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Supplementary Information

The Impact of $nTiO_2$ and GO (Graphene Oxide), and their Combinations, on Freshwater *Chlorella* sp.: A Comparative Study in Lake water and BG 11 Media

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Materials and Methods

Methods S1: Synthesis of GO

The modified Hummer's method was followed using graphite powder (>99.95% purity). The sulphuric acid and phosphoric acid were measured in the volume of 27 mL and 3 mL (9:1 ratio) and stirred continuously for 15 min. 0.225 g of graphite powder was added to the acid mix and followed by the slow addition of $KMnO_4$ (1.32 g). The solution changed to dark green after 6 h of continuous stirring. The removal of excess $KMnO_4$ was done by adding 0.675 mL of H_2O_2 (dropwise), under continuous stirring for 10 min. The solution was allowed to cool down. At the end of the reaction, the material was washed with HCl (10 mL) and deionized water (30 mL) using centrifugation at 5000 rpm for 20 min. The obtained pellet was dried in a hot air oven (for 48 h at 60 $^{\circ}C$) to achieve the powder form of GO for further studies [1].

Methods S2: Chemicals used

Sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4), and hydrochloric acid (HCl) were purchased from Molychem Pvt. Ltd., India. Potassium permanganate ($KMnO_4$) was purchased from Sisco Research Laboratories Pvt. Ltd., India. $nTiO_2$ (Aeroxide P25), Graphite powder, 2′ ,7′ -dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma Aldrich. DMSO (Dimethyl sulfoxide), and hydrogen peroxide (H_2O_2) were purchased from SDFCL (Mumbai). BG-11, Trichloroacetic acid (TCA), and thiobarbituric acid (TBA) were purchased from Hi-Media Pvt. Ltd (Mumbai, India).

Methods S3: Characterization

Pristine form of $nTiO_2$, GO, and their mixtures in both the media *i.e*. BG-11 and lake water were characterized by Field Emission Scanning Electron Microscope (FESEM) (Thermo Fisher FEI Quanta 250 FEG). The surface charge was analysed by zeta potential (90 Plus Particle Size Analyzer, Brookhaven Instruments Corp., USA).

Methods S4: Growth Inhibition

Equation (I) was followed to evaluate the total growth inhibition and shading effect as well.

$$Growth\ Inhibition\ (\%) = 100 - \left(\frac{Test}{Control} * 100\right) \quad \ldots (I)$$

Methods S5

The independent action (IA) model was used to examine the influence of $nTiO_2$, GO and their mixture toxicity. Based on the toxicity (%) brought about by pristine $nTiO_2$ and GO, equation (II) was utilized to determine the predicted toxicity (C_{Exp}) of the mixture.

$$C\ exp = A + B - \left(\frac{A * B}{100}\right) \quad \ldots (II)$$

where A and B, respectively, stand for the individual toxicity of GO and $nTiO_2$.

$$RI = \frac{C\ obs}{C\ exp} \quad \ldots (III)$$

where C_{Obs} stands for the observed toxicity of the mixture of GO and $nTiO_2$. Then, using equation (III), the nature of the interaction between the two nanomaterials were identified using the inhibition ratio (RI). Despite the obtained R_I values, the interaction between $nTiO_2$ and GO

was regarded as additive when the toxicity difference between C_{Obs} and C_{Exp} were statistically insignificant at the $p > 0.05$.

Methods S6: Oxidative stress

DCFH-DA is a non-fluorescent cell permeable dye. In reaction with ROS, this dye is oxidized to a highly fluorescent 2',7'-dichlorofluorescein which is detected. After72 h, 5 ml of algal cell suspension was incubated with 50 µl of DCFH-DA (100 mM) under the dark condition for 30 min. The fluorescence intensity of the samples loaded in 96 well plate (white) was measured at an excitation and emission wavelength of 485 nm and 530 nm respectively using a spectrofluorometer. The generated ROS was calculated with reference to algal cells without treatment [2].

After 72 h of interaction, treated samples were centrifuged at 4 °C for 10 min at 7000 rpm. Cell pellets were further treated with 0.25% TBA (prepared in a 10% TCA solution) and kept in a water bath at 95 °C for 30 min. The heated sample mixtures were quickly placed on ice to stop the reaction and centrifuged at 7000 rpm for 10 min. The supernatant's absorbance containing the released MDA enzyme was measured at 530 nm. By subtracting the absorbance at 600 nm (Microplate spectrophotometer, BIO-RAD xMark™) [3].

Methods S7: Photosynthetic parameters

A photosynthesis yield analyzer (Mini PAM, Heinz Walz, Germany) was used to measure the photochemical quantum yield of the PS II (Y II) system, and electron transport rate (ETR) in the treated and control algal cells. After 72 h of interaction, the control and the treated cells were placed in dark incubation for 30 min followed by addition of 200 µl of sample into the instrument chamber and passing on the high-intensity Actinic light to record the effective quantum yield of PS II (Y II) and ETR of the samples [4].

References

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