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On the toxicity behaviour of composite polyaniline-zinc oxide photocatalytic nanoparticles and their surrogate chemicals

Idil Arslan-Alaton [a,*], Olga Koba-Ucun [a], Miray Bekbolet [b], Nazli Turkten [c], Yunus Karatas [c]

[a] Istanbul Technical University, School of Civil Engineering, Department of Environmental Engineering, Maslak, Istanbul 34469, Turkey
[b] Institute of Environmental Sciences, Bogazici University, Bebek, Istanbul 34342, Turkey
[c] Department of Chemistry, Faculty of Arts and Sciences, Kirsehir Ahi Evran University, Kirsehir 40100, Turkey

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ABSTRACT

The acute toxic effect of home-made polyaniline-zinc oxide (PANI-ZnO) nanocomposite photocatalyst samples was investigated. Toxicity behaviour was compared with that of their surrogates PANI-emeraldine base (PANI-EB), PANI-emeraldine salt (PANI-ES) and zinc oxide (ZnO). The freshwater crustacean *Daphnia magna* reacted more sensitively to the exposure than the marine photobacterium *Vibrio fischeri* and thus higher acute toxicity levels were recorded for *Daphnia magna*. The toxicity response increased with exposure time for both tested organisms. The hydrophobic PANI-EB was non-toxic, whereas ZnO nanoparticles were toxic towards both test organisms and the main reason of the toxicity of the PANI-ZnO composites. The toxicity behaviour changed with ZnO content rather than the PANI-ZnO nanocomposite synthesis type (in-situ polymerization or hybridization method). The acute toxicity also increased when the PANI-ZnO samples were subjected to UV-A (photocatalytic) treatment (conditions:0.25 g/L catalyst; pH=7.0; t = 180 min; I_0 =0.5 W/L). A relationship between the photo-induced Zn(II) release and acute toxicity increase after photocatalytic treatment was evident.

1. Introduction

Water and wastewater decontamination is one of the major challenges of the century since high concentrations of pollutants resulting from a variety of anthropological and industrial activities can have dramatic effects on ecosystems, human life and health. Due to the discharge of toxic and/or persistent organic pollutants into the environment, conventional treatment fail to effectively eliminate these pollutants including pesticides, drugs, hormones, personal care products, surfactants, stabilizers, plasticizers and many others [1]. In the last decades, some advanced treatment methods have been developed and applied to eliminate them using destructive rather than phase-transfer operations and processes. Among these, heterogeneous photocatalytic processes offer an effective and promising water remediation strategy by exploiting visible light energy for the destructive removal of priority pollutants [1,2]. This approach is motivating the development of photocatalytic materials, such as semiconductor materials (TiO_2, ZnO, CdS) and other heterostructured nanocomposites that can be activated even with solar (visible light) energy and are capable of water/wastewater treatment to the mineralization end point [3,4]. So far, an appreciable effort has been put into the synthesis of nanoparticles (NP) with superior photocatalytic properties such as high quantum efficiency and treatment capacity, capability of working with lower bandgap energy and minimum charge recombination. However, the development of highly efficient photocatalysts with superior treatment performance and at the same time minimized ecotoxicological effects on aquatic biota is still in progress and remains a partly un-solved environmental pollution problem [5–7]. Toxicity patterns observed during photocatalytic treatment of micropollutants were also investigated in the recent past. However, researchers have rarely analyzed the potential toxic risks of the photocatalytic NP themselves on aquatic organisms. In fact, it is critical to study the biotoxic effects of these photocatalytic materials to ensure efficient and also ecotoxicologically safe treatment applications. So far, thousands of NP have been manufactured globally and their full-scale applications in a wide range of industries, most prominently including the pharmaceutical, cosmetic, medical and electronic industries have been reported [1,2,8–10]. Among these, polyaniline (PANI) has become an essential polymeric material due to its characteristic electrical properties, modifiable surface chemistry, physical stability and low-cost fabrication methods [11,12]. It is one of the best conductive polymers due to –NH groups present in the chain. PANI is being frequently used in sensor devices, light emitting diodes and rechargeable batteries due to

* Corresponding author.
E-mail address: arslanid@itu.edu.tr (I. Arslan-Alaton).

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its above mentioned unique physicochemical properties [13–17]. On the other hand, shortcomings of conducting polymers are their low dispensation capability, poor chemical stability and mechanical strength [13,14]. Considering its role in photocatalysis, PANI reduces the bandgap of semiconductors thereby enabling the use of near-UV and visible light in photocatalytic processes. PANI is referred to as PANI-emeraldine base (PANI-EB; Fig. S1); however, if doped or protonated, it is called PANI-emeraldine salt (PANI-ES), with the iminic nitrogens protonated by an acid [18]. PANI-ES (hydrochloride salt; Fig. S1) is its most widely used form due to its high stability electrical conductivity owing to its extended conjugated electron system [15]. PANI also increases the visible light absorption capacity of photocatalysts, enhances the mobility of charge carriers and also acts as an electron donor [14,15]. The degradation performance of the PANI-ZnO composite material is attributed to the improved charge separation efficiency at the excited PANI-ZnO interface. PANI has a band gap energy of 2.8 eV which is lower than the band gap energy of ZnO which is 3.44 eV, rendering ZnO an efficient electrons-holes transporter under visible light [19,20]. The above mentioned, unique properties of PANI-ZnO NP composites make them promising candidates for near-UV and visible-light-induced, heterogeneous photocatalysis [19–26]. When aqueous suspensions of PANI-ZnO NP composites are irradiated with light of suitable bandgap energy (typically UV or near-UV light), reactive oxygen speies (ROS) such as $HO^{\bullet}$, $SO_4^{\bullet-}$, $O_2^{\bullet-}$ and $HO_2^{\bullet}$, generated in the presence of O_2 (aeration) and H_2O (aqueous solution), can induce a great variety of photochemical redox reactions [23–26]. In this way, many organic and inorganic molecules can be removed. Related reactions and formation of ROS are shown below [23];.

$$\text{PANI-ZnO} + h\nu \rightarrow \text{PANI-ZnO}(e_{cb}^{-} + h_{vb}^{+}) \quad (1)$$

$$OH^{-} + h_{vb}^{+} \rightarrow HO^{\bullet} \quad (2)$$

$$O_2 + e_{cb}^{-} \rightarrow O_2^{\bullet-} \quad (3)$$

$$O_2^{\bullet-} + H^{+} \rightarrow HO_2^{\bullet} \quad (4)$$

$$2HO_2^{\bullet} \rightarrow O_2 + H_2O_2 \quad (5)$$

$$H_2O_2 + O_2^{\bullet-} \rightarrow HO^{\bullet} + HO^{-} + O_2 \quad (6)$$

On the other hand, the ecotoxicity of NP including PANI is becoming a major concern globally [27–30]. Among the NP, ZnO has attracted considerable attention worldwide because of its wide range of applications in sunscreen products, textiles, paintings and industrial coatings. ZnO is also used as a semiconductor photocatalyst for water and wastewater treatment [1]. Further, ZnO is the major ingredient in diaper rash and cold cream formulations and frequentl applied to treat minor burns and skin irritations as it is known for its antibacterial/antioxidative action [31–33]. The widespread use and applications of ZnO NP have raised serious concerns about their potential to induce adverse environmental effects. Hence, it has become necessary to estimate the level of exposure to humans and ecosystem receptors when determining their potential risks. It has been evidenced that ZnO and other NP's toxicity depends on several factors [32, 34–40] that need to be investigated in more detail. Toxic effects of one kind of NP observed in different studies may be derived from various experimental conditions as well as varied physicochemical properties of the NP samples and therefore it is suggested that even in the case of NP with the same chemical formula, their toxicity requires case-by-case investigation. As a matter of fact, it would be important to test the toxicity of NP to ensure their safe application. Among the available bioassays, the *Vibrio fischeri* (*V. fischeri*) bioluminescence inhibition assay is frequently chosen due to its high reproducibility, speed and sensitivity towards a wide range of pollutants [41]. Its commercial kits are frequently used as standard and practical toxicity tests. On the other hand, the widespread use of the freshwater crustacean *Daphnia magna* (*D. magna*) as a model test organism is attributed to its unique biological characteristics, such as (1) parthenogenetic reproduction, (2) small size and short generation time, (3) wide range of endpoints for ecotoxicological studies, (4) known genome, useful for providing mechanistic information and (5) high sensitivity to environmental contaminants and other potential stressors [34,39,42,43]. Due o the above-indicated reasons, these two test organisms were used to examine the toxicity behaviour of PANI-ZnO composite NP and its surrogate samples in this work.

Considering the above-mentioned issues, the present study aimed at investigating the acute toxicity of composite PANI-ZnO NP that was synthesized using different methods (in-situ polymerization and hybridization), having different zinc contents (1:1 and 1:9 on weight basis) and surrogate chemicals (PANI-ES, PANI-EB and Merck grade ZnO). The acute toxicity behaviour of the selected NP towards *V. fischeri* and *D. magna* was comparatively evaluated. The toxicity effect of these samples was also tested after photocatalytic treatment with UV-A light and compared to that of the original samples. Acute toxicity test results were related to the following parameters; Zn(II), nitrate, sulfate and dissolved organic carbon (DOC) release tests.

2. Materials and methods

2.1. Experimental procedures

The selected NP were characterized and studied for their photocatalytic activity in previous work [25]. The PANI-ZnO NP composite prepared by the in-situ polymerization method is called "PZI", whereas the PANI-ZnO NP composite synthesized using the hybridization method is called "PZS" in this paper. The treatment conditions (pH, concentration, photocatalytic treatment time) being optimized in earlier work [25] were selected as the working conditions in this experimental study. Detailed information about the synthesis method, physicochemical/structural properties of the selected NP composite samples can be found in Table S1 and Figs. S1–2–3 that are presented in the Supporting Information section of this work (Supplementary Materials file) as well as related, previous work [15,25,44,45].

Toxicity tests were performed with the marine photobacterium *V. fischeri* (also called *aliivibrio fischeri*) and the freshwater microcrustacean *D. magna* that were selected as well-known, routine, standard, reliable and rapid bioassays. Acute (short-term) toxicity tests were conducted on so-called PZI (composites prepared by in-situ polymerization method) and PZS (composites prepared by hybridization method) NP with two different ZnO molar ratios (1:1 and 1:9) and their surrogate materials, namely PANI-EB, PANI-ES and Merck-grade ZnO.

In the final part of this experimental study, photocatalytic tests were conducted where the effect of UV-A light on the PANI-ZnO NP composite samples was tested in pure water without pollutant addition. For these runs, distilled-deionized water that was adjusted to pH= 7.0–7.2 with 0.1 N NaOH solution and contained 0.25 g/L PANI-ZnO NP samples were taken into the 500-mL capacity quartz glass photoreactor and subjected to UV-A light radiation. The photoreactor was continuously stirred from its bottom with a magnetic stirrer to keep the photocatalyst is suspension. The eight (8) UV-A lamps were turned on after 30 min (accepted as t = 0 min) to reach continuous light emission ($I_0 = 0.5$ W/L) and an adsorption-desorption equilibrium of the NP suspensions. At t = 0 min and after t = 180 min photocatalytic treatment samples are taken and filtered though 0.22 Millipore micron filters for further instrumental analyses. Detailed information about the photoreactor set-up was already given elsewhere [46].

2.2. Instrumental procedures

The filtrates were analyzed for specific ions (sulfate, nitrate, phosphate), metals (Fe) and dissolved organic carbon (DOC) via ion chromatography Dionex ICS-3000 ion chromatography, Perkin Elmer Aanalyst 300 Atomic Absorption Spectrometry and Total Organic Carbon Analyzer Shimadzu TOC VWP), respectively.

The slight decrease/increase in pH values observed in the tested suspensions when compared to the positive control occurred due the nature of the NP, which in aqueous solution undergo hydrolysis to their corresponding acid/base molecules.

2.3. Bioassays

Appropriate volumes specified for the acute toxicity tests were prepared by adding the catalyst samples into the test medium by means of an automatic pipette forming NP + test medium suspensions. All test samples were continuously stirred with a magnetic stirrer before and during their transfer into the test vials to ensure that the same amount of the NP was taken for each replicate. Toxicity tests were conducted using commercially available test kits and used as received.

2.3.1. V. fischeri

V. fischeri toxicity was determined with a commercial bioassay kit according to the ISO 11348–3 test protocol [47] (ISO 11348–3, 2008). Briefly, the photocatalysts were dissolved in 2% w/v NaCl solution and the pH of the samples were all the time adjusted to pH = 7.0 ± 0.2. The lyophilized bacteria with reconstituted reagents were equilibrated at T = +4 °C for at least 30 min and then stabilized at T = +15 °C for at least 30 min before pipetting a bacteria suspension into the samples. For each dilution level two parallel samples were prepared and the luminescence intensity was recorded in all test tubes, including controls, after t = 15 min and 30 min. Only t = 30 min responses were presented in the main manuscript text, t = 15 min data is available in the Supporting Information section (see Table S.2). Since the NP samples were in particulate form and metals might leach out after a while, the sample preparation time was reduced from the routine t = 24 h to t = 120 min. In order to ensure that no incorrect (positive) intensity inhibition was observed due to the precipitation of the photocatalyst NP, all samples were mixed with the pipet tip right before each luminescence measurement. ZnO test samples were prepared by serial dilution of a 400 mg/L ZnO stock solution in the test medium since a lower and wider concentration range was tested compared to the other test samples. The concentration range for the photocatalyst samples was selected according to previous work and preliminary (toxicity screening) test response. The selected NP concentration ranges for the *V. fischeri* tests are listed below;.

- PANI-ES: 0.125–0.550 g/L
- PANI-EB: 0.125–1.000 g/L
- ZnO: 3.1–200 mg/L
- PANI-ZnO composites: 0.125–0.500 g/L

2.3.2. D. magna

A preliminary *D. magna* acute toxicity screening test was performed for t = 48 h at various concentrations of the NP to determine a suitable concentration range to be used for the *D. magna* acute toxicity assessment. The number of *D. magna* individuals that survived after t = 24 h and 48 h of incubation in the solutions was recorded. Acute toxicity tests with *D. magna* were performed according to the OECD test protocol [48] with the exposure of 20 neonates collected in t = 2–26 h, for t = 48 h. The samples were diluted with ISO synthetic freshwater medium at a controlled temperature of T = 20 ± 2 °C without luminosity and feeding. The toxicity endpoint used in this test was the death/immobility of the test organisms at t = 48 h. The stock NP solutions were prepared at a concentration of 1000 mg/L and their dilutions were from this stock solution in a concentration range of 125–500 mg/L with the exception of ZnO, that was diluted to the 50–200 mg/L concentration range. ZnO, PANI-EB and PANI-ES were used as positive controls. The ISO synthetic freshwater medium was used as a negative control. The quality criteria of the test were completely fulfilled as in the test control immobilization of daphnids did not exceed 10%. The number of immobilized daphnids was recorded at t = 24 h and t = 48 h among 20 test organisms.

2.4. Statistical data analysis

Each test, including a blank control, was conducted in triplicate. Median effective concentrations (EC_{50} values) and corresponding 95% confidence intervals were calculated by regression using Excel and the software of the toxicity test kits. A confidence interval overlap test was used to evaluate if there is significant difference between any two EC_{50} values.

3. Results and discussion

3.1. Toxicity behaviour of the PANI-ZnO composite NP surrogates

In the first part of the study, the acute toxicity of the surrogate NP chemicals was tested. Fig. 1 presents NP concentration (g/L) versus *V. fischeri* inhibition (%) profiles for PANI-ES (a), PANI-EB (b) and ZnO (c) surrogate NP at an exposure time of t = 30 min. Percent relative inhibition results for t = 15 min were provided in Appendix Table S.2 for comparison of toxicity behviour for two incubations times, namely t = 15 min with t = 30 min. From Fig. 1 it can be seen that increasing the PANI-ES concentration from 0.125 g/L to 0.550 g/L raised the inhibitory effect towards *V. fischeri* from − 16% (described as stimulation) up to 77%. An EC_{50} value of 0.44 g/L was calculated for this surrogate NP indicating that an inhibitory effect would be expected in the selected concentration range (Fig. 1a). For PANI-EB on the other hand, no inhibitory effect was observed even when the concentration was increased to 1.00 g/L and relative values were calculated in the − 17% to + 19% range (negative value = stimulative effect; see Fig. 1b). Apparently, the PANI-EB surrogate was non-toxic towards *V. fischeri*. The third surrogate NP (ZnO; Merck grade NP) was tested over a wider concentration range (0.0031–0.2000 g/L) via serial dilution and the resulting inhibitory responses varied between − 30% to + 49% (Fig. 1c) indicating a relatively wide-ranged, moderately toxic response level for the often studied and cited nano-sized ZnO. Upon comparison of Fig. 1 (established for t = 30 min) and Table S.2 (prepared for t = 15 min) it is apparent that generally speaking t = 30 min values were higher and more appropriate for acute toxicity evaluation than t = 15 min, particularly considering the ZnO sample, where only stimulative effects were recorded at the lower incubation time (t = 15 min). In previous work it has been demonstrated that the acute toxicity of ZnO NPs is time-and dose-dependent [49]. Considering these results, it was decided to work with an exposure time of t = 30 min for the *V. fischeri* tests.

Fig. 2 depicts the concentration (g/L) - *D. magna* inhibition (%) profiles of the surrogates NP PANI-ES (a), PANI-EB (b) and ZnO (c) for two exposure times; namely t = 24 h and t = 48 h. According to these test results, per cent death-immobilization rates obtained for PANI-ES increased to 5–10–90% (t = 24 h) and 15–35–100% (t = 48 h) upon elevating the PANI-ES concentrations to 0.125–0.250–0.500 g/L, respectively. From the *D. magna* bioassay it is also evident that the toxic effect of the sample increases with incubation time, resulting in more death and/or immobilized daphnids when extending the incubation time from t = 24 h to t = 48 h (Fig. 2a). As in the case of *V. fischeri* tests, no toxic effect was found for the surrogate PANI-EB; only for the highest tested conentration of 0.500 g/L PANI-EB, a death/immobilization rate of 20% was obtained at an exposure time of t = 48 h. For all other exposure times and sample concentrations, no inhibitory/toxic effect was observed, revealing that PANI-EB was also practically non-toxic towards *D. magna* (Fig. 2b). The surrogate ZnO was tested at 0.050–0.100–0.200 g/L concentrations and percent death/immobilization rates were obtained as 65–70–85% and 85–90–95% for t = 24 h and t = 48 h, respectively, indicating significant toxicity levels, as expected for ZnO NP. From the toxicity test results being established for PANI-ES, PANI-EB and ZnO (Fig. 2c) it can be inferred that the test organisms (*V. fscheri* and *D. magna*) exhibited paralel responses for all tested surrogate NP samples.

In the related scientific literature it has been reported that surface

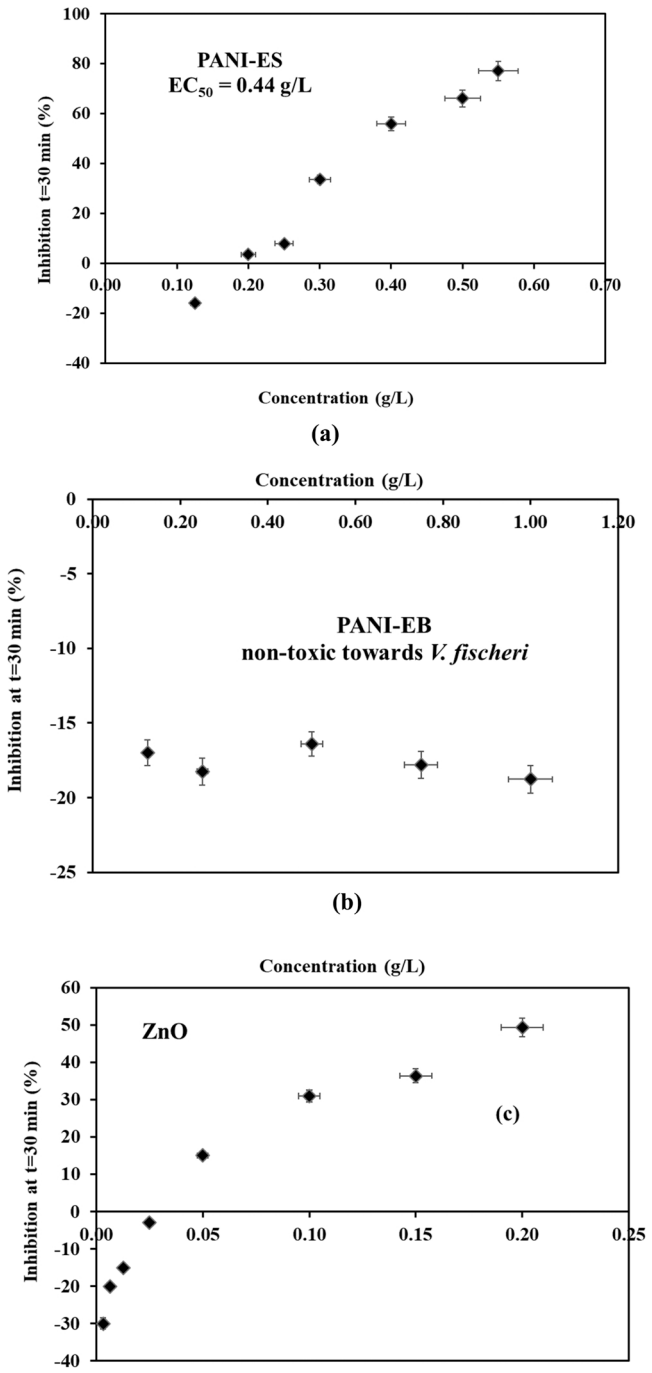

Fig. 1. Percent *V. fischeri* inhibition after exposure to PANI-ES (a), PANI-EB (b) and ZnO (c) for t = 30 min.

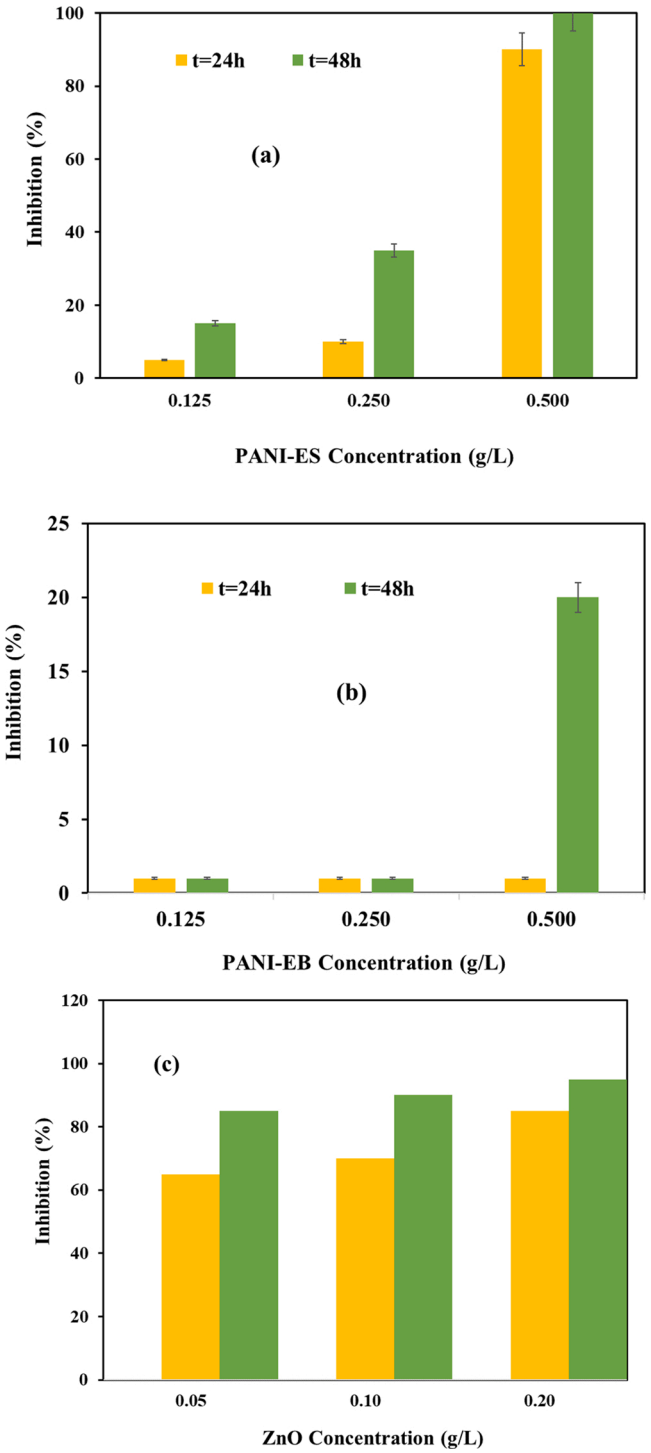

Fig. 2. Percent *D. magna* death and/or immobilization (inhibition) after exposure to PANI-ES (a), PANI-EB (b) and ZnO (c) for t = 24 h and t = 48 h.

hydrophobicity can have tremendous effects on NP toxicity, behavior and fate in aquatic environments [11,12,25]. It was shown that hydrophobic materials such as the PANI-EB samples in the present stuy that possess a negative charge at all pH conditions would not adhere to negatively charged biological substances and thus minimize physical contact and interaction with both test organisms [50]. PANI-ES, an acid salt of PANI-EB, on the other hand, is more hydrophilic and has a zero-point-of charge value of pH = 8.25 [25]. The surface charge of PANI-ES would be close to neutral under the tested pH conditions (= 7–8) that would influence the toxic effects of PAN-ES on *V. fischeri* and particularly *D. magna* [50,51]. It should be noted here that the intimate contact between cells and particles is much more important than the entrance of NP into cells to cause the biotoxic effect. Surface modification with organic chemical groups may also influence the toxicity behaviour of NP [50–52]. Chemical surface modification can hinder the recognition of the NP by the test organism, thus making the NP more easily internalized [31]. It is hence expected that PANI-EB being completely insoluble and stable in aqueous media, could hardly be toxic [12,31]. Thus it seems that prevailing hazards with respect to toxicity on biological systems are associated with hydrophilic, low-molecular-weight components and hence more likely to be expected from PANI-ES and its synthesis chemicals raher than PANI-EB [50–52]. Humpolicek et al. [53] showed that the cytotoxicity of PANI-ES and

PANI-EB as well that of standard, purified PANI differed notably. The cytotoxicity improvement after serial reprotonation-deprotonation steps were attributed to reduced concentration of residual low-molecular-weight byproducts of aniline oxidation entrapped in the PANI samples. Moreover, the slight differences in cytotoxicity of PANI-ES and PANI-EB could be ascribed to the use of HCl during polymer preparation and the presence of Cl^- counter-ions that could negatively influence cell viability and hence contribute to the observed cytotoxicity [53].

The ecotoxicological effects of ZnO NP have been studied over a wide range of test organisms [54–57]. Many factors in the environment including salinity, pH, UV/solar light and the presence of dissolved organic carbon (DOC), can significantly affect the toxicity of ZnO NP [56,57]. For instance, Miao et al. [58] examined the mechanical toxicological pathway of ZnO NPs. The cytosolic entrance and dissolution of ZnO NP lead to an initial elevation in cytosolic Zn(II). Mitochondria sequester excess cytosolic Zn(II), resulting in a high Zn(II) in the mitochondria which on the other hand induces mitochondrial membrane potential collapse, that in turn leads to cell apoptosis and lactate dehydrogenase (LDH) release [58–60]. ZnO NP also induce structural damages. Similarly, Bacchetta et al. [42] evaluated the acute effects of ZnO NP and observed a structural damage of gut epithelial cells of *D. magna*.

3.2. Toxicity behaviour of PANI-ZnO composite NP

The toxic effects of PANI-metal oxide NP composites have rarely been investigated so far. In this study, the major purpose was to examine the acute toxicity behaviour of the PANI-ZnO composite materials. Fig. 3 displays the concentration (0.125–1.000 g/L) - per cent *V. fischeri* inhibition profiles at an exposure time t = 30 min for the PZS-1, PZS-9, PZI-ZnO-1 and PZI-ZnO-9 composite NP. It should be once more noted here that the number following the composite name indicates the "PANI:ZnO percent mass ratio" of the composite sample, whereas the labels "S" and "I" inicate the synthesis method (as hybridization and in-situ polymerization, respectively). From Fig. 3 it is apparent that the inhibitory effect increases in parallel to the elevated sample concentrations and the levels of inhibition do not differ appreciably for PZS-1, PZS-9 and PZI-1 samples. No inhibitory effect was observed for PZI-9 (ranging between −11/−1%) revealing that the PZI-9 composite NP is non-toxic towards *V. fischeri* at least within the concentration range that is typical for treatment applications of this particular photocatalyst type. Calculated EC_{50} values are also given in the Fig. 3 legend for all of the three more toxic PANI-ZnO NP composite samples. According to the calculated EC_{50}

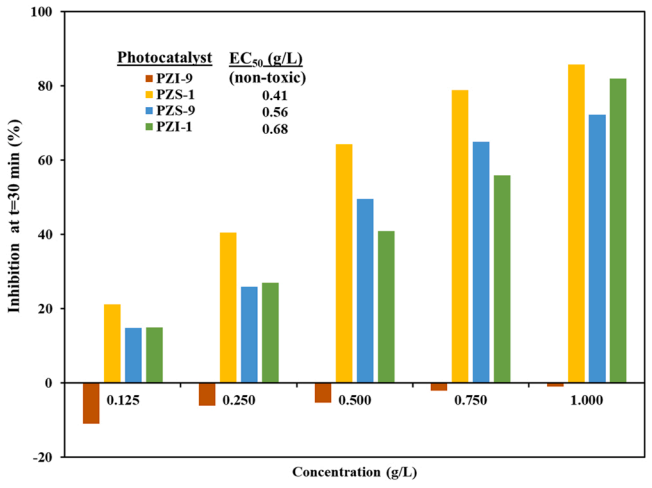

Fig. 3. Percent *V. fischeri* inhibition after exposure to PZS-1, PZS-9, PZI-1 and PZI for t = 30 min. EC_{50} (in g/L) values are also given in the Figure Legend for the examined photocatalysts whenever computable.

values, PZS-1 is most toxic (0.41 g/L), followed by PZS-9 (0.56 g/L) and PZI-1 (0.68 g/L), indicating that they might have some remarkable negative effects on freshwater biota when used at a concentration exceeding 0.25 g/L. Again, the selected concentration range is typical for treatment applications of these composite NP in heterogenous photocatalysis [25,26,45]. On the other hand, it appears that the PZI-9 sample can be safely used considering the *V. fischeri* test results. From the test conducted with *V. fischeri* it can also be inferred that both the synthesis technique (either in-situ polymerization or hybridization) and the ZnO content (PANI:ZnO content of 1:1 or 1:9 on weight basis) of the NP composite samples could speculatively have an effect on their inhibitory effects on aquatic microorganisms.

Fig. 4 shows percent death/immobilization rates obtained for the selected composite NP PZS-1 (a), PZS-9 (b), PZI- 1 (c) and PZI-9 (d), at t = 24 h and t = 48 h exposure time, representing two different synthesis routes and ZnO contents. From the toxicty test results it is apparent that incubation time has a dramatic influence on the death-immobilizaton rates; extending the incubation time from t = 24 h to t = 48 h practically doubles the negative effect of the photocatalyst material. According to the concentration (g/L) - response (%) profiles given in Fig. 4, the increasing order of inhibitory effect can be listed as follows; PZI-9 < PZI-1 < PZS-9 < (≈) PZS-1. Different from the *V. fischeri* test results is the toxicity of PZI-9 towards *D. magna*, speaking for differences in sensitivity and type of the test organism towards different concentrations of various test samples. However, again, the (photo)catalysts prepared by the hybridization method appeared to be more toxic than those prepared by the in-situ polymerization technique.

A key aspect of assessing the risk of applied NP that bear metal oxides/are metal oxide composites is to understand the relationship between their physicochemical characteristics and biological/ecotoxicological effects. The damage caused by these metal oxide/composite NP is usually rather complex and may involve the following factors [54–60]:

i) dissolution / release of metal ions that can be adsorbed by the test organism.

ii) generation of reactive oxygen species (ROS) via photocatalytic reactions.

iii) other particle-, shape-, and size-related effects.

iv) physical or mechanical damage due to the direct contact of the NP with the test organisms.

v) test conditions (exposure mode and time).

vi) environmental conditions (pH, presence of complexing agents, etc).

NP can enter organisms directly or through bioaccumulation and exhibit some toxicity, such as inflammatory responses and cell membrane leakage [55–57]. More information is available for the freshwater crustacean as a higher animal and hence more complicated in morphology. For instance, *Daphnia magna* are non-selective filter--feeders that cannot easily reject unwanted food items or discriminate between particles of similar size and are therefore likely to consume more NP than selective feeders [27,43]. That is, *D. magna* can ingest particles up to approximately 70 μm in diameter, with the minimum size depending on the distance between setulae on thoracic limbs, which do not depend on age or size since the gap is constant and species-specific. Exposure routes of NP to *D. magna* include oral, filtration through thoracic appendices, penetration into brood chamber, and body adsorption [25,26]. After exposure, NP can bioaccumulate under the carapace, on the external body surface, digestive tract and appendages of *D. magna* [32,61]. The digestive tract is one of the dominant uptake sites for NP in *Daphnia* with around 60% of the NP ending up there [31,61,62]. Subsequently, NP have been observed to cross epithelial barriers and move into the endocytic vesicles near the upper cell surface of the microvilli, and from there they translocate to the mitochondria, enterocyte's nuclei, the folded basal plasma membrane, finally reaching the gut muscular layer [60–62]. Negative consequences of NP accumulation in the digestive tract may the decrease in food intake resulting in

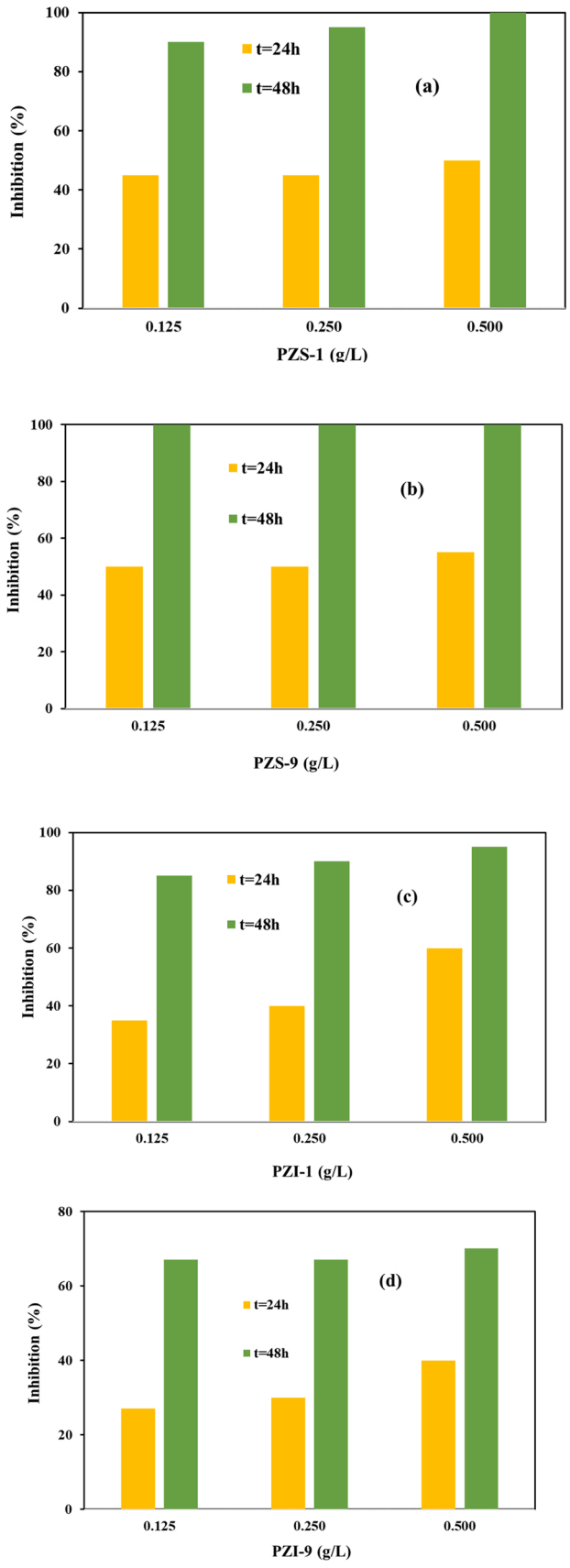

Fig. 4. Percent *D. magna* death and/or immobilization (inhibition) after exposure to PZS-1 (a), PZS-9 (b) PZI-ZnO-1 (c) and PZI-9 (d) for t = 24 h and t = 48 h.

decreased growth and survival [32,33,54,63]. The results of a recent NP uptake test indicated that the *Artemia nauplii* (*A. Nauplii*) were able to uptake NP from saltwater and this uptake was concentration- and time-dependent. At the same time, the uptake test showed that *A. nauplii* were not able to eliminate all of the ingested NP and a fraction accumulated inside their bodies after a depuration period [56].

It is known that the size of the NP is another important parameter that might affect their toxicity. Smaller-sized NP more easily cross cell membranes compared to larger particles [54,57]. Therefore, the toxicity of NP usually increases with decreasing particle size. This has been confirmed by several studies with *D. magna*. For example, Santo et al. [64] found that ZnO NP smaller than 50 nm had an EC_{50}-48 h value of 1.9 mg/L and for those of 50–100 nm the EC_{50}-48 h value was 3.1 mg/L. However, not all NP have a particle size effect on toxicity. It is suggested that 26-nm ZnO NP appeared to have the highest toxicity, while a certain concentration of nano-sized ZnO with the average sizes of 62 nm and 90 nm had the same influence on membrane integrity. Some characteristics of composite NP including preparation method, kind of capping agent, size, and shape may change their effect on living organisms [10,56]. SEM images that were shown in Figs. S2 and S2 for different NP samples investigated in this study also indicated that the smaller particle-sized NP were found to be more acutely toxic than larger-sized particles.

3.3. Toxicity after UV-A treatment of PANI-ZnO composite NP in pure water

An important factor that could affect PANI-ZnO toxicity is exposure to solar or UV light radiation in the presence of NP that might cause Zn (II) photo-dissolution as well as the release of other organic (DOC) and/or inorganic materials such as chloride or nitrate. Hence, the PANI-ZnO NP composite samples were also tested for their toxic effects after t = 180 min exposure to UV-A light in distilled-deionized ("pure") water (reaction conditions: Catalyst concentration = 0.25 g/L; pH = 7.0–7.2; I = 0.5 W/L) in the absence of pollutants to assess the photocatalytic treatment effects on the photocatalyst-induced toxicity. In these samples, DOC, Zn(II) and inorganic ions were measured in the filtrates of the photocatalytic reaction solutions. Fig. 5 comparatively presents *V. fischeri* toxicity (t = 30 min, as percent relative inhibition; 5a), *D. magna* toxicity (t = 48 h, as percent relative inhibition; 5b) together with Zn(II) release results (in mg/L; Fig. 5c) for different composite PANI-ZnO samples at t = 0 min (for the original composite sample) and t = 180 min (UV-A exposure time). From Fig. 5a it can be seen that the *V. fischeri* toxicity increased at t = 180 min compared to t = 0 min for all studied NP composite samples. The most dramatic increase in toxicity values occurred for the PZI-1 sample on *V. fischeri* toxicity that increased from 18.2% to 86.8% relative inhibition after 180 min UV-A treatment of the PANI-ZnO composite samples. The second highest increase in *V. fischeri* toxicity was obtained for PZS-1 increasing from 38% to 64% after 180 min photocatalytic treatment in pure water. In the case of *D. magna*, relative changes in toxicity levels (measured as percent death/immobilization) also increased after photocatalytic treatment (Fig. 5b), however, the degree and change in toxic behaviour differed from those trends observed in the *V. fischeri* bioassays. The increase in toxicity behaviour was most dramatic for PZS-1 (% inhibition increases from 50% to 90% after 180 min-photocatalytic treatment), and changed only slightly for the already quite toxic PZI-1 (from 70% to 75% after photocatalytic treatment). Interestingly, the Zn(II) release was highest (starting from 0.71 mg/L and reaching almost 4.0 mg/L) for the PANI-ZnO composites with the higher ZnO content (1:1 on weight basis) independent from the synthesis techniques. For the other two photocatalysts, Zn(II) release was relatively minor (increasing from approximately 1.4 mg/L to 2.4 mg/L). There was no definite relationship between the toxic effect (% relative inhibition rate) and the amount of Zn(II) released (in mg/L) for the studied catalyst materials, although inhbition and Zn(II) release rates both exhibited an increasing trend

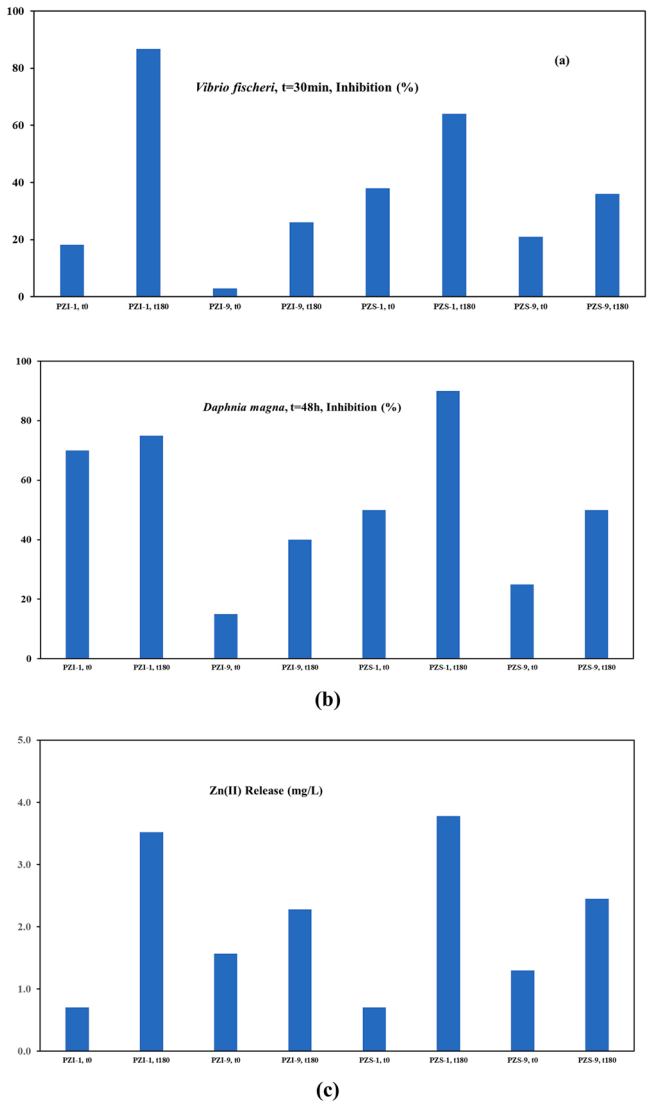

Fig. 5. Percent *V. fischeri* inhibition after t = 30 min exposure (a); Percent *D. magna* death/immobilization (inhibition) after t = 48 h exposure (b) and mg/L Zn(II) concentration (c) before (t = 0 min) and after (t = 180 min) UV-A treatment of PZI-1; PZI-9, PZS-1 and PZS-9 in pure water. PANI-ZnO composite (photocatalyst) concentrations = 0.25 g/L; Light intensity = 0.5 W/L; pH = 7.0.

after photocatalytic treatment of the NP composites as compared to the original catalyst samples (Fig. 5c). Nitrate, sulfate and non-purgable, dissolved organic carbon (DOC) were also followed during the photocatalytic experiments with the PANI-ZnO NP, however, no appreciable change was observed for these parameters (nitrate, sulfate, chloride and non-purgable DOC) after UV-A exposure of the PANI-ZnO composite samples for t = 180 min (see Supporting Table S.3).

Photo-induced toxicity results being comparable with the present data is only available for ZnO NP and reported below. Several scenarios have been proposed for photo-induced toxicity of ZnO; when ZnO, a well-known semiconductor with a band gap energy of 3.37 eV, corresponding to a minimum excitation wavelength of 368 nm, is exposed to radiation with UV-A light, its excitation initiates the generation of ROS [65]. The toxic effect of ROS was first reported for bulk ZnO and more recently for nano-sized ZnO photocatalysts even under solar light radiation [66]. In this way, the application of UV light could accelerate ZnO dissolution and ZnO photo-dissolution, together with photochemical ROS generation. All these factors are expected to have a significant impact on ZnO and ZnO NP toxicity [35,54]. Consequenlty, ROS could also form during PANI-ZnO-mediated heterogenous photocatalysis [65] and affect its toxicity. Besides ROS, HP, that can be measured after photocatalytic treatment as an indicator of photocatalytic reactions (see reaction Eq. 6) as well as the photo-dissolution of ZnO particles from the PANI-ZnO composite to form Zn(II), can be major reasons of toxicity related to NP-mediated heterogenous photocatalysis (reaction Eq. 7);.

$$\text{PANI-ZnO} + 2\ h^{+}_{VB} \rightarrow \text{PANI} + Zn^{2+}(aq) + 1/2\ O_2 \quad (7)$$

Zn(II) ions have a high potency to aquatic organisms. It was shown that the toxicity of ZnO NP for crustaceans and bacteria was due to photo-solubilized Zn(II) ions rather than ZnO NP themselves [66,67]. On the other hand, toxicity mitigation experiments confirmed that photocatalytic generation of ROS could be more toxic to *D. magna* than dissolved Zn(II) in acute and chronic toxicity tests [66]. In fact, ROS attacks damaged the antioxidant system of *D. magna* [65]. Once within the cytoplasm of intestinal cells, the Zn(II) ions can alter the permeability of the mitochondrial membrane, inducing ROS production [42,55,60]. The antibacterial activity of ZnO NP is due to the formation of HP on the surface of the particles. This reaction occurs by hydrolysis in the presence of UV or visible light and generates ROS [65,66]. Moreover, HP is a powerful oxidizing agent, which will attack cell components such as lipids, proteins and the DNA, leading to fatal lesions in the cell. Under these circumstances, the principal toxicity mechanism of ZnO identified in vitro is oxidative stress caused by photochemical ROS production, causing oxidative damage, inflammation and cell death. In particular, one major contributor to oxidative damage is HP which is converted from $O_2^{\bullet-}$ that leaks from the mitochondria [8,59,60]. In another related study, ZnO suspensions exihibited time- and concentration-dependent Zn(II) dissolution profiles during a 48-h time treatment period in the presence artificial and solar UV light. These solubilized Zn(II) ions, which form metal bindings to sulfur-hydrogen groups of proteins, especially in the plasma membranes, inhibit the cell growth of the test organisms imparting toxic responses [54,65]. They also induce active transport inhibition, alter amino acid metabolism and enzyme system disruption. In previous work it was also verified that photo-produced Zn (II) ions induce the formation of morphologically different vascular structures, leading to cell death [66]. Nevertheless, once inside the organism, an increase of NP reactivity could occur due to the high concentration of Zn(II) ions being released inside the organism, thereby causing synergistic effects and changing the osmotic imbalance in the cells, leading to cell damage and eventually death [66–68].

4. Conclusions

In this experimental work for the first time the acute toxicity of home-made PANI-ZnO composite nanomaterials (photocatalysts) with varying PANI: ZnO mass ratios and synthesis techniques was examined using two well-known and routinely used, standard bioassays, namely the *Vibrio fischeri* and *Daphnia magna* acute toxicity (inhibition and death/ immobilization) tests. Toxicity reponses were compared with the toxicities of their surrogate chemicals (PANI-ES, PANI-EB and ZnO). In addition, changes in acute toxicity after UV-A treatment of the PANI-ZnO composites in pure (distilled-deionized) water were studied. Experimental results indicated that some of the selected nanomaterials could have serious negative effects in the aquatic environment and their toxicity might increase after photocatalytic treatment. There were several factors affecting their toxicity behaviour such as i) type of test organism, ii) exposure time, iii) physicochemical properties of the NP (particle size, hydrophobicity, zero point of charge, etc.), iii) synthesis method/technique, iv) ZnO content as well as v) photocatalytic treatment conditions. Important would be to assess possible relationships between these multiple operating factors and establish the synergistic tendencies. In brief, nanomaterials offer several attractive and beneficial ways to remove priority pollutants and toxicants, however, careful assessment of their own biotoxic behaviour and ecotoxicological safety

deserves special attention before their application/implementation in real case studies. The information obtained from the above study will help to improve the understanding of the exotoxicological impacts of PANI-ZnO nanocomposite photocatalysts on freshwater ecosystems.

CRediT authorship contribution statement

Idil Arslan-Alaton: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Supervision, Project administration, Funding acquisition, Writing. **Olga Koba-Ucun:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Writing. **Miray Bekbolet:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation , Visualization, Supervision, Project administration, Funding acquisition, Writing. **Nazli Turkten:** Methodology, Investigation, Resources, Data curation, Visualization, Funding acquisition. **Yunus Karatas:** Methodology, Investigation, Resources, Data curation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apcata.2023.119199.

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