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Technical Note

The forms and bioavailability of phosphorus in integrated vertical flow constructed wetland with earthworms and different substrates

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HIGHLIGHTS

- Addition of earthworms increased the Ex-P, Fe-P, Ca-P, Oc-P, De-P and Org-P content.
- Addition of earthworms into IVFCW increased the bioavailable P content.
- The bioavailable P forms in IVFCW were influenced by type of substrates.

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ABSTRACT

A sequential extraction method was utilized to analyze seven forms of P in an integrated vertical-flow constructed wetland (IVFCW) containing earthworms and different substrates. The aluminum-bound P (Al-P) content was found to be lower, and the occluded P (Oc-P) content was higher in the IVFCW. The addition of earthworms into the influent chamber of IVFCW increased the exchange P (Ex-P), iron-bound P (Fe-P), calcium bound P (Ca-P), Oc-P, detritus-bound (De-P) and organic P (Org-P) content in the influent chamber, and also enhanced P content uptake by wetland plants. A significantly positive correlation between P content of above-ground wetland plants and the Ex-P, Fe-P, Oc-P and Org-P content in the rhizosphere was found ($P < 0.05$), which indicated that the Ex-P, Fe-P, Oc-P and Org-P could be bio-available P. The Ex-P, Fe-P, De-P, Oc-P and Ca-P content of the influent chamber was higher where the substrate contained a mixture of Qing sand and river sand rather than only river sand. Also the IVFCW with earthworms and both Qing sand and river sand had a higher removal efficiency of P, which was related to higher P content uptake by wetland plants and P retained in IVFCW. These findings suggest that addition of earthworms in IVFCW increases the bioavailable P content, resulting in enhanced P content uptake by wetland plants.

1. Introduction

Phosphate occurs in the natural environment in many forms including as ortho-, meta-, pyro-, polyphosphates, and as various phosphorus (P) containing organic compounds; they have been widely used in fertilizers, pesticides, detergents and food additives (Gunduz et al., 2011). Nutrient enrichment of water-bodies through elevated P concentration can lead to eutrophication (Aydin et al., 2010). P can also be found in lake and river sediments in the forms of organic P and inorganic P (Pettersson et al., 1988). The fate of P in sediment ecosystems is influenced by physical, chemical and biological factors, including desorption, dissolution and reduction processes (Zhuang et al., 2014). Tallberg et al. (2008) reported that changes in physical, chemical, and biological conditions can induce release and transformation of organic and inorganic P from river sediments. Therefore, eutrophication may continue or reoccur long after P inputs have ceased due to P remobilization (Søndergaard et al., 1993).

The bio-available P in sediment can be defined as the sum of available P and potential P that can be transformed into an available form by changing physical, chemical and biological processes (Wang et al., 2009). The bioassay is often assumed to give a reliable estimate of the bio-available P, however it is a time consuming method. Therefore, relatively simple chemical extraction methods have been designed to quantify discrete chemical or mineralogical compounds of P in the sediments (Hieltjes and Lijklema, 1980). The

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P in sediments can be classified as different P fractions according to different sequential extraction schemes (Ruttenberg, 1992). The six P forms obtained by sequential extraction methods from Gunduz et al. (2011) and Zhuang et al. (2014) are: (i) exchangeable (Ex-P) or loosely adsorbed P (Ads-P); (ii) aluminum-bound P (Al-P); (iii) iron-bound P (Fe-P); (iv) authigenic apatite plus $CaCO_3$-bound P plus biogenic apatite (Ca-P); (v) detrital apatite plus other inorganic P (De-P) and (vi) organic P (Org-P). Ex-P was the best parameter for the assessment of the bioavailability of P (Tang et al., 2014). Fe-P and Al-P represented P bond to iron and aluminum oxides, and they could support growth of phytoplankton. Zhou et al. (2001) indicated that Fe-P and Al-P could also be used for the evaluation of algal available P. Ca-P is considered to be a major storage fraction of sedimentary P with low mobile potentials (Gomez et al., 1999). Peng et al. (2007) reported that the Ca-P was stable, but that Ca-P can be released from sediment under acid conditions. De-P is mainly derived from magma and metamorphic rocks as well as marine sediments (Ruttenberg, 1992). Org-P could be derived from terrestrial input and biological processes such as the food chain, which may directly affect dissolved P in the primary productivity (Vaalgamaa, 2004).

The different forms and mobility of P are influenced by many factors. For example under low pH conditions Ca-P can be released into the overlying water (Jin et al., 2006) and when dissolved oxygen (DO) is diffused into overlying water, Fe^{2+} may be converted to Fe^{3+}, to form iron oxyhydroxides. The formation of iron oxyhydroxides can cause P absorption, which leads to an increase in the fraction of Fe-P and possibly exchangeable-P (Kraal et al., 2009). Redox potential (Eh) is also an important factor. Iron ions are reduced when Eh is lower, which results in release of P from sediments. Active iron is oxidized and rapidly adsorbs P from the water column when Eh increases, which increases P concentrations in sediments (Smolders et al., 2006). The transformation of amorphous iron oxides into crystalline iron oxides occurs due to penetration of oxygen from the overlying water to the sediments under resuspension, which results in transformation of P from mobile forms to inert forms (Li and Huang, 2010). Particle size is also an important factor in controlling the Ads-P or Ex-P contents in sediments. Sediments with smaller particle size will have stronger adsorption capacity due to greater specific surface area, and the Ads-P contents are higher in fine grained sediments (Zhuang et al., 2014).

Wetlands have often been called "green filters", and between 80% and 90% of the P is retained in the soil or sediment of wetlands (Jiménez-Cárceles and Álvarez-Rogel, 2008). The bio-available P in sediment is influenced by physical and chemical factors such as temperature, pH, water dynamic conditions, bioturbation and the redox characteristics (Chen et al., 2011). Constructed wetlands may remove P in the following ways: by adsorption to substrate; uptake by plant roots or absorption through plant leaves in submerged species; and uptake by microbiota (Vymazal, 2007). Integrated vertical-flow constructed wetlands (IVFCW) are used to purify wastewater in Europe and China because they have the higher hydraulic loading rate and relatively low land area requirements (Perfler et al., 1999; Chang et al., 2012). Due to their important function in matter cycling the addition of earthworms can combine with other methods to purify wastewater. Li et al. (2011) found that constructed wetlands with added earthworms removed 2–5% more N and 12% more P than constructed wetlands without earthworms. However, there is little information on the effect of substrates and earthworms on forms of P and bio-available P, and correlation between forms of P and P uptake by wetland plants in integrated vertical flow constructed wetland. Therefore, the objectives of this study were to (i) determine seven forms of P in IVCW with different substrates by seven sequential extractions; (ii) undertake comparison analysis of P uptake content in IVCW with addition of earthworms and different substrates, and (iii) assess the removal efficiency of P in an IVFCW with the addition of earthworms and different substrates, and its relation with P retained in substrates and uptake by wetland plants.

2. Material and methods

2.1. Materials

River sand and Qing sand were obtained from a local building supply company to be used as two substrates (one a mixture of Qing sand and river sand; the other river sand only). Table 1 summarizes their physical and chemical characteristics. Rice straw, ground to powder, was used as organic matter. Uniform and healthy rhizomes of *Canna indica*, *Iris japonica*, *Acorus calamus* L., *Phragmites australis*, *Zizania caduciflora* and *Typha angustifolia* were used in this study because they are often used in constructed wetlands in order to help purify wastewater. Earthworms (*Eisenia fetida*) were purchased from a local farmers market. *E. fetida* was chosen because it is often used in constructed wetlands (Li et al., 2011).

2.2. Integrated vertical flow constructed wetland setup

The IVFCW with two parallel influent and effluent chambers was designed in Nanjing University of Information Science and Technology. The influent chamber was $60 \times 60 \times 90$ cm (length × width × height), and the effluent chamber was $60 \times 60 \times 60$ cm (length × width × height). They were divided into 3 uniform cells separated by nylon mesh to prevent plant roots from penetrating into adjacent cells (Fig. 1).

Four types IVFCW were designed. (1) The IVFCW without plants (IVFCW I). The 97:3 (v/v) mixture of river sand and organic matter as substrate was added to the influent chamber with 80 cm depth and effluent chamber with 50 cm depth, respectively. (2) The IVFCW with wetland plants (IVFCW II). The same quantity of substrate was added into the influent chamber and effluent chamber of IVFCW, respectively, and then four *C. indica*, four *I. japonica* and five *A. calamus* were planted into influent chamber, and four *P. australis*, five *Z. caduciflora* and four *T. angustifolia* were also planted into effluent chamber. (3) The IVFCW with wetland plants and earthworms (IVFCW III). The same quantity of river sand and plants was added into IVFCW, and then earthworms with density 6 g l^{-1} for the upper layer (30 cm depth) were also added into the influent chamber of the constructed wetland. (4) The IVFCW with a mixture of river sand, Qing sand (V_R:V_Q = 1:1) and organic matter (V_M:V_O = 97:3) as substrate (IVFCW IV). The tested substrate was added into the influent and effluent chambers to maintain the same substrate depth as the previously designed IVFCW. The same quantity of wetland plants and earthworms were also added into IVFCW.

Synthetic domestic wastewater was used in the experiment for minimizing the fluctuation of influent pollutant concentrations. The synthetic wastewater was prepared using: $(NH_4)_2SO_4$, glucose, peptone and KH_2PO_4. The average COD, TN, NH_4-N and TP were 221, 21, 14 and 2.8 mg l^{-1}, respectively. All IVFCW were fed with synthetic wastewater at a rate of 20 L d^{-1} giving a hydraulic retention time of 2 d. Water was sampled from the constructed wetland after 2 d to analyze TP from January to December 2013.

2.3. Measure P fractions

Three uniformly distributed sites were chosen in each cell of the influent and effluent chambers for rhizosphere substrate sampling. Sampling took place in December 2013. The below-ground plants in each sampling site were excavated and substrate released from the roots by hand. Small roots were removed to obtain substrate in