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

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Abstract

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

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

1 Introduction

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

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

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

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

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

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

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

2 Material and Methods

2.1 Characteristics of wood ash and corresponding leachates

Origin and sampling

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

Physico-chemical analyses

Particle size distribution of bottom and fly ash was analysed in the range between 63 µm and 6.3 mm by dry sieving according to Deutsche Industrie Norm 18123 (DIN, 1996). Analysis of loss on ignition (LOI) at 500 °C was performed for bulk ash samples following DIN 18128 (DIN, 2002). Ash sub-samples for each replication and leachate were dried at 30°C for 3-4 days until the weight remained constant, and the particle fraction > 4 mm was removed. Leachates were prepared according to the European Norm (EN) 12457-2 one stage leaching test for granular waste (CEN, 2002) at 10 l/kg water ratio, with 24 h contact time and filtering (Fisherbrand, FB 59031). Fresh leachates were applied in bioassays. Titration of ash leachates was performed with 0.02 N H_2SO_4 to pH 4.

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

Table 1 near here

2.2 Growth inhibition test with *Lemna minor*

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

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

Growth inhibition assay

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

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

Figure 1

2.3 Calculations and statistics

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

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

3 Results

3.1 Physico-chemical characteristics of wood ash

Ash solids

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

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

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

Ash leachates

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

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

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

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

3.2 *Lemna minor* bioassays

3.2.1 Electrical conductivity and pH conditions in the test

Electrical conductivity (ElC) of native ash suspensions in distilled H_2O and Hutner's media differed due to the base electrical conductivity of the nutrient medium itself (1660 ± 160 µS/cm). However, the difference in ElC between ultra-oligotrophic and eutrophic test solutions decreased as ash concentration was increased (Figure 2A, D). The difference in pH values of ultra-oligotrophic and eutrophic medium was substantial and related to the buffer capacity and the slightly acidic pH 5.01 ± 0.28 of Hutner's medium. The difference in pH values decreased with increasing ash concentration. Concentrated suspensions of fly ash in oligotrophic or eutrophic medium displayed higher pH and ElC than respective bottom ash suspensions. Similarly, FA leachates increased ElC and pH more than BA leachates (Fig. 3). To determine potential pH effects on toxicity, another approach was instigated whereby medium was neutralised at the start of the experiment. ElC of neutralized suspensions and dilutions of neutralized leachates in Hutner's medium (Figure 4A, D) displayed the same ash dose dependent increase as their respective native counterparts.

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

Suspended bottom ash solids

When suspended in Hutner's medium, bottom ash concentrations of 1.25, 2.5 and 5 g/L neither impaired nor benefitted the growth of the test organism relative to the corresponding control (Figure 2B, C). However, BA concentrations of 40 g/L ($p < 0.05$, LOEC) and above ($p < 0.001$) significantly decreased biomass growth rates. EC_{10} and EC_{50} for biomass growth rate were 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 ± 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 ± 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

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

Bottom ash leachates

Bottom ash leachate added to Hutner's medium (Figure 3B), and diluted to 0.625 and 1.25 g aeq/L, did not alter the biomass growth rate of *L. minor*. Higher concentrations of 2.5 to 20 g aeq/L BAL improved biomass growth compared to the control, but not significantly. A significant reduction of biomass growth ($p < 0.001$) was observed at 40 g aeq/L (LOEC). Plants exposed to 80 g aeq/L were necrotic. The calculated biomass EC_{10} was 25 g aeq/L, while the 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 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, respectively.

Figure 3 near here

Plants grown on ultra-oligotrophic medium supplemented with BAL (Figure 3B) exhibited significantly higher biomass growth rates in the concentration range between 0.625 to 10 g aeq/L, when compared to the corresponding control. The fastest growth was observed on medium with 2.5 g aeq/L BAL added. The biomass growth EC_{10} for BAL was calculated to be 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 growth rate did not respond to increasing BAL doses in the nutrient deficient medium up to 40 g aeq/L. Plants were found to be necrotic at 80 g aeq/L.

Fly ash leachates

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

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

FAL treatments. In fact, plants were necrotic. The biomass EC_{10} was slightly higher and the EC_{50} was lower compared to the equivalent values using Hutner's medium. Frond growth EC_{10} and EC_{50} were >10 g aeq/L and <20 g aeq/L FAL, respectively.

3.2.3 Neutralized suspensions and leachate dilutions

Lemna minor exposed to the neutralized suspensions of either bottom or fly ash solids in Hutner's medium (Figure 4B, C) maintained biomass and frond growth up to relatively high concentrations. Growth could be observed on Hutner's medium supplemented with 160 g/L bottom ash solids or 80 g/L fly ash solids. Thus, dose-inhibition curves for bottom and fly ash solids were stretched and quite flat, compared to those observed with non-neutralised ash suspensions. For bottom ash suspensions, the biomass EC_{10} and EC_{50} were 9.7 g/L and 74.4 g/L (95% CI: 56.7 to 97.6 g/L), respectively. For fly ash suspensions, the biomass EC_{10} and EC_{50} 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 inhibition at lower bottom ash concentrations, increasing chlorosis at frond edges could be observed in plants on bottom or fly ash suspensions exceeding 10 g/L. Necrosis was not observed with either of the two neutralized ash suspensions.

Figure 4 near here

Low concentrations of neutralized ash leachates added to Hutner's growth medium (Figure 4E, F) had very little impact on biomass and frond growth rates. The shape of the dose-response curve for plants exposed to neutralized BAL displayed an abrupt increase in effect severity above 40 g aeq/L. The biomass growth rate EC_{10} and EC_{50} for neutralised BAL were 51.6 g aeq/L and 87.9 g aeq/L (95% CI: 69 to 112 g/L), respectively. The shape of the dose-response curve for plants exposed to neutralized FAL displayed a slightly more gradual decrease in biomass and frond number growth, and EC_{10} and EC_{50} values were both markedly increased compared to the equivalent values for neutralised BAL.

4 Discussion

4.1 Wood ash solids and corresponding leachates

Wood ash solids

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

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

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

Our data show that the micronutrient and trace element concentrations in BA and FA are distinct, likely implying distinct hazards. Relative enrichment of Fe and Al in bottom ash in this study is higher than reported in Park et al. (2012) and Pöykiö et al. (2009) but matches earlier findings (Narodoslawsky and Obernberger, 1996). B, Cu, Mn and Zn are present in both ashes at levels that were previously reported (Augusto et al., 2008). Mo and Ni contents in the tested Sitka spruce ashes are slightly lower than commonly reported. Among micronutrients, a minimum nutrient content for ash spreading (Haglund, 2008) has been defined for Zn (0.5 g/kg) only. BA supplies 65% of required Zn content, while FA exceeds the requirement by 3.6-fold. Elements of concern, defined in 86/278/EEC (Council of the European Union, 1986), such as Cd, Pb and Zn are enriched in fly ash, as was found by others (Park et al., 2012; Pöykiö et al., 2009). As and Se are also enriched in fly ash, but the partitioning between FA and BA is less pronounced than that reported by Park et al. (2012) and the opposite of what was described for As by Pöykiö et al. (2009). Enrichment prevalence is commonly linked to condensation on fly ash particles (Izquierdo and Querol, 2012; Narodoslawsky and Obernberger, 1996; Pitman, 2006) and also affected by incomplete combustion. Based on the comparison of the chemical composition of Sitka spruce wood ash with a meta-analysis data set of various wood ashes (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 (Etitgni 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 SO_4, 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

4.2 Growth promotion

Wood ash contains a range of plant nutrients as well as contaminants (Table 1). Hytönen (2016) showed that addition of wood ash to a peaty soil increased concentrations of extractable P, K, Ca and Mg in the soil, and this was associated with growth stimulation. In ultra-oligotrophic or eutrophic media the relative contribution of ash-derived plant nutrients to plant growth differs. In the ultra-oligotrophic medium nutrients are virtually absent. Plant growth in the ultra-oligotrophic water control (dotted lines in Figure 2 and Figure 3) is sustained for the short duration of the test by nutrients stored in the plant. Under these conditions, the only nutrients present are those supplied through wood ash. This study shows substantially increased growth rates for *L. minor* in ultra-oligotrophic medium supplemented with ash (Figure 2B, C, E and 3B). In ultra-oligotrophic media, bottom ash solids and leachates, up to concentrations of 2.5 g/L and 10 g aeq/L respectively, increase biomass and frond growth significantly (Figure 2B and 3B). Fly ash stimulates biomass growth when applied as a solid (up to 5 g/L, Figure 2E). Osmotic stress in the ultra-oligotrophic medium (distilled H_2O) control (20 µS/cm) may reduce plant growth performance, thus artificially emphasizing the stimulatory effects mediated by ash. However, this scenario is unlikely, as significant increases in growth occur at ash concentrations that barely affect electrical conductivity (i.e. Fig. 2; compare responses to 0.625 and 1.25 g/L ash). Therefore, growth promoting effects under oligotrophic conditions are likely to be caused by improved nutrient supply. Given the key role that nitrogen plays in mediating plant growth, it might be expected that fly ash leachate should cause a more pronounced growth stimulation. However, Hutner's medium provides 560 mg/L NO_3 and the 10 g aeq/L fly ash dilution only carries 0.7 mg/L NO_x, and therefore the latter is unlikely to significantly resolve nitrogen deficiency. This also implies that even greater stimulation of biomass growth can be expected when ash is supplemented with an N-source, and this is an aspect that has considerable relevance for practical ash applications in, for example, forestry. There have been previous reports of wood ash mediated growth enhancement. For example, Aronsson and Ekelund (2006) showed that growth of the water moss *Fontinalis antipyretica* was increased when the growth medium was supplemented with extracts of crushed wood ash. Nabeela et al., (2015) found that the addition of low concentrations (<1g/kg) of wood ash to the soil stimulates growth of *Brassica napus*, but that toxicity occurs when higher concentrations (>10 g/kg) are used. Bonfim-Silva et al., (2015) found that incorporation of wood ash in the soil led to substantial increases in height, leaf and tiller number of two species of grass. Growth stimulation increased with ash concentration, reaching a plateau at around 12 g/L of wood ash, but no toxicity was apparent at higher concentrations. Other studies did, however, fail to show any growth stimulation by wood ash. For example, Park et al., (2005) failed to show enhanced biomass production by willows (*Salix purpurea*) and it was hypothesised that this was due to the fact

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

4.3 Phytotoxicity

Lemnaceae are an excellent group of model species for ecotoxicity assessment, considered representative for aquatic macrophytes, and also to a lesser extent for vascular plants in general. *Lemna minor* ranks highly for tolerance against a broad range of metal and metalloid elements, although the species is particularly sensitive to Co, Cr and Cu (Wu et al., 2013). *L. minor* is a sentinel species (Dorman et al., 2010) that is commonly used for phytoremediation. This not only suggests that obtained EC_{50} outline an aquatic worst-case scenario but also facilitate the distinction between growth promotion at low ash concentrations and toxicity at higher levels for this study.

When exposed to bottom and fly ash, *Lemna minor* growth performance exhibits distinct dose-response relationships (Figure 2, 3, 4). Fly ash is always more hazardous than bottom ash. Severe toxic effect concentrations (EC_{50}, Table 2) of bottom and fly ash solids are significantly different, regardless of the use of oligotrophic or eutrophic growth conditions or native or neutralised ash applications.

Table 2 near here

Plant nutrition provided by the medium has no substantial effect on the toxicity of native fly ash solids or leachates (Table 2). However, severe toxicity (EC_{50}) is decreased due to pH neutralization (Table 2). For neutralized bottom ash leachates, both EC_{10} and EC_{50} are about 2-fold higher compared to native samples, while the difference is 3-fold for neutralized fly ash leachates. Thus, careful management of pH during fertilization with ash may avert detrimental effects (toxicity) and this can have important management implications for ash spreading.

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

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

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

4.4 Conclusion

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

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Tables and figures

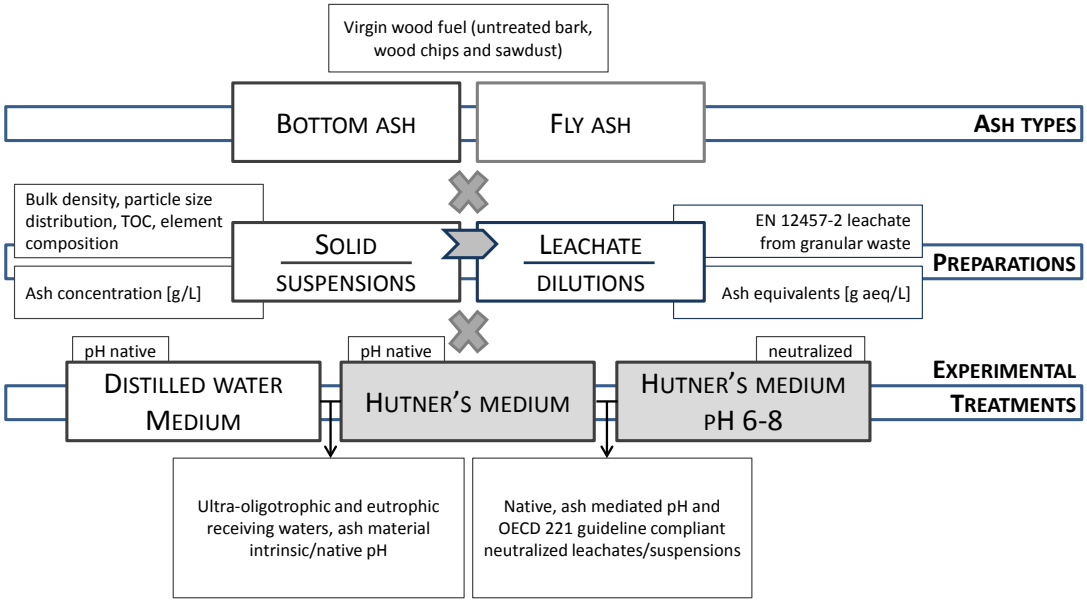
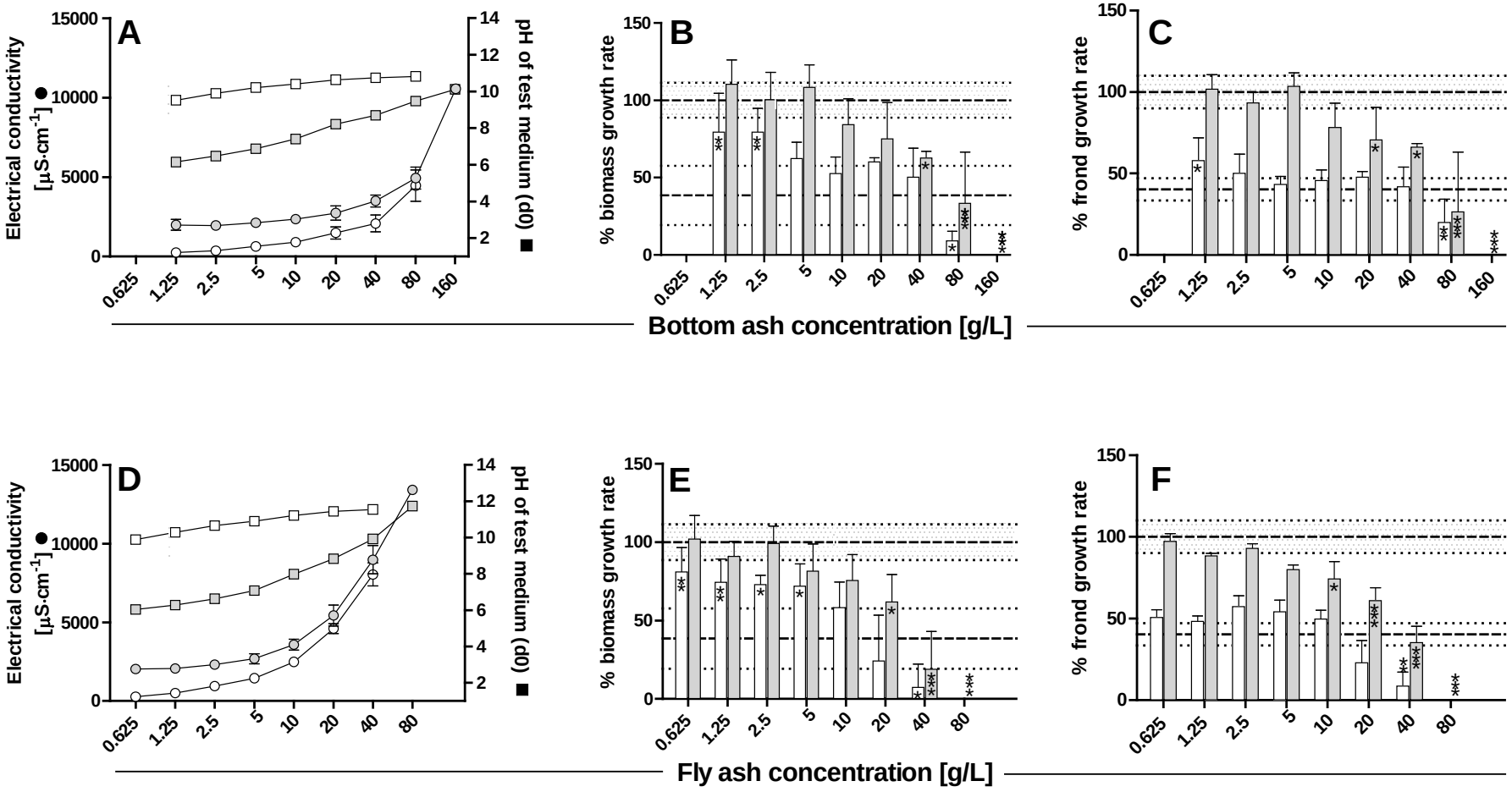


Figure 1: *Lemna minor* biomass (fresh weight) and frond (number) growth rates were quantified following exposure to wood ash suspensions or leachates under ultra-oligotrophic or eutrophic (nutrient medium) conditions. Impacts on *L. minor* growth were also quantified for pH neutralised suspensions and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

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 (NO_2 + NO_3). 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
SO_4				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

735

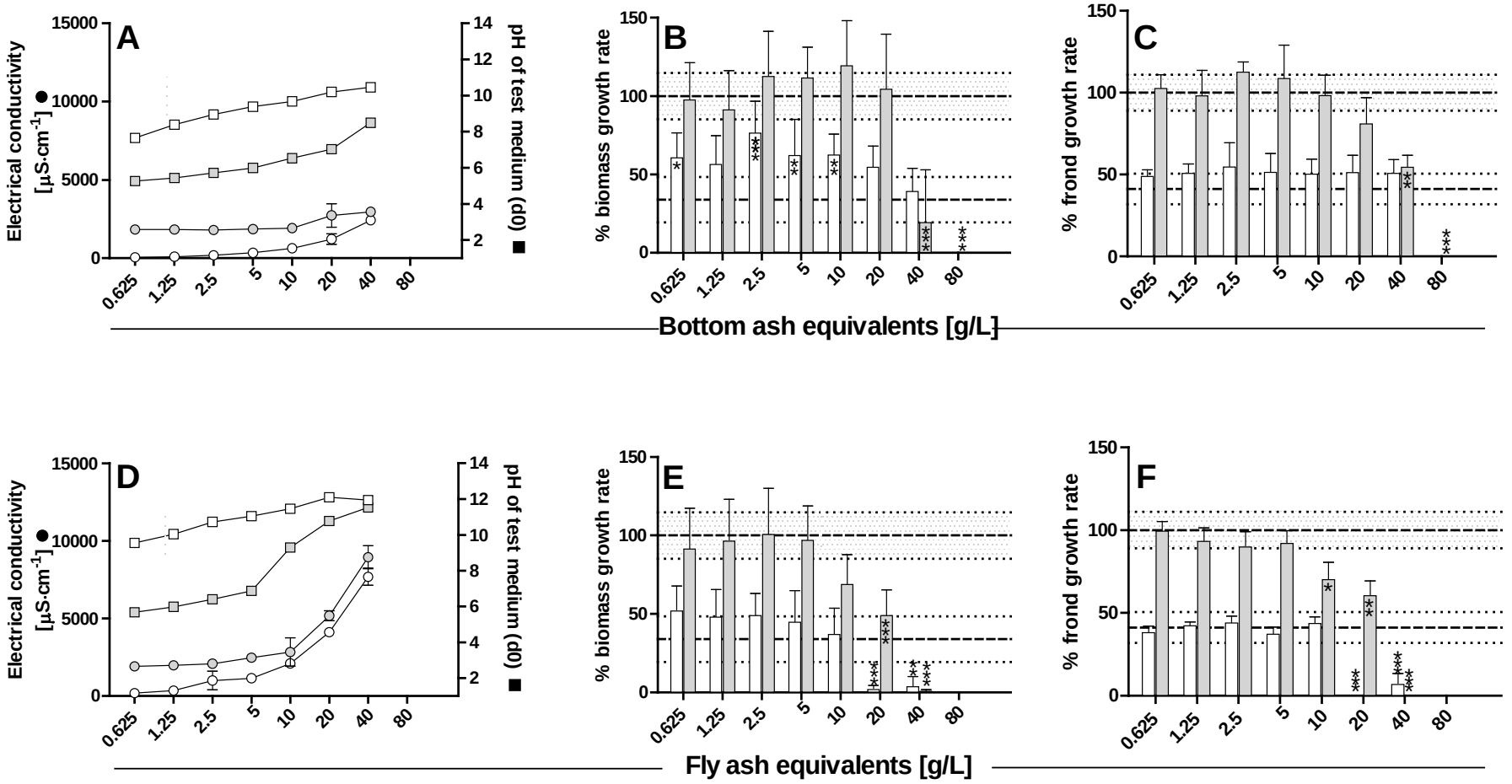

A
B
C
D
E
F
Electrical conductivity [µS·cm-1] ● ■ pH of test medium (d0)
% biomass growth rate
% frond growth rate
Bottom ash concentration [g/L]
Fly ash concentration [g/L]
0.625
1.25
2.5
5
10
20
40
80
160

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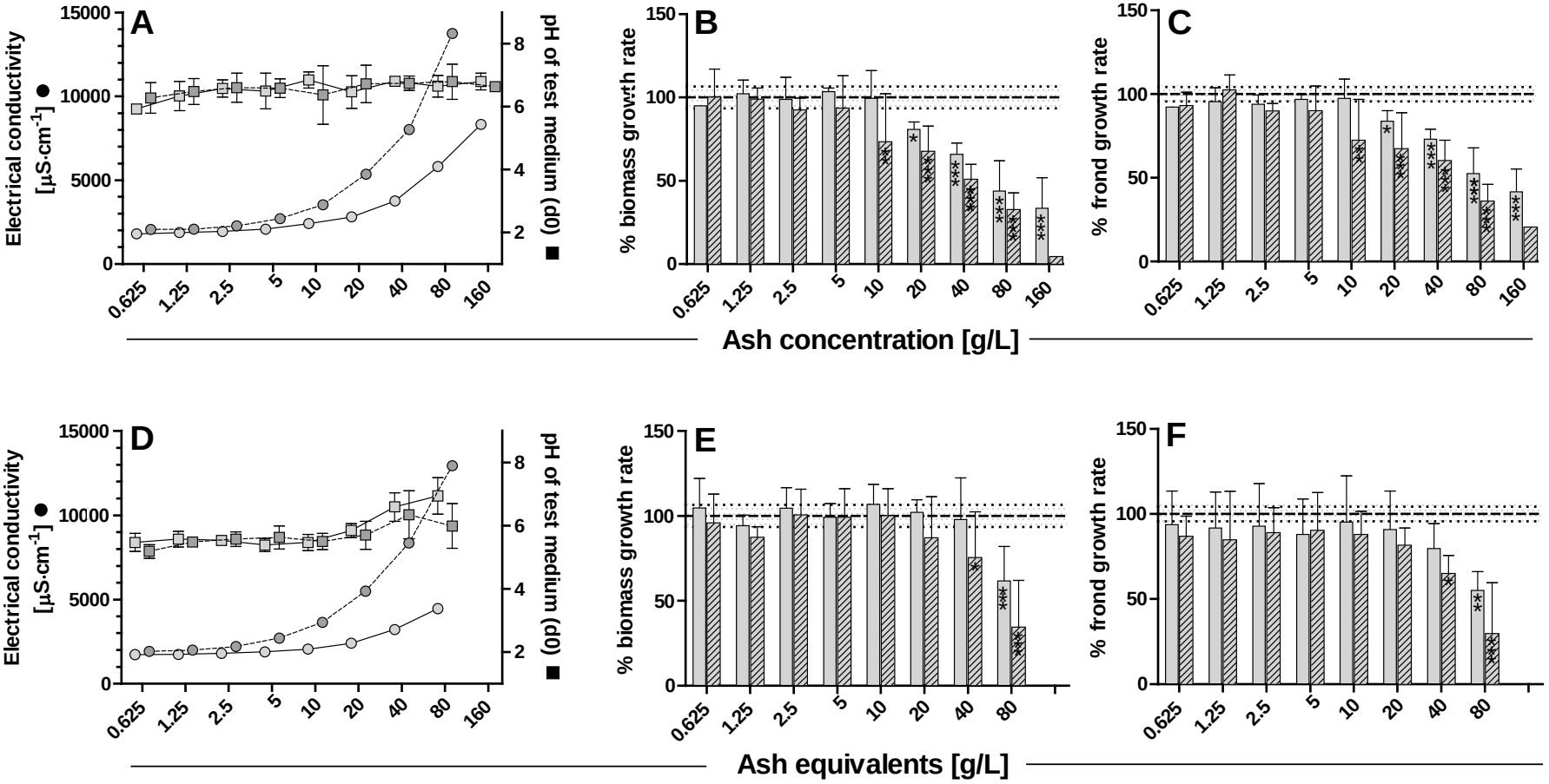
738 **Figure 2: Biomass and frond growth rates of *Lemna minor* exposed to wood ash solid suspensions under two trophic regimes; bottom ash (B-C), fly ash (E-F); ultra-**
739 **oligotrophic medium (empty bars) and Hutner's growth medium (grey bars). Also shown are electrical conductivity and pH (day 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±0.053 day^{-1} and 0.277±0.043 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**
744

745


A
B
C
D
E
F
Electrical conductivity [µS·cm-1] ●
pH of test medium (d0) ■
% biomass growth rate
% frond growth rate
0.625
1.25
2.5
5
10
20
40
80
Bottom ash equivalents [g/L]
Fly ash equivalents [g/L]

746
747

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±0.053 day^{-1} and 0.277±0.043 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**
754


A
B
C
D
E
F
Electrical conductivity [µS·cm⁻¹] ●
pH of test medium (d0) ■
% biomass growth rate
% frond growth rate
Ash concentration [g/L]
Ash equivalents [g/L]
0.625
1.25
2.5
5
10
20
40
80
160

755

756

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) 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 and squares, respectively) supplemented with neutralized ash suspensions (A) or neutralized leachate (D). Biomass and frond growth rates were normalized to Hutner's control growth rates (0.370±0.040 day^{-1} and 0.255±0.061 day^{-1}; respectively), shown as 100%, dashed line with grey SD range. P=0.05; p=0.01; p=0.001

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Table 2: Effect Concentration (EC_{10} and EC_{50}) values with 95% CI calculated for the inhibition of biomass and frond growth rate in respective media

		Bottom ash (95% CI)	Bottom ash leachate (95% CI)	Fly ash (95% CI)	Fly ash leachate (95% CI)
		g/L	g aeq/L	g/L	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)
	EC_{50}	**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
	EC_{50}	**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)
	EC_{50}	**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)
	EC_{50}	**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)
	EC_{50}	**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)
	EC_{50}	**68.3 (**48.2-96.9)	**94.2 (**56.7-156)	**36.7 (**22.2-60.6)	**60.8 (**44.3-83.3)

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