Effect of feeding of cyclopoid copepods (Eucyclop sp.) exposed to engineered titanium dioxide nanoparticles (nTiO$_2$) and Lead (Pb$^{2+}$) on Clarias gariepinus growth and metabolism

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Abstract

Background: The application of Lead (Pb$^{2+}$) and titanium dioxide nanoparticles (nTiO$_2$) in commercial products is on the rise since the development of nanotechnology. The increasing usage of products containing these compounds had led to the rise of their concentration in aquatic environment, but information on the potential risk of co-exposure of these compounds in aquatic environment is still limited. In this study, the effect of feeding Clarias gariepinus with cyclopoid copepods exposed to engineered Pb$^{2+}$ and nTiO$_2$ on growth performance, and proximate composition of Clarias gariepinus was investigated.

Methodology: A chronic (28 days) laboratory bioassay was carried out by feeding C. gariepinus fries with cyclopoid copepods exposed to nTiO$_2$ (7.5, 16.5 μgL$^{-1}$) and Pb$^{2+}$ (6.5, 15 μgL$^{-1}$) alone as well as binary mixtures through dietary uptake.

Results: Our results indicate negative allometric growth ($b < 3$), while the highest condition factor (1.74) was recorded in the control. A significant decreased of specific growth rate (SGR) compared to the control was observed in exposed fish. Some parameters of proximate composition (crude protein, ash, moisture, total lipid) from the fish decreased significantly ($P < 0.05$) with synergistic effect on binary mixture. In contrast, carbohydrate content increased significantly ($P < 0.05$) with synergistic effect on binary mixture.

Conclusion: The present study clearly indicates that the chronic exposure of nTiO$_2$ and Pb$^{2+}$ mixtures caused the delay in the growth performance and changes in the proximate compositions of the fish. This findings raise concern regarding the fate of higher trophic level feeding on primary consumers inhabiting freshwater ecosystems contaminated with nTiO$_2$ and Pb$^{2+}$.

Keywords: Heavy metal, Nanomaterials, Binary mixture, Biochemical composition, Stress
Background

Nanomaterials are defined as engineered materials having nanostructured surface and topography of at least one dimension equal or less than 100 nm (Hagens, Oomen, de Jong, Cassee, & Sips, 2007). All nanomaterials have specific physical, chemical, optical, electrical, catalytical, and mechanical properties. In addition, particles size, number, surface area, charge, shape, and mass determine their biological interactions with consequences on the behavior and responses in the body of organisms (Hagens et al., 2007).

However, nanomaterials such as titanium dioxide have the peculiarity of having unique physicochemical properties including a bright white color, ability to block UV light, and antimicrobial activity (Smijks & Pavl, 2011). TiO₂ nanoparticles exist in three different crystalline structures (anatase, rutile, and brookite) and are widely used as a pigment (in paint, plastic, and paper), in personal care products (sun-screens and toothpastes), and in food such as (ice cream) (Weir, Westerhoff, Fabricius, Hristovski, & Von Goetz, 2012).

Piccinno, Gottschalk, Seeger, and Nowack (2012) documented the estimated worldwide production of titanium dioxide nanoparticles (nTiO₂) at approximately 5000 t/year in 2006–2010 and 10,000 t/year in 2011–2014; with an estimated production by 2025 expected to reach 2.5 million metric tons.

Since the application of nTiO₂ has increased in recent years, it is expected that this nanomaterials will find their way into the aquatic environment (Gottschalk, Sonderer, Scholz, & Nowack, 2009). Based on toxicological studies, the predicted environmental concentration of nTiO₂ in Switzerland waters was reported as 0.7–16 μg L⁻¹ (Mueller & Nowack, 2008). Despite increased usage of product containing nTiO₂, there is very little data reported on their toxicological effects in aquatic environments in Sub-Sahara Africa. Recent research showed evidence of trophic transfer of nTiO₂ via dietary intake from freshwater Daphnia to Zebrafish without biomagnifications of the nanoparticles (Zhu, Chang, & Chen, 2010). Toxic effects of nTiO₂ on aquatic organisms have been well documented in many in vivo and in vitro studies (Iavicoli, Leso, & Bergamaschi, 2012; Liu, Lin, & Zhao, 2013; Tassinari, La Rocca, Stecca, Tait, De Berardis, Ammendolia, Iosi, Di Virgilio, Martinelli, & Maranghi, 2015), but studies on the toxicity of nTiO₂ and heavy metals mixtures are currently sparse (Hartmann, Legros, Von der Kammer, Hofmann, & Baun, 2012; Liu et al., 2013; Zhang, Niu, Li, Zhao, Song, Li, & Zhou, 2010). Although, it is possible that both compounds enter the aquatic systems differently, mixtures formed can demonstrate synergistic adverse effect on fish more than that of each compound alone.

Lead (Pb²⁺) has received critical attention as a major source of pollution worldwide (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). This non-essential metal has been used for more than 800 decades in the manufacturing of glass, pigments, fuel additives, batteries, electronic components, cosmetics, and wine and has been detected in cooking, agricultural, urban, and industrial wastes (United Nation Environmental Programme, 2010). Lead (Pb²⁺) is known to affect growth, development, and reproduction in humans and animals. Lead (Pb²⁺) pollution has become an environmental problem worldwide especially in aquatic environment in which it may reach high-risk levels for aquatic organisms and their consumers including humans (Soto-Jimenez, Arellano-Fiore, Rocha-Velarde, Jara-Marini, & Ruelas-Inzunza, 2011).

Methods

Preparation and characterization of nTiO₂
Titanium (IV) oxide-anatase-rutile nanoparticles (particle size < 21 nm) and PbNO₃ were purchased from Sigma-Aldrich (St. Louis, MO, USA) and further characterization of the metals was done using X-ray diffraction using an Empyrean XRD (Panalytical, The Netherlands) equipped with filtered Cu Kα radiation operated at 40 K, and 40 mA. The XRD patterns were recorded from 10 to 80° with a scanning speed of 0.526° per minute. X-ray diffraction analysis was used to...
confirm the chemical composition and crystal structure of the nTiO$_2$.

TiO$_2$ nanoparticles were rigorously mixed with magnetic stirrer on a plate (VWR Scientific 370, Radnor, PA, USA) using a 1-in bar for 15 min (30° C, 1400 rpm, 50 kHz). In addition, the morphology of nTiO$_2$ aggregates in the test solutions was observed using a scanning electron microscope (Xpert Pro). Particles aggregation of nTiO$_2$ after 24 h was also observed in 10 mg L$^{-1}$ of nTiO$_2$.

**Culture of cyclopoid copepods sp.**

**Test organism**

Cyclopoid copepods sp. were obtained from the National Institute of freshwater and Fisheries Research, (NIFFR), New Bussa, Nigeria and continuously cultured in the Limnology laboratory of the Institute. The culture medium was kept static for 48 h and the cyclopoid copepods sp. were fed daily with cultured Chlorella ellipsoidea contaminated with nTiO$_2$, Pb$^{2+}$ alone, and in combination. The culture was maintained at a constant temperature (25 ± 2 °C) with a natural light-dark cycle.

**nTiO$_2$ and Pb accumulation in cyclopoid copepods sp.**

Cyclopoid copepods sp. were fed for 2 days (ad libitum) with contaminated C. ellipsoidea at a concentration of 1.10$^6$ cells mL$^{-1}$ with the following concentrations of 0 (control), 6.5 µg L$^{-1}$, and 15 µg L$^{-1}$ of Pb; 7.5 µg L$^{-1}$, 16.5 µg L$^{-1}$ TiO$_2$ NPs and four couples of mixtures of (6.5, 7.5); (6.5, 16.5); (15, 7.5); (15, 16.5) µg L$^{-1}$. In this experiment, 35 cyclopoid copepods sp. were placed in each container (1000 mL glass beakers containing 250 mL test solution), and each treatment had 3 replicates. Test containers were monitored every 24 h.

After 48 h of exposure, they were harvested, rinsed with fresh culture medium, and transferred immediately to Clarias gariepinus larvae tanks as fish food.

**Acclimatization and training of C. gariepinus**

Clarias gariepinus larvae (3 days old; 0.33 ± 0.09 g) were hatched at the Hatchery laboratory of NIFFR. The fish were kept in glass aquaria of 50 cm × 50 cm (25 fish per tank) filled with 10 L of freshwater. Before the experiment, fish were acclimatized to the experimental conditions (25 ± 2 °C; 12:12 h light:dark; with daily water change) for at least 1 week. At the same time, C. gariepinus larvae were trained to eat live cyclopoid copepods sp. During acclimatization and training, the health of the fish was observed and recorded. Only healthy fish were selected for the examination described below. All animal protocols in this study were conducted under the supervision and approval of the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

**Trophic transfer of TiO$_2$ NPs and Pb from cyclopoid copepods to Clarias gariepinus**

Trophic transfer experiments consisting of a 28-day uptake period were conducted to determine if the transfer of nTiO$_2$ and Pb from cyclopoid copepods sp. to C. gariepinus can occur.

Cyclopoid copepods sp. exposed to 6.5 µg L$^{-1}$ and 15 µg L$^{-1}$ of Pb; 7.5 µg L$^{-1}$, 16.5 µg L$^{-1}$ nTiO$_2$ and the 4 couples of mixtures of (6.5, 7.5); (6.5, 16.5); (15, 7.5); (15, 16.5) µg L$^{-1}$ for 48 h (as mentioned above) were harvested with a plastic net, rinsed three times using fresh culture medium, and transferred into C. gariepinus aquaria as fish food.

Clarias gariepinus were fed with cyclopoid copepods sp. (ad libitum) in the uptake period and uncontaminated cyclopoid copepods in the depuration period. Twice daily (6 am and 6 pm), the water (in which neither nTiO$_2$ nor Pb$^{2+}$ was added) was renewed; in most cases, the C. gariepinus would consume the entire cyclopoid copepods sp. This static renewal system was used to ensure that the C. gariepinus ate all the food provided and so that none would be washed away.

**Assessment of length-weight relationship and condition factor**

Fish samples were collected for a period of 7 weeks, standard length of the fish was measured to the nearest centimeter, using measuring board. The fish were also weighed to the nearest gram on a sensitive balance. The length-weight relationship and Fulton’s condition factor were calculated according to Le Cren’s, 1951 equations:

1. $W = aL^b$. Then the data were transformed into logarithms prior to calculations. The equation became $\log W = \log a + b\log L$

   Where
   - $W$ = weight of fish (g)
   - $L$ = standard length of fish (cm)
   - $a$ = constant or intercept
   - $b$ = an exponent or slope.

2. Fulton’s condition factor: $K = 100 \frac{W}{L^3}$ where

   - $K$ = condition factor
   - $W$ = weight of fish (g)
   - $L$ = standard length of fish (cm)

   The percentage of survival of C. gariepinus within the duration of the experiment was calculated according to the formula of Zhu et al. (2007) and Wang, Long, Cheng, Liu, & Yan (2015). Percentage survival rate = number of fry that survived × 100 / total number of fry that start the treatment in each aquarium.

3. Specific growth rate (% per week) = (final ln weight-initial ln weight) × 100/experimental days.
Proximate composition
Fish was subjected to several procedures for determination of crude protein, ash, moisture, total lipid (fat), and carbohydrate.

Total crude protein was measured according to Lowry, Rosebrough, Farr and Randall (1951). A stock of Lowry reagent was compounded in a 48:1:1 ratio of Lowry reagents (2% (w/v) anhydrous Na₂CO₃ in 0.1 N NaOH; 1% (w/v) NaK Tartrate tetrahydrate) and (0.5% (w/v) CuSO₄·5H₂O in H₂O), respectively. Samples were then incubated for 10 min at room temperature, absorbance was read at 600 nm, and concentration was determined using standard curve: total protein content = wt. of protein (g) × 100 / wt. of culture (g).

Lipid was extracted according to the method of Bligh and Dyer (1959). A mixture of 2 mL of methanol and 1 mL of chloroform was made and added to 1 g fish biomass. The lower layer was pipetted out and weighed: lipid content (%) = wt. of lipid (g) × 100 / wt. of culture (g).

For carbohydrate determination, 50 μL of 80% phenol and 5 mL of 95% sulfuric acid was added to 1 mL culture supplemented with 1 mL of filtered distilled water, following the method of Dubois, Gilles, Hamilton, Roberts and Smith (1956) using glucose as standard.

DNA isolation, purification, and quantification
Total deoxyribonuclease (DNA) was purified from C. gariepinus tissues using EZNA, tissue DNA kit (OMEGA, USA). The purified DNA was subjected to spectrometry using a Nanodrop 1000. The absorbance of extracted DNA was read at A280nm.

Statistical analysis
All experiments were done in triplicates, and data were recorded as mean and standard deviation (SD). One-way analysis of variance with Tukey’s multiple comparisons was used to detect significant differences among groups. In all data analyses, a P value < 0.05 was considered statistically significant.

Results
Fish fed with contaminated copepods for 4 weeks showed the lowest survival (50%) in combined compounds (Pb (15) + nTiO₂ (16.5)). However, the lowest mean weight (0.15 g) was recorded in nTiO₂ (7.5), while a decrease in mean weight was observed in exposed fish compared to the control; the lowest weight gain (0.15) was concentration dependent (Pb (15)) for single Pb (Table 1).

Weight gain significantly (P < 0.05) decreased compared to the control; the lowest weight gain (0.25 g) was recorded in fish exposed to Pb (15) and the highest (0.42 g) was recorded in fish exposed to Pb (15) + nTiO₂ (16.5). Furthermore, the specific growth rate (SGR) was lowest (3) in fish exposed to Pb (15) and highest (3.8) in fish exposed to Pb (6.5) + nTiO₂ (16.5) (Table 1).

The b value accounting for the allometric growth was negative and less than 3 (b < 3) in all exposed fish indicating disproportionate growth between the size and the weight of fish. However, the maximum b value of 2.09 was recorded from the control indicating that the absence of the compounds in the food was responsible for moderate negative allometric growth (b < 3). The b values in this study were fitted into respective correlation coefficient (r) levels and transformed into linear equations (Table 2). The result showed significant correlation (P < 0.05) of no allometric growth throughout the treatments except for fish fed contaminated copepods nTiO₂ (7.5), Ti (16.5), and Pb (6.5) + nTiO₂ (16.5) (Table 2).

Clarias gariepinus post frys in this study had relatively low allometric growth rate in fish fed with contaminated copepods with b values between −13 and −0.12.

Condition factor (K) of contaminated fish ranged between 0.99 and 1.74 (Table 2); these values were lower than the ideal level of 2.9−4.8 for fish water quality.

Proximate composition of C. gariepinus fed with cyclopoid copepods exposed to Pb and nTiO₂ showed that the protein content significantly (P < 0.05) decreased in exposed fish to contaminated dietary food. A decrease of 83.9%, 82%, 88.5%, 84.37%, 82.38%, 81.75%, 80.98%, and 80.88% was recorded for Pb (6.5), Pb(15), nTiO₂ (7.5), nTiO₂ (16.5), Pb(6.5) + nTiO₂ (7.5), Pb(6.5) + nTiO₂ (16.5), Pb(15) + nTiO₂ (7.5), Pb(15) + nTiO₂ (16.5) μg L⁻¹ respectively. The results showed that there was a synergistic decrease of protein content in binary compounds (Fig. 1a).

Ash content was lowest in binary mixture compared to the control 96.97%, 95.97%, 95.4%, 95.30% for Pb(6.5) + nTiO₂ (7.5), Pb(6.5) + nTiO₂ (16.5), Pb(15) + nTiO₂ (7.5), Pb(15) + nTiO₂ (16.5) μg L⁻¹ respectively. This result showed that there was significant (P < 0.05) decrease of ash content compared to the control (Fig. 1b).

Moisture content in fish significantly (P < 0.05) decreased in single and combined compounds compared to the control, 94.44%, 93.82%, 97.53%, 96.91%, 93.82%, 97.53%, 96.91%, 93.82%, 91.97%, 91.35%, 91.11% for Pb (6.5), Pb(15), nTiO₂ (7.5), Ti(16.5), Pb(6.5) + nTiO₂ (7.5), Pb(6.5) + nTiO₂ (16.5), Pb(15) + nTiO₂ (7.5), Pb(15) + nTiO₂ (16.5) μg L⁻¹ respectively. The moisture content decreased with the increase of concentrations when the compound was used alone. This result showed synergistic effect of binary compounds for moisture content (Fig. 1c).
The highest (91.85) DNA concentration was recorded in nTiO\(_2\) the concentration of the compound of the fed fish. The concentration due to treatment of fish was irrespective of 83.86% for Pb (6.5), Pb(15), nTiO\(_2\) (7.5), and nTiO\(_2\) (16.5) separately with percentages 83.46%, 73.50%, 86.25%, concentration dependent when both compounds were used respectively. After addition of nTiO\(_2\) to Pb\(^{2+}\), the combination of both compounds had the highest concentration (90.3) for Pb(6.5) + nTiO\(_2\) (7.5) exposed fish compared to the control. There was no synergistic effect of the compounds recorded in the present study. However, there was significant (P < 0.05) difference between DNA concentration and the exposed fish compared to the control.

**Discussion**

The result of the present study suggests that Lead (Pb\(^{2+}\)) and nTiO\(_2\) nanoparticles have negative impact on the survivability and growth of *C. gariepinus* which is evidence by numerous other studies indicating the negative effect of heavy metals and nanoparticles on fish (Asharani, Wu, Gong, & Valiyaveettil, 2008; Kim & Kang, 2015; Zhou, Wang, Gu, & Li, 2009). The interaction between the highest concentrations of binary mixture observed in the present study was more prominent for fish that demonstrated low survival rate (50%) indicating that post fries were unable to cope with their hyperactivity due to stress leading to depletion of energy and causing death.

Chojnacki and Sliwinski (2013) reported that the growth of *Leuciscus idus* fish was positively affected by silver nanoparticles served through dietary means. The growth rate is generally retarded in response to toxicant because the allocation of energy for growth is used to compensate for repair (Kim & Kang, 2015). The specific growth rate (SGR) of fish contaminated with single and combined compounds, in this study decreased compared to the control. The effect on growth of fish in the study was not concentration-dependent.

**Table 1** Growth response and survival of *C. gariepinus* fries fed with contaminated zooplankton of Pb and nTiO\(_2\)

| Parameters | Pb (6.5) | Pb (15) | nTiO\(_2\) (7.5) | nTiO\(_2\) (16.5) | Pb(6.5) + nTiO\(_2\) (7.5) | Pb(6.5) + nTiO\(_2\) (16.5) | Pb(15) + nTiO\(_2\) (7.5) | Pb(15) + nTiO\(_2\) (16.5) | Control |
|------------|---------|---------|-----------------|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|---------|
| Percentage survival (%) | 98      | 98      | 96              | 100             | 92                       | 80                       | 100                      | 50                       | 100     |
| Initial mean weight (g) | 0.18 ± 0.007 | 0.19 ± 0.014 | 0.28 ± 0.028 | 0.15 ± 0.007 | 0.24 ± 0.021 | 0.27 ± 0.028 | 0.23 ± 0.042 | 0.22 ± 0.028 | 0.28 ± 0.01 |
| Final mean weight (g) | 0.51 ± 0.26 | 0.44 ± 0.22 | 0.64 ± 0.39 | 0.49 ± 0.39 | 0.61 ± 0.45 | 0.67 ± 0.83 | 0.50 ± 0.44 | 0.64 ± 0.46 | 0.83 ± 0.42 |
| Mean weight gain (g) | 0.33 ± 0.21 | 0.25 ± 0.20 | 0.36 ± 0.49 | 0.34 ± 0.54 | 0.37 ± 0.63 | 0.39 ± 1.2 | 0.36 ± 0.55 | 0.42 ± 0.62 | 0.56 ± 0.23 |
| SGR (%/day) | 3.8* | 3.0* | 2.95* | 3.11* | 3.3* | 4.01* | 3.36* | 3.8* | 4.22* |

Superscripts of the same letter on the same row are not significant (P > 0.05)

**Table 2** Estimated parameters of total length-body weight relationships and condition factor for *C. gariepinus*

| Treated zooplankton diet | Linear equation for each treated diet | a | b | r | P value | K |
|--------------------------|--------------------------------------|---|---|---|---------|---|
| Pb (6.5) | Log\(W = 0.19-0.12\log TL\) | 0.19 | -0.12 | 0.42 | 0.02 | 1.38 |
| Pb(15) | Log\(W = 0.18-13\log TL + 0.22\) | 0.18 | -13 | 0.17 | 0.02 | 1.36 |
| nTiO\(_2\) (7.5) | Log\(W = 0.28-39\log TL\) | 0.28 | -0.39 | 0.38 | 0.06 | 1.37 |
| nTiO\(_2\) (16.5) | Log\(W = 0.40-0.72\log TL\) | 0.40 | -0.72 | 0.70 | 0.09 | 1.77 |
| Pb(6.5) + nTiO\(_2\) (7.5) | Log\(W = 0.23-0.27\log TL\) | 0.23 | -0.27 | 0.67 | 0.02 | 1.17 |
| Pb(6.5) + nTiO\(_2\) (16.5) | Log\(W = 0.60-1.35\log TL\) | 0.60 | -1.35 | 0.68 | 0.32 | 1.44 |
| Pb(15) + nTiO\(_2\) (7.5) | Log\(W = 0.27-0.37\log TL\) | 0.27 | -0.37 | 0.69 | 0.03 | 1.18 |
| Pb(15) + nTiO\(_2\) (16.5) | Log\(W = 0.20-0.17\log TL\) | 0.20 | -0.17 | 0.75 | 0.01 | 0.99 |
| Control | Log\(W = -2.44\log TL-0.36\log TL\) | -0.36 | 2.09 | -2.44 | 0.03 | 1.74 |
This implies that the fish fed with contaminated zooplankton continued its growth though not significant ($P > 0.05$). This result agreed with findings of Chojnacki and Sliwinski (2013) who also documented higher SGR in contaminated fodder with titanium dioxide nanoparticles fed to fish. This was probably related to the initiation of detoxification processes and reduction of the integrity of the cell membrane (Chojnacki & Sliwinski, 2013).

The negative allometric growth between the length and weight of fish in the present study may be due to the presence of single and combined nanoparticles of Pb and TiO$_2$ in the fish diets. These compounds might have caused the disproportionate growth of fish given that dietary Lead (Pb) exposure has been shown to generate a significant inhibition of growth in rockfish (Kim &

### Table 3

| Fish treatments (μg/L) | DNA concentration (ng/μL) |
|------------------------|---------------------------|
| Control                | $102.4^{±}14.21$ (92.4–112.5) |
| Pb(6.5)                | $22.9^{±}2.82$ (20.9–24.9)  |
| Pb(15)                 | $30.9^{±}14.14$ (20–40)     |
| nTiO$_2$ (7.5)         | $91.85^{±}1.20$ (91–92.7)   |
| nTiO$_2$ (16.5)        | $21.2^{±}1.55$ (20.1–22.3)  |
| Pb(6.5) + nTiO$_2$ (7.5) | $90.7^{±}0.42$ (90.4–91)   |
| Pb(6.5) + nTiO$_2$ (16.5) | $48.6^{±}8.98$ (42.3–55)  |
| Pb(15) + nTiO$_2$ (7.5) | $77.6^{±}2.28$ (57.6–97.6) |
| Pb(15) + nTiO$_2$ (16.5) | $72.3^{±}25.03$ (54.6–90) |

Superscripts of the same letter on the same row are not significant ($P > 0.05$).
In addition, hazardous effect of nanoparticles induced growth restriction in Mystusvittatus (Chatterjee, Bhattacharjee, & Lu, 2014). Similar observation was found in the present study for nTiO2; furthermore, the combination of binary compounds also showed relatively low allometric values suggesting that the addition of nTiO2 to Pb causes retardation of growth.

Condition factors (K) values in this study ranged between 0.99 and 1.74 and were lower than the ideal level 2.9–4.8 reported by Bagenal (1978) for maximum growth of freshwater fish. The low (K) values recorded might have been caused by the rapid decrease of biological dissolve oxygen demand in the flow through water system in cultivated fives.

Protein levels in the present study decreased in all other treated fish compared to the control. Protein content below 15% is considered low (FAO, 2007); however, it is treated fish compared to the control. Protein content and lipid content decreased with an increase in concentration of Pb2+ and nTiO2 used alone and subjected to C. gariepinus, indicating that Pb2+ were less harmful to energy stores. The addition of nTiO2 to Pb2+ shows further synergistic decrease of protein content and lipid content suggesting the presence of nTiO2 might have increased the bioavailability of Pb2+ thereby being critically deleterious to energy storage.

Moisture content consistently decreased and varied between 16.2 and 14.76% which was not between the 60 and 80% acceptable levels of moisture content in fish (Gallagher, Harrell, & Rulifson, 1991). In this study, the concentration of compounds used influenced fish moisture level, higher in nTiO2 than Pb2+ that, however, synergistically decreased in combined mixture. The size of the fish (post larvae) could be responsible for the low moisture level as observed in the control. The moisture level in the present study was not in agreement with the 70% moisture content reported by Stanchevaet al. (2013).

Ash content in food could be defined as the measure of the total amount of minerals content present in food item. In this study, ash content decreased in all exposed fish, but further decrease in binary mixtures was also observed probably because of the reduction of minerals levels in the fish. However, the range of ash was between 5.96 and 5.68% indicating low ash content compared to Pseudotolithus elongates and Pseudotolithus typus from the Cameroonian Coast which recorded ash content between 7.7 and 54% (Njinkoue et al., 2016).

Carbohydrate serves as the instant energy source during stress, so it is expected that during exposure, glycogen content in the muscles was broken down to carbohydrate (glucose) via glycogenolysis (Javed & Usmani, 2015). Carbohydrate content in the present study varies between 46.28 and 54.09% and was higher than in the control. This result suggests that carbohydrate level in treated C. gariepinus increased due to chronic stress. The result in this study was in line with the result reported by Javed and Usmani (2015) who reported depletion of glycogen in the liver and muscle of Channa punctatus inhabiting river polluted by thermal power plant effluents.

During stress conditions, fish need more energy to detoxify the pollutants and overcome stress (Amza et al., 2006). In this study, decrease of basic metabolic compounds (protein, lipid, carbohydrate) was observed. This may be due to the need for energy to meet the increasing demand for energy due to stress. The observed depletion of protein, lipid, and ash may have been due to their degradation and probably possible utilization of degraded products for metabolic purpose (Amza et al., 2006).

Assessment of DNA concentration and damage was studied by using several methods. One of the methods is the quantification of DNA that has been carried out using a UV absorbance at 280 with spectrophotometer (Georgiou, Papapostolou, & Grintzalis, 2009). Characterizing the structural integrity of genomic DNA is very important in a variety of biological application (Georgiou et al., 2009). The genomic DNA product derived in this study stem from the exposure of post fries C. gariepinus to Pb and nTiO2 alone and in combination with values ranging between 21.2 and 102.45 ng/μL. The present study indicated low DNA concentrations in exposed fish in comparison with control; DNA concentrations however, were not
Conclusions

The result obtained from this study indicated a synergistic effect of Pb and nTiO₂ on the proximate composition of C. gariepinus. The growth and DNA concentration of C. gariepinus were influenced by exposure of Pb and nTiO₂, a phenomenon that can be used as biomarker for stress in polluted aquatic ecosystems. The role of nTiO₂ in binary mixture with heavy metal in relation to food uptake in food chain and their physiological and metabolic effects on fish need further investigations.

Abbreviations
C. ellipsoidea; Chlorella ellipsoidea; C. gariepinus; Clarias gariepinus; DNA: Deoxyribonuclease; NIFFR: Nigeria Freshwater and Fisheries research; nTiO₂: Titanium dioxide nanoparticles; Pb: Lead; SGR: Specific growth rate; XRD: X-ray diffraction

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Authors’ contributions
The authors designed the research work, carried out laboratory work, and wrote the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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