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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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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 analyses were 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 were 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. Metal accumulation in the AG biomass relative to the total amount entering the system (Table 3) over the eight-month study period ranged from 0.02% (for Cu) to 1.22% (for Zn). 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.

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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 ($\mu g\ L^{-1}$)[1]
Cadmium[2]	0.3	<0.3	$\mu g\ L^{-1}$	1
Chromium	3.0	<0.3	$\mu g\ L^{-1}$	50
Copper	3.0	7.0	$\mu g\ L^{-1}$	1,000
Lead[2]	0.9	<0.9	$\mu g\ L^{-1}$	50
Nickel	1.5	1.9	$\mu g\ L^{-1}$	
Zinc	10	17	$\mu g\ L^{-1}$	1,000

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

[2] Cadmium and lead consistently reported below the LOD

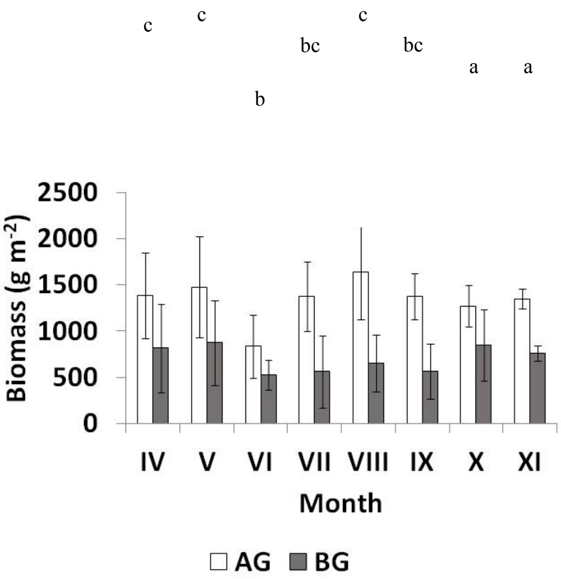

Fig 1. Average amounts of aboveground (AG) and belowground (BG) biomass (inlet and outlet zones combined) in the wetland vegetation during the period of April – November, 2015. Error bars represent the standard deviation. Different letters indicate significant differences between the monthly means at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

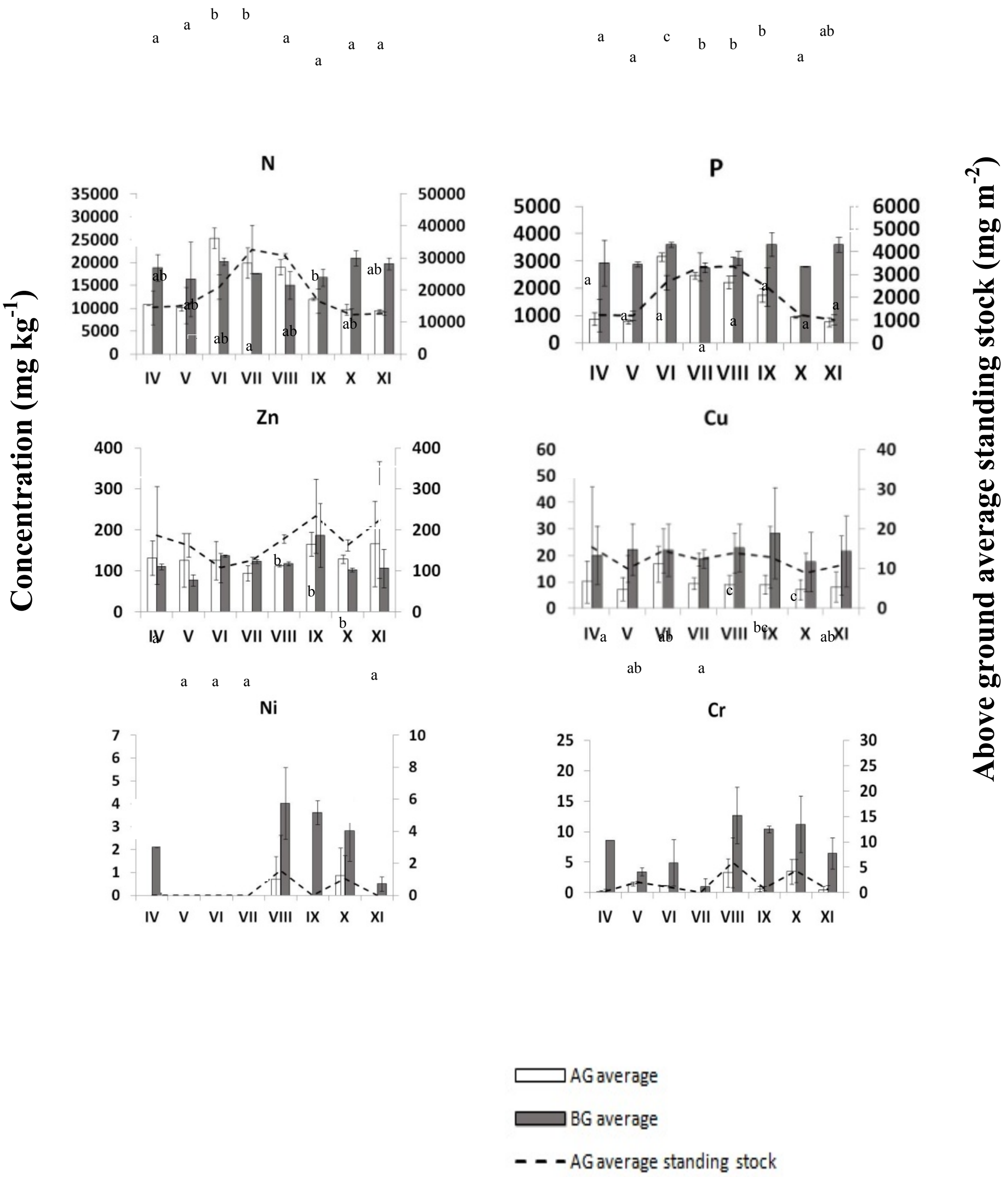

Fig. 2 Comparison of the seasonal variation in aboveground (AG) and belowground (BG) concentrations of nutrients (nitrogen and phosphorus) and metals (zinc, copper, nickel and chromium) (mg kg^{-1}) and aboveground standing stocks (mg m^{-2}) in biomass of *Phragmites australis* during the period April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