Hindawi
Journal of Nanotechnology
Volume 2019, Article ID 7686597, 11 pages
https://doi.org/10.1155/2019/7686597

Research Article

Antagonistic Effects of Sublethal Concentrations of Certain Mixtures of Metal Oxide Nanoparticles and the Bulk (Al_2O_3, CuO, and SiO_2) on Gill Histology in *Clarias gariepinus*

Amaeze Henry Nnamdi, Tam-Miette Dawarri Briggs, Oluwaseun Olusola Togunde, and Henry Ebele Obanya

Ecotoxicology Unit, Department of Zoology, University of Lagos, Lagos, Nigeria

Correspondence should be addressed to Henry Ebele Obanya; henryobanya@rocketmail.com

Received 28 November 2018; Revised 6 February 2019; Accepted 20 May 2019; Published 12 June 2019

Academic Editor: Paresh Chandra Ray

Background. The effect of nanoparticles (NPs) on aquatic environments is poorly studied. *Aim*. This study evaluates the toxicity of joint effects of these different metal nanoparticles and their bulk in mixtures (Al_2O_3, CuO, and SiO_2) on fish using histological biomarker. *Materials and Methods*. The bulk and nano sizes of three salts (Al_2O_3, CuO, and SiO_2) were used. Nanosizes ranged from 25 nm to 100 nm. The juvenile fishes of *Clarias gariepinus* (mean Length: 12.3 ± 3.5 cm; mean weight: 18.52 ± 6.41 g) were used for the acute and chronic toxicity tests. They were exposed to 7 mg/L each of the bulk and nano sizes of the three metallic oxides either singly or in mixtures for 28 days. The basis for the sublethal concentration was that the 96 hr acute toxicity of the varied sizes of the three metallic oxides was nontoxic up to the concentrations of 100 mg/L with no significant mortality at the highest exposure concentrations. The gills were collected for histopathology. *Results*. Of the three metal oxide nanoparticles, SiO was the most toxic, with histopathological alteration index (HAI) of 20.0, followed by nano-CuO (HAI, 10.0) and nano-Al_2O_3 (HAI, 2.0). In single exposure, the gill alterations include high frequencies of erosion of gill lamella (EGL), hypertrophy (HPT), oedema (OD), and necrosis (N). Less damage was observed at the combination of the metal oxide nanoparticles of SiO + Al_2O_3, SiO + CuO and SiO + Al_2O_3 + CuO in equal (1 : 1—HAI, 2 and 6; 1 : 1 : 1—HAI, 6) and unequal ratios (1 : 2—HAI, 16 and 6; 2 : 1—HAI, 8 and 6). Similarly, all bulk combinations were also antagonistic except for the equal ratio of bulk CuO (HAI, 20) and bulk Al_2O_3 (HAI, 10) that gave additive effect with HAI of 32. *Conclusion*. The joint actions of nano Al_2O_3 and CuO with SiO produced a low toxic effect, unlike the high toxicity of their single trials; this also indicates that nano Al_2O_3 and CuO are antagonists. Similarly, among the bulk metal oxides (SiO, Al_2O_3, and CuO), CuO was the most toxic. Bulk SiO and Al_2O_3 are antagonistic on the effects of CuO on the fish gill. There is need to properly document the ecological implications of nanoparticles in the aquatic environment.

1. Introduction

The rapid development of the nanotechnology industry in the last 20 years is yet to reach its potential [1]. This has been fuelled by the unusual properties that materials possess at the nanoscale; however, it is also that these properties are in part fuelling concerns regarding their potential toxicity and ecotoxicology. The engineering material applications of NPs can increase their concentrations in groundwater, soil, and surface water which presents the most significant exposure avenues, pathway, and fate for assessing environmental risks [2].

Nanoparticles like silicon oxide have several applications in the preparation of nanocomposites to enhance thermal resistance and electrical and mechanical properties. Also, it is used in various fields of catalysts, pigment stabilization, electronics, and sensors. Nano-aluminium oxide is used as raw material in solid rocket propellant formulation and explosives. Nano-copper II oxide is used in biomedical applications such as antimicrobial and

plasmonic materials as a component of reforming catalysts.

Studies associated with the use of NPs are limited [3]. Miniaturization of materials is of great interest due to the difference in their physicochemical properties compared to the bulk materials. These properties include colour, solubility, conductivity, and catalytic activity of engineered nanomaterials [4]. In addition to their increased surface area to volume ratio, nanoparticles can serve as contaminant absorbents.

Nanotoxicology explains the concepts of the toxicological basis of NPs on health and the environment [5]. Several studies have reported the harmful effects of NPs and their bulk salts on the biota, but presence of other contaminants and constituent mixture in environment has not been fully studied yet. The pollution of the aquatic ecosystems by NPs has been of global concern [6]. These pollutants including nanoparticles could increase the level of metals in natural water and seriously affect wetland habitats [7]. It was reported that the 95 hr LC_{10} of various nanoparticles for fish ranged from 100 μgL^{-1} to 1 $mg{\cdot}L^{-1}$, while the 95 hr LC_{50} of NPs reach the mg/L range [8]. Expected concentrations of NPs in surface waters range from μgL^{-1} to low $mg{\cdot}L^{-1}$ [8, 9].

The use of fish histopathological alteration index as a tool for classification and categorization of stages of tissue changes relative to stress or chemical exposure has been reported by several ecotoxicologists and scientists [10–13]. This has been used to evaluate the effects of contaminants on the health of fish in the environment and to help establish a causal relation between exposure to toxic substances and the various biological responses [10]. The use of incidence and prevalence of fish diseases as associated with contaminants as indicators of environmental stress provides a definitive biological endpoint for the history of exposure [10, 13, 14].

The fish gill is very sensitive to environmental changes and is easily affected by pollutants at low concentrations [15]. The gills have a large surface area and perform various vital functions such as respiration, osmoregulation, and excretion. Due to the fact that they are external structure in direct contact with the external environment, they are sensitive to chemical and physical changes of the aquatic environment [16]. The gills are the principal sites for gas exchange and other important functions such as ionic and osmotic regulation in addition to acid-base balance; histopathological changes in the structure of these organs involve respiratory disturbances and electrolyte imbalance [16].

Mansouri et al. [15] have reported the most common histopathological anomalies in the gill of common carp such as hyperplasia, oedema, curvature, fusion, aneurism, and necrosis after 10 and 20 days exposure to TiO_2 NPs (10.0 $mg{\cdot}L^{-1}$) and CuO NPs (2.5 and 5.0 $mg{\cdot}L^{-1}$) singly and in mixtures. The mixture effect was reported as synergetic. This study was similar to that of [17] that exposed *Carassius auratus* (goldfish) to mixtures of suspensions of 20 nm size Al_2O_3 and 50 nm size ZnO. Significant morphological alteration such as hyperplasia (with fusion of lamellae) was reported in the gills. Combined interactions within the mixtures could be antagonistic, synergetic, or additive depending on the specific properties and type of oxide NPs such as size and surface area [17]. However, there are limited studies on the joint effects of nanoparticles and their bulk metallic salts on the fish using histological biomarker.

The aim of this study is to evaluate the toxicity of joint effect of different heavy metals in mixtures (Al_2O_3, CuO, and SiO_2) on fish using histological biomarker.

2. Materials and Methods

2.1. Experimental Chemicals and Preparation of Test Solutions. The bulk and nano sizes of three salts (Al_2O_3, CuO, and SiO_2) were procured from Sigma Aldrich. They were stored at room temperatures in the laboratory before use. Nano-Al_2O_3 appeared as grey coloured powder, density of 2.70 g/cm^3, and size of 40 nm with 99.9% purity. Nano-CuO has red to yellow powder, density of 8.94 g/ml at 25°C with size <50 nm. SiO_2 is a brown yellow powder, density of 2.33 g/ml at 25°C with size <100 nm.

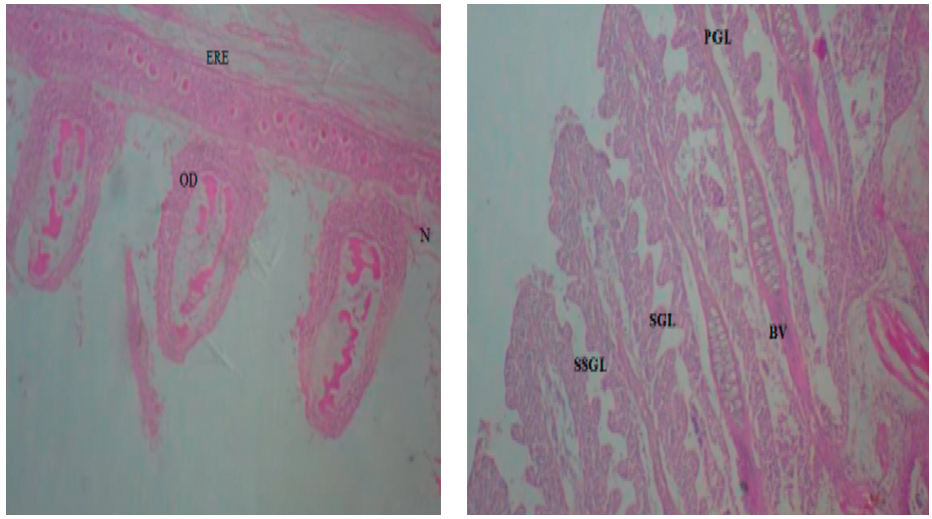
Stock suspensions of the uncoated powder were made using dechlorinated tap water to obtain various concentrations of the test solution. Respective grams of the metallic oxides were dissolved in 1 L of the dechlorinated tap water to obtain appropriate mixtures to obtain stock solutions (Figure 1). Literature search was used to obtain the test concentrations. The concentrations of the test compounds were prepared as single solutions and mixtures of 1 : 1, 1 : 2, 2 : 1, and 1 : 1 : 1 both for bulk and nanoscale oxides.

2.2. Experimental Organisms: Collection and Acclimatization. The African sharp tooth Catfish/African Catfish, *Clarias gariepinus*, was used for this study. It was selected on the basis of ease of culture and known responses to pollutants from existing literatures. The fishes were procured from a fish farm in Akoka, Lagos, and transported in open drums to the Department of Zoology, Laboratory Annex at the Biological Garden. The fishes were transported into large holding tanks (100 L capacity) in the laboratory which were half filled with dechlorinated tap water left open for 24 hours. The juvenile fishes (mean length: 12.3 ± 3.5 cm; mean weight: 18.52 ± 6.41 g) were employed for the sublethal/chronic toxicity tests. Only catfishes of the same batches were used for the experiment.

The fishes were acclimatized in the lab using the 100 L capacity holding tanks at a stocking density of 20 fishes per litre for the fingerlings and 5 fishes per litter for the juveniles. The acclimatization lasted for 7 days after which the bioassay commenced. The acclimatization was conducted under standard laboratory conditions (temp, 26.0 ± 3.0°C; humidity, 75 ± 6%; photoperiod, light: dark, 12 : 12 hours). The bioassay media (dechlorinated tap water) was also of suitable quality (pH: 6.8; D.O, 8.5 mg/L; Salinity, 0.0 ppt).

2.3. Laboratory Bioassay

2.3.1. Acute Toxicity Assay. The acute toxicity test/range finding exercise was conducted over 96 hours with varying concentrations in order to achieve mortality. The bioassay



(a) (b)

Figure 1: (a) Histological section of gill of fish for control experiment showing primary gill lamella (PGL), blunt secondary gill lamella (SSGL), and blood vessel (BV) (H&E stains, ×400). (b) Histological section of gill of fish exposed to nanosize SiO showing total destruction of primary gill lamella (PGL) and secondary gill lamellae (SGL), eroded epithelium (ERE), and area of severe necrosis (N) and oedema (OD) (H&E stains, ×100).

containers were made of plastics (6 m × 4 cm × 4 cm). During the acute toxicity test, the fingerlings were stocked at 10 fishes per litre of water, made up by equivalent concentrations of the stock solution of the bulk and nanoscale heavy metals acting singly. Each setup was in duplicate, i.e., 20 fingerlings per concentrations.

The fishes were exposed to concentrations of 1 mg/L, 10 mg/L, 100 mg/L, 1000 mg/L, and 3000 mg/L as required based on the response of the test organism. The 96-hour acute toxicity of the nano and the bulk metals was found to be nontoxic up to the concentration of 100 mg/L, and no significant mortality was found at the highest exposure concentration.

2.3.2. Chronic Toxicity Assay. The juvenile catfishes were employed in the chronic toxicity tests in which they were exposed to 7 mg/L each of the bulk and nano sizes of the three metallic oxides either singly or in mixtures for 28 days. The chronic toxicity assays were conducted in larger plastics (11 cm × 9 cm × 7.5 cm) using 5 L of water because of the larger sizes of the fishes compared to the acute toxicity test. At the end of the exposure period, the fishes were immobilized by spinal puncture and dissected to collect the gills and preserved for histopathological investigation.

2.3.3. Histopathological Examination. The gills were fixed in 10% formalin, dehydrated in graded ethanol [18], cleared in xylene, embedded in paraffin wax, and sectioned at 5 μm on a rotary microtome. Slides were stained using the haematoxylin and eosin technique for light microscopy [18]. The histopathological changes were evaluated according to [18] and [10], which includes the calculation of the histopathological alteration index (HAI) for each fish using the following formula:

$$\text{HAI} = 1\sum \text{I} + \sum \text{II} + \sum \text{III}. \quad (1)$$

Because I, II, and III correspond to the number of stages of change, the mean HAI was scored on six-point scale: 0 = normal tissue; 2.0 = mild damage to the tissue; 4.0 = moderate damage to the tissue; 6.0 = partially severe damage to the tissue; 8.0 = severe damage to the tissue; 10 = irreparable damage to the tissue. These stages of change and scoring system or scale are given in Table 1.

3. Results and Discussion

3.1. Histopathological Effects and Joint Action of Single and Combined Exposures of Clarias gariepinus on 28-Day Sublethal Concentrations of Nanometallic Salts. The interaction of NPs with other contaminants is dependent on the properties of the NPs such as size, composition, morphology, porosity, aggregation/disaggregation, and aggregate structure. The devastating effects of NPs and bulk are mainly due to the dispersion, persistence, and bioaccumulation and biomagnification potentials in addition to their toxicity in the biological tissues [19].

The fish gill is the primary target organ affected by NPs. Jayaseelan et al. and Griffitt et al. [20, 21] had reported physiological alterations such as dysfunction in osmoregulation, respiratory gas exchange, and body fluid permeability balance. Due to the large superficial area of the epithelium per volume ratio, the organ is more susceptible to effects of contaminants [22]. Histological and biochemical analysis in previous studies revealed the gills to be the primary target organ [10, 11, 21]. The physiological alteration could become visible as histological alteration as revealed by Griffitt et al. [21], who reported that NPs produced hypertrophy of epithelial cells in the gills [21].

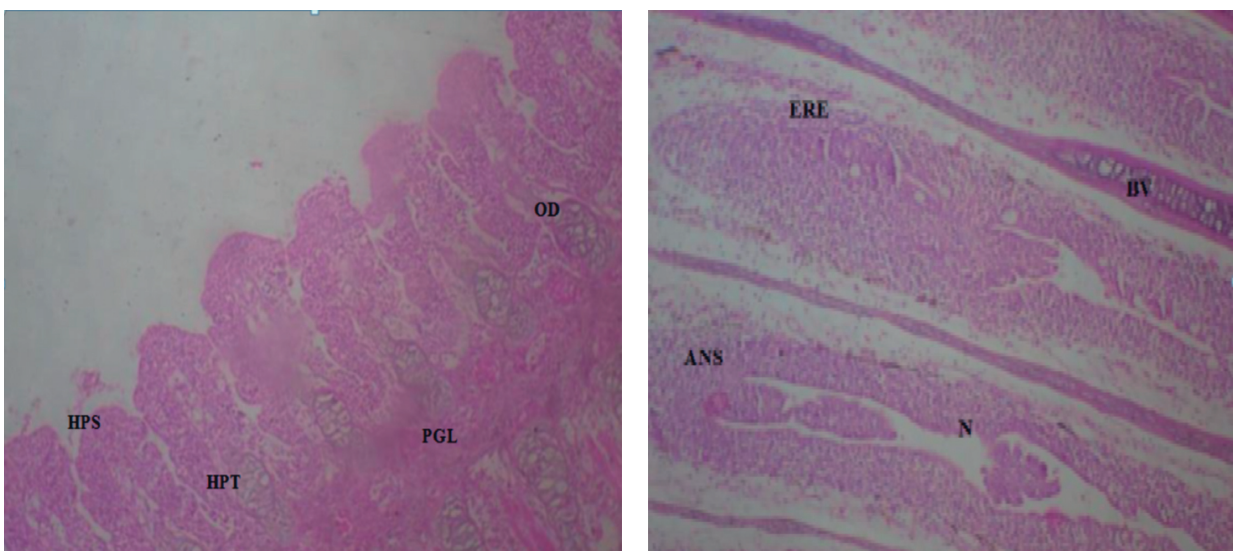
Table 1: Stages of change and histological alterations of the gill.

Alteration score	Score description	Histological alteration
0	Normal tissue	No lesion or any alteration (NT)
2.0	Mild damage	Mild thickening of gill lamella (GL1) Epithelial hyperplasia (EH) Hypertrophy (HPT)
4.0	Moderate damage	Moderate thickening of gill lamella (GL2) Oedema (OD)
6.0	Partially severe damage	Eroded outer operculum (ERO) Epithelial lifting (EPL) Partial fusion of secondary lamella (FSGL1) Erosion of gill lamella (EGL) Shortening of secondary lamella (SSGL) Stunted gill lamella (SGL) Blunt secondary lamella (BSGL) Uncontrolled proliferation of epithelial cells (PEC)
8.0	Severe damage	Severe thickening of gill lamella (GL3) Complete fusion of secondary lamella (FSGL1) Aneurysm (ANS)
10.0	Irreparable damage	Necrosis (N) Total damage to the lamella (TDL)

Table 2: A 28-day single and joint exposure of *Clarias gariepinus* to nanosize metals.

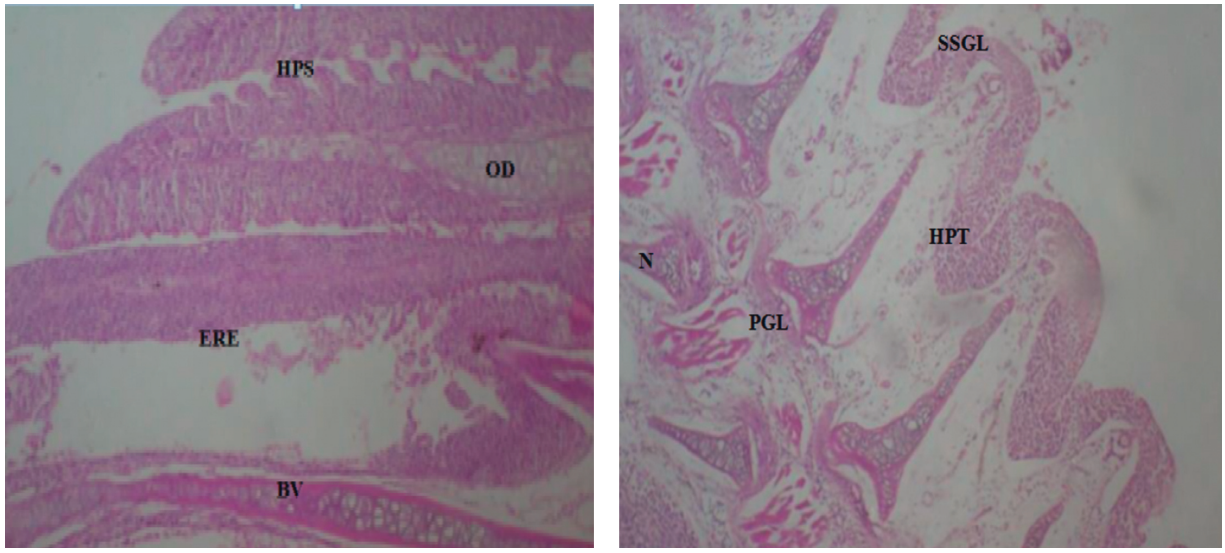
Conc. ratio	Nanometals	Assumed HAI	Toxic level	Actual HAI	Toxic level	Type of joint effect	Gill pathology
Singly	SiO	20.0	High	NA	NA	NA	EGL, hypertrophy
	Al_2O_3	2.0	Low	NA	NA	NA	Oedema
	CuO	10.0	Moderate	NA	NA	NA	Necrosis
1 : 1	$SiO + Al_2O_3$	22.0	High	2.0	Low	Antagonistic	Hypertrophy, SSGL
	SiO + CuO	30.0	High	6.0	Low	Antagonistic	
2 : 1	$SiO + Al_2O_3$	22.0	High	8.0	Low	Antagonistic	EH, SSGL
	SiO + CuO	30.0	High	6.0	Low	Antagonistic	
1 : 2	$SiO + Al_2O_3$	22.0	High	16.0	Moderate	Antagonistic	PEC, EPL, necrosis
	SiO + CuO	30.0	High	6.0	Low	Antagonistic	
1 : 1 : 1	$SiO + Al_2O_3 + CuO$	30 or 22	High	6.0	Low	Antagonistic	EGL, SSGL

Toxic level: HAI: $X < 10$, low; $10 \geq X < 20$, moderate; $X \geq 20$, high.



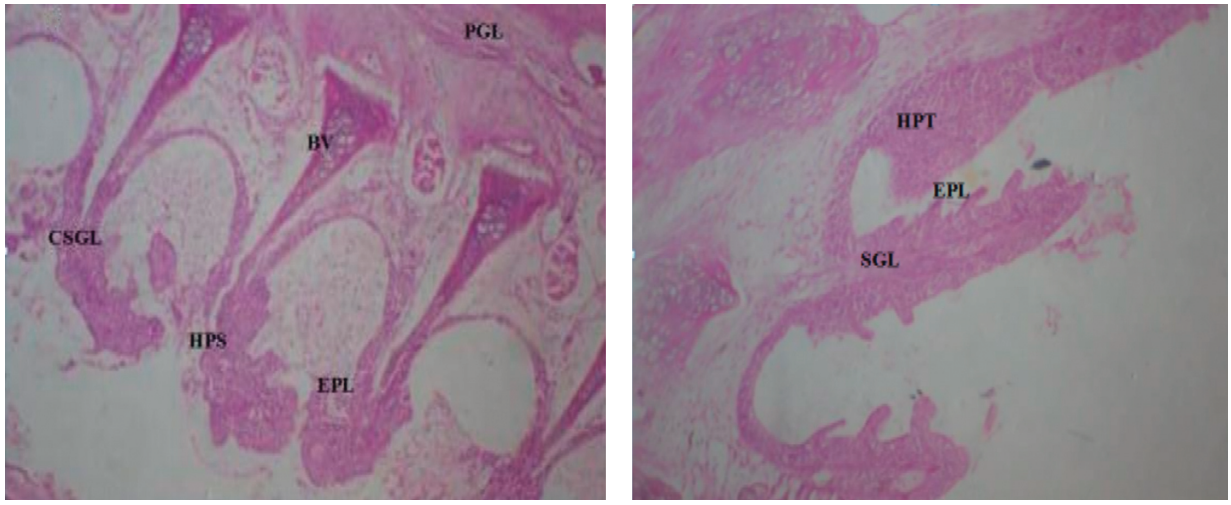
(a) (b)

Figure 2: (a) Histological section of gill exposed to bulk SiO. Deformities such as oedema of secondary gill lamella (OD), hypertrophy (HPT), and epithelial hyperplasia are observed (HPS). Primary gill lamella is also observed (H&E stains, ×400). (b) Histological section of gill exposed to bulk CuO. Total destruction of both primary and secondary lamella is seen. Areas of eroded epithelium (ERE), aneurysm (ANS), necrosis (N), and blood vessel (BV) are also observed (H&E stains, ×100).



(a) (b)

Figure 3: (a) Histological section of gill exposed to nano-CuO showing oedema of primary gill lamella (OD), epithelial hyperplasia (HPS), eroded epithelium (ERE), and the blood vessel (BV) (H&E stains, ×100). (b) Histological section of gill exposed to bulk Al_2O_3 showing primary gill lamella (PGL), shortened secondary gill lamella (SSGL), hypertrophy (HPT), and some areas of mild necrosis (N) (H&E stains, ×400).



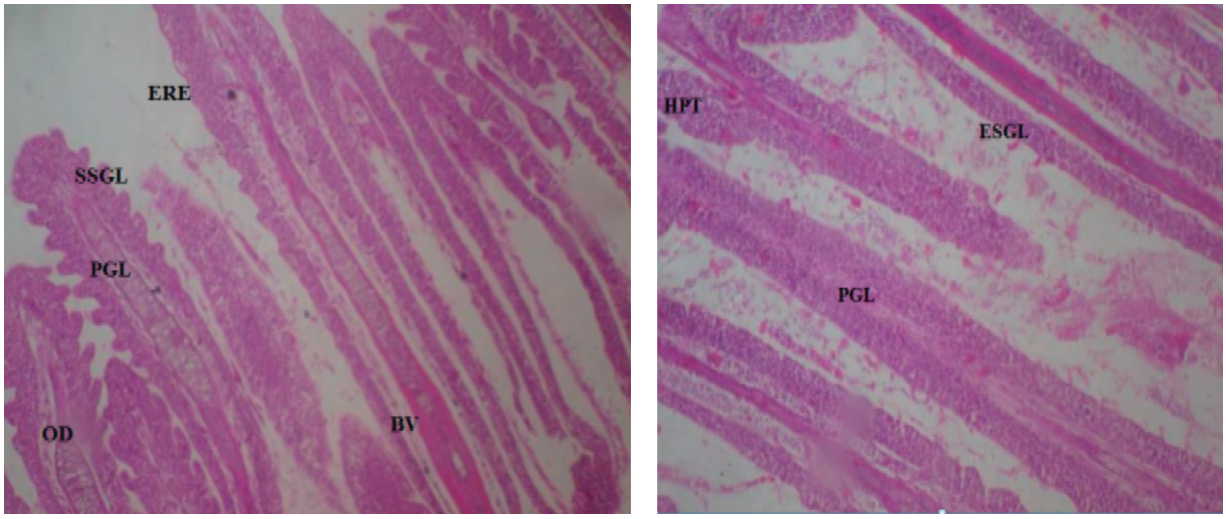
(a) (b)

Figure 4: (a) Histological section of gill exposed to nano-Al_2O_3 showing primary gill lamella (PGL), curved secondary gill lamella (CSGL), epithelial hyperplasia (HPS), epithelial lifting (EPL), and blood vessel (BV) (H&E stains, ×400). (b) Histological section of gill exposed to bulk SiO/CuO (1 : 1) showing secondary gill lamella (SGL), hypertrophy (HPT), and epithelial lifting (EPL) (H&E stains, ×400).

In this study, the most histopathological lesions in fish gills such as hyperplasia, oedema, curvature, shortening and fusion of gill lamellae, aneurism, and necrosis are described and quantified using an index as used in several studies [10–13]. Tables 2 and 4–7 and Figures 1–12 show the histopathological effects and joint action of single and combined exposures of *Clarias gariepinus* to 28-day sublethal concentrations of nanometallic salts. Single exposure to nano-SiO, nano-Al_2O_3 and nano-CuO produced varying effects after 28 days. Nano-SiO was the most toxic, with histopathological alteration index (HAI) of 20.0, followed by nano-CuO (HAI, 10.0) and nano-Al_2O_3 (HAI, 2.0). The gill alterations include high frequencies of erosion of gill lamella (EGL), hypertrophy (HPT), oedema (OD), and necrosis (N).

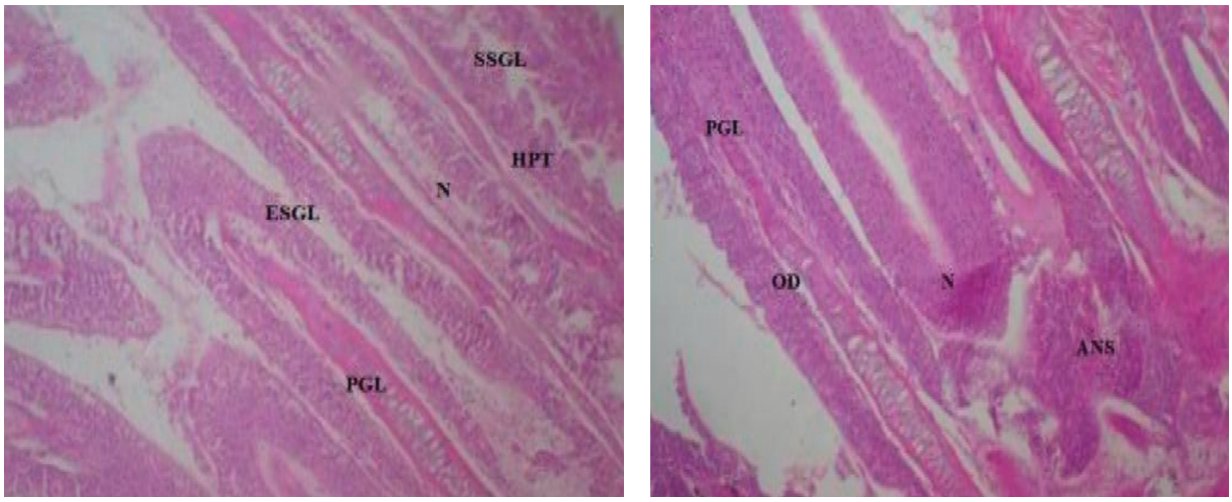
The histopathological alteration index (HAI) showed that the gills are affected by nanoparticles of these metal oxides, and similar damages have been reported in other fish species exposed to Cu NPs [22, 23], TiO_2 NPs [24], and other NPs such as cobalt (III) oxide (Co_2O_3) nanoparticle [24] and colloidal silver nanoparticle [25].

If the effect of the joint exposures of nano-SiO with another nanometallic salt of equal ratio (1 : 1) were to be the addition of individual effect, then the HAI of SiO + Al_2O_3



(a) (b)

Figure 5: (a) Histological section of gill exposed to nano-SiO/CuO (1 : 1) showing primary gill lamella (PGL) shortened secondary gill lamella (SSGL), blood vessel, oedema of primary lamella, and eroded epithelium (H&E stains, ×100). (b) Histological section of gill exposed to nano-SiO/Al_2O_3 (1 : 1) showing primary gill lamella (PGL), eroded secondary gill lamella (ESGL), and hypertrophy (HPT) (H&E stains, ×100).



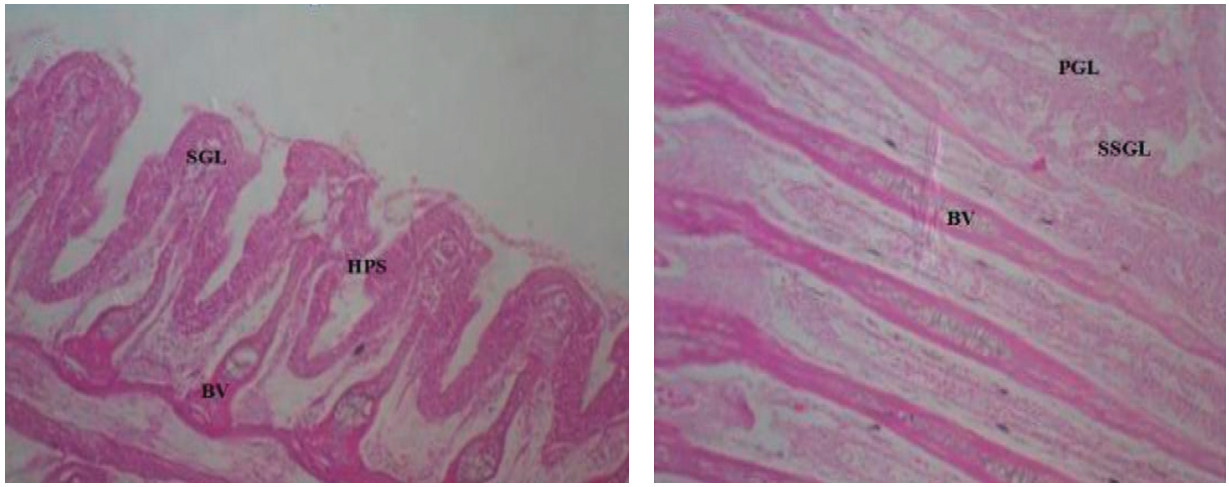
(a) (b)

Figure 6: (a) Histological section of gill exposed to bulk SiO/Al_2O_3 (1 : 1) showing primary gill lamella (PGL), eroded secondary gill lamella (ESGL), and hypertrophy (HPT). Shortened secondary gill lamella (SSGL) and area of necrosis (N) are also observed (H&E stains, ×100). (b) Histological section of gill exposed to bulk Al_2O_3/CuO (1 : 1) showing primary gill lamella (PGL), total destruction of secondary gill lamella, oedema (OD), aneurysm (ANS), and necrosis (N) (H&E stains, ×100).

and SiO + CuO should be 22.0 and 30.0, respectively. The actual combined effects of SiO + Al_2O_3 and SiO + CuO were HAI: 2.0 and 6.0, respectively. The gill alterations include low frequencies of hypertrophy (HPT) and shortening of secondary gill lamella (SSGL). This implies that joint actions of Al_2O_3 and CuO with SiO produced a low toxic effect, unlike the high toxicity of their single trials; this also indicates that Al_2O_3 and CuO are antagonists.

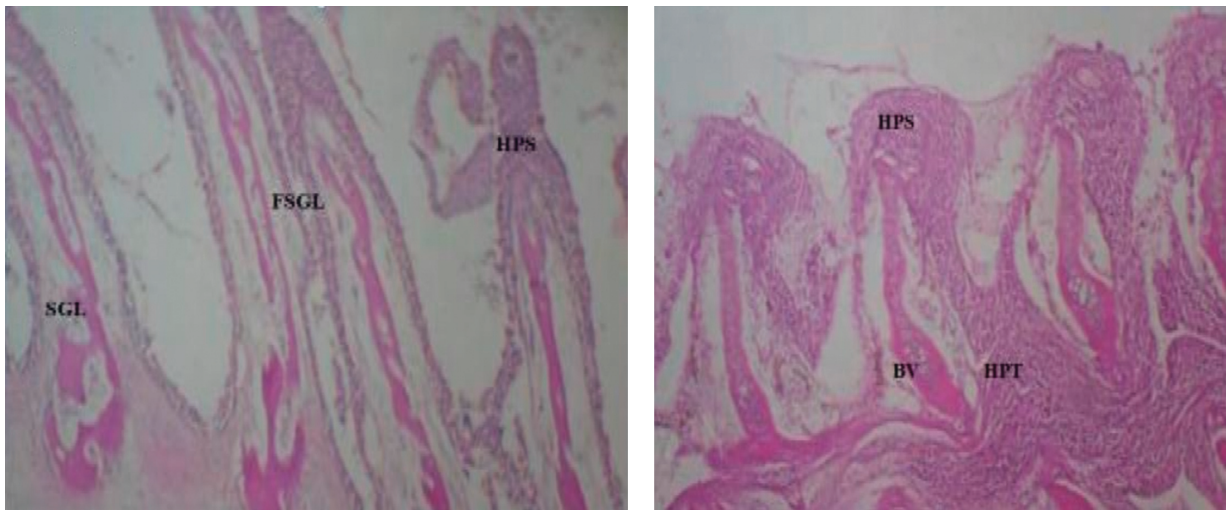
Increasing the concentration of SiO in the mixtures (SiO + Al_2O_3 and SiO + CuO) in ratio 2 to 1 makes no difference in the toxic level. The actual combined effects of SiO + Al_2O_3 and SiO + CuO were HAI: 8.0 and 6.0, respectively. The gill alterations include low frequencies of gill epithelial hyperplasia (EH) and shortening of secondary gill lamella (SSGL).

Increasing the concentrations of Al_2O_3 and CuO in the mixtures (SiO + Al_2O_3 and SiO + CuO) in ratio 1 to 2 still had no change in the toxic level for CuO with HAI of 6.0, but there was moderate toxicity for Al_2O_3 with HAI of 16.0. The gill alterations include moderate frequencies of uncontrolled proliferation of epithelial cells (PEC), gill epithelial lifting (EPL), and necrosis (N).



(a) (b)

Figure 7: (a) Histological section of gill exposed to nano-SiO/Al_2O_3 (2 : 1) showing secondary gill lamella (SGL), blood vessel, and epithelial hyperplasia (HPS) (H&E stains, ×400). (b) Histological section of gill exposed to bulk SiO/Al_2O_3 (2 : 1) showing primary gill lamella, shortened secondary gill lamella (SSGL), and blood vessel (BV) (H&E stains, ×400).



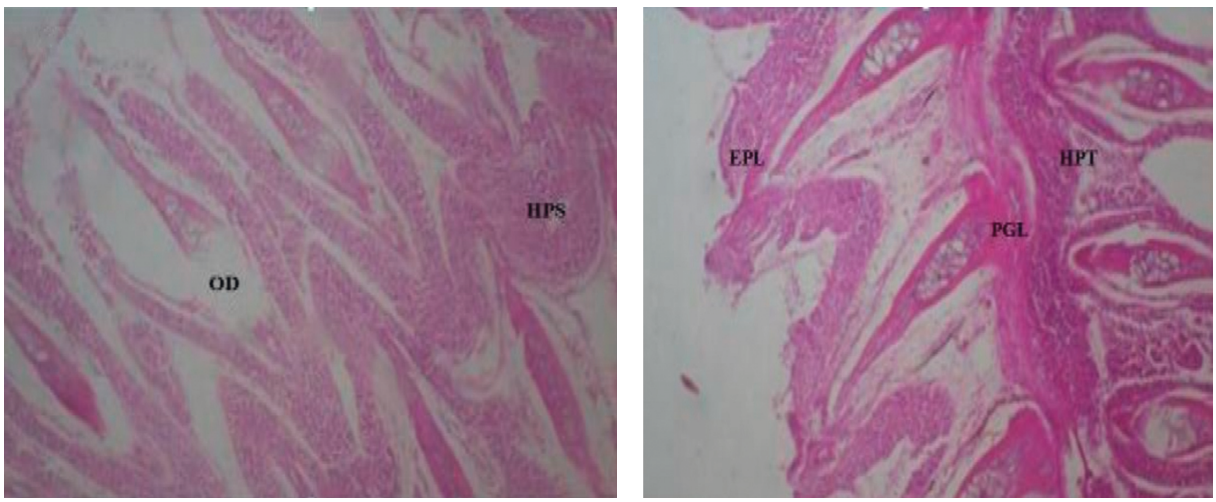
(a) (b)

Figure 8: (a) Histological section of gill exposed to nano-SiO/CuO (2 : 1) showing fused secondary gill lamella (FSGL), secondary gill lamella (SGL), and epithelial hyperplasia (HPS) (H&E stains, ×400). (b) Histological section of gill exposed to bulk SiO/CuO (2 : 1) showing hypertrophy (HPT), blood vessel (BV), and epithelial hyperplasia (HPS) (H&E stains, ×400).

Mixtures of the three nanometallic salts (SiO, Al_2O_3, and CuO) in ratio 1 : 1 : 1 produced low toxic effect (HAI, 6.0) compared to the addition of the individual effect, assumed to be HAI: 30 or 22. The gills have low frequencies of epithelial lifting (EPL) and shortening of secondary gill lamella (SSGL).

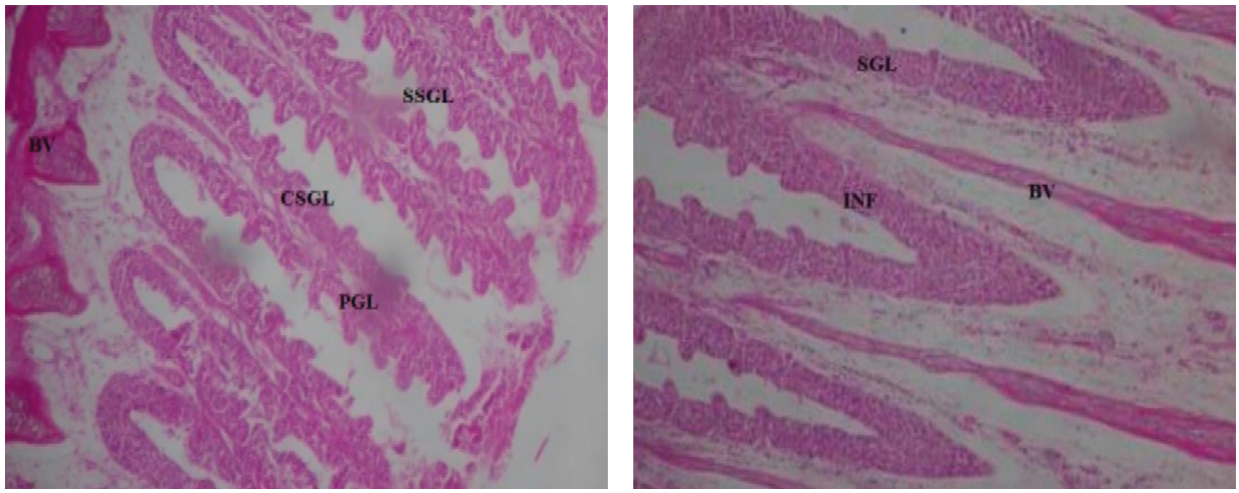
3.2. Histopathological Effects and Joint Action of Single and Combined Exposures of Clarias gariepinus on 28-Day Sublethal Concentrations of Bulk Metallic Salts. Tables 3 and 4–7 and Figures 1–12 show the histopathological effects and joint action of single and combined exposures of *Clarias gariepinus* to 28-day sublethal concentrations of bulk metallic salts. Single exposure to bulk SiO, bulk Al_2O_3, and bulk CuO produced varying effects after 28 days. Bulk CuO was the most toxic, with histopathological alteration index (HAI) of 20.0, followed by bulk Al_2O_3 (HAI, 10.0) and bulk SiO (HAI, 6.0). The gill alterations include high frequencies of erosion of gill lamella (EGL), hypertrophy (HPT), oedema (OD), stunted gill lamella (SGL), aneurysm (ANS), and necrosis (N).

Major alterations in fish under acute and chronic exposure to NPs and their bulk are changes in the morphology of the lamellar epithelium. The epithelium layer of secondary lamella becomes oedema with reduced surface area, resulting in asphyxiation. This change might also indicate acute



(a) (b)

Figure 9: (a) Histological section of gill exposed to bulk Al_2O_3/CuO (2 : 1) showing oedema (OD) and epithelial hyperplasia (HPS) (H&E stains, ×400). (b) Histological section of gill exposed to bulk Al_2O_3/CuO (1 : 2) showing primary gill lamella (PGL), epithelial lifting (EPL), and hypertrophy (HPT) (H&E stains, ×400).



(a) (b)

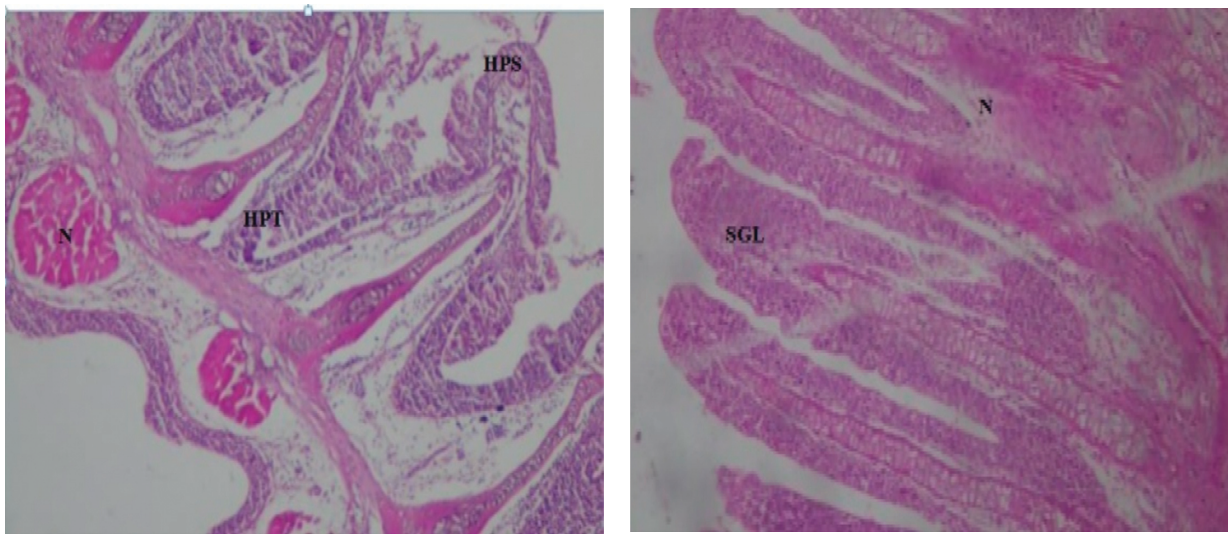
Figure 10: (a) Histological section of gill exposed to nano-Al_2O_3/CuO (1 : 2) showing primary gill lamella (PGL), curved secondary gill lamella (CSGL), and shortened secondary gill lamella (SSGL) (H&E stains, ×400). (b) Histological section of gill exposed to nano-SiO/CuO (1 : 2) showing secondary gill lamella (SGL), inflammation of lamella (INF), and blood vessel (H&E stains, ×400).

inflammation due to the failure of the epithelial sodium pump [26]. The epithelial oedema and fusion affect toxicokinetics and distribution of pollutants in the gill epithelium [27].

Furthermore, changes in the structure of the lamellar epithelium cause changes in the volume of gas and ion exchange [28, 29]. Similar studies by Ostaszewska et al. and Johari et al. [30–32] reported these histological changes such as swelling of goblet cell, villus deformation, hyperplasia, inflammation, necrosis, and vacuoles in the intestinal tissues.

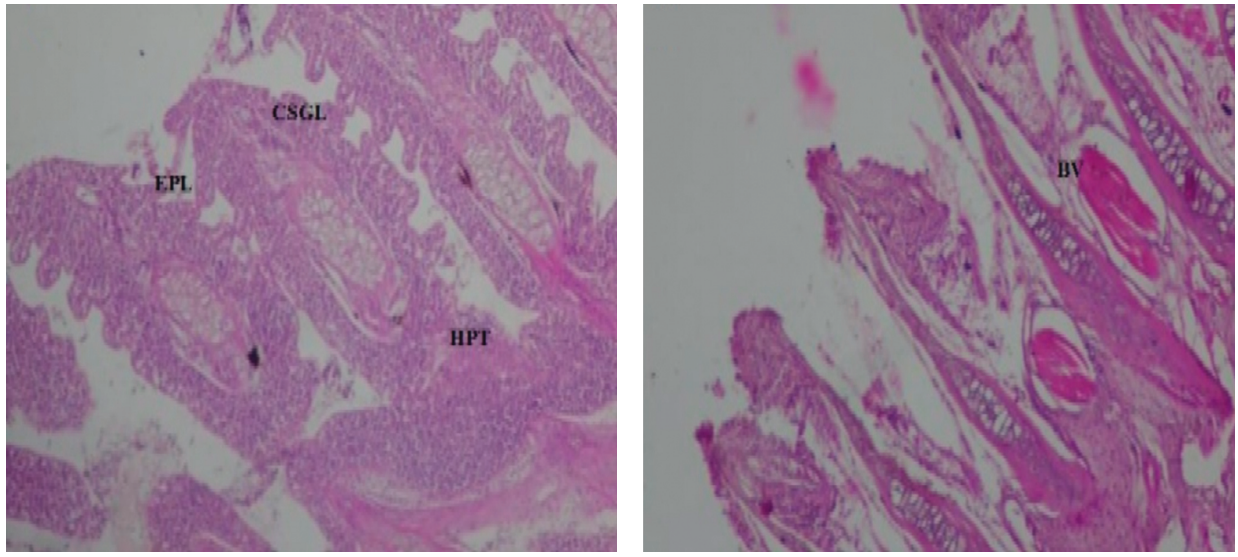
If the effect of the joint exposures of bulk CuO with another bulk metallic salt of equal ratio (1 : 1) were to be the addition of individual effect, then the HAI of CuO + SiO and CuO + Al_2O_3 should be 26.0 and 30.0, respectively. The actual combined effects of CuO + SiO and CuO + Al_2O_3 were HAI: 14.0 and 32.0, respectively. The gill alterations for CuO + SiO include moderate frequencies of hypertrophy (HPT), erosion of gill lamella (EGL), and gill epithelial lifting (EPL). The gill alterations for CuO + Al_2O_3 include moderate frequencies of oedema (OD), aneurysm (ANS), total damage to gill lamella (TDL), and necrosis (N). This implies that joint actions of SiO and Al_2O_3 with CuO produced a low toxic effect, unlike the high toxicity of their single trials; this also indicates that bulk SiO and bulk Al_2O_3 are antagonists.

Increasing the concentration of CuO in the mixtures (CuO + SiO and CuO + Al_2O_3) in ratio 2 to 1 makes no



(a) (b)

Figure 11: (a) Histological section of gill exposed to bulk SiO/CuO (1 : 2) showing epithelial hyperplasia (HPS), necrosis (N), and hypertrophy (HPT) (H&E stains, ×400). (b) Histological section of gill exposed to bulk SiO/Al_2O_3 (1 : 2) showing mild necrosis (N) and secondary gill lamella (SGL). No serious damage is observed (H&E stains, ×400).



(a) (b)

Figure 12: (a) Histological section of gill exposed to nano-SiO/Al_2O_3 (1 : 2) showing curved secondary gill lamella (CSGL), epithelial lifting (EPL), and hypertrophy (HPT) (H&E stains, ×400). (b) Histological section of gill exposed to nano-SiO/Al_2O_3/CuO showing total destruction of gill architecture. Blood vessel is seen (H&E stains, ×400).

difference in the toxic level. The actual combined effects of $CuO + SiO$ and $CuO + Al_2O_3$ were HAI: 6.0 and 6.0, respectively. The gill alterations include low frequencies of gill epithelial hyperplasia (EH), hypertrophy, and necrosis (N).

Increasing the concentrations of SiO and Al_2O_3 in the mixtures ($CuO + SiO$ and $CuO + Al_2O_3$) in ratio 1 to 2 makes no difference in the toxic level for Al_2O_3. The actual combined effects of $CuO + Al_2O_3$ were HAI: 8.0. There was moderate toxicity with SiO with HAI of 14.0. The gill alterations include moderate frequencies of epithelial hyperplasia (EH) and oedema (OD).

Mixtures of the three bulk metallic salts (SiO, Al_2O_3, and CuO) in ratio 1 : 1 : 1 produced low toxic effect (HAI, 12.0) compared to the addition of the individual effect, assumed to be HAI: 26 or 30. The gills have low frequencies of epithelial lifting (EPL) and shortening of secondary gill lamella (SSGL).

Aneurysm is one common alteration in this study. This is the swelling of the blood vessel in the gill tissues, which could

Table 3: A 28-day single and joint exposure of *Clarias gariepinus* to bulk size metals.

Conc. ratio	Bulk metals	Assumed HAI	Toxic level	Actual HAI	Toxic level	Type of joint effect	Pathology
Singly	CuO	20.0	High	NA	NA	NA	Hypertrophy, EGL, OD, SGL, ANS, necrosis
	SiO	6.0	Low	NA	NA	NA	
	Al_2O_3	10.0	Moderate	NA	NA	NA	
1 : 1	CuO + SiO	26.0	High	14.0	Moderate	Antagonistic	Hypertrophy, EGL, OD, EPL, ANS, N, TDL
	CuO + Al_2O_3	30.0	High	32.0	High	Additive	
2 : 1	CuO + SiO	26.0	High	6.0	Low	Antagonistic	EH, necrosis
	CuO + Al_2O_3	30.0	High	6.0	Low	Antagonistic	Hypertrophy
1 : 2	CuO + SiO	26.0	High	14.0	Moderate	Antagonistic	EH, oedema
	CuO + Al_2O_3	30.0	High	8.0	Low	Antagonistic	
1 : 1 : 1	CuO + SiO + Al_2O_3	30 or 26	High	12.0	Low	Antagonistic	EGL, SSGL

Toxic level: HAI: $X < 10$, low; $10 \geq X < 20$, moderate; $X \geq 20$, high.

disturb blood flow [32, 33]. The histological responses in the gills of fish are mostly caused by circulatory disturbances, as aneurism, regressive and progressive changes, and hyperplasia [34, 35].

4. Conclusion

The joint actions of Al_2O_3 and CuO with SiO produced a low toxic effect, unlike the high toxicity of their single trials; this also indicates that Al_2O_3 and CuO are antagonists. Similarly, among the bulk metal oxides (SiO, Al_2O_3, and CuO), CuO was the most toxic. The joint actions of SiO and Al_2O_3 with CuO produced a low toxic effect, unlike the high toxicity of their single exposures. Bulk SiO and bulk Al_2O_3 are antagonistic on the effects of CuO on the fish gill. There is a need to properly document the ecological implications of nanoparticles in the aquatic environment.

Data Availability

All data have been provided in the manuscript. For any further data that may be needed, contact the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Tables 4–7 list data used in calculating and estimating the results summarised in Tables 2 and 3. (*Supplementary Materials*)

References

[1] K. Savolainen, H. Alenius, H. Norppa, L. Pylkkänen, T. Tuomi, and G. Kasper, "Risk assessment of engineered nanomaterials and nanotechnologies—a review," *Toxicology*, vol. 269, no. 2-3, pp. 92–104, 2010.

[2] M. Farré, K. Gajda-Schrantz, L. Kantiani, and D. Barceló, "Ecotoxicity and analysis of nanomaterials in the aquatic environment," *Analytical and Bioanalytical Chemistry*, vol. 393, no. 1, pp. 81–95, 2009.

[3] P. Christian, F. Von der Kammer, M. Baalousha, and T. Hofmann, "Nanoparticles: structure, properties, preparation and behaviour in environmental media," *Ecotoxicology*, vol. 17, no. 5, pp. 326–343, 2008.

[4] S. W. Wijnhoven, S. Dekkers, M. Kooi, W. P. Jongeneel, and W. H. Jong, *Nanomaterials in Consumer Products: Update of Products on the European Market in 2010*, National Institute for Public Health and the Environment, Netherlands, 2010.

[5] R. D. Handy, N. van den Brink, M. Chappell et al., "Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: what have we learnt so far?," *Ecotoxicology*, vol. 21, no. 4, pp. 933–972, 2012.

[6] D. G. McNeil and J. Fredberg, "Environmental water requirements of native fishes in the middle river catchment, kangaroo island, south Australia—a report to the SA department for water," *Aquatic Sciences*, vol. 528, pp. 50–57, 2011.

[7] G. B. Yu, Y. Liu, S. Yu et al., "Inconsistency and comprehensiveness of risk assessments for heavy metals in urban surface sediments," *Chemosphere*, vol. 85, no. 6, pp. 1080–1087, 2011.

[8] R. D. Handy, T. B. Henry, T. M. Scown, B. D. Johnston, and C. R. Tyler, "Manufactured nanoparticles: their uptake and effects on fish-a mechanistic analysis," *Ecotoxicology*, vol. 17, no. 5, pp. 396–409, 2008.

[9] F. Gottschalk, T. Sonderer, R. W. Scholz, and B. Nowack, "Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis," *Environmental Toxicology and Chemistry*, vol. 29, pp. 1036–1048, 2010.

[10] V. Poleksic, M. Lenhardt, I. Jaric et al., "Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (*Acipenser ruthenus Linnaeus*, 1758)," *Environmental Toxicology and Chemistry*, vol. 29, no. 3, pp. 515–521, 2010.

[11] J. K. Saliu, S. A. Oluberu, I. I. Akpoke, and U. D. Ukwa, "Cortisol stress response and Histopathological Alteration Index in *Clarias gariepinus* exposed to sub-lethal concentrations of Qua Iboe crude oil and rig wash," *African Journal of Aquatic Science*, vol. 42, no. 1, pp. 55–64, 2016.

[12] B. Akinsanya, O. U. Utoh, and U. D. Ukwa, "Toxicological, phytochemical and anthelminthic properties of rich plant extracts on *Clarias gariepinus*," *Journal of Basic & Applied Zoology*, vol. 74, pp. 75–86, 2016.

[13] U. D. Ukwa, J. K. Saliu, and A. O. Osibona, "Combined effects of intestinal infestation and extrinsic stress on host energy in *Malapterurus electricus*host-parasite system in Lekki Lagoon, Nigeria," *Iranian Journal of Ichthyology*, vol. 5, no. 1, pp. 43–54, 2018.

[14] J. K. Saliu, B. Akinsanya, U. D. Ukwa, J. Odozie, and Y. Ganiu, "Host condition, parasite interaction and metal accumulation in *Tilapia guineensis* from iddo area of Lagos lagoon, Nigeria," *Iranian Journal of Ichthyology*, vol. 1, no. 4, pp. 286–295, 2014.

[15] B. Mansouri, A. Maleki, S. A. Johari, B. Shahmoradi, E. Mohammadi, and B. Davari, "Histopathological effects of copper oxide nanoparticles on the gill and intestine of common carp (*Cyprinus carpio*) in the presence of titanium dioxide nanoparticles," *Chemistry and Ecology*, vol. 33, no. 4, pp. 295–308, 2017.

[16] C. C. C. Cerqueira and M. N. Fernandes, "Gill tissue recovery after copper exposure and blood parameter responses in the tropical fish *Prochilodus scrofa*," *Ecotoxicology and Environmental Safety*, vol. 52, no. 2, pp. 83–91, 2002.

[17] M. Benavides, P. Coelho, C. Lodeiro, and M. S. Diniz, "Effects of exposure of oxides of nanoparticles (Al_2O_3 and ZnO) singly and mixtures on *Carassius auratus* gills," *Microscopy and Microanalysis*, vol. 21, no. 6, pp. 18-19, 2015.

[18] J. Schwaiger, R. Wanke, S. Adam, M. Pawert, W. Honnen, and R. Triebskorn, "The use of histopathological indicators to evaluate contaminant-related stress in fish," *Journal of Aquatic Ecosystem Stress and Recovery*, vol. 6, no. 1, pp. 75–86, 1997.

[19] M. Golobič, A. Jemec, D. Drobne, T. Romih, K. Kasemets, and A. Kahru, "Upon exposure to Cu nanoparticles, accumulation of copper in the isopod *Porcellio scaber* is due to the dissolved Cu ions inside the digestive tract," *Environmental Science and Technology*, vol. 46, no. 21, pp. 12112–12119, 2012.

[20] C. Jayaseelan, A. Abdul Rahuman, R. Ramkumar et al., "Effect of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia, *Oreochromis mossambicus*," *Ecotoxicology and Environmental Safety*, vol. 107, pp. 220–228, 2014.

[21] R. J. Griffitt, R. Weil, K. A. Hyndman et al., "Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*)," *Environmental Science & Technology*, vol. 41, no. 23, pp. 8178–8186, 2007.

[22] A. Maleki, N. A. Azadi, B. Mansouri, F. Majnoni, Z. Rezaei, and F. Gharibi, "Health risk assessment of trace elements in two fish species of Sanandaj Gheshlagh Reservoir, Iran," *Toxicology and Environmental Health Sciences*, vol. 7, no. 1, pp. 43–49, 2015.

[23] G. A. Al-Bairuty, B. J. Shaw, and R. D. Handy, *Histopathological effects of metal and metallic nanoparticles on the body systems of rainbow trout (Oncorhynchus mykiss)*, Ph.D. thesis, School of Biomedical and Biological Sciences, University of Plymouth, Plymouth, UK, 2013.

[24] L. Song, M. G. Vijver, W. J. G. M. Peijnenburg, T. S. Galloway, and C. R. Tyler, "A comparative analysis on the in vivo toxicity of copper nanoparticles in three species of freshwater fish," *Chemosphere*, vol. 139, pp. 181–189, 2015.

[25] G. Federici, B. Shaw, and R. Handy, "Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects," *Aquatic Toxicology*, vol. 84, no. 4, pp. 415–430, 2007.

[26] B. Mansouri and S. A. Johari, "Effects of short-term exposure to sublethal concentrations of silver nanoparticles on histopathology and electron microscope ultrastructure of zebrafish (*Danio Rerio)* Gills," *Iranian Journal of Toxicology*, vol. 10, no. 1, pp. 15–20, 2016.

[27] R. N. Mitchell and R. S. Cotran, "Cell injury adaptation and death," in *Robbins Basic Pathology*, V. Kumar, R. S. Cotran, and S. L. Robbins, Eds., pp. 3–32, Saunders, New Delhi, India, 2000.

[28] R. Bhuvaneshwari, K. Padmanaban, and R. Babu Rajendran, "Histopathological alterations in muscle, liver and gill tissues of zebrafish *Danio rerio* due to environmentally relevant concentrations of organchlorine pesticides (OCPs) and heavy metals," *International Journal of Environmental Research*, vol. 9, pp. 1365–1372, 2015.

[29] W. Jiraungkoorskul, E. S. Upathama, and M. Kruatrachuea, "Histopathological effects of roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*)," *Science Asia*, vol. 28, pp. 121–127, 2002.

[30] T. Ostaszewska, M. Chojnacki, M. Kamaszewski, and E. Sawosz-Chwalibóg, "Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of Siberian sturgeon," *Environmental Science and Pollution Research*, vol. 23, no. 2, pp. 1621–1633, 2016.

[31] H. Kaya, F. Aydın, M. Gürkan et al., "A comparative toxicity study between small and large size zinc oxide nanoparticles in tilapia (*Oreochromis niloticus*): organ pathologies, osmoregulatory responses and immunological parameters," *Chemosphere*, vol. 144, pp. 571–582, 2016.

[32] S. A. Johari, M. R. Kalbassi, I. J. Yu, and J. H. Lee, "Chronic effect of waterborne silver nanoparticles on rainbow trout (*Oncorhynchus mykiss*): histopathology and bioaccumulation," *Comparative Clinical Pathology*, vol. 24, no. 5, pp. 995–1007, 2015.

[33] F. Flores-Lopes and A. Thomaz, "Histopathologic alterations observed in fish gills as a tool in environmental monitoring," *Brazilian Journal of Biology*, vol. 71, no. 1, pp. 179–188, 2011.

[34] K. S. Rajkumar, N. Kanipandian, and R. Thirumurugan, "Toxicity assessment on haemotology, biochemical and histopathological alterations of silver nanoparticles-exposed freshwater fish *Labeo rohita*," *Applied Nanoscience*, vol. 6, no. 1, pp. 19–29, 2012.

[35] J. C. Van Dyk, M. J. Marchand, G. M. Pieterse, I. E. Barnhoorn, and M. S. Bornman, "Histological changes in the gills ofClarias gariepinus (Teleostei: *clariidae*) from a polluted South African urban aquatic system," *African Journal of Aquatic Science*, vol. 34, no. 3, pp. 283–291, 2009.