Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary information

Combined effects of P25 TiO_2 nanoparticles and disposable face mask leachate on microalgae Scenedesmus obliquus: Analysing the effects of heavy metals

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Materials and methods:

Assessing growth parameters and biochemical assays in algae

Method S1: The fluorescent dye DCFH-DA was used to calculate the total reactive oxygen species (ROS) generated by the treated algal cells. After the algae had been exposed to the toxicants for 72 h, samples were incubated with DCFH-DA dye (50 µL of 100 µM) for 30 mins in the dark. After incubation, the fluorescence intensity of the samples was evaluated using a fluorescence spectrophotometer (Cary Eclipse, G9800A; Agilent Technologies, USA). The excitation and emission wavelengths were 485 nm and 530 nm, respectively (1). The obtained intensities were compared to the intensities of the control algal cells. Under abiotic conditions, there was no apparent increase in the fluorescence intensity.

Method S2: The MDA concentration was determined by subjecting the interacted algal samples to 30 mins of heating in a water bath at a temperature of 95 °C along with the mixture of TBA and TCA (0.25% (w/v) TCA in 10% (w/v) TBA) (2). Following centrifugation, a UV-Vis spectrophotometer (xMARK microplate absorbance spectrophotometer, BIO-RAD) was used to evaluate the absorbance of the supernatants at 532 and 600 nm.

Method S3: The comprehensive method for analyzing superoxide dismutase (SOD) activity can be followed from the study published by Kono in 1978 Kono (27). After incubation, the cell pellet was recovered by centrifuging the sample at 7000 rpm for 10 mins at 4 °C, washed, and then homogenizing it in 0.5 M phosphate buffer before centrifuging it again for 20 mins at 4 °C. Following centrifugation, the following components were added to 100 µl of the supernatant: 50 mM Na_2CO_3, 96 mM nitro tetrazolium blue chloride, 0.6% triton x-100, and 20 mM hydroxylamine hydrochloride. This mixture was then incubated at 37 °C for 20 mins under visible light conditions. Following incubation, the absorbance of these samples was

determined using a UV-spectrophotometer (xMARK microplate absorbance spectrophotometer, BIO-RAD) at 560 nm.

Method S4: To perform the catalase assay, the interacted algal samples were centrifuged at 7000 rpm for 10 mins at 4 °C after being incubated for 72 h. One past study on catalase assay showed the detailed procedure to perform this experiment Romanowska-Duda, Grzesik (28). After centrifugation, they were rinsed with sterile 0.5 M PBS and homogenized. After adding 1 ml of a newly prepared H_2O_2 solution (10 mM) to 2 ml of supernatant, the absorbance at 240 nm was measured for 3 minutes using a spectrophotometer (Hitachi U-2910 Japan). The reaction mixture without H_2O_2 was used as a control.

Method S5: A photosynthesis yield analyzer (Mini PAM, made by Heinz Walz in Germany) was used to detect chlorophyll fluorescence and the electron transport rate in the samples. This was performed to quantify the level of stress that the toxicant was putting on algal photosystem II. After 72 h of exposure, the samples were put in a dark condition for 30 mins. Following that, 200 µl of the interacted algal samples were placed into the chamber of the Photosynthesis Yield Analyzer (Mini PAM), and a high-intensity actinic light was used to evaluate the Fv/Fm ratio and the ETR max value. The ratio of the maximum photochemical quantum yield of the PS II system in treated and control algal cells is denoted by the symbol Fv/Fm, where Fv stands for variable fluorescence, and Fm stands for the maximum fluorescence released by the samples when exposed to high intensities of actinic light. Graphs were used to illustrate these findings compared to the control samples.

Figures:

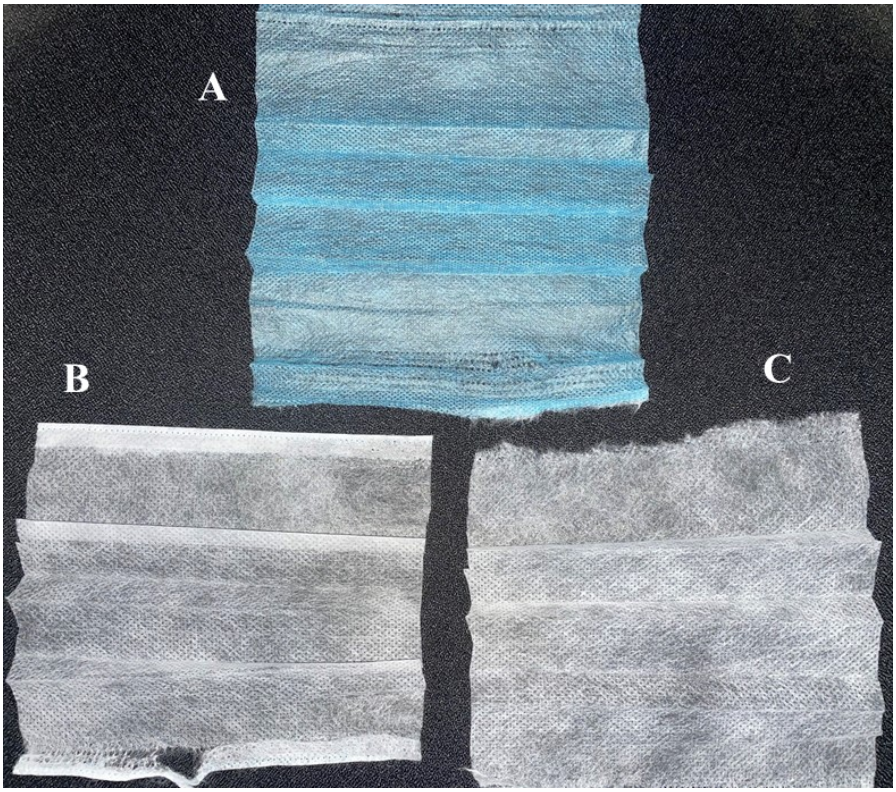

Fig. S1: Different layers of disposable surgical face mask (A) Outer (B) Middle (C) Inner

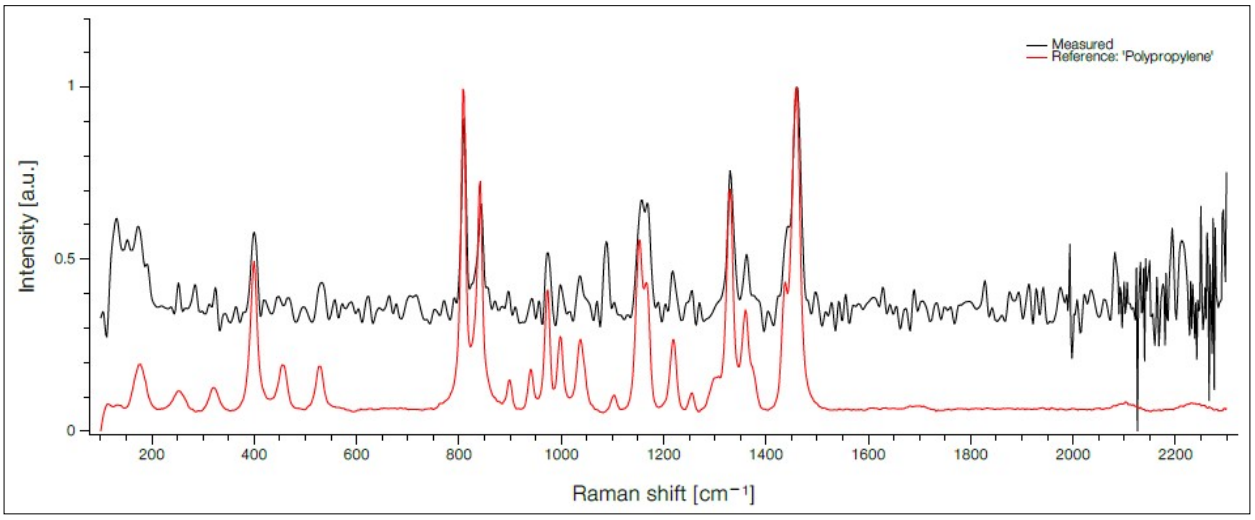

Fig. S2: Raman spectra of leachates from the face mask

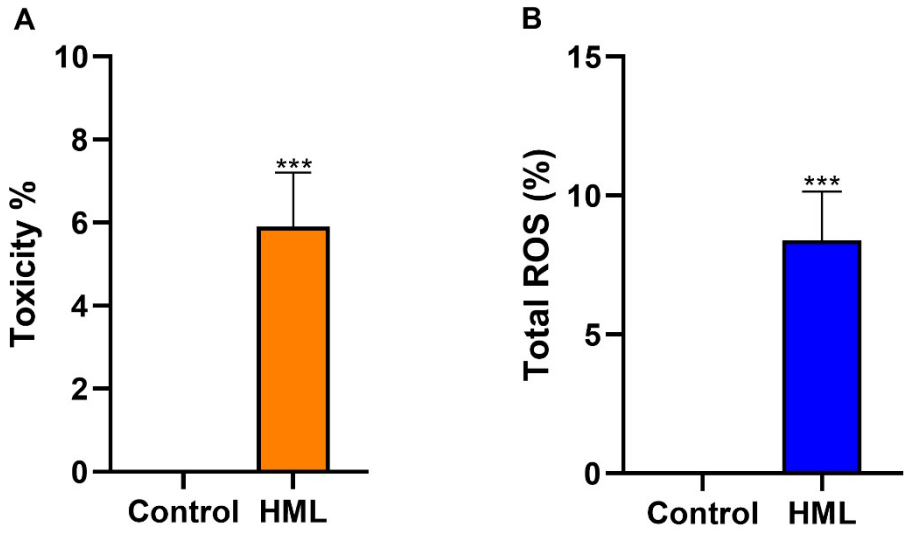

Fig. S3: (A) Toxicity percentage of HML when interacted with *Scenedesmus obliquus* (B) Total ROS produced when interacted with FML. '*' symbolizes a significant difference in percentage observed with respect to control (* = p < 0.001)**

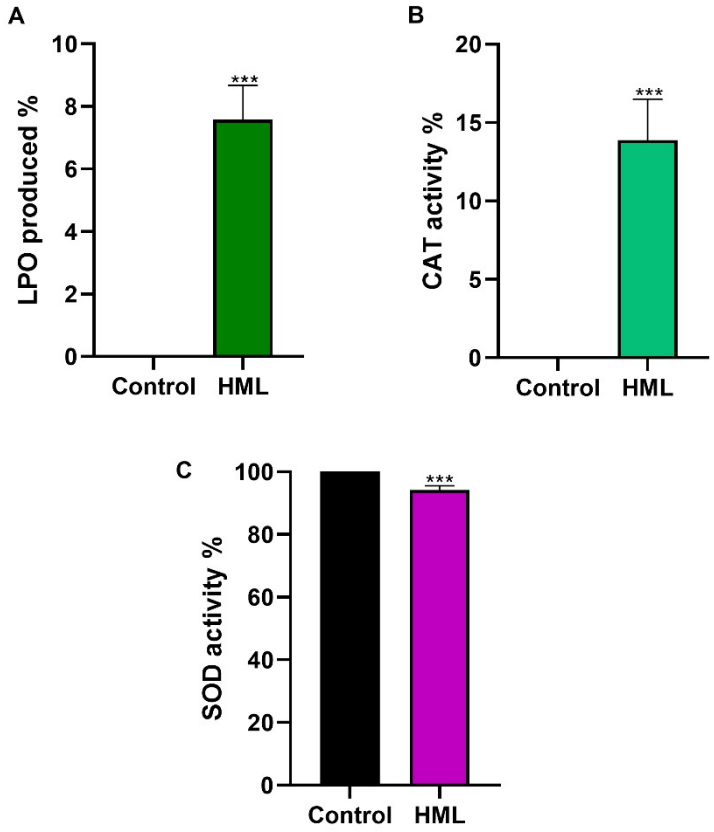

Fig. S4: (A) LPO produced when interacted with HML (B) CAT activity when interacted with HML (C) SOD activity when interacted with HML. '*' symbolizes a significant difference in percentage observed with respect to control (* = p < 0.001)**

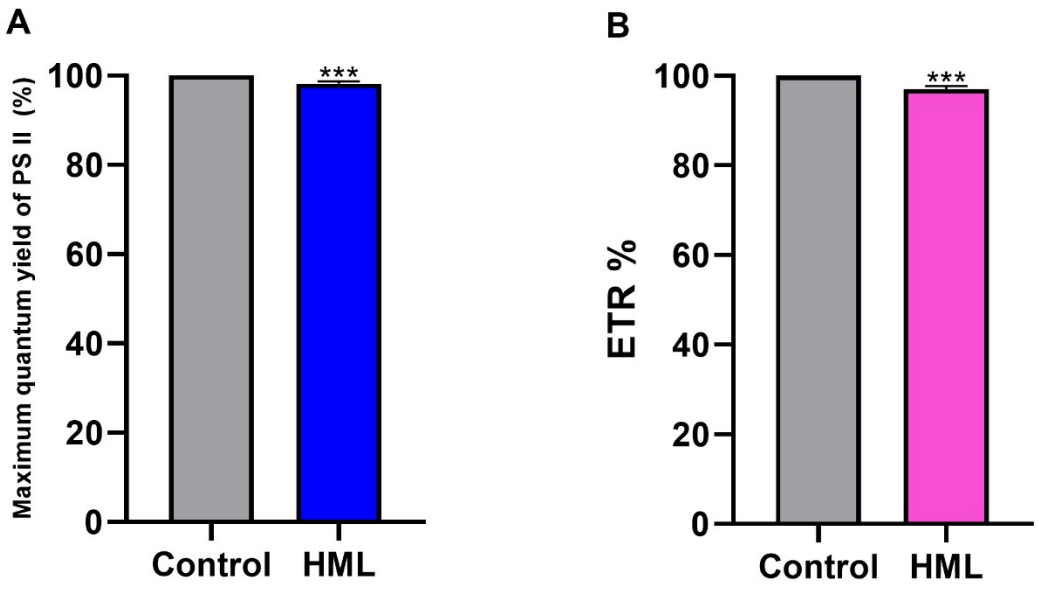

Fig. S5: **Fig. 6**: **(A)** Maximum quantum yield of PSII after exposure to HML **(B)** Electron transport rate after exposure to HML. '*' symbolizes a significant difference in percentage observed with respect to control (*** = $p < 0.001$).

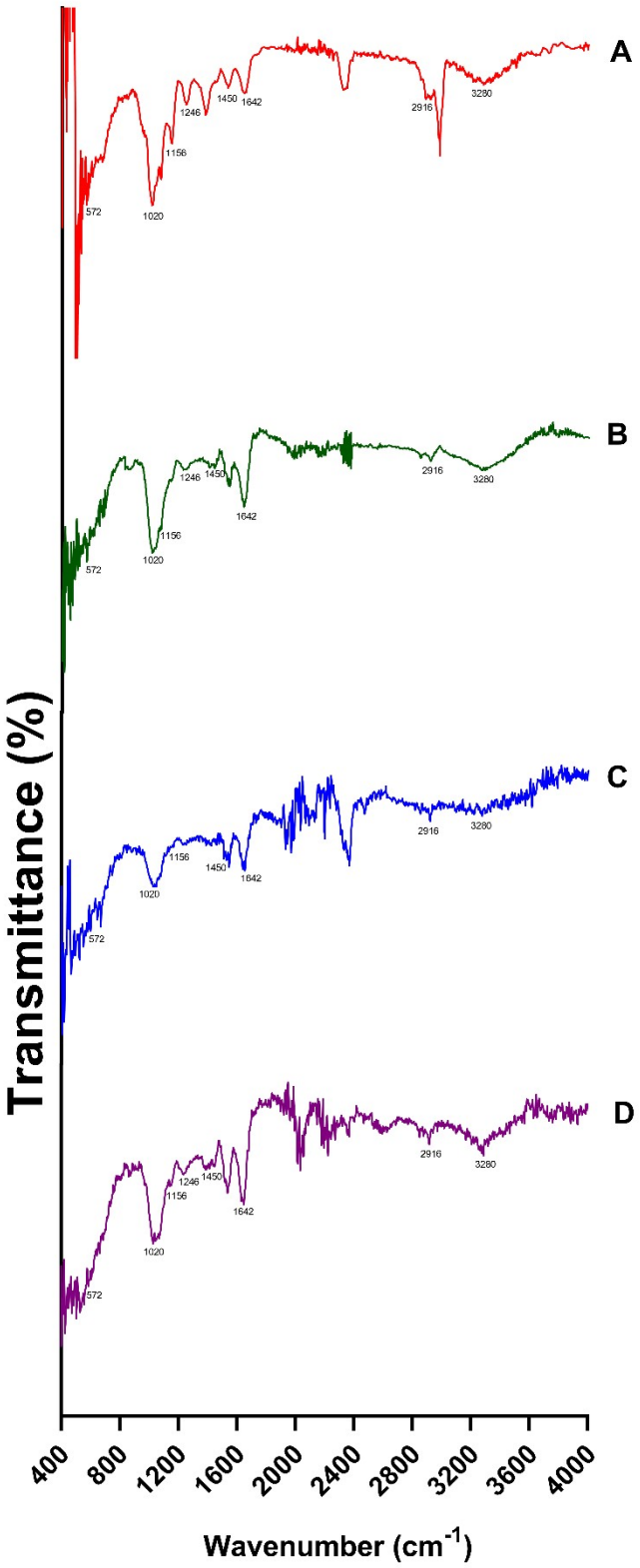

Fig S6: FTIR spectra of algae when treated with (A) Control (B) 4 mg L^{-1} of $nTiO_2$ (C) FML (D) $nTiO_2$ (4 mg L^{-1}) + FML

Tables:

Elemental Symbol	Concentration in ppm (mg/L)
Cd	0.010
Cr	0.006
Cu	0.005
Hg	0.356
Pb	0.006
Sb	0.027
Ti	0.342
Zn	0.015

Table S1: ICP-OES analysis of filtered face mask leachate

Wavenumber (cm^{-1})	Pristine $nTiO_2$ treated	Pristine FML treated	$nTiO_2$ + FML treated	Peak assignment
572	↓	↑↑	↑	C=O bending
850	↑↑	↓	↑	Attributed to the presence of Ti-O-Ti
1020	↑	↑↑	↑	C-O stretching
1156	↓	↑↑	↑	C-O-C stretching

1246	↓	↑	↑	Protein amide III, α-helix structure
1450	↓↓	↑	↑	CH_3 and CH_2 bending
1642	↑	↑	↑	C-O stretching
2916	↓↓	↑	↑	Asymmetric vibrations of nucleic acid
3280	↑	↑	↑	-NH and –OH bond stretching of lipids

Table S2: Spectral changes upon treatment with $nTiO_2$, FML and $nTiO_2$ + FML

↑: Increased change in % Transmittance; ↑↑: Strongly increased change in % Transmittance;

↓: Decreased change in % Transmittance; ↓↓: Strongly change in % Transmittance

References

1. Giri S, Mukherjee A. Ageing with algal EPS reduces the toxic effects of polystyrene nanoplastics in freshwater microalgae Scenedesmus obliquus. Journal of Environmental Chemical Engineering. 2021;9(5):105978.

2. Natarajan L, Jenifer MA, Chandrasekaran N, Suraishkumar G, Mukherjee A. Polystyrene nanoplastics diminish the toxic effects of Nano-TiO2 in marine algae Chlorella sp. Environmental Research. 2022;204:112400.