# TOXIC EFFECT OF TIRE WEAR PARTICLE-DERIVED COMPOUND 6PPD-QUINONE TO *CLARIAS GARIEPINUS* (GSH, $GP_X$ AND SOD)

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**DATE: NOVEMBER, 2025**

## CERTIFICATION

This is to certify that this research titled "**TOXIC EFFECT OF TIRE WEAR PARTICLE-DERIVED COMPOUND 6PPD-QUINONE TO *CLARIAS GARIEPINUS* (GSH, $GP_x$ and SOD) "** was carried out by **"KOLAWOLE MOTUNRAYO MARY (MISS)"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of a Bachelor of Science degree in Environmental Management and Toxicology.

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**Dr. O. OGBEIDE** **DATE**

**(PROJECT SUPERVISOR)**

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Prof. (Mrs) E. T. AISIEN **DATE**

**(HEAD OF DEPARTMENT)**

## DECLARATION

I **"KOLAWOLE MOTUNRAYO MARY (MISS)"** declare that **"TOXIC EFFECT OF TIRE WEAR PARTICLE-DERIVED COMPOUND 6PPD-QUINONE TO *CLARIAS GARIEPINUS* (GSH, $GP_x$ and SOD) "** is my work and that all sources that I have used or quoted have been acknowledged using complete references and that this work has not been submitted before for any other degree at any other University.

_____ _____

**KOLAWOLE MOTUNRAYO MARY** **DATE**

## DEDICATION

This report is dedicated to God Almighty, for his guidance and protection during this project. I also want to dedicate this report to my beloved parents Mr and Mrs Kolawole for their unwavering support, prayers, love and financial assistance throughout my academic journey.

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## Abstract

The tire antioxidant 6PPD reacts with ozone to form 6PPD-quinone (6PPD-Q), a global pollutant known to cause acute mortality in sensitive fish like coho salmon. However, its sublethal effects on resilient, tropical species are poorly understood. This study investigated the oxidative stress

induced by 6PPD-Q in the African catfish, *Clarias gariepinus*. Juveniles were exposed to sublethal concentrations (0-1500 µg/L) for 28 days, and key antioxidant biomarkers were analyzed. Results showed that 6PPD-Q significantly disrupted the fish's antioxidant defense system, suppressing the activity of Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD), indicating a compromised ability to manage oxidative stress. This study provides the first evidence of 6PPD-Q-induced sublethal toxicity in *C. gariepinus*, highlighting that the threat of this tire-derived chemical extends beyond acute lethality to chronic health impairments in tolerant species, underscoring the need for enhanced monitoring and regulation.

**Keywords:** 6PPD-quinone, *Clarias gariepinus*, Oxidative Stress, Antioxidant Enzymes, Sublethal Toxicity, Tire Wear Particles, Environmental Contamination.

# CHAPTER ONE

## 1.0 INTRODUCTION

6PPD-quinone (6PPD-Q) is a toxic chemical formed when 6PPD, an antioxidant used in vehicle tires to prevent rubber degradation from ozone exposure, reacts with ozone in the air. As tires

wear down, particles containing 6PPD-Q are released into the environment and can be washed into water bodies by stormwater runoff, exposing aquatic organisms.6PPD-Q is highly toxic to certain fish species, notably coho salmon, causing rapid mortality at environmentally relevant concentrations. It affects fish by inducing oxidative stress, neurodegeneration, disruption of intestinal barrier function, cardiac issues, and abnormal swimming behavior. Some fish populations affected by 6PPD-Q are culturally and ecologically important and even endangered.Beyond aquatic toxicity, 6PPD-Q bioaccumulates in organisms and has been detected in human urine, serum, and cerebrospinal fluid, raising concerns about potential neurotoxic effects, including links to abnormal protein aggregation seen in Parkinson's disease. Human exposure routes include inhalation of tire wear particles near traffic, ingestion, and contact with rubber products.The increasing prevalence of synthetic rubber additives in urban environments has raised significant concerns regarding their ecological impacts, particularly on aquatic life. In the aquatic environment context,tyre wear is one of the major pollution sources. Despite this fact it remain largely underrepresented in environmental impact assessments. Specifically,pollution stems from tyre and road wear particles(TRWPs) formed by the mechanical abrasion of car tyres against the road during driving and braking (Tamis *et al*.,2021). Up to 12% of the tyre mass can be released into the environment over its lifetime (Rauert *et al.,*2021),and estimated annual tyre wear emissions in different countries can range from 0.2-5.5kg per capital (Baensch-Baltruschat *et al.,*2020).

## 1.2 BACKGROUND OF STUDY

6PPD and 6PPD-Q are commonly found in the ambient environment, present in various environmental matrices such as roadside soils, urban runoff, sediment, and airborne particulate matter (Cao *et al.*, 2022), and are considered key indicators of tire-related pollutant

contamination (Johannessen et al., 2022; Klockner ¨ *et al*., 2021a).6PPD can undergo chemical transformation in the environment, particularly upon interaction with ozone, forming a toxic quinone derivative known as 2-anilino-5-[(4-methylpentan-2yl)amino]cyclohexa-2,5-diene-1,4-dione (6PPDQ) (Hiki *et al*., 2021; Tian *et al*., 2021). 6PPDQ has been detected in airborne particles (Cao *et al*., 2022; Chen *et al*., 2024; Wang *et al*., 2022b), roadside soil (Huang *et al*., 2021), indoor dust (Liu *et al*., 2024a), and water (Johannessen *et al*., 2022a; Rauert *et al*., 2022), indicating its wide distribution in the environment. Humans are exposed to 6PPD and 6PPDQ through various routes, including inhalation of airborne particles, ingestion of contaminated food and water, and dermal contact with rubber products (Ji *et al*., 2022; Jin *et al*., 2023).

### 1.3 Environmental distribution

Once released from the tire rubber surface, 6-PPDQ tends to be transferred to various environments because of its physicochemical properties. 6-PPDQ has been frequently detected in various environmental media, including air, water, soil, dust and sediment (Cao *et al.*, 2022; Zhang *et al*., 2022b; Zeng *et al*., 2023; Cao *et al*., 2023).Thus, individuals are inevitably exposed to 6-PPDQ through various routes, including ingestion, inhalation, and direct contact. Moreover, once they enter the circulatory system, 6-PPDQ can rapidly be distributed within tissues and organs, and have been detected in some organs, such as the liver, lungs, and kidneys, potentially causing corresponding pathological damage (Fang *et al*., 2023; He *et al*., 2023; He *et al*., 2024; Zhang *et al*., 2024a). The 6-PPDQ has been detected in human urine, blood and cerebrospinal fluid samples, indicating its potential risk to human health (Du *et al*., 2022; Fang *et al*., 2024; Zhang *et al*., 2024a).

### 1.4 **Bioavailability of 6-PPDQ to organisms**

During environmental migration, pollutants can be transferred to the food chain, where they bioaccumulate. The gradual accumulation of pollutants may induce toxic effects in environmental organisms. Using in vitro simulations of human digestion experiments, 6-PPDQ was detected in gastrointestinal fluids with a detection frequency of 100%, indicating its bioavailability. Gastrointestinal fluids are formulated according to the human digestive system.There have been numerous studies focusing on the occurrence of 6-PPDQ in the environment and toxic effects on aquatic species. It has reported that 6-PPDQ induces various toxicity in mammals, including hepatotoxicity, pulmonary toxicity, enterotoxicity, neurotoxicity, cardiotoxicity, and reproductive toxicity (Hua and Wang, 2023a). Moreover, 6-PPDQ may be associated with liver damage in humans (Song *et al*., 2024).

## 1.5 Toxicity Concern

The deleterious effects of 6PPD-Q on the environment and human health have raised widespread concern for several reasons. First, 6PPD-Q is speculated to be continuously present in receiving water bodies on a global scale. This is because of the ubiquitous use of its parent compound, 6PPD, in the production of a wide array of rubber products (Hiki *et al*., 2021). Second, 6PPD-Q effects on aquatic organisms include mortality, cardiotoxicity, oxidative stress, and developmental and behavioural toxicity (Tian *et al*., 2021; Varshney *et al*., 2022). Third, 6PPD-Q exhibits moderate environmental stability (Hu *et al*., 2023), which may influence its persistence in the environment.

### 1.5.1 6**PPD-quinone toxicity to plant**

Highly toxic compounds, such as 6PPD-Q, may also be a concern to consumers, as they occur in edible plant root zones. Castan et al. (2023) exposed lettuce plants to TRWPderived compounds including 6PPD-Q. As shown by the recorded biomass and the absence of growth inhibition, lettuce plants did not exhibit phytotoxicity signs. However, 6PPD-Q was siphoned by lettuce roots and translocated to their leaves. The compound concentration (i.e. 6PPD-Q) then continued to increase in the leaves over two weeks, with values reaching 2.19 g/g. 6PPD-Q accumulation in lettuce roots may be more alarming for root vegetables such as carrots and radishes. Generally, this could become a critical issue if the regulatory limits are based only on the original compound concentrations without considering the sum of the parent compound and transformation products, which have unknown toxicities at present.

6PPDQ is a toxic transformation product of 6PPD, a common tire rubber antioxidant, noted for acute toxicity to aquatic organisms. There are studies highlighting species-specific toxicity and metabolism differences, including some fish species tolerant to 6PPDQ by detoxifying it metabolically. However, specific toxicological studies directly mentioning *"Clarias gariepinu*s" with 6PPDQ appear limited or unavailable at high detail level in open sources.

## 1.6 AIM AND OBJECTIVES

This current study provides a critical review of published papers on 6PPD-Q to;

Evaluate its toxicity to aquatic organisms and plants.

Identify the influence of environmental conditions on its levels.

Identify ways to manage its risks.

Summarise its occurence and fate in water,soil and air as influenced by stormwater runoff.

Identify research gaps and future prospects.

**The objectives of this study were to;**

Assess potential hazards to aquatic health from exposure of 6PPD-Q across watersheds of varied urban development

Explore relationships of water chemistry parameter e.g turbidity,chloride,nutrients with 6PPD-Q concentration to better understand factors influencing observed levels

Measure the concentration of 6PPD-Q in urban impacted streams and nearshore receiving water encompassing fall and spring spawning times when fish congregate near and in streams.

## CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 OVERVIEW OF TIRE CHEMICALS AND 6PPDQ

With the growing interest in 6PPD-Q environmental occurence,fate and toxicity,a systematic review of the literature is necessary to critically assess the current state of knowledge and research gaps. This approach will provide a broader understanding of 6PPD-Q ecological and human toxicities;these insights can be used to facilitate the creation of robust environmental policies that are based on insights rooted in scientific understanding.N-(1,3-dimethylbutyl)-N'-p-phenylenediamine(6PPD) is an antioxidant and antiozonant that is used in synthetic rubber production. Specifically,this substance is used to improve rubber durability,thereby reducing chemical and mechanical degradation effects(Varshney *et al*.,2022). This compound is found in tyres at mass fractions ranging from 0.4-2.0%(Johannessen *et al*.,2022a). (Tian *et al.,*2021) recently discoverd a 6PPD transformation product,6PPD-quinone(*Oncorhynchus kisutch*) as they spawn in urban and suburban streams in the U.S. Pacific Northwest.6PPD (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine) is primarily used as an antioxidant and antiozonant in rubber tires, where it protects the tire components from oxidative damage caused by ozone, oxygen, and other environmental factors. Its antioxidant role is crucial in preventing the degradation of rubber materials.

A study found that both 6PPD and 6PPD-Q can enter the circulatory system and affect the nervous system of zebrafish larvae, leading to developmental and behavioral changes as well as cardiotoxicity at high concentrations (Ricarte *et al* 2023). As for toxicity to mammalians, knowledge is still very limited, especially for humans. Fang et al. found that 6PPD-Q oral administration caused damage to the liver tissue of mice via activated inflammation and

disturbed glycolipid metabolism (Fang *et al* 2023), revealing that 6PPD-Q might also pose the potential to cause adverse effects on the human liver.Regarding 6PPD-quinone (6PPDQ), a derivative of 6PPD formed through oxidation, studies reveal it induces oxidative stress by increasing reactive oxygen species (ROS) levels in cells. Exposure to 6PPDQ significantly upregulates oxidative stress-related genes such as catalase, superoxide dismutase, and glutathione peroxidase, indicating its involvement in oxidative damage responses. 6PPDQ can disrupt cellular redox balance, induce oxidative damage, and interfere with metabolic pathways, potentially leading to toxicity, including liver cell toxicity and promotion of lung cancer cell proliferation through mechanisms involving ROS and related signaling pathways.

### 2.2 **Transformation pathways and products of 6PPD**

The transformation of 6PPD is not limited by a singular process but unfolds through a series of interconnected oxidative, photolysis, hydrolysis, microbial metabolism, and catalytic surface reactions, leading to over 32 structurally diverse TPs, with potentially high persistence and toxicity compared to parent compound (Tian *et al.,*2021). These TPs vary significantly depending on environmental conditions, including medium, presence of oxidants, sunlight exposure, microbial activity, and physicochemical properties like pH and temperature (Hua *et al.,*2023).

### 2.3 **Ozonation and oxidative transformation**

Ozonation is one of the most dominant and extensively characterized pathways through which 6PPD transforms into 6PPDQ and a variety of other transformation products (TPs). This process is especially relevant in urban environments where TWPs interact with atmospheric ozone during stormwater runoff or on road surfaces (Li *et al.,*2024). The oxidative reactivity of 6PPD,

stemming from its chemical function as an antioxidant, makes it particularly susceptible to reaction with oxidants like O3, leading to three possible mechanistic pathways shown in Fig. 1.

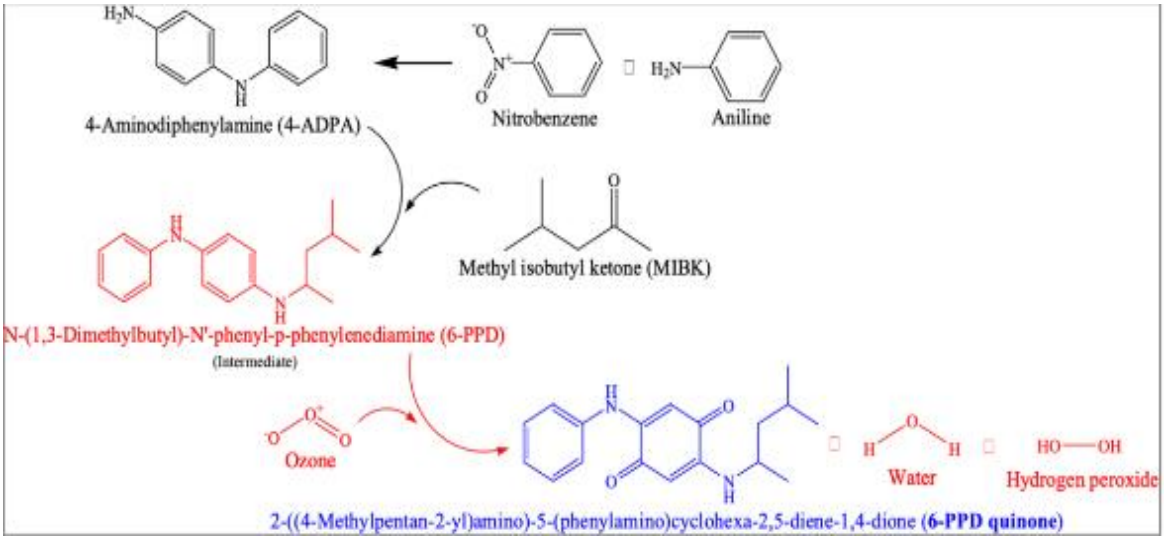


Fig. 2.3.1: Technological synthesis of 6PPD and its transformation to 6PPD-quinone (source: Zhang *et al*. 2022)

**2.4 Environmental Concentrations and Urban Runoff**

The significant presence of 6PPD-quinone in urban stormwater runoff has been well-documented.(Tian *et al*.,2022) reported that during storm events, concentrations of 6PPD-quinone often exceed lethal thresholds for aquatic organisms, particularly in urban waterways. This research emphasizes the importance of monitoring urban runoff for contaminants and suggests that urban land use significantly contributes to the loading of 6PPD-quinone into aquatic ecosystems.(Cao *et al*.,2022) investigated rubber-derived quinones from p-phenylenediamine (PPD) antioxidants, including 6PPD.They found that 6PPD-Q levels (0.21–2.43 g/L) in the runoff water samples from Hong Kong were lower than those reported by (Tian et al.,2021); nevertheless, this range exceeded the LC50 for coho salmon, indicating a

potential risk to aquatic organisms. The reported 6PPD concentrations are similar to those of 6PPD-Q, with values ranging from 0.21–2.71 g/L.

In a recent study,(Kryuchkov *et al*.,2023) found tunnel-wash runoff samples in Norway containing 6PPD-Q at 0.11–0.14 g/L. Runoff samples from artificial turf pitch, which is usually made of rubber granules from discarded car tyres, also contained 6PPD-Q at 0.16 g/L. These studies highlight the geographic variability in the amounts of 6PPD-Q, suggestive of the possibility that 6PPD-Q levels could be influenced by several factors, i.e., land utilisation, infrastructure, population, weather conditions, study sampling design, and chemical transportation dynamics (Challis *et al.*,2021). In future studies, experiments should be designed to consider the effects of these factors on the occurrence and fate of 6PPD

Recent reports have revealed the presence 6PPD-Q in run off water (Cao *et al.,*2022;Tian *et al*.,2021),rivers (Johannessen *et al*.,2022a;Rauert e*t al.*,2021),waste water (Seiwert *et al*.,2022), air particles (Cao *et al.*,2022; Y.J.Zhang *et al*.,2022), and road side soil (Cao *et al*.,2022). Given that tyre production and usage will continue,investigating 6PPD-Q occurence and fate in the environment is critical understand and emphasise its potential ecotoxicological impact.

In another study by (Johannessen *et al*.,2022), a peak 6PPD-Q concentration of 2.30 0.05 g/L was recorded in Don River in Toronto during storm events. These values are considerably higher than those observed by (Rauert et al.,2021, 2022) in their study on the quantification of TRWPs and tyre additive chemicals in Australian surface waters during extreme storms. Tyre additive concentrations increased more than 40 times during storm events, with a 0.088 g/L 6PPD-Q maximum concentration (Rauert *et al.*,2021). However, lower 6PPD-Q levels (<0.00005–0.024 g/L) were detected at 18 of the 21 sampled sites (Rauert *et al*., 2022), with

the highest concentration approximately four times lower than 0.095 g/L, the LC50 for coho salmon (Tian *et al*., 2022).

Recent studies have provided insights into occurence,fate,sources of 6PPD-Q with elevated concentrations of 6PPD-Q found in storm water run off and urban streams(Tian *et al*.,2021;Tian *et al*.,2022;Challis *et al*.,2021;Johannessen *et al*.,2021;Johannessen *et al*.,2022;Rauert *et al*.,2022),snow and snowmelt that accumulates TWP(Seiwert *et al*.,2022),and road dusts(Deng *et al*.,2022;Hiki and Yamamoto,2022).

(Seiwert *et al*.,2022) detected 6PPD and 13 transformation products, including 6PPD-Q, in WWTP influent and effluent during snowmelt, rainfall, and dry weather in Germany. The 6PPD-Q concentrations were generally below the quantification limit except in the influents during snowmelt (0.11 0.04 g/L) and rainfall events (0.05 0.02 g/L). These values were lower than those detected for road runoff (Cao et al., 2022; Challis et al., 2021; Tian et al., 2021), which could be due to runoff dilution with wastewater from households and industry in combined sewer systems (Seiwert *et al*., 2022)

In parallel, another study by (Rauert *et al*.,2022) indicated the widespread occurrence of 6PPD-quinone in particulate matter (PM2.5) derived from urban areas, linking it to potential health risks associated with long-term exposure. Although this research primarily focused on air pollution, it highlights the compound's environmental persistence and its potential to affect both human health and aquatic ecosystems, pointing to a need for integrated studies that address both air and water quality.

## 2.5 Toxicological Study On Fish

### 2.5.1 Acute Toxicity to Aquatic Life

Research by (Tian *et al*.,2020) highlighted the acute toxicity of 6PPD-quinone, specifically its lethal effects on coho salmon, which are often subjected to high concentrations during storm events. This study underscores the urgent need to monitor and regulate tire-derived contaminants, as even low concentrations of 6PPD-quinone have been linked to significant mortality rates among salmon populations. The findings indicate that urban runoff.particularly during heavy rainfall, can lead to toxic concentrations that threaten vulnerable aquatic species.

Further investigations by (Brinkmann *et al*.,2022) expanded on the acute toxicity of 6PPD-quinone by examining its effects on various fish species, including rainbow trout and brook trout. The study revealed that while coho salmon exhibited the highest sensitivity, other species demonstrated varying levels of toxicity, indicating the necessity for comprehensive assessments across multiple taxa. These findings are critical for developing environmental risk management strategies and highlight the complexity of assessing ecological risks associated with 6PPD-quinone.

Another research by (Prosser *et al*.,2023) with four tested species (*Hexagenia.*spp., *Daphnia magna, planorbelle pilsbyri* and *Megalonaias nervosa*) showed the lowest no-observed effect concentration to be 11.4µg/L 6PPD-Q. furthermore Hiki et al.2021 found no acute toxicity in *Daphnia magna* or the amphipod *Hyalella azteca.* Similarly,no acute toxicity was found in test using the standard laboratory test fish species zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*). However,zebrafish used in sublethal effects studies indicated that

behaviour/swimming performance may be impacted at high concentrations.(Ji *et al*.,2021;Varshney *et al*.,2022)

Three studies reported LC10 values in addition to LC50 values. (Lo *et al*.,2023) reported a 24-hour LC10 for coho salmon of 0.021 µg/L and 20.9 µg/L for Chinook salmon, and 24-hour LC50 values of 0.041 µg/L and >67.3 µg/L for coho salmon and Chinook salmon, respectively. (Greer *et al*.,2023a) reported similar results, with a 24-hour LC10 of 0.0292 µg/L for coho salmon and 24.6 µg/L for Chinook salmon, and 24- hour LC50 values of 0.0804 µg/L for coho salmon and 82.1 µg/L for Chinook salmon. (Brinkmann *et al*.,2022) reported a 24-h LC10 of 0.477 µg/L for brook trout and a 96-hour LC10 of 0.8 µg/L for rainbow trout.

Non-salmonid fish species were found to be much more tolerant to 6PPD-quinone than salmonids, with 96-hour NOEC values ranging from 12.7 µg/L for juvenile white sturgeon (Acipenser transmontanous) (Brinkmann *et al*.,2022) to 54 µg/L for zebrafish (Danio rerio) embryos (Hiki *et al*.,2021). In addition, (Varshney et al.,2022) reported 96-hour and 24-hour LC50 values for Zebrafish larvae of 132.92 µg/L and 308.67 µg/L, respectively.

A recent study of zebrafish larvae found a 24-hour exposure to 6PPD-quinone affected the central nervous system causing behavioural shifts, along with alterations in the sleep/wake cycle and electrical conduction of the heart (i.e., increased heartbeat rate) (Ricarte *et al*.,2023). At concentrations of 0.02 µg/L, larvae exhibited exploratory behavior and habituation change; exposure to the highest concentration tested (2 µg/L) altered the wake/sleep cycle and the expression of circadian clock genes (Ricarte *et al*., 2023). Although this study used an acute exposure period, the results suggest there may be sub-lethal effects on fish that are more tolerant of the concentrations of 6PPD-quinone typically found in the environment. (Ricarte *et al*.,2023)

concluded that the changes to zebrafish found in their study could be lethal to individuals in their natural environment; therefore, fish species that appear tolerant to 6PPD-quinone may, in fact, be at risk.

### 2.6 Mechanisms of Toxicity and Oxidative Stress

While much of the research has focused on the acute effects of 6PPD-quinone, there is also a growing interest in understanding the underlying mechanisms of toxicity. (Varshney et al.,2021) explored the toxicological effects of 6PPD and its quinone form in zebrafish larvae, providing insight into how these compounds induce oxidative stress. The study aligns with broader findings that link quinones to the production of reactive oxygen species (ROS), which can lead to cellular damage and contribute to the overall toxicity observed in aquatic organisms.

Moreover, the role of redox-active species, including quinones, in generating oxidative stress has been discussed in the context of ambient particulate matter (Lakey *et al*., 2016). Although this research does not specifically address 6PPD-quinone, it provides a valuable framework for understanding the environmental health implications of such compounds, suggesting that further studies are needed to elucidate the specific pathways through which 6PPD-quinone exerts its toxic effects.

### 2.7 African/Nigeria Context

Over the few past years, there has been a gradual decline in the populations of the fish species. This has been attributed to overfishing and environmental pollution.(Sikoki *et al*.,2013;Oshineye et al.,2000;U.S. Energy Information Administration (EIA).,2005). Some of the pollution sources are from anthropogenic activities such as oil exploration, which include pipeline rupture, oil well blowouts, seepages, tanker accidents, ballast water and refinery wastewater, sabotage of

operational facilities, oil spillage, and gas flaring (Mogaji *et al*.,2018;Farrington *et al*.,2014). These pollutants include PAHs, persistent organic pollutants (POPs), pesticides, metals, and, more recently, plastic waste. This report reviewed available data on contaminants in freshwater and marine fish in Nigeria. It reflects the degree of the environmental pollution in fish consumed in Nigeria with reference to the international maximum levels and the potential risk to human health. The report is divided into four parts based on the nature of the contaminants: (i) Poly Aromatic Hydrocarbons (PAHs); (ii) Persistent Organic Pollutants (POPs); (iii) Metals; (iv) Microplastics.

Studies on PAHs in marine fish are scarce in Nigeria, as the main focus is on environmental PAH pollution rather than on their presence in food. The detrimental effects of these contaminants on fish populations have been reported(Lindén *et al*.,2013;Nwaichi *et al*.,2016).However, the effects of different remediation actions on PAH levels in fish have not been fully documented.Related toxicological studies on *Clarias gariepinus* discuss genotoxic and other pesticide toxicities but do not focus on 6PPDQ specifically. For example, genotoxic evaluations on *Clarias gariepinus* with pesticides and chemical exposures show DNA damage and genetic implications, but not specifically for 6PPDQ.

Table 2.7.1: Summary of *Clarias gariepinus* and their sources assessed for contaminant

PAHs

| Fish species | sample size | location | Analytical method used | Reference | Year published |
|---|---|---|---|---|---|
| *Clarias gariepinus* | - | Benue state | GC/MS NA | Ubwa *et al.2015;*Akinrotimi *et al.2013* | 2015 2013 |
| *Clarias gariepinus* | - | Edo state | GC/MSD GC/FID | Alani *et al.2012;Tongo et al.2017* | 2012, 2017 |

POPs

| Fish species | Sample size | location | Analytical method used | Reference | Year published |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| *Clarius gariepinus* *Tilapia zilli* | 92 | Edo state | GC/ECD GC | Ezemonye et al.,2015;Taiwo et al 2019 | 2015 2019 |
| *clarius gariepinus* *Neochanna diversus* | 92 | Edo state | GC/ECD,GC/MS-MS | Adeyemi et *al*.2009;Ingenbleek *et al*.2019 | 2009 2019 |

Metal Contaminants

| Fish species | Sample size | Location | Analytical method used | Reference | Year published |
|---|---|---|---|---|---|
| *Clarius gariepinus* | - | River Niger | AAS,NA | Nsofor *et al*.2014;USEPA 2001 | 2014 2001 |

| | | | | | |
|---|---|---|---|---|---|
| *Clarius gariepinus* *Tilapia Zilli* | - | Anambra State | AAS,NA | Ibemenuga *et al.*2019;Koppe *et al.*2001 | 2019 2001 |

**2.8 Common fish species used in this study**

Recent studies have found that 6PPDQ is toxic to certain freshwater fish species at relatively low concentrations (Tian *et al*., 2021; Brinkmann *et al*., 2022; Foldvik *et al*., 2022; Lo *et al*., 2023). For example, (Lo *et al*.2023) reported that a 24-h exposure of coho salmon (Oncorhynchus kisutch) to 0.041 μg/L of 6PPDQ would result in mortality of 50% of the juvenile fish (LC50). 6PPDQ has also been implicated in the mass mortality of coho salmon following urban stormwater runoff (Tian *et al*., 2021).

Coho salmon coho salmon,root mean square velocity(URMS)begins with a common progression of behavioral symptoms, which include increased surface swimming, loss of equilibrium and buoyancy, gasping at the surface, and ultimately mortality ( Chow *et al*. 2019;Scholz *et al*. 2011;Tian *et al*.2022)Of the fish species studied thus far, coho salmon are the most sensitive

Zebrafish.Several studies have been conducted to assess the toxicity of compounds from the 6PPD family on zebrafish by analyzing at three distinct developmental stages: embryonic exposure, larval exposure, and adult exposure.In a study, zebrafish embryos were exposed to

varying concentrations of 6PPD from 2 h post-fertilization (hpf) for a duration of 5 days (Peng *et al*., 2022). The findings revealed a notable decrease in hatching rate and voluntary movement of the embryos. Additionally, the length of hatched hatchlings was reduced, indicating significant developmental toxicity (Jiao *et al*., 2024). Further investigations suggested that this phenomenon could be attributed to the impact of 6PPD on the growth hormone/insulin-like growth factor axis and the hypothalamic-pituitary-thyroid axis, leading to disruptions in hormone levels and consequent growth inhibition (Peng *et al*., 2022).Moreover, exposure to 6PPD and 6PPD-Q was found to induce teratogenic effects in zebrafish, with 6PPD causing myopia and 6PPD-Q linked to intestinal dysfunction. These effects are believed to result from abnormal retinoic acid metabolism and increased potassium channel activity, respectively (Zhang *et al*., 2023b)

Following exposure during the larval stage, zebrafish exhibited impaired swim bladder expansion, resulting in a significant decrease in swimming ability. This locomotor toxicity was associated with compromised central nervous system function, including downregulation of biological clock genes and alterations in neurotransmitter profiles (Varshney *et al*., 2022; Ricarte *et al*., 2023).Furthermore, 6PPD was found to potentially induce cardiotoxicity in zebrafish through mechanisms involving oxidative stress, calcium channel inhibition, and myocardial contraction suppression (Fang Y. Jiang *et al*.,2023b;Jiang *et al*., 2024).

In adult zebrafish exposed to 6PPD, motor toxicity manifestations such as reduced swimming speed, shortened swimming distance, increased wandering, and abnormal postures were observed. Fish in the 6PPD group displayed a propensity to remain at the bottom of the tank, indicating an increase in anxiety-related behaviors compared to counterparts in the 6PPD-Q or 4-hydroxy exposed group (Ji *et al.*, 2022). The underlying mechanism may be related to a decrease

in neurotransmitters, such as acetylcholine. These abnormal locomotor behaviors and alterations in neurotransmitter levels resemble those seen in Huntington's disease.

### 2.9 Regulatory Implications and Knowledge Gaps

The findings across these studies collectively underscore the need for updated risk assessments and regulatory measures regarding the environmental impact of 6PPD-quinone. Despite the growing body of evidence highlighting its acute toxicity, there remain significant knowledge gaps regarding its chronic effects, long-term environmental persistence, and the full range of species impacted by its presence in aquatic ecosystems.

Studies on environmental fate and toxicity of 6PPDQ emphasize its global significance as a pollutant impacting fish species but are more commonly studied in salmonids and zebrafish rather than *Clarias gariepinus*.

while there is broad toxicological interest in 6PPDQ and some studies on *Clarias gariepinus* for other toxicants, detailed published toxicological studies specifically on 6PPDQ effects in *Clarias gariepinus* appear scarce or not well documented in publicly available literature in Nigeria as of now

### 2.10 Future research directions should focus on:

1. Long-Term Toxicity Studies: Investigating the chronic effects of 6PPD-quinone exposure on various aquatic organisms and ecosystems.
2. Additional considerations should be incorporated into assessments of 6PPD-Q and related compounds. Studies to date have involved individual species,rather than multispecies tests that include species interactions,such as microcosms/mesocosms

3. Mechanistic Studies: Elucidating the biochemical and physiological pathways through which 6PPD-quinone induces toxicity, particularly in different species.
4. Ecosystem-Level Assessments: Evaluating the broader ecological impacts of 6PPD-quinone on aquatic food webs and ecosystem functions.
5. Regulatory Framework Development: Supporting the establishment of guidelines for monitoring and regulating tire-derived contaminants in urban runoff.
6. Improvements in sampling and analysis,particularly around storage ,may also impact risk assessment calculations as it has been indicated that 6PPD-Q concentrations are reduced under refrigerated short term storage.

Table 2.10.1: Literature Review Summary Table

| Author(s) | Year | Focus Area | Specie studied | Key findings |
|---|---|---|---|---|
| Tian *et al* | 2021 | 6PPDQ toxicity to aquatic organism | Coho salmon | Acute mortality in coho salmon |
| Kryuchkov *et al* | 2023 | 6PPDQ in tunnel-wash runoff | | The study highlighted the geographical variability in the amount of 6PPDQ |
| Ricarte *et al* | 2023 | 24hours exposure of 6PPDQ to zebra fish larvae | Zebra fish larvae | Behaviour shifts,alteration in sleep/wake cycle and electrical conduction of the heart |

| | | | | |
|---|---|---|---|---|
| Brinkman *et al* | 2022 | Expanded on acute toxicity of 6PPDQ | Rainbow brout and brout trout | Coho salmon exhibited the highest sensitivy other species demonstrated vary level of toxicity |

# CHAPTER THREE

## MATERIALS AND METHODS

### 3.1 Chemicals

6PPD-quinone (purity 97.26%) and the isotopically labeled internal standard 6PPD-quinone-D5 were obtained from HPC Standards GmbH (Borsdorf, Germany) and the 6PPD-quinone analytical independent check standard from Cambridge Isotope Laboratories (Cambridge, MA, USA). Stock solutions of 6PPDQ were prepared using dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific, Waltham, MA, USA) to achieve a final solvent concentration of <0.001% (v/v) during exposures. Buffered tricaine methanesulfonate (MS-222) (Syndel's Syncaine, Ferndale, WA, USA) was used for anesthesia (100 mg/L) and euthanasia (250 mg/L).

Plate 1: Image of 6PPD-Q

Source: Ecotoxicology laboratory, University of Benin, Benin city, Edo state

Dimethyl Sulfoxide (DMSO) was obtained from a commercial vendor. The DMSO (Dimethyl sulfoxide; CAS No. 67-68-5) is an organosulfur compound widely used as a solvent in biological and chemical research due to its ability to penetrate biological membranes. It is a polar aprotic solvent, fully miscible with water and many organic solvents. DMSO is known for its anti-inflammatory properties and is used as a vehicle for drug delivery in experimental studies.

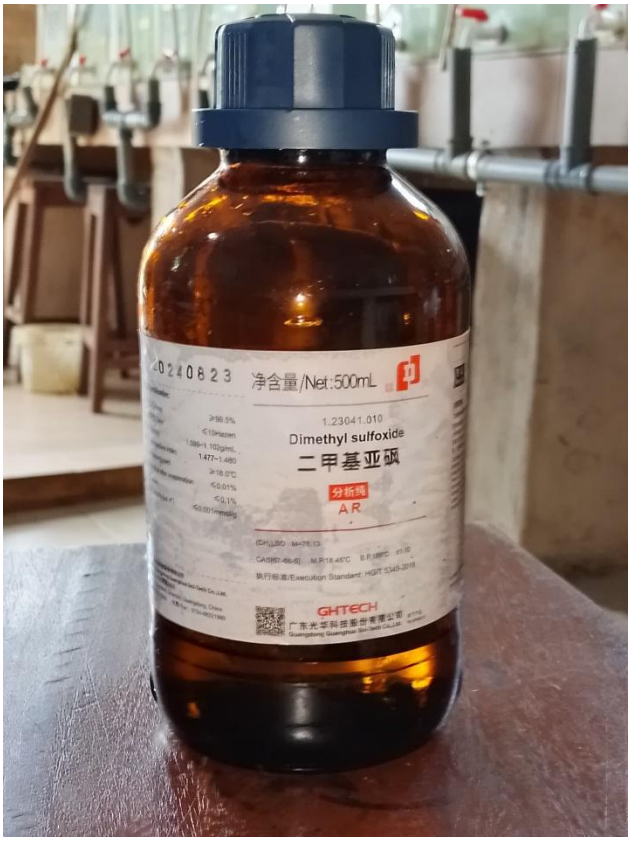


Plate 2: Image of Dimethyl Sulfoxide (DMSO)

Source: Ecotoxicology laboratory, University of Benin, Benin city, Edo state

### 3.2 Test organism

*Clarias gariepinus* (African catfish) is widely distributed in tropical freshwater ecosystems and relished by the tropical region populace (Ogueji *et al.,* 2018). The fish is omnivorous, feeding on invertebrates, fish, small mammals, seeds and fruit (Hastuti *et al*., 2019). According to Ogueji *et al.* (2018), it is also rugged, adaptive to laboratory conditions produces reasonable quantities of blood for haematological parameter estimation. It is among the most widespread of the African freshwater fish (Nguyen and Janssen, 2002).

### 3.3 Source of experimented fish, Acclimatization and Ethical Approval

The experimental fish, *Clarias gariepinus* juveniles were purchased at a fish farm in Benin city, Edo state and transported to the ecotoxicology laboratory of Animal and Environmental Biology, University of Benin, Benin City, Edo state where the experiment was conducted. The average weight and average length of the fish was 24.10 ± 11.51g and 12.51 ± 2.8 cm respectively. The juveniles were acclimatized in a 160L cylindrical plastic tank for two days and fed on a pelleted commercial feed daily. They were however left unfed for the first 24 hours to adapt to a change in the environment before feeding commenced with the fish diet. Approximately, 80% of the water was replaced every two days. They were kept under natural photoperiod. There was 8% mortality during the acclimatization period. Ethical clearance on the use of the fish species was obtained and approved by the Committee on the Ethical Use of Laboratory Animals, Faculty of Life Sciences, University of Benin, Nigeria.

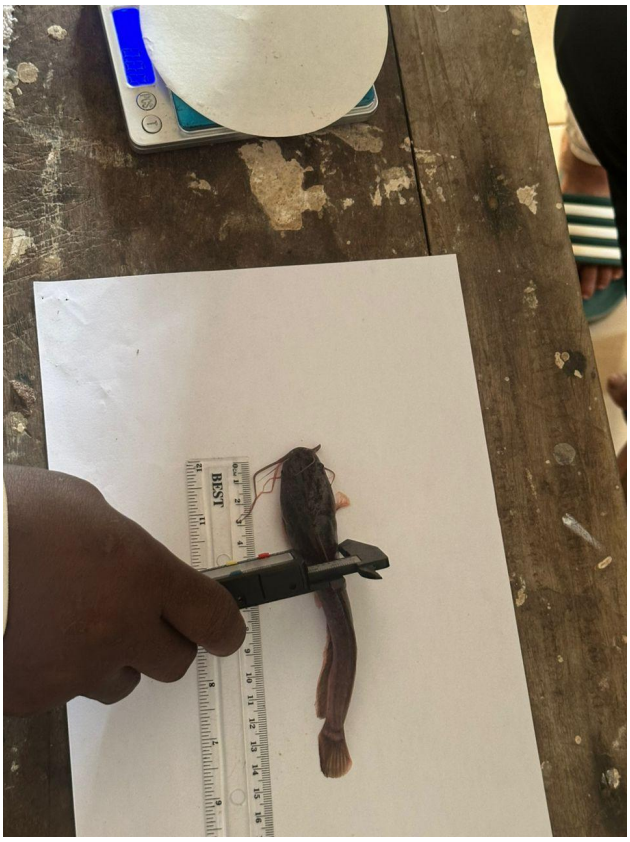


Plate 3: Measurement of the average width of the test organism (*Clarias gariepinus*)

Source: Ecotoxicology Laboratory university of Benin, Benin City, Edo state

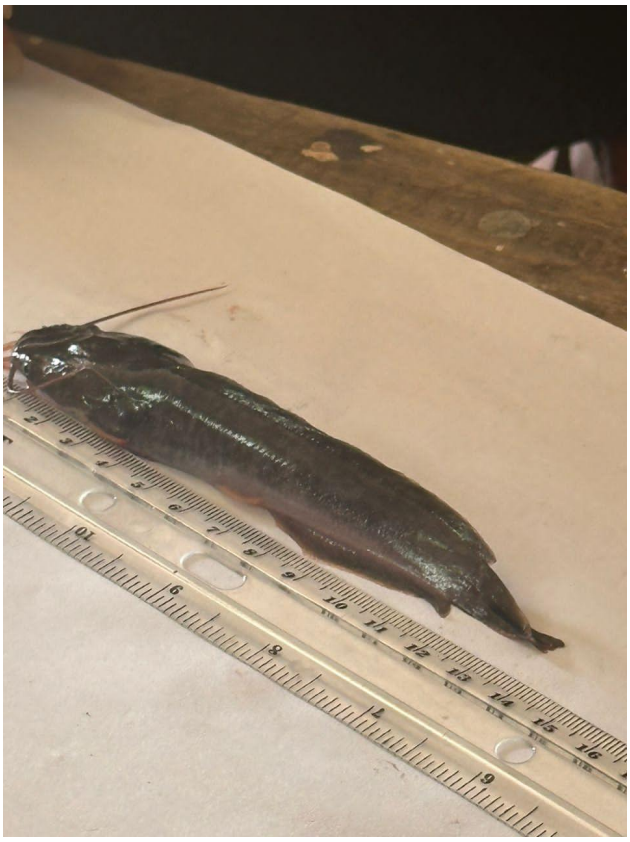

Plate 4: Measurement of the average length of test organism (*Clarias gariepinus*)

Source: Ecotoxicology Laboratory, university of Benin, Benin City, Edo state

Plate 5: Measurement of the average weight of test organism (*Clarias gariepinus*)

Source: Ecotoxicology Laboratory, university of Benin, Benin City, Edo state

### 3.4 Experiment design

A total of 237 *Clarias gariepinus* juveniles used were divided into five treatments which included a positive control (+control), negative control (-control), and three different concentrations of the toxicant) with two replicates. Seven juveniles were stocked into each tank with two replicates treatment. The water used was tap water left to stand for 24 hours.

plate 6: Experimental design

Source: Ecotoxicology laboratory, university of Benin, Benin City, Edo state

### 3.5 Experimental protocol

The experiment was carried out as described in the OECD guidelines for fish sublethal toxicity testing (OECD, 2019). 20g of 6ppd-q was dissolved into 100ml of DMSO to produce a stock solution. The stock solution was used to produce the three different concentrations- 500µg/L, 1000µg/L and 1500µg/L of toxicants introduced into experimental tank of 20litres of water in each tank. The positive control (+control) had 0.0mg/L of toxicants, while the negative control (-control) had 200µL of DMSO. Duplicates were made for each concentration. The test was carried out under static conditions. The duration of the test was 28 days from which started on 19$^{th}$ September 2025 to 17$^{th}$ October 2025, during which the organisms were fed once a day.

### 3.6 Biomarker Analysis

At the end of the exposure periods, a range of biomarkers was evaluated to assess the sublethal effects of 6PPD-q exposure. Glutathione Peroxidase (GPx), Glutathione (GSH), and Superoxide Dismutase (SOD). These biomarkers were chosen for their relevance in detecting hepatotoxicity, oxidative stress, and systemic physiological changes in fish exposed to environmental contaminants like BPA.

#### 3.6.1 Oxidative Biomarker Analysis

##### 3.6.1.1 Determination of Glutathione Peroxidase (GPx)

The activity of glutathione peroxidase (GPx) was determined according to the method described by Ursini *et al.* (2016). This method is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting in a deep brown coloration that can be measured spectrophotometrically at 430 nm. To perform the assay, 0.2 mL of the sample was mixed with 5 mL of phosphate-buffered $H_2O_2$ and 1.5 mL of pyrogallol (ISO9001:2008, Tianjin Kernei Chemical, China). The reaction mixture was allowed to stand at room temperature for 30 minutes, after which the absorbance was measured at 430 nm. The enzyme activity was calculated using this equation 2:

$$\frac{\mathrm{O.}\frac{\mathrm{D}}{\mathrm{min}} \times \mathrm{V}_t \quad \times \mathrm{D}_f}{E \times V_s \times Y} \times 100$$

where O.D is the absorbance of the test sample, is the total reaction volume, is the dilution factor, E is the molar extinction coefficient (12/M/cm), Vs is the sample volume, and Y represents the mg of protein used.

### 3.6.1.2. Determination of Superoxide Dismutase (SOD)

The activity of superoxide dismutase (SOD) was assessed using the method of Paoletti, and Mocali, (1990). This method is based on the ability of SOD to inhibit the auto-oxidation of adrenaline in aqueous solution, which leads to the formation of adrenochrome. The concentration of adrenochrome is measured spectrophotometrically at 420 nm, with the degree of inhibition serving as an indicator of SOD activity. To perform the assay, 0.2 mL of sample homogenate was mixed with 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate. The reaction was initiated by adding 0.3 mL of freshly prepared 0.03 mM adrenaline (Product Code: 11330, MOLYCHEM, Mumbai, India). The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5 mL of carbonate buffer, 0.2 mL of distilled water, and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to adrenochrome formation was monitored every 30 seconds for 120 seconds. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of adrenaline auto-oxidation. The percentage inhibition was calculated using this equation:

Enzyme activity in units per mg of protein was determined using this equation:

$$\frac{O.D_{test} - \mathrm{O.D}_{reference}}{O.D_{test}} \times 100$$

Where Y represents the mg of protein in the sample.

### 3.6.1.3. Determination of Reduced Glutathione (GSH) Concentration

The concentration of reduced glutathione (GSH) was determined using the method of Rahma *et al*.(2006). This assay utilizes 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which reacts with free thiol groups in GSH to form a yellow chromophore that absorbs at 412 nm. To conduct the assay, 1.0 mL of the sample was mixed with 2.5 mL of 10% trichloroacetic acid (TCA) by MOLYCHEM, Mumbia (CAS No: 76-03-9) and centrifuged at 3000 g for 10 minutes. Then, 1.0 mL of the supernatant was reacted with 0.5 mL of Ellman's reagent (0.0189% DTNB and 1% sodium citrate) and 3.0 mL of 0.3 M phosphate buffer (pH 8.0). The resulting yellow coloration was immediately measured at 412 nm. The GSH concentration was determined using equation:

$$= \frac{Atest \times Conc.\,of\,Standard}{Astandard}$$

Where $A_{test}$ is the absorbance of the sample, and $A_{Standard}$ is the absorbance of the standard. The results were expressed as μM GSH/g plasma

## 3.7 Data Analysis

All values were expressed as the mean standard error of the mean (SEM). The difference between groups was analyzed with one-way ANOVA followed by Dunnett's multiple comparison test. The data were analyzed using GraphPad Prism version 8.01. The level of significance was set at $p< 0.05$. Graph analyses were plotted using the GraphPad Prism software and excel.

# CHAPTER FOUR

## 4.0. RESULT

This chapter present the analysis and interpretation data collected as outlined in the previous chapter. The primary objective is to summarize the key findings using descriptive statistics, notably the mean and standard deviation values, which provides essential insight into the central tendencies and variability of the measured variables.

### 4.1. Glutathione (y'-glutamycysteiny lglycine(GSH))Activity

The mean value of GSH activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 7, for +VE control, DMSO (-VE control), 500µg/L,1000µg/L,1500µg/L are as follows; 0.5440 ± 0.01980, 0.5360 ± 0.001414, 0.4975 ± 0.0007071, 0.5440 ± 0.07071, 0.5420 ± 0.008485. There was no significant differences from positive control compared to the other concentrations such as the DMSO, 500µg/L, 1000µg/L, 1500µg/L.

The mean value of GSH activity in C*larias gariepinus* juvenile exposed to 6PPD-Q on day 14, for +VE control, DMSO (-VE control), 500µg/L, 1000µg/L, 1500µg/L are as follows; 0.4705 ± 0.009192, 0.4915 ± 0.02758, 0.5480 ± 0.04950, 0.4700 ± 0.01414, 0.5360 ± 0.02404. There was no significant differences from positive control compared to the other concentrations such as the DMSO, 500µg/L, 1000µg/L, 1500µg/L

The mean value of GSH activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 21, for +VE control, DMSO (-VE control), 500µg/L, 1000µg/L, 1500µg/L are as follows; 0.4915 ± 0.01485, 0.5585 ± 0.01909, 0.5435 ± 0.0007071, 0.4980 ± 0.02546, 0.6235 ± 0.1704. There was

no significant differences from positive control compared to the other concentrations such as the DMSO, 500µg/L, 1000µg/L, 1500µg/L.

The mean value of GSH activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 28, for +VE control, DMSO (-VE control), 500µg/L, 1000µg/L, 1500µg/L are as follows; 0.6780 ± 0.03818, 0.4110 ± 0.04243, 0.5185 ± 0.006364, 0.4730 ± 0.04667, 0.4480 ± 0.02121. There was a slight significant difference between the positive controls compared to the 500µg/L and there was a high significant difference between the positive control compared to the DMSO, 1000µg/L and 1500µg/L concentrations.

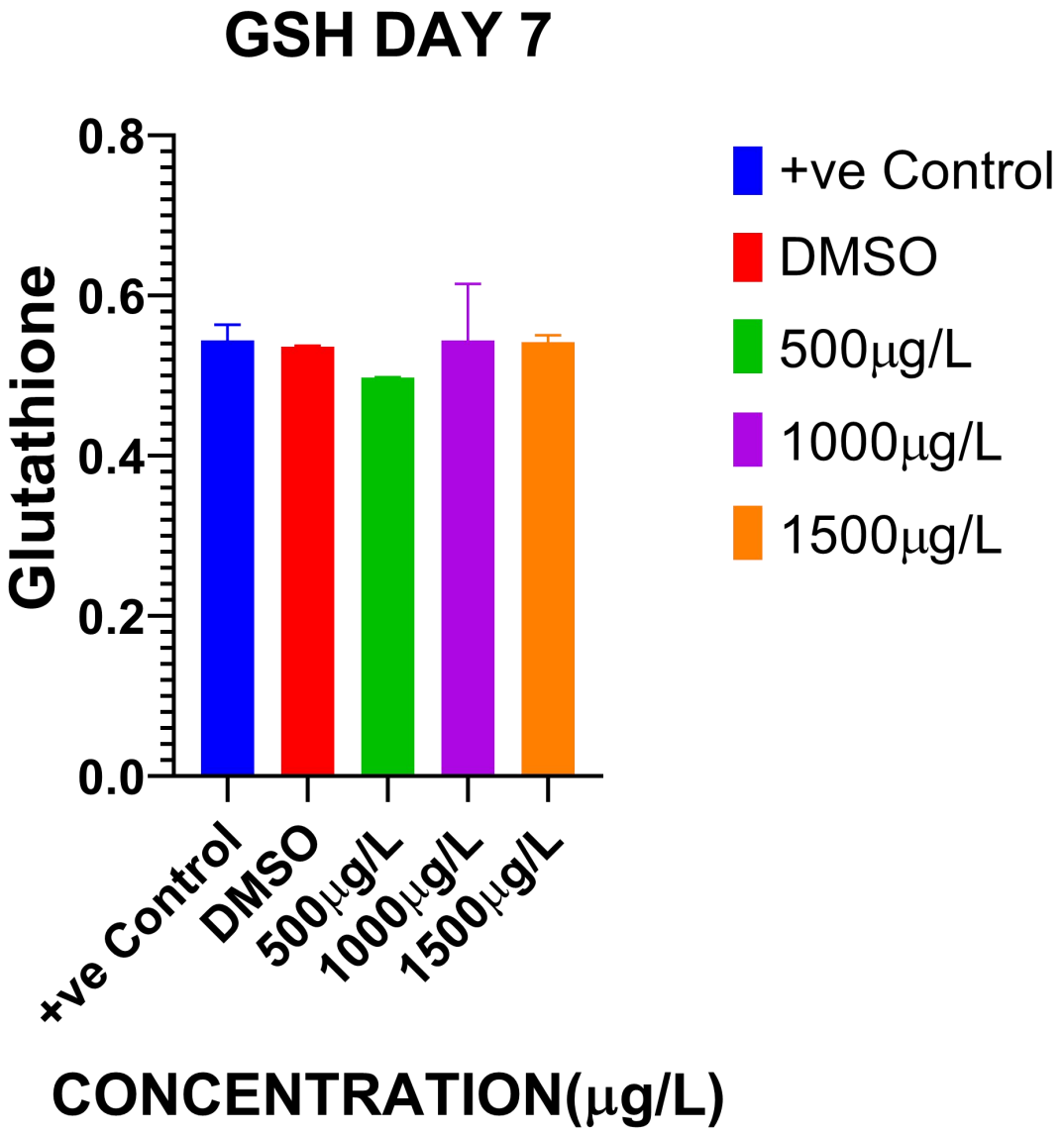


Figure 4.1.1: Mean activity levels of GSH in *Clarias gariepinus* exposed to 6PPD-Q in 7days

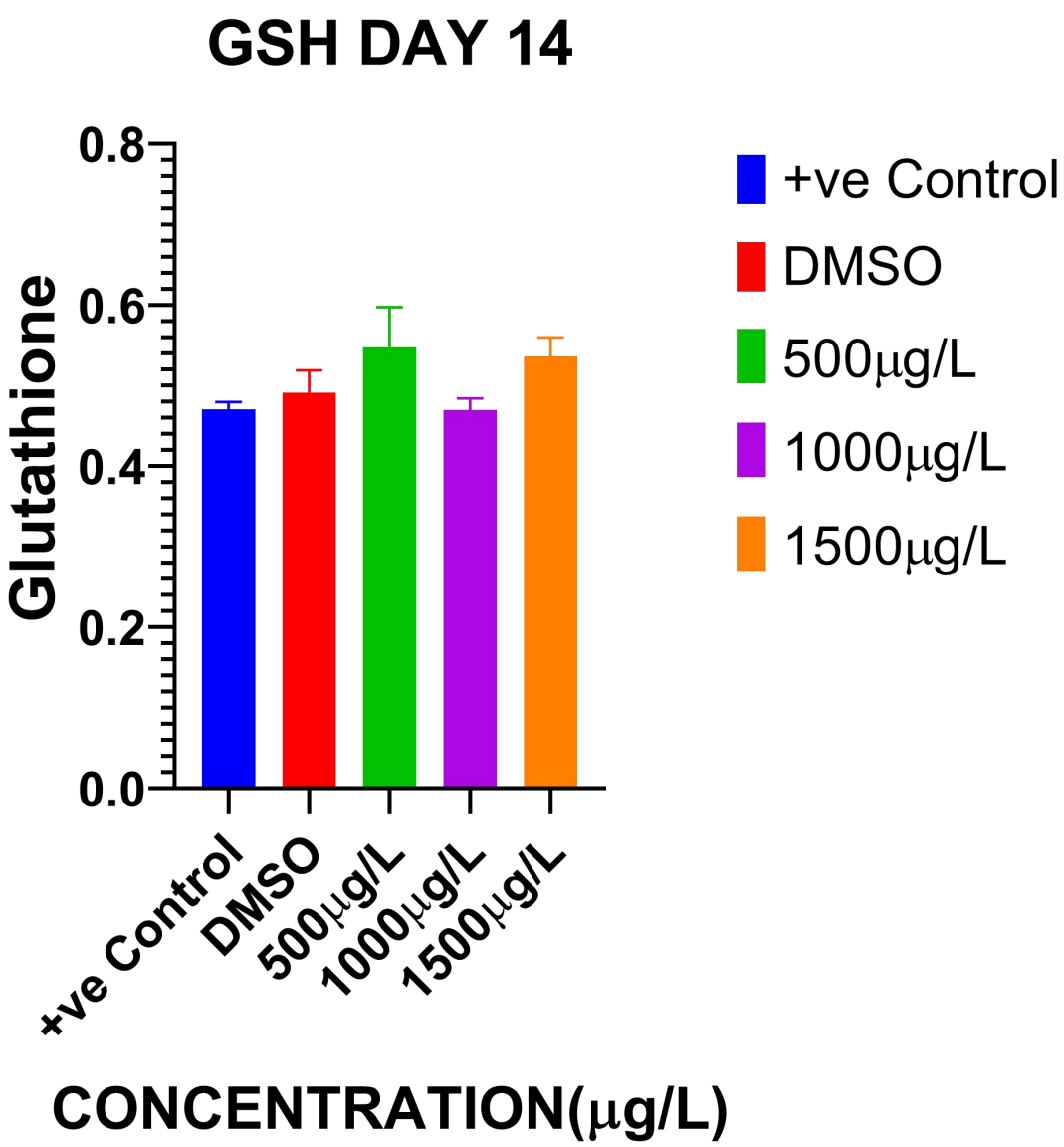


Figure 4.1.2: Mean activity levels of GSH in *Clarias gariepinus* exposed to 6PPD-Q in 14days

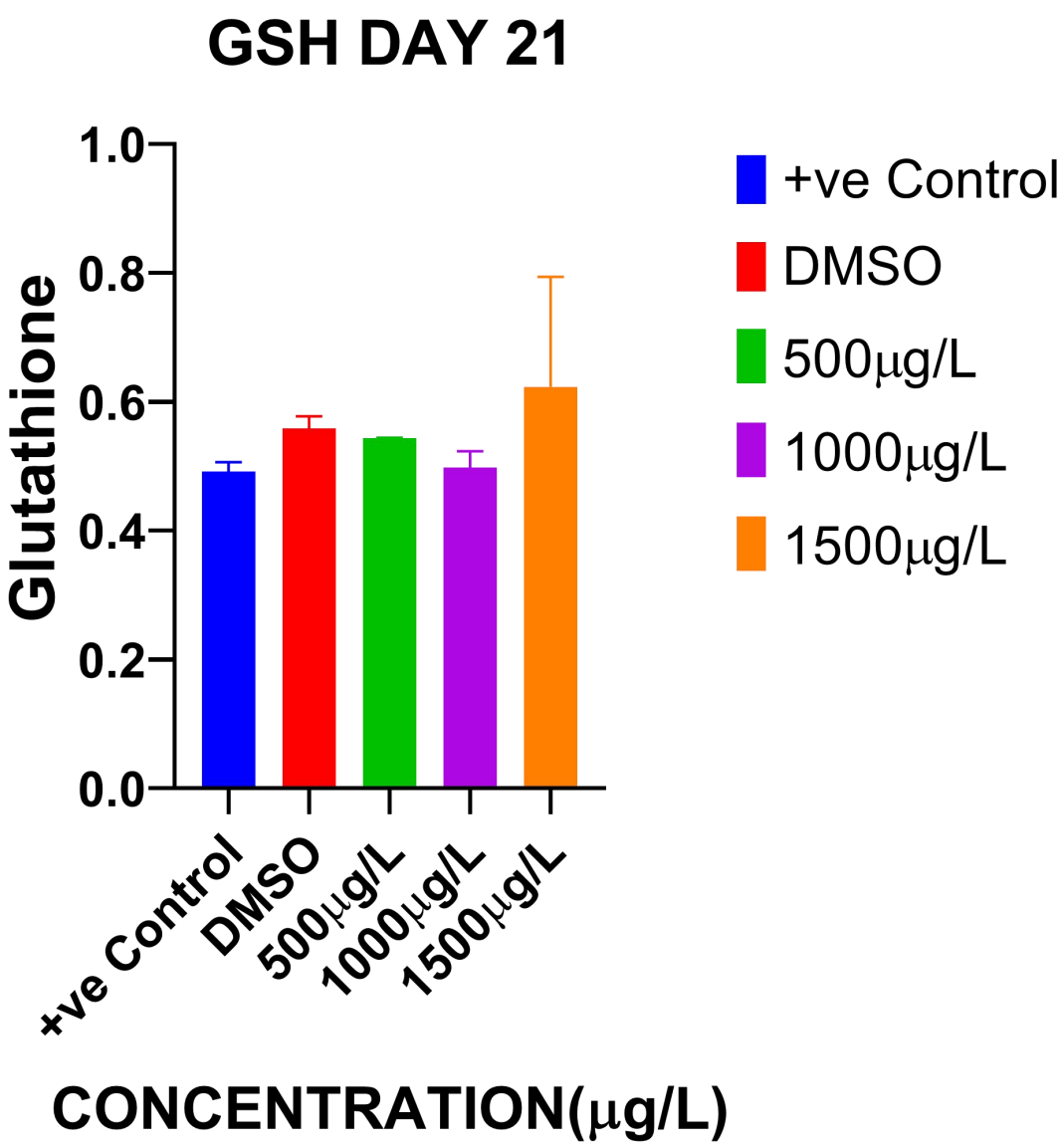


Figure 4.1.3: Mean activity of GSH in *Clarias gariepinus* exposed to 6PPD-Q in 21days

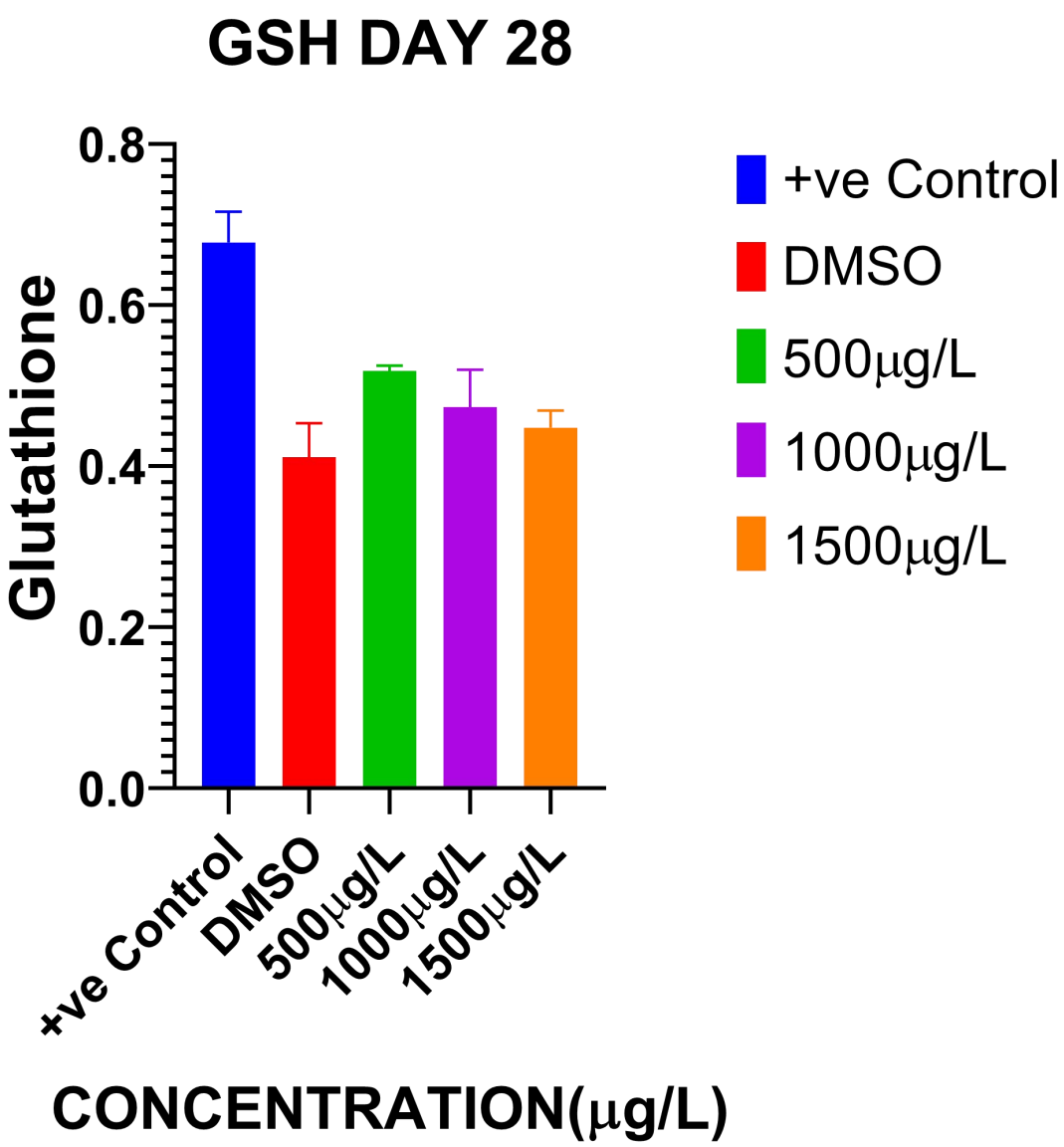


Figure 4.1.4: Mean activity of GSH in *Clarias gariepinus* exposed to 6PPD-Q in 28days

### 4.2. Glutathione Peroxidase ($GP_x$) Activity

The mean value of $GP_x$ activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 7, for +ve control, DMSO (-Ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 1.344 ± 0.2510, 0.9370 ± 0.1485, 0.8700 ± 0.05657, 0.6820 ± 0.04667, 0.6670 ± 0.02828. The data indicate a clear significant differences between the positive control and the 500µg/L, 1000µg/L, 1500µg/L concentrations and no significant difference between the positive control and the DMSO (-ve control).

The mean value of $GP_x$ activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 14, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 1.427 ± 0.1471, 2.964 ± 0.1315, 1.359 ± 0.3132, 0.7805 ± 0.3019, 1.077 ± 0.2765. The mean difference of positive control and the DMSO suggests a statistical significant difference, where as that of the positive control compared to the 500µg/L, 1000µg/L, 1500µg/L concentrations shows no significant difference.

The mean value of $GP_X$ activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 21, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 7.101 ± 0.02051, 2.032 ± 0.01768, 3.400 ± 0.4879, 1.792 ± 0.2404, 1.555 ± 0.2029.

The analysis revealed a substantial mean difference from the positive control compared to the DMSO, 1000µg/L, 1500µg/L concentrations whereas, the positive control compared to the 500µg/L concentration show a bit high mean difference.

The mean value of $GP_x$ activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 28, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 6.407 ± 0.1860, 2.727 ± 0.2807, 3.296 ± 0.3698, 1.331 ± 0.1435, 0.7425 ± 0.04313. The analysis revealed a slight mean

difference between the positive control and the 500µg/L concentration and a substantial statistical mean difference between the positive control and the DMSO, 1000µg/L, 1500µg/L concentrations.

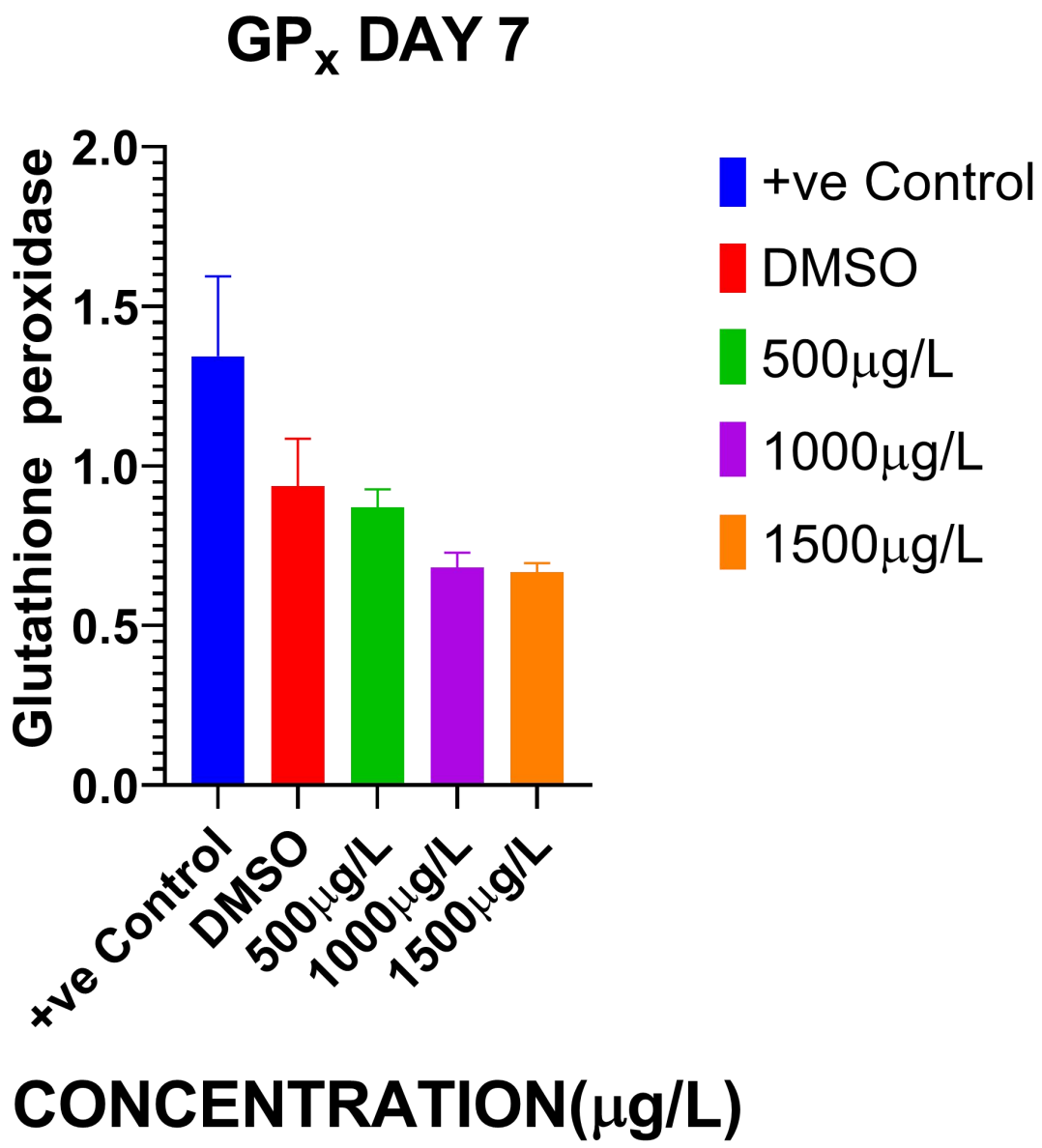


Figure 4.2.1: Mean activity of $GP_x$ in *Clarias gariepinus* exposed to 6PPD-Q in 7days

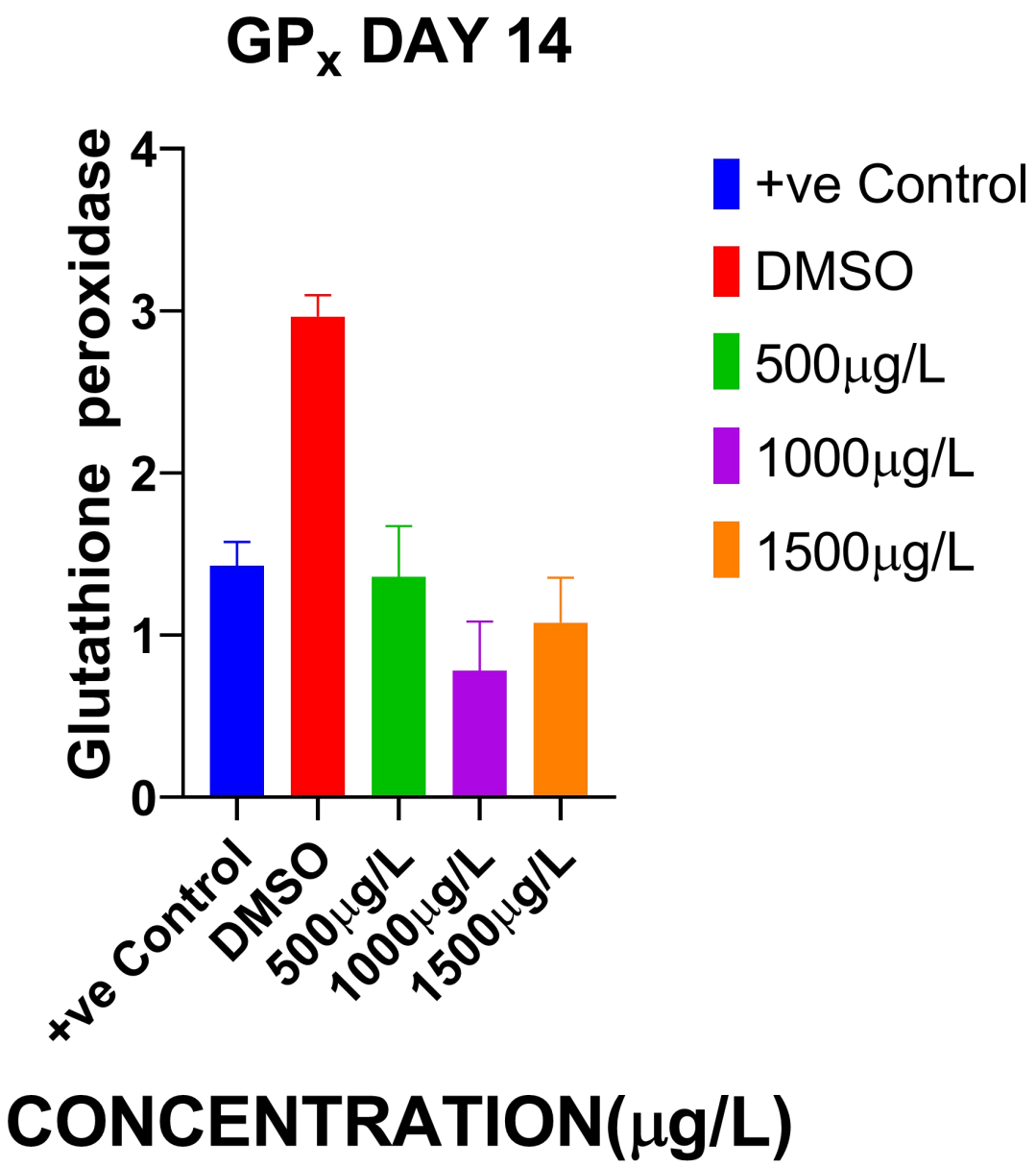


Figure 4.2.2: Mean activity of $GP_x$ in *Clarias gariepinus* exposed to 6PPD-Q in 14days

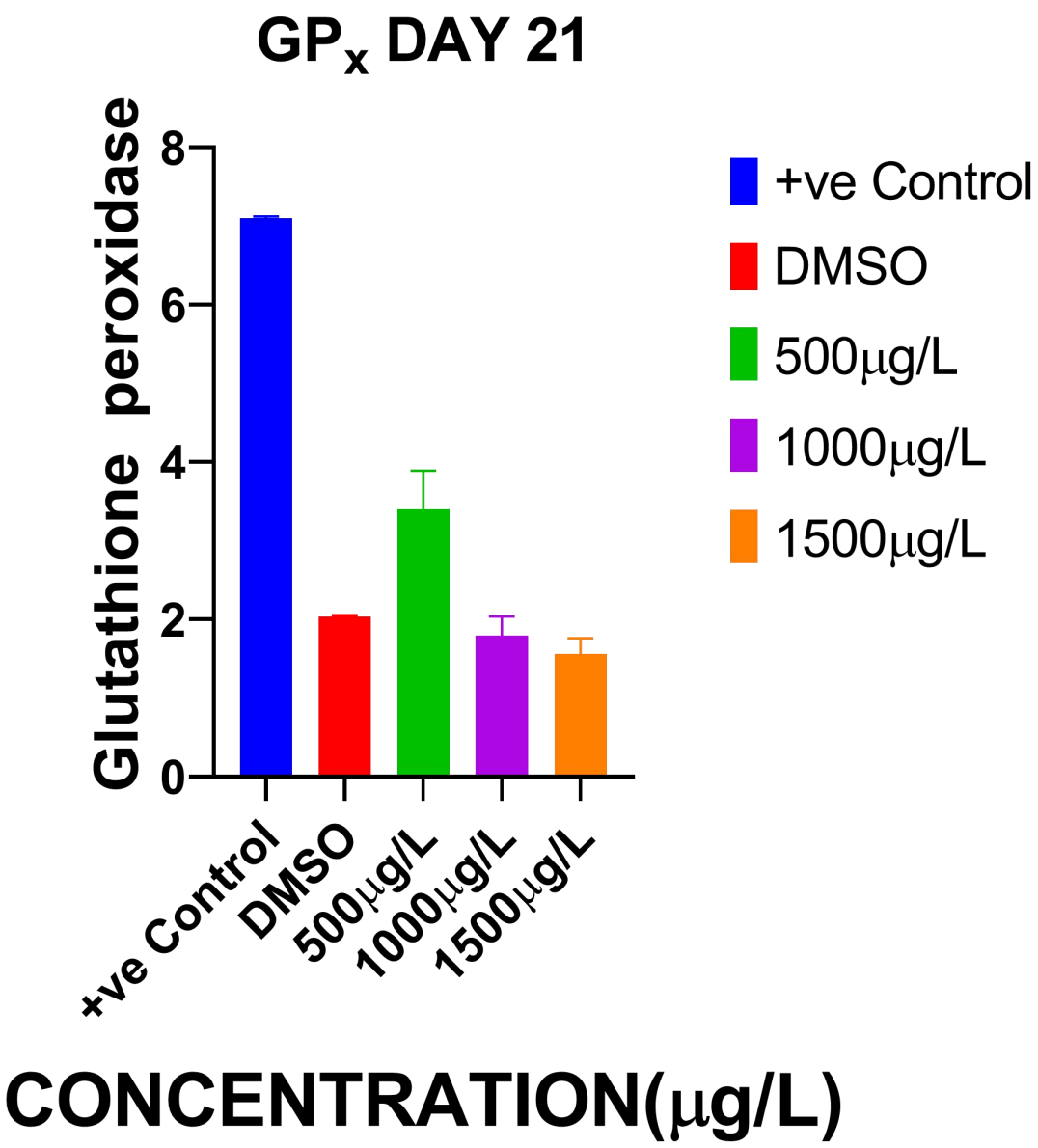


Figure 4.2.3: Mean activity of $GP_x$ in *Clarias gariepinus* exposed to 6PPD-Q in 21days

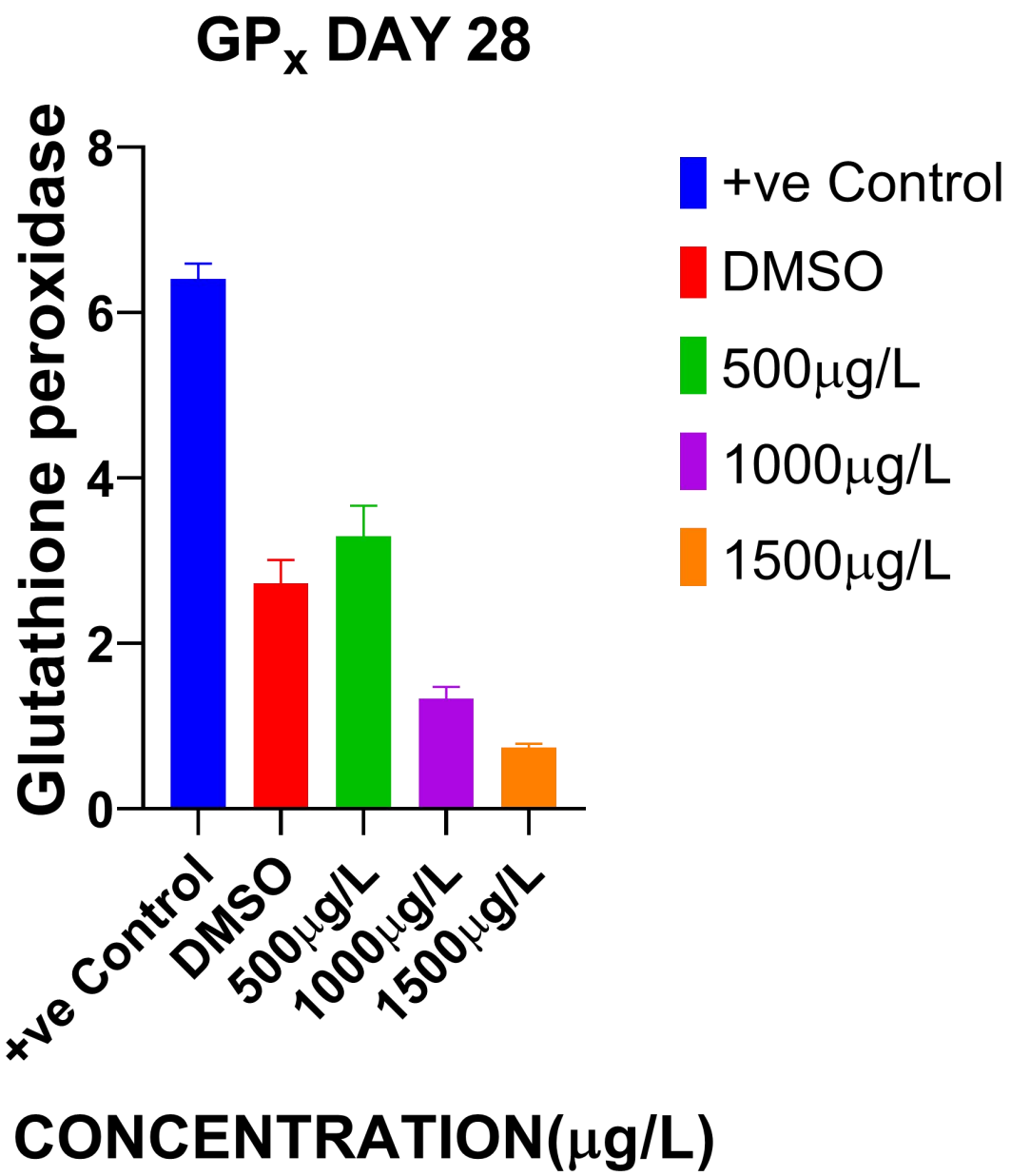


Figure 4.2.4: Mean activity of $GP_x$ in *Clarias gariepinus* exposed to 6PPD-Q in 28days

### 4.3. Superoxide Dismutase (SOD) Activity

The mean value of SOD activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 7, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 0.3754 ± 0.03154, 0.3166 ± 0.03429, 0.3775 ± 0.04815, 0.3367 ± 0.02814, 0.3765 ± 0.01874. The results shows that there was no significant mean differences from the positive control compared to the other concentrations; DMSO, 500µg/L, 1000µg/L, 1500µg/L

The mean value of SOD activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 14, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µrg/L thusly; 1.047 ± 0.07481, 0.6923 ± 0.01846, 0.9014 ± 0.02977, 0.7317 ± 0.1225, 0.6158 ± 0.007637. The results revealed that there was no significant difference between the positive control and the 500µg/L concentration, a slight significant difference between the positive control and the 1000µg/L concentration, a bit high significant difference between the positive control and DMSO, 1500µg/L concentrations.

The mean value of SOD activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 21, for +ve contol, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 1.309 ± 0.07976, 1.187 ± 0.007071, 0.8046 ± 0.1312, 0.7789 ± 0.3024, 0.9736 ± 0.01874. The results revealed that there was a slight significant mean difference between the positive control and the 1000µg/L concentration whereas the positive control compared to the DMSO, 500µg/L, 1500µg/L shows no significant difference.

The mean value of SOD activity in *Clarias gariepinus* juvenile exposed to 6PPD-Q on day 28, for +ve control, DMSO (-ve control), 500µg/L, 1000µg/L, 1500µg/L thusly; 1.138 ± 0.01131, 0.8215 ± 0.01520, 1.105 ± 0.0002828, 0.9299 ± 0.01923, 0.8969 ± 0.1260. The result revealed

that there was no significant mean difference between the positive control and the 500µg/L, there was a slight significant mean difference between the positive control and the 1000µg/L,1500µg/L concentrations, there was a bit high significant mean difference between the positive control group and the DMSO (-ve control) concentration.

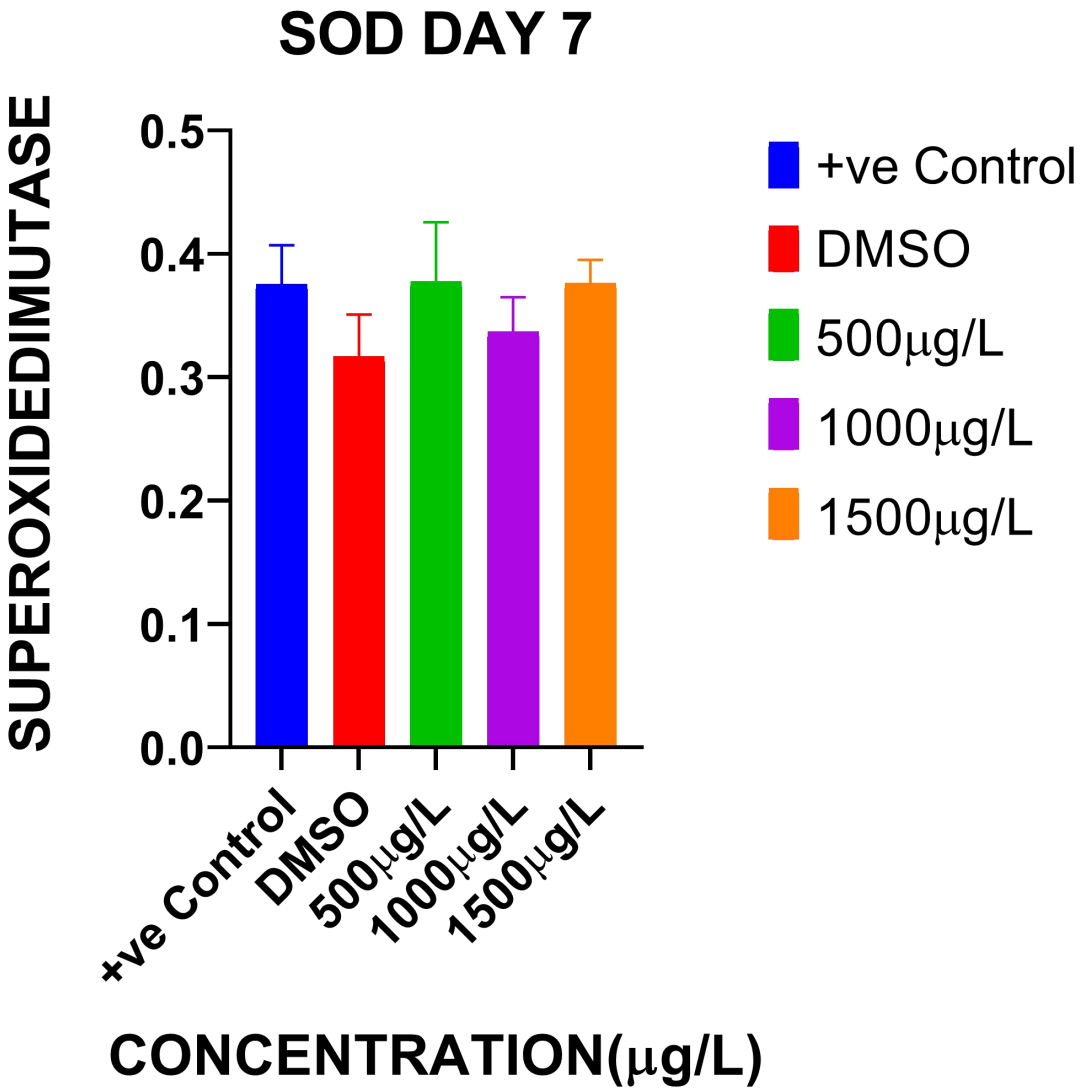


Figure 4.3.1: Mean activity of SOD in *Clarias gariepinus* exposed to 6PPD-Q in 7days

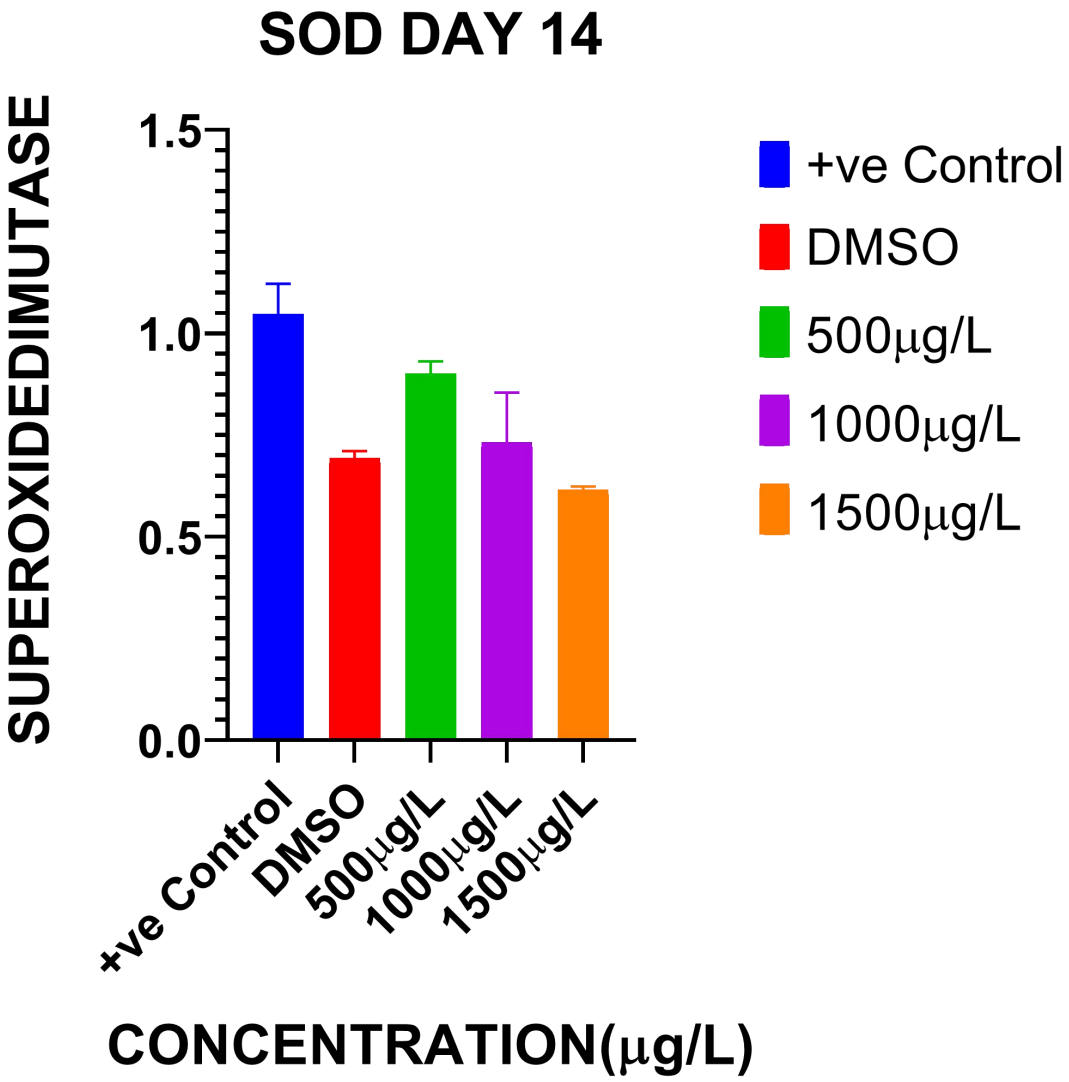


Figure 4.3.2: Mean activity of SOD in *Clarias gariepinus* exposed to 6PPD-Q in 14days

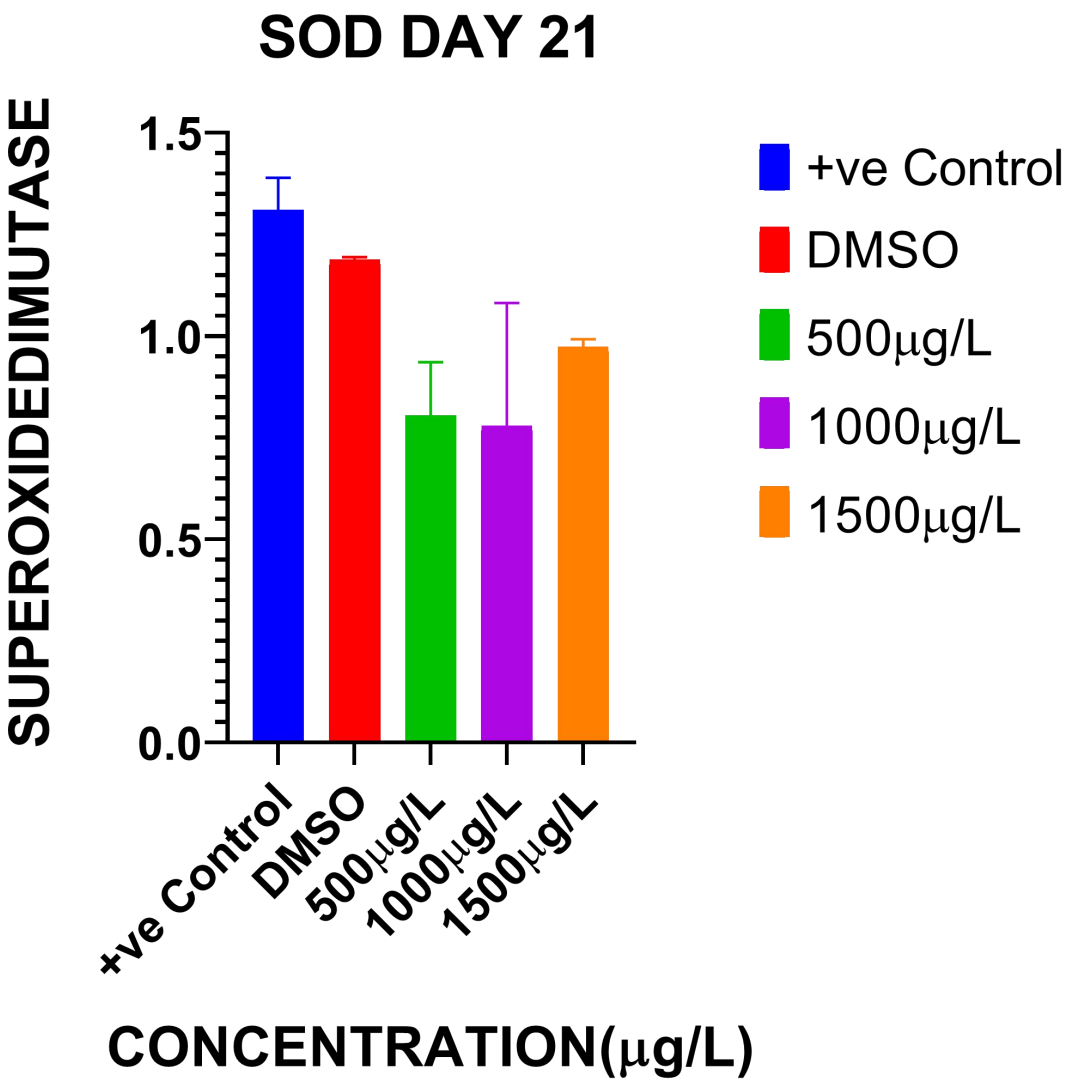


Figure 4.3.3: Mean activity of SOD in *Clarias gariepinus* exposed to 6PPD-Q in 21days

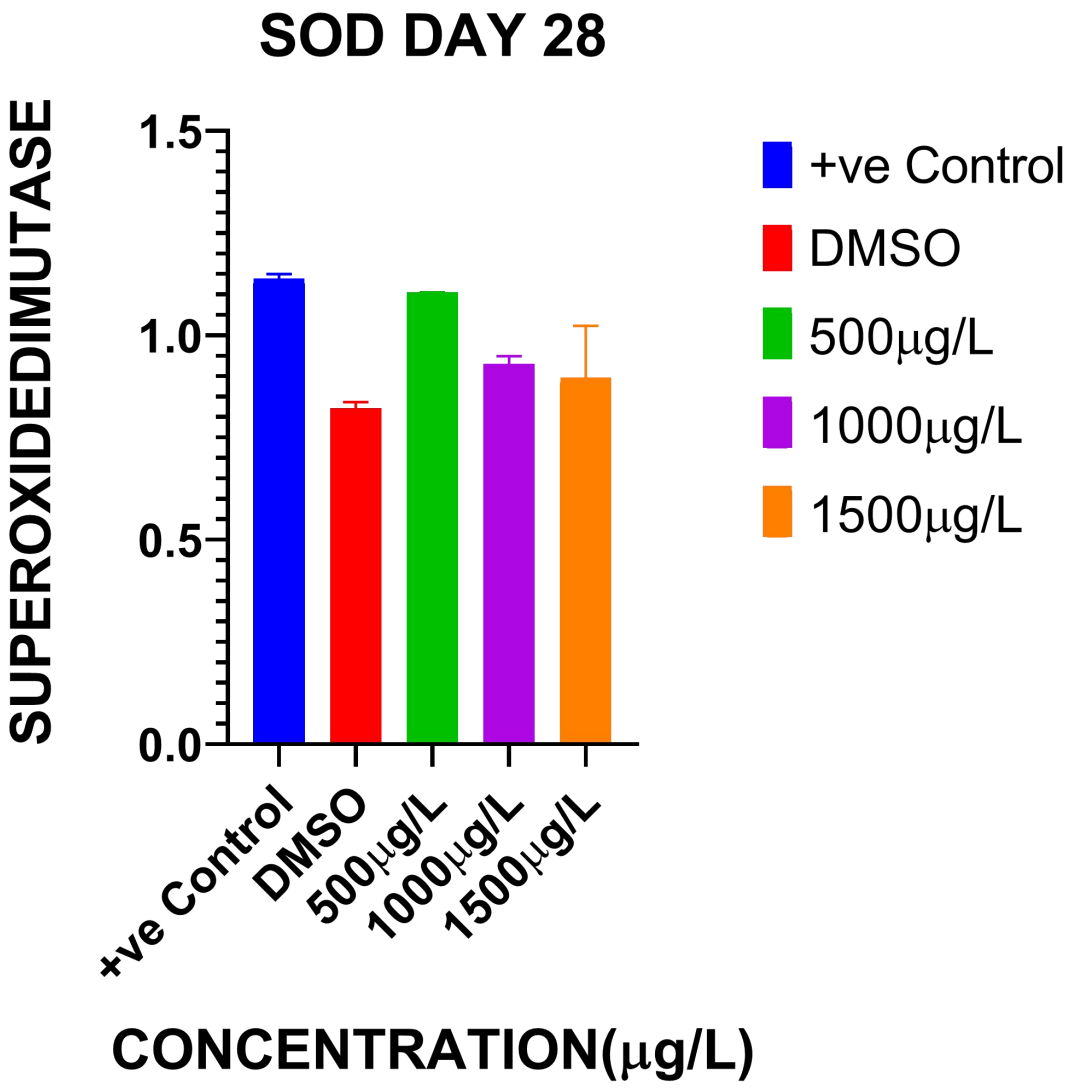


Figure 4.3.4: Mean activity of SOD in *Clarias gariepinus* exposed to 6PPD-Q in 28days

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Discussion

The findings from this study demonstrate that exposure to 6PPD-Q induces significant alterations in the oxidative stress response of *Clarias gariepinus* juveniles, particularly affecting key antioxidant enzymes over a 28-day period. While glutathione (GSH) activity remained largely unchanged during the initial weeks, a significant decrease was observed by day 28 in fish exposed to higher concentrations (1000 and 1500 µg/L) and the DMSO control compared to the positive control. This suggests a delayed oxidative stress effect, possibly due to the cumulative impact of 6PPD-Q exposure.

More pronounced effects were observed in the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD). GPx activity was significantly suppressed across multiple exposure concentrations and time points, indicating a compromised ability to mitigate hydrogen peroxide-induced oxidative damage. Similarly, SOD activity showed variable but significant reductions, particularly at later exposure intervals, reflecting an impaired capacity to neutralize superoxide radicals. These results align with previous studies that have linked 6PPD-Q exposure to oxidative stress in aquatic organisms. For instance, 6PPD-Q has been shown to induce reactive oxygen species (ROS) and cause oxidative damage in species such as zebrafish and *Caenorhabditis elegans* (Ge *et al.*, 2024; Hua *et al.*, 2023b).

The observed enzymatic disruptions are consistent with the known toxic mechanisms of 6PPD-Q, which include oxidative stress, mitochondrial dysfunction, and neurotoxicity (Varshney *et al.*, 2022; Ricarte *et al.*, 2023). Although *Clarias gariepinus* has not been widely studied in the

context of 6PPD-Q toxicity, existing research on other species such as coho salmon and zebrafish has documented similar sublethal effects, including behavioral alterations and metabolic disruption (Tian *et al.*, 2021; Brinkmann *et al.*, 2022). The variability in species sensitivity—with salmonids being highly susceptible and some teleosts like zebrafish showing higher tolerance—highlights the need for taxon-specific risk assessments (Hiki *et al.*, 2021; Lo *et al.*, 2023).

Furthermore, the environmental relevance of the concentrations used in this study (up to 1500 µg/L) must be interpreted with caution. While these levels exceed typical environmental detections (e.g., <2.5 µg/L in urban runoff; Cao *et al.*, 2022), they provide insight into the potential consequences of chronic or episodic exposure in heavily contaminated systems. The presence of 6PPD-Q in diverse environmental matrices—from urban runoff to airborne particles—underscores its persistence and bioavailability (Johannessen *et al.*, 2022; Rauert *et al.*, 2022), reinforcing concerns about its ecological and human health risks.

In conclusion, this study provides evidence of 6PPD-Q-induced oxidative stress in *Clarias gariepinus*, supporting earlier findings in other aquatic species. Future work should focus on chronic low-dose exposures, multi-species ecosystem-level assessments, and the development of regulatory frameworks to mitigate the environmental impact of tire-derived contaminants.

This study investigates the environmental contaminant 6PPD-quinone (6PPD-Q), a transformation product of the tire antioxidant 6PPD, with a focus on its ecotoxicological effects, particularly on the African catfish (*Clarias gariepinus*). The research contextualizes 6PPD-Q as a globally distributed pollutant, prevalent in urban runoff, air, soil, and water bodies due to tire wear, and documents its acute toxicity to sensitive species like coho salmon. A significant

research gap identified is the lack of toxicological data on 6PPD-Q's effects on African aquatic species, such as *C. gariepinus*, which are vital for local ecosystems and food security.

The experimental component of the work exposed *C. gariepinus* juveniles to sublethal concentrations of 6PPD-Q (500, 1000, and 1500 µg/L) over 28 days. The results demonstrated that exposure induced significant oxidative stress, marked by the suppression of key antioxidant enzymes. Glutathione peroxidase ($GP_X$) activity was significantly inhibited across multiple time points, and superoxide dismutase (SOD) activity showed variable but significant reductions, indicating an impaired capacity to manage reactive oxygen species (ROS). Glutathione (GSH) levels also declined significantly by day 28 at higher concentrations, suggesting a cumulative oxidative damage effect.

## 5.2 CONCLUSION

In conclusion, this study confirms that 6PPD-Q is a potent inducer of oxidative stress in *Clarias gariepinus*, corroborating findings in other model species like zebrafish and coho salmon. The observed sublethal biochemical alterations specifically the disruption of the antioxidant defense system highlight a significant toxicological risk even for species considered more tolerant than salmonids. Given the compound's environmental persistence, bioavailability, and widespread occurrence, 6PPD-Q poses a substantial and underappreciated threat to aquatic ecosystems in Nigeria and globally. The findings underscore that the ecological impact of tire-derived chemicals extends beyond acute mortality to include chronic, sublethal health impairments in aquatic organisms.

## 5.3 RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

**Enhanced Environmental Monitoring:** Regulatory agencies in Nigeria and other developing nations should initiate and expand monitoring programs to quantify 6PPD-Q levels in urban runoff, rivers, and lakes, especially those supporting fisheries.

**Species-Specific Risk Assessment:** Further toxicological studies should be conducted on a wider range of locally relevant aquatic species to establish region-specific safety thresholds and understand the full scope of ecological risk.

**Chronic and Multi-Generational Studies:** Research should focus on the long-term, low-dose effects of 6PPD-Q, including impacts on reproduction, growth, and multi-generational health of aquatic organisms.

**Development of Mitigation Strategies:** Investment in and implementation of green infrastructure (e.g., constructed wetlands, filtration systems) is crucial to treat urban stormwater runoff and reduce the load of 6PPD-Q and other tire-derived chemicals entering water bodies.

**Policy and Regulatory Action:** There is an urgent need to develop and enforce environmental regulations and guidelines specifically for 6PPD-Q and related tire wear compounds, informing both water quality standards and sustainable tire manufacturing practices.

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## APPENDICES

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**Appendix A:** List of Acronyms and Abbreviations

| Acronym/Abbreviation | Full Meaning |
|---|---|
| 6PPD | N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine |
| 6PPD-Q / 6PPDQ | 6PPD-quinone |
| DMSO | Dimethyl Sulfoxide |
| GC/MS | Gas Chromatography–Mass Spectrometry |
| GSH | Glutathione |
| GPx | Glutathione Peroxidase |
| OECD | Organisation for Economic Co-operation and Development |
| PAHs | Polycyclic Aromatic Hydrocarbons |
| POPs | Persistent Organic Pollutants |

| Acronym/Abbreviation | Full Meaning |
|---|---|
| ROS | Reactive Oxygen Species |
| SEM | Standard Error of the Mean |
| SOD | Superoxide Dismutase |
| TRWPs | Tyre and Road Wear Particles |
| TWP | Tyre Wear Particles |

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**Appendix B:** Detailed Experimental Data Tables

Table B1: Raw Data for Glutathione (GSH) Activity (µM GSH/g plasma)

*(Representative example; full dataset would be included in the actual appendix)*

| Treatment Group | Replicate | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| +VE Control | 1 | 0.562 | 0.480 | 0.505 | 0.715 |

| Treatment Group | Replicate | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| | 2 | 0.526 | 0.461 | 0.478 | 0.641 |
| DMSO (-VE) | 1 | 0.537 | 0.520 | 0.575 | 0.370 |
| | 2 | 0.535 | 0.463 | 0.542 | 0.452 |
| 500 µg/L | 1 | 0.498 | 0.600 | 0.544 | 0.525 |
| | 2 | 0.497 | 0.496 | 0.543 | 0.512 |
| 1000 µg/L | 1 | 0.610 | 0.485 | 0.525 | 0.520 |
| | 2 | 0.478 | 0.455 | 0.471 | 0.426 |
| 1500 µg/L | 1 | 0.550 | 0.515 | 0.520 | 0.465 |
| | 2 | 0.534 | 0.557 | 0.727 | 0.431 |

Table B2: Raw Data for Glutathione Peroxidase (GPx) Activity

*(Activity in units as defined in Chapter 3)*

| Treatment Group | Replicate | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|

| Treatment Group | Replicate | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| +VE Control | 1 | 1.150 | 1.300 | 7.125 | 6.580 |
| | 2 | 1.538 | 1.554 | 7.077 | 6.234 |
| ... | ... | ... | ... | ... | ... |

Table B3: Raw Data for Superoxide Dismutase (SOD) Activity

*(Activity in units/mg protein as defined in Chapter 3)*

| Treatment Group | Replicate | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| +VE Control | 1 | 0.350 | 1.000 | 1.250 | 1.130 |
| | 2 | 0.401 | 1.094 | 1.368 | 1.146 |
| ... | ... | ... | ... | ... | ... |

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Appendix C: Chemical Safety Data Sheets (SDS) Excerpts

C1: 6PPD-quinone (CAS: N/A) – Key Safety Information

Hazards Identification: Suspected of causing cancer. Very toxic to aquatic life with long-lasting effects.

First Aid Measures: IF INHALED: Remove person to fresh air. IF ON SKIN: Wash with plenty of soap and water. IF IN EYES: Rinse cautiously with water for several minutes. Seek medical attention.

Handling and Storage: Wear protective gloves/protective clothing/eye protection/face protection. Use only in a well-ventilated area. Store locked up. Store in a cool, dry, well-ventilated place.

Stability and Reactivity: Stable under recommended storage conditions. Incompatible with strong oxidizing agents.

C2: Dimethyl Sulfoxide (DMSO) (CAS: 67-68-5) – Key Safety Information

Hazards Identification: Combustible liquid. Causes serious eye irritation.

First Aid Measures: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

Handling and Storage: Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking. Store in a well-ventilated place. Keep container tightly closed