

Supplementary material

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

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1. Methodology

Chemical analysis of ashes

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

Total and soluble concentration of major and trace elements

Total concentrations of major (Si, Al, Fe, Ca, Mg, Na, K) and trace elements (B, As, Cu, Ni, Pb, Cu, Hg) were determined in acid extracts of the samples (9 ml of HNO₃ 65% and 3 ml of HCl 37% added to 0.5 g of ground sample) after microwave digestion (Ethos Plus Milestone microwave) at 200 °C during 50 minutes. After digestion, the extracts were completed to 50 ml with ultrapure water (1:100 sample:solution). Certified soil standards were used to validate the method of trace metal extraction (SRM 2709a, SMR2710a, from NIST, U.S.A.), with a mean recovery rate of >93% (Santín *et al.* 2015).

Leaching tests were carried out according to the methodology proposed by (Hageman 2007). 3 g of unground ash samples were weighed into 125 ml bottles. Then, 60 ml ultrapure water (sample:water ratio 1:20) was added and the bottles were capped and shaken for 5 minutes. After shaking, the contents were allowed to settle for 10 minutes. The leachate was then filtered using a 0.45 µm pore-size nitrocellulose capsule filter. A sub-sample of the filtrate was collected in plastic bottles and refrigerated for ion chromatography analysis. Another sub-sample of the filtrate was acidified to pH < 1.5 with suprapur grade HNO₃ for atomic absorption spectroscopy and inductively coupled plasma mass spectrometry analysis.

Dissolved organic carbon (DOC) was analysed in a loop flow analysis system (Systea). Phosphate (PO₄³⁻) (given as Total Phosphorous - TP), Nitrate (NO₃⁻) and ammonium (NH₄⁺) concentrations in leachate extracts were determined by colorimetry (Kempers 1974) using a Jasco V-630 spectrophotometer. Fluoride (F⁻) concentrations were measured using an ion-selective fluoride electrode (Metrohm 692 pH/Ion Meter). Sulfate and Cl⁻ were determined by ion chromatography (Dionex 4500i system).

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

PAHs Analysis

Ash samples (~ 3 g) were extracted in Soxhlet with a 1:3 acetone:hexane mixture spiked with a mixture of six deuterated PAHs. The extracts were then cleaned-up using aluminium oxide and copper was added to remove sulphur interferences. PAHs were identified with a GC/MS Thermo mod DSQ II (Thermo Electron Corporation, Austin, TX USA). The GC (TRACE GC Ultra) was equipped with a DB-5 MS column (J&W Scientific Folsom, CA; 0.25 mm i.d., 0.25 µm film, 60 m, 5% phenylsubstituted methylpolysiloxane phase) and helium was used as carrier gas. The operating conditions were: held at 50°C for 3 min, ramped to 200°C at 6°C min⁻¹ and finally to 315°C at 4°C min⁻¹ holding that temperature for 15 min. The sample was injected using an on-column injection system with the purge valve activated 30 s after the injection. Transfer line and ion source temperatures were held at 280°C and 220°C, respectively. The MS was operated in the electron impact (EI) mode at 70 eV and the spectrum divided into 8 SIM

windows, each scanned for up to 7 molecular masses, previously optimized, for the corresponding analyses and the deuterated internal standards eluting in this windows. Quantitation of parent and alkylated PAHs was performed using Thermo Scientific™ Xcalibur™ software package.

Chemical and reagents used during analysis (Suprasolv grade acetone, hexane, neutral alumina (70–230 mesh) and anhydrous sodium sulphate for analysis) were obtained from Merck (Darmstadt, Germany). A standard mixture of deuterated PAHs containing naphthalene-d8, biphenyl-d10, anthracene-d10, dibenzophthiophene-d8, pyrene-d10 and benz[a]pyrene-d12 was obtained from CIL (Massachusetts, USA). A PAH mixture containing Naphthalene, Biphenyl, 2-Methylnaphthalene, 1-Methylnaphthalene, 2,3-Dimethylnaphthalene, Acenaphthylene, Acenaphthene, 2,3,6-Trimethylnaphthalene, Fluorene, Dibenzothiophene, Phenanthrene, Anthracene, 4-Methyldibenzothiophene, 2-Methylphenanthrene, 2,8-Dimethyldibenzothiophene, 1,6-Dimethylphenanthrene, Fluoranthene, 2,4,7-Trimethyldibenzothiophene, Pyrene, 1,2,8-Trimethylphenanthrene, 1-Methylpyrene, Benzo(c)phenanthrene, Benz(a)anthracene, Triphenylene, Chrysene, 2-Methylchrysene, 7,12-DimethylB(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(e)pyrene, Benzo(a)pyrene, Perylene, Indeno(1,2,3-c,d)pyrene, Dibenzo(a,h)anthracene, Benzo (g,h,i) perylene was prepared from mixtures from CPA Chem (Bulgaria) and Chiron (Trondheim, Norway).

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

2. Tables

Table 1. Total dry chemical composition of the six ash types tested (mg kg⁻¹).

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

N.D (not detected) represents elements in concentrations <0.01 mg kg⁻¹.

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