Mulkeen, CJ, Williams, CD, Gormally, MJ and Healy, MG

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

http://researchonline.ljmu.ac.uk/id/eprint/6873/

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Mulkeen, CJ, Williams, CD, Gormally, MJ and Healy, MG (2017) Seasonal patterns of metals and nutrients in Phragmites australis (Cav.) Trin. ex Steudel in a constructed wetland in the west of Ireland. Ecological Engineering. 107. pp. 192-197. ISSN 0925-8574

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

C.J. Mulkeen[1,2], C.D. Williams[3], M.J. Gormally[2], and M.G. Healy[1*,]

[1]Civil Engineering, National University of Ireland, Galway, Ireland.
[2]Applied Ecology Unit, School of Natural Sciences, National University of Ireland, Galway, Ireland.
[3]School of Natural Sciences and Psychology, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK.
*Corresponding author: mark.healy@nuigalway.ie

Abstract

An understanding of the seasonal variation in the standing stock of metals and nutrients in emergent vegetation of constructed wetlands (CWs), as well as the amounts present in aboveground (AG) and belowground (BG) biomass, is crucial to their design and management. Given that biomass harvesting is a labour and time consuming operation, a paucity of information currently exists on accumulation and standing stocks in biomass in CWs, in particular in North Western European countries. To address this knowledge gap, this paper examined the seasonal variations of metals and nutrients in *Phragmites australis* (Cav.) Trin. ex Steudel in a CW treating municipal wastewater, with a view to identifying an optimal time for biomass harvesting of the AG vegetation. Although the AG biomass was greatest in August (1,636 ± 507 g m^{-2}), the maximum concentrations and accumulations of metals and nutrients occurred at different times throughout the duration of the study (April to

November). Furthermore, with the exception of zinc and nitrogen, metals and nutrients measured in BG biomass ranged from 66% (phosphorus) to greater than 80% (nickel and chromium) of the AG biomass. This indicates that analysis of only the emergent shoots may significantly underestimate the metal and nutrient uptake and capacity of the plant. In order to effectively target the bulk of metals and nutrients, an AG harvest in late August or September is suggested.

Keywords: constructed wetlands, macrophytes, metals, biomass harvesting

1. Introduction

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

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

Metals are non-biodegradable, and water pollution by metals is a serious environmental problem which is difficult to solve (Keng et al., 2014). In CWs, metals tend to accumulate in the sediments as well as in the plants (Březinová & Vymazal, 2015). While metals in CWs are removed through physical (settling and sedimentation) and chemical (sorption and adsorption) mechanisms, metal uptake by plants has also been identified as the principal removal mechanism for some pollutants, particularly in lightly loaded systems (Březinová & Vymazal, 2015). However, metal content in the roots and shoots of wetland vegetation varies from season to season and there has been no attempt to explain this variability, or to determine optimum conditions for metal uptake by plants in CWs to date (Vymazal and Březinová, 2016). In the context of how we manage CWs, the seasonal variations of metals in macrophytes must be first of all understood, if we intend to expand the use of CWs for treating effluents containing metals in the future.

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

The total storage of a substance in a plant part is called standing stock (Vymazal & Březinová, 2015) and is calculated by multiplying the concentration by biomass per unit area. Vymazal & Březinová (2015) suggest that knowledge of concentrations alone does not provide any information of the translocation or accumulation of metals in a plant without knowing the biomass. In a literature review of metals in AG biomass of *P. australis* by Vymazal & Březinová (2016), the authors theorize that in order to obtain correct accumulation values in a plant, it is necessary to include the biomass values. Biomass harvesting is a labour and time consuming operation, and therefore a paucity of information exists on accumulation and standing stocks in AG biomass in CWs.

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

2. Materials and methods

Site description

The free-water surface constructed wetland (FWS CW) investigated in this study is located in Fenagh, Co. Leitrim, Ireland (54˚1′2″N; 7˚49′43″W). This CW was designed and constructed to cater for a population equivalent (PE) of 400 in 2004, but currently receives wastewater with a PE of 132 (Table 2). Wastewater enters the treatment works at the primary settlement tank, flows by gravity to a rotating biological contactor before entering the CW, where the wastewater undergoes tertiary treatment. The CW has a surface area of 400 m^2, and is lined with a high-density polyethylene liner. The wetland was originally planted with a monoculture of *P. australis*. Vegetation cover in the wetland is 100%, with some occasional bramble (*Rubus fruticosus* agg.), nettle (*Urtica dioica* L.) and willow scrub (*Salix* spp. L.) encroaching onto the reed bed.

Vegetation sampling regime

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

Laboratory analysis

Nitrogen testing was carried out by combustion analysis using a Carla Erba nitrogen analyzer following the Association of Official Analytical Chemists (AOAC) method 990.03 (2005). The instrument was calibrated daily with an atropine standard. Quality control (QC) [National Institute of Standards and Technology (NIST)] tomato leaf check samples were run throughout analysis (every ten samples). Phosphorus, aluminium (Al), boron (B), iron (Fe), manganese (Mn), magnesium (Mg), potassium (K), copper (Cu), zinc (Zn), sulphate (S) and calcium (Ca) were digested using nitric acid and hydrogen peroxide in a CEM Mars microwave system and analysed using a Thermo 65 Duo ICP following P4.3 “Soil, Plant and Water Reference methods for the Western Region” (Gavlak et al., 2003). Check samples were run through the ICP every 50 samples. Cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) were analysed using Inductively Coupled Plasma (ICP) mass spectrometry after digestion with *aqua regia* (1:3 HNO_3: HCl) at 110°C for three hours. Similarly, calibration standards and QC samples were run initially followed by blank, spiked and matrix spiked samples throughout the analysis (every ten samples) for verification purposes. Using these data, the AG and BG biomass and nutrient and metal content for each sampling section were obtained. Standing stocks were calculated as follows: standing stock (g m^{-2}) = concentration (g kg^{-1}) x dry matter (kg m^{-2}).

Statistical analysis:

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

3. Results

3.1 Aboveground and belowground biomass

The average dry AG and BG biomass harvested during the study is presented in Fig. 1. Maximum recorded AG biomass in the study was recorded in August (1,636 g m^{-2}), while biomass was lowest in June (835 g m^{-2}). Belowground biomass which ranged from 523 g m^{-2} to 872 g m^{-2} represented 53% to 62% of the AG biomass respectively. There was a statistically significant ($P = 0.002$) interaction between AG and BG biomass and month of the year.

3.2 Seasonal pattern of metal concentrations and accumulations

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

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

Zinc concentrations were highest in AG organs in September and November (165.2 mg kg^{-1} and 165.6 mg kg^{-1}). Zinc standing stocks were also highest during these months (233.9 mg m^{-2} and 224.3 mg m^{-2}). The highest monthly concentration of Zn was measured in BG organs in September (187 mg kg $^{-1}$), and the lowest was measured in May (77.1 mg kg $^{-1}$). There was no significant ($P > 0.05$) interaction between AG versus BG, and month of the year for the concentration of Zn in *P. australis* biomass throughout the study.

3.3 Seasonal pattern of nutrient concentrations and accumulations

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

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

4. Discussion

Metals enter the environment from natural and anthropogenic sources, and are non-biodegradable, accumulate in the environment, and pose a threat to the environment and human health (Ali et al., 2013). Studies examining the ability of emergent vegetation in CWs to uptake metals and nutrients have commonly examined AG vegetation only or concentrations only. However, the findings of the current study suggest that analysis of only the emergent shoots or concentrations only, may significantly underestimate the metal and nutrient uptake of the plant. With the exception of Zn and N, there were higher concentrations of metals and nutrients in the BG organs of the plant during each month of analysis. Overall, Zn concentrations were cumulatively higher in AG biomass (52%) during April, May, October and November, whereas N concentrations in AG biomass were higher during June, July and August (the typical growing season for *P. australis*). The findings of higher concentrations in BG biomass was similar to other studies (Peverly et al., 1995; Mays & Edwards, 2001; Bragato et al., 2009), and indicates that *P. australis* is prevalently a root bioaccumulator species (Bonanno, 2011). The roots and rhizomes are the immediate points of uptake in plants and, consequently, the concentrations are usually greater in roots in comparison to leaves and other AG organs (Vymazal et al., 2007). The lower concentrations in AG organs in the current study is in agreement with the speculation that plants restrict the movement of metals into their AG plant tissues to avoid the potential toxic effects of high metal concentrations on their photosynthetic organs (Bragato et al., 2006). The reduction of N and P in AG parts in October and November, is known to occur in rhizomatous plants such as *P. australis*, where the nutrients are translocated to and stored in BG organs during winter, and are ready to initiate growth the following season (Chapin III et al., 1990). The concentrations of N and P at the beginning of the study (April and May) are similar to concentrations at the end of the study (October and November), therefore it may be assumed that nutrients are overwintered in BG organs.

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

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

Conclusions

Plant uptake and accumulation is one method of metal and nutrient removal from CWs. With the exception of Zn and N during some months of the study, BG biomass of *P. australis* predominantly contained higher concentrations of metals and nutrients than AG biomass. In order to remove maximum quantities of metals and nutrients, the harvesting process must take place during the period of maximum content of the targeted element in the plant.

Knowledge of the concentrations alone does not provide information on the translocation or accumulation of elements in the plants. In order to maximise the removal of metals and nutrients in CWs, a harvest should take place during the period of maximum accumulation in AG biomass. With this in mind, a harvest in Autumn of AG biomass is suggested based on the results of this study.

Acknowledgements

The authors acknowledge funding from the Irish Environmental Protection Agency (EPA) (Project number 2013-B-PhD-12). With thanks to Leitrim County Council and Irish Water, S. Mulkeen, V. Bacle, C. Jaudoin and C. Teillet.

References

Ali, H., Khan, E. and Sajad, M.A., 2013. Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, *91*(7), pp.869-881.

AOAC International. 2005. Official methods of analysis. 17th ed. Gaithersburg , Md. : AOAC International. http://sfxhosted.exlibrisgroup.com/galway/sfx.gif

Białowiec, A., Davies, L., Albuquerque, A. and Randerson, P.F., 2012. Nitrogen removal from landfill leachate in constructed wetlands with reed and willow: redox potential in the root zone. Journal of Environmental Management, 97, pp.22-27.

Bonanno, G., 2011. Trace element accumulation and distribution in the organs of Phragmites australis (common reed) and biomonitoring applications. Ecotoxicology and Environmental Safety, 74(4), pp.1057-1064.

Bonanno, G. and Giudice, R.L., 2010. Heavy metal bioaccumulation by the organs of Phragmites australis (common reed) and their potential use as contamination indicators. Ecological Indicators, 10(3), pp.639-645

Bragato, C., Brix, H. and Malagoli, M., 2006. Accumulation of nutrients and heavy metals in Phragmites australis (Cav.) Trin. ex Steudel and Bolboschoenus maritimus (L.) Palla in a constructed wetland of the Venice lagoon watershed. Environmental Pollution, 144(3), pp.967-975.

Bragato, C., Schiavon, M., Polese, R., Ertani, A., Pittarello, M. and Malagoli, M., 2009. Seasonal variations of Cu, Zn, Ni and Cr concentration in Phragmites australis (Cav.) Trin ex steudel in a constructed wetland of North Italy. Desalination, 246(1-3), pp.35-44.

Březinová, T. and Vymazal, J., 2015. Evaluation of heavy metals seasonal accumulation in Phalaris arundinacea in a constructed treatment wetland. Ecological Engineering, 79, pp.94-99.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Peverly, J.H., Surface, J.M. and Wang, T., 1995. Growth and trace metal absorption by Phragmites australis in wetlands constructed for landfill leachate treatment. Ecological Engineering, 5(1), pp.21-35.

Schierup, H.H. and Larsen, V.J., 1981. Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in Phragmites australis (Cav.) Trin. Aquatic Botany, 11, pp.197-210.

Shelef, O., Gross, A. and Rachmilevitch, S., 2013. Role of plants in a constructed wetland: current and new perspectives. Water, 5(2), pp.405-419.

Stottmeister, U., Wießner, A., Kuschk, P., Kappelmeyer, U., Kästner, M., Bederski, O., Müller, R.A. and Moormann, H., 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances, 22(1), pp.93-117.

Vymazal, J., Švehla, J., Kröpfelová, L. and Chrastný, V., 2007. Trace metals in Phragmites australis and Phalaris arundinacea growing in constructed and natural wetlands. Science of the Total Environment, 380(1), pp.154-162.

Vymazal, J. and Kröpfelová, L., 2008. Nitrogen and phosphorus standing stock in Phalaris arundinacea and Phragmites australis in a constructed treatment wetland: 3-year study. Archives of Agronomy and Soil Science, 54(3), pp.297-308.

Vymazal, J., Kröpfelová, L., Švehla, J. and Štíchová, J., 2010. Can multiple harvest of aboveground biomass enhance removal of trace elements in constructed wetlands receiving municipal sewage? Ecological Engineering, 36(7), pp.939-945.

Vymazal, J., 2011. Constructed wetlands for wastewater treatment: Five decades of experience. Environmental Science and Technology, 45(1), pp 61-69.

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

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

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

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

Table 1. Metal and nutrient concentrations (mg kg $^{-1)}$ in aboveground and belowground biomass of *Phragmites australis* in natural and constructed wetlands from previous studies

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]

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

[2] N = natural wetland; C = constructed wetland

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

Table 2. Details of site characteristics

Reed bed dimensions			*Area (m^2)*	*PE*	*Volume (m^3)*	*Hydraulic retention time (d)**	*Hydraulic loading rate ($m\ d^{-1}$)**
Length (m)	*Width (m)*	*Depth (m)*					
20	20	0.5	400	400	200	7.3	0.068*

*Based on a mean flow of $27.3 m^3$ per day

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

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

[1] From Subsidiary Leglislation 549.21, 28th June, 2002

[2] Cadmium and lead consistently reported below the LOD

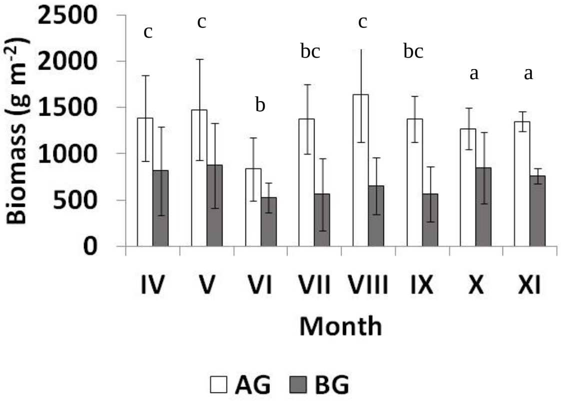

Fig 1. Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and outlet zones combined) in the wetland vegetation during the period of April – November, 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at $P < 0.05$.

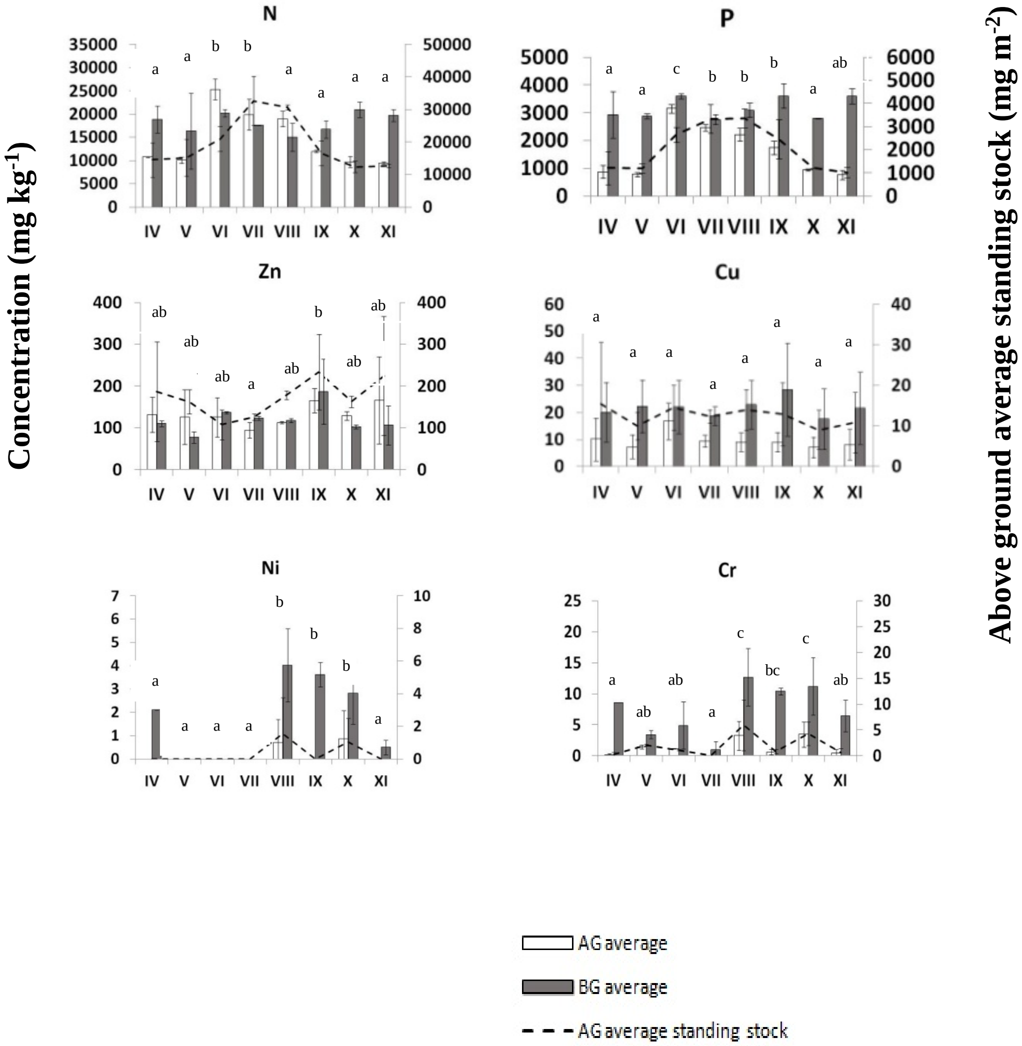

Fig. 2 Comparison of the seasonal variation in aboveground (AG) and belowground (BG) concentrations of nutrients (nitrogen and phosphorus) and metals (zinc, copper, nickel and chromium) (mg kg^{-1}) and aboveground standing stocks (mg m^{-2}) in biomass of *Phragmites australis* during the period April – November, 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at $P < 0.05$.