



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

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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

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10  
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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 m<sup>2</sup>, 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 m<sup>2</sup> 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<sup>-2</sup>). 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 HNO<sub>3</sub>: 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<sup>-2</sup>) = concentration  
161 (g kg<sup>-1</sup>) x dry matter (kg m<sup>-2</sup>).

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<sup>-2</sup>), while  
175 biomass was lowest in June (835 g m<sup>-2</sup>). Belowground biomass which ranged from 523 g m<sup>-2</sup>

176 to 872 g m<sup>-2</sup> 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<sup>-1</sup> for Cr and 4 mg  
185 kg<sup>-1</sup> 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<sup>-1</sup> (Table 3).  
190 Belowground concentrations of Cu ranged from 17.6 mg kg<sup>-1</sup> to 28.5 mg kg<sup>-1</sup>, and were  
191 always higher than AG concentrations, which ranged from 7.1 mg kg<sup>-1</sup> to 16.7 mg kg<sup>-1</sup>.  
192 Aboveground standing stock of Cu was highest early in the growing season in April (15.4 mg  
193 m<sup>-2</sup>). 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<sup>-1</sup>  
197 and 165.6 mg kg<sup>-1</sup>). Zinc standing stocks were also highest during these months (233.9 mg m<sup>-2</sup>  
198 and 224.3 mg m<sup>-2</sup>). The highest monthly concentration of Zn was measured in BG organs in  
199 September (187 mg kg<sup>-1</sup>), and the lowest was measured in May (77.1 mg kg<sup>-1</sup>). 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<sup>-1</sup>), the early growing season  
206 in Ireland, and declined from then to its lowest concentration of 9,463 mg kg<sup>-1</sup> in November.  
207 Nitrogen was lowest in the BG tissues in August (15,000 mg kg<sup>-1</sup>) and highest in October  
208 (20,975 mg kg<sup>-1</sup>). The maximum nitrogen AG standing stock (32.6 g m<sup>-2</sup>) 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<sup>-1</sup>) and steadily declined throughout the  
214 study until November (768 mg kg<sup>-1</sup>). Belowground values for P ranged from 2755 mg kg<sup>-1</sup> in  
215 July to 3605 mg kg<sup>-1</sup> 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<sup>-2</sup> and 3.4 g m<sup>-2</sup>, respectively) and lowest in November  
218 (1 g m<sup>-2</sup>). 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<sup>-1</sup>. However, *P. australis* has the potential to store up to 60 mg kg<sup>-1</sup> of Ni  
263 (Bragato et al., 2006). Chromium content has previously been recorded at 4,825 mg kg<sup>-1</sup> and  
264 827 mg kg<sup>-1</sup> 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<sup>-1</sup>). 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<sup>-2</sup> and 1,346 g m<sup>-2</sup>, 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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464 Table 1. Metal and nutrient concentrations (mg kg<sup>-1</sup>) 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 <sup>1</sup>	Country	Wetland type <sup>2</sup>	Wastewater type	Reference	Max value <sup>1</sup>	Country	Wetland type <sup>2</sup>	Wastewater type	Reference
<b>Cd</b>	2.1	Greece	N		<sup>3</sup>	1.21	Denmark	N		<sup>7</sup>
<b>Cr</b>	118	Italy	C	Municipal	<sup>4</sup>	6.97	Italy	N		<sup>5</sup>
<b>Cu</b>	14.98	Italy	N		<sup>5</sup>	230	UK	C	Mine water	<sup>9</sup>
<b>Ni</b>	60	Italy	C	Municipal	<sup>4</sup>	9.12	Italy	N		<sup>5</sup>
<b>Pb</b>	39	China	C	Mine water	<sup>6</sup>	>2,000	China	C	Mine water	<sup>6</sup>
<b>Zn</b>	217	Denmark	N		<sup>7</sup>	>1,000	China	C	Mine water	<sup>6</sup>
<b>N</b>	26,500	Italy	C	Municipal	<sup>4</sup>	19,100	Czech Republic	C	Municipal	<sup>8</sup>
<b>P</b>	2,200	Czech Republic	C	Municipal	<sup>8</sup>	2,700	Czech Republic	C	Municipal	<sup>8</sup>

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

469 <sup>2</sup> N = natural wetland; C = constructed wetland

470 <sup>3</sup>Obolewski et al. (2011); <sup>4</sup>Bragato et al. (2006) ; <sup>5</sup>Bonanno & Giudice (2010); <sup>6</sup> Deng et al. (2004); <sup>7</sup>Schierup & Larsen (1981); <sup>8</sup>Vymazal & Kröpfelová (2008); <sup>9</sup>Ye 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 (m<sup>2</sup>)</i>	<i>PE</i>	<i>Volume (m<sup>3</sup>)</i>	<i>Hydraulic retention time (d)*</i>	<i>Hydraulic loading rate (m d<sup>-1</sup>)*</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 27.3m<sup>3</sup> 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 ( $\mu\text{g L}^{-1}$ ) <sup>1</sup>
Cadmium <sup>2</sup>	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 <sup>2</sup>	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 <sup>1</sup> From Subsidiary Legislation 549.21, 28<sup>th</sup> June, 2002

497 <sup>2</sup>Cadmium and lead consistently reported below the LOD

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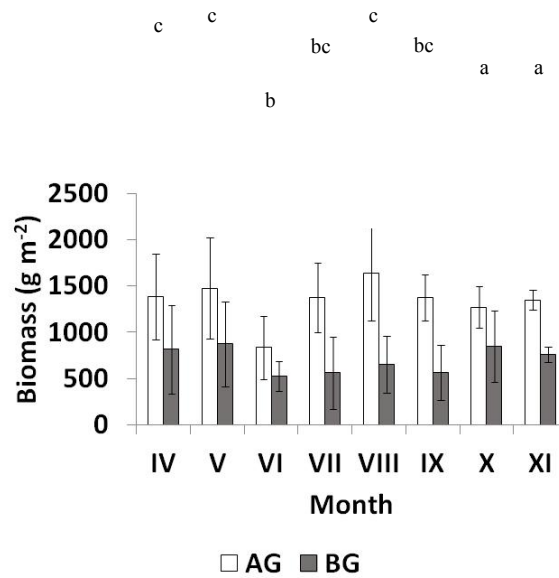
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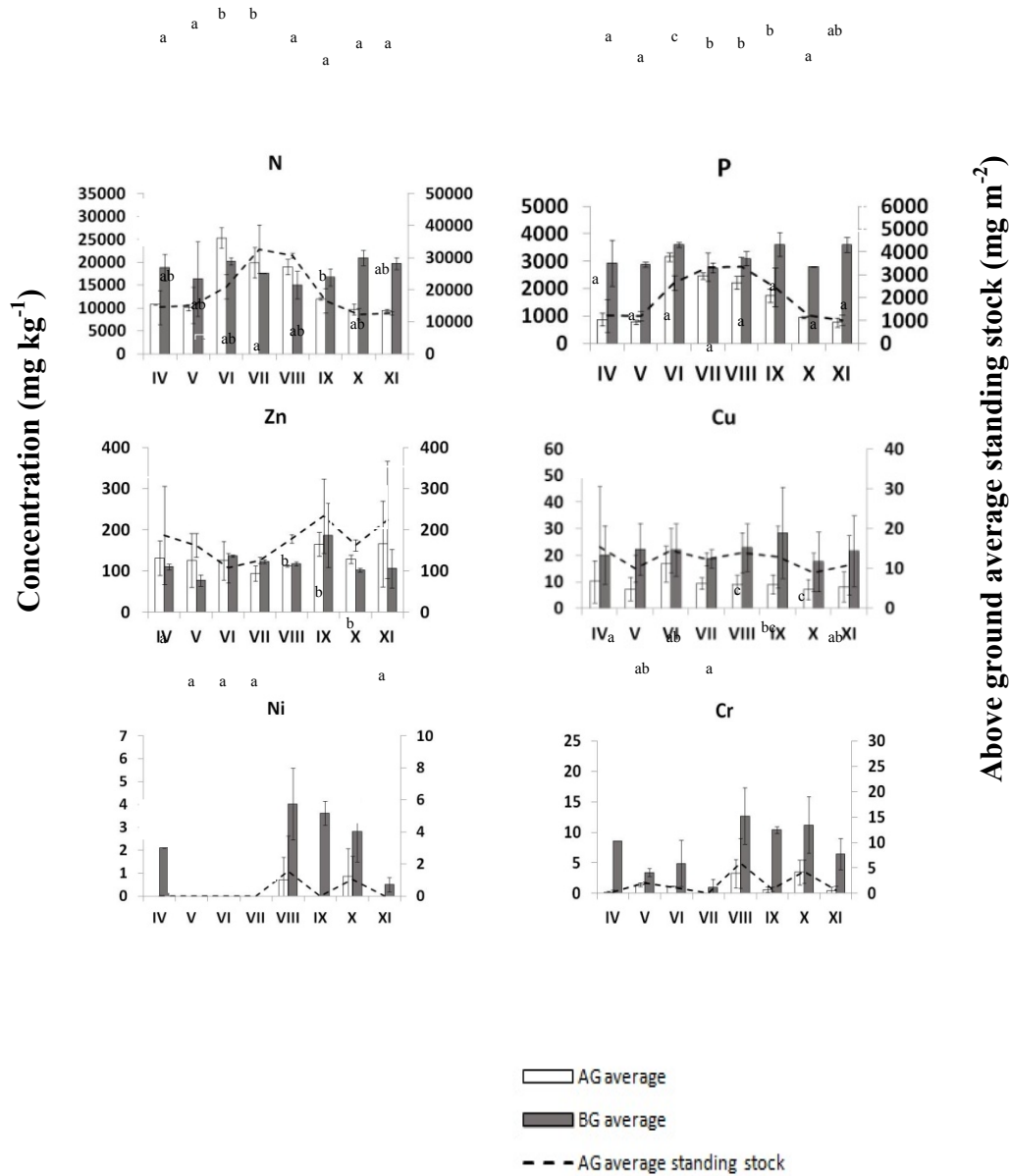
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517 Fig 1. Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and  
518 outlet zones combined) in the wetland vegetation during the period of April – November,  
519 2015. Error bars represent the standard deviation. Different 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) ( $\text{mg kg}^{-1}$ ) and aboveground standing stocks ( $\text{mg m}^{-2}$ ) 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