South African Journal of Chemical Engineering 25 (2018) 128–132

Contents lists available at ScienceDirect

South African Journal of Chemical Engineering

journal homepage: http://www.journals.elsevier.com/south-african-journal-of-chemical-engineering

Chromium remediation and toxicity assessment of nano zerovalent iron against contaminated lake water sample (Puliyanthangal Lake, Tamilnadu, India)

M.I. Niyas Ahamed [a, *], S. Rajeshkumar [b], V. Ragul [a], S. Anand [a], K. Kaviyarasu [a]

[a] Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur, Tamilnadu, India
[b] School of Biosciences and Technology, VIT, Vellore, Tamilnadu, India

ARTICLE INFO

Article history:
Received 28 December 2017
Received in revised form
12 April 2018
Accepted 25 April 2018

Keywords:
Iron nanoparticles
Green synthesis
Heavy metal degradation
E. coli
Groundwater remediation
Toxicity

ABSTRACT

Since its high toxicity and mobility, hexavalent chromium is considered to be a high priority pollutant. This study was performed to carry out a remediation test along with toxicity assessment in a water sample collected from the saturated zone of a historically Cr (VI)-contaminated site known as a Puliyanthangal lake (Tamilnadu, India) using nanoscale zero-valent iron (nZVI). The water samples were examined before and after the nZVI application by means of microbial cultivation tests, phospholipid fatty acid analysis (PLFA) and toxicological tests. The present experimental results revealed that Cr (VI) is considerably adsorbed on nZVI nanoparticles and it could be a cost-effective method for the in situ remediations of Cr (VI). In addition, standard plate count assay showed the dose-dependent decrease in the bacterial cell viability in lake water sample after the addition of nZVI.
© 2018 Published by Elsevier B.V. on behalf of Institution of Chemical Engineers. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Chromium is one of the most abundant heavy metals, causing pollution of groundwater and soil due to its frequent industrial application. Chromium occurs naturally mainly in the trivalent Cr (III) and hexavalent Cr (VI) forms. The majority of its adverse effects is caused by Cr (VI) because of its solubility, mobility and high oxidizing potential leading to generally higher toxicity causing health problems such as liver damage, pulmonary congestion, vomiting and severe diarrhea (Nriagu and Nieboer, 1988). On the other hand, Cr (III) is less reactive and toxic and can be readily precipitated out of solution. Therefore, the majority of in situ treatment methods employed at the present time utilizes geo-fixation of Cr (VI) by its reduction to Cr (III) and formation of insoluble Cr (III) compounds (Jardine et al., 1999). A number of articles have been published to date describing various applications of individual biological or chemical approaches to precipitate chromium into its insoluble Cr(III) form. One of the most promising methods is reduction using iron-based materials such as zero-valent iron and dissolved Fe (II) and solids containing Fe(II) (Barrera-Díaza et al., 2012). Interest has increased over the past few years in using zero-valent iron (Fe0) and its respective nano-scale form to reduce chromium (VI) contamination.

Zero-valent iron (ZVI) is a readily available and low-cost reducing agent that is also used extensively to remove a number of other kinds of contaminants, such as chlorinated compounds, pesticides, and heavy metals e.g. As(V). Although the efficiency of ZVI and especially nano-scale ZVI (nZVI) in reducing the concentrations of Cr(VI) and other pollutants is well documented, only a few works have focused on its ecotoxicity for native organisms in the soil (Ahamed et al., 2016).

This study was performed to carry out a remediation test along with toxicity assessment in the saturated zone of a historically Cr (VI)-contaminated site known as Puliyanthangal Lake (Tamilnadu, India) using nZVI. The water samples were examined before and after the nZVI application by means of microbial cultivation tests, phospholipid fatty acid analysis (PLFA) and toxicological tests.

Based on the available literature, not sufficient work was carried out on this study area Puliyanthangal Lake (Tamilnadu, India) and these combinations of study like remediation test along with toxicity assessment. These phenomena provide novelty of the research investigation.

* Corresponding author.
E-mail address: driniyasahamed@shctpt.edu (M.I.N. Ahamed).

https://doi.org/10.1016/j.sajce.2018.04.004
1026-9185/© 2018 Published by Elsevier B.V. on behalf of Institution of Chemical Engineers. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Materials and methods

2.1. Chemicals

$FeCl_3 \cdot 6H_2O$ (Analytical grade) was procured from SD Fine Chemicals Ltd (India). All other reagents used were of analytical grade.

2.2. Synthesis of nano zero valent iron particles

For the synthesis of nZVI; 0.5406 g $FeCl_3 \cdot 6H_2O$ was dissolved in a 4/1 (v/v) ethanol/water mixture (24 mL ethanol + 6 mL deionized water) and stirred well followed by the addition of 10 mL of *Parthenium hysterophorus* plant extract. Then the sample was incubated in dark for 24 h. After 24 h, the sample was measured for its maximum absorbance using UV–Visible spectrophotometry. The sample was then heat dried to obtain the synthesized nano zero valent iron particles for characterization (Ahamed et al., 2016).

2.3. Characterization of the synthesized nano zerovalent iron particles

Prepared nanoparticles were characterized by means of various physicochemical studies includes; UV–Visible spectroscopy analysis, Fourier transform infrared spectroscopy (FTIR) analysis, scanning electron microscopy (SEM) analysis, transmission electron microscopy (TEM) analysis and Dynamic Light Scattering (DLS) analysis (details in our previous report, Ahamed et al., 2016).

2.4. Chromium remediation test

Water and soil samples were collected from Puliyanthangal Lake (leather industrial site and study area). The initial and final water characterization including determination of pH, temperature, DO, TDS etc., was carried out using water quality analyzer (ELICO-PE 138, India). Metals concentration was determined after acid digestion according to the EPA (US-Environmental Protection Agency) method followed by atomic absorption spectrophotometry (AAS) analysis. The concentration of Cr (VI) was determined by the colorimetric method using diphenyl carbazide after alkaline digestion.

2.5. Phospholipid fatty acid analysis (PLFA)

Chromium contaminated soil samples for PLFA were extracted with a mixed chloroform-methanol–phosphate buffer (1:2:0.8). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck, Germany) and the samples were subjected to mild alkaline methanolysis. The free methyl esters of phospholipid fatty acids were analyzed by gas chromatography-mass spectrometry (456-GC, SCION SQmass detector, Bruker, USA). Similarly, groundwater samples for PLFA analyses were prepared by filtration through microbial filters (0.2 μm). These filters were then extracted, fractionated and analyzed using the above-said method.

2.6. Cytotoxicity assessment

All the experiments were carried out on the dominant bacterial species *E.Coli,* isolated from the lake water and an initial cell count of 5×100 CFU mL^{-1} was maintained throughout the study. Bacteria interacted with three different low concentrations (0.05 ppm, 0.5 ppm, and 1 ppm) of nZVI dispersion.

All the experiments were carried out in parallel in two sets, under light and dark conditions. For light induced studies, test beakers were exposed to UV irradiation through UV lamps (Philips, 15 W, Poland) and for supporting non-illuminated studies, complete darkness was maintained. Control beakers without nZVI addition were kept for both light and dark conditions. Samples were analyzed after an interaction period of 3 h, 6 h, and 24 h respectively.

2.7. Cell viability assessment

Percentage reduction in cellular viability after the interaction period (3 h, 6 h, and 24 h) was determined using the standard plate count (SPC) assay as described by Mossman. (1983). In the recent past, the SPC assay has been employed as a method for analyzing the cellular viability of prokaryotes (Jardine et al., 1999). The decrease in bacterial viability in test samples was calculated with respect to control. Cell cultivability was determined using standard plate count assay on depicts nutrient agar medium.

2.8. Live–dead cells discrimination through fluorescence microscopy

Membrane permeability of treated cells was observed by fluorescence microscopy (Leica, DM-2500) after 3 h, 6 h and 24 h of NP interaction. Cells were stained with Acridine orange (AO) and Ethidium Bromide following the protocol described by Jakopec et al. (2006). 40 To 500 μL of bacterial suspension, 4 μL of AO (15 μg mL^{-1} in PBS) and 4 μL of EtBr (50 μg mL^{-1} in PBS) were added. After 5 min incubation, the cell suspension was centrifuged and the supernatant was discarded to eliminate unbound dyes. The cell pellet was resuspended in 500 μL PBS. The dark condition was maintained to avoid photobleaching of dyes. Fluorescence was detected by the BP 450–490, LP 590 filter; images were captured with a Leica-DFC-295 camera and processed using Leica-Application Suite 3.8.

2.9. Oxidative stress assessment

The fluorescence probe DCFH-DA (2'-7'-Dichlorodihydrofluorescein diacetate) was used to quantify generation of reactive oxygen species (ROS). DCFH-DA is membrane permeable and oxidizes to form the green fluorescent DCF in the presence of cellular esterases and ROS. Intracellular ROS generation was monitored in control and NP interacted bacterial cells at 3 h, 6 h and 24 h following the protocol described by Wang and Joseph with minor modifications (1999). 5 mL of cell suspension was incubated with DCFH-DA with a final concentration of100 μM at 37 °C for 30 min.

Fluorescence was measured using a Spectrofluorometer (SL174, ELICO, India) with excitation and emission wavelengths of 485 nm and 530 nm, respectively. A negative control of nZVI without cells was also analyzed to find out the auto fluorescence of nZVI that may interfere with the DCFH-DA dye.

Superoxide dismutase activity in interacted cells was measured by the method described by Winterbourne et al. using riboflavin as the O_2 generator (Wintherbourn et al., 1975). This method depends on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide, generated by the reaction of photo-reduced riboflavin and oxygen.

Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSH) to reduced glutathione (GSH). This enzyme enables the cell to sustain adequate levels of cellular GSH, which acts as an antioxidant, reacting with free radicals and organic peroxides. The activity of glutathione reductase in interacted cells was monitored spectrophotometrically using a commercially available Glutathione reductase assay kit. Data were reported as percentage release of GSH compared to control.

2.10. Membrane permeability assessment

The level of extracellular Lactate dehydrogenase (LDH) release was assessed as an indicator of membrane permeability and cytotoxicity. The interacted bacterial cell suspension was centrifuged (5000 g, 15 min), and the LDH level in the supernatant was measured following the standard protocol (Brown et al., 2001). To 100 μL of the supernatant, 100 μL of 30 mM sodium pyruvate and 2.8 mL of 0.2 M Tris-HCl was added. 100 μL of 6.6 mM NADH was added prior to use. The rate of decrease in absorbance at 340 nm was recorded using UV–Vis spectroscopy to determine the LDH activity.

The interacted cells stained with EtBr were subjected to spectrofluorometric analysis (Spectrofluorometer, SL174, ELICO) with excitation wavelength 510 nm and emission wavelength 595 nm. The increase in fluorescence intensity was analyzed with respect to control.

2.11. Surface chemistry

The involvement of surface functional groups in *E.Coli*-nZVI interaction was confirmed by Fourier Transform Infrared spectroscopy. 5 mL of the cell suspension after a 3 h interaction period were centrifuged for 10 min at 7000 g. The pellet was washed twice with 1 mL phosphate buffered saline and lyophilized to remove moisture. The dried cells were then subjected to FTIR analysis using KBr pellet (Nicolet 6700 FT-IR Spectrometer, Thermo Scientific Instruments Groups, Madison, Wisconsin).

2.11.1. Statistical analysis

All *in-vitro* toxicity tests were carried out in triplicates, and the data are presented as a mean ± standard error. The data was processed using one-way ANOVA, followed by Dunnett's post-hoc test at $p < 0.05$ for the standard plate count assay. The data for the ROS, SOD, GSH, LDH assay and metal ion analysis were processed through Student's t-test at $p < 0.05$. Significant data points are denoted with the symbol*.

Table 1
Physio-chemical characterization studies.

Physical parameters	Before nZVI Treatment	After nZVI treatment
Atmospheric temperature (°C)	21 ± 1	21 ± 0.5
Water temperature (°C)	24 ± 0.5	25 ± 1
Total Dissolved Solids (ppm)	1200 ± 1	1121 ± 1
Electrical Conductivity (**mS/m**)	50 ± 1	45 ± 1

3. Results and discussion

The water samples were collected from Puliyanthangal Lake and characterized using various physio-chemical characterization studies (Tables 1 & 2). nZVI treatment was significantly reduced the heavy metals present in the lake water sample. The total and hexavalent chromium concentration was found to be 1 mg/L and 0.06 mg/L respectively in the untreated lake water sample. By the results of the nZVI treatment leads to the reduction of both total and hexavalent chromium concentrations in the same sample, which may be due to the reduction property of nZVI. A similar trend was observed in the case of dissolved oxygen, total hardness, calcium, magnesium, nitrate, phosphate and chloride concentrations. In the other hand opposite results were observed after the addition of nZVI, for example, the concentrations of sodium, potassium, iron, ammonia, nitrate, fluoride and sulfate were slightly increased. In addition, the physical parameters like pH, temperature, total dissolved solids (TDS) and electrical conductivity (EC) was not affected by the addition of nZVI.

3.1. PLFA of groundwater samples

The results of PLFA are summarized in Fig. 1. It consists of specific phospholipid fatty acid concentrations in lake water samples. Significant changes in the relative abundances were observed throughout the whole experiment; however, it is not possible to conclude that the amount of Gram + and Gram − bacteria populations in the lake water at the tested site. Very few publications studied the impact of ZVI or nZVI on microbial biota in soils. Because of the rapid turnover of phospholipids. PLFA enables us to quantify living microorganisms (bacteria) via detection of specific fatty acids, also including noncultivable strains (Snajdr et al., 2011). Pawlett et al. (2013) used multiple substrate-induced respiration and PLFA to evaluate the effect of nZVI on microbes. They found that the effect was dependent on the organic matter content and soil mineral type in the three tested unsaturated surface soils. On the other hand, Cullen et al. (2011) used another approach employing tests of ZVI on enzyme activities (dehydrogenase, hydrolase, and ammonia oxidation potential) and these authors reported impacts on the enzyme activities. However, the authors acknowledged that the nZVI interfered with the assay conditions.

Table 2
Physio-chemical characterization studies.

Chemical parameters	Before nZVI Treatment (ppm)	After nZVI treatment (ppm)
pH	7.3 ± 0.5	7.8 ± 0.5
Total Alkalinity (mg/L)	250 ± 0.5	200 ± 0.5
Total Hardness (mg/L)	4814 ± 0.5	3712 ± 0.5
Calcium (mg/L)	279 ± 0.5	270 ± 0.5
Magnesium (mg/L)	328 ± 0.5	317 ± 0.5
Sodium (mg/L)	664 ± 0.5	814 ± 0.5
Potassium (mg/L)	62 ± 0.5	69 ± 0.5
Iron (mg/L)	8 ± 0.5	34 ± 0.5
Free Ammonia (mg/L)	0.73 ± 0.05	0.77 ± 0.05
Nitrite (mg/L)	0.03 ± 0.01	0.02 ± 0.01
Nitrate (mg/L)	258 ± 0.5	287 ± 0.5
Phosphate (mg/L)	7 ± 0.5	1 ± 0.5
Chloride (mg/L)	812 ± 0.5	718 ± 0.5
Fluoride (mg/L)	0.4 ± 0.01	0.6 ± 0.01
Sulfate (mg/L)	228 ± 0.5	837 ± 0.5
Total chromium (mg/L)	1 ± 0.01	0.05 ± 0.01
Hexavalent Chromium (mg/L)	0.6 ± 0.01	Below detection limit
Dissolved Oxygen (O_2 mg/l)	8 ± 0.05	7 ± 0.05

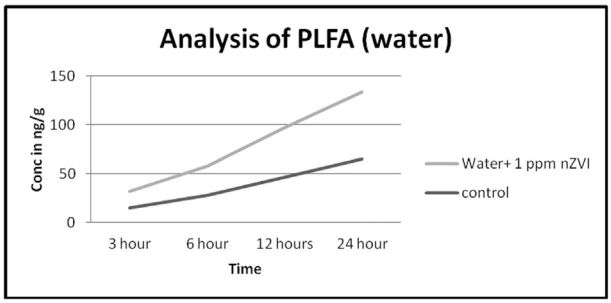

Fig. 1. Analysis of Phospholipid Fatty acid (PLFA).

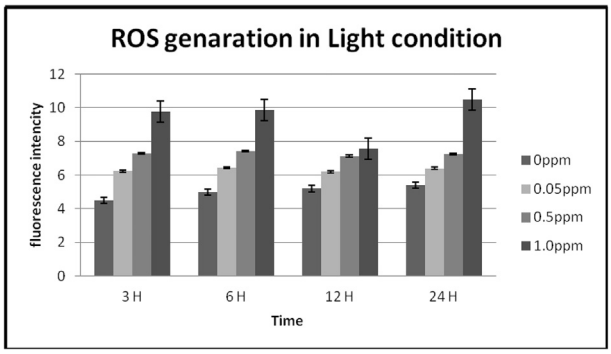

Fig. 4. ROS generation in light condition.

3.2. Cell viablity assessment

A concentration-dependent reduction in cell viability of *E.Coli* upon NP interaction was observed through plate count assay. A plate count assay showed a significant decline in viability of NP interacted cells ($21.3 \pm 1.7\%$ and $23.4 \pm 1.2\%$ under light and dark conditions respectively, 3 h), as compared to control, at 1 ppm concentration (Fig. 2). The cytotoxicity of NPs measured in terms of the viability of cells was found to decrease with time. The difference in toxicity under both test conditions (light and dark) was detected to be insignificant at all the three concentrations (0.05, 0.5 and 1.0 ppm). At lower concentrations, the difference in cell viability under light and dark conditions was found to be insignificance.

3.2.1. Live–dead discrimination through fluorescence microscopy

To visualize the live/dead cells in the sample, we performed AO-EtBr fluorescence staining. Control and NP interacted cells (1 ppm, 3 h) stained with AO and EtBr were visualized under a fluorescence microscope. Live cells appeared green (Fig. 3A), and cells with the compromised membrane, were observed to be bright red in color.

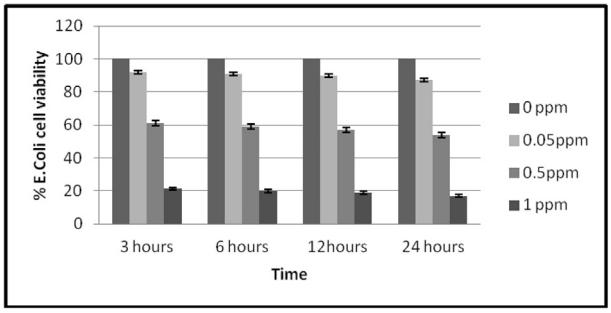

Fig. 2. Cell viability assessment.

Interacted samples were found to be a mixture of green and bright red cells for both lights (Fig. 3B) and dark conditions (Fig. 3C). Some intermediate or injured cells having motility (orange-red) were also observed in dark-treated samples.

The fluorescence microscopy of the NP interacted cells under light and dark conditions explained the combination of live and departed cells. A significant dose-dependent increase in intracellular ROS generation compared to control was noted. The ROS level after 24 h of interaction was significantly higher (Figs. 4 and 5) under light conditions ($10.5 \pm 0.2\%$) as compared to dark conditions ($6.62 \pm 0.2\%$). The NPs were steady against aggregation in clean lake water matrix for a period of 24 h, under both light and dark conditions. However, in the occurrence of bacterial cells, a prominent rate of sedimentation was noted in dark situation. A significant concentration dependent increase in superoxide dismutase level was detected ($0.05 < 0.5 < 1.0$ ppm) for both light and dark conditions compared to control. But opposite results was found for GSH level. The LDH analyses confirmed a statistically significant increase in membrane permeability under dark conditions compared to the light conditions.

3.3. Surface chemistry: FTIR analysis

The FTIR spectra obtained for untreated (control) and nanoparticle treated (test) *E.Coli* cells were compared (Figs. 6 & 7). The FT-IR spectrum may play a major role in the identification of bio and phytocompounds responsible for nanoparticles biosynthesis (Malarkodi and Rajeshkumar, 2017; Rajeshkumar 2017). The spectrum of untreated cells showed the presence of a broadband at 3340 cm^{-1}, which corresponds to vibration of OH– causing enhanced hydration of bacterial cells (Kamnev, 2008). The absorption peaks in the range of 1648–1742 cm^{-1} related to C=O or C–O bonds were noted. The peak corresponding to COO– in

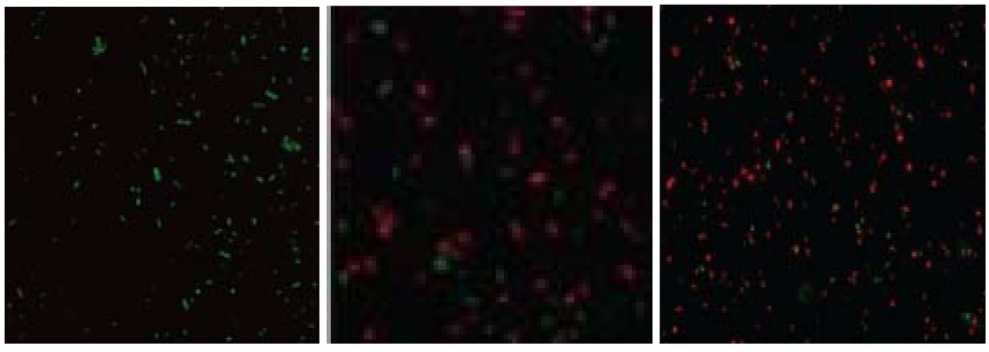

Fig. 3. Fluorescence microscopy Analysis.

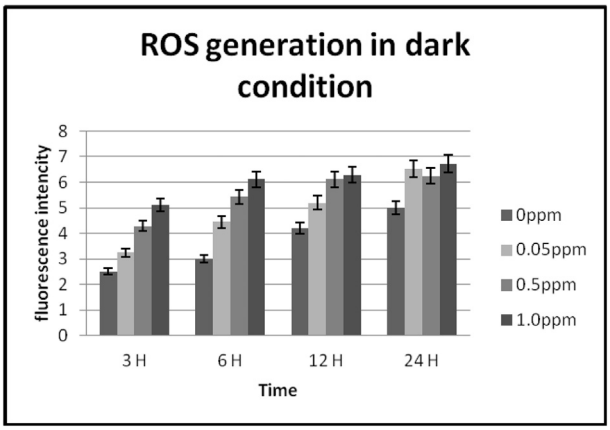

Fig. 5. ROS generation in dark condition.

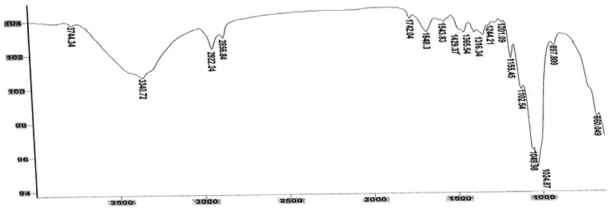

Fig. 6. FTIR analysis of Untreated *E.Coli* Cell.

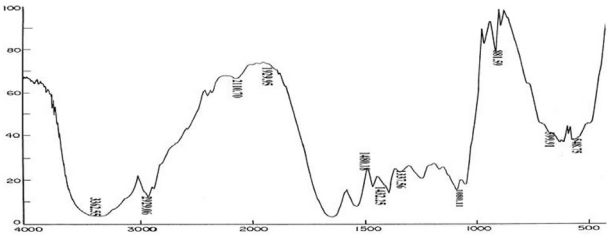

Fig. 7. FTIR analysis of Nanoparticles treated *E.Coli* Cell.

hydrophobic glycerol was observed at 1365 cm−1.45 A characteristic peak attributed to PO_2 – vibrations present exclusively in nucleic acids with a little contribution from phospholipids of cell wall was evident at 1244 cm^{-1}.45 A broad peak corresponding to glycogen units and polysaccharides in the cell wall was noted at the fingerprint region with peak intensity at 660 cm^{-1}.46 After treatment of bacteria with nanoparticles, the bands at 3340, 1742, 1365, 1244 cm^{-1} were shifted to 3362, 1929, 1480, 1089 cm^{-1}respectively. The FTIR studies demonstrated the possible involvement of surface functional groups in the attachment.

4. Conclusions

The present study explores the mechanistic aspects of remediation test along with toxicity assessment in the saturated zone of a historically Cr (VI)-contaminated site known as Puliyanthangal Lake (Tamilnadu, India) using nZVI at low exposure conditions under non-irradiated or dark conditions, and compares with those at irradiated or light conditions. The findings from this study can be extrapolated to study the environmental risk of these nanoparticles given their increasing use as remediation agent. In this context understanding the underlying fundamental chemistry of the nZVI–cell interaction in dark and light conditions will be helpful for future studies.

Acknowledgment

Authors thank the Science and Engineering Research Board (SERB), Govt. of India for the financial support to carry out this project and also thank the Secretary and Principal of Sacred Heart College (Autonomous), Tirupattur for having provided the necessary facilities.

References

Ahamed, I.N., Anbu, S., Vikraman, G., Nasreen, S., Muthukumari, M., Kumar, M.M., 2016. Green synthesis of nano zerovalent iron particles (nZVI) for environmental remediation. Life Sci. Archives 2 (3), 549–554.
Barrera-Diaza, C.E., Lugo-Lugo, V., Bilyeu, B., 2012. A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. J. Hazard Mater 223–224, 1–12.
Brown, D.M., Wilson, M.R., MacNee, W., Stone, V., Donaldson, K., 2001. Toxicol. Appl. Pharmacol. 175, 191–199.
Cullen, L.G., Tilston, E.L., Mitchell, G.R., Collins, C.D., Shaw, L.J., 2011. Assessing the impact of nano- and micro-scale zerovalent iron particles on soil microbial activities: particle reactivity interferes with assay conditions and interpretation of genuine microbial effects. Chemosphere 82, 1675–1682.
Jakopec, S., Dubravcic, K., Polanc, S., Kosmrlj, J., Osmak, M., 2006. Toxicol. Vitro 20, 17–226.
Jardine, P.M., Fendorf, S.E., Mayes, M.A., Larsen, I.L., Brooks, S.C., Bailey, W.B., 1999. Fate and transport of hexavalent chromium in undisturbed heterogeneous soil. Environ. Sci. Technol. 33, 2939–2944.
Kamnev, A.A., 2008. Spectrosc. Amst. 22, 83–95.
Malarkodi, C., Rajeshkumar, S., 2017. Invitro bactericidal activity of bio-synthesized semiconductor nanoparticles against UTI causing pathogens. Synthesis React. Inorg. Metal-Organic, Nano-Metal Chem. 47 (9), 1290–1297.
Mossman, T., 1983. J. Immunol. Methods 65, 55–63.
Nriagu, J., Nieboer, E., 1988. Chromiumin the Natural and Human Environments. Wiley Series, New York.
Pawlett, M., Ritz, K., Dorey, R.A., Rocks, S., Ramsden, J., Harris, J.A., 2013. The impact of zero-valent iron nanoparticles upon soil microbial communities is context dependent. Environ. Sci. Pollut. Res. 20, 1041–1049.
Rajeshkumar, S., 2017. Antioxidant activity of characterized silver nanoparticles synthesized using flower extracts of *Chrysanthemum indicum* Research. J. Biotechnol. 12 (8), 38–43.
Snajdr, J., Cajthaml, T., Valaskova, V., Merhautova, V., Petrankova, M., Spetz, P., Leppanen, K., Baldrian, P., 2011. Transformation of Quercus petraea litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS Microbiol. Ecol. 75, 291–303.
Wang, H., Joseph, J.A., 1999. Free Radic. Biol. Med. 27, 612–616.
Wintherbourn, C., Hawkins, R., Brian, M., Carrell, R., 1975. J. Lab. Clin. Med. 85, 337–341.