Environmental Fate and Toxicity of Zinc Oxide Nanoparticles in Aquatic Ecosystems: A Comprehensive Review

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ABSTRACT	ARTICLE INFO
Zinc oxide nanoparticles (ZnO NPs) are increasingly utilized in agriculture, electronics, and medicine, raising concerns about their environmental fate and toxicity in aquatic ecosystems. This study aims to review the fate, bioaccumulation, and toxicity of ZnO NPs in aquatic ecosystems. This review was conducted by comprehensively analyzing peer-reviewed literature from databases such as Scopus, Web of Science, and PubMed. The finding indicates that key environmental factors, including pH, ionic strength, and DOM, govern ZnO NP fate in aquatic ecosystems. ZnO NPs aggregate in high-salinity environments, whereas acidic conditions enhance dissolution, leading to increased Zn^{2+} ion release and potential toxicity. Smaller ZnO NPs exhibit higher reactivity and bioavailability, increasing their potential for bioaccumulation. Bioaccumulation of ZnO NPs is influenced by concentration, exposure time, and particle size, with smaller nanoparticles more readily absorbed by aquatic organisms. Toxicity varies depending on exposure duration and environmental conditions, with DOM mitigating by reducing Zn^{2+} ion availability. Additionally, ZnO NP exposure has been linked to oxidative stress, developmental abnormalities, and behavioral changes in aquatic organisms. This highlights the need for regulations and tailored risk assessments that account for water chemistry variations to mitigate ecological risks. Future research should focus on long-term impacts, including multi-species interactions and trophic transfer, to improve mitigation strategies.	

INTRODUCTION

In recent years, there has been a significant increase in the production and distribution of NPs. According to BCC Research (2019), the commercial output of NPs increased from approximately 223,060 metric tons in 2014 to around 584,984 metric tons by 2019, with a compound annual growth rate of 21.1%. The global market valuation for NPs grew from $2.0 billion in 2017 to an estimated $7.3 billion by 2022. Among the different types of NPs, metallic nanoparticles have attracted considerable attention due to their unique physicochemical properties and extensive use in industrial, biomedical, and environmental applications (Shaw

et al., 2011). While these NPs offer numerous benefits, concerns regarding their toxicological effects on biological systems have been raised (Rajput et al., 2018).

A critical subset of these novel materials consists of metallic NPs (Shaw et al., 2011), which have been extensively studied and evaluated for their toxicological impacts on biological organisms' activity, abundance, and diversity. Furthermore, due to their antimicrobial properties, they have been utilized as biocidal agents to inhibit or restrict the proliferation of microorganisms (Rajput et al., 2018). In addition to metallic NPs, metal oxide NPs (MeO-NPs) are also prevalent and commonly employed in commercial and industrial applications despite the substantial production of bulk metal oxide products across various commercial and industrial sectors. Among these, ZnO NPs are recognized as one of the most extensively utilized due to their unique structural and functional characteristics, including a wide band gap, high excitonic binding energy (Sabir et al., 2014), stability, photocatalytic activity (Hao et al., 2013), UV absorption properties, and antimicrobial efficacy (Pereira et al., 2019). These attributes have led to their incorporation into a diverse range of consumer products, including paints, coatings, cosmetics, biosensors, plastics, construction materials, and pharmaceuticals (Brun et al., 2014; Wong et al., 2010). The global production of ZnO NPs is estimated to range from 550 to 33,400 tons annually, making them the third most prevalently utilized metal-based NPs (Rajput et al., 2018). However, their widespread use increases the likelihood of environmental exposure, particularly in aquatic ecosystems, through wastewater discharge, direct application, and atmospheric deposition (Vale et al., 2016; Poynton et al., 2019).

Introducing ZnO NPs into aquatic environments raises significant ecological concerns due to their potential toxicity. Studies have demonstrated that these NPs can adversely affect aquatic organisms, including prokaryotes, phytoplankton, crustaceans, protozoa, and fish (Cong et al., 2017). Fish models, particularly *Danio rerio* (zebrafish), have been extensively used to assess the toxic effects of ZnO NPs due to their sensitivity and relevance to environmental monitoring. Research has predominantly focused on the acute toxicity of ZnO NPs, highlighting their detrimental impact on the early developmental stages of zebrafish (Xiong et al., 2011; Zhao et al., 2013). However, chronic toxicity studies remain limited, leaving gaps in understanding the long-term ecological risks associated with ZnO NPs. Comparative toxicity studies indicate that ZnO NPs may pose a more significant environmental threat than other commonly used metal oxide NPs. For instance, Zhu et al. (2008) reported that ZnO NPs exhibited higher toxicity in zebrafish embryos compared to titanium dioxide (TiO_2) and aluminum oxide (Al_2O_3) NPs. Similarly, Bhuvaneshwari et al. (2017) found that ZnO NPs had a lower LC_{50} value (27.62–71.63 mg/L) than TiO_2 NPs (117–120.9 mg/L) in Artemia salina under pre-UV-A and visible light conditions, reinforcing concerns about their potential ecological risks.

Given these concerns, this review aims to assess the environmental and biological impacts of ZnO NPs in aquatic ecosystems. By evaluating existing toxicological data and

identifying knowledge gaps, this article seeks to contribute to a better understanding of the risks associated with ZnO NPs and inform future regulatory and mitigation strategies.

MATERIALS & METHODS

A comprehensive literature search was conducted to gather and critically evaluate relevant studies on the behavior of ZnO NPs in aquatic organisms, focusing on identifying key environmental factors that influence their dynamics and assessing potential ecological risks. To find the most relevant studies, a thorough search was done across several scientific databases, including Web of Science, Scopus, PubMed, and Google Scholar. Only peer-reviewed articles in English were included, with no time limit set, to ensure both early and recent research were considered.

RESULTS AND DISCUSSION

Nanoparticles

No systematic definition of NPs has been accepted worldwide yet. However, according to the International Organization for Standardization (ISO) and the American Society for Testing and Materials (ASTM) standards, NPs are small particles with one or more dimensions, ranging in size from 1 to 100 nm (Ealia & Saravanakumar, 2017). Also, NPs can be defined as particles with at least one dimension between 1 and 100 nm and different characteristics from bulk materials (Miao et al., 2010). These particles can be nanofilms (one dimension), nanowires and nanotubes (two dimensions), or nanoparticles (three dimensions), while nanotechnology is known as the usage of these materials (Handy et al., 2008; Patibandla et al., 2018). With predicted growing global market from $38.5 billion in 2021 to $68.4 billion by 2026 at a compound annual growth rate of 12.2% from 2021 to 2026 (BCC Research, 2021), nanotechnology recently developed as a rapidly growing market with efficient effects on major economic sectors with novel and unique properties, have been implemented in diverse group of consumer goods such as agriculture, cosmetics, electronics, textile, and pharmaceutical (Handy et al., 2008; Li et al., 2013; Rajput, 2018). With an average growth rate of 21.1%, NP consumption is estimated to rise from 225,060 metric tonnes to almost 584,957 metric tonnes from 2014 to 2019 globally (Vale et al., 2016). The hazards and advantages of these new materials have been extensively debated. The advantages of NPs are enormous, and their benefits are still being studied.

Based on their composition, NPs can be classified into the following categories:

1. Carbon-based NPs: These types of NPs include those NPs that contain carbon in their composition, such as fullerenes (C_{60}), carbon nanotubes, carbon nanofibers, carbon black, and graphene (Jeevanadam et al., 2018). Carbon-based NPs have been widely implemented in different areas such as green energy, biosensors, and medical therapy, and due to the combination of the high stability of the buckyball structure, C_{60} has attracted much attention in commercial products. There is more than enough proof suggesting that C_{60} and its compounds exhibit properties that indicate its

potential use in biomedicine, such as apoptosis prevention, neuroprotection, and DNA photocleavage (Wang et al., 2014).

2. Organic-based NPs: These groups of NPs are produced mainly through organic material, excluding carbon-based such as dendrimers, micelles, liposomes, and polymer NPs. Organic NPs have received less attention; despite this, the hundreds of insoluble organic active chemicals employed across numerous product sectors could provide enormous economic potential for organic NPs. Organic NPs, suitable for application in food, are lipid, protein, or polysaccharide-based particles (Peters et al., 2011).

3. Inorganic NPs: Non-carbon NPs are known as inorganic NPs. Inorganic NPs include metal and metal oxide-based NPs. Metal-based NPs are NPs that have been produced from metals to nonmetric sizes using destructive or constructive processes. Although all metals can be transformed into NPs, the most commonly metal-based NPs are aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn). This type of NP has distinct characteristics such as their size, high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical and color, reactivity and sensitivity to environmental factors such as air, moisture, heat and sunlight (Ealia & Saravanakumar, 2017).

4. Metal oxide-based NPs are manufactured to alter the characteristics of metal-based NPs; for instance, Fe NPs in the presence of oxygen rapidly oxidize to iron oxide NPs (Fe_2O_3 NPs) at normal temperature, which increases their reactivity in contrast to Fe NPs. Because of their higher reactivity and efficiency, metal oxide NPs are manufactured. Aluminum oxide (Al_2O_3), cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), silicon dioxide (SiO_2), Titanium oxide (TiO_2), and zinc oxide (ZnO) are the most commonly produced MeO-NPs (Ealia & Saravanakumar, 2017; Rajput et al., 2018).

5. Composite-based NPs: These NPs are produced by combining NPs with other NPs or NPs with bulk materials categorized under this type of NPs (such as hybrid nanofibers) (Jeevanandam et al., 2018). Due to their highly π conjugated polymeric chains and exceptional electrical characteristics that encompass the entire insulator semiconductor metal range, composite-based NPs have subsequently gained significant interest in nano-science and nanotechnology. Also, because of their reversible doping/deduping process, unusual conducting mechanism, and controllable chemical and electrochemical characteristics, they can be recognized not just as good molecular wires for the creation of nano-devices, but also as optimizing materials for the construction of sensing platforms with high sensitivity (Guo et al., 2009).

Depending on their origin, NPs can also be classified into natural NPs and synthetic NPs. Colloids in freshwater, volcanic dust in the atmosphere, and soil erosion are particles with nanoscale that have been present naturally for hundreds of years. Mechanical processing, automotive combustion, and smoke can release NPs into the environment that are synthesized through physical, chemical, and biological processes or a combination of these processes (Handy et al., 2008; Jeevanandam et al., 2018).

Different types of NPs are implemented in different products. For instance, MeO-NPs are among the most used NPs used in diverse commercial products (for instance, environmental and industrial applications) (Yu et al., 2011), pharmacy, medical technologies, food industry or renewable energy, in water clearing and desalination, energy production, green chemistry (García-Gómez et al., 2020), as fluorescent biological labels, in the diagnosis, monitoring and destruction of tumors, in the detection of pathogens and proteins, in gene expression and phagokinetic studies, and magnetic resonance imaging contrast enhancement (Patibandla et al., 2018), fuels, and sports clothes (Waalewijn-kool et al., 2013), sewage treatment, sunscreens (Li et al., 2018). Around 1814, consumer goods produced by nanotechnology were available in over 20 countries in 2014 (Jeevanandam et al., 2018).

Properties and Usage of Zinc Oxide Nanoparticles

Zinc oxide, represented by the molecular formula ZnO, exists as a white powder and is characterized by its limited solubility in aqueous environments. Zinc (Zn) and oxygen (O_2) occupy the second and sixth groups of the periodic table, respectively. ZnO is conventionally classified as an II-VI semiconductor within materials science. It exhibits remarkable properties such as high transparency, elevated electron mobility, a broadband gap, and robust luminescence at room temperature; it finds extensive applications in transparent electrodes for liquid crystal displays, energy-efficient or heat-reflective windows, and various electronic devices. Zinc oxide NPs are renowned for their wide band gap semiconductor property, possessing a band gap energy of 3.3 eV at ambient temperature (Sabir et al., 2014).

Among various NPs, ZnO NPs are recognized for their efficient application within the nanoscale range, characterized by a substantial band-gap and significant excitonic binding energy (Sabir et al., 2014), alongside distinctive attributes including remarkable stability, anticorrosive properties, and photocatalytic capabilities (Hao et al., 2013), as well as their non-migratory nature, fluorescence, piezoelectric properties, light absorption, and scattering of ultraviolet radiation (Li et al., 2018), in addition to their diverse nanostructural forms (Bai et al., 2010), and their demonstrated antimicrobial efficacy (Pereira et al., 2019). The notable band-gap of 3.37 eV at ambient temperature and the multifaceted characteristics of ZnO encompass a blend of physical properties—such as comparatively high electrical and thermal conductivity, optical absorption in the ultraviolet spectrum, and exceptional thermal stability at elevated temperatures—and chemical properties, exemplified by stability under neutral pH conditions, mild antibacterial activity, and capacity for ultraviolet radiation blocking (Omar et al., 2014).

Zinc oxide NPs have been extensively utilized in various consumer products, including but not limited to paints, ultraviolet (UV) filters, biosensors, paper, plastics, ceramics, construction materials, rubber, power electronics, coatings, animal feed, and the photocatalytic degradation processes of textiles and printed materials. Moreover, ZnO NPs exhibit considerable potential in the realm of oil pollution remediation, pigments, semiconductor applications, photovoltaic devices, and wastewater treatment (Wong et al., 2010; Bai et al., 2010; Yu et al., 2011; Hao et al., 2013; Li et al., 2018). In addition, ZnO NPs serve as crucial components in a diverse array of personal care products, such as sunscreens and cosmetics. Furthermore, ZnO NPs can be utilized as antibacterial agents in medicine. For example, various morphologies of ZnO NPs, including nanorods, nanosheets, and nanoflowers, have demonstrated efficacy in inhibiting the proliferation of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Concurrently, ZnO NPs are also employed as anti-cancer agents, fungicides, and biomedical applications. Additionally, ZnO NPs represent promising candidate materials for photovoltaic cells and electrical sensors, commercial sun care formulations, lasers, light-emitting diodes, field-effect transistors (FETs), field emission devices, piezoelectric nanogenerators, bioimaging agents, biosensors, drug delivery systems, and in ointments, as well as in coatings and pigment formulations relevant to materials science and optics (Wong et al., 2010; Chen et al., 2014; Omar et al., 2014; Du et al., 2017; Yung et al., 2017; Pereira et al., 2019; Poynton et al., 2019). Simultaneously, the composition of ZnO NPs finds widespread application in agriculture as pesticides or fertilizers (García-Gómez et al., 2020) and in soil remediation processes; for instance, ZnO NPs have been utilized for the detection of chlorinated phenols through the quenching of visible emission on semiconductor films (Waalewijn-Kool et al., 2013).

Zinc oxide NPs, with a global annual production ranging from 550 to 33,400 tons (Rajput et al., 2018), represent the third most extensively produced NPs on an annual basis, attributable to their diverse applications and are followed only by silicon dioxide NPs (SiO2 NPs) and titanium dioxide NPs (TiO2 NPs) (García-Gómez et al., 2020). The combination of TiO2 NPs, which effectively block UV-A radiation, and ZnO NPs, which obstruct both UV-A and UV-B radiation, is commonly employed in the formulation of sunscreens that provide enhanced UV protection (Pereira et al., 2019). Nevertheless, utilizing ZnO NPs may soon exceed that of TiO2 NPs, given their capacity to absorb both UV-A and UV-B radiation, thereby delivering superior protection and enhanced opaqueness (Wong et al., 2010).

Source and Fate of Zinc Oxide Nanoparticles

Although the existing data regarding the environmental concentrations of NPs are presently scarce, it is evident that numerous sources of NPs exist within aquatic ecosystems, and these NPs are anticipated to be assimilated by a diverse array of aquatic organisms. Based on their origins, sources of NPs can be classified into three distinct categories: (1) incidental NPs, which are inadvertently generated from industrial processes such as automotive exhaust emissions and certain geological events like wildfires; (2) manufactured NPs, which humans

deliberately create to exhibit specific characteristics suitable for intended applications; and (3) natural NPs, which are produced by natural processes and have been present in the environment since the inception of life on Earth (Jeevanandam et al., 2018). Nonetheless, the distinction between naturally occurring, accidental, and manufactured sources of NPs can occasionally lead to confusion.

Approximately 69,000 metric tons of NPs are anticipated to be emitted. They may permeate aquatic ecosystems during various stages of the NP lifecycle, including processing, transportation, consumer usage, recycling, and final disposal (Caballero-Guzman & Nowack, 2016; Gupta et al., 2017).

Zinc oxide NPs can enter the environment, particularly aquatic systems, through three primary pathways: (1) wastewater discharge, (2) direct application, and (3) atmospheric deposition (Vale et al., 2016). During the processing phase, it is estimated that merely 0-2% of NPs will likely be released into the ecosystems, notwithstanding the wastewater treatment plants (WWTPs) operation. Conversely, nearly 90-95% of NPs, including ZnO NPs, TiO_2, and SiO_2, extensively utilized in various products, are projected to be discharged into the environment via WWTPs. Nevertheless, over 4% of these effluents are believed to enter aquatic ecosystems without any treatment measures. Simultaneously, NPs may also infiltrate aquatic environments through consumer products such as textiles, rubber, cosmetics, electronics, sunscreens, plastics, and pharmaceuticals. For instance, the global production volume of MeO-NPs, including TiO_2, ZnO, and Fe_2O_3, for skincare applications is estimated to reach 103 tons. Furthermore, researchers in Italy have calculated that at least 25% of the sunscreen applied to the skin will likely be washed off during bathing and swimming, indicating that approximately 250 tons of these NPs may be introduced into aquatic environments. Nonetheless, Wong et al. (2010) have suggested that at least 25% (about 250 tons of NPs) of the sunscreen applied would plausibly be discharged into aquatic ecosystems during similar activities. However, a minimal quantity of NPs is released into the environment through catalysts, electronic devices, and comparable products (Callaghan & MacCormack, 2017) (Figure 1).
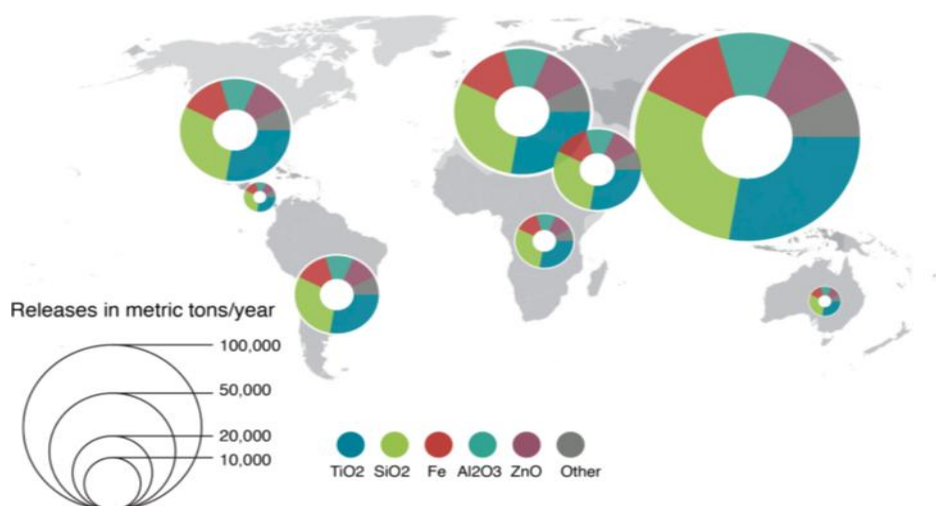
Figure 1: Regional distribution of estimated NPs releases to all compartments: air, water, soil, and landfills (Keller & Lazareva, 2014)

Environmental concentrations of ZnO NPs have been documented to range from 3.1 to 31 μg/kg in soil and from 76 to 760 μg/L in aquatic environments (Rajput et al., 2018). Concurrently, research indicates that effluent from wastewater treatment facilities contains the highest concentrations of ZnO NPs, measured at 0.3 to 0.4 μg/L (Poynton et al., 2019). Nonetheless, these concentrations are projected to increase, given the rising frequency of products incorporating ZnO NPs and their subsequent release into the ecosystem.

Environmental Transformation of Zinc Oxide Nanoparticles

The final destination for MeO-NPs is primarily expected to be aquatic ecosystems, where these nNPs experience a series of physical, chemical, and biological transformations, resulting in new particles that possess distinct properties compared to their initial states. The environmental alteration of MeO-NPs can be shaped by their unique characteristics, such as significant specific surface area, superparamagnetic traits, likelihood of dissolution, and enhanced sorption capacity compared to other types of nanoparticles. Nonetheless, it is primarily the diverse physical, chemical, and biological processes that contribute to the environmental transformation of MeO-NPs (Amde et al., 2017). The physical and chemical alterations of ZnO NPs transpire upon their release into the environment, particularly within aquatic settings, thereby modifying their environmental fate and toxicity toward aquatic organisms. Such alterations reduced bioavailability and toxicity; however, instances of increased bioaccumulation and toxicity have been documented in certain scenarios. These transformations encompass dissolution, aggregation/agglomeration, and sedimentation, contingent upon the physicochemical characteristics of ZnO NPs and the environmental conditions post-release.

Aggregation/Agglomeration of Zinc Oxide Nanoparticles

One of the most significant influences on the fate of MeO-NPs within aquatic ecosystems is the formation of NPs clusters, a phenomenon called aggregation/agglomeration. Nanoparticles can undergo two distinct types of aggregation within the environment. Homo-

aggregation occurs when the agglomeration results from interactions among identical NPs. Conversely, hetero-aggregation arises when interactions occur with disparate components present in the environment. Hetero-aggregation appears more prevalent than homo-aggregation due to various environmental chemical constituents (Amde et al., 2017). These modifications result in alterations to the extensive range of size distributions of ZnO NPs through the formation of aggregates; some investigations have indicated that such aggregations can increase the size of ZnO NPs to levels ten times larger than that of the original ZnO NPs (Hao et al., 2013; Rajput et al., 2018).

The aggregation behavior of ZnO NPs is intrinsically linked to various factors, including the presence of DOM within the surrounding environment (Brun et al., 2014), the methodology employed for dispersion (Hao et al., 2013), as well as pH and ionic strength (Li et al., 2018). Additionally, the physicochemical characteristics of NPs, such as their particle size and shape (Shaw & Handy, 2011), alongside surface properties, including surface modifications (Yung et al., 2017), also play a critical role. Notably, lower levels of aggregation for ZnO NPs have been documented at a pH of 7.45, while enhanced aggregation is anticipated at a pH of 8.65 (Amde et al., 2017). Furthermore, Wong et al. (2010) elucidated that the aggregation and size distribution of TiO_2 NPs could be influenced by the ionic strength and pH of NaCl solutions, which may ultimately affect their bioavailability to aquatic organisms. Concurrently, Poynton et al. (2019) reported a rapid formation of aggregates of ZnO NPs in NaCl suspensions exceeding 0.9 ppt. In the same study, it was noted that elevated ionic strength and alkaline pH could promote the aggregation of ZnO NPs. In contrast, the presence of DOM may confer stabilization to the NP suspension.

Nevertheless, Miao et al. (2010) indicated that the formation of aggregates of ZnO NPs occurred within a similar micrometer size range in both deionized water and synthetic seawater. Moreover, Hao et al. (2013) demonstrated that aggregation was likely to occur within the suspension as the surface charge of ZnO NPs neared neutrality. Simultaneously, the chosen dispersion method also significantly influences the aggregation behavior of ZnO NPs in suspension. For instance, ultrasonic dispersion achieved uniform dispersion of NPs, thereby mitigating their aggregation up to a certain threshold within the medium. Consequently, Bai et al. (2010) reported that particle concentrations and exposure duration affect the size distribution of NPs in E3 medium, with their size distributions nearly transcending the nanoscale range.

Furthermore, alterations to the surface characteristics also impact the aggregation behavior of ZnO NPs. Specifically, Yung et al. (2017) compared the aggregation behavior of coated versus uncoated ZnO NPs. They found that the aggregates of uncoated ZnO NPs were more substantial, with elevated concentrations of ionic Zn^{2+} compared to those of the coated ZnO NPs at equivalent concentrations.

Depending on the characteristics of the water, humic substances can either hinder or facilitate the aggregation of ZnO NPs, potentially leading to the stabilization or destabilization of these NPs in suspension. As a result, temporal fluctuations in water

chemistry, including variations in the abundance and composition of humic substances, can significantly influence the physico-chemical properties of ZnO NPs and their subsequent degradation products (Callaghan & MacCormack, 2017). The common physical transformations that MeO-NPs undergo in aquatic environments are presented in Figure 2.
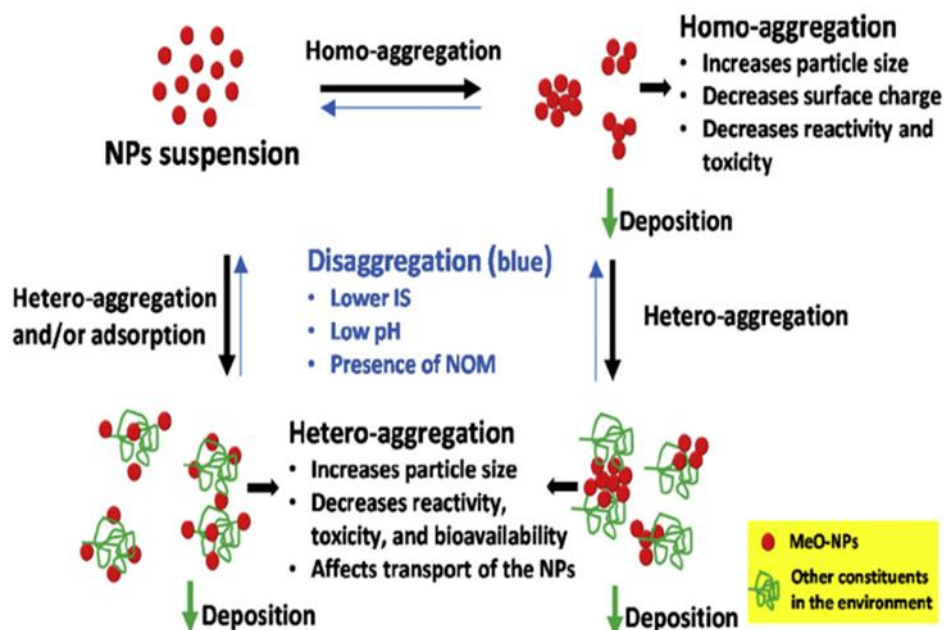
Homo-aggregation

NPs suspension → Homo-aggregation
- Increases particle size
- Decreases surface charge
- Decreases reactivity and toxicity

Deposition

Hetero-aggregation and/or adsorption

Disaggregation (blue)
- Lower IS
- Low pH
- Presence of NOM

Hetero-aggregation

Hetero-aggregation
- Increases particle size
- Decreases reactivity, toxicity, and bioavailability
- Affects transport of the NPs

Deposition | Deposition

- MeO-NPs
- Other constituents in the environment

Figure 2: Common physical transformations that MeO-NPs undergo in the aquatic environment (Amade et al., 2017)

Sedimentation of Zinc Oxide Nanoparticles

The aggregation of ZnO NPs within aquatic ecosystems is likely to promote their deposition from suspension to the substrate of water bodies. Given the increased aggregation and subsequent sedimentation of NPs, estuarine and aquatic sediments have been identified as potential final endpoints for various NPs (Rajput et al., 2018). Recent investigations have documented varying sedimentation rates for distinct MeO-NP categories. For example, the sedimentation rate of cerium dioxide (CeO_2), exceeding 95% within seven days, has been more rapid than that of ZnO NPs (Amde et al., 2017). Most sedimentation for ZnO NPs commenced upon their introduction into the aquatic environment, occurring predominantly within a 24-hour timeframe, after which the sedimentation rate diminished over time (Yu et al., 2011).

Furthermore, Poynton et al. (2019) demonstrated that 97% of dispersed ZnO NPs in aqueous environments settled out, whereas approximately 2% of Zn^{2+} ions remained in solution. Numerous factors can potentially influence the sedimentation of ZnO NPs, including pH, temperature of the water, particle size, zeta potential, ionic strength, and density (Yu et al., 2011). For instance, Poynton et al. (2019) indicated that ZnO NPs transformed by phosphates exhibited a diminished sedimentation rate, attributable to the increased mobility of ionic zinc due to phosphate ions. Conversely, DOM may enhance the sedimentation processes of ZnO

NPs by immobilizing metallic constituents. An increase in the salinity of the suspension could also potentially augment the sedimentation processes of ZnO NPs.

Dissolution of Zinc Oxide Nanoparticles

Zinc oxide is characterized as an amphoteric oxide, readily dissolving in both acidic and basic environments. The amphoteric nature of ZnO, along with the formation of hydroxide coatings on its surface ($\equiv$M–OH) in the presence of water, similar to other metal oxides, elevates its charge due to the physicochemical sorption of water molecules, enabling it to interact with both H^+ and OH^- ions. Nonetheless, a heightened dissolution rate has been documented at a pH of 3.4 for ZnO NPs. Additionally, a time-dependent reduction in pH has been observed when ZnO is dissolved in aqueous solutions (Degan & Kosec, 2000; Tang et al., 2002; Omar et al., 2014).

Another critical factor that may impact the dissolution of ZnO NPs in the aquatic environment is ionic strength, with increased dissolution rates for ZnO NPs predicted at reduced ionic strength. Li et al. (2018) conducted a comparative analysis of the dissolution of ZnO NPs in freshwater and saltwater, revealing that ZnO NPs exhibited enhanced solubility in saltwater relative to freshwater; however, due to the increase in ionic strength, the concentration of Zn^{2+} was found to be lower in saltwater than in freshwater at equivalent concentrations. Consequently, the sedimentation rate of ZnO NPs in saltwater surpasses that in freshwater, and this phenomenon may elucidate the observed decline in Zn^{2+} concentration over time in saltwater, whereas an increase is noted in freshwater. Moreover, Li et al. (2013) established that dissolution was contingent upon concentration for ZnO NPs, mainly when the initial concentration of NPs was excessively high. Similarly, Hao et al. (2013) reported the concentration-dependent dissolution for both ZnO NPs and zinc oxide bulk particles (ZnO BPs), with ZnO BPs demonstrating a greater concentration of Zn^{2+} in comparison to an equivalent concentration of ZnO NPs. In other terms, ZnO NPs manifested a reduced solubility in contrast to ZnO BPs, which may be attributable to a heightened aggregation rate of ZnO NPs relative to ZnO BPs, thereby diminishing the release of Zn^{2+} diffusion from the particle surface and impeding ion dissolution, thereby underscoring the significance of aggregation in the dissolution process of ZnO NPs. Concurrently, Wong et al. (2010) indicated a superior solubility rate for ZnO NPs compared to ZnO BPs in saltwater, suggesting that this could be ascribed to their smaller size, increased surface area, and curvature. Additionally, Bai et al. (2010) reported a temporal dependence of the solubility of ZnO NPs in the E3 medium. Significant transformations of NPss following their release into the aquatic environment are illustrated in Figure 3.
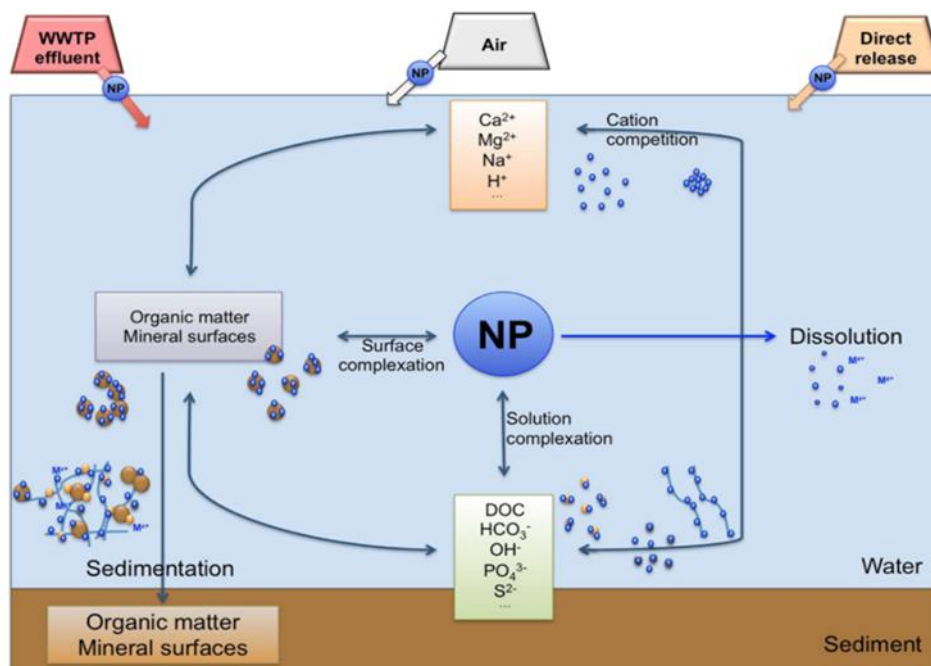
Figure 3: Physicochemical transformation of NPs when released into the aquatic environment (Vale et al., 2016)

Bioaccumulation of Zinc Oxide Nanoparticles

Following exposure, NPs may accrue and disseminate throughout various tissues, resulting in oxidative damage and histopathological alterations. The characteristics of NPs diverge significantly from those of BPs composed of identical materials; this distinction can be primarily attributed to their elevated surface area and reactivity, potentially leading to increased bioavailability (Kahru & Dubourguier, 2010). Hao et al. (2013) noted that the bioaccumulation patterns of ZnO NPs and ZnO BPs were disparate, a phenomenon that may stem from the capacity of ZnO NPs to penetrate the circulatory system and internal tissues, thereby facilitating greater accumulation and distribution of ZnO NPs within fish organs. Subsequently, it was proposed that the liver and gills could serve as preferential target organs for ZnO NPs, while the intestine may be the principal site for ZnO BPs; the authors also illustrated the potential for the entry of ZnO NPs through the compromised epithelial cell membrane by documenting the presence of dark aggregates accumulating on the mucus of chloride cells. Concurrently, previous investigations have established that exposure to TiO_2 NPs exacerbates hepatic stress and induces liver damage, resulting in lipidosis in species such as carp (Hao et al., 2009) or zebrafish (Handy et al., 2008). Wang et al. (2011) further demonstrate that the liver could be a target organ for ZnO NPs due to the synthesis of numerous metal-sulfur proteins within hepatic tissue, a process stimulated by NPs.

The concentration of ZnO NPs has been identified as a critical determinant influencing the bioaccumulation of ZnO NPs in zebrafish (Hou et al., 2019). Simultaneously, a concentration-dependent accumulation of ZnO NPs has been documented by Shaw & Handy (2011) and Yu et al. (2011). Moreover, earlier investigations have indicated that the duration of ZnO NPs exposure significantly impacts their accumulation in ichthyic organisms. For example, Hao et al. (2013) reported a time-dependent accumulation of ZnO NPs. However,

there remains a lack of data to substantiate the time-dependent accumulation of MeO-NPs in fish.

Additionally, it was indicated that ZnO NPs can be distributed across various organs, particularly affecting the gastrointestinal system, heart, brain, yolk, and liver following uptake. Conversely, Pereira et al. (2019) discovered that ZnO NPs were concentrated in the gastrointestinal tract of zebrafish larvae, suggesting that the developmental stages of fish could modulate the absorption, accumulation, and distribution of ZnO NPs. Furthermore, ZnO NPs have been documented to accumulate in the gills, liver, and brain, as well as to adsorb onto the chorion membrane, subsequently accumulating in oil droplets and being transferred to the yolk and gallbladder of embryos (Chen et al., 2014).

Toxicity of Zinc Oxide Nanoparticles

Zinc oxide NPs have been shown to exhibit significant toxicity across various algal species, affecting their growth and cellular processes. Eco-toxicity assessments reveal that ZnO NPs induce oxidative stress, mitochondrial disorganization, and apoptosis-like cell death in species like *Prorocentrum cordatum* at concentrations as low as 0.6 mg/L (Shoman et al., 2024). Environmental factors, such as nutrient concentrations, further influence this toxicity, as demonstrated by *Chlorococcum* sp., which experiences reduced growth under low and high nitrate conditions when exposed to ZnO NPs (Tzanakis et al., 2023). Additionally, certain species exhibit varied responses to ZnO NP exposure, with diatoms like *Phaeodactylum tricornutum* being particularly vulnerable, while organisms like *Artemia salina* display higher resistance (Xu et al., 2023). Freshwater microalgae, such as *Scenedesmus obliquus*, show significant sensitivity to ZnO NPs, especially in combination with other metal oxides, with reactive oxygen species (ROS) playing a key role in inducing cellular stress (Das et al., 2023). Moreover, genera like *Bracteacoccus* and *Lobosphaera* are highly susceptible to Zn, with exposure to critical concentrations leading to mortality (Maltsev et al., 2021).

Toxicity studies of ZnO NPs on aquatic invertebrates remain limited, with most focusing on freshwater crustaceans such as *Daphnia magna* and *Thamnocephalus platyurus*. These studies have reported comparable EC/LC$_{50}$ values, with *D. magna* and *T. platyurus* showing 48 h LC$_{50}$s of 3.20 mg/L and 0.18 mg/L, respectively, for ZnO NPs (Heinlaan et al., 2008; Blinova et al., 2010). Despite these acute toxicity findings, there is a notable lack of data on chronic toxicity in *D. magna*. Meanwhile, Fabrega et al. (2012) found that 1 mg/L of waterborne ZnO NPs increased mortality and adversely affected growth and reproduction in the marine amphipod *Corophium volutator*. Different invertebrate species demonstrate varying sensitivity to ZnO NPs, with *D. magna* often showing the highest sensitivity and other organisms, such as *Phaeodactylum tricornutum*, exhibiting significant vulnerability with LC$_{50}$ values ranging from 0.36 to 95.6 mg/L across species (Bordin et al., 2023; Xu et al., 2023). Additionally, ZnO NPs have been shown to cause similar toxic effects to ionic zinc, with sub-lethal impacts observed in species like *Paracentrotus lividus* (Prato et al., 2021).

Aquatic vertebrate species exhibit diverse responses to ZnO NP pollutants, highlighted by eco-toxicity assessments across multiple studies. For example, the obscure puffer (*Takifugu obscurus*) significantly reduces hatching rates and survival due to oxidative stress caused by ZnO NP exposure, especially at higher concentrations (Tang et al., 2024). Similarly, goldfish (*Carassius auratus*) experience severe oxidative stress and histopathological alterations in metabolic organs, with reduced blood cell counts and enzyme activities (Ghafarifarsani et al., 2022). Zebrafish are commonly used in toxicity studies among aquatic vertebrates due to their well-established biology and regulatory approval as a model organism. Studies have shown that ZnO NPs can severely impact zebrafish across life stages. For instance, Zhu et al. (2008) demonstrated that ZnO NPs showed higher toxicity in the early life stage of zebrafish. Meanwhile, a much lower 96 h LC_{50} (4.9 mg/L and 1.793 mg/L) in distilled and Milli-Q© R water for ZnO NPs have been reported (Zhu et al., 2008; Xiong et al., 2011), which possibly raised the importance of water chemistry on the toxicity of ZnO NPs. Similarly, Amin et al. (2021) reported three different LC_{50} values (0.643 mg/L, 1.333 mg/L, and 2.370 mg/L) for ZnO NPs in the early life stage of Javanese medaka in the ultra-pure, deionized, and dechlorinated tap water. While numerous studies have focused on acute toxicity, chronic toxicity assessments of ZnO NPs remain scarce despite evidence indicating serious threats to aquatic environments. For instance, after 21 days of exposure to ZnO NPs, the heart rate and mortality rate of Javanese medaka increased while the hatching rate decreased, and a series of abnormalities were observed in our previous study (Amin et al., 2024). Similar results were also observed after exposing marine medaka, zebrafish, and yellow stripe goby (Bai et al., 2010; Cong et al., 2017; Li et al., 2018; Amin et al., 2024)

A range of factors, including concentration, time, aggregation, size, and environmental conditions, influence ZnO NPs' toxicity. Studies have shown concentration-dependent effects, with Zhu et al. (2008) reporting that while zebrafish embryos were unaffected at 0.5 mg/L, higher concentrations increased toxicity. Similarly, Bai et al. (2010) found that mortality and abnormalities in zebrafish embryos occurred at 50 and 100 mg/L ZnO NPs. Time-dependent toxicity has also been observed, as Li et al. (2018) reported increased mortality in Yellow stripe goby embryos between four and five days post-exposure at 25 and 50 mg/L. Aggregation plays a critical role, as smaller aggregates and Zn^{2+} are more likely to penetrate cells and induce toxicity, whereas larger aggregates are less toxic (Bai et al., 2010; Jeevanandam et al., 2018). While some studies highlight size-dependent toxicity, particularly for smaller ZnO NPs (Appierot et al., 2009), others, like Adams et al. (2006), observed no size-dependent effects in *Bacillus subtilis*. The sensitivity to ZnO NPs also varies across species and life stages, with zebrafish larvae showing higher susceptibility than embryos (Wehmas et al., 2015). Environmental factors further influence ZnO NP toxicity, as changes in bioavailability and bioaccumulation often result from the dissolution of MeO-NPs, releasing toxic metal ions into aquatic environments (Waalewijn-Kool et al., 2011). Auffan et al. (2009) and Bai et al. (2010) identified the release of metal ions as a key contributor to NPs toxicity, particularly Zn^{2+} in the case of ZnO NPs (Franklin et al., 2007; Heinlaan et al., 2008). Interestingly, Callaghan & MacCormack (2019) found that humic acid significantly reduced

the toxicity of Ag NPs in aquatic species by preventing Ag^+ ion release. Although ZnO NP toxicity is often attributed to dissolved Zn^{2+}, size-dependent toxicity may play a more prominent role, as shown in studies by Li et al. (2018), who reported size-dependent toxic effects in juvenile carp despite lower Zn^{2+} release compared to BPs (Hao et al., 2013). Additionally, the generation of ROS is a recognized mechanism of NP toxicity, leading to oxidative stress and damage to proteins, lipids, and DNA, which in turn causes tissue damage, immune responses, and developmental abnormalities in aquatic organisms (Chen et al., 2014; Brun et al., 2014; Callaghan & MacCormack, 2017).

CONCLUSION

The extensive use of ZnO NPs in various industries necessitates a thorough understanding of their environmental fate and toxicity in aquatic ecosystems. This review highlights that the interactions of ZnO NPs with environmental factors, such as pH, ionic strength, and DOM, significantly influence their behavior, bioaccumulation, and toxicity. The evidence indicates that the physicochemical properties of ZnO NPs and the surrounding environmental conditions dictate their ecological impact. Therefore, ongoing research is essential to elucidate the long-term consequences of ZnO NPs on aquatic life and inform regulatory policies to minimize their potential harm. By advancing our knowledge in this area, we can better safeguard aquatic ecosystems from the adverse effects of ZnO NPs and promote sustainable practices in their use.

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