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Polystyrene nanoplastics and wastewater displayed antagonistic toxic effects due to the sorption of wastewater micropollutants

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Abstract

The knowledge about the interaction of nanoplastics with other aquatic pollutants and their combined effects on biota is very scarce. In this work, we studied the interaction between polystyrene nanoplastics (PS NPs) (30 nm) and the micropollutants in a biologically treated wastewater effluent (WW). The capacity of PS NPs to sorb micropollutants was studied as well as their single and combined toxicity towards three freshwater organisms: the recombinant bioluminescent cyanobacterium, *Anabaena* sp. PCC 7120 CPB4337; the duckweed, *Spirodela polyrhiza* and the cladoceran, *Daphnia magna*. The endpoints were the inhibition of bioluminescence, the growth inhibition of the aquatic plant and the immobilization of *D. magna* after 24, 72 and 48 h of exposure, respectively. Combination Index (CI)-isobologram method was used to quantify mixture toxicity and the nature of interactions. PS NPs sorbed a variety of chemicals present in WW as micropollutants in a range of tens of ng/L to μg/L. It was found that those pollutants with positive charge were the main ones retained onto PS NPs, which was attributed to the electrostatic interaction with the negatively charged PS NPs. Regarding the toxicological effects, single exposure to PS NPs affected the three tested organisms. However, single exposure to WW only had a negative impact on the cyanobacterium and *S. polyrhiza* with no observed toxicity to *D. magna*. Regarding PS NPs-WW combined exposure, a reduction of toxicity in comparison with single exposure was observed probably due to the sorption of micropollutants onto PS NPs, which resulted in lower bioavailability of the micropollutants. In addition, the formation of PS NPs-WW heteroaggregates was observed which could result in lower bioavailability of PS NPs and sorbed micropollutants, thus lowering toxicity. This study represents a near-realistic scenario approach to the potential sorption of wastewater pollutants onto nanoplastics that could alter the toxicological effect on the biota.

Keywords: Polystyrene nanoplastics; Wastewater micropollutants; Sorption; Toxicological interactions; Freshwater organisms; Joint toxicity

1. Introduction

The existing body of evidence on the distribution, fate, and effects of the smaller size fraction of plastic debris, called nanoplastics (NPs, size <1 μm or <100 nm, depending on the classification) is very scarce (Besseling et al., 2019; Gigault et al., 2018). NPs potentially represent the most hazardous fraction of plastic fragments due to their physicochemical properties such as high surface-to-volume ratio and hydrophobicity (Koelmans et al., 2015; Gaylarde et al., 2021). The detection of NPs in natural environments is still challenging because of the lack of suitable analytical methods for assessing their presence and concentration in real matrixes (Cai et al., 2021; Wang et al., 2021). Accordingly, only a few authors reported results on the presence of NPs in natural environments: Ter-Halle et al. (2017) found the presence of several populations of highly polydisperse particles on the nanoscale (1 to 1000 nm) in the North Atlantic Subtropical Gyre. They calculated the mean relative proportion of each type of nanoplastic according to the anthropogenic pyrolytic fingerprint but could not give absolute concentration numbers. The study by Materić et al., 2020 on micro- and nanoplastics in Alpine Snow used thermal desorption-proton transfer reaction-mass spectrometry and could quantify micro-nanoplastics in the ng/mL range with a maximum value of 23.6 ± 3.0 ng/mL for PET micro-nanoplastics in filtered snow. Furthermore, the observed increase of degradation and fragmentation of larger fractions in the environment, could indicate that the number of NPs in natural ecosystem is much higher than that of MPs (Sorasan et al., 2021).

As microplastics (MPs, size < 5mm), NPs also can be classified as primary, if originally manufactured in that size, or secondary if they are the consequence of the weathering (photodegradation, mechanical abrasion, hydrolysis, or biodegradation) of MPs (Gaylarde et al.,

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2021). Wastewater treatments plants (WWTP) represent an important source of entry of NPs in the environment because the current systems do not retain nanoparticles (Frehland et al., 2020; Xiong et al., 2020). Therefore, NPs are expected to be released to freshwater ecosystems though effluent discharge although no data are still available on their concentration in WWTP effluents (Murray and Örmeci, 2020; Pico et al., 2019). Other additional terrestrial sources are tyre abrasion and the use of sewage sludge as soil amendment (Auta et al., 2017; Edo et al., 2020; Peng et al., 2020).

Several negative effects of NPs in organisms belonging to different trophic levels have been described, including responses on growth, reproduction, feeding, metabolic activities, immunity, or behavioural disorders (Chae and An, 2017; Agathokleous et al., 2021; Ramasamy and Palanisamy, 2021; Redondo-Hasselerharm et al., 2021; TamayoBelda et al., 2021). Interestingly, Kögel et al. (2020) showed that the lower fraction of plastic particles (< 10 μm) presents higher toxicity than larger ones. Previous studies have shown the capacity of NPs (24-51 nm) to be transferred to higher trophic levels via food chains (Mattsson et al., 2015; Chae et al., 2018). Specifically on aquatic organisms representative of low levels of the trophic web, several authors reported inhibitory effects on the growth and pigments of *Dunaliella tertiolecta*, *Anabaena* sp. PCC7120, *Pseudokirchneriella subcapitata*, *Scenedesmus obliquus* and *Chaetoceros neogracile* (Besseling et al., 2014; Sjollema et al., 2016; Nolte et al., 2017; González -Férnandez et al., 2020, Tamayo-Belda et al., 2021), or effects on survival, reproduction and defence system on *Daphnia pulex* and *Daphnia magna* (Besseling et al., 2014; Mattsson et al., 2017; Liu et al., 2020b). Compared to MPs, NPs exhibit greater affinities towards contaminants, which represents a risk of increased transfer to the biota (Ma et al., 2016). The lower size of NPs may favor the internalization of sorbed pollutants potentially inducing enhanced toxic responses (Bhargava et al., 2018).

The risk assessment of pollutant mixtures has been reported as an imperative and emerging concern topic (Yu et al., 2019; Jacob et al., 2020). A certain body of information is available about the interaction between MPs and pollutants, such as heavy metals, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ethers, and pharmaceuticals and personal care products (Chae and An, 2017; Wang et al., 2020b; Gaylarde et al., 2021; González-Pleiter et al., 2021; Tang, 2021; Verdú et al., 2021; Xiang et al., 2022). However, very limited research has been performed on the joint toxicity exerted by chemical pollutants and NPs. Ma et al., (2016) showed that the presence of polystyrene (PS) NPs increased the bioaccumulation and bioconcentration factor of phenanthrene in *Daphnia magna*. Synergistic effects were also observed in zebrafish embryos exposed to combinations of NPs (PS, 50-500 nm) and silver (Lee et al., 2019) and combinations of NPs (50 nm) with 2.2', 4.4'-tetrabromodiphenyl ether (BDE-47) and triclosan on marine rotifer *Brachionus koreanus* (Jeong et al., 2018). On the contrary, Bellingeri et al. (2019) did not observe that PS-COOH NPs (87-106 nm) altered the toxicity of copper to *Raphidocelis subcapitata,* whereas Zhang et al. (2018) showed that the presence of nPSNH2 NPs (200 nm), decreased the toxicity of glyphosate to the cyanobacterium *Microcystis aeruginosa*. An antagonistic effect was also observed by Trevisan et al. (2019), who studied the toxic effects of a mixture of PAHs and PS NPs (44 nm) on zebrafish and by Lian et al. (2020) in the co-exposure of wheat (*Triticum aestivum* L.) to PS NPs and cadmium; also by Wang et al. 2020a who reported lower toxicity and bioaccumulation of ibuprofen in the presence of PS NPs (600 nm) on freshwater microalgae *Chlorella pyrenoidosa*.

The aim of this work was to shed light on the interaction and combined effects of NPs with chemical pollutants under realistic exposure scenarios. For it, we studied the capacity of PS NPs to sorb the pollutants present in a real WWTP effluent, as well as their joint toxicity to the aforementioned freshwater organisms by means of the combination index (CI)-isobologram method (Rodea-Palomares et al., 2010). Up to our knowledge this is the first combined toxicity study between NPs and other pollutants using real wastewater. In this study we chose three freshwater organisms: two primary producers, a cyanobacterium, *Anabaena* sp., CPB4337 and a vascular plant, *Spirodela polyrhiza* which are at the base of aquatic food webs, and the cladoceran crustacean *Daphnia magna* as a primary consumer. Because of their ecological relevance in the aquatic food webs and sensitivity to different substances, these three organisms have been previously used in a number of ecotoxicology studies (Böcük et al., 2013; González-Pleiter et al., 2019; Greenberg et al., 1992; Martín-de-Lucía et al., 2017; Valimaña-Traverso et al., 2019; Wang et al., 2021).

2. Experimental section

2.1. Materials and physicochemical characterization

Wastewater (WW) was collected from the treated effluent of a conventional activated sludge WWTP located near Madrid (Spain). The plant treats a mixture of industrial and domestic wastewater with a nominal capacity of 3000 m3/h. WW was filtered using a Millipore Stainless 47 mm pressure holder by 0.7 μm glass fibre filters (Whatman® glass microfiber filters, Grade GF/F) and kept frozen (-20 °C) in glass bottles until runs and analyses. The main quality parameters were recorded. Conductivity and pH were measured during sample collection (Crison MM40+ and pH 25+). Total organic carbon (TOC) was analysed using a TOC analyser (Shimadzu, TOC-VCSH). Nutrients were quantified by ion chromatography with a Metrohm 930 Compact IC Flex apparatus. The main water quality

data are summarized in Table S1 (Supplementary Material, SM).

Polystyrene nanoplastics (PS NPs) of 30 nm (latex aqueous suspensions, 5000 series, refractive index 1.59 at 589 nm, density 1.05 g/cm3) were acquired from Thermo Scientific. From the commercial stock, a 1:100 dilution was prepared in Milli-Q® water with a concentration of 10 g/L, which was stored at 4 ºC until use. Prior to any determinations and experiments, PS NPs suspensions were sonicated for 30 s in an ultrasonic bath (Fisher Scientific FB15060) to avoid possible aggregates (PS NPs size as determined by DLS after ultrasonication was 24.2 ± 1.3 μm). The experimental micro-FTIR spectra of PS NPs is presented in Fig. S1 (SM). The particle size and ζ-potential of PS NPs suspensions, in ultrapure water, biological media (microalgae, plant and *D. magna* culture media) and WW, were determined by dynamic light scattering (DLS) and electrophoretic light scattering (Zetasizer Nano ZS, Malvern Instruments) at room temperature using 200 mg/L PS NPs suspensions

2.2. Adsorption studies and analysis of micropollutants

The analysis of micropollutants was performed in: i) raw WW, ii) WW ultrafiltered through Sartorius Vivaspin 20 (50 kDa) polyethersulfone ultrafiltration centrifuge tubes, and iii) methanol extracts of the filters after filtrating suspensions of PS NPs in contact with WW. The contact between WW and PS NPs was performed using different concentrations of NPs (10, 50, 100 and 200 mg/L) for 24 h, in the darkness, at room temperature, and under constant stirring (100 rpm). We chose NPs concentrations in this range as lower NP concentrations did not allow to detect and quantify micropollutants such as those present in WW (Martin de Lucia et al., 2017). The detection and quantification of micropollutants were performed following the steps specified in the Supplementary Text (SM).

2.3. Experimental design

The individual and combined toxicity of PS NPs and WW was assayed using a full factorial experimental design as described below. To study the individual effect of PS NPs suspensions, a wide range of concentrations, namely 0, 12.5, 25, 50, 100 and 200 mg/L were tested. Prior to use the stock was sonicated as described in the previous section. In addition, ultrafiltrates of PS NPs suspensions were tested to check whether dissolved chemicals potentially present, such as additives or surfactants included in commercial stocks, could cause negative effects on the studied organisms. For it, PS NPs suspensions at the higher tested concentration were filtered through 50 kDa (removing all the particles larger than ~5 nm). Previously homogenised by magnetic stirring, WW was serially diluted using distilled water (dH2O) to obtain five dilutions in the 0.0625-1 range (0.0625, 0.125, 0.25, 0.5 and 1 expressed as dilution factors, d.f.; which corresponded to 1:16, 1:8, 1:4, 1:2 and 1:1 of WW-dH2O dilutions respectively). To test the effect of the combination, binary mixtures were performed as shown in Fig. S2 (SM). In total 35 treatments and control were conducted.

2.4. Bioassays

The recombinant bioluminescent cyanobacterium *Anabaena* sp. PCC 7120 strain CPB4337 (hereinafter *Anabaena* CPB4337) was used in this work as toxicity reporter based on the inhibition of its constitutive luminescence caused by the presence of a toxic substance (Rodea-Palomares et al., 2010). This recombinant strain bears in the chromosome a Tn5 derivative with *luxCDABE* from the luminescent terrestrial bacterium *Photorhabdus luminescens* (Fernández-Pinas and Wolk, 1994; Martín-deLucía et al., 2018; Martín-de-Lucía et al., 2017). *Anabaena* CPB4337 was routinely cultured and maintained in sterilized conditions in Allen & Arnon medium diluted eight-fold supplemented with 5 mM of nitrate (Table S2, SM) and 10 mg/mL of neomycin sulphate (Rodea-Palomares et al., 2010) (hereinafter AA/8 +N) in 100 mL in 250 mL Erlenmeyer flasks. The organism was grown under continuous light (ca. 60 μmol photons m^2 s^{-1}) on a rotary shaker (135 rpm) at 25 ºC for 72 h, when midlogarithmic phase is reached. The luminescence inhibition-based toxicity bioassays were conducted in 25 mL Erlenmeyer glass flasks in a final volume of 10 mL for each treatment (PS NPs, WW, and their binary mixtures, as it is described in the previous section). Non-treated cells were used as control. Each treatment was tested by triplicate. *Anabaena* CPB4337 cells were centrifuged and washed twice and were inoculated to reach a final cell density at OD750nm of 0.2. The bioluminescent inhibition was measured after 24 h of exposure as follows: 100 mL of each sample were transferred to an opaque white 96 well microtiter plate and bioluminescence was recorded in a Centro LB 960 luminometer during 10 min (González-Pleiter et al., 2013). A confocal microscope (Leica TCS SP5 system, Germany) was used to visualize cells of *Anabaena* CBP4337 exposed to 200 mg/L PS NPs, WW dilution 1:1 and the binary combination of them. The excitation laser was set at 488 nm and emission filter at 665 nm for chlorophyll *a*.

The aquatic plant *S. polyhriza* (duckweed) acute test was performed using Duckweed Toxkit F™ kit (MicroBioTests) according to both the manufacturer's instructions and the International Standard ISO 20227: 2017. The duckweed toxicity test was prepared by sprouting the turions for 72 h in standardised Steinberg medium (Table S3, SM), under controlled conditions (25 °C; continuous 6000 lux-light photoperiod; IBERCEX chamber, Spain). The exposure experiment was carried out in transparent 5 mL sterile glass beakers, filled with 2 mL per beaker of each tested sample (different PS NPs concentrations, WW

dilutions, and their binary mixture, as described in the preceding section), which were inoculated with one fresh, healthy, and uniform frond sized duckweed subsequently incubated for 72 h (25 °C; continuous 6000 lux-light photoperiod). Non-treated plants were used as control Each treatment was tested by triplicate. The grown fronds of the duckweeds were digitally recorded at 0 and 72 h. Growth inhibition (%) of the aquatic plant was determined by area measurement of the first frond using digital image processing (Image J software, National Institute of Health, USA). Chlorophyll fluorescence of its components (bud, leave, and root) was slso evaluated by confocal imaging (Leica TCS SP5 system, Germany, λex/λem 488/595-700 nm).

The aquatic cladoceran crustacean *Daphnia magna* was used in order to test the acute immobilization assay following the protocol described in OECD TG 202 (OECD, 2004). The assays were conducted using a commercial test kit (Daphtoxkit F). The medium used was the Standard Freshwater (ISO formula as shown in Table S4, SM), adjusted at pH 7.3 and aerated. Neonates were obtained by ephippia hatching, incubated at 21 ºC under continuous illumination (6000 lux) for 72-80 h. 2 h prior to the beginning of acute tests, neonates < 24 h old were fed with a suspension of *Spirulina* microalga. The test was performed in 25 mL glass beakers with 10 mL of each treatment suspensions (PS NPs, WW, and their binary mixtures). Non-treated organisms were used as control. Each treatment was run by quadruplicated (single exposures) or triplicate (binary mixture exposures) according to the experimental design explained before. In each beaker, five neonates were added and incubated in darkness for 48 h at 21 ºC. The toxicity was evaluated observing the mobility of the neonates. The individuals were considered immobile when they were unable to swim after a gentle stimulation with the tip of a Pasteur pipette for 15 s. Micrographs were captured using an Olympus CX41 microscope with digital colour camera (Olympus, DP20) at 200 mg/L PS NPs, non-diluted WW, and their binary mixture.

2.5. Evaluation of toxicity parameters

Bioluminescence inhibition (%), plant growth inhibition (%), and daphnids immobilization (%) after the different exposures were calculated for each PS NPs concentration and for each WW dilution. The median effective concentration (EC50) values for PS NPs or WW dilution individually, which were those causing 50 % inhibition with respect to the non-treated control, as well as the corresponding confidence intervals, were obtained by non-linear fitting to parametric functions (3 or 4-parameters logistic function). To assess the significance of differences between treatments, one-way analysis of variance (ANOVA) and post-hoc Tukey's HSD test were performed (previously, assumptions of normality were checked). When $p < 0.05$, the difference was considered statistically significant. To evaluate the mixture toxicity, data were analyzed using Compusyn software, which uses the combination index (CI)-isobologram method (Chou and Talalay, 1984). The effect of the nanopolymer mixture was determined using the median-effect equation based on the mass action law. (Chou, 1976). For each effect level, the value of the combination index can be calculated according to the general CI equation:

$$(CI)_x{}^n = \sum_{j=1}^{n} \frac{(D)_j}{(D_x)_j} = \sum_{j=1}^{n} \frac{(D_x)_{1-n}\left(\frac{(D)_j}{\sum_1^n (D)}\right)}{(D_m)_j \left(\frac{(f_{ax})_j}{1-(f_{ax})_j}\right)^{1/mj}}$$

In this equation, D is the concentration of chemical , D_m is the EC50, f_a is the fraction affected by concentration D (e.g 0.25 correspond to 25 % of inhibition) and f_u is the unaffected fraction (f_a =1- f_u), m is the coefficient of the sigmoidicity of the dose-effect curve (being m=1, hyperbolic; m > 1 sigmoidal and m < 1 negative sigmoidal), $(CI)_x{}^n$ is the combination index of n chemicals for a given f_a, $(D_x)_{1\text{-}n}$ is the sum of the doses of the n compounds that exert x % inhibition of the endpoint studied in combination; $((D)_j/\sum(D))$ is the proportion of the dose of each n compounds that exert x % of the endpoint; $(D_m)j((f_{ax})_j/1 - (f_{ax})_j)^{1/mj}$ is the dose of each drug separately that exerts the inhibition of x %. From the equation CI < 1, CI = 1 and CI > 1 indicates synergism, an additive effect and antagonism, respectively.

2.6. Quality Assurance/Quality Control assessment

A complete description of Quality Assurance/Quality Control assessment (QA/QC) based on de Ruijter et al. (2020) and a QA/QC evaluation score is provided as Table S5 (SM).

3. Results and discussion

3.1. Physicochemical characterization

The physicochemical properties of PS NPs suspensions for each WW dilution and culture medium are shown in Table S6 for contact time corresponding to the maximum exposure of each bioassay. Measurements in distilled water (pH 6.5) were also performed. In distilled water, the average size of the hydrodynamic diameter measured by DLS was 25.0 ± 0.1 nm, indicating that PS NPs did not aggregate. The DLS size of the background colloid without PS NPs was 354 ± 36 nm in WW, and 1247 ± 256 nm, 408 ± 90 and not detectable (by DLS) in AA/8+N, Steinberg, and Standard Freshwater, respectively. Table S6 shows the existence of aggregates reaching the micron-size range for the lowest WW dilutions (higher WW concentrations), both, for Steinberg and Standard Freshwater media. In Standard Freshwater medium, DLS sizes could not be recorded in suspensions with higher WW concentrations because it reached the upper limit of detection of the equipment. Regarding ζ-potential, surface charge was negative due to the well-known fact that plain PS latexes bears a net negative

charge as a result of the interaction of the polymer phase with water (Kamel et al., 1981). Furthermore, and consistent with the surface charge of background media (WW, -20.1 ± 4.3; AA/8+N, -27.7 ± 1.3; Steinberg, -4.6 ± 2.1; Freshwater, -5.6 ± 1.5), the results showed that PS NPs generally contributed to an increase of the negative charge of colloids.

The aggregation of nanoparticles in aquatic environments has been previously reported (Surette and Nason, 2019). The Derjaguin-Landau-Verwey-Overbeek (DVLO) theory explains the aggregation behaviour of aqueous dispersions, focusing on the effect of the forces of attraction and repulsion and points out the relevance of ionic strength, so the stability of the nanoparticles lowers when the ionic strength increases due to the compression of the double layer that increases the particle attachment efficiency (Shams et al., 2020; Sharma et al., 2021). The conductivity values for all WW-dH2O dilutions are reported in Table S7 (SM). Furthermore, in AA/8+N medium the presence of PSNPs aggregates was not observed. A possible explanation is the presence of Na2EDTA in AA/8+N culture medium (and, to a lower extent, in Steinberg medium). Na2EDTA acts as anticoagulant and stabilizer, so reducing the tendency of colloids to aggregate (Martinez-Andrade et al., 2018). Shams et al. (2020), studied the aggregation kinetics and stability of PS NPs (28 nm) over a wide range of water chemical compositions, and showed the relevance of ionic strength and salt types on their stability. Accordingly, $CaCl_2$ destabilized PS NPs more than NaCl and $MgCl_2$ because of the specific adsorption capacity of Ca^{2+} ions called “bridging effect”. Standard Freshwater was the culture medium with the higher Ca^{2+} ions concentration, which explained the higher aggregation observed. Singh et al. (2019) reported that PS NPs were less stable in seawater in comparison to river water and groundwater. The aggregation pattern is also consistent with the presence of natural organic matter from WW (TOC 9.6 mg/L) because NP suspensions become unstable in the presence of natural colloids.

3.2. Sorption of WW micropollutants onto PS NPs

The analytical method used in this work was able to detect and quantify 44 compounds present in WW. Table S8 (SM) lists their main characteristics together with their concentration in the raw WW used in this work. Most compounds detected were pharmaceuticals belonging to several therapeutic classes like antibiotics (ciprofloxacin), anti-inflammatories (ketorolac, acetaminophen), contrast agents (iomeprol, iopamidol), antidepressants, beta-blockers, and several intermediate metabolites, among other compounds like the ubiquitous stimulant caffeine. The compounds found in higher concentrating (> 10 μg/L) were ciprofloxacin (fluoroquinolone antibiotic), famotidine (histamine-2 antagonist against stomach acid), flecainide (antiarrhythmic), iomeprol (contrast agent), ketorolac (nonsteroidal anti-inflammatory drug).

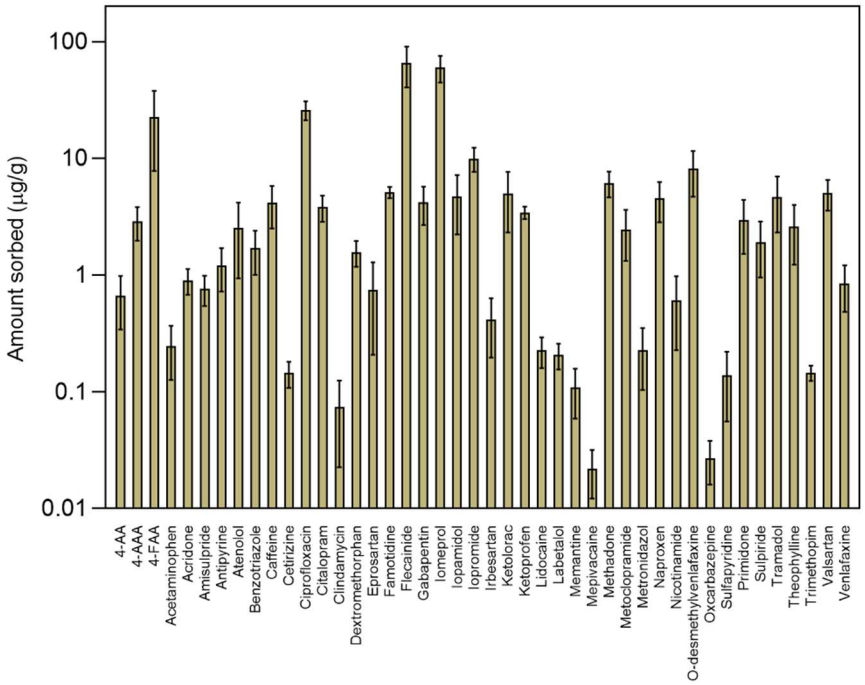

Figure 1. Amount of identified micropollutants sorbed on PS NPs (μg/g) after 24 h of contact with WW. Results are presented as mean values ± SD.

Fig. 1 shows the amount of the different compounds sorbed per unit mass of PS NPs calculated from contact experiments performed using PS NPs concentrations in the 10-200 mg/L range as indicated in the experimental section. Fig. S3 (SM) represents the retention percentage of each compound with respect to their concentration in WW. The average sorption reported was 17 % with huge differences among compounds. While mepivacaine, oxcarbazepine or famotidine were essentially not retained (< 2 %), acridone, cetirizine,

citalopram, dextromethorphan, and methadone were totally or almost totally sorbed onto PS NPs. Some physiochemical properties can explain the capacity of sorption of pollutants onto plastic particles, which include surface hydrophobicity, the molecular structure of solute and polymer, and surface charge (Torres et al., 2021). Our data showed that compounds with positive charge were preferentially sorbed over those neutral or negatively charged (Fig. 2). The charge for all compounds is given in Table S6 and was calculated using the Herderson-Hasselbalch equations. This result is in agreement with the negative charge of the PS NPs (Table S6). Paul et al. (2014), Zhao et al. (2015) and Li et al. (2018, 2019) suggested that electrostatic interactions are the main sorption mechanism for charged compounds. Sharma et al. (2021) showed that negatively charged particles with small size are more likely to be covered by adsorbed cations. Besides, the presence of benzene rings in PS chemical structure could explain the wide capacity of PS to sorb aromatic pollutants due to the relatively strong π-π interactions (Hüffer and Hofmann, 2016; Uber et al., 2019; Wang et al., 2019). Several results indicated that π-π interactions explained the binding of PAH and PCBs onto PS NPs (Liu et al., 2016; Velzeboer et al., 2014). Finally, Liu et al. (2020a) suggested that electrostatic interaction, together with hydrophobicity and hydrogen bonding could explain the sorption of the drugs atorvastatin and amlodipine to PS NPs.

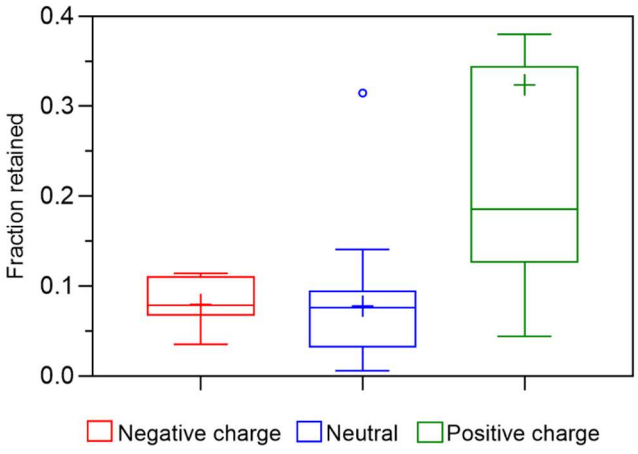

Figure 2. Fraction retained of WW micropollutants onto PS NPs according to their charge (as shown in Table S6).

3.3. Effect of PS NPs, WW, and their binary mixture on Anabaena CPB4337, *S. polyrhiza*, and *D. magna*

The results of bioluminescence inhibition of *Anabaena* CPB4337 after 24 h of single exposure to PS NPs and WW as well as exposed to their binary combination are shown in Fig. 3 and Table S9. As shown in Fig. S4 A (SM) no significant effects were found when the toxicity of supernatant of PS NPs suspensions were tested indicating that the PS NPs-free suspensions were not toxic to any of the three organisms ($p > 0.05$, Tukey's HSD test). The inhibition of constitutive bioluminescence follows a dose-response relationship with an EC50 value for PS NPs of 58.3 ± 12.7 mg/L as shown in Fig. 4 A and Table S10 (SM). This result agrees with the EC50 value for growth inhibition previously reported by Tamayo-Belda et al. (2021), who worked with the same *Anabaena* species and fluorescent PS NPs (30 nm). Wan et al. (2021) studied the effect of PS NPs (500 nm) towards two freshwater microalgae, *Chlorella* sp. and *R. subcapitata* and reported in both cases EC50 > 99 mg/L for chlorophyl *a* content decrease after 16 days of exposure. Mao et al. (2018) exposed *Chlorella pyrenoidosa* to 100 mg/L (100 nm) for 30 days, reporting a 38.5 % of growth inhibition and Reynolds et al. (2020) reported a 33.7 % growth reduction in *Raphidocelis subcapitata* exposed 72 h to PS NPs (100 nm). Other authors also reported low toxicity of NPs on microalgae (Wan et al., 2019). The dose-response curve for WW shows that the EC50 corresponded to a WW dilution factor (d.f.) of 0.34 ± 0.03 (Fig. 4 B and Table S10). Rosal et al. (2009) and Martín de Lucía et al. (2017) also evaluated the toxicity of wastewater on *Anabaena* CPB4337 using as toxicity endpoint bioluminescence inhibition and reported EC50 values after 24 h or exposure of 0.66 and 0.94, respectively, which are higher that the value found in this study, although it should be taken into account that the pollutants present in those effluents and their concentrations might be different and that might explain the lower toxicity in comparison with the results presented here. Table S11 (SM) lists the literature toxicological data reported for which toxicological data were available of the pollutants detected in water samples in this work. Although it is difficult to make predictions about toxicity in real samples, such as the wastewater samples because of their complexity due to pollutant interactions, some of the compounds detected have already been reported as toxic for bacteria and microalgae (EC50 <1mg/L), according to The EU Directive 93/67/EEC (Commission of the European Communities 1996). Ciprofloxacin, citalopram, or clindamycin are some examples. Besides, it has been previously pointed out that complex pharmaceutical mixtures tended to synergistic effects on organisms such as *Anabaena* CPB 4337 and *R. subcapitata* (Gonzalez-Pleiter et al., 2013). The results shown in Fig. 3 and Table S9 (SM) correspond to the toxic effect of binary mixtures of PS NPs and WW towards the cyanobacterium. The results showed that PS NPs-WW mixtures generally tended to a lower toxicity than single PS NPs toxicity with the increase of WW dilution. These interactions also displayed a general tendency to toxicity mitigation with respect to the single toxicity for WW in the highest WW concentrations (1:2 and 1:1 dilutions).

Although several studies use the term “synergy” or “antagonism” to broadly refer to the toxicological interactions of mixtures, the use of mathematical

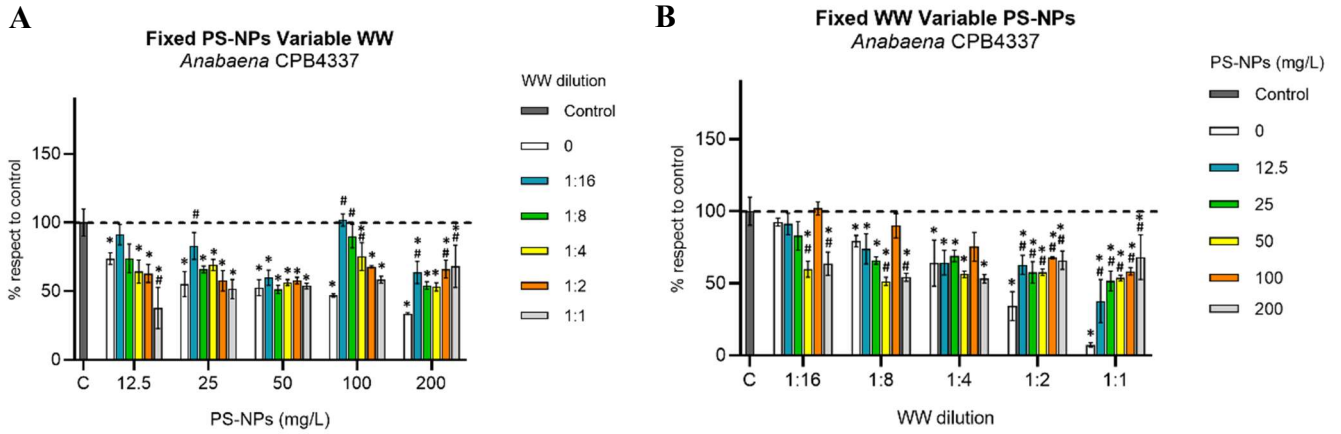

Figure 3. Effect of fixed PS NPs and variable WW concentrations (A), and fixed WW and variable PS NPs concentrations (B) in the bioluminescence of *Anabaena* CBP4337 after 24 h of exposure. Results are expressed as percentage ± SD with respect to control (non-exposed cells either to PS NPs or WW); an asterisk (*) represents significant differences with respect to control, a hash (#) represents significant differences with respect to single exposure to PS NPs (A) or WW (B) ($p < 0.05$).

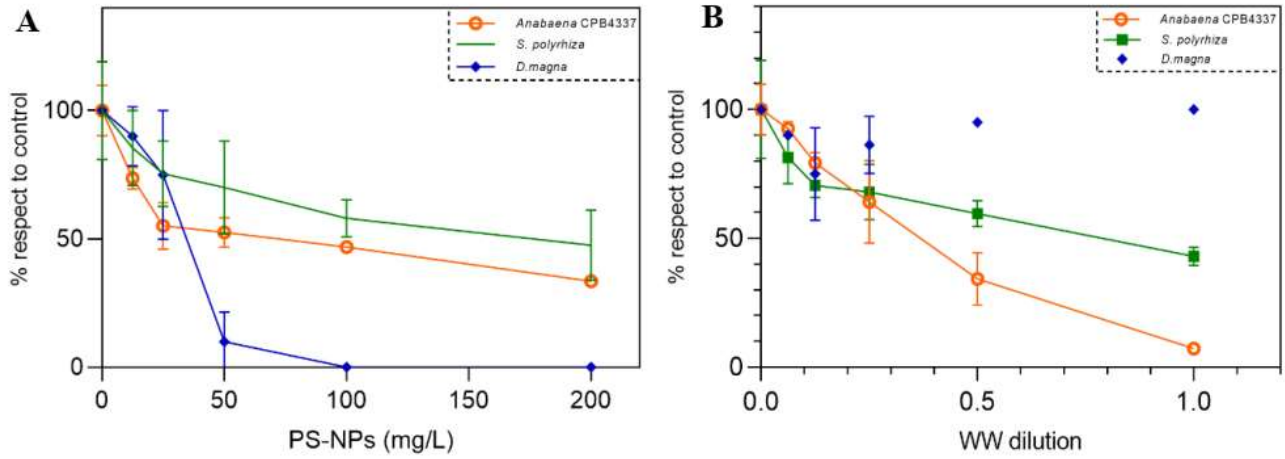

Figure 4. Dose-response curves for PS NPs (A) and WW (B) of *Anabaena* CPB4337, *S. polyrhiza* and *D. magna* at 24 h, 72 h and 48 h of exposure respectively, in terms of the studied endpoints (inhibition of bioluminescence in *Anabaena* CPB4337, growth in *S. polyrhiza* and mobility (*D. magna*)

models supporting such type of conclusions is required (Chou, 2006). We used the CI-isobologram model to assess mathematically the presence of synergistic or antagonistic mixture effects. Fig. 5 shows the CI-f_a plot for the toxicological interactions between PS NPs and WW for *Anabaena* CPB4337 bioassays. The CI-fa plot clearly shows an antagonistic interaction ($CI > 1$) running over the whole experimental range of affected fractions. This means that the individual effects of each stressor, PS NPs, and WW, was higher or even much higher than that of their combination. Other studies showed antagonistic interaction for nanoparticle-chemical pollutant combinations with a tendency towards lower antagonism, additivity or even synergy at the higher affected fractions. Specifically, antagonism was found by Martín-de-Lucía et al. (2017 and 2018) for the binary mixtures of between wastewater and inorganic nanoparticles (SiO_2, SiO_2–NH_2, TiO_2, Fe_3O_4 and graphene oxide) to *Anabaena* CPB4337 and *Chlamydomonas reinhardtii*, and by Tamayo-Belda et al. (2021) for PS NPs and PAMAM dendrimers to *Anabaena* sp. PCC7120**.**

Our results showing the capacity of PS NPs to withdraw pollutants from wastewater may explain the lower toxicity observed in PS NPs-WW mixtures towards the cyanobacterium because of the lower bioavailability of sorbed compounds. Li et al. (2020a) observed an antagonistic effect when the marine microalgae *Chlorella pyrenoidosa* was exposed to PS plastic particles of 0.55 µm and dibutyl phthalate (DBP), which was attributed to a decreased DBP bioavailability as well as the aggregation of particles at high concentrations. Zhang et al. (2018) reported lower toxicity of a binary mixture of nPS-NH_2 NPs (200 nm) and glyphosate in comparison to the single toxicity of glyphosate to the freshwater cyanobacterium *Microcystis aeruginosa*, caused by glyphosate sorption onto NPs. A similar result was obtained by Wang et al. (2020a) for the joint effect of ibuprofen and PS NPs (600 nm) to *C. pyrenoidosa* growth rate.

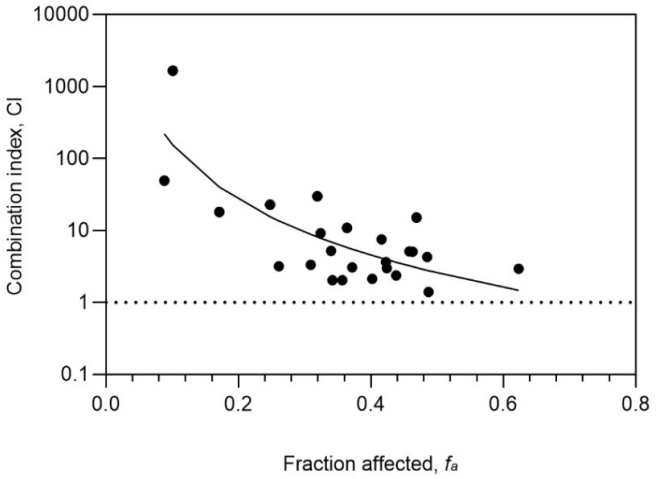

Figure 5. Combination index-fa plot for *Anabaena* CPB 433. CI values are plotted as function of the fraction affected (f_a) (fractional inhibition of bioluminescence) within the experimental range. CI < 1 indicates synergism; CI = 1 additivity and CI >1 antagonism.

Confocal images of cells exposed to PS NPs did not show any apparent cellular damage, and chlorophyll *a* autofluorescence did not differ from non-exposed cells. However, clear heteroaggregation of PS NPs and microalgae was observed (Fig. S5, SM). Previous studies showed heteroaggregation when microalgae were exposed to MPs/NPs (Khoshnamvand et al., 2021; Larue et al., 2021; Prata et al., 2019; Wan et al., 2019). The confocal micrographs in Fig. S5 (SM) clearly showed shorter filaments when cells were exposed to WW. Tamayo et al. (2019) also reported a lower length in *Anabaena* sp. PCC7120 filaments upon exposure to PAMAM dendrimers and Rodea-Palomares et al. (2011) when *Anabaena* CPB4337 was exposed to CeO_2 nanoparticles. Furthermore, binary mixtures resulted also in clear heteroaggregation and, although long filaments were visible indicating limited toxicity, some cells in the filaments did not show their characteristic chlorophyll fluorescence, which could be related to the mechanical damage of some cell envelopes by the heteroaggregates. Tamayo et al. (2021) observed a similar effect in the same cyanobacterium exposed to a mixture of PS NPS and PAMAM dendrimers.

The effect of PS NPs, WW, and their combinations towards the growth of the aquatic plant *S. polyrhiza*, is shown in Fig. 6 and Table S12 (SM). For this organism the EC50 was 170 ± 14 mg/L and 0.94 ± 0.27 for PS NPs and WW, respectively (Fig. 4 and Table S10, SM). No significant effects were found when PS NPs-free suspensions were tested as shown in Fig. S4 B (SM) ($p > 0.05$, Tukey's HSD test). As for combined toxicity, when *S. polyrhiza* was exposed to low PS NPs (12.5, 25 and 50 mg/L) and low WW dilutions (1:16, 1:8), clear hormesis was observed, with plant growth significantly higher when exposed to PS NPs-WW combinations than to PS NPs and WW separately and also with respect to controls (Fig. 6). Exceptionally, the combination between the highest PS NPs (200 mg/L) and the lowest WW (1:16) dilution resulted in a significant higher toxicity ($p < 0.05$). For 1:2 and 1:1 WW dilutions in all the binary combinations the growth was significantly higher than when the plant was exposed to WW individually (Fig. S6 B, SM); the adsorption of WW micropollutants onto NPs was probably the main reason of the observed decrease of toxicity in these binary mixtures.

Fig. 7 shows the CI-f_a plot for PS NPs and WW mixtures to *S. polyrhiza*. CI-fa plot displayed an antagonistic interaction (CI > 1) essentially over the entire experimental range of affected fractions with a tendency towards synergism at higher fa values (f_a > 0.7). Therefore, most binary mixtures exhibited lower or much lower negative effects than PS NPs and WW separately towards the plant. Previous studies analysing the type of toxic interactions to *S. polyrhiza*, found moderate antagonism for the joint exposure to duloxetine and econazole (Valimaña-Traverso et al., 2019).

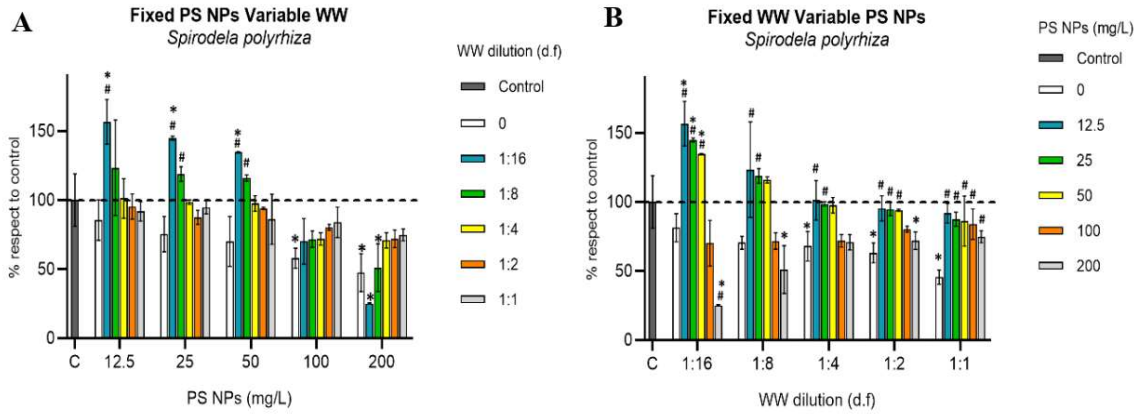

Figure 6. Effect of constant PS NPs and variable WW concentrations (A), and constant WW and variable PS NPs concentrations (B) to the growth of *S. polyrhiza* after 72 h of exposure. Results are expressed as percentage ± SD with respect to control (non-exposed plants neither PS NPs nor WW); an asterisk (*) represents significant differences with respect to control, a hash (#) represents significant differences with respect to single exposure to PS NPs (A) or WW (B) ($p < 0.05$).

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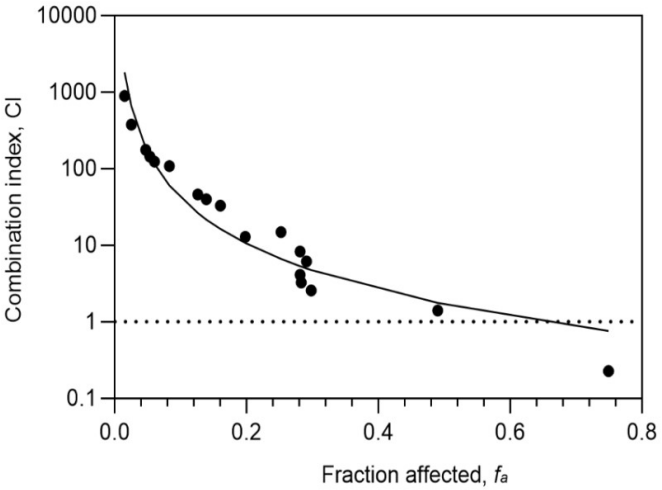

Figure 7. Combination index-f_a plot for *S. polyrhiza*. CI values are plotted as function of the fraction affected (f_a) (fractional inhibition of bioluminescence) within the experimental range. CI < 1 indicates synergism; CI = 1 additivity and CI >1 antagonism.

Few studies have investigated the effects of NPs in aquatic plants. Plant elongation growth was the main parameter studied. Dovidat et al. (2020) reported that fluorescent PS NPs (50 nm) were externally sorbed onto the roots of *S. polyrhiza,* without significantly affecting their growth after 120 h exposure. In another Lemnaceae, *Lemna minor*, Kalčíková et al. (2017) reported reduced root formation because of the mechanical blockage of pores due to the presence of PS microbeads (< 600 μm). Such mechanical blockage could result in a reduction of nutrient acquisition, with potential sorption of ions onto plastic particles that could impair plant nutritional status. van Weert et a. (2019) reported small effects in the shoot-to-root ratio and in shoot length of the sediment-rooted macrophytes *Myriophyllum spicatum* and *Elodea* sp. exposed to high concentrations of PS NPs (50-190 nm).

According to the physicochemical characterization of PS NPs and WW, the antagonism could be explained by the sorption of pollutants from wastewater onto PS NPs, thereby withdrawing them from the medium, and making them less bioavailable for the plant, as in the case of the cyanobacterium *Anabaena* CPB4337 described above. However, it should be taken into account that the concentrations of NPs used in the experiment was in the mg/L range which could be considered high (as indicated above, this range was chosen because at lower NP concentrations no sorbed micropollutants could be detected). This may be a limitation when extrapolating these results to a real specific environment if lower NP concentrations are present. However, as already indicated, the detection of NPs in natural environments is still challenging, as there is a need for developing suitable analytical methods for assessing their presence and concentration in real matrices (Cai et al., 2021; Wang et al., 2021). Likewise, the aggregation of PS NPs in contact with WW could also promote a reduction in the toxicity of PS NPs due to the sequestration of the non-aggregated PS NPs. Previous studies reported the importance of adsorption capacity of nanoparticles to reduce the toxicity of other pollutants to plants. Sun et al. (2019) informed that ZnO nanoparticles decreased cadmium concentration in the culture medium, which resulted in a toxicity decrease to the duckweed species *Lemna minor*. To our knowledge, the observed hormesis effect of a binary mixture of pollutants with nanoplastics has not been reported before. Wastewater-related hormesis is usually attributed to the presence of essential nutrients for plant growth (Jager et al., 2013). Nanomaterial-induced hormesis has also been reported sometimes in plants, although it is a phenomenon scarcely documented (Agathokleous et al., 2019; Rai et al., 2018; Pullagurala et al., 2018).

The higher toxicity observed for the combined exposure to 200 mg/L and the lowest WW dilution (1:16) could be attributed to the sorption of pollutants onto PS NPs which could become internalized and translocated into the plant body as suggested by Ke et al. (2021). The absence of aggregation observed for this binary combination (Table S6, SM) is compatible with that explanation. Hartmann et al. (2010) also reported size-dependent growth inhibition of *R. subcapitata* exposed to TiO_2 nanoparticles and Cd, with higher toxicity for 30 nm compared to 300 nm TiO_2 nanoparticles. Fig. S6 (SM) shows confocal images of leaves, buds, and roots of *S. polyrhiza* after exposure to PS NPs, WW, and their binary mixture. Generally, a decrease in autofluorescence of chlorophyll *a*, was observed for the joint exposure to PS NPs and WW. Furthermore, the lower toxicity of the binary mixture resulted in higher chlorophyll *a* autofluorescence, particularly in the buds, compared to single exposures. The loss of chlorophyll *a* autofluorescence of *Cucumis sativus* L. exposed to 100 and 700 nm PS NPs was reported by Li et al. (2020b).

The effect to the mobility of *D. magna* neonates exposed to PS NPs and WW individually and in mixtures is shown in Fig. 4 Table S13 (SM). The EC50 for PS NPs was 32.4 ± 2.4 mg/L, but no significant effect on mobility was observed after WW exposure (Fig. 4). In fact, a tendency to lower toxicity was observed for increasing WW concentration. The toxicity reported in the literature for the wastewater pollutants identified in this work was generally low to *D. magna* (Table S11, SM). The low toxicity of WW to *D. magna* agrees with data reported elsewhere (Han et al., 2006; Ra et al., 2008; Rodriguez et al., 2006; Verma, 2008; Verma, 2010; Villegas-Navarro et al., 1997). The toxic effect of NPs in *D. magna* neonates has been widely studied. Pikuda et al. (2018), reported the effect of fluorescent PS NPs (20 nm) on the mortality of neonates after 48 h exposure and reported LC50 of 15.7 mg/L. Ma et al. (2016) determined EC50 of 15.13 ± 3.34 mg/L for 48-h acute toxicity of PS NPs (50 nm). In both cases the authors indicated that the

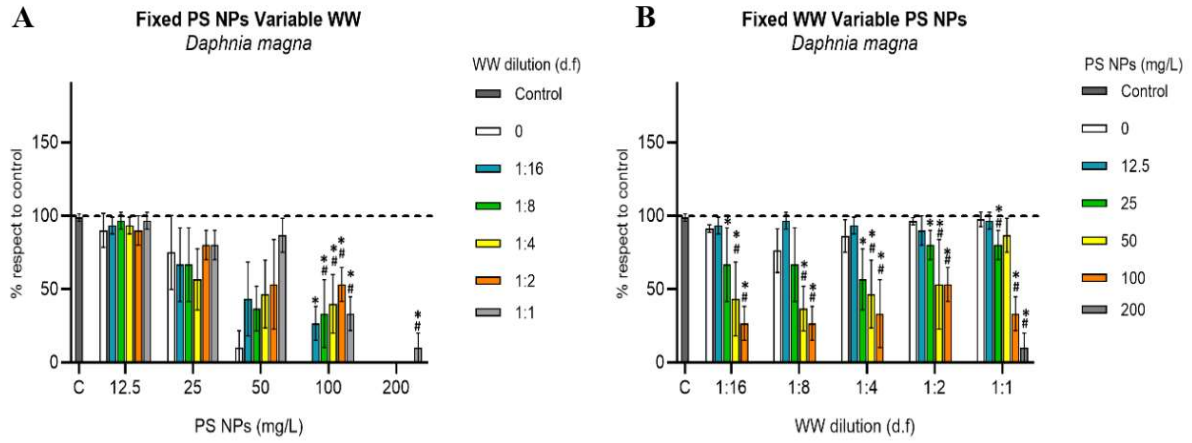

Figure 8. Effect of constant PS NPs and variable WW concentrations (A), and constant WW and variable PS NPs concentrations (B) to the mobility of *D. magna* after 48 h of 1013 exposure. Results are expressed as percentage ± SD with respect to control (non-exposed neonates either PS NPs or WW); an asterisk (*) represents significant differences with respect to control, a hash (#) represents significant differences with respect to single exposure to PS NPs (A) or WW (B) ($p < 0.05$).

toxicity could be due to the surfactant of other additives added to commercial PS stocks. However, in this work no significant negative effects were observed when organisms were exposed to PS NPs-free suspension in the whole range of tested concentrations, indicating that if any surfactant or additive was added to the commercial stock, it did not cause any harm to *D. magna* (Fig S4 C, SM).

The effect of PS NPs and WW combinations is shown in Fig. 8 A and B. Regarding constant PS-NPs and variable WW (Fig. 8 A), no significant differences ($p > 0.05$) were observed with respect to single PS NPs exposure, except at the highest concentrations of PS NPs (100 and 200 mg/L), for which a significant decrease in toxicity was observed for the entire range of WW concentrations. The observed toxicity decrease was lower for higher WW concentration. For constant WW and variable PS-NPs, the mixture toxicity increased when the PS NPs also increased ($p < 0.05$) with respect to the single exposure to WW (Fig. 8 B). Although a lower toxicity was found when *D. magna* was exposed to the mixture as compared to single exposure to PS NPs, the type of mixture interaction could not be quantified using the CI-isobologram method because no dose-dependent toxic response was observed for WW exposure of neonates.

Due to the absence of toxicity of WW to *D. magna*, the observed decrease in the combined toxicity with respect to that of PS NPs could be explained because of the aggregation of PS NPs driven by the conductivity increase in WW-containing mixtures. Besides, the presence of organic carbon in the wastewater (TOC: 9.6 mg/L, see Table S1) could represent a source of energy for the neonates. For 200 mg/L the effect could be only observed for non-diluted WW probably because of the high toxicity of PS NPs at this concentration. Therefore, in this organism, PS NPs single toxicity and the interaction of PS NPs with physicochemical characteristics of the culture medium and wastewater, as well as wastewater nutritional status most probably determined the observed combined toxicity. The lower toxicity in binary mixtures containing nanoparticles at high concentrations due to particle aggregation and sedimentation was also observed before (Martín de Lucía et al., 2019).

The observed toxicity was in good agreement with the physical damages observed in optical micrographs of daphnids (Fig. S7, SM). The images illustrated the high accumulation of PS NPs aggregates onto the thoracopod of the organism, with a subsequent loss of body integrity. In single exposure to WW, no structural damage was observed but, interestingly, the brown colour of the gastrointestinal tract could indicate the accumulation of dissolved organic matter from WW, which would represent an extra energy source. In binary exposure (PS NPs + WW), the adhesion of PS NPs aggregates to the body of daphnids was also observed, but to a lower extent, indicating a decrease of toxicity when *D. magna* was exposed to WW-PS NPs mixtures. Although NPs ingestion seems to be the main mechanism to interact with invertebrates like *D. magna*, the ingestion of NPs could not be the only cause of the immobilization of the neonates (Wan et al., 2019). Ma et al. (2016) also reported that PS NPs accumulate on the surface of thoracopods, thereby limiting their capacity to swim and feed. The impact of negatively charged nanoparticles on the reproduction and body size of *D. magna* was observed before associated to agglomerates adhered to the exoskeleton that could block nutrient absorption or impair swimming with subsequent energy imbalance (Bozich et al., 2014).

Conclusions

In this work, the ability of PS NPs to sorb micropollutants from a wastewater effluent was studied for the first time. Our results revealed that PS NPs

could retain a variety of chemicals belonging to different classes of pharmaceutical compounds. Although a wide variety of chemicals were detected in different concentrations and with percentages of retention, those with positive charge were mostly sorbed onto PS NPs. Electrostatic interactions between the positive charge of these compounds and the negatively charged surface of PS NPs could facilitate their binding. Besides, the single and joint toxicity of PS NPs and WW on three organisms was assessed. Antagonism was the predominant mixture interaction. Lower toxicity was generally observed for the combined exposure of organisms to PS NPs and WW in comparison to the toxicity resulting from single exposure to PS NPs or WW. The binding of micropollutants contained in WW onto PS NPs and their subsequent removal from the solution may explain the decrease in toxicity to the three tested organisms under combined PS NPs and WW exposure. In addition, the formation of PS NPs aggregates was observed when the particles were put in contact with WW because of the high WW ionic strength and composition (Ca2+ ions concentration) of culture media. The formation of heteroaggregates would result in lower bioavailability of PS NPs and sorbed micropollutants. WW was not toxic to *D. magna* probably due the presence of organic matter that could represent an energy source for the neonates; however, combined exposure to PS NPs and WW resulted in lower toxicity than PS NPs single exposure probably due to the formation of the observed large aggregates and the nutritional status of WW. This study represents a near-realistic scenario approach to the potential sorption of pollutants present on WW onto nanoplastics that could alter the toxicological effect on the biota.

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References

Agathokleous, E., Feng, Z., Iavicoli, I., Calabrese, E.J., 2019. The two faces of nanomaterials: A quantification of hormesis in algae and plants. Environment International 131, 105044.

Agathokleous, E., Iavicoli, I., Barceló, D., Calabrese, E.J., 2021. Micro/nanoplastics effects on organisms: A review focusing on 'dose'. Journal of Hazardous Materials 417, 126084.

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environment International 102, 165-176.

Bellingeri, A., Bergami, E., Grassi, G., Faleri, C., Redondo-Hasselerharm, P., Koelmans, A.A., Corsi, I., 2019. Combined effects of nanoplastics and copper on the freshwater alga *Raphidocelis subcapitata*. Aquatic Toxicology 210, 179-187.

Besseling, E., Redondo-Hasselerharm, P., Foekema, E.M., Koelmans, A.A., 2019. Quantifying ecological risks of aquatic micro- and nanoplastic. Critical Reviews in Environmental Science and Technology 49, 32-80.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic Affects Growth of S. obliquus and Reproduction of D. magna. Environmental Science & Technology 48, 12336-12343.

Bhargava, S., Chen Lee, S.S., Min Ying, L.S., Neo, M.L., Lay-Ming Teo, S., Valiyaveettil, S., 2018. Fate of Nanoplastics in Marine Larvae: A Case Study Using Barnacles, *Amphibalanus amphitrite*. ACS Sustainable Chemistry & Engineering 6, 6932-6940.

Böcük, H., Yakar, A., Türker, O.C., 2013. Assessment of *Lemna gibba* L. (duckweed) as a potential ecological indicator for contaminated aquatic ecosystem by boron mine effluent. Ecological Indicators 29, 538-548.

Bozich, J.S., Lohse, S.E., Torelli, M.D., Murphy, C.J., Hamers, R.J., Klaper, R.D., 2014. Surface chemistry, charge and ligand type impact the toxicity of gold nanoparticles to *Daphnia magna*. Environ. Sci. Nano 1, 260-270.

Cai, H., Xu, E.G., Du, F., Li, R., Liu, J., Shi, H., 2021. Analysis of environmental nanoplastics: Progress and challenges. Chemical Engineering Journal 410, 128208.

Commission of the European Communities (1996) Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances. Part II: Environmental Risk Assessment. Office for Official Publications of the European Communities, Luxembourg

Chae, Y., An, Y.-J., 2017. Effects of micro- and nanoplastics on aquatic ecosystems: Current research trends and perspectives. Marine Pollution Bulletin 124, 624-632.

Chae, Y., Kim, D., Kim, S.W., An, Y.-J., 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Scientific Reports 8, 284.

Chou, T.C., 1974. Relationships between inhibition Constants and Fractional Inhibition in Enzyme-Catalyzed Reactions with Different Numbers of Reactants, Different Reaction Mechanisms, and Different Types and Mechanisms of Inhibition. Molecular Pharmacology 10, 235.

Chou, T.C., 2006. Theoretical Basis, Experimental Design, and Computerized Simulation of

Synergism and Antagonism in Drug Combination Studies. Pharmacological Reviews 58, 621.
Chou, T.C., Talalay, P., 1984. Quantitative Analysis of Dose-Effect Relationships: The Combined Effects of Multiple Drugs or Enzyme Inhibitors. Advances in enzyme regulation 22, 27-55.
de Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T., Koelmans, A.A., 2020. Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review. Environmental Science & Technology 54, 11692-11705.
Dovidat, L.C., Brinkmann, B.W., Vijver, M.G., Bosker, T., 2020. Plastic particles adsorb to the roots of freshwater vascular plant *Spirodela polyrhiza* but do not impair growth. Limnology and Oceanography Letters 5, 37-45.
Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. Environmental Pollution 259, 113837.
Fernández-Pinas, F., Wolk, C.P., 1994. Expression of *luxCD-E* in *Anabaena* sp. can replace the use of exogenous aldehyde for in vivo localization of transcription by *luxAB*. Gene 150, 169-174.
Frehland, S., Kaegi, R., Hufenus, R., Mitrano, D.M., 2020. Long-term assessment of nanoplastic particle and microplastic fiber flux through a pilot wastewater treatment plant using metal-doped plastics. Water Research 182, 115860.
Gaylarde, C.C., Baptista Neto, J.A., da Fonseca, E.M., 2021. Nanoplastics in aquatic systems - Are they more hazardous than microplastics? Environmental Pollution 272, 115950.
Gigault, J., Halle, A.T., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: What is a nanoplastic? Environmental pollution 235, 1030-1034.
González-Fernández, C., Le Grand, F., Bideau, A., Huvet, A., Paul-Pont, I., Soudant, P., 2020. Nanoplastics exposure modulate lipid and pigment compositions in diatoms. Environmental Pollution 262, 114274.
González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I., Leganés, F., Rosal, R., Boltes, K., Marco, E., Fernández-Piñas, F., 2013. Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implications for environmental risk assessment. Water Research 47, 2050-2064.
González-Pleiter, M., Pedrouzo-Rodríguez, A., Verdú, I., Leganés, F., Marco, E., Rosal, R., Fernández-Piñas, F., 2021. Microplastics as vectors of the antibiotics azithromycin and clarithromycin: Effects towards freshwater microalgae. Chemosphere 268, 128824.
González-Pleiter, M., Tamayo-Belda, M., Pulido-Reyes, G., Amariei, G., Leganés, F., Rosal, R., Fernández-Piñas, F., 2019. Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments. Environmental Science: Nano 6, 1382-1392.
Greenberg, B.M., Huang, X.-D., Dixon, D.G., 1992. Applications of the aquatic higher plant *Lemna gibba* for ecotoxicological assessment. Journal of Aquatic Ecosystem Health 1, 147-155.
Han, G.H., Hur, H.G., Kim, S.D., 2006. Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: Occurrence and toxicity to *Daphnia magna*. Environmental Toxicology and Chemistry 25, 265-271.
Hartmann, N.B., Von der Kammer, F., Hofmann, T., Baalousha, M., Ottofuelling, S., Baun, A., 2010. Algal testing of titanium dioxide nanoparticles—testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology, 269, 190-197.
Hüffer, T., Hofmann, T., 2016. Sorption of non-polar organic compounds by microsized plastic particles in aqueous solution. Environmental Pollution 214, 194-201.
Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of virgin micro- and nanoplastics on fish: Trends, meta-analysis, and perspectives. Environmental Science & Technology 54, 4733-4745.
Jager, T., Barsi, A., Ducrot, V., 2013. Hormesis on life-history traits: is there such thing as a free lunch? Ecotoxicology 22, 263-270.
Jeong, C.-B., Kang, H.-M., Lee, Y.H., Kim, M.-S., Lee, J.-S., Seo, J.S., Wang, M., Lee, J.-S., 2018. Nanoplastic Ingestion Enhances Toxicity of Persistent Organic Pollutants (POPs) in the Monogonont Rotifer *Brachionus koreanus* via Multixenobiotic Resistance (MXR) Disruption. Environmental Science & Technology 52, 11411-11418.
Kalčíková, G., Žgajnar Gotvajn, A., Kladnik, A., Jemec, A., 2017. Impact of polyethylene microbeads on the floating freshwater plant duckweed *Lemna minor*. Environmental Pollution 230, 1108-1115.
Kamel, A.A., Ma, C.M., El-Aasser, M.S., Micale, F.J., Vanderhoff, J.W., 1981. Concerning the origin of charge at the polystyrene particle/water interface. Journal of Dispersion Science and Technology, 2, 315-330.
Ke, M., Ye, Y., Zhang, Z., Gillings, M., Qu, Q., Xu, N., Xu, L., Lu, T., Wang, J., Qian, H., 2021. Synergistic effects of glyphosate and multiwall carbon nanotubes on Arabidopsis thaliana physiology and metabolism. Science of the Total Environment 769, 145156.
Khoshnamvand, M., Hanachi, P., Ashtiani, S., Walker, T.R., 2021. Toxic effects of polystyrene nanoplastics on microalgae *Chlorella vulgaris*: Changes in biomass, photosynthetic pigments and morphology. Chemosphere 280, 130725.

Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A. M., Sanden, M., 2020. Micro-and nanoplastic toxicity on aquatic life: Determining factors. Science of the Total Environment, 709, 136050.

Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Nanoplastics in the Aquatic Environment. Critical Review, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 325-340.

Larue, C., Sarret, G., Castillo-Michel, H., Pradas del Real, A.E., 2021. A Critical Review on the Impacts of Nanoplastics and Microplastics on Aquatic and Terrestrial Photosynthetic Organisms. Small 17, 2005834.

Lee, W.S., Cho, H.-J., Kim, E., Huh, Y.H., Kim, H.-J., Kim, B., Kang, T., Lee, J.-S.,

Jeong, J., 2019. Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos. Nanoscale 11, 3173-3185.

Li, J., Zhang, K., Zhang, H., 2018. Adsorption of antibiotics on microplastics. Environmental Pollution 237, 460-467.

Li, Y., Li, M., Li, Z., Yang, L., Liu, X., 2019. Effects of particle size and solution chemistry on Triclosan sorption on polystyrene microplastic. Chemosphere 231, 308-314.

Li, Z., Li, X., Zhou, H., Chi, T., Li, W., Yang, K., 2020a. Combined effect of polystyrene microplastics and dibutyl phthalate on the microalgae *Chlorella pyrenoidosa*, Environmental Pollution, 257, 113604.

Li, Z., Li, R., Li, Q., Zhou, J., Wang, G., 2020b. Physiological response of cucumber (*Cucumis sativus* L.) leaves to polystyrene nanoplastics pollution. Chemosphere 255, 127041.

Lian, J., Wu, J., Zeb, A., Zheng, S., Ma, T., Peng, F., Tang, J., Liu, W., 2020. Do polystyrene nanoplastics affect the toxicity of cadmium to wheat (*Triticum aestivum* L.)? Environmental Pollution 263, 114498.

Liu, L., Fokkink, R., Koelmans, A.A., 2016. Sorption of polycyclic aromatic hydrocarbons to polystyrene nanoplastic. Environmental Toxicology and Chemistry 35, 1650-1655.

Liu, P., Lu, K., Li, J., Wu, X., Qian, L., Wang, M., Gao, S., 2020a. Effect of aging on adsorption behavior of polystyrene microplastics for pharmaceuticals: Adsorption mechanism and role of aging intermediates. Journal of Hazardous Materials 384, 121193.

Liu, Z., Cai, M., Wu, D., Yu, P., Jiao, Y., Jiang, Q., Zhao, Y., 2020b. Effects of nanoplastics at predicted environmental concentration on *Daphnia pulex* after exposure through multiple generations. Environmental Pollution 256, 113506

Ma, Y., Huang, A., Cao, S., Sun, F., Wang, L., Guo, H., Ji, R., 2016. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environmental Pollution 219, 166-173.

Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q., Li, H., 2018. Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period. Chemosphere 208, 59-68.

Martín-de-Lucía, I., Campos-Mañas, M.C., Agüera, A., Leganés, F., Fernández-Piñas, F., Rosal, R., 2018. Combined toxicity of graphene oxide and wastewater to the green alga *Chlamydomonas reinhardtii*. Environmental Science: Nano 5, 1729-1744.

Martín-de-Lucía, I., Campos-Mañas, M.C., Agüera, A., Rodea-Palomares, I., PulidoReyes, G., Leganés, F., Fernández-Piñas, F., Rosal, R., 2017. Reverse Trojanhorse effect decreased wastewater toxicity in the presence of inorganic nanoparticles. Environmental Science: Nano 4, 1273-1282.

Martínez-Andrade, J.M., Avalos-Borja, M., Vilchis-Nestor, A.R., Sánchez-Vargas, L.O., Castro-Longoria, E., 2018. Dual function of EDTA with silver nanoparticles for root canal treatment–A novel modification. PLOS ONE 13, e0190866.

Materić, D., Kasper-Giebl, A., Kau, D., Anten, M., Greilinger, M., Ludewig, E., van Sebille, E., Röckmann, T., Holzinger, R., 2020. Micro- and nanoplastics in Alpine snow: A new method for chemical identification and (semi)quantification in the nanogram range. Environmental Science & Technology 54, 2353-2359.

Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environmental Science & Technology 49, 553-561.

Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Scientific Reports 7, 11452.

Murray, A., Örmeci, B., 2020. Removal Effectiveness of Nanoplastics (< 400 nm) with Separation Processes Used for Water and Wastewater Treatment. Water 12.

Nolte, T.M., Hartmann, N.B., Kleijn, J.M., Garnæs, J., van de Meent, D., Jan Hendriks, A., Baun, A., 2017. The toxicity of plastic nanoparticles to green algae as influenced by surface modification, medium hardness and cellular adsorption. Aquatic Toxicology 183, 11-20.

OECD, 2004. Test No. 202: *Daphnia* Sp. Acute Immobilisation Test. Test No. 202. OECD Publishing, Paris.

Paul, T., Liu, J., Machesky, M.L., Strathmann, T.J., 2014. Adsorption of zwitterionic fluoroquinolone antibacterials to goethite: A charge distribution-multisite complexation model. Journal of Colloid and Interface Science 428, 63-72.

Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: Source, distribution and threats - A review. Science of the Total Environment 698, 134254.

Pico, Y., Alfarhan, A., Barcelo, D., 2019. Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies. TrAC Trends in Analytical Chemistry 113, 409-425.

Pikuda, O., Xu, E.G., Berk, D., Tufenkji, N., 2019. Toxicity assessments of micro- and nanoplastics can be confounded by preservatives in commercial formulations. Environmental Science & Technology Letters 6, 21-25.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. Effects of microplastics on microalgae populations: A critical review. Science of the Total Environment 665, 400-405.

Pullagurala, V.L.R, Adisa, I.O., Rawat, S., Kim, B., Barrios, A.C., Medina-Velo, I.A., Hernández-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2018. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environmental Pollution 241, 1175-1181.

Ra, J.S., Lee, B.C., Chang, N.I., Kim, S.D., 2008. Comparative Whole Effluent Toxicity Assessment of Wastewater Treatment Plant Effluents using *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 80, 196-200.

Rai, P.K., Kumar, V., Lee, S., Raza, N., Kim, K.-H., Ok, Y.S., Tsang, D.C.W., 2018. Nanoparticle-plant interaction: Implications in energy, environment, and agriculture. Environment International 119, 1-19.

Ramasamy, B.S.S., Palanisamy, S., 2021. A review on occurrence, characteristics, toxicology and treatment of nanoplastic waste in the environment. Environmental Science and Pollution Research 28, 43258-43273.

Redondo-Hasselerharm, P.E., Vink, G., Mitrano, D.M., Koelmans, A.A., 2021. Metaldoping of nanoplastics enables accurate assessment of uptake and effects on *Gammarus pulex*. Environmental Science: Nano 8, 1761-1770.

Reynolds, A., Giltrap, D.M., Chambers, P.G., 2021. Acute growth inhibition & toxicity analysis of nano-polystyrene spheres on *Raphidocelis subcapitata*. Ecotoxicology and Environmental Safety 207, 111153.

Rodea-Palomares, I., Petre, A.L., Boltes, K., Leganés, F., Perdigón-Melón, J.A., Rosal, R., Fernández-Piñas, F., 2010. Application of the combination index (CI)-isobologram equation to study the toxicological interactions of lipid regulators in two aquatic bioluminescent organisms. Water Research 44, 427-438.

Rodríguez, P., Martínez-Madrid, M., Cid, A., 2006. Ecotoxicological assessment of effluents in the Basque country (Northern Spain) by acute and chronic toxicity tests using *Daphnia magna* straus. Ecotoxicology 15, 559-572.

Rosal, R., Rodea-Palomares, I., Boltes, K., Fernández-Piñas, F., Leganés, F., Gonzalo, S., Petre, A., 2010. Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms. Environmental Science and Pollution Research 17, 135-144.

Shams, M., Alam, I., Chowdhury, I., 2020. Aggregation and stability of nanoscale plastics in aquatic environment. Water Research 171, 115401.

Sharma, V.K., Ma, X., Guo, B., Zhang, K., 2021. Environmental factors-mediated behavior of microplastics and nanoplastics in water: A review. Chemosphere 271, 129597.

Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H.S., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquatic Toxicology 170, 259-261.

Singh, N., Tiwari, E., Khandelwal, N., Darbha, G.K., 2019. Understanding the stability of nanoplastics in aqueous environments: effect of ionic strength, temperature, dissolved organic matter, clay, and heavy metals. Environmental Science: Nano 6, 2968-2976.

Sorasan, C., Edo, C., González-Pleiter, M., Fernández-Piñas, F., Leganés, F., Rodríguez, A., Rosal, R., 2021. Generation of nanoplastics during the photoageing of low-density polyethylene. Environmental Pollution 289, 117919.

Sun, S., Li, S., Sun, C., Cao, W., Hu, C., Zhao, Y., Yang, A., 2019. Effects of ZnO nanoparticles on the toxicity of cadmium to duckweed *Lemna minor*, Science of the Total Environment 662, 697-702.

Sun, H., Jiao, R., Wang, D., 2021. The difference of aggregation mechanism between microplastics and nanoplastics: Role of Brownian motion and structural layer force. Environmental Pollution 268, 115942.

Surette, M.C., Nason, J.A., 2019. Nanoparticle aggregation in a freshwater river: the role of engineered surface coatings. Environmental Science: Nano 6, 540-553.

Tamayo-Belda, M., González-Pleiter, M., Pulido-Reyes, G., Martin-Betancor, K., Leganés, F., Rosal, R., Fernández-Piñas, F., 2019. Mechanism of the toxic action of cationic G5 and G7 PAMAM dendrimers in the cyanobacterium *Anabaena* sp. PCC7120. Environmental Science: Nano 6, 863-878.

Tamayo-Belda, M., Vargas-Guerrero, J.J., Martín-Betancor, K., Pulido-Reyes, G., González-Pleiter, M., Leganés, F., Rosal, R., Fernández-Piñas, F., 2021. Understanding nanoplastic toxicity and their interaction with engineered cationic nanopolymers in microalgae by physiological and proteomic approaches. Environmental Science: Nano 8, 2277-2296.

Tang, K.H.D., 2021. Interactions of Microplastics with Persistent Organic Pollutants and the Ecotoxicological Effects: A Review. Tropical Aquatic and Soil Pollution 1, 24-34.

Ter Halle, A., Jeanneau, L., Martignac, M., Jardé, E., Pedrono, B., Brach, L., Gigault, J., 2017. Nanoplastic in the North Atlantic subtropical gyre. Environmental Science & Technology 51, 13689-13697.

Torres, F.G., Dioses-Salinas, D.C., Pizarro-Ortega, C.I., De-la-Torre, G.E., 2021. Sorption of chemical contaminants on degradable and non-degradable microplastics: Recent progress and research trends. Science of the Total Environment 757, 143875.

Trevisan, R., Voy, C., Chen, S., Di Giulio, R.T., 2019. Nanoplastics Decrease the Toxicity of a complex PAH mixture but impair mitochondrial energy production in developing zebrafish. Environmental Science & Technology 53, 8405-8415

Uber, T.H., Hüffer, T., Planitz, S., Schmidt, T.C., 2019. Sorption of non-ionic organic compounds by polystyrene in water. Science of the Total Environment 682, 348-355.

Valimaña-Traverso, J., Amariei, G., Boltes, K., García, M.Á., Marina, M.L., 2019. Stability and toxicity studies for duloxetine and econazole on *Spirodela polyrhiza* using chiral capillary electrophoresis. Journal of Hazardous Materials 374, 203-210.

van Weert, S., Redondo-Hasselerharm, P.E., Diepens, N.J., Koelmans, A.A., 2019. Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes, Science of the Total Environment 654, 1040-1047.

Velzeboer, I., Kwadijk, C.J.A.F., Koelmans, A.A., 2014. Strong sorption of PCBs to nanoplastics, microplastics, carbon Nanotubes, and fullerenes. Environmental Science & Technology 48, 4869-4876.

Verdú, I., González-Pleiter, M., Leganés, F., Rosal, R., Fernández-Piñas, F., 2021. Microplastics can act as vector of the biocide triclosan exerting damage to freshwater microalgae. Chemosphere 266, 129193.

Verma, Y., 2008. Acute toxicity assessment of textile dyes and textile and dye industrial effluents using *Daphnia magna* bioassay. Toxicology and Industrial Health 24, 491-500.

Verma, Y., 2010. Toxicity assessment of dye containing industrial effluents by acute toxicity test using *Daphnia magna*. Toxicology and Industrial Health 27, 41-49.

Villegas-Navarro, A., Santiago, M.R., Pérez, F.R., Torres, R.R., Abularach, T.D., Reyes, J.L., 1997. Determination of LC50 from *Daphnia magna* in treated industrial waste waters and non-treated hospital effluents. Environment International 23, 535-540.

Wan, J.-K., Chu, W.-L., Kok, Y.-Y., Lee, C.-S., 2019. Distribution of Microplastics and Nanoplastics in Aquatic Ecosystems and Their Impacts on Aquatic Organisms, with Emphasis on Microalgae, in: de Voogt, P. (Ed.), Reviews of Environmental Contamination and Toxicology Volume 246. Springer International Publishing, Cham, pp. 133-158.

Wan, J.-K., Chu, W.-L., Kok, Y.-Y., Lee, C.-S., 2021. Influence of polystyrene microplastic and nanoplastic on copper toxicity in two freshwater microalgae. Environmental Science and Pollution Research 28, 33649-33668.

Wang, J., Liu, X., Liu, G., Zhang, Z., Wu, H., Cui, B., Bai, J., Zhang, W., 2019. Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. Ecotoxicology and Environmental Safety 173, 331-338.

Wang, F., Wang, B., Qu, H., Zhao, W., Duan, L., Zhang, Y., Zhou, Y., Yua, G., 2020a. The influence of nanoplastics on the toxic effects, bioaccumulation, biodegradation and enantioselectivity of ibuprofen in freshwater algae *Chlorella pyrenoidosa*. Environmental Pollution 263, 114593.

Wang, T., Wang, L., Chen, Q., Kalogerakis, N., Ji, R., Ma, Y., 2020b. Interactions between microplastics and organic pollutants: Effects on toxicity, bioaccumulation, degradation, and transport. Science of the Total Environment 748, 142427.

Wang, J., Zhao, X., Wu, F., Niu, L., Tang, Z., Liang, W., Zhao, T., Fang, M., Wang, H., Wang, X., 2021. Characterization, occurrence, environmental behaviors, and risks of nanoplastics in the aquatic environment: Current status and future perspectives. Fundamental Research 1, 317-328.

Xiang, Y., Jiang, L., Zhou, Y., Luo, Z., Zhi, D., Yang, J., Lam, S.S., 2022. Microplastics and environmental pollutants: Key interaction and toxicology in aquatic and soil environments. Journal of Hazardous Materials 422, 126843.

Xiong, Y., Zhao, J., Li, L., Wang, Y., Dai, X., Yu, F., Ma, J., 2020. Interfacial interaction between micro/nanoplastics and typical PPCPs and nanoplastics removal via electrosorption from an aqueous solution. Water Research 184, 116100.

Yu, F., Yang, C., Zhu, Z., Bai, X., Ma, J., 2019. Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. Science of the Total Environment 694, 133643.

Zhang, Q., Qu, Q., Lu, T., Ke, M., Zhu, Y., Zhang, M., Zhang, Z., Du, B., Pan, X., Sun, L., Qian, H., 2018. The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa* growth. Environmental Pollution 243, 1106-1112.

Zhao, Y., Gu, X., Li, S., Han, R., Wang, G., 2015. Insights into tetracycline adsorption onto kaolinite and montmorillonite: experiments and modeling. Environmental Science and Pollution Research 22, 17031-1704

Supplementary Material

Polystyrene nanoplastics and wastewater displayed antagonistic toxic effects due to the sorption of wastewater micropollutants

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on growth of the *Daphnia magna* after 48 h of exposure. Results are expressed as percentage ± SD of mobility with respect to control (non-exposed neonates = 100) (n=4 in single exposure, n=3 in combined exposure). ND, not significantly different from zero.

Figure S1. Micro-FTIR spectra of PS NPs.

Figure S2. Experimental design of the binary mixture combinations of PS NPs-WW, used in this study. (1 in WW dilution axis represents 1:1 WW-dH_2O dilution.)

Figure S3. Percentage of pollutant retention onto PS NPs

Figure S4. Effect of PS NPs-free suspensions (50 kDa ultrafiltrates) to *Anabaena* CPB4337 bioluminescence (A), *S. polyrhiza* growth (B) and *D. magna* mobility (C) on bioluminescence, after 24, 72 and 48 h, respectively. Results are expressed as percentage ± SD with respect to control (non-exposed organisms). X-axes represent the concentration of PS-NPs suspensions before ultrafiltration. No significant differences were found ($p < 0.05$, Tukey's HSD test).

Figure S5. Confocal microscopy images of *Anabaena* CPB4337 exposed to PS NPs, WW, and their binary mixture (PS NPs+WW). Red color indicates the autofluorescence of chlorophyll *a*. Scale bar represents 25 μm.

Figure S6. Representative confocal microscopy images of the *S. polyrhiza* exposed to PS NPs, WW, and their binary mixture (PS NPs + WW). Red colour indicates the autofluorescence of chlorophyll *a*. Scale bar represents 50 μm.

Figure S7. Microscopy images of the *D. magna* exposed to PS NPs, WW, and their binary mixture (PS NPs + WW). Scale bar represents 20 μm.

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Supplementary Text: Detection and quantification of micropollutants

The detection and quantification of micropollutants were performed by ultrahigh-pressure liquid chromatography (ExionLC AC, Sciex, Foster city, CA, USA) coupled to mass spectrometry detection, using a quadrupole-linear ion trap analyser (UHPLC-QqLIT-MS/MS) also from Sciex (5500 QTRAP™). A Luna Omega Polar column (100 × 2.1 mm, 1.6-µm particle size, Phenomenex, Torrance, CA, USA) was used for the chromatographic separation. The mobile phase consisted of 0.1% of formic acid in Milli-Q water (eluent A) and methanol (eluent B) at a flow rate of 0.3 mL/min. The applied gradient was as follows: 5% (B) (kept 1 min); from 5% (B) to 100% (B) in 7 min; this composition was held for 4 min and then changed to 5% (B) within 0.5 min; finally, this gradient was hold for 5 min more to equilibrate the system. The total analysis time was 17.5 min. The injection volume was 10 µL. The column oven temperature was 30ºC and the autosampler was operating at 15ºC.

The electrospray source (Turbo IonSpray) operated in the positive mode (ESI+) for all the compounds with the following settings: ion spray voltage (IS), 5500 V; source temperature, 550ºC; CAD gas, medium; ion source gas 1 (GS1), 50 psi; ion source gas 2 (GS2), 50 psi and curtain gas, 20 (arbitrary units). Nitrogen was used as a nebulizer gas, curtain gas and collision gas. The multiple reaction monitoring (MRM) mode and the Scheduled MRM™ Algorithm were used for the acquisition of the samples. An MRM detection window of 40 s was applied, and the target scan time was of 1 s. To confirm the analytes, the criteria indicated by the European Union guidelines for pesticide residue analysis (SANTE/12682/2019): two SRM transitions with the expected retention time and an appropriate ion ratio (tolerance ±30%).

Quantification of the detected analytes was accomplished by matrix-matched standard calibration for WW samples (standards prepared from 10 to 2000 ng/L). Blank subtraction and sample dilution was applied when necessary. Methanol samples from nanoparticle washing were quantified using solvent calibration. All the samples were spiked with an injection standard of ^{13}C-caffeine. For data acquisition and processing, Analyst 1.7.1. and Sciex OS (both from Sciex) were used, respectively.

Reference: European Commission, 2019. Guidance document on analytical quality control and method validation procedures for pesticides residues and analysis in food and feed. Document No. SANTE/12682/2019. European Commission Directorate-General for Health and Food Safety. https://www.eurl-pesticides. eu/userfiles/file/EurlALL/AqcGuidance _ SANTE _ 2019 _12682.pdf. Accessed October 2021.

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Table S1. Main wastewater parameters (0.7 µm filtered samples)

pH		7.5
Conductivity (mS/cm)		0.78
TOC (mg/L)		9.6
Cations (mg/L)	Sodium	42.7
	Calcium	35.3
	Magnesium	11.5
Anions (mg/L)	Fluoride	0.37
	Chlorite	0.03
	Chloride	89.6
	Chlorate	0.08
	Nitrite	0.08
	Nitrate	16.4
	Bromide	0.1
	Phosphate	1.92
	Sulphate	70.1

Table S2. Composition of Allen and Arnon medium eight-fold diluted and supplemented with nitrate as used in bioassays.

	Macroelements	Concentration (mM)
AA/8+N medium	KNO_3	0.78
	$NaNO_3$	0.78
	$CaCl_2 \cdot 2H_2O$	0.0625
	NaCl	0.5
	K_2HPO_4	0.25
	$MgSO_4 \cdot 7H_2O$	0.125
	Microelements	Concentration (µM)
	H_3BO_3	5.781
	$ZnSO_4 \cdot 7H_2O$	0.0956
	$MnCl_2 \cdot 4H_2O$	1.136
	MoO_3	0.156
	$FeSO_4 \cdot 7H_2O$	8.645
	NH_4VO_3	0.0245
	$CoCl_2$	0.021
	$CuSO_4 \cdot 5H_2O$	0.0395
	Na_2EDTA	9.59

Table S3. Components of Steinberg medium used in *S. polyhriza* bioassays.

	Macroelements	Concentration (mM)
Steinberg medium	KNO_3	3.46
	$Ca(NO_3)_2 \cdot 4H_2O$	1.25
	KH_2PO_4	0.66
	K_2HPO_4	0.072
	$MgSO_4 \cdot 7H_2O$	0.41
	Microelements	Concentration (µM)
	H_3BO_3	1.94
	$ZnSO_4 \cdot 7H_2O$	0.63
	$Na_2MoO_4.2H_2O$	0.18
	$MnCl_2 \cdot 4H_2O$	0.91
	$FeCl_3 \cdot 6H_2O$	2.81
	Na_2EDTA	4.03

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Table S4. Components of Standard Freshwater medium used in *D. magna* bioassays.

		Concentration (mM)
Freshwater medium	$NaHCO_3$	0.771
	$CaCl_2 \cdot 2H_2O$	2
	$MgSO_4 \cdot 7H_2O$	0.5
	KCl	0.077

Table S5. Quality Assurance/Quality Control assessment, based on de Ruijter et al. (de Ruijter et al. 2020).

Technical quality assessment		
Particle characterization		Score
1. Particle size	NPs size was measured by DLS (Zetasizer Nano ZS, Malvern Instruments) in distilled water, and different media used for bioassays as it is indicated in Table S6.	2
2. Particle shape	Microspheres according to manufacturer	1
3. Polymer type	Polystyrene (PS). This material was checked by micro-FTIR (Perkin-Elmer Spotlight 200 Spectrum Two apparatus with mercury cadmium telluride (MCT) detector), whose spectra are shown in Fig. S1**.**	2
4. Source of NP	Commercial Latex Microspheres Suspension (5000 series) manufactured by Thermo Scientific, (Fremont, California, USA) Catalog Number: 5003A. Lot number: 216608	2
5. Data reporting	PS NPs concentrations in this study were reported as mass and expressed in mg/L	1
Experimental Design		Score
6. Chemical purity	Commercial stock, containing a minimal surfactant concentration (data not reported by the manufacturer) was diluted 1/100 in Milli-Q® water. In addition, to check that the remaining surfactant concentration or dissolved chemical potentially present were negligible in terms of ecotoxicological effects, the toxicity of supernatants (PS NPs suspension was filtered through 50 kDA) and were tested (Fig. S4).	1
7-8. Laboratory preparation/verification of contamination	To minimize possible NPs contamination during the manipulation and test exposure, all the instruments were previously washed with Milli-Q® water. Glass materials were also used to perform the test and keep the WW and PS NPs solutions (Erlenmeyer flask, beakers, bottles…). Cotton lab coats were also used. The preparation and manipulation of stock solutions and bioassays took place in laminar flow hoods.	2+2
9. Verification of exposure	From a commercial stock 10 % w/w (15 mL, density 1.05 g/cm^3), a working stock 1/100 diluted in MilliQ® water was prepared, resulting in 10000 mg/L. Serially different test concentrations were prepared based on it.	1
10. Homogeneity of exposure	Prior to bioassays and characterization measurements PS NPs suspension was ultrasonicated during 30s (ultrasonic bath Fischer Scientific FB15060) and WW was magnetically stirred. Particle and colloid background size (n=3) measured by DLS ensured good homogenization (Table S6).	2
11. Exposure assessment	PS NPs contact with three organisms tested in biossays was demonstrated by confocal microscopy (Leica TCS SP5 system) in *Anabaena* CBP4337 (Fig. S5) and *S. polyrhiza* (Fig. S6), and optical microscopy (Olympus CX41 microscope) in *D. magna* (Fig. S7).	2
12. Replication	Minimum three replicates for statistical rigor were performed for bioassays and characterization measures.	2

Applicability in ecological risk assessment		
Applicable for Risk Assessment		Score
13. Endpoints	Three endpoints were considered according to the organism. So, bioluminescence inhibition, growth inhibition and immobility were the endpoint studied for *Anabaena* CBP4337, *S. polyrhiza*, and *D. magna*, respectively.	2
14. Presence of natural (food) particles	Two photoautotrophic organisms (*Anabaena* CBP4337 *and S. polyrhiza*) and one heterotrophic (*D. magna*) organism were used in bioassays. Because the test consisted in short-time exposures for acute toxicity evaluation, the heterotrophic organism (*D. magna*) was not fed (OECD 202). Due to the WW exposure in single WW toxicity test and PS NPs-WW mixture toxicity test, extra natural organic matter contained in WW (filtered by 0.7 μm) was present in these two assays. TOC was measured (Table S.1).	1
15-16. Reporting of effect thresholds and quality of dose-response relationship	Dose-responses curves were reported (Fig. 4). A total of six NPs concentrations (0, 12.5, 25, 50, 100 and 200 mg/L) were tested. EC_{50} values were estimated according to non-linear fitting to parametric functions (3 or 4-parameters logistic function). Microsoft Office 365 Excel and IBM IBM SPSS Statistics 26 software were used for calculations, reporting values, standard deviation and 95 % confidence intervals.	2+2
Ecological Relevance		Score
17. Concentration range tested	Five PS NPs concentrations (12.5, 25, 50, 10 and 200 mg/L) were tested according to previous laboratory experiments that evaluated NPs toxicity in organisms from low trophic levels (Mattsson et al., 2017; Sjollema et al., 2016; Tamayo-Belda et al., 2021). This range was also tested as lower NP concentrations did not allow to detect and quantify micropollutants such as those present in WW (Martin de Lucia et al., 2017). Environmental concentrations are still unknown due to actual technical limitations (Cai et al., 2021; Wang et al., 2021)	1
18. Aging and biofouling	In order to resemble realistic scenarios, NPs were incubated with real WW samples from a treated effluent (24-72 h depending on bioassay)	2
19. Diversity of NPs tested	Only one type of polymer with one size range (polystyrene NPs, 30 nm) was tested.	0
20. Exposure time	The exposure time employed to bioassays was 24 h (*Anabaena* CBP4337), 72 h (*S. polyrhiza*) and 48 h (*D. magna*) following standard guidelines (OECD 201, ISO Standard 20227, OECD 202, respectively) recommended for acute toxicity tests.	0
	Total Score	30

References (Table S5)

de Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T., Koelmans, A.A., 2020. Quality Criteria for Microplastic Effect Studies in the Context of Risk Assessment: A Critical Review. Environmental Science & Technology 54, 11692-11705.

Table S6. Physicochemical properties of PS NPs suspensions (200 mg/L) for the different WW dilutions and culture medium at the maximum exposure times of each bioassay (24 h for *Anabaena* CPB4337, 72 h for *S. polyrhiza* and 48 h for *D. magna*). Distilled water is also included. ND, non-detectable.

		Distilled water (pH=6.5)		AA/8 +N médium (pH=7.5)		Steinberg medium (pH=6.5)		Standard Freshwater medium (pH=7.2)	
		d_{DLS}(nm)	ζ-potential (mV)	d_{DLS}(nm)	ζ-potential (mV)	d_{DLS}(nm)	ζ-potential (mV)	d_{DLS}(nm)	ζ-potential (mV)
WW dilution	0	25.9 ± 2.0	-33.4 ± 1.8	23.8 ± 2.5	-30.6 ± 3.7	30.5 ± 4.4	-23.3 ± 0.5	1104 ± 265	-20.0 ± 1.4
	1:16	-	-	23.8 ± 1.8	-29.1 ± 1.7	29.8 ± 2.6	-22.8 ± 1.4	1393 ± 57	-21.3 ± 0.4
	1:8	-	-	26.0 ± 2.8	-29.0 ± 1.7	78.2 ± 12.5	-22.7 ± 0.8	1573 ± 279	-19.6 ± 2.1
	1:4	-	-	28.2 ± 0.4	-26.9 ± 0.5	1265 ± 813	-21.5 ± 1.3	2336 ± 246	-20.3 ± 0.4
	1:2	-	-	24.0 ± 1.5	-23.8 ± 0.6	1380 ± 135	-20.8 ± 1.1	ND	-20.2 ± 0.7
	1:1	-	-	52 ± 12	-23.4 ± 0.9	1694 ± 625	-18.6 ± 0.7	ND	-16.0 ± 2.0

Table S7. Conductivity (mS/cm) for each WW dilution (1:16, 1:8, 1:4, 1:2 and 1:1 which are equivalent to 0.0625, 0.125, 0.25, 0.5 and 1 WW-dH_2O d.f.) and culture medium combinations according to bioassay treatments.

Conductivity (mS/cm)		Water	AA/8 +N medium	Steinberg medium	Freshwater medium
WW dilution	0	0.035 ± 0.001	0.87 ± 0.04	0.85 ± 0.06	0.61 ± 0.01
	1:16	-	0.98 ± 0.03	0.93 ± 0.01	0.67 ± 0.02
	1:8	-	1.06 ± 0.06	0.98 ± 0.02	0.69 ± 0.01
	1:4	-	1.08 ± 0.03	1.1 ± 0.01	0.74 ± 0.1
	1:2	-	1.25 ± 0.03	1.25 ± 0.03	0.85 ± 0.02
	1:1	-	1.53 ± 0.04	1.5 ± 0.01	1.00 ± 0.05

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Table S8. Concentrations and physicochemical properties of pollutants detected in wastewater. (D_{ow} is the pH-dependent or apparent octanol-water distribution coefficient, which considers both the dissociation constant of acidic of basic solutes, given by pK_a, and the pH of the medium.)

Nº	Compound	Concentration (ng/L)	CAS number	Molecular formula	$logK_{ow}$	pK_a (1)	pK_a (2)	pK_a (3)	Acid/Basic	$logD_{ow}$ (pH 7.8)	Charge (+/-)	Main Use
1	4-Aminoantipyrine (4-AA)	588	83-07-8	$C_{11}H_{13}N_3O$	-0.07	4.9			Weakly basic	-0.07	0.00	Metabolite of aminopyrine
2	4-Acetyl aminoantipyrine (4-AAA)	4255	83-15-8	$C_{13}H_{15}N_3O_2$	-0.10	12.8			Weakly acidic	-0.10	0.00	Metabolite of metamizole
3	4-Formyl aminoantipyrine (4-FAA)	8049	1672-58-8	$C_{12}H_{13}N_3O_2$	0.11	12.7			Weakly acidic	0.11	0.00	Metabolite of aminopyrine
4	Acetaminophen	156	103-90-2	$C_8H_9NO_2$	0.46	9.4			Weakly acidic	-1.15	-0.02	Analgesic
5	Acridone	143	578-95-0	$C1_3H_9NO$	5.28	5.6			Weakly basic	5.28	0.01	Antiviral
6	Amisulpride	194	53583-79-2	$C1_7H_{27}N_3O_4S$	1.06	9.4			Basic	-0.52	0.97	Antipsychotic
7	Antipyrine	460	60-80-0	$C_{11}H_{12}N_2O$	0.38	1.4			Weakly basic	0.38	0.00	Analgesic
8	Atenolol	801	29122-68-7	$C_{14}H_{22}N_2O_3$	0.16	9.6			Basic	-1.65	0.98	β-blocker
9	Benzotriazole	1386	95-14-7	$C_6H_5N_3$	1.44	8.4			Weakly basic	1.3	0.79	Antifungal
10	Caffeine	2542	58-08-2	$C_8H_{10}N_4O_2$	-0.07	0.8			Neutral	-0.07	0.00	Stimulant
11	Cetirizine	108	83881-51-0	$C_{21}H_{25}N_2ClO_3$	1.70	3.57	7.6		Zwitterionic	-2.53	-0.63	Antihistaminic
12	Ciprofloxacin	19440	85721-33-1	$C_{17}H_{18}FN_3O_3$	0.28	6.1	8.7		Zwitterionic	-1.49	-0.09	Antibiotic
13	Citalopram	162	59729-33-8	$C_{20}H_{21}FN_2O$	3.74	9.7			Basic	1.83	0.99	Antidepressant
14	Clindamycin	100	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	2.16	7.8			Basic	1.86	0.49	Antibiotic
15	Dextromethorphan	39	125-71-3	$C_{18}H_{25}NO$	3.97	9.9			Basic	1.92	0.99	Antitussives
16	Eprosartan	1080	133040-01-4	$C_{23}H_{24}N_2O_4S$	3.67	3.1	3.8	7.1	Zwitterionic	-0.38	-1.84	Angiotensin II receptor antagonists
17	Famotidine	14700	76824-35-6	$C_8H_{15}N_7O_2S_3$	-0.64	6.7			Weakly Basic	-0.67	0.07	Antiulcer
18	Flecainide	14613	54143-55-4	$C_{17}H_{20}N_2F_6O_3$	3.78	9.3			Basic	2.27	0.97	Antiarrhythmic
19	Gabapentin	2657	60142-96-3	$C_9H_{17}NO_2$	-1.10	3.7	10.7		Zwitterionic	-5.25	0.00	Anticonvulsant
20	Iomeprol	34678	78649-41-9	$C_{17}H_{22}I_3N_3O_8$	-2.79	11.4			Weakly acidic	-2.79	0.00	Contrast agent
21	Iopamidol	3213	60166-93-0	$C_{17}H_{22}I_3N_3O_8$	-2.42	10.7			Neutral	-2.42	0.00	Contrast agent
22	Iopromide	3565	73334-07-3	$C_{18}H_{24}I_3N_3O_8$	-2.05	11.1			Weakly acidic	-2.05	0.00	Contrast agent
23	Irbesartan	884	138402-11-6	$C_{25}H_{28}N_6O$	5.31	4.1	4.3		Weakly basic	5.31	0.00	Angiotensin II receptor antagonists
24	Ketorolac	10676	74103-06-3	$C_{15}H_{13}NO_3$	2.32	3.5			Weakly basic	-1.98	0.00	Anti-inflammatory
25	Ketoprofen	2057	22071-15-4	$C_{16}H_{14}O_3$	3.12	5.9			Acidic	1.25	-0.99	Anti-inflammatory
26	Labetalol	55	36894-69-6	$C_{19}H_{24}N_2O_3$	3.09	9.3			Basic	1.58	0.97	Beta blocker

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27	Lidocaine	258	137-58-6	$C_{14}H_{22}N_2O$	2.26	8.0			Basic	1.84	0.62	Local anaesthetic
28	Memantine	43	19982-08-2	$C_{12}H_{21}N$	3.28	10.3			Basic	0.81	1.00	NMDA receptor antagonist
29	Mepivacaine	189	96-88-8	$C_{15}H_{22}N_2O$	1.95	7.7			Basic	1.70	0.44	Local anaesthetic
30	Methadone	6	76-99-3	$C_{21}H_{27}NO$	3.93	9.2			Basic	2.51	0.96	Synthetic Opioid
31	Metoclopramide	325	364-62-5	$C_{14}H_{22}ClN_3O_2$	2.62	9.2			Basic	1.20	0.96	Antiemetic
32	Metronidazole	427	443-48-1	$C_6H_9N_3O_3$	-0.02	2.5			Weakly basic	-0.02	0.00	Antibiotic
33	Naproxen	2008	22204-53-1	$C_{14}H_{14}O_3$	3.18	4.2			Acidic	-0.47	-1.00	Anti-inflammatory
34	Nicotinamide	390	98-92-0	$C_6H_5NO_2$	-0.37	13.3			Weakly acidic	-0.37	0.00	Vitamin
35	O-desmethylvenlafaxine	2753	142761-12-4	$C_{16}H_{25}NO_2$	0.21	9.5	10.7		Basic	-1.45	0.98	Antidepressants
36	Oxcarbazepine	189	28721-07-5	$C_{15}H_{12}N_2O_2$	1.11	-0.7			Neutral	1.11	0.00	Antiepileptic
37	Primidone	713	125-33-7	$C_{12}H_{14}N_2O_2$	0.91	12.3			Weakly acid	0.91	0.00	Antiepileptic
38	Sulfapyridine	73	144-83-2	$C_{11}H_{11}N_3O_2S$	0.35	2.3	8.4		Basic	0.35	0.00	Antibiotic
39	Sulpiride	518	15676-16-1	$C_{15}H_{23}N_3O_4S$	0.57	9.1			Basic	-0.77	0.95	Antipsychotic
40	Theophylline	542	58-55-9	$C_7H_8N_4O_2$	-0.02	8.8			Basic	-1.07	0.91	Bronchodilator/ Vasodilator
41	Tramadol	2277	27203-92-5	$C1_6H_{25}NO_2$	3.01	9.4			Basic	1.39	0.98	Analgesic
42	Trimethoprim	199	738-70-5	$C_{14}H_{18}N_4O_2$	0.91	7.1			Basic	0.83	0.17	Antibiotic
43	Valsartan	2275	137862-53-4	$C_{24}H_{29}N_5O_3$	4.00	4.0	4.6		Acidic	0.20	-1.00	Antihypertensives
44	Venlafaxine	337	93413-69-5	$C_{17}H_{27}NO_2$	3.20	9.4			Basic	1.59	0.98	Antidepressant

*Wastewater at pH 7.8

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Table S9. Single toxicity of PS NPs (12.5, 25, 50, 100 and 200 mg/L) and WW (1:16, 1:8, 1:4, 1:2 and 1:1 which are equivalent to 0.0625, 0.125, 0.25, 0.5 and 1 WW dilution factors, d.f) and their combined toxicity on bioluminescence of the *Anabaena* CPB4337 after 24 h of exposure. Results are expressed as percentage of bioluminescence inhibition ± SD with respect to control (non-exposed cells = 100) (n=3).

Anabaena CPB4337		PS NPs (mg/L)					
		0	12.5	25	50	100	200
WW dilution	0	100 ± 9.9	73.6 ± 4.2	55.1 ± 9.1	52.6 ± 5.7	35.2 ± 20.2	33.5 ± 0.7
	1:16	92.5 ± 2.7	91.2 ± 7.5	82.9 ± 9.8	59.8 ± 5.5	101.8 ± 4.5	63.6 ± 8.1
	1:8	79.3 ± 3.9	73.9 ± 10.4	65.8 ± 2.6	51.3 ± 2.3	90.9 ± 8.5	54.2 ± 2.8
	1:4	64.1 ± 15.8	64.3 ± 8.5	69.1 ± 4.0	56.2 ± 2.2	75.2 ± 10.0	53.1 ± 3.0
	1:2	34.2 ± 10.1	62.8 ± 6.6	57.6 ± 7.4	57.7 ± 2.4	67.6 ± 0.8	65.9 ± 6.4
	1:1	7.2 ± 1.6	37.7 ± 15.0	51.5 ± 7.0	53.7 ± 2.0	58.4 ± 2.5	58.1 ± 10.4

Table S10. Dose-response parameters for bioluminescence inhibition at 24 h, growth at 72 h, and immobilization at 48 h of *Anabaena* CPB4337, *S. polyrhiza* and *D. magna*, respectively, after individual exposure to PS NPs (mg/L) and WW (d.f).

Organism	Compound	EC_{50}	SD	95 % Confidence Interval	r^2
Anabaena CPB4337	PS NPs	58.3	12.7	31.0-85.5	0.92
	WW	0.34	0.03	0.26-0.41	0.94
S. polyrhiza	PS NPs	170	14	124-215	0.98
	WW	0.94	0.27	0.075-1.81	0.96
D. magna	PS NPs	32.4	2.4	27.5-37.4	0.94
	WW	-	-	-	-

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Table S11. Toxicity reported in the literature for the wastewater pollutants identified in this work.

Nº	Compound	Taxon	Species	Toxicological Endpoint	LC_{50}/EC_{50} (mg/L)	Reference
4	Acetaminophen	Bacteria	*Vibrio fischeri*	Luminescence inhibition (15 min)	567.5	Kim et al. 2007
				Luminescence inhibition (30 min)	650	Henschel et al. 1997
		Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (96 h)	> 240	Wang et al. 2015
			Scenedesmus dimorphus			
			Stichococcus bacillaris			
			Chlorella vulgaris			
			Chlamydomonas reinhardtii			
			Scenedesmus subspicatus	Growth inhibition (72 h)	134	Henschel et al. 1997
			Lemna minor	Number of fronds (7 d)	446.6	Nunes et al. 2014
		Animal	*Daphnia magna*	Mortality (48 h)	2.831	Damasceno de Oliveira et al. 2016
					40	Du et al. 2016
8	Atenolol	Bacteria	*Vibrio fischeri*	Luminescence inhibition (30 min)	1304	Escher et al. 2006
		Algae	*Desmodesmus subspicatus*	Photosynthetic yield (24 h)	1335	Escher et al. 2006
				Growth inhibition (72 h)	620	Cleuvers 2005
		Animal	*Thamnocephalus platyurus*	Immobilization (24 h)	>100	Kim et al. 2009
9	Benzotriazole	Bacteria	*Vibrio fischeri*	Luminescence inhibition (5 min)	41.13	Cancilla et al. 1997
				Luminescence inhibition (15 min)	41.65	Cancilla et al. 1997
		Algae	*Desmodesmus subspicatus*	Growth (72 h)	1.18*	Seeland et al. 2012
		Plant	*Lemna minor*	Growth (7 d)	3.94*	Seeland et al. 2012
		Animal	*Daphnia magna*	Mortality (48 h)	93.3	Giraudo et al. 2017
				Immobility (48 h)	107	Seeland et al. 2012
					155.4	Durjava et al. 2013
			Ceriodaphnia dubia	Mortality (48 h)	102	Pillard et al. 2001
10	Caffeine	Bacteria	*Vibrio fischeri*	Luminescence inhibition (5 min)	671.9	Calleja et al. 1994

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				Luminescence inhibition (30 min)	1244.3	Lomba et al. 2020
		Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	N.E 150	Zarrelli et al. 2014
			Raphidocelis subcapitata	Growth inhibition (72 h)	870.3	Lomba et al. 2020
		Animal	*Daphnia magna*	Immobilization (24 h)	1078.9	Lomba et al. 2020
					684	Lilius et al. 1994
12	Ciprofloxacin	Bacteria	*Vibrio fischeri*	Luminescence inhibition (30 min)	114.2**	Li et al. 2014
				Luminescence inhibition (5 min)	>5.9	Hernando et al.
				Luminescence inhibition (15 min)	>5.9	Hernando et al.
				Luminescence inhibition (30 min)	>5.9	Hernando et al.
			Microcystis aeruginosa	Growth inhibition (5 d)	0.017	Robinson et al. 2005
				Growth inhibition (7 d)	0.005	Halling-Sorensen et al. 2000
			Anabaena flos-aquae	Growth inhibition (72 h)	0.102	Ebert et al. 2011
		Algae	*Chlorella vulgaris*	Growth inhibition (96 h)	20.6	Nie et al. 2008
			Pseudokirchneriella subcapitata	Growth inhibition (72 h)	2.97	Halling-Sorensen et al. 2000
					6.7	Yang et al. 2008
					18.7	Robinson et al. 2005
			Desmodesmus subspicatus	Growth inhibition (72 h)	>0.008	Ebert et al. 2011
13	Citalopram	Bacteria	*Raphidocelis subcapitata*	Growth inhibition (72 h)	3.3	Minguez et al. 2016
		Algae		Growth inhibition (48 h)	1.6	Christensen et al. 2007
		Animal	*Daphnia magna*	Immobilization (48 h)	30.1	Minguez et al. 2014
14	Clindamycin	Bacteria	*Anabaena flos-aquae*	Growth inhibition (72 h)	0.0296	Coors et al. 2017
		Algae	*Raphidocelis subcapitata*	Growth inhibition (72 h)	0.01	Villain et al. 2016
		Animal	*Daphnia sp.*	Immobilization (48 h)	0.07	Mankes and Silver 2016
17	Famotidine	Animal	*Thamnocephalus platyurus*	Immobilization (24 h)	>100	Kim et al. 2009
21	Iopamidol	Animal	*Lampsilis siliquoidea*	Mortality (7 d)	>101	Gilroy et al. 2017

22	Iopromide	Bacteria	*Vibrio fischeri*	Luminescence inhibition (15 min)	>10000	Steger-Hartmann et al. 1998
				Luminescence inhibition (30 min)	>10000	Vandenbergh et al. 2003
				Luminescence inhibition (30 min)	>10000	Steger-Hartmann et al. 1998
		Algae	*Scenedesmus subspicatus*	Growth inhibition (72 h)	>10000	Steger-Hartmann et al. 1998
		Animal	*Daphnia magna*	Immobilization (48 h)	>10000	Gyllenhammar et al. 2009
				Immobilization (24 h)	>10000	Steger-Hartmann et al. 1998
23	Irbesartan	Algae	*Raphidocelis subcapitata*	Growth inhibition (72 h)	>100	Minguez et al. 2016
		Animal	*Daphnia magna*	Immobilization (48 h)	>100	Minguez et al. 2016
25	Ketoprofen	Bacteria	*Vibrio fischeri*	Luminescence inhibition	19.3	Farré et al. 2001
				Luminescence inhibition	15.6	Farré et al. 2001
		Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	49.30	Minguez et al. 2016
		Animal	*Daphnia magna*	Immobilization (48 h)	32.93	Minguez et al. 2016
27	Lidocaine	Bacteria	*Vibrio fischeri*	Luminescence inhibition (30 min)	4754.3	Lomba et al. 2020
		Algae	*Scenedesmus vacuolatus*	Growth rate (24 h)	108-145	Neuwoehner and Escher, 2011
			Raphidocelis subcapitata	Growth inhibition (72 h)	194.3	Lomba et al. 2020
		Animal	*Daphnia magna*	Immobilization (24 h)	308.8	Lomba et al. 2020
32	Metronidazole	Algae	*Chlorella sp.*	Growth inhibition (72 h)	38.8	Lanzky and Halting-Sørensen et al. 1997
			Pseudokirchneriella subcapitata	Growth inhibition (72 h)	39.1	Lanzky and Halting-Sørensen et al. 1997
		Animal	*Daphnia magna*	Immobility (48 h)	>1000	Wollenberger et al. 2000
33	Naproxen	Bacteria	*Anabaena flosaquae*	Growth inhibition (72 h)	12.3	Straub and Stewart, 2007
			Vibrio fischeri	Luminescence inhibition (30 min)	42.95	DellaGreca et al. 2004

		Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	44.4	Villain et al. 2016
				Growth inhibition (72 h)	31.82	Zuccato et al. 2001
			Desmodesmus subspicatus	Growth inhibition (72 h)	625.5	Cleuvers, 2004
		Plant	*Lemna minor*	Growth inhibition (7 d)	24.2	Cleuvers, 2003
		Animal	*Daphnia magna*	Immobilization (48 h)	174	Cleuvers, 2003
36	Oxcarbazepine	Animal	*Daphnia magna*	Mortality (48 h)	1.5	Wang et al. 2018
38	Sulfapyridine	Bacteria	*Vibrio fischeri*	Luminescence Inhibition (30 min)	>50	Białk-Bielińska et al. 2011
		Algae	*Scenedesmus vacuolatus*	Growth inhibition (24 h)	5.28	Białk-Bielińska et al. 2011
		Plant	*Lemna minor*	Growth inhibition (7 d)	0.46	Białk-Bielińska et al. 2011
39	Sulpiride	Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	99.8	Watanabe et al. 2016
		Animal	*Ceriodaphnia dubia*	Reproduction (8 d)	>100	Watanabe et al. 2016
40	Theophylline	Animal	*Daphnia pulex*	Immobility (24 h)	327.9	Lilius et al. 1994
			Daphnia magna	Immobility (24 h)	155.19	Lilius et al. 1994
41	Tramadol	Animal	*Daphnia magna*	Mortality (24 h)	170	Le et al. 2011
42	Trimethoprim	Bacteria	*Anabaena variabilis*	Growth inhibition (6 d)	11	Ando et al. 2007
			Nostoc sp. PCC7120	Growth inhibition (6 d)	53	Ando et al. 2007
			Microcystis aeruginosa (NIES-44)	Growth inhibition (6 d)	150	Ando et al. 2007
			Microcystis wesenbergii (NIES-107)	Growth inhibition (6 d)	>200	Ando et al. 2007
			Synechococcus sp. (PCC 7002)	Growth inhibition (6 d)	> 200	Ando et al. 2007
			Anabaena cylindrica (NIES-19)	Growth inhibition (6 d)	> 200	Ando et al. 2007
			Anabaena flos-aquae (ATCC 29413)	Growth inhibition (6 d)	>200	Ando et al. 2007
			Vibrio fischeri	Luminescence inhibition (30 min)	> 0.28	van der Grinten et al. 2010

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			Microcystis aeruginosa	Growth inhibition (7 d)	112	Halling-Sorensen et al. 2000
		Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (24 h)	> 9	van der Grinten et al. 2010
				Growth inhibition (72 h)	56.01	Minguez et al. 2016
					110	Halling-Sorensen et al. 2000
					80.3	Eguchi et al. 2004
					40	Yang et al. 2008
		Animal	*Daphnia magna*	Immobilization (48 h)	>100	Minguez et al. 2016
43	Valsartan	Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	>100	Minguez et al. 2016
		Animal	*Daphnia magna*	Immobilization (48 h)	>100	Minguez et al. 2016
44	Venlafaxine	Algae	*Pseudokirchneriella subcapitata*	Growth inhibition (72 h)	47.58	Minguez et al. 2016
		Plant	*Polystichum setiferum*	Mitochondrial activity	0.01***	Feito et al. 2013
				growth (DNA content) (48 h)	0.001***	Feito et al. 2013
		Animal	*Daphnia magna*	Immobilization (48 h)	141.28	Minguez et al. 2014

NE = No effect at "x" (mg/L)
* EC_{10} (mg/L)
** EC_{20} (mg/L)
*** LOEC (µg/L)

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References (Table S11)

Ando, T., Nagase, H., Eguchi, K., Hirooka, T., Nakamura, T., Miyamoto, K., Hirata, K., 2007. A novel method using cyanobacteria for ecotoxicity test of veterinary antimicrobial agents. Environmental Toxicology and Chemistry 26, 601-606.

Białk-Bielińska, A., Stolte, S., Arning, J., Uebers, U., Böschen, A., Stepnowski, P., Matzke, M., 2011. Ecotoxicity evaluation of selected sulfonamides. Chemosphere 85, 928-933.

Calleja, M.C., Persoone, G., Geladi, P., 1994. Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Archives of Environmental Contamination and Toxicology 26, 69-78

Cancilla, D.A., Holtkamp, A., Matassa, L., Fang, X., 1997. Isolation and characterization of Microtox®-active components from aircraft de-icing/anti-icing fluids. Environmental Toxicology and Chemistry 16, 430-434.

Christensen, A.M., Faaborg-Andersen, S., Flemming, I., Baun, A., 2007. Mixture and single-substance toxicity of selective serotonin reuptake inhibitors toward algae and crustaceans. Environmental Toxicology and Chemistry 26, 85-91.

Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicology Letters 142, 185-194.

Cleuvers, M., 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicology and Environmental Safety 59, 309-315.

Cleuvers, M., 2005.Initial risk assessment for three β-blockers found in the aquatic environment. Chemosphere**,** 59, 199-205.

Coors, A., Vollmar, P., Sacher, F., Thoma, A. 2017. Joint effects of pharmaceuticals and chemicals regulated under REACH in wastewater treatment plant effluents–Evaluating concepts for a risk assessment by means of experimental scenarios. Umwelt Bundesamt, 61/2017, 1-128

de Oliveira, L.L.D., Antunes, S.C., Gonçalves, F., Rocha, O., Nunes, B., 2016. Acute and chronic ecotoxicological effects of four pharmaceuticals drugs on cladoceran *Daphnia magna*. Drug and Chemical Toxicology 39, 13-21.

DellaGreca, M., Brigante, M., Isidori, M., Nardelli, A., Previtera, L., Rubino, M., Temussi, F., 2003. Phototransformation and ecotoxicity of the drug Naproxen-Na. Environmental Chemistry Letters 1, 237-241.

Du, J., Mei, C.-F., Ying, G.-G., Xu, M.-Y., 2016. Toxicity Thresholds for diclofenac, acetaminophen and ibuprofen in the water flea *Daphnia magna*. Bulletin of Environmental Contamination and Toxicology 97, 84-90.

Durjava, M.K., Kolar, B., Arnus, L., Papa, E., Kovarich, S., Sahlin, U., Peijnenburg, W., 2013. Experimental assessment of the environmental fate and effects of triazoles and benzotriazole. Alternatives to Laboratory Animals 41, 65-75.

Escher, B.I., Bramaz, N., Richter, M., Lienert, J., 2006. Comparative ecotoxicological hazard assessment of beta-blockers and their human metabolites using a mode-of-action-based test battery and a QSAR approach. Environmental Science & Technology 40, 7402-7408

Ebert, I., Bachmann, J., Kühnen, U., Küster, A., Kussatz, C., Maletzki, D., Schlüter, C., 2011. Toxicity of the fluoroquinolone antibiotics enrofloxacin and ciprofloxacin to photoautotrophic aquatic organisms. Environmental Toxicology and Chemistry 30, 2786-2792.

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y.S., Goto, K., Hirata, K., Miyamoto, K., Yoshimura, H., 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere 57, 1733-1738.

Farré, M.l., Ferrer, I., Ginebreda, A., Figueras, M., Olivella, L., Tirapu, L., Vilanova, M., Barceló, D., 2001. Determination of drugs in surface water and wastewater samples by liquid chromatography–mass spectrometry: methods and preliminary results including toxicity studies with *Vibrio fischeri*. Journal of Chromatography A 938, 187-197.

Feito, R., Valcárcel, Y., Catalá, M., 2013. Preliminary data suggest that venlafaxine environmental concentrations could be toxic to plants. Chemosphere 90, 2065-2069.

Gilroy, È.A.M., Gillis, P.L., King, L.E., Bendo, N.A., Salerno, J., Giacomin, M., de Solla, S.R., 2017. The effects of pharmaceuticals on a unionid mussel (*Lampsilis siliquoidea*): An examination of acute and chronic endpoints of toxicity across life stages. Environmental Toxicology and Chemistry 36, 1572-1583.

Gyllenhammar, I., Holm, L., Eklund, R., Berg, C., 2009. Reproductive toxicity in *Xenopus tropicalis* after developmental exposure to environmental concentrations of ethynylestradiol. Aquatic Toxicology 91, 171-178

Halling-Sørensen, B., Lützhøft, H.C.H., Andersen, H.R., Ingerslev, F., 2000. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. Journal of Antimicrobial Chemotherapy 46, 53-58.

Henschel, K.P., Wenzel, A., Diedrich, M., Fliedner, A., 1997. Environmental hazard assessment of pharmaceuticals. Regulatory Toxicology and Pharmacology 25, 220-225.

Hernando, M.D., De Vettori, S., Martínez Bueno, M.J., Fernández-Alba, A.R., 2007. Toxicity evaluation with *Vibrio fischeri* test of organic chemicals used in aquaculture. Chemosphere 68, 724-730.

Kim, J.-W., Ishibashi, H., Yamauchi, R., Ichikawa, N., Takao, Y., Hirano, M., Koga, M., Arizono, K., 2009. Acute toxicity of pharmaceutical and personal care products on freshwater crustacean *Thamnocephalus platyurus* and fish *Oryzias latipes*. The Journal of Toxicological Sciences 34, 227-232.

Kim, Y.S., Taura, T., 2007. Design creativity workshop, Proceedings of the 6th ACM SIGCHI conference on Creativity & cognition. Association for Computing Machinery, Washington, DC, USA, p. 275.

Le, T.-H., Lim, E.-S., Lee, S.-K., Park, J.-S., Kim, Y.-H., Min, J., 2011. Toxicity evaluation of verapamil and tramadol based on toxicity assay and expression patterns of Dhb, Vtg, Arnt, CYP4, and CYP314 in *Daphnia magna*. Environmental Toxicology 26, 515-523.

Li, M., Wei, D., Du, Y., 2014. Acute toxicity evaluation for quinolone antibiotics and their chlorination disinfection processes. Journal of Environmental Sciences 26, 1837-1842.

Lilius, H., Isomaa, B., Holmström, T., 1994. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. Aquatic Toxicology 30, 47-60.

Lomba, L., Lapeña, D., Ros, N., Aso, E., Cannavò, M., Errazquin, D., Giner, B., 2020. Ecotoxicological study of six drugs in Aliivibrio fischeri, *Daphnia magna* and *Raphidocelis subcapitata*. Environmental Science and Pollution Research 27, 9891-9900.

Mankes R.F., Silver C.D., 2016 Hospital Antibiotic Wasting and Evaluation of Potential Ecologic Effects. Annals of Environmental Science and Toxicology 1, 012-022.

Minguez, L., Farcy, E., Ballandonne, C., Lepailleur, A., Serpentini, A., Lebel, J.-M., Bureau, R., Halm-Lemeille, M.-P., 2014. Acute toxicity of 8 antidepressants: What are their modes of action? Chemosphere 108, 314-319.

Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., Halm-Lemeille, M.-P., 2016. Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern France. Environmental Science and Pollution Research 23, 4992-5001.

Neuwoehner, J., Escher, B.I., 2011. The pH-dependent toxicity of basic pharmaceuticals in the green algae Scenedesmus vacuolatus can be explained with a toxicokinetic ion-trapping model. Aquatic Toxicology 101, 266-275.

Nie, X., Wang, X., Chen, J., Zitko, V., An, T., 2008. Response of the freshwater alga *Chlorella vulgaris* to trichloroisocyanuric acid and ciprofloxacin. Environmental Toxicology and Chemistry 27, 168-173.

Nunes, B., Pinto, G., Martins, L., Gonçalves, F., Antunes, S.C., 2014. Biochemical and standard toxic effects of acetaminophen on the macrophyte species Lemna minor and Lemna gibba. Environmental Science and Pollution Research 21, 10815-10822.

Pillard, D.A., Cornell, J.S., DuFresne, D.L., Hernandez, M.T., 2001. Toxicity of benzotriazole and benzotriazole derivatives to three aquatic species. Water Research 35, 557-560.

Robinson, A.A., Belden, J.B., Lydy, M.J., 2005. Toxicity of fluoroquinolone antibiotics to aquatic organisms. Environmental Toxicology and Chemistry 24, 423-430.

Seeland, A., Oetken, M., Kiss, A., Fries, E., Oehlmann, J., 2012. Acute and chronic toxicity of benzotriazoles to aquatic organisms. Environmental Science and Pollution Research 19, 1781-1790.

Steger-Hartmann, T., Länge, R., Schweinfurth, H., 1999. Environmental Risk Assessment for the Widely Used Iodinated X-Ray Contrast Agent Iopromide (Ultravist). Ecotoxicology and Environmental Safety 42, 274-281.

Straub, J.O., Stewart, K.M., 2007. Deterministic and probabilistic acute-based environmental risk assessment for naproxen for Western Europe. Environmental Toxicology and Chemistry 26, 795-806.

van der Grinten, E., Pikkemaat, M.G., van den Brandhof, E.-J., Stroomberg, G.J., Kraak, M.H.S., 2010. Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of antibiotics. Chemosphere 80, 1-6.

Vandenbergh, G.F., Adriaens, D., Verslycke, T., Janssen, C.R., 2003. Effects of 17-ethinylestradiol on sexual development of the amphipod *Hyalella azteca*, Ecotoxicology and Environmental Safety., 54, 216–222.

Villain, J., Minguez, L., Halm-Lemeille, M.-P., Durrieu, G., Bureau, R., 2016. Acute toxicities of pharmaceuticals toward green algae. mode of action, biopharmaceutical drug disposition classification system and quantile regression models. Ecotoxicology and Environmental Safety 124, 337-343.

Wang, D. C. Y., Chu, W. L., Kok, Y. Y. 2015. Assessment of Paracetamol (acetaminophen) toxicity in microalgae. Polish Journal of Environmental Studies 24 (2), 735–741

Wang, T., Huang, Z.-X., Miao, H.-F., Ruan, W.-Q., Ji, X.-P., Sun, F.-B., Zhao, M.-X., Ren, H.-Y., 2018. Insights into influencing factor, degradation mechanism and potential toxicity involved in aqueous ozonation of oxcarbazepine (CHEM46939R1). Chemosphere 201, 189-196.

Watanabe, H., Tamura, I., Abe, R., Takanobu, H., Nakamura, A., Suzuki, T., Hirose, A., Nishimura, T., Tatarazako, N., 2016. Chronic toxicity of an environmentally relevant mixture of pharmaceuticals to three aquatic organisms (alga, daphnid, and fish). Environmental Toxicology and Chemistry 35, 996-1006.

Wollenberger, L., Halling-Sørensen, B., Kusk, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40, 723-730.

Yang, L.-H., Ying, G.-G., Su, H.-C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*. Environmental Toxicology and Chemistry 27, 1201-1208.

Zarrelli, A., DellaGreca, M., Iesce, M.R., Lavorgna, M., Temussi, F., Schiavone, L., Criscuolo, E., Parrella, A., Previtera, L., Isidori, M., 2014. Ecotoxicological evaluation of caffeine and its derivatives from a simulated chlorination step. Science of The Total Environment 470-471, 453-458.

Zuccato, E., Bagnati, R., Fioretti, F., Natangelo, M., Calamari, D., Fanelli, R., 2001. Environmental Loads and Detection of Pharmaceuticals in Italy, in: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 19-27.

https://doi.org/10.1016/j.scitotenv.2022.153063

Table S12. Single toxicity of PS NPs (12.5, 25, 50, 100 and 200 mg/L) and WW (1:16, 1:8, 1:4, 1:2 and 1:1 which are equivalent to 0.0625, 0.125, 0.25, 0.5 and 1 WW dilution factors, d.f) and their combined toxicity on growth of *S. polyrhiza* after 72 h of exposure. Results are expressed as percentage of growth ± SD with respect to control (non-exposed plants = 100, n=3).

Spirodela polyrhiza		PS NPs (mg/L)					
		0	12.5	25	50	100	200
WW dilution	0	100 ± 19	85 ± 14	75.4 ± 12.7	70 ± 18	58.0 ± 7.2	47.5 ± 13.7
	1:16	81 ± 10	157 ± 16	144.8 ± 1.4	134.7 ± 0.4	70 ± 17	25.1 ± 0.4
	1:8	70.5 ± 4.7	123 ± 35	118.9 ± 5.3	116.0 ± 2.2	71.7 ± 5.9	51 ± 17
	1:4	68 ± 11	101 ± 14	98.5 ± 1.6	97.5 ± 5.6	71.9 ± 4.6	70.9 ± 5.5
	1:2	63.2 ± 7.2	95.3 ± 9.1	94.7 ± 4.7	94.0 ± 0.8	80.2 ± 2.1	71.9 ± 4.3
	1:1	45.6 ± 5.1	91.7 ± 6.9	87.4 ± 5.1	86 ± 18	84 ± 17	74.8 ± 4.3

Table S13. Single toxicity of PS NPs (12.5, 25, 50, 100 and 200 mg/L) and WW (1:16, 1:8, 1:4, 1:2 and 1:1 which are equivalent to 0.0625, 0.125, 0.25, 0.5 and 1 WW dilution factors, d.f) and their combined toxicity on growth of the *Daphnia magna* after 48 h of exposure. Results are expressed as percentage ± SD of mobility with respect to control (non-exposed neonates = 100) (n=4 in single exposure, n=3 in combined exposure). ND, not significantly different from zero.

Daphnia magna		PS NPs (mg/L)					
		0	12.5	25	50	100	200
WW dilution	0	100 ± 2.5	90 ± 12	75 ± 31	10 ± 12	ND	ND
	1:16	91 ± 2.5	93 ± 10	76 ± 26	35 ± 26	27 ± 12	ND
	1:8	76 ± 15	96.0 ± 8.9	72 ± 23	37 ± 20	33 ± 23	ND
	1:4	86 ± 11	93 ± 10	60 ± 20	43 ± 29	40 ± 20	ND
	1:2	96 ± 2.5	90 ± 17	80 ± 18	53 ± 30	53 ± 12	ND
	1:1	98 ± 5	96.7 ± 8.2	80 ± 14	85 ±10	33.0 ± 12	10 ± 11

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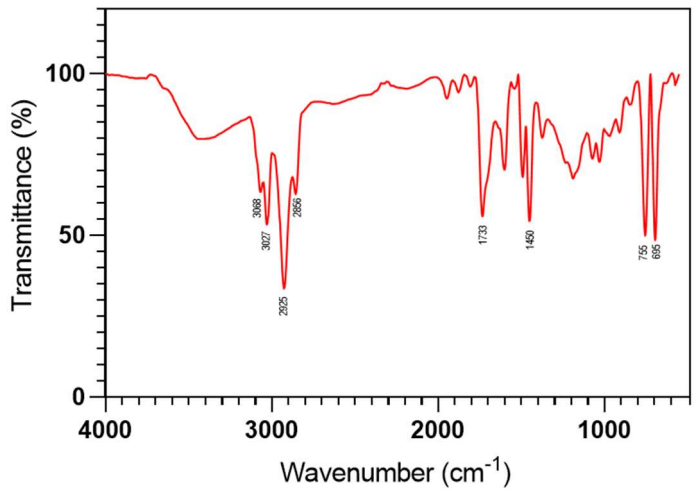

Figure S1. Micro-FTIR spectra of PS NPs.

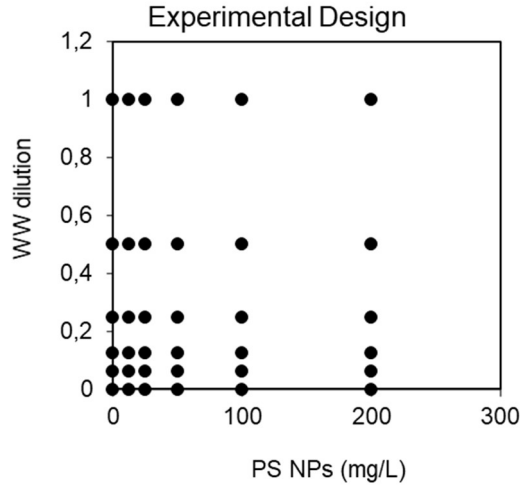

Figure S2. Experimental design of the binary mixture combinations of PS NPs-WW, used in this study. (1 in WW dilution axis represents 1:1 WW-dH_2O dilution, d.f.)

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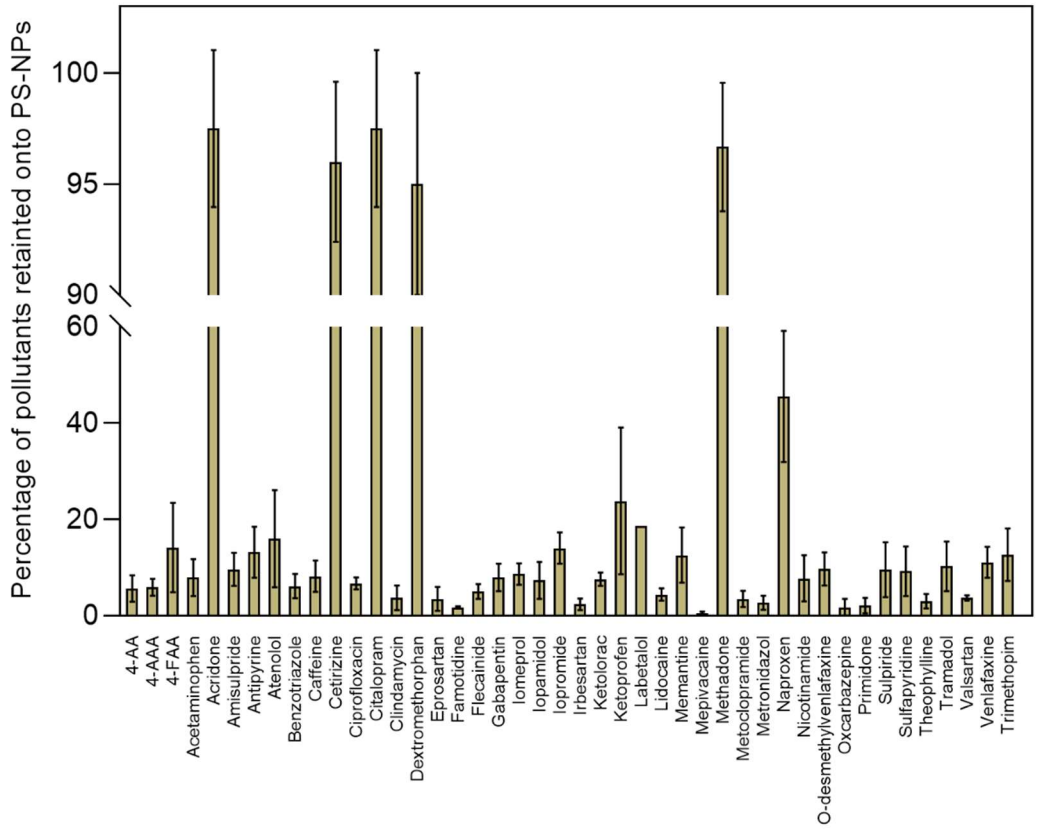

Figure S3. Percentage of pollutant retention onto PS NPs.

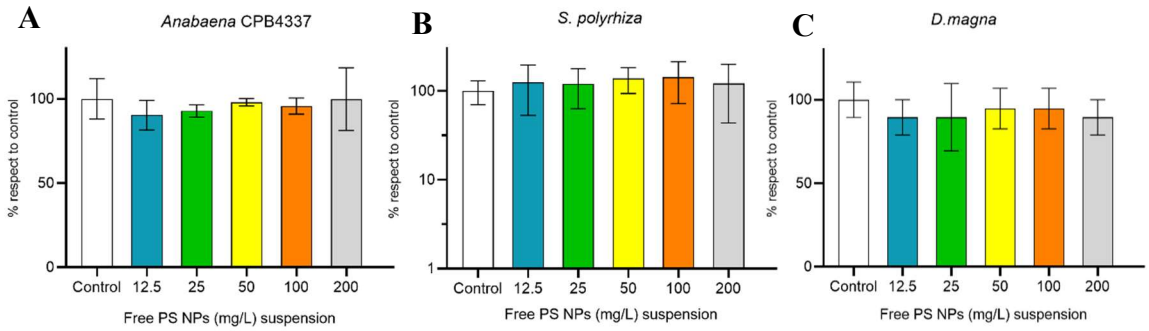

Figure S4. Effect of PS NPs-free suspensions (50 kDa ultrafiltrates) to *Anabaena* CPB4337 bioluminescence (A), *S. polyrhiza* growth (B) and *D. magna* mobility (C) on bioluminescence, after 24, 72 and 48 h, respectively. Results are expressed as percentage ± SD with respect to control (non-exposed organisms). X-axes represent the concentration of PS NPs suspensions before ultrafiltration. No significant differences were found ($p < 0.05$, Tukey's HSD test)

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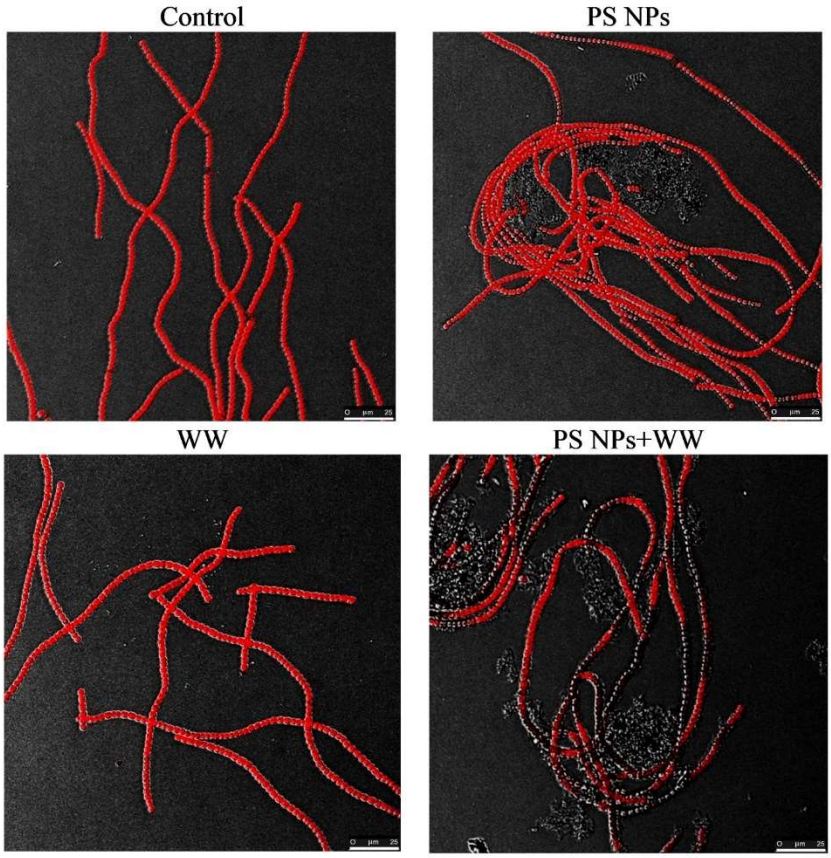

Figure S5. Confocal microscopy images of *Anabaena* CPB4337 exposed to PS NPs, WW, and their binary mixture (PS NPs+WW). Red color indicates the autofluorescence of chlorophyll *a*. Scale bar represents 25 μm.

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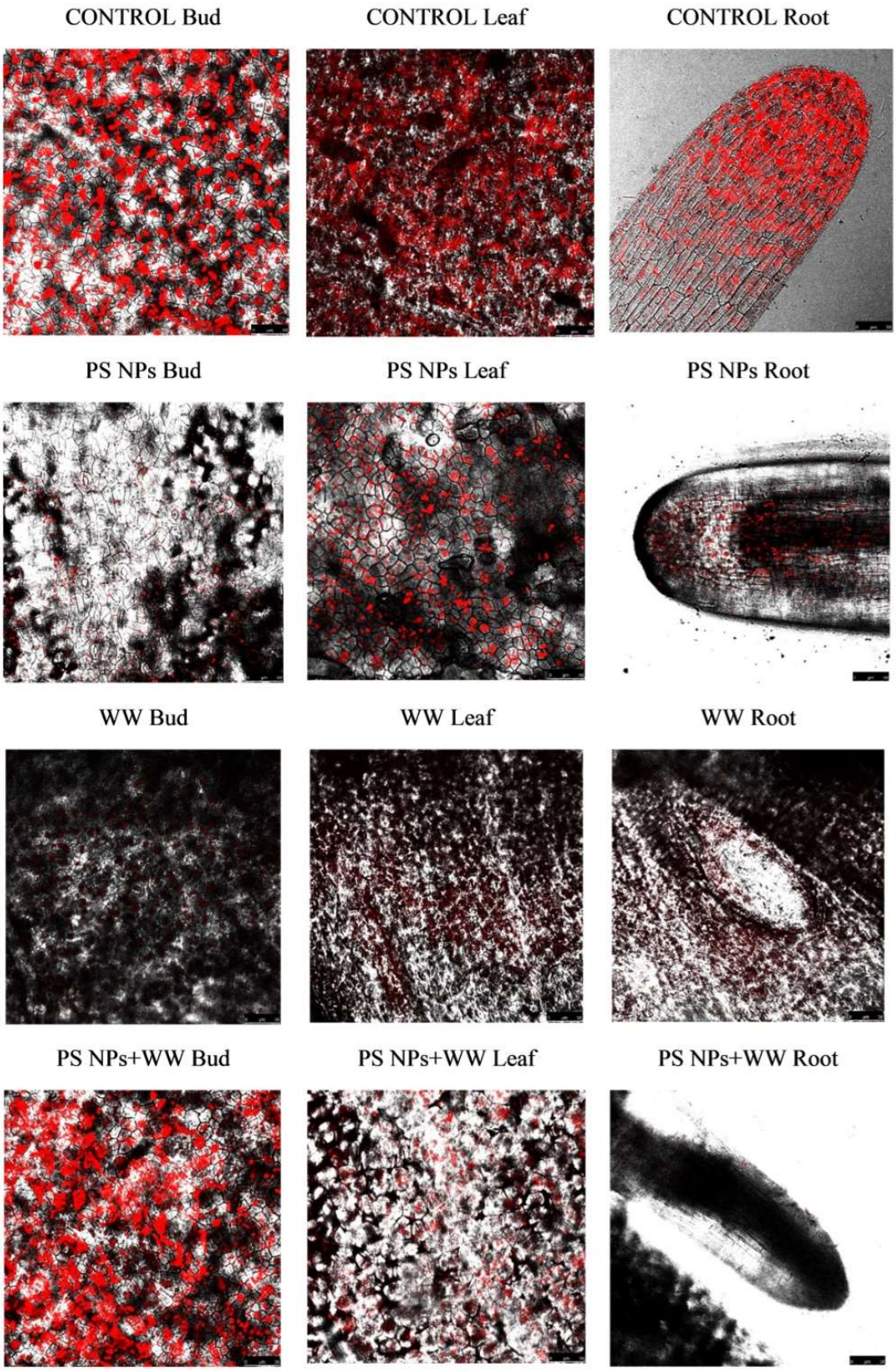

Figure S6. Representative confocal microscopy images of the *S. polyrhiza* exposed to PS NPs, WW, and their binary mixture (PS NPs+WW). Red colour indicates the autofluorescence of chlorophyll *a*. Scale bar represents 50 µm

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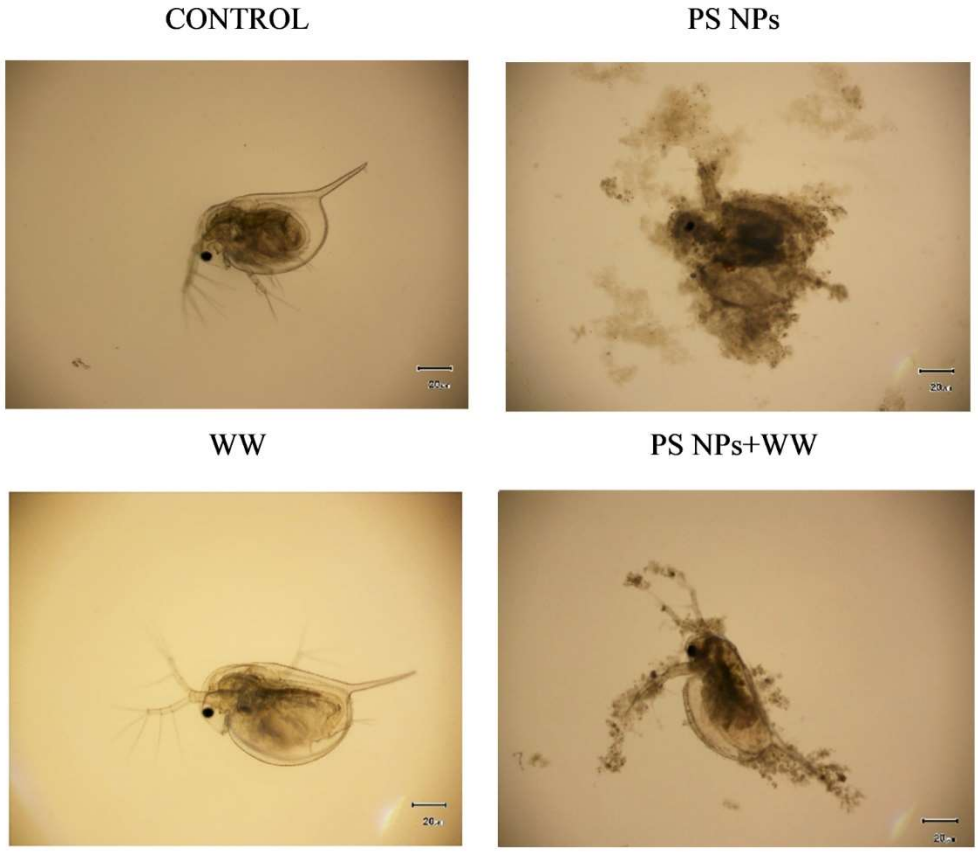

Figure S7. Microscopy images of the *D. magna* exposed to PS NPs, WW, and their binary mixture (PS NPs + WW). Scale bar represents 20 µm.