



Swansea University  
Prifysgol Abertawe



## Cronfa - Swansea University Open Access Repository

---

This is an author produced version of a paper published in:  
*International Journal of Wildland Fire*

Cronfa URL for this paper:  
<http://cronfa.swan.ac.uk/Record/cronfa50894>

---

### **Paper:**

Harper, A., Santin, C., Doerr, S., Froyd, C., Albin, D., Otero, X., Viñas, L. & Pérez-Fernández, B. (2019). Chemical composition of wildfire ash produced in contrasting ecosystems and its toxicity to *Daphnia magna*. *International Journal of Wildland Fire*  
<http://dx.doi.org/10.1071/WF18200>

---

This item is brought to you by Swansea University. Any person downloading material is agreeing to abide by the terms of the repository licence. Copies of full text items may be used or reproduced in any format or medium, without prior permission for personal research or study, educational or non-commercial purposes only. The copyright for any work remains with the original author unless otherwise specified. The full-text must not be sold in any format or medium without the formal permission of the copyright holder.

Permission for multiple reproductions should be obtained from the original author.

Authors are personally responsible for adhering to copyright and publisher restrictions when uploading content to the repository.

<http://www.swansea.ac.uk/library/researchsupport/ris-support/>

1 Manuscript draft for a Special Issue on Forests – Flames and Faucets in the International  
2 Journal of Wildland Fire. <https://www.publish.csiro.au/wf>

3

## 4 **Chemical composition of wildfire ash produced in contrasting** 5 **ecosystems and its toxicity on *Daphnia magna*.**

6

7 Ashleigh R. Harper<sup>1</sup>, Cristina Santin<sup>1,2</sup>, Stefan H. Doerr<sup>1</sup>, Cynthia A. Froyd<sup>2</sup>, Dania Albini<sup>2</sup>,  
8 Xose Luis Otero<sup>3</sup>, Lucia Viñas<sup>4</sup>, Begoña Pérez-Fernández<sup>4</sup>

9

10

11 <sup>1</sup> Department of Geography, Swansea University, Singleton Park, Swansea, SA2 8PP, UK.

12 <sup>2</sup> Department of Biosciences, Swansea University, Singleton Park, Swansea, SA2 8PP, UK.

13 <sup>3</sup> Department of Edaphology and Agricultural Chemistry. Faculty of Biology. University of  
14 Santiago de Compostela, 15782 Santiago de Compostela, Spain

15 <sup>4</sup> Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Vigo, Subida a Radio  
16 Faro 50, 36390 Vigo, Spain

17

18 Corresponding author A. Harper, E-mail: [ashleigh.r.harper@hotmail.co.uk](mailto:ashleigh.r.harper@hotmail.co.uk)

19

### 20 **Abstract**

21 It is well established in the world's fire-prone regions that wildfires can considerably change  
22 the hydrological dynamics of freshwater catchments. Limited research, however, has focused  
23 on the potential impacts of wildfire ash toxicity on aquatic biota. Here we assess the chemical  
24 composition and toxicity of ash generated from wildfires in six contrasting vegetation types  
25 distributed globally (UK grassland, Spanish pine forest, Spanish heathland, USA chaparral,  
26 Australian eucalypt forest and Canadian spruce forest). Acute (48 h) immobilisation tests were  
27 conducted on the aquatic macroinvertebrate *Daphnia magna*, a species extensively used in  
28 ecological and toxicological studies as a sensitive indicator of aquatic contaminants. We found  
29 significant differences between the chemical composition and toxicity of these ash types. The  
30 UK and Spanish ash types had no detectable toxicity on *Daphnia magna* whereas, the

31 Australian eucalypt (48 h EC<sub>50</sub>\*) (\*9.5 g L<sup>-1</sup>), USA chaparral (\*20 g L<sup>-1</sup>) and Canadian spruce  
32 (\*26 g L<sup>-1</sup>) ash all caused significant toxicity (immobilisation). The principle characteristics of  
33 the latter ash types were their high pH, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> and conductivity levels. Elevated water-  
34 soluble and total concentrations of metals (e.g. Mn, Fe, Zn, Pb, Cu and As) and total polycyclic  
35 aromatic hydrocarbons (PAHs) were not linked to toxicity.

## 36 **Introduction**

37 Fires are a natural process in many habitat types worldwide (Bixby *et al.* 2015), but they can  
38 be a social and environmental concern, potentially impacting public health, safety,  
39 infrastructure, biodiversity, land-use, water and air pollution (Bladon *et al.* 2014; Brito *et al.*  
40 2017). Fire activity is projected to increase in many locations and ecotypes as a result of climate  
41 and societal changes, making the full understanding of their impacts crucial (Scholze *et al.*  
42 2006; Chen *et al.* 2018).

43 During wildland fires, combustion of fuels releases a wide range of organic and inorganic  
44 components into the atmosphere, but also concentrates some of them into wildfire ash left on  
45 the ground (Bodí *et al.* 2014). Fresh wildfire ash typically consists of mineral materials and  
46 charred organic components, is non-cohesive, has a low density, and is not attached to the soil,  
47 which facilitates its mobilisation and transportation by post-fire water and wind erosion (Bodí  
48 *et al.* 2014; Abraham *et al.* 2017). The release of soluble elements and particulate matter from  
49 eroded ash and underlying soil into aquatic systems following fires can cause increases in water  
50 turbidity, pH, organic matter, suspended sediment, conductivity and a depletion of dissolved  
51 oxygen, among other (Smith *et al.* 2011; Tsai *et al.* 2017). Ash is however, not usually  
52 examined as a distinct part of the post-fire sediment and few laboratory studies have  
53 characterised the composition of wildfire ash (Bodí *et al.* 2014).

54 The majority of the general studies into the effects of wildfire on water quality have focused  
55 on runoff amounts and nutrient levels and only more recently has increasing research attention  
56 been given to pyrolytic substances, chemical elements and biological reactivity (Shakesby and  
57 Doerr 2006; Campos *et al.* 2012; Silva *et al.* 2015). Key areas receiving particular attention as  
58 a result of their environmental concern are the production and mobilisation of polycyclic  
59 aromatic hydrocarbons (PAHs) and heavy metals (e.g. Vila-Escalé *et al.* 2007; Campos *et al.*  
60 2012; Oliveira-Filho *et al.* 2018). Both present major biological concern due to their  
61 carcinogenic potential, persistence within ecosystems and tendency to bio-accumulate (Smith  
62 *et al.* 2011; Chen *et al.* 2018). These contaminants are thought to have complex impacts on  
63 water quality and the biological effects of this in aquatic systems have been observed to persist  
64 across long spatial and temporal scales (Earl and Blinn 2003; Costa *et al.* 2014).

65 Ash has also begun to receive increasing recognition as a source of diffuse contamination in  
66 freshwater systems with detrimental impacts on both lake and stream biota, including fish (e.g.  
67 Nunes *et al.* 2017; Oliveira-Filho *et al.* 2018; Gonino, Branco, *et al.* 2019), amphibians (Pilliod

68 *et al.* 2003), macroinvertebrates (Brito *et al.* 2017) and algae (Campos *et al.* 2012) have all  
69 been observed. Highly variable impacts of ash contamination on freshwater biota have been  
70 reported between different ecosystems, types of ash, fires and species (Smith *et al.* 2011; Silva  
71 *et al.* 2015; Oliveira-Filho *et al.* 2018). Campos *et al.* (2012) and Silva *et al.* (2015) for  
72 example, found no significant impact of eucalypt ash on the planktonic crustacean *D. magna*  
73 reproduction or immobilisation rates over chronic (21 day) and acute (48 h) exposures,  
74 respectively. Toxicity was, however, observed on several lower trophic level species in these  
75 studies, the bacteria *Vibrio fischeri*, algae *Pseudokirchneriella subcapitata* and the macrophyte  
76 *Lemna minor*. A similar study by Brito *et al.* (2017) tested toxicity over acute exposures (48 h)  
77 of three types of ash from the Brazilian *Cerrado* ecoregion on the planktonic crustacean  
78 *Ceriodaphnia dubia*, the fish *Danio rerio* and the mollusc *Biomphalaria glabrata* and found  
79 that all ash types caused toxicity to *C. dubia*, none impacted *B. glabrata* and only one type was  
80 toxic for *D. rerio*. At higher trophic levels negative impacts of Brazilian sugar cane ash have  
81 also been observed on several native fish species (*Astyanax lacustris*, *Moankhausia* and *M.*  
82 *forestii*) over 24 h acute exposures but not for two non-native fish species (*Oreochromis*  
83 *niloticus* and *Poecilia reticulata*) (Gonino *et al.* 2019). These studies demonstrate the  
84 variability and complexity of influencing factors in relation to the impacts of ash contamination  
85 on aquatic biota, highlighting the limited breadth of available research in this area (Hallema *et*  
86 *al.* 2018).

87 To enhance our understanding of the impacts of ash contamination on aquatic biota, this study  
88 aimed to (1) determine the chemical composition of wildfire ash produced in six contrasting  
89 ecosystems, (2) examine the ecotoxicological effect of these ash types on the freshwater  
90 indicator species *Daphnia magna* and (3) evaluate the relationship between chemical  
91 composition and observed toxicity and its implications for the relative water contamination  
92 potential of ash produced in these differing ecosystems. To the best of our knowledge this  
93 constitutes the first ecotoxicology assessment allowing the direct comparison of the  
94 composition and toxicity of ash from several globally distributed contrasting ecosystems.

95

## 96 **Materials and methods**

### 97 *Ash samples*

98 Six composite ash samples were collected after wildland fires, prior to any rainfall, in each of  
99 the selected ecosystems types (Table 1): Australian eucalypt forest (AUS), USA chaparral

100 (USA), Canadian spruce forest (CAN), Spanish heathland (URIA), Spanish pine forest (SPA),  
101 UK grassland (UK). Fire and vegetation characteristics are summarised in Table 1. Each  
102 composite ash samples was sieved through a 1 mm mesh before chemical characterisation or  
103 use in the bioassays.

104

#### 105 *Chemical characterisation*

106 Chemical characterisation of the six ash types collected was undertaken to determine the total  
107 and water-soluble concentrations of major (Ca, Cl<sup>-</sup>, Mg, Na, Si, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>) and trace elements  
108 and compounds (Al, B, Cu, F<sup>-</sup>, Fe, Ni, NH<sub>4</sub><sup>+</sup>, As, Cd, Hg, Pb and PO<sub>4</sub><sup>3-</sup>), in addition to pH,  
109 dissolved organic carbon (DOC) and electric conductivity. This characterisation was  
110 undertaken using established methods (Plumlee *et al.* 2007; Santín *et al.* 2015, 2018; see  
111 supplementary material for full details).

112 The concentrations of 35 PAHs were also determined according to Pérez-Fernández *et al.*  
113 (2015) and Viñas *et al.* (2009) with a GC/MS Thermo mod DSQ II (Thermo Electron  
114 Corporation, Austin, TX USA). The first step consisted of a 10 h Soxhlet extraction with a 1:3  
115 acetone:hexane mixture. The extract was then treated overnight with activated copper for  
116 elemental sulphur removal and then cleaned using column chromatography with deactivated  
117 alumina (see supplementary material for full details).

118

#### 119 *Daphnia toxicity testing*

120 Ecotoxicological assays consisting of acute ash exposures (48 h) were conducted using the  
121 planktonic crustacean *Daphnia magna*. This species is extensively used in ecological and  
122 toxicological studies as a sensitive indicator of the effects of contaminants on aquatic biota  
123 (OECD 2004; USEPA 2016). *Daphnia* sp. are also particularly relevant to freshwater lentic  
124 ecosystems (lakes, reservoirs and ponds) and ideal for investigating contamination potential in  
125 downstream waterbodies (Robinson and Thorn 2009; Nikinmaa 2014).

126 A monoclonal starter culture of *D. magna* was obtained from a long-term (2 year) rearing  
127 program. The new culture was reared and maintained according to recommended guidelines  
128 (OECD 2004; USEPA 2016), under controlled temperature (20±2°C), light conditions (uniform  
129 illumination of cool-white type, approx. 5000 lux; photoperiod 16<sup>L</sup>:8<sup>D</sup>) and fed every 2 days

130 with a distilled suspension of *Pseudokirchneriella subcapitata* at approximately 0.1-0.2 ml per  
131 *Daphnid*/day.

132 To produce the test solutions, each ash sample was combined with a culture medium (synthetic  
133 hardwater medium – ASTM 1996) at the ratio 1:10 (mass:volume) (e.g. 100 g of ash in 1 L of  
134 medium). The samples were then homogenised in an orbital shaker for 4 h and stored at 4°C  
135 (max 24 h) before using in the ecotoxicological assays.

136 The acute toxicity tests were conducted according to the OECD 202 (OECD 2004) guidelines,  
137 with the exception of full pH adjustment. pH was not adjusted to control levels (pH  $7.2 \pm 0.2$ )  
138 in the bioassays to reproduce as close to natural conditions as possible, given pH is one of the  
139 most important factors affecting the toxicity and bioavailability of elements to freshwater  
140 species (Franklin *et al.* 2000). OECD 202 guidelines acknowledge that tests should be carried  
141 out without the adjustment of pH, where values are within pH 6-9 at the highest test  
142 concentration (OECD 2004). It is crucially important that pH adjustment does not cause  
143 significant changes to the test substances and due to the complex and varying compositions  
144 and reactivity of wildfire ash, potential interactions are unclear. Little is known to date on  
145 wildfire ash concentrations in water bodies, therefore, a wide range of ash concentrations was  
146 tested, trying to represent the potential variability of different natural scenarios. Six different  
147 concentrations of the ash-medium solutions were used during testing (3.12, 6.25, 12.5, 25, 50,  
148  $75 \text{ g L}^{-1}$ ), plus four controls per concentration.

149 Tests were initiated using new-borns of less than 24 h old, originating from the 3<sup>rd</sup> – 5<sup>th</sup> brood  
150 of the culture. For each ash type 150 daphnids were used. This sample size was divided into  
151 five individuals per test vessel for each concentration with four replicates and one control per  
152 concentration. The test was conducted for 48 h and the immobilisation of neonates was  
153 documented at 24 h and 48 h. Immobilisation of neonates is defined here as individuals not  
154 able to swim within 15 s of gentle agitation of the test vessel. During this period the same  
155 temperature ( $20 \pm 2^\circ\text{C}$ ) and photoperiod (photoperiod, 16<sup>L</sup>:8<sup>D</sup>) conditions as during rearing were  
156 maintained. *D. magna* were not fed during the acute exposure (USEPA 2016).

157

### 158 *Statistical analysis*

159 The water-soluble (leachates) chemical composition results were subjected to principle  
160 component analysis (PCA) (RStudio *version 5.4.1*) to identify constituents most strongly

161 correlated with the different ash types. This approach to assessing the characteristic  
162 components in a given sample is widely used in environmental research when dealing with  
163 complex datasets (Brito *et al.* 2017). The leachates data were chosen for this analysis, as  
164 opposed to the total elements data, because this is likely the most bioavailable fraction and,  
165 therefore, the most likely to have impacted the *Daphnia* over an acute exposure.

166 To identify thresholds in the *D. magna* toxicity results and in agreement with standard  
167 procedures (Musset 2006) the data were subjected to single factor analysis of variance tests  
168 (RStudio *version 5.4.1*). Where significant results were identified post-hoc Dunnetts analysis  
169 was used to test if the response at each concentration was significantly different to the control  
170 groups and therefore, identify critical thresholds (lethal concentrations) in the response  
171 relationships. This enables the effect concentrations ( $EC_{10}$  = concentration at which 10% of  
172 individuals are immobilised and  $EC_{50}$  = concentration at which 50% of individuals are  
173 immobilised) for each ash to be interpolated, along with the lowest observed effect  
174 concentration (LOEC) (Musset 2006). A significance level of 5% (0.05) was used in all  
175 statistical tests.

176

## 177 **Results**

### 178 *Ash chemistry*

179 The total elemental composition of the six ash types overall contained a number of potential  
180 contaminants, but in highly variable concentrations (Sup. Material Table 1). The most abundant  
181 element in all samples was Ca (range = 11,800 – 177,000 mg kg<sup>-1</sup>) with Al (range = 1320 –  
182 22,600 mg kg<sup>-1</sup>) and Fe (range = 979 – 30,600 mg kg<sup>-1</sup>) both present in high concentrations  
183 throughout. The elements found in the lowest total concentrations were: As (range = 0.46 –  
184 9.67 mg kg<sup>-1</sup>), Cd (range = 0.17 – 1.13 mg kg<sup>-1</sup>) and Hg (range = 0 – 0.05 mg kg<sup>-1</sup>) (Sup.  
185 Material Table 1).

186 pH and electrical conductivity (EC), measured in the leachates, notably varied across ash  
187 samples, with pH levels ranging from moderately alkali in the UK ash (7.9), to strongly alkali  
188 in the USA ash (11.2). Equally, EC levels varied greatly from 233  $\mu\text{S cm}^{-1}$  in the SPA ash to  
189 3880  $\mu\text{S cm}^{-1}$  in the AUS ash. High pH and EC values were both characteristic features of the  
190 ash types producing immobilisation of *D. magna* tested (see *Acute toxicity test* section). pH  
191 within the bioassays themselves however, were notably less variable (7.31-9.08, Tables 4 &

192 5), likely due to differences in dilution between leachates and the bioassay testing, and the  
193 addition of the culture medium in the latter.

194 The water-soluble (leachate) composition of the ash types were also highly variable (Table 2)  
195 with the most abundant components being  $\text{SO}_4^{2-}$  (range = 1203 – 10,180  $\text{mg kg}^{-1}$ ),  $\text{Cl}^-$  (range =  
196 228 – 1509  $\text{mg kg}^{-1}$ ) and Na (range = 17 – 3893  $\text{mg kg}^{-1}$ ). The minor metal and metalloids  
197 elements were similarly the components found in the lowest concentration in the leachates; Cd  
198 (range = 0 – 7  $\mu\text{g kg}^{-1}$ ), Ni (range = 60 – 844  $\mu\text{g kg}^{-1}$ ), Zn (range = 0 – 140  $\mu\text{g kg}^{-1}$ ) and Hg  
199 (range = 1 – 2  $\mu\text{g kg}^{-1}$ ) (Table 2).

200 Some soluble elements occurred in particularly high levels, highlighting the variation in  
201 element content within the ash (Table 2). For example, in the UK sample,  $\text{PO}_4^{3-}$  (620  $\text{mg kg}^{-1}$ )  
202 and metals such as Fe (4378  $\mu\text{g kg}^{-1}$ ) and Mn (9292  $\mu\text{g kg}^{-1}$ ) were notably high in comparison  
203 to the other ash types. There were also notably high levels of, for example, Ca (5864  $\text{mg kg}^{-1}$ )  
204 and  $\text{SO}_4^{2-}$  (32,289  $\text{mg kg}^{-1}$ ) in the CAN sample; B (85  $\text{mg kg}^{-1}$ ) and Na (3893  $\text{mg kg}^{-1}$ ) in the  
205 AUS sample; and Cu (5158  $\mu\text{g kg}^{-1}$ ) and As (329  $\mu\text{g kg}^{-1}$ ) in the URIA sample (Table 2).

206 The water-soluble concentrations of each element were relatively low when compared to the  
207 total dry concentration within each ash type (Table 2). On average, the proportions of water-  
208 soluble Al, Pb, Mn, Fe, Zn were < 1% total dry weight; As, Si, Ca, P, Ni, Cu, Cd were < 5%  
209 and Mg was < 10%. The levels of Na (2 – 77%) and Hg (5 – 57%) solubility were highly  
210 variable and are clearly the most overall soluble of the components analysed.

211 PCA identified three primary components explaining 79% of the total leachates dataset  
212 variance (PC1 = 41%; PC2 = 23%; PC3 = 15%) (Table 3). PC1 is most strongly positively  
213 correlated with Mn, Fe, Zn, As, Pb and  $\text{PO}_4^{3-}$  levels and most strongly negatively correlated  
214 with pH, EC,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , Hg and  $\text{SO}_4^{2-}$  (Table 3 and Figure 1). A biplot of the standardised PC1  
215 and PC2 values (Figure 1) shows which components best characterised each ash type and pH,  
216 EC,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ , Hg and  $\text{SO}_4^{2-}$  were most closely correlated with the three ash types producing  
217 significant immobilisation of *D. magna*. Whilst, Al, Cu, Ni,  $\text{NH}_4^+$ , As, Fe, Mn,  $\text{PO}_4^{3-}$ , Pb, Cd  
218 were more closely correlated with the three non-toxic ash types (Figure 1).

219 Thirty five PAHs were analysed across the ash types including the sixteen United States  
220 Environmental Protection Agency (EPA) priority PAHs, which provide the focus of the  
221 following discussion (Table 4). The total concentration of these priority contaminants ranged  
222 from 1155 – 14,078  $\text{ng g}^{-1}$  ash, the highest total being found in the UK ash originating from an  
223 upland grassland ecosystem in South Wales ( $\sum 16$  EPA PAHs: 12,336  $\text{ng g}^{-1}$  ash) (Table 4).

224 Notably high PAHs concentration were also found in the CAN ( $\sum 16$  EPA PAHs: 7,486 ng g<sup>-1</sup>)  
225 and the SPA ash ( $\sum 16$  EPA PAHs: 4,393 ng g<sup>-1</sup> ash) (Table 4).

226 The proportion of the methylated and non-methylated PAHs was very similar in all the samples  
227 with around three times more non-methylated PAHs in each ash type, except the USA ash  
228 which contained over 15 times the amount of non-methylated PAHs (Table 4). There was also  
229 a predominance of 2-ring PAHs in all the samples. Generally, the quantity of each ring type  
230 decreases sequentially with the number of rings, 2 > 3 > 4 > 5 & 6 with the exception of the  
231 USA sample, which had a relatively similar quantity of 3, 4, 5 & 6 ring PAHs. The predominant  
232 2-ring PAHs in all samples was Naphthalene. Phenanthrene was the most common 3-ring  
233 PAHs, except in the UK sample where it was Acenaphthylene. All three of these abundant  
234 PAHs (naphthalene, phenanthrene and acenaphthylene) are classified as EPA priority  
235 contaminants (Table 4).

236

#### 237 *Acute toxicity test*

238 High levels of *D. magna* immobilisation were recorded at both 24 and 48 h exposure for three  
239 of the six ash types tested: AUS, USA and CAN ( $p < 0.001$  for all three ash types) (Figure 2;  
240 Table 5 & 6). The response relationships identify the AUS ash as the most toxic, with a 100%  
241 immobilisation of *D. magna* individuals at less than 25 g ash L<sup>-1</sup> within the first 24 hours of  
242 exposure (Table 5; Figure 2). The immobilisation effect of both the North American ash  
243 samples (USA and CAN) were relatively similar, with 48 h EC<sub>50</sub> being achieved at 20 and 26  
244 g ash L<sup>-1</sup> respectively, despite the notably different source vegetation (Table 6; Figure 2). In  
245 contrast, no significant immobilisation occurred in response to the remaining three ash types  
246 (URIA, SPA and UK) (Tables 5 & 6). The UK ash did not produce any observable  
247 immobilisation across any of the test concentrations after 48 h of exposure. The Spanish  
248 samples (URIA, SPA) only produced small rates of immobilization at the highest  
249 concentrations (Tables 5 & 6).

250

## 251 **Discussion**

### 252 *Overall ash chemical properties*

253 The total concentration of each element within the six ash types showed a wide variability (Sup.  
254 Material Table 1). These variations might be explained by the accumulative capacity of the  
255 different vegetation types, taking up different levels of elements from the soil and surrounding  
256 environment (Peralta-Videa *et al.* 2009; Brito *et al.* 2017). Fire dynamics (e.g. burn  
257 temperature) and soil properties are also important features in the composition of elements  
258 within ash (Pitman 2006; Bodí *et al.* 2014; Chen *et al.* 2018). In general, oxides and hydroxides  
259 of particularly Ca, Mg, Si and P tend to be abundant in wildfire ash (Pereira and Úbeda 2010;  
260 Silva *et al.* 2015) as found in the ash tested here (Sup. Material Table 1).

261 Overall, water solubility of the studied elements in all ash types is low (<20% except for Na  
262 and Hg). This agrees with previous findings (Khanna *et al.* 1994; Santín *et al.* 2015; Silva *et*  
263 *al.* 2015; Brito *et al.* 2017). The most abundant compounds in all leachates were  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  and  
264  $\text{Na}^+$  (Table 2), likely due to them forming very soluble salts (i.e. sulphates or chlorides). These  
265 components are thus commonly found in high concentrations in the dissolved residuals of ash  
266 (Freitas and Rocha 2011; Santín *et al.* 2015) (Table 2). In contrast, heavy metals such as Cd,  
267 Ni and Zn showed the lowest concentration in the leachates due to being relatively insoluble in  
268 alkaline (pH: 8-10) conditions, precipitating mainly as hydroxides (Brookins 1989; Weiner and  
269 Group 2007). These results are similar to those found in other studies assessing post-fire runoff  
270 and ash leachates in a range of ecosystem types (Jung *et al.* 2009; Pereira *et al.* 2011) and in  
271 agreement with the general trend of alkali (Na, K) > alkaline (Ca, Mg) >> heavy metals (Pb, Cd  
272 and Hg) found by Santín *et al.* (2015) in eucalypt forest ash.

273

#### 274 *Ash types and element solubility*

275 Despite the overall similarities in ash solubility in the ash leachates, there are also substantial  
276 variations among the ash types, making their chemical profiles notably different. Brito *et al.*  
277 (2017), assessing Brazilian *Cerrado* ash types, also found there were little qualitative  
278 differences in the overall composition of the different ash tested, but large variations in the  
279 concentration of the chemical elements between sampling areas.

280 The PCA analysis carried out allowed detection of key differences in the composition of the  
281 ash types studied here. The UK ash leachate has a distinctly soluble profile in comparison to  
282 the others. PCA analysis shows a number of heavy metals (Mn, Fe, Zn and Pb) and  $\text{PO}_4^{3-}$  to  
283 be characteristic elements of the UK ash leachate (Figure 1). This leachate shows high  
284 concentrations of soluble Fe, Mn and  $\text{PO}_4^{3-}$  in comparison to the other ash types (Table 2). The

285 pH (7.9) of the UK leachate was 1 to 3 units lower than the extracts from the other samples  
286 (Table 2). These less alkaline conditions favour the solubility of metals and P compared to the  
287 other samples where the metals tend to precipitate as hydroxide for values above 8-9 and the  
288 phosphate as hydroxyapatite for pH values  $> 8.5$  (for example see: Diaz *et al.* 1994; Stumm  
289 and Morgan 2013).

290 A characteristic component of the CAN sample (identified by PCA, Figure 1) was the high  
291 levels of soluble Ca, despite the total concentration in dry ash being relatively similar to that  
292 of the AUS, SPA and USA ash (Table 2). It is unclear why the solubility of Ca is notably higher  
293 in the CAN ash in comparison to the other ash types (Jung *et al.* 2009; Brito *et al.* 2017), but  
294 that may be responsible for the reduced  $\text{PO}_4^{3-}$  levels ( $1.2 \text{ mg kg}^{-1}$ ) in the CAN leachate as P has  
295 a tendency to precipitate in the presence of Ca (Diaz *et al.* 1994). This P-Ca interaction may  
296 influence algal and cyanobacterial growth (and thus, eutrophication) by regulating P levels in  
297 freshwater systems (Bladon *et al.* 2008; Blake *et al.* 2009). In the broader context, Ca is not  
298 normally considered hazardous, but can significantly influence the overall toxicity of ash  
299 eluates (e.g. its strong relationship with  $\text{SO}_4^{2-}$  leaching) (Mount *et al.* 1997; Tian *et al.* 2018).  
300 Stiernström *et al.* (2013) even propose that Ca might be one of the key elements responsible  
301 for the ecotoxicity of ash eluates on the crustacean *Nitocra spinipes*, despite Ca not being  
302 classified as individually ecotoxic. The high Ca concentration CAN ash tested here also  
303 produced significant immobilisation of *D. magna* over the 48 h exposure.

304 For the AUS ash sample, the levels of soluble B and Na are higher than in the other ash types  
305 (Figure 1, Table 2). These elements are often found in high concentrations in ash leachates  
306 (Jung *et al.* 2009; Pereira *et al.* 2011), particularly B in other eucalyptus ash tested (Freitas and  
307 Rocha 2011). High  $\text{Na}^+$  levels in freshwater systems can present an issue for water purification  
308 processes as they cannot be removed using conventional methods (Smith *et al.* 2011). Unlike  
309 reported by Silva *et al.* (2015), where the principle potential toxic components of their eucalypt  
310 ash were Mn and Zn, neither of these elements were found in the eucalypt (AUS) ash analysed  
311 here. This further highlights the differences in ash composition comparing individual fire  
312 events and ecosystem types (Bodí *et al.* 2014).

313 In the URJA ash, the most defining components were Cu, Al, Ni,  $\text{NH}_4^+$  and As (Figure 1). This  
314 ash contained comparatively high concentrations of, particularly, soluble Cu ( $5158 \text{ } \mu\text{g kg}^{-1}$ )  
315 and the carcinogen As ( $329 \text{ } \mu\text{g kg}^{-1}$ ). Similar elevated soluble levels of Cu have, however, been  
316 found in mixed eucalyptus ash ( $\text{Cu} = 5100 - 6200 \text{ } \mu\text{g kg}^{-1}$ ) by Santín *et al.* (2015). The reason

317 for the significantly higher solubility rate of Cu in this heathland ash (URIA = 12.9%, range  
318 excluding URIA = 0.49 – 2.01%) is worth further consideration to identify areas or components  
319 likely to increase the risk of Cu contamination. The concentration of As, although elevated in  
320 the URIA (and UK) sample here, have been reported in higher quantities in a number of other  
321 wildfire ash samples (e.g. 4000 - 7300  $\mu\text{g kg}^{-1}$  in Santin *et al.* (2015); 42000  $\mu\text{g kg}^{-1}$  in Silva *et*  
322 *al.* (2015)) and despite being above the 0.01  $\text{mg L}^{-1}$  World Health Organisations drinking-water  
323 guideline (World Health Organization 2011) it does not appear to cause significant  
324 immobilisation of *D. magna* in the URIA or UK ash.

325 The SPA ash has a relatively insoluble overall profile with notably high concentrations of the  
326 metals Al, Fe, Zn, Cu, Pb and the metalloid As in the dry ash (Sup. Material 1) but limited, to  
327 no, leaching of Al, Fe, Zn and Pb into the water-soluble composition (Table 2). Despite this,  
328 Cd presented as a distinct principle component of the SPA ash with a comparatively high  
329 soluble concentration (7  $\mu\text{g kg}^{-1}$ ) and as the only sample to register a solubility percentage of  
330 greater than 1% (2.85%). Similar dry quantities of Cd were recorded by Brito *et al.* (2017)  
331 assessing Brazilian *Cerrado* ash types (0.1-0.3  $\text{mg kg}^{-1}$ ) but Cd solubility was lower in these  
332 ash types (<0.01%).

333

#### 334 *PAHs composition*

335 The organic fraction of ash may also contain organic contaminants of biological concern (Vila-  
336 Escalé *et al.* 2007; Chen *et al.* 2018). The data available on PAHs release following fire,  
337 however, is relatively limited (Vila-Escalé *et al.* 2007; Kim *et al.* 2011; Campos *et al.* 2012;  
338 Rey-Salgueiro *et al.* 2018).

339 The concentrations of PAHs found in the ash analysed here are also widely variable, with a  
340 range of 1155  $\text{ng g}^{-1}$  in the AUS ash to 14,078  $\text{ng g}^{-1}$  in the UK ash (16 U.S. Protection Agency  
341 (EPA) priority PAHs) (Table 4). The values contained within the ash tested here are  
342 substantially higher than those presented by Olivella *et al.* (2006) testing wildfire ash from pine  
343 and oak forests ( $\Sigma 12$  PAHs: 1- 19  $\text{ng g}^{-1}$  ash). The lowest concentration, found in the AUS ash  
344 type ( $\Sigma 16$  EPA PAHs: 1155  $\text{ng g}^{-1}$  ash), was of a comparable level to those found by Silva *et*  
345 *al.* (2015), assessing dry wildfire ash in a predominantly eucalypt ecosystem in Portugal ( $\Sigma 16$   
346 EPA PAHs: 1100  $\text{ng g}^{-1}$  ash). The full range of PAH concentrations found here are within the  
347 range of 1000-50,000  $\text{ng g}^{-1}$  ( $\Sigma 16$  EPA PAHs) found by Santín *et al.* (2017) analysing PAHs  
348 in pine forest floor and wood under wildfire charring and slow-pyrolysis.

349 The UK ash shows a much higher PAHs concentration than the other types (Table 4). It is  
350 unclear why this is the case as no other research has been conducted on the PAHs composition  
351 of wildfire ash originating from comparable grassland ecosystems. The type of fuel and  
352 variations in combustion temperatures and oxygen availability are thought to strongly affect  
353 the concentration and type of PAHs in ash (Enell *et al.* 2008; Rey-Salgueiro *et al.* 2018). Chen  
354 *et al.* (2018) found that PAHs concentrations were significantly higher in black wildfire ash  
355 (moderate burn severity) in comparison to white wildfire ash (severe burn severity). This was  
356 also true of the ash types tested here with the darker (dark grey-black) ash samples (UK, URIA,  
357 CAN) containing a much higher concentration of PAHs than the lighter (light grey-white)  
358 samples (AUS, USA, SPA) (Table 4). Although, variations in combustion completeness could  
359 be related to PAHs content here, the proportion of methylated PAHs in the UK ash is similar  
360 to that of the other samples tested (Table 4). The proportion of methylated/total PAHs is usually  
361 considered an indicator of combustion completeness as during combustion the methylated  
362 component of the compound is lost first (Keiluweit *et al.* 2012) (Table 4).

363 The high presence of low molecular weight and therefore, greater volatility PAHs (i.e. Nap and  
364 Phe) in the ash tested here may seem contradictory as it can be expected that these compounds  
365 would be lost during a fire. It is, however, likely that these PAHs preferentially re-condense in  
366 the ash layer and are retained in microporous structures of pyrogenic material (Santín *et al.*  
367 2017). Other studies support this idea, reporting high concentrations of Nap and Phe (Kim *et al.*  
368 *et al.* 2011) or Nap, Chry, BaA and Acy (Campos *et al.* 2012) from wood burning. Ash studies  
369 of beech and similar species (Bundt *et al.* 2001) were dominated by Nap and, to a slight extent,  
370 by BghiPer, BbF, BkF, Chry, Triph and Phe.

371 Caution is required when making comparisons between the PAHs values across studies as there  
372 are important variations in the methodologies employed. Some studies examine PAHs in ash  
373 (Enell *et al.* 2008; Silva *et al.* 2015) or sediment (Olivella *et al.* 2006; Kim *et al.* 2011) and  
374 others in stream water (Olivella *et al.* 2006), pond water (Vila-Escalé *et al.* 2007), runoff water  
375 (Campos *et al.* 2012) or aqueous extracts (Enell *et al.* 2008; Silva *et al.* 2015) meaning  
376 concentration and compositional differences are to be expected. It is likely the high to very  
377 high PAHs concentrations recorded in the ash studied here would be dramatically reduced if  
378 the leachable fraction of the samples was tested, as opposed to total concentrations, therefore,  
379 making the portion more accessible to interact with aquatic fauna lower (Frišták *et al.* 2019).

380

381 *Implications for toxicology*

382 The wildfire ash analysis conducted here not only demonstrates the high variability in the  
383 concentration of chemical components of ash produced in contrasting ecosystems (Table 2),  
384 but also the differences in its potential toxic effects in aquatic systems (Table 5 & 6).  
385 Significant toxicity was observed on *D. magna* over the acute exposures for three of the six ash  
386 types tested: AUS, USA and CAN (Figure 2; Table 5 & 6). Ash type and composition,  
387 therefore, seems crucial to the level of toxicity on cladoceran species, as also demonstrated  
388 previously (Campos *et al.* 2012; Silva *et al.* 2015; Brito *et al.* 2017).

389 The combination of the chemical data with the *D. magna* immobilisation results highlights a  
390 number of possible relationships (Figure 1). The PCA identified pH and EC as two of the  
391 parameters strongly characteristic of the three ash types causing significant *D. magna*  
392 immobilisation (AUS, USA, CAN) (Table 3, Figure 1). It is well established that extreme  
393 values of pH and EC have a detrimental impact on zooplankton species (Mount *et al.* 1997;  
394 Franklin *et al.* 2000; Silva *et al.* 2015). The pH values in the bioassays themselves, however,  
395 were notably lower and less variable than in the leachate results used during the PCA analysis  
396 and within a range thought acceptable for the survival of *D. magna* and similar cladoceran  
397 species (OECD 2004) (Table 5 & 6). Crucially however, the relationship between pH and  
398 immobilisation is very similar between the leachates and bioassays pH results with higher pH  
399 values, characteristic of the ash types, producing immobilisation in *D. magna*. This perhaps  
400 suggests that pH has an indirect effect on *D. magna* immobilisation as pH can also influence  
401 the dissolution of elements from ash into water and therefore the relative toxic potential of  
402 other ash components (Fedje *et al.* 2010). Low pH values, for example, encourage the leaching  
403 of oxyanion-forming (As, B, Cr, Sb and V) and cation-forming elements (Ca), and neutral pH  
404 greatly reduces the leaching of amphoteric elements (e.g. Al, Cd, Cu, Pb and Zn) (Fedje *et al.*  
405 2010). The more neutral pH of the UK sample, however, does not seem to have reduced the  
406 leaching of Al, Cd, Cu and Pb. pH has an inconsistent relationship with toxicity, and, often,  
407 results are difficult to interpret (Wilde *et al.* 2006; Silva *et al.* 2015).

408 The influence of key nutrients on *D. magna* immobilisation is perhaps less well established  
409 (Smith *et al.* 2011) (Figure 1), as ions such as, Cl<sup>-</sup> and NO<sub>3</sub><sup>-</sup> are required at minimum levels to  
410 support aquatic life. However, the PCA also identified high concentrations of Cl<sup>-</sup> and NO<sub>3</sub><sup>-</sup> as  
411 being key characteristic components of the three toxic ash types, particularly the more toxic  
412 AUS and USA ash (Table 3 and Figure 1). Many anthropogenic (e.g. oil/gas production,

413 irrigation methods and industrial/agricultural processes) and natural (e.g. sediment pore waters  
414 and burning) circumstances have been shown to increase nutrient concentrations to toxic levels  
415 (e.g. Hoke *et al.* 1993; Ferreira *et al.* 2005; Mast and Clow 2008). Scott and Crunkilton (2005)  
416 demonstrated  $\text{NO}_3^-$  produces immobilisation of *D. magna* at  $462 \text{ mg L}^{-1}$  with no observable  
417 effect concentration at  $358 \text{ mg L}^{-1}$ . Similarly, Mount *et al.* (1997) estimated a concentration of  
418  $1000 - 2000 \text{ mg L}^{-1}$  as the concentration of  $\text{Cl}^-$  required to produce  $\text{EC}_{50}$  in *Ceriodaphnia*  
419 *dubia*. This suggests despite the correlations between  $\text{NO}_3^-$  and  $\text{Cl}^-$  with the toxic ash types  
420 found here, the relatively low quantities of these components alone are not likely capable of  
421 causing the observed toxicity (Table 2). The limited number of studies focusing on  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  
422  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  exports after fire have found maximum levels sampled in ash fall well below  
423 recommended limits (Smith *et al.* 2011).

424 The relatively high PAHs concentrations found in the ash tested here appear to produce no  
425 observable toxicity on *D. magna* and furthermore, higher PAHs concentration seem to be  
426 associated with reduced toxicity. It has to be noted that PAHs concentrations were only  
427 determined in bulk ash samples. PAHs have limited solubility in water, particularly of the  
428 larger ring size PAHs ( $> 3$  rings) (Chen *et al.* 2018). The lack of relationship between high  
429 levels of PAHs and toxicity found here and in other studies (Campos *et al.* 2012; Silva *et al.*  
430 2015) raise questions about the bioavailability of PAHs in this context. In an assessment of the  
431 methylated PAHs composition of sludge-derived pyrogenic material, Frišták *et al.* (2019)  
432 found during pyrolysis methylated aromates mainly bind to insoluble carbon fractions or get  
433 trapped in microporous structures of pyrogenic material and, therefore, are unlikely to be  
434 bioavailable and hazardous to freshwater systems. This may be one reason why the PAHs  
435 concentrations are not associated with toxicity in *D. magna* here. The potential more subtle and  
436 longer term impacts of PAHs on aquatic biota such as, reductions in the rate of growth,  
437 metabolic activity, reproduction or increased mutation and cancer risk (Hellou *et al.* 2006;  
438 Campos *et al.* 2012) were beyond the scope of this study. Potential synergistic, antagonistic  
439 and additive effects of the complex and variable PAHs composition of the ash types tested  
440 could also not be ruled out as a source of toxicity. Further research should be conducted  
441 investigating if these levels of total PAHs pose a greater water contamination risk from a wider  
442 ecological or drinking water perspective.

443 Despite the variations in ash composition and the subsequent significant differences in *D.*  
444 *magna* immobilisation, it is difficult to isolate the primary causes of toxicity. In addition to the  
445 most likely, if indirect, influential parameters, pH and electrical conductivity, there are also

446 likely components that are not necessarily toxic by themselves, but could be variables  
447 influencing toxicity in certain concentrations (e.g. DOC, Na, Ca and Mg) (Freitas and Rocha  
448 2011; Simplício *et al.* 2016). Physical characteristics of the ash types may also be a possible  
449 cause of immobilisation as variations in particle size and distribution of the suspended  
450 particulate matter in the unfiltered samples used could have compromised the food intake and  
451 locomotive ability of *D. magna* leading to immobilisation or death (Brito *et al.* 2017). Even  
452 when using a standardised laboratory approach, as employed here, it remains difficult to  
453 untangle the effects of such components from those caused by other variables in such complex  
454 samples (Wilde *et al.* 2006; Silva *et al.* 2015; Brito *et al.* 2017).

455 Earlier work has suggested macroinvertebrate species such as *D. magna* are less sensitive to  
456 contamination than lower trophic species (Campos *et al.* 2012) and thus, the effects of ash  
457 contamination on these higher trophic organisms are expected to be primarily indirect through  
458 the propagation of toxicity across the food chain via bottom-up, bioaccumulation processes  
459 (Abrantes *et al.* 2008). A few notable studies have demonstrated this premise with no  
460 observable effect of ash toxicity on daphnid survival or reproduction rates over both acute (48  
461 h) and chronic (21 day) exposures, but significant impacts have been observed on lower trophic  
462 species (bacteria, algae and macrophytes) (Campos *et al.* 2012; Silva *et al.* 2015).  
463 Understanding the mechanisms influencing the bioaccumulation/availability of ash  
464 contaminants in freshwater systems should thus be a focus of future research. The results  
465 presented here, along with other studies, appear to justify the concerns around the impacts of  
466 wildfire ash on aquatic biota and water quality even without the assessment of bioaccumulation  
467 processes (Campos *et al.* 2012; Brito *et al.* 2017).

468

## 469 **Conclusion**

470 The chemical characterisation of the six wildfire ash types shows an overall similar  
471 composition of elements, but significant variations in the concentration, reactivity and  
472 solubility of these elements. Solubility of all elements was low for all ash types comparing the  
473 total and leachate characterisation data.

474 Our results demonstrate significant immobilisation of *D. magna* over acute exposure (48 h) to  
475 three of the six ash types (AUS, USA and CAN). The principle characteristics of these ash  
476 types producing immobilisation, derived from PCA, were high values of pH, EC, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, Hg  
477 and SO<sub>4</sub><sup>2-</sup>. None of these components, however, appear likely to have directly caused the

478 immobilisation response (ecotoxicity) observed. It is perhaps more likely that these  
479 components, and possible others (e.g. Ca), have contributed indirectly to the observed toxicity.  
480 Elevated water-soluble concentrations of metal and metalloid contaminants (Mn, Fe, Zn, Pb,  
481 Cu and As) did not produce any significant inhibition and tended to be characteristic of the  
482 non-toxic ash types. The total PAHs concentrations were also not linked to significant  
483 inhibition. It continues to prove difficult to identify specific causes of toxicity in aquatic biota  
484 using test substances as complex and variable as wildfire ash.

485 Combining the detailed chemical characterisation of the ash types with the ecotoxicology  
486 results helps to provide further insight into the composition and variations in ash produced in  
487 contracting ecosystems and potential implications of wildfire ash contamination on the  
488 environment. A detailed understanding of the interactions and impacts of metals, nutrients and  
489 PAHs in different ecosystem types is essential for evaluating the pollution risk of fires and for  
490 informing management. The results presented here justify the concerns around the down-  
491 stream contamination potential of ash in certain ecosystems on aquatic biota and highlight the  
492 need for a greater understanding of possible direct/indirect chemical causalities. Further  
493 research is therefore, required in order to (i) identify and predict conditions creating certain  
494 chemical signatures in ash and (ii) to investigate the specific direct (or indirect) causality of  
495 toxicity in key groups of aquatic species.

496 **Acknowledgments**

497 This work was supported by a Swansea University, College of Science PhD studentship  
498 (Harper), a *Leverhulme Trust Research Fellowship* RF-2016-456\2 (Doerr) and a *Ser Cymru*  
499 *Fellowship* supported by European Union's 2020 research and innovation programme under  
500 the Marie Skłodowska-Curie grant agreement N. 663830 (Santin) and NERC grant  
501 NE/R011125/1. Xose Luis Otero was supported by CRETUS strategic group  
502 (AGRUP2015/02).

503 We would also like to acknowledge and thank A. Cerdà and M. Bodi for kindly providing the  
504 Spanish pine forest (SPA) ash sample.

505

506 **Conflicts of interest**

507 The authors declare no conflicts of interest.

508

509

510 **References**

- 511 Abraham J, Dowling K, Florentine S (2017) Risk of post-fire metal mobilization into surface  
512 water resources: A review. *Science of the Total Environment* **599**, 1740–1750.  
513 doi:10.1016/j.scitotenv.2017.05.096.
- 514 Abrantes N, Pereira R, Soares AMVM, Gonçalves F (2008) Evaluation of the  
515 ecotoxicological impact of the pesticide Lasso® on non-target freshwater species,  
516 through leaching from nearby agricultural fields, using terrestrial model ecosystems.  
517 *Water, Air, and Soil Pollution* **192**, 211–220. doi:10.1007/s11270-008-9648-5.
- 518 Bixby RJ, Cooper SD, Gresswell RE, Brown LE, Dahm CN, Dwire KA (2015) Fire effects  
519 on aquatic ecosystems: an assessment of the current state of the science. *Freshwater*  
520 *Science* **34**, 1340–1350. doi:10.1086/684073.
- 521 Bladon KD, Emelko MB, Silins U, Stone M (2014) Wildfire and the future of water supply.  
522 *Environmental Science and Technology* **48**, 8936–8943. doi:10.1021/es500130g.
- 523 Bladon KD, Silins U, Wagner MJ, Stone M, Emelko MB, Mendoza CA, Devito KJ, Boon S  
524 (2008) Wildfire impacts on nitrogen concentration and production from headwater  
525 streams in southern Alberta's Rocky Mountains. *Canadian Journal of Forest Research*  
526 **38**, 2359–2371. doi:10.1139/X08-071.
- 527 Blake WH, Wallbrink PJ, Droppo IG (2009) Sediment aggregation and water quality in  
528 wildfire-affected river basins. *Marine and Freshwater Research* **60**, 653–659.  
529 doi:10.1071/mf08068.
- 530 Bodí MB, Martin DA, Balfour VN, Santín C, Doerr SH, Pereira P, Cerdà A, Mataix-Solera J  
531 (2014) Wildland fire ash: Production, composition and eco-hydro-geomorphic effects.  
532 *Earth-Science Reviews* **138**, 503. doi:10.1016/j.earscirev.2013.12.007.
- 533 Boelter AM, Lamming FN, Farag AM, Bergman HL (1992) Environmental effects of saline  
534 oil-field discharges on surface waters. *Environmental Toxicology and Chemistry* **11**,  
535 1187–1195. doi:10.1002/etc.5620110815.
- 536 Brito DQ, Passos CJS, Muniz DHF, Oliveira-Filho EC (2017) Aquatic ecotoxicity of ashes  
537 from Brazilian savanna wildfires. *Environmental Science and Pollution Research* **24**,  
538 19671–19682. doi:10.1007/s11356-017-9578-0.
- 539 Brookins DG (1989) Eh-pH diagrams for geochemistry. *Geochimica et Cosmochimica Acta*

- 540       **53**, 763. doi:10.1016/0016-7037(89)90020-3.
- 541 Bundt M, Krauss M, Blaser P, Wilcke W (2001) Forest Fertilization with Wood Ash: Effect  
542       on the Distribution and Storage of Polycyclic Aromatic Hydrocarbons (PAHs) and  
543       Polychlorinated Biphenyls (PCBs). *Journal of Environmental Quality* **30**, 1296–1304.
- 544 Campos I, Abrantes N, Vidal T, Bastos AC, Gonçalves F, Keizer JJ (2012) Assessment of the  
545       toxicity of ash-loaded runoff from a recently burnt eucalypt plantation. *European*  
546       *Journal of Forest Research* **131**, 1889–1903. doi:10.1007/s10342-012-0640-7.
- 547 Chen H, Chow AT, Li XW, Ni HG, Dahlgren RA, Zeng H, Wang JJ (2018) Wildfire Burn  
548       Intensity Affects the Quantity and Speciation of Polycyclic Aromatic Hydrocarbons in  
549       Soils. *ACS Earth and Space Chemistry* **2**, 1262–1270.  
550       doi:10.1021/acsearthspacechem.8b00101.
- 551 Costa MR, Calvão AR, Aranha J (2014) Linking wildfire effects on soil and water chemistry  
552       of the Marão River watershed, Portugal, and biomass changes detected from Landsat  
553       imagery. *Applied Geochemistry* **44**, 93–102. doi:10.1016/j.apgeochem.2013.09.009.
- 554 Diaz OA, Reddy KR, Moore PA (1994) Solubility of inorganic phosphorus in stream water as  
555       influenced by pH and calcium concentration. *Water Research* **28**, 1755–1763.  
556       doi:10.1016/0043-1354(94)90248-8.
- 557 Earl SR, Blinn DW (2003) Effects of wildfire ash on water chemistry and biota. *Freshwater*  
558       *Biology* **48**, 1015–1030.
- 559 Enell A, Fuhrman F, Lundin L, Warfvinge P, Thelin G (2008) Polycyclic aromatic  
560       hydrocarbons in ash: Determination of total and leachable concentrations.  
561       *Environmental Pollution* **152**, 285–292. doi:10.1016/j.envpol.2007.06.055.
- 562 Ferreira AJD, Coelho COA, Boulet AK, Lopes FP (2005) Temporal patterns of solute loss  
563       following wildfires in Central Portugal. *International Journal of Wildland Fire* **14**, 401–  
564       412. doi:10.1071/wf05043.
- 565 Franklin NM, Stauber JL, Markich SJ, Lim RP (2000) pH-dependent toxicity of copper and  
566       uranium to a tropical freshwater alga (*Chlorella* sp.). *Aquatic Toxicology* **48**, 275–289.  
567       doi:10.1016/S0166-445X(99)00042-9.
- 568 Freitas EC, Rocha O (2011) Acute and chronic effects of sodium and potassium on the  
569       tropical freshwater cladoceran *Pseudosida ramosa*. *Ecotoxicology* **20**, 88–93.

- 570 doi:10.1007/s10646-010-0559-z.
- 571 Frišták V, Laughinghouse HD, Packová A, Graser M, Soja G (2019) Monitoring of  
572 methylated naphthalenes in sludge-derived pyrogenic carbonaceous materials.  
573 *Chemosphere* **217**, 456–462. doi:10.1016/j.chemosphere.2018.11.030.
- 574 Gonino G, Branco P, Benedito E, Ferreira MT, Santos JM (2019) Short-term effects of  
575 wildfire ash exposure on behaviour and hepatosomatic condition of a potamodromous  
576 cyprinid fish, the Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864). *Science of*  
577 *the Total Environment* **665**, 226–234. doi:10.1016/j.scitotenv.2019.02.108.
- 578 Gonino GMR, Figueiredo BRS, Manetta GI, Zaia Alves GH, Benedito E (2019) Fire  
579 increases the productivity of sugarcane, but it also generates ashes that negatively affect  
580 native fish species in aquatic systems. *Science of the Total Environment* **664**, 215–221.  
581 doi:10.1016/j.scitotenv.2019.02.022.
- 582 Hallema DW, Sun G, Caldwell P V., Norman SP, Cohen EC, Liu Y, Bladon KD, McNulty  
583 SG (2018) Burned forests impact water supplies. *Nature Communications* **9**, 1307.  
584 doi:10.1038/s41467-018-03735-6.
- 585 Hellou J, Leonard J, Collier TK, Ariese F (2006) Assessing PAH exposure in feral finfish  
586 from the Northwest Atlantic. *Marine Pollution Bulletin* **52**, 433–441.  
587 doi:10.1016/j.marpolbul.2005.11.012.
- 588 Hoke RA, Giesy JP, Zabik M, Unger M (1993) Toxicity of Sediments and Sediment Pore  
589 Waters from the Grand Calumet River-Indiana Harbor, Indiana Area of Concern.  
590 *Ecotoxicology and Environmental Safety* **26**, 86–112. doi:10.1006/eesa.1993.1042.
- 591 Ingersoll CG, Dwyer FJ, Burch SA, Nelson MK, Buckler DR, Hunn JB (1992) The use of  
592 freshwater and saltwater animals to distinguish between the toxic effects of salinity and  
593 contaminants in irrigation drain water. *Environmental Toxicology and Chemistry* **11**,  
594 503–511. doi:10.1002/etc.5620110408.
- 595 Jung HY, Hogue TS, Rademacher LK, Meixner T (2009) Impact of wildfire on source water  
596 contributions in Devil Creek, CA: Evidence from end-member mixing analysis.  
597 *Hydrological Processes* **23**, 183–200. doi:10.1002/hyp.7132.
- 598 Karlfeldt Fedje K, Ekberg C, Skarnemark G, Steenari BM (2010) Removal of hazardous  
599 metals from MSW fly ash-An evaluation of ash leaching methods. *Journal of Hazardous*

- 600 *Materials* **173**, 310–317. doi:10.1016/j.jhazmat.2009.08.094.
- 601 Keiluweit M, Kleber M, Sparrow MA, Simoneit BRT, Prahlg FG (2012) Solvent-extractable  
602 polycyclic aromatic hydrocarbons in biochar: Influence of pyrolysis temperature and  
603 feedstock. *Environmental Science and Technology* **46**, 9333–9341.  
604 doi:10.1021/es302125k.
- 605 Keith LH (2015) The Source of U.S. EPA’s Sixteen PAH Priority Pollutants. *Polycyclic*  
606 *Aromatic Compounds* **35**, 147–160. doi:10.1080/10406638.2014.892886.
- 607 Khanna PK, Raison RJ, Falkiner RA (1994) Chemical properties of ash derived from  
608 Eucalyptus litter and its effects on forest soils. *Forest Ecology and Management* **66**,  
609 107–125. doi:10.1016/0378-1127(94)90151-1.
- 610 Kim EJ, Choi SD, Chang YS (2011) Levels and patterns of polycyclic aromatic hydrocarbons  
611 (PAHs) in soils after forest fires in South Korea. *Environmental Science and Pollution*  
612 *Research* **18**, 1508–1517. doi:10.1007/s11356-011-0515-3.
- 613 Mast MA, Clow DW (2008) Effects of 2003 wildfires on stream chemistry in Glacier  
614 National Park, Montana. *Hydrological Processes* **22**, 5013–5023. doi:10.1002/hyp.7121.
- 615 Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM (1997) Statistical models to  
616 predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and  
617 *Pimephales promelas* (fathead minnows). *Environmental Toxicology and Chemistry* **16**,  
618 2009–2019. doi:10.1897/1551-5028(1997)016<2009:SMTPTT>2.3.CO;2.
- 619 Musset ML (2006) Current Approaches in the Statistical analysis of Ecotoxicity data.
- 620 Nikinmaa M (2014) ‘An introduction to aquatic toxicology.’ (Elsevier)
- 621 Nunes B, Silva V, Campos I, Pereira JL, Pereira P, Keizer JJ, Gonçalves F, Abrantes N  
622 (2017) Off-site impacts of wildfires on aquatic systems — Biomarker responses of the  
623 mosquitofish *Gambusia holbrooki*. *Science of the Total Environment* **581–582**, 305–313.  
624 doi:10.1016/j.scitotenv.2016.12.129.
- 625 OECD (2004) OECD Guideline for testing of chemicals - *Daphnia* sp., acute immobilisation  
626 test.
- 627 Oliveira-Filho EC, Brito DQ, Dias ZMB, Guarieiro MS, Carvalho EL, Fascineli ML, Niva  
628 CC, Grisolia CK (2018) Effects of ashes from a Brazilian savanna wildfire on water, soil

- 629 and biota: An ecotoxicological approach. *Science of the Total Environment* **618**, 101–  
630 111. doi:10.1016/j.scitotenv.2017.11.051.
- 631 Olivella MA, Ribalta TG, De Febrer AR, Mollet JM, De Las Heras FXC (2006) Distribution  
632 of polycyclic aromatic hydrocarbons in riverine waters after Mediterranean forest fires.  
633 *Science of the Total Environment* **355**, 156–166. doi:10.1016/j.scitotenv.2005.02.033.
- 634 Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J (2009) The  
635 biochemistry of environmental heavy metal uptake by plants: Implications for the food  
636 chain. *International Journal of Biochemistry and Cell Biology* **41**, 1665–1677.  
637 doi:10.1016/j.biocel.2009.03.005.
- 638 Pereira P, beda X, Martin D, Mataix-Solera J, Guerrero C (2011) Effects of a low severity  
639 prescribed fire on water-soluble elements in ash from a cork oak (*Quercus suber*) forest  
640 located in the northeast of the Iberian Peninsula. *Environmental Research* **111**, 237–247.  
641 doi:10.1016/j.envres.2010.09.002.
- 642 Pereira P, Úbeda X (2010) Spatial distribution of heavy metals released from ashed after a  
643 wildfire. *Journal of Environmental Engineering and Landscape Management* **18**, 13–22.  
644 doi:10.3846/jeelm.2010.02.
- 645 Pérez-Fernández B, Viñas L, Franco MÁ, Bargiela J (2015) PAHs in the Ría de Arousa (NW  
646 Spain): A consideration of PAHs sources and abundance. *Marine Pollution Bulletin* **95**,  
647 155–165. doi:10.1016/j.marpolbul.2015.04.028.
- 648 Pilliod DS, Bury RB, Hyde EJ, Pearl CA, Corn PS (2003) Fire and amphibians in North  
649 America. *Forest Ecology and Management* **178**, 163–181. doi:10.1016/S0378-  
650 1127(03)00060-4.
- 651 Pitman RM (2006) Wood ash use in forestry - A review of the environmental impacts.  
652 *Forestry* **79**, 563–588. doi:10.1093/forestry/cpl041.
- 653 Plumlee GS, Martin D a, Hoefen T, Kokaly R, Eckberg A, Meeker GP, Adams M, Anthony  
654 M, Lamothe PJ (2007) Preliminary Analytical Results for Ash and Burned Soils from  
655 the October 2007 Southern California wildfires.
- 656 Rey-Salgueiro L, Martínez-Carballo E, Merino A, Vega JA, Fonturbel MT, Simal-Gandara J  
657 (2018) Polycyclic Aromatic Hydrocarbons in Soil Organic Horizons Depending on the  
658 Soil Burn Severity and Type of Ecosystem. *Land Degradation and Development* **29**,

- 659 2112–2123. doi:10.1002/ldr.2806.
- 660 Robinson L, Thorn I (2009) ‘Toxicology and Ecotoxicology in Chemical Safety Assessment.’  
661 doi:10.1002/9781444305494.
- 662 Santín C, Doerr SH, Merino A, Bucheli TD, Bryant R, Ascough P, Gao X, Masiello CA  
663 (2017) Carbon sequestration potential and physicochemical properties differ between  
664 wildfire charcoals and slow-pyrolysis biochars. *Scientific Reports* **7**, 11233.  
665 doi:10.1038/s41598-017-10455-2.
- 666 Santín C, Doerr SH, Otero XL, Chafer CJ (2015) Quantity, composition and water  
667 contamination potential of ash produced under different wildfire severities.  
668 *Environmental Research* **142**, 297–308. doi:10.1016/j.envres.2015.06.041.
- 669 Santín C, Otero XL, Doerr SH, Chafer CJ (2018) Impact of a moderate/high-severity  
670 prescribed eucalypt forest fire on soil phosphorous stocks and partitioning. *Science of*  
671 *the Total Environment* **621**, 1103–1114. doi:10.1016/j.scitotenv.2017.10.116.
- 672 Scholze M, Knorr W, Arnell NW, Prentice IC (2006) A climate-change risk analysis for  
673 world ecosystems. *Proceedings of the National Academy of Sciences* **103**, 13116–13120.  
674 doi:10.1073/pnas.0601816103.
- 675 Scott G, Crunkilton RL (2005) Acute and chronic toxicity of nitrate to fathead minnows  
676 (pimephales promelas), ceriodaphnia dubia and daphnia magna. *Environmental*  
677 *Toxicology and Chemistry* **19**, 2918. doi:10.1897/1551-  
678 5028(2000)019<2918:aacton>2.0.co;2.
- 679 Shakesby RA, Doerr SH (2006) Wildfire as a hydrological and geomorphological agent.  
680 *Earth-Science Reviews* **74**, 269–307. doi:10.1016/j.earscirev.2005.10.006.
- 681 Silva V, Pereira JL, Campos I, Keizer JJ, Gonçalves F, Abrantes N (2015) Toxicity  
682 assessment of aqueous extracts of ash from forest fires. *Catena* **135**, 401–408.  
683 doi:10.1016/j.catena.2014.06.021.
- 684 Simplício N, Muniz D, Rocha F, Martins D, Dias Z, Farias B, Oliveira-Filho E (2016)  
685 Comparative Analysis between Ecotoxicity of Nitrogen-, Phosphorus-, and Potassium-  
686 Based Fertilizers and Their Active Ingredients. *Toxics* **5**, 2. doi:10.3390/toxics5010002.
- 687 Smith HG, Sheridan GJ, Lane PNJ, Nyman P, Haydon S (2011) Wildfire effects on water  
688 quality in forest catchments: A review with implications for water supply. *Journal of*

- 689 *Hydrology* **396**, 170–192. doi:10.1016/j.jhydrol.2010.10.043.
- 690 Stiernström S, Lindé M, Hemström K, Wik O, Ytreberg E, Bengtsson BE, Breitholtz M  
691 (2013) Improved understanding of key elements governing the toxicity of energy ash  
692 eluates. *Waste Management* **33**, 842–849. doi:10.1016/j.wasman.2012.12.008.
- 693 Stumm, W. and Morgan J. (2013) ‘Aquatic chemistry: chemical equilibria and rates in natural  
694 waters.’ doi:10.5860/choice.33-6312.
- 695 Tian Q, Guo B, Nakama S, Sasaki K (2018) Distributions and Leaching Behaviors of Toxic  
696 Elements in Fly Ash. *ACS Omega* **3**, 13055–13064. doi:10.1021/acsomega.8b02096.
- 697 Tsai KP, Uzun H, Karanfil T, Chow AT (2017) Dynamic Changes of Disinfection Byproduct  
698 Precursors following Exposures of *Microcystis aeruginosa* to Wildfire Ash Solutions.  
699 *Environmental Science and Technology* **51**, 8272–8282. doi:10.1021/acs.est.7b01541.
- 700 USEPA - United States Environmental Protection Agency (2016) EPA - OCSP 850.1010:  
701 Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids.
- 702 Vila-Escalé M, Vegas-Vilarrúbia T, Prat N (2007) Release of polycyclic aromatic compounds  
703 into a Mediterranean creek (Catalonia, NE Spain) after a forest fire. *Water Research* **41**,  
704 2171–2179. doi:10.1016/j.watres.2006.07.029.
- 705 Viñas L, Franco MA, González JJ (2009) Polycyclic aromatic hydrocarbon composition of  
706 sediments in the Ría de Vigo (NW Spain). *Archives of Environmental Contamination*  
707 *and Toxicology* **57**, 42–49. doi:10.1007/s00244-008-9230-6.
- 708 Weiner ER, Group F (2007) ‘Applications of Environmental Aquatic A Practical Guide.’  
709 doi:10.1201/9781420008371.
- 710 Wilde KL, Stauber JL, Markich SJ, Franklin NM, Brown PL (2006) The effect of pH on the  
711 uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp.).  
712 *Archives of Environmental Contamination and Toxicology* **51**, 174–185.  
713 doi:10.1007/s00244-004-0256-0.
- 714 World Health Organization (2011) Guidelines for drinking-water quality - 4th edition.  
715 doi:10.1016/S1462-0758(00)00006-6.
- 716

717 **Table 1. Fire and vegetation characteristics of the six ash types used in this study.**

Sample name	Location	Vegetation cover	Burn description
Australian eucalypt (AUS)	West of Sydney (33°52'14" S; 150°36'01" E)	Open, dry sclerophyll forest with a dense shrubby undergrowth. Key species; ironbark ( <i>Eucalyptus fibrosa</i> ), stringybarks ( <i>Eucalyptus eugenioides</i> , <i>Eucalyptus oblonga</i> ), <i>Banksia</i> sp., <i>Leptospermum</i> sp., <i>Acacia</i> sp. and <i>Petrophile</i> sp..	Moderate to high severity prescribed fire in 2014. Fire did not affect tree canopy but complete combustion of understory fuels and mostly fine ash, light in colour was generated (Santín <i>et al.</i> 2018).
USA chaparral (USA)	South western California (34°25'14" N; 119°30'39" W)	Mixed chaparral with the dominant species including coast live oak ( <i>Quercus agrifolia</i> ), Toyon ( <i>Heteromeles arbutifolia</i> ), coyote brush ( <i>Baccharis pilularis</i> ), holly-leaf cherry ( <i>Prunus ilicifolia</i> )	Large-scale, extreme high-severity wildfire (Thomas fire) in 2018, achieving almost complete combustion of above surface fuel.
Canadian spruce (CAN)	Northwest Territories (61°34'55" N; 117°11'55" W)	Very dense tree canopy comprised of mostly black spruce ( <i>Picea mariana</i> ) and jack pines ( <i>Pinus banksiana</i> ) with very little understory vegetation with the exception of young spruce and moss species.	Very high intensity experimental crown fire in 2015, all fine fuels aboveground were consumed. The forest floor was only slight affected (<1cm depth of burn).
Spanish heathland (URIA)	North western Spain (43°6'17" N 6°50'52" W)	Dominant species included, heather ( <i>Calluna vulgaris</i> ), western gorse ( <i>Ulex gallii</i> ) and a variety of Erica sp. (e.g. <i>Erica tetralix</i> ).	Extreme hot and dry conditions producing a severe experimental wildfire in 2017. Combustion completeness very high (most fine fuel consumed).
Spanish pine forest (SPA)	Eastern Spain (40°18'36" N; 1°01'59" W)	Forested area dominated by Aleppo pine ( <i>Pinus halepensis</i> ).	High-severity wildfire producing a very high level of combustion completeness. All surface fuel consumed.
UK grassland (UK)	South Wales (51°50'11" N; 3°25'44" W) and (51°41'02" N; 3°38'37" W)	Upland graminoid dominant areas. Dominant vegetation in these species-poor areas consisted of purple moor-grass ( <i>Molinia caerulea</i> ), soft rush ( <i>Juncus effuses</i> ), mat-grass ( <i>Nardus stricta</i> ) and heath plait-moss ( <i>Hypnum jutlandicum</i> ).	Moderate severity wildfires in 2018. Consumed most above surface fuel and litter, but did not penetrate soil surface. Composite from two fires created in weight ratio 2:1 (g) of ash from site one and site two, respectively.

718

719 **Table 2. Water-soluble chemical composition of the six ash types obtained by leaching**  
 720 **tests.**

721 Solubility of elements provided in brackets as a percentage (%) of the total ash composition.  
 722 Electrical conductivity (E.C.) given in  $\mu\text{S cm}^{-1}$ . N.D (not detected) represents components  
 723 with quantities  $<0.01 \text{ mg kg}^{-1}$ . The symbol (-) is used to denote values not able to be  
 724 calculated due to the dry weight of the component not being tested for or the value being 0.

		Ash types											
		AUS		USA		CAN		URIA		SPA		UK	
mg kg <sup>-1</sup>	<b>pH</b>	11.1	-	11.2	-	10.3	-	10.3	-	9.1	-	7.9	-
	<b>E.C</b>	3880	-	2570	-	2500	-	1505	-	233	-	293	-
	<b>Al</b>	0	-	4	(0.02)	0	-	20	(0.2)	0	-	0	-
	<b>Si</b>	45	(2.17)	182	(8.81)	27	(1.54)	133	(5.59)	25	(1.13)	27	(1.86)
	<b>Ca</b>	55	(0.03)	136	(0.06)	5864	(3.60)	580	(1.97)	1101	(0.83)	114	(0.97)
	<b>PO<sub>4</sub><sup>3-</sup></b>	10	(0.65)	10	(0.06)	1	(0.01)	27	(0.35)	10	(0.17)	620	(7.50)
	<b>NH<sub>4</sub><sup>+</sup></b>	8	-	9	-	0	-	33	-	4	-	20	-
	<b>DOC</b>	496	-	130	-	1331	-	1272	-	93	-	198	-
	<b>Cl<sup>-</sup></b>	1509	-	1494	-	1139	-	955	-	230	-	228	-
	<b>NO<sub>3</sub><sup>-</sup></b>	207	-	232	-	206	-	104	-	24	-	26	-
	<b>SO<sub>4</sub><sup>2-</sup></b>	4065	-	10180	-	32289	-	5600	-	3370	-	1203	-
	<b>B</b>	85	-	17	-	6	-	12	-	4	-	1	-
	<b>Na</b>	3893	(77.2)	831	(18.1)	860	(27.6)	1766	(49.6)	17	(1.49)	148	(22.3)
	<b>Mg</b>	377	(3.81)	26	(0.12)	3067	(25.6)	328	(5.13)	232	(4.23)	172	(6.36)
μg kg <sup>-1</sup>	<b>F<sup>-</sup></b>	340	-	3260	-	460	-	5080	-	9300	-	800	-
	<b>Mn</b>	0	-	0	-	68	(0.01)	656	(0.07)	136	(0.04)	9292	(0.65)
	<b>Fe</b>	205	-	643	-	553	(0.06)	2172	(0.03)	406	-	4378	(0.06)
	<b>Ni</b>	0	-	0	-	0	-	844	(3.84)	0	-	59	(0.37)
	<b>Cu</b>	423	(2.01)	280	(0.54)	198	(0.68)	5158	(12.9)	147	(0.49)	340	(0.68)
	<b>Zn</b>	0	-	0	-	0	-	0	-	0	-	140	(0.08)
	<b>As</b>	18	(1.17)	26	(1.12)	6	(1.21)	329	(7.40)	102	(1.05)	259	(5.96)
	<b>Cd</b>	1	(0.42)	0	-	1	(0.59)	0	-	7	(2.83)	2	(0.18)
	<b>Hg</b>	1	(44.9)	2	(57.4)	2	(10)	1	(5.96)	1	(17.9)	1	(4.91)
	<b>Pb</b>	16	(0.05)	7	(0.02)	3	(0.01)	5	(0.01)	7	(0.01)	64	(0.06)

725

726 **Table 3. Relative contribution of the 24 water-soluble ash constituents and parameters to**  
 727 **four of the significant principle components of the six ash types derived from principle**  
 728 **components analysis.**

729 Cumulative proportion (%) of the variance explained by each principle component also  
 730 provided.

	<b>PC1</b>	<b>PC2</b>
731		
732	<b>pH</b>	-0.29
	<b>E.C</b>	-0.02
733	<b>Al</b>	0.00
	<b>Si</b>	-0.10
734	<b>Ca</b>	-0.13
	<b>PO<sub>4</sub><sup>3-</sup></b>	0.27
735	<b>NH<sub>4</sub><sup>+</sup></b>	0.15
	<b>DOC</b>	-0.12
736	<b>Cl<sup>-</sup></b>	-0.27
	<b>NO<sub>3</sub><sup>-</sup></b>	-0.27
737	<b>SO<sub>4</sub><sup>2-</sup></b>	-0.18
	<b>B</b>	-0.14
	<b>Na</b>	-0.16
	<b>Mg</b>	-0.14
	<b>F<sup>-</sup></b>	0.05
	<b>Mn</b>	0.28
	<b>Fe</b>	0.27
	<b>Ni</b>	0.04
	<b>Cu</b>	0.02
	<b>Zn</b>	0.27
	<b>As</b>	0.23
	<b>Cd</b>	0.11
	<b>Hg</b>	-0.19
	<b>Pb</b>	0.26
	<b>Cumulative prop. (%)</b>	0.41

738 **Table 4. Concentration and composition of PAHs found in each ash type (ng g<sup>-1</sup>).**

739 PAHs displayed followed with the notation \* are U.S. Environmental Protection Agency

740 priority PAHs (Keith 2015).

PAH (ng/g)	Ash type					
	AUS	USA	CAN	URIA	SPA	UK
Naphthalene*	744.9	1148.6	4540.3	2861.4	1147.4	8010.9
Biphenyl	293.5	654.3	1851.1	1953.3	1019.1	1677.6
Acenaphthylene*	75.2	9.7	377.3	323.7	28.1	3337.2
Acenaphthene*	13.2	1.9	84.5	44.3	9.6	198.2
Fluorene*	18.1	3.4	99.7	104.0	26.7	380.4
Dibenzothiophene	4.7	3.5	45.6	11.9	7.7	29.2
Phenanthrene*	140.5	121.2	1049.5	487.4	170.5	1131.8
Anthracene*	19.1	10.4	126.3	76.1	15.6	193.2
Fluoranthene*	36.1	27.5	285.6	128.1	26.7	262.2
Pyrene*	37.3	16.9	215.8	112.9	20.7	257.8
Benzo(c)phenanthrene	2.9	3.0	12.7	9.7	2.9	12.7
Benz(a)anthracene*	11.9	8.4	32.3	23.9	4.4	35.3
Triphenylene	7.7	53.7	44.6	16.0	7.1	14.7
Chrysene*	14.3	26.5	38.9	27.4	7.3	36.3
Benzo(b)fluoranthene*	16.3	29.2	335.0	83.1	7.9	95.4
Benzo(k)fluoranthene*	4.2	8.9	92.8	25.0	3.0	32.3
Benzo(e)pyrene	11.3	44.1	291.8	90.9	8.8	57.3
Benzo(a)pyrene*	7.2	6.1	74.9	31.7	2.4	34.2
Perylene	3.4	1.3	20.1	14.8	1.9	13.2
Indeno (1,2,3-c,d) pyrene*	5.2	5.5	30.2	15.9	1.5	27.0
Dibenzo(a,h)anthracene*	1.9	2.6	10.5	14.8	0.9	5.2
Benzo (g,h,i) perylen*	9.6	18.2	92.5	33.0	2.8	40.7
2-Methylnaphtalene	225.7	66.9	1702.3	751.4	274.3	2118.2
1-Methylnaphthalene	168.2	34.3	1204.2	683.7	318.7	1663.5
2,3-Dimethylnaphthalene	43.2	7.1	388.0	138.0	166.8	235.0
2,3,6-Trimethylnaphthalene	19.4	3.4	140.5	50.0	20.9	80.0
4-Methyldibenzothiophene	7.9	1.0	32.5	49.5	32.8	35.0
2-Methylphenanthrene	21.2	6.3	154.3	87.7	44.7	153.6

2,8-Dimethyldibenzothiophene	3.0	0.7	14.1	12.0	5.2	29.4
1,6-Dimethylphenanthrene	31.9	5.5	147.2	109.0	51.5	120.0
2,4,7-Trimethyldibenzothiophene	0.3	0.1	0.8	1.0	0.5	3.3
1,2,8-Trimethylphenanthrene	8.4	2.1	67.2	47.8	43.4	35.5
1-Methylpyrene	9.8	1.4	24.5	31.3	11.3	32.9
2-Methylchrysene	3.8	1.7	4.5	5.9	1.9	6.4
7,12-DimethylB(a)A	2.9	0.5	1728.4	64.2	90.9	12.5
<b><math>\Sigma</math>16 PAHS</b>	1155	1445	7486	4393	1476	14078
<b><math>\Sigma</math>35 PAHS</b>	2024	2336	15360	8521	3586	20408
<b><math>\Sigma</math> Methylated</b>	546	131	5608	2031	1063	4525
<b><math>\Sigma</math> Non-methylated</b>	1479	2205	9752	6489	2523	15883
<b>% Methylated</b>	27	6	37	24	30	22

741

742 **Table 5. Immobilisation percentage of *Daphnia magna* at 24 h.**

743 Estimates of Lowest Observed Effect Concentration (LOEC) (Dunnetts test;  $p < 0.05$ ),  $EC_{10}^*$   
 744 and  $EC_{50}$  ( $g L^{-1}$ ). One-way analysis of variance p-values also provided, testing if observed  
 745 immobilisation of each ash type was significantly different to the control.  $*EC_x$  = the  
 746 concentration of substance required to produce x% (10 or 50) of the test individuals to  
 747 become immobilised. The symbol (-) is used to denote values not able to be calculated.

	Concentration ( $g L^{-1}$ )							pH	LOEC ( $g L^{-1}$ )	EC <sub>10</sub> ( $g L^{-1}$ )	EC <sub>50</sub> ( $g L^{-1}$ )	p value
	Control	3.12	6.25	12.5	25	50	75					
<b>AUS</b>	0	5	10	75	100	100	100	8.81	6.25	6.25	11	<0.001
<b>USA</b>	0	0	0	5	35	100	100	8.78	25	14	30	<0.001
<b>CAN</b>	0	0	0	5	10	100	100	8.23	50	25	37	<0.001
<b>URIA</b>	0	0	0	0	0	0	5	8.17	-	-	-	0.451
<b>SPA</b>	0	0	0	0	0	5	0	7.88	-	-	-	0.451
<b>UK</b>	0	0	0	0	0	0	0	7.56	-	-	-	-

748

749

750 **Table 6. Immobilisation percentage of *Daphnia magna* at 48 h.**

751 Estimates of Lowest Observed Effect Concentration (LOEC) (Dunnetts test;  $p < 0.05$ ),  $EC_{10}^*$   
 752 and  $EC_{50}$  ( $g L^{-1}$ ). One-way analysis of variance p-values also provided, testing if observed  
 753 immobilisation of each ash type was significantly different to the control.  $*EC_x$  = the  
 754 concentration of substance required to produce x% (10 or 50) of the test individuals to  
 755 become immobilised. The symbol (-) is used to denote values not able to be calculated.

	Concentration ( $g L^{-1}$ )							pH	LOEC ( $g L^{-1}$ )	EC <sub>10</sub> ( $g L^{-1}$ )	EC <sub>50</sub> ( $g L^{-1}$ )	p value
	Control	3.12	6.25	12.5	25	50	75					
<b>AUS</b>	0	10	15	85	100	100	100	8.93	6.25	5.5	9.5	<0.001
<b>USA</b>	0	0	5	5	65	100	100	9.08	6.25	14	20	<0.001
<b>CAN</b>	0	0	0	5	40	100	100	7.81	25	14	26	<0.001
<b>URIA</b>	0	0	0	0	0	0	10	8.03	-	-	-	0.451
<b>SPA</b>	0	0	0	0	0	5	0	7.58	-	-	-	0.451
<b>UK</b>	0	0	0	0	0	0	0	7.31	-	-	-	-

756

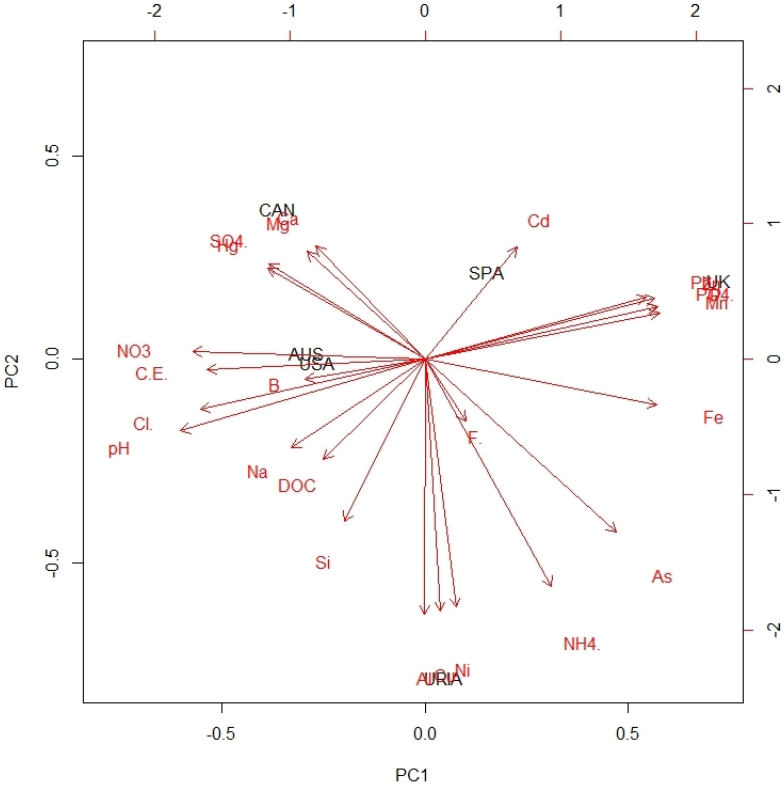


Fig.1: Representation of the ordination in the first two axes (PC1 and PC2) produced by principle component analysis (PCA) of the water-soluble chemical composition of the six ash types studies.

248x210mm (96 x 96 DPI)

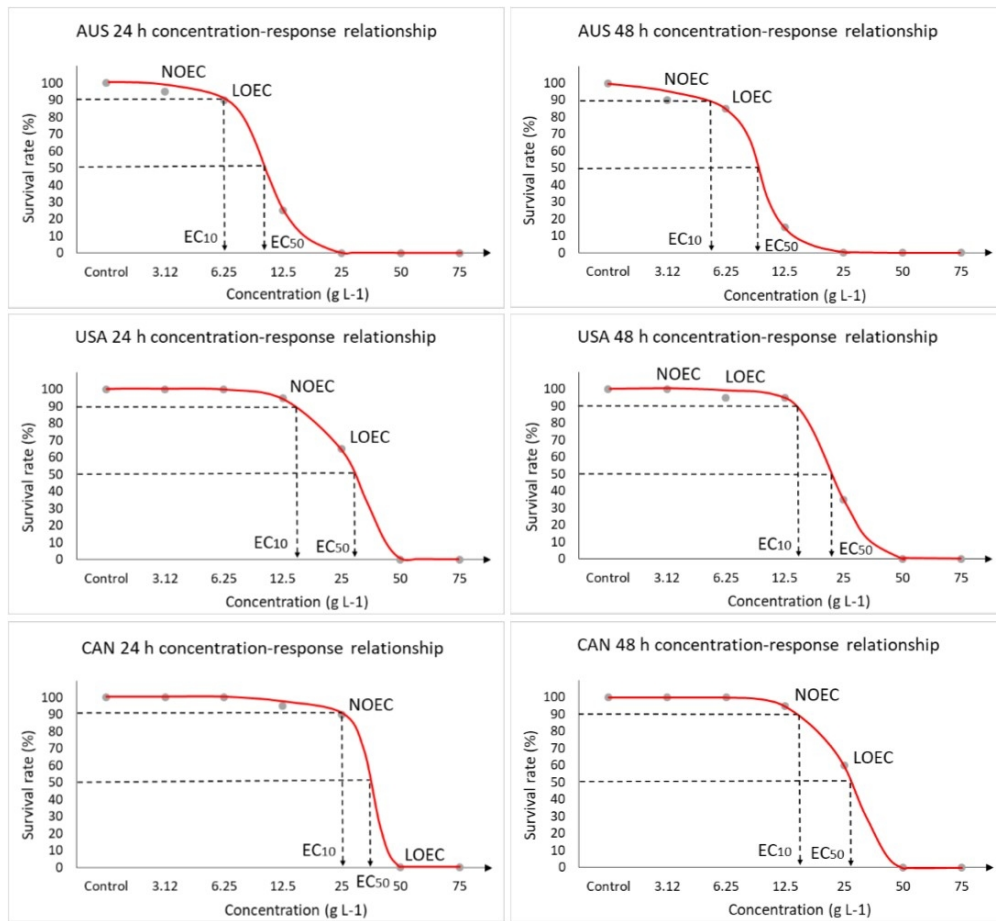


Fig. 2: Concentration response relationship after 24 and 48 hours of exposure. NOEC = no observed effect concentration, LOEC = lowest observed effect concentration, EC<sub>10</sub> = concentration at which 10% of daphnids are immobilised, EC<sub>50</sub> = concentration at which 50% of daphnids are immobilised.

184x169mm (150 x 150 DPI)

## 1 **Supplementary material**

2

### 3 1. Methodology

#### 4 *Chemical analysis of ashes*

5 pH and EC measurements were conducted on a 1:20 sample:water ratio solution (unground  
6 sample) after shaking for 5 minutes and allowing to settle for another 10 minutes (same  
7 procedure than for the leaching tests below but without filtering). pH was measured with a  
8 Crison micropH 2000 pH meter, with buffer solutions of pH 4, 7 and 9 and EC was measured  
9 with a Crison GLP 31 apparatus, previously calibrated with a 0.1 M KCl solution.

10

#### 11 *Total and soluble concentration of major and trace elements*

12 Total concentrations of major (Si, Al, Fe, Ca, Mg, Na, K) and trace elements (B, As, Cu, Ni,  
13 Pb, Cu, Hg) were determined in acid extracts of the samples (9 ml of HNO<sub>3</sub> 65% and 3 ml of  
14 HCl 37% added to 0.5 g of ground sample) after microwave digestion (Ethos Plus Milestone  
15 microwave) at 200 °C during 50 minutes. After digestion, the extracts were completed to 50 ml  
16 with ultrapure water (1:100 sample:solution). Certified soil standards were used to validate the  
17 method of trace metal extraction (SRM 2709a, SMR2710a, from NIST, U.S.A.), with a mean  
18 recovery rate of >93%) (Santín *et al.* 2015).

19 Leaching tests were carried out according to the methodology proposed by (Hageman 2007).  
20 3 g of unground ash samples were weighed into 125 ml bottles. Then, 60 ml ultrapure water  
21 (sample:water ratio 1:20) was added and the bottles were capped and shaken for 5 minutes.  
22 After shaking, the contents were allowed to settle for 10 minutes. The leachate was then filtered  
23 using a 0.45 µm pore-size nitrocellulose capsule filter. A sub-sample of the filtrate was  
24 collected in plastic bottles and refrigerated for ion chromatography analysis. Another sub-  
25 sample of the filtrate was acidified to pH < 1.5 with suprapur grade HNO<sub>3</sub> for atomic absorption  
26 spectroscopy and inductively coupled plasma mass spectrometry analysis.

27 Dissolved organic carbon (DOC) was analysed in a loop flow analysis system (Systea).  
28 Phosphate (PO<sub>4</sub><sup>3-</sup>) (given as Total Phosphorous - TP), Nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>)  
29 concentrations in leachate extracts were determined by colorimetry (Kempers 1974) using a  
30 Jasco V-630 spectrophotometer. Fluoride (F<sup>-</sup>) concentrations were measured using an ion-

31 selective fluoride electrode (Metrohm 692 pH/Ion Meter). Sulfate and Cl<sup>-</sup> were determined by  
32 ion chromatography (Dionex 4500i system).

33 Major and trace elements in acid and leachates extracts were determined using Inductively  
34 Coupled Plasma Mass Spectrometry (ICP-MS) using a VARIAN 820-MS ICP-MS  
35 spectrometer, except; Fe, Si and Al that were determined by atomic absorption (PerkinElmer  
36 Spectrometer 1100B).

37

### 38 *PAHs Analysis*

39 Ash samples (~ 3 g) were extracted in Soxhlet with a 1:3 acetone:hexane mixture spiked with  
40 a mixture of six deuterated PAHs. The extracts were then cleaned-up using aluminium oxide  
41 and copper was added to remove sulphur interferences. PAHs were identified with a GC/MS  
42 Thermo mod DSQ II (Thermo Electron Corporation, Austin, TX USA). The GC (TRACE GC  
43 Ultra) was equipped with a DB-5 MS column (J&W Scientific Folsom, CA; 0.25 mm i.d., 0.25  
44 µm film, 60 m, 5% phenylsubstituted methylpolysiloxane phase) and helium was used as carrier  
45 gas. The operating conditions were: held at 50°C for 3 min, ramped to 200°C at 6°C min<sup>-1</sup> and  
46 finally to 315°C at 4°C min<sup>-1</sup> holding that temperature for 15 min. The sample was injected  
47 using an on-column injection system with the purge valve activated 30 s after the injection.  
48 Transfer line and ion source temperatures were held at 280°C and 220°C, respectively. The MS  
49 was operated in the electron impact (EI) mode at 70 eV and the spectrum divided into 8 SIM  
50 windows, each scanned for up to 7 molecular masses, previously optimized, for the  
51 corresponding analyses and the deuterated internal standards eluting in this windows.  
52 Quantitation of parent and alkylated PAHs was performed using Thermo Scientific™  
53 Xcalibur™ software package.

54 Chemical and reagents used during analysis (Suprasolv grade acetone, hexane, neutral alumina  
55 (70–230 mesh) and anhydrous sodium sulphate for analysis) were obtained from Merck  
56 (Darmstadt, Germany). A standard mixture of deuterated PAHs containing naphthalene-d8,  
57 biphenyl-d10, anthracene-d10, dibenzophthiophene-d8, pyrene-d10 and benz[a]pyrene-d12 was  
58 obtained from CIL (Massachusetts, USA). A PAH mixture containing Naphthalene, Biphenyl,  
59 2-Methylnaphthalene, 1-Methylnaphthalene, 2,3-Dimethylnaphthalene, Acenaphthylene,  
60 Acenaphthene, 2,3,6-Trimethylnaphthalene, Fluorene, Dibenzothiophene, Phenanthrene,  
61 Anthracene, 4-Methyldibenzothiophene, 2-Methylphenanthrene, 2,8-  
62 Dimethyldibenzothiophene, 1,6-Dimethylphenanthrene, Fluoranthene, 2,4,7-

63 Trimethyldibenzothiophene, Pyrene, 1,2,8-Trimethylphenanthrene, 1-Methylpyrene,  
64 Benzo(c)phenanthrene, Benz(a)anthracene, Triphenylene, Chrysene, 2-Methylchrysene, 7,12-  
65 DimethylB(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(e)pyrene,  
66 Benzo(a)pyrene, Perylene, Indeno(1,2,3-c,d)pyrene, Dibenzo(a,h)anthracene, Benzo (g,h,i)  
67 perylene was prepared from mixtures from CPA Chem (Bulgaria) and Chiron (Trondheim,  
68 Norway).

69  
70 For the quality assurance and quality control of analysis (QA/QC), reagents blanks were  
71 analyzed and recovery procedures checked to assure that no contamination or losses occurred  
72 during extraction or other analytical procedures. Blanks result in no detectable PAHs  
73 concentrations and average recovery of PAHs ranged from 55 to 114 % for Acenaphthylene  
74 and Acenaphthene, respectively. The method detection limits (LOD) for individual PAHs  
75 calculated using the signal-noise ratio standard deviation were in the range of 0.25 to 2.62 ng  
76 g<sup>-1</sup> d.w. A minimum of five levels of a PAHs mixture standards were run with every batch of  
77 samples to build the linear regression curves by plotting the peak area ratios versus each PAH  
78 concentration. Four different sediments used in the lab in various intercalibration exercises  
79 organised by Quality Assurance of Information for Marine Environmental Monitoring  
80 (QUASIMEME) were used as internal reference materials (Viñas *et al.* 2009; Pérez-Fernández  
81 *et al.* 2015).

## 82 2. Tables

83

84 **Table 1. Total dry chemical composition of the six ash types tested (mg kg<sup>-1</sup>).**

85

	Ash type					
	AUS	USA	CAN	URIA	SPA	UK
87 <b>Al</b>	7000	22600	1320	10000	32800	2805
88 <b>Si</b>	2079	2068	1782	2376	2255	1595
89 <b>Ca</b>	177000	215000	163000	29400	133000	11800
90 <b>TP</b>	477	5342	5826	2418	1866	2645
91 <b>Na</b>	5043	4603	3113	3563	1123	663
92 <b>Mg</b>	9900	22000	12000	6400	5500	2700
93 <b>Mn</b>	510	710	830	1000	320	1430
94 <b>Fe</b>	4300	19100	979	8600	30600	7100
95 <b>Ni</b>	16	99	15	22	32	16
96 <b>Cu</b>	21	52	29	40	30	50
97 <b>Zn</b>	144	112	144	101	172	181
98 <b>As</b>	1.57	2.37	0.46	4.45	9.67	4.35
99 <b>Cd</b>	0.17	0.21	0.22	0.18	0.26	1.13
100 <b>Hg</b>	N.D	0.05	N.D	0.01	0.01	0.02
101 <b>Pb</b>	35	38	24	35	59	112

102 N.D (not detected) represents elements in concentrations <0.01 mg kg<sup>-1</sup>.

103

104

105

106

107

108

109 **References**

- 110 Hageman PL (2007) US Geological Survey field leach test for assessing water reactivity and  
111 leaching potential of mine wastes, soils, and other geologic and environmental materials.  
112 doi:10.3133/tm5D3.
- 113 Kempers AJ (1974) Determination of sub-microquantities of ammonium and nitrates in soils  
114 with phenol, sodiumnitroprusside and hypochlorite. *Geoderma* **12**, 201–206.  
115 doi:10.1016/0016-7061(74)90068-8.
- 116 Pérez-Fernández B, Viñas L, Franco MÁ, Bargiela J (2015) PAHs in the Ría de Arousa (NW  
117 Spain): A consideration of PAHs sources and abundance. *Marine Pollution Bulletin* **95**,  
118 155–165. doi:10.1016/j.marpolbul.2015.04.028.
- 119 Santín C, Doerr SH, Otero XL, Chafer CJ (2015) Quantity, composition and water  
120 contamination potential of ash produced under different wildfire severities.  
121 *Environmental Research* **142**, 297–308. doi:10.1016/j.envres.2015.06.041.
- 122 Viñas L, Franco MA, González JJ (2009) Polycyclic aromatic hydrocarbon composition of  
123 sediments in the Ría de Vigo (NW Spain). *Archives of Environmental Contamination*  
124 *and Toxicology* **57**, 42–49. doi:10.1007/s00244-008-9230-6.

125