Toxicity of polystyrene microplastics in freshwater algae Scenedesmus obliquus: Effects of particle size and surface charge

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1. Introduction

Microplastics (MPs) are recognized as contributor to the biodiversity loss and can significantly affect the aquatic biota [1]. MPs can come to the environment from both primary and secondary sources. Polyethylene (PE) and polypropylene are the most often found polymers in water samples, followed by polystyrene (PS) and polycarbonate (PC), with microplastic concentrations ranging ten orders of magnitude (1 \( \times \) \( 10^{-5} \) - \( 10^9 \) particles/liter) [2]. PS is derived from styrene monomer and is amorphous in nature. It has low specific weight, great transparency and minimal shrinkage. In addition to that it is also easy to manufacture industrially and can be used in various household materials and cosmetics [3]. It is available in several forms, including expanded polystyrene (EPS), general-purpose polystyrene (GPPS), high-impact polystyrene (HIPS) and extruded polystyrene (XPS) [4]. Personal care products, industrial abrasives, plastic pellets/flakes used as raw material in plastic fabrication are the main contributors to primary plastic pollution [5]. PS can also be generated from secondary sources due to degradation/fragmentation of larger plastics. These pieces come from fishing nets, films, fibers, industrial inputs, consumer goods, and household appliances [6]. The usage patterns indicate that the release of MPs in the aquatic habitats will rise over time and they will stay as persistent pollutants [7]. Once MPs enter the aquatic environment, they are subjected to various physical and chemical interactions including biofouling, weathering, and the integration of secondary pollutants. Depending on the characteristics, the plastic fragments are often found in various compartments of the aquatic ecosystem [8].

Phytoplankton is a major primary producer in freshwater food chain [9]. In freshwater system species richness, diversity, and abundance of phytoplankton play a critical role in maintaining the ecological balance. These indicators also provide necessary information regarding pollution level of particular aquatic ecosystem [9]. Previous studies reported that MPs would affect freshwater microalgae in concentration and size-dependent manner [10]. Sjollema et al., (2016) observed negative effect of uncharged and negatively charged microplastics of sizes 0.05, 0.5 and 6 \( \mu \)m on three different types of microalgae, D. tertiolecta,

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https://doi.org/10.1016/j.toxrep.2022.10.013
Received 22 August 2022; Received in revised form 8 October 2022; Accepted 24 October 2022
Available online 25 October 2022
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C. vulgaris, T. pseudonana [11]. Yokota et al. conducted a literature review that revealed primary producer–microplastic interactions can alter algal growth, photosynthetic efficiency, morphology, possibly via adhesion or transfer of adsorbed pollutants from microplastics. Wang et al., (2020) observed that both pristine and aged polyvinyl chloride microplastics (PVC MPs) would affect the growth rate and chlorophyll content of *Chlamydomonas reinhardii* [12]. Xiao et al., (2020) investigated negative consequences of PS- MPs in freshwater microalgae, *Euglena gracilis* [13]. They noted that PS-MPs (0.1 and 5 µm) strongly suppressed algal growth in a concentration-dependent manner. Zhang et al., (2016) also revealed that PVC MPs inhibited the growth of *Sel-letonema costatum* [14]. In another work [15] the impact of PS of varied surface charges in *Scenedesmus obliquus* was assessed. The researchers reported reduced cell viability, photosynthetic efficiency and increased oxidative stress in the cells interacted with polystyrene nano plastics of 200 nm size with different surface charges. But they did not examine the effects of particles with different sizes or concentrations. Liu et al., (2019) reported, PS nano plastics (size: 100 nm) of different charges, (plain, aminated and carboxylated) caused growth inhibition and increase in the oxidative stress in *S. obliquus* [16]. But they did not explore the effects of varied sizes of PS nanoparticles on microalgae. Similarly, Huang et al., 2019 observed decreased cell viability and photosynthetic efficiency of *S. obliquus* upon interaction with three sizes of PS beads (0.1, 0.5, 1, and 2 µm) combined with different charges of PS (negative, positive and fluorescence tagged). But the size of PS-bead size was limited only to 2 µm. They did not study the effects of larger PS particles.

This necessitates a detailed study on the effects of both surface charge (plain, Aminated and Carboxylated) and size (lowest: 1 µm, highest: 12 µm) of MPs in freshwater microalgae. So, the major objective of the work was to explain the combined effects of size and surface charge of MPs in a freshwater alga, *Scenedesmus obliquus*. The major findings from this work will provide further cues in understanding the consequences of MP contamination in aquatic environment. A nuanced experimental approach has been taken to answer the following questions: (a) how polystyrene MPs of 1 and 12 µm sizes would affect growth, biochemical and photosynthetic parameters and (b) to what extent the toxic effects would depend on the surface charge of the particles (Plain-PS, COOH-PS, NH$_2$-PS). The study involved assessment of growth parameters, photosynthetic and metabolic indicators, and oxidative stress parameters along with the activities of the crucial antioxidant enzymes.

### 2. Materials and methods

#### 2.1. Chemicals

Polystyrene MPs of 1 and 12 µm size with different functionalization [Carboxyl-PS (COOH-PS); Aminated-PS (NH$_2$-PS); Nonfunctionalized-PS (Plain-PS)] were purchased from Corpruscular, Inc., USA. Dihy- droethidium (DHE) and Aminophenyl fluorescein (APF) were acquired from Invitrogen™, Molecular Probes®, CA, USA. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydroxylamine hydrochloride, and dimethyl sulfoxide were purchased from Hi-Media Pvt. Ltd (Mumbai, India). Nitroblue tetrazolium chloride (NBT) and hydrogen peroxide solution (H$_2$O$_2$, 30% w/v) were procured from SDGFL, Mumbai, India. 2',7'-Dichlorofluorescin diacetate (DCFDA), were purchased from Sigma–Aldrich Missouri (USA).

#### 2.2. Preliminary characterization of PS in the exposure medium

Field Emission-Scanning electron microscope (FE-SEM) was used to determine and confirm the size and shape of PS. Aliquots of PS solution (1 mg/L) was kept on a piece of a glass slide, air-dried, sputter-coated with gold, and visualized through an electron microscope (operating voltage: 10.00 kV; working distance range: 9.4–10.7 mm) (Thermo Fisher FEI Quanta 250 FEG). To elucidate the charge on the MPs (1 and 12 µm), Zeta potential was evaluated at 0th h for all three differently functionalized MPs dispersed in lake water (90 Plus Particle Size Analyzer; software: Particle Size Analyzer, Brookhaven Instruments Corp., USA).

#### 2.3. Culturing of *Scenedesmus obliquus*

The test organism used in this study was unicellular green freshwater algae, *Scenedesmus obliquus*, isolated from VIT lake, Vellore (12°58’10”N, 79°9’37”E). Sub-culturing and the maintenance of the *Scenedesmus obliquus* have been provided in the supplementary information (Method S1).

#### 2.4. Assessing the cell toxicity in algae

##### 2.4.1. Assessing cell viability

To determine the effects of two different sized and differently func- tionalized MPs, cell viability was evaluated by counting the cells. The detailed procedure has been provided in the supplementary information (Method S2).

##### 2.4.2. Assessment of oxidative stress generated in algae

To estimate the oxidative stress generated in the control and treated algal cells, total ROS, superoxide radical, and hydroxyl radical generation were assessed. Followed by, antioxidant assays, Superoxide Dismutase (SOD) and Catalase were performed to determine the enzymatic activity in the algal cells. The total ROS production was measured using a cell membrane-permeable fluorescent dye, H$_2$DCFDA [17] (Method S3). Dihydroethidium (DHE) (DHE$^+$) is a membrane-permeable dye that has been used to monitor superoxide radical production in algal cells [18] (Method S4). Aminophenyl fluorescein (APF) is a dye that reacts with hydroxyl radicals to produce intense green fluorescence [19] (Method S5). The SOD assay was performed by the method described previously [20]. A series of chemical mixture (Na$_2$CO$_3$ buffer (50 mM, pH 10), 96 mM Nitro tetrazolium blue chloride (NBT), 0.6% Triton X-100, and 20 mM hydroxylamine hydrochloride) were used to perform SOD activity. The detailed protocol has been provided in the supplementary information (Method S6). Catalase assay was performed following the protocol mentioned in the previous report [21] (Method S7). To perform CAT activity 10.8 mM H$_2$O$_2$ solution (2 mL) and 50 mM - pH 7 potassium phosphate buffer (100 µL) was being treated with the algal supernatant. The detailed protocol has been provided in the supplementary information.

##### 2.4.3. Estimation of photosynthetic parameters

The algal samples treated with PS were incubated in dark for 15 min. The dark-adapted samples (100 µL) were loaded into the Photosynthesis Yield Analyzer (Mini PAM, Heinz Walz, Germany). High-intensity actinic light was passed through the sample to record the ratio of variable fluorescence (F$_v$) to maximum fluorescence (F$_m$) of the sample [11]. This ratio is the effective photochemical quantum yield of PS II.

##### 2.4.4. Metabolic activity

To assess the metabolic activity of the treated and control algal cells esterase and mitochondrial membrane potential assays were performed. According to the procedure reported elsewhere [22], fluorescein diacetate (FDA) was employed to quantify esterase activity and Rhodamine 123 (Rh123) was used to measure mitochondrial membrane potential (ΔΨm) [23]. The detailed protocol has been provided in the supplementary information (Method S8 and S9).

##### 2.4.5. Determination of membrane integrity

To determine membrane integrity, lipid peroxidation (LPO) was performed and SYTOX green was used as a fluorescence label. The LPO assay was used to determine the quantity of malondialdehyde (MDA), an end product of lipid peroxidation [24] (Method S10). The membrane...
integrity of PS-treated *Scenedesmus* sp. was determined using SYTOX green, a fluorescent nucleic acid binding dye that does not penetrate healthy cells [25]. The detailed protocol has been provided in the supplementary information (Method S11).

### 2.5. Statistical analysis

All the measurements were carried out in triplicate (n = 3) to demonstrate the statistical difference. Entire data is presented as mean ± standard deviation. To check the significant difference, Two-way ANOVA was performed with Bonferroni post-test using Graph Pad Prism 8.

### 3. Results

#### 3.1. Characterisation of PS

FE-SEM micrographs of 1 and 12 µm PS of different charges (plain, aminated and carboxylated) showed that all the MPs are spherical in shape (Supplementary Fig. S1). 1 µm PS showed the average size of 1.825 ± 0.011, 1.847 ± 0.007, and 1.851 ± 0.004 µm for Plain-PS, COOH-PS, and NH₂-PS, respectively. Whereas 12 µm particles showed an average size of 10.603 ± 0.020, 13.046 ± 0.414, and 10.576 ± 0.194 µm for Plain-PS, COOH-PS, and NH₂-PS, respectively. The surface charge details of the MPs dispersed in lake water are tabulated in Table 1.

#### 3.2. Toxicological responses in algae

**3.2.1. Cell viability**

The toxicological effects of 1 and 12 µm PS in terms of cell viability are shown in Fig. 1. Upon exposure to increasing concentrations of PS (1 and 12 µm) dose-dependent effects at 1 and 10 mg/L was noted (p < 0.001) for all the three charges. The highest decrease in the cell viability was observed in NH₂-PS treated algal cells followed by COOH-PS and Plain-PS.

In case of 12 µm size, a significant difference compared to the control samples was found for 1 and 10 mg/L NH₂-PS, 10 mg/L COOH-PS, and 10 mg/L Plain-PS treated samples (p < 0.001). When compared to 1 µm PS treated samples, cell viability in the algal cells treated with 12 µm PS significantly increased (p < 0.001) at 1 and 10 mg/L concentrations.

**3.2.2. Oxidative stress: Total ROS, superoxide radical, hydroxyl radical**

Fig. 2 illustrates the overall ROS levels in PS-treated algal samples. Based on the results, the highest ROS production was observed in 1 µm COOH-PS treated samples followed by NH₂-PS and Plain-PS. It is observed that all the treatment groups experienced enhanced ROS levels (p < 0.001) in comparison with the control groups except for 0.1 mg/L PS treated cells. In 1 µm PS treated samples, a dose-dependent increase in ROS was found at all the charges of PS, and the difference between the surface charges was significant (p < 0.001).

When compared to 1 µm PS, 12 µm PS treated algal cells generated less ROS (Fig. 2). In this case, the highest ROS production was observed in COOH-PS treated samples followed by NH₂-PS and Plain-PS.

Furthermore, high significant differences in ROS generation between 1 and 12 µm PS treated samples was observed for 1 and 10 mg/L concentrations (p < 0.001).

**3.2.3. Antioxidant enzyme activity**

The activation of the SOD enzyme by 1 and 12 µm PS in algae is depicted in Fig. 5. The increase in enzyme activity was dose dependent. All the test groups demonstrated a significant increase in SOD activity when compared to the control (p < 0.001) except for 0.1 mg/L 1 µm Plain-PS treated algal cells (p > 0.05). When the charges and their corresponding concentrations are compared between 1 and 12 µm PS treated samples, a significant reduction in SOD activity is noticed in case of 12 µm PS (p < 0.001). For both the sizes of MPs, a significant difference in SOD activity was found between Plain-PS and NH₂-PS across the concentration range (p < 0.001).

In case of CAT activity, both 1 and 12 µm PS treated samples demonstrated a significant increase concerning the control sets (p < 0.001) (Fig. S2). The highest CAT activity was observed in NH₂-PS followed by COOH-PS and Plain-PS treated algal samples. When compared to 1 µm PS treated algal cells, the large decline in CAT activity in 12 µm PS treated algal cells was observed (p < 0.001). This difference was found for all three charges and their respective concentrations.

**3.2.4. Effects on photosynthetic apparatus of algal cells: Maximum quantum yield of PS II and ETR**

A clear dose-dependent response was noted in the yield of PS II after treating with the PS MPs (Fig. 6). A significant difference between the effects by Plain-PS and NH₂-PS (p < 0.001) for all the concentrations was evident for 1 µm PS. In case of 12 µm PS treated samples, a significant decrease was noticed only in case of 1 and 10 mg/L concentrations. Likewise, when compared with 1 µm PS, 12 µm PS significantly enhanced the PS II yield at 1 and 10 mg/L concentrations for all the charges (p < 0.001). This enhancement in the yield was observed for NH₂-PS followed by COOH-PS and Plain-PS (p < 0.001).

A decrease in the electron transfer rate in the algal cells treated with 1 µm PS was significant at all the concentrations when compared to control (p < 0.001) (Fig. S3). However, 12 µm PS exhibited significant reduction only at 1 and 10 mg/L (p < 0.001). For both the sizes of MPs, a significant difference in ETR was found between Plain-PS and NH₂-PS at all the concentrations (p < 0.001).

**3.2.5. Effect on metabolic activity: Esterase activity and ΔΨm**

The reduction in metabolic activity can be confirmed by a decline in FDA activity. The intensity of green fluorescence significantly decreased in the algal cells exposed to 1 µm PS, when compared to control (p < 0.001) (Fig. 7). However, such significant reduction in FDA fluorescence intensity was observed for 1 and 10 mg/L concentrations of...
Fig. 1. Change in the cell viability of *S. obliquus* when compared to control (A) for Plain-PS interacted samples (*n* = 3), (B) for COOH-PS interacted samples (*n* = 3), (C) for NH$_2$-PS interacted samples. Note: ‘α’, γ, δ’ indicates significant difference represented significance between 1 and 12 µm MPs interacted samples (*α* = *p* < 0.001, γ = *p* < 0.05, δ = *p* > 0.05); ‘***’ indicates significant difference between test and control samples.

Fig. 2. Change in total ROS generation in *S. obliquus* relative to control (A) In case of Plain-PS interacted algae samples (*n* = 3), (B) In case of COOH-PS interacted algae samples (*n* = 3), (C) In case of NH$_2$-PS interacted samples. Note: ‘α, β, δ’ indicates significant difference represented significance between 1 and 12 µm MPs interacted samples (*α* = *p* < 0.001, β = *p* < 0.01, δ = *p* > 0.05); ‘***’, ‘**’ and ‘*’ indicates significant difference between test and control samples (‘***’ = *p* < 0.001, ‘**’ = *p* < 0.01, ‘*’ = *p* < 0.05).
12 µm PS treated algal samples when compared to control. The increase in esterase activity in the algal cells treated with 12 µm PS was significant, when compared to 1 µm PS (p < 0.001).

The exposure to 1 µm PS provoked a significant reduction (p < 0.001) in Rh123 fluorescence at all the concentrations except 0.1 mg/L Plain-PS (Fig. S4). However, 12 µm PS treated algal cells revealed no significant reduction in Rh123 fluorescence when compared to the control cells (p > 0.05) except at the highest concentration i.e., 10 mg/L (p > 0.001). Moreover, hyperpolarization, i.e., an increase in $\Delta\Psi_m$ was observed in 12 µm PS when compared to 1 µm PS at

**Fig. 3.** Change in superoxide radical production in *S. obliquus* relative to control (A) in case of Plain-PS interacted algae samples (n = 3), (B) in case of COOH-PS interacted algae samples (n = 3), (C) In case of NH$_2$-PS interacted algae samples. Note: ‘α’ indicates significant difference represented between 1 and 12 µm MPs interacted samples (α = p < 0.001); ‘***’ indicates significant difference between test and control samples.

**Fig. 4.** Change in hydroxyl radical production in *S. obliquus* relative to control (A) in case of Plain-PS interacted algae samples (n = 3), (B) in case of COOH-PS interacted algae samples (n = 3), (C) in case of NH$_2$-PS interacted algae samples. Note: ‘α, δ’ indicates significant difference represented between 1 and 12 µm MPs interacted samples ($\alpha = p < 0.001, \delta = p > 0.05$); ‘***’ indicates significant difference between test and control samples.
Fig. 5. Change in SOD activity in S. obliquus relative to control (A) in case of Plain-PS interacted algae samples (n = 3), (B) in case of COOH-PS interacted algae samples (n = 3), (C) in case of NH$_2$-PS interacted algae samples. Note: ‘α’ indicates significant difference represented between 1 and 12 µm MPs interacted samples (α = p < 0.001); ‘***’ indicates significant difference between test and control samples.

Fig. 6. Change in Maximum quantum yield of PS II in S. obliquus relative to control (A) for Plain-PS interacted samples (n = 3), (B) for COOH-PS interacted samples (n = 3), (C) for NH$_2$-PS interacted samples. Note: ‘α, γ’ indicates significant difference represented significance between 1 and 12 µm MPs interacted samples (α = p < 0.001, γ = p < 0.05); ‘***’ indicates significant difference between test and control samples.
concentrations, 1 and 10 mg/L. This difference was also found to be significant \(p > 0.001\).

### 3.2.6. Effects on the membrane integrity

Fig. 8 displays the MDA content in algal cells interacted with 1 and 12 µm PS. Interaction with 1 µm PS produced a significantly higher amount of MDA for all the charges when compared to control samples.
(p < 0.001). The highest MDA content was observed in NH2-PS treated samples and the least production was noted in Plain-PS treated algal samples. When compared between two varied sizes, a significant reduction in MDA content can be noted in 12 µm PS for all the charges and their respective concentrations (p < 0.001). This again shows reduced toxic effects of 12 µm PS compared to 1 µm PS.

SYTOX Green was used to label the algal cells to distinguish the cells with damaged or permeabilized plasma membrane. The fluorescence intensity of the dye was stronger when exposed to 1 µm PS of three distinct charges of PS at all concentrations except for 0.1 mg/L Plain-PS, when compared to control (p < 0.001) (Fig. 5S). Whereas 12 µm PS treated algal samples demonstrated a significant increase in fluorescence intensity only at higher concentrations when compared to control (p < 0.001). A significant difference in fluorescence intensity was observed between the two sizes for all the charges of MPs (p < 0.001).

4. Discussion

MPs pose deleterious effects to algae and may result in biocumulation and magnification, by entering the food chain [26]. To comprehend potential toxic effects of nanoparticles in aqueous test medium examining the effects of their surface charge is pertinent [27]. The low zeta potential values of the MPs suggest that the weak electrostatic repulsion between neighboring particles may lead to poor stabilization. So, they might tend to agglomerate.

PS-MPs of size 1 µm showed a dose dependent decline in cell viability irrespective of the charges. This may be due to the interaction of algal cell wall with the charged MPs. Adsorption of the particles on algae cell wall would damage the cell membrane facilitating particle internalization [28]. The MPs with effectively large surface area are readily ingested by algal cells resulting in toxicity [24]. MPs of size 12 µm did not exert any notable toxic effects on algae. Since algal cell walls have smaller pore sizes and are selectively permeable the entry of the large sized particles into the cells is restricted [29].

MPs irrespective of their sizes and charge are known to elicit oxidative stress in algae [11]. It is noted, 1 µm MPs generated more ROS compared to 12 µm MPs. Similar trends have been noticed for superoxide and hydroxyl radical generation too. Larger sizes of MPs tend to trigger lesser amount of oxidative stress than the smaller sized ones because small sizes can penetrate the cell membrane easily [30]. These reactive species can trigger damage to the cell membrane by interfering with the polyunsaturated fatty acids present in the algal cell membrane. This reveals a positive association between ROS production and lipid peroxidation [31]. In the previous studies 1 and 2 µm PS were found to be internalized by *Platymonas helgolandica* var. *tsingtaoensis* after 72 h of incubation but MPs of 3.0–5.0 µm size were found adhering to the cell surface only. The positively functionalized MPs (PS-NH2) caused more ROS production than negatively functionalized MPs (PS-COOH). Similar observations were confirmed by the previous researchers too [30,32,33].

MP-generated ROS enhances the activity of the antioxidant enzymes, SOD, and CAT in the cells, which serve as a defense mechanism to combat the oxidative stress [12]. Previous reports also revealed activation of anti-oxidant enzymes in *Microcystis aeruginosa* [34] and *Euglena gracilis* [13] after treating with MPs. SOD activity in the algal cells was more than the control sets for both 1 and 12 µm MPs treatment groups. CAT activity increased with the concentration of MP in the plain MP treated algal cells, but the activity was suppressed with an increasing concentration of PS-NH2. PS-COOH treated cells. An improved CAT activity suggests better amelioration of oxidative stress in the cells. Stress beyond the threshold of an organism’s tolerance levels can result in the decrease or even inhibition of the enzyme activity leading to the accumulation of ROS and further oxidative damage to cells [35].

Photosynthesis is an important biochemical process of algae, which maintains the O2/CO2 balance and any disturbances in this process may affect the ecosystem adversely. Fv/Fm, a vital indicator for photosystem II (PSII) activity, is measured to identify stress-induced damage to the photosynthetic machinery. It is noted that induced oxidative stress impaired the photosynthetic machinery to a varied extent in algae. Previous research by Wu and colleagues showed that the Fv/Fm ratio declined with increasing nanoplastics concentration [36]. In this study, 1 µm MP reduced the quantum yield of PSII in a dose-dependent manner whereas 12 µm MP showed lesser damage to quantum yield. The electron transfer rate also followed a similar trend. It has been hypothesized that the PSNPs stress slows down the PS II electron transport rate. This causes an increased buildup of electrons, which in turn amplifies the photoinhibition and subsequent rise in reactive oxygen species (ROS). The production of ROS in the cells prevents the synthesis of chlorophyll, which results in a significant decrease in the yield of photosynthetic reactions [36]. In a previous study 1 mm PS-MPs exposure led to inhibition of the Fv/Fm ratio in *C. pyrenoidosa* [37]. Liu et al., 2020 reported that *S. obliquus* exposed to several types of polystyrene particles, (0.1, 0.5, 1, and 2 µm) showed a differential response to the size and charges of the particles.

Membrane integrity is strongly associated with the cell viability, which is regulated by mitochondrial and chloroplast functions [38]. It was observed that 1 µm PS led to greater reduction in mitochondrial membrane potential (MMP) than 12 µm PS. Enhanced MMP values in the 12 µm PS treated algal cells indicated enhanced mitochondrial function. Esterase activity is related to the algal cell metabolism. Loss of membrane integrity and decreased cell metabolism may lead to increased esterase activity [33,39].

5. Conclusion

Plastic waste generation and its improper disposal are increasing every day, making MP pollution a chronic worldwide concern, more so for aquatic ecosystems. In the present context, this study has enormous potential to depict the differences in reaction of aquatic algae to MPs of varying size and charges. However, this study comes with certain impediments like, the MP uptake or translocation was not determined and the molecular mechanism underlying the stress response was not elucidated. This creates room for future investigations to focus on deciphering the underlying molecular framework and signaling events associated with MP induced stress and the long-term effect of MP deposition in the aquatic environment.

CRediT authorship contribution statement

**Lokeshwari Natarajan:** Investigation, Methodology, Visualization, Formal analysis, Writing – original draft. **Soupm Das:** Investigation, Methodology, Formal analysis. **Swarnali Dey:** Formal analysis, Writing – review and editing. **N. Chandrasekaran:** Formal analysis, Resources. **Rita Kundu:** Resources, Supervision, Writing – review and editing. **Subhabrata Paul:** Supervision, Investigation, Writing – review & editing. ** Amitava Mukherjee:** Conceptualization, Supervision, Project administration, Writing – review and editing.

**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.

**Acknowledgements**

The research was supported by Department of Biotechnology, West Bengal [project no. 76 (Sanc.) – BT/P/Budget/RD-14/2017, Date
Toxicology Reports 9 (2022) 1953–1961

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is grateful to UGC for providing fellowship to him. Authors also would like to acknowledge Vellore Institute Technology (VIT), Vellore, India for the Field Emission Scanning Electron Microscopy facility used in this study.

Appendix A: Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.10.013.

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