Influence of substrate depth and particle size on phosphorus removal in a surface flow constructed wetland

Cui Lijuan, Li Wei, Zhou Jian, Zhang Yan, Zhang Manyin, Lei Yinru, Kang Xiaoming, Zhao Xinsheng and Pan Xu

ABSTRACT

Substrate adsorption is one of the main processes by which redundant phosphorus is removed from wastewater in surface flow constructed wetlands (SFCWs). The physical properties of the substrate, such as depth and particle size, will influence the amount of phosphorus adsorption. This study was carried out in a long-running intermittent inflow constructed wetland that covered a total area of 940.4 m² in the Shunyi District of Beijing, China. We investigated how the concentrations of four phosphorus fractions, namely calcium phosphate (CaP), iron phosphate (FeP), adsorbed phosphorus (AdsP), and organic phosphorus (OP), varied between the surface (0–10 cm) and subsurface (10–20 cm) substrate and among the different substrate particle sizes. The total phosphorus concentrations in the substrate ranged from 154.97 to 194.69 mg/kg; CaP accounted for more than 80% of the total phosphorus content. The concentrations of OP were significantly higher in the surface layer than in the subsurface layer, but the concentrations of inorganic phosphorus were not significantly different between the two layers. The CaP, AdsP, and OP adsorption capacities were greater for small-sized substrate particles than for large-sized substrate particles. The results from this study provide a theoretical basis for the construction of constructed wetlands.

Key words | constructed wetland, dynamic process, inorganic phosphorus, organic phosphorus, phosphorus removal, wastewater treatment

INTRODUCTION

As an effective alternative to traditional treatment technologies, constructed wetlands based on the ecological functions of natural wetlands have gradually evolved, and are increasingly used for treating different kinds of wastewater because of their low construction costs, low energy consumption, ease of operation, and low maintenance requirements (Wood et al. 2008; Liu et al. 2010; Li et al. 2014; Gachango et al. 2015; Sudarsan et al. 2015). Constructed wetland systems have been used extensively for sewage treatment and landscaping applications in China since the 1990s (Zhu et al. 1991; Liu et al. 2010). Recent research on constructed wetlands has mainly focused on water purification (Fey et al. 1999; Ávila et al. 2014), hydraulic characteristics (Mexicano et al. 2013), choice of substrate (Ge et al. 2015), hydraulic retention time (Dzakpasu et al. 2015), and plant selection and configuration (Wang & Sample 2013). Few studies, however, have examined how the physical properties of the substrates, such as substrate depth and size, influence pollutant removal in constructed wetlands.

In the wetland ecosystem nutrient cycling process, phosphorus, an essential nutrient for wetland plants, provides a range of biological nutrients for the system. However, excessive phosphorus will cause water eutrophication, the main cause for the proliferation of plankton and other symptoms of water pollution (Jaanus & Pellikka 2003). Spatial and temporal variations in the concentrations and distribution of phosphorus will affect the wetland ecosystem nutrient balance (Spiess 2011; Weaver & Wong 2011; Li et al. 2015). Therefore, the process of phosphorus adsorption by bottom sludge is particularly important in wetlands (Wang & Sample 2013). Early studies showed that wetland substrate and vegetation were major long-term storage pools for phosphorus (Cooke 1992; Hiley 1995; Drizo et al. 2008). Phosphorus removal in surface flow constructed wetlands
(SFCWs) occurs through chemical precipitation, adsorption, and assimilation by vegetation and biofilms that form in the substrate, and the radicular system of the vegetation (Sousa et al. 2004; Sekhon & Blumbla 2016). The amount of phosphorus retained in the substrate depends on the phosphorus concentrations in the water column and the interactions between the water column and soil biochemical processes, which include adsorption/desorption, precipitation, mineralization of organic phosphorus (OP), and diffusion of phosphorus from the soil (Matagi et al. 1998). Therefore, the physicochemical properties of the substrate will determine the adsorption of phosphorus in wastewater. Previous studies have reported that more than 80% of the phosphorus in constructed wetlands was removed by substrate adsorption, and that phosphorus must be removed from water to prevent eutrophication (Vymazal 2010; Jia et al. 2014; Sudarsan et al. 2015). It is known that SFCWs are not effective at adsorbing phosphorus over the long term, reasons for which have been proposed in previous studies, as follows: (1) the root biomass fills the pore spaces of the substrate, directing wastewater flow deeper in the wetland media (Breen & Chick 1995); (2) the litter formed by decomposing vegetation remains on the surface of the substrate and so the substrate does not interact with the wastewater (Kyanbadde et al. 2005); and (3) the substrate media usually used does not contain sufficient concentrations of Ca²⁺, Fe³⁺, or Al³⁺ to actively adsorb phosphorus (Vymazal 2004).

Phosphorus adsorption and removal in constructed wetlands are influenced by many factors, including the content of metal ions, the forms of phosphorus, pH, salinity (Wang et al. 2010), temperature, vegetation (Drizo et al. 2008), and various other environmental factors. Recent studies on the substrates of constructed wetlands have mainly focused on the phosphorus sorption capacities and physicochemical properties of different substrate materials (Börling et al. 2004; Ren et al. 2007; Cui et al. 2008). However, few studies have examined the influence of substrate size and depth on phosphorus removal in existing SFCWs. This study was carried out in an intermittent inflow SFCW in Beijing, China. We examined the concentrations and distribution of four forms of phosphorus using stratified sampling.

**MATERIALS AND METHODS**

**Research area introduction**

The research SFCW was in the Beijing Wildlife Rescue & Rehabilitation Center, Shunyi District, China. In the central part of the facility, there is a constructed lake that is supplied mainly by groundwater and aquaculture wastewater. The lake frequently supports large numbers of waterfowl, and its water self-purification ability is weak, which causes serious water eutrophication. To improve the water quality, we built an SFCW close to the constructed lake in May, 2008, into which water is pumped from the lake for 3 hours every day (Figure 1). The SFCW is 326.2 m long, has an average depth of 0.4 m, and covers a total area of 940.4 m². It is divided into three parts, including upstream, midstream, and downstream. The upstream, midstream, and downstream areas are 140.6 m, 107.4 m, and 78.2 m long, respectively, and have areas of 326.9 m², 371.5 m² and 242.0 m², respectively. The substrate of the wetland is a mixture of sand and normal soil collected locally.

**Sample collection**

We established three sampling points in the SFCW, one in the upstream, one in the midstream, and one in the downstream area in May 2013. At each of these sampling points, we collected three samples of both surface (0–10 cm) and subsurface (10–20 cm) substrate that each weighed more than 100 g. We collected a total of 18 samples, and these were placed in polyethylene bags and returned to the laboratory. The samples were stored at 4 °C and were air-dried naturally at 4 °C for more than 2 weeks. They were then sieved through a 2-mm nylon sieve to remove coarse debris and stones.

The sampled substrate material was divided into three particle diameter classes, namely those with a diameter...
Φ > 63 μm, Φ < 2 μm, 2 μm < Φ < 63 μm, referred to as large (L), small (S), and middle (M) respectively. To obtain the L-sized particles, we weighed 100 g of dried substrate from which impurities had been removed into a beaker. We then added distilled water and mixed the samples. The substrate was pressed through a 250-mesh sieve, and rinsed then added distilled water and mixed the samples. The substrate material with a particle Φ > 63 μm was obtained by drying the collected substrate residues at 30 °C for 1.5 hours in a desiccator. The sieved substrate was then divided into three equal parts. Each part was placed into a graduated cylinder to which 1 L of distilled water was added and mixed well. We based our experiments on Stokes theory of particle sedimentation (Lu 1980; Kyambadde et al. 2005) and the particle settling table for specific temperatures, which shows the relationship between the settlement depth and settling time for particles of a certain diameter. We marked a tag line on the graduated cylinder, and withdrew the mixed solution above the tag line after 10 min. Then we added distilled water up to the 1-L scale line in the graduated cylinder, continued stirring, and drew the mixed solution up to the tag line again. We repeated this procedure until the water was clear. The water samples collected from the graduated cylinder were shaken for 30 min, filtered with filter paper, and the filtrates remaining in the filter were dried for 1.5 hours at 30 °C. Using this method, we obtained the substrates with Φ < 2 μm (S). We then collected the residual solution from below the tag line marked earlier on the graduated cylinder. We also filtered the solution and dried the residue. From this step, we obtained the target substrates with particle diameters 2 μm < Φ < 63 μm (M).

**Extraction and determination of phosphorus**

In this study, we extracted calcium phosphate (CaP), iron phosphate (FeP), adsorbed phosphorus (AdsP), and OP from the substrate samples in the three size categories. (1) To extract CaP, we thoroughly mixed the extracted substrates with 98.3% concentrated sulfuric acid, centrifuged the mixed solution at 2,263.95 × g for 8 min in a centrifuge, extracted the supernatant liquid, and determined the content of CaP. The centrifugal radius (Cr) was 10 cm and centrifugal speed (Cs) was 4,500 rpm; we calculated the centrifugal force (xg) with the formula: Cr × 11.18 × 10⁻⁶ × Cs². (2) To extract FeP, the residue of (1) was placed into a 50 ml centrifuge tube. We then added 12.5 ml of saturated sodium chloride solution, rinsed it twice, and then centrifuged the residual substrate. We then mixed the cleaned residue with 25 ml of sodium hydroxide solution, centrifuged the solution at 2,263.95 × g for 10 min, and determined the FeP content in the supernatant. (3) To extract the AdsP, we dried the collected samples at 30 °C for 1.5 h. We then put 0.5 g of dried sample and 25 ml of ammonium chloride into a 50 ml centrifuge tube. We centrifuged the solution at 1,369.55 × g for 10 min, and determined the FeP content in the supernatant. We also centrifuged the solution at 2,263.95 × g for 30 min at a constant temperature of 25 °C. Finally, we determined the content of AdsP in the supernatant by ammonium molybdate spectrophotometry. (4) To extract OP, we soaked the substrate sample in 20 ml NaHCO₃ solution that contained a small amount of non-phosphorus activated carbon. Then, the mixed solution was shaken at 4.47 × g for 30 min at a constant temperature of 25 °C. The extracted solution was placed in a 25 ml colorimetric tube after passing through a 0.45 μm filter. Finally, we determined the OP content by molybdenum antimony spectrophotometry at 700 nm (Swift & Sparks 1996; Vlessidis et al. 2004). (5) Total phosphorus concentrations in the substrate materials were calculated as the total of the CaP, FeP, AdsP, and OP concentrations.

**Data analysis**

We used two-way analysis of variance (ANOVA) for a series of linear comparisons (Sokal & Rohlf 1981). To test the effects of substrate depth (0–10 cm and 10–20 cm) and flow position (including upstream, midstream, and downstream) on adsorption of the four forms of phosphorus (CaP, FeP, Abs_P, and OP) to the SFCW substrate, we ran a two-way ANOVA in which substrate depth and flow position were treated as fixed effects. Then, we tested the effects of particle size (including Φ > 63 μm, 2 μm < Φ < 0.63 μm, and Φ > 0.63 μm) and flow position on phosphorus adsorption by the SFCW substrate in the same way. To meet the requirements of homoscedasticity and normality, some data (marked in the tables) were square root transformed before analysis. All analyses were conducted using SPSS 22.0 (SPSS, Chicago, IL, USA). Effects were considered significant at p < 0.05.

**RESULTS**

**Phosphorus adsorption in the SFCW**

The total concentrations of phosphorus in the SFCW substrate ranged from 154.97 to 194.69 mg/kg. The mean concentrations of total phosphorus were higher in the upstream than in the downstream area (Table 1). However, there were
no significant differences among the concentrations of the substrate from the upstream, midstream, and downstream areas. CaP was the dominant form, and accounted for more than 80% of the total phosphorus content. OP accounted for about 10%. The concentrations of the other forms, AdsP and FeP, were lower (Table 1).

**Influence of substrate depth on substrate phosphorus adsorption**

The total phosphorus concentrations in the SFCW differed significantly with substrate depth. The phosphorus concentrations in the surface layer (0–10 cm) were 20% greater than in the subsurface layer (10–20 cm, Figure 2(a)). Similar to the total phosphorus concentrations, the concentrations of OP decreased as the substrate depth increased \((F = 24.31, p < 0.001, \text{Figure 2(d) and Table 2})\); the concentrations of CaP, FeP, and AdsP were not significantly different at different depths \((p > 0.05, \text{Table 2})\). The results of two-way ANOVA showed that the concentrations of FeP and OP \((p > 0.05, \text{Table 2})\), but not the concentrations of CaP and AdsP \((p = 0.045 \text{and } p = 0.032, \text{Table 2})\), were significantly different at different positions in the SFCW. There were no significant interaction effects between the two treatments.

**Influence of substrate size on substrate phosphorus adsorption**

The phosphorus adsorption capacity of the substrate decreased significantly as the particle size in the SFCW

### Table 1

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>TP mg/kg</th>
<th>OP mg/kg</th>
<th>CaP mg/kg</th>
<th>FeP mg/kg</th>
<th>AdsP mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream-1</td>
<td>194.69 ± 26.71</td>
<td>30.14 ± 3.51</td>
<td>162.47 ± 29.18</td>
<td>0.38 ± 0.14</td>
<td>1.70 ± 0.66</td>
</tr>
<tr>
<td>Midstream-1</td>
<td>179.55 ± 16.35</td>
<td>37.52 ± 12.48</td>
<td>139.50 ± 16.31</td>
<td>0.38 ± 0.08</td>
<td>2.15 ± 1.02</td>
</tr>
<tr>
<td>Downstream-1</td>
<td>161.78 ± 20.95</td>
<td>32.09 ± 9.13</td>
<td>128.43 ± 12.15</td>
<td>0.41 ± 0.09</td>
<td>0.85 ± 0.81</td>
</tr>
<tr>
<td>Upstream-2</td>
<td>167.19 ± 9.21</td>
<td>19.56 ± 7.75</td>
<td>145.92 ± 1.47</td>
<td>0.67 ± 0.21</td>
<td>1.04 ± 0.32</td>
</tr>
<tr>
<td>Midstream-2</td>
<td>154.97 ± 16.81</td>
<td>10.65 ± 10.51</td>
<td>142.75 ± 7.83</td>
<td>0.43 ± 0.35</td>
<td>1.14 ± 0.16</td>
</tr>
<tr>
<td>Downstream-2</td>
<td>138.51 ± 9.26</td>
<td>8.49 ± 6.16</td>
<td>129.07 ± 14.14</td>
<td>0.34 ± 0.13</td>
<td>0.61 ± 0.05</td>
</tr>
</tbody>
</table>

-1 means surface substrate (0–10 cm).

-2 means subsurface substrate (10–20 cm).
increased. The substrate phosphorus adsorption capacity was strongest, especially that of CaP, AdsP, and OP, when the particle diameter was <2 μm (the F category) (Figure 3). There were no significant differences between the substrate phosphorus adsorption capacities of M and L. Different particle sizes had no significant effect on the concentrations of FeP in the substrate (p = 0.241, Table 3).

**DISCUSSION**

Phosphorus forms in the substrate of the SFCW were mainly inorganic, including CaP, FeP, and AdsP (Table 1). The dominant form, CaP, accounted for more than 80% of the total phosphorus content, and is more stable than other phosphorus forms. This form dominated because the wetland plants have a low capacity for using CaP. As wetland plants decompose, dissolved phosphorus and calcium ions combine, settle, and are adsorbed and accumulated by the substrate (Jiang et al. 2009). Previous studies have shown that salinity and temperature are the environmental variables that most commonly influence sediment-water exchange of phosphate, and also inhibit the potential for CaP desorption in SFCW (Zhang & Huang 2011). Our results show that CaP was mainly concentrated in the substrate with smaller particle sizes (Φ < 2 μm, Figure 3); the substrate with the smallest particle size has a bigger surface area and a stronger adsorption capacity, which promotes metal ion reactions that form phosphorus compounds by precipitation (Zhu et al. 2004). As the sampling depth increased, the content of CaP decreased (Figure 2), which was related to the decrease in the total phosphorus concentrations in the substrate.

The content of FeP did not change significantly as the particle size of the substrate changed (Figure 3(b)); this may have been related to elevated concentrations of secondary Fe and manganese (Mn) oxides (Zhang et al. 2004). Plants can influence the FeP transformation process in the substrate by altering the internal dissolved oxygen environment, and can also increase the content of FeP by

### Table 2

<table>
<thead>
<tr>
<th>Trait</th>
<th>Position (P) F2,12</th>
<th>Depth (D) F1,12</th>
<th>P * D F2,12</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaP</td>
<td>4.05 0.045</td>
<td>0.26 0.622</td>
<td>0.64 0.546</td>
<td></td>
</tr>
<tr>
<td>FeP</td>
<td>1.37 0.291</td>
<td>0.39 0.543</td>
<td>1.34 0.298</td>
<td></td>
</tr>
<tr>
<td>AdsP</td>
<td>4.68 0.032</td>
<td>3.98 0.069</td>
<td>0.53 0.604</td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>0.47 0.638</td>
<td>24.31 &lt; 0.001</td>
<td>1.45 0.272</td>
<td></td>
</tr>
</tbody>
</table>

*Means data were transformed to root square.
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Table 3 | Effects of substrate size (Φ > 63 μm, 2 μm < Φ < 0.63 μm, and Φ < 0.63 μm) and flow position on the contents of P determined by two-way ANOVA. F, p, and DF are given, and values of p < 0.05 are in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Position (P)</th>
<th>Particle size (D)</th>
<th>P * D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaP</td>
<td>3.41</td>
<td>0.055</td>
<td>17.86</td>
</tr>
<tr>
<td>FePa</td>
<td>2.94</td>
<td>0.078</td>
<td>1.54</td>
</tr>
<tr>
<td>AdsP</td>
<td>3.76</td>
<td>0.043</td>
<td>39.01</td>
</tr>
<tr>
<td>OP</td>
<td>0.04</td>
<td>0.961</td>
<td>180.15</td>
</tr>
</tbody>
</table>

*Means data were transformed to root square.

delivering oxygen to the roots, because the phosphorus binding capacity of Fe and Mn oxides is stronger under aerobic conditions than under anaerobic conditions in the substrate. In addition, reduced water mobility has a negative effect on the oxygen flux in the water, which results in release of the FeP phosphate anion.

The content of OP decreased as the substrate depth (F = 24.31, p < 0.001, Figure 2(d) and Table 2) and substrate particle size both increased (Figure 5). This may be explained by the fact that the spatial distribution of OP in constructed wetland substrates is strongly influenced by the content of OP in the upper part of the plant (Liu et al. 2006). The plant residues are not cleared or harvested in the winter, and OP is released from these residues after microbial decomposition and deposition on the surface of the substrate, thereby contributing to the higher OP content in the surface substrate relative to that in the subsurface substrate (Figure 2(d)). The water inflow to the constructed wetland is intermittent, which may have promoted mineralization of OP; it may also have contributed to increases in the OP content in the surface substrate (Gleyzes et al. 2002). The high content of OP in the substrate with the smallest particle size may reflect weak OP mineralization reactions, which are related to the substrate pH. Previous studies have reported that increases in the pH of the substrate have a negative effect on the migration and permeation of OP (Li & Yang 2008), and lead to high concentrations of OP in the substrate.

CONCLUSIONS

In our study, we found that the concentrations of OP were almost 20% higher in the surface layer than in the subsurface layer, and that the concentrations of inorganic phosphorus (including CaP, FeP and AdsP) were not significantly different between the 0–10 cm and 10–20 cm layers. The CaP, AdsP, and OP adsorption capacities were greater for small-sized substrate particles than for large-sized substrate particles.

Our results indicate that the different forms of phosphorus were correlated with both the substrate depth and size. This study provides useful information that can be used to support decisions about substrate selection for constructed wetlands.

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