



# The influence of media type on removal of arsenic, iron and boron from acidic wastewater in horizontal flow wetland microcosms planted with *Phragmites australis*



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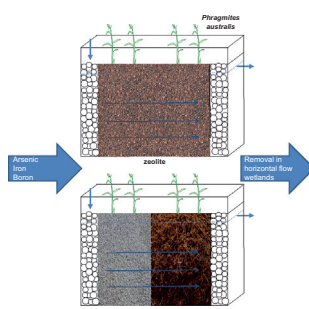
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## HIGHLIGHTS

- Horizontal flow wetlands were employed to remove As, Fe and B from acidic water.
- Two types of wetland media: (1) zeolite (2) limestone and cocopeat were tested.
- Both wetland types presented high removal of As and Fe.
- Pollutants were retained in wetland media rather than accumulated in plants.
- Alternative media appear as an effective way to enhance As and Fe removal.

## GRAPHICAL ABSTRACT



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## ABSTRACT

In this study, horizontal flow wetland microcosms were used to test the effectiveness of two media types: (1) zeolite and (2) a series of limestone and cocopeat in the removal of arsenic (As), boron (B) and iron (Fe) from contaminated acidic waters such as those associated with mine waste. The wetlands were operated under a hydraulic loading of 30 mm/d, to treat acidic water with As = 2.3 mg/L, Fe = 97.3 mg/L and B = 30.8 mg/L at pH 2 ± 0.2. Both media were highly effective in the removal of As and Fe. The zeolite wetlands removed 99.9%, 96.1% and 12% of As, Fe and B respectively, whereas the removal efficiencies of the limestone/cocopeat wetlands were 99.8%, 87.3% and 17%. The contribution of plant uptake to As, Fe and B removal in both wetland types was almost negligible (<3% in all cases). The results confirm the key role of the wetland media in fostering specific removal processes. These processes include As co-precipitation with Fe due to pH adjustment provided by limestone, and As and Fe removal facilitated by the cation exchange capacity of the zeolite. Limestone/cocopeat wetlands may offer a more suitable treatment, given the neutral pH achieved and the slightly higher B removal, but zeolite wetlands are able to achieve lower concentrations of Fe, despite the acidic water in the treated effluent.

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## 1. Introduction

The presence of arsenic (As) in aquatic environments is a world-wide problem [1], with As in drinking water known to cause bladder, lung and non-melanoma skin cancer [2]. In Bangladesh alone, over 100 million people drink water with arsenic levels of up to 1 mg/L, which is 100 times the World Health Organisation drinking water guideline value [3].

Most of the As contamination in waters worldwide are the result of As mobilisation under natural conditions [4]. However, anthropogenic activities can also mobilise As. The exploitation of gold and base-metal deposits is a principal cause of As contamination of surface drainage and groundwater in many countries, and metal mine discharges have degraded many rivers around the world [5]. The use of phosphorus in fertilizers plays a role in As mobility in soil and crops, given that the chemical behaviour of phosphate is similar to that of arsenate, competing in plant uptake [6].

While treatment of As-contaminated drinking water is a world-wide challenge, surface water sources for non-drinking purposes, such as irrigation or protection of aquatic ecosystems, may also require treatment. In this case, conventional drinking water treatment technologies may not be suitable, due to the required hydraulic loading rates. There is also a need for onsite technologies that can simultaneously remove other metals/metalloids, because As-contaminated waters may contain other pollutants. For example, surface waters in Northern Chile are also rich in boron (B), antimony (Sb) and iron (Fe) [7,8]. The presence of other metals (e.g. Fe) may affect As removal and thus must be taken into account in designing appropriate treatment systems.

Oxidation, co-precipitation, adsorption, ion exchange and membrane processes are the major techniques employed to remove arsenic from water. However, variations in arsenic speciation and the characteristics of the water to be treated make it difficult to treat using just one of these processes. Indeed, in surface waters, As is mainly present as As(V), whereas in groundwater, As is mainly present as As(III). If As-contaminated waters have acidic pH values, As occurs in dissolved form, however As may still be found as a dissolved species over a broad pH range (1–12) [9]. For this reason, more than one technology is often required [10]. While some of these treatment methods are quite simple, the disadvantage associated with co-precipitation/sorption methods is the large production of toxic sludge [1]. Membrane methods generate toxic wastewater and require high maintenance [3].

Constructed wetlands are considered a reliable water treatment technology [11] and have the potential to remove metals and metalloids [12]. However, few studies have investigated their potential to remove As, with the focus of constructed wetlands research for metal removal instead being on the treatment of acid mine drainage, targeting pollutants such as iron and manganese [13]. In these instances, surface flow wetlands (SF) have been the predominant treatment of choice [14].

Some studies have investigated the application of subsurface flow wetlands (SSF) to treat arsenic and metal contaminated water with promising results. Buddhawong et al. [15] found that subsurface flow wetlands were more effective than surface flow wetlands in removing As and Zn, attributing this observation to the combined effect of supporting media (gravel), the presence of Fe and the role of vegetation. Furthermore, to enhance As and metal removal in SSF wetlands, the use of alternative media has been proposed [16], but testing of these media has been limited. Some studies have been performed in vertical subsurface flow wetlands (VSSFs) with alternative wetland media to enhance As and metal removal, where mainly aerobic processes occur. One study identified alternative media to enhance As removal in VSSFs under acidic conditions [17] and another [18] confirmed the key role of a

suitable substrate for As removal in subsurface flow constructed wetlands. No study to date has employed alternative media in horizontal subsurface flow wetlands (HSSFs) for this purpose.

Horizontal subsurface flow wetlands have been most commonly used to treat urban and domestic wastewater [19]. Little is known about their efficiency to remove high quantities of metals and metalloids, such as those found in acid mine drainage [20]. Indeed, few studies have investigated As removal by HSSFs (e.g. Rahman et al. [21]). Since HSSFs have limited oxygen transfer [14], it may be hypothesised that As in these systems may be removed by sulfur or iron precipitation under reducing conditions, if these elements are present. It is also likely that iron-oxidising bacteria play a critical role in Fe and As cycling in such systems [22].

Given the multiple factors that affect As removal in SSF wetlands and the small number of studies performed under specific environmental and operation conditions (e.g. As and other pollutant levels, pH, hydraulic loading, type of flow, vegetation, substrate), there is a need to better understand the performance of such systems, and the influence of key design parameters, including the substrate and the role of vegetation.

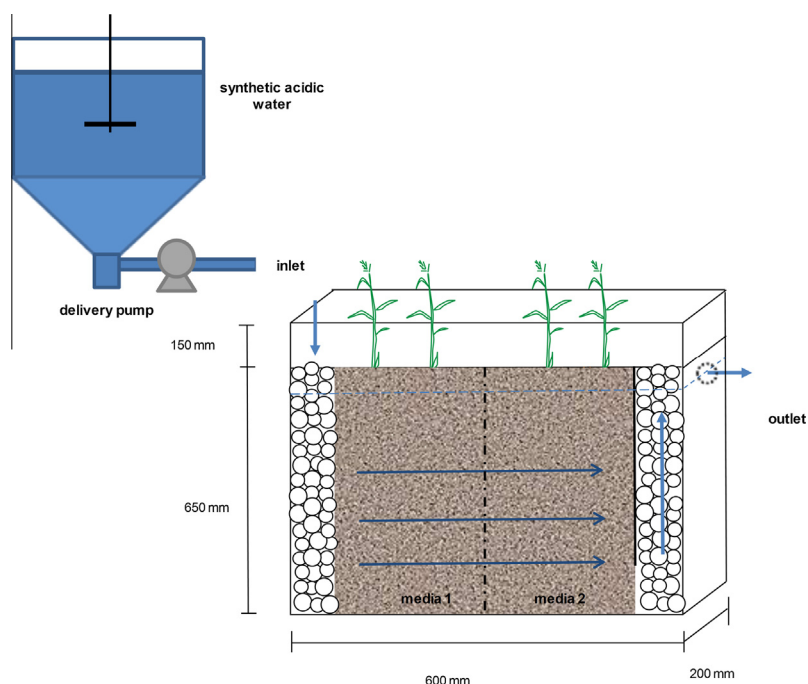
This study aims to test the effectiveness of alternative media (zeolite, limestone and cocopeat) in laboratory-scale HSSF wetland microcosms. These media have been tested in isolation in vertical flow subsurface wetlands and were found to enhance As, Fe and B removal [17,23], thus demonstrating their potential. This current study focused on As, Fe and B removal from highly contaminated water similar to that found in the Azufre River in Northern Chile, using these three media types planted with *Phragmites australis*, a species widely used in constructed wetlands. The key removal mechanisms and the environmental factors that affect these mechanisms were identified. These findings can be used to develop the design of subsurface flow constructed wetlands targeting the removal of As, Fe and B from acidic water. It is important to note that this study is restricted to examining the role of media type, and thus does not investigate the variability in performance and processes due to species selection.

## 2. Materials and methods

### 2.1. The wetland cells

Six horizontal flow wetlands cells were built using 12 mm thick PVC, with one side of each wetland built with clear PVC to enable observation of water, substrate and plant roots. This side was kept covered by black LDPE plastic sheeting when not being observed, to prevent light-induced artefacts. Each wetland had an inlet and an outlet (65 and 100 mm long, respectively), which contained gravel 20–40 mm size. Fig. 1 illustrates the main characteristics of the wetland cells, which were located in a greenhouse shelter (covered roof but open walls) at Monash University, Clayton Campus. Previous research on lab-scale HSSFs [24] informed the dimensions of the wetlands cells of this study, which are presented in Fig. 1.

Two types of wetland columns were built, using (i) zeolite and (ii) limestone/cocopeat as the main supporting media. In the limestone/cocopeat wetlands, media 1 was limestone and media 2 was cocopeat (Fig. 1), thus meaning that the outflow of the limestone section was the inflow of the cocopeat section. Thus, outflow samples from these wetlands represented the combined effect of both media. Each wetland media had three replicates, operated as individual treatment units. Young *P. australis* plants were obtained from a local nursery and planted in December 2011, five months before the experiments started. In each wetland, approximately 10 stems were planted and submerged in tap water to allow them to establish in their new environment. *Phragmites* was selected for



**Fig. 1.** Diagram of the experimental wetland mesocosms. In zeolite wetlands, media 1 and media 2 were zeolite, whereas in limestone/cocopeat wetlands, media 1 was limestone and media 2 was cocopeat.

this and previous studies (i.e. [17,23]) given its tolerance to acidic conditions [25] and its common use in practice.

## 2.2. Synthetic wastewater

Synthetic wastewater resembling pollutant levels in the Azufre River, Northern Chile, was prepared. This river was chosen as a model because of its high levels of arsenic under acidic conditions. The river has other contaminants from different natural and anthropogenic sources, mainly influenced by the Tacora volcano and the legacy of sulfur mining on its crater [26]. Because the main concern for the Azufre is the presence of As and B, given their high levels, little information exists regarding the levels of other metals such as Cu, Mn, Pb and Zn [27]. Thus, this study focused on the presence of As and B, as well as that of Fe, due to its role in As speciation. Representative synthetic wastewater was prepared using tap water with the following reagents added per litre of water: 3 mL 1000 mg/L arsenic standard solution (arsenic acid  $\text{As}_2\text{O}_5$  in  $\text{H}_2\text{O}$ ), 30 mL 1000 mg/L boron standard solution (boric acid  $\text{H}_3\text{BO}_3$  powder in  $\text{H}_2\text{O}$ ), 0.5 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.425 mL  $\text{H}_2\text{SO}_4$  (95–97% Merck ISO grade). As a result, the concentrations of the target pollutants in the synthetic feed were (average  $\pm$  standard deviation):  $2.6 \pm 0.5$  mg/L As,  $30.8 \pm 6.2$  mg/L B, and  $97.3 \pm 14.0$  mg/L Fe. The resulting pH value was  $2.0 \pm 0.2$  and as such, As, Fe and B occurred predominantly in the dissolved fraction. Typical analysis of Melbourne's water (representing the quality of the tap water which was used as a base for the synthetic water) indicates the following ranges for hardness and alkalinity: 15–44 and 9.9–26.6 mg/L as  $\text{CaCO}_3$  respectively. Arsenic levels are below 0.005 mg/L and Fe levels fluctuate between 0.01 and 0.08 mg/L, whilst B is not analysed and assumed to be below detection limits [28].

## 2.3. Operation of the wetland system

The synthetic wastewater was stored in a 500 L polyethylene feed tank with a continuous stirrer and pumped to each wetland

column at an average rate of 150 mL/h, resulting in a hydraulic loading rate of 30 mm/d and a hydraulic retention time of approximately 11 days. The design loading rate was chosen to reflect loading rates commonly used in HSSF wetlands for As treatment [20,21]. The wetlands were incrementally dosed every 72 min over a 24 h period. Each dosing event took 18 min. This type of dosing regime was necessary given the very small flow rates, to ensure that the volumes applied could be accurately maintained to their specification. In all wetland columns, a water level of 0.6 m was maintained using an overflow outlet hose (Fig. 1). The mean outflow rate was  $144.9 \pm 13.9$  mL/h from lime-peat (limestone/cocopeat) wetlands, and  $129.5 \pm 14.4$  mL/h from zeolite wetlands. Outflow rates were measured for every sampling event.

Dosing commenced on May 7, 2012 whilst sampling and analysis commenced on 28 May, 2012 and lasted for 22 weeks. This period encompassed the autumn, winter and spring seasons (in the southern hemisphere) which ensured a range of seasonal conditions were included. A longer experimental period was not feasible given the available resources and timeframe (a common constraint in lab-scale constructed wetlands experiments, e.g. [16,24]).

## 2.4. Sampling and analysis

### 2.4.1. Water quality measurements

Water samples were collected from the inlet (dosing hose) and outlet (outlet hose) of each wetland replicate, three times per week for the first two weeks, once a week for the following six weeks, and then fortnightly for the remaining fourteen weeks. Sampling was carried out by placing a PVC container in the outlet hose of each replicate, allowing it to fill up and to obtain an approximate sample volume of 300 mL, sufficient for analyses of total metals, dissolved metals and *in situ* parameters. Separate samples were taken for total and dissolved metal analysis, with analysis undertaken according to Standard Method 3120B/3125B accordingly [29]. For the measurement of dissolved pollutants, the samples were filtered (through 0.45  $\mu\text{m}$  cellulose acetate papers) and

acidified to pH <2 with concentrated nitric acid (65%) immediately after sampling. The analyses of As, B and Fe concentrations were carried out for both unfiltered and filtered samples to obtain total and dissolved values. The analyses of other parameters were carried out for unfiltered samples after sampling: dissolved oxygen, pH and temperature were measured using HACH 51970 and HACH 51910 probes that were connected to a Sension 378 meter and they were calibrated before every measurement. An ORP Tetr10 probe was used to measure redox potential (Eh). Sulfate concentrations were determined using HACH DR5000 UV/VIS spectrophotometer based on Standards Methods [29].

The concentrations of As, B and Fe were determined by ICP-OES and ICP-MS (if the parameter analysed was below the OES detection limit) in a NATA (National Association of Testing Authorities, Australia, <http://www.nata.asn.au/>) accredited laboratory. All QC procedures were based on Standard Methods [29]. ICP-OES detection limits were 0.1 mg/L for As, 0.05 mg/L for B and 0.05 mg/L for Fe. ICP-MS detection limits were 0.001 mg/L for As, 0.02 mg/L for B and 0.02 mg/L for Fe. Since *in situ* parameters in outlet samples may not be representative of the environmental conditions inside the wetland cells, *in situ* parameters were measured continuously at the bottom of one replicate from each of the limestone/cocopeat (abbreviated to “LP”) and zeolite (“Z”) groups: Cell 2LP (lime–peat cell) from limestone/cocopeat wetlands, and Cell 3Z (zeolite cell) from zeolite wetlands. Dissolved oxygen (DO), pH, temperature and redox potential were measured using four probes that were connected to a Campbell CR1000 data logger. The probes used were Sensorex CS511-L, CS525 ISFET, 109SS (Campbell Scientific, Australia), and a submersible redox sensor (TPS Pty Ltd., Australia) respectively. These probes took measurements every five minutes for the entire experimental period. The probes were calibrated before the beginning of the experiments and then in the middle of the experiments. The purpose of measuring *in situ* parameters was to infer how the pollutants are retained in the wetland bed by associating these measurements with removal mechanisms (e.g. low dissolved oxygen levels indicate anaerobic processes). However, given the complexity of the reactions and the environmental conditions occurring in the wetland cells, these measurements should be used only as a reference for this purpose. They may not accurately represent the environmental conditions in the entire wetland given their particular location and the likely existence of micro-zones with different environmental conditions. Removal of the probes for calibration resulted in significant alterations in measured DO and ORP, potentially due to recalibration, but most likely due to disturbance of the system. These results should therefore be considered together with the above information.

#### 2.4.2. Analysis of media

Media samples were collected and analysed to determine the pollutant concentrations in the wetland media. At the end of the experiments, all wetland cells were drained at the same rate with which they had been dosed (150 mL/h) to avoid disturbance to the media. While they were being drained, media samples were collected from nine locations: inlet, middle and outlet at three different horizontal levels: top, middle and bottom of each wetland. This was done by locating three points in the middle of each media section (inlet, middle and outlet), and then digging carefully using a small shovel to obtain an approximate volume of 150 mL. Media samples were dried at 40 °C until constant weight was achieved and the metal concentrations in media samples from Cell 2LP, Cell 4LP, Cell 9LP (all three lime–peat wetland replicates), Cell 3Z and Cell 7Z (two out of the three zeolite wetland replicates) were analysed in the same NATA accredited laboratory described above.

#### 2.4.3. Analysis of plants

Plants samples were obtained and analysed to estimate the contribution of plant uptake to the removal of the target pollutants. Upon completion of the experiment, all plants were collected and divided into two groups, depending on their physical location in each wetland: inlet or outlet. They were separated into shoots and roots. Samples were dried at 55 °C until constant weight was achieved [30]. The total mass of shoots and roots in Cell 3Z was 39 and 47.6 g respectively, whereas in Cell 2LP was 40.2 and 87.9 g respectively (dry weight). Metal concentrations in these samples were determined in a NATA accredited laboratory by USEPA methods 3051A and 3060A.

#### 2.5. Statistical analysis

##### 2.5.1. Performance of the wetland system

To analyse the performance of the two wetland systems, statistical tests were performed using PASW Statistics 19 and a significance level of  $\alpha = 0.05$ . The Kolmogorov–Smirnov test was first performed to check data normality.

The influence of the wetland media type was statistically assessed by one way ANOVA. Rather than a simple comparison between the two media types, the inlet concentrations and the outlet concentrations of each media type were compared, in order to detect significant differences between inlet and outlet concentrations that are influenced by any media type (i.e. to determine if the concentrations in the outlet are lower than those in the inlet). The same analysis was undertaken for the environmental parameters (pH, ORP, DO, sulfate). When a significant difference attributed to the media types was found (ANOVA  $p < 0.05$ ), multiple comparison post hoc tests were performed to elucidate differences between the individual media: Tukey’s test was applied when the assumption of homogeneity of variances was satisfied (as determined by Levene’s test), or Games–Howell’s test when this assumption was not satisfied.

If the assumption of normality was not achieved, Kruskal–Wallis analysis was performed instead of ANOVA. In these cases, when significant difference was found (Kruskal–Wallis  $p < 0.05$ ), Mann–Whitney post hoc test were performed to distinguish the mean differences between treatment pairs (Mann–Whitney  $p < 0.05$ ).

##### 2.5.2. Correlation between pollutant levels

Spearman rank  $\rho$  correlation coefficient was obtained to determine possible relationships between the removal of the target pollutants and the environmental factors (including pH, Eh and concentrations of Fe). Spearman  $\rho$  was used instead of Pearson’s correlation due to the non-normal distribution of most of the data (determined by Kolmogorov–Smirnov test).

### 3. Results

#### 3.1. Overall performance

As presented in Table 1, both media types were highly effective in removing As and Fe, but not very effective in removing B. The target pollutants were mostly remained in dissolved form in the effluent for both media types.

Regarding the environmental parameters, pH and Eh were the key parameters most affected by media type. Although pH was raised by both media types, it reached an almost neutral value (6.95 median value) in the limestone/cocopeat wetlands, whereas it was still acidic in the zeolite wetlands (4.1 median value) (Table 1). Redox potential decreased to negative values in the limestone/cocopeat wetlands, whereas it was still positive in the zeolite wetlands.

**Table 1**

Median inflow and outflow levels of the target pollutants, and median inflow and outflow levels of monitored environmental parameters. Values in brackets indicate the 5th and 95th percentiles. The data consist of a total of fifty-seven measurements (three replicates multiplied by nineteen data sets).

Parameter (unit)		Inflow		Limestone/cocopeat		Zeolite	
		Level	Average removal (%)	Level	Average removal (%)	Level	Average removal (%)
As (mg/L)	Dissolved	2.7 [1.4,3]	0.002 [0.005,0.012]	99.8	0.001 [0.005,0.004]	0.001 [0.005,0.0042]	99.9
	Total	2.8 [1.4,3]	0.002 [0.005,0.015]				
Fe (mg/L)	Dissolved	89 [81,121]	7.8 [0.31,25.2]	87.3	1.1 [0.005,12.4]	1.7 [0.005,12.4]	96.1
	Total	91 [82,130]	8.8 [1.56,28.2]				
B (mg/L)	Dissolved	30 [19.8,30.4]	25 [19.8,30.4]	15.9	27 [19.8,32]	28 [21.8,32.2]	11.5
	Total	31 [21,31.2]	26 [21,31.2]				
pH		2 [1.7,2.4]	6.95 [6.5,7.4]		4.1 [2.9,6]		
T (°C)		16.1 [14.0,22.2]	15.35 [13.0,22.3]		15.35 [12.4,21.8]		
DO (mg/L)		12.61 [7.65,16.2]	10.87 [7.20,14.25]		12.25 [8.01,15.92]		
Eh (mV)		425 [395,450]	-37 [-47.2, 59.6]		315 [99.8,461]		
SO <sub>4</sub> (mg/L)		684 [508.8,845.3]	648.4 [474.7,840.6]		684 [411.2,814.8]		

### 3.2. Removal trends

#### 3.2.1. Arsenic

Arsenic removal efficiencies were always greater than 99.8% for both wetland types (Table 1). As presented in Fig. 2, the zeolite media produced on average lower As concentrations than limestone/cocopeat wetlands throughout the experiment: 0.018 versus 0.037 mg/L. Interestingly, As levels in the outflow from both treatments were not significantly different (Mann–Whitney  $p = 0.297$  for total As, Mann–Whitney  $p = 0.458$  for dissolved As), despite the significant difference between inflow and outflow concentrations for both wetland designs (Kruskal–Wallis  $p < 0.001$  for total and dissolved As). Furthermore, no temporal trend in As removal was present for either type of wetland, with the outflow levels remaining almost constant throughout the experiment.

#### 3.2.2. Iron

Zeolite wetlands were more effective than limestone/cocopeat wetlands in removing Fe, confirming the capability of the system to significantly change the concentration of Fe (Kruskal–Wallis  $p < 0.001$  for both total and dissolved Fe).

In contrast to As, Fe levels in the outflow differed significantly between media types (Mann–Whitney  $p < 0.001$  for both total and dissolved Fe). In fact, Fig. 2 shows that Fe levels in the outflow from zeolite wetlands were considerably lower than those in the outflow from limestone/cocopeat wetlands at the beginning of the experiments, but became similar towards the end of the experiment. On the other hand, Fe levels in the outflow from limestone/cocopeat wetland tended to decrease through time, after being quite elevated at the start of the experiment.

#### 3.2.3. Boron

There were only moderate reductions in boron levels through the wetland system. Boron concentrations were reduced on average by 17% in limestone/cocopeat wetlands, and by 12% in zeolite wetlands. While B concentrations in the outflow from both media types were significantly lower than those in the inflow (ANOVA  $p < 0.001$ ), the difference between media types was not significant (Games–Howell  $p = 0.155$ ). Fig. 2 shows that no discernible temporal trends were observed throughout the experimental period.

### 3.3. Environmental parameters in outflow samples

As shown in Table 1, pH was significantly affected by the media type (ANOVA  $p < 0.001$ ). The outflow pH from limestone/cocopeat wetlands was significantly higher than that from zeolite wetlands (Games–Howell  $p < 0.001$ ). Importantly, the pH values in the outflow from the zeolite wetlands were statistically higher than those in the inflow (Games–Howell  $p < 0.001$ ).

Similarly to pH, redox potential was significantly affected by media type (Kruskal–Wallis  $p < 0.001$ ), with Eh levels in the outflow from limestone/cocopeat wetlands being significantly lower than those in the inflow (Mann–Whitney  $p < 0.001$ ) and than those in the outflow from zeolite wetlands (Mann–Whitney  $p < 0.001$ ). Although Eh levels in the outflow from the zeolite wetlands did not decrease as markedly as those from limestone/cocopeat wetlands (Table 1), they were still significantly different to those in the inflow (Mann–Whitney  $p < 0.001$ ).

Although DO was also affected by the media type (ANOVA  $p = 0.002$ ), DO levels in the outflow from zeolite wetlands were not significantly different to those in the inflow (Tukey's  $p = 1$ ). On the other hand, DO levels in the outflow from limestone/cocopeat wetlands were significantly lower than those from zeolite wetlands (Levene's  $p = 0.003$ ) and to those in the inflow (Levene's  $p = 0.031$ ).

Neither sulfate nor temperature were significantly affected by any of the wetland types (ANOVA  $p = 0.517$ , Kruskal–Wallis  $p = 0.4$ , respectively).

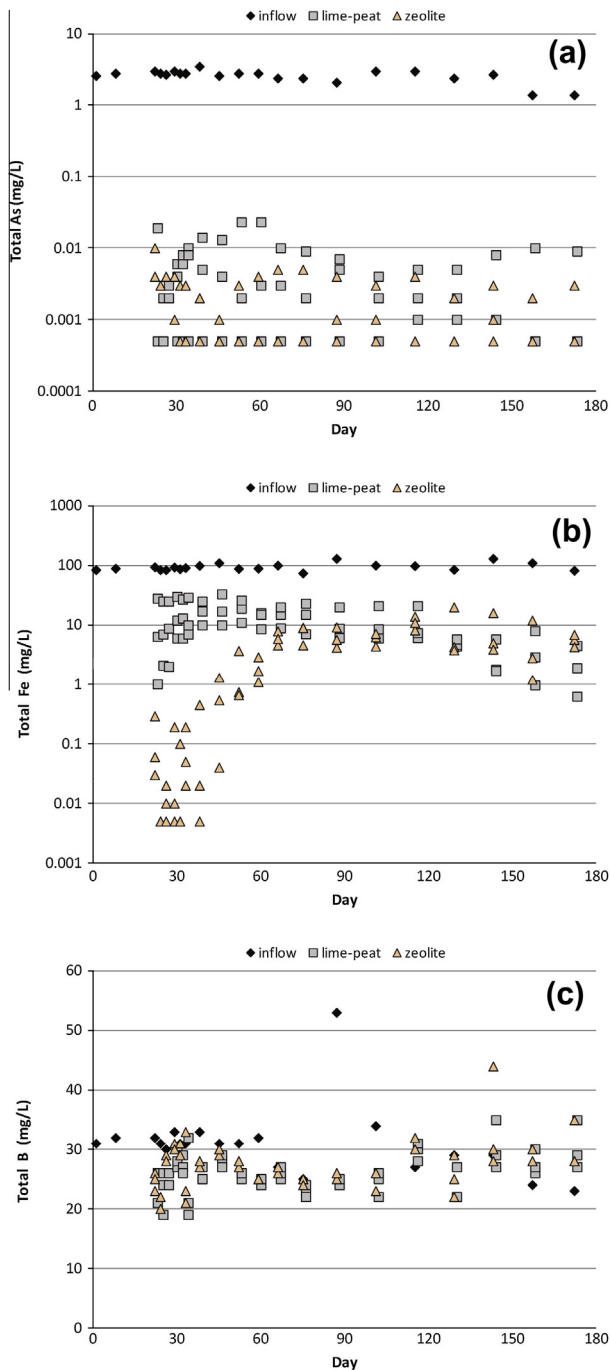
### 3.4. Environmental parameters within wetland cells

Both limestone/cocopeat (Cell 2LP) and zeolite (Cell 3Z) wetland cells presented a very similar trend in temperature, showing the effect of season (Fig. 3). While a statistically significant difference between them was found (Mann–Whitney  $p < 0.001$ ), the actual difference in temperature (13.1 °C in the limestone/cocopeat cell and 13.07 °C in the zeolite wetland) is unlikely to play a role in the observed wetland performance.

Neutral and stable pH values were measured in Cell 2LP (Fig. 3), whereas in Cell 3Z pH values were lower than 5 and slightly fluctuating. As expected, a significant difference between cells 2LP and 3Z pH values was found (Mann–Whitney  $p < 0.001$ ).

Redox potential in Cell 2LP reached negative values quickly once the experiments started, and they remained close to -400 mV (Fig. 3). On the other hand, Eh values in Cell 2LP decreased from positive to zero values for around half the duration of the experiment (until the probe was taken out for recalibration, as noted in the Methods). Following recalibration, Eh levels started to oscillate between 0 and 400 mV. The type of media significantly affected redox potential (Mann–Whitney  $p < 0.001$ ).

As shown in Fig. 3, DO values were low (<4 mg/L) in both wetland cells. The lime–peat cell presented lower DO values than the zeolite cell for most of the experiment, except at the beginning. The DO values for the lime–peat cell increased after the probe was taken out, whilst for the zeolite cell the DO values decreased. The type of media played a significant role in dissolved oxygen levels (Mann–Whitney  $p < 0.001$ ), being higher in the zeolite than in the limestone/cocopeat wetland.



**Fig. 2.** Total concentration of As (a), Fe (b) and B (c) in the inflow and outflow from the six wetland cells. Y axis is presented in log scale on As and Fe graphs. The surge in B inflow may correspond to analytical inaccuracies, as the inflow concentration should be similar to that of the previous sampling date.

### 3.5. Relationships between pollutant removal and environmental parameters

Table 2 presents Spearman's  $\rho$  coefficient for significant correlations between the different water quality and environmental parameters monitored in outflow samples, in each wetland group. A strong correlation was observed between Eh and Fe levels in zeolite wetlands. A significant positive correlation between As and Fe outflow concentrations from limestone/cocopeat wetlands was also observed. Conversely, a significant negative correlation was observed between As and Fe concentrations in the outflow from

zeolite wetlands. Iron concentrations decreased when pH increased in both wetland types.

### 3.6. Plant uptake

The uptake of As, Fe and B by plants is shown in Fig. 4. Plants located in the inlet accumulated higher levels of As than those in the outlet, regardless of media type. However, inlet plants in the lime-peat cell accumulated more As in roots than in shoots, whereas inlet plants in the zeolite cell accumulated more As in shoots than in roots. Plants located in the outlet accumulated similar levels of As in both wetland cells, in both roots and shoots. The total mass of As taken up by plants corresponded to 0.11% and 0.09% of the total As loaded into the limestone/cocopeat and zeolite cell, respectively.

Iron uptake by plants was also higher in plants located in the inlet of the two wetland cells. However, roots accumulated much higher levels of Fe than did shoots in plants at the outlet in both wetland types, with the root/shoot concentrations ratio being higher than 100. Of the total Fe influent mass, 2.05% and 0.66% was taken up by the *Phragmites* in the limestone/cocopeat and zeolite cell, respectively.

Again, the same pattern of uptake was observed for B (Fig. 4). Furthermore, B was mainly present in shoots rather than roots, in both wetland media types. However, plant uptake in the limestone/cocopeat wetland was considerably higher than uptake in the zeolite wetland. The proportion of B taken up by plants was 0.1% and 0.02% of the loaded B in limestone/cocopeat and zeolite cell respectively.

### 3.7. Metal retention in wetland media

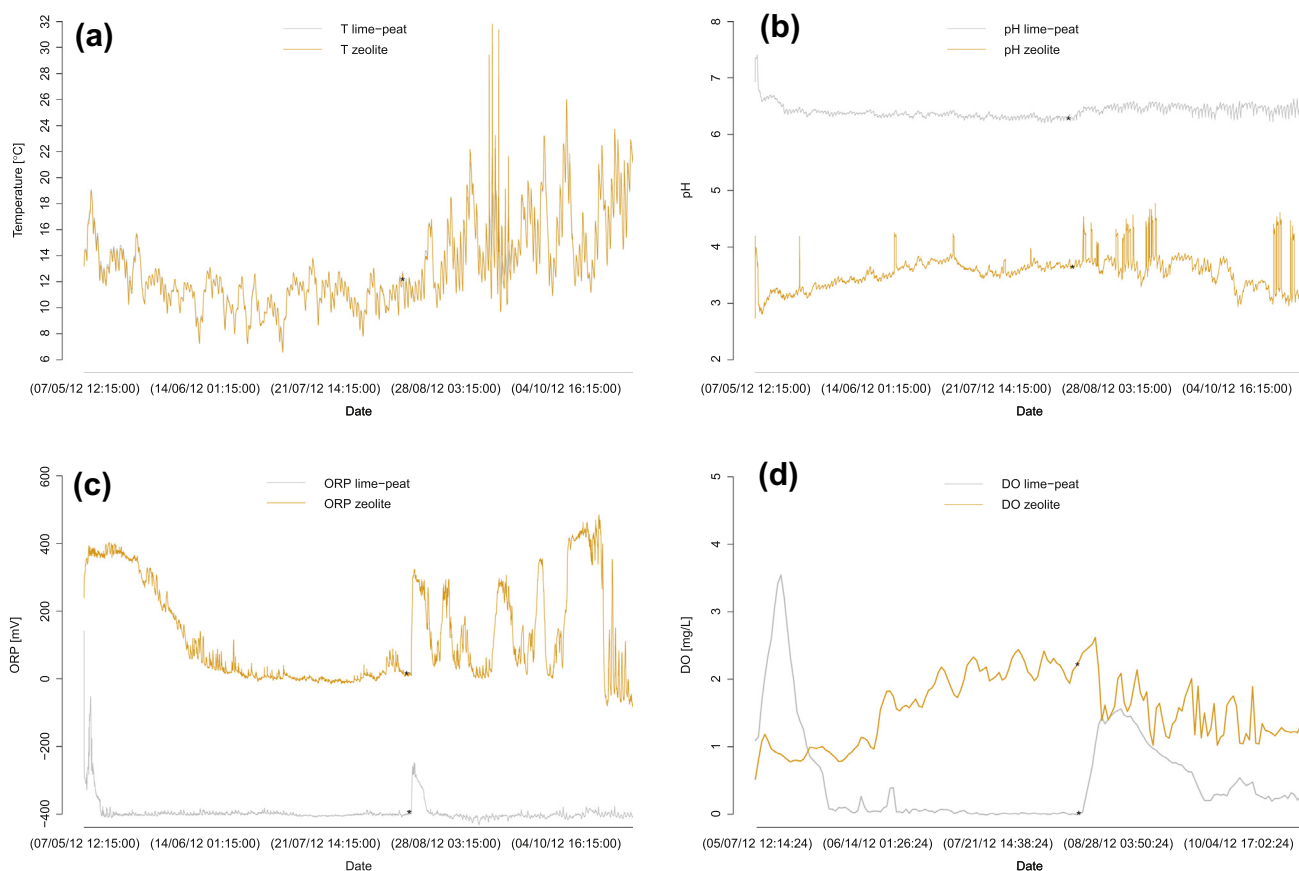
Zeolite wetlands had higher concentrations of As in the media at the end of the experiment than did limestone/cocopeat wetlands, which had As concentrations below detection in all but one replicate. The As concentration in the zeolite media tended to decrease towards the outlet and towards the bottom (Table 3).

A similar trend was observed for Fe, with concentrations in zeolite media higher than in limestone/cocopeat wetlands and tending to decrease towards the outlet and the bottom.

The cocopeat component of the limestone/cocopeat wetlands presented the highest concentrations of boron: between 200 and 350 mg/kg. Conversely, in the limestone section, B was not detected. In addition, B concentrations higher than 10 mg/kg were only detected in the upper section of one zeolite wetland (Cell 7Z).

### 3.8. Mass balances

Mass calculations considering nine equal volumetric sections in each wetland with the same concentration (as per Table 3) showed that As and Fe were mainly retained in the zeolite media, whereas B was mainly retained in the cocopeat media. For example, to estimate the mass of As in the top-inlet section of Cell 2LP (35.1 mg as per Table 4), the As concentration in that section (5 mg/kg) was multiplied by the mass of limestone in the section (which in-turn was calculated from the section volume ( $0.13 \times 0.2 \times 0.18 \text{ m}^3$ ) multiplied by the limestone density ( $1540 \text{ kg/m}^3$ ). Under this assumption, zeolite media retained all the influent As. The mass of As retained in the zeolite was 16% higher than the influent mass, suggesting the presence of errors due to sampling or analysis. The mass of As retained in the limestone/cocopeat wetland cannot be calculated accurately (Table 4), as arsenic was detected only in few sections. These facts suggest that the homogeneity assumption may not represent the actual trend of pollutant retention, although



**Fig. 3.** Temperature (a), pH (b), ORP (c) and DO (d) profiles in one replicate from each group throughout the whole experimental period. The \* symbol indicates where the probes were taken out for re-calibration.

**Table 2**

Correlation coefficients (Spearman's  $\rho$ ) for the relationships between measured water quality parameters, in both limestone/cocopeat and zeolite wetlands.

	DO (mg/L)	T (°C)	pH	Eh (mV)	SO <sub>4</sub> (mg/L)	As (mg/L)	Fe (mg/L)	B (mg/L)
<i>Limestone/cocopeat</i>								
DO (mg/L)	1							
T (°C)	-0.536**	1						
pH			1					
Eh (mV)			0.449***	1				
SO <sub>4</sub> (mg/L)					1			
As (mg/L)			-0.319*			1		
Fe (mg/L)			-0.606***	-0.346**		0.410***	1	
B (mg/L)		0.408**						1
<i>Zeolite</i>								
DO (mg/L)	1							
T (°C)	-0.433**	1						
pH	0.513***	-0.468***	1					
Eh (mV)	-0.494**	0.344**	-0.838***	1				
SO <sub>4</sub> (mg/L)		0.278*	-0.308*		1			
As (mg/L)						1		
Fe (mg/L)	-0.386*	0.362**	-0.764***	0.738***		-0.454***	1	
B (mg/L)		0.28						1

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .

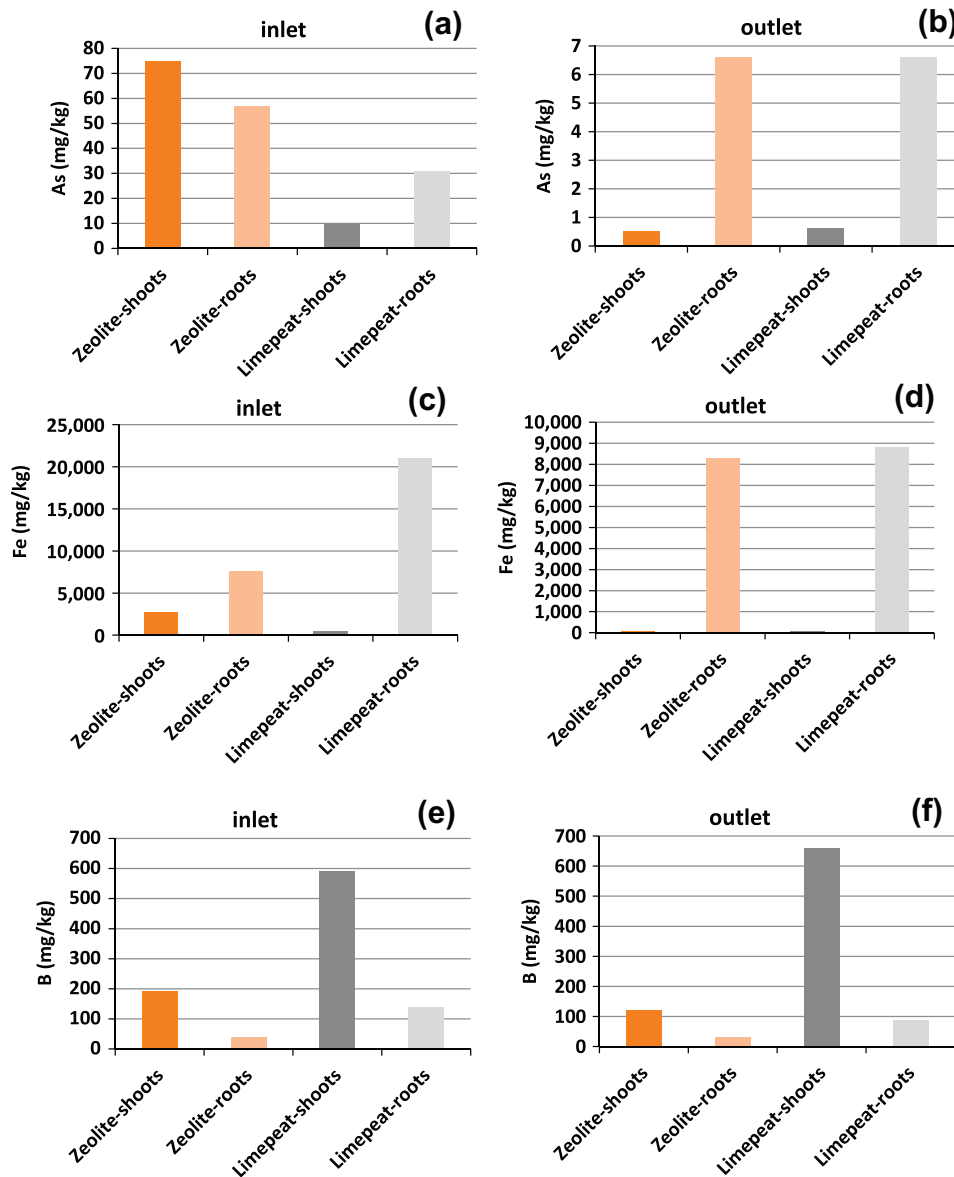
the afore-mentioned sampling and analysis errors may also have contributed to these uncertainties.

Iron behaved differently to As, with 95% of the influent Fe in the limestone/cocopeat cell detected in the media. However, this percentage is probably overestimated due to the Fe content in limestone, which has a typical value of 0.33% [31]. Since the limestone and cocopeat used in this study could not be analysed for the Fe content, the values in Table 3 represent the total Fe

measured in each medium rather than the concentration of Fe resulting solely from the retention of influent Fe.

On the other hand, the Fe content in pure zeolite was found to be 3600 mg/kg Fe (as Fe<sub>2</sub>O<sub>3</sub>). Therefore, the concentrations in Table 3 for this component correspond to the difference between the laboratory measurements for total Fe and the content of Fe in pure zeolite.

The boron concentrations in cocopeat in Table 3 represent total B content, assuming that the cocopeat did not contain any B in its



**Fig. 4.** Pollutants accumulation in plants in both wetland cells: As concentration in plants located at (a) inlet and (b) outlet; Fe concentration in plants located at (c) inlet and (d) outlet; B concentration in plants located at (e) inlet and (f) outlet. Note the different scales on the As (a and b) and Fe (c and d) graphs.

original state. Given the nature of cocopeat, whose main components are carbon, nitrogen and phosphorus, it is expected that its B content would be negligible.

## 4. Discussion

### 4.1. The role of wetland media

Results from this study demonstrate the potential of limestone, cocopeat and zeolite as supporting media in HSSF wetlands for the removal of As, Fe and B from acidic water. The zeolite wetlands had a marginally higher removal rate of As and Fe than the limestone/cocopeat wetlands. The higher pH resulting from the limestone, reducing and anaerobic conditions to trigger As–Fe coprecipitation and/or Fe–As–S precipitation, were not sufficient to achieve a significantly improved performance over that of zeolite.

#### 4.1.1. Limestone/cocopeat wetlands

The design of these hybrid wetlands was based on the capability of limestone to raise pH, thereby causing Fe precipitation and As

co-precipitation, and the capability of cocopeat to sorb B, resulting in optimal removal performance. The lack of sampling between the two distinct compartments reduces our ability to infer the behaviour of the individual components, although previous research can be used to infer the relative roles of the limestone and cocopeat [17,23]. The plant response to the different media may also be considered if both media are to be compared, as the particular characteristics of each of them (for example pH) affects As and metal retention in the rhizosphere. The positive correlation between the removal of As and Fe (Table 2) suggests the occurrence of Fe precipitation and As coprecipitation. In addition, as B has a high affinity for organic matter, and the cocopeat has potential for removing B, it was hypothesised that the initial pH adjustment (provided by the limestone section of the wetland) would improve the removal of B. As such, B removal was (slightly) higher than that of the zeolite wetlands. A higher pH may enhance this difference, as it has been found that B removal by zeolite is improved at higher pH levels (10–11; [32]). In addition, the adsorption capacity of cocopeat for B may have decreased due to the presence of As and Fe, as organic matter is also able to sorb As and Fe [33]. Therefore,



**Table 3**

Pollutant concentrations (mg/kg) in nine different locations of the wetland cells. Middle samples between inlet and outlet in limestone/cocopeat cells correspond to limestone media.

Wetland	Location	Limestone/cocopeat wetlands								
		As			Fe			B		
		Inlet	Middle	Outlet	Inlet	Middle	Outlet	Inlet	Middle	Outlet
Cell 2LP	Top	5	<5	<5	990	1100	3200	<10	<10	350
	Middle	5	<5	<5	1100	1100	990	<10	<10	260
	Bottom	<5	<5	<5	1000	890	1800	<10	<10	320
Cell 4LP	Top	13	59	31	1100	2000	3600	<10	<10	200
	Middle	7	9	18	870	840	2700	<10	<10	230
	Bottom	10	5	<5	2200	1200	2100	<10	<10	250
Cell 9LP	Top	<5	<5	<5	910	1200	5900	<10	<10	330
	Middle	15	6	<5	1500	1100	2400	<10	<10	350
	Bottom	<5	<5	<5	870	930	2100	<10	<10	250
<i>Zeolite wetlands</i>										
Cell 3Z	Top	150	80	77	3900	2800	2900	<10	<10	<10
	Middle	31	17	12	2300	1200	900	<10	<10	<10
	Bottom	11	9	8	1900	1100	1200	<10	<10	<10
Cell 7Z	Top	230	57	15	5700	7400	2000	21	10	<10
	Middle	58	21	13	2400	2000	2000	<10	<10	<10
	Bottom	17	11	11	2800	1400	2100	<10	<10	<10

**Table 4**

Estimated mass of pollutants (mg) detected in the zeolite (Cell 3Z), limestone and cocopeat media (Cell 2LP). Middle samples between inlet and outlet in limestone/cocopeat cell correspond to limestone media, whereas outlet samples correspond to cocopeat media. Calculations were based on data presented in Table 3, considering that that concentration was homogeneous throughout the media located in the corresponding volumetric section of the wetland.

Location	Limestone/cocopeat wetland									
	As			Fe			B			
	Inlet	Middle	Outlet	Inlet	Middle	Outlet	Inlet	Middle	Outlet	
Top	36.0	<36.0	<36.0	7135.1	7927.9	1531.1	<4.84	<4.8	167.5	
Middle	36.0	<36.0	<36.0	7927.9	7927.9	473.7	<4.8	<4.8	124.4	
Bottom	<36.0	<36.0	<36.0	7207.2	6414.4	6414.4	<4.8	<4.8	153.1	
Total mass per section	≥72.0	–	–	22,270.3	22,270.3	2866.1	–	–	445	
Total mass in media			≥70.0			47,406.6			≥445	
Total mass dosed			1687.7			61,402.1			19,915.0	
Total mass in plants			1.8			1298.0			20.8	
<i>Zeolite wetland</i>										
Top	731.0	411.8	337.7	20,077.2	14,414.4	12,719.4	<51.48	<51.48	<43.86	
Middle	159.6	87.5	52.6	11,840.4	6177.6	3947.4	<51.48	<51.48	<43.86	
Bottom	56.6	46.3	35.1	9781.2	5662.8	5263.2	<51.48	<51.48	<43.86	
Total mass per section	947.2	545.7	425.4	41,698.8	26,254.8	21,930.0	–	–	–	
Total mass in media			1918.4			89,883.6			–	
Total mass dosed			1659.4			60,373.0			19,581.3	
Total mass in plants			1.5			414.1			3.5	

it is likely that a number of sorption sites have been taken up by As and Fe instead of B.

#### 4.1.2. Zeolite wetlands

Zeolite was employed for its capacity to remove As [34], Fe [35] and B [32]. The negative correlation between removal rates of As and Fe suggests that As and Fe are being removed via different mechanisms: Fe cations can be exchanged by Ca, Na or K cations, whereas As anions can be exchanged by aluminol or silanol hydroxyl groups [34]. Additionally, As concentrations in the outflow remained mostly constant through time, whereas Fe concentrations increased (refer to Figs. 2 and 3), indicating a likely saturation of Fe-exchange sites. A previous study [17] reported a positive correlation between the removal of As and Fe from acidic water in vertical subsurface flow wetlands, inferring that both pollutants were removed by a common mechanism. As such, different specific sorption mechanisms cannot be discarded, as As can be attracted by Fe already exchanged on zeolite sites [36], meaning that the removal of Fe facilitates the removal of As.

The wetland media also affected the outflow rates. Outflow rates were lower in the zeolite wetlands than in the limestone/

cocopeat wetlands (129.5 versus 144.9 mL/h), despite the fact that all wetlands received the same inlet rate (150 mL/h). This observation is attributed to the physical characteristics of the media, such as particle size, porosity and compaction. Other factors such as plant density and subsequent differences in evapotranspiration are considered to have a minor role. As a result of the differences in flow rate, the detention time was slightly higher in zeolite wetlands. Higher detention times are known to improve metal removal efficiency in wetlands [37] and this may explain the higher pollutant removal rates in zeolite wetlands.

#### 4.2. The role of vegetation

Our results demonstrate that roots of *Phragmites* have greater As retention than shoots and thus play an important role in accumulating As. Few studies have employed *Phragmites* in constructed wetlands for As removal. One example from Vymazal et al. [38] reported plant uptake concentrations considerably lower than those reported in our study, but the As levels in the inflow were also much lower than in this study. The As content in the plants for this study is within the range reported in the literature for other types

of plants (e.g. [39,16]), but for different levels of As in the inflow. In Ye et al.'s study [16], As concentration in the inflow was 0.46 mg/L, and As concentration was 16.5 mg/kg in shoots and 110.1 mg/kg in roots. In Singhakant et al.'s study [39], As concentration in the inflow was 4.7 mg/L, and As concentrations were in the range of 0.5–4 mg/kg in the shoots and 5–40 mg/kg in the roots. This suggests that apart from inflow concentrations, other factors affect plant uptake of As, such as the length of the experiments, the type of vegetation [40] and the type of substrate (Fig. 4). The immature state of the wetland system may also explain the high plant uptake values.

The fact that plants located in the inlet acquired more As than those towards the outlet demonstrates the effective removal within the inlet section. As such, lower concentrations of As are transmitted to the outlet (and lower) sections of the wetland and therefore less As is available for plant uptake in these locations. The higher uptake by plants in the zeolite cell may be explained by the higher bioavailability of As, which can still be dissolved given the acidic pH in the cell (Fig. 3). Iron was also mainly removed in the inlet of both wetland types (Fig. 4), but in this case the plant uptake was higher in the limestone/cocopeat wetland cell. It is possible that the neutral pH enhances iron plaque formation in the roots [41], since Fe precipitates easily at neutral pH. Biological precipitation may also occur. Roots in this cell developed an intense orange colour, which is characteristic of iron precipitates. Since these iron precipitates were attached to the roots, all Fe detected here was considered as Fe uptaken by roots. Plant roots in the zeolite cell did not develop this orange colour.

Other studies have also reported the minor contribution of plant uptake to the overall removal of As [16,42], Fe [43] and B [16], despite observed high accumulation of the pollutants in the roots. However, there is a need for further research regarding the indirect effect of vegetation on removal processes [19], as it has been reported that vegetated systems are more effective than unvegetated systems in the removal of As [15]. This suggests that mechanisms other than direct uptake play a more important role, such as the interaction with the granular medium and microorganisms, and the provision of organic matter [19]. The direct effect of vegetation also requires further investigation. In particular, the use of accumulators [12] shows potential, although most of the studies performed on this topic have been on dryland plants. To date, no emergent wetland plants have been identified as hyperaccumulators, suggesting that the main known role of plants in the wetland context is the provision of organic matter through dieback and the provision of organic compounds via root exudation [44]. However, plants may also influence As removal through two other mechanisms: (1) Arsenic hyperaccumulators produce more root exudates under As stress, and this may help them to accumulate more As; (2) changes in the DOC concentration in the rhizosphere affect microbial population [45], which may play a significant role in As speciation.

Iron also accumulated at a greater rate in roots as compared to stems or leaves, consistent with previous studies [16,43]. However, Fe levels detected in plants were higher than those typically reported in the literature (200 and 2000 mg/kg (ibid)), but they are comparable with those observed by Ye et al. [43], who reported 41,318 mg/kg Fe in roots of cattail. Boron displayed a different behaviour, being primarily accumulated in the shoots. Ye et al. [16] reported a similar finding in different plants, for similar influent B concentrations. However, in another study, B accumulated more in roots than in shoots [43]. Boron is essential for plant growth [46]. Higher levels of B in shoots than in roots may be a result of B translocation across a concentration gradient, since once in the shoots B may accumulate to the point where water is lost through stomata in the leaf [47].

The fact that B accumulation was similar in plants located in the inlet and the outlet suggests that there was not such a strong

removal gradient across the system as occurred with As and Fe. Higher accumulation of B in plants in the limestone/cocopeat wetland contradicts the findings of several authors who suggest that higher soil pH decreases boron uptake by plants (e.g. [48]). Under alkaline conditions the borate ion  $B(OH)_4^-$  rather than  $B(OH)_3$  predominates, and borate has higher affinity for soils, increasing B sorption on soil. Additionally, it has been found that B uptake decreases in neutral conditions [49]. As such, further research is required to understand the boron uptake mechanisms and the related factors, as the impacts of the different media could be dwarfing the impact seen by different pH values.

#### 4.3. Pollutant removal mechanisms

Arsenic may be removed via several mechanisms in a constructed wetland, with precipitation and sorption being the most important [50]. High levels of As and Fe in the wetland media, low contribution of plant uptake and the very small decrease of sulfate levels in the outflow all suggest that the key removal mechanisms are chemical precipitation of iron and arsenic in the limestone/cocopeat wetlands, and sorption in the zeolite wetlands (rather than biological precipitation of sulfides). Biological processes may still occur, particularly in the limestone/cocopeat wetlands, given the suitable conditions for sulfate reducing bacteria such as neutral pH, presence of organic matter, low dissolved oxygen levels and reducing conditions. As presented in Section 3.4, limestone/cocopeat wetlands had lower DO and Eh levels than the zeolite wetlands, which could suggest the presence of anaerobic bacteria. In fact, preliminary results which screened the outflow for the bacterial community using metagenomics (by studying the genetic material derived from water samples) suggested the existence of different bacterial communities in both zeolite and limestone/cocopeat wetlands (data not shown). Among these, the order *Desulfobacterales* (strictly anaerobic sulfate reducing bacteria) was found in both types of wetlands. However, the contribution of biological precipitation to the overall removal cannot be calculated with the experimental data of this study. Future studies should focus on the role of these communities in the performance of wetland systems. It has been suggested that metal sulfide precipitation may be more desirable than metal oxide precipitation due to the generation of alkalinity and the higher density of metal sulfides [51]. Thus, it may be worth enhancing this removal mechanism (instead of Fe oxides precipitation) in constructed wetlands by facilitating the development of sulfate reducing bacteria.

Arsenic can also be removed by sorption onto calcite [52], the main component of the limestone employed in this study. This mechanism cannot be disregarded, although the low levels of As detected in the limestone media samples suggest that As is being removed elsewhere, as shown by mass balance calculations (Table 4). Furthermore, two samples collected from the inlet (next to the gravel inlet zone, in the upper and the lower part) of Cell 4LP presented 1600 and 250 mg/kg As and 45,000 and 8000 mg/kg Fe, respectively. These are all considerably higher than those detected in the adjacent location of the wetland cell (Table 3). These results suggest that the retention of both As and Fe is heterogeneous and occurs mainly in the inlet of the limestone/cocopeat wetlands, as soon as the water comes into contact with the limestone section. It is likely here that Fe precipitates are formed, capturing As. These particles may also be trapping B, since the B concentration in the upper section of the inlet was elevated. Arsenic and iron may also be retained in the cocopeat section, and their availability may be affected by the degradation of organic matter.

Boron was primarily removed by sorption in the cocopeat substrate, which is not surprising given the capacity of organic matter

to sorb boron [53]. On the other hand, very low levels of B in the wetland media suggest that the sorption capacity of limestone and zeolite media is low. In addition, Fe oxides are mainly trapping As and not B. Reports of B removal by Fe oxides indicate that higher pH is required to increase B removal [54]. Plant uptake might also play a role, but lower B concentrations would be required [55].

#### 4.4. Recommendations for the removal of the target pollutants in constructed wetlands

Both wetland types performed effectively in removing As and Fe, with limestone/cocopeat slightly less effective in removing Fe. The most appropriate system to be implemented will depend on the specific requirements for the treated effluent, and also other considerations such as the cost of media and maintenance requirements. Trapping As and Fe in the inlet section of the wetlands can reduce maintenance costs by allowing maintenance to be restricted to the inlet zone.

Boron removal remains a challenge and although organic materials such as cocopeat are promising, more research is needed to increase B removal in constructed wetlands. A second stage of treatment could be implemented, under alkaline conditions and where B sorption by organic matter and/or iron/aluminium oxides and/or clay minerals is encouraged. This second stage may be effective, since As would have been removed already. A hydroponic system using an accumulator species such as duckweed might also work, but this has only been recommended for B concentrations below 2 mg/L [55], and thus would require further investigation in circumstances where B concentrations are elevated.

Results from this study therefore confirm the key role of media in the removal of As and metals from acidic water, and the minor role of plant uptake despite the elevated concentrations in plants tissues. Combinations of media appear to be an effective way of optimising the removal of different pollutants in individual wetland cells. Further research into possible configurations of the media is required. For example, a bed of limestone, followed by a bed of cocopeat and then of zeolite would be worth testing. Further research is also needed to confirm the indirect role of vegetation in the performance of the system, and the presence and effect of microorganisms. Future studies should focus on the role of microbial communities in the removal processes, together with the speciation of the pollutants in the solid phase using advanced techniques such as X-ray diffraction (XRD), X-ray spectroscopy, or sequential extraction procedures [56]. Other methods such as determination of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) could also be employed for bottom sediments [57]. This information is crucial to fully understand the fate of the pollutants in the wetland matrix and therefore optimise the design of constructed wetlands for the removal of As, Fe and B from contaminated water.

## 5. Conclusions

- The effectiveness of alternative wetland media was demonstrated in HSSF wetlands: limestone/cocopeat and zeolite wetlands showed excellent removal efficiencies for As (99.8% and 99.9% respectively) and Fe (87.3% and 96.1%) for a hydraulic loading rate of 30 mm/d.
- Limestone/cocopeat wetlands could be a more suitable option depending on the requirements for the final effluent, given their capacity to raise pH. However, Fe removal was higher in the zeolite wetlands.
- The key removal mechanisms in limestone/cocopeat wetlands were pH-related Fe oxide precipitation, which caused As co-precipitation, as well as B sorption by cocopeat. In zeolite wetlands, As and Fe were removed by ion-exchange.

- Plant uptake therefore played a minor role in the overall removal of the target pollutants, as the accumulation of As, Fe and B in plants were 0.11%, 2.05% and 0.1% of the total mass loading in limestone/cocopeat wetlands, and 0.9%, 0.66% and 0.02% in zeolite wetlands, respectively.
- Metal accumulation in the wetland media indicated that As and Fe removal is heterogeneous: in limestone/cocopeat wetlands As and Fe were mainly removed in the inlet section (i.e. the limestone component), whereas in zeolite wetlands the removal decreased towards the bottom and the outlet sections of the cells.

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