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Influence of plant species and submerged zone with carbon addition on the removal of metals by stormwater biofilters

Zhenhua Zhang[a,b,*], Zed Rengel[b], Tania Liaghati[c], Antonietta Torre[c], Kathy Meney[d]

[a]*Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, 50 Zhongling Street, Nanjng 210014, China*
Tel. +61 8 6488 2557; Fax: +61 8 6488 1050; email: zhenhua.zhang@uwa.edu.au
[b]*School of Earth and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia*
[c]*Department of Water, Western Australia, 168 St Georges Terrace, Perth, WA 6000, Australia*
[d]*Syrinx Environmental Pty Ltd, 12 Monger St., Perth, WA 6000, Australia*

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ABSTRACT

The metal removal in stormwater biofilters may be influenced by the type of vegetation and the presence of a submerged zone (SZ) with carbon (C) addition under wet-dry seasonal climatic patterns. A glasshouse experiment using two plant species (*Baumea juncea* and *Melaleuca lateritia*) with/without SZ and C addition, and three planting treatments (*Baumea rubiginosa*, *Juncus subsecundus*, and no-plant as control) with SZ and C addition was conducted to investigate the metal removal from the stormwater in biofilter columns. After 20 months of growth, plant growth was better in the presence of SZ than absence. The removal of copper (Cu) and lead (Pb) significantly increased in the biofilters with SZ, but Zn removal was not significantly influenced regardless of type of vegetation. Although the metals accumulated differently in the various plant species, it was not possible to discriminate relative performance in terms of metal removal among the plant species. Dissolved oxygen (DO) and pH in the outflows were significantly influenced by the type of vegetation and the presence of SZ. Hence, further study is needed to elucidate the different adsorption and retention of metals in media in relation to variations of redox and pH in biofilters under wet/dry seasonal climatic patterns. Furthermore, studies under the field conditions are needed to verify the findings.

Keywords: Bioretention systems; Metal; Plant growth; Wastewater; Wet/dry season

1. Introduction

There is an increasing need to utilize stormwater for nonpotable requirements, thus reducing a demand on potable sources [1]. However, it is necessary to treat stormwater before use due to potential deleterious impacts on human and environmental health associated with the use of stormwater containing pollutants such as metals (even at trace concentrations) [2].

Biofilters (also called biofiltration systems, bioretention systems or rain gardens) are becoming widely used for treating stormwater in urban areas [3–5]. A typical biofilter comprise a vegetated swale or basin,

*Corresponding author.

overlaying a filter medium that drains to the underlying soil *in situ* or to a drainage pipe at the bottom of the system, depending on the site conditions [4,6]. The presence of plants is one of the most prominent features of biofilters and their presence distinguishes constructed biofilters from the unplanted media (such as soil-only) filters [7,8]. The removal efficiency of pollutants (including metals) from stormwater in biofilters relies strongly on vegetation and its symbiotic relationships with microorganisms [9].

There is a large number of native plant species in the southwest of Western Australia (WA) inhabiting areas that are permanently or seasonally inundated, and contain mineral or organic soil and eutrophic to oligotrophic water [10]. Each of these species is potentially of interest for use in stormwater biofilters. Although some plant species have adapted to both drought and waterlogging stresses in wet/dry climate conditions, they might suffer water stress during the long dry periods under Mediterranean wet-dry climatic conditions. Even though the variations of species in plant growth and pollutant accumulation have been documented by Zhang et al. [11–13], it remains unclear (i) whether there are significant differences in efficiency of metal removal among the species; and (ii) which species in terms of plant growth and metal removal is the most suitable for use in biofilters under the wet/dry climatic conditions.

A submerged zone (SZ) (or saturation zone) is a design modification of drainage configuration that involves the creation of a SZ in biofilters [14]. Although the design modification could play a key role in plant survival during long dry periods under the wet/dry climatic conditions, and enhance nitrogen (particularly NO_3-N) removal in biofilters [15–17], it would affect the environmental conditions such as pH and redox in media, resulting in different plant growth, mobilization/immobilization of metals and performance of biofilters [18]. However, few studies have been conducted to test for either possible positive or negative impacts of a SZ on metal removal [14,18].

The objectives of this study were to characterize (1) the differences in the removal of metals (Cd, Cu, Pb, and Zn) among the plant species (including no-plant as control); and (2) the impacts of a SZ with C addition on metal removal in stormwater biofilters under the wet/dry seasonal dosing conditions.

2. Materials and methods

2.1. Experimental setup and procedure

The details of experimental setup and procedure were described by Zhang et al. [15]. Briefly, total thirty-five biofilter columns (300 mm in diameter, 800 mm in height) were constructed from PVC stormwater pipes. The media in the columns consisted of three layers: (1) the top 300-mm soil layer (<2 mm; Gingin sandy loam soil); (2) the 200-mm river sand (<4 mm) transition layer; and (3) the bottom 100-mm fine gravel (mean diameter 5 mm) drainage layer. Properties of Gingin sandy loam soil (top 300-mm soil layer) were listed in Table 1. In 25 of 35 columns, a 300-mm SZ was created; in these columns, 200 g and 100 g per column of C source (jarrah woodchips) was added into the transition layer (200-mm river sand) and drainage layer (bottom 100-mm fine gravel), respectively. The total of seven treatments with five replicates were tested. There were two plant species (*Baumea juncea* and *Melaleuca lateritia*) with/without SZ and C (jarrah woodchips) addition, in addition to two plant species (*Baumea rubiginosa* and *Juncus subsecundus*) and a no-plant as control with SZ and C (jarrah woodchips) addition. The seedlings of plant species were collected from the local nursery and

Table 1
Properties of Gingin sandy loam soil used for biofilter media

Property	Amount
General	
pH ($CaCl_2$)	5.4
pH (H_2O)	6.4
Electrical conductivity (EC), $dS\,m^{-1}$	0.012
Sulfur (S), $mg\,kg^{-1}$	8.2
Total organic carbon, $g\,kg^{-1}$	3.2
Hydraulic conductivity, $mm\,h^{-1}$	173
Nitrogen	
Total nitrogen, $g\,kg^{-1}$	0.22
Nitrate nitrogen, $mg\,kg^{-1}$	2
Ammonium nitrogen, $mg\,kg^{-1}$	1
Phosphorus	
Total phosphorus, $g\,kg^{-1}$	0.12
Exchangeable phosphorus, $mg\,kg^{-1}$	12
Phosphorus retention index	5.2
Phosphorus buffering index	27.7
Total heavy metals	
Cadmium (Cd), $mg\,kg^{-1}$	<0.0007
Copper (Cu), $mg\,kg^{-1}$	1.3
Lead (Pb), $mg\,kg^{-1}$	1.8
Zinc (Zn), $mg\,kg^{-1}$	1.7
Particle size	
Coarse sand (200–2,000 μm), $g\,kg^{-1}$	873
Fine sand (20–200 μm), $g\,kg^{-1}$	79
Silt (2–20 μm), $g\,kg^{-1}$	19
Clay (<2 μm), $g\,kg^{-1}$	29

transplanted into the columns in March 2009. There were six plants of *B. juncea* or *B. rubiginosa*, three plants of *J. subsecundus* or five plants of *M. lateritia* per column based on the initial plant size. The soil surface was covered with a 30-mm layer of alkathene beads to minimize soil disturbance when the water was added. Plants were watered (with tap water) as required for 4 months to allow establishment.

The details on how to calculate the values of the inflow were described by Zhang et al. [15]. Briefly, the column dosing volume was initially calculated based on the estimated annual inflow volume. The inflow that the biofilter would receive on an annual basis (taking into account initial losses such as evaporation et al.) was calculated and partitioned according to the dosing frequency. This methodology resulted in a required dosing volume of 30 L (per dose per column), based on the average annual rainfall (729 mm) for Perth during 1975–2007 and allowing bypass of runoff in excess of the design storm. As the 30 L dosing volume was considered impractical, it was decided to triple the concentration of pollutants and reduce the dosing volume to 10 L. This approach resulted in the total dosing load matching the annual load of pollutants that would be received from the catchment by the biofilter.

As natural stormwater was not available in the required quantity (and with the required consistency to ensure appropriate experimental control), the synthetic stormwater was used (Table 2), made from local soil (passed through a 300-μm sieve) and chemicals mixed with de-ionized water to achieve the three-fold higher concentrations than those in the most concentrated stormwater measured in the Swan–Canning catchment drainage system of Western Australia.

The stormwater dosing started on 6 July 2009 after approximately four months of plant growth in the columns. To achieve consistent input concentrations for each column, the dosing was delivered as two lots of 5 L volume. Each column was dosed with 10 L of the synthetic stormwater twice per week from the start to September 2009 (wet seasonal dosing-1, as per wet season schedule) and once per fortnight from October 2009 to April 2010 (dry seasonal dosing, as per dry season schedule), then twice per week from May 2010 to October 2010 (wet seasonal dosing-2, as per wet season schedule). The total dosing for the wet and dry seasons was 78 and 15 times, respectively.

2.2. Sampling and measurements

The details of plant growth monitoring and harvest as well as the stormwater sampling were described by Zhang et al. [15]. Briefly, the total plant shoot number (excluding mature and dead shoots) and the maximum shoot height were measured in each column at monthly intervals. The plants were harvested after approximately 20 months of plant growth. Shoots were cut just above the soil surface, and their base was washed with de-ionized water to remove any adhering sediments. The below-ground biomass (rhizomes including stem base and root) in one of five replicates was separated from soil by washing with running tap water, rinsing with de-ionized water three times and collecting plant material onto a mesh. All samples were dried to constant weight at 70˚C for five days in a forced-air cabinet, weighed for dry weight (DW) and ground to pass a 0.75-mm mesh. The water samples of inflow and outflow were collected monthly.

Table 2
The pollutant inflow target concentrations (mg L^{-1}, except for pH) and sources of pollutants in the synthetic stormwater

Pollutant	Inflow concentration	Sources	CAS number for chemical used
Total suspended solids (TSS)	39.6	From soil	
Total nitrogen (TN)	4.02	From other N additives	
NO_x-N	1.08	KNO_3	7757-79-1
NH_4-N	0.90	NH_4Cl	12125-02-9
Dissolved organic nitrogen (DON)	2.04	Yeast extract	
Total phosphorus (TP)	0.51	From other P additives	
Total dissolved phosphorus (TDP)	0.24	KH_2PO_4	7778-77-0
Copper (Cu)	0.012	$CuSO_4$	7758-98-7
Lead (Pb)	0.009	$Pb(NO_3)_2$	10099-74-8
Zinc (Zn)	0.165	$ZnCl_2$	7646-85-7
Cadmium (Cd)	0.0003	$CdCl_2$	10108-64-2
pH	6.0		

The water samples for pH and dissolved oxygen (DO) were measured at the first and last sampling runs. The water samples for metal analysis were analyzed in the first four-month sampling runs, then approximately in three-monthly intervals (i.e. total eight sampling runs) during the 16-month wet/dry seasonal dosing period. The pH and DO in samples of water were measured immediately after the samples were taken. Sub-samples required for the analysis of metals were supplemented with a few drops of nitric acid. The pH was measured using a combination glass membrane electrode with a Calomel internal reference (Cyberscan 20 pH meter, Eutech Instruments, Singapore). The DO was measured using a membrane electrode with a galvanic probe (OAKTON DO 300 waterproof portable meter, Eutech Instruments Pte Ltd./Oakton Instruments, Singapore), which has the built-in temperature sensor in the probe and calibrated to 25°C for all samples. The metals were measured by ICP-OES (Optima 5300DV, PerkinElmer, Shelton, USA) after nitric acid digestion [19].

The concentrations of total metals in plant tissues were determined by ICP-OES (Optima 5300DV, PerkinElmer, Shelton, USA) after digesting plant material in a mixture of concentrated nitric and perchloric acids [20].

2.3. Data calculation

The relative maximum shoot height growth rate (RGR_{height}), expressed as $mm\,m^{-1}\,d^{-1}$ or relative shoot number increase rate (RGR_{shoot}), expressed as shoot $1{,}000^{-1}\,d^{-1}$, was calculated to describe the capability of plant growth during the four stages of experiment (i.e. plant establishment from March to June 2009, wet seasonal dosing-1 from July to September 2009, dry seasonal dosing from October 2009 to April 2010 and wet seasonal dosing-2 from May to October 2010).

$$RGR_{height} = (\ln \text{final shoot height} - \ln \text{initial shoot height})/\text{days during a stage} \quad (1)$$

$$RGR_{shoot} = (\ln \text{final shoot number} - \ln \text{initial shoot height})/\text{days during a stage} \quad (2)$$

The data were reported for Cu, Pb, and Zn, but not for Cd, due to non-detectable Cd in the water samples of outflows as well as in plant samples.

Metal removal efficiency was calculated using the formula (3):

$$\text{Removal efficiency } (\%) = (1 - C_o/C_i) \times 100 \quad (3)$$

where C_o and C_i were metal concentrations in the outflow and inflow, respectively.

Total metal accumulation in plants, expressed as mg column^{-1}, was calculated as follows:

$$\text{Total metal accumulation} = \text{metal concentration in aboveground tissues} \times \text{aboveground DW} + \text{metal concentration in belowground tissues} \times \text{belowground DW} \quad (4)$$

The percentage of input metal accumulated in plants was calculated as:

$$\text{Percentage of input metal } (\%) = (\text{total metal accumulation at harvesting} - \text{total metal accumulation at transplanting}) / \text{total input from the stormwater} \times 100 \quad (5)$$

2.4. Statistical analyses

All statistical tests were performed using IBM® SPSS® version 19. Two-way ANOVA was used to test significant interaction between the plant species and the presence/absence of SZ. When no significant interaction was detected, two-way ANOVA was applied to determine significances between treatments and the sampling runs on metal removal and DO, and between treatments and experiment stages on RGR_{height} and RGR_{shoot}, while one-way ANOVA was used to determine significances of the treatment effect on the other parameters if applicable. Least significant difference (LSD) was used to test for significant differences between means.

3. Results

3.1. Plant growth

The capabilities of plant growth varied greatly among the plant species during the different stages of experiment (Fig. 1 and Table 3). The RGR_{height} and RGR_{shoot} were significantly ($p \leqslant 0.05$) influenced by the interaction of treatments and experiment stages (Table 3). The RGR_{height} and RGR_{shoot} were much higher during the three-month wet seasonal dosing-1 (from July to September 2009) compared with the other stages of experiment (i.e. plant establishment from March to June 2009, dry seasonal dosing from October 2009 to April 2010 and wet seasonal dosing-2

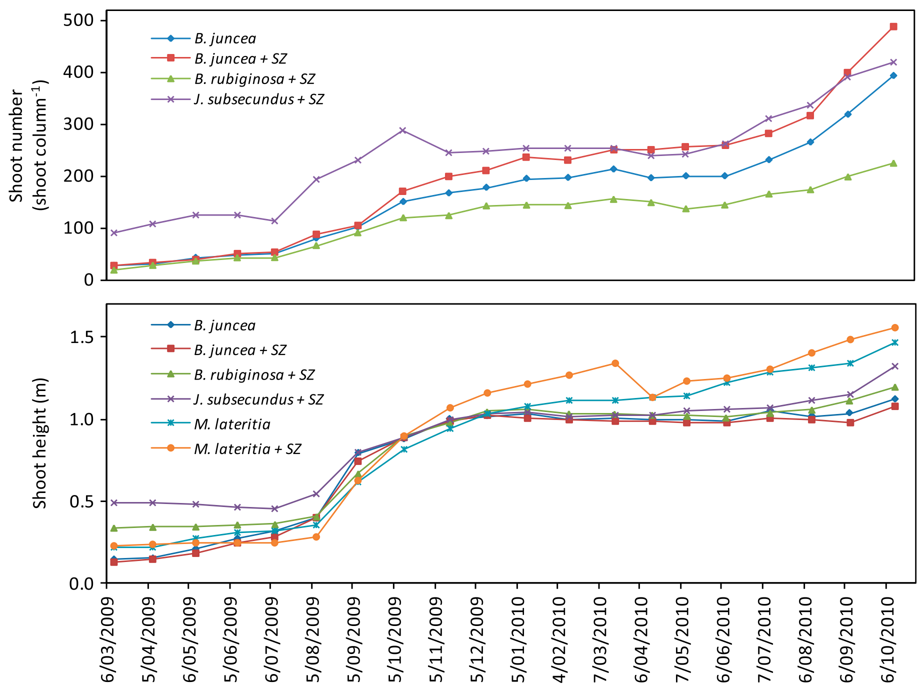

Fig. 1. The variation in plant shoot numbers (except for *M. lateritia* with/without SZ due to the absence of new shoots) and the highest shoot height for various plant treatments after transplanting to the columns during 20 months of plant growth (i.e. plant establishment from March to June 2009, wet seasonal dosing-1 from July to September 2009, dry seasonal dosing from October 2009 to April 2010 and wet seasonal dosing-2 from May to October 2010).

Table 3
The relative maximum shoot height growth rate (RGR_{height}) and relative shoot number increase rate (RGR_{shoot}) in the different plant treatments during the four stages of experiment (means ± SE, $n = 5$)

Treatment	Experiment stage*			
	Plant establishment	Wet seasonal dosing-1	Dry seasonal dosing	Wet seasonal dosing-2
RGR_{height} (mm m^{-1} d^{-1})				
B. juncea	6.3 ± 0.3b**A***	10.8 ± 0.5aC	0.6 ± 0.2cAB	0.6 ± 0.1cA
B. juncea + SZ	6.7 ± 0.4bA	12.0 ± 0.9aB	0.5 ± 0.2cB	0.5 ± 0.3cA
B. rubiginosa + SZ	0.5 ± 0.3bC	9.7 ± 0.4aD	0.6 ± 0.1bAB	0.8 ± 0.2bAB
J. subsecundus + SZ	1.1 ± 0.2bC	7.0 ± 0.2aE	0.6 ± 0.1bAB	1.4 ± 0.1bA
M. lateritia	3.2 ± 0.6bB	9.9 ± 0.5aCD	1.5 ± 0.2cA	1.4 ± 0.3cA
M. lateritia + SZ	0.9 ± 0.2bC	13.8 ± 0.2aA	1.1 ± 0.3bAB	1.7 ± 0.2bA
*RGR_{shoot} (shoot $1{,}000^{-1}$ d^{-1})****				
B. juncea	5.3 ± 0.5bB	11.3 ± 0.8aAB	1.2 ± 0.2c A	3.8 ± 0.2bA
B. juncea + SZ	5.4 ± 0.6bB	12.4 ± 1.0aA	1.8 ± 0.2dA	3.6 ± 0.3cAB
B. rubiginosa + SZ	7.0 ± 0.8bA	10.9 ± 0.8aAB	1.0 ± 0.3cA	2.2 ± 0.2cB
J. subsecundus + SZ	1.9 ± 0.2bC	9.8 ± 0.8aB	−0.9 ± 0.2cB	3.0 ± 0.2bAB

*Plant establishment (from March to June 2009), wet seasonal dosing-1 (from July to September 2009), dry seasonal dosing (from October 2009 to April 2010) and wet seasonal dosing-2 (from May to October 2010).
**Means (± SE, $n = 5$) followed by the same lowercase letter within rows are not significantly different according to LSD ($p \leqslant 0.05$).
***Means (± SE, $n = 5$) followed by the same capital letter within columns in RGR_{height} or RGR_{shoot} are not significantly different according to LSD ($p \leqslant 0.05$).
****No data for *M. lateritia* with/without SZ due to the absence of new shoots.

from May to October 2010), while the lowest RGR_{shoot} was recorded during the dry seasonal dosing. A negative RGR_{shoot} for *J. subsecundus* was observed during the dry seasonal dosing because some shoots of this species started to reach maturity after 8 months of growth (with the old shoot not being counted).

The growth of *B. juncea* and *M. lateritia* was relatively better in the treatment with SZ than without. The shoot number of *B. juncea* and the maximum shoot height of *M. lateritia* were slightly great in the treatments with SZ than without after 20-month growth (Fig. 1).

It is worth noting that plant growth was affected only slightly by a severe hail storm on 23 March 2010. The average shoot height in *M. lateritia* with SZ dropped in April 2010 because some shoots in three of five replicates were damaged by the storm (Fig. 1). Due to storm damage to the glasshouse that had housed plants, they were moved into another glasshouse, in which they had the same growth conditions as before.

3.2. Metal removal

The removal of Cu and Pb varied significantly ($p \leqslant 0.05$), but not Zn removal (between 91 and 99%) among the sampling runs during the 16-month wet/dry seasonal dosing period. The removal of Cu increased with time from 68% to nearly 100%, whereas the removal of Pb varied from the lowest 15% to the highest 100% among the sampling runs during the experimental period (Fig. 2).

No significant interaction between the plant species and the presence/absence of SZ was detected for the metal removal. The significant differences ($p \leqslant 0.05$) in the removal of Cu and Pb were detected between the treatments with SZ and without, but not Zn removal, with 96% Zn (averaged over all treatments) being removed. The removal of Cu and Pb was significantly higher in the treatments with SZ (92 and 81% of Cu and Pb removed, respectively) than without (87 and 67% of Cu and Pb removed, respectively). The significantly lowest removal of Cu and Pb was recorded in *M. lateritia* without SZ, but there was no significant difference between the planted

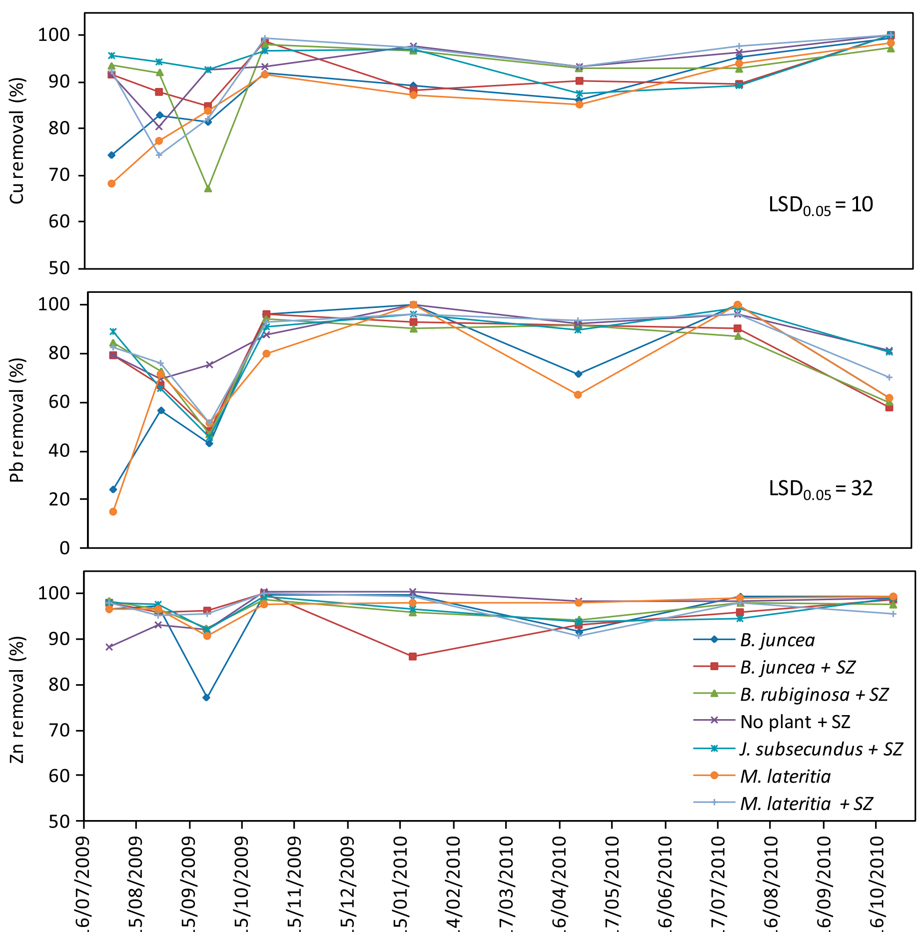

Fig. 2. The variation in the removal of Cu, Pb, and Zn in different treatments during the 16-month wet/dry seasonal doing period (wet seasonal dosing-1 from July to September 2009, dry seasonal dosing from October 2009 to April 2010 and wet seasonal dosing-2 from May to October 2010). Note: different scales in the removal of various metals and nonsignificant difference in Zn removal.

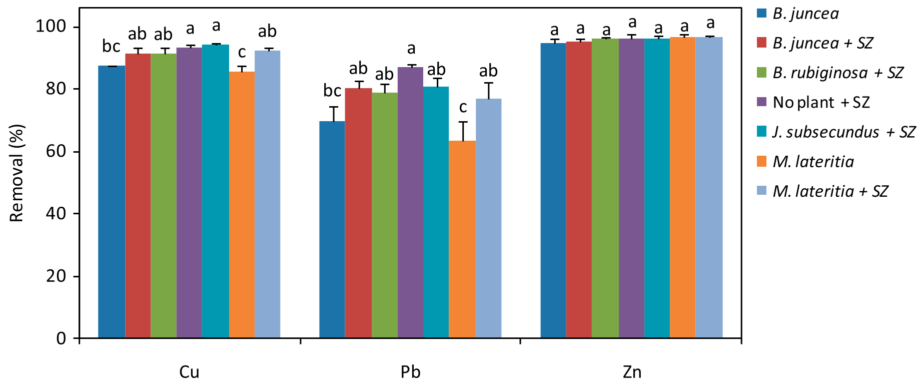

Fig. 3. Average removal of Cu, Pb, and Zn as influenced by different treatments. Bars with the same letter for a metal are not significantly different according to LSD ($p \leqslant 0.05$).

treatments without SZ. No significant difference in the metal removal was detected among the treatments with SZ regardless of presence/absence of plants (Fig. 3).

3.3. Dissolved oxygen (DO) in outflows

No significant interaction between the plant species and the presence/absence of SZ was detected in DO. The concentration of DO was significantly lower ($p \leqslant 0.05$) in the treatments with SZ than without, but there was no significant difference between the two planted treatments (*B. juncea* and *M. lateritia*) without SZ (Table 4).

A significant interaction between the treatment and sampling runs was observed for the concentration of DO in outflows. The significant differences ($p \leqslant 0.05$) in DO were detected among the treatments and between the first and last sampling runs (Table 4). The concentration of DO among the planted treatments with SZ was significantly ($p \leqslant 0.05$) higher in the last than first sampling run, but the opposite was true in the planted treatment without SZ. The concentration of DO between the first and the last sampling runs was not significantly different in the no-plant treatment with SZ. The highest concentration of DO was in *J. subsecundus* among the treatments with SZ, and the lowest in the no-plant treatment with SZ (except for the first sampling run).

3.4. pH in outflows

The significant interaction between the plant species and the presence/absence of SZ was detected in pH at the last sampling run, but not at the first sampling run. The significantly lower ($p \leqslant 0.05$) pH at the last sampling run was observed in *B. juncea* with SZ compared with no SZ, but no significant difference was observed between *M. lateritia* with and without SZ (Table 4).

The significant difference ($p \leqslant 0.05$) in pH was observed between the planted/unplanted treatments.

Table 4
The DO and pH in the outflows in the different treatments between the first (31 July 2009) and last sampling runs (26 October 2010)

Treatment	DO (mg L^{-1})		pH	
	First sampling run	Last sampling run	First sampling run	Last sampling run
B. juncea	7.6 ± 0.1a*A**	6.2 ± 0.1aB	6.6 ± 0.04ab	5.3 ± 0.02b
B. juncea + SZ	2.2 ± 0.2cB	3.2 ± 0.3cA	6.5 ± 0.10bc	4.8 ± 0.03d
B. rubiginosa + SZ	2.3 ± 0.1bcB	3.1 ± 0.2cA	6.4 ± 0.05c	4.8 ± 0.06d
No plant + SZ	2.2 ± 0.2cA	1.8 ± 0.1dA	6.7 ± 0.03a	5.5 ± 0.02a
J. subsecundus + SZ	2.8 ± 0.2bB	4.1 ± 0.1bA	6.4 ± 0.02c	5.1 ± 0.05c
M. lateritia	7.4 ± 0.2aA	6.3 ± 0.1aB	6.6 ± 0.07ab	5.0 ± 0.07c
M. lateritia + SZ	2.2 ± 0.1cB	2.7 ± 0.1cA	6.6 ± 0.10ab	5.0 ± 0.03c

*Means (± SE, $n = 5$) followed by the same lowercase letter within columns are not significantly different according to LSD ($p \leqslant 0.05$).
**Means (± SE, $n = 5$) followed by the same capital letter within rows are not significantly different according to LSD ($p \leqslant 0.05$).

The highest pH among the treatments was in the no-plant treatment with SZ and the lowest in *B. rubiginosa* with SZ regardless of the sampling runs (Table 4).

3.5. Concentrations and accumulation of metals in plants

The significant interaction between the plant species and the presence/absence of SZ was detected in the concentration and accumulation of Pb in aboveground tissues, but not in the concentrations and accumulation of Cu and Zn. The concentration and accumulation of Pb were significantly lower ($p \leqslant 0.05$) in the aboveground *M. lateritia* with SZ than without. The concentrations and accumulation of Cu and Zn in the aboveground tissues were significantly different ($p \leqslant 0.05$) between the planted treatments. The concentrations and accumulation of Cu, Pb, and Zn were greatly higher in belowground than aboveground tissues (Table 5).

The different metal accumulation was observed among the plant species (Table 5). The relatively more difference was observed in the accumulation of Cu and Zn, but less in Pb among the plant species. The highest accumulation in plants was for Zn, followed by Cu and Pb. The ranges of total metal accumulation by plants were from 5.5 mg per column in *B. juncea* with SZ to 13.8 mg per column in *B. rubiginosa* with SZ for Cu, 0.88 mg per column in *M. lateritia* with SZ to 1.8 mg per column in *M. lateritia* without SZ for Pb and 24.0 mg per column in *B. juncea* with SZ to 43.9 mg per column in *J. subsecundus* with SZ (Table 5). The percentage of input metal accumulated in plants varied for the different metals and plant species. The highest percentage of input metal accumulated in plants was for Cu, followed by Zn and Pb (Table 5).

4. Discussion

4.1. Plant species influencing metal removal

Plants are the most notable feature of biofilters, and their presence has been reported to improve pollutant removal [21]. In the present study, the metal removal was not significantly different between the planted and nonplanted treatments with SZ (Fig. 3), as also observed by others [22]. For instance, there was no significant difference in the average metal concentration of effluents between the presence of

Table 5
The concentrations and accumulation of metals in plants and percentage of the input amount of metal (14 mg Cu, 9 mg Pb, and 151 mg Zn per column) accumulated in plants after 20 months of plant growth

Treatment	Concentration (mg kg^{-1})		Accumulation (mg $column^{-1}$)			Percentage of input
	Aboveground	Belowground	Aboveground	Belowground	Total	
Cu						
B. juncea	2.2 ± 0.3b**	33.9	0.27 ± 0.05c[a]	5.9	6.2	44
B. juncea + SZ	1.9 ± 0.2b	26.2	0.29 ± 0.04c	5.2	5.5	39
B. rubiginosa + SZ	1.6 ± 0.4b	40.4	0.29 ± 0.06c	13.5	13.8	99
J. subsecundus + SZ	4.9 ± 0.6a	46.9	1.49 ± 0.19a	10.8	12.3	87
M. lateritia	5.5 ± 0.3a	66.7	0.67 ± 0.04b	8.3	9.0	64
M. lateritia + SZ	4.8 ± 0.3a	57.4	0.67 ± 0.02b	7.5	8.2	58
Pb						
B. juncea	1.9 ± 0.2a	5.0	0.22 ± 0.02abc	0.88	1.1	12
B. juncea + SZ	2.3 ± 0.7a	4.1	0.39 ± 0.15a	0.80	1.2	13
B. rubiginosa + SZ	0.8 ± 0.1b	3.2	0.15 ± 0.03bc	1.1	1.3	14
J. subsecundus + SZ	0.4 ± 0.1b	5.1	0.10 ± 0.04c	1.2	1.3	14
M. lateritia	2.7 ± 0.4a	11.7	0.33 ± 0.04ab	1.5	1.8	20
M. lateritia + SZ	0.3 ± 0.2b	6.5	0.04 ± 0.02c	0.84	0.88	10
Zn						
B. juncea	54.0 ± 4.7bc	103.3	6.5 ± 0.5c	18.1	24.6	16
B. juncea + SZ	44.6 ± 5.6 cd	88.1	6.6 ± 0.6c	17.4	24.0	16
B. rubiginosa + SZ	32.7 ± 1.5d	60.6	6.1 ± 0.2c	20.3	26.4	17
J. subsecundus + SZ	70.9 ± 8.1ab	97.6	21.4 ± 2.0a	22.5	43.9	28
M. lateritia	86.5 ± 5.3a	135.9	10.6 ± 0.5b	16.9	27.5	18
M. lateritia + SZ	80.7 ± 11.8a	212.1	11.0 ± 1.0b	27.6	38.6	25

*Means (± SE, $n = 5$) with the same letter within columns for each metal are not significantly different according to LSD ($p \leqslant 0.05$).

plants and soil-only treatments [22]. These studies indicated that the processes in the media of biofilters involved in binding of metals were more important than did the immediate plant-related processes (such as root exudation and uptake). In addition to plant accumulation, there are a number of other processes that may be significant in terms of metal removal, including chelation or complexation by organic matter, biofilm and thus sequestration or sedimentation and retention in the media [23]. The relatively low loading of metals and likely high metal adsorption by the media could have contributed to a lack of significant difference in the metal removal between the treatments with the presence and absence of plants. Adsorption is considered the most significant mechanism of metal removal in biofilters, and accretion into the sediments is recognized as the principal process in the removal of heavy metals from stormwater in natural and constructed biofilters [24,25].

Most studies showed that the choice of species can be important for the removal efficiency of pollutants (including metal) [8,22]. In the present study, plants exhibited significantly different capability in metal accumulation and partitioning between shoots and roots (Table 5). However, it was not possible to discriminate the relative performances in terms of the metal removal either among the four plant species with SZ or between the two plant species without SZ (Fig. 3), as found by others. For example, the type of grass species (*Panicum virgatum*, *Kentucky-31* and *Bromus ciliates*) did not affect the removal of the metals (Cd, Cu, Pb, and Zn) in the laboratory bioretention systems [24].

In the current study, the metal accumulated in plants mostly remained in the belowground tissues. The amount of metals sequestered in the aboveground biomass represented less than 10% for Cu, 4% for Pb, and 14% for Zn with respect to the input metal, respectively. The relatively high percentages of the input amount of metals (such as Cu) accumulated in the plant species (Table 5), but the high values might have been caused by the accumulation of metals in plants during the 4-month plant establishment stage (i.e. from March to June 2009), in which there was no input metals from the stormwater to the column, whereas plants could have taken up metals from the media (soil) of the biofilters and accumulated them in plants to some extent.

Plants not only take up metals from media directly, but also release oxygen and exudates (such as organic acid anions, phytometallophores, phytochelatins etc.) from roots, thus indirectly affecting metal removal by acidifying and/or oxidizing the rhizosphere [9]. In the present study, it was observed that the concentration of DO in the outflows significantly increased, while the pH significantly decreased in the presence of plants. The DO and pH were also influenced significantly ($p \leqslant 0.05$) by the various plant species (Table 4). These variations could cause the changes in redox and pH in the media of the planted biofilters, particularly in rhizosphere, resulting in different adsorption and retention of metals in biofilters [26].

4.2. Impact of a SZ with C addition on plant growth and metal removal

Plant growth is mostly related to the availability of nutrients and water in media [27,28]. The plant growth significantly ($p \leqslant 0.05$) increased with a SZ in the present study (Table 3). The relatively better plant growth and higher biomass in the planted treatments with SZ compared to no SZ was probably due to increased amounts of nutrients and water in SZ with C addition [15]. In practical application, a SZ could provide plants for buffering against long dry periods under Mediterranean climatic pattern [14]. Compared with the absence of a SZ, the aboveground biomass of *B. juncea* and *M. lateritia* with SZ increased by 26 and 14% and the belowground biomass by 13 and 5%, while the ratio of belowground to aboveground biomass decreased by 10 and 8%, respectively [15]. Water deficits could promote greater relative allocation of photosynthates to root growth, allowing plants maximize the absorption and minimize the loss of water [29].

The removal of Cu and Pb in the biofilters significantly ($p \leqslant 0.05$) increased with a SZ, but not on Zn removal in the present study (Fig. 3). This was partly agreed with Blecken et al. [14]. The presence of SZ enhanced removal of heavy metals (Cu, Pb, and Zn) and the presence of both SZ and C had the most consistent and practically important effect on Cu [14]. The significantly lower concentrations of DO in the outflows were observed in the treatments with SZ compared with non-SZ in the present study (Table 4). Anoxic conditions in media increased metal sorption into sediments compared to oxic conditions [30]. It is possible that the reducing conditions in the biofilters with SZ caused metal complexation and decreased mobility in the media [31]. Moreover, the introduction of C sources could support metal retention by forming metal-organic matter complexes. The previous report has indicated that Cu might have a strong affinity for organic matter in media. The organic matter might immobilize Pb via specific adsorption reactions [32]. Nevertheless, more research is required to understand the impact of SZ with C addition on metal removal in biofilters due to very complex processes influencing metal behavior in media.

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