (99.5% purity) under the trade name Formolon® 622 (CAS no. TH1764490248) was procured from a polymer manufacturing firm in Enugu state, Nigeria. Particle size was determined using optical microscopy (Nikon Eclipse E100, China) and an automated laser particle size analyser (Microtrac S3500, 0.02–2800 μm, Germany). Small sample (200 mg/g-kg^{-1}) of PVC powder was fed into the dry dispersion device (Turbotrac) to deliver dispersed samples to the measuring cell in the Microtrac optical bench for particle size analysis. The statistical data regarding the percentage of particle size in various ranges was determined from the instrument's software and the average particle size obtained was 95.41 ± 4.23 μm. The average size of PVC microparticle obtained in the present study was found to be within the environmental relevant size ranges (< 5 mm) found to be ingested by aquatic biota in the wild (Plastic Team, 2016).

2.3. Ethical statement

All experimental procedures were performed in compliance with the standards described by the institution of animal welfare act in line with the National Environmental Standard Regulations Enforcement Agency (NESREA) Act of Nigeria on the protection of animals against cruelty.

2.4. Diet preparation and spiking

Four (4) iso-nitrogenous and iso-lipidoneous test diets were prepared for this experiment. Diets were formulated with the following ingredients; fish meal 40%, soybean meal 15%, corn meal 15%, fish oil 1.0%, wheat offal 15%, vitamin premix 4%, tapioca starch 5% and mineral premixes 5%. Different percentage inclusion of PVC microparticles such as 0.5%, 1.5%, 3.0% and 0.0% (control) were added to the diets as treatments. It is important to state the PVC resin particles added to diets do not contribute any nutrient. Although the amount of microplastic of plastic polymer ingested by fish in the wild is not known (Jovanović, 2017; Jovanovic et al., 2018; Pellini et al., 2018), however, the PVC dietary inclusion levels hypothetically reflects low, medium and high exposure presumably. Jovanovic et al. (2018) hypothesized that daily consumption of micro plastics in the aquatic environment was about 0.3% which translate to 3.33 g kg^{-1} feed during dietary exposure in a laboratory experiment. Dietary administration of up to 100–500 mg kg^{-1} PVC microplastics have been tested with European Sea bass (*Dicentrarchus labrax* L.) in an in vitro experiment (Espinosa et al., 2019). Also, dietary exposure effect of polyethylene microplastic (up to 10% of the diet) in Medaka fish has been evaluated in a laboratory experiment (Rochman et al., 2013). Formulated fish diets were mechanically mixed and pelletized into 3 mm diameter size using a locally fabricated machine. Pelleted diets were air dried at 25 °C for 24 h and stored at 4 °C in the refrigerator until used. The approximate composition of formulated diets is as follows; 50.10% crude protein, 15.90% crude fat, 5.20% crude fiber, 10.25% crude ash, 5.0% and 13.55% nitrogen free extract.

2.5. Experimental procedure

PVC resin particle is highly insoluble in water and most conventional organic solvents; hence the dietary exposure method was used for the study. The dietary exposure pattern was adopted from the procedure of Jovanovic et al. (2018) and guideline of OECD (2012a, 2012b) 305. A total of 180 *C. gariepinus* (25.15 ± 1.34 g and 13.05 ± 1.46 cm) were randomly assigned to four treatment diets (control, 0.5%, 1.5% and 3.0%) in a completely randomised design (CRD) pattern. Each treatment contained 45 fish and was further randomised into triplicates of 15 fish per replicate. Fish specimens were fed 3% body biomass daily in two portions (morning and evening). The feeding trial lasted for 45 days (long term) known as the uptake (exposure) period/phase (fish were fed PVC spiked diets) (Jovanovic et al., 2018). After the uptake phase, the remaining fishes were fed control diet, which lasted for 30 days (OECD, 2012a, 2012b), known as the depuration phase. The Experimental tank water was closely monitored daily in order to maintain the optimal water quality conditions. Tank water was siphoned with hose and replaced with clean dechlorinated tap water every three days and this was done to ensure the removal of faecal materials and feed remnants that may undermine the physico-chemical properties of culture media. Tank water was sampled weekly and water quality parameters were estimated using water quality kit (ProLab™, Florida). Values obtained are presented as mean ± SE as follows; temperature 27.47 ± 0.35 °C; pH 6.97–7.23; dissolved oxygen 5.20 ± 0.23 mg/L; conductivity 42.71 Us/cm and ammonia 0.04 mg/L.

2.6. Procedure for blood collection

Three fish per replicate were collected every 15 days interval for blood sampling during the uptake phase while at the end of the depuration phase; the same number (3) of fish were also sampled for blood collection. The fish were randomly caught using hand net and euthanized with tricaine methane sulfonate (MS 222) at 50.0 mg/l to minimize stress. The method for blood collection was done following the procedure of Bello et al. (2014). Sterilised 3G needles and syringes of 5 ml were used to collect blood from fish. At first, the ventral cavity for fish was wiped with dry tissue paper to avoid contamination with mucus during blood collection. At a distance of 3–4 cm from the genital opening of each fish, the needle was inserted at a right angle to the vertebral column of the fish. Under gentle aspiration, 3 ml of blood was taken using the heparinized syringe and gently withdrawn and transferred to a heparinized tube for haematological analysis.

2.7. Haematological analysis

2.7.1. Total erythrocyte count (red blood cell) estimation

Hendricks solution was used for the erythrocyte count. Neubauer's chamber haemocytometer was prepared and blood drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool to adjust the volume to exactly 0.5 mark. The pipette was filled to 101 mark with the diluting fluid and shaken for 30 min to ensure mixing. The diluted suspension of cells then drawn into the chamber. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer were counted with 4 mm objectives and ×40 eyepiece microscope. The number of cells was multiplied by ×10, which gave the total number of cells per cubic millimeter (mm^3) of blood (Hesser, 1960).

2.7.2. Total leucocytes count (white blood cell) estimation

Leucocytes count was estimated after diluting the blood with WBC diluting fluid (1:20 v/v) as described by Houston (1990). Total of 0.02 ml of the blood was drawn up to the 0.5 mark on the stem of a white cell blood was pipetted into a small test tube and 0.38 ml of the dilution fluid was added. A few drops of the diluted blood were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted using a 4 mm objective lens at 40× magnification. The number of cells was multiplied 10× to obtain the total number of leucocytes per cubic millimeter (mm^3) of blood (Houston, 1990).

2.7.3. Packed cell volume (haematocrit) estimation

Determination of packed cells volume was carried out by micro-Westergren method as described in Blaxhall and Daisley (1973). The well mixed sampled blood from the heparinized was drawn into micro-haematocrit tube, 75 mm long, and 1.1–1.2 mm internal diameter. The tubes were centrifuged for 5 min. The reading is made with the aid of a micro-haematocrit reader and expressed as the volume of the erythrocytes per 100 cm^3.

2.7.4. Haemoglobin content estimation

Haemoglobin content of blood samples was determined using cynomethaemoglobin method described by Briggs and Bain (2011), using Drabkins reagent that converts the haemoglobin and carboxyhaematoblobin to cynomethaemoglobin.

2.7.5. Red cell indices estimation

The absolute values made up of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the results of RBC, haemoglobin and PCV/(Ht) (Dacie and Lewis, 2011; Iheanacho et al., 2018).

$$\mathrm{MCV}(\mu m^3) = \frac{\mathrm{PCV}\% \times 10}{\mathrm{RBC}(\mathrm{cells\ mm}^3)}$$

$$\mathrm{MCH}(\mathrm{pg{\cdot}Cell}^{-1}) = \frac{\mathrm{Hb}(\mathrm{g}/100\,\mathrm{ml}) \times 10}{\mathrm{RBC}(\mathrm{cells\ mm}^3)}$$

Table 1
Two-way anova statistics for haematological indices of *Clarias gariepinus* exposed to poly vinyl chloride microparticles.

Source	RBC		Hb		PCV		WBC		MCV		MCH		MCHC	
	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Corrected model	4.035	0.000	5.869	0.000	3.755	0.001	8.387	0.000	6.895	0.000	8.624	0.000	1.888	0.064
Intercept	5116.905	0.000	9780.357	0.000	5043.840	0.000	2846.074	0.000	2884.537	0.000	3254.909	0.000	4083.715	0.000
Treatment	0.997	0.407	2.220	0.105	6.431	**0.002***	26.216	**0.000***	27.991	**0.000***	1.267	0.302	3.219	**0.036***
Duration	17.778	**0.000***	25.388	**0.000***	8.492	**0.000***	9.933	**0.000***	3.614	**0.024***	40.94	**0.00***	0.480	0.699
Treatment * duration	0.467	0.886	0.580	0.804	1.284	0.283	1.928	0.083	0.957	0.492	0.304	0.968	1.914	0.086
Adjusted R squared	0.492		0.608		0.468		0.702		0.653		0.709		0.221	

RBC = red blood cell; Hb = haemoglobin; PCV = packed cell volume; WBC = white blood cell; MCV = mean cell volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration. Asterisk (*) denote significance ($p < 0.05$).

$$\text{MCHC}(\text{g}/100\,\text{ml}) = \frac{\text{Hb}(\text{g}/100\,\text{ml}) \times 100}{\text{Ht}\%}$$

2.7.6. Leucocyte differentials count estimation

Two drops of blood were placed on a slide and made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol and then stained with Giemsa's stain and 170 buffer distilled water (Hesser, 1960). It was allowed to stand for 20–30 min after which the slide was washed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope.

2.8. Tissue collection and preparation for enzyme analysis

After blood sampling from fish (three per replicate), the fish were further sacrificed for tissue sampling for each sampling days (15, 30, 45 and 30-d depuration period). Brain and gill were carefully collected using spatula to avoid damage of tissues after evisceration; hence rinsed in a physiological saline to remove traces of blood. Tissue samples from each tank were pooled together, weighed and homogenized in a chilled sucrose of 0.25 M (1:19 w/v).The homogenate was separated into two portions; of which one was used for lipid peroxidation (LPO) analysis, while the other portion was further centrifuged at 12,000 $\times g$ for 15 min, at 4 °C to obtain the supernatant that was used for antioxidant enzymes and acetylcholinesterase analysis.

2.9. Antioxidant enzymes assay

Catalase activity was estimated spectrophotometrically at 250 nm according to the method of Beutler (1984). The enzyme activity was expressed as μmole of hydrogen peroxide decomposed/minute/mg of protein. Superoxide dismutase (SOD) activity was measured spectrophotometrically at 480 nm according to the method of Misra and Friedovich (1972). One unit of superoxide dismutase activity was expressed as the amount of protein required for 50% of inhabitation of epinephrine autoxidation/minute. The activity of glutathione peroxidase (GPx) was measured spectrophotometrically at 412 nm according to the method of Beutler (1984), and the values were given as μg/min/mg protein.

2.10. Lipid peroxidation (LPO) assay

The measurement of thiobarbituric acid reactive substance (TBARS) measured as malondialdehyde content of the tissues was estimated according to the method of Erdelmeier et al. (1998). The concentration of TBARS was measured spectrophotometrically at 525 nm and its activity was expressed as nanomoles of TBARS mg^{-1} protein.

2.11. Assay of acetyl cholinesterase (AChE)

The AChE activity was determined at 412 nm according to the method of Ellman et al. (1961). The activity was expressed as μmole of acetylcholine hydrolysed mg protein^{-1} min^{-1}.

2.12. Statistical analysis

Data generated are presented as mean ± standard deviation (SD), and were analysed using SPSS IBM version 25.0 computer program (SPSS Inc. Chicago, Illinois, USA). All data were preliminary tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk test) and homogeneity (Levene test) prior to analysis. Variations in the means were subjected to two-way analysis of variance (ANOVA), to check for significance among treatments, along the periods of exposure and interactions between the treatments and exposure periods. Statistical significance was declared at $p < 0.05$ using post hoc Duncan multiple range test (DMRT).

3. Result

3.1. Haematology

The result on haematological profile of *C. gariepinus* juvenile exposed to graded concentrations of PVC is presented in Tables 1 and 2. In the present study, PVC induced no significant effect on the RBC counts of the exposed groups when compared to the control ($p = 0.407$), but caused a significant change considering the different exposure time ($p = 0.000$). However, there was no significant interaction effect between the treatment and duration ($p = 0.886$). PVC orchestrated the increase in RBC counts in a time-dependent manner, but the depuration phase showed that RBC counts reduced significantly than 30th and 45th exposure periods. In the present study, variations in Hb contents among the exposed groups were insignificant ($p = 0.105$), relative to the control, but differed significantly, comparing the different exposure duration sampling ($p = 0.000$). However, the interaction effect was not significant ($p = 0.804$). The changes in PCV values among the exposed groups was observed to be significant ($p = 0.002$), when compared to the control. A similar trend was observed regarding the exposure durations ($p = 0.000$), but the interaction effect was not significant ($p = 0.283$). PCV contents of the exposed groups decreased significantly in a concentration and duration dependent manner. With regards to WBC counts, a significant difference was observed among the treatments ($p = 0.000$) and between the different exposure periods ($p = 0.000$), while the interaction effect was not significant ($p = 0.083$). WBC counts were significantly higher in the control than the exposed groups, but however decreased significantly in a time-dependent pattern. Post exposure assessment revealed that WBC count increased significantly when compared to the 45th day exposure duration. Information regarding MCV showed that there was a significant difference among the exposed groups ($p = 0.000$) with respect to the control, as well as the different exposure periods ($p = 0.024$), while the interaction effect was observed to be insignificant ($p = 0.492$). Exposure to PVC microparticles induced a significant decrease in MCV content in a concentration and duration-dependent pattern (Table 2). Changes in MCH values among treatments was not significant ($p = 0.302$) when compared to the control, but was

Table 2
Post-hoc test on haematological profile of *Clarias gariepinus* exposed to polyvinyl chloride microparticle spiked diet.

	Parameter					MCH (pg/cell)	MCHC (%)
	RBC (10^6 cells/mm^3)	PCV (%)	Hb (g/dl)	WBC (10^3 cells/mm^3)	MCV (fl/cell)		
Treatment (%)							
Control	2.98 ± 0.37^a	49.17 ± 2.69^a	21.94 ± 2.46^a	10.61 ± 2.26^a	167.56 ± 24.83^a	75.29 ± 16.43^a	44.78 ± 5.88^b
0.5	2.83 ± 0.37^a	42.33 ± 5.68^b	21.34 ± 1.95^{ab}	8.23 ± 2.52^b	153.41 ± 36.08^{ab}	77.28 ± 15.89^a	50.91 ± 5.37^a
1.5	3.01 ± 0.51^a	42.00 ± 6.55^b	20.48 ± 2.03^b	8.58 ± 1.44^b	143.99 ± 39.11^b	70.56 ± 17.82^a	49.54 ± 6.69^a
3.0	2.92 ± 0.35^a	41.67 ± 4.68^b	20.81 ± 2.94^{ab}	8.73 ± 1.57^b	145.43 ± 30.66^b	72.87 ± 17.79^a	49.95 ± 4.55^a
Duration (days)							
15	2.50 ± 0.23^c	47.00 ± 3.95^a	23.47 ± 1.54^a	11.53 ± 1.12^a	190.13 ± 0.06^a	94.78 ± 10.56^a	50.16 ± 4.12^a
30	3.19 ± 0.35^a	42.58 ± 6.84^{bc}	20.12 ± 1.67^b	8.08 ± 1.66^{bc}	133.57 ± 17.48^c	63.74 ± 8.34^c	48.33 ± 8.33^a
45	3.23 ± 0.23^a	40.00 ± 6.73^c	18.67 ± 1.56^c	7.23 ± 1.37^c	123.73 ± 18.82^c	57.93 ± 4.99^c	47.69 ± 7.57^a
30 d-depuration	2.83 ± 0.25^b	45.58 ± 2.61^{ab}	22.31 ± 1.07^a	8.86 ± 0.88^b	162.98 ± 21.72^b	79.57 ± 8.77^b	49.01 ± 2.49^a

RBCs = red blood cells, PCV = packed cell volume, Hb = haemoglobin, WBCs = white blood cells, MCHC = mean corpuscular haemoglobin concentration, MCH = mean corpuscular haemoglobin, and MCV = mean corpuscular volume. Data (n = 9) are presented as mean ± SD. Means with dissimilar alphabet superscript across column are significantly different ($p > 0.05$). fl = flucolitre; pg = picogram.

observed to be significant ($p = 0.000$) between the different exposure duration sampling. MCH content increased significantly in a progressive manner with a corresponding increase in the exposure period, but the interaction effect was not significant ($p = 0.968$). In the current study, MCHC value was observed to be significantly different ($p = 0.036$) among treatments with reference to the control. However, no significant change was seen in MCHC contents of PVC exposed fish with regards to the different duration samplings, as well as the interaction effect. MCHC content of the control was observed to be significantly lower than the exposed groups.

Information on the effect of PVC on leukocyte cell differentials of *C. gariepinus* are presented in Tables 3 and 4. In terms of lymphocyte count, insignificant change ($p = 0.267$) was observed among the exposed groups as well as the different sampling period ($p = 0.965$), but the interaction effect was significant ($p = 0.008$). Similarly, the changes in neutrophil counts among PVC exposed groups was not significant ($p = 0.660$) with reference to the control. However, a significant difference ($p = 0.056$) was detected with regards to neutrophil sampled at different exposure durations, likewise the interaction effect ($p = 0.003$). A significant and duration-dependent decrease in neutrophil counts was detected. Information on monocyte counts revealed that there was no significant difference ($p = 0.505$) among PVC exposed groups relative to the control, likewise between the different exposure periods ($p = 0.084$) and also the interaction effect ($p = 0.827$). In a nutshell, PVC had no significant effect on monocytes in the exposed fish.

3.2. Antioxidant enzymes activity

The effect of PVC microparticles on brain antioxidant enzymes activities, lipid peroxidation and acetylcholinesterase response in *C. gariepinus* are presented in Tables 5 and 6. With regards to GPx activity, a significant difference ($p = 0.000$) was observed among the exposed groups relative to the control, and as well as the different exposure durations ($p = 0.011$), but there was no significant interaction effect ($p = 0.420$). The activity of GPx declined significantly in 0.50% PVC exposed group than the control. Moreover, GPx activity decreased progressively with time as shown in the present study. However, the GPx activity measured at the end of the depuration period was not significantly different from 30th and 45th day exposure period. The activity of SOD varied significantly ($p = 0.015$) among PVC exposed group with respect to the control, as well as the different exposure durations ($p = 0.003$), but the interaction between treatment and exposure duration was not significant ($p = 0.880$). The activity of SOD reduced significantly in 0.5% PVC exposed fish when compared to the control, but however, showed a biphasic trend between the different exposure periods. The activity of CAT changed significantly ($p = 0.001$)

Table 4
Post-hoc test on leukocyte differentials (%) of *Clarias gariepinus* exposed to polyvinyl chloride spiked diet.

	Parameter		
	Lymphycytes	Neutrophils	Monocytes
Treatment (%)			
Control	67.92 ± 2.58^a	29.92 ± 2.99^a	1.50 ± 0.52^a
0.5	67.58 ± 2.43^a	30.92 ± 2.50^a	1.25 ± 0.62^a
1.5	67.50 ± 2.71^a	30.75 ± 3.08^a	1.17 ± 0.58^a
3.0	67.67 ± 3.31^a	30.67 ± 2.06^a	1.17 ± 0.72^a
Duration (days)			
15	66.75 ± 2.58^a	31.91 ± 2.35^a	1.33 ± 0.65^{ab}
30	67.50 ± 2.55^a	29.83 ± 2.51^b	1.58 ± 0.67^a
45	68.08 ± 2.78^a	29.75 ± 3.08^b	1.25 ± 0.45^{ab}
30 d-depuration	68.33 ± 2.10^a	30.75 ± 2.22^a	0.92 ± 0.51^b

Data (n = 9) are presented as mean ± SD. Means with dissimilar alphabet superscript across column are significantly different ($p > 0.05$).

Table 3
Two-way anova statistics for leukocyte differentials of *Clarias gariepinus* exposed to poly vinyl chloride microparticles.

Leukocyte diff	Lymphocytes		Neutrophil		Monocyte	
Source	F	P-value	F	P-value	F	P-value
Corrected model	2.175	0.032	2.831	0.007	0.974	0.502
Intercept	50,963.787	0.000	10,248.043	0.000	206.722	0.000
Treatment	1.378	0.267	2.798	0.660	0.796	0.505
Duration	0.090	0.965	0.538	**0.056***	2.426	0.084
Treatment * duration	3.135	**0.008***	3.607	**0.003***	0.549	0.827
Adjusted R squared	0.273		0.369		0.008	

Asterisk (*) denote significance ($p < 0.05$).

Table 5
Two-way anova statistics for antioxidant enzymes, lipid peroxidation and acetylcholinesterase activities in brain and gill of *Clarias gariepinus* exposed to poly vinyl chloride microparticles.

Source	GPX		SOD		CAT		LPO		AChE	
	F	P-value	F	P-value	F	P-value	F	P-value	F	P-value
Brain										
Corrected model	3.094	0.004	2.216	0.029	3.257	0.002	72.361	0.000	2.215	0.029
Intercept	6970.672	0.000	9403.863	0.000	17,015.823	0.000	17,078.758	0.000	6801.072	0.000
Treatment	7.936	**0.000***	4.071	**0.015***	6.795	**0.001***	5.137	**0.005***	3.020	**0.044***
Duration	4.364	**0.011***	5.583	**0.003***	4.060	**0.015***	351.021	**0.000***	8.010	**0.000***
Treatment * duration	1.057	0.420	0.475	0.880	1.810	0.105	1.883	0.091	0.016	1.000
Adjusted R squared	0.401		0.280		0.419		0.958		0.279	
Gill										
Corrected model	8.463	0.000	2.137	0.035	1.250	0.289	37.313	0.000	4.743	0.000
Intercept	14,236.496	0.000	12,363.597	0.000	10,178.534	0.000	7112.428	0.000	10,474.740	0.000
Treatment	6.720	**0.001***	2.115	0.118	1.901	0.149	1.635	0.201	0.138	0.937
Duration	26.506	**0.000***	6.970	**0.001***	2.524	0.075	178.119	**0.000***	19.449	**0.000***
Treatment * duration	3.031	**0.010***	0.533	0.839	0.608	0.781	2.270	**0.043***	1.376	0.240
Adjusted R squared	0.704		0.266		0.074		0.946		0.544	

Values marked with asterisk (*) denotes significant difference ($p > 0.05$). GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; LPO = lipid peroxidation; AChE = acetylcholinesterase.

Table 6
Post-hoc test on brain acetylcholinesterase, lipid peroxidation and anti-oxidant enzymes activities (mmol min^{-1} mg $protein^{-1}$) in *Clarias gariepinus* exposed to polyvinyl chloride microparticle spiked diet.

	Parameter				
	GPx	SOD	CAT	LPO	AChE
Treatment (%)					
Control	23.52 ± 2.13^{a}	39.92 ± 3.37^{a}	16.14 ± 0.70^{a}	4.06 ± 0.31^{b}	0.45 ± 0.04^{ab}
0.5	20.14 ± 1.39^{c}	36.10 ± 2.87^{b}	14.89 ± 0.79^{b}	4.37 ± 1.12^{a}	0.44 ± 0.39^{b}
1.5	22.87 ± 2.82^{ab}	38.34 ± 2.64^{ab}	16.21 ± 1.19^{a}	4.38 ± 1.19^{a}	0.48 ± 0.06^{a}
3.0	21.64 ± 1.47^{bc}	38.66 ± 3.06 ^{a}	16.13 ± 1.17^{a}	4.25 ± 0.95^{a}	0.46 ± 0.04^{ab}
Duration (days)					
15	23.32 ± 3.00^{a}	38.01 ± 3.21^{b}	16.26 ± 1.18^{a}	3.86 ± 0.31^{c}	0.48 ± 0.03^{a}
30	22.54 ± 1.46^{ab}	40.86 ± 2.72^{a}	15.50 ± 0.70^{b}	4.27 ± 0.21^{b}	0.48 ± 0.04^{a}
45	20.85 ± 1.05^{c}	36.44 ± 2.27^{b}	15.34 ± 1.06^{b}	5.93 ± 0.31^{a}	0.41 ± 0.39^{b}
30 d-depuration	21.46 ± 2.76^{bc}	37.70 ± 3.18^{b}	16.26 ± 1.18^{a}	3.02 ± 0.24^{d}	0.46 ± 0.05^{a}

Values are presented as mean (n = 9) ± SD. Means with dissimilar alphabet superscript across columns are significantly different ($p > 0.05$). GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; LPO = lipid peroxidation; AChE = acetylcholinesterase.

among the exposed group as well as the different exposure periods ($p = 0.015$), but the interaction effect was no significant ($p = 0.105$). CAT activity in the brain reduced significantly ($p < 0.05$) in 0.5% PVC exposed fish with reference to the control. Similarly, CAT activity decreased on the 30th–45th day PVC exposure periods but afterwards enhanced significantly at the end the depuration period. LPO levels in fish differed significantly among treatments ($p = 0.005$) likewise the different exposure periods ($p = 0.000$) but interaction effect was not significant ($p = 0.091$). The control had the lowest LPO level when compared to the PVC exposed fish. Notably, PVC significantly increased LPO levels in a duration-dependent manner. However, LPO in exposed fish decreased significantly ($p < 0.05$) at the end of the depuration period. The brain AChE activity differed substantially among the exposed groups ($p = 0.044$) and a similar trend was observed comparing the AChE activity sampled at different exposure periods ($p = 0.000$), however there was no significant interaction effect ($p = 1.000$). Brain AChE activity reduced significantly 0.5% PVC exposed fish when compared to 1.5% PVC treated fish, but not significantly different ($p > 0.05$) from the control. However, it was observed that AChE activity reduced substantially on the 45th day exposure period but increased afterwards at the end of the depuration period.

The activities of gill antioxidant enzymes, LPO and acetylcholinesterase in juvenile *C. gariepinus* exposed to graded concentrations of PVC microparticles are shown in Tables 5 and 7. GPx activity varied significantly ($p = 0.001$) among treatments as well as the different exposure durations ($p = 0.000$). The interaction effect was also observed to be significant ($p = 0.010$). PVC significantly provoked GPx activity of 1.5% exposed group compared to the other exposed groups and the control. Assessment between different exposure periods revealed that the activity of GPx reduced substantially on the 30th and 45th day compared to the 15th day exposure, but the post exposure assessment showed that its activity increased significantly than the 30–45th exposure periods. The activity of SOD varied insignificantly ($p = 0.118$) among PVC exposed groups, but was significant between exposure durations ($p = 0.001$) However, there was no significant interaction effect ($p = 0.839$). PVC had no significant effect on SOD activity the exposed fish with respect to the control, but its activity showed a biphasic trend between the exposure periods. The changes in CAT activity was not substantial among the exposed groups ($p = 0.149$), as well as the different exposure periods ($p = 0.075$), and the interaction effect ($p = 0.781$). Notwithstanding, CAT activity was lowest in 3.0% PVC treated fish and was observed to be minimal on the 45th day exposure period, but increased at the end of the depuration phase. Changes in LPO levels was observed to be insignificantly different ($p = 0.201$) among treatments, but was significant ($p = 0.000$) between the different exposure durations as well as the interaction effect ($p = 0.043$). PVC had not significant effect on LPO levels of the exposed groups irrespective of the different concentrations, but

Table 7
Post-hoc test on gill acetylcholinesterase, lipid peroxidation and anti-oxidant enzymes activities (mmol min^{-1} mg $protein^{-1}$) in *Clarias gariepinus* exposed to polyvinyl chloride microparticle spiked diet.

	Parameter				
	GPx	SOD	CAT	LPO	AChE
Treatment (%)					
Control	23.92 ± 2.03^{b}	40.85 ± 2.49^{ab}	16.69 ± 1.07^{ab}	4.39 ± 1.55^{a}	0.51 ± 0.06^{a}
0.5	24.10 ± 1.39^{b}	38.95 ± 3.26^{b}	17.01 ± 1.05^{ab}	4.71 ± 1.32^{a}	0.50 ± 0.05^{a}
1.5	25.74 ± 3.26^{a}	41.27 ± 2.94^{a}	17.43 ± 1.03^{a}	4.53 ± 1.43^{a}	0.49 ± 0.60^{a}
3.0	23.26 ± 1.89^{b}	39.76 ± 2.72^{ab}	16.35 ± 1.48^{b}	4.65 ± 1.15^{a}	0.49 ± 0.33^{a}
Duration (days)					
15	26.89 ± 2.45^{a}	40.15 ± 2.56^{b}	17.29 ± 1.49^{a}	4.04 ± 0.27^{c}	0.54 ± 0.03^{a}
30	23.46 ± 1.57^{c}	42.74 ± 2.54^{a}	16.68 ± 0.64^{ab}	4.50 ± 0.62^{b}	0.51 ± 0.37^{a}
45	21.94 ± 0.88^{d}	38.12 ± 2.27^{b}	16.20 ± 0.63^{ab}	6.57 ± 0.49^{a}	0.44 ± 0.32^{b}
30 d-depuration	24.74 ± 2.25^{b}	39.83 ± 2.54^{b}	17.29 ± 1.20^{a}	3.16 ± 0.21^{d}	0.52 ± 0.05^{a}

Values are presented as mean (n = 9) ± SD. Means with dissimilar alphabet superscript across columns are significantly different ($p > 0.05$). GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; LPO = lipid peroxidation; AChE = acetylcholinesterase.

elevated the LPO levels of the fish with a corresponding increase in the exposure time (duration-dependent pattern). However, LPO levels in fish decreased significantly at the end of the post exposure trial. In Table 5, the activity of AChE varied significantly ($p = 0.000$) between the different exposure durations, but was not significant ($p = 0.937$) among the exposed groups when compared to the control. However, there was no significant interaction effect ($p = 0.240$). PVC induced no significant effect on gill AChE activities of exposed groups with respect to the control, but reduced the AChE level of the exposed fish on the 45th day exposure. At the end of the depuration period, the activity of AChE enhanced more than the 45th exposure period.

4. Discussion

The present study assessed the biological effect of polyvinyl chloride microparticles in *C. gariepinus* through a battery of oxidative stress biomarkers (SOD, GPx, CAT), haematology, lipid peroxidation and neurotoxicity biomarker (AChE). The size of PVC microparticles (95.41 ± 4.23 μm) used in the laboratory experiment was within the range (1 nm–5 mm) reported to have been detected in the aquatic systems (wild) (Plastic Europe, 2015; Espinosa et al., 2019).

4.1. Haematology

Haematological indices are essential biomarkers for assessing pathological conditions and physiological changes in animals (Ogueji et al., 2018). The time dependent increases in RBC counts observed in PVC exposed fish as revealed in the present study suggested a defensive mechanism action against the toxicity effect of PVC microparticles. According to Sopinka et al. (2016), changes (increase or decrease) in the contents of erythrocytes and haematocrit could be part of defence response to stress especially environmental or toxicant induced stress. However, the depuration trial showed that homeostasis was restored to the normal physiological levels in fish following a down-scale of RBC counts. In the present study, Hb content decreased progressively in a time-dependent pattern and this could be attributed to marked impairment of erythropoiesis elicited by the adverse effect of PVC, leading to the possible inhibition of Hb biosynthesis. Yaji et al. (2018a) stated that the reduction of Hb and RBC in animals were obvious consequence of erythropoietic impairment which might result to the disruption of erythrocyte maturation. This scenario in most cases is notable when aquatic animals are exposed to chemical stressors (Yaji et al., 2018a). Increase in Hb content at the end of the depuration phase suggested that homeostasis was restored. The concentration and duration- dependent decreases in PCV values could be attributed to possible interruption of hematopoietic process (Okoro et al., 2019) caused by PVC microparticles. PVC orchestrated the inhibition of WBC production in the exposed groups as revealed in the present study, resulting to a condition known as leucopoenia. The degeneration of WBC counts was also duration dependent. WBCs are known to play a defensive immunological role in the system of organisms (Jeney, 2017). Red cell indices (MCV, MCH and MCHC) are important biomarkers that provide useful information on haemoglobin content and sizes of red bloods cells (Ogueji et al., 2018), thus used to diagnose anaemic status in animals (Yaji et al., 2018b). Changes (elevation and/or reduction) in red cell indices outside the normal physiological range in most cases denote microcytic or macrocytic anaemic conditions (Dacie and Lewis, 2011). In the present study, microcytic anaemic condition was evident in exposed groups following the progressive and time dependent reductions in MCV and MCH contents.

Leukocyte differentials are sensitive biomarkers of environmental stress which serve as first line of defence against foreign invasions into the system of organisms (Davis et al., 2008; Jovanović, 2017). Based on the findings of the present study, PVC exerted an adverse effect on neutrophil counts in treated groups (Tables 3 and 4) but had no significant effect on lymphocyte and monocyte cells. Espinosa et al. (2018) reported a significant reduction of phagocytes in PVC microplastic exposed fish (*S. auratus*). Greven et al. (2016) reported an elevation of neutrophil counts in polycarbonate and polystyrene nanoplastics exposed fish.

4.2. Antioxidant enzymes activities

Antioxidant enzymes are sensitive biomarkers of oxidative stress (Ajima et al., 2017). Antioxidant enzymes under normal conditions detoxify and eliminate reactive oxygen species (ROS) and other pro-oxidants from the cells (Egea et al., 2017; Ajima et al., 2017). Oxidative stress ensues when there is disparity between the generation and elimination of ROS particularly if the production of ROS supplants the antioxidant system (Ajima et al., 2017). The pathophysiological consequence of oxidative stress involves notable changes in the functions of the antioxidant system which could result to cell and tissue damage (Song et al., 2006). SOD is an antioxidant enzyme responsible for catalyzing the dismutation of superoxide anion into hydrogen peroxide and thus, the first enzyme associated in antioxidant defence line (Song et al., 2006). Fluctuations of SOD activity in the brain and gill organs of PVC exposed groups like wise at different exposure periods (Tables 6) suggested the need to balance the surpassed level of superoxide into a less damaging hydrogen peroxide (Ribeiro et al., 2017) in order avert cellular oxidative injury resulting from PVC microparticle exposure. In another study, Liver SOD activity was reported not to reduce substantially in European sea bass (*D. labrax*) exposed to 100–500 mg kg^{-1} PVC microplastic (Espinosa et al., 2019). Lu et al. (2016) reported that the dietary uptake and accumulation of microplastic induced oxidative

stress by altering SOD and CAT levels in the gill and liver of Zebra fish, *Danio rerio*. Ribeiro et al. (2017) reported a significant elevation of SOD activity in the gill of *Scrobicularia plana* exposed to plastic microparticles. GPx enzyme plays a defensive role by decreasing hydroperoxides to alcohols which in turn protect the animal against oxidative dysfunction (Ajima et al., 2017). The time-dependent inhibition of GPx activity in the brain (Table 6) and gill (Table 7) organs could be credited to an increased generation of hydroperioxides levels in the respective organs (Ajima et al., 2017; Ribeiro et al., 2017) suspected to have been caused by the adverse effect of PVC toxicant. Browne et al. (2013) reported that PVC microplastic induced an oxyradical generation and oxidative stress in *Avenicola marina* and lugworms. Jeong et al. (2017) reported that microplastic spike diets fed to marine copepod (*Paracyclopina nana*) elicited oxidative stress by altering the activities of GPx, SOD and GR. Avio et al. (2015) also reported that increased production of ROS caused an inhibition of GPx activity in the liver of *M. galloprovincialis* exposed to microparticles. GPx activity returned (increased) to the normal physiological level in the gill except for the brain of treated fish at the end of the depuration period.

Catalase activity is an important biomarker for assessing toxicity in fish, which plays a defensive role against exogenous source of superoxide by ensuring the removal of free hydrogen perioxide radicals (Regoli and Giuliani, 2014). In the present study, CAT activity in the brain of exposed groups was inhibited on the 30th–45thday exposure (Table 6), and could be as a result of the influx of superoxide radicals in the brain (Ribeiro et al., 2017). However, CAT levels enhanced after the 45th day exposure, indicating that its activity was restored to normalcy in the respective organs at the end of the duration period. In our current study, the activity of gill CAT was not significantly suppressed in PVC exposed fish, suggesting that the gill CAT is seemingly not be the antioxidant defence mechanism used by *C. gariepinus* to respond to PVC toxicity. Espinosa et al. (2019) stated that there was no significantly inhibition in the activity of CAT in PVC microplastic exposed fish (*D. labrax*). However, the inhibition of CAT activity was reported in annelid worm (*Hedieste diversicolor*) exposed to PVC spiked sediments (Gomiero et al., 2018). Barboza et al. (2018) demonstrated that uptake of microplastic induced oxidative stress and damage in *D. labrax* juvenile. On a general note, Espinosa et al. (2018) stated that PVC microplastic induced oxidative stress by impairing cellular innate immune activities in European sea bass *D. labrax*. LPO is a useful biomarker of oxidative damage and loss of cell function linked to oxidative stress in aquatic organisms (Ajima et al., 2017). LPO reaction is usually caused by ROS generation, resulting to peroxidation of unsaturated fatty acids (Barboza et al., 2018). Elevations of LPO levels in the brain (Table 6) and gill (Table 7) of PVC exposed groups with increased exposure duration could be attributed to the generation of free ROS that attacked the lipid membranes and consequently induced oxidative dysfunction in exposed fish (Li et al., 2016). Gomiero et al. (2018) reported increased LPO levels in PVC exposed annelid worm. Ribeiro et al. (2017) reported an increased level of LPO in the digestive gland of microplastic exposed bivalves (*S. plana*). LPO levels decreased in the organs at the end of the depuration period, indicating that the fish recuperated from PVC microplastic exposure.

Acetylcholinesterase (AChE) is a sensitive biomarker of neurotoxicity in organisms challenged with toxicants and adverse environmental conditions (Ajima et al., 2017). AChE plays an essential role in acetylcholine deactivation at nerve ending and ensures the proper functioning of neuromuscular system (Ribeiro et al., 2017). AChE activity levels denote the physiological state of the nervous system (Pala and Serdar, 2018). The progressive and time-dependent inhibition of AChE activity in the gill of the treated fish suggested an adverse consequence in cholinergic neurotransmission, and probably in nervous and neuromuscular function (Ribeiro et al., 2017). The activity of AChE was also significantly inhibited in the brain of the exposed fish on the 45th day exposure, hence may suggest a marked acetylcholine accumulation in the brain (Pala and Serdar, 2018), which may probably result to neurotoxicity in the exposed fish. However, AChE activity in the organs enhanced at the end of the depuration period indicating that the fish recovered from the exposure of PVC microparticles. According to Glusczak et al. (2006), inhibition in AChE activity is often orchestrated by the accumulation of acetylcholine in the synapse which resultantly alters the functions of the nervous system. Ribeiro et al. (2017) reported AChE inhibition in the gill of *S. plana* exposed to microplastic. Oliveira et al. (2013) observed oxidative damage caused by the inhibition in AChE activity in the brain of common Goby juvenile (*Pomatoschistus microps*) exposed to pyrene virgin plastic microplastic spiked diet.

5. Conclusion

We conclude by saying that PVC microparticles exposure significantly affected the health of *C. gariepinus* by promoting oxidative stress, neurotoxicity and lipid peroxidation. These conditions tend to be more pronounced with increase in PVC exposure time. Moreover, the depuration time appeared to be efficient for recuperation in the fish after PVC microparticle exposure in the brain and gill tissues. Additional studies are needed to comprehend the mechanism of action of PVC toxicant, bioaccumulation potential and its implication in fish physiology.

Declaration of competing interest

Authors declare that there is no conflicting interest for the manuscript.

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