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The role of algal EPS in reducing the combined toxicity of BPA and polystyrene nanoparticles to the freshwater algae *Scenedesmus obliquus*

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ABSTRACT

Both Bisphenol A (BPA) and polystyrene nanoplastics (PSNPs) are routinely found in several consumer products such as packaging materials, flame retardants, and cosmetics. The environment is seriously endangered by nano- and microplastics. In addition to harming aquatic life, nanoplastics (NPs) also bind to other pollutants, facilitating their dispersion in the environment and possibly promoting toxicity induced by these pollutants. The toxic effects of polystyrene nanoplastics (PS-NPs) and BPA were examined in this study, as well as the combined toxic impacts of these substances on the freshwater microalgae *Scenedesmus obliquus*. In addition, the exopolymeric substances (EPS) secreted by algae will interact with the pollutants modifying their physicochemical behaviour and fate. This work aimed to investigate how algal EPS alters the combined effects of BPA and PSNPs on the microalgae Scenedesmus obliquus. The algae were exposed to binary mixtures of BPA (2.5, 5, and 10 mg/L) and PSNPs (1 mg/L of plain, aminated, and carboxylated PSNPs) with EPS added to the natural freshwater medium. Cell viability, hydroxyl and superoxide radical generation, cell membrane permeability, antioxidant enzyme activity (catalase and superoxide dismutase), and photosynthetic pigment content were among the parameters studied to determine the toxicity. It was observed that for all the binary mixtures, the carboxylated PSNPs were most toxic when compared to the toxicity induced by the other PSNP particles investigated. The maximum damage was observed for the mixture of 10 mg/L of BPA with carboxylated PSNPs with a cell viability of 49%. When compared to the pristine mixtures, the EPS-containing mixtures induced significantly reduced toxic effects. A considerable decrease in reactive oxygen species levels, activity of antioxidant enzymes (SOD and CAT), and cell membrane damage was noted in the EPS-containing mixtures. Reduced concentrations of the reactive oxygen species led to improved photosynthetic pigment content in the cells.

1. Introduction

Bisphenol A (BPA) is a rapidly growing industrial chemical mostly employed in the polymerization of polycarbonate (hard, clear plastic used in bottles), epoxy resins (used for the inner lining of food cans and dental fillings), and also in the production of polyester carbonate, polysulfones, polyetherimides, etc. (Beausoleil et al., 2018). The production of BPA-based polymers has increased over the past ten years, reaching an amount of nearly 6.5 million metric tonnes annually (Jandegian et al., 2015). With the increasing usage of BPA in various products it has emerged as a major contaminant in the aquatic environment. As an endocrine disruptor, BPA can cause toxic effects in aquatic organisms throughout the food chain (Ben Ouada et al., 2018).

Owing to the rapid growth of the world economy over the past few years, plastic consumption has increased significantly. Global plastic production is estimated to touch 300 million metric tonnes per year, of which 13 to 86 million metric tonnes are emitted into rivers and oceans. It is estimated that the volume of discarded plastic will increase up to 250 million metric tonnes by the end of 2025 (Enfrin et al., 2019). The ever-increasing abundance of submicron-sized plastic particles in freshwater bodies raises serious concerns about their bio-accumulation in the organisms leading to the possibility of trophic transfer (Alimi et al., 2018). The breakdown of large-sized particles by natural processes (UV photodegradation, hydrolysis, mechanical abrasion, and

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biodegradation) generates a large volume of secondary microplastics and nanoplastics in aquatic bodies (MPs and NPs) (Thompson et al., 2009). The various facial cleansers seem to be the most dominant sources of primary nanoplastics (NPs) in aquatic bodies (Hernandez et al., 2017). Among the various plastic particles, polystyrene nanoplastics (PSNPs) are frequently employed in ecotoxicity studies (Liu et al., 2019).

Microalgae are the primary producers at the base of the aquatic food chain. Microalgae have a significant impact on the structure and operation of the entire freshwater environment. So, they are useful in monitoring water quality as a biological indicator (Naselli-Flores and Padisák, 2022). A recent report on the growth inhibition of BPA in two extremophilic microalgae, *Picocystis* sp., and *Graesiella* sp., showed that the growth was suppressed significantly with increasing concentrations of BPA (Ben Ouada et al., 2018). After 4 days of exposure, the specific growth rate of BPA-treated microalgae *Cylindrospermopsis raciborskii* and *Scenedesmus quadricauda* decreased significantly with the EC_{50} for *C. raciborskii* and *S. quadricauda* noted at 9.66 ± 0.05 and 13.23 ± 0.07 ppm respectively (Xiang et al., 2018).

A dose-dependent toxicity of PS-NH_2 nanoplastics (90, 200, and 300 nm) was recorded in *Chlorella vulgaris* (Khoshnamvand et al., 2021). Our previous work with plain, carboxylated, and aminated PSNPs in *Scenedesmus obliquus* revealed significant toxic effects of the three differently surface-modified PS in terms of growth inhibition, ROS generation, membrane damage, disruptions in the photosynthetic apparatus (Giri and Mukherjee, 2021). The toxicity of a mixture of multiple ENPs is a new area of study that presents both opportunities and difficulties. In light of the ongoing emergence of numerous new ENPs, the workload associated with assessing and predicting the mixture toxicity of various ENPs will increase. A mechanistic understanding has not yet been explored, particularly regarding the interaction behaviour between various particles in mixtures of NPs. Joint interactions for those novel materials are still poorly understood. Investigations into the combined toxicity of hybrid NMs will need to continue, especially at concentrations that are relevant to the environment (Zhang et al., 2022). So far only one study assessed the combined effects of plastic microparticles and BPA in microalgae. The combined toxicity assessment of BPA (1 mg/L and 10 mg/L) and PS (5 mg/L) microspheres in *Chlorella pyrenoidosa* revealed a concentration-dependent increase in growth inhibition after 8 days of exposure to both BPA and BPA + PS. The results clearly showed that PS and BPA together had more harmful effects than BPA or PS alone (He et al., 2022). There are no studies on the effects of BPA and PSNPs in a freshwater microalga so far.

Along with nanoplastics and BPA, natural organic matter (NOM) also coexists in aquatic systems. NOM can be classified into two major categories: autochthonous and allochthonous compounds depending on whether they are formed inside or outside the water bodies before being released into it. Extracellular polymeric substances (EPS) are excreted by aquatic organisms in response to stress, and they are among the major constituents of autochthonous NOM (Gutierrez et al., 2013). EPS are often made up of a complicated high-molecular-weight (HMW) mixture of various biopolymers (Xiao and Zheng, 2016). The interaction(s) among emerging pollutants and various components of the ecosystem in aquatic environments have recently gained increased attention. NPs in aquatic bodies will inevitably interact with NOM, primarily EPS. The sorbed NOM layer on the NPs is known as the eco-corona or the environmental corona. These nano-bio interactions can control the colloidal behaviour of NPs. They can either induce a stabilizing effect by introducing electro-steric hindrances or may cause aggregation through interparticle complex/bridge formation. These bio-nano interactions will greatly affect the bioavailability and biological impacts of the NPs by altering the physico-chemical properties of the NPs (Nasser et al., 2020).

It is therefore worthwhile to examine how the presence of NOM, especially EPS, will affect the toxicity of PSNPs-BPA mixtures in freshwater algae. Although EPS-PSNPs interactions and consequent biological effects have recently been investigated, most of the studies involve PSNP as a single pollutant. In our previous study the growth inhibition of PSNPs in *Scenedesmus obliquus* was significantly reverted in the presence of EPS and the results were correlated with the related decline in ROS generation, antioxidant enzyme activity, and membrane damage (Giri and Mukherjee, 2021). PSNPs coexist with other emerging pollutants in realistic aquatic bodies. The effects of NOM (EPS) on the mixture of NPs and other pollutants deserve critical attention since the sorption behaviour and carrier effect of the particles can be altered significantly by these biopolymers. To the best of the authors' knowledge, the present study is the first ever to elucidate how the addition of algal EPS to a freshwater medium modifies the toxic effects of the combination of BPA and PSNPs in freshwater algae.

The main aim of this work was to examine the role of EPS in altering the toxic potential of mixtures of different surface-modified PSNPs (plain, aminated, and carboxylated) with BPA in *Scenedesmus obliquus*, a freshwater microalga. The different binary mixtures in this study included 2.5 mg/L of BPA with 1 mg/L of plain, aminated and carboxylated PSNPs, 5 mg/L of BPA with 1 mg/L of plain, aminated and carboxylated PSNPs and finally 10 mg/L of BPA with 1 mg/L plain, aminated and carboxylated PSNPs. For studying the impact of EPS on these binary mixtures, 1 mg/L of extracted algal EPS from the same alga was added to the exposure medium and various biochemical parameters were measured after a 72-h exposure period. The methodology was designed to closely resemble the conditions of real-world exposure. Filtered and sterilized natural freshwater was employed as the exposure medium as in our previous studies (Bhuvaneshwari et al., 2015; Chakraborty et al., 2021; Giri and Mukherjee, 2021; Roy et al., 2021). Cell viability, intracellular ROS levels, and antioxidant enzyme activity (SOD and CAT) were measured. In addition, the photosynthetic pigment content and membrane integrity were assessed.

2. Methods

2.1. Chemicals used

The chemicals used for performing the various experiments in this study, including Bisphenol A, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), triton x-100, and sodium carbonate (Na_2CO_3) were purchased from Sigma Aldrich. The polystyrene nano plastics (PSNPs) used in this study (Plain, aminated, carboxylated) were of 200 nm size and were procured at a concentration of 25 mg/ml from Corpuscular, Inc., USA. Dihydroethidium (DHE), 5 mM, Aminophenyl fluorescein (APF), 5 mM, and SYTOX Green, 5 mM were acquired from Invitrogen™, Molecular Probes®, CA, USA. MTT (3- [4, 5-dimethylthiazol- 2-yl]- 2,5-diphenyltetrazolium bromide), hydroxylamine hydrochloride, and dimethyl sulfoxide (DMSO) were obtained from Hi-Media Pvt. Ltd (Mumbai, India). Lastly. Nitroblue tetrazolium chloride (NBT) and hydrogen peroxide solution (H_2O_2- 30% w/v) were purchased from SDFCL, Mumbai, India.

2.2. Preparation and characterization of the mixture of BPA and PSNPs

Stock solutions of carboxylated, aminated, and plain PSNPs were prepared in a concentration of 100 mg/L in deionized water. To obtain a homogeneous suspension, the NPs were dispersed with the help of a bath sonicator for 15 min. Similarly, a stock solution of 200 mg/L BPA was prepared in acetone. 2.5, 5, and 10 mg/L of BPA and 1 mg/L of various surface-modified PSNPs were mixed and sonicated in a bath sonicator to prepare the binary mixtures. The final concentration of BPA in the interaction medium was measured spectrophotometrically using an UV spectrophotometer at 230 nm (Ioan et al., 2007). For the EPS interaction studies, 5 mg/L of EPS (equivalent TOC concentration) solutions were mixed with the freshwater medium to get a final concentration of 1 mg/L EPS in all the various types of PSNPs-BPA combinations.

The 90 Plus Particle Size Analyzer, Brookhaven Instruments Corp., USA, was employed to examine the changes in mean hydrodynamic

diameter (MHD) and surface charge at 0 h and 72 h.

2.3. Isolation and culture maintenance

Scenedesmus obliquus, an environmentally dominant and easy-to-grow alga, was used for all experiments in this study. These algae were isolated from the lake in VIT, Vellore (12°58′10" N, 79°9′ 37"E). The sterilized BG-11 medium, which was purchased from Hi-Media Pvt. Ltd., was used to subculture the isolated algal cells. This subculture was preserved in a chamber with a controlled temperature (I.L.E. Co., India) at 23 ± 2 °C. To facilitate proper culture growth, a photoperiod of 16 h was specifically maintained by illuminating the cultures with a white fluorescent lamp with a 3000 lx illumination (Philips TL-D Super 80, linear fluorescent lamp, India) (Roy et al., 2021).

2.4. Collection and filtration of lake water

This work used freshwater as an interaction medium to simulate environmental conditions. The water for the current study came from the Lake at VIT University in Vellore, Tamil Nadu, India. Before being used as an interaction medium, this lake water went through a series of filtration processes. The water was initially passed through blotting paper twice to remove the majority of the large particulate matter and unwanted materials. Following that, the filtrate was screened through Whatman no. 1 (pore size- 11 μm) filter paper in order to remove any remaining suspended matter. Ultimately, after a series of filtrations, the collected filtrate was sterilized in an autoclave at 121 °C for 15 min. Following a series of filtrations and sterilization steps the collected lake water was referred to as the 'freshwater medium'. This filtered and sterilized lake water was used as the interaction medium throughout the current study.

2.5. Extracellular polymeric substance (EPS) extraction and characterization

The procedures for EPS extraction and characterization were followed from our previous work (Giri and Mukherjee, 2021). The extracted EPS was added to the freshwater medium after characterization and TOC quantification. This was referred to as the "EPS-containing medium" in the rest of the manuscript. The detailed procedures are included in the Supplementary information (Methodology section).

2.6. Interactions of algal samples with the binary mixtures of BPA and PSNPs

An algal culture in the late log phase was taken, centrifuged, and set at an optical density of 0.5 for the interaction studies. The optimal algal cell optical density was achieved, and the cells were maintained for a 72-h interaction with the mixture of BPA and different surface-modified PSNPs. For the binary mixture, contaminants were mixed at 2.5, 5, and 10 mg/L of BPA and 1 mg/L (plain, aminated, and carboxylated) PSNPs in the interaction medium. Finally, filtered, sterilized lake water was added to bring the total volume to 5 ml. In EPS-interacted samples, EPS was added to the interaction medium along with the binary mixture of the contaminants. The above interactions were studied under visible light at 23 °C and the exposure media were kept static for 72 h. During toxicity testing, the OECD guidelines (2004) were strictly followed. For the experiments, the algal samples were kept in each combination with a control that did not include the contaminant mixtures. To achieve statistical significance in the data, all experiments were carried out in triplicate (n = 3). The control tests with the three concentrations of BPA were also run to compare their effects with that of the binary mixtures.

2.7. Evaluation of cell viability

At the end of the incubation period, the samples were analyzed to get the cell viability using the MTT dye. Freshly prepared dye at a concentration of 5 mg/ml was made in sterile PBS buffer (pH-7.4). From the interacted samples, 500 μl of the cells were collected, and to that 20 μl of the dye was added and kept under dark incubation for 4 h. Once the incubation period was over, the samples were centrifuged at 8000 rpm, and the supernatant was discarded. To the cell pellet, 200 μl of DMSO was added and the pellet was dissolved in it. The absorbance of this was measured at 570 nm using an ELISA plate reader (xMARK microplate absorbance spectrophotometer, BIO-RAD) (Suman et al., 2015). In comparison to the control, the percentage of cell viability of treated samples was calculated.

2.8. Assessment of oxidative stress indicators

2.8.1. Superoxide radical generation

The blue fluorescent dye dihydroethidium (DHE) detects the superoxide radical produced within the algal cells. The interacted as well as the control samples after incubation were centrifuged at 4000 rpm for 20 min at 4 °C and the supernatant was discarded. To the collected cell pellets, 10 mM of the DHE dye was added to the 96-well plate and kept in the dark for incubation for 30 min. In the end, the fluorescence intensity was measured for all samples at 480 nm excitation and 570 nm emission using a spectrofluorometer (Cary Eclipse fluorescence spectrophotometer, model G9800A; Agilent Technologies, USA) (Owusu-Ansah et al., 2008).

2.8.2. Hydroxyl radical generation

The generation of hydroxyl radicals (•OH) in algal cells was measured using APF (Aminophenyl fluorescein) dye. At the end of the incubation, the samples (interacted and control) were centrifuged for 20 min at 4 °C at 4000 rpm. The supernatant was discarded and the cell pellet was collected. These cell pellets were then resuspended in 10 mM of APF dye in the 96-well plate and it was kept under dark incubation for 30 min. After the dark incubation was over, the samples were measured for their fluorescence intensity at 480 nm excitation and 570 nm emission using a spectrofluorometer (Cary Eclipse fluorescence spectrophotometer, model G9800A; Agilent Technologies, USA) (Setsukinai et al., 2003).

2.8.3. Total ROS generation

Using the cell-permeable fluorescent dye (ROS indicator), DCFH-DA, the production of total intracellular reactive oxygen species (ROS) was measured in this test. All treated (pristine mixtures and EPS-interacted mixtures) and control samples were incubated in the dark for 30 min with 100 μM DCFH-DA after 72 h of interaction. After that, the samples' fluorescence was measured with a spectrofluorometer (Agilent Technologies, USA, Cary Eclipse fluorescence spectrophotometer, model G9800A) at 485 nm for excitation and 530 nm for emission (Roy et al., 2018).

2.9. Antioxidant enzyme assays

After 72 h of interaction with binary mixtures and EPS-interacted binary mixtures, the activity of Superoxidase dismutase (SOD) in algae was measured using riboflavin. The cell pellet was collected after incubation by centrifugation at 7000 rpm for 10 min at 4 °C, washed and homogenized with 0.5 M phosphate buffer, and centrifuged for another 20 min at 13,000 rpm at 4 °C. After centrifugation, 100 μL of supernatant was mixed with 50 mM Na2CO3, 96 mM nitro tetrazolium blue chloride, 0.6% triton x-100, and 20 mM hydroxylamine hydrochloride and incubated at 37 °C for 20 min under visible light conditions. Finally, their absorbance at 560 nm was measured using a microplate reader (xMARK microplate absorbance spectrophotometer, BIO-RAD) (Kono, 1978).

After 72 h of treatment, the catalase enzyme activity of the algal cells was measured by first centrifuging the cells at 7000 rpm for 10 min at

4 °C. Then, the collected cells were homogenized in sterile 0.5 M PBS before being centrifuged at 13,000 rpm for 20 min at 4 °C. After this, to 2 ml of the supernatant, 1 mL of freshly made H2O2 solution (10 mM) was added and the absorbance decline was measured at 240 nm for 3 min using a spectrophotometer (Evolution 220, Thermo Fischer Scientific, USA) (Chakraborty et al., 2021).

2.10. Evaluation of membrane integrity

SYTOX green, a fluorescent nucleic acid binding stain that is impermeable to living healthy cells with intact cell membranes, was used in this study to determine membrane integrity in algae treated with the pristine mixture as well as EPS-interacted mixtures. Following the 72-h interaction period, 200 μL of all interacted samples (both control and treated) were incubated for 40 min in a 96-well plate with 0.1 μM of the dye. A spectrofluorometer (Cary Eclipse fluorescence spectrophotometer, model G9800A; Agilent Technologies, USA) with excitation and emission wavelengths of 485 ± 20 nm and 516 ± 20 nm, respectively, was used to measure the intensity of green fluorescence (Natarajan et al., 2021).

2.11. Photosynthetic pigment content analysis

Using 95% ethanol as a reference, the concentration of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) was determined spectrophotometrically. For these tests, 4.5 ml of an algal suspension was centrifuged at 4000 rpm for 15 min, the supernatant was taken out, and the pellet was then given 3 ml of 95% ethanol and glass beads. Before being centrifuged for 3 min at 4000 rpm, the suspension was vortexed. The supernatant's absorbance was immediately determined at wavelengths 665, 649, and 470 nm. The pigment concentrations were calculated using the equations

chlorophyll *a* (Chl a) = 13.95 A665–6.88 A649;

chlorophyll *b* (Chl b) = 24.96 A649–7.32 A665;

carotenoids (Car) = (1000 A470–2.05 Chl a–114.8 Chl b)/245;

and calculated for algal suspension density (Filová et al., 2021).

2.12. Statistical analysis

All the experiments in this work were carried out in triplicates (n = 3). The data are presented as mean ± SD (standard deviation). In Graph Pad Prism 5, a two-way ANOVA test with a Bonferroni post-test was used to test statistical significance for different test samples versus controls. Data with $p < 0.05$ was considered statistically significant (at the 95% confidence level, $p < 0.05$ denoted significance).

3. Results

3.1. Characterization of the medium and colloidal stability of PSNP and BPA mixtures

The constituents of the EPS-containing medium were identified from the FTIR spectra. The spectra indicate the presence of two dominant peaks at 3304 cm^{-1} and 1638 cm^{-1}, and a minor peak at 2143 cm^{-1} representing the –NH bond and –C═C stretching, and –N═C═N

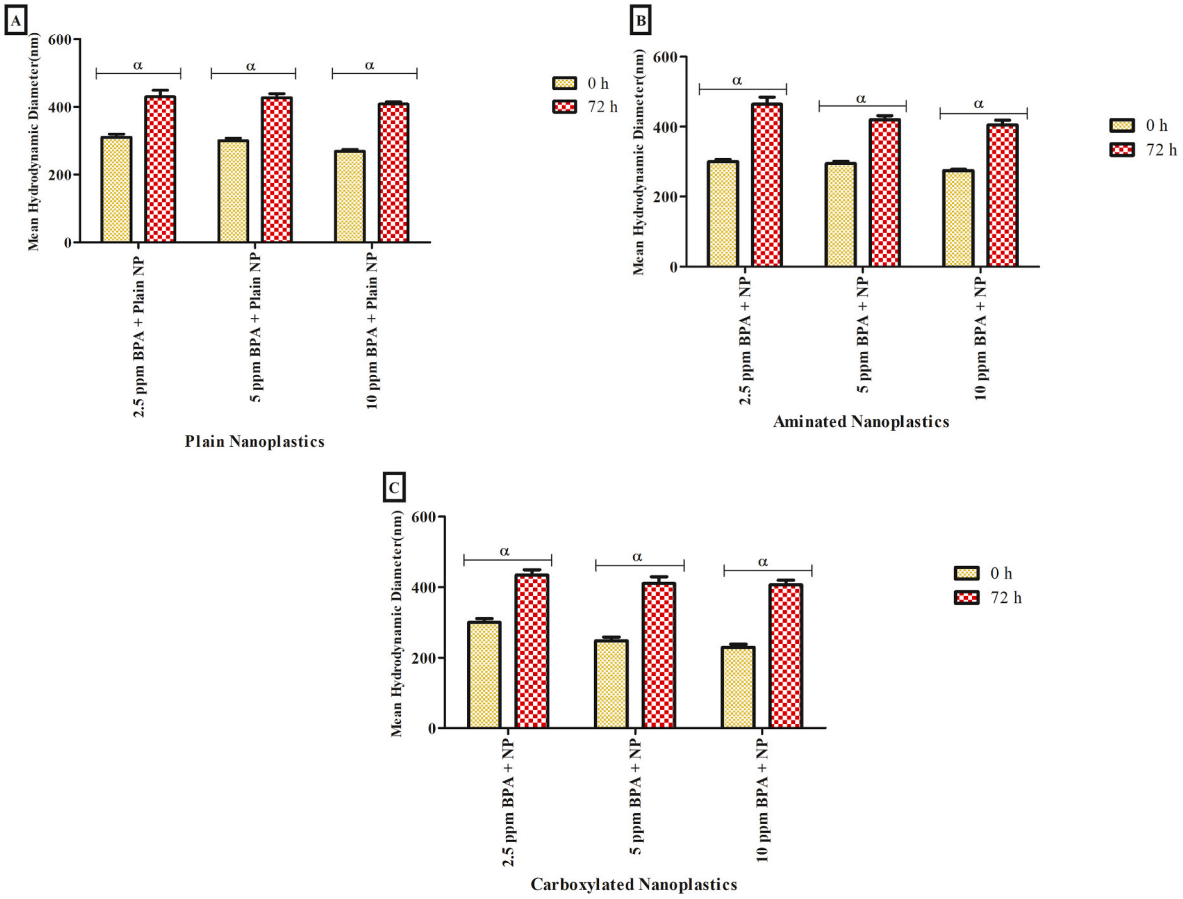

Fig. 1. Mean Hydrodynamic diameter (MHD) of the binary mixture of BPA at concentrations of 2.5, 5, and 10 mg/L with 1 mg/L of (A) Plain PSNPs, (B) Aminated PSNPs and (C) Carboxylated PSNPs. The level of the significant increase in the MHD was represented by '***' ($p < 0.001$). All the experiments were performed in triplicates.

stretching, respectively (Giri and Mukherjee, 2021). HR-LCMS of the medium containing EPS revealed the presence of various organic compounds including oligopeptide chains, fatty acids, and triacylglycerols (Giri and Mukherjee, 2021).

The changes in the mean hydrodynamic diameters (MHD) of the three types of PSNPs in the EPS-containing medium with various concentrations of BPA during the 72 h interaction period are presented in Fig. 1. A significant increase in the MHDs was noticeable for all three combinations of PSNPs and BPA at the end of the 72 h incubation period. When compared to deionized water (Fig. S1, Supplementary information) the aggregation was significantly enhanced in the EPS-containing medium. The changes in the zeta potential values of the PSNPs-BPA mixtures in the EPS-containing medium and the deionized water over the 72 h interaction period are provided in Table 1.

The measured concentrations of BPA were 2.09 mg/l, 3.67 mg/l, and 6.29 mg/l in the EPS-containing medium: 2.25 mg/L, 3.92 mg/L, and 9.02 mg/L in the freshwater medium (without EPS), for nominal values of 2.5 mg/l, 5 mg/l, and 10 mg/l, respectively. This demonstrates a significant decline in the available concentration of BPA in the presence of EPS.

3.2. Growth inhibition effects

The effects of three doses of BPA on the algal cell viability can be deduced from Fig. S2. There was a concentration dependent decline in the cell viability as the concentration of BPA increased. Fig. 2 depicts the effects of BPA and PSNPs mixtures on growth inhibition of *Scenedesmus obliquus*. Compared to the control, a significant increase ($p < 0.001$) in the growth inhibition was observed for all pristine mixtures (2.5 mg/l, 5 mg/l, 10 mg/l BPA + 1 mg/l plain, carboxylated, aminated PSNPs). The effect was dose-dependent in nature. Among all the treatment groups the maximum inhibitory effect was observed for the mixture consisting of 10 mg/l BPA and 1 mg/l carboxylated PSNPs.

While comparing the three different surface modified PSNPs the maximum inhibition was noted for the mixture of (BPA + carboxylated PSNPs) while (BPA + plain PSNPs) and (BPA + aminated PSNPs) showed less intense toxic effects.

Compared to the pristine mixtures, there was a significant decline in the growth inhibitory effects in the presence of EPS. The decreased toxic effect was highly significant for the mixture of (10 mg/l BPA + plain PSNPs) with EPS when compared to the respective pristine mixtures ($p < 0.001$). Similarly, in comparison with the pristine mixtures (2.5 mg/l, 5 mg/l BPA + plain PSNPs) and (2.5 mg/l BPA + carboxylated PSNPs) the EPS containing mixtures showed a significantly ($p < 0.01$) reduced growth inhibition. To evaluate the effects of acetone on algal cells, the highest volume of acetone (0.25 ml in 5 ml) used in the BPA solutions was added with the algal cells. It was noted that acetone did not induce any growth inhibition in the algal cells.

Table 1
Zeta Potential values for the binary mixtures in deionized water and EPS-containing medium at 0 h and 72 h.

Name of Sample	Zeta Potential in Deionized water at 0 h	Zeta Potential in Deionized water at 72 h	Zeta Potential in EPS-containing medium at 0 h	Zeta Potential in EPS-containing medium at 72 h
2.5 ppm BPA+1 ppm Plain PSNPs	−14.04 ± 2.09 mV	−10.96 ± 2.00 mV	−18.47 ± 3.83 mV	−23.55 ± 2.87 mV
2.5 ppm BPA+1 ppm Aminated PSNPs	−11.07 ± 2.17 mV	−10.94 ± 2.63 mV	−12.23 ± 1.35 mV	−21.18 ± 3.39 mV
2.5 ppm BPA+1 ppm Carboxylated PSNPs	−14.98 ± 1.86 mV	−15.94 ± 1.83 mV	−22.39 ± 2.57 mV	−24.73 ± 1.72 mV
5 ppm BPA+1 ppm Plain PSNPs	−16.40 ± 1.71 mV	−15.67 ± 1.28 mV	−23.75 ± 3.45 mV	−22.48 ± 1.51 mV
5 ppm BPA+1 ppm Aminated PSNPs	−15.91 ± 3.34 mV	−12.02 ± 2.47 mV	−22.59 ± 2.83 mV	−21.96 ± 1.58 mV
5 ppm BPA+1 ppm Carboxylated PSNPs	−15.27 ± 3.50 mV	−18.07 ± 3.64 mV	−24.54 ± 4.53 mV	−24.99 ± 2.44 mV
10 ppm BPA+1 ppm Plain PSNPs	−12.85 ± 1.11 mV	−16.99 ± 1.80 mV	−18.03 ± 7.92 mV	−15.29 ± 5.02 mV
10 ppm BPA+1 ppm Aminated PSNPs	−12.22 ± 1.92 mV	−11.05 ± 1.66 mV	−12.22 ± 4.07 mV	−9.44 ± 3.18 mV
10 ppm BPA+1 ppm carboxylated PSNPs	−14.67 ± 1.01 mV	−16.01 ± 4.73 mV	−24.13 ± 6.23 mV	−18.20 ± 4.11 mV

3.3. Generation of oxidative stress

The total ROS, superoxide, and hydroxyl radical generation in the cells after treatment with BPA is represented graphically in Fig. S3. Quite similar to the trend in the cell viability, the oxidative stress parameters showed an increment in a concentration dependent manner, where 10 mg/l of BPA showed the highest increase in total ROS, superoxide, and hydroxyl radical production. The superoxide radical generation in the cells interacted with the pristine and EPS containing mixtures is shown in Fig. 3. Compared to the control, the superoxide radical generation by the pristine mixture of (2.5 mg/l BPA+ 1 mg/l PSNPs) was observed to be the least among the three concentrations of BPA. The superoxide radical generation was significantly enhanced with respect to the control samples in case of the mixtures (5 mg/l and 10 mg/l BPA + 1 mg/l PSNPs) ($p < 0.001$).

When comparing between the superoxide radical production for three types of PSNPs, the superoxide radical production was maximum for the mixture of (BPA + carboxylated PSNPs) whereas (BPA + plain PSNPs) and (BPA + aminated PSNPs) resulted in reduced radical generation.

Compared to the pristine mixtures a significant decrease in the superoxide radical generation was noted when EPS was added to the medium. In the presence of EPS, the decrease in superoxide radical generation was highly significant ($p < 0.001$) for the mixtures of (2.5 mg/l, 10 mg/l BPA + plain PSNPs), (2.5 mg/l, 10 mg/l BPA + aminated PSNPs), and (10 mg/l BPA + carboxylated PSNPs), when compared to the respective pristine mixtures.

Fig. 4 shows the changes in the hydroxyl radical generation after treating the algae with the pristine and EPS containing mixtures. The hydroxyl radical generation of the pristine mixture of (2.5 mg/l BPA + PSNPs) was the lowest among the three pristine mixtures. The maximum increase in the radical generation was observed for the mixture of (10 mg/l BPA + 1 mg/l PSNPs) ($p < 0.001$).

When the effects of three different surface modified PSNPs are compared, the enhancement of hydroxyl radical generation by the mixture (BPA + carboxylated PSNPs) was maximum. The hydroxyl radical generation by the different surface modified PSNPs too followed a similar trend as the cell viability and superoxide radical generation in the cells.

Compared to the pristine mixtures, there was a significant decrease in hydroxyl radical generation in the mixtures containing EPS. In the presence of EPS the decline in hydroxyl radical generation was highly significant ($p < 0.001$) for all the three mixtures when compared to their respective pristine counterparts.

The changes in intracellular ROS generation by the pristine and EPS

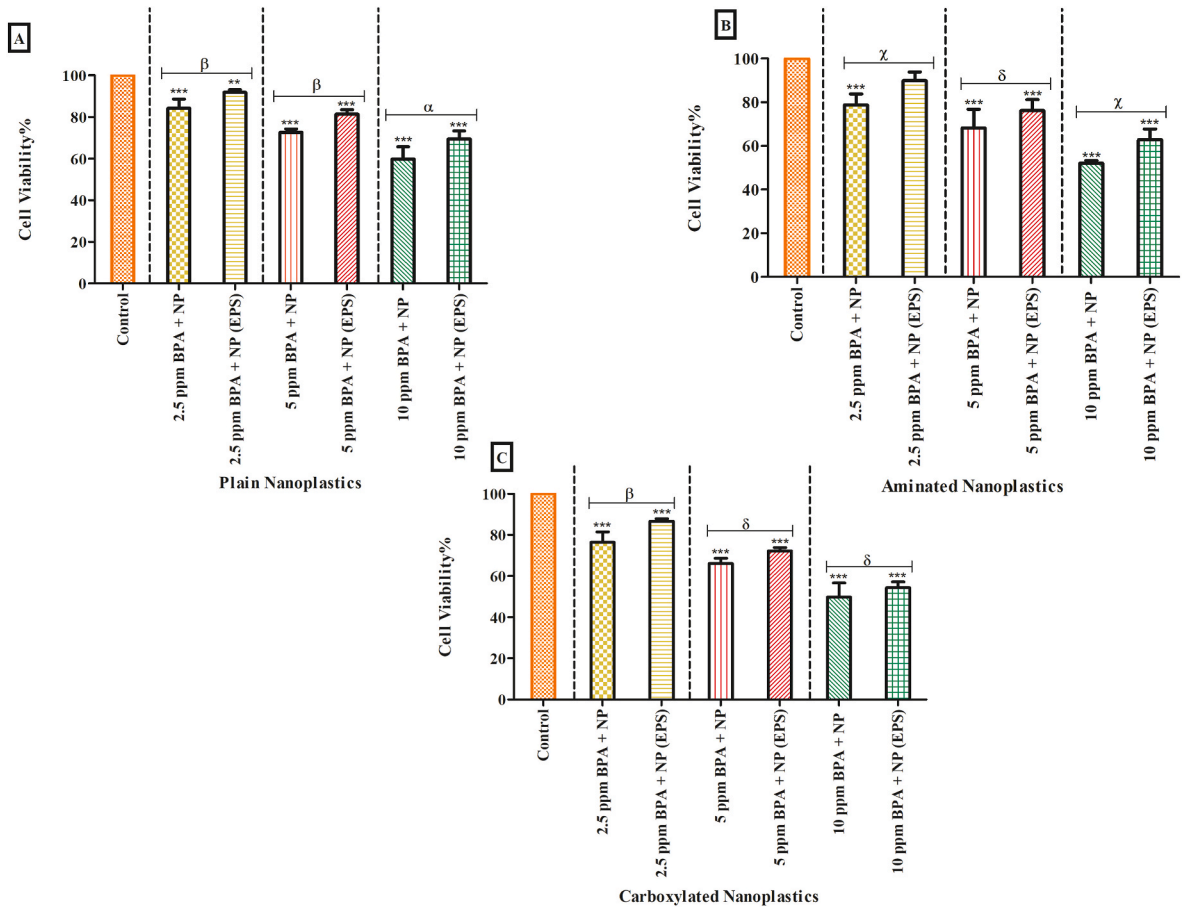

Fig. 2. The comparison of cell viability for 2.5, 5, and 10 mg/l BPA +1 mg/l of (A) plain PSNPs (B) Aminated PSNPs, and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'α, β, γ, δ' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups ($\alpha = p < 0.001$, $\beta = p < 0.01$, $\gamma = p < 0.05$, and δ = no significance).

containing mixtures are shown in Fig. S7 (supplementary information). Among the treated samples, the maximum ROS generation was noted for the mixture of (10 mg/L BPA+ 1 mg/L PSNPs) and it was highly significant ($p < 0.001$) in comparison with the control.

While comparing the three different surface modifications of the PSNPs, the maximum ROS generation was observed for the mixture of (BPA + carboxylated PSNPs) whereas (BPA + plain PSNPs) and (BPA + aminated PSNPs) showed less ROS generation. Thus, like the growth inhibition of microalgae, the total ROS generation in different surface modified PSNPs mixtures followed the order of plain PSNPs < aminated PSNPs < carboxylated PSNPs.

Compared to the pristine mixtures, there was a significant decrease in the total ROS generation when EPS was added to the medium. In presence of EPS the decline in ROS generation was highly significant for the mixtures of (2.5 mg/L, 5 mg/L, 10 mg/L BPA + plain PSNPs), (2.5 mg/L, 5 mg/L BPA + aminated PSNPs), and (2.5 mg/L BPA + carboxylated PSNPs), when compared to the respective pristine mixtures ($p < 0.001$). Similarly (5 mg/L BPA + carboxylated PSNPs) mixture in the presence of EPS showed a significant decrease ($p < 0.01$) in ROS generation. For the combinations (10 mg/L BPA + aminated PSNPs) and (10 mg/L BPA + carboxylated PSNPs) also addition of EPS resulted in a decrease in the ROS generation, although this decrease was less significant ($p < 0.05$) in comparison with cells treated with the pristine mixtures.

3.4. Antioxidant enzyme activity

The changes in the antioxidant activities (SOD and catalase) in the algal cells when interacted with BPA are represented in Fig. S4. Like the results of cell viability and oxidative stress parameters, the antioxidant enzyme activities of the BPA treated samples also showed an increase in the dose dependent manner. Fig. 5 represents the increase in the activity of superoxide dismutase (SOD) enzyme for the pristine and EPS containing mixtures. The treated cells showed a significantly higher ($p < 0.001$) SOD activity in comparison with the control for all the pristine as well as the EPS containing mixtures. The increase in the SOD activity for the various surface modified PSNPs in the mixtures was observed to follow the order: plain < aminated < carboxylated PSNPs. Irrespective of the different surface modifications of the PSNPs, the SOD enzyme activity was significantly reduced ($p < 0.001$) for the EPS containing mixtures when compared to the pristine ones.

Fig. 6 shows the changes in the catalase enzyme activity after treating the cells with the pristine and EPS containing mixtures. Compared to the control, the catalase enzyme activity for the pristine mixture (10 mg/L BPA + PSNPs) treated group was observed to be the highest amongst the three concentrations of BPA. Only for the (5 mg/L and 10 mg/L BPA + 1 mg/L PSNPs) mixtures the catalase activity was significantly higher with respect to the control group ($p < 0.001$). Comparing between the three different surfaces modified PSNPs, the enhancement of catalase activity was shown mostly by the mixture of (BPA + carboxylated PSNPs), whereas the (BPA + plain PSNPs) and (BPA + aminated PSNPs) mixtures showed less catalase activity.

Compared to the pristine mixtures, there was a significant drop ($p < 0.001$) in catalase activity in the presence of EPS for the (5 mg/L, 10 mg/L BPA + plain PSNPs), (10 mg/L BPA + aminated PSNPs) and (10 mg/L BPA + carboxylated PSNPs) mixtures, with respect to the corresponding

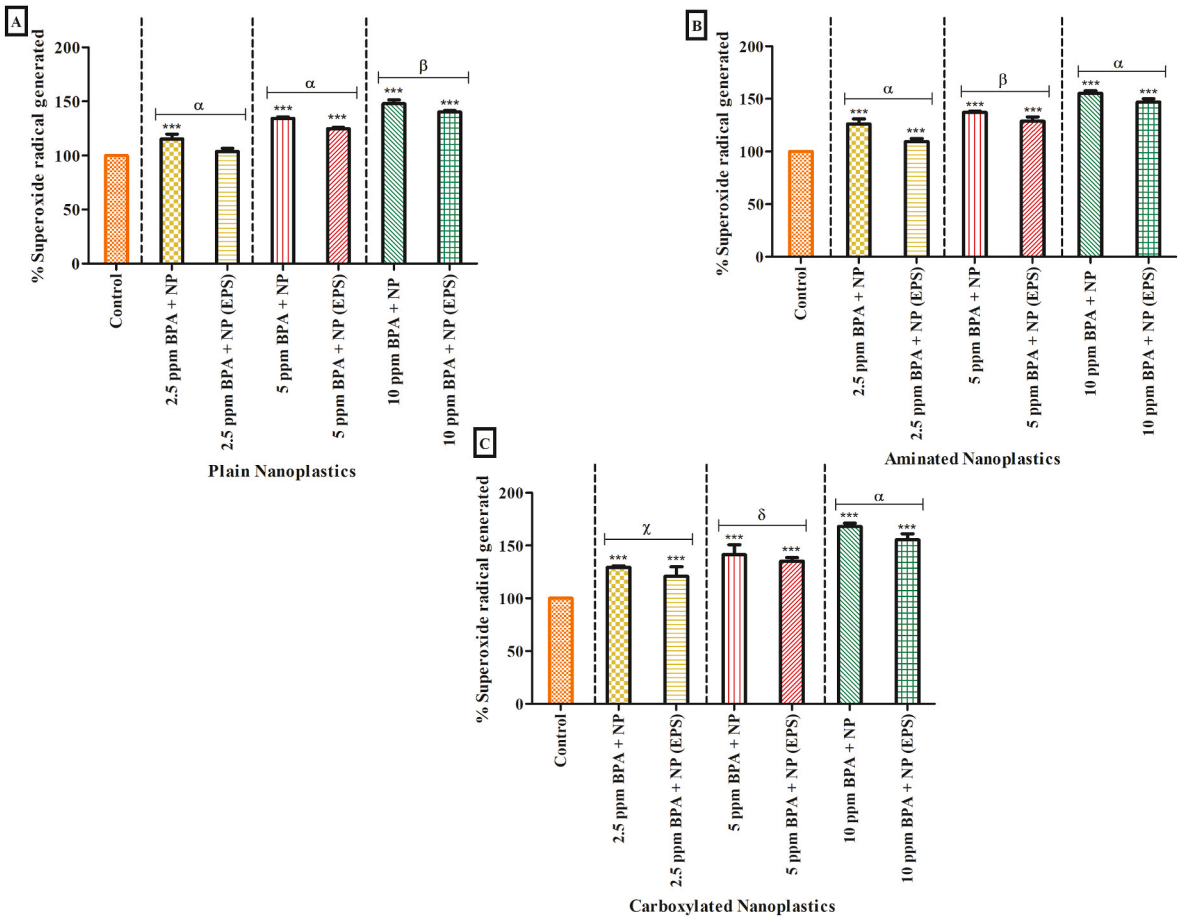

Fig. 3. The comparison of superoxide radical generation for 2.5, 5, and 10 mg/l BPA + 1 mg/l of (A) plain PSNPs (B) Aminated PSNPs and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'α, β, γ, δ' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups (α = $p < 0.001$, β = $p < 0.01$, γ = $p < 0.05$, and δ = no significance).

pristine mixtures. For the mixtures of (5 mg/L BPA + 1 mg/L aminated and carboxylated PSNPs) the addition of EPS decreased the catalase activity slightly ($p < 0.05$) compared to the pristine mixtures.

3.5. Cell membrane permeability

The cell membrane permeability analysis of the algae treated with BPA is presented in Fig. S5. The cell membrane permeability increased in the treated cells in a dose dependent manner with10 mg/L of BPA causing the highest impact. The changes in the cell membrane permeability of the pristine and EPS containing mixtures treated samples are graphically represented in Fig. S8. While comparing the different PSNPs, the mixture of (10 mg/L BPA + carboxylated PSNPs) showed a significant increase in the membrane permeability. When compared with the pristine treated samples, only the EPS containing mixture of (2.5 mg/L BPA + plain and carboxylated PSNPs) induced a moderate decrease ($p < 0.05$) in the membrane permeability.

3.6. Photosynthetic pigment content

The effects of BPA alone on the photosynthetic pigment production in the treated algal cells are represented in Fig. S6. In line with the results observed for oxidative stress parameters, antioxidant enzyme activities and membrane permeability, the photosynthetic pigments also showed a decline in the concentration dependent manner, where 10 mg/L of BPA caused the maximum decline in the photosynthetic pigment synthesis. The changes in photosynthetic pigment content for the pristine and EPS containing mixture treatments are represented in Fig. 7. When compared to the pristine groups, the EPS containing mixtures of (10 mg/L BPA + plain, aminated, and carboxylated PSNPs) induced a slight increase ($p < 0.05$) in the chlorophyll *a* pigment production. However, the EPS containing mixtures of (2.5 and 5 mg/L BPA + PSNPs) showed no significant change in the chlorophyll *a* pigment content compared to their pristine counterparts.

The variations in chlorophyll *b* pigment content for the pristine and EPS containing mixture treatments are shown in Fig. S9 (supplementary information). In comparison to the pristine treatments, the EPS containing mixtures, (2.5 mg/L and 10 mg/L BPA + PSNPs) and (5 mg/L BPA + aminated and carboxylated PSNPs), showed no significant increase in chlorophyll *b* content. When compared to the pristine groups, the EPS containing mixtures of (5 mg/L BPA + plain PSNPs) showed a significant increase ($p < 0.01$) in the chlorophyll *b* content.

Fig. S10 (supplementary information) demonstrates the changes in carotenoid content in the cells treated with the pristine and EPS containing mixtures. In comparison with the control, all the pristine and EPS containing groups produced significantly less carotenoid pigment ($p < 0.001$). While comparing the different surface modified PSNPs, the (10 mg/L BPA + carboxylated PSNPs) mixture produced the least pigment content among the three concentrations of BPA.

In comparison to the pristine treatments, the EPS mixtures, (2.5 and 5 mg/L BPA + PSNPs) as well as (10 mg/L BPA + carboxylated PSNPs) treated cells showed no significant increase in carotenoid pigment content. However, only the EPS containing (10 mg/L BPA + plain and aminated PSNPs) mixture resulted a moderate increase ($p < 0.05$) in the

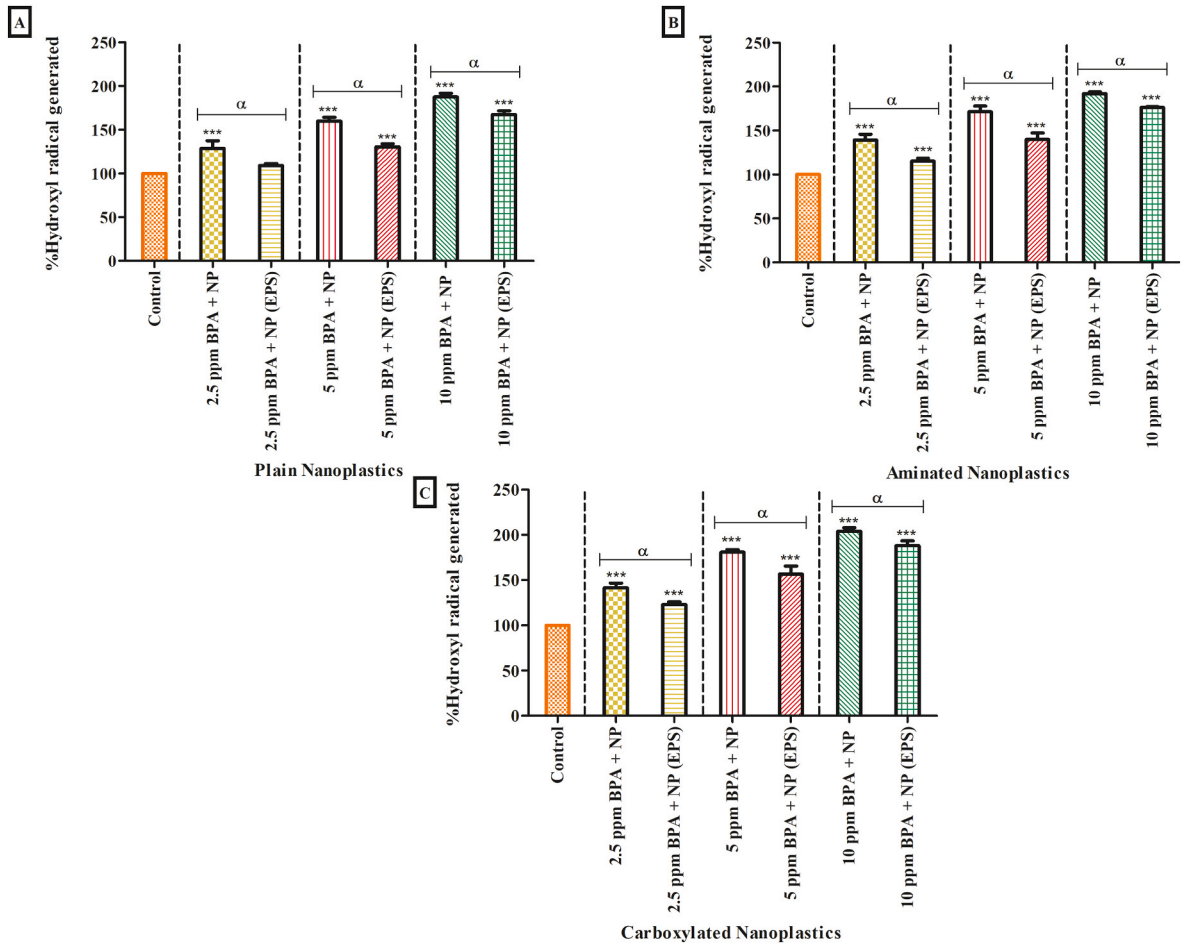

Fig. 4. The comparison of hydroxyl radical generation for 2.5, 5, and 10 mg/l BPA + 1 mg/l of (A) plain PSNPs (B) Aminated PSNPs and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'α, β, γ, δ' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups (α = $p < 0.001$, β = $p < 0.01$, γ = $p < 0.05$, and δ = no significance).

carotenoid content compared to the pristine mixture.

4. Discussion

The adsorption of emerging micropollutants like BPA on PSNPs has already been described by previous researchers and they noted that these plastics possess an extremely high sorption capacity for BPA due to hydrophobic interactions (Xiong et al., 2020). The adsorption of BPA on the surface of PSNPs mostly involves dispersion effects along with electrostatic effects. Dispersion effects (38–49%) and electrostatics effects (43–50%) interact to drive the adsorption mechanism of nano Polyethylene terephthalate (PET) on BPA; specifically, dispersion effects predominate the inner surface adsorption while electrostatics energies predominate the outer surface adsorption (Cortés-Arriagada, 2021). The large specific surface area of the nano-sized plastics coupled with the fact that the benzene ring structure of PS provides the possibility of binding through π–π interactions making them a strong sorbent for BPA (Xu et al., 2014). So PSNPs can function as a carrier for BPA and induce significant toxic effects in aquatic organisms. The role of PSNPs as carriers for BPA in Danio rerio has already been highlighted by Chen et al. (2017) (Chen et al., 2017).

From Fig. 1 (and Fig. S1) it is clear that the addition of EPS led to significant increases in the mean hydrodynamic size of PSNPs in BPA + PSNPs mixtures irrespective of the BPA concentrations and the types of PSNPs. The addition of PSNPs to the different concentrations of BPA results in changes in the hydrodynamic diameter of the BPA particles in the medium. The addition of EPS in the medium results in an increase in the hydrodynamic diameter of the particles. The presence of NOM in freshwater is known to enhance the formation of hetero-aggregates among this particles (Liu et al., 2014). The fibrillar nature of the algal EPS facilitates the process (Grassi et al., 2020). The negatively charged groups of the EPS facilitate the formation of an eco-corona on the aminated PSNPs. This increases the hydrodynamic diameter. Also, the peptide groups of the EPS provided the necessary moieties for binding to the surface of the carboxylated PSNPs, causing an increase in the hydrodynamic diameter. Similar findings were shared in another study where PSNPs acquired eco-corona layers of natural organics (Saavedra et al., 2019). The possible mechanism (s) of eco-corona formation on plastic particles can involve different types of interactions such as electrostatic, hydrophobic, and Van der Waals force-based (Chakraborty et al., 2021). The aggregation of the PSNPs exacerbates gravitational settling phenomena, and this decreases the bioavailability of the particles. There have been several previous reports supporting enhanced aggregation of PSNPs as induced by algal EPS (Junaid and Wang, 2021). This would also greatly restrict the available contact area for adsorbing BPA, thus minimizing the vector effect of NPs. After EPS adsorption the electrostatic repulsion between the algal cells and the PSNPs (with BPA in the mixture) could increase due to the negative charge on both surfaces. This would also restrict hetero-aggregation between the PSNPs and algal cells (Kang et al., 2009). Both these factors contributed to reducing the toxic potential of BPA-PSNP mixtures by the algal EPS.

Upon increasing the concentration of BPA in the mixture with PSNPs

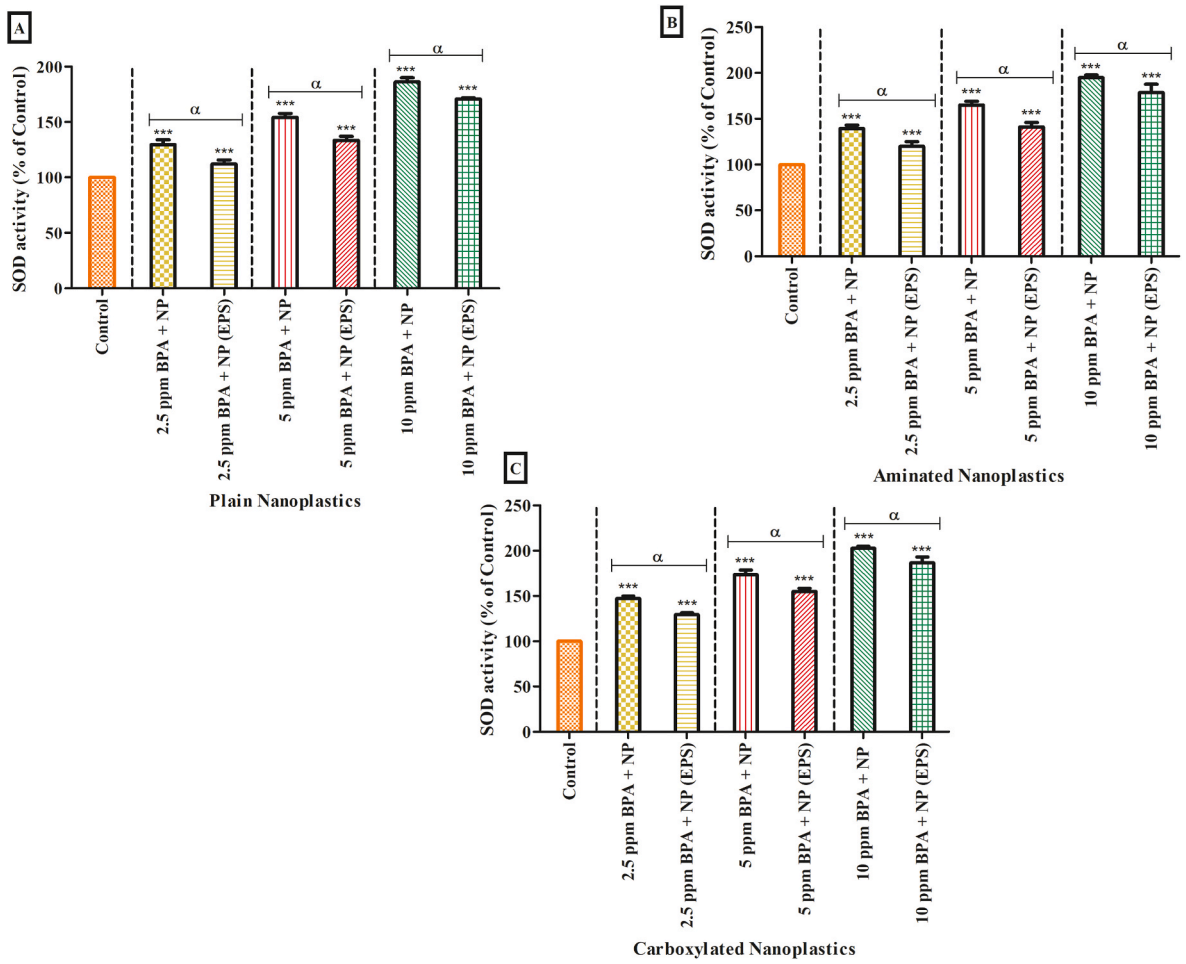

Fig. 5. The comparison of superoxide dismutase enzyme activity for 2.5, 5, and 10 mg/l BPA + 1 mg/l of (A) plain PSNPs (B) Aminated PSNPs and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'α' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups ($\alpha = p < 0.001$).

the growth inhibition increased, demonstrating a clear dose-response trend. A similar concentration-dependent reduction in the growth of *C. mexicana* and *C. vulgaris* was observed in another study (Ji et al., 2014). The authors proposed that high BPA concentrations caused inhibition of cell division and an increase in cell size. In a prior study after 48, 96, and 144 h of exposure to BPA, *S. obliquus* demonstrated dose-dependent toxicity (range: 1.6–50 mg/L) (Li et al., 2017). Another group of researchers (He et al., 2022) observed that co-exposure of 1 mg/L BPA + 5 mg/L PSNPs and 10 mg/L BPA + 5 mg/L PSNPs induced growth inhibition in *Chlorella pyrenoidosa*. In the current work the addition of EPS significantly reduced the overall toxicity of the BPA-PSNP mixtures. A similar result was observed in a previous study (Gattullo et al., 2012), where NOM addition to BPA (2 mg/L and 4 mg/L) stimulated algal cell division in the freshwater green alga *Monoraphidium braunii*. This positive effect of NOM was attributed to its ability to supply inorganic and organic nutrients to algae (Gattullo et al., 2012). In our previous study it was also observed that ageing in EPS containing medium prior to interaction led to a significant reduction in the toxicity of PSNPs in *Scenedesmus obliquus* (Giri and Mukherjee, 2021).

A number of intracellular reactive oxygen species (ROS) like the hydroxyl radical (OH), the superoxide anion radical (O_2^-), singlet oxygen (O), and hydrogen peroxide (H_2O_2) are produced as a result of various abiotic environmental stressors (Li et al., 2008). In the current work treatment of algae with three BPA-PSNP mixtures caused intracellular ROS generation that scaled with concentrations of BPA. Overproduction of ROS may inflict severe damage to the cellular organelles. ROS generation disrupts physiological and metabolic processes by attacking vital molecules like polyunsaturated fatty acids, proteins, and nucleic acids (Ali et al., 2016). In a related study a significant rise in the level of intracellular superoxide anion radicals ($O2^{\cdot-}$) was noticed in *C. reinhardtii* cells after treating with BPA (Esperanza et al., 2020). Superoxide dismutase (SOD) and catalase (CAT) are known to protect the cells from oxidative damage by scavenging ROS. In algae the overexpression of these enzymes has been associated with defence against BPA stress (Azizullah et al., 2022). The antioxidant enzyme activities (SOD and catalase) were found to rise in similar fashion like oxidative radicals after treatment with the BPA-PSNPs mixtures in the current study. Improved activities of SOD and catalase was recently observed in algal species *C. pyrenoidosa* and *S. obliquus* on exposure to BPA at concentrations of 25 and 50 mg/L (Zhang et al., 2014). The observed decrease in growth inhibition by the BPA-PSNP mixture after adding EPS is correlated well with decreased intracellular ROS generation and antioxidant enzyme activity. It is already reported by researchers that short-range ROS generation may be reduced by the presence of an organic coating on nanomaterials (Lawrence et al., 2016; Liu et al., 2015). Gao et al. also reported that algal EPS attenuated oxidative stress induced by TiO_2 NPs in a freshwater microalga *Chlorella pyrenoidosa* (Gao et al., 2018).

Oxidative stress generated by BPA-PSNP mixtures leads to lipid peroxidation, which causes the toxicant to alter the fatty acid profile of algae. This can potentially affect cell membrane functions and metabolic

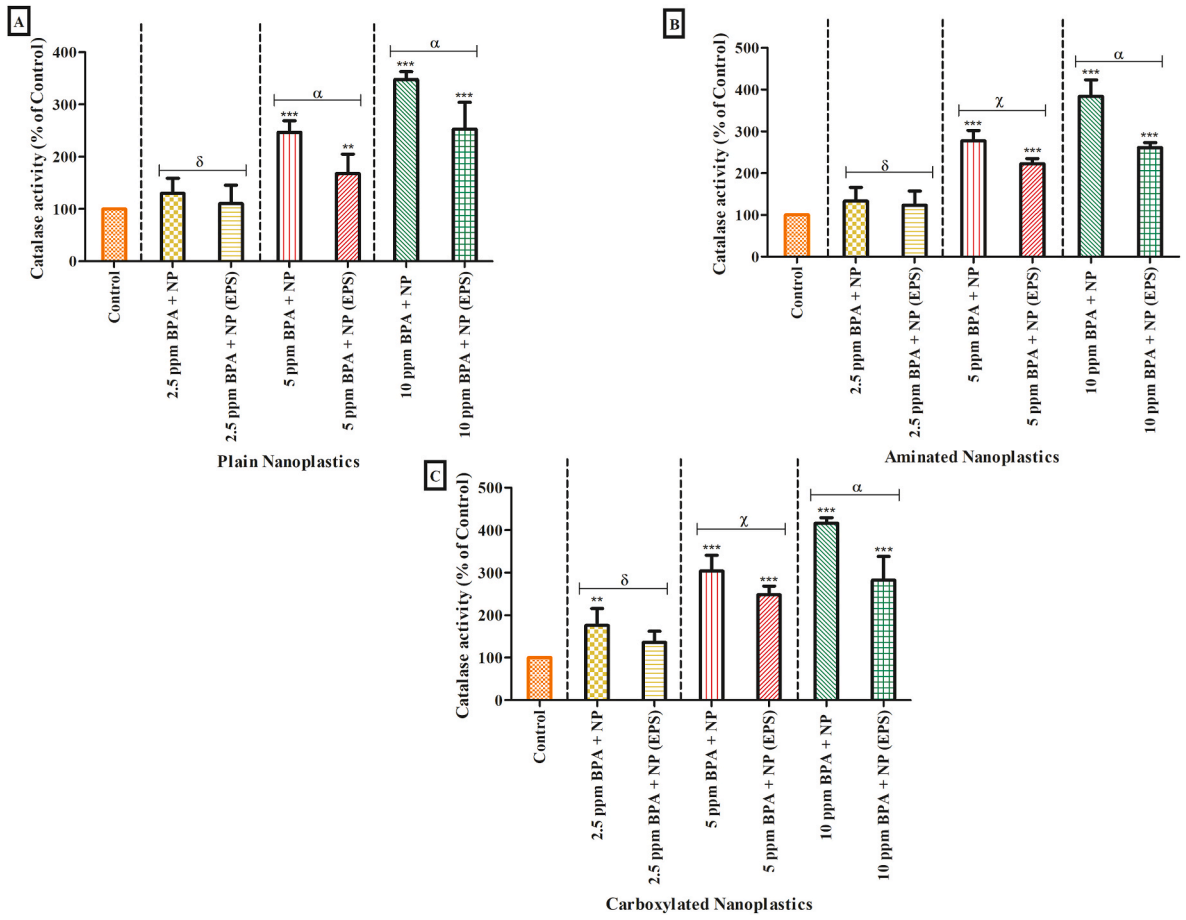

Fig. 6. The comparison of catalase enzyme activity for 2.5, 5, and 10 mg/l BPA + 1 mg/l of (A) plain PSNPs (B) Aminated PSNPs and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'α, γ, δ' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups ($\alpha = p < 0.001$, $\gamma = p < 0.05$, and δ = no significance).

processes in the algae (Li et al., 2008). In the current study, we observed increasing membrane damage with increasing concentrations of BPA in the mixtures. In another study, *P. tricornutum* cells displayed significant changes in membrane permeability when exposed to BPA for 24 h. This was related to the overproduction of ROS causing a decreased stability of the membrane (Seoane et al., 2021). BPA exposure induced lipid peroxidation in *Picocystis* and *Graesiella* and the observed effects increased with BPA concentration and exposure period (Ben Ouada et al., 2018). Similar to our findings on growth inhibition and oxidative stress generation, the algal EPS was able to attenuate the membrane damage too (Fig. S4). This has a direct relation to the observed decline in oxidative stress parameters.

Photosynthetic pigments, chlorophyll, and carotenoids are considered indicators of the photosynthetic functions of algae (Lu et al., 2018). Variations in the pigmentation system are regarded as a defense mechanism in response to various types of abiotic stresses (Zhang et al., 2018). In the current work, a dose-dependent decrease in the chlorophyll *a*, *b*, and carotenoid contents was noticed as the BPA concentration increased in the mixtures with PSNPs. In a recent study, it was observed that the chlorophyll content of *C. pyrenoidosa* declined as the concentrations of PS and BPA increased in the mixtures of BPA with PS microspheres (He et al., 2022). With the addition of EPS, the pigment content showed a revival for all the different treatment groups in the present work. This result correlated well with the ROS, antioxidant enzymes, and membrane permeability results. When EPS was added to the mixtures the accumulation of ROS was attenuated significantly, restoring photosynthetic efficiency by reclaiming the chlorophyll content.

The results of the growth inhibition, ROS generation, lipid peroxidation, and photosynthetic pigment content showed similar trends regarding the effects of various PSNPs. The maximum effect was observed for the mixtures containing carboxylated NPs followed by the aminated NPs, while the plain NPs showed significantly reduced toxic effects. This may be related to the enhanced sorption of BPA on the carboxylated and aminated NPs in the mixtures enhancing the carrier effect.

Fig. 8 portrays the proposed mechanism of action for the toxic effects of the BPA-PSNPs mixture in *Scenedesmus obliquus* and how EPS attenuated the impact. Summarizing the results, the BPA-PSNPs mixtures induced the generation of oxidative radicals and an increase in the antioxidant enzyme activity. The results from the interaction of BPA alone with the algal cells clearly show that oxidative stress was the main mode of action. This was confirmed by the increment in the total ROS, oxidative radical generation, and antioxidant enzyme activity. In the case of the mixtures also an identical trend was observed in the variations of various biological responses although the effects were more severe. The oxidative stress generated by the superoxide and hydroxyl radicals produced due to the mixture BPA-PSNPs entering the algal cells might lead to damage in the nuclear membrane. The damage in the nuclear membrane will lead to damage in the DNA which was shown by the increase in the intensity of the SYTOX Green fluorescence. The PSNPs probably acted as a vector for the BPA and enhanced its toxic potential. It is well known that hydrophobic organic pollutants in the environment can adsorb on plastics' hydrophobic surfaces. As a result, plastics may act as a "Trojan Horse" effect for the uptake of contaminants by aquatic organisms. All aquatic environments contain natural organic

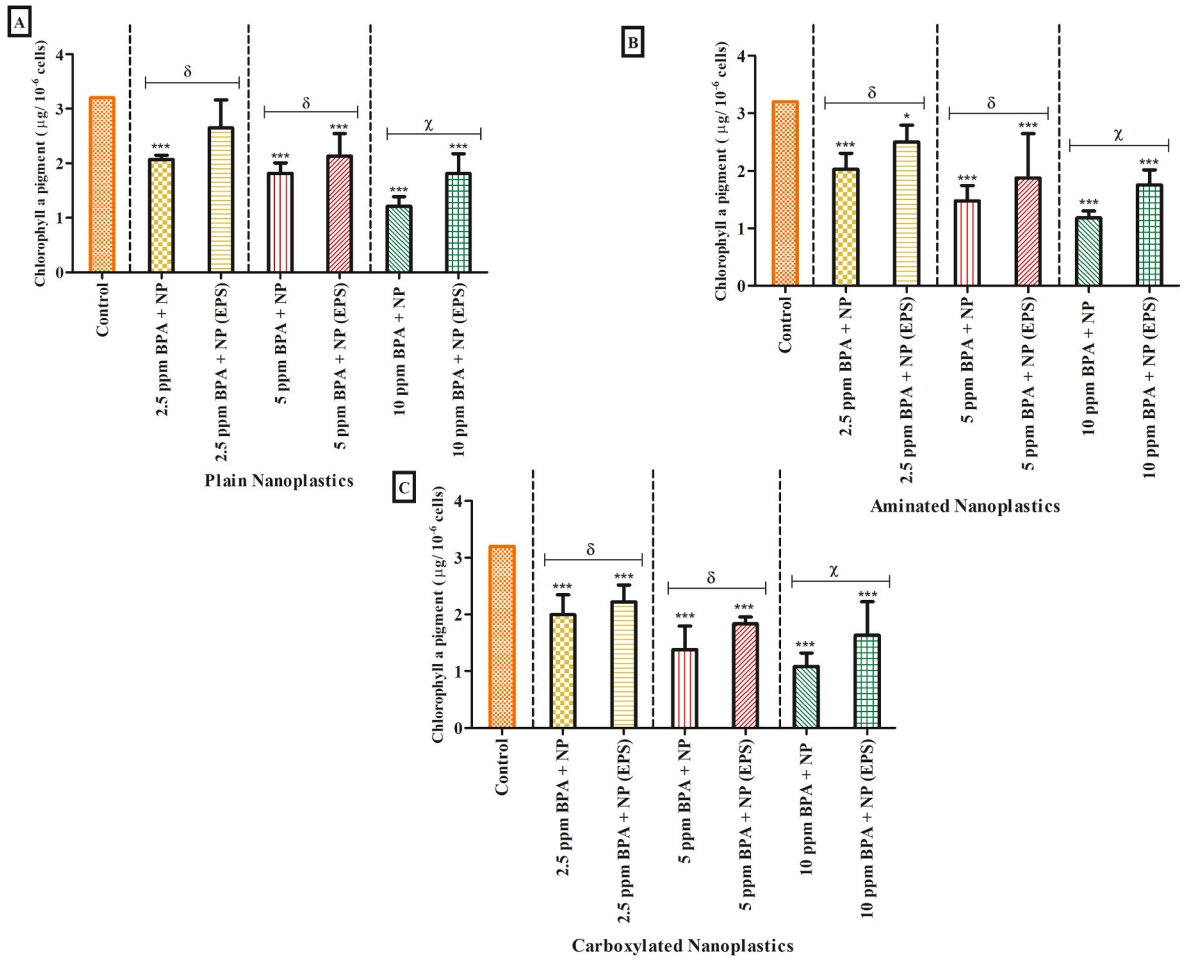

Fig. 7. The comparison of chlorophyll "a" pigment content for 2.5, 5, and 10 mg/l BPA + 1 mg/l of (A) plain PSNPs (B) Aminated PSNPs and (C) Carboxylated PSNPs in the presence and absence of EPS. The level of significance for algal cells treated with BPA + PSNPs with respect to control is marked with '***' ($p < 0.001$), 'γ, δ' indicates a significant difference between pristine and EPS interacted BPA + PSNPs treatment groups ($\gamma = p < 0.05$, and δ = no significance).

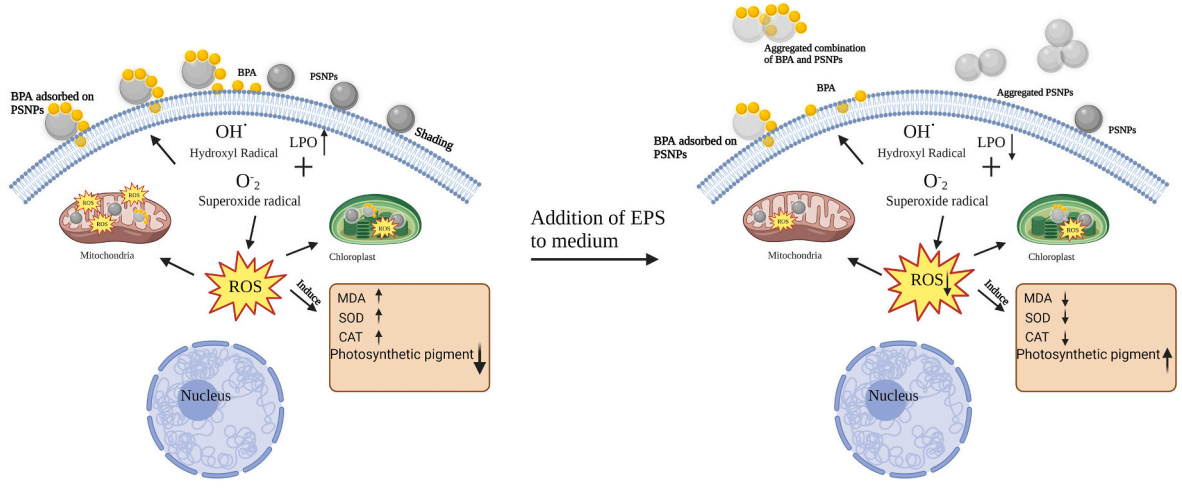

Fig. 8. A schematic diagram elucidating the mechanism of toxicity of the binary mixture of BPA + PSNPs and how the addition of EPS alleviates the toxic potential of the mixtures.

matter (NOM), which can interact with organic pollutants and nanoplastics. However, there is still little information on how NOM affects the "Trojan Horse" effects of nanoplastics for hydrophobic organic pollutants. When the algal EPS was added to the mixtures, the homo aggregation of PSNPs was facilitated leading to the settling of the large flocs. This decreases the bioavailability of the NPs and the available contact surface for adsorbing BPA. This would lessen the carrier effect of the PSNPs. In addition, the EPS around the NPs can function as an effective barrier to hetero-aggregation between the NPs and algal cells, further attenuating the toxicity.

5. Conclusions

This study highlights the role of EPS in reducing the toxic effects of BPA-PSNP mixtures to *Scenedesmus obliquus*. BPA alone was found to exert a toxic potential towards the agal cells in a dose dependent manner. The major mode of action for BPA toxicity was the induction of excessive oxidative stress within the treated algal cells. A concentration dependent increase in oxidative stress parameters and decline in growth indicators was observed for the binary mixtures of BPA and PSNPs, in similar lines with the toxic action of BPA alone. Co-exposure to PSNPs and BPA induced considerable growth inhibition, reactive oxygen species generation, lipid peroxidation leading to membrane damage, and reduced photosynthetic efficacy in the algae. The algal EPS reduced bioavailability of the PSNPs and thus overall toxic effects of the mixtures. The surface modifications of PSNPs influenced their bioreactivity.

Freshwater presents a complex ecosystem containing numerous engineered nanoparticles, natural organic matter, in addition to various emerging and legacy pollutants. The presence of multiple other pollutants and natural colloids may influence the interactions between EPS, BPA and PSNPs in aquatic bodies. Furthermore, the composition and physicochemical behaviour of algal biopolymers from different species may differ. Further research is to be directed towards understanding the combined effects of various emerging pollutants and natural organic matter on different freshwater organisms. The work can also be extended to multiple trophic levels to assess the risk of these pollutants in aquatic ecosystems.

Credit author statement

Sayani Giri: Conceptualization, Investigation, Methodology, Visualization, Formal analysis, Writing-Original Draft; **Abisha Christy Christudoss::** Investigation, Methodology, Writing-Original Draft; **N. Chandrasekaran:** Formal analysis, Resources; **Willie J.G.M. Peijnenburg:** Formal analysis, Writing- Review and editing; **Amitava Mukherjee:** Conceptualization, Methodology, Formal analysis, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2023.107664.

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