RECENT STUDIES ON THE EFFECT OF TiO_2-NPs ON MARINE BIVALVES: UNVEILING POTENTIAL THREATS AND ECOTOXICOLOGICAL IMPLICATIONS

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SUMMARY

This review examines recent studies investigating the impact of TiO_2 nanoparticles (NPs) on marine bivalves, shedding light on potential threats and ecotoxicological implications. As TiO_2 NPs become ubiquitous in industrial and consumer products, concerns arise about their effects on crucial marine ecosystem components. The analysis delves into bioaccumulation, cellular responses and its consequences, emphasizing the need for understanding the intricate interactions between TiO_2 NPs and marine bivalves. By unveiling emerging threats and ecotoxicological implications, this article aims to inform scientists, policymakers, and stakeholders, guiding future research and facilitating measures to mitigate potential risks to marine ecosystems.

KEYWORDS

TiO_2 NPs, Marine molluscs, Oxidative stress, Bioaccumulation

1. INTRODUCTION

Marine bivalves are vital for marine ecosystem health, serving roles as filter feeders, scavengers, and prey, contributing to nutrient cycling and food webs. Concerns arise as engineered nanomaterials, particularly ubiquitous TiO_2 NPs, are increasingly used in various products, raising worries about their impact on marine life. Researchers are investigating the effects of TiO_2 NPs due to their wide application and unique physicochemical properties. The alarming rise in water contamination by TiO_2 NPs has prompted studies, revealing that their photocatalytic characteristics can induce harmful effects in organisms under sunlight exposure, including dysregulated cell signaling, cell damage, genotoxicity, inflammation, apoptosis, DNA damage, immune responses, and fibrosis (Sayes et al., 2006; Montiel-Dávalos et al., 2012; Shi et al., 2013; Fu et al., 2014).

Recent efforts have explored the impact of TiO_2 NPs on various aspects of marine mollusc biology and ecology (Liu et al., 2019 & Rocha et al. 2015). Xu et al. investigated the accumulation of NPs containing Ti, Cu, Zn, and Ag in different marine bivalves (Xu et al., 2020). NPs were detected in all five species studied, including oysters, mussels, scallops, clams, and ark shells, with the typical particle size of NPs ranging to 40-110 nm. NPs accumulated in all species due to their smaller size. Clams accumulated less NPs compared to other species. Gills and digestive glands were the primary sites of NP accumulation in all bivalves. Along with it few smaller NPs were found in the adductor muscle of every species. This study highlights the potential risk of NP exposure for various bivalves, with NPs posing the greatest concern due to their higher accumulation. The bioaccumulation of TiO_2 NPs in mussels that are consumed as food elevates the potential risks to both marine ecosystems as well as human health (Gallocchio et al., 2020). However, the majority of recent research has focused exclusively on bivalves among molluscs when investigating the impact of TiO_2 NPs on marine molluscs, it strongly suggests the detrimental effects of TiO_2 NPs exposure on marine ecosystems (Pikula et al., 2020; Li et al., 2021).

This review explores recent studies on TiO_2 nanoparticle interactions with molluscs, offering a comprehensive overview of exposure routes and their cellular effects. Through critical analysis, it identifies key research areas, aiming to provide valuable insights for scientists, policymakers, and stakeholders concerned about the ecotoxicological impact of TiO_2 NPs on marine ecosystems.

2. EFFECT ON *MYTILUS GALLOPROVINCIALIS*

Bivalve molluscs exposed to TiO_2 NPs experience oxidative stress, immunotoxicity, neurotoxicity, and genotoxicity, along with observable behavioural and physiological alterations (Abdel-Latif et al., 2020). Furthermore, the toxic impact of TiO_2 NPs on bivalve molluscs is influenced by interactions with various environmental pollutants, including metals and organic contaminants.

Exposure of habitats to the widely used antifouling agent diuron poses significant environmental toxicity, particularly for marine invertebrates (Bouzidi et al., 2021A). Concurrently, NPs function as carriers for organic pollutants in marine ecosystems, impacting the bioaccumulation and toxicity in exposed organisms. Exposure to diuron alone and diuron-ZnO NPs at 100 µg/L significantly reduced filtration capacity and respiration rates in the mussel *Mytilus galloprovincialis* compared to the control group. However, no significant effects were observed with TiO_2 NPs and diuron-TiO_2 NPs co-exposure. Diuron-TiO_2 NPs co-exposure diminished the activities of antioxidant enzymes compared to diuron alone, indicating induced physiological and oxidative pathways with varied responses in *M. galloprovincialis.*

Bouzidi et al. investigated the combined and individual effects of polycyclic aromatic hydrocarbons (PAHs) and nanoparticles (ZnO and TiO_2 NPs) on Mediterranean mussels *M. galloprovincialis* (Bouzidi et al., 2023). PAHs had a greater impact on physiology and biochemistry compared to NPs. Mussel tissues accumulated Ti and Zn from the respective nanoparticles, suggesting potential trophic transfer concerns. Interestingly, the presence of NPs influenced the uptake and toxicity of PAHs in mussels. Acetylcholinesterase (AChE) activity was affected by both contaminants and organ type, with gills being more sensitive to NPs than digestive glands. AChE (E.C.3.1.1.7) is a specialized enzyme with a dual role in hydrolyzing the neurotransmitter ACh and participating in the maturation and regeneration of neurons. Consequently, AChE activity is commonly considered an indicator of neurotoxicity (Jebali et al., 2013). ZnO NPs caused a significantly greater decrease in AChE activity compared to TiO_2 NPs in gills. The authors propose that the observed AChE inhibition might be linked to the generation of hydroxyl radicals by NPs, leading to the oxidative breakdown of PAHs. Additionally, the decrease in some PAH concentrations in the presence of NPs could be attributed to photocatalytic degradation (Bouzidi et al., 2021B).

Gallocchio et al. investigated the combined effects of TiO_2 NPs and non-dioxin-like polychlorinated biphenyls (ndl-PCBs) on *M. galloprovincialis* (Gallocchio et al., 2023). TiO_2 NPs themselves didn't affect the overall accumulation of ndl-PCBs in the mussels. However, combining TiO_2 NPs with ndl-PCBs significantly increased the toxic effects on the mussels compared to ndl-PCBs alone. After a depuration period, most TiO_2 NPs were eliminated, while their presence led to a greater elimination of ndl-PCBs compared to the ndl-PCB-only group. Although TiO_2 NPs may not directly increase pollutant accumulation, it interact with existing pollutants like ndl-PCBs and enhance their toxicity and depuration.

Building on their previous research, this group investigated the impact of gold-doped TiO_2 and ZnO NPs on *M. galloprovincialis* (Bouzidi et al., 2024). Undoped NPs caused membrane damage, neurotoxic effects, and antioxidant enzyme activity change in the gills and digestive glands, depending on concentration. Notably, gold-doped nanoparticles affected AChE activity in mussel tissues, suggesting additional neurotoxicity in the digestive gland at 100 µg/L compared to undoped counterparts. This finding implies that gold-doped TiO_2 NPs, in particular, exhibit stronger neurotoxic tendencies towards *M. galloprovincialis.*

Leite et al. studied how two different forms of TiO_2 crystal (rutile and anatase) impact the health of *M. galloprovincialis* (Leite et al., 2020A). Both forms caused similar levels of titanium accumulation in the mussels, but their effects differed. Rutile exposure maintained the mussels' metabolism and triggered effective detoxification, preventing cellular damage. In contrast, anatase exposure increased metabolism and oxidative stress, leading to cellular damage despite increased antioxidant defenses. This suggests that the toxicity of TiO_2 NPs in mussels is more related to their crystal structure than size and that anatase poses a greater risk due to its oxidative potential. Whereas at 18°C rutile TiO_2 NPs didn't show lipid proliferation or cellular damage on *Mytilus galloprovincialis* (Leite et al., 2020B).

3. EFFECT ON *MYTILUS CORUSCUS*

Hu et al. investigated how elevated temperature and different crystal structures of TiO_2 NPs affect the gut microbiome of mussels in *Mytilus coruscus* (Li et al., 2024A). Mussels were exposed to various forms of TiO_2 NPs (rutile, anatase, P25) at 20°C and 28°C for 14 days. Compared to 20°C, NP agglomeration increased at 28°C. Exposure to TiO_2 NPs increased mussel mortality and altered the relative abundance of gut microbiome such as *Bacteroidetes, Proteobacteria, Firmicutes*. Interestingly, mussels exposed to anatase NPs associated with lower mortality and showed increased levels of potentially beneficial symbionts (Tenericutes, Fusobacteria). Finally, the combined stress from warming and TiO_2 NPs might heighten the risk of infection by pathogenic bacteria in mussels.

Liu et al. investigated how three metal oxide (TiO_2, ZnO, and Fe_2O_3) NPs impact the production and strength of byssal threads in the thick-shelled mussel *M. coruscus* (Shi et al., 2020). Byssal threads are crucial for the attachment of bivalves to a solid surface. While TiO_2 NPs had the least impact, it still caused a significant decrease in overall byssal attachment strength (20-30%). Interestingly, TiO_2 NPs didn't affect toughness or breaking stress, suggesting a different mechanism of action compared to the other nanoparticles. The weakening properties of byssal threads after exposure to NPs could be attributed to downregulated gene expression of either precursor collagen proteins, mussel foot proteins, and proximal thread matrix proteins or their combined effects.

Similarly, Wang group investigated how short-term dietary exposure to warming, TiO_2 NPs, and their combination affects the byssal thread performance of mussels *M. coruscus* (Li et al. 2023). Both temperature and TiO_2 NPs exposure weakened the mechanical strength of byssal threads. Surprisingly, the number and length of byssal threads increased with exposure. The gene expression analysis revealed the upregulation of proteins involved in byssus production, suggesting a compensatory response. However, combined exposure of warming and TiO_2 NPs downregulated key collagen genes, indicating potential limits to this response. This suggests that while mussels initially try to adapt to stress by producing more byssal threads, prolonged exposure, especially in combination, compromise their attachment ability and survival.

Kong et al. tested the dynamic changes in digestive enzyme activities in *M. coruscus* exposed to combined ocean acidification (OA) and TiO_2 NPs (Kong et al., 2019). During exposure, low pH is an important factor in the activity of digestive enzymes of M. coruscus, especially pepsin, trypsin and lipase. The digestive capacity of *M. coruscus* is highly sensible to the hypercapnia environment, which is obvious in this global climate change scenario. The variations in results could be attributed to the accumulation of TiO_2 NPs in the mussels' digestive gland, leading to immunotoxicity, oxidative stress, and cellular damage to proteins, membranes, and DNA (D'Agata et al., 2014). Consequently, this heightened sensitivity of digestive enzyme activities to acidification is observed. Additionally, there is an increase in activity was observed in digestive enzymes at the beginning of the exposure period; indicating that OA initially stimulates the mussels, because the optimal pH of digestive enzymes is lower than the actual pH in the digestive gland.

Subsequently, the collective impact of TiO_2 NPs and OA on the specific dynamic action (SDA) of mussels M. coruscus was examined (Shang et al., 2020). Under conditions of seawater acidification or exposure to TiO_2 NPs, various metabolic parameters such as standard metabolic rate, aerobic metabolic scope, SDA slope, and SDA showed a significant decrease. Conversely, peak metabolic rate, time to peak, and SDA duration exhibited a noteworthy increase. Furthermore, the adverse effects of ocean pH and TiO_2 NPs were interactive as evident in SDA parameters, except for time to peak and SDA. Consequently, the combined influence of TiO_2 NPs and low pH could negatively impact the digestive metabolism of mussels.

TiO_2 NPs has the potential to serve as a carrier for organic compounds like pentachlorophenol (PCP), thereby presenting a possible risk to marine ecosystems. Sun et al. investigated how PCP and TiO_2 NPs affect the energy metabolism of *Mytilus coruscus* in the presence of a predator *Portunus trituberculatus* (Sun et al., 2024). Both pollutants and NPs disrupt the cellular energy allocation of mussels, leading to decreased energy reserves and increased consumption. This forces predators to consume more prey, potentially impacting the entire ecosystem. The study highlights the long-term consequences of pollutants on marine ecosystems, including weakened health, reduced biodiversity, and disrupted predator-prey interactions.

Wang group further added the details on the interactive effects of TiO_2 NPs, PCP, and predation risk on antioxidant and immune responses in mussels (Wei et al., 2023). Individual exposure to TiO_2 NPs or PCP disrupted the antioxidant system and immune function, causing stress. Larger TiO_2 NPs (≈100 nm) induced higher toxicity than smaller ones (25 nm). Combining TiO_2 NPs and PCP worsened antioxidant imbalances and immune stress compared to individual exposures. Additionally, the predation risk further amplified the negative effects of combined stressors. The study highlights how multiple environmental stressors can have synergistic impacts, with TiO_2 NPs exacerbating the toxicity of PCP and predation risk further magnifying these effects.

Meanwhile, anthropogenic CO_2 emissions have caused a reduction of seawater pH. Hu et al. investigated how ocean acidification and TiO_2 NPs exposure interact to affect the health of mussels *M. coruscus* (Hu et al., 2017). Both high CO_2 and TiO_2 NPs exposure harmed the mussels' health, but TiO_2 NPs had a stronger negative impact. This was evident in reduced activity, feeding efficiency, and growth potential. Interestingly, combining high CO_2 and TiO_2 NPs exposure produced synergistic effects, further worsening the mussels' condition. Even at low CO_2 levels, TiO_2 NPs can significantly harm mussels, and the two stressors together can have even more severe consequences.

4. EFFECT ON OTHER BIVALVES

Xia et al. investigated to assess the neurotoxic effects of TiO_2 NPs on marine scallops, *Chlamys farreri* (Xia et al., 2017). An unintentional discovery was revealed during the examination of various biomarkers in *C. farreri*, which involved a significant increase in acetylcholinesterase (AChE) activity in the scallops after 14 days of exposure to TiO_2 NPs. Therefore, the observed change in AChE activity strongly suggests notable neurotoxic effects of TiO_2 NPs on the scallop *C. farreri*. TiO_2 NPs induced severe oxidative stress and neurotoxicity in the gill and digestive glands of the scallops. A time-dependent activity pattern was observed with these biomarkers after exposure to TiO_2 NPs. Additionally, histopathological alterations such as dysplasia and necrosis were observed in the digestive gland and gill of the scallop, possibly attributed to oxidative damage induced by exposure to TiO_2 NPs. These findings highlight the potential of the integrated biomarker response (IBR) approach in providing evidence for the risk assessment of nanoparticles on marine.

In this context, Liu group demonstrated the neurotoxic effect of TiO_2 in blood clam *Tegillarca granosa* (Guan et al., 2018). This neurotoxicity is characterized by an increase in in vivo neurotransmitter concentrations, the suppression of acetylcholinesterase (AChE) activity, and the downregulation of genes associated with neurotransmitters. These effects have the potential to disrupt various physiological processes. The quantitative polymerase chain reaction (qPCR) results indicated a noteworthy suppression in the expression of GABAT, MAO, and AChE after 96 hours of exposure to TiO_2 NPs. GABAT, MAO, and AChE are modulatory enzymes responsible for neurotransmitters dopamine (DA), gamma-aminobutyric acid (GABA), and acetylcholine (ACh), respectively. These enzymes play a role in decomposing corresponding neurotransmitters through hydrolysis (Jebali et al., 2013). The inhibited activity of these modulatory enzymes, including AChE studied in this research, may impede the deprivation of neurotransmitters, leading to an increase in their in vivo concentrations.

Marin et al. studied the effects of zinc oxide ZnO, TiO_2, and C_{60} fullerene (FC-60) NPs, alone and in the mixture, on the clam *Ruditapes philippinarum* (Marisa et al., 2022). Clams were exposed to 1 μg/L concentrations of each nanoparticle for 7 days. The haemolymph biomarker study demonstrated the increased bioaccumulation of all nanoparticles in all tissues, linked to oxidative stress and damage to proteins, lipids, and DNA. The proteomic analysis revealed changes in protein abundance and damage, particularly in the cytoskeleton and energy metabolism pathways. The NPs mixture exhibited the most adverse effect on the digestive gland compared to gill, showing increased Ti and FC-60 accumulation but reduced Zn accumulation compared to single exposures.

Li et al. evaluate the physiological responses to TiO_2 NPs exposure in pearl oysters *Pinctada fucata martensii* (Li et al., 2024B). Pearl oysters exposed to TiO_2 NPs for 14 days showed reduced activity of digestive enzymes like protease, amylase, and lipase, suggesting hindered energy metabolism. It also enhanced the antioxidant response by increasing the activity of enzymes like CAT, SOD, and GPx, indicating oxidative stress. TiO2 NPs exposure leads to heightened activity of lysozyme, ACP, and AKP, implying immune system activation. Consequently, an impaired biomineralization was observed implying irregular nacreous layer structure in shells, potentially affecting pearl quality. Even after 7 days without exposure, recovery of cells was limited and many effects persisted, particularly for higher concentrations, which indicates that TiO2 NPs have negative impacts on pearl oyster health and pearl quality**.** They disrupt digestion, stress the immune system, and hinder nacre formation, even at low concentrations.

Concerns regarding the environmental impact of TiO2 nanoparticles (NPs) extend to their influence on the toxicity and bioavailability of arsenic (As), disrupting the equilibrium of As accumulation and speciation in organisms. Qian et al. (2020) investigated the effects of TiO2 NPs on the bioaccumulation and biotransformation of arsenate (As(V)) in *Perna viridis* mussels. (Qian et al., 2020) TiO2 NP exposure increased arsenate bioconcentration, likely due to As adsorption onto TiO2 NPs. Notably, it impeded the biotransformation of inorganic to organic As-species, resulting in inorganic As-enriched mussels. TiO2 NPs hindered As methylation in mussels, evidenced by suppressed glutathione S-transferase (GST) activity and glutathione S-conjugates (GSH) content, indicative of impaired As metabolism. Adsorption on TiO2 NPs altered arsenic distribution and reduced biotransformation, intensifying arsenic toxicity in marine organisms by limiting its methylation and increasing its inorganic form.

The Fernandes group investigated the correlation between exposure time and the toxic effects of copper oxide (CuO) and TiO_2 NPs in both pure form as well as modified NPs with differentially functionalized surface such as (polyethylene glycol [PEG], carboxyl [COOH], and ammonia [NH_3]) in the bivalve *Mytilus spp* (Connolly et al., 2022). High-throughput in vitro testing during Tier 1 (exposure of 48 hours) of this toxicity assessment revealed that CuO NPs induced cytotoxic effects on the lysosomes of hemocytes in mussels, with the hazard potential ranking as CuO PEG > CuO COOH > CuO NH_3 > CuO core. In contrast, TiO_2 NPs were not found to be cytotoxic compared to CuO NPs. Genotoxicity was observed in mussel hemocytes and gill cells after in vivo exposure to CuO NPs (upto 48 hours). Longer in vivo exposures (48 hours to 21 days) in Tier 2 unveiled subacute and chronic oxidative outcomes of both CuO and TiO_2 NPs, with lipid peroxidation occurring in some cases (specifically with core TiO_2 NPs). In Tier 3, bioaccumulation of these NPs (mainly in gills for Cu and in digestive glands for Ti) was observed, leading to an increase in superoxide dismutase (SOD) levels in cells and causing severe toxic effects in the organism through oxidative stress responses in the respective glands.

Pikula et al. compared the toxic effects of 10 different nanoparticles, including TiO_2 NPs, on the hemocytes (blood cells) of three marine bivalve species, namely, *Crenomytilus grayanus, Modiolus modiolus, and Arca boucardi* (Pikula et al., 2020). Short-term exposure of these NPs to the hemocytes followed by estimation of viability and changes in polarization of cell membranes through flow cytometry exhibited that metal-based nanoparticles were the most toxic to all three species and each species showed varying sensitivity to different nanoparticle types. Additionally, NPs induced rapid cell membrane depolarization, indicating an early toxic response. This study raises concerns about the potential chronic threat of even "safe" nanoparticles to aquatic organisms.

5. CONCLUSION

This review highlights recent findings and notable concerns regarding the effects of bioaccumulation of TiO_2 NPs in marine bivalves and their ecosystem. While TiO_2 NPs may not exhibit acute toxicity, their photocatalytic property induces intracellular oxidative stress. TiO_2 NPs have a natural tendency to accumulate and settle at the bottom, but a substantial fraction may persist in the water column, posing risks to resident organisms. The compiled evidence underscores the widespread impact of TiO_2 NPs on bivalves, revealing their potential toxicity in terms of growth inhibition, and cellular functions. The intricate interplay between TiO_2 NPs and organic pollutants along with atmospheric conditions such as pH and temperature play an crucial role on the effects on marine life. Internalization of TiO_2 NPs into cells affects cellular structures, unveiling complex molecular mechanisms.

The cumulative findings strongly support the need for comprehensive monitoring programs, regulatory frameworks, and environmentally friendly alternatives to mitigate potential ecological risks associated with the widespread use of TiO_2 NPs. Embracing sustainable practices and environmental stewardship, ongoing research efforts are crucial to unravel the nuances of TiO_2 NPs toxicity in marine aquatics and guide informed conservation strategies for protecting valuable marine ecosystems.

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