Environmental Health Engineering and Management Journal 2019, 6(1), 73–80
http://ehemj.com
doi 10.15171/EHEM.2019.08

Environmental Health Engineering and Management Journal

Original Article

Environmental Health Engineering and Management

Open Access Publish Free

Physicochemical transformation of ZnO and TiO_2 nanoparticles in sea water and its impact on bacterial toxicity

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Abstract

Background: The enormous properties of metal oxide nanoparticles make it possible to use these nanoparticles in a wide range of products. As their usage and application continue to expand, environmental health concerns have been raised. In order to understand the behavior and effect of metal oxide nanoparticles in the environment, comprehensive and comparable physicochemical and toxicological data on the environmental matrix are required. However, the behavior and effect of nanoparticles in the real environmental matrix, e.g. sea water, are still unknown.

Methods: In this study, the effects of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on the bacteria (gram positive-*Bacillus subtilis, Staphylococcus aureus*/gram-negative *Escherichia coli*, and *Pseudomonas aeruginosa*) in sea water were investigated. Furthermore, to better understand the behavior of the toxicity, surface chemistry, sedimentation, dissolution, particle size, and zeta potential of the nanoparticles dispersed in the sea water matrices were investigated using Fourier-transform infrared spectrometry (FTIR), ultraviolet–visible (UV-VIS) spectrophotometry, graphite furnace atomic absorption spectrometer (GFAAS), and dynamic light scattering (DLS), respectively.

Results: The environmental matrix had a significant influence on physicochemical behavior of the tested nanoparticles. Besides, the inhibition of tested bacteria was observed against ZnO and TiO_2 nanoparticles in the presence of sea water, while there was no inhibition in the controlled condition.

Conclusion: The results demonstrate that surface chemistry with exposure to the sea water can have a significant role on the physicochemical properties of nanoparticles and their toxicity.

Keywords: Nanoparticle toxicity, Titanium dioxide, Zinc oxide, Sea water, Physicochemical properties, Matrix effect

Citation: Baysal A, Saygin H, Ustabasi GS. Physicochemical transformation of ZnO and TiO_2 nanoparticles in sea water and its impact on bacterial toxicity. Environmental Health Engineering and Management Journal 2019; 6(1): 73–80. doi: 10.15171/EHEM.2019.08.

Article History:
Received: 26 December 2018
Accepted: 29 January 2019
ePublished: 17 February 2019

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Introduction

Nanoparticles (NPs) offer unique mechanical, chemical, electrical or optical properties and are used in a broad spectrum of applications, such as industrial, consumer, and medical products. With increase of the production and use of NPs, much attention has been drawn to evaluate the potential risks of these particles to the environment and human health (1-5).

The key aspect for understanding the potential risks of NPs to the environment is the type of environmental system (e.g. water, soil, and air) and its composition. Several techniques and studies are available that can provide information on the physicochemical properties and toxicological effects of metal oxide NPs, but most of the environmental studies have been conducted under artificial or controlled laboratory conditions. In order to determine whether NPs are toxic to a specific species and understand the toxicity mechanisms, most of the studies do not account for real conditions or environmental matrix (4-11). In particular, the effect of environmental matrix (matrix effect) on the physicochemical properties (e.g. surface chemistry) of the NPs and its contribution to the toxicity has been mostly ignored in the environmental studies. Peng et al (4) and Hsiung et al (12) showed that Cl^- and SO_4^{2-} ions in the water samples can promote the agglomeration of NPs and also reported the presence of the capping molecules on the surface of the ZnO NPs. However, their effect on the surface chemistry and toxicity has not been investigated in detail.

The toxicity of metal oxide NPs has been investigated in mammalian cell lines, microorganisms (bacteria, yeats, fungi, etc), plants, etc, in the literature to find their toxic

or ecotoxic effects using various methods such as classical methods (viability or inhibition assay), molecular-based techniques, or spectrometric techniques (bioluminescence assay, etc) (13-16). One of the most recognized organisms to investigate the toxicity of NPs are bacteria. Bacteria play many critical roles in the ecosytem and some bacteria (e.g. *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus*) can be found in the seawater through natural or anthropogenic sources (17). Also, it reflects natural or anthropogenic sources contributions in the ecosystem. The relationship between bacteria and NPs may provide significant information about the impact of NPs on the environment, and at the same time, signify that bacteria are good test models to assess the NPs toxicity at the cellular level in the ecosystem (18-20). Wide range of studies have investigated the toxic and nontoxic effect of NPs on bacteria. These studies tried to explain the inhibition of NPs by size, dissolution, and agglomeration (20-28). According to these studies, not only NPs characteristics (e.g. composition, size, and shape), but also the ionic strength and pH of the environmental matrix can influence their aggregation or dissolution and thus, alter toxicity (26-28). Some studies indicate that the released metal ions of the metal oxide NPs are the major cause of toxicity, however, other studies show that the dissolved ions were the major sources of toxicity (23-25). On the other hand, although there is no clear understanding of the effects of particle size on toxicity, most published results prove that the toxicity increases with decrease of particle size (20-22). In most of the ecotoxicity studies on the relationship between the physicochemical properties and toxicity data, the effect of environmental matrix (exposure/environmental media or matrix effect) has been disregarded. While some limited studies have investigated the effect of environmental media by soil or aquatic ecosystem, the effect of sea water on the physicochemical properties and the bacteria inhibition has not been investigated and further studies are required on this issue.

The aim of the study was to investigate the physicochemical transformation of some metal oxide NPs (ZnO and TiO_2 NPs) in the sea water and also to evaluate the NPs toxicity towards gram-negative bacteria (*E. coli* and *P. aeruginosa*) and gram-positive bacteria (*B. subtilis* and *S. aureus*) under exposure to various concentrations of the sea water.

Materials and Methods

Reagents

The zinc oxide (ZnO) and titanium oxide (TiO_2) NPs were obtained from Torrecid-Turkey and Nanografi-Turkey in two different sizes for each NP. All chemicals were of analytical grade (Merck, Germany; Fluka, Switzerland). The sizes of the NPs were 120 and 400 nm for ZnO, and 45 and 150 nm for TiO_2, respectively.

The model organisms were gram-negative bacteria (*E. coli* (*E. coli*) ATCC 25922 and *P. aeruginosa* ATCC 27853) and gram-positive bacteria (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923). They were acquired from the American Type Culture Collection (ATCC) (Manassas, USA). Cultures were activated at 37°C in darkness overnight using nutrient agar (peptone from meat: 5.0 g/L, meat extract: 3.0 g/L, agar-agar:12.0 g/L) obtained from Merck (Product number 1.05450, Merck KGaA Darmstadt, Germany).

Sampling and characterization of the sea water

To find the effect of environmental matrix or exposure media on the physicochemical properties and toxicity behavior of the NPs, sea water was used at two different concentrations. The sea water was collected from Florya Beach-Istanbul, Turkey, and kept in sterile polyethylene tubes. Direct application of sea water was considered as high concentration (H-sea water) and 1:10 dilution of sea water with ultrapure water considered as low concentration (L-sea water). Some physicochemical properties of the sea water samples are shown in Table 1.

Preparation and characterization of nanoparticles

To investigate the effect of environmental matrix on the physicochemical properties of the NPs, 5.0 mg of the NPs was treated in one liter of the low and high concentration of sea water during 24 hours, then, the environmental matrix was removed and dried until full evaporation in a vacuum oven. All measurements were repeated at least five times. For the control, NPs were treated with ultrapure water using above-mentioned procedure. Then, control and sea water treated NPs were investigated by their particle size, zeta potential, surface chemistry, dissolution, and sedimentation.

The surface chemistry of NPs was investigated using Fourier-transform infrared (FTIR) spectrometry (Perkin Elmer). The FTIR analysis was acquired in the range of 4000 to 650 cm^{-1} to investigate the effect of environmental matrix on the surface chemistry of control and treated NPs.

The particle size and zeta potential of the NPs in suspensions were measured via dynamic light scattering (DLS) using Zetasizer Nano ZS instruments (Malvern,

Table 1. Some physicochemical properties of the tested sea water samples and control (N = 3, SD <10%)

Matrix	Sulfate (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)	Conductivity (mV)
Control (ultrapure water)	Not detected	Not detected	Not detected	Not detected	22.5
L-sea water	274.6	Not detected	Not detected	4.1	-48.8
H-sea water	2848.3	Not detected	Not detected	38.9	-97.5

UK) at 25°C at a scattering angle of 173° using a 4 mW He-Ne laser. Control and treated NPs were sonicated for 5 minutes and placed in Standard Malvern zeta potential disposable capillary cells and polystyrene cuvettes for zeta potential and size measurements, respectively.

To evaluate the NPs sedimentations, NPs dispersions were prepared using similar protocols used for the preparation of the NPs. The sedimentation rate (A/A_0) was determined by monitoring the optical absorbance (at 372 and 378 nm for Zn and Ti, respectively) as a function of time, during an interval of 0 and 24 hours, which indicates A_0 and A, by ultraviolet–visible (UV–VIS) spectrophotometry (Libra S70 UV-VIS spectrophotometer, BioChrom, Cambridge, UK). All measurements were performed at 25°C in square cuvettes with 1 cm light path; the center of the light beam struck the cuvette 1.5 cm above its bottom.

The released ion concentration in the samples was adapted from Suman et al (29) and measured by graphite furnace atomic absorption spectrometer (GFAAS, Varian Gmbh). The suspensions of ZnO and TiO_2 were prepared by dispersing the NPs in sea water in a bath sonicator for 30 minutes to break possible aggregates as much as possible and mildly mixing during 24 h. Then, dissolution rates (C/C_0) were calculated at 24 h (C) and 0 (C_0).

Toxicity assessment

The bacterial toxicity of the NPs was assessed using colony counting method (18,20,30-32). Firstly, in order to examine the role of the NPs on the bacterial viability, the controlled conditions were applied. For this purpose, 5 mg/L NPs was applied and incubation time was tested between 0 and 24 hours in the controlled condition, in which nutrient agar was prepared using ultrapure water. After the exposure/incubation time, colony-forming units (CFUs) were counted in each test unit. The percentage of the susceptibility was calculated using the following equation: (N/No)*100, where *N* is the agar media with NPs employed as a sample and *No* is the agar media without NPs employed as a control; the non-inhibitory duration chosen for the further analysis is 24 hours. To investigate the effect of sea water as an environmental matrix, the procedure on the controlled condition was adapted and 2% agar solution was prepared using different concentrations of sea water and 5 mg/L NPs (N). The 2% agar medium was prepared using different concentrations of sea water without NPs and employed as a control for the environmental matrix (No). Cultures of each of the microorganisms were prepared at 37°C in darkness overnight using nutrient broth, and 100 µL of culture was used to inoculate agar Petri dishes with specific concentrations of NPs. The test units were then placed in an incubator at a controlled temperature of 37°C in darkness. The toxicity was evaluated by comparing the number of CFUs on the nutrient agar plates after 24-hour of exposure. Each concentration (e.g., treatment) was repeated five times.

Statistical analysis

The ANOVA with post hoc Tukey was used to evaluate the difference between the control and each treatment, as well as different treatments. Statistical significant level was considered at $P < 0.05$. Data were analyzed using Spearman correlation (two-tailed test) by SPSS version 17.0.

Results

To investigate the structure and stability of the ZnO and TiO_2 NPs under influence of sea water, some physicochemical properties of these NPs were evaluated by DLS, FTIR, UV-VIS, and GFAAS after 24 hours of sea water exposure.

Figure 1 shows the surface chemistry of the NPs obtained in controlled condition and different concentrations of sea water by FTIR spectroscopy. As can be seen in the FTIR spectrum of the tested NPs, there is a weak or no absorption band in control. With the treatment of different concentrations of sea water, strong broad band (3550-3200 cm^{-1}), Strong stretching (1650 and 1150-1085 cm^{-1}) were observed on the NPs surfaces, and these represented O-H, N-H, C=O, and C-N groups, respectively. The results also indicated that hydroxylation was the dominant surface functional groups with the exposure of the sea water and this was independent from the type of metal oxide. In addition to the induction of new functional groups on the NPs surfaces, the intensities were increased with increase of the concentration of sea water. According to matrix characterization (Table 1), the nitrogen-related group on the NPs surface was formed by the detection of the ammonia in sea water.

The sedimentation behavior of the NPs in the environmental matrices was investigated by UV-VIS (Figure 2). There was no consistent sedimentation behavior between NPs and matrix, unless the rate of sedimentation decreased with increase of the sea water concentration.

The release rate of Zn and Ti from the control and seawater was evaluated by GFAAS analysis. Table 2 shows the ion release rates from NPs after 24 hours of sea water treatment. The sea water treatment showed the effects of dissolution on NPs. The release rate of Zn ions from both Zn NPs was high in low concentration of sea water compared to the high concentration of sea water. However, the release rate of Ti ions from Ti NPs increased with increase of the sea water concentration.

The particle size and zeta potential of the NPs in the control and sea water matrix are shown in Table 3. Zn1 were negatively charged and Zn2 were positively charged in control, however, zeta potentials became more negative with increase of the sea water concentration, and both Zn NPs were charged negatively after exposure of sea water. While the zeta potential of Zn1 became slightly negative with increase of sea water concentration, the zeta potential of Zn2 became significantly negative with increase of sea water concentration. Both tested Ti NPs were negatively

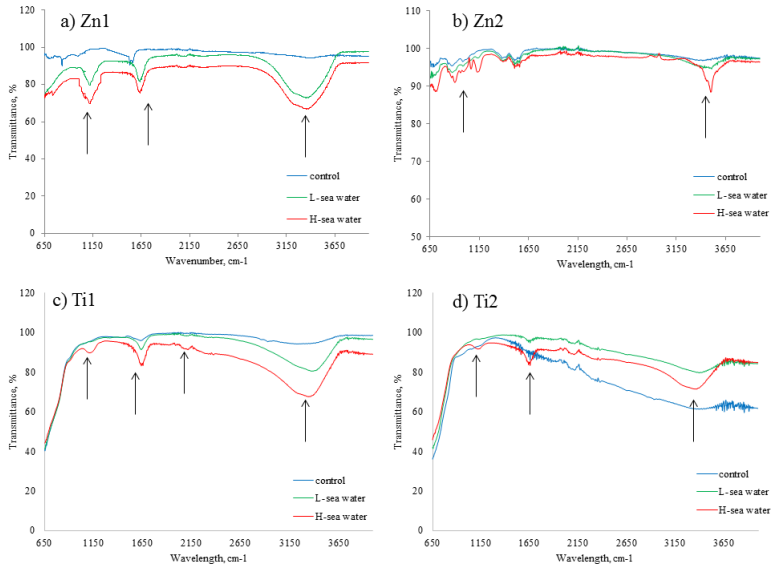

Figure 1. FTIR spectrum of the tested nanoparticles under controlled condition at low and high concentration of sea water (nanoparticle concentration: 5 mg/L, exposure duration: 24 h, N=3, L-sea water: low concentration of sea water, H-sea water: high concentration of sea water).

charged in control. In the sea water, the negativity of Ti1 was significantly decreased. On the other side, the negativity of Ti2 increased with increase of the sea water concentration. Furthermore, the particle size of Zn NPs was significantly decreased with increase of the sea water concentration. However, particle size of Ti NPs increased in the sea water. On the other hand, with increase of the sea water concentration, the particle size of all tested NPs decreased.

Figure 3 shows the results of the inhibition rate of bacteria exposed to tested NPs under exposure of various concentrations of sea water and controlled condition. While there was no toxicity for the tested NPs in controlled condition, different toxicity patterns were found with the matrix effect of sea water. Although both gram-negative bacteria had high tolerance to tested Zn NPs in sea water, toxicity effect was observed for gram-positive bacteria (Figure 3a-b). According to the results of the exposure concentration of sea water, the inhibitory effect of ZnO NPs on gram-positive microorganisms increased by increase of the sea water concentration. In addition, among gram-positive bacteria, *B. subtilis* was more vulnarable than *S. aureus*.

The toxicity of Ti NPs to bacteria in sea water was also investigated and it was revealed that the viability decreased in both gram-positive and gram-negative microorganisms (Figure 3c-d). The high tolerance of the gram-negative bacteria was found against Ti NPs in the low concentration of sea water, while viability of gram-positive bacteria decreased in the high concentration of sea water. In low concentration of sea water, almost the inhibition of 90% and 10-15% was observed in *B. Subtilis* and *S. aureus*, respectively. On the other hand, when the concentration of sea water increased, the inhibition also increased in gram-negative bacteria.

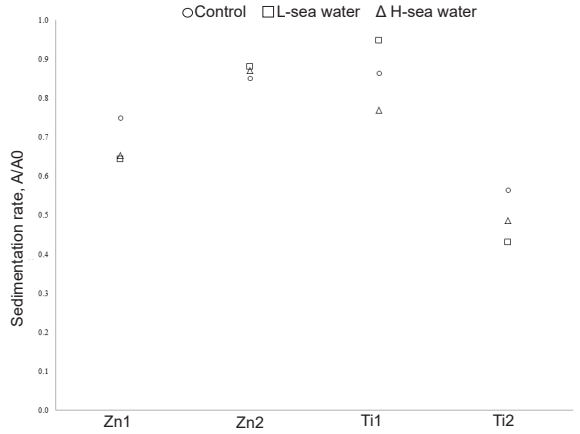

Figure 2. Sedimentation rate of the tested metal oxide NPs. L-sea water: low concentration of sea water, H-sea water: high concentration of sea water. A: absorbance at 24 h, A_0: absorbance at 0. N = 3, SD < 10%.

Discussion

Environmental evaluations on the physicochemical behavior and toxicity of the NPs confirmed the role of pH, electrolyte composition, and the presence of organic matter in the aggregation or stabilization of NPs. However, there is no consensus about these parameters and very few

Table 2. The dissolution rate of NPs in control and in different concentrations of sea water using GFAAS

Nanoparticle	Control	L-sea Water	H-sea Water
Zn1	0.14	0.73	0.38
Zn2	0.21	0.65	0.43
Ti1	0.32	0.41	0.45
Ti2	0.27	0.53	0.57

Table 3. Particle size and zeta potential of the tested nanoparticles under controlled condition and sea water

Matrix	Zn1		Zn2		Ti1		Ti2	
	Size (d.nm)	Zeta Potential (mV)	Size (d.nm)	Zeta potential (mV)	Size (d.nm)	Zeta Potential (mV)	Size (d.nm)	Zeta Potential (mV)
Control	1470.0	-19.2	1160.0	9.9	353.0	-27.5	1038.0	-3.8
L-sea water	340.3	-18.7	901.5	-19.4	1059.1	-7.8	1251.0	-11.5
H-sea water	200.4	-21.3	104.5	-20.7	911.8	-7.9	927.2	-12.2

Nanoparticle concentration: 5 mg/L, exposure duration: 24 h L-sea water: low concentration of sea water, H-sea water: high concentration of sea water (N=5, SD<5%).

studies have focused on the structure and stability of the NPs in the environmental matrix and their role on toxicity (11,13,30-32).

In order to understand the influence of environmental matrix on the physicochemical properties of NPs, formation or loss of surface functional groups is a key parameter (33,34). However, surface chemistry has been mostly ignored in the environmental assessments. On the other hand, it is reported that NP surfaces can sorb to some co-ions through the oxide atoms or capping agents on the particle surface by the O_2, H_2O, or UV implementation on the matrix (31,32,35,36). According to the FTIR spectrum (Figure 1) and matrix chemical characterization (Table 1), the NPs surfaces were coated with the functional groups in the presence of nitrogen-related compounds and organic species in the sea water, and these results indicate the importance of the environmental matrix on the surface chemistry of the NPs.

Also it was revealed that sedimentation behavior of NPs can be changed because it is dominantly affected by the sea water properties, e.g. pH and electrolytes (4,33,34,37). Decreasing of the sedimentation rate can be explained by the forces existing between the particles, and these forces depend on the formation or loss of the functional groups on the NPs surfaces (Figure 2). Furthermore, the sedimentation rate can be reduced by the high concentration of organic compounds and low conductivity in the high concentration of sea water, as explained by by some studies (4,33,34,37).

Dissolution of NPs is an important property that influences their toxicity or environmental impact. Both solubility and rate of dissolution are related to the surface chemistry, particle size, surrounding media and its properties (e.g. pH) (4,26,30,37). The presence of anions in the sea water was supposed to serve as binding ligands, thus, promoting the dissolution of tested NPs (Table 2). Another possible explanation is the internalization of the particles by the formation of –OH groups (4,26,30,37).

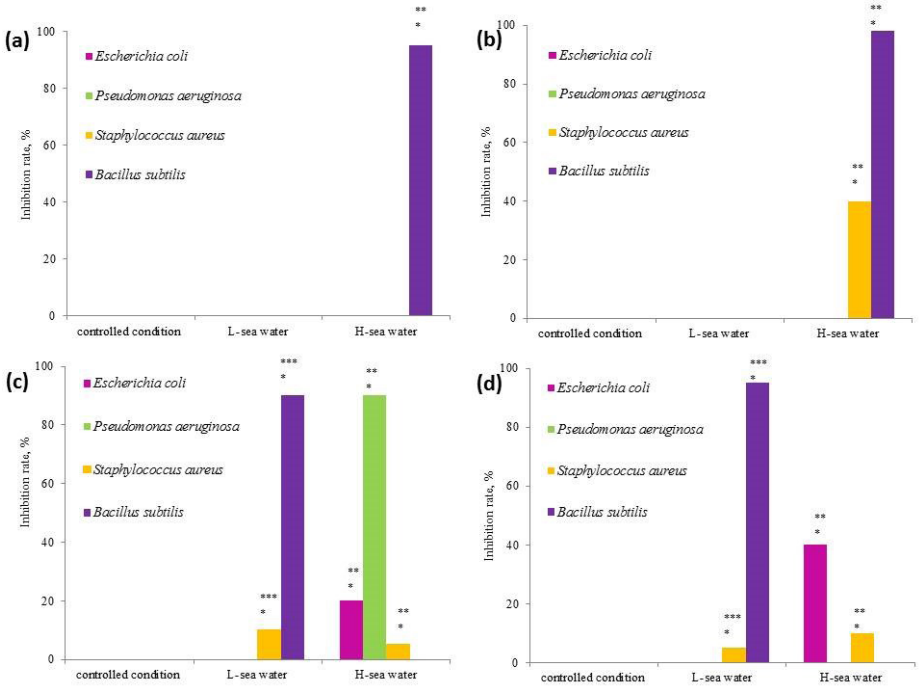

Figure 3. Inhibition rate of bacteria exposed to the tested nanoparticles under exposure of various concentration of sea water and controlled conditions. (a) Zn1, (b) Zn2, (d) Ti1, (e) Ti2. Different Arabic letters for the bars indicate statistically significant results. *In relation to control (P<0.05); **In relation to low concentration of sea water ($P < 0.05$); ***In relation to high concentration of sea water ($P < 0.05$). (nanoparticle concentration: 5 mg/L, exposure duration: 24 h, N=5, L-sea water: low concentration of sea water, H-sea water: high concentration of sea water)

Zeta potentials can give information about the agglomeration and functional groups on the surface (13). Different patterns on the zeta potentials can be due to the formation of new functional groups on the NPs surfaces. The formation of –OH groups on the Zn2 and Ti2 increased the negativity of zeta potential. On the other hand, the FTIR spectrums (Figure 1) and zeta potential (Table 3) showed that not only –OH groups but also C-N and N-H groups influenced the zeta potential of Zn1 and Ti1 surfaces. Furthermore, coating/functionalization capacity of NPs surfaces and sea water composition can influence the zeta potential. However, further studies on this issue are required. Particle size and zeta potential are negatively correlated, and this result was approved in the present study, except for Zn1. The results show that other parameters can be considered for the internalization of Zn1. Furthermore, sea water affected the Zn NPs as an internalized matrix, contrarily, it triggered the agglomeration of Ti NPs. All the results indicate that sea water has effect on the stability and agglomeration of NPs. There was no inhibition in the controlled condition, however, the inhibition was observed in the presence of sea water for the tested NPs (Figure 3). The inhibition of the *B. subtilis* can be mainly due to the different charges between Zn NPs and the bacterium due to the increased negativity of Zn NPs and decreased particles size (31,32). Also, *S. aureus* showed viability loss against Zn2 due to the high negative surface charge and low particle size of Zn2 in the sea water. The inhibition differences between the tested Zn NPs was related to the particle size, so that the internalization of Zn NPs increased the inhibition degree and diversificated the inhibited gram-positive bacteria. The difference of inhibition between gram-positive bacteria can be due to use of a set of enzymes to make teichoic acid (38,39). Besides, different number of phosphate unit were used to make cell wall teichoic acids (39). *S. aureus* has more glycerol and ribitol chains on teichoic acids polymers, which resulted in less toxic effect compared to *B. subtilis*. Therefore, while the main inhibition reason seems to be bacteria cell envelope, the background of the inhibition and bias can be caused by the functionalization of the surfaces using environmental matrix.

The viability of species had different patterns against Ti NPs in sea water compared to Zn NPs (Figure 3c-d). For example, high inhibition of *B. subtilis* and viability loss of *S. aureus* towards Ti NPs were observed in low concentration of sea water that can be mainly caused by the agglomeration. However, in high concentration of sea water, no inhibition of *B. subtilis* and changes of *S. aureus* viability was obtained by decreasing the particle size. Furthermore, viability loss of gram-negative bacteria (*E. coli* and *P. aeruginosa*) can be obtained by the dissolution of Ti NPs in high concentration of sea water. These results showed that agglomeration and dissolution of Ti ions are the two main factors for the inhibition of bacteria.

All the obtained results also proved that aggregation and stability are not the main effective factors. Aggregation or stability can be affected by the surface functionalization using environmental matrix and its composition. Although the effect of the surface functionalization of NPs using environmental matrix has been identified, it is important to determine which chemical groups can influence the inhibition of bacteria.

Conclusion

Ecotoxicity studies confirmed the role of different physicochemical properties on the NPs toxicity. However, surface functionalization of NPs by the environmental matrix has not been explained. This study showed that physicochemical characteristics of NPs are strongly related to the environmental matrix and its composition. Also content/composition of matrix is an important factor on the NPs fate and behavior in the aquatic environment. According to the results, the surface of NPs is functionalized by different chemical groups and there is a correlation between functionalization and inhibition. Also, surface functionalization influences zeta potential, sedimentation, dissolution, as well as particle size. The functionalization degree or structure of the NPs surface in such complex and heterogeneous systems is more challenging, therefore, further investigations are still needed.

Acknowledgments

The authors would like to gratitude Prof. Filiz Altay, Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, and Prof. Mustafa Ozyurek, Chemistry Department, Faculty of Engineering, Istanbul University, providing DLS and FTIR instruments.

Ethical issues

This study does not contain any studies with human participants or animals performed by any of the authors. The authors certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published separately elsewhere.

Competing interests

The author declare that they have no competing interests.

Authors' contributions

All authors contributed in data collection, analysis, and interpretation. All authors critically reviewed, refined, and approved the manuscript.

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