# RECENT FINDINGS IN ADVERSE EFFECTS OF $TiO_2$ NPs IN MARINE ALGAE AND ZOOPLANKTONS: A THREAT TO MARINE ECOSYSTEMS

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## SUMMARY

The rapid advancement of nanotechnology has boosted the applications of $TiO_2$ nanoparticles ($TiO_2$ NPs) in various industries, resulting in their release into marine environments. This review article provides a comprehensive overview of recent findings on the adverse effects of $TiO_2$ NPs in marine algae and zooplankton. Special attention is given to the underlying mechanisms of toxicity, including oxidative stress, genotoxicity, and disruptions in cellular processes. This review consolidates recent scientific evidence to underscore the emerging concerns surrounding the adverse effects of $TiO_2$ NPs in marine aquatics, emphasizing the urgency of further research and the implementation of precautionary measures to protect marine ecosystems from potential harm.

## KEYWORDS

$TiO_2$ NPs, Algae, Zooplankton, Oxidative stress, ROS

## 1. INTRODUCTION

Titanium dioxide in the form of nanoparticles ($TiO_2$ NPs) has become an important part of our daily lives in drug delivery, medical care, biosensors, cosmetics, paints and coatings, plastics, skin care products, food, water, and medicine (Vance et al., 2015). The inclusion of surface-modified nano-$TiO_2$ in acrylic nanocomposite paint significantly enhances its antifouling performance, making it particularly valuable for coatings on the exteriors of ships (Zhang et al., 2016). Nanoparticulate $TiO_2$ exhibits phototoxicity to cells in vitro, leading to its utilization in wastewater disinfection (Theron et al., 2008; Zhang et al., 2010) and exploration as an anti-cancer agent (Rozhkova et al., 2009). Given its widespread application and production, $TiO_2$ NPs rank among the most emergent contaminants, potentially posing broad ecological impacts in marine ecosystems (Sendra et al., 2017a; Sendra et al., 2017b; Miller et al., 2012). $TiO_2$ NPs are found at higher concentrations in surface waters (3.0 ng $L^{-1}$ in freshwater, 0.30 ng $L^{-1}$ in seawater) compared to sediments (1.2 ng $kg^{-1}$ in freshwater, 0.39 ng $kg^{-1}$ in marine environments), indicating a preference for suspension in water columns over settling (Boxall et al., 2007; Gottschalk et al., 2015).

The concerning escalation of $TiO_2$ NPs contamination in water has captured the attention of researchers, prompting an exploration into its impact on organisms. With its photo-catalytic properties, $TiO_2$ NPs can generate various adverse effects in organisms when exposed to sunlight, including dysregulated cell signalling, cell damage, altered cell motility, genotoxicity, DNA damage, pulmonary inflammation, apoptosis, abnormal immune responses, and fibrosis (Sayes et al., 2006; Montiel-Dávalos et al., 2012; Shi et al., 2013; Fu et al., 2014). Therefore, $TiO_2$ NPs are classified as "hazardous" against organisms such as algae, bacteria, crustaceans, fish, nematodes and yeast, with LC50 values ranging from 10 to 100 mg $mL^{-1}$ (Kahru & Dubourguier, 2010). Consequently, numerous ecotoxicological review articles have explored the impact of $TiO_2$ NPs on marine organisms, uncovering their toxicity to species such as invertebrates, cyanobacteria, and polychaetes (Shah et al., 2017; Baker et al., 2014; Canesi & Corsi 2016).

Despite existing research, ongoing publications highlight the need for a resource to keep researchers updated and organized in this rapidly evolving field. In this context, we have provided a summary of recent articles, particularly those published after 2017, detailing the adverse effects of $TiO_2$ NPs on marine organisms (Figure 1).

## 2. EFFECT ON ALGAE

Biofouling poses a significant challenge in marine water systems, affecting various components like immersed leisure vessels, marine sensors, heat exchangers, and ship hulls. This issue arises due to the adherence of barnacles,

 

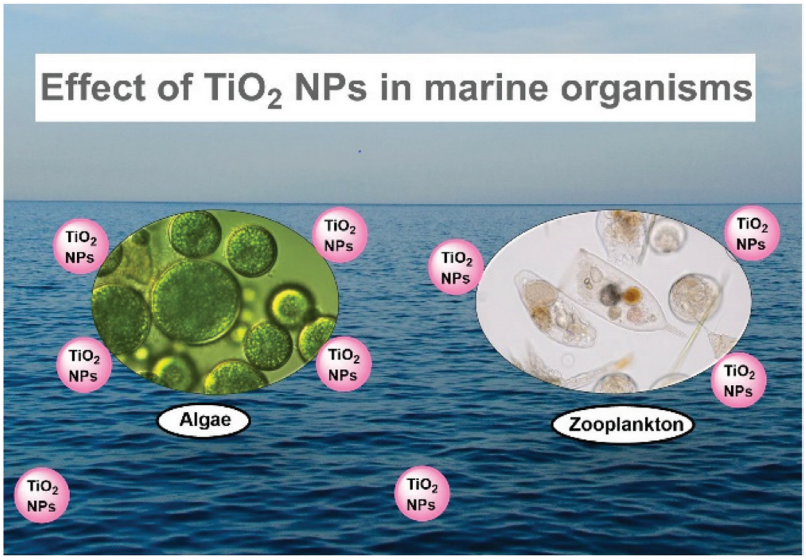


Figure 1. $TiO_2$ NPs in marine aquatics: A threat to ocean ecosystem

mussels, bacteria, as well as micro and macroalgal species. To combat biofouling, films and membranes incorporating metal nanoparticles have been utilized. The incorporation of nanoparticles (NPs) into antifouling membranes serves two primary purposes (Kim & Van der Bruggen, 2010; Xu et al., 2009). Firstly, it facilitates bonding between polymer chains, solvents, and the NP surface, creating membranes embedded with nanoparticles. These modifications on film and membrane play an important role in ultrafiltration and nanofiltration, providing high selectivity and permeability for gas separation. Secondly, nanoparticles can be modified to appear hydrophilic and contain functional groups to reduce membrane fouling by aquatic organisms.

In practice, nanoparticles of various metal oxides including titanium, silver, silicate, attapulgite, zeolite, carbon nanotubes, and graphene are blended with polymer paints to enhance the effective antifouling activity of the material (Kumar et al., 2019; Kumar et al., 2020; Kumar et al., 2021). Due to its photo-catalytic activity, $TiO_2$ NPs can generate a substantial amount of reactive oxygen species (ROS) when exposed to light of UV or near-UV wavelengths. A study focused on biofouling inhibition in the marine microalgal species *Dunaliella salina*, reveals that $TiO_2$ NPs can produce ROS in exposure to near ultra-violate radiation, which can brutally damage algal cells, preventing slime formation (Natarajan et al., 2018). The mechanism underlying cell damage involves oxidative radicals interacting with the cell membrane, leading to increased permeability and the leakage of small molecules and ions from the cell. Ultimately, this process results in the degradation of cellular components and, consequently, cell death. The accumulation of nanoparticles in the cell membrane contributes to toxicity through cell wall damage and hetero-agglomeration.

In a study on *Dunaliella tertiolecta*, $TiO_2$ NPs did not hinder cell growth or influence cellular pigment content at concentrations of up to 10 mg $L^{-1}$ over a 72-hour exposure (Morelli et al., 2018). Rapid aggregation and sedimentation of $TiO_2$ NPs above a certain concentration efficiently limited their toxicity to *D. tertiolecta*. Intracellular ROS generation increased with $TiO_2$ NP exposure, coinciding with a temporary rise in catalase enzyme (CAT) activity, crucial for scavenging harmful oxygen radicals. The subsequent decline in ROS generation aligned with CAT activity restoration. $TiO_2$ NPs interacting with exopolymeric substances (EPS) released by microalgae, particularly binding to proteins, revealed their role in marine environment stability and enjoyment.

Hu et al. investigated the effects of $TiO_2$ NPs on marine microalgae *I. galbana* (Hu et al., 2018). Exposure to up to 500 mg/L showed minimal harm to algal cells in size and reproduction. High $TiO_2$ NP levels partially obstruct light, reducing chlorophyll production in algae. Aggregated $TiO_2$ particles could adsorb on the surface of algae, forming $TiO_2$–algae complexes that might engage in ligand-to-metal charge transfer reactions, leading to the oxidation of algal cell walls through ROS generation. ROS generation increased with $TiO_2$ NP levels, potentially contributing to an overall elevation in phototoxicity (Miller et al., 2012).

A recent study by Xia et al. explored how ocean acidification (OA) alters the effects of $TiO_2$ NPs on marine microalgae (Xia et al., 2018). They exposed the microalga *Chlorella vulgaris* to TiO2 NPs under two simulated OA conditions (pH 7.77 and 7.47) and compared it to a control group with normal pH. Under acidic conditions, the algae exposed to $TiO_2$ NPs suffered significantly higher levels of oxidative stress, indicating cellular damage and an intensified

inhibition of algal cell growth. This was likely due to a synergistic effect between the inherent stress of OA and the stress induced by the NPs on algal cells. The piercing toxicity was attributed to increased internalization of NPs by cells, stemming from a reduction in aggregation and the elevated level of suspended NPs in acidified seawater. Consequently, the findings suggest that OA could raise the threat of NPs to marine ecosystems.

In their investigation into the prolonged exposure of $TiO_2$ NPs on *Chlorella sp.* microalgae under UV-A irradiation, the Mukherjee group observed a reduction in toxicity due to NP aggregation and sedimentation in aquatic environments (Thiagarajan et al., 2019a). The three-cycle experiment involved setting $TiO_2$ NPs at 62.6 mM in the first cycle, leading to substantial aggregation and a subsequent decrease in bioavailability. This aggregation, attributed to self-interactions (homo-aggregation) and interactions with algal cells (hetero-aggregation), resulted in gravitational sedimentation. Cellular viability, initially at 24.2 ± 2.5% after cycle I, increased to 96.5 ± 2.8% after cycle III during continuous exposure. The internalisation of titanium (Ti) was found to be dependent on particle size, with smaller-sized particle aggregates able to traverse the algal cell wall. Notably, $TiO_2$ NPs exhibited a higher degree of internalisation compared to extracted $TiO_2$ and bulk particles with sub-micron and micron sizes. The overall impact on *Chlorella sp.* followed the sequence: $TiO_2$ NPs > Extracted $TiO_2$ > Bulk $TiO_2$, indicating a direct proportionality between $TiO_2$ toxicity and the concentration of Ti within the cell (Thiagarajan & Ramasubbu, 2022).

In their investigations on the impact of secondary pollutants on P-25 $TiO_2$ NPs in seawater algae *Chlorella sp.,* Thiagarajan et al. (2019b, 2019c) explored differently functionalized polystyrene microplastics (PMPs) (Thiagarajan et al., 2019b) and antibiotic-tetracycline (TC) (Thiagarajan et al., 2019c). The study focused on $NH_2$-functionalized PMP ($NH_2$-PMP), COOH-functionalized PMP (COOH-PMP), and plain PMP. Plain PMP and $NH_2$-PMP heightened the toxicity of $TiO_2$ NPs, while COOH-PMP mitigated it. The toxic mechanisms of plain PMP and $NH_2$-PMP involved the generation of hetero-aggregates, potentially damaging algal cell membranes and facilitating $TiO_2$ NPs internalization. Another possible mechanism suggested that caveolae on the algal cell surface adsorbed MPs, limiting nutrient uptake and restricting contact with the external environment. $NH_2$-PMP's higher affinity to lipid bilayers led to a decline in algal growth. Conversely, the interaction between COOH-PMP and $TiO_2$ NPs resulted in aggregation and sedimentation, producing an antagonistic effect. TC did not induce toxicity at lower $TiO_2$ NP concentrations but significantly enhanced toxicity at a threshold concentration of 4 mg $L^{-1}$, consistent with antioxidant enzyme activity and oxidative stress caused by ROS generation in Chlorella sp. from chloroplast and mitochondria.

In contrast to microplastics (MPs), differently functionalized polystyrene nanoplastics (PNPs) rapidly associated with $TiO_2$ NPs, leading to hetero-aggregation between various particle types that exacerbated aggregate formation, increased particle settling and reduced bioavailability of substance in the medium (Thiagarajan et al., 2022a). In photosynthetic seawater organisms including microalgae, PNPs and $TiO_2$ NPs induce cellular oxidative stress in cells. However, when $TiO_2$ NPs and PNPs act in combination, the declined bioavailability of toxic substances results in reduced ROS generation within the cells. ROS not only affect algal photosynthesis but also cause algal membrane lipid peroxidation. Therefore, mixture of PNPs with $TiO_2$ NPs helps in reduction of ROS generation significantly which mitigates the observed toxic effects. Furthermore, when PNPs are combined with nano-$TiO_2$, there is an observed decline in antioxidant enzyme activities. Consequently, $TiO_2$ NPs in the presence of PNPs exert significantly reduced cytotoxic effects on *Chlorella sp.* compared to the effects observed with corresponding pristine particles.

In a distinct investigation, the toxicity effects of four nanohybrids (NHs) namely, ZnO-fuesed carbon nanotubes (CNTs), ZnO-fuesed graphene oxide (GO), $TiO_2$- fuesed GO, and $TiO_2$-fuesed CNT were examined on *Thalassiosira pseudonana*, a marine diatom (Baek et al., 2020). The in vitro evaluation of adverse effects of the synthesized NHs on *T. pseudonana* revealed increased toxicity with higher concentration and prolonged exposure time of NH. The order of toxicity significance was found to be ZnO-GO > ZnO-CNT > $TiO_2$-GO > $TiO_2$-CNT. NHs based on graphene oxide (GO) exhibited less aggregation and better dispersion compared to those based on carbon nanotubes (CNTs). The well-dispersed NHs are likely to possess more reactive sites and larger surface areas. The observed toxicity of NHs is thought to result from the generation of ROS, damage to cell membranes caused by NH attachment, and the shading effect of NHs preventing algae photosynthesis.

Baharlooeian et al. investigated the toxic effects of $TiO_2$ NPs and bulk $TiO_2$ on the marine diatom *Chaetoceros muelleri* (Baharlooeian & Haq, 2020). The chlorophyll a content in samples exposed to both $TiO_2$ NPs and bulk $TiO_2$ exhibited a rapid initial decrease compared to the control between third to sixth days, followed by a gradual reduction. Notably, bulk $TiO_2$ demonstrated lower toxicity in comparison to $TiO_2$ NPs. The decline in chlorophyll content due to exposure to both nano and bulk $TiO_2$ suggests the occurrence of shading effects. Therefore, the content of photosynthetic pigments such as chlorophyll a serves as a sensitive and efficient indicator of toxicity and the growth status of algae. Furthermore, the results indicated that the antioxidant values in samples treated with NPs were higher than those treated with bulk $TiO_2$ compared to the control. This further suggests elevated

 

oxidative stress, signifying the increased toxicity of $TiO_2$ NPs compared to bulk $TiO_2$.

The potential harm of TiO2 nanoparticles (NPs) to marine microalgae is intensified by heightened UV radiation due to their photocatalytic properties. Zhu et al. explored the bioavailability and toxic effects of $TiO_2$ NPs on *Chlorella pyrenoidosa* under UV-B radiation (Zhu et al., 2022). Despite UV-B radiation accelerating $TiO_2$ NP settling, the remaining suspended particles had smaller hydrodynamic diameters, leading to more pronounced growth inhibition, indicating a size-related effect. A dose-dependent antagonistic interaction between $TiO_2$ NP concentrations and UV-B radiation reduced extracellular polymeric substances (EPS) production, facilitating increased entry of $TiO_2$ NPs into algal cells. Internalized $TiO_2$ NPs under UV-B radiation-induced intracellular ROS, causing lipid peroxidation and severe cellular damage. The combination of $TiO_2$ NPs and UV-B radiation inhibited photosynthesis in *C. pyrenoidosa*, attributed to the dual impact of oxidative damage and shading by NPs. In summary, the diminished protection of algal cells by EPS contributed to enhanced $TiO_2$ NP internalization, identified as the primary mechanism behind heightened toxicity under UV-B radiation conditions.

Bameri et al. investigated the impact of $TiO_2$ NPs on the microalgae *Tetraselmis suecica*, crucial for the ecosystem (Bameri et al., 2023). Concentrations ranging from 5 to 400 mg/L were applied for 10 days, revealing growth inhibition and decreasing chlorophyll a and b content with higher $TiO_2$ NP concentrations. The shading effect from nanoparticles reduced photosynthetic productivity due to limited light penetration. Internalized $TiO_2$ NPs induced ultrastructural damage to chloroplasts, potentially deactivating enzymes and pigments in the thylakoid system, leading to impaired photosynthesis and metabolism. Protein and lipid content significantly declined on the tenth day, inversely correlated with increasing $TiO_2$ NP concentrations. Overall, $TiO_2$ NPs adversely affected *Tetraselmis suecica* stocks, highlighting their toxic impact on this ecologically important microalga (Middepogu et al., 2018).

The study by Zhu et al. explored the molecular mechanisms of $TiO_2$ NP toxicity on Chlorella pyrenoidosa under UV-B radiation (Zhu et al., 2024). $TiO_2$ NPs intensified growth inhibition and internalized into cells, damaging the cell membrane. Transcriptome analyses revealed protective responses including EPS secretion and upregulation of photosynthesis and energy metabolism genes. Despite ROS generation, antioxidant genes were not upregulated, indicating a compensatory response by the algae to maintain vitality under dual stress.

## 3. EFFECT ON ZOOPLANKTON

Lu et al. investigated the impact of $TiO_2$ NPs on the toxicity of phenanthrene (Phe) and cadmium ($Cd^{2+}$) towards the marine zooplankton *Artemia salina* (Lu et al., 2018). While $TiO_2$ NPs showed minimal toxicity on *A. salina*, they significantly influenced the mortality and immobility induced by $Cd^{2+}$ and Phe. At a lower concentration of 5 mg $L^{-1}$, $TiO_2$ NPs enhanced $Cd^{2+}$ and Phe toxicity by 57.5% and 2.0%, suggesting a synergistic effect attributed to increased bioaccumulation. Conversely, at 400 mg/L, $TiO_2$ NPs reduced Phe and $Cd^{2+}$ toxicity by 24.5% and 57.1%, indicating an antagonistic effect likely caused by severe aggregation and reduced specific volume adsorption capacity of $TiO_2$ NPs. The higher impact of $TiO_2$ NPs on $Cd^{2+}$ toxicity compared to Phe may result from $Cd^{2+}$ desorption in *A. salina's* intestine and its bridging effect on $TiO_2$ NPs' aggregation/accumulation behaviour.

*Artemia salina*, as non-selective filter feeders with remarkable reproductive capacity, are highly sensitive to low contaminant concentrations and environmental stress. Investigating potential toxic modulation, the Mukherjee group studied interactions between $TiO_2$ NPs and Cr(VI) on *A. salina* (Thiagarajan et al., 2020). In artificial seawater, $TiO_2$ NPs agglomerated into micron-sized particles, settling rapidly, and Cr(VI) addition further enhanced agglomeration by sorbing onto $TiO_2$ NPs. Mortality data indicated antagonistic toxicity effects from both mixture groups, with $TiO_2$ NPs preventing mixture synergy by adsorbing Cr(VI), resulting in an antagonistic effect. Mortality depended on initial $TiO_2$ NPs uptake, and Cr(VI) adsorption to increasing $TiO_2$ NPs concentrations reduced Cr(VI) bioavailability, diminishing $TiO_2$ NPs toxicity. Conversely, strong Cr(VI) adsorption to $TiO_2$ NPs and its photocatalytic conversion to Cr(III) at the gut's acidic pH decreased Cr(VI) toxicity with increasing $TiO_2$ NPs concentrations (Matouke & Mustapha, 2019).

Microplastics (MPs) and $TiO_2$ NPs pose a threat to aquatic ecosystems, particularly in their combined impact on algae and sea plankton. Thiagarajan et al. observed toxic effects in *A. salina* when exposed to MPs alone or in conjunction with $TiO_2$ NPs (Thiagarajan et al., 2021). The presence of $TiO_2$ NPs heightened toxicity in *A. salina*, which was dose-dependent, indicating a correlation between $TiO_2$ NPs uptake and mortality. The internalization of $TiO_2$ NPs increased by approximately 1-fold in the presence of plain and $NH_2$-functionalized MPs, while a 1.4-fold decrease was observed with COOH-functionalized MPs. The combination of $NH_2$-functionalized and plain MPs with nano-$TiO_2$ further enhanced toxicity, suggesting an additive effect through ingestion or internalization, possibly due to electrostatic interaction with nauplii surfaces. The accumulation of $TiO_2$ NPs and MPs in the gut was identified as a major contributor to toxicity in zooplanktons like *A. salina* (Varó et al., 2019). Similarly, the ingestion of $TiO_2$ NPs, and the antibiotic tetracycline (TC) resulted in mortality, likely induced by oxidative stress, increased ROS production, and lipid peroxidation in *A. salina* exposed to $TiO_2$ NPs (Thiagarajan et al., 2022b). However, the role of ROS and lipid peroxidation in *A.*

*salina* following TC exposure lacks substantial literature evidence. Interestingly, the toxicity of $TiO_2$ NPs, MPs, TC, and their combination was found to be lower when exposure occurred through foodborne routes compared to waterborne routes, highlighting potential variations in toxicity mechanisms based on exposure pathways.

In this context, Ignoto et al. assessed the spermiotoxic effects of two different form of $TiO_2$ NPs namely $TiO_2$ sol-gel and $TiO_2$-rGO on both the vitality and motility of spermatozoa in *Paracentrotus lividus* (Ignoto et al., 2023). The results indicated that both the NPs exhibit detrimental effects on the vitality and motility of spermatozoa with increasing exposure time across all concentrations of NPs. The toxicity of $TiO_2$-rGO exhibited a consistent increase with exposure times (30 and 60 minutes) and concentrations (10, 20, and 40 µg/mL) of $TiO_2$, with the most pronounced damage occurring with prolonged exposure. Whereas, exposure to $TiO_2$ sol-gel NPs demonstrated higher toxicity, even at the concentration of 10 µg/mL, regardless of exposure time.

## 4. CONCLUSION

This review highlights the adverse impacts of $TiO_2$ NPs in marine environments, emphasizing their threat to ecosystems. While not acutely toxic, $TiO_2$ NPs induce oxidative stress due to their photocatalytic property. $TiO_2$ NPs tends to accumulate and settle to the bottom, yet a significant fraction of NPs may remain in the water column, posing risks to organisms residing there. Studies consistently highlight the potential toxicity of $TiO_2$ NPs and their interactions with other pollutants, leading to cumulative adverse effects. Understanding these interactions is crucial to comprehend the threat $TiO_2$ NPs pose to marine ecosystems. Moreover, the potential for bioaccumulation and the activation of defense mechanisms in marine organisms in response to $TiO_2$ NPs exposure highlight the complexity of ecological consequences.

Comprehensive monitoring, regulatory frameworks, and eco-friendly alternatives are crucial to mitigate ecological risks from widespread $TiO_2$ NP use. Ongoing research is essential to understand toxicity nuances, informing conservation strategies for sustainable practices and protecting marine ecosystems.

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