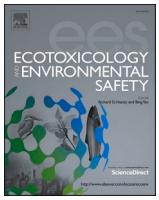
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The first comprehensive study evaluating the ecotoxicity and biodegradability of water-soluble polymers used in personal care products and cosmetics

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ABSTRACT

Water-soluble polymers (WSPs) are organic materials that have been used for decades in various applications as part of paints, coatings, adhesives, washing agents, pharmaceuticals, personal care products and cosmetics. However, their ecotoxicity, biodegradability, and overall impact on the environment are still unknown. In this study four polyacrylic acid- based WSPs (three in the solid state and one in the liquid state), which are widely used in cosmetic industry, were tested in terms of their ecotoxicity and biodegradability. The ecotoxicity tests were performed using aquatic plant *Lemna minor*, microalga *Pseudokirchneriella subcapitata*, crustacean *Daphnia magna*, bacterium *Allivibrio fischeri,* and a mixed bacterial culture of activated sludge (with heterotrophic and nitrifying microorganisms tested separately). All four WSPs had low or moderate effects on the tested organisms at several endpoints. However, the liquid WSP had a specific toxic effect on the bioluminescence of *Allivibrio fischeri* and the oxygen consumption of nitrifying microorganisms – 100 mg/L caused 73% and 88% inhibition, respectively. Therefore, some WSPs capable of inhibiting nitrifying microorganisms could have implications for the nitrification process in wastewater treatment plants and aquatic ecosystems, despite 100 mg/L being a high tested concentration and probably difficult to reach in wastewater. All investigated WSPs were not biodegradable; therefore, their persistence in the environment could be expected.

1. Introduction

Plastics are truly exceptional materials that have pervaded nearly all aspects of modern life. They are suitable for an enormously broad range of applications due to their excellent mechanical properties, light weight, stability, durability, and inertness (Raddadi and Fava, 2019); therefore, plastics production dramatically increased from less than 2 million tons in 1950 (Zalasiewicz et al., 2016) to more than 368 million tons annually today (Plastics Europe, 2020). The drawback of this success story is the generation of massive amounts of plastic waste. As a consequence, plastics contaminate aquatic and terrestrial habitats worldwide, making plastics one of the most significant anthropogenic pollutants (Geyer et al., 2017). However, it was not until the discovery of microplastics (i.e., plastic particles in size between 1 and 1000 μm (Hartmann et al., 2019)) that plastics in the environment received considerable attention from scientists and society (SAPEA, 2019). The widespread occurrence of microplastics and the number of effects on various organisms across ecosystems have raised many concerns; research on microplastics has started increasing exponentially (Rozman and Kalčikova, 2022; Zhang et al., 2020). However, another form of plastic pollution has been overlooked by environmental scientists: water-soluble polymers (WSPs).

WSPs are organic materials that can be modified through dispersion or dissolution in water and become gelled, stabilized, concentrated, or emulsified (Ammar et al., 2019). They have been intensively used for different applications; they are used as dispersing agents in paints, coatings, fertilizers (mainly polyethylene oxide (PEO), polypropylene oxide (PPO), and polyethylene glycol (PEG)), as conditioners in soils to prevent erosion (polyacrylamide (PAM)), as flocculants in wastewater treatment processes (PAM and polyethylene imide (PEI)), in the textile industry for surface modifications (PEG), and they are also ingredients in pharmaceuticals (polyvinylpyrrolidone (PVP) and PEO), personal care products and cosmetics (polyacrylic acid (PAA)) (Huppertsberg et al., 2020; Patil and Ferritto, 2013; Rivas et al., 2018). Although the exact annual production of WSPs is unknown, it has been estimated to be several million tons in Europe (Arp and Knutsen, 2020; Huppertsberg

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et al., 2020). After use, many WSPs are discharged into the sewer system (e.g., WSPs in personal care products and cosmetics) or directly released into the environment (e.g., washed out of paints and coatings or used directly with pesticides and fertilizers on crops) (Huppertsberg et al., 2020).

PAAs are the most commonly used WSPs in personal care products and cosmetics, mostly as thickeners. They are typically used as cross-linked homopolymers of acrylic acid (known as carbomers) and cross-linked copolymers with acrylic acid and C10–C30 alkyl acrylates. For a cross-linking agent, allyl sucrose or allyl pentaerythritol is usually used (Brady et al., 2017). PAAs are available in solid state or as dispersions and emulsions. At low pH, they have low viscosity; after neutralization, the viscosity increases, and a gel is formed. WSPs such as polyacrylic copolymers thicken by charge-induced chain extension, chain entanglement, and hydration (Patil and Ferritto, 2013).

Although WSPs used in personal care products and cosmetics have a great potential to occur in the environment, their effects and fate have not yet been systematically studied. Limited information is available on their concentrations in the environment (however, see the recent monitoring study on PEG (Wang et al., 2021)) and their toxicological profile is based on the results of animal studies to evaluate possible effects on humans (e.g. in Carbomers, 1990; Suka et al., 2021). Knowledge on the ecotoxicity of WSPs is limited as most studies only investigated PAM, which is commonly used as a flocculant in wastewater treatment processes (Buczek et al., 2017; Duggan et al., 2019; Tabra et al., 2020; Weston et al., 2009). Therefore, ecotoxicological data on WSPs used in personal care products and cosmetics (including PAAs) are still lacking (Duis et al., 2021; Huppertsberg et al., 2020).

In this context, the aim of this study was to provide novel knowledge regarding the aquatic hazard of four commercially available WSPs used in personal care products and cosmetics. Their biodegradability under common environmental conditions and aquatic ecotoxicity was assessed by testing several organisms from different trophic levels: primary producers (algae and plants), consumers (crustaceans), and decomposers (bacteria). Such data are essential to evaluate whether WSPs represent a potential threat to the aquatic environment.

2. Materials and methods

2.1. Water-soluble polymers

In this study, four different WSPs extensively used in personal care products and cosmetics were tested. They were kindly provided by Milnica.si, Ljubljana, Slovenia (a seller of cosmetic ingredients). Three of them were solid in the form of a white powder (A, B, and C) and one was in the form of a white liquid (D); according to the MSDS specifications, it contained 30% *(w/w)* solids. WSP A and C were labeled as carbomers (cross-linked homopolymer of polyacrylic acid), the B as acrylates/C10–30 alkyl acrylate cross-polymer (cross-linked copolymer of polyacrylic acid), and D as an acrylates copolymer (lightly cross-linked acrylate copolymer). According to the regulatory summary provided by the manufacturer, WSPs A, B, and C can contain up to 1000, 500, and 1000 ppm of unreacted acrylic acid monomer, respectively.

2.2. Characterization

Prior to the ecotoxicity and biodegradability testing, Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) were determined according to ISO (6060, 8245), respectively. Solid samples A, B, and C were diluted with deionised water to the final concentration of 200 and 1000 mg/L for COD and TOC analysis, respectively. D was diluted by factors of 1000 and 1500 for COD and TOC analysis, respectively. The pH of each WSP solution (diluted with deionized water) at a concentration of 100 mg/L was measured using a FiveEasy™ Plus pH/mV bench meter (Mettler Toledo, Switzerland).

Fourier transform infrared (FTIR) spectroscopy was used for chemical characterization. Samples were recorded with a PerkinElmer Spectrum Two FT-IR spectrometer (PerkinElmer, USA) equipped with an UATR module (Dimond) from 4000 to 500 cm^{-1}, where background and ATR corrections of the spectra were used. The resolution of the spectrometer was 2 cm^{-1}. Spectra of WSP D were recorded in liquid and solid form (dried to a constant mass at 70 °C, detailed information can be found in S1, Supplementary Information). In addition, WSP D was examined for the possible presence of nanoparticles in suspension using a field-emission scanning electron microscope (FE-SEM) Zeiss ULTRA plus (Zeiss, Germany) (detailed information can be found in S2, Supplementary Information).

2.3. Ecotoxicity testing

Ecotoxicity testing was performed with several aquatic organisms. The specifications of each ecotoxicity test are provided in Table 1. All tests were initially performed with 100 mg/L of each WSP as recommended by (OECD, 2019) and used in other studies (Jemec Kokalj et al., 2018; Rozman et al., 2021; Zong et al., 2021); if the toxic effect exceeded 60%, several lower concentrations were tested; if possible, EC_{50} values were calculated. All ecotoxicity tests were acute and performed according to standard procedures.

2.3.1. Aquatic plant Lemna minor

The ecotoxicity test with a laboratory culture of duckweed (*L. minor*) was performed according to the standard procedure (Table 1). Briefly, 10 fronds were added to 50 mL Steinberg culture medium with dissolved WSP (100 mg/L). Due to the low pH of the prepared A, B, and C WSP solutions and the difficulties in adjusting the pH to higher values, the pH of the control test (with no sample) was adjusted to lower values (4.20 ± 0.05, 0.05 M HCl), which is still tolerable by duckweed (McLay, 1976). The pH of the D WSP solution (100 mg/L) and the control sample were comparable (5.60 ± 0.05). The ecotoxicity test lasted 7 days under controlled conditions (24 ± 1 °C, 7000 ± 500 lx, and a ratio of light/dark of 16/8 h). At the end of the test, inhibition of the specific growth rate, root length and chlorophyll *a* and *b* contents were determined, as described in our previous studies (Kalčíková et al., 2016, 2017).

2.3.2. Microalga Pseudokirchneriella subcapitata

The microalga *P. subcapitata* was purchased as Algaltoxkit F (Microbiotests, Belgium) and the ecotoxicity test was conducted following the manufacturer's instructions. Briefly, tested WSPs were diluted in the growth medium, where pH was adjusted to 8.10 ± 0.10 with 0.05 M NaOH. Microalgae were added to the test solution to obtain an initial concentration of $1{\cdot}10^4$ cells/mL. Tests were performed in test vials in an incubator at a constant temperature of 23 ± 1 °C and a constant light of 10000 ± 100 lx. The ecotoxicity test lasted 72 h. Absorbance at 670 nm (optical density (OD)) was measured every 24 h with Varian Cary 50 UV-Vis spectrophotometer (Agilent Technologies, USA).

2.3.3. Crustacean Daphnia magna

Water fleas (*D. magna*) originated from a permanent laboratory culture and the ecotoxicity test followed the standard procedure (Table 1). Ten juvenile daphnids (less than 24 h old) were used for the tested concentration and exposed for 48 h. However, the addition of WSPs A, B, and C into the ISO medium (ISO, 6341, 2012) caused immediate precipitation or they quickly formed a gel after adjusting pH to higher values. Therefore, we were able to test only the D WSP, which did not precipitate up to pH values required for daphnia ecotoxicity testing (pH = 6–8).

2.3.4. Bacterium Allivibrio fischeri

The bioluminescence inhibition test with freeze-dried bacterium *A. fischeri* (previously named *Vibrio fischeri*) was performed according to the standard procedure (Table 1). A stock solution of each WPS was

Table 1
Summary of ecotoxicological experiments.

Investigated organism	Sample	Concentration (mg/L)	End points	Number of replicates per sample	Reference
Aquatic plant duckweed *Lemna minor*	A	100	Specific growth rate inhibition; Root length inhibition; Chlorophyll *a* and *b* contents reduction	4	(OECD, 2006)
	B	100			
	C	100			
	D	100			
Microalga *Pseudokirchneriella subcapitata*	A	100	Specific growth rate inhibition	3	(OECD, 2011)
	B	100			
	C	100			
	D	100			
Crustacean water flea *Daphnia magna*	D	100	Immobility	4	(ISO, 6341, 2012)
Bacterium *Allivibrio fischeri*	A	100	Inhibition of bioluminescence	3	(ISO, 11348-3, 2007)
	B	100			
	C	100			
	D	12.5, 25, 50 and 100			
Activated sludge heterotrophic microorganisms	A	100	Inhibition of oxygen consumption	5	(ISO, 8192, 2007)
	B	100			
	C	100			
	D	100			
Activated sludge nitrifying microorganisms	A	100	Inhibition of oxygen consumption	5	(ISO, 8192, 2007)
	B	100			
	C	100			
	D	100			

prepared (200 mg/L) in 2% NaCl. At the beginning of the test, 0.5 mL of each stock solution was added to 0.5 mL of bacterial suspension (final concentration of WPSs = 100 mg/L). Due to the low volume of the suspension, the pH was checked by pH strips (Macherey-Nagel, Germany), being always 7–8. Bioluminescence was measured prior to and after (30 min) the exposure time with a LUMIStox luminometer (Dr. Lange, Germany). Liquid sample D showed a high inhibition toward bacteria at concentration of 100 mg/L; therefore, additional concentrations (12.5, 25, and 50 mg/L) were prepared and tested to calculate the 30-min EC_{50} value. The test was repeated twice.

2.3.5. Activated sludge

The effect of WSPs on the microorganisms of the activated sludge was assessed by measuring the inhibition of oxygen consumption. The test was performed according to a standard procedure (Table 1). Briefly, activated sludge was obtained from a regional municipal wastewater treatment plant and 1500 mg/L was used for the test. The microorganisms of the activated sludge were exposed to the WSP for 30 min; afterwards, the oxygen consumption was measured with an oxygen sensor Seven2Go™ DO portable meter (Mettler Toledo, Switzerland). The oxygen consumption was measured for heterotrophic and nitrifying microorganisms separately, a specific inhibitor of nitrification (N-allylthiourea (ATU)) was added to the system, and the consumption of oxygen was monitored only for heterotrophic microorganisms. The consumption of oxygen by nitrifying microorganisms was calculated as the difference between oxygen consumption by all and by heterotrophic microorganisms of the activated sludge.

2.4. Biodegradability testing

The aerobic biodegradability test was performed according to ISO (9408, 1999) to evaluate the ultimate aerobic biodegradability by determining the oxygen demand in a closed respirometer. The concentrations of WSPs in the system were chosen to avoid any toxic impact on the activated sludge microorganisms (the inoculum in the biodegradability tests, based on the results of the toxicity test in Section 2.3.5) and to ensure an initial COD of 100 mg/L. Each WSP was dissolved in deionized water with the addition of buffers and inoculum and stirred in a closed flask. Since the oxygen consumption can be driven by both heterotrophic and nitrifying microorganisms, ATU was added to suppress nitrification. Therefore, the determined oxygen consumption was the result of biodegradation of only organic substances. The consumption of oxygen was determined by the change in pressure in a flask (OxiTop®, WTW, Germany). Treatments containing (i) abiotic control without inoculum, but with the addition of $HgCl_2$ to inhibit any naturally occurring microorganisms, and (ii) reference biodegradable compound (sodium acetate, COD = 100 mg/L) to confirm the activity of microorganisms under test conditions were also prepared. The degradation was followed over a period of 28 days at 20 ± 1 °C in the dark. The amount of oxygen consumed by heterotrophic microorganisms for the degradation of organic compounds (after correction by comparison with the blank test) is expressed as a percentage of the initial COD.

To evaluate the ability of microorganisms to survive and degrade readily biodegradable compounds in the presence of WSPs, the biodegradability test was repeated under the same conditions as described above, but instead of oxygen consumption, we monitored the concentration of Dissolved Organic Carbon (DOC) according to ISO (7827, 2010). To each WSP, the reference compound sodium acetate was added (COD = 100 mg/L) and its degradation was monitored over a period of 28 days.

2.5. Data analysis

Results are presented as the arithmetic mean ± standard deviation. Normality of data was tested using the Shapiro-Wilk test. Statistical significance of differences between the control group and the exposed group was determined using the Mann–Whitney *U* test; differences were considered significant if $p < 0.05$ (Table S1) using OriginPro 2021b software (OriginLab Corp. Northampton, MA, USA).

3. Results

3.1. Characterization

COD, TOC, and pH were determined prior to toxicity testing. COD values were comparable for the solid WSPs (A, B, and C); however, the liquid WSP D exhibited a significantly higher COD (Table 2). TOC measurements were comparable for samples B and C, but sample A had a TOC nearly ten times higher. Similar to the COD results, the TOC of sample D was also much higher compared with the solid samples. In general, the pH of the WSPs was acidic, showing similar pH values. Liquid sample D had a slightly higher pH than the other samples (Table 2).

Table 2
Chemical oxygen demand (COD; mg/g), total organic carbon (TOC; mg/g) and pH of WSPs solutions (100 mg/L).

Parameter/Sample	A	B	C	D[a]
COD (mg/g) (n = 3)	1393 ± 24	1214 ± 4	1279 ± 10	454080 ± 1034
TOC (mg/g) (n = 3)	306.3 ± 8.4	40.6 ± 0.8	48.3 ± 0.3	634.0 ± 2.1
pH[b] (/) (n = 3)	4.74 ± 0.01	4.81 ± 0.01	5.01 ± 0.01	5.73 ± 0.02

[a] calculated for 30% (w/w) of solids,
[b] 100 mg/L solution in deionised water

The FTIR spectra of WSP A, B, and C (Fig. 1) were similar to each other and comparable to the previous FTIR spectra obtained for a solid polyacrylic acid based WSP (Sahoo et al., 2011). Similarly, the FTIR spectrum of WSP D was comparable to the spectrum of a liquid WSP of polyacrylic acid (Al-Awady et al., 2017). The peaks observed in all four samples were also comparable to the peaks in the FTIR spectrum of polyacrylic acid from the database (John Wiley and Sons, 2020b). The absorption band in the range 2983–2929 cm^{-1} represented OH stretching vibration and intramolecular hydrogen bonding. The high absorption peak seen between 1718 and 1700 cm^{-1} in the spectrum of each sample represented the carbonyl (C=O) bond stretching (Sahoo et al., 2011). The prominent absorption peaks in the range from 1383 to 1472 cm^{-1} represented the stretching of the carbonyl bond (CO) and the bending of the OH bend, while absorption peaks between 1259 and 1020 cm^{-1} were assigned to coupling between in-plane OH bending and CO stretching of neighboring carboxyl groups. Absorption peaks between 799 and 851 cm^{-1} were attributed to out-of-plane of C=CH bending (Al-Awady et al., 2017; John Wiley and Sons, 2020b; Sahoo et al., 2011). Additional absorption peaks in the FTIR spectrum of liquid WSP D (3383, 1640, and 748 cm^{-1}) were similar to peaks in the FTIR spectrum for water (John Wiley and Sons, 2020a), which was confirmed by the FTIR spectrum of dried WSP D (Fig. S1). FE-SEM images of WSP D revealed nanoparticles within the suspension (Fig. S2).

3.2. Ecotoxicity testing

Ecotoxicity testing was performed with four species (*L. minor* , *D. magna*, *P. subcapitata,* and *A. fischeri*) and a mixed bacterial culture (heterotrophic and nitrifying microorganisms). Low inhibition against duckweed *L. minor* was observed for all tested WSPs (Table 3). All WSPs had a statistically significant effect (Table S1) on duckweed's specific growth rate compared with the respective control (Fig. 2A); however, only WSPs A and B had a significantly negative effect on root length (Fig. 2B): root length was reduced by 33% and 25%, respectively. Chlorophyll *a* content in *L. minor* was significantly (Table S1) reduced when exposed to WSPs A, B, and D (Fig. 2C), but a significant effect (Table S1) on chlorophyll *b* content was observed only with WSP D (Fig. 2D).

WSPs A, B, and C had a significant (Table S1) effect on the specific growth rate of microalgae *P. subcapitata* (Table 3). The specific growth rate in control was 0.966 ± 0.133 day^{-1}, while in the presence of WSPs A, B, C, and D it was 0.445 ± 0.019, 0.507 ± 0.046, 0.565 ± 0.019 and 0.806 ± 0.082 day^{-1} respectively.

The acute toxicity test with *D. magna* was performed only with WSP D because other WSPs were not soluble in the ISO medium at a neutral pH. The liquid sample D had no negative effect on daphnids after 48 h (Table 3, Table S1).

The solid WSPs (A, B, and C) had no negative effect on the bioluminescence intensity of *A. fischeri*, whereas the liquid WSP D caused 73% inhibition (Table 3, Table S1). Further testing allowed us to calculate the 30-min EC_{50} of WSP D as 48 ± 1 mg/L.

Similarly, no WSP affected the heterotrophic microorganisms of activated sludge, whereas the WSP D strongly inhibited the consumption of oxygen by nitrifying microorganisms (Table 3, Table S1).

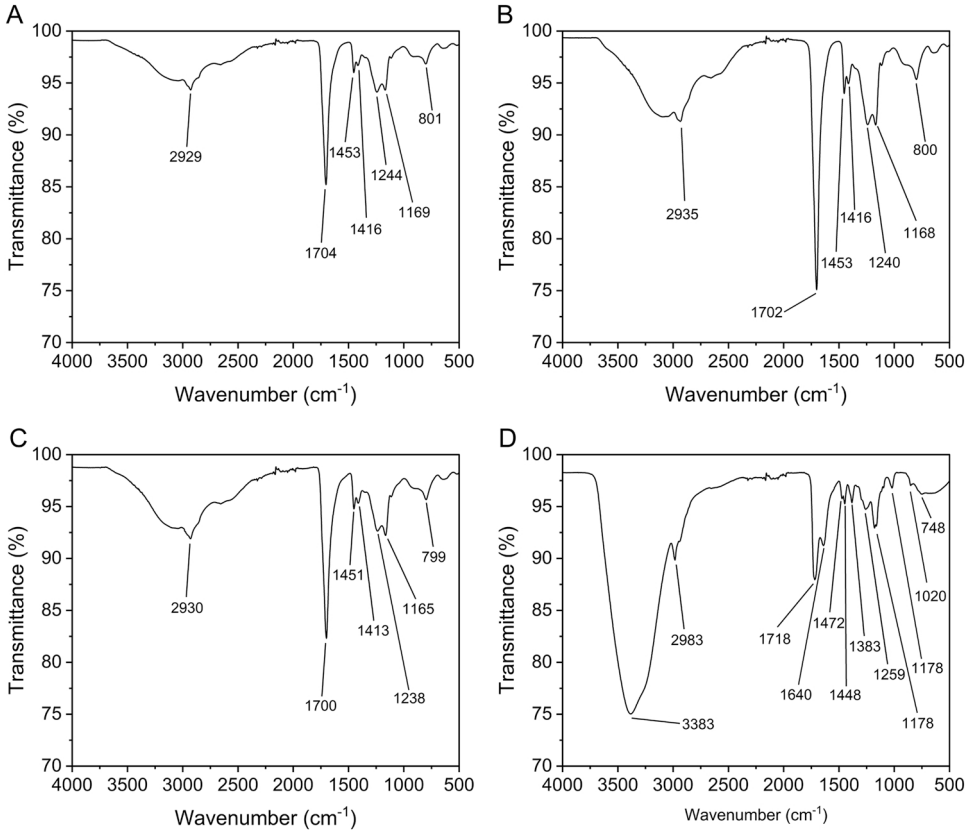


Fig. 1. FTIR spectra of investigated WSPs A, B, C, and D.

Table 3
Effects of 100 mg/L of WSPs A, B, C, and D on various organisms.

WSP	Inhibition (%)								
	L. minor				*P. subcapitata*	*D. magna*	*A. fischeri*	Activated sludge	
								HET MO	NIT MO
	Specific growth rate	Root length	Chl *a*	Chl *b*	Specific growth rate	Mobility	Bioluminescence	Oxygen consumption	
A	27 ± 4*	33 ± 7*	37 ± 10*	23 ± 14	54 ± 2*	/	0 ± 0	< 10	< 10
B	22 ± 3*	25 ± 9*	25 ± 9*	0 ± 0	47 ± 5*	/	4 ± 1	< 10	< 10
C	10 ± 4*	5 ± 3	32 ± 2	0 ± 0	42 ± 2*	/	7 ± 1	< 10	< 10
D	22 ± 4*	0 ± 0	59 ± 6*	44 ± 16*	16 ± 9	0 ± 0	73 ± 1*	< 10	88 ± 17*

/ – inhibition was not determined; * – statistical difference in comparison to control (Table S1); HET MO – heterotrophic microorganisms; NIT MO – nitrifying microorganisms, Chl *a* – chlorophyll *a,* Chl *b* – chlorophyll *b*. Calculated inhibitions were ranked into four classes: no inhibition (<10%) (green), low inhibition (10–40%) (yellow), moderate inhibition (41–60%) (orange) and high inhibition (>60%) (red).

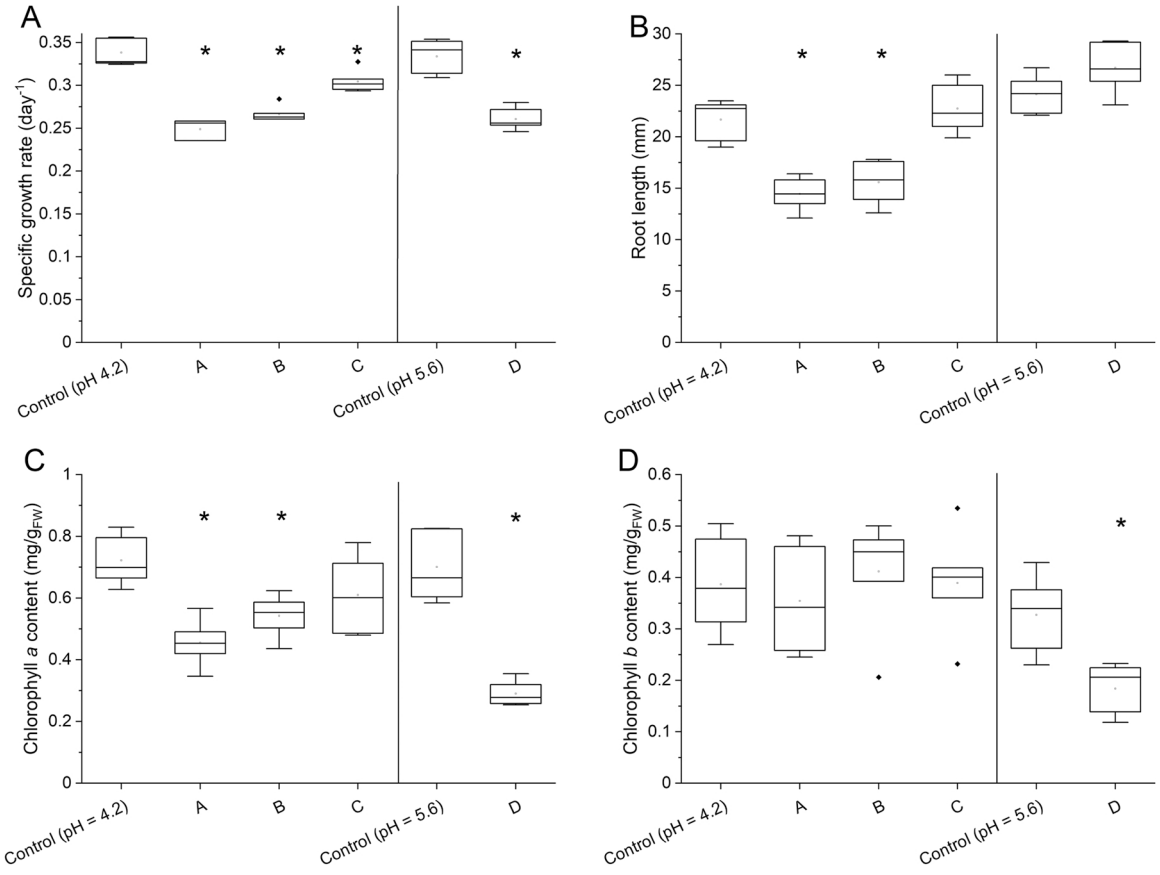


Fig. 2. Specific growth rate (A), root length (B), chlorophyll *a* content (C), and chlorophyll *b* content (D) of *L. minor* exposed to WSPs. A statistically significant difference in comparison to the control is marked with *($p < 0.05$). Outliers are shown as ■.

3.3. Biodegradability

The results of biodegradability testing for all investigated WSPs and the reference compounds (sodium acetate) are shown in Fig. 3. The reference compound degraded more than 60% in the first 6 days and reached 85% after 28 days, whereas the degradation of the WSPs did not proceed (<5%) as well as abiotic degradation.

Additional tests with reference compound directly added into WSP test vessels confirmed the activity of microorganisms under the test conditions: the reference compound was always degraded about 50% in the presence of WSPs A, B, and C, and almost 100% in the presence of WSP D and without any WSP (detailed data not shown).

4. Discussion

WSPs have been used in many applications for decades, but unlike other chemicals used on a daily basis, they have escaped the attention of environmental scientists. As a result, knowledge is limited about their behavior, fate, and impact on the environment. Not much is known about their annual production, as polymers are not part of Regulation,

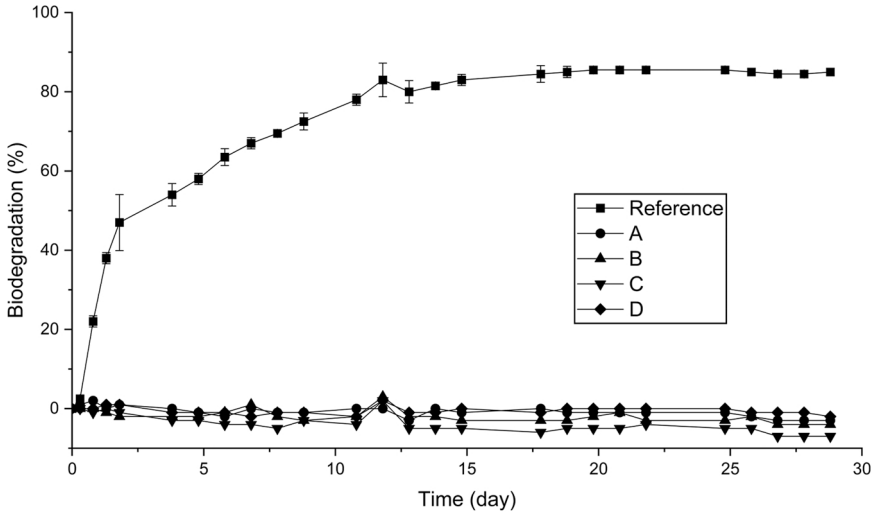


Fig. 3. Biodegradability of investigated WSPs A, B, C, and D and the reference compound (sodium acetate).

Evaluation, Authorization and Restriction of Chemicals (REACH) legislation, because they are considered as low-concern chemicals. However, the Plastic Soup Foundation was one of the first organizations to draw attention to the massive use of WSPs in personal care products and cosmetics. They highlighted that WSPs (also called "liquid microplastics") pose the same threat to the environment as cosmetic microbeads, which have been recently banned in many countries. The most commonly used WSPs in personal care products and cosmetics are carbomers (cross-linked polyacrylic acid), cyclopentasiloxane, and acrylic (co)polymers (Plastic Soup Foundation, 2020). Cyclopentasiloxane belongs to the group of cyclic methyl siloxanes and are highly volatile, therefore, after application, they are released into the atmosphere (Montemayor et al., 2013). However, carbomers and acrylic (co)polymers (which are present in 40% of all cosmetic products (CodeCheck, 2020)) are, after the application of a cosmetic product, washed into sewage and have the potential to enter the aquatic ecosystems via effluents from wastewater treatment plants (Mairinger et al., 2021). Although some WSPs such as PAM, used as flocculants in wastewater treatment or as stabilizers for erosion control in agriculture, have been previously investigated (Buczek et al., 2017; Duggan et al., 2019; Tabra et al., 2020; Weston et al., 2009), the information regarding the ecotoxicity of WSPs used in personal care products and cosmetics is scant; to the best of our knowledge this is the first study focused specifically on their ecotoxicity and biodegradability.

The WSPs investigated in this study were acrylic-acid-based polymers and are often used in personal care products and cosmetics as thickeners. They have an acidic pH when they are dissolved in water, but when pH is raised, they transform into gels (Patil and Ferritto, 2013). WSPs A, B, and C had virtually identical FTIR spectra (Fig. 1), but WSP A differs significantly in TOC value. It is common for different WSPs to contain additives or/and remaining solvents and therefore, their composition and ecotoxicity can vary widely (Weston et al., 2009). In this study, no additional peaks were indicated in the FTIR spectrum of WSP A, so it is possible that it contains some unreacted acrylic acid, as also stated by manufacturer (Section 2.1). WSP D is also based on polyacrylic acid, but it is prepared in liquid form and the water content caused peaks overlap. The spectrum of dried WSP D (Fig. S1) did not reveal any other components, but volatile additives such as solvents could evaporate during drying. On the other hand, FE-SEM imaging showed that WSP D was essentially a suspension of polyacrylic acid-based nanoparticles, which are increasingly used personal care products and cosmetics (Somasundaran et al., 2005).

The acute ecotoxicity of the studied WSPs varied across tested species as well as depended on the respective WSP (Table 2). However, WSPs A, B, and C showed a similar ecotoxicity pattern (Table 3); they had no measurable effects on microorganisms of activated sludge and the bacterium *A. fischeri,* and a low, but statistically significant effect on duckweed (*L. minor*) for at least of one endpoint (specific growth rate, root length, chlorophyll *a* or chlorophyll *b* content) (Fig. 1). The reason for the low and non-specific inhibition of plant growth is probably the adhesion of these WSPs to the plant surface. Polyacrylic-based WSPs are commonly used as bioadhesives (Cozens et al., 2021) and they form hydrogen bonding with polysaccharides and proteins under acidic conditions (Sahoo et al., 2011; Wang et al., 2009), which was predominant in the duckweed test. The bioadhesion of WSPs to plant tissues and the effect on chlorophylls was also observed by Kuboi and Fujii (1985). The authors investigated several WSPs used as flocculants in wastewater treatment and suggested that positively charged WSPs adhere to negatively charged root surfaces and cause inhibition of root growth, and chlorosis is related to the increase of Cu concentration near the roots due to Cu interactions with the WSPs. These conclusions are also supported by the results of Tabra et al. (2020), who investigated the effects of cationic polyacrylamides on the germination of terrestrial plants.

Moreover, the microalga *P. subcapitata* was the most affected organism by WSPs A, B, and C (≈50% inhibition). Bioadhesion could also play a role in inhibiting algal growth, but it is more plausible that the increased toxicity toward algae results from the presence of unreacted acrylic acid, which is considered highly toxic to microalgae, as the 72-h EC_{50} for *P. subcapitata* is 0.13–0.21 mg/L but expresses lower toxicity to other aquatic species (e.g., the 48-h EC_{50} for *D. magna* is 95 mg/L) (Sverdrup et al., 2001). When the ecotoxicity of various types of WSPs (polyacrylamides used for erosion control in agriculture) was tested on *P. subcapitata* (previously named *S. capricornutum*), the authors suggested that the polymer itself has no effect on algae, but the toxicity depends on the product formulation, as oil- and water-based polyacrylamides contained additives, such as emulsifiers and surfactants that increase toxicity (Weston et al., 2009).

WSP D showed a significantly different ecotoxicological profile compared to WSPs A, B, and C, probably because WSP D was the only product in the form of a nanoparticle suspension. Its effect on duckweed specific growth rate and root length was minimal, while it significantly affected chlorophylls (Fig. 2, Table 3). WSP D caused chlorosis and the fronds of the plant were smaller and thinner, as is typical for duckweed exposed to solvents (Hu et al., 2017). The microalga *P. subcapitata* was not strongly affected, probably due to the absence of the unreacted acrylic acid monomer in WSP D (Section 2.1). The results showed that WSP D had no negative effects on *D. magna* (Table 3). Similarly, water-based polyacrylamide had no adverse effects on the crustacean

Ceriodaphnia dubia in the chronic toxicity tests (LC_{50} > 100 mg/L). However, when *C. dubia* was exposed to oil-based polyacrylamide (LC_{50} = 0.3 mg/L) and granular polyacrylamide (LC_{50} = 28.7 mg/L), high toxicity was observed, which was attributed to the additives present and the high viscosity of the solution, respectively (Weston et al., 2009).

WSP D was nontoxic to the heterotrophic microorganisms of the activated sludge but was the only WSP tested in this study that strongly inhibited oxygen consumption by nitrifying microorganisms and bioluminescence of *A. fischeri*. Nitrifying bacteria obtain their energy for growth from the oxidation of ammonia initiated by the ammonia monooxygenase enzyme. There are many organic and inorganic compounds that inhibit this enzyme (Keener and Arp, 1993), but one of the well-known inhibitors is allylthiourea, which targets enzyme action and chelates copper in the active site, ultimately hindering its function (Tatari et al., 2017). Juliette et al. (1993) found that allylsulfide specifically and effectively inactivated ammonia monooxygenase, whereas a variety of organic sulphides did not. Furthermore, the bioluminescence of *A. fischeri* is induced by enzyme catalysis via the enzymatic action of luciferase, which is a flavin-dependent monooxygenase (Tinikul et al., 2020). It is therefore plausible that liquid WSP D specifically inhibited monooxygenase in the tested bacteria due to the presence of compounds with an allyl group, e.g., allyl sucrose or allyl pentaerythritol, which are typically used as cross-linking agents in the production WSPs for personal care products and cosmetics (Brady et al., 2017).

All tested WSPs were poorly biodegradable under the test conditions; therefore, their biodegradation in common environmental conditions is not expected. The additional experiment showed that the reference compound (sodium acetate, a readily biodegradable compound) was partially degraded in the presence of WSPs A, B, and C, while the biodegradation of the reference compound in the presence of WSP D was comparable to that of the reference compound alone. Since the investigated WSPs did not show any acute effects on the heterotrophic microorganisms of activated sludge (Table 3) (which were also used in the biodegradability test as inoculum), it is possible that during the biodegradability test (28 days) the WSPs adhered to the cells of activated sludge, as previously shown for some other WSPs (Tabra et al., 2020), and modified their ability to degrade organic compounds. This argument is supported by the previously discussed bioadhesion of WSP A, B, and C to plant tissues. Similarly, they can adhere to cells of activated sludge. On the other hand, the bioadhesion of WSP D may be lowered due to its increased pH that leads to dissociation of the carboxylic acid groups and the loss of its hydrogen bonding capability (Wang et al., 2009).

5. Conclusions

Plastic pollution deserves special attention because a large quantity of plastic waste leaks into the environment, causing significant economic and ecological damage. Therefore, in the last decades, considerable attention has been paid to the development of better and sustainable plastic waste management, strategies, and regulations. As a result, plastic waste collection and recycling rates have increased significantly in many EU countries. However, water-soluble polymers (WSPs) have been excluded from this success story. They are not monitored or included in current EU plastics strategies or circular economy action plans. Although it may seem that WSPs do not pose a risk to the environment due to their low ecotoxicological profile, our study showed the opposite. One WSP investigated in our study significantly affected nitrifying bacteria, which are key microorganisms in the nitrogen cycle. However, the possible implications for nitrification process are uncertain because WSPs concentrations in wastewaters and in the environment have not yet been investigated. In addition, our findings confirm the poor biodegradability of all the WSPs tested, but their actual fate in the environment remains unclear. Therefore, there is an urgent need to extend polymer research to soluble polymers and to evaluate their long-term effects as they may have the same critical environmental implications as solid ones.

CRediT authorship contribution statement

Ula Rozman: Investigation, Conceptualization, Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Gabriela Kalčíková:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.113016.

References

Al-Awady, M.J., et al., 2017. Enhanced antimicrobial effect of berberine in nanogel carriers with cationic surface functionality. J. Mater. Chem. B 5, 7885–7897.
Ammar, S., et al., 2019. Chapter 2 - Polymers-based nanocomposite coatings. In: Nguyen Tri, P., et al. (Eds.), Nanomaterials-Based Coatings. Elsevier, pp. 9–39.
Arp, H.P.H., Knutsen, H., 2020. Could we spare a moment of the spotlight for persistent, water-soluble polymers? Environ. Sci. Technol. 54, 3–5.
Brady, J., et al., 2017. Chapter 7 - polymer properties and characterization. In: Qiu, Y., et al. (Eds.), Developing Solid Oral Dosage Forms, Second edition.,. Academic Press, Boston, pp. 181–223.
Buczek, S.B., et al., 2017. Acute toxicity of polyacrylamide flocculants to early life stages of freshwater mussels. Environ. Toxicol. 36, 2715–2721.
Carbomers, F.R. o t S.A. o, 1990. J. Am. Coll. Toxicol. 1, 109–141.
CodeCheck, 2020, The invisible danger: hidden polymers in cosmetic products. ⟨https://codecheck-app.com/codecheck-study-the-invisible-danger-hidden-polymers-in-cosmetic-products/⟩.
Cozens, E.J., et al., 2021. Comparative adhesion of chemically and physically crosslinked poly(acrylic acid)-based hydrogels to soft tissues. Eur. Polym. J. 146, 110250.
Duggan, K.L., et al., 2019. Effects of cationic polyacrylamide and cationic starch on aquatic life. J. Hazard., Toxic., Radioact. Waste 32.
Duis, K., et al., 2021. Environmental fate and effects of water-soluble synthetic organic polymers used in cosmetic products. Environ. Sci. Eur. 33, 21.
Geyer, R., et al., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782.
Hartmann, N.B., et al., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047.
Hu, L.-X., et al., 2017. Biochemical alterations in duckweed and algae induced by carrier solvents: Selection of an appropriate solvent in toxicity testing. Environ. Toxicol. Chem. 36, 2631–2639.
Huppertsberg, S., et al., 2020. Making waves: water-soluble polymers in the aquatic environment: an overlooked class of synthetic polymers? Water Res. 181.
ISO 6060, 1989, Water quality – Determination of the chemical oxygen demand.
ISO 6341, 2012, Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) – Acute toxicity test.
ISO 7827, 2010, Water quality – Evaluation of the "Ready", "Ultimate" Aerobic Biodegradability of Organic Compounds in an Aqueous Medium – Method by Analysis of Dissolved Organic Carbon (DOC).
ISO 8192, 2007, Water quality – Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation.
ISO 8245, 1999, Water quality – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).
ISO 9408, 1999, Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer.
ISO 11348–3, 2007, Water quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminiscent Bacteria Test), Part 3: Method using freeze-dried bacteria.

Jemec Kokalj, A., et al., 2018. Screening study of four environmentally relevant microplastic pollutants: Uptake and effects on Daphnia magna and Artemia franciscana. Chemosphere 208, 522–529.
John Wiley & Sons, I.S., SpectraBase Compoud ID=Cz3wO46i3MG SpectraBase Spectrum ID=H7KdKI7UNSz, 2020a. ⟨https://spectrabase.com/spectrum/H7KdKI7UNSz⟩.
John Wiley & Sons, I.S., SpectraBase Compound ID=IOwmSnAEiGm SpectraBase Spectrum ID=Fy4UfVgaAPC, 2020b. ⟨https://spectrabase.com/spectrum/Fy4UfVgaAPC⟩.
Juliette, L.Y., et al., 1993. Inhibition of ammonia oxidation in nitrosomonas europaea by sulfur compounds: thioethers are oxidized to sulfoxides by ammonia monooxygenase. Appl. Environ. Microbiol. 59, 3718–3727.
Kalčíková, G., et al., 2016. The impact of humic acid on chromium phytoextraction by aquatic macrophyte Lemna minor. Chemosphere 147, 311–317.
Kalčíková, G., et al., 2017. Impact of polyethylene microbeads on the floating freshwater plant duckweed Lemna minor. Environ. Pollut. 230, 1108–1115.
Keener, W.K., Arp, D.J., 1993. Kinetic studies of ammonia monooxygenase inhibition in nitrosomonas europaea by hydrocarbons and halogenated hydrocarbons in an optimized whole-cell assay. Appl. Environ. Microbiol. 59, 2501–2510.
Kuboi, T., Fujii, K., 1985. Toxicity of cationic polymer flocculants to higher plants. Soil Sci. Plant Nutr. 31, 163–173.
Mairinger, T., et al., 2021. Characterization of water-soluble synthetic polymeric substances in wastewater using LC-HRMS/MS. Water Res. 190, 116745.
McLay, C.L., 1976. The effect of pH on the population growth of three species of duckweed: Spirodela oligorrhiza, Lemna minor and Wolffia arrhiza. Freshw. Biol. 6, 125–136.
Montemayor, B.P., et al., 2013. Accounting for intended use application in characterizing the contributions of cyclopentasiloxane (D5) to aquatic loadings following personal care product use: Antiperspirants, skin care products and hair care products. Chemosphere 93, 735–740.
OECD, 2006, Test No. 221: Lemna sp. Growth Inhibition Test.
OECD, 2011, Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test.
OECD, 2019, Test No. 203: Fish, Acute Toxicity Test.
Patil, A., Ferritto, M.S., 2013. Polymers for Personal Care and Cosmetics. American Chemical Society, Washington, DC.
Plastic Soup Foundation, 2020, Liquid microplastics in cosmetics are the new battleground . ⟨https://www.plasticsoupfoundation.org/en/2020/03/battle-on-the-so-called-liquid-microplastics-in-cosmetics/⟩.
Plastics Europe, 2020, Plastics - the Facts 2020. ⟨https://www.plasticseurope.org/application/files/5716/0752/4286/AF_Plastics_the_facts-WEB-2020-ING_FINAL.pdf⟩.
Raddadi, N., Fava, F., 2019. Biodegradation of oil-based plastics in the environment: Existing knowledge and needs of research and innovation. Sci. Total Environ. 679, 148–158.
Rivas, B.L., et al., 2018. Water-soluble and insoluble polymers, nanoparticles, nanocomposites and hybrids with ability to remove hazardous inorganic pollutants in water. Front. Chem. 6, 320.
Rozman, U., Kalčikova, G., 2022. Seeking for a perfect (non-spherical) microplastic particle – the most comprehensive review on microplastic laboratory research. J. Hazard. Mater. 424, 127529.
Rozman, U., et al., 2021. An extensive characterization of various environmentally relevant microplastics – Material properties, leaching and ecotoxicity testing. Sci. Total Environ. 773, 145576.
Sahoo, S., et al., 2011. FTIR and Raman spectroscopy as a tool for analyzing sustained release hydrogel or cirpofloxacin/carbopol polymer. Int. J. Pharm. Sci. Res. 2, 268–277.
SAPEA, 2019, A scientific perspective on microplastics in nature and society. SAPEA, evidence review report, 10.26356/microplastics.
Somasundaran, P., et al., 2005. Surfactants, polymers and their nanoparticles for personal care applications. Int. J. Cosmet. Sci. 27, 135–136.
Suka, M., et al., 2021. Single intratracheal administration of cross-linked water-soluble acrylic acid polymer causes acute alveolo-interstitial inflammation and the subsequent fibrotic formation possibly via the TGF-β1 pathway in the lung of rats. Toxicology 448, 152647.
Sverdrup E., L., et al., 2001. Comparative toxicity of acrylic acid to marine and freshwater microalgae and the significance for environmental effects assessments. Chemosphere 45, 653–658.
Tabra, K., et al., 2020. Evaluation of phytotoxic effects of cationic polyacrylamide polymers: implication for the use of sludges as organic soil amendments in assisted phytostabilization. Int. J. Phytoremediat. 22, 1068–1074.
Tatari, K., et al., 2017. Challenges in using allylthiourea and chlorate as specific nitrification inhibitors. Chemosphere 182, 301–305.
Tinikul, R., et al., 2020. Chapter Fourteen - Bacterial luciferase: Molecular mechanisms and applications. In: Chaiyen, P., Tamanoi, F. (Eds.), The Enzymes. Academic Press, pp. 427–455.
Wang, T., et al., 2009. pH dependence of the properties of waterborne pressure-sensitive adhesives containing acrylic acid. ACS Appl. Mater. Interfaces 1, 631–639.
Wang, Z., et al., 2021. Advances in ultra-trace analytical capability for micro/nanoplastics and water-soluble polymers in the environment: fresh falling urban snow. Environ. Pollut. 276, 116698.
Weston, D.P., et al., 2009. Toxicity of anionic polyacrylamide formulations when used for erosion control in agriculture. J. Environ. Qual. 38, 238–247.
Zalasiewicz, J., et al., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17.
Zhang, Y., et al., 2020. Global trends and prospects in microplastics research: A bibliometric analysis. J. Hazard. Mater. 400, 123110.
Zong, X., et al., 2021. Effects of polystyrene microplastic on uptake and toxicity of copper and cadmium in hydroponic wheat seedlings (Triticum aestivum L.). Ecotoxicol. Environ. Saf. 217, 112217.