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6 Seasonal patterns of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a
7 constructed wetland in the west of Ireland

8
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17

18 **Abstract**

19 An understanding of the seasonal variation in the standing stock of metals and nutrients in
20 emergent vegetation of constructed wetlands (CWs), as well as the amounts present in
21 aboveground (AG) and belowground (BG) biomass, is crucial to their design and
22 management. Given that biomass harvesting is a labour and time consuming operation, a
23 paucity of information currently exists on accumulation and standing stocks in biomass in
24 CWs, in particular in North Western European countries. To address this knowledge gap, this
25 paper examined the seasonal variations of metals and nutrients in *Phragmites australis* (Cav.)
26 Trin. ex Steudel in a CW treating municipal wastewater, with a view to identifying an
27 optimal time for biomass harvesting of the AG vegetation. Although the AG biomass was

28 greatest in August ($1,636 \pm 507 \text{ g m}^{-2}$), the maximum concentrations and accumulations of
29 metals and nutrients occurred at different times throughout the duration of the study (April to
30 November). Furthermore, with the exception of zinc and nitrogen, metals and nutrients
31 measured in BG biomass ranged from 66% (phosphorus) to greater than 80% (nickel and
32 chromium) of the AG biomass. This indicates that analysis of only the emergent shoots may
33 significantly underestimate the metal and nutrient uptake and capacity of the plant. In order to
34 effectively target the bulk of metals and nutrients, an AG harvest in late August or September
35 is suggested.

36

37 **Keywords:** constructed wetlands, macrophytes, metals, biomass harvesting

38

39 1. Introduction

40 Constructed wetlands (CWs) are gaining in popularity for the treatment of municipal
41 (Vymazal, 2011) and industrial wastewaters, including, *inter alia*, landfill leachate (Bulc,
42 2006; Białowiec et al., 2012), tannery industry wastewaters (Calheiros et al., 2012), highway
43 runoff (Gill et al., 2014), effluents from wineries (Grismer et al., 2003), aquaculture
44 wastewater (Lin et al., 2005), mine wastewater (O’Sullivan et al., 2004), wastewaters
45 containing estrogens, androgens and hormones (Cai et al., 2012; Vymazal et al., 2015), and
46 pharmaceutical and personal care products (Matamoros et al., 2009). Numerous studies
47 measuring wetland treatment performance with and without vegetation have concluded
48 almost invariably, that wetland performance is better when plants are present (Kadlec and
49 Knight, 1996). Wetland macrophytes are highly productive plants and possess several
50 functions in relation to wastewater treatment (Brix, 2003) such as flow resistance and
51 particulate trapping (Kadlec and Wallace, 2009), nutrient uptake (Shelef et al., 2013), and
52 insulation, particularly in colder climates. In addition to this, the vegetation in CWs has the

53 ability to tolerate high concentrations of nutrients and metals, as well as to accumulate them
54 in their plant tissues (Stottmeister et al., 2003).

55

56 The selection of plant species for CWs requires careful consideration, as the vegetation must
57 be capable of surviving the potential toxic effects of wastewater and its variability (Maine et
58 al., 2009). The Common Reed, *Phragmites australis*, (Cav.) Trin. ex Steudel, is used
59 worldwide for the treatment of domestic and industrial wastewaters in CWs (Du Laing et. al,
60 2003). Investigations of the uptake and seasonal variations in storage capacities of nutrients
61 in *P. australis* and other plants such as *Typha latifolia* L. have been undertaken in CWs under
62 Irish climatic conditions (Healy et al., 2007; Mustafa and Scholz, 2011). However, a paucity
63 of information exists on metal cycling and accumulation by vegetation, in particular in CWs
64 of North Western European countries. Such information is important in the future design and
65 operation of CWs, particularly when the efficacy of CWs regarding nutrient and metal
66 removal from wastewaters is being assessed.

67

68 Metals are non-biodegradable, and water pollution by metals is a serious environmental
69 problem which is difficult to solve (Keng et al., 2014). In CWs, metals tend to accumulate in
70 the sediments as well as in the plants (Březinová & Vymazal, 2015). While metals in CWs
71 are removed through physical (settling and sedimentation) and chemical (sorption and
72 adsorption) mechanisms, metal uptake by plants has also been identified as the principal
73 removal mechanism for some pollutants, particularly in lightly loaded systems (Březinová &
74 Vymazal, 2015). However, metal content in the roots and shoots of wetland vegetation varies
75 from season to season and there has been no attempt to explain this variability, or to
76 determine optimum conditions for metal uptake by plants in CWs to date (Vymazal and
77 Březinová, 2016). In the context of how we manage CWs, the seasonal variations of metals in

78 macrophytes must be first of all understood, if we intend to expand the use of CWs for
79 treating effluents containing metals in the future.

80

81 Maximum recorded metal concentrations from international studies in above and
82 belowground (BG) biomass of *P. australis* are presented in Table 1. Macrophytes are known
83 to take up metals from the environment but largely accumulate these in the BG organs, such
84 as the roots and rhizomes (Peeverly et al., 1995). The generally lower concentrations of metals
85 in aboveground (AG) organs of macrophytes (stems and leaves) may be attributable to metal
86 tolerance, where it has been suggested that macrophytes limit high metal concentrations in
87 the photosynthetic organs of the plant (Bragato et al., 2006). The levels of metals in AG
88 organs may vary seasonally in response to plant growth dynamics, metal levels and
89 availability in the surrounding waters (Larsen & Schierup, 1981; Schierup & Larsen, 1981).
90 The possibility of harvesting of the AG vegetation as a means of wetland management and
91 removal of metals from the system has previously been suggested (Bragato et al., 2006;
92 Březinová & Vymazal, 2015). However, a dearth of information currently exists on
93 macrophyte management in CWs, including best practices for harvesting.

94

95 The total storage of a substance in a plant part is called standing stock (Vymazal &
96 Březinová, 2015) and is calculated by multiplying the concentration by biomass per unit area.
97 Vymazal & Březinová (2015) suggest that knowledge of concentrations alone does not
98 provide any information of the translocation or accumulation of metals in a plant without
99 knowing the biomass. In a literature review of metals in AG biomass of *P. australis* by
100 Vymazal & Březinová (2016), the authors theorize that in order to obtain correct
101 accumulation values in a plant, it is necessary to include the biomass values. Biomass

102 harvesting is a labour and time consuming operation, and therefore a paucity of information
103 exists on accumulation and standing stocks in AG biomass in CWs.

104

105 With this in mind, the current study aims to evaluate the seasonal variations of metals as well
106 as nutrients (nitrogen (N) and phosphorus (P)) in AG and BG biomass of *P. australis* in a CW
107 receiving municipal wastewater in a temperate oceanic climate in the west of Ireland, with a
108 view to: (1) investigating the efficacy of metal and nutrient removal via biomass harvesting
109 of AG vegetation; and (2) identifying an optimal period for biomass harvesting. The results
110 of this study may inform how a wetland treating industrial wastewaters or effluents with high
111 concentrations of metals may be managed in the future. We focus on a north western
112 European context, but many of our suggestions may be suitable for other environmental
113 contexts.

114

115 **2. Materials and methods**

116

117 **Site description**

118 The free-water surface constructed wetland (FWS CW) investigated in this study is located in
119 Fenagh, Co. Leitrim, Ireland (54°1'2"N; 7°49'43"W). This CW was designed and constructed
120 to cater for a population equivalent (PE) of 400 in 2004, but currently receives wastewater
121 with a PE of 132 (Table 2). Wastewater enters the treatment works at the primary settlement
122 tank, flows by gravity to a rotating biological contactor before entering the CW, where the
123 wastewater undergoes tertiary treatment. The CW has a surface area of 400 m², and is lined
124 with a high-density polyethylene liner. The wetland was originally planted with a
125 monoculture of *P. australis*. Vegetation cover in the wetland is 100%, with some occasional

126 bramble (*Rubus fruticosus* agg.), nettle (*Urtica dioica* L.) and willow scrub (*Salix* spp. L.)
127 encroaching onto the reed bed.

128

129 **Vegetation sampling regime**

130 Sampling and analysis of vegetation was undertaken between April and November 2015.
131 Aboveground and BG biomass of *P. australis* were sampled monthly in the inlet and outlet
132 zones (5 m from the inlet and outlet edges) of the CW. During each sampling time, four 0.25
133 m² quadrats were placed into each of the inlet and outlet zones of the wetland using a
134 randomized block design. All shoots were clipped at ground level within each of the eight
135 quadrats. The BG biomass was completely dug out to a depth of 0.3 m from within the same
136 quadrats. Upon delivery to the laboratory, the BG samples were thoroughly washed with
137 potable water to remove all sediment and gravel. The washing was performed in large
138 containers to minimize loss of hairy roots. The AG biomass consisted of stems, leaves and
139 flowers combined, and the BG biomass consisted of roots and rhizomes combined. All
140 samples of AG and BG biomass were then dried in a 70°C oven (after Vymazal et al., 2010)
141 until samples reached constant weight, and the total dry biomass was calculated (g biomass
142 m⁻²). Aboveground and BG samples were then ground in a mill and a subsample was tested in
143 the laboratory. This process was repeated monthly.

144 **Laboratory analysis**

145 Nitrogen testing was carried out by combustion analysis using a Carla Erba nitrogen analyzer
146 following the Association of Official Analytical Chemists (AOAC) method 990.03 (2005).
147 The instrument was calibrated daily with an atropine standard. Quality control (QC)
148 [National Institute of Standards and Technology (NIST)] tomato leaf check samples were run
149 throughout analysis (every ten samples). Phosphorus, aluminium (Al), boron (B), iron (Fe),
150 manganese (Mn), magnesium (Mg), potassium (K), copper (Cu), zinc (Zn), sulphate (S) and

151 calcium (Ca) were digested using nitric acid and hydrogen peroxide in a CEM Mars
152 microwave system and analysed using a Thermo 65 Duo ICP following P4.3 “Soil, Plant and
153 Water Reference methods for the Western Region” (Gavlak et al., 2003). Check samples
154 were run through the ICP every 50 samples. Cadmium (Cd), chromium (Cr), nickel (Ni) and
155 lead (Pb) were analysed using Inductively Coupled Plasma (ICP) mass spectrometry after
156 digestion with *aqua regia* (1:3 HNO₃: HCl) at 110°C for three hours. Similarly, calibration
157 standards and QC samples were run initially followed by blank, spiked and matrix spiked
158 samples throughout the analysis (every ten samples) for verification purposes. Using these
159 data, the AG and BG biomass and nutrient and metal content for each sampling section were
160 obtained. Standing stocks were calculated as follows: standing stock (g m⁻²) = concentration
161 (g kg⁻¹) x dry matter (kg m⁻²).

162

163 **Statistical analysis:**

164 A full factorial (i.e. including first order interaction) Two-way ANOVA and Tukey (HSD)
165 post hoc tests (P <0.05) were used for statistical analysis of biomass along with metal and
166 nutrient concentration of *P. australis*. The two independent variables were *month* and *AG*
167 *versus BG* with dependent variables being various metal and nutrient concentrations, and
168 biomass. All significant values were reported at alpha $P < 0.05$. All analyses were conducted
169 on SPSS version 24.

170

171 **3. Results**

172 **3.1 Aboveground and belowground biomass**

173 The average dry AG and BG biomass harvested during the study is presented in Fig. 1.
174 Maximum recorded AG biomass in the study was recorded in August (1,636 g m⁻²), while
175 biomass was lowest in June (835 g m⁻²). Belowground biomass which ranged from 523 g m⁻²

176 to 872 g m⁻² represented 53% to 62% of the AG biomass respectively. There was a
177 statistically significant ($P = 0.002$) interaction between AG and BG biomass and month of the
178 year.

179

180 **3.2 Seasonal pattern of metal concentrations and accumulations**

181 Average Cd and Pb concentrations in the influent wastewater were below the limit of
182 detection (LOD) during the study (Table 3), and likewise were not detected in either the AG
183 or BG biomass. Both Cr and Ni concentrations were lower in AG than BG, or were below the
184 LOD (Fig. 2). Belowground values for both peaked in August (12.7 mg kg⁻¹ for Cr and 4 mg
185 kg⁻¹ for Ni). The BG organs cumulatively held > 80% of the total Ni and Cr in the plant as a
186 whole. The interactions between AG versus BG, and month of the year were significant ($P <$
187 0.05), with respect to the concentrations of both Ni and Cr in the biomass of *P. australis*.

188

189 The average influent Cu concentration measured during the study was 7 µg L⁻¹ (Table 3).
190 Belowground concentrations of Cu ranged from 17.6 mg kg⁻¹ to 28.5 mg kg⁻¹, and were
191 always higher than AG concentrations, which ranged from 7.1 mg kg⁻¹ to 16.7 mg kg⁻¹.
192 Aboveground standing stock of Cu was highest early in the growing season in April (15.4 mg
193 m⁻²). No significant ($P > 0.05$) interactions occurred between months and AG versus BG, for
194 the concentration of Cu in the biomass.

195

196 Zinc concentrations were highest in AG organs in September and November (165.2 mg kg⁻¹
197 and 165.6 mg kg⁻¹). Zinc standing stocks were also highest during these months (233.9 mg m⁻²
198 and 224.3 mg m⁻²). The highest monthly concentration of Zn was measured in BG organs in
199 September (187 mg kg⁻¹), and the lowest was measured in May (77.1 mg kg⁻¹). There was

200 no significant ($P > 0.05$) interaction between AG versus BG, and month of the year for the
201 concentration of Zn in *P. australis* biomass throughout the study.

202

203 **3.3 Seasonal pattern of nutrient concentrations and accumulations**

204 Concentrations and AG standing stocks of N and P are presented in Fig. 2. Nitrogen
205 concentrations in the AG tissues peaked in June (25,338 mg kg⁻¹), the early growing season
206 in Ireland, and declined from then to its lowest concentration of 9,463 mg kg⁻¹ in November.
207 Nitrogen was lowest in the BG tissues in August (15,000 mg kg⁻¹) and highest in October
208 (20,975 mg kg⁻¹). The maximum nitrogen AG standing stock (32.6 g m⁻²) was measured in
209 July. The AG biomass cumulatively contained almost half (44%) of the total N accumulated
210 in the CW. The interaction between AG versus BG and month of the year was significant
211 ($P < 0.05$) with respect to the concentration of N in the biomass of *P. australis*.

212

213 Concentrations AG of P peaked in June (3156 mg kg⁻¹) and steadily declined throughout the
214 study until November (768 mg kg⁻¹). Belowground values for P ranged from 2755 mg kg⁻¹ in
215 July to 3605 mg kg⁻¹ in September. Belowground biomass cumulatively accounted for two
216 thirds of the total P accumulated within the wetland. The highest AG standing stock of P was
217 recorded in July and August (3.3 g m⁻² and 3.4 g m⁻², respectively) and lowest in November
218 (1 g m⁻²). Similar to N, there was a significant interaction ($P < 0.05$) between AG versus BG
219 and month of the year for P concentrations in the study.

220

221 **4. Discussion**

222 Metals enter the environment from natural and anthropogenic sources, and are non-
223 biodegradable, accumulate in the environment, and pose a threat to the environment and
224 human health (Ali et al., 2013). Studies examining the ability of emergent vegetation in CWs

225 to uptake metals and nutrients have commonly examined AG vegetation only or
226 concentrations only. However, the findings of the current study suggest that analysis of only
227 the emergent shoots or concentrations only, may significantly underestimate the metal and
228 nutrient uptake of the plant. Metal accumulation in the AG biomass relative to the total
229 amount entering the system (Table 3) over the eight-month study period ranged from 0.02%
230 (for Cu) to 1.22% (for Zn). With the exception of Zn and N, there were higher concentrations
231 of metals and nutrients in the BG organs of the plant during each month of analysis. Overall,
232 Zn concentrations were cumulatively higher in AG biomass (52%) during April, May,
233 October and November, whereas N concentrations in AG biomass were higher during June,
234 July and August (the typical growing season for *P. australis*). The findings of higher
235 concentrations in BG biomass was similar to other studies (Peeverly et al., 1995; Mays &
236 Edwards, 2001; Bragato et al., 2009), and indicates that *P. australis* is prevalently a root
237 bioaccumulator species (Bonanno, 2011). The roots and rhizomes are the immediate points of
238 uptake in plants and, consequently, the concentrations are usually greater in roots in
239 comparison to leaves and other AG organs (Vymazal et al., 2007). The lower concentrations
240 in AG organs in the current study is in agreement with the speculation that plants restrict the
241 movement of metals into their AG plant tissues to avoid the potential toxic effects of high
242 metal concentrations on their photosynthetic organs (Bragato et al., 2006). The reduction of N
243 and P in AG parts in October and November, is known to occur in rhizomatous plants such as
244 *P. australis*, where the nutrients are translocated to and stored in BG organs during winter,
245 and are ready to initiate growth the following season (Chapin III et al., 1990). The
246 concentrations of N and P at the beginning of the study (April and May) are similar to
247 concentrations at the end of the study (October and November), therefore it may be assumed
248 that nutrients are overwintered in BG organs.

249

250 The current study was carried out in a lightly loaded system with a small PE (Table 2).
251 Previous studies have suggested that uptake by plants in AG and BG organs, is significant
252 only under low loading conditions (Brix, 1997), similar to that of the CW in the current
253 study. Zinc was the only metal to be present in higher concentrations in AG biomass during
254 some months of the study which was similar to Pevery (1995) and Schierup and Larsen
255 (1981), where higher concentrations of Zn were found in AG plant parts and stems. Zinc
256 plays an essential role in plant nutrition and enzymatic processes (Bonanno & Giudice,
257 2010). The higher concentrations of Zn in AG tissues may have occurred due to its essential
258 function in the formation of indole acetic acid, a plant hormone which is manufactured in the
259 stems of plants (Schierup and Larsen, 1981). Unlike Zn, which is essential to plant growth,
260 Ni and Cr are regarded as elements which are toxic to plants (Bonanno & Giudice, 2010).
261 Nickel was only detected in August and October in the AG biomass (Fig. 2), and at levels
262 lower than 5 mg kg⁻¹. However, *P. australis* has the potential to store up to 60 mg kg⁻¹ of Ni
263 (Bragato et al., 2006). Chromium content has previously been recorded at 4,825 mg kg⁻¹ and
264 827 mg kg⁻¹ in the roots and shoots of *P. australis* in a pot study using tannery wastewater
265 (Calheiros et al., 2008) and values found in this study were significantly lower than this
266 threshold level. Significant quantities of N were detected in the AG tissues of *P. australis* (up
267 to 25,338 mg kg⁻¹). Nitrogen removal from a CW is greatly facilitated by the plant uptake
268 through the root system of *P. australis*. June, July and August are the growing season for *P.*
269 *australis* in Ireland; therefore, higher quantities of N were found in the AG biomass during
270 these months. In addition to this, AG biomass was lowest in June (Fig. 1), the typical early
271 growing season for *P. australis* in Ireland. At this point, the majority of dead plant growth
272 from the previous year has fallen away and new shoots are appearing. The AG biomass
273 values in April and November are similar (1,384 g m⁻² and 1,346 g m⁻², respectively), which

274 leads us to believe that these values may be typical of the biomass values throughout the
275 winter season. However, further studies are needed to verify this.

276

277 Common reed is a traditional building material which is widely used in roofs, and insulation
278 blocks made from reed are highly valued in eco-friendly construction (Maddisson et al.,
279 2009). With this in mind, harvesting of the AG biomass of macrophytes has been suggested
280 by many researchers as an option for nutrient and metal removal in CWs (Bragato et al.,
281 2006; Vymazal et al., 2010; Vymazal & Březinová, 2015). In order to maximise removal, the
282 harvesting process needs to take place during a period of maximum content of the targeted
283 element in the plant. However, based on the results of this study, under temperate maritime
284 climatic conditions, metals and nutrients follow different seasonal patterns, and it is difficult
285 to identify an optimum time for harvest to obtain maximum removal of all nutrients and
286 metals at the same time based on the concentrations only. Therefore, if harvesting is to be
287 considered as an option, it will be necessary to prioritise between maximising the removal of
288 specific nutrients and metals. Furthermore, the effects of frequent harvesting on the regrowth
289 success of *P. australis* also needs to be evaluated (Maddisson et al., 2009). However, the
290 results of standing stocks of each metal and nutrient measured in the study, would suggest a
291 harvest in Autumn (late August or September) may capture the maximum contents of most
292 nutrients and metals in the AG biomass. This could result in the removal of between 0.6 g
293 (Ni) and 71.2 g (Zn) based on a harvest in August. The ability of *P. australis* to accumulate
294 metals and nutrients in AG biomass under such climatic conditions provides strong
295 encouragement for CW applications in industrial settings. Further work is needed to
296 investigate the translocation and accumulation of metals to the AG tissues, and the
297 implications of harvesting in terms of regrowth success in CWs treating industrial
298 wastewaters.

299

300 **Conclusions**

301 Plant uptake and accumulation is one method of metal and nutrient removal from CWs. With
302 the exception of Zn and N during some months of the study, BG biomass of *P. australis*
303 predominantly contained higher concentrations of metals and nutrients than AG biomass. In
304 order to remove maximum quantities of metals and nutrients, the harvesting process must
305 take place during the period of maximum content of the targeted element in the plant.
306 Knowledge of the concentrations alone does not provide information on the translocation or
307 accumulation of elements in the plants. In order to maximise the removal of metals and
308 nutrients in CWs, a harvest should take place during the period of maximum accumulation in
309 AG biomass. With this in mind, a harvest in Autumn of AG biomass is suggested based on
310 the results of this study.

311

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324 **References**

- 325 Ali, H., Khan, E. and Sajad, M.A., 2013. Phytoremediation of heavy metals—concepts and
326 applications. *Chemosphere*, 91(7), pp.869-881.
- 327 AOAC International. 2005. Official methods of analysis. 17th ed. Gaithersburg , Md. :
328 AOAC International. <http://sfxhosted.exlibrisgroup.com/galway/sfx.gif>
- 329 Białowiec, A., Davies, L., Albuquerque, A. and Randerson, P.F., 2012. Nitrogen removal
330 from landfill leachate in constructed wetlands with reed and willow: redox potential in
331 the root zone. *Journal of Environmental Management*, 97, pp.22-27.
- 332 Bonanno, G., 2011. Trace element accumulation and distribution in the organs of *Phragmites*
333 *australis* (common reed) and biomonitoring applications. *Ecotoxicology and*
334 *Environmental Safety*, 74(4), pp.1057-1064.
- 335 Bonanno, G. and Giudice, R.L., 2010. Heavy metal bioaccumulation by the organs of
336 *Phragmites australis* (common reed) and their potential use as contamination
337 indicators. *Ecological Indicators*, 10(3), pp.639-645
- 338 Bragato, C., Brix, H. and Malagoli, M., 2006. Accumulation of nutrients and heavy metals in
339 *Phragmites australis* (Cav.) Trin. ex Steudel and *Bolboschoenus maritimus* (L.) Palla
340 in a constructed wetland of the Venice lagoon watershed. *Environmental*
341 *Pollution*, 144(3), pp.967-975.
- 342 Bragato, C., Schiavon, M., Polese, R., Ertani, A., Pittarello, M. and Malagoli, M., 2009.
343 Seasonal variations of Cu, Zn, Ni and Cr concentration in *Phragmites australis* (Cav.)
344 Trin ex steudel in a constructed wetland of North Italy. *Desalination*, 246(1-3), pp.35-
345 44.
- 346 Březinová, T. and Vymazal, J., 2015. Evaluation of heavy metals seasonal accumulation in
347 *Phalaris arundinacea* in a constructed treatment wetland. *Ecological Engineering*, 79,
348 pp.94-99.

349 Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands?. Water
350 Science and Technology, 35(5), pp.11-17.

351 Brix, H., 2003. Plants used in constructed wetlands and their functions. - In: Dias, V. &
352 Vymazal, J. (Eds.): Proceedings of the 1st International Seminar on the Use of
353 Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands, May 8-10,
354 2003, Lisbon, Portugal. pp. 81-109.

355 Bulc, T.G., 2006. Long term performance of a constructed wetland for landfill leachate
356 treatment. Ecological Engineering, 26(4), pp.365-374.

357 Cai, K., Elliott, C.T., Phillips, D.H., Scippo, M.L., Muller, M. and Connolly, L., 2012.
358 Treatment of estrogens and androgens in dairy wastewater by a constructed wetland
359 system. Water Research, 46(7), pp.2333-2343.

360 Calheiros, C.S., Rangel, A.O. and Castro, P.M., 2008. The effects of tannery wastewater on
361 the development of different plant species and chromium accumulation in *Phragmites*
362 *australis*. Archives of environmental contamination and toxicology, 55(3), pp.404-
363 414.

364 Calheiros, C.S., Quitério, P.V., Silva, G., Crispim, L.F., Brix, H., Moura, S.C. and Castro,
365 P.M., 2012. Use of constructed wetland systems with *Arundo* and *Sarcocornia* for
366 polishing high salinity tannery wastewater. Journal of Environmental
367 Management, 95(1), pp.66-71.

368 Chapin III, F.S., Schulze, E.D. and Mooney, H.A., 1990. The ecology and economics of
369 storage in plants. Annual Review of Ecology and Systematics, 21(1), pp.423-447.

370 Deng, H., Ye, Z.H. and Wong, M.H., 2004. Accumulation of lead, zinc, copper and cadmium
371 by 12 wetland plant species thriving in metal-contaminated sites in
372 China. Environmental Pollution, 132(1), pp.29-40.

373 Du Laing, G., Tack, F.M. and Verloo, M.G., 2003. Performance of selected destruction
374 methods for the determination of heavy metals in reed plants (*Phragmites*
375 *australis*). *Analytica Chimica Acta*, 497(1), pp.191-198.

376 Gavlak, R.G., Horneck, D.A., Miller, R.O., Kotuby-Amacher, J., 2003. Soil, Plant, and
377 Water Reference Methods for the Western Region. 2nd edition. WREP-125.

378 Gill, L.W., Ring, P., Higgins, N.M. and Johnston, P.M., 2014. Accumulation of heavy metals
379 in a constructed wetland treating road runoff. *Ecological Engineering*, 70, pp.133-139.

380 Grismer, M.E., Carr, M.A. and Shepherd, H.L., 2003. Evaluation of constructed wetland
381 treatment performance for winery wastewater. *Water Environment Research*, 75(5),
382 pp.412-421.

383 Healy, M.G., Newell, J. and Rodgers, M., 2007. Harvesting effects on biomass and nutrient
384 retention in *Phragmites australis* in a free-water surface constructed wetland in
385 western Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy*,
386 107B(3), pp. 139-145.

387 Kadlec, R.H. and Knight, R.L., 1996. *Treatment Wetlands*. CRC Press, Boca Raton, Florida,
388 USA.

389 Kadlec, R.H. and Wallace, S.D., 2009. *Treatment Wetlands*, second ed., CRC Press, Boca
390 Raton, Florida, USA.

391 Keng, P.S., Lee, S.L., Ha, S.T., Hung, Y.T. and Ong, S.T., 2014. Removal of hazardous
392 heavy metals from aqueous environment by low-cost adsorption
393 materials. *Environmental Chemistry Letters*, 12(1), pp.15-25.

394 Larsen, V.J. and Schierup, H.H., 1981. Macophyte cycling of zinc, copper, lead and cadmium
395 in the littoral zone of a polluted and a non-polluted lake. II. Seasonal changes in
396 heavy metal content of above-ground biomass and decomposing leaves of *Phragmites*
397 *australis* (Cav.) Trin. *Aquatic Botany*, 11, pp.211-230.

398 Lin, Y.F., Jing, S.R., Lee, D.Y., Chang, Y.F., Chen, Y.M. and Shih, K.C., 2005. Performance
399 of a constructed wetland treating intensive shrimp aquaculture wastewater under high
400 hydraulic loading rate. *Environmental Pollution*, 134(3), pp.411-421.

401 Maddison, M., Soosaar, K., Mairing, T. and Mander, Ü., 2009. The biomass and nutrient and
402 heavy metal content of cattails and reeds in wastewater treatment wetlands for the
403 production of construction material in Estonia. *Desalination*, 246(1-3), pp.120-128.

404 Maine, M.A., Sune, N., Hadad, H., Sánchez, G. and Bonetto, C., 2009. Influence of
405 vegetation on the removal of heavy metals and nutrients in a constructed
406 wetland. *Journal of Environmental Management*, 90(1), pp.355-363.

407 Matamoros, V., Arias, C., Brix, H. and Bayona, J.M., 2009. Preliminary screening of small-
408 scale domestic wastewater treatment systems for removal of pharmaceutical and
409 personal care products. *Water Research*, 43(1), pp.55-62.

410 Mays, P.A. and Edwards, G.S., 2001. Comparison of heavy metal accumulation in a natural
411 wetland and constructed wetlands receiving acid mine drainage. *Ecological
412 Engineering*, 16(4), pp.487-500.

413 Mustafa, A. and Scholz, M., 2011. Nutrient accumulation in *Typha latifolia* L. and sediment
414 of a representative integrated constructed wetland. *Water, Air, & Soil
415 Pollution*, 219(1-4), pp.329-341.

416 Obolewski, K., Skorbiłowicz, E., Skorbiłowicz, M., Glińska-Lewczuk, K., Astel, A.M. and
417 Strzelczak, A., 2011. The effect of metals accumulated in reed (*Phragmites australis*)
418 on the structure of periphyton. *Ecotoxicology and Environmental Safety*, 74(4),
419 pp.558-568.

420 O'Sullivan, A.D., Murray, D.A. and Otte, M.L., 2004. Removal of sulfate, zinc, and lead
421 from alkaline mine wastewater using pilot-scale surface-flow wetlands at Tara Mines,
422 Ireland. *Mine Water and the Environment*, 23(2), pp.58-65.

- 423 Peverly, J.H., Surface, J.M. and Wang, T., 1995. Growth and trace metal absorption by
424 *Phragmites australis* in wetlands constructed for landfill leachate
425 treatment. *Ecological Engineering*, 5(1), pp.21-35.
- 426 Schierup, H.H. and Larsen, V.J., 1981. Macrophyte cycling of zinc, copper, lead and
427 cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability,
428 uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin. *Aquatic*
429 *Botany*, 11, pp.197-210.
- 430 Shelef, O., Gross, A. and Rachmilevitch, S., 2013. Role of plants in a constructed wetland:
431 current and new perspectives. *Water*, 5(2), pp.405-419.
- 432 Stottmeister, U., Wießner, A., Kusch, P., Kappelmeyer, U., Kästner, M., Bederski, O.,
433 Müller, R.A. and Moormann, H., 2003. Effects of plants and microorganisms in
434 constructed wetlands for wastewater treatment. *Biotechnology Advances*, 22(1),
435 pp.93-117.
- 436 Vymazal, J., Švehla, J., Kröpfelová, L. and Chrástný, V., 2007. Trace metals in *Phragmites*
437 *australis* and *Phalaris arundinacea* growing in constructed and natural
438 wetlands. *Science of the Total Environment*, 380(1), pp.154-162.
- 439 Vymazal, J. and Kröpfelová, L., 2008. Nitrogen and phosphorus standing stock in *Phalaris*
440 *arundinacea* and *Phragmites australis* in a constructed treatment wetland: 3-year
441 study. *Archives of Agronomy and Soil Science*, 54(3), pp.297-308.
- 442 Vymazal, J., Kröpfelová, L., Švehla, J. and Štichová, J., 2010. Can multiple harvest of
443 aboveground biomass enhance removal of trace elements in constructed wetlands
444 receiving municipal sewage? *Ecological Engineering*, 36(7), pp.939-945.
- 445 Vymazal, J., 2011. Constructed wetlands for wastewater treatment: Five decades of
446 experience. *Environmental Science and Technology*, 45(1), pp 61-69.

447 Vymazal, J., Březinová, T. and Koželuh, M., 2015. Occurrence and removal of estrogens,
448 progesterone and testosterone in three constructed wetlands treating municipal sewage
449 in the Czech Republic. *Science of the Total Environment*, 536, pp.625-631.

450 Vymazal, J. and Březinová, T., 2015. Heavy metals in plants in constructed and natural
451 wetlands: concentration, accumulation and seasonality. *Water Science and
452 Technology*, 71(2), pp.268-276.

453 Vymazal, J. and Březinová, T., 2016. Accumulation of heavy metals in aboveground biomass
454 of *Phragmites australis* in horizontal flow constructed wetlands for wastewater
455 treatment: A review. *Chemical Engineering Journal*, 290, pp.232-242.

456 Ye, Z.H., Baker, A.J.M., Wong, M.H. and Willis, A.J., 2003. Copper tolerance, uptake and
457 accumulation by *Phragmites australis*. *Chemosphere*, 50(6), pp.795-800.

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464 Table 1. Metal and nutrient concentrations (mg kg⁻¹) in aboveground and belowground biomass of *Phragmites australis* in natural and
 465 constructed wetlands from previous studies.

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Element	Aboveground					Belowground				
	Max value ¹	Country	Wetland type ²	Wastewater type	Reference	Max value ¹	Country	Wetland type ²	Wastewater type	Reference
Cd	2.1	Greece	N		³	1.21	Denmark	N		⁷
Cr	118	Italy	C	Municipal	⁴	6.97	Italy	N		⁵
Cu	14.98	Italy	N		⁵	230	UK	C	Mine water	⁹
Ni	60	Italy	C	Municipal	⁴	9.12	Italy	N		⁵
Pb	39	China	C	Mine water	⁶	>2,000	China	C	Mine water	⁶
Zn	217	Denmark	N		⁷	>1,000	China	C	Mine water	⁶
N	26,500	Italy	C	Municipal	⁴	19,100	Czech Republic	C	Municipal	⁸
P	2,200	Czech Republic	C	Municipal	⁸	2,700	Czech Republic	C	Municipal	⁸

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468 ¹ Maximum values are based on the maximum concentration values reported in the papers reviewed throughout this study

469 ² N = natural wetland; C = constructed wetland

470 ³Obolewski et al. (2011); ⁴Bragato et al. (2006) ; ⁵Bonanno & Giudice (2010); ⁶ Deng et al. (2004); ⁷Schierup & Larsen (1981); ⁸Vymazal & Kröpfelová (2008); ⁹Ye et al.
 471 (2003)

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479 Table 2. Details of site characteristics

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<i>Reed bed dimensions</i>			<i>Area (m²)</i>	<i>PE</i>	<i>Volume (m³)</i>	<i>Hydraulic retention time (d)*</i>	<i>Hydraulic loading rate (m d⁻¹)*</i>
<i>Length (m)</i>	<i>Width (m)</i>	<i>Depth (m)</i>					
20	20	0.5	400	400	200	7.3	0.068*

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482 *Based on a mean flow of 27.3m³ per day

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493 Table 3. Average concentrations of metals in inflow wastewater entering the constructed
 494 wetland at Fenagh during the study period (April – November, 2015) (n = 3)

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Metals (total)	Limit of Detection (LOD)	Average result (n = 3)	Units	Limits in surface water ($\mu\text{g L}^{-1}$) ¹
Cadmium ²	0.3	<0.3	$\mu\text{g L}^{-1}$	1
Chromium	3.0	<0.3	$\mu\text{g L}^{-1}$	50
Copper	3.0	7.0	$\mu\text{g L}^{-1}$	1,000
Lead ²	0.9	<0.9	$\mu\text{g L}^{-1}$	50
Nickel	1.5	1.9	$\mu\text{g L}^{-1}$	
Zinc	10	17	$\mu\text{g L}^{-1}$	1,000

496 ¹ From Subsidiary Legislation 549.21, 28th June, 2002

497 ²Cadmium and lead consistently reported below the LOD

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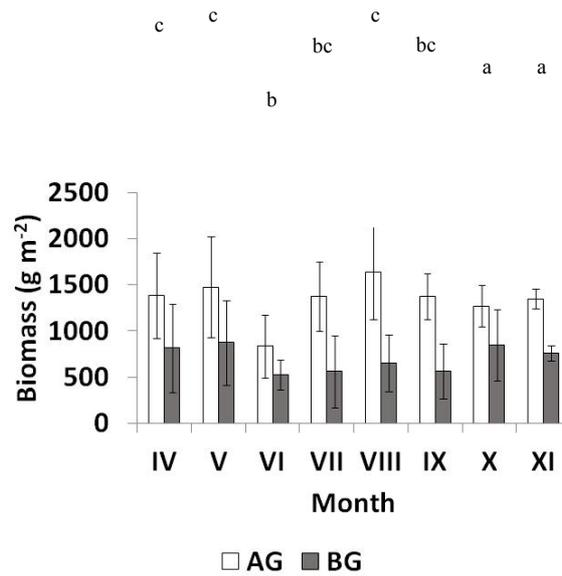
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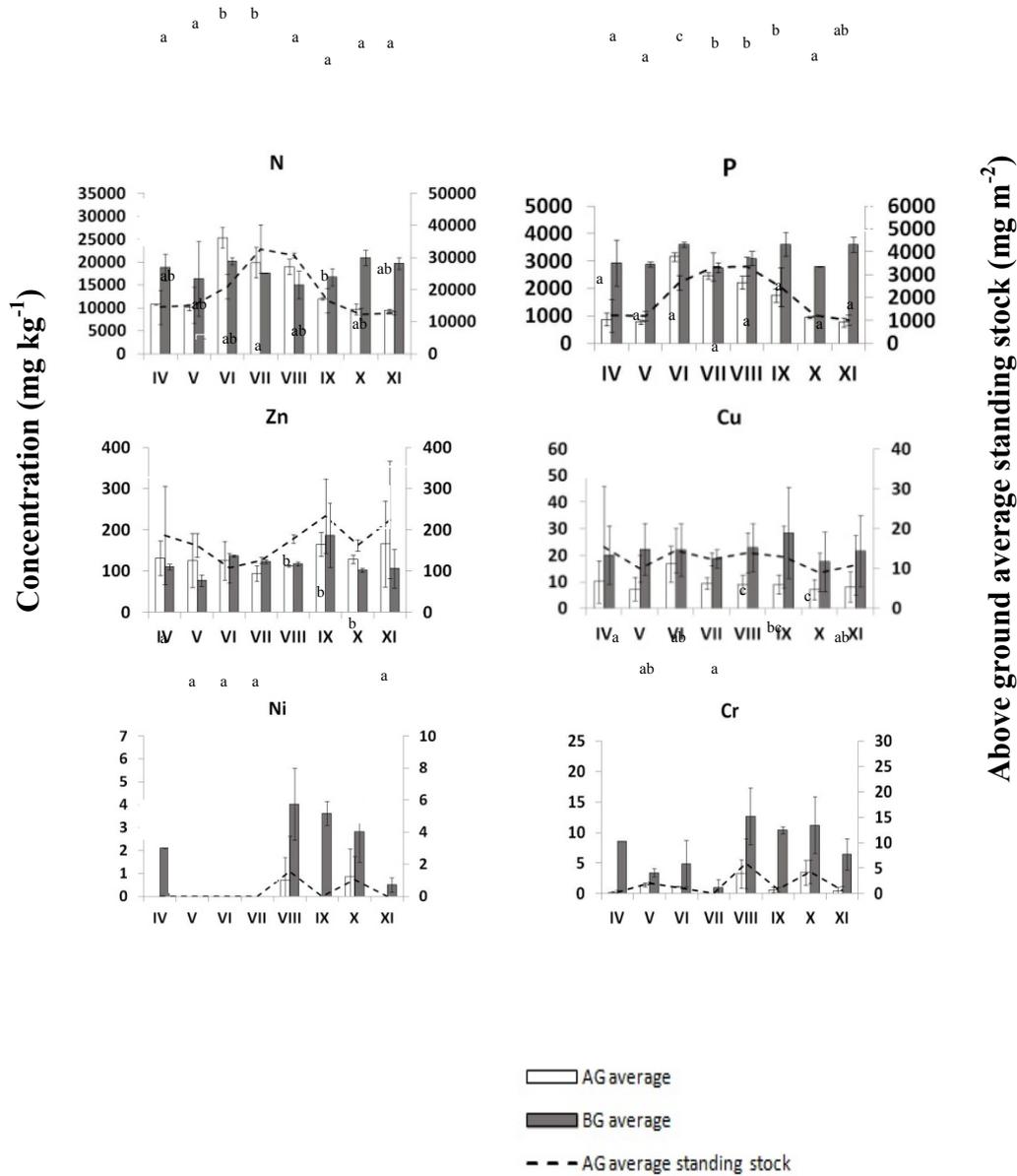
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517 Fig 1. Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and
 518 outlet zones combined) in the wetland vegetation during the period of April – November,
 519 2015. Error bars represent the standard deviation. Different letters indicate significant
 520 differences between the monthly means at $P < 0.05$. (For the significance of above versus
 521 below ground and the above versus below ground x month interaction, see the text of the
 522 results section).

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526 Fig. 2 Comparison of the seasonal variation in aboveground (AG) and belowground (BG)
 527 concentrations of nutrients (nitrogen and phosphorus) and metals (zinc, copper, nickel and
 528 chromium) (mg kg^{-1}) and aboveground standing stocks (mg m^{-2}) in biomass of *Phragmites*
 529 *australis* during the period April – November, 2015. Error bars represent the standard
 530 deviation. Different letters indicate significant differences between the monthly means at $P <$
 531 0.05 . (For the significance of above versus below ground and the above versus below ground
 532 x month interaction, see the text of the results section).
 533