

Chemical composition of wildfire ash produced in contrasting ecosystems and its toxicity to *Daphnia magna*

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Abstract. It is well established in the world's fire-prone regions that wildfires can considerably change the hydrological dynamics of freshwater catchments. Limited research, however, has focused on the potential impacts of wildfire ash toxicity on aquatic biota. Here, we assess the chemical composition and toxicity of ash generated from wildfires in six contrasting vegetation types distributed globally (UK grassland, Spanish pine forest, Spanish heathland, USA chaparral, Australian eucalypt forest and Canadian spruce forest). Acute (48 h) immobilisation tests were conducted on the extensively studied aquatic macroinvertebrate *Daphnia magna*, a sensitive indicator of aquatic contaminants. We found significant differences between the chemical composition and toxicity of these ash types. The UK and Spanish ash had no detectable toxicity to *Daphnia magna*, whereas the Australian eucalypt, USA chaparral and Canadian spruce ash all caused significant toxicity (immobilisation). The principal characteristics of the latter ash types were their high pH, and NO_3^- , Cl^- and conductivity levels. Elevated water-soluble and total concentrations of metals (e.g. Mn, Fe, Zn, Pb, Cu and As) and total polycyclic aromatic hydrocarbons (PAHs) were not linked to toxicity.

Additional keywords: bioassays, ecotoxicology, polycyclic aromatic hydrocarbons (PAH), wildfire impacts.

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Introduction

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

During wildland fires, combustion of fuels releases a wide range of organic and inorganic components into the atmosphere but also concentrates some of them into wildfire ash left on the ground (Bodí *et al.* 2014). Fresh wildfire ash typically consists of mineral materials and charred organic components, is non-cohesive, has a low density, and is not attached to the soil, which facilitates its mobilisation and transportation by post-fire water and wind erosion (Bodí *et al.* 2014; Abraham *et al.* 2017). The release of soluble elements and particulate matter from eroded ash and underlying soil into aquatic systems following fires can

cause increases in water turbidity, pH, organic matter, suspended sediment and conductivity and depletion of dissolved oxygen, among other effects (Smith *et al.* 2011; Tsai *et al.* 2017). Ash is however not usually examined as a distinct part of the post-fire sediment and few laboratory studies have characterised the composition of wildfire ash (Bodí *et al.* 2014).

The majority of the general studies into the effects of wildfire on water quality have focused on runoff amounts and nutrient levels and only more recently has increased research attention been given to pyrolytic substances, chemical elements and biological reactivity (Shakesby and Doerr 2006; Campos *et al.* 2012; Silva *et al.* 2015). Key areas receiving particular attention as a result of concern about their environmental effects are the production and mobilisation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (e.g. Vila-Escalé *et al.* 2007; Campos *et al.* 2012; Oliveira-Filho *et al.* 2018). Both present major biological concern owing to their carcinogenic potential, persistence within ecosystems and tendency to bio-accumulate (Smith *et al.* 2011; Chen *et al.* 2018). These contaminants are

Table 1. Fire and vegetation characteristics of the six ash types used in the present 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 understorey 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 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, extremely high-severity wildfire (Thomas fire) in 2018, achieving almost complete combustion of above-surface fuel
Canadian spruce (CAN)	North-west Territories (61°34'55"N; 117°11'55"W)	Very dense tree canopy comprising mostly black spruce (<i>Picea mariana</i>) and jack pines (<i>Pinus banksiana</i>) with very little understorey vegetation with the exception of young spruce and moss species	Very-high-intensity experimental crown fire in 2015, all fine fuels above ground were consumed. The forest floor was only slightly affected (<1 cm depth of burn)
Spanish heathland (URIA)	North-western Spain (43°6'17"N; 6°50'52"W)	Dominant species including heather (<i>Calluna vulgaris</i>), western gorse (<i>Ulex gallii</i>) and a variety of <i>Erica</i> sp. (e.g. <i>Erica tetralix</i>)	Extreme hot and dry conditions produced 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 produced 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 consisting of purple moor-grass (<i>Molinia caerulea</i>), soft rush (<i>Juncus effusus</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

thought to have complex impacts on water quality and the biological effects of these in aquatic systems have been observed to persist across long spatial and temporal scales (Earl and Blinn 2003; Costa *et al.* 2014).

Ash has also begun to receive increasing recognition as a source of diffuse contamination in freshwater systems and detrimental impacts on both lake and stream biota, including fish (e.g. Nunes *et al.* 2017; Oliveira-Filho *et al.* 2018; Gonino *et al.* 2019a), amphibians (Pilliod *et al.* 2003), macroinvertebrates (Brito *et al.* 2017) and algae (Campos *et al.* 2012) have all been observed. Highly variable impacts of ash contamination on freshwater biota have been reported between different ecosystems, types of ash, fires and species (Smith *et al.* 2011; Silva *et al.* 2015; Oliveira-Filho *et al.* 2018). Campos *et al.* (2012) and Silva *et al.* (2015), for example, found no significant impact of eucalypt ash on the planktonic crustacean *Daphnia magna* reproduction or immobilisation rates over a chronic (21 day) and acute (48 h) exposures respectively. Toxicity was, however, observed on several lower trophic level species in these studies, the bacteria *Vibrio fischeri*, algae *Pseudokirchneriella subcapitata* and the macrophyte *Lemna minor*. A similar study by Brito *et al.* (2017) tested toxicity over acute exposures (48 h) of three types of ash from the Brazilian Cerrado ecoregion on the planktonic crustacean *Ceriodaphnia dubia*, the fish *Danio rerio* and the mollusc *Biomphalaria glabrata* and found that all ash types caused toxicity to *C. dubia*, none affected *B. glabrata* and only one type was toxic for *D. rerio*. At higher trophic levels, negative impacts of Brazilian sugarcane ash have also been

observed on several native fish species (*Astyanax lacustris*, *Moenkhausia bonita* and *M. forestii*) over 24-h acute exposures but not for two non-native fish species (*Oreochromis niloticus* and *Poecilia reticulata*) (Gonino *et al.* 2019b). These studies demonstrate the variability and complexity of influencing factors in relation to the impacts of ash contamination on aquatic biota, highlighting the limited breadth of available research in this area (Hallema *et al.* 2018).

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

Materials and methods

Ash samples

Six composite ash samples were collected after wildland fires, before any rainfall, in each of the selected ecosystems types (Table 1): Australian eucalypt forest (AUS), USA chaparral (USA), Canadian spruce forest (CAN), Spanish heathland

(URIA), Spanish pine forest (SPA), UK grassland (UK). Fire and vegetation characteristics are summarised in Table 1. Each composite ash sample was sieved through a 1-mm mesh before chemical characterisation or use in the bioassays.

Chemical characterisation

Chemical characterisation of the six ash types collected was undertaken to determine the total and water-soluble concentrations of major (Ca, Cl^- , Mg, Na, Si, SO_4^{2-} , NO_3^-) and trace elements and compounds (Al, B, Cu, F^- , Fe, Ni, NH_4^+ , As, Cd, Hg, Pb and PO_4^{3-}), in addition to pH, dissolved organic carbon (DOC) and electric conductivity. This characterisation was undertaken using established methods (Plumlee *et al.* 2007; Santín *et al.* 2015, 2018; see supplementary material for full details).

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

Daphnia toxicity testing

Ecotoxicological assays consisting of acute ash exposures (48 h) were conducted using the planktonic crustacean *Daphnia magna*. This species is extensively used in ecological and toxicological studies as a sensitive indicator of the effects of contaminants on aquatic biota (OECD 2004; USEPA 2016). *Daphnia* spp. are also particularly relevant to freshwater lentic ecosystems (lakes, reservoirs and ponds) and ideal for investigating contamination potential in downstream waterbodies (Robinson and Thorn 2005; Nikinmaa 2014).

A monoclonal starter culture of *D. magna* was obtained from a long-term (2-year) rearing program. The new culture was reared and maintained according to recommended guidelines (OECD 2004; USEPA 2016), under controlled temperature ($20 \pm 2^\circ\text{C}$) and light conditions (uniform illumination of cool-white type, approximately 5000 lx; photoperiod 16 h light : 8 h dark) and fed every 2 days with a distilled suspension of *Pseudokirchneriella subcapitata* at ~ 0.1 – 0.2 mL per *Daphnid* per day.

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

The acute toxicity tests were conducted according to the OECD 202 (OECD 2004) guidelines, with the exception of full pH adjustment. pH was not adjusted to control levels ($\text{pH } 7.2 \pm 0.2$) in the bioassays to reproduce as close to natural conditions as possible, given pH is one of the most important factors affecting the toxicity and bioavailability of elements to freshwater species (Franklin *et al.* 2000). OECD 202 guidelines acknowledge that tests should be carried out without the adjustment of pH where values are within pH 6–9 at the highest test

concentration (OECD 2004). It is crucially important that pH adjustment does not cause significant changes to the test substances and owing to the complex and varying compositions and reactivity of wildfire ash, potential interactions are unclear. Little is known to date on wildfire ash concentrations in water bodies; therefore, a wide range of ash concentrations was tested, trying to represent the potential variability of different natural scenarios. Six different concentrations of the ash–medium solutions were used during testing (3.12, 6.25, 12.5, 25.0, 50.0, 75.0 g L^{-1}), plus four controls per concentration.

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

Statistical analysis

The water-soluble (leachates) chemical composition results were subjected to principal component analysis (PCA) (*RStudio version 5.4.1*) to identify constituents most strongly correlated with the different ash types. This approach to assessing the characteristic components in a given sample is widely used in environmental research when dealing with complex datasets (Brito *et al.* 2017). The leachates data were chosen for this analysis, as opposed to the total elements data, because this is likely the most bioavailable fraction and, therefore, the most likely to have affected the daphnia over an acute exposure.

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

Results

Ash chemistry

The total elemental composition of the six ash types overall contained several potential contaminants, but in highly variable concentrations (Supplementary material Table S1). The most abundant element in all samples was Ca (range 11 800–177 000 mg kg^{-1}) with Al (range 1320–22 600 mg kg^{-1}) and Fe (range 979–30 600 mg kg^{-1}) both present in high concentrations throughout. The elements found in the lowest total

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

Solubility of elements provided in brackets as a percentage (%) of the total ash composition. Electrical conductivity (*EC*) given in $\mu\text{S cm}^{-1}$. The symbol (–) is used to denote values not able to be calculated owing to the dry weight of the component not being tested or the value being 0

		Ash types											
		AUS		USA		CAN		URIA		SPA		UK	
(mg kg ⁻¹)	pH	11.1	–	11.2	–	10.3	–	10.3	–	9.1	–	7.9	–
	<i>EC</i>	3880	–	2570	–	2500	–	1505	–	233	–	293	–
	Al	0	–	4	(0.02)	0	–	20	(0.2)	0	–	0	–
	Si	45	(2.17)	182	(8.81)	27	(1.54)	133	(5.59)	25	(1.13)	27	(1.86)
	Ca	55	(0.03)	136	(0.06)	5864	(3.60)	580	(1.97)	1101	(0.83)	114	(0.97)
	PO ₄ ^{3–}	10	(0.65)	10	(0.06)	1	(0.01)	27	(0.35)	10	(0.17)	620	(7.50)
	NH ₄ ⁺	8	–	9	–	0	–	33	–	4	–	20	–
	DOC	496	–	130	–	1331	–	1272	–	93	–	198	–
	Cl [–]	1509	–	1494	–	1139	–	955	–	230	–	228	–
	NO ₃ [–]	207	–	232	–	206	–	104	–	24	–	26	–
	SO ₄ ^{2–}	4065	–	10 180	–	32 289	–	5600	–	3370	–	1203	–
	B	85	–	17	–	6	–	12	–	4	–	1	–
	Na	3893	(77.2)	831	(18.1)	860	(27.6)	1766	(49.6)	17	(1.49)	148	(22.3)
	Mg	377	(3.81)	26	(0.12)	3067	(25.6)	328	(5.13)	232	(4.23)	172	(6.36)
(μg kg ⁻¹)	F [–]	340	–	3260	–	460	–	5080	–	9300	–	800	–
	Mn	0	–	0	–	68	(0.01)	656	(0.07)	136	(0.04)	9292	(0.65)
	Fe	205	–	643	–	553	(0.06)	2172	(0.03)	406	–	4378	(0.06)
	Ni	0	–	0	–	0	–	844	(3.84)	0	–	59	(0.37)
	Cu	423	(2.01)	280	(0.54)	198	(0.68)	5158	(12.9)	147	(0.49)	340	(0.68)
	Zn	0	–	0	–	0	–	0	–	0	–	140	(0.08)
	As	18	(1.17)	26	(1.12)	6	(1.21)	329	(7.40)	102	(1.05)	259	(5.96)
	Cd	1	(0.42)	0	–	1	(0.59)	0	–	7	(2.83)	2	(0.18)
	Hg	1	(44.9)	2	(57.4)	2	(10)	1	(5.96)	1	(17.9)	1	(4.91)
	Pb	16	(0.05)	7	(0.02)	3	(0.01)	5	(0.01)	7	(0.01)	64	(0.06)

concentrations were: As (range 0.46–9.67 mg kg⁻¹), Cd (range 0.17–1.13 mg kg⁻¹) and Hg (range 0–0.05 mg kg⁻¹) (supplementary material Table S1).

pH and electrical conductivity (*EC*) measured in the leachates notably varied across ash samples, with pH levels ranging from moderately alkaline in the UK ash (7.9), to strongly alkaline in the USA ash (11.2). Equally, *EC* levels varied greatly from 233 $\mu\text{S cm}^{-1}$ in the SPA ash to 3880 $\mu\text{S cm}^{-1}$ in the AUS ash. High pH and *EC* values were both characteristic features of the ash types producing immobilisation of *Daphnia magna* tested (see *Acute toxicity test* section). pH within the bioassays themselves, however, was notably less variable (7.31–9.08), likely due to differences in dilution between leachates and the bioassay testing, and the addition of the culture medium in the latter.

The water-soluble (leachate) composition of the ash types was also highly variable (Table 2), with the most abundant components being SO₄^{2–} (range 1203–10 180 mg kg⁻¹), Cl[–] (range 228–1509 mg kg⁻¹) and Na (range 17–3893 mg kg⁻¹). The minor metal and metalloids elements were similarly the components found in the lowest concentration in the leachates: Cd (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 (range 1–2 $\mu\text{g kg}^{-1}$) (Table 2).

Some soluble elements occurred in particularly high levels, highlighting the variation in element content within the ash (Table 2). For example, in the UK sample, PO₄^{3–} (620 mg kg⁻¹) and metals such as Fe (4378 $\mu\text{g kg}^{-1}$) and Mn (9292 $\mu\text{g kg}^{-1}$) were notably high in comparison with the other ash types. There

were also notably high levels of, for example, Ca (5864 mg kg⁻¹) and SO₄^{2–} (32 289 mg kg⁻¹) in the CAN sample; B (85 mg kg⁻¹) and Na (3893 mg kg⁻¹) in the AUS sample; and Cu (5158 $\mu\text{g kg}^{-1}$) and As (329 $\mu\text{g kg}^{-1}$) in the URIA sample (Table 2).

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

PCA identified three primary components explaining 79% of the total leachates dataset variance (PC1 = 41%; PC2 = 23%; PC3 = 15%) (Table 3). PC1 is most strongly positively correlated with Mn, Fe, Zn, As, Pb and PO₄^{3–} levels and most strongly negatively correlated with pH, *EC*, NO₃[–], Cl[–], Hg and SO₄^{2–} (Table 3 and Fig. 1). A biplot of the standardised PC1 and PC2 values (Fig. 1) shows which components best characterised each ash type and pH, *EC*, NO₃[–], Cl[–], Hg and SO₄^{2–} were most closely correlated with the three ash types producing significant immobilisation of *D. magna*, whereas Al, Cu, Ni, NH₄⁺, As, Fe, Mn, PO₄^{3–}, Pb, Cd were more closely correlated with the three non-toxic ash types (Fig. 1).

Thirty-five PAHs were analysed across the ash types including the 16 USEPA priority PAHs, which provide the focus of the following discussion (Table 4). The total concentration of these

Table 3. Relative contribution of the 24 water-soluble ash constituents and parameters to four of the significant principal components (PCs) of the six ash types derived from principal components analysis

Cumulative proportion (%) of the variance explained by each principle component also provided. *EC*, electrical conductivity; *DOC*, dissolved organic carbon

	PC1	PC2
pH	−0.29	−0.11
EC	−0.26	−0.02
Al	0.00	−0.40
Si	−0.10	−0.26
Ca	−0.13	0.18
PO ₄ ^{3−}	0.27	0.08
NH ₄ ⁺	0.15	−0.36
DOC	−0.12	−0.16
Cl [−]	−0.27	−0.08
NO ₃ [−]	−0.27	0.01
SO ₄ ^{2−}	−0.18	0.15
B	−0.14	−0.03
Na	−0.16	−0.14
Mg	−0.14	0.17
F [−]	0.05	−0.10
Mn	0.28	0.07
Fe	0.27	−0.07
Ni	0.04	−0.39
Cu	0.02	−0.40
Zn	0.27	0.10
As	0.23	−0.27
Cd	0.11	0.18
Hg	−0.19	0.14
Pb	0.26	0.10
Cumulative proportion (%)	0.41	0.64

priority contaminants ranged from 1155 to 14 078 ng g^{−1} ash, the highest total being found in the UK ash originating from an upland grassland ecosystem in south Wales (\sum 16 EPA PAHs: 12 336 ng g^{−1} ash) (Table 4). Notably high PAHs concentrations were also found in the CAN (\sum 16 EPA PAHs: 7486 ng g^{−1}) and the SPA ash (\sum 16 EPA PAHs: 4393 ng g^{−1} ash) (Table 4).

The proportion of the methylated and non-methylated PAHs was very similar in all the samples with approximately three times more non-methylated PAHs in each ash type, except the USA ash, which contained over 15 times the amount of non-methylated PAHs (Table 4). There was also a predominance of two-ring PAHs in all the samples. Generally, the quantity of each ring type decreases sequentially with the number of rings, 2 > 3 > 4 > 5 and 6 with the exception of the USA sample, which had a relatively similar quantity of three-, four-, five- and six-ring PAHs. The predominant two-ring PAH in all samples was naphthalene. Phenanthrene was the most common three-ring PAH, except in the UK sample where it was acenaphthylene. All three of these abundant PAHs (naphthalene, phenanthrene and acenaphthylene) are classified as EPA priority contaminants (Table 4).

Acute toxicity test

High levels of *D. magna* immobilisation were recorded at both 24- and 48-h exposure for three of the six ash types tested: AUS, USA and CAN (*P* < 0.001 for all three ash types) (Fig. 2; Tables 5

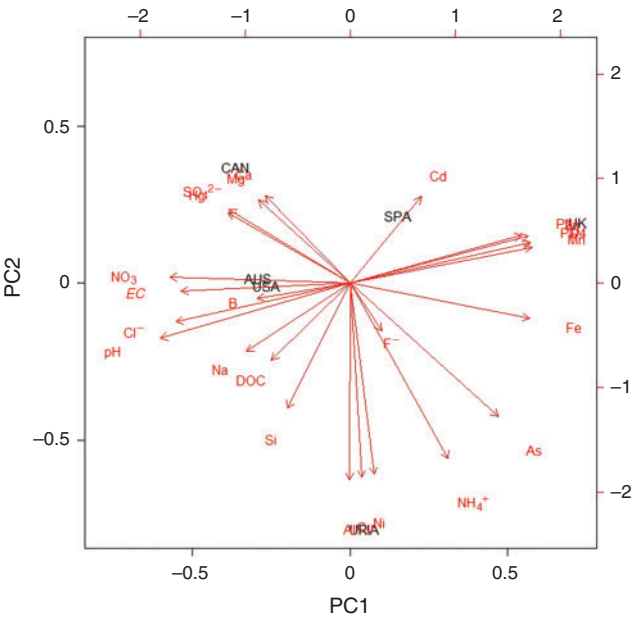


Fig. 1. Representation of the ordination in the first two axes (PC1 and PC2) produced by principal component analysis (PCA) of the water-soluble chemical composition of the six ash types studied. *EC*, electrical conductivity.

and 6). The response relationships identify the AUS ash as the most toxic, with a 100% immobilisation of *D. magna* individuals at less than 25 g ash L^{−1} within the first 24 h of exposure (Table 5; Fig. 2). The immobilisation effect of both the North American ash samples (USA and CAN) were relatively similar, with 48-h EC₅₀ being achieved at 20 and 26 g ash L^{−1} respectively, despite the notably different source vegetation (Table 6; Fig. 2). In contrast, no significant immobilisation occurred in response to the remaining three ash types (URIA, SPA and UK) (Tables 5 and 6). The UK ash did not produce any observable immobilisation across any of the test concentrations after 48 h of exposure. The Spanish samples (URIA, SPA) only produced low rates of immobilisation at the highest concentrations (Tables 5 and 6).

Discussion

Overall ash chemical properties

The total concentration of each element within the six ash types showed a wide variability (Supplementary material Table S1). These variations may be explained by the accumulative capacity of the different vegetation types, taking up different levels of elements from the soil and surrounding environment (Peralta-Videa et al. 2009; Brito et al. 2017). Fire dynamics (e.g. burn temperature) and soil properties are also important features in the composition of elements within ash (Pitman 2006; Bodí et al. 2014; Chen et al. 2018). In general, oxides and hydroxides of Ca, Mg, Si and P particularly tend to be abundant in wildfire ash (Pereira and Úbeda 2010; Silva et al. 2015) as found in the ash tested here (Supplementary material Table S1).

Overall, the water solubility of the studied elements in all ash types was low (<20% except for Na and Hg). This agrees with previous findings (Khanna et al. 1994; Santín et al. 2015; Silva et al. 2015; Brito et al. 2017). The most abundant ions in all

Table 4. Concentration and composition of polycyclic aromatic hydrocarbons (PAHs) found in each ash type
PAHs with the notation † are US Environmental Protection Agency priority PAHs (Keith 2015)

PAH (ng g ⁻¹)	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)perylene†	9.6	18.2	92.5	33.0	2.8	40.7
2-Methylnaphthalene	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-Dimethylbenz(a)anthracene	2.9	0.5	1728.4	64.2	90.9	12.5
Σ16 PAHS	1155	1445	7486	4393	1476	14 078
Σ35 PAHS	2024	2336	15 360	8521	3586	20 408
ΣMethylated	546	131	5608	2031	1063	45 25
ΣNon-methylated	1479	2205	9752	6489	2523	158 83
% Methylated	27	6	37	24	30	22

leachates were SO_4^{2-} , Cl^- and Na^+ (Table 2), likely owing to them forming very soluble salts (i.e. sulfates or chlorides). These components are thus commonly found in high concentrations in the dissolved residue of ash (Freitas and Rocha 2011; Santín *et al.* 2015) (Table 2). In contrast, heavy metals such as Cd, Ni and Zn showed the lowest concentration in the leachates owing to being relatively insoluble in alkaline (pH 8–10) conditions, precipitating mainly as hydroxides (Weiner 2012). These results are similar to those found in other studies assessing post-fire runoff and ash leachates in a range of ecosystem types (Jung *et al.* 2009; Pereira *et al.* 2011) and in agreement with the general concentration trend of alkali (Na, K) > alkaline (Ca, Mg) >> heavy metals (Pb, Cd and Hg) found by Santín *et al.* (2015) in eucalypt forest ash.

Ash types and element solubility

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

The PCA analysis allowed detection of key differences in the composition of the ash types studied here. The UK ash leachate has a distinctly soluble profile in comparison with the others. PCA analysis shows several heavy metals (Mn, Fe, Zn and Pb) and PO_4^{3-} to be characteristic elements of the UK ash leachate (Fig. 1). This leachate shows high

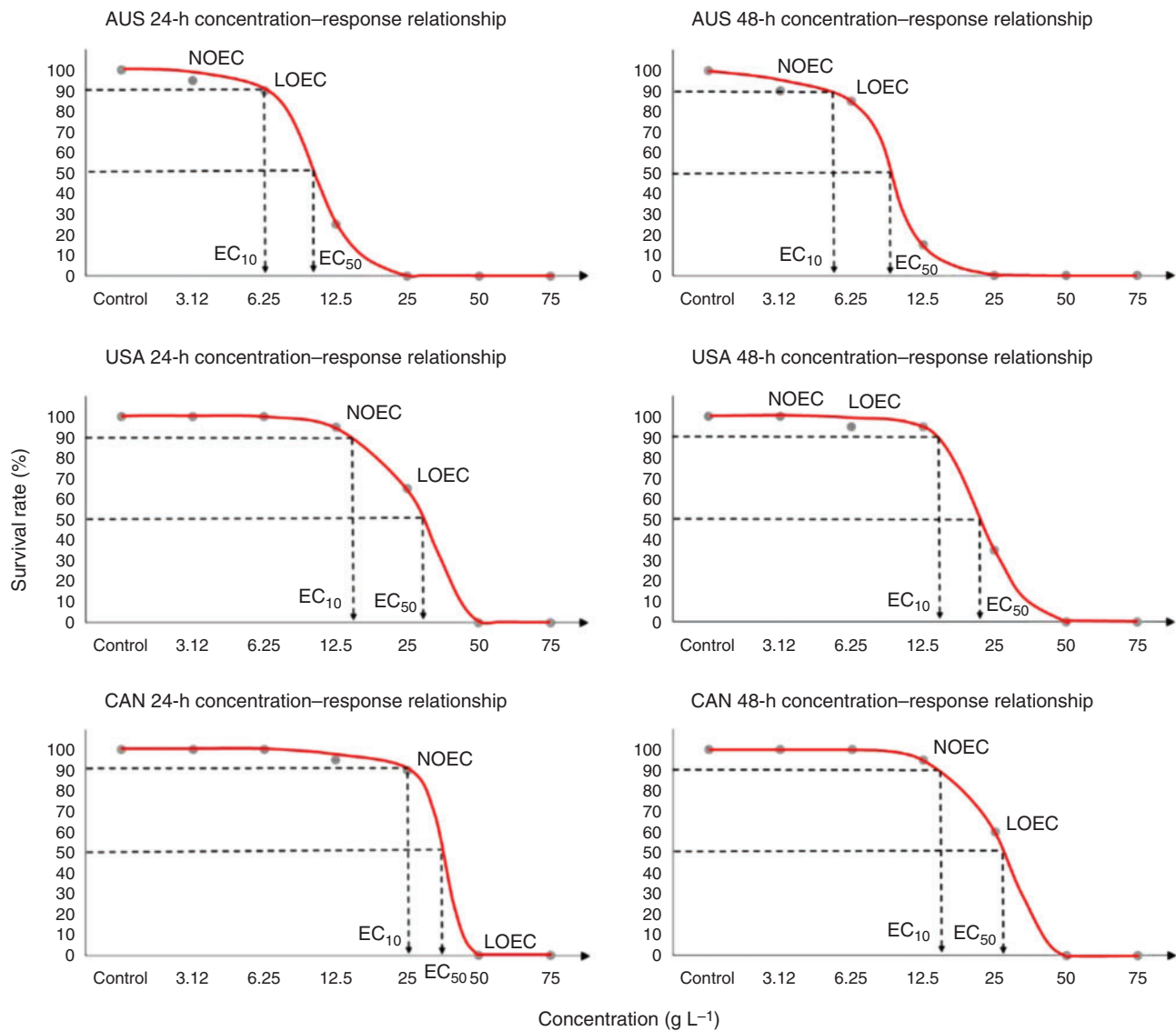


Fig. 2. Concentration response relationship after 24 and 48 h of exposure. NOEC, no observed effect concentration; LOEC, lowest observed effect concentration; EC₁₀, effect concentration at which 10% of daphnids are immobilised; EC₅₀, effect concentration at which 50% of daphnids are immobilised.

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

Estimates of Lowest Observed Effect Concentration (LOEC) (Dunnett’s test; $P < 0.05$), EC₁₀ and EC₅₀ (g L⁻¹). One-way analysis of variance P values also provided, testing if observed immobilisation of each ash type was significantly different to the control. Effect concentration (EC_x) refers to the concentration of substance required to produce x% (10 or 50) of the test individuals immobilised. The symbol (–) is used to denote values not able to be calculated

	Concentration (g L ⁻¹)							pH	LOEC (g L ⁻¹)	EC ₁₀ (g L ⁻¹)	EC ₅₀ (g L ⁻¹)	<i>P</i> value
	Control	3.12	6.25	12.5	25	50	75					
AUS	0	5	10	75	100	100	100	8.81	6.25	6.25	11	<0.001
USA	0	0	0	5	35	100	100	8.78	25	14	30	<0.001
CAN	0	0	0	5	10	100	100	8.23	50	25	37	<0.001
URIA	0	0	0	0	0	0	5	8.17	–	–	–	0.451
SPA	0	0	0	0	0	5	0	7.88	–	–	–	0.451
UK	0	0	0	0	0	0	0	7.56	–	–	–	–

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

Estimates of Lowest Observed Effect Concentration (LOEC) (Dunnett's test; $P < 0.05$), EC_{10} and EC_{50} ($g\ L^{-1}$). One-way analysis of variance P values also provided, testing if observed immobilisation of each ash type was significantly different to the control. Effect concentration (EC_x) refers to the concentration of substance required to produce $x\%$ (10 or 50) of the test individuals immobilised. The symbol (–) is used to denote values not able to be calculated

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

concentrations of soluble Fe, Mn and PO_4^{3-} in comparison with the other ash types (Table 2). The pH (7.9) of the UK leachate was 1 to 3 units lower than the extracts from the other samples (Table 2). These less alkaline conditions favour the solubility of metals and P compared with the other samples where the metals tend to precipitate as hydroxides for pH values above 8–9 and the phosphate as hydroxyapatite for pH values >8.5 (for example see: Diaz *et al.* 1994; Stumm and Morgan 1995).

A characteristic component of the CAN sample (identified by PCA, Fig. 1) was the high levels of soluble Ca, despite the total concentration in dry ash being relatively similar to that of the AUS, SPA and USA ash (Table 2). It is unclear why the solubility of Ca is notably higher in the CAN ash in comparison with the other ash types (Jung *et al.* 2009; Brito *et al.* 2017), but it may be responsible for the reduced PO_4^{3-} levels ($1.2\ mg\ kg^{-1}$) in the CAN leachate as P has a tendency to precipitate in the presence of Ca (Diaz *et al.* 1994). This P–Ca interaction may influence algal and cyanobacterial growth (and thus, eutrophication) by regulating P levels in freshwater systems (Bladon *et al.* 2008; Blake *et al.* 2009). In the broader context, Ca is not normally considered hazardous, but can significantly influence the overall toxicity of ash eluates (e.g. its strong relationship with SO_4^{2-} leaching) (Mount *et al.* 1997; Tian *et al.* 2018). Stiernström *et al.* (2013) even propose that Ca might be one of the key elements responsible for the ecotoxicity of ash eluates on the crustacean *Nitocra spinipes*, despite Ca not being classified as individually ecotoxic. The CAN ash tested here produced significant immobilisation of *Daphnia magna* over the 48-h exposure also potentially influenced by its high Ca concentration.

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

In the URIA ash, the most defining components were Cu, Al, Ni, NH_4^+ and As (Fig. 1). This ash contained comparatively high concentrations of soluble Cu ($5158\ \mu g\ kg^{-1}$) and the carcinogen As ($329\ \mu g\ kg^{-1}$). Similar elevated soluble levels of Cu have, however, been found in mixed eucalyptus ash (Cu 5100 – $6200\ \mu g\ kg^{-1}$) by Santín *et al.* (2015). The reason for the significantly higher solubility rate of Cu in this heathland ash (URIA 12.9%, range excluding URIA 0.49–2.01%) is worth further consideration to identify areas or components likely to increase the risk of Cu contamination. The concentration of As, although elevated in the URIA (and UK) sample here, has been reported as higher in several other wildfire ash samples (e.g. 4000 – $7300\ \mu g\ kg^{-1}$ in Santín *et al.* (2015); $42\ 000\ \mu g\ kg^{-1}$ in Silva *et al.* (2015)) and despite being above the $0.01\ mg\ L^{-1}$ World Health Organization's drinking-water guideline (World Health Organization 2011), it did not appear to cause significant immobilisation of *D. magna* in the URIA or UK ash.

The SPA ash has a relatively insoluble overall profile with notably high concentrations of the metals Al, Fe, Zn, Cu, Pb and the metalloid As in the dry ash (Supplementary material S1) but limited to no leaching of Al, Fe, Zn and Pb into the water-soluble extract (Table 2). Despite this, Cd presented as a distinct principal component of the SPA ash with a comparatively high soluble concentration ($7\ \mu g\ kg^{-1}$) and as the only sample to register a solubility percentage greater than 1% (2.85%). Similar dry quantities of Cd were recorded by Brito *et al.* (2017) assessing Brazilian Cerrado ash types (0.1 – $0.3\ mg\ kg^{-1}$) but Cd solubility was lower in these ash types ($<0.01\%$).

PAHs composition

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

The concentrations of PAHs found in the ash analysed here are also widely variable, with a range of $1155\ ng\ g^{-1}$ in the AUS ash to $14\ 078\ ng\ g^{-1}$ in the UK ash (16 USEPA priority PAHs) (Table 4). The values contained within the ash tested here are substantially higher than those presented by Olivella *et al.* (2006) testing wildfire ash from pine and oak forests ($\Sigma 12$ PAHs: 1 – $19\ ng\ g^{-1}$ ash). The lowest concentration,

found in the AUS ash type ($\sum 16$ EPA PAHs: 1155 ng g^{-1} ash), was of a comparable level with those found by Silva *et al.* (2015) assessing dry wildfire ash in a predominantly eucalypt ecosystem in Portugal ($\sum 16$ EPA PAHs: 1100 ng g^{-1} ash). The full range of PAH concentrations found here are within the range $1000\text{--}50\,000 \text{ ng g}^{-1}$ ($\sum 16$ EPA PAHs) found by Santín *et al.* (2017) analysing PAHs in pine forest floor and wood under wildfire charring and slow pyrolysis.

The UK ash shows a much higher PAHs concentration than the other types (Table 4). It is unclear why this is the case as no other research has been conducted on the PAHs composition of wildfire ash originating from comparable grassland ecosystems. The type of fuel and variations in combustion temperatures and oxygen availability are thought to strongly affect the concentration and type of PAHs in ash (Enell *et al.* 2008; Rey-Salgueiro *et al.* 2018). Chen *et al.* (2018) found that PAHs concentrations were significantly higher in black wildfire ash (moderate burn severity) in comparison with white wildfire ash (high burn severity). This was also true of the ash types tested here with the darker (dark grey-black) ash samples (UK, UR1A, CAN) containing a much higher concentration of PAHs than the lighter (light grey-white) samples (AUS, USA, SPA) (Table 4). Although variations in combustion completeness could be related to PAHs content here, the proportion of methylated PAHs in the UK ash is similar to that of the other samples tested (Table 4). The proportion of methylated/total PAHs is usually considered an indicator of combustion completeness as during combustion, the methylated component of the compound is lost first (Keilueit *et al.* 2012) (Table 4).

The high presence of low-molecular-weight and therefore, greater-volatility PAHs (i.e. Naphthalene (Naph) and Phenanthrene (Phe)) in the ash tested here may seem contradictory as it can be expected that these compounds would be lost during a fire. It is, however, likely that these PAHs preferentially recondense in the ash layer and are retained in microporous structures of pyrogenic material (Santín *et al.* 2017). Other studies support this idea, reporting high concentrations of Naph and Phe (Kim *et al.* 2011) or Naph, Chrysene (Chry), Benz(a)anthracene and Acenaphthylene (Campos *et al.* 2012) from wood burning. Ash studies of beech and similar species (Bundt *et al.* 2001) were dominated by Naph and by Benzo(ghi)perylene, Benzo(b)-fluoranthene, Benzo(k)fluoranthene, Chry, Triphenylene and Phe but at lower concentrations.

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

Implications for toxicology

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

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

The influence of key nutrients on *D. magna* immobilisation is perhaps less well established (Smith *et al.* 2011) (Fig. 1), as ions such as Cl^- and NO_3^- are required at minimum levels to support aquatic life. However, the PCA also identified high concentrations of Cl^- and NO_3^- as being key characteristic components of the three toxic ash types, particularly the more toxic AUS and USA ash (Table 3 and Fig. 1). Many anthropogenic (e.g. oil and gas production, irrigation methods and industrial and agricultural processes) and natural (e.g. sediment pore waters and burning) conditions have been shown to increase nutrient concentrations to toxic levels (e.g. Hoke *et al.* 1993; Ferreira *et al.* 2005; Mast and Clow 2008). Scott and Crunkilton (2000) demonstrated NO_3^- produces immobilisation of *D. magna* at 462 mg L^{-1} with no observable effect concentration at 358 mg L^{-1} . Similarly, Mount *et al.* (1997) estimated a concentration of $1000\text{--}2000 \text{ mg L}^{-1}$ as the concentration of Cl^- required to produce EC_{50} in *Ceriodaphnia dubia*. This suggests despite the correlations between NO_3^- and Cl^- with the toxic ash types found here, the relatively low quantities of

these components alone are not likely capable of causing the observed toxicity (Table 2). The limited number of studies focusing on Na^+ , Cl^- , SO_4^{2-} and NO_3^- exports after a fire have found maximum levels sampled in ash fall well below recommended safe limits (Smith *et al.* 2011).

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

Despite the variations in ash composition and the subsequent significant differences in *D. magna* immobilisation, it is difficult to isolate the primary causes of toxicity. In addition to the most likely, if indirect, influential parameters pH and EC, there are also likely components that are not necessarily toxic by themselves but could be variables influencing toxicity at certain concentrations (e.g. DOC, Na, Ca and Mg) (Freitas and Rocha 2011; Simplicio *et al.* 2016). Physical characteristics of the ash types may also be a possible cause of immobilisation as variations in particle size and distribution of the suspended particulate matter in the unfiltered samples used could have compromised the food intake and locomotive ability of *D. magna*, leading to immobilisation or death (Brito *et al.* 2017). Even when using a standardised laboratory approach, as employed here, it remains difficult to untangle the effects of such components from those caused by other variables in such complex samples (Wilde *et al.* 2006; Silva *et al.* 2015; Brito *et al.* 2017).

Earlier work has suggested macroinvertebrate species such as *D. magna* are less sensitive to contamination than lower trophic species (Campos *et al.* 2012), and thus the effects of ash contamination on these higher trophic organisms are expected to be primarily indirect through the propagation of toxicity across the food chain via bottom-up bioaccumulation processes (Abrantes *et al.* 2008). A few notable studies have demonstrated this premise with no observable effect of ash toxicity on daphnid survival or reproduction rates over both acute (48 h) and chronic (21 day) exposures, but significant impacts have been observed

on lower trophic species (bacteria, algae and macrophytes) (Campos *et al.* 2012; Silva *et al.* 2015). Understanding the mechanisms influencing the bioaccumulation and availability of ash contaminants in freshwater systems should thus be a focus of future research. The results presented here, along with other studies, appear to justify the concerns around the impacts of wildfire ash on aquatic biota and water quality even without the assessment of bioaccumulation processes (Campos *et al.* 2012; Brito *et al.* 2017).

Conclusion

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

Our results demonstrate significant immobilisation of *Daphnia magna* over acute exposure (48 h) to three of the six ash types (AUS, USA and CAN). The main characteristics of these ash types producing immobilisation, derived from PCA, were high PC values of pH, EC, NO_3^- , Cl^- , Hg and SO_4^{2-} . None of these components, however, appear likely to have directly caused the immobilisation response (ecotoxicity) observed. It is perhaps more likely that these components, and possible others (e.g. Ca), have contributed indirectly to the observed toxicity. Elevated water-soluble concentrations of metal and metalloid contaminants (Mn, Fe, Zn, Pb, Cu and As) did not produce any significant inhibition and tended to be characteristic of the non-toxic ash types. The total PAHs concentrations were also not linked to significant inhibition. It continues to prove difficult to identify specific causes of toxicity in aquatic biota using test substances as complex and variable as wildfire ash.

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

Conflicts of interest

The authors declare no conflicts of interest.

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