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Nanotoxicological Assessment of Titanium Dioxide Nanoparticles on *Colurella adriatica*

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Abstract
This research aimed to investigate the potential toxicity of titanium dioxide nanoparticles (TiO_2NPS) to a freshwater planktonic rotifer, *Colurella adriatica*. Titanium dioxide (TiO_2) nanoparticles (NPS) have gained significant attention in various fields owing to their unique physicochemical properties. They constitute approximately 70% of the global pigment-manufacturing volume. The TiO_2 crystal forms include anatase and rutile, with the former being more chemically reactive. According to previous studies, the higher the surface area of the nanoparticles, the more toxic they become. *Colurella adriatica* was selected as the test species and acute exposure toxicity testing was conducted in reconstituted water. Acute concentrations of TiO_2 NPS were 0.01, 0.05, 0.10, 0.50, and 1.00 mg/l, with an LC_{50} of 0.0075 mg/l determined. Results from the acute toxicity test revealed a dose-dependent lethal effect of TiO_2 NPs on Colurella adriatica, with mortality reaching 85.67% at 1.00 mg/L after 96 hours. This suggests that TiO_2 NPs may disrupt freshwater ecosystems, warranting further investigation into their environmental impact. This paper recommends that future research should prioritize the development of strategies to prevent and/or mitigate the accumulation of TiO2 NPS in freshwater ecosystems and their impact on aquatic organisms, among others.

Keywords: Titanium dioxide, Nanoparticles, *Colurella adriatica*, Acute-toxicity, Lethal concentration

Introduction

Titanium dioxide (TiO_2) is a naturally occurring mineral extensively used in a range of industrial and consumer goods. Recognized for its white hue, it serves as a pigment in paints, coatings, plastics, and beauty products (Ukaeje & Bandyopadhyay, 2024). Additionally, TiO_2 has been employed in sunscreens for UV protection and in food items for whitening purposes (Lim et al., 2021; Shaltout et al., 2022). The distinctive attributes and potential uses of TiO_2 nanoparticles have recently garnered increasing interest in diverse sectors (Dedman et al., 2021). Every year, approximately 3.7 billion kilogrammes of titanium dioxide (TiO_2), a white pigment known for its high refractive index and brightness, is used worldwide (Ali & Alwan, 2023). It accounts for 70% of the global pigment production volume (Bergamaschi et al., 2022) and is among the top five nanoparticles used in consumer products (Zhang et al., 2023). TiO_2 is also used by people in making paper, inks, medications, pharmaceuticals, and toothpaste (Harris et al., 2023; Ramesh et al., 2023; Abbas et al., 2023). Nanoparticles are particles that are smaller than 100 nm in size. Nanomaterials occur naturally or are introduced into the environment as synthetic materials. According to previous studies, the surface area of nanoparticles can expand, making the substance more poisonous (Babazadeh et al., 2021). Because of their superior catalytic activity, TiO_2 nanoparticles (NP_S) have become more popular in industrial and consumer applications in recent years. The enhancement in catalytic activity was attributed to their reduced size, resulting in a higher surface area per unit mass, thus facilitating more efficient catalytic reactions.

Concerns have been expressed that the same features of TiO_2 nanoparticles may cause distinct bioactive problems in life (Wang et al., 2020). The rapid increase in the number of published studies demonstrates that there is considerable concern regarding the safety of TiO_2 nanoparticles. These researchers extensively explored some animal models with numerous exposure modes of administration, such as inhalation, cutaneous exposure, intratracheal instillation, oral gavage, and intragastric, intraperitoneal, or intravenous injection. Previous studies (Rashid et al., 2021; Autthawong et al., 2021; Zhang et al., 2023) have shown that TiO_2 NP_S are more hazardous than fine Particles. Juarez-Maldonado (2022) demonstrated that nano-sized Al_2O_3 may accumulate and influence the reproduction and behaviour of *Eisenia fetida*, albeit at elevated levels remains to be determined in the natural environment. This indicates that titanium oxide nanoparticles may infiltrate the food chain and cause poisoning in animals (Herrera-Rodríguez et al., 2023). These results underscore the necessity for additional investigations on nanoparticle ecotoxicity, particularly their species-specific effects in aquatic ecosystems. Therefore, it is necessary to investigate the influence of titanium dioxide nanoparticles on zooplankton, *Colurella adriatica* Ehrenberg, 1831, and their effect on the aquatic food chain.

Colurella adriatica is a planktonic rotifer that belongs to the genus Lepadellidae and lives in freshwater, marine, brackish, and terrestrial settings. Its widespread application establishes it as a principal model for toxicity, ecology, and evolutionary biology studies. Rotifers offer exceptional suitability as model organisms for ecotoxicological research owing to their rapid cultivation in laboratory settings and capacity to yield sensitive test results. Moreover, the health of rotifer populations serves as a dependable indicator of water quality, as evidenced by previous research (Hashimshony et al., 2024). As zooplankton, they contribute to the survival of a tiny ecosystem that feeds and grows few fish. Rotifers are freshwater or brackish water creatures that do not serve as natural food for marine fish (Branco et al., 2023). However, they have a variety of advantageous characteristics that make them suitable for rearing freshwater fish larvae (Aidos et al., 2020; Soleh et al., 2023). Rotifers ranging from 200 to 350 µm are holoplankton, indicating that they are constant zooplankton inhabitants who are continuously in motion (Luo & Segers, 2020). Titanium dioxide nanoparticles smaller than 25 nm can pass through the cell membranes of freshwater zooplankton via canal water discharge caused by anthropogenic activities such as toothpaste usage, paint cracks, and plastics. Because zooplankton are not membrane-bound, they have access to organelles and deoxyribonucleic acid (DNA) inside the cell. This is dangerous because nanoparticles can carry harmful substances (Mohamed et al., 2023; Ferrante et al., 2023). Nanoparticles accumulate because of their ability to penetrate membranes, potentially increasing their toxicity (Pogribna et al., 2020). Research has demonstrated the acute toxicity of titanium dioxide nanoparticles (TiO_2 NPs) to rotifers, key planktonic organisms in freshwater ecosystems. Dong et al. (2020) investigated TiO_2 NP effects on *Brachionus calyciflorus*, revealing impaired survival, reproduction, and physiological responses (e.g., antioxidant activity, swimming speed). Findings are relevant to closely related species like *Colurella adriatica*. Similarly, Das and Mukherjee (2024) highlighted TiO_2 NP toxicity in both the rotifer *B. calyciflorus* and the alga *Scenedesmus obliquus*, though humic acid mitigated some effects. Collectively, these studies underscore the ecological risks posed by TiO_2 NPs to aquatic organisms, warranting further investigation into their long-term environmental impacts.

Statement of the Problem

Nigeria's economy relies heavily on natural resources, including freshwater ecosystems. Evaluating the ecological ramifications of TiO_2 NP exposure is imperative to promote sustainable development practices that strike a balance between economic progress and environmental preservation. Nigeria's aquatic ecosystems, such as rivers, lakes, and coastal areas, may have unique environmental conditions that can interact with TiO_2 NP_S in ways that differ from those of other regions. Understanding how these nanoparticles behave and impact local aquatic organisms is crucial for their effective environmental management. A diverse range of aquatic species, many of which may be vulnerable to the effects of TiO_2 NP_S exist in aquatic bodies in Nigeria. Research, such as this study, can help identify which species are most at risk and develop targeted conservation efforts to protect biodiversity in Nigerian waters. TiO_2 NP_S can bioaccumulate in aquatic organisms and eventually enter the food chain. Research on the ecological consequences of TiO_2 NP exposure can provide insights into the potential human health risks associated with consuming contaminated fish or water from Nigerian aquatic environments. Furthermore, understanding the ecological consequences of TiO_2 NP exposure is essential for developing appropriate regulatory frameworks to protect the aquatic ecosystems in Nigeria. The findings of this study can inform policymakers of the need for specific guidelines or restrictions on the use of TiO_2 NP_S to prevent environmental harm. Available data on the effects of TiO_2 NPs on Nigerian aquatic environments remains sparse. This research can help fill these knowledge gaps and provide a more comprehensive understanding of the potential risks associated with these nanoparticles, as well as support the development of mitigation strategies to minimize the impact of TiO_2 NP_S on aquatic ecosystems in Nigeria. This could include measures, such as wastewater treatment technologies or guidelines for the safe disposal of TiO_2-containing products. Expanding scientific knowledge in this area is essential for protecting Nigeria's aquatic environments and promoting sustainable development across generations

Aim and Objectives of the Study

The aim of the study was to investigate nanotoxicological assessment of titanium dioxide nanoparticles on *colurella adriatica*. Specifically, the study sought to:

1. Determine the acute toxicological effects of titanium dioxide nanoparticles (TiO_2 NPs) on the freshwater planktonic rotifer species, *Colurella adriatica*.
2. Investigate the concentration-dependent effects of TiO_2 NP_S on the mortality rate of *Colurella adriatica*.
3. Establish a lethal concentration (LC_{50}) of TiO_2 NPS for *Colurella adriatica* after 96 hours of exposure.

Research Questions

The research questions that guided the study were as follows:

1. What are the concentration-dependent and time-variable acute toxicity effects of titanium dioxide nanoparticles (TiO_2 NPs) on the freshwater rotifer Colurella adriatica
2. What is the relationship between titanium dioxide nanoparticle (TiO_2 NP_S) concentration and mortality rates in *Colurella adriatica*, and how does this relationship vary across different concentrations and exposure durations?
3. What is the lethal concentration (LC_{50}) of titanium dioxide nanoparticles (TiO_2 NP_S) for *Colurella adriatica* after 96 hours of exposure, and how does this concentration compare with the LC_{50} values reported for other aquatic organisms?

Materials and Methods

Sources of Titanium dioxide nanoparticles and test organisms.

Dry anatase-phase TiO_2 nanopowder (Sigma-Aldrich, St. Louis; CAS 637254) with a manufacturer-reported particle size of <25 nm served as the test material. *C. adriatica* cysts (Brineshrimp Direct, Utah) hatched in reconstituted water was used to establish experimental populations.

Culture of Algae as a Source of Food

Chlorella vulgaris, an alga, was used to feed the zooplankton. *Chlorella vulgaris*, a green alga, was cultivated in an old basal medium. Algal progress was tracked by recording blooms or reductions in growth to take necessary action. The optimal cultivation temperature was maintained between 20 °C and 31 °C using electric fans, and a pH of 8.0. The algae *Chlorella vulgaris* contains most vitamins, except vitamin C, and is extremely easy to culture, which is why it was used in this study.

Dilution water

Reconstituted freshwater was meticulously prepared using high-quality distilled water, with the addition of 96 mg NaHCO3, 60 mg CaSO4.2H2O, 60 mg MgSO4.7H2O, and 4 mg KCl per litre, following the protocol outlined by Peltier and Weber (1985). The mixture was stirred constantly for 24 hours to ensure thorough homogenization. Subsequently, the pH of the solution was carefully adjusted to 7.5, employing a combination of strong hydrochloric acid and sodium hydroxide. It is imperative to note that this diluted water maintains its efficacy for seven days.

Rotifer Cyst Hatching

The cysts were carefully arranged in a shallow, wide dish, commonly known as a petri dish, which offers an optimal surface-area-to-volume ratio, ensuring efficient oxygen exchange. A transparent lid was used to prevent evaporation and maintain clarity. The liquid medium utilized for hatching was reconstituted in freshwater following the methodology described by Arimoro (2006). Approximately 20 ml of the reconstituted water was meticulously dispensed into a Petri dish, providing an ideal environment for the hatching of 1000–5000 eggs. Rotifer cysts were placed in diluted water and incubated at 25ºC with 7000lux illumination to induce hatching. Hatching began after approximately 24 hours, and after 48 hours, over half of the cysts hatched. It was critical to start collecting the hatched test organisms after 50% of the cysts were hatched during the acute toxicity test because feeding was not performed during the test. Food deprivation led to death after approximately 32 hours of testing at 25 °C.

Collection of *Colurella adriatica* into a Petri dish

To transfer *Colurella adriatica*, which was less than 100µm in size, a micropipette and magnification were used. Newborns, distinguishable by their white colouration against a contrasting dark background, were carefully observed under a stereomicroscope set to 15X magnification. To ensure optimal visibility, the environment was dimmed to create a dark backdrop while sufficient illumination was provided. Using a micropipette, the test organisms were carefully collected and transferred to Petri plates containing the designated toxicant concentrations while under meticulous observation using a stereo microscope.

Acute toxicity tests

Acute toxicity testing of titanium dioxide nanoparticles (TiO_2 NPs) was performed using an adapted OECD Guideline 2004 protocol. The experimental design included five TiO_2 NP concentrations (0.01, 0.05, 0.10, 0.5, and 1.0 mg/L) and a blank control, with all treatments replicated three times. Test organisms (<24-hr-old neonates of *Colurella adriatica*) were exposed in groups of 10 individuals per 20 mL test solution (Petri dishes) under static conditions for 96 hours. During the exposure period, organisms were not fed to standardize metabolic responses. The hydrogen content of the test medium (pH 7.5) was determined at the beginning and end of the test. *Colurella adriatica*, which was unable to swim after 15 seconds of mild agitation in the test container, was termed immobile. Heartbeats were monitored under a light microscope (4× magnification), and those that stopped were considered dead.

Results

Acute toxicity of titanium dioxide nanoparticles on *Colurella adriatica*

The mortality of *Colurella adriatica* exposed to an acute concentration of titanium dioxide nanoparticles is presented in Table 1. The mortality rates were dose-dependent. The mortality rates increased when the TiO_2 NP concentrations increased. At 24 hours, the highest mortality rates were 19.67% at 1.00 mg/l^{-1} concentration, followed by 15.33% at 0.50 mg/l^{-1}, 13.00% at 0.10 mg/l^{-1}, 6.77% at 0.05 mg/l^{-1}, and 4.33%, 0.01 mg/l^{-1}. A significant difference ($p<0.05$) was observed between the control and the concentration of 1.00 mg/L after 24 hours of exposure. After 48 hours, the highest percentage mortality was recorded at 25.33% for the 1.00 mg/l^{-1} concentration, while the lowest was 7.67% for the 0.01 mg/l^{-1} concentration. Significant differences ($p<0.05$) were noted between the control and the 1.00 mg/l^{-1} concentration after 48 hours of exposure. At 72 hours, the highest percentage of mortality was observed at 42.00% for the 1.00 mg/l^{-1} concentration, with the lowest at 13.00% for the 0.01 mg/l^{-1} concentration. Again, significant differences ($p<0.05$) were found between the control and the 1.00 mg/l^{-1} concentration after 72 hours of exposure. By 96 hours, the lowest mortality rate was 23.00% for the 0.01 mg/L concentration, while the highest was 85.67% at a 1.00 mg/l^{-1} concentration. A statistically significant difference ($p < 0.05$) was observed between the control group and the 1.00 mg L^{-1} exposure group following 96 hours of treatment.

Table 1: Percentage (%) mortality of *Colurella adriatica* exposed to acute concentrations of titanium dioxide nanoparticles.

Time (h)	Conc. (mgl^{-1})					
	0.00	**0.01**	**0.05**	**0.10**	**0.50**	**1.00**
24*	1.33±0.33^{e}	4.33±0.88^{de}	7.67±0.88^{cd}	13.00±1.15^{bc}	15.33±1.45^{ab}	19.67±0.88^{a}
48*	2.67±0.33^{e}	7.67±0.33^{d}	10.67±1.20^{cd}	13.67±1.45^{c}	22.00±1.00^{b}	25.33±2.60^{a}
72*	4.33±0.88^{e}	13.00±1.15^{d}	26.67±1.76^{c}	35.33±0.88^{b}	38.00±2.65^{ab}	42.00±2.08^{a}
96*	6.00±1.53^{e}	23.00±1.53^{d}	37.00±1.53^{c}	54.67±0.88^{b}	68.00±1.15^{b}	85.67±1.20^{a}

1. Values are presented as means ± standard error
2. The superscripts a, b, c, d, and e are used to show how the means differ along the rows per 24 h.

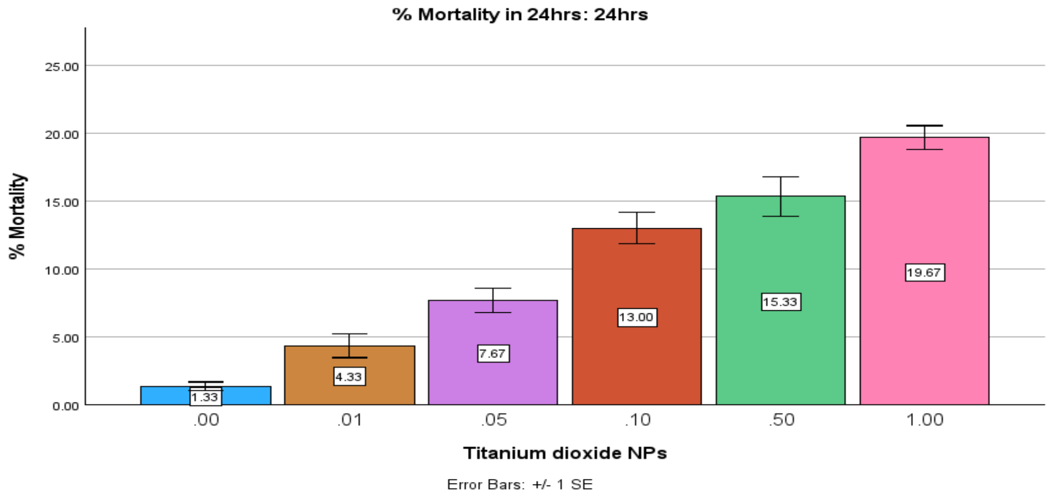

Figure 1: Mortality of Colurella adriatica exposed to TiO_2 NPS at acute dose-dependent rates after 24 h

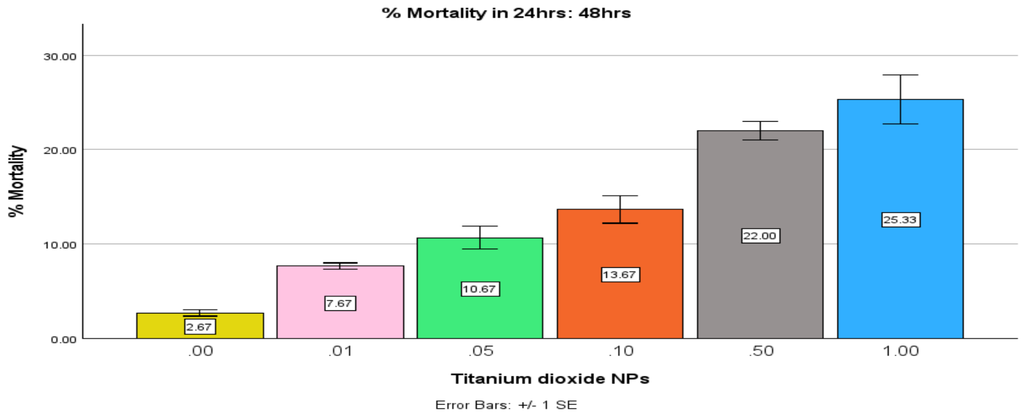

Figure 2: Mortality of Colurella adriatica exposed to TiO_2 NPS at acute dose-dependent rates after 48 hours.

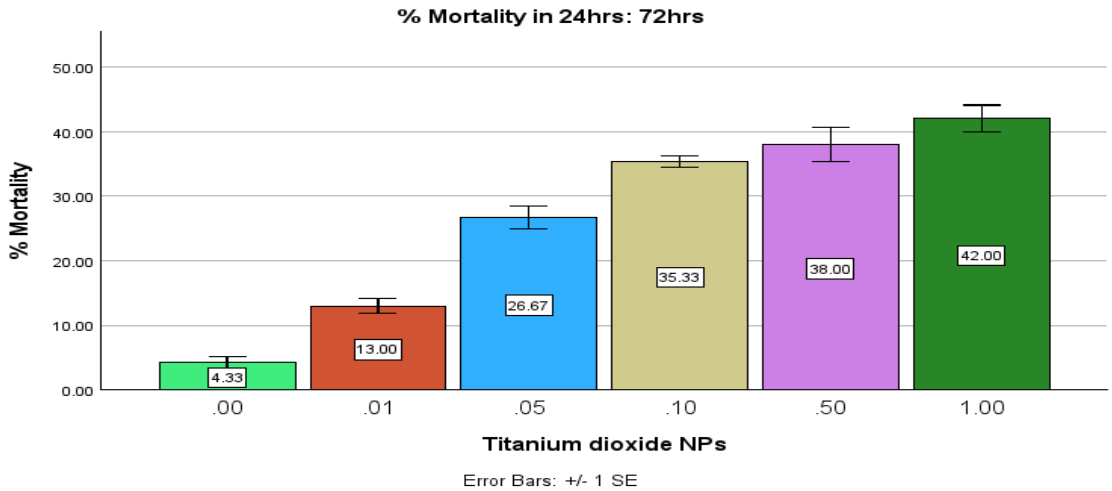

Figure 3: Mortality of Colurella adriatica exposed to TiO_2 NPS at acute dose-dependent rates after 72 hours.

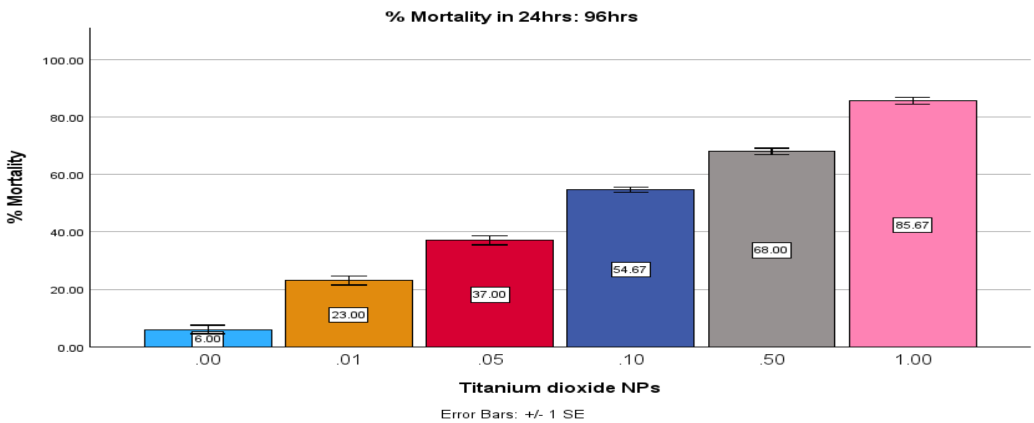

Figure 4: Mortality of Colurella adriatica exposed to TiO_2 NPS at acute dose-dependent rates after 96 hours.

Discussion

After 96 hours, *Colurella adriatica* mortality rates varied between concentrations, with the highest rate at 1.00 mg/l^{-1} of <25 nm TiO_2 nanoparticles (85.67%) and the lowest at 0.01 mg/l^{-1} of TiO_2 nanoparticles (23.00%). As a result, prolonged exposure of a water body to TiO_2 nanoparticles at these concentrations for more than 96 hours may lead to significant mortality rates in the aquatic ecosystem, affecting various organisms, such as phytoplankton, zooplankton, fingerlings, and small fish (Zhu et al., 2021; Zheng et al., 2023). The studies conducted by Ambroziak et al. (2020), Manchwari et al. (2022), and Bîrleanu et al. (2022) have also reported that TiO_2 nanoparticles (with sizes ranging from 25 nm to <100 nm) at concentrations below 3 mg/l^{-1} had minimal effects on Daphnid immobilization. TiO_2 has also been shown to enhance *D. magna* mortality as TiO_2 concentration increases (Kazemi et al., 2022). The toxicity of the test organisms increased dramatically when the duration was extended from 24 to 96 hours, resulting in an LC_{50} (0.075 mg/L). This demonstrated that exposure time significantly influenced the toxicological effects of TiO_2 NP_S on *Colurella adriatica*. This is in line with studies by Smith (2017), Grande et al. (2022) and Ferrante et al. (2023). Furthermore, the toxicity of TiO_2 nanoparticles under pre-illumination by simulated sunlight was found to be higher than that of non-illuminated TiO_2 nanoparticles. Research conducted by Li et al. (2019), Zeng et al., (2020) and Yang et al. (2023) corroborates this finding. In this study, the toxicity of NPs increased with exposure length, indicating that they may also play a key role in the toxicity mediated by NPs. Babaei et al. (2022) also observed that nanoparticles can travel through the ecosystem and affect fish at the top of the food chain. In a similar vein, Jones and Johnson (2019) studied the acute toxicity of TiO_2 nanoparticles on *Colurella adriatica* in a controlled laboratory setting. The researchers subjected *Colurella adriatica* to varied concentrations of TiO_2 nanoparticles and found dose-dependent effects on mortality and reproductive behaviour. The results showed that exposure to high concentrations of TiO_2 nanoparticles increased death rates and impaired reproductive success in *Colurella adriatica* populations.

Titanium dioxide nanoparticles exhibited toxicity to *Colurella adriatica*, with mortality reaching 85.67% as the concentration of the toxicant increased from 0.01 mg/l^{-1} to 1.00 mg/l^{-1}. After 96 hours of exposure, the LC_{50} was determined to be 0.075 mg/l^{-1}. These findings indicate that the release of TiO_2 NPs into aquatic environments may represent a risk to the health of rotifers, zooplankton and other aquatic creatures, as well as having negative impacts on them. It also may threaten the rotifers' fertility (population growth rate) by lowering their reproductive rates when the concentrations and duration of exposure to the toxicant, TiO_2, rise. As nanomaterials (NMs) grow more prevalent in everyday life, it is clear that the consequences of NMs, particularly TiO_2 NPs, on the environment have become a major source of worry. Furthermore, the release of NPs into freshwater ecosystems may put humans at risk of exposure through drinking water and food chains. This finding can be used to demonstrate the dose-dependent effects of Titanium oxide NPs on the survival, growth, and reproduction of *Colurella adriatica*. The acute toxicity of titanium dioxide nanoparticles (TiO_2 NPs) to *Colurella adriatica* poses substantial ecological risks for Nigerian freshwater ecosystems. As a keystone planktonic rotifer, *C. adriatica* plays a vital role in food web dynamics and nutrient cycling. Our findings demonstrate that TiO_2 NP exposure significantly impairs rotifer survival, which may trigger trophic cascades by reducing prey availability for higher organisms (Matouke et al., 2021). Such nanoparticle-induced population declines could destabilize aquatic community structures, as evidenced in other freshwater systems (Hou et al., 2020; Ganguly & Candolin, 2023). Furthermore, TiO_2 NP contamination may compromise water quality parameters (Rylsky et al., 2023), potentially affecting essential ecosystem services, including drinking water provision, agricultural use, and recreational activities. These findings underscore the need for stringent regulation of nanoparticle discharges in vulnerable aquatic habitats.

Furthermore, the toxicity of TiO_2 nanoparticles (NPs) to aquatic organisms is critically dependent on their physicochemical characteristics, particularly particle size, surface area, and coating properties (Chen & Li, 2020). Nanoparticles with smaller diameters and larger surface-area-to-volume ratios demonstrate enhanced toxicity due to increased biological reactivity and cellular uptake potential. While our acute toxicity studies with *Colurella adriatica* provide crucial baseline data on immediate nanoparticle effects, these findings represent just one component of comprehensive ecological risk assessment. A complete understanding of TiO_2 NP impacts requires complementary investigations including chronic exposure studies to identify sublethal and transgenerational effects, field-based validations to determine environmental bioavailability under natural conditions, and computational modelling to predict ecosystem-scale consequences. For Nigerian freshwater ecosystems, where *C. adriatica* serves dual roles as both a keystone species in food web dynamics and a sensitive bioindicator of water quality, addressing these knowledge gaps becomes particularly urgent. Implementing initiative-taking conservation strategies that combine targeted nanoparticle research, systematic environmental monitoring programmes, and evidence-based regulatory frameworks will be essential for safeguarding aquatic biodiversity and maintaining ecosystem services in vulnerable freshwater habitats.

Conclusion

In conclusion, this research has shed light on the acute toxicity of titanium dioxide nanoparticles (NPs) on *Colurella adriatica* Ehrenberg, 1831, providing valuable insights into the potential environmental impacts of these widely used nanoparticles. Through systematic experimentation and analysis, we have demonstrated that titanium dioxide NPs pose a significant threat to the survival and physiological integrity of *Colurella adriatica*, highlighting the importance of careful consideration and regulation in their use and disposal. These findings underscore the necessity for further research aimed at understanding the mechanisms underlying nanoparticle toxicity and developing effective mitigation strategies to safeguard aquatic ecosystems. As we continue to advance our understanding of nanoparticle toxicity, we must integrate this knowledge into regulatory frameworks and industry practices to ensure the sustainable coexistence of nanotechnology and environmental health.

Recommendations

Based on the findings from the study, the recommendations are as follows:

1. The acute toxicity of titanium dioxide nanoparticles in *Colurella adriatica* raises concerns about the wider impact on aquatic ecosystems and biodiversity. Adequate measures should be taken to prevent the use and wanton disposal of titanium dioxide nanoparticles.
2. There should be risk assessment and regulatory decision-making on the use and emission of titanium dioxide nanoparticles.
3. The complex challenges of nanoparticles require interdisciplinary collaboration between scientists, policymakers and stakeholders in the industry. This study recommends the integration of the expertise of various fields to develop an effective strategy for assessing and managing the risks of nanoparticles.
4. To protect the health of the environment, it is essential to educate the public about potential risks associated with nanoparticles and promote responsible use and disposal practices.
5. This study provides a foundation for future research on nanoparticle toxicity, including investigations into the mechanisms of toxicity, long-term effects on aquatic organisms, and potential interactions with other environmental stressors. It is essential to continue advancing our understanding of nanoparticle behaviour and its impacts on natural ecosystems.

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