

# Inactivation of Heterotrophic Bacteria in Well Water Using ZVI, TiO<sub>2</sub> and ZnO Nanoparticles

Mohammad Ali Zazouli<sup>a</sup>, Marjan Safarpour<sup>\*b</sup>, Farzaneh Veisi<sup>b</sup>, Mahdieh Alam Gholilou<sup>b</sup>

<sup>a</sup>Department of Environmental Health, School of Health and Health Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

<sup>b</sup>Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran.

\*Correspondence should be addressed to Mr.MarjanSafarpour, Email: m21\_safarpour@yahoo.com

## A-R-T-I-C-L-E-I-N-F-O

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## A-B-S-T-R-A-C-T

**Background & Aims of the Study:** The heterotrophic bacteria are widely used as a water microbial pollution index for drinking water. The aim of this study was to investigate the effect of metallic nanoparticles such as Zero Valent Iron (ZVI), Titanium dioxide (TiO<sub>2</sub>) and Zinc oxide (ZnO) on Heterotrophic Bacteria inactivation in well water.

**Materials & Methods:** We performed an experimental-laboratory study that the effect of nanoparticles type Zero valent iron (ZVI), Titanium Dioxide (TiO<sub>2</sub>) and Zinc oxide (ZnO) in constant contact time (30 min) and nanoparticles dose (1 g/L) was investigated on heterotrophic bacteria inactivation.

**Results:** The results showed that TiO<sub>2</sub> was detected more effective than ZnO and ZVI. The HPC inactivation after 30 min of retention time by TiO<sub>2</sub>, ZnO and ZVI nanoparticles were 71.5, 50 and 36.4 as percent, respectively. The maximum bacteria inactivation was 98.82% in the presence of TiO<sub>2</sub> nanoparticles.

**Conclusions:** It is concluded that nanoparticles used in this study could be effectively used to increase the efficiency of removing heterotrophic bacteria from water and can be considered for microorganisms' inactivation.

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## Background

There are several microbial indicators for assessment of the microbiological safety of drinking water. One of the standard methods for controlling of microbiological water quality in the water treatment systems is heterotrophic plate counts that described as colony-forming units (CFU) (1). High HPC is threatening to human health and may cause gastrointestinal diseases (2). In recent years, a lot of discussion has been raised about the effects of the common disinfectant in water, such as: carcinogenic effects, forming harmful disinfection byproducts (DBPs) (3,4) and microorganisms

resistant that the need for research to find alternatives disinfection has increased (5). The rapid development of nanotechnology and its wide use in the environmental field due to high reactivity and the large surface area has attracted the attention of many studies (4,6-9). Nanotechnology has introduced different types of nanomaterial including Titanium dioxide (TiO<sub>2</sub>), silver nanoparticles, etc to water industry that can have antimicrobial properties (10-12). The several studies have been carried out on the use of TiO<sub>2</sub> in the removal of contaminants from aquatic environments (4,10,13,14). The research showed that titanium dioxide can be effective in removal of different

types of pollutants including microorganisms (bacteria, viruses, cysts, fungi, algae and protozoa), due to effect of OH radicals, hydrogen peroxide, superoxide anions and metal ions (15-17). In recent years, some studies have been performed on the microbial quality of water, for instance Maness et al. (18) and Cho et al. (13) investigated that inactivation of E.coli in the presence of the TiO<sub>2</sub> photocatalytic disinfection. Nowadays nZVI due to high reduction capacity, high efficiency, abundance, cheapness and also their unique atomic, molecular and chemical properties was used in the treatment of contaminated water (19,20). In addition, one of the important features nZVI is high reduction capacities that have been considered by many scientists in various fields (21). Diao et al. (22) reported that complete inactivation was achieved both for B. Subtilis var. Niger and P. fluorescens when treated with NZVI particles. Also, antibacterial properties of ZnO have been much attention.

Zhang et al. (23) reported that photocatalytic degradation and inactivation of Escherichia coli can be obtained by ZnO/ZnAl<sub>2</sub>O<sub>4</sub>.

According to literature review there is no research about the effect of nanoparticles on heterotrophic bacteria inactivation.

#### Aims of the study:

The aim of this study was to evaluate the ability of nanoparticles to heterotrophic bacteria inactivation in water.

### Materials & Methods

#### Materials:

Zero-valent iron powder and Zinc oxide nanoparticles were purchased from Research Institute of Petroleum Industry of Iran (RIPI). Titanium dioxide was purchased from Iranian nanomaterials Pioneers Company. All nanoparticles were used without any more treatment. Table 1 shows the properties of nanoparticles, according to manufactures.

Table1) characteristics of nanoparticles

Nanoparticles	Particle sizes (nm)	Purity (%)	Specific surface areas (m <sup>2</sup> /g)	Color
ZVI	8-18	65	59-79	black
TiO <sub>2</sub>	10-25	>99	200-240	white
ZnO	10-30	>99	20-60	White

#### Nanoparticles:

The morphology of ZnO and ZVI nanoparticles were determined in the RIPI by X ray diffraction (XRD) pattern model PW1840 Philips, Transmission Electron Microscopy (TEM) model Bruker AT-210 and Scanning Electron Microscopy (SEM) model S360 Cambridge instrument UK. The TEM image of TiO<sub>2</sub> was determined by Transmission Electron Microscopy model Hu-12A.

#### Sampling of water:

A total of 32 samples were collected from eight sites from several deep wells supplying water to the Mazandaran University of Medical Sciences located in Khazar road, Sari, Iran. Sampling was conducted between the hours of 9 am to 1 pm. Water treated with a plain chlorination. Water sampling was done according to the

Standard Methods for the Examination of Water and Wastewater, method 9060 A (24).

#### Heterotrophic bacteria inactivation experiments:

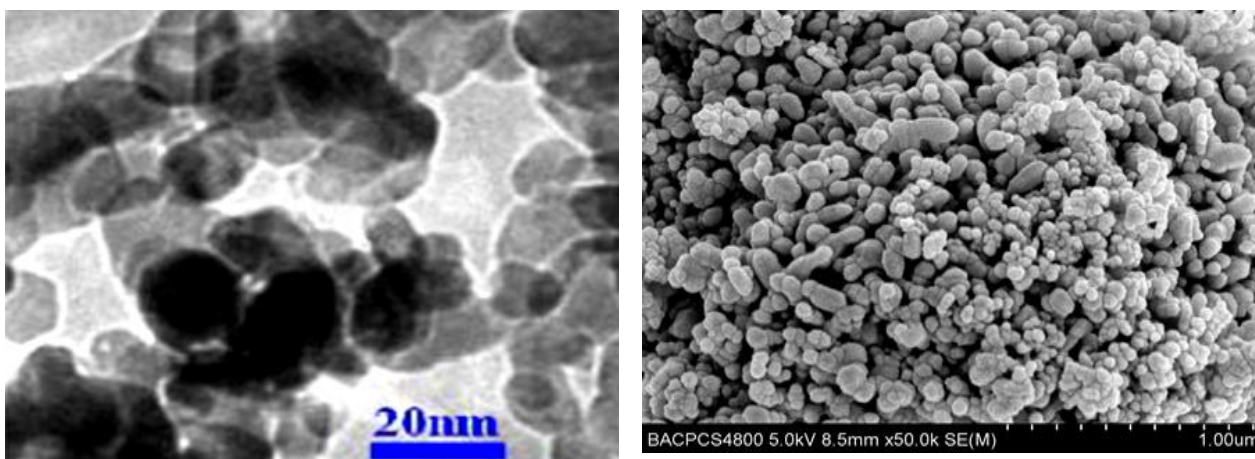
All equipment used in the experiments to be sterilized in an autoclave before the tests. In the laboratory; every eight collected samples were divided into four parts. One sample was examined without any treatment from each sites the control. Based on the literature review (10,22,25) 1g/L certain nanoparticles (ZVI, TiO<sub>2</sub> and ZnO) were added into the water and shaken with a rate of 200 rpm using a horizontal shaker (Sibata) for 30 min. After the required time, the suspension was centrifuged for 30 minute at 4000 rpm. After the centrifugation, the samples were filtered using

membrane filter with 0.45  $\mu\text{m}$  pore size (24). To avoid the filtration effect on the test results, the samples were not filtered before to test. The Standard Methods for the Examination of Water and Wastewater, method 9215c (Spread Plate Method) was used for analysis of HPC (24). R<sub>2</sub>A culture medium (Merck) was prepared and then sterilized in an autoclave at 121 °C and then 0.1-0.5 mL of sample volume was spread onto the surface of R<sub>2</sub> agar medium and incubated for 48 h at 35°C. The results were analyzed at the end.

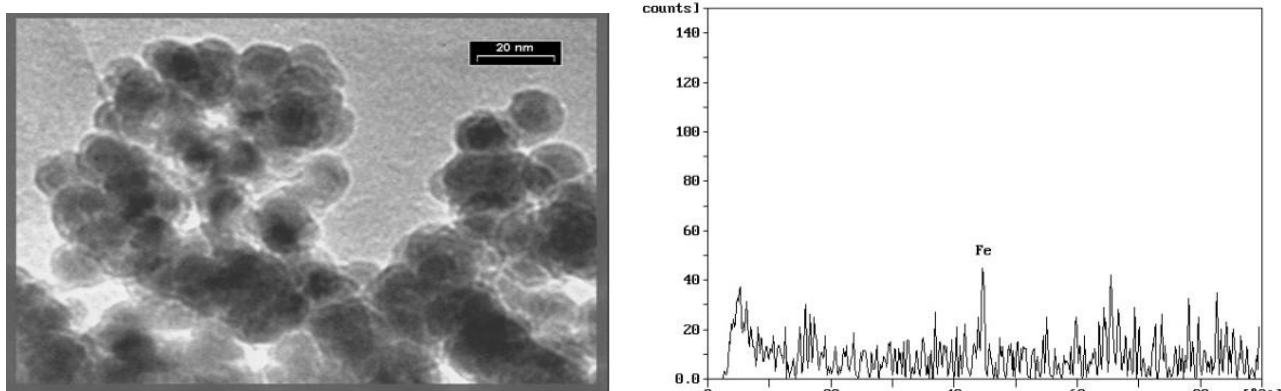
## Results

### Characterization of nanoparticles:

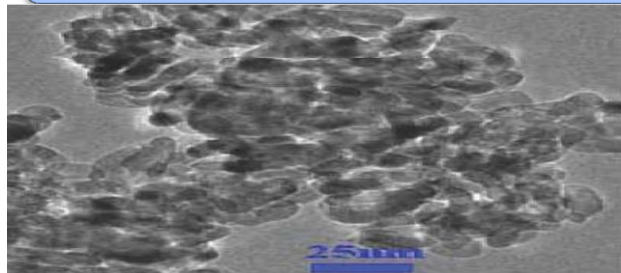
The images of XRD, TEM and SEM of used nanoparticles are shown in Figures 1 to 3. In the Figure 2 (B), the main peaks are characteristic peaks of iron and it should be noted that the samples were amorphous because they were prepared through liquid method. Therefore, only iron index peaks are evident in the 44.7 area.



(A) (B)  
Figure 1) (A) TEM images and (B) SEM image of ZnO



(A) (B)  
Figure 2) (A) TEM images and (B) XRD pattern of nZVI

Figure 3) TEM images of TiO<sub>2</sub>**Effect of TiO<sub>2</sub>:**

TiO<sub>2</sub> have a significant effect on heterotrophic bacteria inactivation. Maximum efficiency is observed in the experiment No.7 that the number of bacteria is reduced from eighty-five to one after treatment with TiO<sub>2</sub> nanoparticle. The heterotrophic bacterial inactivation efficiency by different nanoparticles was listed in table 2 based on this table remarkable amounts of bacteria (98.8 %) are destroyed by TiO<sub>2</sub>. It is noteworthy that this high efficiency has been created no UV radiation and activation of nano. An antibacterial property of the TiO<sub>2</sub> has been attributed to two mechanisms: generation reactive oxygen species (ROS), damage to various parts of the cell (4).

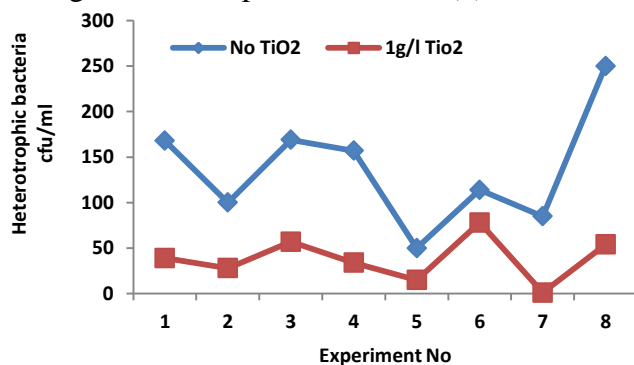
Figure 4) Effect of TiO<sub>2</sub> on the removal of heterotrophic bacteria at 30 min

Table 2) Heterotrophic bacterial inactivation

nanoparticles	Heterotrophic bacterial inactivation (%)		
	Min	Mean	Max
ZVI	6.5	36.4	67.1
ZnO	13.6	50	91.8
TiO <sub>2</sub>	31.6	71.5	98.8

**Effect of ZnO:**

The highest efficiency of ZnO nanoparticles in the removal of heterotrophic bacteria was 91.8% that close to TiO<sub>2</sub> efficiency. In addition

to the above-mentioned mechanisms for titanium dioxide, production of Zn ions and H<sub>2</sub>O<sub>2</sub> are effective on toxicity of ZnO (4,26).

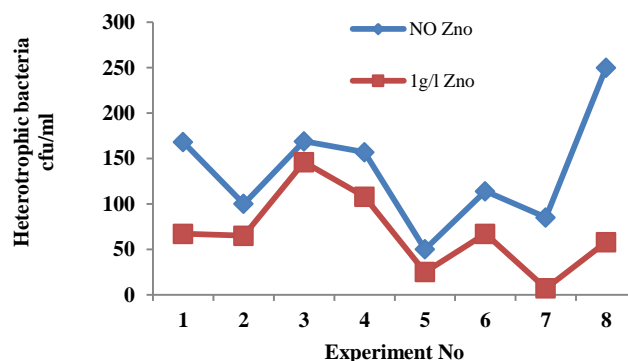


Figure 5) Effect of ZnO on the removal of heterotrophic bacteria at 30 min

**Effect of nZVI:**

A number of studies have shown that iron nanoparticles have antibacterial properties (22,27,28). The average removal of heterotrophic bacteria was obtained 36.4% at 8 experiments by nZVI. Average removal of ZnO and TiO<sub>2</sub> nanoparticles was estimated 5 and 71.5 percent, respectively. Mechanisms of nZVI in destroying bacteria are: physical impaired of the cells, production fenton conditions, generation oxidative stress (29).

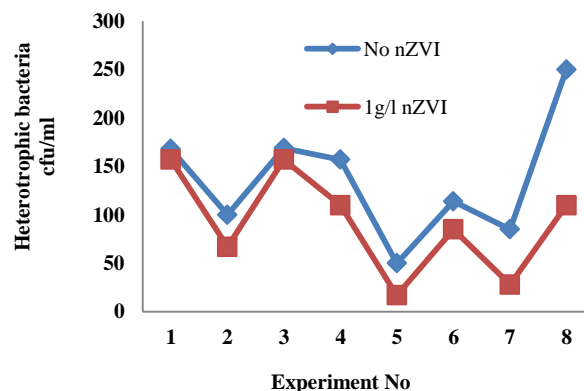


Figure 6) Effect of nZVI on the removal of heterotrophic bacteria at 30 min

**Discussion**

The results of this study are different with results reported by Adams et al. who

demonstrated antibacterial activity of ZnO nanoparticles higher than of TiO<sub>2</sub> and SiO<sub>2</sub> in the removal of Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* (25). Similar results were reported by Rezaei-Zarchi *et al.* (30) they found that nano-TiO<sub>2</sub> has proven to be more efficient antibacterial agent as compared to nano-CdO. Moreover, Kasemets *et al.* (31) have used of ZnO, CuO and TiO<sub>2</sub> to evaluate the toxic effect of nanoparticles to *Saccharomyces cerevisiae*. Their results showed that nano and bulk TiO<sub>2</sub> were not toxic and nano CuO was about 60-fold more toxic than bulk CuO to *Saccharomyces cerevisiae* that was not similar to this research. Aruoja *et al.* (14) showed that ZnO nanoparticles compared with CuO and TiO<sub>2</sub> are most toxic to microalgae *Pseudokirchneriella subcapitata*. Their research results are not close to data obtained by present study. In another study conducted by Sawai *et al.* for the study of antibacterial metallic oxide powders (32). The results indicated that ZnO powder was the least effective against *E. coli* among the, MgO and CaO powder. Additionally, Dasari *et al.* (33) stated that the LC<sub>50</sub> values was in the order of ZnO < CuO < TiO<sub>2</sub> < Co<sub>3</sub>O<sub>4</sub> under light condition, their results showed that ZnO were the most toxic among the tested nanoparticles, which is not in accordance with present findings. The toxicity of nanoparticulate and bulk ZnO, Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> to the nematode *Caenorhabditis elegans* was studied by Wang *et al.* (34). They reported that ZnO has minimum LC<sub>50</sub> among the tested nanoparticles that are different with this research results. Various studies demonstrated the mechanism of ZnO on a wide range of bacteria (35-38). In a study was done by Leet *et al.* on the effect of nZVI on the *Escherichia coli* (28). It was reported that *Escherichia coli* are inactivated in the presence of nZVI rapidly (28). Also Diao *et al.* realized that 95%, 80% inactivation of *B. subtilis* var. niger at nZVI concentrations of 1, 0.1 mg/ml (22) which was much higher than the values obtained in this study. The effects of ZVI

nanoparticles on a natural river water bacterial community were studied by Barnes *et al.* (27). They reported that the addition of 100 mg/L ZVI nanoparticles to aerobic river water was not toxic to the indigenous river water bacterial community.

## Conclusion

This is the first study on the effects of various nanoparticles on heterotrophic bacteria that is an important indicator in drinking water. The results showed that the nanoparticles can be used in of heterotrophic bacteria inactivation in drinking water with further investigation of their fate in the distribution network and its impact on human health and the environment and may be a perfect alternative to traditional methods of water disinfection. The HPC inactivation after 30 min of retention time by TiO<sub>2</sub>, ZnO and ZVI nanoparticles were 71.5, 50 and 36.4 as percent, respectively. Table 2 shows the heterotrophic bacterial inactivation efficiency by different nanoparticles. TiO<sub>2</sub> was more effective than nano ZnO and nano ZVI.

## Footnotes

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### Conflict of Interest:

The authors declared no conflict of interest.

## References

1. Zazouli MA, Safarpour Ghadi M, Veisi A, Habibkhani P. Bacterial Contamination in Bottled Water and Drinking Water Distribution Network in Semnan, 2012. *J Mazandaran Univ Med Sci* 2013;22(1):151-9. (Full Text in Persian)

2. Edberg SC, Allen MJ. Virulence and risk from drinking water of heterotrophic plate count bacteria in human population groups. *Int J Food Microbiol* 2004;92(3):255-63.
3. Shang K, Ai S, Ma Q, Tang T, Yin H, Han H. Effective photocatalytic disinfection of *E. coli* and *S. aureus* using polythiophene/MnO<sub>2</sub> nanocomposite photocatalyst under solar light irradiation. *Desalination*. 2011;278(1-3):173-8.
4. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, et al. Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Res* 2008;42(18):4591-602.
5. Bao Q, Zhang D, Qi P. Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection. *J Colloid Interface Sci* 2011;360(2):463-70.
6. Zazouli MA, Dianatitilaki R, Safarpour M. Nitrate Removal from Water by Nano zero Valent Iron in the Presence and Absence of Ultraviolet Light. *J Mazandaran Univ Med Sci* 2014;24(113):151-61. (Full Text in Persian)
7. Zazouli MA, Ebrahimzadeh MA, Yazdani Charati J, Shiralizadeh Dezfoli A, Rostamali E, Veisi F. Effect of Sunlight and Ultraviolet Radiation in the Titanium Dioxide (TiO<sub>2</sub>) Nanoparticles for Removal of Furfural from Water. *J Mazandaran Univ Med Sci* 2013;23(107):126-38. (Full Text in Persian)
8. Dyanati-Tilaki RA, Zazouli MA, Yazdani J, Alam-Ghaliloo M, Rostamali E. Degradation of 4-chlorophenol by sunlight using catalyst of zinc oxide. *J Mazandaran Univ Med Sci* 2014;23(2):196-201. (Full Text in Persian)
9. Nadafi K, Vosoughi M AA, Borna MO, Shirmardi M, Mbbb V, Amm A, MOuuu B, Mppp S. Reactive Red 120 dye removal from aqueous solution by adsorption on nano-alumina. *J Water Chem Technol* 2014;36(3):125-33.
10. Lydakis-Simantiris N, Riga D, Katsivela E, Mantzavinos D, Xekoukoulotakis NP. Disinfection of spring water and secondary treated municipal wastewater by TiO<sub>2</sub> photocatalysis. *Desalination*. 2010;250(1):351-5.
11. Choi O, Deng KK, Kim N-J, Ross Jr L, Surampalli RY, Hu Z. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res* 2008;42(12):3066-74.
12. Hadei M, Aalipour M, Mengli Zadeh N, Pourzamani H. Ethylbenzene Removal from Aqueous Solutions by Nano Magnetic Particles. *Arch Hyg Sci* 2016;5(1):22-32.
13. Cho M, Chung H, Choi W, Yoon J. Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO<sub>2</sub> photocatalytic disinfection. *Water Res* 2004;38(4):1069-77.
14. Aruoja V, Dubourguier H-C, Kasemets K, Kahru A. Toxicity of nanoparticles of CuO, ZnO and TiO<sub>2</sub> to microalgae *Pseudokirchneriella subcapitata*. *Sci Total Environ* 2009;407(4):1461-8.
15. Mori M, Sugita T, Mase A, Funatogawa T, Kikuchi M, Aizawa K, et al. Photodecomposition of humic acid and natural organic matter in swamp water using a TiO<sub>2</sub>-coated ceramic foam filter: potential for the formation of disinfection byproducts. *Chemosphere* 2013 Jan;90(4):1359-65.
16. Chong MN, Jin B, Saint CP. Bacterial inactivation kinetics of a photo-disinfection system using novel titania-impregnated kaolinite photocatalyst. *Chem Eng J* 2011;171(1):16-23.
17. Zhang L, Bai X, Tian H, Zhong L, Ma C, Zhou Y, et al. Synthesis of antibacterial film CTS/PVP/TiO<sub>2</sub>/Ag for drinking water system. *Carbohydr Polym* 2012;89(4):1060-6.
18. Matsunaga T, Tomoda R, Nakajima T, Wake H. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol Letters* 1985;29(1-2):211-4.
19. Noubactep C, Caré S. On nanoscale metallic iron for groundwater remediation. *J Hazard Mater* 2010;182(1-3):923-7.
20. Xiong Z, Zhao D, Pan G. Rapid and complete destruction of perchlorate in water and ion-exchange brine using stabilized zero-valent iron nanoparticles. *Water Res* 2007;41(15):3497-505.
21. Huang YH, Zhang TC, Shea PJ, Comfort SD. Effects of oxide coating and selected cations on nitrate reduction by iron metal. *J Environ Q* 2003 32(4):1306-15.
22. Diao M, Yao M. Use of zero-valent iron nanoparticles in inactivating microbes. *Water Res* 2009;43(20):5243-51.
23. Zhang L, Yan Jh, Zhou MJ, Yu Yp, Liu Y, Liu Yn. Photocatalytic degradation and inactivation of *Escherichia coli* by ZnO/ZnAl<sub>2</sub>O<sub>4</sub> with heteronanostructures. *Trans Nonferrous Metals Soc China* 2014;24(3):743-9.
24. APHA, AWWA, AEF. Standards Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association/American Water Works Association/ Water Environmental Federation. Washington, DC; 2005.
25. Adams LK, Lyon DY, Alvarez PJJ. Comparative eco-toxicity of nanoscale TiO<sub>2</sub>, SiO<sub>2</sub>, and ZnO water suspensions. *Water Res* 2006;40(19):3527-32.
26. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl<sub>2</sub> to a fresh water microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. *Environ Sci Technol* 2007;41(24):8484-90.

27. Barnes RJ, van der Gast CJ, Riba O, Lehtovirta LE, Prosser JI, Dobson PJ, et al. The impact of zero-valent iron nanoparticles on a river water bacterial community. *J Hazard Mater* 2010;184(1-3):73-80.
28. Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL. Bactericidal Effect of Zero-Valent Iron Nanoparticles on *Escherichia coli*. *Environ Sci Technol* 2008 2008/07/01;42(13):4927-33.
29. Auffan M, Achouak W, Rose J, Roncato MA, Chanéac C, Waite DT, et al. Relation between the Redox State of Iron-Based Nanoparticles and Their Cytotoxicity toward *Escherichia coli*. *Environ Sci Technol* 2008 2008/09/01;42(17):6730-5.
30. Rezaei-Zarchi S, Javed A, Javeed Ghani M, Soufian S, Barzegari Firouzabadi F, Bayanduri Moghaddam A, et al. Comparative Study of Antimicrobial Activities of TiO<sub>2</sub> and CdO Nanoparticles against the Pathogenic Strain of *Escherichia coli*. *Iran J Pathol* 2010;5(2):83-89.
31. Kasemets K, Ivask A, Dubourguier HC, Kahru A. Toxicity of nanoparticles of ZnO, CuO and TiO<sub>2</sub> to yeast *Saccharomyces cerevisiae*. *Toxicol Vitro* 2009;23(6):1116-22.
32. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. *J Microbiol Methods* 2003 Aug;54(2):177-82.
33. Dasari TP, Pathakoti K, Hwang HM. Determination of the mechanism of photoinduced toxicity of selected metal oxide nanoparticles (ZnO, CuO, Co<sub>3</sub>O<sub>4</sub> and TiO<sub>2</sub>) to *E. coli* bacteria. *J Environ Sci* 2013;25(5):882-8.
34. Wang H, Wick RL, Xing B. Toxicity of nanoparticulate and bulk ZnO, Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> to the nematode *Caenorhabditis elegans*. *Environ Pollut* 2009 Apr;157(4):1171-7.
35. Gordon T, Perlstein B, Houbara O, Felner I, Banin E, Margel S. Synthesis and characterization of zinc/iron oxide composite nanoparticles and their antibacterial properties. *Colloids Sur A Physicochem Eng Asp* 2011;374(1-3):1-8.
36. Huang Z, Zheng X, Yan D, Yin G, Liao X, Kang Y, et al. Toxicological Effect of ZnO Nanoparticles Based on Bacteria. *Langmuir* 2008;24(8):4140-4.
37. Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS Microbiol Lett* 2008 Feb;279(1):71-6.
38. Jafari, A. J., Dehghanifard, E., Kalantary, R. R., Gholami, M., Esrafil, A., Yari, A. R., & Baneshi, M. M. (2016). Photocatalytic degradation of aniline in aqueous solution using zno nanoparticles. *Environmental Engineering & Management Journal (EEMJ)*, 15(1).