

1 **Smoked cigarette butt leachate impacts survival and behaviour of freshwater**  
2 **invertebrates**

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9 **Abstract**

10 Smoked cigarette filters a.k.a. “butts”, composed of plastic (e.g. cellulose acetate) are one of  
11 the world’s most common litter items. In response to concerns about plastic pollution,  
12 biodegradable cellulose filters are being promoted as an environmentally safe alternative,  
13 however, once smoked, both contain toxins which can leach once discarded. The impacts of  
14 biodegradable butts as littered items on the receiving environment, in comparison with  
15 conventional butts has not yet been assessed. A freshwater mesocosm experiment was used to  
16 test the effects of leachate from smoked cellulose acetate versus smoked cellulose filters at a  
17 range of concentrations (0, 0.2, 1 and 5 butts L<sup>-1</sup>) on the mortality and behaviour of four  
18 freshwater invertebrates (*Dreissena polymorpha*, *Polycelis nigra*, *Planorbis planorbis* and  
19 *Bithynia tentaculata*). Leachate derived from 5 butts L<sup>-1</sup> of either type of filter caused 60-100%  
20 mortality to all species within 5 days. Leachate derived from 1 butt L<sup>-1</sup> of either type resulted  
21 in adults being less active than those exposed to no or 0.2 butts L<sup>-1</sup> leachate. Cigarette butts,  
22 therefore, regardless of their perceived degradability can cause mortality and decreased activity  
23 of key freshwater invertebrates and should always be disposed of responsibly.

24 **Key words:** smoking, cigarette butts, leachate, molluscs, platyhelminth.

25 **Capsule:** As litter in enclosed aquatic habitats, conventional and biodegradable cigarette butts  
26 have the same effects causing mortality and behavioural changes to invertebrates.

27

## 28 **1. Introduction**

29 Cigarette butts (used cigarette filters) are the most common form of personal litter worldwide  
30 due to the majority (>75%) of smokers littering them after use (Patel et al. 2013). Each year,  
31 ~6 trillion cigarettes are smoked globally, possibly resulting in an estimated deposition of ~4.5  
32 trillion used cigarette butts in the environment (Novotny and Slaughter 2014). Despite their  
33 prevalence as litter in the environment, the effects of cigarette butts on marine, freshwater and  
34 terrestrial habitats is still vastly understudied. The majority (~90%) of cigarette filters are  
35 composed of cellulose acetate (Pauly et al. 2002), a type of plastic which is not readily  
36 biodegradable, but can break down into smaller pieces and persist as microplastics and  
37 nanoplastics (Chevalier et al. 2018). Cellulose acetate itself can cause environmental impacts  
38 as litter, with some studies finding that even unsmoked plastic filters can cause a detrimental  
39 effect on the receiving ecosystem, for example, decreasing plant growth (Green et al. 2019)  
40 causing mortality to fish (Slaughter et al. 2011) and amphibians (Lawal and Ologundudu 2013).  
41 In response to concerns about plastic, alternative materials, including pure, unbleached  
42 cellulose, are being promoted for use in cigarette filters instead of cellulose acetate plastic.  
43 These alternative filters have been described as “green”, “biodegradable” and “environmentally  
44 friendly” giving the impression that these items would be benign as litter (Amos et al. 2017).  
45 There is, however, no research providing evidence of their level of toxicity as litter items nor  
46 any research comparing their effects with that of the cellulose acetate butts.

47 As litter, cigarette butts present a unique combination of physical and chemical contamination.  
48 Once smoked, cigarette butts contain thousands of chemicals including nicotine, polycyclic  
49 aromatic hydrocarbons and heavy metals which, once entering an aquatic environment, can

50 leach out into the surrounding water (Moerman and Potts 2011; Roder Green et al. 2014;  
51 Dobaradaran et al. 2019). Such leachates are likely to pose a greater threat to lotic habitats that  
52 can have slow rates of water turnover such as ponds, low energy streams or rockpools than to  
53 habitats where the rate of water replacement is rapid (e.g. the ocean and in fast flowing streams  
54 and rivers). Indeed, leachate from smoked cigarette butts can be lethal for freshwater organisms  
55 such as microalgae, including *Raphidocelis subcapitata* (Bonanomi et al. 2020), water fleas,  
56 including *Ceriodaphnia dubia* (Warne et al. 2002, Micevska et al. 2006), *Daphnia magna*  
57 (Register 2000), fish including *Pimephales promelas* (Slaughter et al. 2011) and amphibians  
58 including *Hymenochirus curtipes* and *Clarias gariepinus* (Lawal and Ologundudu 2013).  
59 Although mortality often occurs at high concentrations of cigarette butt leachate ( $> 1$  butt  $L^{-1}$ ),  
60 sublethal impacts at lower, more environmentally realistic concentrations ( $<0.2$  butts  $L^{-1}$ ) have  
61 been observed, including mutagenic effects (Montalvão et al. 2019), developmental retardation  
62 (Lee and Lee 2015, Parker and Rayburn 2017) and alterations to behaviour (Booth et al. 2015;  
63 Wright et al. 2015). Such sublethal effects are often overlooked by policymakers, but may  
64 invoke important cascading ecological effects (Relyea and Hoverman 2006).

65 To explore toxicological effects of leachate from smoked cigarette butts at incremental  
66 concentrations, four different aquatic invertebrate species were studied in a controlled  
67 environment. The selected organisms included *Dreissena polymorpha* (Pallas 1771) (zebra  
68 mussel), *Polycelis nigra* (Müller 1774) (a flatworm), *Planorbis planorbis* (Linnaeus 1758)  
69 (ramshorn snail) and *Bithynia tentaculata* (Linnaeus 1758) (faucet snail). These were chosen  
70 as model organisms as each are commonly found in pond ecosystems across Europe and the  
71 UK and fulfil a range of ecosystem functions (as e.g. detritivores, grazers, filter feeders,  
72 predators and prey organisms). Here, lethal (mortality) and sublethal (behaviour) effects were  
73 measured in response to leachate derived from smoked cigarettes with either conventional  
74 cellulose acetate filters or biodegradable cellulose filters. The hypothesis tested was that

75 alternative, cellulose cigarette butts would not cause the same lethal and sublethal effects as  
76 conventional, cellulose acetate cigarette butts on the aquatic invertebrates.

77

## 78 **2. Materials and methods**

### 79 *2.1. Preparation of leachate from smoked cigarette filters*

80 Cigarettes were rolled manually using standard cigarette papers to an average ( $\pm$  S.E.) of 0.543  
81  $\pm$  0.002 g per cigarette of a leading brand of tobacco in the UK, with either a cellulose acetate  
82 or a cellulose (unbleached) filter. All cigarettes were smoked using a hand-operated vacuum  
83 pump with silicone tubing attached to the filter of the cigarettes. After lighting, approximately  
84 30 ( $\pm$  1) ml of air was drawn in, simulating a draft and each cigarette was smoked for a total  
85 inhalation volume of  $\sim$ 600 ml per cigarette, thereby emulating a similar total inhalation volume  
86 of cigarettes smoked by humans ( $585 \pm 245$ ) ml; McBride et al. 1984). Cigarettes were smoked  
87 until 2 mm from the edge of the filter and stubbed out in an aluminium tray. Any remaining  
88 tobacco was removed, leaving the filter with the cigarette paper attached. A stock solution of  
89 leachate from each type of filter used (cellulose and cellulose acetate) was prepared separately  
90 by soaking 14 smoked butts in 1 L of fresh, filtered (20  $\mu$ m) rainwater obtained from an  
91 artificial pond in glass volumetric flasks and gently agitating (100 rpm) on an orbital shaker  
92 for 18 h at room temperature ( $\sim$ 18  $^{\circ}$ C). Rainwater was chosen to represent how cigarettes butts  
93 may experience leaching when exposed to precipitation in the environment. Furthermore,  
94 rainwater resembles pond water more closely than media such as distilled water. Rainwater has  
95 been shown to also leach potential contaminants from cigarette butts (e.g. Koutela et al. 2020).

### 96 *2.2. Mesocosm set-up and experimental design*

97 The experiment was carried out in a temperature and light controlled facility at the Portaferry  
98 Marine Laboratory with a 12/12 h light/dark cycle. Mesocosms were set up in the laboratory,

99 using conical glasses (86 mm diameter at top, 65 mm diameter at bottom) that were filled with  
100 rainwater (400 ml), extracted from the same artificial pond as the test organisms, and left to  
101 settle without any added leachate for 24 h before the experimental exposures were initiated.  
102 On day 1 (19<sup>th</sup> March, 2020) of the experiment, treatments were randomly assigned to  
103 mesocosms and corresponding leachate was added by removing the required volume of water  
104 and substituting with 5.7, 28.6 or 142.8 ml of stock leachate representing incremental  
105 concentrations based on 0.2, 1 or 5 smoked butts L<sup>-1</sup> of either cellulose or cellulose acetate  
106 smoked filters. The experimental organisms including *D. polymorpha*, *P. nigra*, *P. planorbis*  
107 and *B. tentaculata* were harvested using a net from an artificial pond (1.4 x 2.1 x 0.9 m). One  
108 individual of each species was added to each mesocosm along with five *B. tentaculata* juveniles  
109 thereby creating representative communities of similar densities to those found in the sampled  
110 pond (Table S1). A treatment with no added leachate served as a control. Therefore, the  
111 experiment consisted of an asymmetric design with 2 fixed factors; “Butts” (2 levels; cellulose  
112 versus cellulose acetate filters) and “Concentration” (3 levels; 0.2, 1 and 5 butts L<sup>-1</sup> added as  
113 leachate). Each treatment was replicated using 5 separate mesocosms (n = 5, N = 35) (Figure  
114 1). Water temperature within the mesocosms had an average pH of 8.13 (± 0.02), salinity <  
115 0.05 ppt and was maintained at 15 (± 0.42) °C throughout the experiment.

116 The experiment was repeatedly sampled every 24 h for a total of 120 h. At each sampling  
117 occasion, mortality was recorded and a number of behavioural observations were recorded into  
118 categories including (i) filtering or (ii) closed for the bivalves, (i) moving, (ii) open (antennae  
119 and foot extended) or (iii) closed (antennae and foot withdrawn into shell) for the gastropods  
120 and (i) moving, (ii) open (body elongated) or (iii) closed (body compressed into a spherical  
121 shape) for the flatworms. Observations were made in real time by the same observer each time.  
122 Due to the high mortality rate in the 5 butts L<sup>-1</sup> treatments, behavioural observations were only  
123 recorded for mesocosms exposed to 0, 0.2 or 1 butt L<sup>-1</sup>.

124

### 125 2.3. Statistical analysis

126 Mortality data was categorised into a mortality scale ranging from 0 to 5 with “0” meaning no  
127 mortality at the end of the experiment and “1”, “2”, “3”, “4” and “5” meaning that death  
128 occurred after >120, 96, 72, 48 and 24 hours respectively. In this way, the higher the number,  
129 the more rapidly the animal died representing a more lethal effect. The survival of juvenile *B.*  
130 *tentaculata* was converted to percentage out of 5 which were still alive at each time point.  
131 Mortality and juvenile survival were analysed using asymmetrical ANOVA (see e.g. Green et  
132 al. 2016 for more details) to account for a single set of control units for the two experimental  
133 levels Butt and Concentration. The survival of juvenile *B. tentaculata* was analysed separately  
134 for each time point to avoid complications involved with repeated measures. Univariate data  
135 were screened for normality and homogeneity of variance to check assumptions of ANOVA  
136 and any necessary transformations are where appropriate. Statistical analyses were done using  
137 R V.3.6.2 (R Core Team 2019).

138 To test effects of leachate on the behaviour over the duration of the experiment, the behavioural  
139 data over the course of the 5 days was pooled and analysed mirroring the univariate analysis  
140 except with only 2 levels of leachate concentration (0.1 and 1 butt L<sup>-1</sup>) instead of three due to  
141 the removal of the 5 butts L<sup>-1</sup> treatment. Multivariate ANOVA was done on Bray-Curtis  
142 dissimilarities of untransformed data with 9999 permutations under the reduced model using  
143 Type I SS using the vegan package v2.5-2 (Oksanen et al. 2019). The asymmetric analysis was  
144 done by fitting each main effect (‘Butt’ and ‘Concentration’) in turn with a Type I (sequential)  
145 SS model, swapping the order of the terms and combining the results of these 2 analyses. The  
146 multivariate behaviour data were visualised using a non-metric multidimensional scaling  
147 ordination approach reflecting the dissimilarity matrix used for the PERMANOVA with  
148 variables with a Pearson’s correlation  $R > 0.6$  overlain as vectors. SIMPER was used to

149 elucidate which behaviours were driving the significant differences between treatments  
150 (contributing >5% to the dissimilarity) found by PERMANOVA analysis. Note that  
151 behavioural data is a sublethal response variable so data from either of the 5 butts L<sup>-1</sup> treatments  
152 was omitted since there was a high instance of mortality in these treatments. The nMDS and  
153 the SIMPER analyses were generated using Primer V6.1.13 (PRIMER-e, Plymouth, UK).

154

### 155 **3. Results**

#### 156 *3.1. Effects of leachate from smoked cigarette butts on mortality of aquatic invertebrates.*

157 At 5 butts L<sup>-1</sup> most of *D. polymorpha*, *P. planorbis*, *B. tentaculta* and *P. nigra* died after 72  
158 hours of exposure on average (Figure 1), which was significantly (Table 1) different from  
159 mesocosms treated with 1 butt L<sup>-1</sup> (Concentration [5 vs 1 butt L<sup>-1</sup>]: P < 0.001), 0.2 butt L<sup>-1</sup>  
160 (Concentration [5 vs 0.2 butt L<sup>-1</sup>]: P < 0.001) or mesocosm with no leachate (Concentration [5  
161 butt L<sup>-1</sup> vs control]: P < 0.001). There was no significant difference between survival of the test  
162 organisms based on leachate derived from cellulose versus cellulose acetate butts (Table 1).

163 Significantly fewer juvenile *B. tentaculata* survived in mesocosms with either 5 butts L<sup>-1</sup> of  
164 cellulose acetate or cellulose butts compared with controls with less than 20% surviving even  
165 after just 24 h (Table 1, Concentration [Control vs 5 cellulose butts L<sup>-1</sup>]: P < 0.001,  
166 Concentration [control vs 5 cellulose acetate butts L<sup>-1</sup>]: P < 0.001 for each time point). After  
167 48 and 72 h, survival with 1 cellulose acetate butt L<sup>-1</sup> was ~50% which was significantly lower  
168 than in the Controls (Control vs 1 cellulose acetate butt L<sup>-1</sup>: P < 0.001 at 48 and 72 h). At the  
169 same time points (48 and 72 h) 1 cellulose butt L<sup>-1</sup> did not have a significant effect on survival  
170 (Control vs 1 cellulose butt L<sup>-1</sup>: P = 0.690). After 120 h, however, there were no differences  
171 between cellulose acetate and cellulose butts and 1 butt L<sup>-1</sup> of either type caused survival to  
172 drop to ~30% (Table 1, Figure 2). In addition, by 120 h, survival decreased with increasing  
173 concentration of leachate with 100% survival at 0.2 butts L<sup>-1</sup>, ~30% at 1 butt L<sup>-1</sup> and <5% at 5

174 butts L<sup>-1</sup> (post-hoc tests for concentration at 120 h; 0.2 vs 1: P < 0.001, 0.2 vs 5: P < 0.001 and  
175 1 vs 5: P < 0.001).

176

### 177 3.2. Sub-lethal effects of leachate from smoked cigarette butts on aquatic invertebrates.

178 Behaviour of the surviving individuals did not significantly differ (Figure 3) regardless of the  
179 source of the leachate (Butt [cellulose vs cellulose acetate], P = 0.458). The concentration of  
180 leachate, however, did significantly alter patterns of behaviour. In particular, the mesocosms  
181 exposed to 1 butt L<sup>-1</sup> exhibited different types of behaviour compared to those in mesocosms  
182 with 0.2 butts L<sup>-1</sup> or no leachate (Concentration [control vs 1 butt L<sup>-1</sup>]: P < 0.003 and [0.2 vs 1  
183 butt L<sup>-1</sup>]: P = 0.002). These differences were mostly due to a greater occurrence of movement  
184 or filtering in the case of *D. polymorpha* (accounting for ~40% of the variation in the  
185 multivariate pattern), and less occurrence of being in a closed state (accounting for ~38% of  
186 the variation in the multivariate pattern), of all four species in mesocosms without leachate or  
187 with 0.2 butts L<sup>-1</sup> leachate compared with those in leachate from 1 butt L<sup>-1</sup> (Figure 3).

188

## 189 4. Discussion

190 Cigarette butt leachate derived from biodegradable (i.e. cellulose) filters was equally as  
191 detrimental to freshwater pond invertebrates as leachate derived from conventional (i.e.  
192 cellulose acetate) filters. Leachate from 5 butts L<sup>-1</sup> derived from either type of butt was lethal  
193 to ~60% of adult *P. nigra*, *P. planorbis* and *B. tentaculata* and to ~40% of adult *D. polymorpha*  
194 within 48 hours. This is similar, albeit less lethal, to the results of Booth et al. (2015) who found  
195 100% mortality of two species of marine gastropod (*Austrocochlea porcata* and *Nerita*  
196 *atramentosa*) after 24 hours of continuous exposure to leachate from 5 butts L<sup>-1</sup>, but 100%  
197 mortality of a third species (*Bembecium nanum*) did not occur until 150 hours.

198 In the current study, mortality of adults was low at exposure to leachate from 1 butt L<sup>-1</sup>  
199 equivalent and no animals died during the experimental period in mesocosms with no or just  
200 0.2 butts L<sup>-1</sup> equivalent of leachate. Juvenile *B. tentaculata*, however, were more sensitive to 1  
201 butt L<sup>-1</sup> than their adult counterparts with only ~30% of juveniles surviving after 120 h of  
202 exposure versus ~80% of adults. This is not surprising given that early life stages of  
203 invertebrates are typically more sensitive to toxicants and hence are often prioritised for use in  
204 ecotoxicological studies (Mohammed 2013). For example, early life stage (ELS) tests (such as  
205 OECD 2018) are widely conducted to estimate toxicity for the registration of industrial  
206 chemicals, pesticides, biocides, and pharmaceuticals. Early life stages are also important  
207 ecologically because a reduction in successful recruitment can result in changes to population  
208 dynamics over the longer term and cause shifts in freshwater biodiversity and ecosystem  
209 functioning (Strayer and Malcom 2012).

210 It is important to measure sublethal responses to contaminants as these may be ecologically  
211 important, for example, movement facilitates feeding, predator avoidance, reproduction and  
212 migration and so can link effects on individuals to a population level (Bayley et al. 1997). Even  
213 though there was little mortality of adults at 1 butt L<sup>-1</sup> of leachate, significant alterations to  
214 behaviour did occur whereby the test animals were less active. It is likely that this indicates  
215 that they were under stress and in the longer-term this may have led to mortality (Rubach et al.  
216 2011). In a study by Wright et al (2015), a marine polychaete (*Hediste diversicolor*) was also  
217 found to be less active, decreasing burrowing in response to >2 butts L<sup>-1</sup> leachate. Alteration  
218 to behaviour also occurred in marine gastropods exposed to 1.25 butts L<sup>-1</sup>, but this differed  
219 depending on the species (Booth et al. 2015). Lee and Lee (2015) found contrasting effects at  
220 increasing concentrations of cigarette butt leachate, with significantly increased heart rates and  
221 accelerated embryonic development at lower concentrations (0.2 - 2 butts L<sup>-1</sup>), but lower heart  
222 rates and suppressed development at high concentrations (5 - 10 butts L<sup>-1</sup>). In addition,

223 Montalvão et al. (2019) found that freshwater mussels, *Anadontites trapesialis*, exposed to  
224 leachate from smoked cigarette butts accumulated heavy metals in their tissues and experienced  
225 mutagenetic effects even at low environmentally relevant concentrations ( $<0.2$  butts  $L^{-1}$ ),  
226 although the treatments were pseudo-replicated. Therefore, the response over time to sublethal  
227 toxicity may manifest in factors such as reproduction or growth performance, important for  
228 population sustainability and warrants further investigation.

229 We currently know very little about how the toxicity of cigarette butts may change over time  
230 when in the environment, but recent research indicates that butts continue to exude toxic  
231 chemicals into the air at least 1 week after being extinguished (Gong et al. 2020). Furthermore,  
232 Bonanomi et al. (2020) found that cellulose acetate cigarette butts remained toxic to the  
233 microalga *Raphidocelis subcapitata* after 5 years of degradation in the terrestrial environment.  
234 Whether or not cellulose cigarette butts also remain toxic for this length of time is unknown  
235 but should be a priority of future work in order to ascertain comparative effects of these  
236 different filter materials. International testing standards designed to evaluate the  
237 biodegradability of materials for use in cigarette butts do not test biodegradation after smoking,  
238 therefore are not environmentally realistic and when smoked, cellulose cigarette butts  
239 deposited as litter in the environment can also persist for years (Joly and Coulis 2018).

240

## 241 **Conclusion**

242 Overall, leachate from either type of butt at 5 butts  $L^{-1}$  caused mortality of most of the  
243 individuals in the experiment. Additionally, at 1 butt  $L^{-1}$ , both types of butt had a lethal effect  
244 on juvenile snails and reduced the activity levels of all four species of invertebrate. This  
245 emphasises that, once smoked, cigarette filters, biodegradable or not, therefore are likely to  
246 have a detrimental effect on the environment due to toxins concentrated from smoking tobacco.

247 Filters manufactured of cellulose, once smoked, can pose the same ecological threat as  
248 conventional cellulose acetate butts if they become litter in an enclosed water body such as a  
249 lake or pond. Considering their lack of rapid biodegradation in terrestrial habitats and their  
250 toxic effects in freshwater habitats, any shift to cellulose cigarette filters should be  
251 accompanied with the same plans for their appropriate post-use disposal as those made from  
252 cellulose acetate.

253

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257

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341 **Tables and figures**

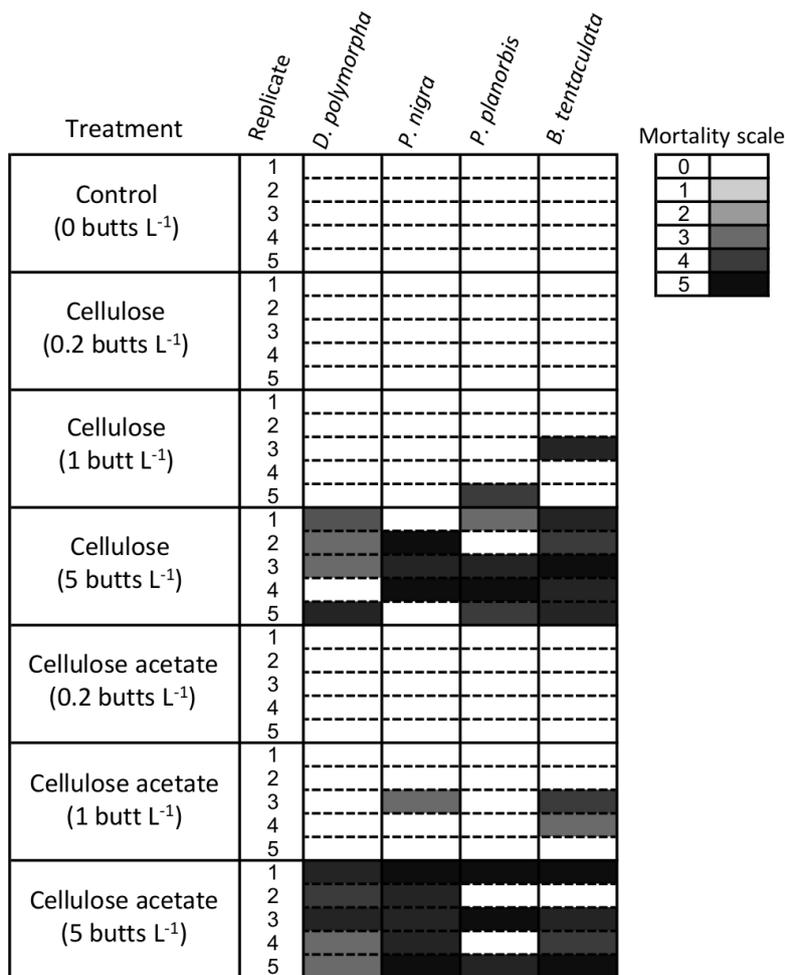
342 **Table 1.** Results of asymmetrical ANOVA for (a) the lethality of leachate to each species  
 343 throughout the experiment and (b) the survival of juvenile *B. tentaculata* at each time point  
 344 (from 24 to 120 h). *d.f.* = degrees of freedom, *F* = F-ratio and *p* = p-value. Significance at  $\alpha <$   
 345 0.05 and is indicated by values in **bold**.

(a)										
Source of variation	d.f.	<i>D. polymorpha</i>		<i>P. nigra</i>		<i>P. planorbis</i>		<i>B. tentaculata</i>		
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	
Treatment (one way)	6	<b>8.63</b>	<b>&lt;0.001</b>	<b>15.72</b>	<b>&lt;0.001</b>	<b>4.47</b>	<b>0.003</b>	<b>10.64</b>	<b>&lt;0.001</b>	
Control vs others	1	1.93	0.175	3.72	0.064	2.27	0.143	<b>5.31</b>	<b>0.029</b>	
Butt (B)	1	1.27	0.297	2.62	0.090	0.07	0.930	0.72	0.497	
Concentration (C)	2	<b>22.37</b>	<b>&lt;0.001</b>	<b>40.85</b>	<b>&lt;0.001</b>	<b>11.97</b>	<b>&lt;0.001</b>	<b>27.82</b>	<b>&lt;0.001</b>	
B x C	2	1.27	0.297	1.85	0.176	0.24	0.791	0.72	0.497	
Residuals	52									

(b)											
Source of variation	d.f.	24 h		48 h		72 h		96 h		120 h	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Treatment (one way)	6	<b>10.64</b>	<b>&lt;0.001</b>	<b>39.31</b>	<b>&lt;0.001</b>	<b>54.67</b>	<b>&lt;0.001</b>	<b>50.37</b>	<b>&lt;0.001</b>	<b>35.47</b>	<b>&lt;0.001</b>
Control vs others	1	<b>5.31</b>	<b>0.029</b>	<b>27.53</b>	<b>&lt;0.001</b>	<b>44.16</b>	<b>&lt;0.001</b>	<b>48.26</b>	<b>&lt;0.001</b>	<b>54.40</b>	<b>&lt;0.001</b>
Butt (B)	1	0.72	0.497	2.60	0.092	2.71	0.084	1.13	0.338	0.70	0.504
Concentration (C)	2	<b>27.82</b>	<b>&lt;0.001</b>	<b>96.10</b>	<b>&lt;0.001</b>	<b>132.88</b>	<b>&lt;0.001</b>	<b>122.19</b>	<b>&lt;0.001</b>	<b>76.21</b>	<b>&lt;0.001</b>
B x C	2	0.72	0.497	5.47	0.010	6.33	0.005	3.67	0.039	2.29	0.120
Residuals	52										

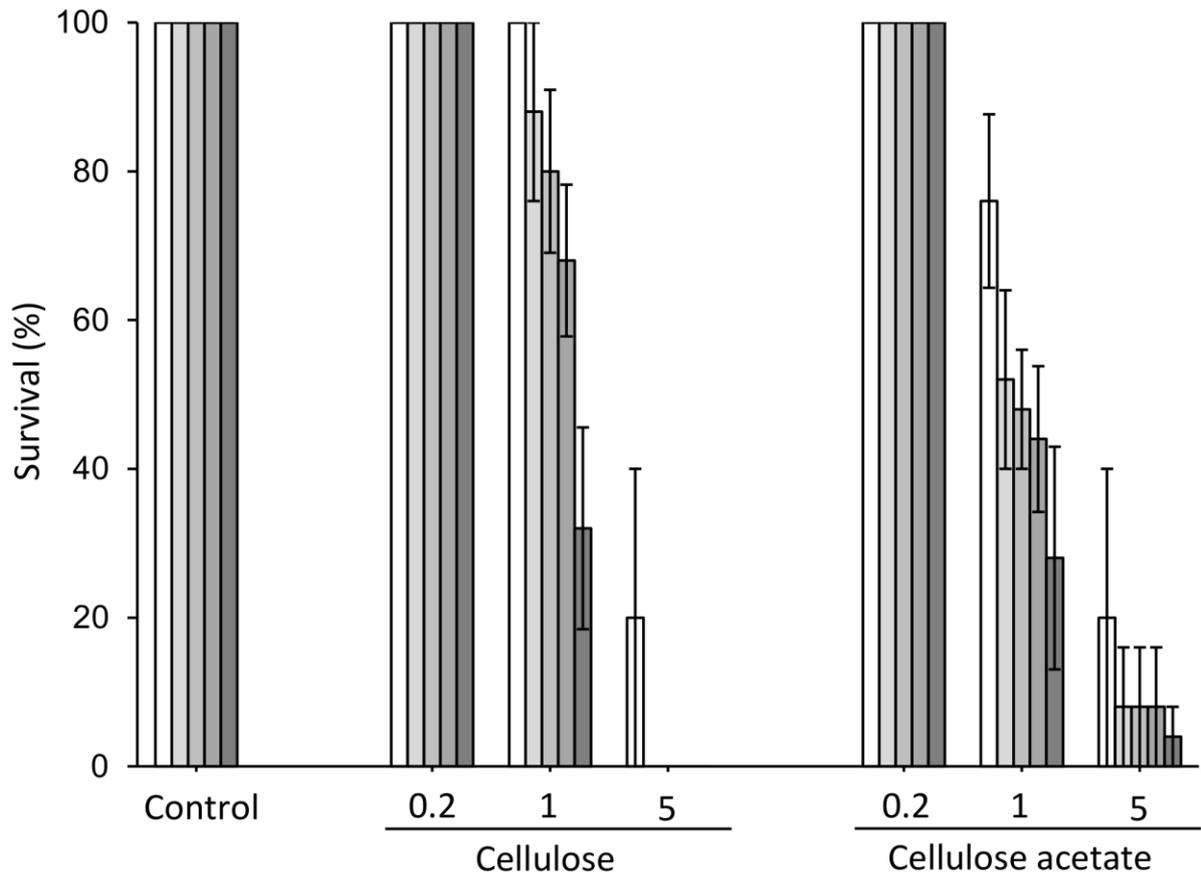
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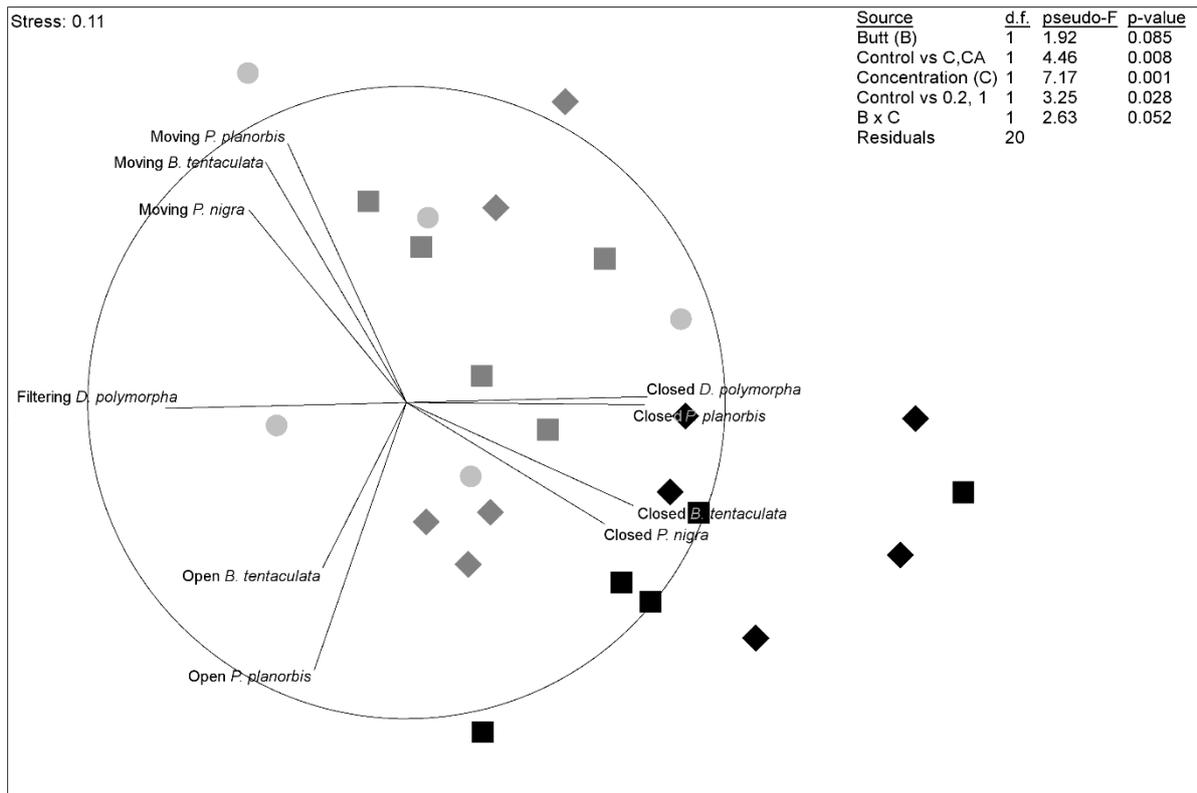
348 **Figure 1.** Heatmap showing the lethality of leachate derived from smoked cigarettes butts  
 349 made from either cellulose or cellulose acetate filters on *D. polymorpha*, *P. nigra*, *P. planorbis*  
 350 and *B. tentaculata* for each replicate mesocosm. Mortality scale is shown and is based on the  
 351 time taken for death to occur, i.e. the darker the cell, the higher the mortality in a replicate  
 352 mesocosm, with 0 the least lethal (no deaths within 120 h) and 5 the most lethal (died within 24  
 353 h).

354



355

356 **Figure 2.** Survival (%) out of 5 individual juvenile *B. tentaculata* snails in either rainwater  
 357 without leachate (Control) and leachate from 0.2, 1 or 5 cellulose, or cellulose acetate butts L<sup>-1</sup>  
 358 <sup>1</sup> at 24 (□), 48 (□), 72 (□), 96 (■) and >120 (■) hours of exposure. Data are mean ± SEM, n  
 359 = 5.



360

361 **Figure 3.** Non-metric multidimensional scaling diagram of the behaviour exhibited by all

362 species pooled over the 5 days of the experiment exposed to either no leachate (○) or to leachate

363 from 0.2 (◇) or 1 (◆) cellulose butts L<sup>-1</sup> or to 0.2 (▣) or 1 (■) cellulose acetate butts L<sup>-1</sup>.

364 Vectors are overlain for behaviours classifications correlated to the multivariate pattern at  $r >$

365 0.6. Included are results of the asymmetric PERMANOVA analysis, with associated pseudo-F

366 values and observed p-values based on 9999 permutations of the data.