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ORIGINAL ARTICLE

Nano-sized natural organic matter interacts with bisphenol A and decreases cytotoxicity to human cells

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Abstract

While the toxicity of pollutants has been rather well explored in simple laboratory conditions, there is little knowledge on their real toxicity in natural environments and living organisms because pollutants are often interacting and trapped into organic matter. Because of these interactions, their real concentrations can also be underestimated. Here we studied the nature, intensity, and strength of the interactions between bisphenol A and nano-sized natural organic matter. The bioavailability and toxicity of the complexed bisphenol A were tested with human colon adenocarcinoma cell lines. Results show that that interaction of bisphenol A with organic matter reduces bisphenol A cytotoxicity. Moreover, the bisphenol A-organic matter interaction is weak in the first hour then very stable after 24 h. Once formed, the bisphenol A-organic matter complex escapes detection and, as a consequence, the levels of pollutants in organic-rich media is most probably underestimated. The mechanism of interaction involves hydrophobic and π-stacking forces inside the core of nano-sized organic matter.

Keywords Emerging contaminant · Cytotoxicity · Fluorescence spectroscopy · Nanoparticle · Pollutant mobility

Introduction

Natural organic matter (NOM), ubiquitous in aquatic systems, comprises a complex mixture of organic components. The percentage and distribution of the different compounds are extremely variable and very site-specific (Filella 2009). NOM can be considered a heterogeneous ligand whose interaction with organic and inorganic contaminants (Chen et al. 2019; Pontoni et al. 2021; Lichtfouse 2024) strongly affect their fate in aqueous solutions (Zularisam et al. 2006; Dryer et al. 2008; Yan et al. 2014, 2018; Chen et al. 2019, 2022), and their bioavailability and toxicity towards living organisms (Pontoni et al. 2022).

This paper investigated the interaction of NOM's low molecular weight fraction, called nano-NOM, supposed to be the most reactive fraction of NOM (Trubetskaya et al. 2016; Kong et al. 2021), with bisphenol A and its consequences on mobility and cytotoxicity. Bisphenol A is one of the most diffused pollutants. At very low doses (ng/L) it can be extremely harmful to living organisms (Muhamad et al. 2016; Morin-Crini et al. 2022), and therefore it has to be detected even at low concentrations. As nanoparticles are frequently removed during purging operations required for analytical determinations, the amount of bisphenol A bound to nanoparticles could be undetectable in water samples, so its concentration could be underestimated.

While many studies describe the behavior of bisphenol A in biological systems, the consequences of nano-NOM and bisphenol A interactions are completely unknown.

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Here we propose a novel approach to evaluate the nature of these interactions and their effects on the environmental quality. In detail, we analyze the physicochemical properties of nano-NOM and bisphenol A complexes. The hypothesis that nano-NOM may modulate the effect of bisphenol A was tested in human colon adenocarcinoma cell lines as model for gastrointestinal tract.

Experimental

Detailed information on reagents and glassware used in this study was provided in Supplementary Material (Sect. S1.1). Nano-NOM was extracted and diluted from Sphagnum peat through a multi-step filtration process (Sects. S1.2, S1.3).

Experiments were conducted in triplicates to investigate the interaction between nano-NOM and bisphenol A. Set1 of experiments aimed at evaluating the effectiveness of the interactions. Different amounts of nano-NOM (100, 500, and 1000 mg/L) were dissolved either in 50 mg/L bisphenol A solution or in deionized water and placed in a beaker under magnetic stirring. After 24-h stirring, samples were subject to high-performance liquid chromatography (HPLC) and high-performance size exclusion chromatography (HPSEC) analysis, details are described in Sect. S1.4.

Set 2 aimed at evaluating the interaction intensity and stability. 5 mL of a solution containing 100 mg/L of nano-NOM and 50 mg/L of bisphenol A was injected into the inner part of a dialysis membrane immersed in a 40 mL working volume glass reactor, containing ultrapure water. The solution was injected immediately after having prepared the solution (instant condition), measuring bisphenol A concentration in the outer part (at 1, 2, 3, 4, 5, and 24 h), whereas the presence of bisphenol A signals in the inner part was also checked (at 24 h). The experiment was repeated, injecting the solution into the inner part of the membrane 24-h after its preparation (stable condition) to evaluate differences due to the development of fast and slow interactions between bisphenol A and nano-NOM.

Set 3 aimed at investigating the nature of the interactions. Fluorescence excitation-emission spectra were recorded as described in Sect. S1.5. Once identified excitation-emission properties of both bisphenol A and nano-NOM, the fluorescence of bisphenol A and nano-NOM mixtures were recorded and analyzed. Samples were prepared by adding increasing amounts of nano-NOM (0–45 mg/L) to a water solution containing 2.25 mg/L of bisphenol A (Table S2, experiments 13–19). Control samples containing pure bisphenol A (0–2.25 mg/L) or pure nano-NOM (0–45 mg/L) were prepared (Table S1, experiments 1–12).

For cytotoxicity assays, the growth profiles of the human colon adenocarcinoma cell lines under different conditions (Sect. S1.6) were monitored using the Incucyte apparatus.

Results and discussion

Interaction between nano-sized natural organic matter and bisphenol A

Set1 chromatograms are reported in Fig. 1. Surprising was the presence of signals in the chromatograms of samples containing only nano-NOM, as nano-NOM samples had been dialyzed to remove small soluble compounds. Most likely, the interaction with the organic eluent e.g., methanol/water, was able to disrupt some interactions occurring at the nano-NOM level and small organic fragments detached from the nano-NOM complex structure. Bisphenol A signal disappeared in the presence of nano-NOM, and fragments' peaks reduced despite the initial nano-NOM concentration, suggesting that interaction occurred.

Mobility and stability of nano-sized natural organic matter and bisphenol A systems

Table S2 and Fig. S1 summarize the results of set2. In the instant condition, a considerable amount of free bisphenol A passed through the membrane. Bisphenol A concentration slowly increased in the outer part of the reactor, up to 7 mg/L after 5 h. After 24 h, the free bisphenol A signal in the outer part of the reactor slightly disappeared. In the stable condition, less bisphenol A was released to the outer part and lasted only 1 h, being almost undetectable after 2 h (Table S2). These results suggested that the interaction between nano-NOM and bisphenol A was very weak in the instant condition and became stronger in the stable condition. The inner part signals after 24 h showed consistency with the results presented in Fig. 1, as most of the bisphenol A peaks disappeared again (Fig. S1; red line). Chromatograms recorded in the outer part of the reactor after 1 h reaction time (Fig. S1) presented a clear bisphenol A peak, which disappeared after 24 h and was substituted by a different signal. The stable complex formed in 24 h (stable condition) both masked bisphenol A presence and modified the nano-NOM structure.

HPSEC experiments were carried out to evaluate the impact of bisphenol A addition on the nano-NOM aggregation state (Fig. 1d,e). Three main peaks (Fig. 1d), for a nominal molecular weight of 173, 127, and 119 KDa, were observed. Once bisphenol A was added to the solution (Fig. 1e), two main effects were observable: (i) nano-NOM was aggregated due the interaction with bisphenol A, and peak #1 appeared at 1.6 min meaning high weight, far above the column separation range; (ii) some fragments were detached from the initial structure and peak #5

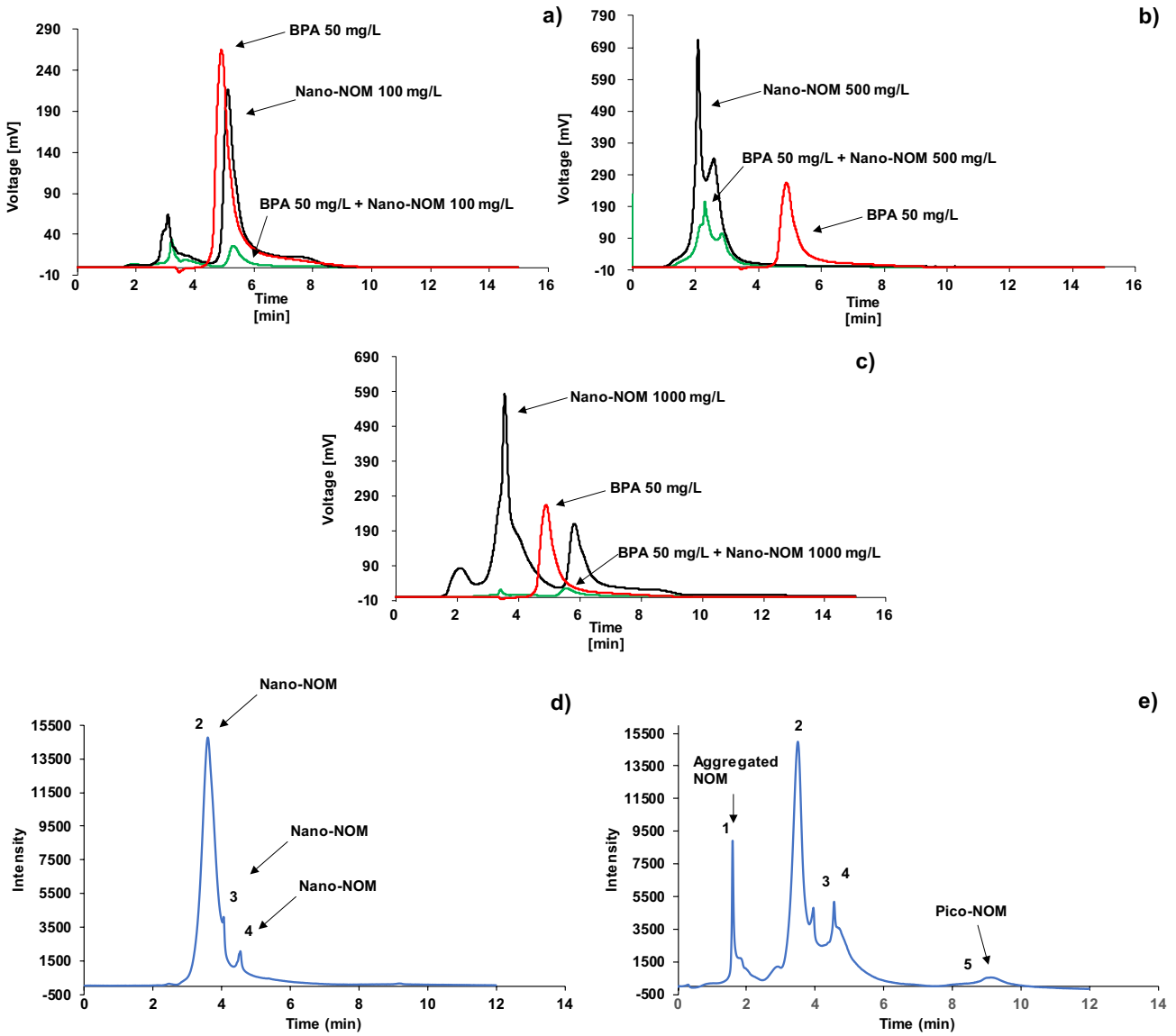

Fig. 1 High-performance liquid chromatography (HPLC) and high-performance size exclusion chromatography (HPSEC) analysis reveals peculiar profiles of bisphenol A (BPA, red line) or nano-sized natural organic matter (Nano-NOM, black line) and their mixtures (green line). **a**–**c** HPLC of BPA 50 mg/L after 24 h interaction with: Nano-NOM 100 mg/L (**a**); Nano-NOM 500 mg/L (**b**); Nano-NOM 1000 mg/L (**c**). **d**–**e**: HPSEC of Nano-NOM 100 mg/L (peaks #2, #3, #4) (**d**); BPA 50 mg/L with Nano-NOM 100 mg/L after 24 h, the appearance of Aggregated NOM (peak #1) and Pico-NOM fragments (peak #5) are highlighted (**e**)

appeared at 9.13 min covering a gaussian-like distribution of nominal weights between 9.1 and 3.4 kDa.

The following mechanism could explain the obtained results: (i) weak bonds characterized the initial interaction stage. These bonds were reversible, and the equilibrium was established between complexed and free bisphenol A; (ii) the different concentrations of bisphenol A inside and outside the membrane provoked the diffusive migration of bisphenol A towards the outer part of the reactor; (iii) as a consequence of the interaction, nano-NOM molecules were partially broken and released pico-fragments (pico-NOM) which passed through the membrane; (iv) pico-NOM complexed the bisphenol A in the outer part masking its presence.

Properties of nano-sized natural organic matter and bisphenol A systems

The interaction between nano-NOM and bisphenol A was also studied by set3 experiments, as reported in Fig. 2. Pure bisphenol A (Table S1, experiment 5, Fig. 2a) presented two evident peaks at excitation (220 nm)/emission (310 nm) (BPA1) and excitation (273 nm)/emission (305 nm) (BPA2). In turn, pure

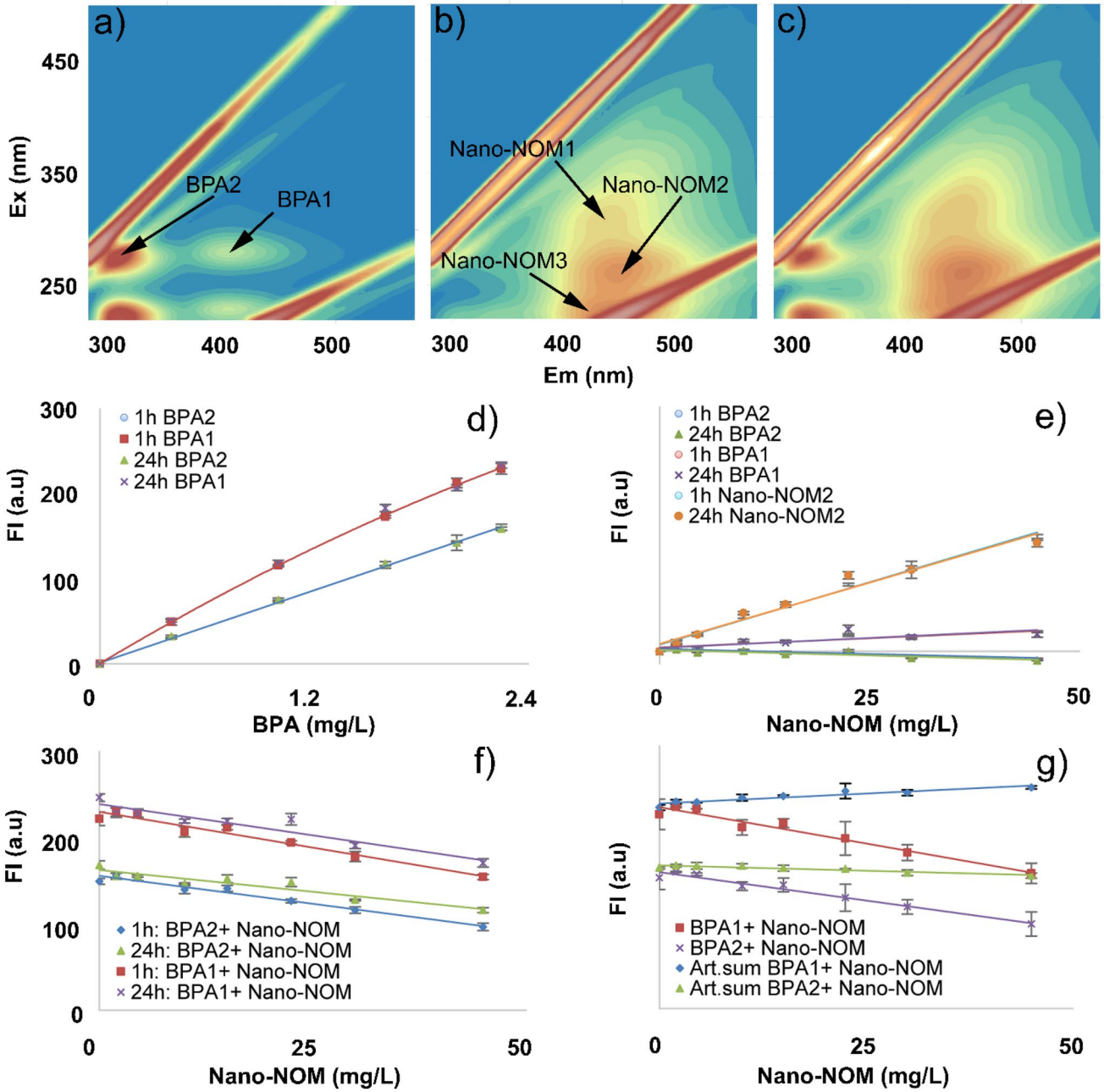

Fig. 2 Fluorescence properties of bisphenol A (BPA) and nano-natural organic matter (Nano-NOM). **a–c** Exemplary three dimensional excitation emission spectra: BPA 2.25 mg/L with two spectral peak areas marked as BPA1: excitation (220 nm)/emission (310 nm), BPA2: excitation (273 nm)/emission (305 nm) (**a**); Nano-NOM 45 mg/L with three spectral areas marked as Nano-NOM1: excitation (304 nm)/emission (435 nm), Nano-NOM2: excitation (260 nm)/emission (445 nm), Nano-NOM3: excitation (225 nm)/emission (435 nm) (**b**); their mixture: bisphenol A 2.25 mg/L + Nano-NOM 45 mg/L (**c**). **d–g** Stability of BPA solutions in time measured as fluorescence intensity (FI) for spectral areas BPA1 and BPA2 after 1 and 24 h (**d**); change of FI in time for increasing concentration (0–45 mg/L) of pure Nano-NOM measured at spectral area BPA1, BPA2 and Nano-NOM2 (**e**); quenching FI of BPA measured at spectral areas BPA1 and BPA2 after addition of increasing concentration (0–45 mg/L) of Nano-NOM (**f**); measured signals for linear quenching of BPA1 and BPA2 areas with increasing Nano-NOM concentration versus artificial sum of unmixed bisphenol A and Nano-NOM (**g**)

nano-NOM (Table S1, experiment 12, Fig. 2b) presented two clear peaks at excitation (304 nm)/emission (435 nm) (NOM1) and excitation (260 nm)/emission (445 nm) (NOM2) and a third peak at excitation (225 nm)/emission (435 nm) (NOM3) that partly overlapped with the Raman band. Peak intensities increased according to concentration and remained constant with increasing reaction time (Fig. 2d, e), confirming that pure solutions were stable. Therefore, any deviations from

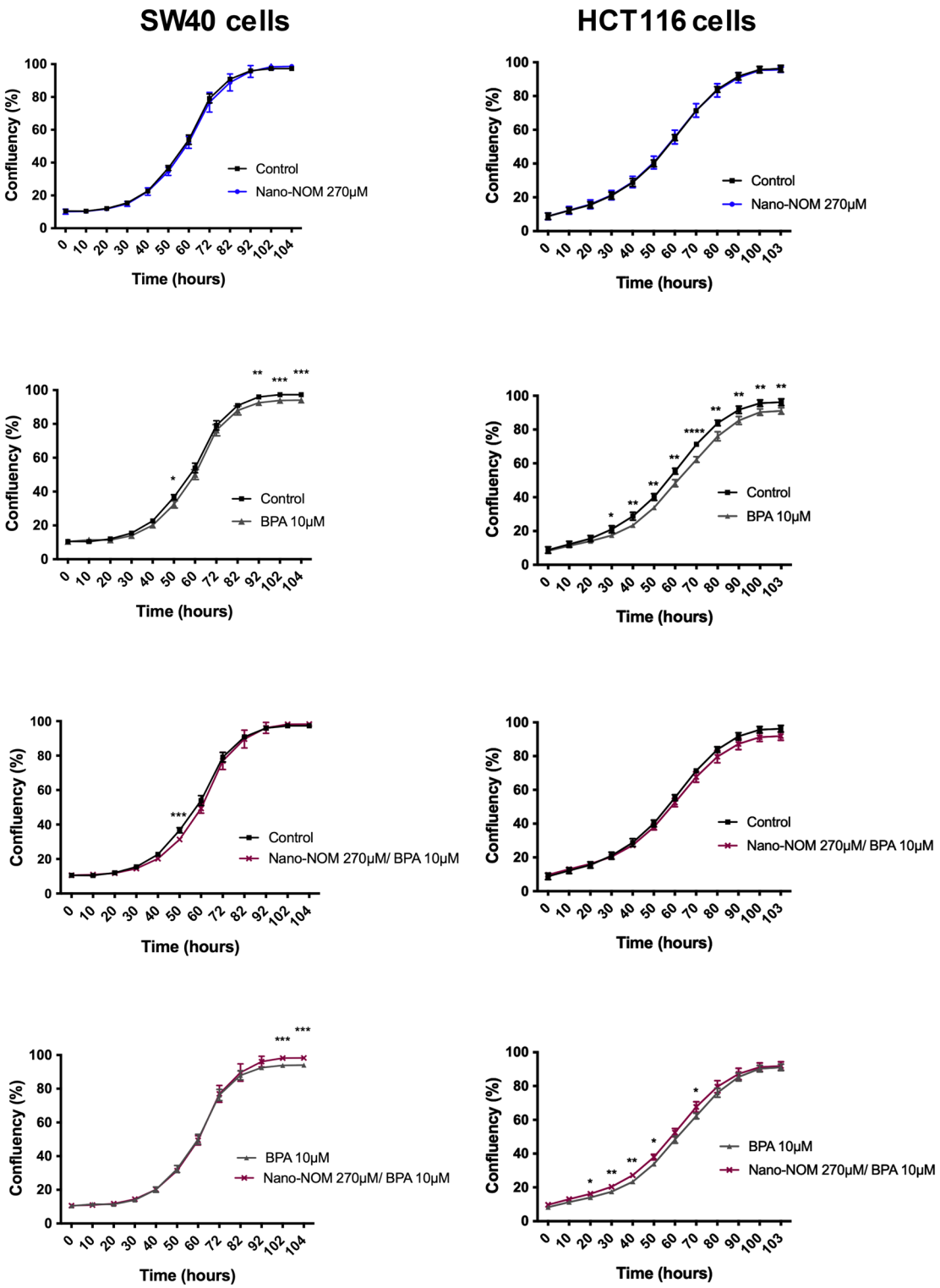

Fig. 3 Growth profile of SW480 cells and HCT116 cells is differently affected by the presence of bisphenol A (BPA) and Nano-sized natural organic matter (Nano-NOM). Growth curves of SW480 (left panels) and HCT116 (right panels) in the presence of bisphenol A (BPA) and Nano-NOM at the concentrations indicated. At each time point, the percentage of cell confluency was quantified as a direct measure of cell proliferation. The values were automatically measured in living cultures and represented as means ± SD of biological replicates ($N = 6$). *$p < 0.05$; **$p < 0.01$ by multiple unpaired t-test and Holm–Sidak correction

the observed trend resulting in the fluorescence spectra of the mixed solution could be attributed to the chemical interactions between bisphenol A and nano-NOM. No signal was measured in the fluorescence spectra of pure nano-NOM solutions in the regions corresponding to bisphenol A fluorescence peaks. Indeed, the fluorescence signal in the BPA1 and BPA2 regions was dramatically quenched in the mixed sample (Fig. 2f). Signal intensity decreased with increasing nano-NOM concentration, in both 1 and 24 h reaction times experiments (Fig. 2f) and followed a linear trend. The linear trend suggested that nano-NOM's capacity to interact with bisphenol A was not saturated, probably because of non-stoichiometric reactions. It is reasonable to assume the establishment of hydrophobic and π-stacking forces between bisphenol A and nano-NOM inner core moieties. The formation of complexes was further confirmed by the comparison of the fluorescence signal of the mixed samples with the fictitious fluorescence signal calculated as their algebraic sum (Fig. 2g). Obtained results indicated an effective interaction between bisphenol A and nano-NOM and the formation of stable complexes.

Cytotoxicity study

To test the relevance of the above observations in a biological system, the growth profiles of SW480 and HCT116 human colon adenocarcinoma cell lines were analyzed (Ryu et al. 2017; Qu et al. 2018).

The cells were maintained in control condition or treated with nano-NOM, bisphenol A, and nano-NOM and bisphenol A complexes. Under control condition, after a short lag of approximately 24 h, the cells grew exponentially until they reached a stable plateau at late time points (Fig. 3; Fig. S2). In both cell lines, nano-NOM alone did not affect cell growth while bisphenol A alone displayed an opposite effect when supplemented at 2µM or 10 µM (Fig. 3; Fig. S2). Indeed, bisphenol A (2 µM) significantly stimulated cell growth at late time points and bisphenol A (10 µM) significantly repressed it, starting from early time points (Fig. 3; Fig. S2). Interestingly, nano-NOM and bisphenol A complexes almost completely reverted the effects of bisphenol A alone.

Our results indicated that nano-NOM blocks the effect of bisphenol A at both concentrations. It could be assumed that nano-NOM, complexing bisphenol A, reduced the bioavailability of this latter, exerting a protective role against its activity.

Conclusion

This work demonstrates for the first time a novel interaction between nano-NOM, the ubiquitous and "water-soluble" nano-sized fraction of NOM, and bisphenol A in water solutions. This stable interaction changes the chemical features of each component making bisphenol A undetectable in water bodies. In the simplified biological system nano-NOM complex affected bisphenol A toxicity. This is a crucial starting point to study the effect of nano-NOM interaction with other emerging contaminants in biological systems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10311-024-01711-9.

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Declarations

Conflicts of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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