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Fluorescent nanoplastics increase the toxic effects of Graphene oxide nanoparticles in freshwater algae *Scenedesmus obliquus*

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ABSTRACT

The increased usage of Graphene oxide (GO) in various industrial applications led to their entry into freshwater systems. Other secondary contaminants like nanoplastics (NPs) often co-exist with GO in the environment. This study examines the possible role of fluorescent nanoplastics (FNPs) in modifying the toxic effects of GO on freshwater algae *Scenedesmus obliquus*. Selected concentrations of GO (0.1, 1, and 10 mg L^{-1}) were combined with a fixed concentration of FNPs (1 mg L^{-1}) to perform combinational toxicity tests on algae. FNPs significantly enhanced the toxic effects of GO in the mixtures in comparison with the pristine GO. In addition to the cytotoxic effects, oxidative stress parameters like total ROS generation and malondialdehyde (MDA) production also increased in the case of the combined pollutants. The antioxidant enzymatic activities like catalase (CAT) and superoxide dismutase (SOD) in the cells were also assessed. Algal exposure to the pristine pollutants and their mixture led to a notable decrease in photosynthetic activities in the cells, with the mixed pollutants aggravating the loss of activity. The interactive toxic effects of the contaminants when present in mixtures were evaluated using Abbotts' Independent action modelling. Furthermore, optical microscopic images revealed the morphological changes in the algal cells after exposure to the contaminants both in the pristine and combined forms.

1. Introduction

Plastic pollution is a global issue [28]. Microplastics (MPs) are plastic particles smaller than 5 mm, while nanoplastics (NPs) are smaller, ranging from 1 to 100 nm. When larger plastic particles break down, they create smaller fragments of particles [47]. They can be found in various environmental strata, and their source can be traced back to the plastics used in cosmetics, skin care products, and dental paste [22]. Both deliberate and unintentional releases of tiny plastics may lead to the pollution of freshwater environments [46]. Nanoplastics, due to their tiny size, can evade the usual treatment methods in existing wastewater treatment facilities [45]. Consequently, the ubiquitous presence of nanoplastics in freshwaters and their possible impact on freshwater creatures might affect the typical functioning of the ecosystem.

Algae act as primary producers in aquatic habitats. Consequently, some environmental stresses may undermine the integrity and operation of the ecosystem by affecting algae. The impact of nanoparticles on several aquatic organisms, including microalgae, has been previously investigated [10,41,48]. Heinlaan et al. [28] documented growth suppression in *Raphidocelis subcapitata* when exposed to NPs (26 nm) at 100 mg L^{-1} concentration. In another study, Hazeem et al. [27] examined the impact of differently charged NPs (20 and 50 nm) on *Chlorella vulgaris*. They observed increased cell death and reduced chlorophyll-a content due to NP exposure.

Graphene oxide (GO) is a highly studied 2-dimensional carbon nanomaterial (CNM) due to its wide range of applications in biosensors, electrical devices, and drug administration [39,42]. By 2026, the annual output of items made from graphene is projected to reach 3800 tonnes. Due to the fast expansion of industrial uses for GO, its concentration has significantly increased in both aquatic and terrestrial habitats. The concentration of GO in wastewater or at industrial discharge sites exceeds 10 mg L^{-1} [2]. Graphene oxide (GO), recognized for its remarkable adsorption properties, has seen a rising use in agriculture to improve nutrient delivery and serve as a pesticide, eventually entering freshwater habitats via agricultural runoff [7,31]. Previous studies found that various concentrations of GO (1, 10, 25, 50, 75, 100, and 125 mg mL-1) were toxic to algae *Porphyridium purpureum* [40]. Malina et al. [36] conducted a study to investigate the impact of GO at various concentrations (up to 200 mg L^{-1}) on algae and cyanobacteria. They

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found that weakly oxidized GO may function as a "nano-blade," leading to physical harm to algal cells. A recent study by Bytešníková et al. [11] also demonstrated that freshwater algal cells *Chlamydomonas reinhardtii* decreased their viability and showed increased oxidative stress upon exposure to high concentrations of GO.

The most regularly found forms of micro/nano-plastics in freshwater systems are polypropylene, polyethylene, polystyrene, and PP&A (polyesters, polyamide and acrylics). Among them, polystyrene (57 %) is frequently used as a representative NP in toxicological research [23]. FNPs may have a major impact on the photosynthetic activity of aquatic species mainly microalgae. The associated dyes reduce light penetration and may be hazardous to some aquatic organisms [30]. Hence, it is essential to investigate the inherent toxicity of fluorescently labelled NPs on freshwater algae to enhance the accuracy of NP toxicity assessments. NPs have the potential to absorb and accumulate harmful pollutants in the environment, including hydrophobic chemicals and poisonous metal ions. This may change the toxicity of these pollutants by affecting the sorption capacity and uptake of the aquatic species [6].

Just a few studies have examined the combined effects of NPs and nanoparticles with other pollutants in algae. Researchers examined the synergistic effects of copper and carboxylated PS NPs on microalgae *Raphidocelis subcapitata*. Differences in growth inhibition were not observed between exposure to copper alone and exposure to copper in conjunction with carboxylated PS NPs [5]. Another research group investigated the toxic effects of the binary mixture of Ag nanoparticles (~10 nm) and PS NPs (~20 nm) on two freshwater microalgae, *Chlamydomonas reinhardtii* and *Ochromonas danica* [29]. Exposure to the binary mixtures for 24 h resulted in a synergistic increase in toxic effects in both freshwater algae. Yesilay et al. [49] observed a reduction in the toxic effects of NPs when combined with GO on marine algae *Picochlorum sp*. They also observed that pre-incubation with GO leads to the formation of GO coating on the algal cells, thereby reducing the toxic effects of polystyrene NPs. A recent study by Cao et al. [13] reported that Cd^{2+} adsorbed on the surface of NPs caused toxicity to freshwater algae *E. gracilis*.

There is a lack of research discussing the interaction between plastics and other nanomaterials and their impact on primary species like algae in freshwater ecosystems. Hence, it is essential to conduct a thorough investigation of the combined effects of nanoplastics and nanoparticles in freshwater algae. This study is the first work to investigate the influence of nanoplastics on the toxic impact of GO in freshwater organisms. In this study, we hypothesized that FNPs would enhance the toxic effects of GO nanoparticles on freshwater algae *Scenedesmus obliquus*. Our experimental design included an algal cell viability assessment based on exposure to GO and FNPs + GO. Furthermore, oxidative stress assessment, antioxidant enzyme activities, and photosynthetic efficiency were also determined. The interaction type between GO and FNPs was determined by applying Abbott's Independent action modelling. In addition, optical light microscopic images of the algal cells were analyzed to observe the morphological changes and aggregation patterns.

2. Materials and methods

2.1. Materials used

The contaminants used in this study are (i) Graphene Oxide, which was synthesized in our lab by following previously published protocols from our lab [14,21], and (ii) Fluorescent polystyrene nanoplastics (size: between 100 and 200 nm), which were purchased from Corpuscular, Inc., USA. The chemicals and materials used for this research are 2′, 7′ dichlorofluorescein diacetate (DCFH-DA), purchased from Sigma Aldrich, USA. Thiobarbituric acid (TBA), hydroxylamine hydrochloride, trichloroacetic acid (TCA), and dimethyl sulfoxide were all obtained from Hi-Media Pvt. Ltd., which is located in Mumbai, India. Nitroblue tetrazolium chloride (NBT) and the hydrogen peroxide solution (H_2O_2, 30 % w/v) were purchased from SDFCL in Mumbai, India.

This study used lake water as the freshwater matrix, similar to our previous studies. Lake water was obtained from VIT, Vellore's lake. The collected lake water was strained several times and sterilized before using for the experiments. The details of the filtration and maintaining the aseptic conditions of the lake water were shown in our previous study [17].

2.2. Stock solution preparation of GO and FNPs

For this research, the stock solutions of GO were prepared in deionized water followed by ultrasonication using a probe-Sonicator for 20 mins to ensure even dispersion of the particles (100 mg L^{-1}) [35]. FNPs solution having a concentration of 100 mg L^{-1} was prepared by dispersing in deionized water and bath sonicated for 15 mins [20].

2.3. Characterization of GO and FNPs

To determine the shape, structure and size of the particles, the sonicated solutions of GO and FNPs were subjected to Field Emission Scanning electron microscope (FE-SEM, Thermo Fisher FEI Quanta 250 FEG).

2.4. Test organisms

The present research used *Scenedesmus obliquus*, an ecologically dominating and easily cultivable alga. This alga was isolated from the lake in VIT, Vellore, located at a latitude of 12°58′10″ N and a longitude of 79°9′37″ E. The isolated algal culture was subsequently sub-cultured utilizing the decontaminated BG-11 medium obtained from Hi-Media Pvt. Ltd. The culture was preserved in a temperature-regulated chamber (I.L.E. Co., India) at a constant temperature of 23 ± 2 °C. The cultures were illuminated with a white fluorescent light (Philips TL-D Super 80, linear fluorescent lamp, India) to maintain a photoperiod of 16 h and an illumination of 3000 lx. This was done to promote the optimal development of the cultures [43].

2.5. Algal interactions with pristine particles and binary combinations

Algal cells from the late log phase were harvested and centrifuged to collect the pellet. The pellets were resuspended in lake water till the optical density (O.D) reached 0.5. The algal cells then interacted with pristine GO (0.1, 1, and 10 mg L^{-1}), pristine FNPs (1 mg L^{-1}), and their mixtures (0.1 mg L^{-1} GO + 1 mg L^{-1} FNPs, 1 mg L^{-1} GO + 1 mg L^{-1} FNPs, and 10 mg L^{-1} GO + 1 mg L^{-1} FNPs). The working concentrations were chosen based on the EC_{50} value. Two concentrations below and one above the EC50 value were selected for the toxicity assays. The concentration of FNPs was 1 mg L^{-1}. In the end, the volume of the mixture was increased to 5 mL by adding more autoclaved lake water to the glass beaker and kept for incubation under visible light conditions for 72 h. Throughout the study, controls, i.e., algal samples without the contaminants, were kept along with the experiments. OECD guidelines were followed while performing the toxicity testing and other biochemical assays [38].

Following 72 h of incubation, a decrease in cell viability was assessed by counting the viable cells on a hemocytometer under an optical microscope. Additional details are mentioned in the Supplementary materials (Method S1).

2.6. Nile red leachate toxicity

The Nile red leaching test was conducted using the methodology outlined by Schiavo et al. [44] and Lee et al. [32]. The detailed and complete methods for the toxicity of leachates can be found in our previous study [20].

2.7. Biochemical analysis

The total ROS generated by the algal samples was analyzed by following the protocol mentioned [16]. The complete methodology has been discussed in the Supplementary material (Method S2).

MDA production of the algal samples was analyzed by following the protocol mentioned [18]. The complete methodology has been discussed in the Supplementary material (Method S3).

The catalase (CAT) and Superoxide dismutase (SOD) activity of the treated and the control algal samples were assessed by the protocol mentioned in Debroy et al. [21]. The complete methodology has been discussed in the Supplementary material (Method S4).

To quantify the photochemical yield of the PS II system and the electron transport rate (ETR_{max}) in both the treated and control algal cells, a photosynthetic yield analyzer called Mini PAM (Heinz Walz; Germany) was used. The Supplementary material has discussed the detailed methodology (Method S5).

2.8. Statistical analysis

All experiments were performed in triplicates to demonstrate the statistical significance of the data (n=3). All the data are provided as the mean value plus or minus the standard deviation (SD). GraphPad Prism 6 was used for a two-way ANOVA test with a Bonferroni post-test. The purpose was to determine the statistical significance between various test samples and controls. Data with a p-value less than 0.05 was deemed statistically significant. A p-value less than 0.05 indicates 95 % significance.

Abbott's statistical Independent action model analyzed the interactions between two nanoparticles in the mixture, explicitly looking at synergism, antagonism, or addition. This model is often used to determine the impact of toxic particles on a typical cause of death. For mixtures with only two particles, this approach is the most effective for comparing observed and anticipated growth inhibitions [1]. The detailed methodology of Abbott's modelling is shown in the supplementary file (Method S6).

3. Results

3.1. Physical characterization of the particles

The FE-SEM analysis of GO showed that it had a sheet-like morphology with layer-to-layer spacing and a large surface area. The FNPs were spherical with a diameter of approximately 120 nm (Fig. 1B).

The zeta potential values for pristine GO (0.1, 1, and 10 mg L^{-1}) were found to be −12.67, −28.95, and −38.47 mV, respectively. The zeta potential value for FNPs (1 mg L^{-1}) was 3.98 mV.

3.2. Cell viability determination

The impact of pristine GO, pristine FNPs, and their combinations with *Scenedesmus obliquus* is presented in Fig. 2. Reduced viability of algal cells was noted when exposed to increasing concentrations of pristine GO (0.1, 1, 10 mg L^{-1}) and 1 mg L^{-1} of pristine FNPs. Furthermore, an enhanced reduction in the viability of the algal cell was noted when exposed to the binary mixtures compared to the individual pollutants alone. The decrease in the viability was significant ($p<0.001$) compared to the control's. In addition, binary mixture (for 1 and 10 mg L^{-1}) exposed algal samples showed a significant viability decrease when compared to the pristine GO counterparts ($p<0.001$).

Independent action modelling was carried out to describe the interaction between GO and FNPs after their exposure (Table S1). The R_I value increased in tandem with the concentration of GO but was not statistically significant. Therefore, an additive type of interaction was seen for all concentrations of the combination.

3.3. Toxic effects of leachates

The amount of Nile red dye leachates released from FNPs, both in the presence and absence of GO, was measured using the Nile red standard curve. A concentration of 0.1 mg L^{-1} of dye was released into the freshwater matrix due to pristine FNPs and GO-FNPs mixtures. The dye leached from 1 mg L^{-1} FNPs + 0.1 mg L^{-1} GO did not have any noticeable harmful effects on the algae, as determined by statistical analysis ($p>0.05$). Nevertheless, a significant reduction ($p<0.001$) in the viability of algal cells was seen for 1 mg L^{-1} FNPs + 10 mg L^{-1} GO mixture (Fig. S1).

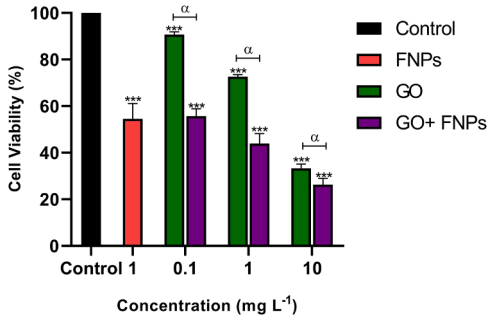

Fig. 2. Decrease in the viability of algal cells (%) when exposed to pristine GO, pristine FNPs and their binary combinations. Note: '*' represents the level of significance compared to control; 'α' represents a significant difference between pristine particles and binary combinations ($p<0.001$).

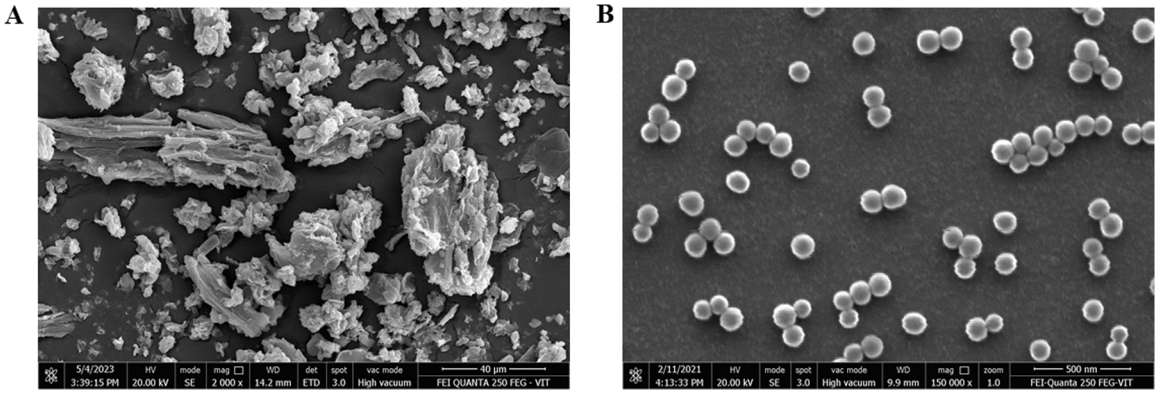

Fig. 1. Field Emission Scanning electron microscopic images of **(A)** GO **(B)** FNPs.

3.4. Physical changes and aggregation patterns of Scenedesmus obliquus

Following 72 h of interaction, algal cell aggregation was not observed in the control group (Fig. S2–A). However, when the algal samples were exposed to pristine GO (10 mg L^{-1}) (Fig. S2-B), pristine FNPs (1 mg L^{-1}) (Fig. S2-C), and a combination of both (10 mg L^{-1} GO + 1 mg L^{-1} FNPs) (Fig. S2-D), a reduction in cell density and an increase in agglomeration were noticed.

3.5. Total ROS generation and MDA production

Fig. 3A represents the total ROS generated by the algal samples exposed to the contaminants. A significant increment in ROS generation was noted when exposed to increasing concentrations of pristine GO and pristine FNPs compared to the control ($p<0.001$). The ROS generation of the algal samples was further enhanced significantly upon exposure to the contaminants' binary mixture compared to that of the pristine GO counterparts ($p<0.001$).

An increase in MDA production of the algal cells was also observed when exposed to increasing concentrations of pristine GO (Fig. 3B). MDA production increased when the algal cells were exposed to pristine FNPs, too. The increase in MDA production of the algal cells treated with pristine contaminants was significant ($p<0.001$) compared to control. MDA production in the algal cells was further enhanced when exposed to the binary mixture compared to pristine GO counterparts ($p<0.001$).

3.6. Antioxidant activity

An increase in CAT activity of the algal cells was observed when exposed to increasing concentrations of pristine GO (Fig. 4A). CAT activity also increased when the algal cells were exposed to pristine FNPs. The increased CAT activity of the algal cells treated with pristine contaminants was significant in comparison to the control ($p<0.001$). CAT activity of the algal samples was further enhanced when exposed to the binary mixture. This increase was statistically significant compared to the control group and pristine GO counterparts ($p<0.001$).

A decrease in SOD activity of the algal cells was observed when exposed to increasing concentrations of pristine GO (Fig. 4B). SOD activity also decreased when the algal cells were treated with pristine FNPs. The drop in SOD activity was significant in comparison to control samples ($p<0.001$). The SOD activity of the algal samples was further reduced when exposed to the binary mixture. The mixture-treated algal samples showed significantly ($p<0.001$) reduced SOD activity in comparison to both the control group and pristine GO counterparts.

3.7. Photosynthetic parameters

Fig. 5A represents the quantum yield of PSII of the algal cells after exposure to the contaminants. A concentration-wise decrease in Φm was noted for the algal samples upon exposure to the increasing pristine GO concentration. A reduction of Φm was also observed upon exposure to pristine FNPs. The decrease was observed to be more significant in comparison to the untreated samples ($p<0.001$). Φm decreased further when the algal cells were exposed to the mixture. The decrease was observed to be more significant in comparison to control and pristine GO counterparts ($p<0.001$).

Fig. 5B represents the ETR_{max} of the algal cells after exposure to the contaminants. A dose-wise decrease in ETR_{max} was observed for the algal samples upon exposure to the increasing concentration of pristine GO. A reduction of ETR_{max} was also observed upon exposure to pristine FNPs. The decrease was statistically significant compared to control ($p<0.001$). ETR_{max} further decreased when the algal cells were exposed to the binary mixture. The decrease was statistically significant compared to control and pristine GO counterparts ($p<0.001$).

4. Discussion

GO showed dose-dependent toxic effects on freshwater algae *Scenedesmus obliquus*. GO has a large surface area, which provides abundant binding sites for the algal cells. When the algal cells get coated with GO, the cell viability may decrease due to the limited entry of light and nutrients into the cells. Yin et al. [50] observed pristine rGO, as well as metals-modified rGO, caused microalgal cell death by getting adsorbed onto the algal cells (*Scenedesmus obliquus* and *Chlamydomonas reinhardtii*), which corroborates our findings. A recent study by Debroy et al. [21] reported the maximum adverse effects of GO on *Chlorella sp.* among GO, rGO, and graphene nanoparticles. Furthermore, the attachment of FNPs onto the algal surface may also block light and nutrients, endangering the cells. This phenomenon was observed in one of our previous studies [19]. A binary mixture of GO nanoparticles and FNPs further enhances the toxic effects. FNPs get adsorbed onto GO and might form hetero-aggregates with algal cells. The adsorption of GO may cause damage to algal cell membranes, allowing FNPs or other pollutants to enter the cells. This can damage cell organelles and eventually lead to cell death [49]. Additive toxic effects were observed for all the combinations due to the binding of GO and FNPs on the algal cells, which damage the cell membrane, thus increasing the permeability of FNPs [29]. In recent studies, the toxicities resulting from the combined exposure to MPs/NPs and other nanomaterials have been found to have different effects. Some studies have reported synergistic effects, where the toxicities are enhanced when both are present [4]. Other studies

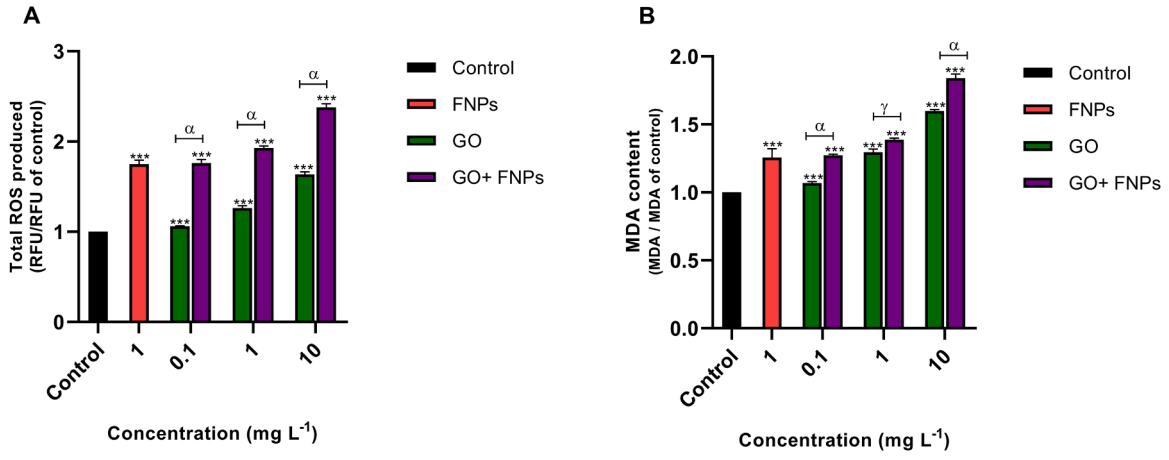

Fig. 3. (A) Total ROS produced by algal cells **(B)** MDA produced by algal cells when exposed to pristine GO, pristine FNPs and their binary combinations. Note: '*' represents the level of significance compared to control; 'α' represents a significant difference between pristine particles and binary combinations ($p<0.001$).

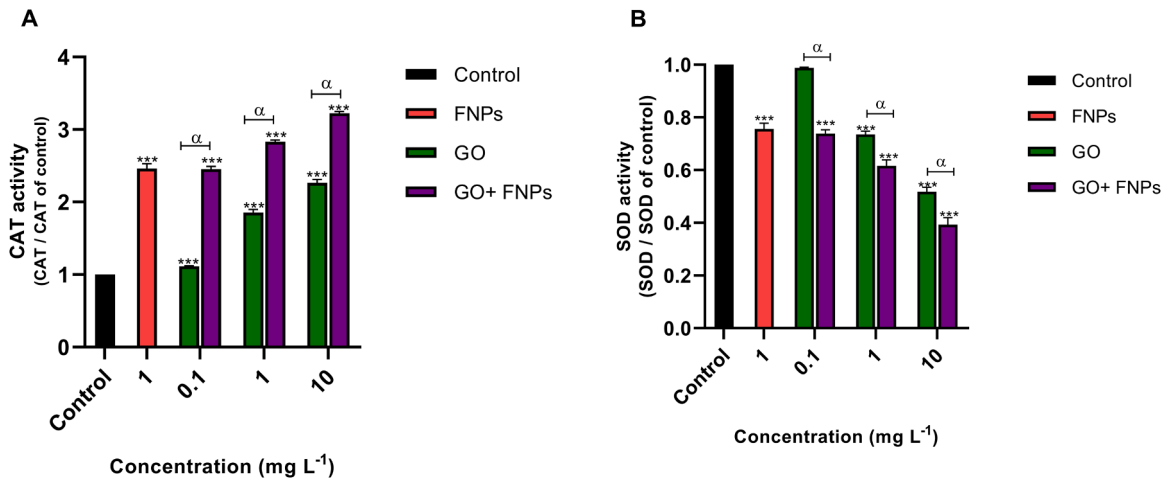

Fig. 4. **(A)** CAT activity **(B)** SOD activity of the algal cells when exposed to pristine GO, pristine FNPs and their binary combinations. Note: '*' represents the level of significance compared to control; 'α' represents a significant difference between pristine particles and binary combinations ($p<0.001$).

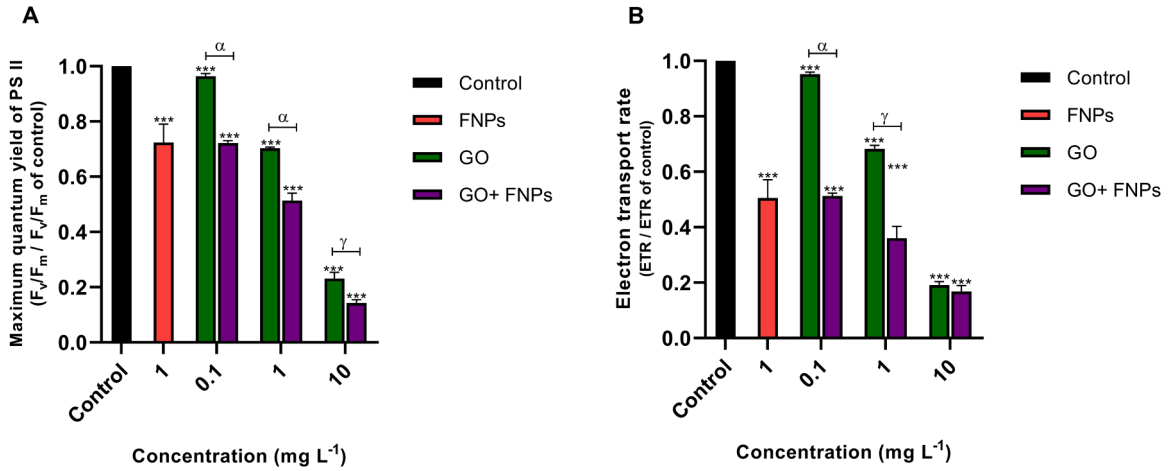

Fig. 5. **(A)** Maximum quantum yield of PSII **(B)** Electron transport rate of the algal cells after exposure to pristine GO, pristine FNPs, and their binary combinations. Note: '*' represents the level of significance compared to control; 'α' represents a significant difference between pristine particles and binary combinations ($p<0.001$).

have found antagonistic effects, where the toxicities are reduced [34]. Some studies have observed additive effects, where the toxicities remain unchanged [33]. According to the results of leachate toxicity experimental studies, the highest concentration mixture was hazardous to *Scenedesmus obliquus*. This may be due to the degradation of NPs in the presence of GO, resulting in the discharge of the leachates [3].

Both GO and FNPs are prone to producing oxidative radicals that are harmful to algal cells. These reactive species can potentially target and destroy the bonds between carbon atoms, the ester linkages between glycerol and fatty acids found in the phospholipid of membranes, resulting in significant damage to the cell membrane. This elucidates the direct associations between the generation of reactive oxygen species (ROS) and lipid peroxidation or MDA production [8]. Our previous study found that upon exposure to FNPs, algal cells can increase MDA production, corroborating our findings [20]. Yin et al. [50] also documented graphene's harmful effects on the cell membrane of *Scenedesmus obliquus*. The current study demonstrated exacerbated MDA production in the cells treated with GO and FNPs. A molecular dynamics investigation in a previous report revealed that graphene can remove membrane lipid layers in two ways: (i) by directly piercing the membrane when it is in the correct formal positioning or (ii) by attaching to the bilayer surface and thereby covering the inverted phospholipids [15]. In line with our research, Yin et al. [51] reported a rise in the concentration of MDA in freshwater algae *S. obliquus* after exposure to GO. A prior investigation examining the impact of GO in conjunction with ZnO on the algal species *Gymnodinium* documented a rise in lipid peroxidation [24]. GO sheets can get adsorbed onto the cell membrane due to its large surface area. The adsorbed GO may disrupt the cell membrane, enabling the entry of additional contaminants into the cells. These contaminants then interact with important molecules, inhibiting photosynthetic activities, electron transport chains, and other normal cell activities, causing toxic effects and cell death [26]. This may explain the observed decline in photosynthetic activities of the algal cells upon exposure to pristine GO, pristine FNPs, and their binary mixtures in the current work. Reduced light exposure due to adsorbed GO and FNPs onto the surface of algal cells also could have caused decreased photosynthetic responses. Yesilay et al. [49] also observed a decrease in photosynthetic activities and chlorophyll upon exposure of microalgae *Picochlorum sp.* to GO (0.5 mg L^{-1}) and polystyrene NPs (size: 20 nm) combined for 5 days.

The reduced SOD enzyme activity in the interacted algal cells suggests that the ROS radicals are hindering the SOD enzyme generated [25]. SOD enzymes have greater efficacy in neutralizing ROS radicals, whereas CAT facilitates the conversion of H_2O_2 into H_2O and O_2. SOD and CAT levels may be increased to counterbalance an excessive amount of ROS generation. Contrary to the former conclusion, increasing the

dosage of pristine GO and the combined mixture directed a simultaneous decline and increase in SOD and CAT activity, respectively. The enzymatic activity like SOD in algae may be initiated to encounter the ROS generated, leading to the conversion of radicals into H_2O_2. The generated H_2O_2 during disproportionation can get combined with the existing intracellular H_2O_2 and has the potential to degrade the SOD enzyme, resulting in a decrease in its activity [20]. In contrast, catalase reacts to the overproduction of H_2O_2 by increasing its activity to eliminate it. In a recent research conducted by Zhu et al. [53], it was revealed that the activity of SOD in microalgae *Gymnodinium* increased when exposed to 4.5 g L^{-1} of graphene quantum dots (size: 10 nm). The SOD activity enhanced even further when the microalgae were exposed to graphene quantum dots combined with different concentrations (1, 10, and 20 mg L^{-1}) of ZnO. Zhang et al. [52] found that treating *Scenedesmus obliquus* with GO over 72 h increased CAT activity. Nazari et al. [37] observed a rise in CAT activity when *Chlorella vulgaris* was exposed to a combination of silver-reduced graphene oxide (Ag-rGO).

An analysis of the relations between the biochemical indicators for different exposure conditions was conducted using a heat map (Fig. 6). The rise in ROS levels correlated with the production of MDA and the increase in CAT activity. The antioxidant enzymes were activated to improve cell tolerance against the excessive accumulation of GO and FNPs and the production of MDA. In addition, there is a strong correlation between the rise in oxidative stress and the decline in photosynthetic activities. It became evident that the impairment of photosynthetic parameters leads to an escalation in oxidative stress, including the generation of ROS radicals and the production of MDA. The study conducted by Cai et al. [12] found that when cells are exposed to high concentrations of ZnO nanoparticles, they can produce excessive oxidative stress radicals. These radicals are known to significantly hinder the activity of epiphytic biofilms. Future research on the combined harmful effects of GO and NPs on microalgae should concentrate on understanding their interactions in natural ecosystems. Investigating how these compounds affect microalgal growth, photosynthesis, and nutrient intake will give crucial information about possible issues in aquatic food networks. Real-world applications might include evaluating the environmental dangers of nanoparticles in water treatment operations, where GO is often employed, or forecasting the consequences of plastic pollution on primary producers. By investigating synergistic or antagonistic harmful effects, researchers may provide recommendations for the safe use of nanomaterials in industry, the preservation of the environment, and the maintenance of ecosystem stability.

5. Conclusion

In this study, we demonstrated that fluorescent nanoplastics modify and develop the toxic effects of graphene oxide in freshwater algae *Scenedesmus obliquus*. We noted an increase in oxidative stress, such as ROS generation and MDA production, upon exposure to the contaminants. Furthermore, to counteract the oxidative stress generated, an increase in CAT activity and a decrease in SOD activity were also observed. The results of this study also highlighted that the mixture of GO and FNPs caused more morphological damage to *Scenedesmus obliquus* than the pristine contaminants. Given the possible hazards, environmental policy should emphasize stricter limits on the discharge of nanoparticles and plastic debris into waterways. Remediation measures might involve developing filtering devices that collect GO and nanoplastics before they reach natural ecosystems and advocating environmentally friendly alternatives to these materials for industrial use. Proactive steps will assist in reducing long-term environmental problems while encouraging the proper usage of nanotechnology.

CRediT authorship contribution statement

Sampriti Giri: Writing – original draft, Validation, Methodology, Investigation. **Amitava Mukherjee:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **Janmey Shah:** Writing – original draft, Validation, Methodology, Investigation. **Soupam Das:** Writing – original draft, Validation, Methodology, Investigation, Conceptualization.

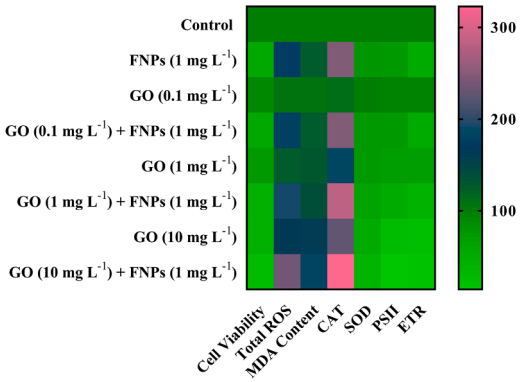

Fig. 6. Heat map representation of the effects of various concentrations of pristine GO, pristine FNPs and their binary combinations on the various biochemical activities of *Scenedesmus obliquus* after 72 h of exposure.

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.

Acknowledgment

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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.toxrep.2024.101759.

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