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2 **Wood ash residue causes a mixture of growth promotion**
3 **and toxicity in *Lemna minor***

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25

26 **Abstract**

27 The use of wood as a sustainable biofuel results in the generation of residual wood ash. The ash
28 contains high amounts of plant macronutrients such as phosphorus, potassium, calcium as well
29 as several micronutrients. To explore the potential use of wood ash as a fertiliser, the growth
30 enhancing properties of Sitka spruce (*Picea sitchensis* Bong.) wood ash were contrasted with
31 the potential toxic action, using common duckweed (*Lemna minor* L.) as a model test species.
32 The growth of *L. minor* exposed to wood bottom and fly ash solids and corresponding leachates
33 was assessed in ultra-oligotrophic and eutrophic media. Ash solids and leachates were also
34 tested as neutralized preparations. Suspended ash solids promoted *L. minor* growth up to
35 concentrations of 2.5-5 g/L. Leachates promoted growth up to 10 g ash equivalents per litre,
36 but for bottom ash only. Beneficial effects of wood ash were most pronounced on ultra-
37 oligotrophic medium. However, on such nutrient-deficient medium severe inhibition of *L.*
38 *minor* biomass and frond growth was observed at relatively low concentrations of fly ash (EC_{50}
39 = 14 g/L). On standard, eutrophic medium, higher concentrations of fly ash (EC_{50} = 21 g/L), or
40 neutralized fly ash (EC_{50} = 37 g/L) were required to impede growth. Bottom ash, or neutralized
41 bottom ash retarded growth at concentrations of 51 g/L and 74 g/L (EC_{50}), respectively, in
42 eutrophic medium. It appears that phytotoxicity is due to the elemental composition of the ash,
43 its alkaline character, and possible interactions between these two properties. Growth
44 promotion was due to the substantial content of plant nutrients. This study underlines the
45 importance of the receiving environment (nutrient status and pH) in determining the balance
46 between toxicity and growth promotion, and shows that the margin between growth promoting
47 and toxicity inducing concentrations can be enlarged through ash neutralization.

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51 **Keywords:** Wood ash, ash suspension, ash leachate, solid waste, toxicity, growth promotion,
52 pH effect

53

54 **1 Introduction**

55 The increased use of biofuels as a component of sustainable energy portfolios, results in
56 increased ash production (Demirbas et al., 2009; James et al., 2012; Kuba et al., 2008; Thurdin
57 et al., 2006; Vassilev et al., 2010). This ash consists mostly of inorganic mineral matter, together
58 with smaller amounts of char and organic mineral solids, as well as fluid to gaseous inclusions
59 of both inorganic and organic matter (Vassilev et al., 2013). In order to dispose of large amounts
60 of wood ash, numerous potential after-use options for these complex materials have been
61 proposed and are practised. *Inter alia*, these include the use of ash for soil fertilization,
62 production of construction materials and sorbents as well as the use of ash for element/mineral
63 recovery (Vassilev et al., 2013). Notwithstanding the valuable plant nutrient content of ash, the
64 bulk of biomass energy ashes is still defined as waste and often disposed of in landfill. Yet,
65 given the increasing scarcity of commercial stocks of some plant nutrients (e.g. phosphate),
66 nutrient recovery needs to be considered.

67
68 Minerals contained in ashes originate from bio-accessible sources. Thus, returning such ashes
69 to the original ecosystem is considered by some as re-cycling. Indeed, wood ash applications
70 to, especially, temperate forest ecosystems have been trialled, and impacts on the soil and trees
71 have been assessed (i.e. Mandre et al., 2004; Augusto et al., 2008; Santalla et al., 2011). It has
72 been argued that ash from untreated biofuels (as opposed to timber treated with paint and/or
73 other preservatives) poses a comparatively low contaminant risk to the environment (Demeyer
74 et al., 2001; Emilsson, 2006; Koppejan and van Loo, 2012). However, the chemical
75 composition of biomass ashes can be extremely variable (Pitman, 2006; Vassilev et al., 2010),
76 and some ashes have been shown to contain a considerable contaminant burden (Pöykiö et al.,
77 2009; Vassilev et al., 2010). Therefore, neither the fertilising-value nor the environmental
78 toxicity of wood ash can be assumed without case assessment.

79
80 Modern biomass and solid fuel fired power plants produce two major residue fractions; bottom
81 ash (BA) and fly ash (FA). Additional precipitation techniques (i.e. cyclone or bag filters) allow
82 for further partitioning of the FA. Although different ash types accrue in separate parts of the
83 furnace, the waste streams are commonly combined and both ashes are collected in a single
84 waste bay. As a result few studies distinguish the two prime ash types (Park et al., 2012; Poykio
85 et al., 2011; Steenari et al., 1999). Rather, the literature on wood ash composition and recycling
86 describes either the composite material (Augusto et al., 2008; Demeyer et al., 2001; Eitigni and
87 Campbell, 1991; Pitman, 2006; Someshwar, 1996), or just one ash fraction (Aronsson and
88 Ekelund, 2006; Pöykiö et al., 2009; Steenari and Karlfeldt Fedje, 2010). Data on both the
89 toxicity and growth promoting potential of these distinct types of ashes from clean (i.e. un-

90 treated) wood fuel are scarce. Such data are important to inform policies for the recycling of
91 clean wood ash (i.e. see Emilsson, 2006; Haglund, 2008).

92

93 Standardised ecotoxicological testing of the impacts of ash on terrestrial organisms is common
94 practice, and typically involves testing the mobile fraction, for example water based ash
95 leachates (Barbosa et al., 2013; CEN, 2002; Jenner and Janssen-Mommen, 1993; Lapa et al.,
96 2002; Tsiridis and Samaras, 2006; Wadge and Hutton, 1987). Wood ash leachates may naturally
97 occur following heavy rain and flooding and in a “worst case” scenario can be leached into
98 downstream waterbodies. Similarly, suspended, solid wood ash can end up in the aquatic
99 environment. Given the complexity of ash, leached compounds may not be the only ones
100 determining environmental effects. Mineral, as well as organic matter from ash, have also been
101 shown to adsorb and precipitate dissolved elements and compounds, thus potentially altering
102 the native nutrient balance in the soil (Chirenje et al., 2006; Chojnacka and Michalak, 2009).

103

104 Standard aquatic toxicological testing has been used to quantify wood ash impacts on a range
105 of species (Barbosa et al., 2013; Stiernström et al., 2011). However, standardised testing with
106 photoautotrophic models (i.e. plants and algae) is based on supplying non-limiting nutrient
107 levels to a media, which will therefore mask any growth stimulating effect of ash. The
108 additional use of a nutrient-poor medium allows the assessment of such growth stimulating (i.e.
109 fertilizing) properties. The alkaline pH of wood ash creates a further dilemma for
110 ecotoxicological assessments. The validity of standardised toxicological test results is typically
111 conditional upon the pH being within the defined range of the test organism tolerance.
112 Therefore, the pH of non-neutral waste extracts is commonly adjusted to pH 6-8 (Lapa et al.,
113 2002; OECD, 2006; Römbke et al., 2009). This practice is inadequate when assessing the
114 toxicity of highly alkaline ash to be reintroduced to the natural environment, as any pH
115 dependent risk will be underestimated, while pH dependent changes in solubilisation and
116 speciation may be promoted (Barbosa et al., 2013).

117

118 This study set out to assess growth stimulating and toxic effects of clean wood ash on the
119 primary producer *Lemna minor* (L.). The study assesses these effects under different trophic
120 conditions, using both native and pH neutralized solid ash and ash leachate (Figure 1), to
121 generate a comprehensive overview of the potential impacts of ash recycling on this plant
122 species. Results will be discussed in the context of recent wood ash recycling recommendations.

123

124

125 **2 Material and Methods**

126 **2.1 Characteristics of wood ash and corresponding leachates**

127 *Origin and sampling*

128 The wood ash was collected from the conveyors of a 3.8 thermal MW rotating grate wood
129 boiler, located at a commercial sawmill in Co. Cork, Ireland. The wood-fuel comprised a
130 mixture of Sitka spruce sawdust, wood chips and bark shavings (sawmill wood processing
131 residues) which was burned at 700-800°C. The wood burned in the boiler was sourced locally
132 in south-west Ireland. Bottom ash (BA) accrues below the firing grates at the base of the boiler.
133 This type of ash contains heavy, large constituents such as clinker agglomerates and chunks of
134 char in addition to small, powderous particles. Fly ash (FA) was collected from the post-furnace
135 filter system where it had been transported with the flue gas. In contrast to bottom ash, fly ash
136 consists of powderous, light weight ash and small char particles. Ash samples were stored in
137 opaque 50 L barrels (HDPE, with clamp top lid) in a sheltered area at ambient temperature.

138

139 *Physico-chemical analyses*

140 Particle size distribution of bottom and fly ash was analysed in the range between 63 µm and
141 6.3 mm by dry sieving according to Deutsche Industrie Norm 18123 (DIN, 1996). Analysis of
142 loss on ignition (LOI) at 500 °C was performed for bulk ash samples following DIN 18128
143 (DIN, 2002). Ash sub-samples for each replication and leachate were dried at 30°C for 3-4 days
144 until the weight remained constant, and the particle fraction > 4 mm was removed. Leachates
145 were prepared according to the European Norm (EN) 12457-2 one stage leaching test for
146 granular waste (CEN, 2002) at 10 l/kg water ratio, with 24 h contact time and filtering
147 (Fisherbrand, FB 59031). Fresh leachates were applied in bioassays. Titration of ash leachates
148 was performed with 0.02 N H₂SO₄ to pH 4.

149

150 Chemical analyses of bulk solids (*aqua regia* extractable elements) and corresponding leachate
151 (water leachable elements and nutrient compounds), biological (BOD) and chemical oxygen
152 demand (COD) were performed by UKAS accredited (#0754) National Laboratory Services
153 (NLS, Leeds, UK). Total metal and metalloid content was determined by ICP-OES from *aqua*
154 *regia* digested reflux extractions. Water leachable concentrations were measured using ICP-
155 OES or MS in standard waste leachates generated according to British Standard (BS) EN
156 12457-2 with distilled water (10 l/kg). The relative mobility of elements was calculated as the
157 leachable proportion of an elements content in the parent solid.

158

159
160

Table 1 near here

161 **2.2 Growth inhibition test with *Lemna minor***

162 *Lemna minor* (L.) (Lemnaceae) is an aquatic macrophyte with close to ubiquitous distribution.
163 This species is commonly used in single substance phytotoxicity tests (OECD, 2006) as well as
164 for water quality assessment of wastewaters and leachates (Jenner and Janssen-Mommen, 1993;
165 Mackenzie et al., 2003). *L. minor* is described as a sentinel species for ash settling ponds of
166 coal fired power plants (Dorman et al., 2010). *L. minor* has a broad pH optimum and tolerates
167 conditions between pH 3-4 and 10.5 (McLay, 1976). *L. minor* single frond lifespan is reported
168 to be 31.3 days during which period it asexually produces daughter fronds (Lemon et al., 2001).

169

170 Axenic specimens were from University College Cork, School of Biological, Earth and
171 Environmental Sciences laboratory stocks. These stocks originated in the Blarney area of
172 southwest Ireland. The *Lemna minor* strain has been registered in the Rutgers Duckweed Stock
173 Cooperative (RDSC) database as strain number 5500 “Blarney”, and been subjected to detailed
174 genetic analysis (Horemans et al., 2015). *L. minor* was cultured on half-strength Hutner’s
175 medium (Lahive et al., 2011) in 1 L crystallizing dishes (Pyrex) covered with watch glasses.
176 Growth medium was renewed every two weeks and the laboratory culture stock was continued
177 from a sub-sample of its precursor. Culturing and bioassays were conducted using a 16/8 h
178 photoperiod (light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$) at $21 \pm 2 \text{ }^\circ\text{C}$.

179

180 *Growth inhibition assay*

181 Growth inhibition tests with *L. minor* were conducted following OECD guideline 221 (OECD
182 2006) recommendations. Effects of solid ash (i.e. ash suspensions) and ash leachates (according
183 to the EN 12457-2) on plant growth were tested, using a medium of either half-strength Hutner’s
184 or distilled water (Figure 1). Solid ash suspension gradients of Bottom Ash (BA) or Fly Ash
185 (FA) were prepared by pouring medium onto the appropriate weight of dry ash sample (particles
186 >4 mm excluded) followed by a 24 h suspension period. Bottom Ash Leachates (BAL) and Fly
187 Ash Leachates (FAL) test solutions were obtained as dilutions of fresh ash leachate in Hutner’s
188 medium (with appropriately reduced water content) or distilled water. Leachate concentrations
189 are expressed as ash equivalents per litre (g aeq/L). Test suspensions and leachates compliant
190 with the pH 6-8 guideline criterion (neutralized) were prepared by adjusting the medium to
191 pH 6.1 ± 0.7 using H_2SO_4 , after 24 h contact with the solid sample. Measurements of pH
192 (resolution 0.001) in the test medium were taken at the beginning and the end of the 7 d
193 experimental period while electrical conductivity (EIC, resolution 0.1 $\mu\text{S/cm}$) was determined

194 after the test (Multi 3420 SET G, WTW). Exposure vessels were 300 ml magentas (HDPE)
195 with punctured lids and cotton wool plugs. Clean test vessels were autoclaved prior to being
196 filled with the test dilutions and suspensions and afterwards to ensure batch sterility.

197

198

Figure 1

199 **2.3 Calculations and statistics**

200 Ash NPK content ratios were calculated as % wt of elemental N and assuming all P and K were
201 present as P_2O_5 and K_2O respectively. Enrichment factors for solids (EF_s , $[FA] \cdot [BA]^{-1}$, Pöykiö
202 et al., 2009) were calculated as ratio between fly and bottom ash *aqua regia* extractable solid
203 concentrations. Likewise, enrichment factors for leachate (EF_L , $[FAL] \cdot [BAL]^{-1}$) were based on
204 BS EN 12457-2 extract concentrations (Table 1). These measured concentrations represent a
205 tenth of total water soluble amounts and relative element mobility was calculated as ratio of
206 total soluble amount in 10 L to *aqua regia* extractable concentration per kg.

207

208 Biological endpoints of the *Lemna minor* exposure studies (Figure 1) were average specific
209 growth rates (OECD, 2006) for biomass fresh weight and frond number after 7 days.
210 Exponential growth rates (RGR) were calculated for increases in weight or frond number, using
211 the natural logarithm (Paolacci et al., 2016). Statistical analysis was performed with Graph Pad
212 Prism 5 (Graph Pad Software, La Jolla, USA). For plotting in Figures 3-4, *L. minor* growth in
213 each replication was normalized to the average growth rates in the controls (half-strength
214 Hutner's medium, SD shown as grey band). In ultra-oligotrophic medium, the ash treatment
215 exhibiting the best growth response was used for normalization and calculation of 10 and 50%
216 growth reduction Effect Concentrations (EC_{10} and EC_{50} , respectively). Significant difference to
217 the controls for No Observed Effect Concentrations (NOEC) and Lowest Observed Effect
218 Concentrations (LOEC) determination in each experiment were tested by one-way ANOVA
219 with Dunnett's post-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The number of replicates per
220 treatment was 4-5, with twice that number of control vessels.

221

222 **3 Results**

223 **3.1 Physico-chemical characteristics of wood ash**

224 *Ash solids*

225 Dried bulk samples of bottom and fly ash had similar bulk densities (BA; $0.27 \pm 0.04 \text{ g/cm}^3$
226 and FA; $0.21 \pm 0.001 \text{ g/cm}^3$). Loss on Ignition (LOI) was twice as high for fly ash compared to

227 bottom ash ($44.7 \pm 1.84\%$ and $25.1 \pm 4.05\%$, respectively), indicating higher levels of
228 combustible residue in fly ash. The average particle diameter of BA was 0.91 ± 0.08 mm, whilst
229 FA was much finer (0.19 ± 0.01 mm). Gravel sized particles due to ash melting (>2 mm) were
230 exclusive to BA. Removal of clinker and dross (>4 mm sized fractions, 15% of bottom ash),
231 strictly combustion products and not ash, resulted in a rather similar particle size distribution.
232 The sieved material was used to determine elemental content and biological impacts.

233

234 The N:P:K-ratios (% wt) of bottom ash (0.1:2.7:6.9) were slightly lower than those of fly ash
235 (0.2:2.8:8.6). A higher N concentration in fly ash was noted. P, K, Ca, Mg, Na and Mn contents
236 were similar in bottom and fly ash, and enrichment factors (EF_s expressed as fly ash over bottom
237 ash concentration) varied between 0.75 and 1.25 (Table 1). Fe and Al displayed relative
238 enrichment in BA compared to FA. Plant micronutrients were present in both ash types although
239 Zn, B, Mo were enriched in fly ash while Fe and Ni tended to present in higher concentrations
240 in bottom ash. Among non-essential trace elements, Ba showed a strong enrichment in bottom
241 ash (EF_s 0.06), while the heavy metal elements Co and Cr were slightly more abundant in
242 bottom ash. FA, in contrast, contained relatively higher amounts of Cd (EF_s 5.99), Pb, As and
243 Se (EF_s 1.39).

244

245 *Ash leachates*

246 Bottom ash leachates (BAL) exhibited both a lower pH and conductivity (pH 10.6 ± 0.18 and
247 3.6 ± 2.3 mS/cm, respectively) than those of fly ash (pH 11.5 ± 0.11 and 12.7 ± 6.43 mS/cm).
248 Titration to pH 4 yielded two equivalence points for bottom ash, BAL required
249 0.006 meq H_2SO_4 /ml for titration to pH 7. Fly ash leachate (FAL) exhibited only one
250 equivalence point and required 3.7-fold more sulphuric acid to neutralize. BAL had a greyish
251 brown tint, FA aqueous eluates were clear. Biological Oxygen Demand (BOD) was <1.4 mg/L
252 for both leachates. Chemical Oxygen Demand was very similar for the two types of ash
253 (46.2 ± 19.6 mg/L and 49.7 ± 1.26 mg/L in BAL and FAL respectively), although variability
254 was a magnitude higher for bottom ash leachates.

255

256 No ammonical N was detected (< 0.5 mg/L) in either ash leachate. FAL contained at least 6-
257 fold more total oxidized nitrogen (TON, Nitrate and Nitrite) than leachate of bottom ash
258 (Table 1). Orthophosphate was detectable only in BAL (2.2 mg/L). In terms of elemental
259 composition, the difference between the leachates was striking. BAL was enriched with P (EF_L
260 0.04), Mg (EF_L 0.18), V (EF_L 0.25), As (EF_L 0.34), B (EF_L 0.39) and Cu (EF_L 0.48). Fly ash
261 leachate contained relatively more K (EF_L 4.47), Ca (EF_L 36.6), Zn (EF_L 623) as well as
262 Al, Sr, Ba, Se, Ti, K, Cr, Mo, Pb and Na. Finally, saliferous chloride (EF_L 11.2) and sulphate

263 (EF_L 13.9) concentrations in fly ash leachate were more than an order of magnitude greater than
264 those in bottom ash leachate.

265

266 Relative mobility of elements (Table 1) was different for the two ash types. Some 21.3 and
267 23.1% of K and Na, respectively, were leached from BA into BAL. In comparison, 76.6 and
268 57.4% of K and Na, respectively, leached from FA into FAL. Other particularly mobile
269 elements in bottom ash were B, V, As, Cr, and Se. Strongly mobile elements in fly ash were
270 Cr, Ca, V, B, Ba, Sr, Se and Zn. Mo was entirely transferred into solution in the case of both
271 ashes.

272 **3.2 *Lemna minor* bioassays**

273 **3.2.1 Electrical conductivity and pH conditions in the test**

274 Electrical conductivity (EIC) of native ash suspensions in distilled H₂O and Hutner's media
275 differed due to the base electrical conductivity of the nutrient medium itself
276 (1660 ± 160 μS/cm). However, the difference in EIC between ultra-oligotrophic and eutrophic
277 test solutions decreased as ash concentration was increased (Figure 2A, D). The difference in
278 pH values of ultra-oligotrophic and eutrophic medium was substantial and related to the buffer
279 capacity and the slightly acidic pH 5.01 ± 0.28 of Hutner's medium. The difference in pH values
280 decreased with increasing ash concentration. Concentrated suspensions of fly ash in
281 oligotrophic or eutrophic medium displayed higher pH and EIC than respective bottom ash
282 suspensions. Similarly, FA leachates increased EIC and pH more than BA leachates (Fig. 3).
283 To determine potential pH effects on toxicity, another approach was instigated whereby
284 medium was neutralised at the start of the experiment. EIC of neutralized suspensions and
285 dilutions of neutralized leachates in Hutner's medium (Figure 4A, D) displayed the same ash
286 dose dependent increase as their respective native counterparts.

287

288 **3.2.2 *Lemna minor* growth on native ash solids and leachates under** 289 **differing trophic conditions**

290 *Suspended bottom ash solids*

291 When suspended in Hutner's medium, bottom ash concentrations of 1.25, 2.5 and 5 g/L neither
292 impaired nor benefitted the growth of the test organism relative to the corresponding control
293 (Figure 2B, C). However, BA concentrations of 40 g/L (p < 0.05, LOEC) and above (p < 0.001)
294 significantly decreased biomass growth rates. EC₁₀ and EC₅₀ for biomass growth rate were
295 10.1 g/L and 50.9 g/L (95% CI: 33 to 78.5 g/L, Table 2), respectively. The LOEC for frond

296 growth was lower than for biomass growth (20 g/L, $p < 0.05$) but EC_{10} and EC_{50} values were
297 similar.

298

299 When cultured under conditions of extreme nutrient scarcity (ultra-oligotrophic medium),
300 *L. minor* biomass average growth rates were just $38.5 \pm 19.2\%$ of those achieved in Hutner's
301 medium (lower dotted line in figure 2B). However, under these conditions the addition of solid
302 BA at concentrations of 1.25 and 2.5 g/L strongly stimulated biomass growth ($p < 0.01$). In fact,
303 the addition of these low concentrations of BA to the ultra-oligotrophic medium resulted in
304 biomass growth responses that were similar ($79.5 \pm 19.2\%$) to those achieved on the Hutner's
305 control medium. Frond growth rates (Figure 2C) were significantly stimulated at 1.25 g/L BA
306 ($p < 0.05$) only.

307

308 Higher concentrations of BA added to ultra-oligotrophic medium impaired the growth of plants
309 (Figure 2B, C). Up to BA concentrations of 40 g/L biomass and frond growth rates decreased
310 gradually, however at a concentration of 80 g/L BA both growth rates declined markedly
311 ($p < 0.05$). Compared to Hutner's medium, the biomass EC_{10} was higher on ultra-oligotrophic
312 medium.

313

314

Figure 2 near here

315

316 *Suspended Fly ash solids*

317 High concentrations of fly ash suspensions added to Hutner's medium caused negative effects
318 on plant growth (Figure 2E, F). These inhibitory effects occurred at lower concentrations than
319 observed for bottom ash, the plateau stage of the dose-response relationship in Hutner's
320 medium spanned the FA concentrations from 0.625 to 2.5 g/L. Significant reductions of
321 biomass growth occurred at 20 g/L ($p < 0.05$) and 40 g/L ($p < 0.01$) FA. The biomass growth
322 EC_{10} and EC_{50} in Hutner's medium were 8.6 g/L and 20.5 g/L (95% CI: 15.6 to 27.1 g/L,
323 Table 4), respectively. Again the LOEC for frond growth (10 g/L, $p < 0.05$) was lower but the
324 EC_{10} and EC_{50} values were the same as for biomass growth.

325

326 When low concentrations of solid FA were added to an ultra-oligotrophic medium growth was
327 stimulated (Figure 2E). Compared to the control, a significantly stronger growth response was
328 observed in medium with 0.625 and 1.25 g/L added FA ($p < 0.01$). Biomass of plants grown on
329 medium with 2.5 and 5 g/L fly ash also increased faster than the corresponding control
330 ($p < 0.05$). A significant reduction of biomass growth occurred at 40 g/L FA ($p < 0.05$, LOEC).
331 Biomass EC_{10} and EC_{50} for FA suspensions in ultra-oligotrophic medium were both slightly

332 lower than in Hutner's medium. The stimulation of frond growth was not significant. The
333 LOEC for frond growth was 40 g/L ($p < 0.01$) and EC_{10} and EC_{50} values were very similar to
334 the values found in FA supplemented Hutner's medium.

335

336 *Bottom ash leachates*

337 Bottom ash leachate added to Hutner's medium (Figure 3B), and diluted to 0.625 and 1.25 g
338 aeq/L, did not alter the biomass growth rate of *L. minor*. Higher concentrations of 2.5 to
339 20 g aeq/L BAL improved biomass growth compared to the control, but not significantly. A
340 significant reduction of biomass growth ($p < 0.001$) was observed at 40 g aeq/L (LOEC). Plants
341 exposed to 80 g aeq/L were necrotic. The calculated biomass EC_{10} was 25 g aeq/L, while the
342 EC_{50} for BAL was 33.1 g aeq/L (95% CI: 20.2 to 54.2 g aeq/L). The frond growth LOEC was
343 also 40 g aeq/L, while the EC_{10} and EC_{50} for BAL were 14.8 g aeq/L and 36.9 g aeq/L,
344 respectively.

345

346 Figure 3 near here

347

348 Plants grown on ultra-oligotrophic medium supplemented with BAL (Figure 3B) exhibited
349 significantly higher biomass growth rates in the concentration range between 0.625 to 10 g
350 aeq/L, when compared to the corresponding control. The fastest growth was observed on
351 medium with 2.5 g aeq/L BAL added. The biomass growth EC_{10} for BAL was calculated to be
352 25.9 g aeq/L. The EC_{50} was 43.9 g aeq/L (95% CI: 34.3 to 56.3 g aeq/L). In contrast, the frond
353 growth rate did not respond to increasing BAL doses in the nutrient deficient medium up to
354 40 g aeq/L. Plants were found to be necrotic at 80 g aeq/L.

355

356 *Fly ash leachates*

357 Fly ash leachate diluted in Hutner's growth medium (Figure 3B) did not significantly affect the
358 biomass or frond growth rates in the concentration range between 0.625 and 5 g aeq/L.
359 However, a significant reduction of biomass growth, compared with the control, was observed
360 at 20 (LOEC) and 40 g aeq/L ($p < 0.001$). The EC_{10} and EC_{50} were 6.92 g aeq/L and 17.8 g aeq/L
361 (95% CI: 13.2 to 24.1 g aeq/L), respectively. The LOEC for frond growth was lower
362 (10 g aeq/L, $p < 0.05$) than the one for biomass growth, but EC_{10} and EC_{50} values were very
363 similar.

364

365 When ultra-oligotrophic medium was supplemented with fly ash leachate, no significant plant
366 growth stimulation was observed, neither of biomass nor frond growth. Significant decreases
367 ($p < 0.001$) in biomass and frond growth rate were observed for the 20 (LOEC) and 40 g aeq/L

368 FAL treatments. In fact, plants were necrotic. The biomass EC₁₀ was slightly higher and the
369 EC₅₀ was lower compared to the equivalent values using Hutner's medium. Frond growth EC₁₀
370 and EC₅₀ were >10 g aeq/L and <20 g aeq/L FAL, respectively.

371

372 **3.2.3 Neutralized suspensions and leachate dilutions**

373 *Lemna minor* exposed to the neutralized suspensions of either bottom or fly ash solids in
374 Hutner's medium (Figure 4B, C) maintained biomass and frond growth up to relatively high
375 concentrations. Growth could be observed on Hutner's medium supplemented with 160 g/L
376 bottom ash solids or 80 g/L fly ash solids. Thus, dose-inhibition curves for bottom and fly ash
377 solids were stretched and quite flat, compared to those observed with non-neutralised ash
378 suspensions. For bottom ash suspensions, the biomass EC₁₀ and EC₅₀ were 9.7 g/L and 74.4 g/L
379 (95% CI: 56.7 to 97.6 g/L), respectively. For fly ash suspensions, the biomass EC₁₀ and EC₅₀
380 were 4.49 g/L and 37.1 g/L (95% CI: 25.9 to 53.3 g/L), respectively. Despite the lack of growth
381 inhibition at lower bottom ash concentrations, increasing chlorosis at frond edges could be
382 observed in plants on bottom or fly ash suspensions exceeding 10 g/L. Necrosis was not
383 observed with either of the two neutralized ash suspensions.

384

385 Figure 4 near here

386

387 Low concentrations of neutralized ash leachates added to Hutner's growth medium (Figure 4E,
388 F) had very little impact on biomass and frond growth rates. The shape of the dose-response
389 curve for plants exposed to neutralized BAL displayed an abrupt increase in effect severity
390 above 40 g aeq/L. The biomass growth rate EC₁₀ and EC₅₀ for neutralised BAL were 51.6 g
391 aeq/L and 87.9 g aeq/L (95% CI: 69 to 112 g/L), respectively. The shape of the dose-response
392 curve for plants exposed to neutralized FAL displayed a slightly more gradual decrease in
393 biomass and frond number growth, and EC₁₀ and EC₅₀ values were both markedly increased
394 compared to the equivalent values for neutralised BAL.

395 **4 Discussion**

396 **4.1 Wood ash solids and corresponding leachates**

397 *Wood ash solids*

398 Fast growing, and commercially important Sitka spruce (*Picea sitchensis* Dong.) is considered
399 to be a promising biofuel species for parts of western Europe. Combustion of this species
400 generates >2% w/w of ash (Owens and Cooley, 2013). Biomass ashes from grate fired boilers

401 contain substantial amounts of charred organic fuel residuals (Emilsson, 2006; Tollin, 2000).
402 In this study, highest levels of organic combustible residues were found in fly ash (44.7%), and
403 this value is within the commonly reported range of 7 to 50% (Someshwar, 1996). The average
404 particle diameter for FA is also similar to a reported FA particle size of 0.23 mm (Etitgni and
405 Campbell, 1991). BA generated in an industrial size furnace is coarser due to its clinker and
406 dross content. However, the removal of large molten agglomerates minimised the difference in
407 particle size distribution between FA and BA.

408

409 Contents of plant macronutrients in the two types of ashes differ only slightly. Bottom and fly
410 ash thus appear equally suited as sources of these essential elements. Levels of P and K found
411 in this study are above median concentrations reported for generic wood ash (Augusto et al.,
412 2008) and exceed cited literature values in Park et al. (2012) markedly. Minimum limit
413 concentrations are set for plant nutrients in wood ash as a prerequisite for ash application in
414 forests (Emilsson, 2006; Haglund, 2008; Pitman, 2006). Nutrients P, K and Mg in both bottom
415 and fly ash exceed required minimal concentrations of these plant nutrients. Thus, based solely
416 on plant nutrient contents, both ashes could be considered for land application. Nevertheless,
417 we note that wood ashes used in this study contain only about half as much Ca as expected from
418 medians reported in a meta-study of wood ashes by Augusto et al. (2008), and do not fully meet
419 the Swedish Ca requirements for wood ash of 125 g/kg (Haglund, 2008).

420

421 Our data show that the micronutrient and trace element concentrations in BA and FA are
422 distinct, likely implying distinct hazards. Relative enrichment of Fe and Al in bottom ash in this
423 study is higher than reported in Park et al. (2012) and Pöykiö et al. (2009) but matches earlier
424 findings (Narodoslawsky and Obernberger, 1996). B, Cu, Mn and Zn are present in both ashes
425 at levels that were previously reported (Augusto et al., 2008). Mo and Ni contents in the tested
426 Sitka spruce ashes are slightly lower than commonly reported. Among micronutrients, a
427 minimum nutrient content for ash spreading (Haglund, 2008) has been defined for Zn (0.5 g/kg)
428 only. BA supplies 65% of required Zn content, while FA exceeds the requirement by 3.6-fold.
429 Elements of concern, defined in 86/278/EEC (Council of the European Union, 1986), such as
430 Cd, Pb and Zn are enriched in fly ash, as was found by others (Park et al., 2012; Pöykiö et al.,
431 2009). As and Se are also enriched in fly ash, but the partitioning between FA and BA is less
432 pronounced than that reported by Park et al. (2012) and the opposite of what was described for
433 As by Pöykiö et al. (2009). Enrichment prevalence is commonly linked to condensation on fly
434 ash particles (Izquierdo and Querol, 2012; Narodoslawsky and Obernberger, 1996; Pitman,
435 2006) and also affected by incomplete combustion. Based on the comparison of the chemical
436 composition of Sitka spruce wood ash with a meta-analysis data set of various wood ashes
437 (Augusto et al., 2008), bottom ash from un-treated Sitka spruce can be considered above

438 average quality for its high content of P and K, and its low content of the toxic metals As and
439 Pb (below reported minimum values). In comparison, fly ash from *P. sitchensis* also has
440 relatively high levels of K and P, but contains above median levels of undesired Cd.
441 Furthermore, contents of the elements As, Pb, Cd, Cr, Hg, Ni and V in bottom and fly ash from
442 untreated Sitka spruce sawmill residues remain below published maximum allowable
443 concentrations (Haglund, 2008).

444

445 *Wood ash leachates*

446 The leachate from granular ash waste serves as a model for mobilization of ash constituents in
447 water. Given the use of distilled water as an eluent, the pH conditions during mobilization are
448 essentially determined by the alkaline pH of the wood ash. The titration profile of BAL exhibits
449 the same carbonate-like characteristics as published for general wood ash (Eitgni and
450 Campbell, 1991). This profile is, however, distinct from that of FAL which shows a strong
451 hydroxide presence. The alkalinity of FAL is 3.6 times higher than that of BAL. Conductivity
452 tests also reveal a much higher ionic strength and quantity of readily dissolvable components
453 in FAL.

454

455 Striking quantitative differences were observed in the concentrations of dissolved, plant
456 nutrients in leachate of the two ash types. Oxidized N is exclusive to FAL, while
457 orthophosphates are mostly dissolved in BAL (Table 1). The small amount of N in wood ash
458 is usually associated with unburned biomass and is largely insoluble, although small amounts
459 of N condensed on FA particle surfaces can be mobile (Demeyer et al., 2001; Someshwar,
460 1996). Small amounts of P and Mg are found in BAL, these elements are likely bound to the
461 silicate matrix (Izquierdo and Querol, 2012) and appear immobile in the case of fly ash. Among
462 macro elements, the leachates are quantitatively distinct in K, Ca, Na, Al, and SO₄, levels which
463 leach more readily from FA. Unsurprisingly, the saliferous alkali metals K and Na are the most
464 mobile elements in one stage leachates from both types of wood ash. Differences in particle
465 size and element composition of the two ash types are likely to contribute to distinct leaching
466 behaviour of nutrients and hazardous substances in the ash (Stiernström et al., 2014; Tsiridis
467 and Samaras, 2006; Wadge and Hutton, 1987). Thus, although nutrient contents of the solid
468 ashes are rather similar for bottom and fly ash, leachates prove to be distinct, with consequences
469 for plant growth.

470

471 4.2 Growth promotion

472 Wood ash contains a range of plant nutrients as well as contaminants (Table 1). Hytönen (2016)
473 showed that addition of wood ash to a peaty soil increased concentrations of extractable P, K,
474 Ca and Mg in the soil, and this was associated with growth stimulation. In ultra-oligotrophic or
475 eutrophic media the relative contribution of ash-derived plant nutrients to plant growth differs.
476 In the ultra-oligotrophic medium nutrients are virtually absent. Plant growth in the ultra-
477 oligotrophic water control (dotted lines in Figure 2 and Figure 3) is sustained for the short
478 duration of the test by nutrients stored in the plant. Under these conditions, the only nutrients
479 present are those supplied through wood ash. This study shows substantially increased growth
480 rates for *L. minor* in ultra-oligotrophic medium supplemented with ash (Figure 2B, C, E and
481 3B). In ultra-oligotrophic media, bottom ash solids and leachates, up to concentrations of
482 2.5 g/L and 10 g aeq/L respectively, increase biomass and frond growth significantly (Figure
483 2B and 3B). Fly ash stimulates biomass growth when applied as a solid (up to 5 g/L, Figure
484 2E). Osmotic stress in the ultra-oligotrophic medium (distilled H₂O) control (20 µS/cm) may
485 reduce plant growth performance, thus artificially emphasizing the stimulatory effects mediated
486 by ash. However, this scenario is unlikely, as significant increases in growth occur at ash
487 concentrations that barely affect electrical conductivity (i.e. Fig. 2; compare responses to 0.625
488 and 1.25 g/L ash). Therefore, growth promoting effects under oligotrophic conditions are likely
489 to be caused by improved nutrient supply. Given the key role that nitrogen plays in mediating
490 plant growth, it might be expected that fly ash leachate should cause a more pronounced growth
491 stimulation. However, Hutner's medium provides 560 mg/L NO₃ and the 10 g aeq/L fly ash
492 dilution only carries 0.7 mg/L NO_x, and therefore the latter is unlikely to significantly resolve
493 nitrogen deficiency. This also implies that even greater stimulation of biomass growth can be
494 expected when ash is supplemented with an N-source, and this is an aspect that has considerable
495 relevance for practical ash applications in, for example, forestry. There have been previous
496 reports of wood ash mediated growth enhancement. For example, Aronsson and Ekelund (2006)
497 showed that growth of the water moss *Fontinalis antipyretica* was increased when the growth
498 medium was supplemented with extracts of crushed wood ash. Nabeela et al., (2015) found that
499 the addition of low concentrations (<1g/kg) of wood ash to the soil stimulates growth of
500 *Brassica napus*, but that toxicity occurs when higher concentrations (>10 g/kg) are used.
501 Bonfim-Silva et al., (2015) found that incorporation of wood ash in the soil led to substantial
502 increases in height, leaf and tiller number of two species of grass. Growth stimulation increased
503 with ash concentration, reaching a plateau at around 12 g/L of wood ash, but no toxicity was
504 apparent at higher concentrations. Other studies did, however, fail to show any growth
505 stimulation by wood ash. For example, Park et al., (2005) failed to show enhanced biomass
506 production by willows (*Salix purpurea*) and it was hypothesised that this was due to the fact

507 that growth was predominantly limited by nitrogen. The diverse responses to wood ash
508 supplementation emphasise that wood ash mediated growth promotion is conditional upon the
509 receiving environment, and growth promotion is more likely on acidic soils low in K, Ca and/or
510 Mg (Augusto et al., 2008; Santalla et al., 2011). For example, Moilanen et al., (2013) showed
511 strong wood ash induced growth of Scots pine on peat substrate suffering P and K deficiency.
512 Our data confirm the growth stimulation potential of wood ash solids, and to a lesser extent
513 leachates, under controlled conditions, and show how these relate to the receiving environment.

514 **4.3 Phytotoxicity**

515 Lemnaceae are an excellent group of model species for ecotoxicity assessment, considered
516 representative for aquatic macrophytes, and also to a lesser extent for vascular plants in general.
517 *Lemna minor* ranks highly for tolerance against a broad range of metal and metalloid elements,
518 although the species is particularly sensitive to Co, Cr and Cu (Wu et al., 2013). *L. minor* is a
519 sentinel species (Dorman et al., 2010) that is commonly used for phytoremediation. This not
520 only suggests that obtained EC₅₀ outline an aquatic worst-case scenario but also facilitate the
521 distinction between growth promotion at low ash concentrations and toxicity at higher levels
522 for this study.

523

524 When exposed to bottom and fly ash, *Lemna minor* growth performance exhibits distinct dose-
525 response relationships (Figure 2, 3, 4). Fly ash is always more hazardous than bottom ash.
526 Severe toxic effect concentrations (EC₅₀, Table 2) of bottom and fly ash solids are significantly
527 different, regardless of the use of oligotrophic or eutrophic growth conditions or native or
528 neutralised ash applications.

529

530 Table 2 near here

531

532 Plant nutrition provided by the medium has no substantial effect on the toxicity of native fly
533 ash solids or leachates (Table 2). However, severe toxicity (EC₅₀) is decreased due to pH
534 neutralization (Table 2). For neutralized bottom ash leachates, both EC₁₀ and EC₅₀ are about 2-
535 fold higher compared to native samples, while the difference is 3-fold for neutralized fly ash
536 leachates. Thus, careful management of pH during fertilization with ash may avert detrimental
537 effects (toxicity) and this can have important management implications for ash spreading.

538

539 Phytotoxicity of wood ash and wood ash leachates can potentially be caused by several different
540 factors individually, as well as through interactions. Two main factors include toxicity of single
541 elements and adverse effects of extreme pH of the medium. Here we have explored these
542 factors, and assessed their potential role in causing phytotoxicity.

543

544 (i) We reviewed the literature for threshold and toxicity concentrations (EC_{10} and EC_{50}) for
545 single elements, and compared these concentrations with those present in wood ash and/or ash
546 leachate (Table 1). Naumann et al. (2007) rank the toxicity of toxic metals to *Lemna minor*
547 (based on thresholds as EC_{10}) as Ag^+ (6-14 $\mu g/L$) > Cd^{2+} > Hg^{2+} > Cr(VI) > Zn^{2+} > Cu^{2+} > Ni^{2+}
548 > Co^{2+} > Tl^+ > As (3-12 mg/L). Based on lowest reported EC_{50} values for metal and metalloid
549 elements under standard test conditions (Davis et al., 2002; Duester et al., 2011; Naumann et
550 al., 2007; Simmons, 2012; Wang, 1990; Wu et al., 2013), bottom ash leachate contains 0.44
551 toxic units (TU, as presented in Horvat et al., 2007) in contrast to 9.75 TU in fly ash leachate.
552 In bottom ash leachate, the toxic units are linked to the presence of the elements B, Cr and Cu
553 which make up 38.3, 31.9 and 21.3% of total TU, respectively. The elements Zn, As, Co, Tl
554 and Ni with 2.49, 2.26, 1.12, 0.91 and 0.59% further contribute to the overall TU load. In fly
555 ash the situation is essentially different. In fly ash 92.4% of total TU are contributed by
556 dissolved Zn while Cr, Co and B accounts for 4.46, 0.97 and 0.67%, respectively TU. Thus,
557 elemental contamination theoretically causes less than 50% effect of the toxic effect of bottom
558 ash leachate, while fly ash leachate, even when diluted 10-fold, may still reduce growth by
559 nearly 50%.

560

561 (ii) In eutrophic medium the reduction of *L. minor* growth coincides with a 10-fold decrease in
562 H^+ ion concentration from pH 7 to 8, irrespective of ash type and form of introduction. High
563 pH values were associated with a near total cessation of growth (Figure 2 and 3). *Lemna minor*
564 is reported to survive in the pH range between 3-4 and 10.5 (McLay, 1976). Growth optima
565 derived from regressions of average frond number growth rates place the pH optimum for
566 growth between 6.2 and 6.9 (McLay, 1976). The growth rate for fronds is reduced to an average
567 of about 80 and 50% of optimum growth at pH 9 and 10, respectively (McLay, 1976). In this
568 study it was found that when medium was neutralised following ash-addition (i.e. less alkaline
569 pH) toxicity decreased and in most cases both EC_{10} and EC_{50} values increased. Thus, we
570 conclude that the alkalinity of wood ash contributes to its phytotoxicity. However, the pH effect
571 is rather complex. For example, the addition of 1.25 g/L bottom ash to oligotrophic medium
572 increases the pH of the medium to 9.5, but this was associated with a marked stimulation of
573 growth (Figure 2B,C) consistent with other findings (Aronsson and Ekelund, 2006). Therefore,
574 it is concluded that observed ash toxicity is unlikely to be due to just the alkalinity of the
575 medium. Rather, it appears that at higher ash concentrations a toxicity threshold is approached
576 due to a combination of exposure to contained contaminants, and the alkaline nature of the ash.

577

578 **4.4 Conclusion**

579 Any wood ash after-use strategy has to reconcile the opposing aims of preventing contaminants
580 from re-entering ecosystems and recycling of beneficial plant nutrients. It is, in principle,
581 feasible to return minerals to the place where they were extracted from the soil by trees. This
582 study demonstrates both the plant growth promoting, as well as the toxic characteristics of wood
583 ash that fulfils the minimal element content criteria for spreading as a fertiliser. It is argued that
584 phytotoxicity is due to both the elemental composition of the ash, its alkaline character, and
585 possible interactions between these two factors. In turn, growth promotion is due to the
586 substantial content of plant growth nutrients. This study shows that the margin between growth
587 promotion and toxicity incurring concentrations can be enlarged through ash neutralisation.
588 Thus, the receiving environment (nutrient status and pH) determines the balance between
589 toxicity and growth promotion, and needs to be considered in any ash spreading strategy.

590

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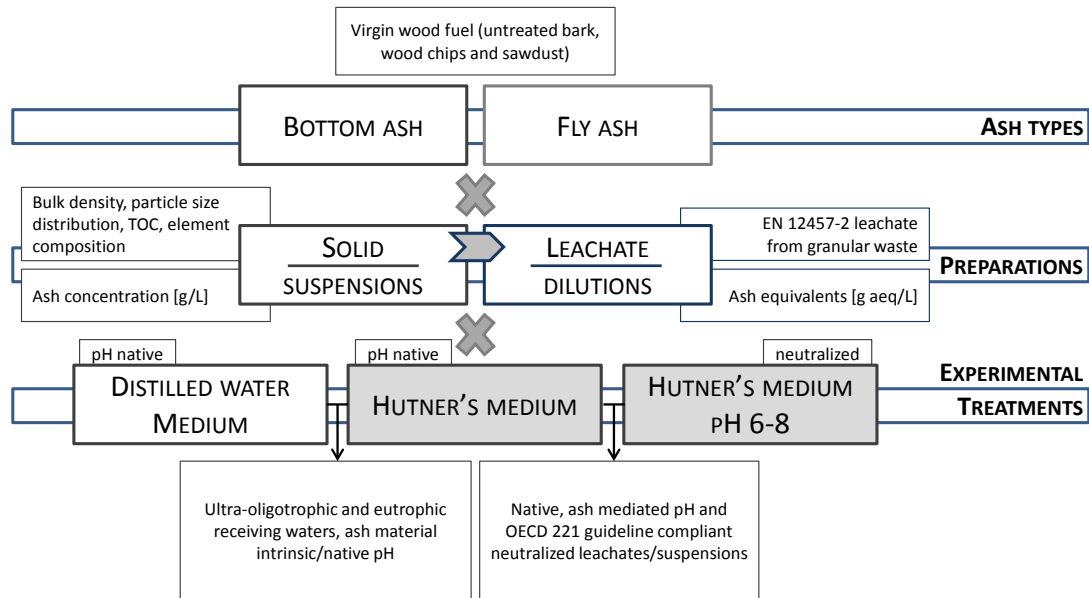
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728 **Tables and figures**

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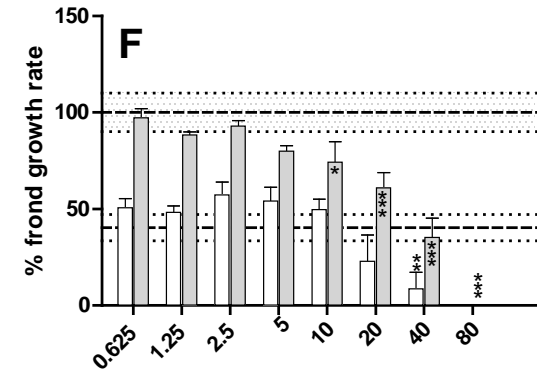
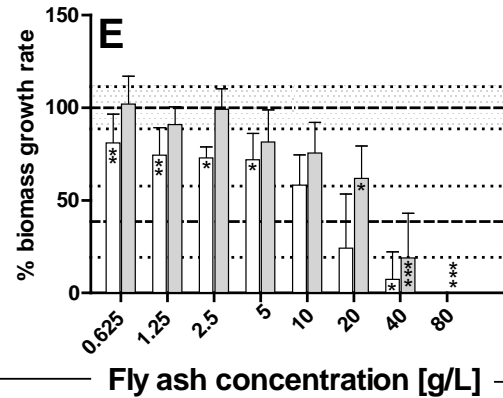
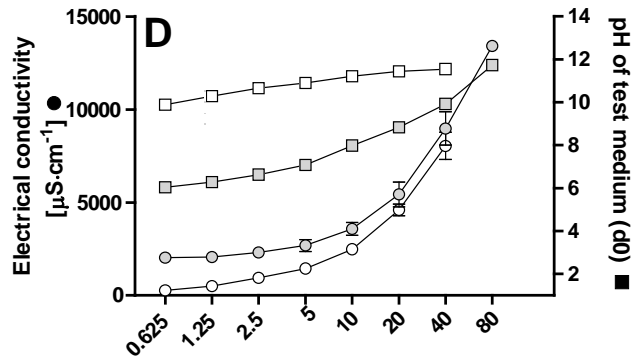
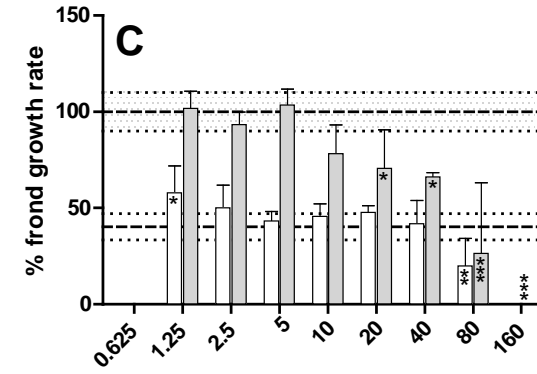
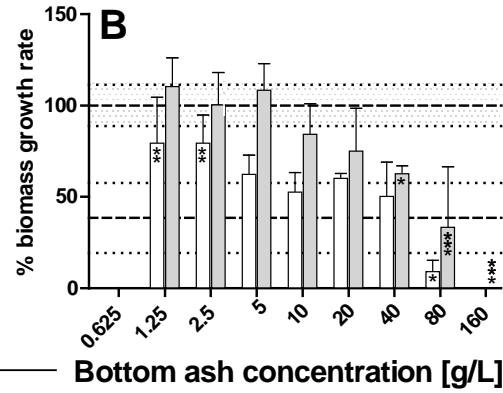
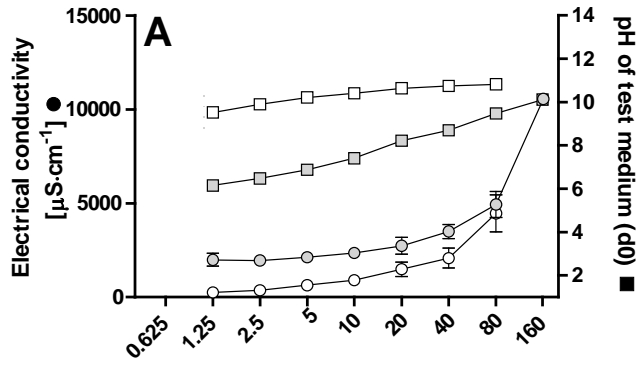
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Figure 1: *Lemna minor* biomass (fresh weight) and frond (number) growth rates were quantified following exposure to wood ash suspensions or leachates under ultra-oligotrophic or eutrophic (nutrient medium) conditions. Impacts on *L. minor* growth were also quantified for pH neutralised suspensions and leachates under eutrophic conditions. All experiments comprised four independent replicates.

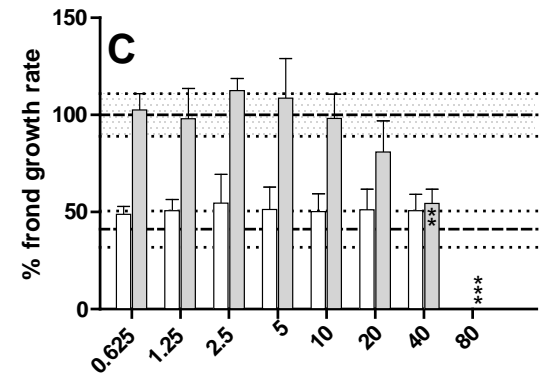
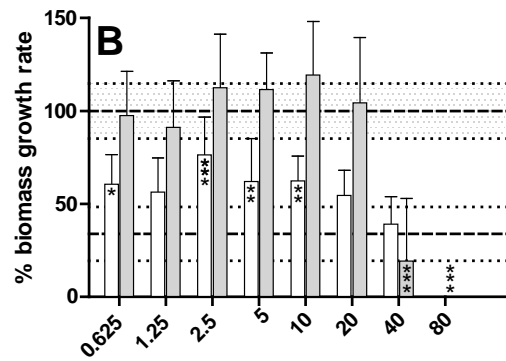
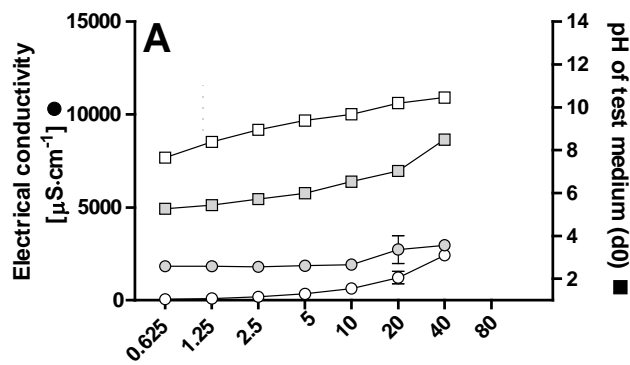
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Table 1: Element analysis of wood bottom and fly ash solids and corresponding BS EN 12457-2 leachates (100 g ash extracted with 1 L distilled water) with relative mobility and enrichment factors. Relative mobility of elements was calculated as the ratio of total soluble amount in 10 L leachate to the *aqua regia* extractable amount per kg; enrichment factors for solids (EF_S) and for leachates (EF_L) were calculated as the ratio between fly and bottom ash *aqua regia* extractable concentrations, and extract concentrations, respectively. TON: Total Oxidized Nitrogen (NO₂ + NO₃). Shown are average ± Standard Deviation (SD), n=4; no SD is given when the analyte was detected only once.

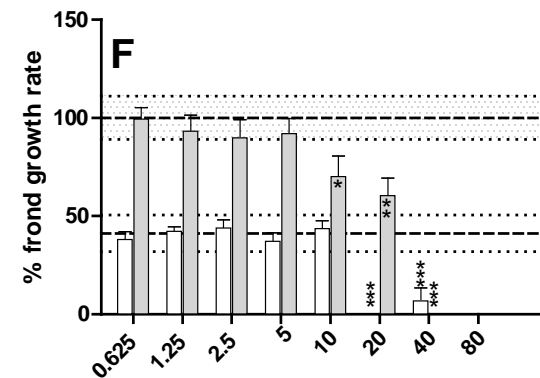
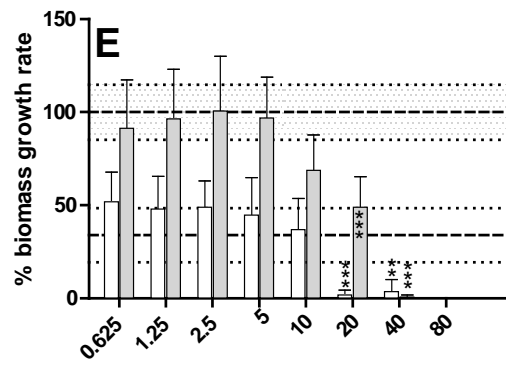
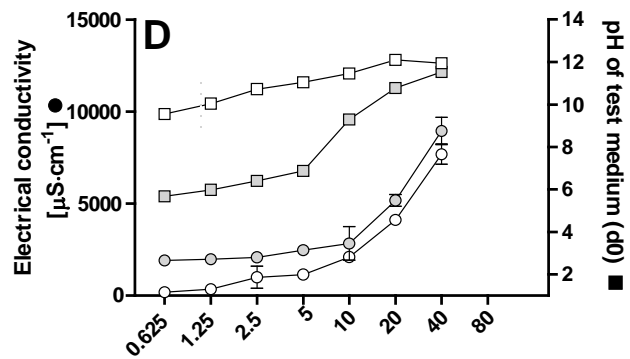
	Bottom ash		Bottom ash leachate		relative mobility	Fly ash		Fly ash leachate		relative mobility	EF _S	EF _L				
	g/kg	±	mg/L	±	ppm	g/kg	±	mg/L	±	ppm						
N	0.66	±	0.24			1.72	±	0.18			2.62					
NH ₃ -N			<0.5					<0.5								
NO ₂ -N			<0.1					0.63	±	0.01		6.32				
TON			<1					6.98	±	0.19		6.98				
P	11.8	±	1.34	1.87	±	0.39	1583	12.4	±	0.49	0.08	61	1.05	0.04		
PO ₄ ³⁻			2.19	±	0.22			<0.5						0.23		
K	57.2	±	9.73	1217	±	306	212855	71.1	±	3.48	5445	±	320	765823	1.24	4.47
Ca	113	±	11.5	11.9	±	4.46	1052	98.0	±	5.3	437	±	280	44636	0.86	36.6
Mg	16.5	±	1.86	1.65	±	0.08	999	15.7	±	0.67	<0.3		<191	0.95	0.18	
Na	4.32	±	0.54	99.9	±	16.6	231192	3.70	±	0.06	212	±	12.4	574425	0.86	2.13
Fe	11.1	±	0.97	<0.03			<27	5.05	±	0.10	<0.03		<59	0.45		
Al	13.4	±	1.61	0.03	±	0.03	25	6.32	±	0.13	1.96		3104	0.47	58.1	
Mn	11.9	±	1.27	0.02			14	10.5	±	0.44	<0.01		<10	0.88		
Cl			111	±	41.3						1243	±	20.8		11.2	
SO₄			297	±	12.4						4133	±	92.4		13.9	
	mg/kg		µg/L			mg/kg		µg/L								
Sb			5.12	±	1.43			<20								
As	2.66	±	0.15	23.4		87887	5.44	±	0.05	8.05		14798	2.04	0.34		
Ba	1228	±	64.0	19.2	±	4.19	157	72.9	±	12.0	247	±	116	33938	0.06	12.9
Be	0.35	±	0.03	<20		<566572	0.16	±	0.02	<20		<1277955	0.44			
B	105	±	6.28	2015	±	430	192042	192	±	1.71	789	±	901	41147	1.83	0.39
Cd	1.62	±	0.13	0.19		1146	9.67	±	0.25	<2		<2069	5.99			
Cr	19.0	±	1.91	81.7	±	17.4	42930	14.0	±	0.60	254	±	21.0	181900	0.73	3.11
Co	8.75	±	0.59	1.04		1189	6.24	±	0.17	<20		<3208	0.71			
Cu	84.0	±	26.0	8.89	±	3.89	1058	76.5	±	1.3	4.26	±	0.31	557	0.91	0.48
Pb	11.5	±	1.53	<2		<1743	38.5	±	0.90	4.45	±	0.29	1156	3.35	2.22	
Li	12.1	±	1.10				7.40	±	0.31				0.61			
Hg	<0.2			<0.01		<500	<0.2			<0.01		500				
Mo	<1			104	±	24.9	<1041000	1.68		243	±	43.5	1447917		2.34	
Ni	17.0	±	1.26	<1		<589	12.9	±	0.21	<10		<7737	0.76			
Se	3.01	±	0.68	8.53		28362	4.18	±	0.75	99.5		238181	1.39	11.7		
Ag	<1						1.41									
Sr	817	±	83.5	73.2	±	21.0	896	725	±	83.5	1858	±	1013	25649	0.89	25.4
Tl	3.95			<1		<2532	4.23			<10		<23641	1.07			
Sn	1.45			<2		<13793	1.72			<40		<232558	1.19			
Ti	719	±	49.5	8.15	±	1.03	113	290	±	24.6	78.1	±	12.5	2691	0.40	9.58
V	18.6	±	1.49	183	±	13.2	98522	10.9	±	0.24	45.5		41935	0.58	0.25	
Zn	325	±	25.2	5.29		163		1833	±	58.0	3296	±	2191	17989	5.64	623



738 **Figure 2: Biomass and frond growth rates of *Lemna minor* exposed to wood ash solid suspensions under two trophic regimes; bottom ash (B-C), fly ash (E-F); ultra-**
739 **oligotrophic medium (empty bars) and Hutner's growth medium (grey bars). Also shown are electrical conductivity and pH (day 0) of ultra-oligotrophic medium**
740 **(empty circles and squares, respectively) and half-strength Hutner's growth medium (grey circles and squares, respectively) supplemented with bottom ash (A) or**
741 **fly ash (D). Biomass and frond growth rates were normalized to Hutner's control growth rates ($0.363 \pm 0.053 \text{ day}^{-1}$ and $0.277 \pm 0.043 \text{ day}^{-1}$; respectively), shown as**
742 **100%, dashed line with grey SD range. Also shown is the growth rate on distilled water without added ash (dashed line with clear SD range). $P=0.05$; $p=0.01$;**
743 **$p=0.001$**
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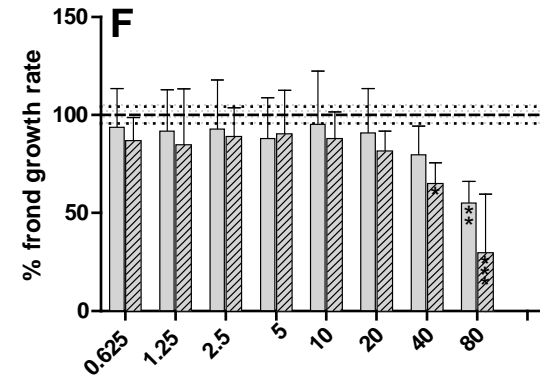
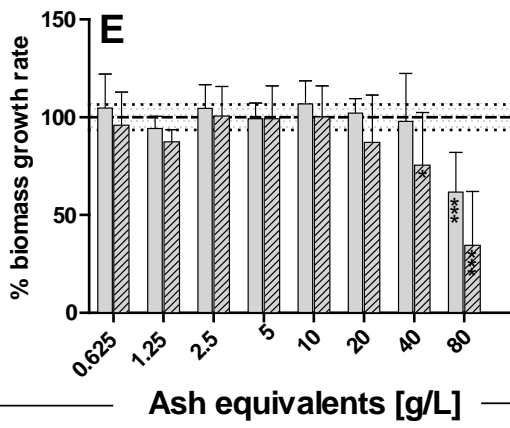
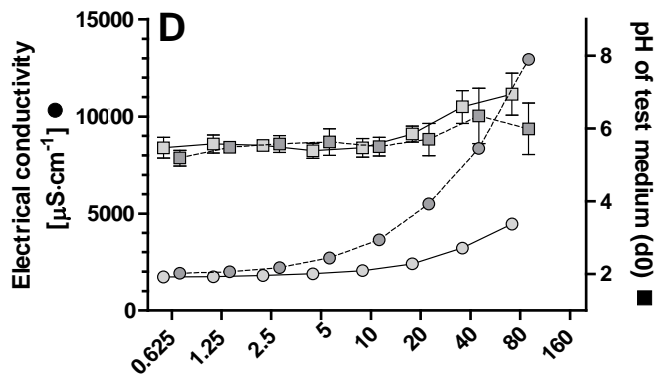
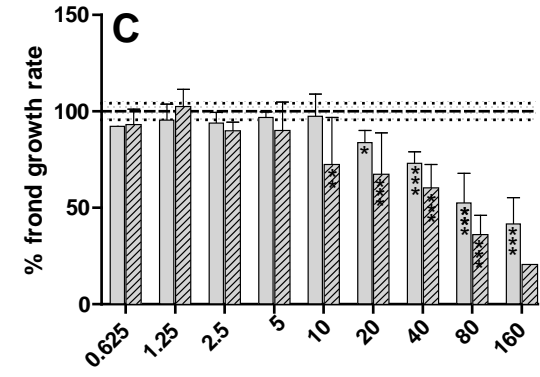
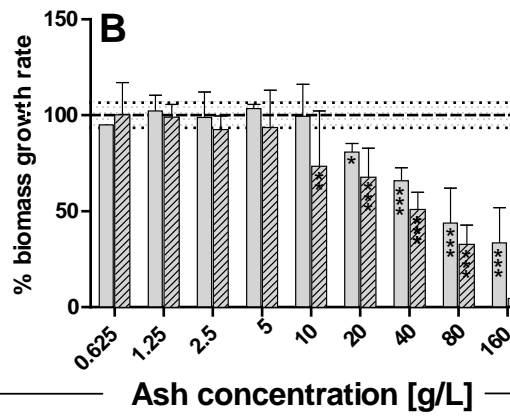
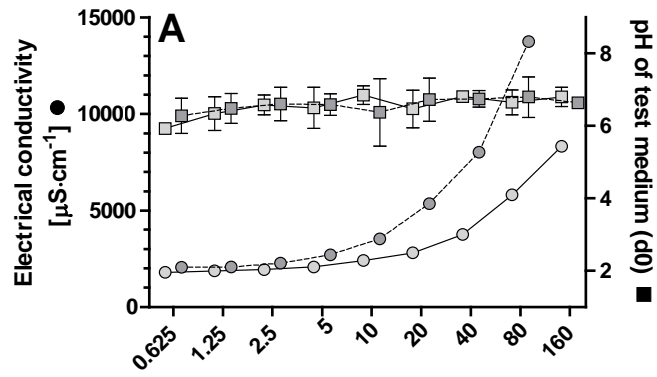


Bottom ash equivalents [g/L]



Fly ash equivalents [g/L]

748 **Figure 3: Biomass and frond growth rates of *Lemna minor* exposed to wood ash leachate dilutions under two trophic regimes; bottom ash (B-C), fly ash (E-F); ultra-**
749 **oligotrophic medium (empty bars) and half-strength Hutner's growth medium (grey bars). Also shown are electrical conductivity and pH (day 0) of ultra-oligotrophic**
750 **medium (empty circles and squares, respectively) and half-strength Hutner's growth medium (grey circles and squares, respectively) supplemented with bottom (A) or**
751 **fly ash (B) leachate. Biomass and frond growth rates were normalized to Hutner's control growth rates ($0.363 \pm 0.053 \text{ day}^{-1}$ and $0.277 \pm 0.043 \text{ day}^{-1}$; respectively), shown**
752 **as 100%, dashed line with grey SD range. Also shown is the growth rate on distilled water without added ash (dashed line with clear SD range). $P=0.05$; $p=0.01$;**
753 **$p=0.001$**
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757 **Figure 4: Biomass and frond growth rates of *Lemna minor* exposed to neutralized wood bottom (light grey bars) and fly ash (striped bars) solid suspensions (B-C)**
758 **and leachate dilutions (E-F) in Hutner's growth medium. Also shown are electrical conductivity and pH (day 0) of half strength Hutner's growth medium (circles**
759 **and squares, respectively) supplemented with neutralized ash suspensions (A) or neutralized leachate (D). Biomass and frond growth rates were normalized to**
760 **Hutner's control growth rates ($0.370 \pm 0.040 \text{ day}^{-1}$ and $0.255 \pm 0.061 \text{ day}^{-1}$; respectively), shown as 100%, dashed line with grey SD range. P=0.05; p=0.01; p=0.001**

Table 2: Effect Concentration (EC₁₀ and EC₅₀) values with 95% CI calculated for the inhibition of biomass and frond growth rate in respective media

		Bottom ash (95% CI) g/L	Bottom ash leachate (95% CI) g aeq/L	Fly ash (95% CI) g/L	Fly ash leachate (95% CI) g aeq/L
native sample in distilled water (ultra-oligotrophic) medium					
Biomass growth rate	EC ₁₀	28.5 (16.3-50)	25.9 (13.5 -49.5)	6.44 (3.15-13.1)	8.03 (4.79-13.4)
	EC₅₀	35.4 (22.3-56.2)	43.9 (34.3-56.3)	14.2 (10.7-18.7)	12.5 (8.67-17.9)
Frond growth rate	EC ₁₀	41.9 (22.1-79)	>40	8.03 (1.53-42.2)	>10
	EC₅₀	52 (33.2-81.5)	<80	18.1 (12.1-27.1)	<20
native sample in Hutner's (eutrophic) medium					
Biomass growth rate	EC ₁₀	10.1 (3.67-28.1)	25 (8.18-76.6)	8.6 (4.28-17.3)	6.92 (3.25-14.7)
	EC₅₀	50.9 (33-78.5)	33.1 (20.2-54.2)	20.5 (15.6-27.1)	17.8 (13.2-24.1)
Frond growth rate	EC ₁₀	13.3(5.1-34.3)	14.8 (9.82-22.2)	8.6 (4.28-17.2)	8.33 (3.87-17.9)
	EC₅₀	42.9 (30.8-60)	36.9 (29.7-45.7)	21.8 (16.8-28.3)	19.6 (14.1-27.2)
neutralized sample Hutner's medium					
Biomass growth rate	EC ₁₀	9.7 (3.56-26.5)	51.6 (27.7-96.4)	4.49 (1.06-19.1)	26.7 (13.6-52.3)
	EC₅₀	74.4 (56.7-97.6)	87.9 (69-112)	37.1 (25.953.3)	61.6 (48.984.5)
Frond growth rate	EC ₁₀	9.71 (3.56-26.5)	35.7 (12.8-99.1)	4.5 (1.06-19.1)	24.8 (12.4-49.7)
	EC₅₀	68.3 (48.2-96.9)	94.2 (56.7-156)	36.7 (22.2-60.6)	60.8 (44.3-83.3)