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Phosphate uptake and translocation in a tropical *Canna*-based constructed wetland

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Abstract

Introduction: Considering the problem of eutrophication of the water bodies, phosphate removal from water has emerged as a research of topical interest. The present study aims to investigate the efficacy of *Canna* lily-based constructed wetland to remove phosphate from wastewater. The translocation of phosphate in plant tissue and its biochemical transformation in sediments is also studied to understand its accumulation and recirculation within the system.

Results: The removal of phosphate stabilized at around 50% in the present study and plant uptake was found to be the major removal mechanism. Average removal was 167 mg/m^2 day for total phosphate and 84 mg/m^2 day for available phosphate for an initial loading rate of 200 mg/m^2 day and 85 mg/m^2 day, respectively, at a HRT of 24 h. Most of the phosphate concentrated in above ground tissue of plant and its relative accumulation was maximum in flowers. Fractionation of phosphate in sediments confirmed removal by sediments with an accumulation of apatite phosphate (Ca and Mg bound), but release of non-apatite form (Fe and Al bound).

Conclusion: The study concludes that *Canna*-based constructed wetland can be an effective tool for phosphate removal from wastewater and sediments particularly under tropical conditions. Regular harvesting of above ground tissue of *Canna* can result in nutrient export from the system, whereas autochthonous addition may result in recirculation.

Keywords: Phosphate removal, *Canna lily*, Wetland, Sediments, Wastewater

Introduction

Phosphate is a critical parameter to establish the wholesomeness of water in respect of its quality and treatment strategy. Conventional treatment of wastewater removes a significant fraction of suspended impurities and organic carbon, but the removal of nutrients like nitrogen and phosphorus remains limited. Advanced treatment techniques like chemical precipitation, ion exchange, and reverse osmosis are either energy or cost intensive or result in production of secondary sludge (Joshi and Srivastava 2006; Haritash et al. 2015). In such a case, discharge of partially treated wastewater may result in nutrient excess and eutrophication in water bodies (Yadav et al. 2015). Phosphate being an integral component of food and plant waste, fertilizers, detergents etc. is continuously discharged in wastewater, and it reaches to water bodies through various routes leading to a eutrophic state of water body every 10–15 years (Jeppesen et al. 2005). Measures towards restoration of such eutrophic water bodies include chemical application of lime, alum, and ferric salt and introduction of planktons, macrophytes (Gao et al. 2009), and higher organisms to restore a functional biological cycling of phosphorus (Jeppesen et al. 2007). Application of constructed wetlands (CWs) for stabilization of secondarily treated wastewater (Vymazal 2007), and bioretention systems for stormwater, prior to discharge in a water body, has resulted in substantial removal and mobilization of nutrients and restoration of functional nutrient cycling (Sack 2013; Hseih and Davis 2005). Regular harvesting of fish or plants from an under-restoration water body results in export of nutrients, but on-site death and decomposition may lead to recirculation of nutrients in the same environment. Phosphorus being a key limiting nutrient for algal growth in freshwater regulates the biochemical health of a water body (Padma and Nair 2010). Most of the studies report that total phosphorus concentration may not be adequate to determine the ecological risks associated with it. The retention or release of phosphate and its bioavailability are regulated by the chemical state of phosphorus and environmental conditions (Zhang et al. 2012). The release of bound phosphorus to free soluble

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form, as regulated by pH and redox conditions, is reported in several studies (Kim et al. 2003). Many authors have reported phosphate removal from wastewater by *Phragmites*, *Typha*, *Eichhornia*, etc., but the ultimate treatment/disposal of such plants by composting is difficult. Whereas *Phragmites* and *Typha* are hard-stem plants, *Eichhornia* is an invasive weed and is difficult to harvest. In such a case, *Canna lily*, a plant with soft tissue, is identified as a potential candidate for phosphate removal without compromising the treatment efficiency, aesthetics, and option of its composting. Some studies on *Canna spp*. have reported its ability to remove nitrogen and phosphorus efficiently (Polomski et al. 2007), as a result of its higher evapotranspiration rates against other ornamental plants. The rate of dry weight accumulation was also reported high owing to high rate of nutrient accumulation in plant tissue. Analysis of different plant parts confirmed maximum storage in shoots and roots. Other important factors responsible for nutrient removal are sediments and microorganisms. Gravel-based sediments and plant roots offer suitable substrate for microbial growth. Root exudates in the form of carbohydrates, amino-acids, enzymes, etc. offer oxidizing conditions and support nitrifying conditions during decomposition of organic matter (Gersberg et al. 1986). The chemistry of sediments too compliments the removal of nutrients, especially phosphate. Fragmented limestone waste used as packing material in a phragmites-based CW demonstrated removal efficiency of around 62% for phosphate (Mateus et al. 2012). Calcium and magnesium present in sediments can bind to phosphate to immobilize it temporarily under alkaline conditions, whereas iron and aluminium can dominantly remove phosphate at neutral pH (VanBeusekom and DeJonge 1997). Thus, pH plays an important role in regulating availability of phosphate in an aquatic system. Reducing/acidic conditions may favour resolubilization of phosphate, making a water body prone to eutrophication. Therefore, it is important to monitor the total load, and fractionation of phosphate in a water body to comment upon its trophic status, and to determine the actual efficiency of plants towards removal of phosphate. The present study, therefore, aimed to study the removal of phosphate by plants and sediments, to characterize the different fractions of phosphate in sediments and water, and to quantify the distribution of phosphate in different plant tissues during phytoremediation.

Methods

The study was carried out in a pilot-scale vertical-flow constructed wetland cell constructed in the campus of Delhi Technological University during April–May 2013 (summer season). The temperature profile during the period was monitored along with other meteorological conditions (Table 1) to relate the uptake of nutrients to prevalent weather conditions. The CW cell was packed with a 35-cm-thick layer of sand-gravel substrate. The cell had dimensions of length 1.1 m, width 0.8 m, and depth 0.45 m and rectangular tank made up of brick and masonry. The influent was fed from the top, and effluent was collected from an outlet placed opposite to the inlet at the bottom of the tank (Fig. 1). The supporting matrix consisted of sand- and gravel-packed bed with a height of 0.35 m corresponding to a total volume of 0.325 m^3. The bulk density of the packing medium was 1.82 g/cm^3, and d_{10}, d_{30}, and d_{60} computed from particle size distribution were 0.42, 2.36, and 9.0 mm, respectively. The supporting matrix was classified as well-graded medium with a value of 21.43 for coefficient of uniformity (C_u) and 1.47 for coefficient of curvature (C_c). The void ratio was determined as 0.77 with porosity of 0.44. The classification of packing medium represented conditions suitable for easy percolation of water and penetration of plant roots. The CW cell was vegetated with 90 (stem count) healthy plants of *Canna* lily with an average shoot length of 43.2 cm. The initial concentration of phosphate in different tissues of plant (roots, stem, leaves, and flowers) was determined to compare it with the final plant-tissue phosphate level to confirm uptake by plants and subsequent accumulation as a process of removal. The CW cell was initially flushed with water at a hydraulic loading rate (HLR) of 400 L/day for 5 days, and later, the plants were irrigated, on daily basis, with HLR of 30 L/day of simulated wastewater. Synthetic wastewater was prepared by dissolving 21.74 mg of potassium-dihydrogen-orthophosphate in groundwater to obtain a concentration of 5.0 mg/L of PO_4^{3-}-P. The *Canna*-based CW cell was fed with this synthetic wastewater on daily basis (for 30 days) maintaining the hydraulic retention time (HRT) of 24 h on plug-flow basis. The concentration of available (AP) and total phosphate (TP) was determined regularly (for 30 days) in the influent and effluent using standard methods (APHA) (Eaton et al. 1995). Phosphate present in dissolved form was determined as available phosphate, and the sample was acid-digested for extraction of TP. Different fractions of phosphate, i.e. apatite phosphate (bound to Ca and Mg), non-apatite inorganic phosphate (NAIP bound to Fe, Al, and Mn), organic phosphate (OP-bound to biomass), were determined in the sediments, in triplicate ($n = 3$), before initiating the experiments and after the completion using standard prescribed procedure (Williams et al. 1980). The total phosphate accumulated in different plant tissues was also determined initially and finally in five ($n = 5$) samples of each tissue. All the experiments were carried out using analytical (AR) grade chemicals and double glass distilled water in triplicates. The glassware and plasticware used in the experiments were soaked in 0.3% HCl and rinsed with deionized water prior to use. The meteorological

Table 1 Concentration of total and available phosphate and its removal rate in *Canna*-based constructed wetland

Day	Total phosphate (TP) (mg/l)		Removal rate (mg/m^2 day)	Available phosphate (AP) (mg/l)		Removal rate (mg/m^2 day)
	Influent	Effluent		Influent	Effluent	
1	8.43	0.92	252.62	2.12	0.92	76.16
2	5.28	1.19	158.04	0.97	0.28	34.89
3	4.67	0.66	139.90	2.02	0.28	72.74
4	4.56	0.82	136.55	2.12	0.30	76.34
5	6.64	0.41	199.08	2.03	0.31	73.10
6	6.33	0.99	189.60	2.00	0.46	71.97
7	6.17	1.01	184.80	3.26	0.36	117.43
8	6.66	1.24	199.43	4.09	0.77	147.23
9	6.30	1.32	188.60	4.51	0.95	162.32
10	7.50	1.83	224.45	4.64	1.37	166.88
11	6.00	2.52	179.24	4.15	1.72	149.10
12	4.81	2.48	143.55	2.14	1.03	76.84
13	6.21	3.27	185.32	2.26	1.08	81.16
14	4.31	1.99	128.70	2.23	1.27	80.02
15	7.07	2.12	211.46	1.75	1.05	62.78
16	5.84	1.59	174.72	1.76	0.97	63.16
17	7.40	2.62	221.21	1.72	0.74	61.79
18	5.84	2.49	174.45	1.20	0.73	43.04
19	5.21	1.39	155.88	2.00	1.00	71.81
20	6.89	2.12	206.06	2.04	1.12	73.21
21	4.51	2.09	134.67	2.10	1.10	75.38
22	5.32	2.72	158.78	2.01	1.15	72.12
23	4.68	1.42	139.97	2.21	1.22	79.31
24	3.91	1.88	116.74	1.71	1.04	61.34
25	3.12	1.05	93.28	1.47	0.83	52.75
26	5.01	2.23	149.63	2.09	1.20	74.99
27	4.11	1.70	122.79	2.05	1.18	73.55
28	3.87	1.80	115.56	2.01	1.15	72.12
29	5.33	2.56	159.13	3.59	1.80	128.89
Mean	5.59	1.74	167.04	2.35	0.94	84.57
SD (±)	1.26	0.71	37.65	0.95	0.40	34.19

observations during the study were obtained from the weather station of Delhi Pollution Control Committee (DPCC). Statistical analysis of the obtained results was done over MS-Excel software to calculate mean, standard deviation, removal rate, etc.; to plot the normalized curve for removal efficiency; and to plot the graphs.

Results and discussion

Based on the values of ambient temperature and solar insolation during the study, it was observed that the study area represents tropical to sub-tropical weather conditions which favour optimum activity and growth of vegetation. Average ambient temperature was 30 °C ranging from 24.5 °C (minimum) to 40.2 °C (maximum) during the study. Such conditions promote good rate of uptake of nutrients and hence maximum efficiency towards removal. In order to highlight the role of vegetation and sediments in removal of phosphate from wastewater, this paper discusses the uptake, transformation, and translocation of different chemical forms of phosphate in detail.

Phosphate removal from wastewater

Significant removal of phosphate from wastewater in a *Canna*-based constructed wetland was observed in the

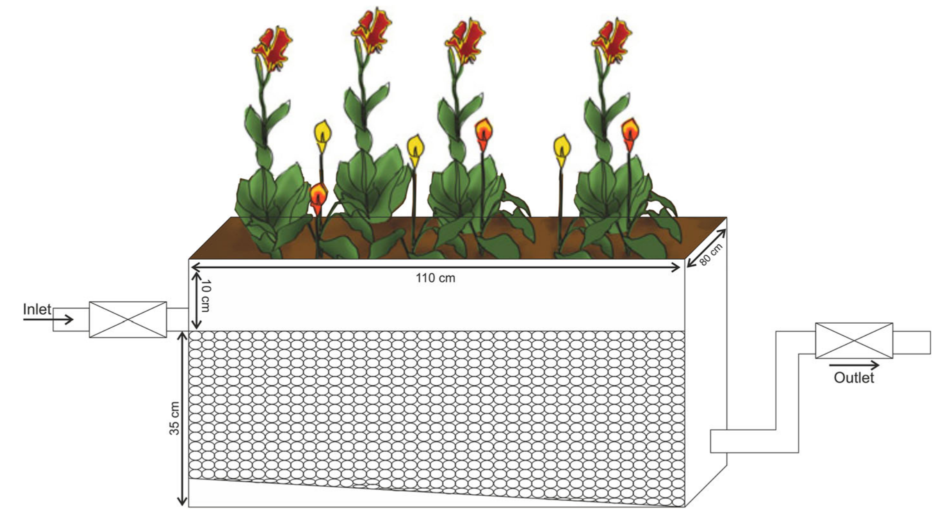

Fig. 1 Schematic description of configuration of constructed wetland cell used in the study

present study. The percent removal of TP was slightly higher than the removal of available phosphate (AP) (Table 1). Average removal of TP was around 68% as against 57% for AP. Percent TP removal was initially higher and went decreasing to stabilize at around 55% in the later stage (after 20 days) (Fig. 2a). On the other hand, the percent removal of AP ranged from 39 to 89% with an average value of 57%. It was low for first 2 days but increased to about 86% on the third day and fluctuated around the same value for 10 days. Later, it decreased and stabilized around 45% by the 20th day (Fig. 2b). Chemical transformation of bound phosphate to orthophosphate (AP) by microorganisms and enzymes secreted by plant roots results in higher removal efficiency for TP. Percent removal of P has been reported in the similar range (24.1–61.55) in another study on nutrient removal from wastewater by 15 wetland species including *Canna* (Jiang et al. 2011). In the present study, average removal rate of TP (167 mg/m^2 day) was higher than that of AP (84 mg/m^2 day) as against the loading rate of 200 mg/m^2 day for TP and 85 mg/m^2 day for AP. In a similar study on treating dairy industry wastewater using a *Canna*-based CW (DeBusk et al. 1995), removal rate of 173 mg/m^2 day has been reported. The present

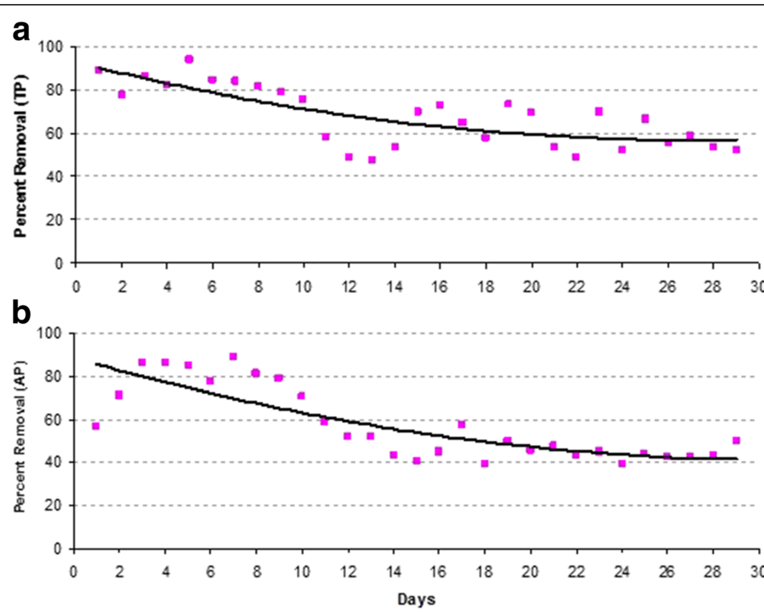

Fig. 2 Percent removal (curve represents normalized removal) of total phosphate (**a**), and available phosphate (**b**) from wastewater in Canna-based constructed wetland

study reports slightly higher removal rates owing to the climatic differences and chemical composition of wastewater in the study. The major mechanisms for phosphate removal are reported as uptake by plants and adsorption over supporting matrix in the study and its efficiency is dependent on plant-uptake rate and sediment binding. In the present study, initially higher percent removal of phosphate could be attributed to higher activity of plants and sediments in the initial phase as reported in other studies too (Haritash et al. 2015). Later, the decrease in removal efficiency is observed in wetlands and bioretention systems with saturation of sediments (Kadlec and Knight 1996).

Phosphate in plant tissue

Since removal of phosphate depends on plant uptake, it is important to study the accumulation of phosphate in various plant tissues. It was determined in various parts (roots, shoots, stem, and flowers) of *Canna* to ascertain its point of accumulation and investigate the possibility of recirculation. Maximum increase in phosphate concentration was observed in leaves, followed by flowers, roots, and stem respectively. The tissue phosphate concentration increased to about 1.7, 1.2, 22, and 4.2 times that of initial phosphate concentration in roots, shoots, leaves, and flowers, respectively (Fig. 3). It confirms that phosphate is taken up by the plants and is accumulated throughout the tissue via metabolism. Similar results have been reported by Polomski et al. (2007) in a study to remove nutrients from nursery run-off in a lab-scale gravel-based system employing seven garden plants individually. The study reported 40% more accumulation of P in shoots than roots at an influent level of 6.77 mg/L phosphate. In a similar study (Jiang et al. 2011), 15 wetland plants were observed to accumulate higher concentration of phosphate in shoots than roots with maximum accumulation by *Canna* among the plants. If relative fraction of initial and final phosphorus in different tissues is compared, flowers accumulated relatively more concentration, and it would result in recirculation of phosphate in the form of organic phosphate (OP) with the fall of flowers into the wetland system. Observations similar to the fact have been reported by Menon and Holland (2014) in a vegetated constructed wetland. In the present study, the higher concentration and overall accumulation of phosphate is observed in stem, leaves, and flowers (above ground tissue), and its recirculation via leaf fall, plant death, and degradation can be avoided by harvesting the above ground tissue at regular intervals, thus facilitating nutrient export from the wetland. Since the nutrients taken up by the plant take part in growth and metabolism, it is important to monitor the physical growth of wetland plants. In the present study, the root length, shoot length, and plant density were found to increase by a factor of 3.69, 3.67, and 2.04, respectively. The shoot-root ratio remained almost the same throughout the study indicating that there was no significant effect over the plant health. It stresses on application of *Canna*-based wetland systems for the removal of nutrients from wastewater.

Phosphate in sediments

Since adsorption of phosphate by sediments is a major removal pathway, its removal and fractionation was studied in the supporting matrix before initiating the experiments and after the completion of study. An increase in the concentration of total phosphate (TP) was observed from 5.14 to 5.88 mg/g confirming to its adsorption by sediments (Fig. 4). Since the root system of *Canna* is strong and extensive, it removes phosphate from sediments too. As a result, the increase of phosphorus in sediments is not as significant. This strengthens the idea

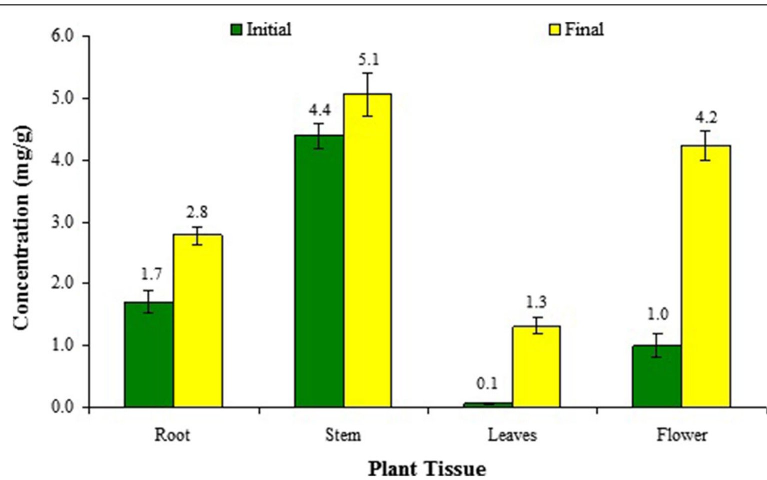

Fig. 3 Average concentration of phosphorus (mgP/g) in different plant tissues of Canna before and after the treatment (*n*=5)

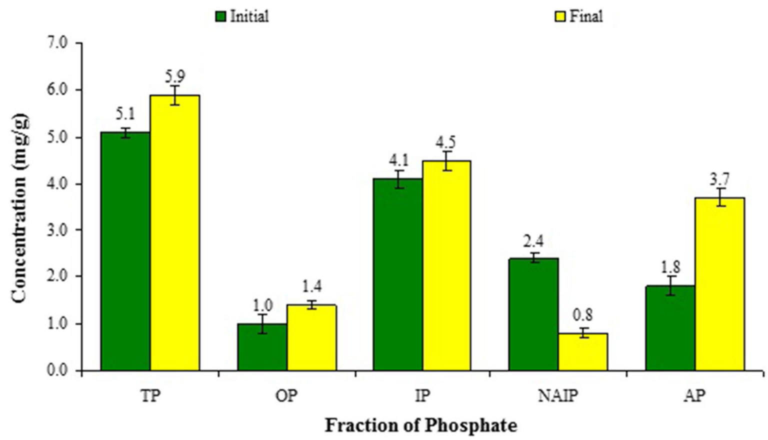

Fig. 4 Average concentration (mg/g) of different fractions of phosphate in sediments before and after the treatment (*n*=3)

that the removal of P in *Canna*-based wetland is predominantly by plant uptake, and sediments too play a role in it. Apart from it, the chemical composition and mineral profile of sediments may also be the reason for relatively less adsorption over sediments. The fraction of organic phosphate (OP) was observed to increase from 1.0 to 1.37 mg/g indicating to the role of microorganisms and organic matter in phosphate removal via biomass accumulation. Some addition of biomass as a result of leaf fall or dead tissue of old roots may also be the reason for an increase of OP in sediments. The concentration of inorganic phosphate (IP) too increased in sediments indicating chemical binding/precipitation of phosphate with its binding to Ca and Mg (AP) or Fe, Al, and Mn (NAIP). The Ca and Mg bound phosphate (AP) increased during the study indicating the binding of phosphate to Ca and Mg present in sediments. Similar observations have been presented by Gu and Dreschel (2008) in a constructed wetland in Florida. The NAIP concentration decreased during the study from 2.37 to 0.76 mg/g. It may be attributed to release of phosphate bound to Fe, Al, and Mn in sediments during higher oxygen demand of wetland for respiration. During high oxygen demand, ferric bound phosphate is released to water with reduction of Fe^{3+} to Fe^{2+} because iron is an alternate terminal electron acceptor during deficiency of dissolved oxygen (Kortmann and Rich 1994). Studies have confirmed that AP will be trapped in sediments for a longer duration and is regarded as non-available. It will not be released to water easily. On the other hand, NAIP can be released to water column easily particularly during summers depending on the redox conditions present (Zhang et al. 2012). Apart from it, the phosphorus concentration in sediments in rooted macrophyte-based system decreases since the plants use sediment too as source of phosphorus (Carignan and Kalff 1980). It further strengthens the objective of using a *Canna*-based wetland since it not only removes phosphate from wastewater but also removes a fraction of NAIP. Based on the results obtained, it was observed that major plant uptake of phosphate is from wastewater and sediment NAIP during oxygen-stressed conditions.

Conclusions

Based on the observations of the present study, *Canna lily*-based constructed wetland is found to be an effective treatment option for phosphate from wastewater. The removal of sediment bound phosphate is also facilitated by *Canna*, but the available phosphate accumulates in sediments which may be released in hypolimnion if pH is acidic or conditions are reducing. Apart from it, though phosphate is translocated to all the tissues, its relative accumulation is observed to be more in flowers which may cause recirculation. Most of the fraction of removed phosphate is accumulated in above ground tissue, and hence, regular harvesting of aerial tissue can result in nutrient export. Considering the nutrient accumulation of harvested tissue, it may be used as raw material for composting if ultimate disposal is aimed at. Within the sediments, AP is accumulated whereas NAIP is released. It confirms easy release of Fe and Al bound phosphate to water though the redox conditions play a crucial role in it. The study concludes that *Canna lily* removes phosphorus not only from wastewater but also from sediments. Hence, *Canna lily*-based wetlands are effective in phosphate removal from wastewater especially under tropical conditions.

Authors' contributions

AKH designed the study, participated in the experimental work, and drafted the manuscript. SD carried out the analysis of phosphate in wastewater and plant. AS did the analysis of sediments and helped prepare the wetland cell for the study. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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