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In-situ and ex-situ EPS-corona formation on ZnO QDs mitigates their environmental toxicity in the freshwater microalgae *Chlorella* sp.

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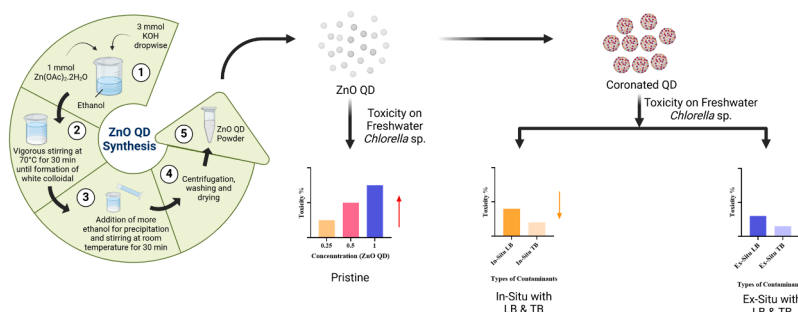
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HIGHLIGHTS

- Eco-corona formation on ZnO QDs didn't change any fluorescence properties of QDs.
- Both LB and TB-EPS individually resulted in the decline of toxicity of ZnO QDs.
- Ex-situ corona formation was more effective to reduce the toxicity on algae.
- Eco-corona formation reduced the uptake of the QDs.
- Decline in the uptake of ZnO QDs reduced oxidative stress.

GRAPHICAL ABSTRACT



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ABSTRACT

The current work seeks to understand how the interactions between ZnO QDs and extracellular polymeric substances (EPS) may vary based on the types of EPS (loosely and tightly bound) and modes of eco-corona formation (*In-situ* or *ex-situ*). *In-situ* eco-corona refers to formation of an EPS layer on the QDs during the interactions with the algae whereas the *ex-situ* condition refers to forming the layer before the interactions. ZnO QDs were added at 0.25, 0.5, and 1.0 mg/L concentrations for pristine, *in-situ*, and *ex-situ* corona treatments with the cells. Pristine ZnO QDs induced significant oxidative stress in algal cells, as evident from increased levels of reactive oxygen species (ROS), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase activity. This decreased the photosynthetic efficiency and caused significant growth inhibition in algae. In contrast, both the *in-situ* and *ex-situ* corona treatments with loosely bound and tightly bound EPS reduced the oxidative stress, improved the photosynthetic efficiency, and diminished growth inhibition effects. This study asserts the importance of EPS in reducing the toxicity of ZnO QDs, while maintaining the fluorescence activity. This ensures the sustainable usage of the ZnO QDs without any harm to aquatic ecosystems.

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1. Introduction

Semiconductor quantum dots (QDs), characterized by their size- and composition-dependent emission wavelengths, broadband excitation spectrum, and photostability, hold significant potential as tools for imaging and drug delivery systems [1–4]. CdSe and CdTe, the most used QDs, have been extensively studied in view of their high-quality and versatile optical properties. However, the use of cadmium-containing QDs is limited by their toxicity, owing to their Cd^{2+} content and ability to generate ROS [5,6]. Significant efforts have recently been made towards developing alternative semiconductors composed of less toxic metals, while maintaining desirable optical properties [7]. The substantial usage of ZnO QDs at various places, it is inevitably released into the environment. ZnO QDs are used in various fields including biosensing, bioimaging, optoelectronics, and photovoltaics. Mainly the ZnO based particles are used in quantum dot light-emitting diodes (QLEDs), photodetectors, UV lasers detectors, and high-efficiency solar cells. It is also used to mineralize industrial wastewater and in dyeing factories [8–11]. ZnO is a low-toxicity and cost-effective material with potential uses in biomedical products and therapeutics [12]. Recent research indicates that zinc oxide quantum dots (ZnO QDs) exhibit potential as pH-responsive pore-blockers and drug delivery systems. These investigations have evidenced the effective transport of doxorubicin (DOX) to cancer cells via ZnO quantum dots, leading to efficient drug release inside intracellular compartments and enhanced cytotoxicity [13]. The discharge into the environment of quantum dots (QDs) may transpire at several stages of the supply chain associated with QD goods, encompassing upstream activities such as the design and manufacture of QD materials, midstream processes involving QD intermediate products, and downstream phases related to QD products, as well as disposal at the product's end of life. The lost quantum dots are expelled jointly with other by-products, effluent, and refuse. The updated figures indicate that 0.057–1.14 metric tonnes of quantum dots are discharged into the environment each year due to synthesis and primary manufacturing processes [14]. Hence, they are prone to enter the aquatic environment, resulting in toxicity for aquatic organisms.

The toxicity of ZnO QDs could be related to the release of Zn^{2+} ions and the strong electrostatic interactions between ZnO QDs and the negatively charged cell membranes. The interactions with the cell membranes may generate a substantial amount of reactive oxygen species (ROS) under visible light exposure [15,16]. ZnO QDs at concentrations of 125, 500, 1000, and 2000 mg L^{-1} were shown to exhibit strong antibacterial effects against *S. agalactiae* WJYT1 [17]. In another study, lettuce was exposed to concentrations of ZnO QDs ranging from 50 to 500 mg L^{-1} to assess their impact on antioxidant capacity, biomass, and nutritional quality. Treatment with 500 mg L^{-1} ZnO QDs resulted in ROS levels, adversely affecting nutrient absorption, soluble protein content, and chlorophyll levels, consequently reducing plant biomass [8]. In a previous study, it was reported that cytotoxicity of ZnO QDs (25, 50, 100, 200, and 400 mg L^{-1}) were assessed on the HeLa and HEK-293T cells [18]. Despite a few previous studies investigating the toxic effects of ZnO QDs on bacteria and plants, their impact on microalgae remains poorly understood. It is crucial to understand how ZnO QDs interact with these organisms and how they elicit toxic responses. Hence in this study we focused on elucidating the toxic impacts of ZnO QDs on freshwater algae as the model organism.

Microalgae play a significant role as the primary producers in aquatic ecosystems and serve as the basic components of the food chain [19,20]. They are characterized by their wide distribution, simple cellular structure, rapid growth, cost-effectiveness, and high adaptability. Due to these attributes, microalgae are commonly utilized as model organisms for evaluating the toxicity of diverse pollutants [21,22]. Freshwater *Chlorella* sp. is widely recognized for its sensitivity to environmental changes and pollutants, making it suitable for studying the effects of ZnO QDs on aquatic ecosystems.

Numerous risk assessment studies have aimed to evaluate the

toxicity of QDs on different model organisms in aquatic ecosystems [23]. Upon discharge into the environment, engineered nanoparticles (ENPs) experience homo-agglomeration, which is the interaction among ENPs, or hetero-agglomeration, which involves interactions between ENPs and organic or inorganic colloids [24]. Due to the presence of various fractions of components in the extracellular polymeric substances (EPS), it can attract the ENMs (engineered nanomaterials) through the formation of various non-covalent bonds, such as Hydrogen bonds, Van der Waals forces, and sometimes ionic interactions as well. These facilitate them in the accumulation of the ENMs, thereby forming large aggregates of the ENMs. The cause is attributed to the presence of aromatic and aliphatic monomers in the protein fractions (PN), hydrophobic chains in the polysaccharide components (PS), aromatic nuclei containing phenolic and carboxylic groups of humic-like substances (HS), and an abundance of polar functional groups in EPS [25]. In addition, it is already reported that, NOMs can be found in the range of 0.5–30 mg/L in the aquatic environment [26,27]. The EPS and aromatic chemicals can combine to produce a molecular cluster through various noncovalent interactions. Noncovalent interactions (e.g., electrostatic interaction, π - π stacking, hydrogen bonding interaction etc.) are significantly weaker in energy than covalent bond formation by one to two orders of magnitude [28]. EPS present in marine algal culture matrices has been observed to mitigate the toxicity of CdSe QDs [29]. EPS is ubiquitous in aquatic environments and plays a significant role in mediating the interactions between ENPs and organisms. Understanding these interactions is instrumental to assess the environmental fate and impact of ZnO QDs. In a previous study, the influence of eco-corona formation on the toxicity of CdSe/ZnS QDs in the freshwater alga *Scenedesmus obliquus* was studied. The oxidative stress markers in the algal cells (LPO, SOD, and CAT) were in correlation with the cytotoxicity results, which all increased upon interaction with the QDs. Aging in the EPS medium led to the formation of the eco-corona layer, which led to enhancement in the algal cell viability and a decrease in the oxidative stress parameters [23]. However, to the best of our knowledge, there have been no previous studies on the influence of algal EPS on the toxic effects of ZnO QDs in freshwater algae, *Chlorella* sp.

This research investigates the toxic effects of ZnO QDs on the freshwater algae *Chlorella* sp., focusing on the mechanisms that induce oxidative stress and diminish photosynthetic efficiency. The study proposes that the application of an extracellular polymeric substance (EPS) corona on QDs—either through direct formation in the exposure medium (in-situ) or via pre-coating prior to exposure (ex-situ)—may alleviate these adverse effects. To our knowledge, this represents the first comprehensive examination of ZnO QD toxicity at notably low concentrations (up to 1.0 mg/L) within this model organism. The main innovations of the study include demonstrating the role of both loosely and tightly bound EPS in reducing QD toxicity; comparing the protective effects of in-situ versus ex-situ EPS corona formation; and using filtered and sterilized lake water as the exposure medium to better replicate real-world conditions. The toxicity assessment of both pristine and EPS-coated QDs encompassed evaluations of growth inhibition, oxidative stress, activities of antioxidant enzymes, photosynthetic performance, zinc uptake, and the toxicity attributed to leached ions. The findings indicate that the formation of an EPS corona significantly mitigates QD toxicity, thereby presenting a sustainable strategy to lessen environmental impacts while maintaining the functional capabilities of QDs for applications, such as imaging.

2. Materials and methods

2.1. Used chemicals, media and synthesis of ZnO QD

Details of the used chemicals in the present work are given in the [Supplementary Information](#) (M1S1). Lake water (VIT lake, Vellore (12°58'10" N, 79°9' 37"E), India) was used as interaction media. The lake water (LW) media was used to mimic the natural environmental

conditions without changing the physicochemical properties as mentioned in our previous studies [30,31]. The collected water underwent a series of filtrations, firstly with a blotting paper for two times followed by a Whatman no. 1 filter paper (three times) with a pore size to the μm -range to eliminate any bigger particles that were present. The filtered LW was subsequently sterilised utilising an autoclave at a temperature of 121 °C for a duration of 15 min to eliminate any undesired proliferation of microorganisms and cooled to room temperature and utilised as the medium for the interaction. The noted pH of the used lake water in the current work was 6.9 and the other physicochemical characteristics of the lake water is reported in our previous study [31] and are detailed in the supplementary section M2S2. The synthesis of ZnO QDs was performed adopting the previous study by Saber et al. (2023). The synthesis protocol is detailed in the [Supplementary Information](#) (M2S2).

2.2. Characterization and stock preparation of ZnO QD

The synthesized pristine QDs were characterized by using fluorescence spectrophotometer, UV Visible spectrophotometer, XRD, FTIR, HR-TEM (with SAED pattern), and FESEM (with EDS, and mapping). The surface charge and the wettability were confirmed by zeta potential analysis and a goniometer, respectively. The details of various characterization techniques are included in the [Supplementary Information](#) (M3S3). One hundred mg/L of stock suspension was prepared for the pristine ZnO QDs in the milli-Q water by using ultrasonication (130 W, 20 kHz; Sonics, USA) for 15 min. Working concentrations were fixed at 0.25, 0.5, and 1.0 mg/L of ZnO QDs.

2.3. Test organism

The current study utilised the freshwater microalgae *Chlorella* sp. as a model organism. The *Chlorella* sp. was obtained from the VIT lake, Vellore, India. The isolation procedure was based on the methodology established by Sadiq et al. [32]. To promote optimal growth, the algal sub-cultures were grown in 200 mL Erlenmeyer flasks containing BG-11 media. For the proper algal growth, a temperature of 23 ± 2 °C was maintained with a light-dark cycle of 16 h. A white fluorescent lamp (3000 lx illumination; Philips TL-D Super 80, linear fluorescent lamp, India) was employed as a light source [33].

2.4. Extraction and characterization of algal EPS

Freshwater algal cells in the exponential growth phase were centrifuged at 7000 rpm at 4 °C for 10 min. Cell pellets were then dispersed in LW to reach the optical density of 0.5 at 610 nm in colorimeter. To check the maximum production of EPS, the algal samples were placed under fluorescent visible light conditions (3000 lux) for various time intervals of 24, 48, and 72 h followed by centrifugation at 13,000 rpm at 4 °C for 10 min [30]. The supernatant was collected and passed through a 0.45 μm filter followed by analysis of the total organic carbon content (TOC) by utilizing a TOC analyser (Model TOC-L, Shimadzu, Japan). TOC analysis revealed that the samples incubated for 24 h were able to produce the maximum yield. This extracted EPS was named as loosely bound EPS (LB-EPS). After the collection of LB-EPS, the algal pellet was further treated with 0.1 M phosphate buffer and kept for 20 min of incubation at 70 °C in a water bath. After cooling, the suspension was centrifuged at 13,000 rpm for 15 min followed by filtration of the supernatant through a 0.45 μm filter. This extracted EPS was named as tightly bound EPS (TB-EPS) [34]. Extracted EPS (LB, TB-EPS) were characterized by using FTIR. Surface charge and wettability were checked by using a zeta potential analyser and a goniometer. Protein and carbohydrate content of LB and TB-EPS were also checked. Details of the EPS characterization are provided in the [supplementary information](#) (M3S3).

2.5. In-situ and ex-situ corona formation and confirmation

Pristine QDs at 0.25, 0.5, and 1.0 mg/L were used for both *in-situ* and *ex-situ* corona formation. EPS concentration for LB and TB-EPS treatments were fixed at 5 mg/L. In brief, for *in-situ* corona formation, 5 mg/L of LB-EPS was added in the interaction media, in the presence of algal cells and 0.25, 0.5, and 1.0 mg/L of QDs [30]. For *ex-situ* corona formation, 5 mg/L of LB-EPS was added in the interaction media with 0.25, 0.5, and 1.0 mg/L of QDs in abiotic conditions [34]. Additionally, for the *in-situ* and *ex-situ* corona formation with TB-EPS, similar steps were followed like in the case of LB-EPS. The corona formation was confirmed in the presence of both the type of EPS (5 mg/L) in the *in-situ* and *ex-situ* form for the highest concentration (1.0 mg/L) of QDs used. FTIR, 3D-EEM, protein, and carbohydrate estimation were performed to confirm the corona formation over the QDs [35]. For FTIR, the spectral range was recorded from 350 to 4500 cm^{-1} . Transmittance data was converted into the absorbance spectra (deconvoluted) and the processing of the data was performed by the OriginPro 2024b software. The same software was used to identify the secondary derivative for 1200 – 1800 cm^{-1} spectral range and the data was plotted by following Gaussian band profiles. Details are provided in the [supplementary information](#) (M3S3).

2.6. Experimental setup

Healthy algal cells belonging to the exponential growth phase, were selected. The cells were centrifuged for 10 min at 4 °C at 7000 rpm. After centrifugation, the cell pellets were collected and re-suspended in the LW medium. The optical density (OD) of the suspension was adjusted to 0.5 at 610 nm in the LW medium. The algae and ZnO QDs at 0.25, 0.5, and 1.0 mg/L were allowed to interact in the following experimental groups: (i) pristine ZnO QDs; (ii) coronated ZnO QDs with LB-EPS in *in-situ* form; (iii) coronated ZnO QDs with LB-EPS in *ex-situ* form; (iv) coronated ZnO QDs with TB-EPS in *in-situ* form; (v) coronated ZnO QDs with TB-EPS in *ex-situ* form; and (vi) without any treatments (control group). The interaction was performed at a temperature of 25 ± 2 °C, keeping an equal overall volume of 5 mL for a duration of 72 h. The experiments were conducted under visible light conditions with an intensity of 3000 lux (Philips). The cell viability evaluation in this study involved analysing a control setup and 5 mg/L of LB and TB-EPS was employed ([Fig. S1](#)). In comparison to the control group, the decrease in cell viability was minimal and statistically insignificant. Therefore, it was not conducted for additional assays. The toxicity assessment in the present study was conducted in accordance with the OECD guideline (Test No. 201: Alga, Growth Inhibition Test) [36].

2.7. Effects on fluorescence intensity

To check the effects of the interaction media (LW) on the synthesized ZnO QD, the fluorescence intensity was checked in LW. In addition, to check the fluorescence intensity after the *in-situ* and *ex-situ* corona formation in the presence of LB, and TB-EPS, fluorescence spectroscopy was performed. To check the *in-situ* coronated particle's intensity, the sample was passed through 0.1 μm membrane filter and the filtrate was used.

2.8. Evaluation of EC_{50} and cytotoxicity

After a 72-hour duration of the interaction, the extent of growth inhibition was examined by counting the live cells with the help of a haemocytometer under an optical microscope (Zeiss Axiostar Microscope, USA) [37]. The detailed steps of the procedure used are provided in the supplementary section (M4S4). The EC_{50} of the pristine ZnO QD was determined by testing several concentrations in a concentration dependent manner. Furthermore, the EC_{50} value of ZnO QD was

determined in the presence of both types of EPS in their *in-situ* and *ex-situ* form. More details can be found in the [Supplementary Information](#) (M4S4). In addition, toxicity of the Zn^{2+} -ions was also assessed for the 1.0 mg/L concentration of ZnO QD in pristine and coronated forms.

2.9. Cellular uptake of Zn by algal cells

Cellular accumulation of Zn by the algal cells was analysed by adapting the protocol followed by Djearmane et al. [38]. Additional details can be found in the [Supplementary Information](#) (M5S5).

2.10. Evaluation of oxidative stress

The protocol followed by Debroy et al. was followed to assess the total ROS and malondialdehyde (MDA) production [39]. The details are given in the [supplementary information](#) (M6S6).

2.11. Estimation of photosynthetic activity

The protocol followed by Giri and Mukherjee and Lee et al. was adapted to measure the effective quantum yield of photosystem II (Y(II)) and the electron transport rate (ETR) values [40,41]. More details are included in the [Supplementary Information](#) (M7S7).

2.12. Estimation of antioxidant enzyme

Antioxidant enzyme viz. superoxide dismutase (SOD), and catalase activities were calculated following the steps adapted from Giri and Mukherjee [40]. Additional details can be accessed from the [Supplementary Information](#) (M8S8).

2.13. Data analysis

All experimental procedures were performed in triplicate ($n = 3$). The data are reported as mean $\pm$ SD (standard deviation). A normality

assessment was applied to all the generated data, followed by two-way ANOVA test with Bonferroni post-test using GraphPad Prism 8 to verify the statistical significance among various test groups in contrast to the control. Graphs were generated using GraphPad Prism 8 and OriginPRO software. To elucidate the impact of pristine and coronated QDs on the biological parameters, cluster heatmap and Pearson correlation matrix analysis were carried out.

3. Results

3.1. Characterization of QDs and EPS

Fig. 1A-i represented HR-TEM images of the pristine QD, and **A-ii** the SAED pattern of the pristine QDs. The QDs were dot-shaped and spherical in nature, with the size around 5 nm. The SAED pattern showed the crystalline nature of the synthesized ZnO QDs. **Fig. S2** showed the FESEM image of pristine ZnO QDs. The EDX spectrum revealed the presence of Zn, O, and K in the QDs. The presence of K might be due to the synthesis process by using KOH.

The UV-Visible spectra (**Fig. S3**) showed a shoulder at around 340 nm. This confirms formation of ZnO QDs. In addition, the photoluminescence spectra (**Fig. S4**) at λ_{ex} 340 nm, revealed a broad emission peak at λ_{em} 400–550 nm. The pristine ZnO QDs emitted light when exposed to UV light in dark conditions (**Fig. S5**), demonstrating their inherent fluorescence. This observation confirmed that the synthesized QDs possessed the ability to absorb UV light and emit it as visible light. The zeta potential of the pristine ZnO QD was -8.85 ± 3.29 mV. The crystallinity of the synthesized ZnO QDs was evaluated by XRD analysis (**Fig. S6**). The shape and intensity of the major diffraction peaks present between 10° and 90° ascertain the high crystallinity of the products. The diffraction peaks at 89.67° , 67.98° , 62.93° , 56.67° , 47.65° , 36.05° , 34.68° , and 31.89° corresponded to the (203), (112), (103), (110), (102), (101), (002), and (100) planes, respectively. FTIR spectral scan ($350\text{--}4500\text{ cm}^{-1}$) examined the surface functional groups of QDs (**Fig. S7**). The presence of broad bands of ZnO at 421 and 852 cm^{-1} were

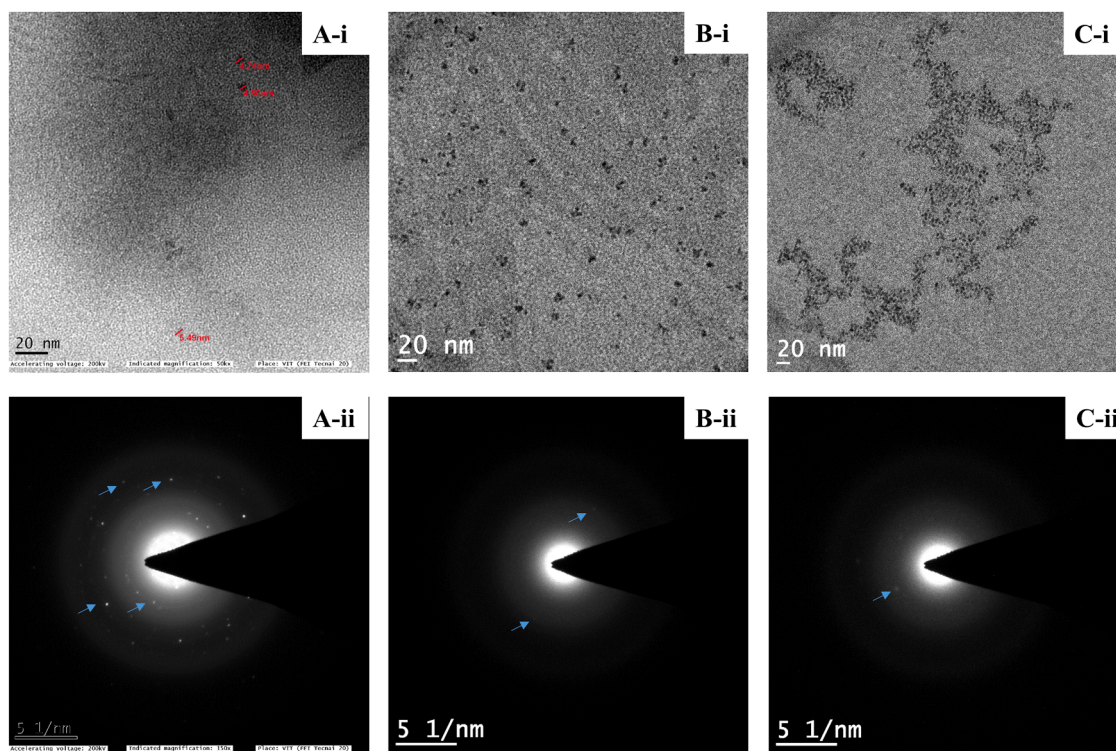


Fig. 1. HR-TEM of (A-i): Pristine ZnO QDs, (A-ii): SAED pattern of pristine ZnO QDs, (B-i): ZnO QD in the presence of LB-EPS, (B-ii): SAED pattern of ZnO QD in the presence of LB-EPS, (C-i): ZnO QD in the presence of TB-EPS, (C-ii): SAED pattern of ZnO QD in the presence of TB-EPS.

noted. The spectra included weak bands at 1656 and 1440 cm^{-1} possibly due to the asymmetric and symmetric vibration of an acetate group (COO^-) bonded to zinc ions [42].

The extracted EPS was also characterized by various analytical methods. The total sugar content (490 nm) in the extracted LB-EPS and TB-EPS was 6.55 ± 0.03 and $15.33 \pm 0.01 \text{ mg/L}$, while the protein content (595 nm) was 13.11 ± 0.05 , $18.77 \pm 0.01 \text{ mg/L}$ for LB-EPS and TB-EPS, respectively. The total organic carbon content equalled 52.8 ± 4.2 and $61.4 \pm 5.8 \text{ mg/L}$ for LB-EPS and TB-EPS, respectively. The 3D-EEM (Fig. 2A, B) spectra confirmed the presence of protein moieties, amino acids, and humic-like substances. 3D-EEM also revealed that the fluorescence intensity of TB-EPS was higher with compared to LB-EPS. Regions 'a' and 'b' show the protein moieties and humic like substances [35,43]. Fig. S8 revealed the FTIR spectra of LB and TB-EPS samples. The presence of protein peaks, amide I at 1616 cm^{-1} ($\text{C}=\text{C}$) and 1633 cm^{-1} ($\text{C}=\text{O}$) [44] was noted. The peak at around 1052 cm^{-1} confirms the presence of glycosidic linkage of polysaccharide ($\text{C}-\text{O}$) [45]. The peak at $2009\text{--}2121 \text{ cm}^{-1}$ reveals the presence of COO^- symmetric stretching. In addition, the broad peak in the range of $3031\text{--}3613 \text{ cm}^{-1}$ revealed the presence of O-H stretching (polysaccharides) [46].

3.2. Changes in the physicochemical and optical characteristics upon the in-situ and ex-situ corona formation on ZnO QDs

HR-TEM (Fig. 1. B-i, C-i) images clearly showed the *ex-situ* corona formation in the presence of both EPS. It also confirms the aggregation of the particles. This aggregation was possibly a result of eco-corona formation. The HR-TEM images of QDs in the presence of LB-EPS (Fig. 1 B-i) clearly showed the aggregation of the particles with a layer of LB-EPS. Furthermore, QDs in the presence of TB-EPS (Fig. 1 C-i) also induced formation of QD aggregates with a layer of TB-EPS. Upon corona formation, the changes in the SAED pattern were also noted in the presence of LB (Fig. 1 B-ii) and TB-EPS (Fig. 1 C-ii). Upon the *in-situ* and *ex-situ* corona formation, the changes in the wettability and surface charge were noticed (Table 1). Addition of EPS rendered the ZnO QDs slightly hydrophilic compared to the pristine QDs. A similar trend was

Table 1

Contact angle and zeta potential values of ZnO QD in pristine, and coronated form.

Materials	Contact angle		Zeta potential	
	Average ($^\circ$) value	SD	Average (mV)	SD
0.25 mg/L ZnO QD	31.30	0.95	−13.05	4.5
0.25 mg/L ZnO QD (<i>In-situ</i> corona with LB)	27.37	0.81	−11.33	4.39
0.25 mg/L ZnO QD (<i>Ex-situ</i> corona with LB)	26.23	0.31	−8.28	1.04
0.25 mg/L ZnO QD (<i>In-situ</i> corona with TB)	25.67	0.55	−3.42	1.33
0.25 mg/L ZnO QD (<i>Ex-situ</i> corona with TB)	23.93	1.32	−3.37	3.07
0.5 mg/L ZnO QD	38.07	1.29	−16.7	1.05
0.5 mg/L ZnO QD (<i>In-situ</i> corona with LB)	37.57	0.87	−16.28	3.77
0.5 mg/L ZnO QD (<i>Ex-situ</i> corona with LB)	36.27	1.50	−15.29	1.94
0.5 mg/L ZnO QD (<i>In-situ</i> corona with TB)	35.10	0.92	−8.15	2.33
0.5 mg/L ZnO QD (<i>Ex-situ</i> corona with TB)	33.60	1.30	−7.22	2.99
1 mg/L ZnO QD	59.53	0.59	−17.24	3.02
1 mg/L ZnO QD (<i>In-situ</i> corona with LB)	55.13	0.80	−17.09	4.3
1 mg/L ZnO QD (<i>Ex-situ</i> corona with LB)	54.87	0.49	−16.76	3.64
1 mg/L ZnO QD (<i>In-situ</i> corona with TB)	52.83	1.33	−12.09	1.27
1 mg/L ZnO QD (<i>Ex-situ</i> corona with TB)	46.17	0.55	−10.34	2.76

noted for *in-situ* and *ex-situ* corona formation for LB and TB-EPS. Most interestingly, in the presence of TB-EPS in case of *ex-situ* corona formation, ZnO QD showed highly hydrophilic nature compared to the pristine forms. It is noted from the surface charge results (Table 1) that ZnO QDs indicated less colloidal stability. Notably, the presence of TB-EPS made them more unstable compared to LB-EPS.

The changes in the total protein (Fig. S9) and carbohydrate content

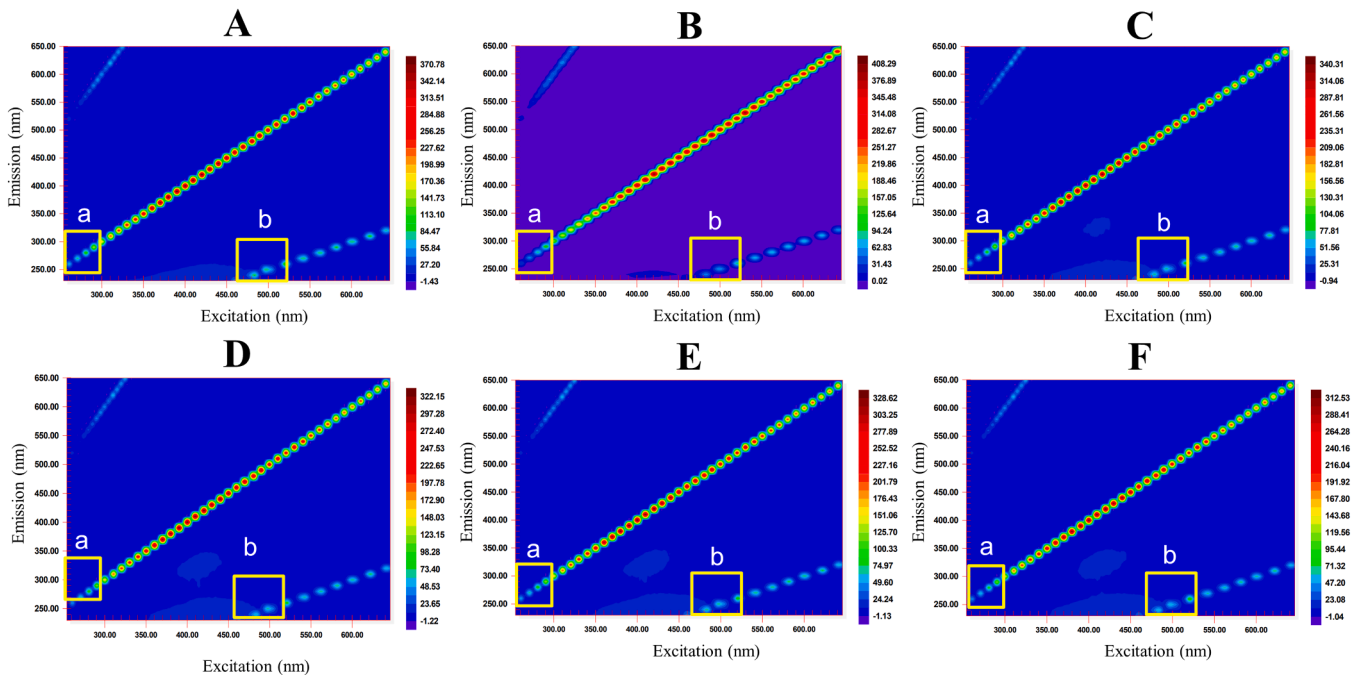


Fig. 2. 3D-EEM of (A): LB-EPS, (B): TB-EPS, (C): LB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*in-situ*), (D): LB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*ex-situ*), (E): TB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*in-situ*), (F): TB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*ex-situ*).

(Fig. S10) for *ex-situ* corona formation were evaluated. The highest protein adsorption was noted in the *ex-situ* treatment for LB, and TB-EPS in the presence of 1.0 mg/L of QDs. A similar trend was noted for the carbohydrate content. The adsorption of proteins and carbohydrates was saturated over time for all the treatment groups, with the maximum adsorption after 72 h. The 3D-EEM images (Fig. 2 C–F) revealed a decline in the fluorescence intensity in the presence of LB, and TB-EPS for *in-situ* and *ex-situ* corona formation. The initial intensity of the LB, and TB-EPS was ~ 370 and ~ 408 RFU. Upon the adsorption of EPS on the QDs, the intensity of the EPS was found to decline for all the test groups for *in-situ*, and *ex-situ* treatments with 1.0 mg/L of ZnO QDs. The maximum decrease in the fluorescence intensity was noted for the *ex-situ* treatment group in the presence of TB-EPS (~ 312 RFU). The noted trend for the fluorescence intensity followed: *in-situ* treatment of 1.0 mg/L ZnO QD in LB-EPS > *ex-situ* treatment of 1.0 mg/L ZnO QD in LB-EPS > *in-situ* treatment of 1.0 mg/L ZnO QD in TB-EPS > *ex-situ* treatment of 1.0 mg/L ZnO QD in TB-EPS.

Fluorescence quenching analysis (Fig. S4) indicated that the fluorescence intensity of ZnO QDs remained consistent when dispersed in lake water medium (Fig. S4B), showing no significant difference compared to their dispersion in Milli-Q water. Furthermore, the interaction of QDs with LB and TB-EPS (Fig. S4C) in case of both *in-situ* and *ex-situ* corona treatments, did not significantly alter the fluorescence intensity of the QDs. This suggests that *in situ* and *ex situ* corona formation with LB and TB-EPS did not impact the intrinsic fluorescence properties of the QDs.

Fig. 3 depicted the FTIR spectrum of the EPS and the QDs with sorbed EPS in case of *in-situ* and *ex-situ* forms of corona formation. The absorption spectrum ($1200 - 1800 \text{ cm}^{-1}$) of the secondary derivatives revealed the changes in the proteins present, more specifically in the

amide I, II, and III bands. For the LB-EPS (Fig. 3A), the presence of peaks at 1351 and 1435 cm^{-1} clearly indicates the presence of amide III. Similarly, peaks at 1481 and 1541 cm^{-1} reveal the presence of amide II, and the peaks at 1618 cm^{-1} confirm the presence of amide I in the LB-EPS. Upon corona formation in the presence of LB-EPS, the changes in the peaks corresponding to the amide I, II, and III bands confirm the adsorption of EPS over the ZnO QDs. Upon the *in-situ* (Fig. 3B), *ex-situ* corona formation (Fig. 3C), in the presence of LB-EPS, changes in the amide peaks were noted. Peaks at 1618 cm^{-1} confirm the presence of the amide I in the *in-situ* corona formation by LB-EPS. Furthermore, peaks at 1481 and 1544 cm^{-1} confirm the presence of amide II. A peak at 1354 cm^{-1} also clearly indicates the presence of amide III. Similarly, peaks at 1618 cm^{-1} confirm the amide I and peaks at 1481 and 1544 cm^{-1} confirm the presence of amide II in the *ex-situ* LB-EPS. Additionally, a peak at 1351 cm^{-1} also clearly indicates the presence of amide III. The changes confirmed the corona formation. Fig. 3D showed the presence of amide I (1650 cm^{-1}), amide II (1524 cm^{-1}), amide III (1277 , 1343 cm^{-1}) in the TB-EPS. The changes in the amide peaks were noted upon the *in-situ* (Fig. 3E) and *ex-situ* (Fig. 3F) corona formation in the presence of TB-EPS too. The peaks at 1656 cm^{-1} confirm the presence of the amide I in the *in-situ* TB-EPS. Furthermore, the peaks at 1294 and 1360 cm^{-1} also clearly indicate the presence of amide III. Similarly, for the *ex-situ* TB-EPS peaks at 1659 cm^{-1} confirm the amide I in the *ex-situ* TB-EPS. Additionally, peaks at 1291 and 1357 cm^{-1} also clearly indicate the presence of amide III.

3.3. Growth inhibition

The EC_{50} values of ZnO QDs in the pristine, *in-situ*, and *ex-situ* coronated forms towards the *Chlorella* sp. were evaluated. In the

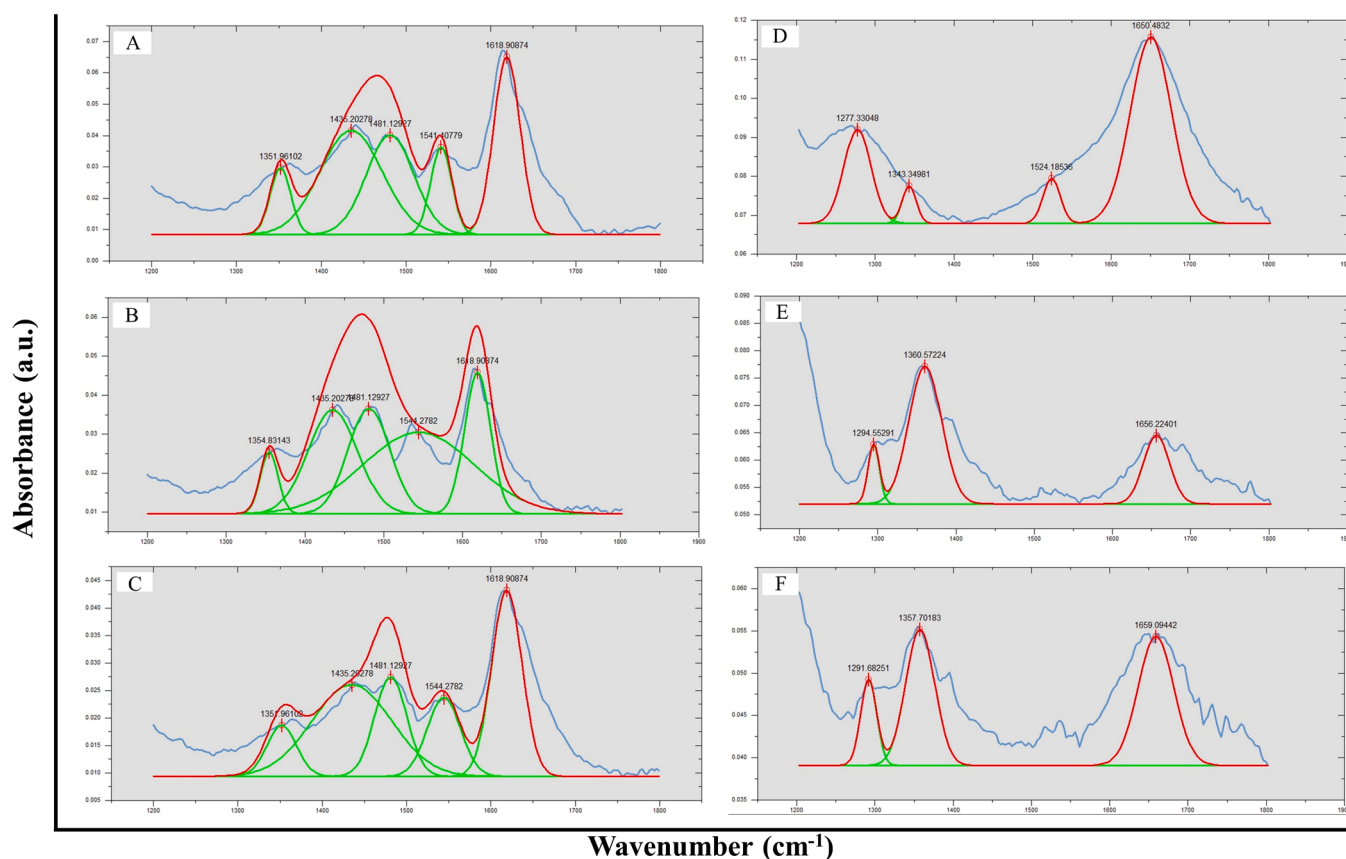


Fig. 3. FTIR absorbance spectra of (A): LB-EPS, (B): LB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*in-situ* corona), (C): LB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*ex-situ* corona), (D): TB-EPS, (E): TB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*in-situ* corona), (F): TB-EPS interacted mixture with 1.0 mg/L ZnO QDs (*ex-situ* corona).

presence of TB-EPS for *in-situ* and *ex-situ* coronated QDs, the EC₅₀ values were 2.14, and 2.64 mg/L, as compared to 1.25 mg/L for pristine QDs. For *in-situ*, and *ex-situ* LB-EPS coronated QDs, the EC₅₀ concentrations were noted at 1.41, and 1.66 mg/L.

The growth inhibition results (Fig. 4A) revealed a concentration dependent increment in growth inhibition for the pristine ZnO QD treatments. All the pristine QD treatments showed increased growth inhibition, significantly higher than the control treatments. The *in-situ* coronated QDs with LB-EPS and TB-EPS showed a decline in growth inhibition for all the concentrations of QDs compared to their pristine counterparts. Similar results were observed for the *ex-situ* corona treatments. In case of the *in-situ* corona formation over 0.25 and 1.0 mg/L ZnO QDs, the decline in the growth inhibition was statistically significant ($p < 0.001$) for TB-EPS corona compared to LB-EPS corona formation. A similar trend ($p < 0.001$) was noted for the *ex-situ* treatment group also, where it was revealed that *ex-situ* TB-EPS was more effective to reduce the growth inhibition compared to *ex-situ* LB-EPS. In addition, it was also noted that the *ex-situ* coronated treatment groups were more effective in reducing the growth inhibition when compared to the *in-situ* treatment groups. The growth inhibition trend followed, pristine ZnO QDs > *in-situ* LB-EPS coronated ZnO QDs > *ex-situ* LB-EPS coronated ZnO QDs > *in-situ* TB-EPS coronated ZnO QDs > *ex-situ* TB-EPS coronated ZnO QDs. Additionally, the cell viability evaluation involved treatment groups, where only 5 mg/L of LB and TB-EPS were employed (Fig. S1). Compared to the control the decrease in cell viability was minimal and statistically insignificant.

Table 2 revealed the leached Zn⁺² concentration from the ZnO QDs at 1.0 mg/L in pristine and coronated form (*in-situ*, and *ex-situ* corona). The leaching was the highest (0.256 ± 0.051 mg/L) when the QDs were present in their pristine form. Upon corona formation, the leaching of Zn⁺² ions was significantly reduced (0.169 ± 0.009 mg/L). The amount of Zn⁺² ions released at the highest concentration (1.0 mg/L) of ZnO QDs, was observed to generate toxicity in the cells (Fig. 4B). However, the growth inhibition caused by the ions leached was significantly reduced ($p < 0.001$) from that by the pristine ZnO QDs. Similar results were observed for the *in-situ* and *ex-situ* LB and TB coronated ZnO QDs compared to their released ion treatments, respectively.

Table 2 revealed the uptake of Zn in the cells for all the treatment groups exposed to the highest concentration (1.0 mg/L) of ZnO. It was noted that the uptake of Zn was the highest for the pristine QD groups. More interestingly, the uptake was reduced by 60 % for the treatment group of 1.0 mg/L *ex-situ* TB-EPS coronated ZnO QDs. The noted trend

Table 2

Concentration of leached Zn⁺² ions in the interaction media and uptake of Zn by the algal cells.

	Leached ions (mg/L)		Uptake of QDs by algal cells (mg/L)		Available QDs in the media (mg/L)	
	Average	SD	Average	SD	Average	SD
1 mg/L QD (Pristine)	0.256	0.051	0.688	0.028	0.467	0.010
1 mg/L QD (<i>In-situ</i> corona LB)	0.216	0.003	0.502	0.024	0.487	0.033
1 mg/L QD (<i>In-situ</i> corona TB)	0.193	0.075	0.449	0.046	0.604	0.052
1 mg/L QD (<i>Ex-situ</i> corona LB)	0.169	0.005	0.402	0.002	0.663	0.004
1 mg/L QD (<i>Ex-situ</i> corona TB)	0.169	0.009	0.259	0.065	0.695	0.023

All data were collected at 72 h

for Zn uptake was pristine 1.0 mg/L ZnO QDs > 1.0 mg/L *in-situ* LB-EPS coronated ZnO QDs > 1.0 mg/L *in-situ* TB-EPS coronated ZnO QDs > 1.0 mg/L *ex-situ* LB-EPS coronated ZnO QDs > 1.0 mg/L *ex-situ* TB-EPS coronated ZnO QDs.

3.4. Oxidative stress parameters

Fig. 5A showed the influence of ZnO QDs on intracellular ROS generation in their pristine and coronated forms. A concentration-dependant increment for the pristine ZnO QD treatments was found, where the highest concentration (1.0 mg/L) of pristine QDs treated cells showed the highest ROS production (~364 %). All the pristine QD treatments induced an increment in ROS production significantly higher than the control treatments. The *in-situ* coronated test groups with LB and TB-EPS showed a decline in total ROS generation for all concentrations of ZnO QDs compared to the pristine groups. Similar results were observed for all *ex-situ* coronated treatments too. For the *in-situ* treatment groups containing 0.25 and 1.0 mg/L ZnO QDs, the observed ROS generation declined ($p < 0.001$) more for the TB-EPS (~136 and ~280 % for 0.25 and 1.0 mg/L, respectively) treatment groups compared to the LB-EPS (~167 and ~345 % for 0.25 and 1.0 mg/L, respectively) groups. A similar trend ($p < 0.001$) was noted for the *ex-situ* treatment groups. It was also noted that the *ex-situ* coronated treatment groups were more effective in reducing the ROS generation compared to the *in-situ* treatment groups.

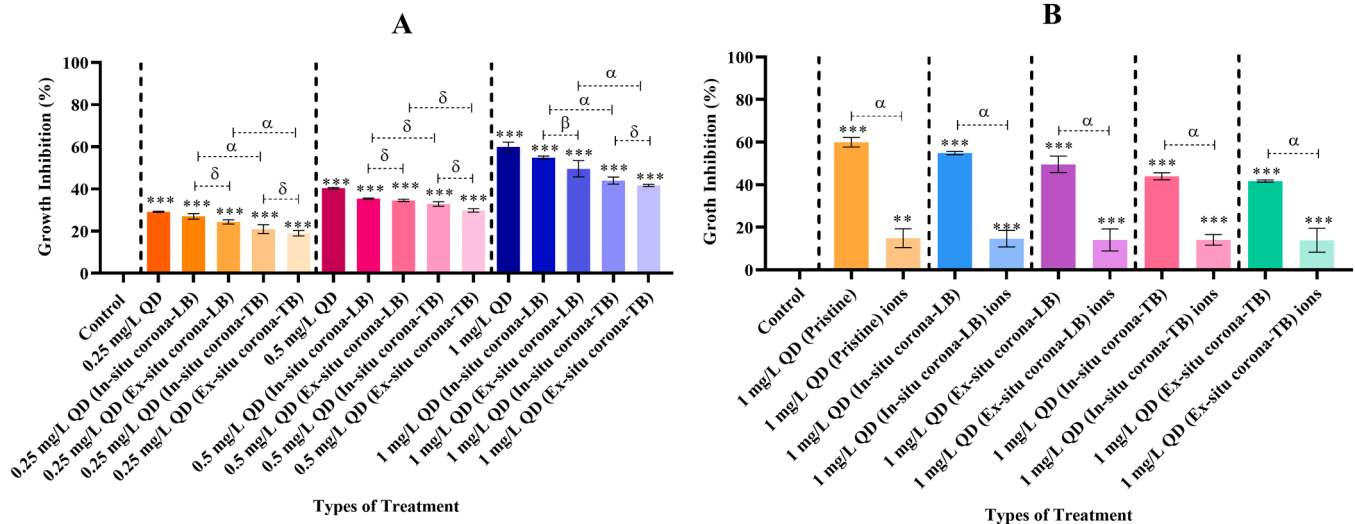


Fig. 4. Growth inhibition of microalgae in the presence of (A): pristine QDs, and coronated QDs; (B): leached Zn⁺² ions from 1.0 mg/L ZnO QD (** represents the mathematical significance with respect to control, **** = $p < 0.001$; Greek character represents the mathematical significant between the test groups, α , β , δ = $p < 0.001$, $p < 0.01$, and $p > 0.05$, respectively).

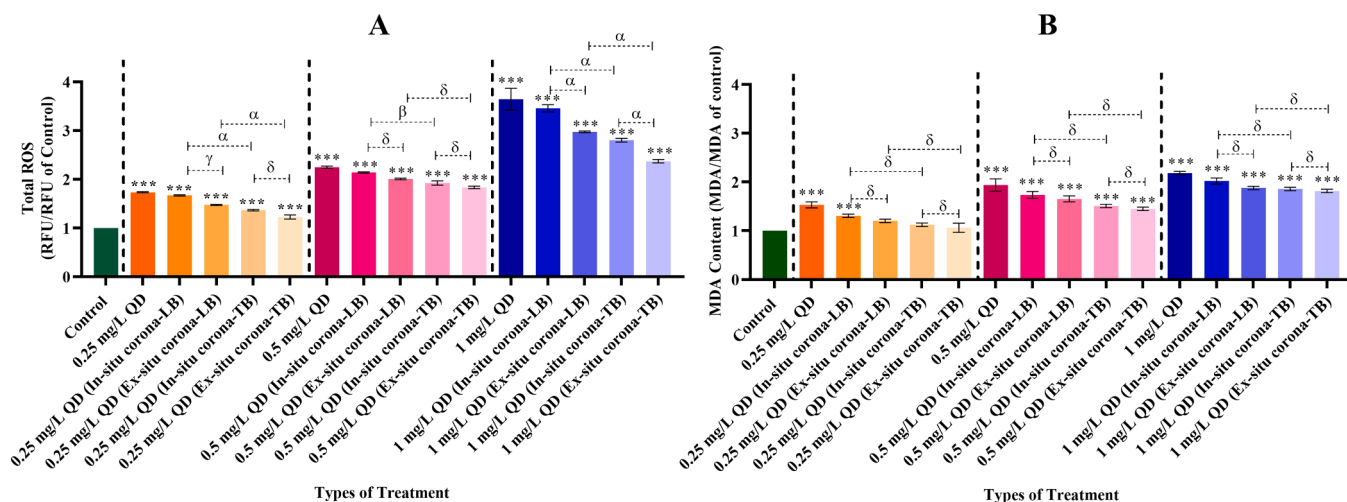


Fig. 5. Oxidative stress generation of microalgae in the presence of pristine QDs, and coronated QDs, (A): total ROS generation; (B): MDA content production. (** represents the mathematical significance with respect to control, **** = $p < 0.001$; Greek character represents the mathematical significant between the test groups, α , β , γ , δ = $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p > 0.05$, respectively).

Fig. 5B depicted the effects of ZnO QDs on MDA production. A concentration dependant increment was noted for the pristine ZnO QD treatments, where the highest concentration of pristine QDs treated cells

revealed significantly highest MDA production (~218 %). All the pristine QD groups showed the increment in MDA production, significantly higher than the control treatments. The in-situ coronated test groups

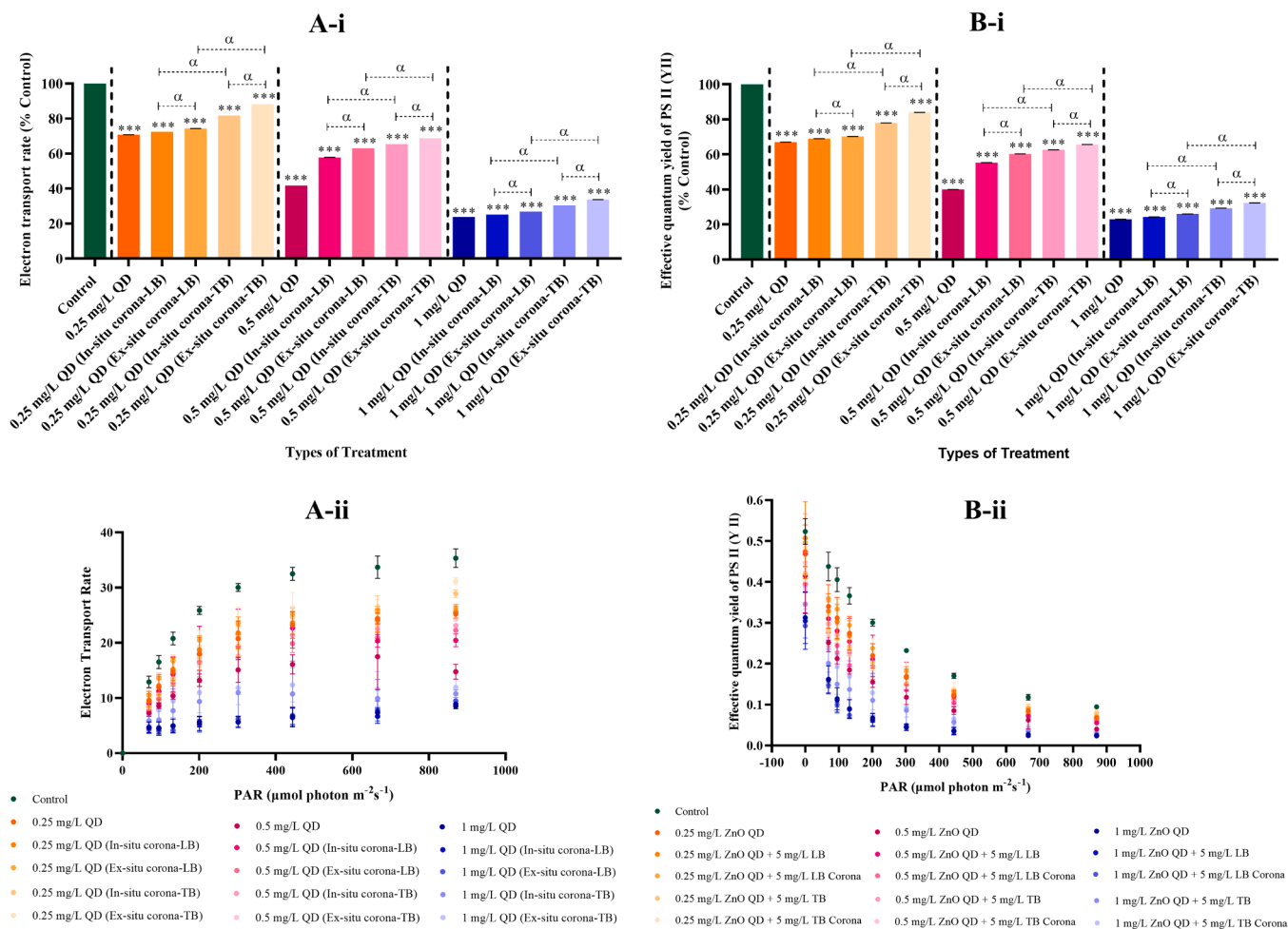


Fig. 6. Photosynthesis activity of microalgae in the presence of pristine QDs and coronated QDs, (A-i): electron transport rate (%); (A-ii): fast light response curve of ETR; (B-i): effective quantum yield of PS (II) Y II (%); (B-ii): fast light response curve of effective quantum yield of PS (II) Y II. (** represents the mathematical significance with respect to control, **** = $p < 0.001$; Greek character represents the mathematical significant between the test groups, α = $p < 0.001$).

with LB and TB-EPS revealed a decline in MDA production for all the concentrations of ZnO QDs tested as compared to their pristine forms. Similar results were observed for the *ex-situ* coronated treatments. For all the treatment sets including 0.25, 0.5, and 1.0 mg/L ZnO QDs, observed MDA production declined more ($p > 0.05$) for the *in-situ* TB-EPS treatment groups than the LB-EPS treatment groups. A similar trend ($p > 0.05$) was noted for the *ex-situ* treatment groups, where it was noted that *ex-situ* TB-EPS was more effective to reduce the MDA production compared to *ex-situ* LB-EPS treatment groups. Comparing between the *in-situ* and *ex-situ* coronated test groups, the *ex-situ* coronated groups caused more decline in MDA production.

3.5. Antioxidant enzyme activities

Figure S11 depicted the effects of ZnO QDs on antioxidant activity viz. SOD (Fig. S11A) and catalase (Fig. S11B) in the algal cells. The enzyme activities were increased significantly ($p < 0.001$) in a concentration dependent manner for the pristine ZnO QD treatment groups, where the 1.0 mg/L of pristine QDs treated algal cells showed the highest activity. The coronated test groups for the *in-situ* treatment with LB and TB-EPS showed a decline in the antioxidant enzyme activity for ZnO QD concentrations when compared to the pristine forms. A similar pattern was noted for all the *ex-situ* coronated treatment groups. For all the treatment groups including 0.25, 0.5, and 1.0 mg/L ZnO QDs, the SOD activity declined ($p < 0.001$) for the *in-situ* TB-EPS treatment groups compared to the *in-situ* LB-EPS treatment groups. A similar trend ($p > 0.05$) was noted for the *ex-situ* treatment groups, where it was noted that *ex-situ* TB-EPS was more effective in reducing the SOD and catalase activity than the *ex-situ* LB-EPS groups.

3.6. Photosynthetic parameters

Figs. 6A-i, A-ii and 6B-i, B-ii depicted the effects of pristine and coronated forms of ZnO QDs on the ETR, and quantum yield of PS (II) (Y (II)) towards the freshwater *Chlorella* sp. Both parameters revealed a concentration dependent decline for the pristine ZnO QD treatments, where 1.0 mg/L (~23 % ETR, and Y (II) of PS (II)) of pristine QDs treated cells showed the highest decline in ETR and in the quantum yield of PS (II) (Y (II)). All the pristine QD treatments revealed the reduction in ETR and quantum yield of PS (II) (Y (II)), significantly lower than in case of the control treatments. The fast and light response curve followed a similar trend. The *in-situ* coronated test groups showed increments for ETR and quantum yield of PS (II) (Y (II)) for all the concentrations of ZnO QDs compared to their pristine groups. A similar trend was noted for all the *ex-situ* coronated test groups. For the test groups containing 0.25 and 1.0 mg/L ZnO QDs, the observed ETR and quantum yield of PS (II) (Y (II)) significantly increased ($p < 0.001$) for *in-situ* TB-EPS treatment groups compared to the *in-situ* LB-EPS treatment groups. A similar increase ($p < 0.001$) was noted for the TB-EPS treatment groups. In addition, for the *ex-situ* coronated treatment groups the increase in the ETR and quantum yield of PS (II) (Y (II)) was more compared to the *in-situ* treatment groups.

4. Discussion

The 3D-EEM of the LB and TB-EPS revealed the presence of polysaccharides and humic substances along with the presence of aromatic proteins containing tryptophan-like substances [35,47]. There was a marked decline in protein and humic-rich zones after EPS interaction, confirming corona formation in the mixtures. The FTIR scans of the LB and TB-EPS showed the presence of a broad absorption region around 1280, 1390, 1710, and 1575 cm^{-1} , likely indicating the secondary structure of the proteins due to the presence of amide bands [47]. The *in-situ* and *ex-situ* corona treatment of LB and TB EPS with QDs led to the presence of the amide bands in the spectra. This reasserts the formation of eco-corona on the ZnO QDs, which indicates the interaction of amide

bond (-C(=O)-NH-) with Zn. In case of *ex-situ* treatment, the carboxylic, hydroxyl, and amide groups of the LB and TB EPS caused QDs to fuse and aggregate by promoting bridging through multiple interactions and reducing their effective surface area. The fibrillar nature of EPS initially triggers nanoparticle attachment, leading to complete aggregation over time [48]. The SAED pattern of the pristine QD showed the presence of small dots in a circular pattern. It revealed that the ZnO nanocrystals exhibit high crystallinity with a hexagonal wurtzite structure, perfectly matching the seven diffraction rings of bulk ZnO [49]. The formation of an eco-corona possibly led to particle aggregation and reduced the number of dots in the pattern. This indicated a decline in the crystallinity of the QDs. Since the LB and TB-EPS were hydrophilic in nature, *ex-situ* coronated QDs became more hydrophilic by forming hydrogen bonds, and electrostatic interactions with EPS [50]. The zeta potential results also indicated the decline in colloidal stability of the coronated QDs. Increased hydrophilicity and decreased colloidal stability led to the agglomeration and settling of QDs in the medium. This reduced their direct interactions with the algal cells.

The exposure of algal cells to ZnO QDs resulted in substantial growth inhibition. Similar results were observed in the microalgae *Scenedesmus obliquus*, where treatments with high concentration of Carbon Quantum Dots (CQDs) or longer-term exposure significantly impacted algal growth [51]. CdSe/ZnS QDs caused substantial growth inhibition of *Desmodesmus communis*, especially after 24 h of exposure in the Lake Balsys water [52]. The addition of EPS in the interaction medium (*in-situ* corona) as well as the interaction of EPS with the QDs before the interactions with the algae (*ex-situ* corona) led to diminished growth inhibition. Similarly protein corona formations were observed for nano-quantum dots (CdSe@ZnS) with extracellular proteins in an activated sludge system [47]. The eco-corona, acting as a natural shield, not only altered the physical-chemical behavior of the QDs but also minimized direct damage to algae as noted in the preceding section.

Investigations of protein coronas of NPs [TiO_2 (TNPs) and ZnO (ZNPs)] and bacterial proteins isolated from activated sludge indicated that TNPs exhibited stronger affinity than ZNPs and that distinct processes played a part in the aggregation development of these two kinds of nanoparticles [53]. In another study, after removing EPS, algae collected more silver, around 7.41- and 1.25-fold of those in the algae *Chlorella vulgaris*, with EPS for AgNPs and AgNO_3 treatments, respectively [54]. Another study demonstrated that the presence of EPS on cell surface increased heteroaggregation between nTiO_2 and algal cells (*Chlorella pyrenoidosa*), and generated greater nTiO_2 deposition on the algal surface; however, algal cell incorporation of nTiO_2 was restricted by the presence of EPS [55]. Thus, these studies show that EPS effectively mitigates the potential toxic impacts of the particles in the aquatic environment, which was similar to our results.

The amount of leached Zn^{+2} ions from the QDs was quite low. Hence the ionic contribution to toxicity was lower than that from pristine QDs at 1.0 mg/L concentration. Similar results were observed in another study where the release of Zn^{+2} ions was shown to have no significant effects on the toxicity of ZnO QDs in *E. coli* strain MG1655 [56]. This release of Zn^{+2} ions was reduced even further upon the addition of both LB as well as TB-EPS to form an *in-situ* as well as an *ex-situ* corona. This can be attributed to the interaction of QDs with EPS, thereby forming a layer on their surface that may restrict the release of Zn^{+2} ions. Similar results were observed in another study, where toxicity of CdSe QDs was reduced upon treatment with loosely bound and tightly bound EPS [57]. The uptake of Zn^{+2} ions by algal cells was significantly high when the cells were treated with the pristine ZnO QDs. This accumulation might cause significant damage to the cells by limiting their nutrient uptake, subsequently affecting the photosynthetic activity of the algal cells [58]. However, for the *in-situ* as well as *ex-situ* corona treatment groups with both the LB and TB EPS a decline in the uptake of Zn^{+2} ions were noted. A similar decline in the Cd^{+2} ions uptake upon the treatment of the algal cells with eco-coronated functionalized CdSe/ZnS QDs was noticed in a previous study on the microalgae *Scenedesmus obliquus* [59].

The functionalized QDs can adhere to the algal cell by non-covalent interactions, such as electrostatic, hydrogen bonding, and hydrophobic contact. These interactions can lead to lipid peroxidation and an increase in MDA generation [60]. In a previous study the interaction of GO (graphene oxide) QDs caused substantial ROS and MDA generation in the blue-green algae *Microcystis aeruginosa* [61]. Interactions with LB and TB-EPS in the *in-situ* and *ex-situ* treatments resulted in a decline in intracellular ROS generation. These results correlated well with the growth inhibition results and can be related to the induced aggregation of the particles leading to decreased interaction and uptake by the cells. In this study pristine ZnO QDs reduced photosynthetic parameters like Y (II), ETR, and the light response curve in a dose-dependent manner. This reduction aligned quite well with the observed growth inhibition, increased ROS levels, MDA content, and elevated SOD and catalase activities. The effects of CdSe/ZnS quantum dots (0.01–0.1 μM) on *Chlorella vulgaris* beads revealed a dose-dependent decline in the ETR [62]. The treatment of the cells with *in-situ* and *ex-situ* coronated ZnO QDs showed recovery effects in Y(II) and ETR. EPS addition significantly decreased ROS.

QDs are known to disturb cellular redox equilibrium in algae [59]. In this work too, treatment with pristine ZnO QDs resulted in an increase in the SOD and catalase enzyme activities. The treatments with CQDs at the

concentration of 200 mg/L resulted in the elevation of SOD enzyme activity in the treated *S. obliquus* cells [51]. Similar results were also observed while treating *M. aeruginosa* cells after 96-h exposure with GO QDs too [61]. The eco-corona plays a significant role in alleviating oxidative stress, as evidenced by the reduced activity of antioxidant enzymes (SOD and CAT). This was observed in case of algal cells treated with the coronated carboxyl- and amine CdSe/ZnS QDs, where both the SOD and catalase enzyme activities reduced after corona formation [59].

The utilization of a cluster heatmap, as shown in Fig. 7A, and correlation mapping (Fig. 7B) allowed us to analyze various aspects of this study to explore the correlations among the key biological markers. The analysis via a cluster heatmap indicated that the parameters related to photosynthesis, specifically Y (II) of PS II and ETR, were closely grouped together across all the treatments. Additionally, there was a noticeable grouping of total ROS with catalase activity and MDA levels with SOD activity. A unique clustering was observed between the groups comprising MDA, SOD activity, and total ROS with catalase activity. Moreover, a direct grouping was identified between growth inhibition and photosynthetic metrics. Hence, the escalation in ROS and MDA levels adversely affected the growth and photosynthetic efficiency of the cells, while augmenting the activity of antioxidant enzymes. This

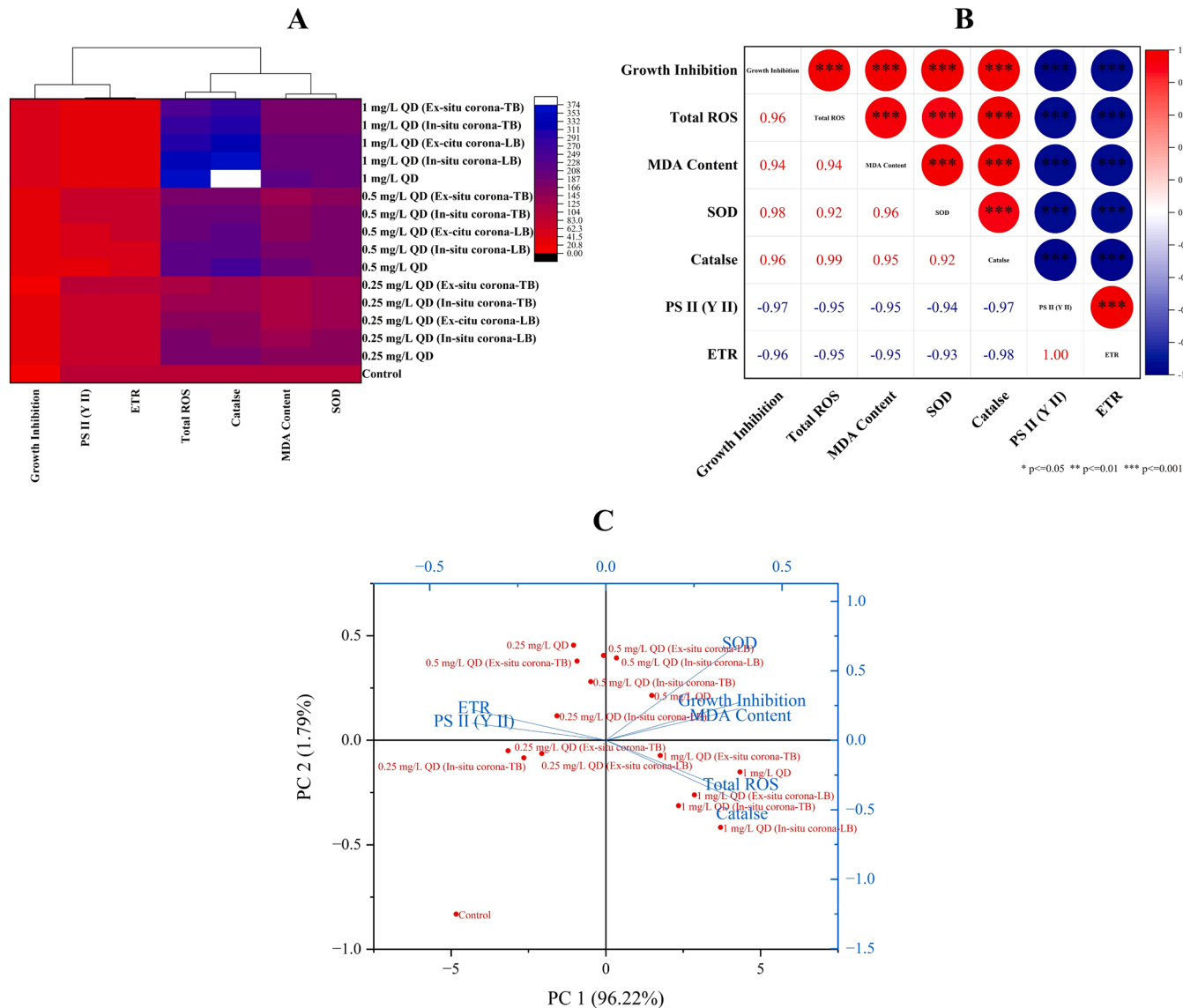


Fig. 7. (A): Clustered heatmap, (B): Pearson correlation analysis, (C): PCA analysis of pristine and coronated QDs.

observation is echoed in recent research studies, that noted similar clustering patterns involving oxidative stress, cell density, and chlorophyll content when algae were exposed to a mixture flame retardant and ZnO NP [63]. The Pearson modeling analysis revealed a positive link among growth inhibition, ROS, MDA, SOD, and catalase activity, with statistically significant correlations ($p < 0.001$) across all treatment groups. Conversely, a considerable negative relationship was identified between growth inhibition and both Y II (PS II) and electron transport. A negative association was also noted between total ROS, ETR, and PS II (Y II). Moreover, there was a significant association between ROS, MDA, SOD, catalase activity, ETR, and Y II (PS II) within the treatment groups. This suggests that enhanced oxidative stress might contribute to reduced ETR and a decline in Y II in the PS II system, leading to increased activity of antioxidant enzymes. Similarly, a recent study has shown clear association between cell density, oxidative stress, and chlorophyll content [63].

The PCA aimed to determine the correlations between various biological parameters in algae for pristine, *in-situ*, and *ex-situ* corona treatments of LB and TB EPS ZnO QDs (Fig. 7C). Components PC1 and PC2 accounted for 98 % of data variability. The growth inhibition, SOD activity and MDA content were grouped together in component 1. The cellular damage indicators, such as ROS and catalase, were clustered in components 1 and 2, showing that an increase in these parameters correlated well with increased cell damage. The growth inhibition in case of 1.0 mg/L and 0.5 mg/L pristine, *in-situ* LB, TB EPS treatments and *ex-situ* LB, and TB EPS treatments all fell under these two components of the graph. The extent of growth inhibition in these two doses was so high in comparison to the other treatment groups that even corona formation by LB and TB EPS could not ensure complete recovery of the cells. The PS II (Y(II)) and ETR were in component 4. This shows that these parameters shared a negative correlation with the growth inhibition. The treatment groups, pristine QDs 0.25 mg/L, 0.5 mg/L *ex-situ* TB coronated QDs and 0.25 mg/L *in-situ* LB EPS coronated QDs fell into this component, confirming the recovery of the growth inhibitory effects by these treatments. The other treatments, such as control, 0.25 mg/L *in-situ* TB EPS, *ex-situ* LB EPS, and *ex-situ* TB EPS coronated QDs treatments, fell in a separate component 3. This means that these treatments did not correlate with any of the biological parameters. ROS, MDA generation, along with SOD and catalase production, positively correlated with the cell damage parameters as expected. However, the interactions with EPS notably reduced these toxic effects.

5. Conclusion

This study underscores the protective role of extracellular polymeric substances (EPS), naturally occurring biopolymers, in mitigating the toxic effects of ZnO QDs in primary producers like algae in aquatic environment. The pristine ZnO QDs induced growth inhibition, triggered oxidative stress generation, and decreased photosynthetic efficiency in the algal cells. However, the interaction of ZnO QDs with LB and TB EPS, for both *in-situ* and *ex-situ* corona formation, significantly reduced oxidative stress and improved algal health. This could be linked to enhanced aggregation and reduced bioavailability of the coronated QD, possibly reducing their toxic impact. This research underscores the significance of natural biopolymers, specifically EPS, in lessening the environmental risks linked to engineered nanomaterials. The study sheds light on effective strategies to decrease the negative impacts of nanomaterials in aquatic ecosystems, especially in freshwater areas where ZnO quantum dots (QDs) are becoming more common. Importantly, the results reveal that EPS plays a vital role in reducing the toxicity of ZnO QDs while maintaining their natural fluorescence. This dual capability not only ensures the safe use of ZnO QDs but also supports their sustainable application, thereby minimizing environmental harm. Consequently, EPS-coated ZnO QDs represent a promising approach to decrease material toxicity without sacrificing performance, contributing to the long-term sustainability of these innovative

materials. The current investigation establishes a foundation for exploring the effects of both *in-situ* and *ex-situ* corona formation on various nanoparticles.

Environmental implications

This research demonstrates the potential risks posed by ZnO QDs to microalgae, key primary producers in aquatic food webs, by highlighting their ability to induce oxidative stress, inhibit growth, and reduce photosynthetic efficiency. The study highlights the influence of algal EPS in LB and TB forms to modify the toxicity of the nanomaterials by reducing the oxidative stress and cellular uptake of QDs. The findings emphasize the role of EPS corona in reducing the toxicity of ZnO quantum dots at the same time maintaining their fluorescence properties and ensuring their safe and sustainable applications.

CRediT authorship contribution statement

Chandan Maity: Writing – review & editing, Formal analysis. **Mrudula Pulimi:** Writing – review & editing, Supervision, Methodology, Investigation. **Abhinav Sinha:** Writing – original draft, Validation, Methodology, Investigation. **Abhrajit Debroy:** Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **Willie J.G.M. Peijnenburg:** Writing – review & editing, Validation, Formal analysis. **AMITAVA MUKHERJEE:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.137034.

Data availability

Data will be made available on request.

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