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Submicro- and nanoplastics: how much can be expected in water bodies?

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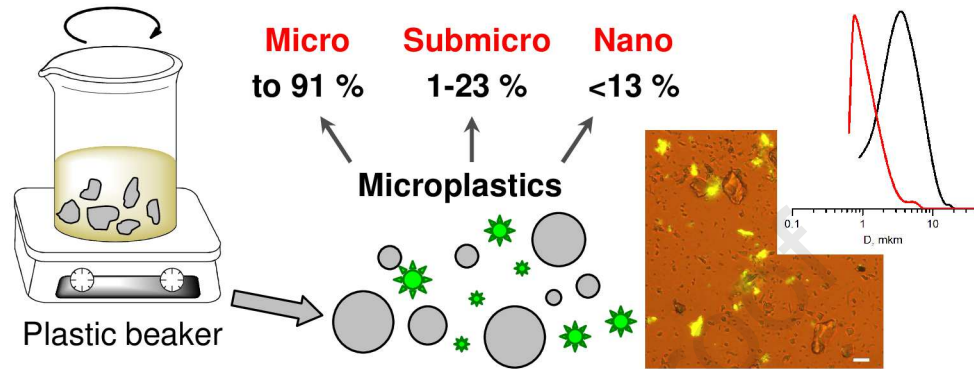
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**Graphical abstract****Submicro- and nanoplastics: how much can be expected in water bodies?**

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**Submicro- and nanoplastics: how much can be expected in water bodies?**

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**Abstract**

Plastic particles smaller than 1  $\mu\text{m}$  are considered to be highly dangerous pollutants due to their ability to penetrate living cells. Model experiments on the toxicity of plastics should be correlated with actual concentrations of plastics in natural water. We simulated the natural destruction of polystyrene, polyvinyl chloride, and poly(methyl methacrylate) in experiments on the abrasion of plastics with small stones. The plastics were dyed in mass with a fluorescent dye, which made it possible to distinguish plastic particles from stone fragments. We found that less than 1% of polystyrene and polyvinyl chloride were converted to submicron size particles. In the case of more rigid poly(methyl methacrylate), the fraction of such particles reaches 11%. The concentration of particles with a diameter less than 1  $\mu\text{m}$  in the model experiments was from 0.7 (polystyrene) to 13 mg/L (poly(methyl methacrylate)), and when transferring the obtained data to real reservoirs, these values should be reduced by several orders of magnitude. These data explain the difficulties associated with the search for nanoplastics in natural waters. The toxicity of such particles to hydrobionts in model experiments was detected for concentrations greater than 1 mg/L, which is unrealistic in nature. Detectable and toxic amounts of nano- and submicron plastic particles in living organisms can be expected only in the case of filter-feeding organisms, such as molluscs, krill, sponges, etc.

30 *A capsule:*

31 The actual concentration of nanoplastics in natural waters should be several  
32 orders lower than in the model toxicological experiments with hydrobionts.

33

34 *Keywords:* nanoplastics; water pollution; simulation; polystyrene; polyvinyl  
35 chloride; poly(methyl methacrylate)

### 36 **1. Introduction**

37 Over the last half a century the number of plastics and their role in human  
38 life has been steadily growing, which in its turn leads to the growing problem of  
39 environmental pollution by plastic waste and products of its destruction. Plastic  
40 materials can decompose in the natural environment under the influence of  
41 many factors with the formation of smaller particles. In modern studies of  
42 environmental pollution with plastics, plastic particles are usually divided into:  
43 macroplastic ( $> 25$  mm), mesoplastic (25-5 mm) and microplastic ( $< 5$  mm).  
44 (Duarte, 2018; European Commission, 2019; Frias and Nash, 2019; Young and  
45 Elliott, 2016). In most of the works the size of microplastic particles varies from  
46 0.3 to 5 mm. The lower limit of this range can be explained by the fact that the  
47 minimum size of meshes in networks for sampling is usually 300-350  $\mu\text{m}$ .  
48 (Oliveira and Almeida, 2019; Peng et al., 2020). In the report of Scientific Advisors  
49 of the European Commission for 2019 (Environmental and Health Risks of  
50 Microplastic Pollution) term "microplastics" was assigned to particles  $< 5$  mm,  
51 including particles  $< 50$   $\mu\text{m}$ , which cannot be seen with the naked eye, as well as  
52 nanoscale particles ( $< 100$  nm) (European Commission, 2019). In its turn,  
53 nanoplastics, according to different sources, are limited to a range of either 1 to  
54 100, or 1 to 1000 nm (Brandelli, 2020; EFSA Panel on Contaminants in the Food  
55 Chain (CONTAM), 2016). Gigault et al. in 2018 defined nanoplastics as  
56 "unintentionally produced (as a result of the decomposition and production of  
57 plastic objects) and colloidal particles ranging in size from 1 to 1000 nm" (Gigault

58 et al., 2018). This size range is the same as that given for colloidal particles in the  
59 IUPAC definition (McNaught and Wilkinson, 1997). Particles of such small size are  
60 actively involved in the Brownian movement, which keeps them dispersed in the  
61 water column and facilitates interaction with microorganisms. Besides,  
62 nanoparticles of about 100 nm in size, in particular polystyrene nanoparticles,  
63 demonstrate a high degree of internalization into cells and, therefore, pose a  
64 serious threat to the health of living organisms (Brandelli, 2020). IUPAC  
65 Recommendations 2012 (Vert et al., 2012) describe nanoparticles as "Particle of  
66 any shape with dimensions in the  $1 \times 10^{-9}$  and  $1 \times 10^{-7}$  m range".

67 When classifying small particles of plastic, that is, micro- and even more  
68 nanoplastics, it is worth considering that such particles can not only get into the  
69 organs of living organisms with food, water and air, but also penetrate through  
70 the membrane inside the cell. A number of cells can capture particles up to 3  $\mu$ m  
71 due to macropinocytosis and phagocytosis. (Foroozandeh and Aziz, 2018; Zauner  
72 et al., 2001). Particular attention should be paid to particles below 500 nm,  
73 because they have access to the universal pathway into various types of cells  
74 through the endocytosis mechanism (Kasper et al., 2011; Manzanares and Ceña,  
75 2020; Pezzoli et al., 2017; Wang et al., 2017). In this regard, we propose to define  
76 nanoplastics as particles ranging in size from 1 to 500 nm, and particles from 500  
77 nm to 1 microns to be classified as submicroplastics, as the effect of Brownian  
78 forces on them remains significant enough to slow down sedimentation in the  
79 water column.

80 Considering the high potential danger of nano and submicroplastics for  
81 living organisms, active work is being done with model hydrobionts cultivated  
82 with different concentrations of plastic particles (Al-Thawadi, 2020; Chang et al.,  
83 2019; Natarajan et al., 2020). Over the last 5 years, the number of works which  
84 can be found by keyword "nanoplastic" in the Web of Science database has  
85 increased from 6 to 73. Reliable toxic effects were observed at concentrations

86 above 1-10 mg/L, with commercial preparations of unclear composition often  
87 used as model particles (Kim et al., 2020; Lei et al., 2018; Sendra et al., 2019).  
88 Moreover, when these preparations were purified from admixtures, e.g. sodium  
89 azide, their  $EC_{50}$  increased to a level  $> 100$  mg/L (Heinlaan et al., 2020), and even  
90 more than 500 mg/L as in the case of 86 – 125 nm pure poly(methyl  
91 methacrylate) nanoparticles which gave no observable toxicity at concentrations  
92 up to 500 mg/L to 1000 mg/L for *Daphnia magna* (Booth et al., 2016). In this  
93 regard, the question arises - how close are the model experiments to the real  
94 situation that occurs when plastic products get into water bodies?

95 There are a lot of works on the degradation of plastics affected by different  
96 weathering factors (Chubarenko et al., 2020; Da Costa et al., 2018; Song et al.,  
97 2017; Sun et al., 2020), but only a few of them are devoted to formation of nano  
98 and submicroplastics under conditions close to natural (Astner et al., 2019; Ekvall  
99 et al., 2019; Lambert and Wagner, 2016). When studying plastic particles in  
100 natural waters or in products of destruction of household plastics in model  
101 experiments, the difficult task is to distinguish between plastic and terrigenous  
102 particles, and these particles may be in an association. It has been suggested to  
103 use the chromatography-mass spectrometric method (Sullivan et al., 2020; Ter  
104 Halle et al., 2017) to determine the content of plastic particles, which allows to  
105 estimate the ratio of different types of plastics, but when estimating the  
106 concentration of plastics, the results should be considered more as semi-  
107 quantitative. A certain information about the nature of the particles can be  
108 obtained using electron microscopy methods with elemental analysis, but in this  
109 case there is still uncertainty in the analysis of associates, which may also contain  
110 biogenic organic substances.

111 In this work, we used industrial plastics such as polystyrene (PS), polyvinyl  
112 chloride (PVC), and poly(methyl methacrylate) (PMMA) modified by the  
113 introduction of water-insoluble fluorescent dye into the plastic mass. These are

114 widely used polymers, for example, in the production of disposable cutlery,  
115 pipes, clothing, construction elements, and therefore these plastics are an  
116 important part of polymer waste. The other reason is the ease of coloring them  
117 in a block compared to another common plastic - polyethylene. The obtained  
118 fluorescent materials were mechanically destroyed under conditions simulating  
119 natural ones. The presence of fluorescence in the obtained micro-, submicro- and  
120 nanoparticles made it possible to identify them using fluorescence microscopy  
121 and to determine the content by spectrofluorimetric methods.

## 122 **2. Material and methods**

### 123 *2.1. Chemicals*

124 Acetone, dichloromethane, and tetrahydrofuran (THF) (reagent grade) were  
125 purchased from Alfa Aesar (Thermo Fisher Scientific Inc.). PS (MW 192 kDa),  
126 fluorescence dye dibenzylfluorescein, and sodium dodecyl sulfate (SDS) were  
127 purchased from Merck KGaA or Thermo Fisher Scientific Inc. Disposable  
128 spectrophotometric cuvettes (BRAND GMBH + CO KG, Germany) were used as a  
129 source of PMMA. PVC (MW 1600 kDa, measured in THF at 25 °C (de Vries et al.,  
130 1971)) was from OJSC Usoliekhimprom, Usolye-Sibirskoe, Russia. THF was  
131 refluxed with  $\text{LiAlH}_4$  and distilled under argon. Acetone and dichloromethane  
132 were distilled before use. Bottled Baikalian deep water (Grachev et al., 1995) was  
133 applied in model abrasion experiments. Stones from the coastal zone of Lake  
134 Baikal (Listvennichny Bay, Russia) were used for simulating friction stress of  
135 plastic in natural conditions. The stones were thoroughly cleaned using a  
136 household detergent (Fairy) and then washed ten times with distilled and  
137 deionized water.

### 138 *2.2. Preparation of fluorescent plastics*

139 Fluorescently labeled plastic was obtained by dissolving polymers in organic  
140 solvents (THF for PS and PVC, acetone for PMMA) with addition of  
141 dibenzylfluorescein dye (0.25% of the polymer weight). The polymer



142 concentrations in solutions were 20.4, 13.3, and 24.9% wt % for PS, PVC and  
143 PMMA, respectively. The obtained polymer solutions were spread over the inner  
144 surface of 1000 mL polystyrene beakers (diameter 105 mm, height 140 mm)  
145 followed by air drying. The procedure was repeated multiple times. Finally, the  
146 beakers were dried to have a constant mass. The thickness of the fluorescent film  
147 was estimated to be 0.79, 0.78, and 0.38 mm for PS, PVC, and PMMA,  
148 respectively, (Table 1-SM) based on the mass of the dried layer of polymer and  
149 the surface area covered. Further abrasion experiments result in erasing 1-3% of  
150 the plastic thickness, so the films are thick enough to neglect their thickness.

### 151 *2.3. Mechanoconstruction of fluorescent plastics*

152 Ten stones (Table 1) and 200 ml of Baikol water were placed into every  
153 beaker and shaken for 96 hours at 180 rpm. This shaking speed ensures that the  
154 stones move along the bottom of the beaker without splashing water. After  
155 shaking, the liquid was poured out from the stones, and in the case of PS, there  
156 was foam from the plastic particles on the surface, which was collected  
157 separately. The poured dispersions were centrifuged at 100 g for 30 minutes, the  
158 sediment was separated and the supernatant was centrifuged again at 4000 g for  
159 30 minutes. The obtained sediments were examined with a fluorescent optical  
160 microscope to determine the size of the particles. Particle sizes in dispersions  
161 after centrifugation were analyzed with the dynamic light scattering (DLS) before  
162 and after filtering through syringe filters with pore diameters of 1.2 and 0.45  $\mu\text{m}$ .

163 The plastic content was determined at each stage of the process. For this  
164 purpose the sediments were dried, weighed and extracted with THF (PS and PVC)  
165 or acetone (PMMA) followed by recording fluorescence. The concentrations of  
166 microplastics were calculated from calibration curves for the fluorescence of the  
167 dye in the corresponding solvents (Fig. 1-SM,  $\lambda_{\text{ex}}=450\text{ nm}$ ).

168 Plastic content in the dispersions was determined by fluorometry using  
169 calibration dispersions of polymers (Fig. 2-SM) precipitated from organic

170 solutions (THF for PS and PVC, acetone for PMMA) into an aqueous SDS solution  
171 similar to the method given in (Miletić et al., 2010). A solution of the polymer (30  
172 mg) and dibenzylfluorescein (0.25% of the polymer weight) in 10 ml THF (acetone  
173 for PMMA) was added by drops to an intensively stirred (2200 rpm) SDS solution  
174 (140 ml, 71.4 mg/L) during 20 minutes. Then stirring was continued for additional  
175 10 minutes. The resulting turbid mixture was passed through a cotton wool filter  
176 to remove large pieces of polymer. The filtrate was treated with ultrasound for  
177 10 minutes and centrifuged at 20,000 g for 30 minutes. The supernatant was  
178 carefully removed and the sediment was resuspended in distilled water by  
179 ultrasound treatment for 30 minutes followed by filtration through a 1.2  $\mu\text{m}$  filter  
180 to obtain the final dispersion of nanoparticles.

181 The polymer concentration in the obtained dispersions was determined  
182 using UV spectroscopy for PS and IR spectroscopy for PVC and PMMA. In the case  
183 of PS and PMMA, the gravimetric method was also applied. PS dispersions were  
184 air dried and dissolved in methylene chloride to record UV spectra. The  
185 concentrations were found using the calibration curve (Fig. 3-SM). PVC  
186 concentrations were found by FTIR spectroscopy (Fig. 4-SM and 5-SM) using  
187 calibration curves for signal intensity ratios of  $1253\text{ cm}^{-1}$  (PVC) and  $2118\text{ cm}^{-1}$  (CN  
188 group of  $\text{K}_3[\text{Fe}(\text{CN})_6]$ ). To prepare the calibration mixtures, PVC and  $\text{K}_3[\text{Fe}(\text{CN})_6]$   
189 (internal standard) were mixed in different ratios, thoroughly ground with KBr,  
190 and pressed into pellets. The PVC dispersion was mixed with  $\text{K}_3[\text{Fe}(\text{CN})_6]$  solution,  
191 dried, mixed with KBr, thoroughly ground and pressed into pellets for the IR  
192 spectroscopy. The concentration of PMMA in the dispersions was found similarly  
193 to PVC using calibration curves for bands at  $1147\text{ cm}^{-1}$  (PMMA) and  $2118\text{ cm}^{-1}$   
194 ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ). (Figure 5-SM). The values measured by gravimetry, UV (PS) and IR  
195 (PMMA) matched with 10% accuracy.

#### 196 2.4. Equipment

197 A BIOSAN OS-20 orbital shaker was applied for shaking beakers during  
198 mechanical destruction of the plastics. Reference dispersions of polymer  
199 nanoparticles were prepared using an overhead stirrer MIULAB with the speed  
200 range of 50-2200 rpm.

201 Absorption, excitation and emission spectra were measured with SM-2203  
202 spectrofluorimeter (CJSC Spectroscopy, Optics and Lasers – Modern  
203 Developments, Republic of Belarus, Minsk). A pulsed xenon lamp was used as an  
204 excitation source in the device. The excitation wavelength of the luminescence  
205 was  $450\pm 0.4$  nm. Spectra of calibration solutions and tested samples were  
206 measured during the same session of the device.

207 Dynamic light scattering (DLS) experiments were performed with the LAD-  
208 079 device designed at the Institute of Thermophysics (Novosibirsk, Russia). Light  
209 scattering from 540 nm laser was measured at  $90^\circ$  angle. IR spectra were  
210 recorded with Infracum FT-801 device (LLC Simex, Novosibirsk, Russia), in pellets  
211 with KBr.

212 The scanning electron microscope FEI Quanta 200 and transmission electron  
213 microscope LEO 906E were applied to study plastic dispersions. The samples  
214 were diluted with water, placed on aluminum plates or grids for microscopy, and  
215 dried in the air. Light and fluorescent microscopy was performed with MOTIC AE-  
216 31T inverted microscope with a HBO 103 W/2 OSRAM mercury lamp with a blue  
217 filter (450 nm). The CCIS Plan Achromats objectives were used with magnification  
218 x20 (LWD, N.A. 0.4), x40 (LWD, N.A. 0.6) and AmScope Plan Achromats x100 (Oil,  
219 N.A. 1.25). Spatial resolution at wavelength 450 nm was: for x100 - 220 nm, x40 –  
220 458 nm, and x20 – 686 nm. Spatial resolution was calculated as  $R=0.61\cdot\lambda/N.A.$ ,  
221 where  $\lambda$  – wavelength of the light, N.A. - numerical aperture of the objective  
222 (Sheppard, 1988). The smallest measured particles were 300 nm in size.

### 223 **3. Results and discussion**

224 Beakers coated with a layer of appropriate polymer inside were applied to  
225 simulate mechanical destruction of plastics in water reservoirs. To create a  
226 coating of the required plastic on the inner surface of the beakers we used  
227 solutions of plastics colored with dibenzylfluorescein (Fig. 1, Table 1-SM). The  
228 fluorescent dye is insoluble in water and is not leached from plastics. This was  
229 confirmed by centrifuging model dispersions of plastics at 50,000 g, which  
230 resulted in particle sedimentation and disappearance of fluorescence (Fig. 2).  
231 Free dibenzylfluorescein gives characteristic bright fluorescent structures in  
232 water, which are easy distinguishable from fluorescent plastic particles (Fig. 6-  
233 SM). The material of the beakers was chosen as polystyrene because of its good  
234 adhesion to the selected plastics. In the case of glass or steel beakers, the  
235 polymer layer quickly detaches from the walls and floats in water, having poor  
236 contact with stones.

237 Smooth stones 10-15 mm in diameter and water were placed into beakers,  
238 and the beakers were shaken on an orbital shaker for 96 hours (Table 1). The size  
239 and number of stones were chosen so that when shaking, they could move along  
240 the bottom of the beaker, abrading the plastic surface. The experimental design  
241 provides significantly better conditions for the accumulation of fine plastic  
242 particles in the water compared to natural conditions. Intense mechanical  
243 abrasion in a water body is possible under certain disturbances (surf, storm, etc.),  
244 but the same disturbance leads to the distribution of plastic particles throughout  
245 the water body, preventing their concentration in the abrasion zone.

246 The stones' friction against the fluorescent plastic and against each other  
247 resulted in dispersions consisting of both organic plastic particles and particles of  
248 mineral nature. Only in case of PS, a layer of poorly wetted particles was  
249 observed on the water surface (Fig. 3 B). The obtained dispersions were divided  
250 into several fractions by a series of centrifugation and filtration operations (Fig.  
251 3):

- 252 - sediment 1 was obtained after centrifugation at 100 g;  
253 - the obtained dispersion 1 was centrifuged at 4000 g, resulting in sediment 2 and  
254 dispersion 2;  
255 - the dispersion 2 was filtrated with 1.2 and after that with 0.45  $\mu\text{m}$  filters giving  
256 rise to dispersions 3 and 4 respectively.

257 The size of plastic particles in sediments and PS foam was characterized by  
258 light and fluorescence microscopy. The use of fluorescent dye allows effective  
259 detection of microplastics with an optical microscope and easily distinguish them  
260 from mineral particles by combining modes of visible light and fluorescence (Fig.  
261 4). The stability of the fluorescent dye during the experiment was confirmed by  
262 the stability of the fluorescence of the nanoparticle calibration dispersion under  
263 the same conditions (Fig. 7-SM). The content of plastics in sediments was  
264 determined by means of solution fluorometry after polymer extraction. Particle  
265 size of dispersions 2 and 3 was estimated using DLS (Fig 5 and Fig. 8-SM).  
266 Fluorescence microscopy was also used for dispersion 3 containing submicron  
267 particles. The microscopy and DLS data do not match because the dispersion  
268 contains mineral particles and optical microscopy is not suitable for sizes below  
269 300 nm. The dispersion 4 after 0.45  $\mu\text{m}$  filter contained too few particles to be  
270 studied by the DLS method, therefore, the SEM and TEM methods were applied  
271 in this case (Fig. 9-SM and 10-SM) and particle size was calculated from SEM data.  
272 However, it should be kept in mind that these methods do not distinguish  
273 between plastic and mineral particles originating from stones but SEM and TEM  
274 data confirm the adequacy of the filtering procedure. Plastics content in the  
275 dispersions was measured by direct fluorometry of dispersion by means of  
276 calibration with synthetic fluorescent nanoparticles. The mass of mineral  
277 particles obtained in the abrasion process was determined by the mass of the  
278 sediment after removal of plastic by organic solvents. In the case of dispersion 2,  
279 sediment after strong centrifugation (50,000 g, 1 hour) was used.

280 According to the optical microscopy data, in the case of the experiment with  
281 PS, the layer of particles that have floated up on the solution surface consists of  
282 associates and individual particles of average size 25  $\mu\text{m}$  (Fig. 11-SM, Table 2-  
283 SM).

284 Particles of 5-10  $\mu\text{m}$  size were found in the sediments 1 after centrifugation  
285 at 100 g (Fig. 4, and 6-8 and). The particle sizes in the sediments 2 after  
286 centrifugation at 4000 g are approximately 2-3 times smaller than after 100 g  
287 (Fig. 6-8, Table 2-SM), e.g. 4.3 / 8.2  $\mu\text{m}$  for PS, 3.3 / 10.1  $\mu\text{m}$  for PVC, and 2.0 / 5.8  
288  $\mu\text{m}$  for PMMA.

289 Data on fractional composition of plastic after mechanical destruction are  
290 summarized in Fig. 8 and Table 2-SM. It can be seen that in the case of PS most of  
291 the fragmented plastics has floated to the solution surface and this is a  
292 microplastic with an average particle size of 25  $\mu\text{m}$ . The formation of floating PS  
293 particles is probably due to the low density of this polymer (1.04  $\text{g}/\text{cm}^3$ )  
294 compared with PMMA (1.18) and PVC (1.39) (Brandrup et al., 1999). When the  
295 stones are frictioned against the surface of PVC, mainly particles of 3 to 10  
296 microns in size are formed. PMMA has shown the highest propensity to form  
297 stable micro- and nanoparticles in the dispersion from the plastics studied.  
298 Almost half of all particles remained in dispersion 1 after centrifugation at 100 g,  
299 and 10.8% of all particles remained in dispersion 2 after centrifugation at 4000 g,  
300 of which half are nanoparticles with a diameter below 450 nm according to the  
301 filtration procedure (dispersion 4, Table 2-SM). Moreover, according to SEM data  
302 (Fig. 9-SM), most of these particles have a diameter of about 110 nm. The reason  
303 for this may be that PMMA is a more rigid polymer. According to the Rockwell  
304 method (Brandrup et al., 1999) the hardness of polymers is 80-100, 60-84 and 65-  
305 85 for PMMA, PS and PVC, respectively. Ultramicroindentation (185, 164, 149  
306 MPa) (Calleja et al., 2004) and AFM nanoindentation (187.1, 167.5 and 86.9 MPa)  
307 (Jee and Lee, 2010) data for the same polymers confirms high hardness of

308 PMMA. When stones are stressed or frictioned against a PMMA surface, the  
309 probability of breaking off small particles is greater than with less rigid PS and  
310 PVC. Softer polyethylene (LDPE, 22.4 MPa (Jee and Lee, 2010) is expected to  
311 produce negligible amounts of these particles. In addition, the formation of a  
312 higher percentage of nanoplastics in the case of PMMA is probably due to its  
313 lower hydrophobicity compared with PS and PVC (water contact angle for PMMA  
314  $70.9^\circ$ , for PVC  $85.6^\circ$ , and  $87.4^\circ$  for PS) (Moshonov and Avny, 1980; Panzer, 1973;  
315 Westerdahl et al, 1974). In terms of hydrophobicity, PS and PVC do not differ  
316 much from each other, and the greater number of PS particles floating on the  
317 solution surface can be explained by the combined effect of hydrophobicity and  
318 lower PS density.

319 The abrasion of the plastic samples with stones produced mineral particles  
320 of 200-280 mg for sediment 1, 140-170 mg for sediment 2, and 20-30 mg for  
321 dispersion 2. These values are one to two orders of magnitude greater than the  
322 mass of plastic particles (Table 2-SM), indicating the difficulty of finding small  
323 plastic objects in nature.

324 Based on the simulation of mechanical destruction of plastics in the near-  
325 bottom zone, we have shown that microplastics are mainly formed. In the case of  
326 PS and PVC, a small number of nanoplastics were found, while PMMA showed a  
327 much greater ability to form submicro- and nanoplastics. The concentrations of  
328 nanoparticles (<450 nm) in dispersions of nanoplastic were 0.7, 1.3 and 13.1  
329 mg/L for PS, PVC and PMMA, respectively. It should be noted that in our  
330 experiment, plastic abrasion was carried out under a 12 cm layer of water for  
331 four days. In natural conditions, the possible concentration of nanoplastics will be  
332 lower due to the following factors: i) incomplete coverage of the bottom of the  
333 reservoir with the original plastic; ii) distribution of nanoplastics over the entire  
334 water column; iii) removal of plastics by water streams; iv) continuous 24-hour  
335 mechanical abrasion at the bottom under the water column is unlikely. If we

336 assume that 5% of the bottom is contaminated with plastic at a depth of 5 m, the  
337 concentration of nanoplastics will not exceed 0.015 mg/L if the particles are  
338 evenly distributed. Obviously, under real conditions, the effectiveness of plastic  
339 abrasion will be much lower than in the experiment, and the removal of  
340 nanoplastics by streams into the zone of greater depths will reduce its  
341 concentration by several orders of magnitude. In this paper we consider only  
342 mechanical destruction of plastics and neglect photo, chemical (oxidative) and  
343 biological destruction, which are also important for the degradation of plastics in  
344 nature. On the other hand, the smallest particles (submicro- and nanoparticles)  
345 under the influence of these factors mainly decompose to non-polymeric  
346 substances (Bianco et al., 2020; Sun et al., 2020; Zhu et al., 2020), and therefore  
347 in the first approximation we assume that mechanical destruction is the most  
348 important factor in the formation of  $<1 \mu\text{m}$  plastic particles.

349 The study of nanoplastics in natural reservoirs raises several questions: How  
350 to find plastic nanoparticles in natural water? Can the concentration of these  
351 nanoparticles be measured? What biological effects are possible from  
352 nanoplastics? When answering the first question, we should keep in mind that  
353 the concentration of total suspended matter in marine and freshwater bodies  
354 ranges from 0.01 to  $>3 \text{ mg/L}$  in coastal areas (Chebykin et al., 2010; Chester,  
355 1990) which is higher than expected concentration of the nanoplastic particles.  
356 Nanoplastics is not a unique organic substance in water, living organisms and the  
357 products of their destruction can be an obstacle to the detection of nanoplastic  
358 particles. Thus, the search for individual particles is almost impossible, but one  
359 can try to estimate the total concentration of synthetic plastics in different  
360 filtration fractions, for example, using mass spectrometry methods.

361 When discussing the biological impacts of nanoplastics, the number of  
362 specimens of hydrobionts should be compared with the expected amount of  
363 plastic particles in the same volume of water. A brief literature analysis



364 (Annenkova, 2013; Dabrowska et al., 2020; Kakizaki, et al., 2011; Stonik and  
365 Orlova, 2013; Vorobyeva, 2018;) shows that one million unicellular organisms *per*  
366 liter is a realistic estimate. With an assumed plastics particle diameter of 250 nm,  
367 one million particles per liter corresponds to a plastics concentration of 0.01  
368  $\mu\text{g/L}$ . Considering our data and reasonings, this concentration also seems  
369 realistic. Therefore, a comparable number of plastic nanoparticles and  
370 hydrobionts in natural reservoirs can be expected. Plastics are inert materials,  
371 and it is difficult to expect visible biological effects when one living cell meets a  
372 single plastic nanoparticle. Of course, these nanoparticles can concentrate  
373 hazardous compounds on the surface that increase their toxicity, but in any case,  
374 we assume that the concentration of nanoplastics in toxicological tests should be  
375 several orders of magnitude lower than 1 mg/L. On the other hand, some  
376 organisms filtrate a lot amount of water (Rahman, 2019 and refs. from this  
377 review), e.g. sponges (Porifera) "filter up to 900 times their body volume of water  
378 per hour, recycling nutrients and coupling a pelagic food supply with benthic  
379 communities" (Ludeman et al., 2017). One liter sponge can thus harvest 6.5 mg of  
380 nanoplastic in one month from 0.01  $\mu\text{g/L}$  dispersion. Since sponges capture  
381 substance from the environment with a certain oscula, this nanoplastic can be  
382 found near the oscula, and blocking the oscula can lead to visible sponge  
383 suppression.

#### 384 **4. Conclusions**

385 Thus, when abrasion of polystyrene and polyvinyl chloride under conditions  
386 simulating mechanical destruction of plastics in water reservoirs, less than 1% of  
387 the plastic is converted to submicron size particles. In the case of more rigid  
388 poly(methyl methacrylate), the fraction of such particles reaches 11%. The  
389 maximum concentration of particles with a diameter less than 1  $\mu\text{m}$  in the model  
390 experiments was 13 mg/L, and when transferring the obtained data to real  
391 reservoirs, this value should be reduced by several orders of magnitude. In this

392 regard, it is obvious that it is possible to detect nanoplastics in the natural  
393 environment in noticeable amounts that have a clear effect on hydrobionts, only  
394 directly at the surface of plastic waste. The accumulation of detectable amounts  
395 of nano and submicron plastic particles in living organisms can be expected only  
396 in the case of filter-feeding organisms, such as molluscs, krill, sponges, etc.

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### 403 **Declaration of competing interest**

404 The authors declare no conflicts of interest.

### 405 **CRediT authorship contribution statement**

406 **Vadim V. Annenkov:** Conceptualization, Methodology, Writing - Review &  
407 Editing, Funding acquisition. **Elena N. Danilovtseva:** Project administration, Data  
408 Curation, Writing - Review & Editing. **Stanislav N. Zelinskiy:** Methodology,  
409 Investigation, Writing - Original Draft. **Viktor A. Palshin:** Methodology,  
410 Investigation, Formal analysis, Writing - Original Draft.

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603

604 Table 1

605 Stones from the coastal zone of Lake Baikal-used for mechanical destruction of  
606 the plastics

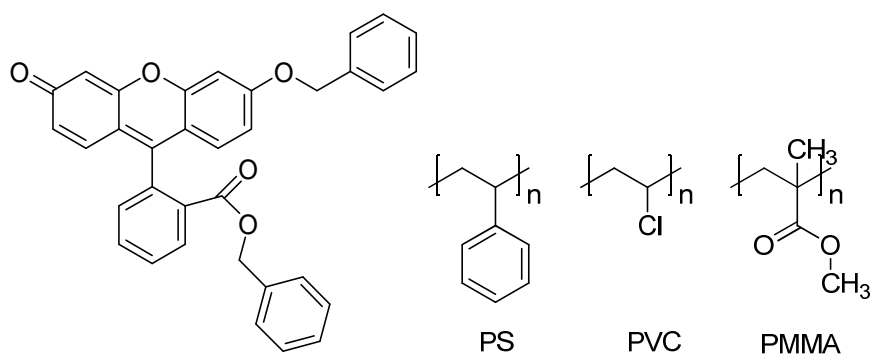
Plastic	Stones	
	Size, mm	Weight, g
PS	12.2±2.3	2.68±1.75
PVC	10.7±0.9	1.37±0.28
PMMA	14.6±1.8	3.98±1.39

607

608

Journal Pre-proof



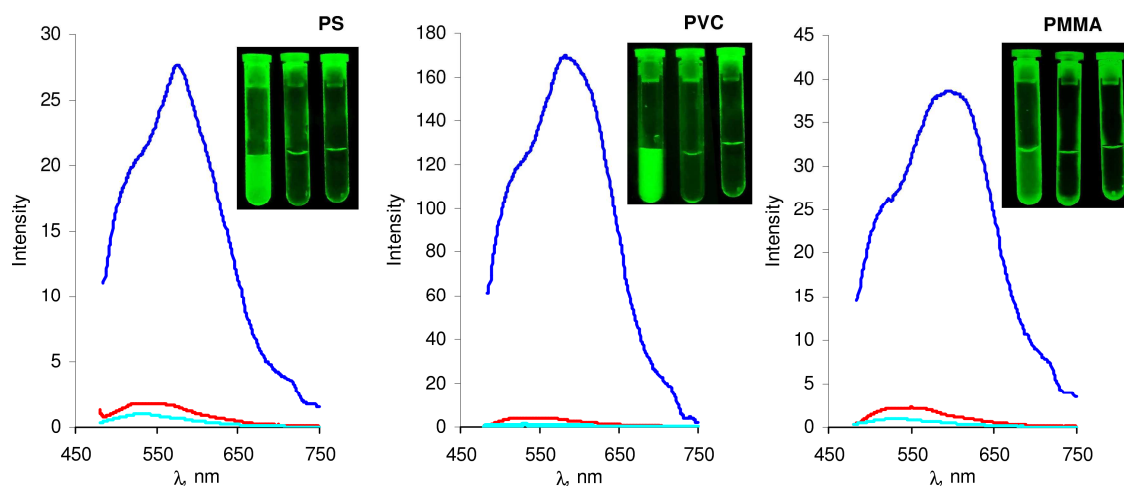


609

610 Fig. 1. Structures of dibenzylfluorescein and polymers

611

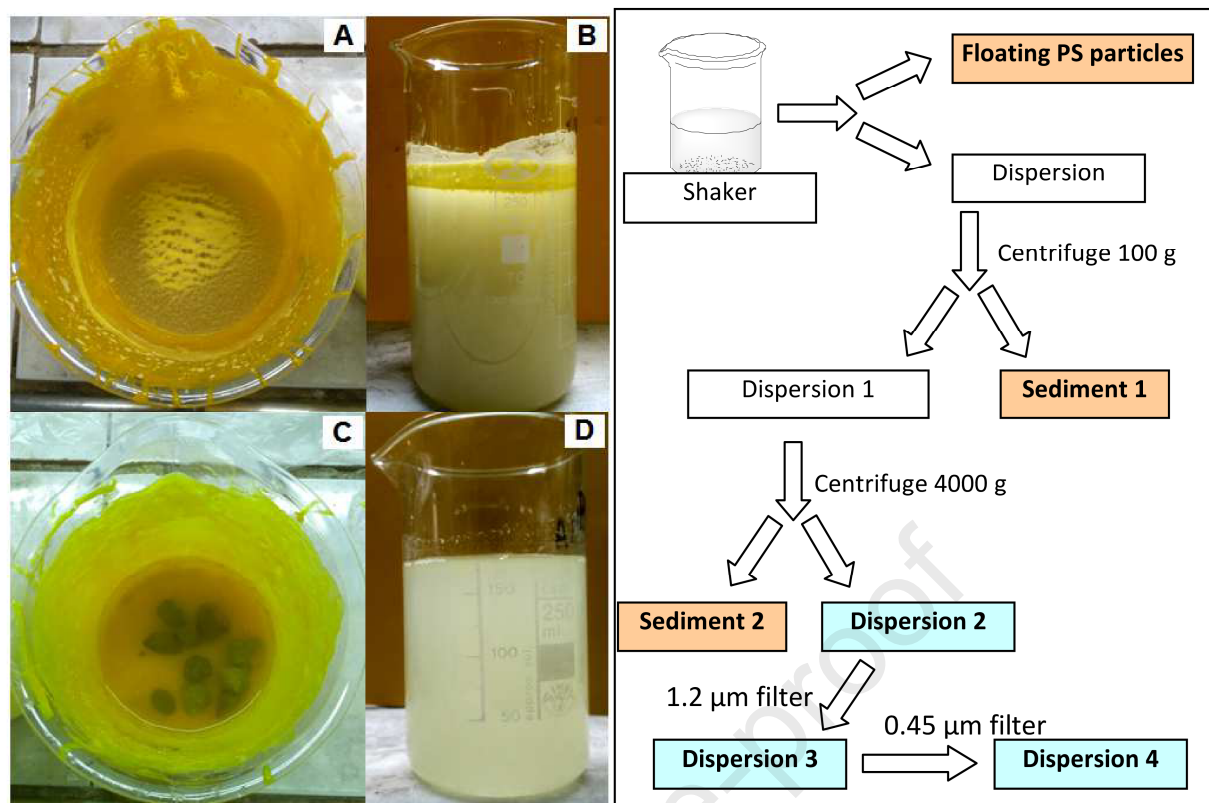
Journal Pre-proof



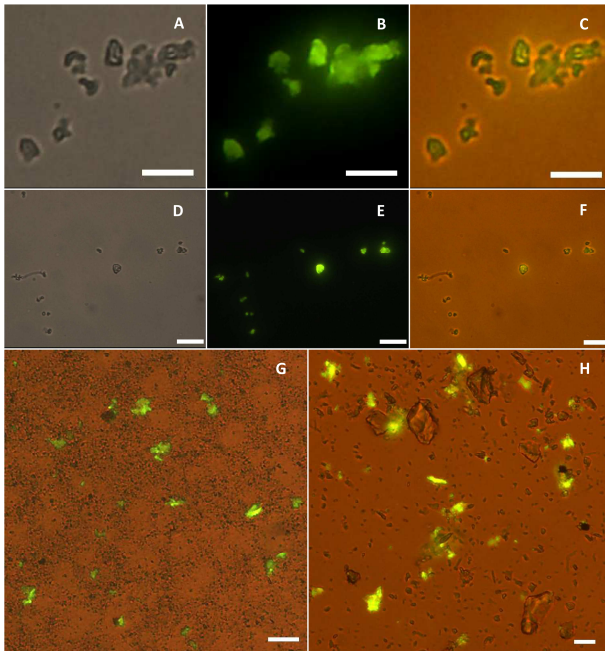
612

613 Fig. 2. Fluorescence spectra of the dispersions of plastic particles that were  
614 prepared as calibration standards in measuring the plastic content of dispersions  
615 #2-4. Blue - spectra of initial dispersions, red - spectra after centrifugation at  
616 50,000 g, 1 hour and light blue - spectra of the cuvette with water. Excitation –  
617 450 nm. The insertions are images of vials with initial dispersions, solutions after  
618 centrifugation, and water (from left to right), visualized on a UV transilluminator  
619 operated at 254 nm. Polymer concentrations: PS – 821, PVC – 518 and PMMA –  
620 311 mg/L. Dibenzylfluorescein – 0.25% from the polymer mass.

621



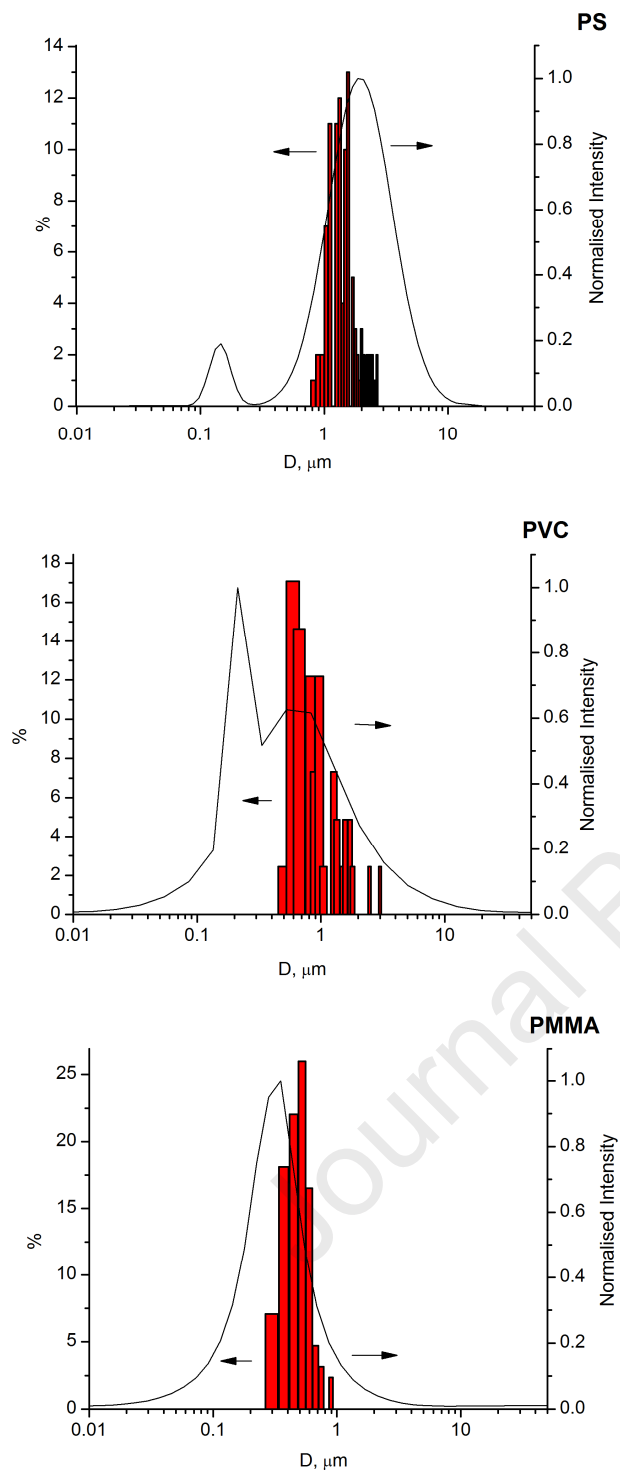
622  
 623 Fig. 3. Pictures of model systems after shaker (A and C) and poured suspensions  
 624 (B and D). On the right - scheme of separation of samples into fractions of plastic  
 625 particles, PS - A, B; PMMA - C, D. Floating PS particles - layer of polystyrene  
 626 particles floating on solution surface, Sediment 1 - sediment after centrifugation  
 627 at 100 g, 30 min; Sediment 2 - sediment after centrifugation at 4000 g, 30 min.  
 628 Dispersion 2 - liquid phase after centrifugation at 4000 g. Dispersions 3 and 4  
 629 were obtained by filtering Dispersion 2 with 1.2 and 0.45  $\mu\text{m}$  filters, respectively.  
 630



631

632 Fig. 4. Light (A and D), fluorescent (B and E), and combined (C, F, G, and H)  
633 microscopy images of sediments 1 obtained after centrifugation at 100 g. Green  
634 fluorescence arises from plastic particles, while dark objects are terrigenous  
635 particles from stones. PMMA: A – F and H, PS: G. Scale: 50  $\mu\text{m}$  - G, 10  $\mu\text{m}$  - D-F  
636 and H, 5  $\mu\text{m}$  - A-C.

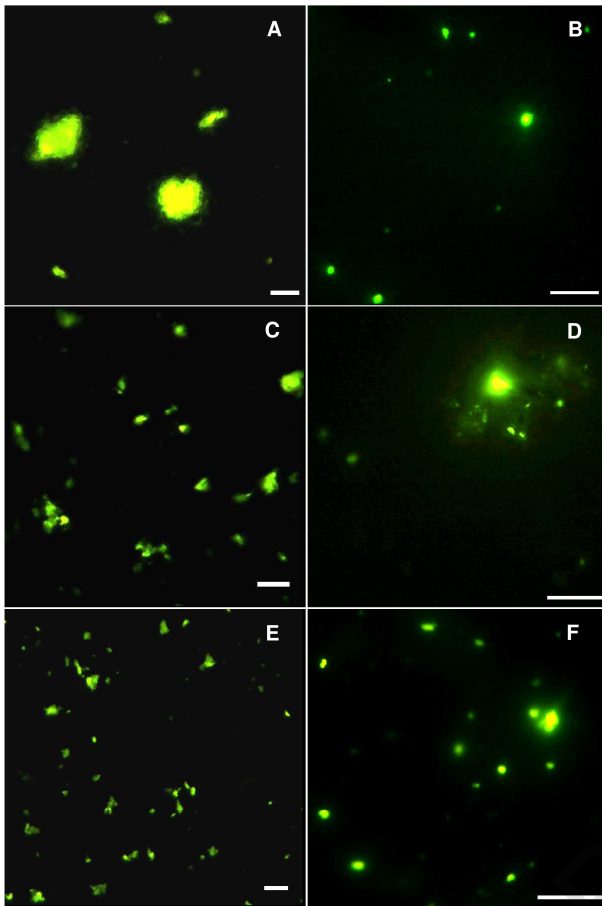
637



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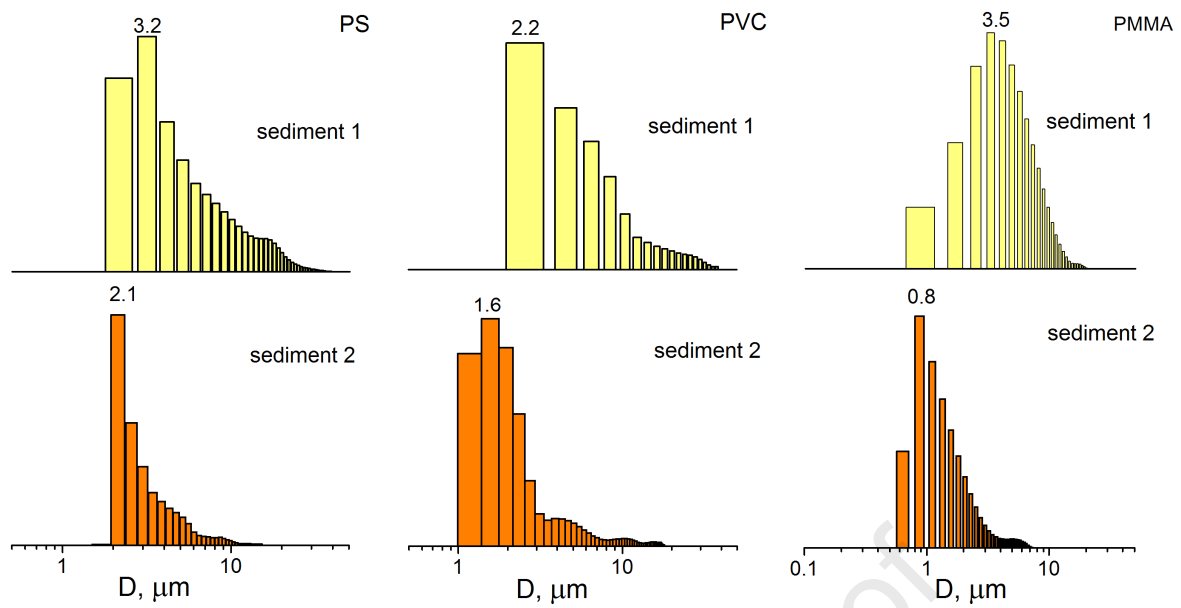
639 Fig. 5. Particle size in dispersion 3 according to DLS and fluorescence microscopy.

640



641  
642 Fig. 6. Fluorescent images of microplastic particles of PS (A, B), PVC (C, D) and  
643 PMMA (E, F) in sediments after centrifugation at 100 g (A, C, E) and 4000 g (B, D,  
644 F). Scale: 10 μm.

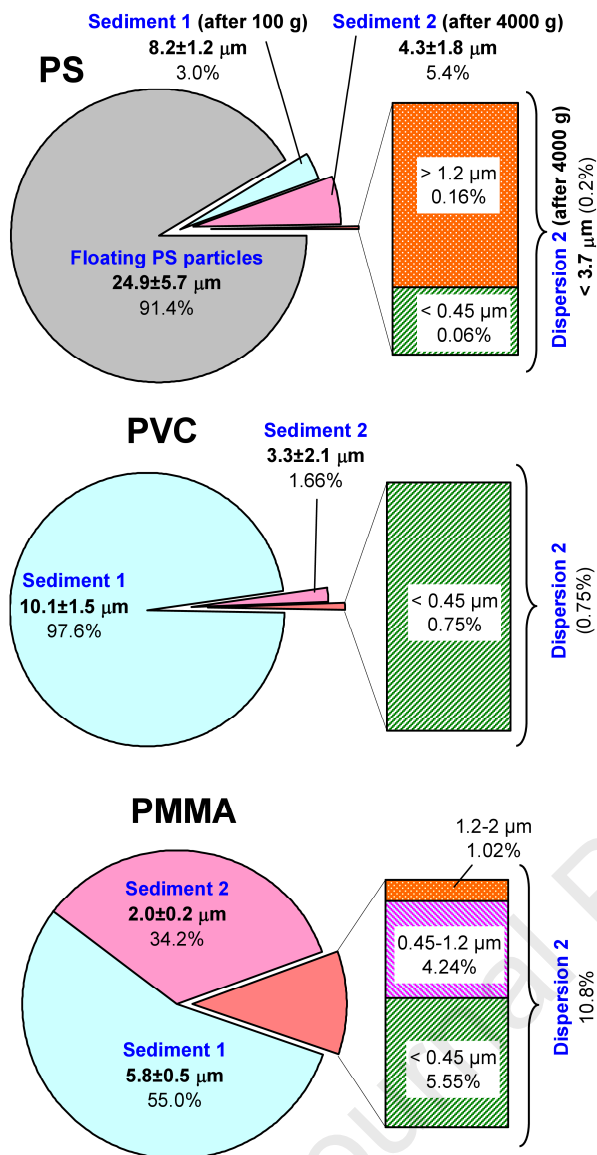
645



646

647 Fig. 7. Size distribution of microplastic particles in sediments after centrifugation  
648 at 100 (sediment 1) and 4000 g (sediment 2). Curves plotted from data for 250  
649 particles.

650



651  
 652 Fig. 8. Fractional composition of plastic particles after mechanical destruction.  
 653 Floating PS particles - layer of polystyrene particles floating on solution surface,  
 654 Sediment 1 - sediment after centrifugation at 100 g, Sediment 2 - sediment after  
 655 centrifugation at 4000 g, Dispersion 2 - in solution after centrifugation at 4000 g.  
 656 The diagrams on the right show the distribution of nanoplastics in suspensions  
 657 after 4000 g centrifugation and filtration. All the plastic that was "erased" from  
 658 the beaker surface is taken as 100%. Signatures: >1.2  $\mu\text{m}$  - plastic particles which  
 659 have not passed through the filter 1.2  $\mu\text{m}$ , <0.45  $\mu\text{m}$  - particles have passed  
 660 through the 0.45  $\mu\text{m}$  filter, and 0.45-1.2  $\mu\text{m}$  - particles have passed through the  
 661 1.2  $\mu\text{m}$  filter less <0.45  $\mu\text{m}$  particles.



How much nanoplastic can we expect in water bodies?

Fluorescent staining allowed simulation of plastic abrasion in an aqueous medium.

Concentration of submicrometer particles was from 0.7 (PSt) to 13 mg/L (PMMA)

The data for real reservoirs should be several orders of magnitude lower

Detectable amount of nanoplastics is expected in filter-feeding organisms only

Journal Pre-proof

**Declaration of interests**

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof