Interactive toxicity effects of metronidazole, diclofenac, ibuprofen, and differently functionalized nanoplastics on marine algae *Chlorella variabilis*

Supplementary Information

Tables:

Table S1. Composition of Artificial Sea Water per 1000 ml

Chemicals	Grams
Sodium Chloride	26.29
Potassium chloride	0.74
Calcium chloride	0.99
Magnesium chloride	6.09
Magnesium sulphate heptahydrate	3.94

Table S2. Preparation of Conway medium

Table S2.1 Preparation of stock 1 per 100 ml

Chemicals	Grams
Zinc chloride	2.1
Cobalt chloride	2.0
Ammonium molybdate	0.9
Copper sulphate	2.0

Table S2.2 Preparation of stock 2 per 100ml

Chemicals	Milligrams
Vitamin B12	10
Vitamin B6	10

Table S2.3 Preparation of stock 3 per L

Chemicals	Grams
Iron chloride hexahydrate	1.3
Manganese chloride	0.36
Boric chloride	33.6
Ethylenediaminetetraacetic acid	45.0
Sodium dihydrogen phosphate dehydrate	20.0
Sodium nitrate	100

Materials and Methods

Methods S1: Chemical used

Metronidazole, Diclofenac sodium salts, Ibuprofen, and 2′,7′ -dichlorofluorescein diacetate (DCFH-DA) were purchased from Sigma Aldrich. Aminated (NH_2) and carboxylated (COOH) polystyrene nanoplastics (600 nm) were purchased from Corpuscular, Inc, USA. DMSO (Dimethyl sulfoxide), Hydrogen peroxide (H_2O_2), and nitroblue tetrazolium (NBT) dye were purchased from SDFCL (Mumbai). Triton-X-100, Hydroxylamine hydrochloride, Trichloroacetic acid (TCA), and thiobarbituric acid (TBA) were purchased from Hi-Media Pvt. Ltd (Mumbai, India).

Method S2: Determination of observed concentration of pharmaceutical products

The observed concentration of PPs was determined using ultra-performance liquid chromatography (UPLC). The samples were filtered using a 0.22 µm filter before undergoing analysis. A C18 1.7µm (2.1 × 150 mm) column was utilized to separate and quantify all PPs. In the case of metronidazole, the mobile phase was isocratically eluted at a flow rate of 0.5 mL/min, consisting of water: acetonitrile (90:10, v/v) [1]. Similarly, for diclofenac, a mobile phase of 0.05 M acetate buffer (pH 2.5) and acetonitrile (50:50, v/v) was employed with a flow rate of 0.5 ml/min [2]. Likewise, the mobile phase for ibuprofen was a mixture of acetonitrile and water (pH 3 adjusted by diluted acetic acid). The separation system comprised 50% acetonitrile and 50% H2O, with a flow rate of 0.2 mL/min [3].

Methods S3: Independent action modeling

The expected toxicity (C_{Exp}) of the mixture was determined by combining the individual toxicities of PPs and PSNPs using equation (1).

$$C_{Exp} = P+N – (P*N/100) \quad (1)$$

In the equation, P and N represent the respective toxicity induced by PPs and PSNPs. Once C_{Exp} is calculated, the ratio of inhibition (R_I) is determined using the provided equation (2).

$$R_I = \text{Observed Toxicity } (C_{Obs}) / \text{Expected Toxicity } (C_{Exp}) \quad (2)$$

Where C_{Obs} represent the observed toxicity resulting from the combination of PPs and PSNPs. The nature of the interaction is determined based on the computed value of R_I. If the R_I value is equal to 1, the interaction is considered additive. If the calculated R_I value is less than 1, the interaction is deemed antagonistic. Conversely, if the R_I value obtained is greater than 1, the interaction is characterized as synergistic.

To validate the computed R_I value, statistical significance between C_{Obs} and C_{Exp} values was assessed using Two-way ANOVA (Bonferroni post-test). Despite the obtained R_I values, the interaction between PPs and PSNPs was regarded as additive when the toxicity difference between C_{Obs} and C_{Exp} was statistically insignificant ($p > 0.05$) [4].

Figures

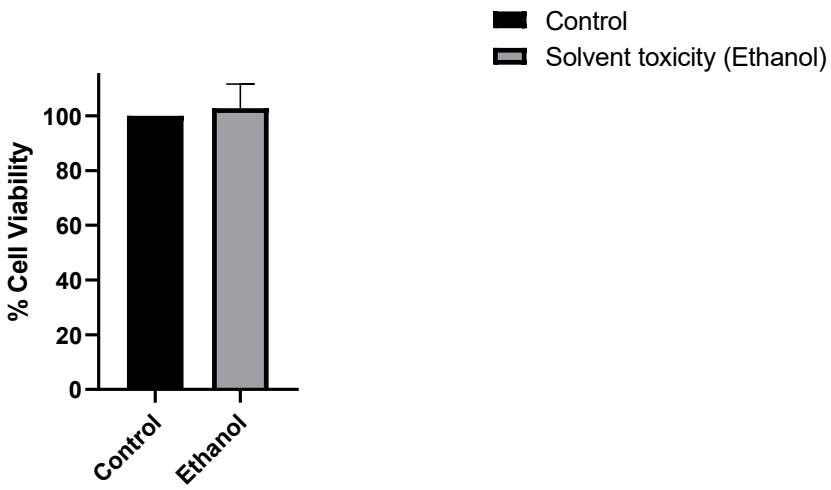

Fig. S1. Percentage cell viability on exposure to 0.05% of ethanol

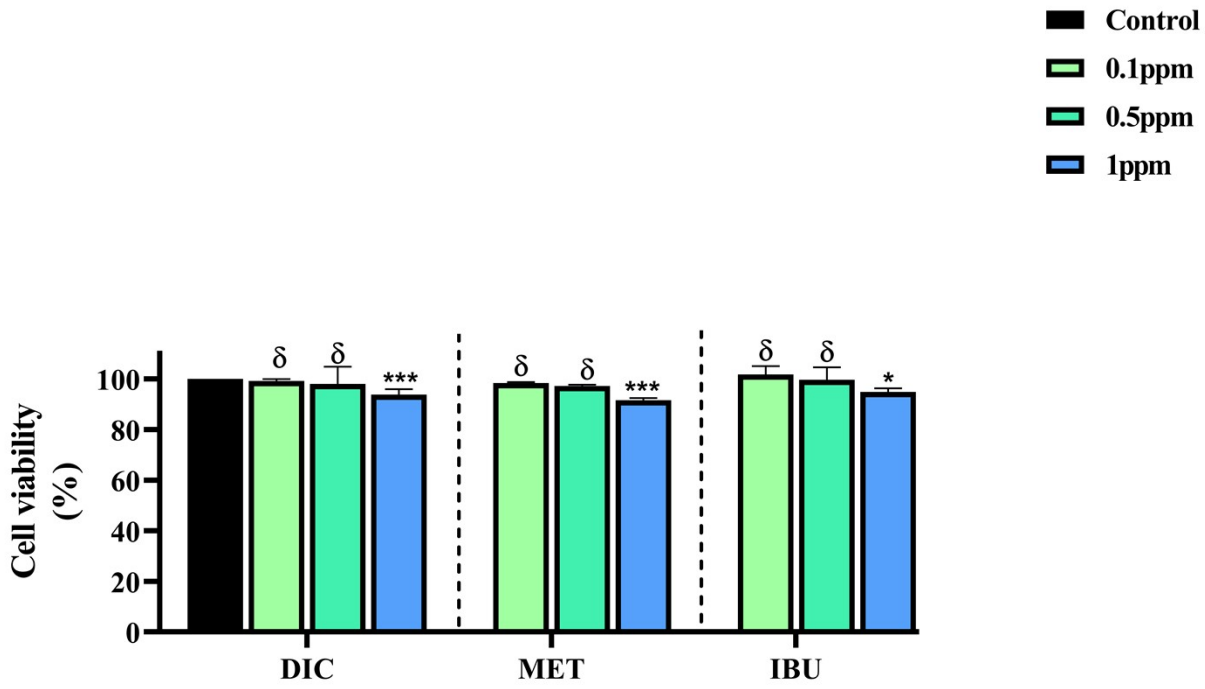

Fig. S2 Percentage cell viability of algal cells on exposure to metronidazole (MET), diclofenac (DIC), and ibuprofen (IBU). Note: '*' represents the significant difference between the control and test groups; δ represents no significance when compared to control group

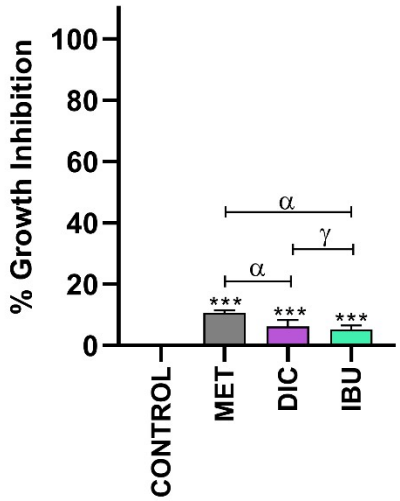

Fig. S3 Percentage growth inhibition of algal cells on exposure to metronidazole (MET), diclofenac (DIC), and ibuprofen (IBU). Note: '*' represents the significant difference noted between the control and test groups. The significant difference between the pristine NPs and the binary mixtures of NPs with PPs are represented using 'α,' and 'γ' ($\alpha = p < 0.001$) and ($\gamma = p < 0.05$)

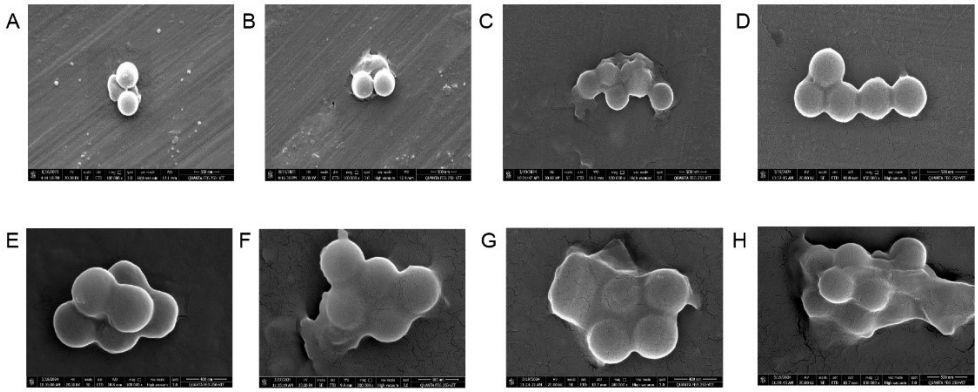

Fig. S4 FE-SEM images of (A) pristine NH_2 PSNPs; (B) pristine COOH PSNPs; (C) combination of NH_2 PSNPs + MET; (D) NH_2 PSNPs + DIC; (E) NH_2 PSNPs + IBU; (F) COOH PSNPs + MET; (G) COOH PSNPs + DIC; (H) COOH PSNPs + IBU

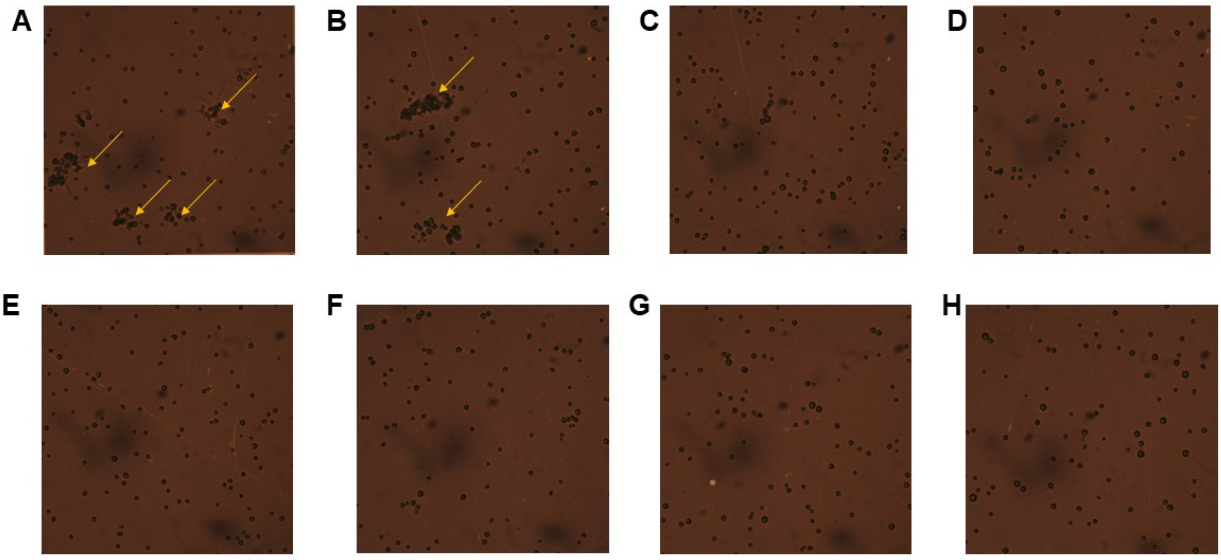

Fig. S5 Optical microscopy image of algal cells interacted with (A) pristine NH_2 PSNPs; (B) pristine COOH PSNPs; (C) combination of NH_2 PSNPs + MET; (D) NH_2 PSNPs + DIC; (E) NH_2 PSNPs + IBU; (F) COOH PSNPs + MET; (G) COOH PSNPs + DIC; (H) COOH PSNPs + IBU

References

1 G. A. Soares, G. S. Rodrigues, L. P. Buranello, R. B. de Oliveira and J. R. de Arruda Miranda, Pharmacomagnetography assessment of the prokinetic effect on metronidazole absorption, *Journal of Pharmacy and Pharmacology*, 2023, rgad088.

2 E. M. Elzayat, M. F. Ibrahim, A. A. Abdel-Rahman, S. M. Ahmed, F. K. Alanazi and W. A. Habib, A validated stability-indicating UPLC method for determination of diclofenac sodium in its pure form and matrix formulations, *Arabian Journal of Chemistry*, 2017, **10**, S3245–S3254.

3 A. A. Ahmed-Anwar, M. A. Mohamed, A. A. Farghali, R. Mahmoud and M. E. M. Hassouna, Green UPLC method for estimation of ciprofloxacin, diclofenac sodium, and ibuprofen with application to pharmacokinetic study of human samples, *Sci Rep*, 2023, **13**, 17613.

4 V. Thiagarajan, V. Iswarya, A. J. P., R. Seenivasan, N. Chandrasekaran and A. Mukherjee, Influence of differently functionalized polystyrene microplastics on the toxic effects of P25 TiO2 NPs towards marine algae Chlorella sp., *Aquatic Toxicology*, 2019, **207**, 208–216.