Enhanced removal of nutrients and heavy metals from domestic-industrial wastewater in an academic campus of Hanoi using modified hybrid constructed wetlands

Mai Huong, Dan-Tam Costa and Bui Van Hoi

ABSTRACT

Vietnam, like many developing countries, is facing serious water quality issues due to discharging wastewaters without treatment or with improper treatment, which can constitute a potential risk for aquatic ecosystems, food safety and human health. Hybrid constructed wetlands with four substrate layers (HCW) and modified hybrid constructed wetland (MHCW-1 and MHCW-2) with seven substrate layers were designed to evaluate the enhanced treatment capacity for wastewaters. To this end, we carried out an outdoor experiment at the Vietnam Academy of Science and Technology, Vietnam to treat its wastewaters from April to August 2019. All constructed wetland units were planted with reed *Phragmites australis* and cyperus *Cyperus alternifolius;* and specifically wetland MHCW-2 was cultured with earthworm *Perionys excavates.* Results indicated that MHCW-1 and MHCW-2 with seven substrate layers had higher removal efficiencies of NO_3^--N, TKN and TP than HCW system. More substrate layers in MHCW-1 and MHCW-2 also resulted in increase of Cu and Pb removal efficiencies, with 73.5%, 79.4%, 71.5% and 67.8%, respectively. Particularly, earthworm addition in MHCW-2 was more efficient in decreasing the concentrations of biochemical oxygen demand (BOD_5), with removal efficiency over 70%.

Key words | earthworms, heavy metals, nutrients, organics, plants, removal efficiency

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HIGHLIGHTS

- Enhanced wastewater quality of academic campus before discharging into the environment.
- More substrate layers increased the removal efficiencies of NO_3^-–N, TKN and TP.
- BOD_5 was effectively removed by modified hybrid constructed wetland which has earthworm addition.
- Cu and Pb were effectively removed in the constructed wetlands which have more substrate layers.

INTRODUCTION

Vietnam, like many developing countries, is facing serious water quality issues due to discharging wastewaters of industrial, agricultural and municipal activities, which can constitute a potential risk for aquatic ecosystems, food safety and human health. The treatment technologies such as bioreactors, membrane filtration, nanotechnology and biodegradable material have been developed for the removal of nutrients, organic pollutants and heavy metals (Singh & Thomas 2012; Bavandpour *et al.* 2018). However, due to the relatively high construction and operation cost for treating

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complex wastewaters, developing an effective and cost-efficient system for the removal of nutrients and heavy metals remains a critical issue. Particularly, mixing industrial and domestic wastewater that contains both nutrients and heavy metals increases the complexity of wastewaters because of their interactions and variability of the physio-chemical forms. Therefore, there is an urgent need to develop and implement efficient and cheap treatments to depurate the increasing amounts of wastewaters released in the environment. The wastewaters from Vietnam Academy of Science and Technology (VAST) are known to mix releases from laboratories and from domestic activities. Although they have been pretreated using biological methods combined with physical–chemical treatment, the concentrations of some nutrients and heavy metals are still high and do not meet discharge requirements (QCVN 14:2008/BTNMT; QCVN 40:2011/BTNMT). Therefore, the need to enhance drained water treatment efficiency has become an important issue.

Constructed wetlands (CWs) are wastewater treatment systems designed to utilize the natural processes and interaction of wetland plants, bacteria and substrates in a controlled environment (Vymazal 2013). In terms of the complexity of the processes occurring, constructed wetlands create a more diverse environment compared to conventional treatment technologies (Vymazal 2013). Thus, constructed wetlands are now attracting more attention and are considered promising for being an environmentally-friendly method with low investment and operating cost for treating wastewater (Wang *et al.* 2017). Over the past few decades, CWs have proved to be functional in treating domestic municipal wastewaters (Kivaisi 2001), agricultural effluents (Wood *et al.* 2007), landfill leaches (Justin & Zupančič 2009), and industrial sewage waters (Guittonny-Philippe *et al.* 2015). In the wetlands, the processes of eliminating nutrients and heavy metals from domestic–industrial wastewaters are determined by interacting processes, which include retention in substrates, plant uptake and microbial metabolism (Bilgin *et al.* 2014).

The selection of plants is an important matter regarding wetlands. Selected plants must be tolerant to toxicity and changes in the entering wastewater characteristics. *Phragmites australis* and *Cyperus alternifolius* are freshwater wetland plants, which have been widely used in CWs (Ebrahimi *et al.* 2013; Rezania *et al.* 2019). The heavy metals and nutrient removal efficiencies by CW vegetated with *P. australis* and *C. alternifolius* were studied and discussed. According to Rezania *et al.* (2019), *P. australis* is a great plant for phytoremediation of contaminants due to its ability to accumulate the different types of heavy metals, while the plant *C. alternifolius* had high efficiency in the removal of nutrients and organic matter of wastewater by its rhizomes through the growing season (Ebrahimi *et al.* 2013; Bilgin *et al.* 2014).

Earthworms are widely used to treat wastewater in CW systems due to their functions in decomposing of biodegradable materials, as well as increasing enzyme activity and nitrification potential (Xu *et al.* 2015). *Perionyx excavatus* is an epigeic earthworm found commonly over a large tropical area and stays active in the surface layers. *P. excavatus* was well known for breaking down and processing organic wastes (Biruntha *et al.* 2013). Earthworms can grind the silt and sand particles, increasing the total specific surface area, which enhances the ability to 'adsorb' the organics and inorganics from the wastewater (Suthar 2007).

According to the hydraulic conductivity and flow paths, various CW designs were developed using different materials as substrate for CWs. Traditionally, wetlands had been constructed with local soils as substrate. However, there was a problem with overland flow and short-circulating of the wastewater between inlet and outlet because of the low hydraulic conductivity of soils. To overcome this disadvantage, the selection of materials with high removal capacity of contaminants plays a crucial role in CW design. The materials used as substrate in CWs should have good hydraulic characteristics and high pollutant removal capacity, and be cheap and locally available, such as sand, gravel, rice straw and wood chips (Wu *et al.* 2015; Xu *et al.* 2015)

The present study aimed to examine the effectiveness of different types of substrate arrangement in hybrid constructed wetlands (HCWs) to treat the wastewater of an academic campus using plants (*P. australis* and *C. alternifolius*) with and without earthworm *P. excavatus*. Thus, one classic hybrid CW with four layers of substrates and two modified hybrid constructed wetlands with seven layers of substrates were designed to confirm the scientific hypothesis that: (1) when wastewater passes through more layers of materials/substrates, particles, pollutants and bacteria in the wastewater can be trapped on the surface or between the gaps of the materials/substrates; (2) more layers can increase the diversity in filtering that may lead to improved efficiency of the treatment. In the modified hybrid CW with earthworm addition, it was expected that it would improve the pollutant removal, because earthworms can contribute as a biological process (bioturbation) in combination with the physical process of wetland to remove pollutants. The finding could provide appropriate design of HCWs for wastewater treatment and significance of substrate arrangement for optimization of CWs.

MATERIALS AND METHODS

Hybrid constructed wetlands and operation

One HCW and two modified hybrid constructed wetlands (MHCW) were built on campus, Vietnam Academy of Science and Technology, Hanoi, Vietnam. Table 1 described the characteristics of HCWs. Those constructed wetlands were exposed to natural conditions and were made of concrete. A waterproof cement (SIKA, Switzerland) has been applied in two layers to ensure waterproofness for all HCWs. The outside of walls is covered with regular cement. Each pilot dimension was 2.0 × 2.0 × 1.5 m (length × width × height) in total. Total height of the filter was 150 cm with 60 cm saturated with water and 120 cm of filtering materials in total. As a filter media, woodchip, gravel and quartz sand (filtration sand) were selected for all CW systems. Figure 1 provides a schematic diagram of wetland dimensions and operational arrangement.

An outlet made from a 90 cm PVC pipe was placed in the middle of each tank to connect the outlet. Ventilation pipes had then been set up to increase the circulation of air and oxygen in the pilots. About 90 mm drains were implemented on three sides of each tank after filling each tank with 60 cm of filtering material. A ventilation pipe went up and has been cut to be at least 15 cm above the last layer (15 cm above woodchip). They were covered with a PVC T pipe to avoid falling of materials inside the ventilation pipe. Overflow spots were created with a PVC pipe of 20 cm diameter. It has been cut to be at least 10 cm above the last layer (10 cm above woodchip). Partition walls have been installed by cutting and using a magnesium oxide panel. Dimensions of partition walls were 1.95 × 0.65 m.

Substrate characteristics and arrangement

Pilot 1 (HCW), which was a classical design of hybrid constructed wetland, was designed with four layers of substrates (Figure 2). The surface layer of the pilot HCW consisted of a 5-cm-thick woodchip, which was used as the mulch for the plants in the wetlands. The following was 40 cm of filtration sand (gain diameter of **Ø** 2/4 mm). The lower horizontal layer of bed was 20 cm thick and consists of medium gravel with a granulation range of **Ø** 6–10 mm. Underneath, there was a layer of a 60-cm-thick coarse gravel (gain diameter ranging from **Ø** 20 to 40 mm).

Pilots 2 and 3, which were modified HCWs (MHCW-1 and MHCW-2), were made with seven layers and consisted of imitating a riverbank matrix. MHCW-1 and MHCW-2 pilots were modified regarding the thickness and arrangement of layers from HCW pilot (Figure 3). The thickness of materials was different from that of the HCW; it was designed with seven layers for each pilot. The first layer was a 5-cm-thick woodchip. The second and fifth layers consisted of 20 cm of filtration sand (granulation range of **Ø** 2–4 mm). The third and sixth layers were 20 cm of medium gravel (gain diameter of **Ø** 6/10). The first and seventh layers were coarse gravel (gain diameter of **Ø** 20–40 mm) and those layers was filled into the filters 2 and 3 with a thickness of 20 cm.

Both HCW and MHCWs were planted with two plants. Half of wetland surface was planted with reed *P. australis* and half with cyperus *C. alternifolius* with 5 plants per m^2

Table 1 | Characteristics of hybrid constructed wetlands

Parameters	**HCW**	**MHCW-1**	**MHCW-2**
Length × width × height	2.0 × 2.0 × 1.5 m	2.0 × 2.0 × 1.5 m	2.0 × 2.0 × 1.5 m
Surface area	4 m^2	4 m^2	4 m^2
Number of substrate layers	4	7	7
Plant species (5 plants/m^2 for each plant species)	*Phragmites australis* and *Cyperus alternifolius*	*Phragmites australis* and *Cyperus alternifolius*	*Phragmites australis* and *Cyperus alternifolius*
Earthworm species (5 kg m^{-3})	–	–	*Perionys excavatus*
Depth of filter	1.2 m	1.2 m	1.2 m
Saturated with water	0.6 m	0.6 m	0.6 m
Average flow	0.1 m^3 day^{-1}	0.1 m^3 day^{-1}	0.1 m^3 day^{-1}
HLR (hydraulic loading rate)	0.17 m day^{-1}	0.17 m day^{-1}	0.17 m day^{-1}

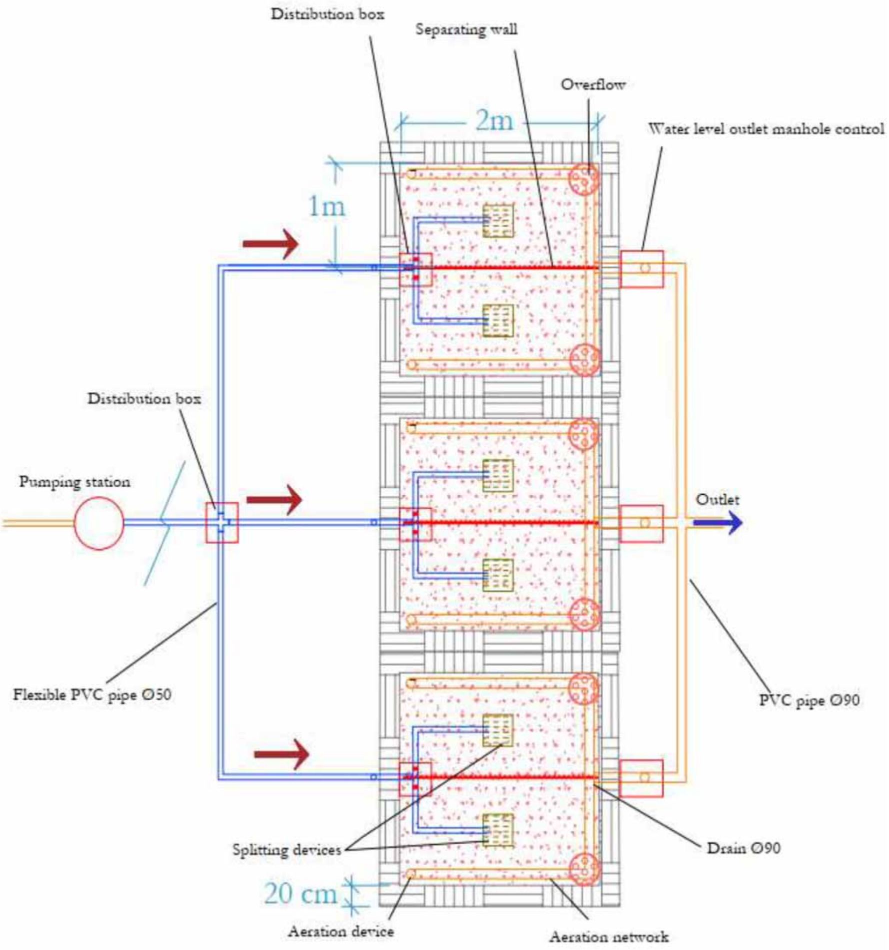

Figure 1 | General scheme of hybrid constructed wetland system.

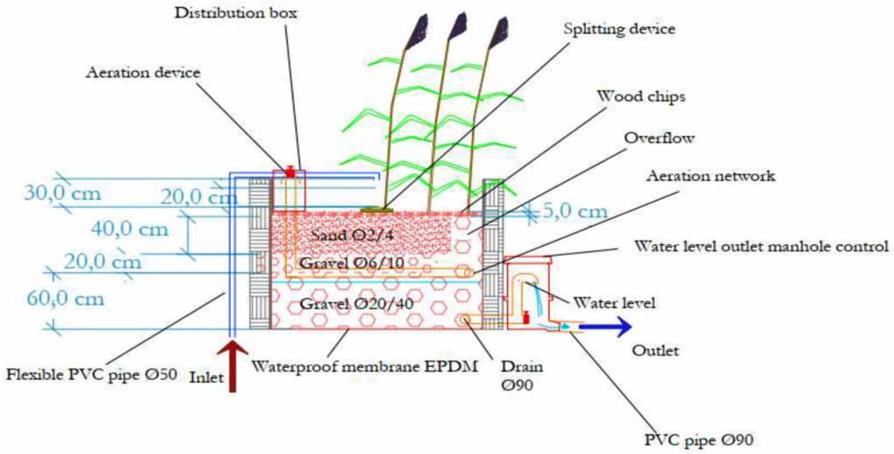

Figure 2 | Operational arrangement of the experimental hybrid wetland system.

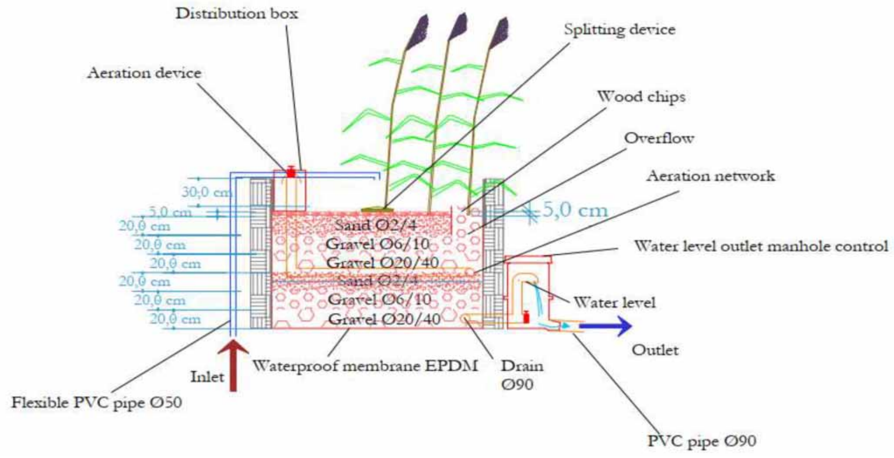

Figure 3 | Operational arrangement of the experimental modified hybrid wetland system (MHCW-1 and MHCW-2).

for each plant species. Earthworm *P. excavatus* was added into the filter of MHCW-2 with a density of 5 kg m^{-3}.

Wastewater sources and maintenance of constructed wetlands

The wastewater was collected from the campus of VAST. Wastewater from baths, showers, wash basins, and research institutes was collected separately and drained into a common settling tank. The acclimation period lasted 90 days (from January to March 2019) for plants (*P. australis* and *C. alternifolius*) and earthworm *P. excavatus* (juvenile stage) prior to experimentation. After acclimation, the experimental period lasted 153 days (from April to August 2019). Wetlands during the acclimation and experimental periods were fed with wastewater collected from research buildings of VAST. Wastewater was pumped from the adjacent stabilization tank to filters every day. The wastewater was supplied to the HCW with a frequency of six cycles per day (feeding every 4 hours) and with the capacity of 100 liters per filter per cycle. Hydraulic residence time was 7 days in each wetland to optimize the removal efficiency of pollutants by physical and biological processes. The growth status of plants, such as sprouting, survival and wilting, was recorded during the acclimation and experimental periods.

Sampling and analysis

During this period, wastewater samples were taken biweekly from the inlet pipe (INLET) and outlet of each filter (HCW, MHCW-1 and MHCW-2). Forty water samples of INLET, HCW and MHCWs were collected and tested in this experiment. The pH was measured and recorded using a multiparameter instrument (Hanna Hi-98,194). The samples were refrigerated at 4 °C in labelled polystyrene bottles for chemical analysis.

The water samples were further used for the physio-chemical and biological parameters as per standard methods for the examination of water and wastewater (APHA 2012). Chemical oxygen demand (COD) was measured using a closed reflux chromate titrimetric method. Biochemical oxygen demand (BOD_5) was measured using the 5-day incubation method. Total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), phosphate (PO_4^{3-}-P) and total phosphate (TP) were analyzed using a spectrometer (UV Spectrophotometer, UV-1800). Total suspended solid (TSS) was determined by the standard paper filtration and direct gravimetric method.

For chemical analysis of Cu, Cr and Pb, a 50-mL water sample was immediately acidified to a final concentration of 5% nitric acid (nitric acid $\geq$65%, Sigma-Aldrich). Samples were then analyzed by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS, Thermofisher Scientific). The standard solutions were prepared from a multielement calibration solution (Astasol-Mix M010, Analytika, Czech Republic). The samples were diluted in a 3% final nitric acid solution made from nitric acid 65%, TraceMetal™ Grade – Fisher Chemical, with 1:3 (v/v) for ICP-MS analysis. Quantification limits were 0.5 μg L^{-1} for Cu, 1.0 μg L^{-1} for Cr and 0.1 μg L^{-1} for Pb.

The removal efficiency of each parameter was calculated using Equation (1) as below:

$$\text{Removal efficiency (\%)} = \frac{Cin - Cout}{Cin} * 100 \qquad (1)$$

where: Cin = influent concentration (mg L^{-1}); $Cout$ = effluent concentration (mg L^{-1})

The removal loading rate was determined using the expression given in Equation (2):

$$\text{Removal loading rate} = q\ (Cin - Cout) \quad (2)$$

where: Removal loading rate (mg m^{-2} day^{-1}); q = hydraulic loading rate (m day^{-1}).

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8.3.0. An analysis of variance (ANOVA) test (Tukey's test) was used to determine statistical differences of the experimental results among treatments at 95% confidence level. All data was expressed as Mean ± S.E (standard error).

RESULTS AND DISCUSSION

Nutrient removal efficiency

The results of the HCW and MHCW systems after 5 months of operation showed that the nutrient removal efficiency and removal loading rate differed depending on the modified HCWs (Figures 4 and 5). The concentrations of NO_3^--N, TKN, PO_4^{3-}-P and TP of HCW and MHCWs were significantly lower compared with the INLET samples (Table 2).

Throughout the monitoring period, NH_4^+-N concentrations in HCW and MHCWs were lower than those in INLET samples (Table 2). NH_4^+-N removal reached efficiencies above 66% (1.99–2.05 mg NH_4^+-N m^{-2} day^{-1}) in all CWs and has shown no significant difference between HCW and MHCWs ($p > 0.05$) (Figure 4). This result was probably due to the uptake of NH_4^+-N by the plants *australis* and *C. alternifolius* in HCW and MHCWs (Saeed *et al.* 2018).

Although a significant reduction in the NO_3^--N concentration was found between inlet and outlets of HCW and MHCWs, removals of NO_3^-- N were low, only 34.2% for HCW, 32.5% for MHCW-1 and 41.8% for MHCW-2. This result showed that the removal efficiency of NO_3^--N in MHCW-2 reached 41.8% (with removal loading rate of 2.48 mg m^{-2} day^{-1}) and was significantly higher than those in HCW and MHCW-1 ($p < 0.05$) (Figure 4). This is consistent with stated results in the literature in that mostly NH_4^+-N and partly organic nitrogen are converted to NO_3^--N in aerobic conditions (Vymazal 2013; Vymazal & Kröpfelová 2015). Processes of ammonification and nitrification-denitrification also aid in the removals of NH_4^+-N and NO_3^--N (Cooper 1996). However, it is difficult to maintain the optimal conditions for the complex processes of nitrification and denitrification in HCW.

The highest removal of TKN was achieved in MHCW-2 (41.3%), followed by MHCW-1 (34.2%), and lowest in HCW (24.9%). The removal loading rate of TKN in HCW was 1.77 mg m^{-2} day^{-1}, which was significantly lower than those in MHCW-1 (2.1 mg m^{-2} day^{-1}) ($p < 0.05$) and in MHCW-2 (2.85 mg m^{-2} day^{-1}) ($p < 0.05$). Comparatively, three systems showed low removal of TKN as compared with those in the literature (Nguyen *et al.* 2017; Haydar *et al.* 2020). The low removal in the TKN could possibly be due to no further uptake of TKN by the plants. Furthermore, the presence of organic matters (such as rice husk, coco peat, wood mulch, and biochar) is also proportional to the TN removal due to media oriented internal C generation (Saeed & Sun 2011; Saeed *et al.* 2012; Tee *et al.* 2012; Zhou *et al.* 2017). However, both HCW and MHCWs had lower amounts of organic matters, having only 5 cm of woodchip

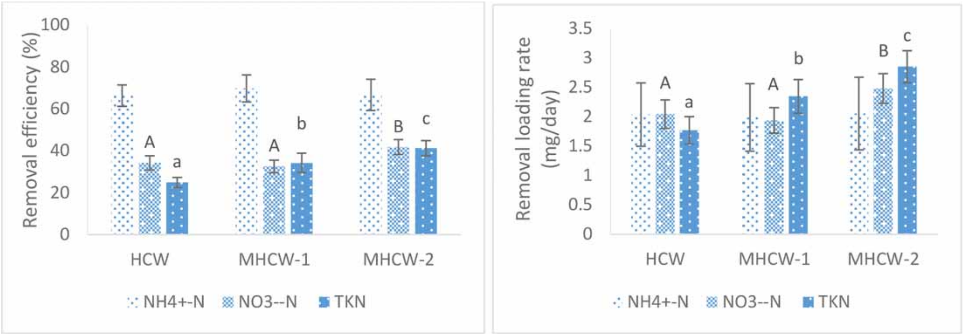

Figure 4 | Removal efficiency (%) and removal loading rate (mg m^{-2} day^{-1}) of NH_4^+-N, NO_3^--N and total Kjeldahl nitrogen through different modified constructed wetlands. *Different letters indicate statistically significant differences among HCW and MHCWs (Tukey test, $p < 0.05$).*

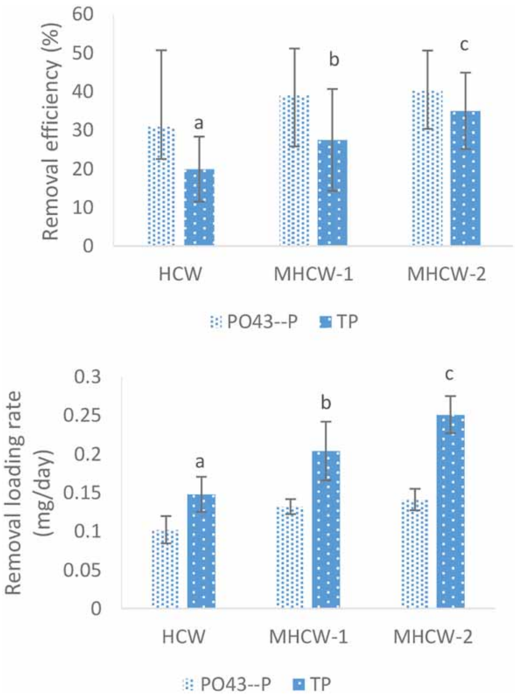

Figure 5 | Removal efficiency (%) and removal loading rate (mg m^{-2} day^{-1}) of PO_4^{3-}-P and total phosphorus through different modified constructed wetlands. *Different letters indicate statistically significant differences among HCW and MHCWs (Tukey test, $p < 0.05$).*

layer in the surface layer of the filters. Thus, low concentration of organic carbon could negatively affect the removal of TKN (Nguyen *et al.* 2017).

All data taken into account, the concentrations of PO_4^{3-}-P and TP treated by HCW and MHCWs significantly decreased ($p < 0.05$) (Table 2). Although the PO_4^{3-}-P removal rates in this study showed no significant differences between MHCW-1 and MHCW-2, the removal rate of TP in MHCW-2 (35%; 0.25 mg m^{-2} day^{-1}) was considerably higher than those in MHCW-1 (27.5%; 0.2 mg m^{-2} day^{-1}) and HCW (19.9%; 0.15 mg m^{-2} day^{-1}) ($p < 0.05$). Previous studies demonstrated that phosphorus is removed from wastewater by various processes such as sorption, precipitation and plant uptake (Vymazal 2007). In addition, the presence of earthworms can result in some phosphorus being converted to form that are plant available (Suthar 2006), because microorganisms in the intestinal organ of the earthworms have the ability to convert insoluble P into soluble forms (Adhami *et al.* 2014). Thus, some TP was probably released in forms available to plant uptake in MHCW-2.

In general, MHCW-2 showed highest efficiency in removing NO_3^--N, TKN and TP from wastewater. Previous studies demonstrated that the presence of earthworms can increase TN and TP removal efficiencies in wastewater (Xu *et al.* 2015). Thus, it can be explained that the earthworm *P. excavatus* in MHCW-2 had efficient biological potential for conversion of insoluble N and P into soluble forms, which are high-value useful plant growth media (Suthar 2006, 2007). In addition, the reed *P. australis* and cyperus *C. alternifolius* had high efficiency in the removal of NO_3^--N, TKN and TP (Ebrahimi *et al.* 2013; Shahi *et al.* 2013; Bilgin *et al.* 2014; Rezania *et al.* 2019; Hu *et al.* 2020).

Organic matter and suspended solid removal efficiencies

TSS concentrations significantly differed between the influents of INLET and effluents of HCW and MHCWs ($p < 0.05$) (Table 2). The efficiency for TSS reached 77.6% in HCW, 78.3% in MHCW-1 and 81.1% in MHCW-2 (Figure 7). Therefore, there were no significant differences in the effluents TSS removal between HCW and MHCWs ($p > 0.05$). The concentrations of COD significantly decreased after treatments by HCW and MHCWs

Table 2 | Concentrations (mg L^{-1}) of environment parameters (mean ± standard error) in INLET and the modified constructed wetlands (HCW, MHCW-1 and MHCW-2)

Filters	NH_4^+-N	NO_3^--N	TKN	PO_4^{3-}-P	TP	COD	BOD_5	TSS
INLET	22.5 ± 1.2	41.1 ± 1.2	48.9 ± 3.3	2.4 ± 0.1	5.1 ± 0.4	185.4 ± 13.7	149.7 ± 14.6	26.8 ± 2.5
HCW-1	8.2 ± 3.1**	26.8 ± 1.2****	36.5 ± 2.5***	1.7 ± 0.2****	4.1 ± 0.3**	42.2 ± 4.9****	95.6 ± 12.2****	6.4 ± 1.6****
MHCW-1	8.6 ± 3.7**	27.5 ± 1.0****	32.5 ± 3.4***	1.5 ± 0.1****	3.7 ± 0.3**	41.9 ± 3.7****	68.7 ± 14.7****	6.5 ± 1.9****
MHCW-2	8.1 ± 3.4**	23.8 ± 1.3****	28.9 ± 2.8***	1.4 ± 0.1****	3.4 ± 0.3**	39.1 ± 4.1****	43.1 ± 9.4****	5.3 ± 1.6****
Vietnam's standard[a]	10	50	–	10	–	150	50	100

Note: Asterisks indicate statistically significant differences between INLET and HCW and MHCWs (** for $p < 0.01$; *** for $p < 0.001$; **** for $p < 0.0001$).

[a] Vietnam's technical regulation on domestic wastewater discharge quality (QCVN 40:2011/BTNMT). –: undefined.

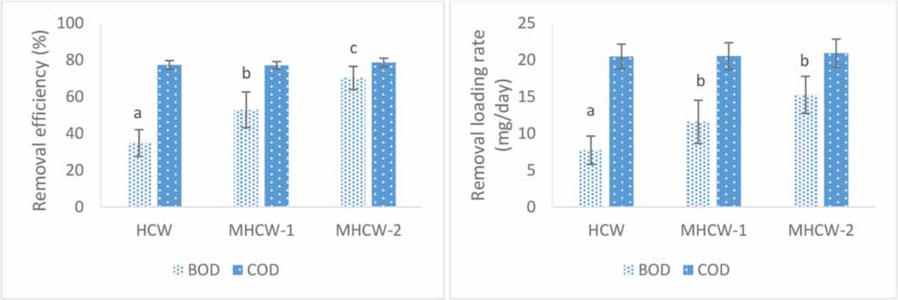

Figure 6 | Removal efficiency (%) and removal loading rate (mg m^{-2} day^{-1}) of COD and BOD through different modified constructed wetlands. *Different letters indicate statistically significant differences among HCW and MHCWs (Tukey test, $p < 0.05$).*

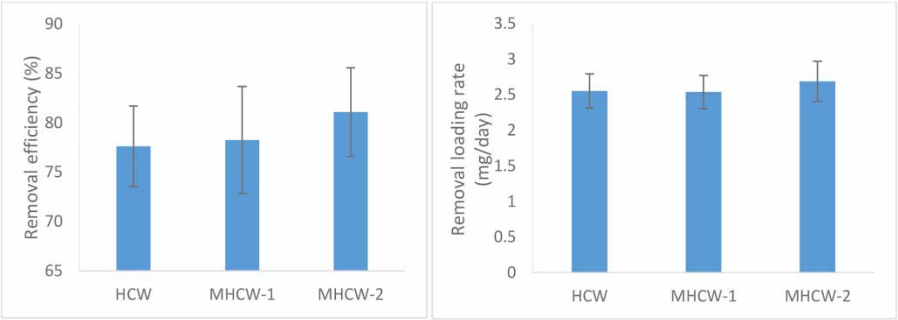

Figure 7 | Removal efficiency (%) and removal loading rate (mg m^{-2} day^{-1}) of TSS through different hybrid constructed wetlands.

(Table 2). Average COD concentrations in HCW and MHCWs were below 50 mg L^{-1}, which were significantly lower than INLET ($p < 0.0001$). The average COD removal efficiencies in HCW and MHCWs were lower than 80% (Figure 6); an insignificant difference was obtained between the three filter units of this study ($p > 0.05$). The results are in line with the COD removal values for floating treatment wetlands reported by Chen *et al.* (2016), whereas they are lower than the concentration reductions reported by Ali *et al.* (2018) for HCWs (80%) and Hu *et al.* (2020) for sludge treatment wetlands (>98%). The presence of earthworms in MHCW-2 unit did not improve the treatment efficiency for COD, indicating that the low COD removal efficiency was mainly due to physical processes such as sedimentation and filtration (Maucieri *et al.* 2016). In addition, a positive relationship between COD concentration and water turbidity was proved in the previous study by Barco & Borin (2017). The relatively high removal efficiencies of TSS could result in the relatively high COD removal efficiencies (Ali *et al.* 2018). In this study, TSS was removed from 77.6 to 81.1% by HCW and MHCW units, and 77–78.5% COD was also removed by those units.

In comparison with Vietnam's discharge limits (QCVN 40:2011/BTNMT and QCVN 14:2008/BTNMT) for industry and domestic wastewater (50 mg L^{-1} of BOD_5), the average effluent of BOD_5 in MHCW-2 was less than 45 mg L^{-1} and suitable for disposal into water bodies that are used for agricultural purposes or the equivalent. The results are in line with the removals reported in literature for BOD_5 removal in HCWs (Nguyen *et al.* 2017). In contrast, BOD_5 values in HCW and MHCW-1 were above Vietnamese's discharge standard for wastewater into environment (above 50 mg L^{-1}). As a consequence, the best removal performance was seen in the MHCW-2 (70.2%), followed by MHCW-1 with 52.9% and only 34.8% for HCW (Figure 6). By ANOVA test (Tukey's test), there were significant differences in the removal efficiency and removal loading rate of BOD_5 between the HCW and MHCWs ($p < 0.05$). It is suggested that earthworms can enhance the microbial activities, hydraulic conductivity and natural aeration by granulating the clay particles during the vermicomposting process (Suthar 2007; Sinha *et al.* 2008). However, the removals of BOD_5 of both HCW and MHCWs were low, according to the previous studies (Vymazal & Kröpfelová 2015; Ali *et al.* 2018; Haydar *et al.* 2020). The low removal efficiency of BOD_5 could be explained due to the long hydraulic residence time (HRT) to be set up for those filters. BOD_5 efficiencies in this study are similar to those found by

Ahmad *et al.* (2013), who reported that an increase in HRT led to a reduction in BOD_5 removal.

Heavy metal removal efficiency

The results showed that the concentrations of Cu and Pb in HCW and MHCWs were significantly lower ($p < 0.05$) than those in INLET, indicating that those wetlands had efficiently removed Cu and Pb from VAST wastewaters (Table 3). In HCW and MHCWs, the Cu concentrations ranged from 0.5 to 1.2 mg L^{-1}, which were within the permissible limit in Vietnam (2.0 mg L^{-1}; QCVN 40:2011/BTNMT). Although the removal loading rates for Cu showed no significant differences between three HCW and MHCWs ($p > 0.05$), effective removals of Cu in MHCW-1 (73.5%; 0.28 mg m^{-2} day^{-1}) and MHCW-2 (79.4%; 0.31 mg m^{-2} day^{-1}) were significantly higher than that obtained in HCW (48%; 0.22 mg m^{-2} day^{-1}) ($p < 0.05$). The removal efficiencies of Cu in this study are in agreement with those obtained from CW treating natural gas storage produced water (Kanagy *et al.* 2008). This result indicates that more substrate layers in MHCWs resulted in increased removal efficiency of Cu. However, it seems that the presence of the earthworm *P. excavatus* in MCHW-2 did not increase the effective removal of Cu from wastewater.

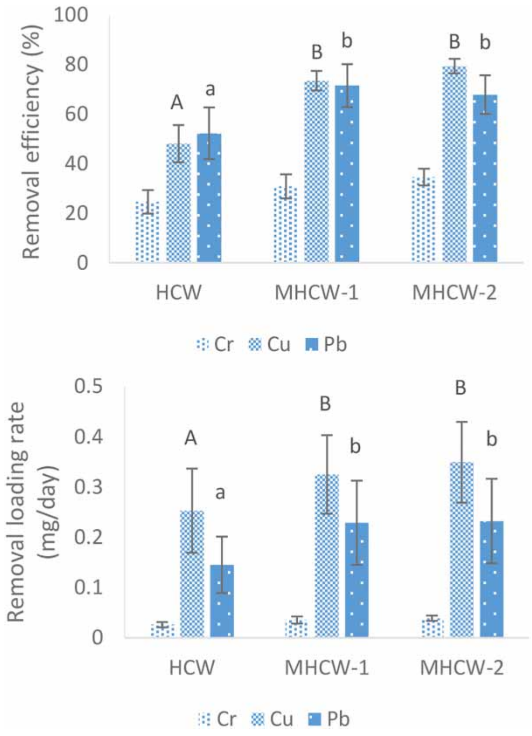

Figure 8 | Removal efficiency (%) and removal loading rate (mg m^{-2} day^{-1}) of heavy metals through different modified constructed wetlands. *Different letters indicate statistically significant differences among HCW and MHCWs (Tukey test, $p < 0.05$).*

Similarly, HCW was significantly less effective for Pb removal (52%) ($p < 0.05$) as compared with MHCW-1 (71.5%) and MHCW-2 (67.9%) (Figure 8), indicating that the number of substrate layers did affect the absorbing capacity of Pb from wastewater of those wetlands. The results also showed that there were insignificant differences in removal efficiencies between MHCW-1 and MHCW-2 ($p > 0.05$). Previous study proved that Pb was removed mainly by the roots of the plants and poorly by earthworms (Das & Osborne 2018). Although Pb concentration was over four times higher in the INLET samples (2.2 mg L^{-1}) and reduced to 0.6 mg L^{-1} in HCW and MHCW-1 (without earthworms), it is slightly above the permissible limit set for industry wastewaters in Vietnam (0.5 mg L^{-1}) (Table 3). The high Pb concentration of INLET wastewaters could reduce the microbe population in both plants and earthworms, leading to a decreased removal efficiency of Pb (Das & Osborne 2018). In addition, the plants used for wastewater treatment would show variable degrees of uptake and accumulation of Pb. According to the study of Khan *et al.* (2009), *P. australis* showed lower Pb uptake and accumulation compared with other plants such as *Pistia stratiotes*, *Typha latifolia* and *Ceratophyllum demersum*. Moreover, the *C. alternifolius* plant in this study has high efficiency in the removal of chemical parameters such as TSS, COD, NO_3^--N and NH_3-N, and PO_4^{3-}-P (Shahi *et al.* 2013).

Table 3 | Heavy metal concentrations (mean ± standard error) of raw wastewater (INLET) and of constructed wetlands throughout the experiment period (mg L^{-1})

Filters	**Cr**	**Cu**	**Pb**
INLET	0.8 ± 0.06	3.0 ± 0.7	2.2 ± 0.7
HCW	0.6 ± 0.06	1.2 ± 0.2*	1.2 ± 0.6*
MHCW-1	0.5 ± 0.04	0.7 ± 0.2**	0.6 ± 0.2**
MHCW-2	0.5 ± 0.04	0.6 ± 0.1**	0.6 ± 0.2**
Vietnam's standard[a]	1.1[b]	2.0	0.5

Asterisks indicate statistically significant differences between INLET and OUTLETs (HCW and MHCWs) (*for $p < 0.05$; **for $p < 0.01$).

[a] Vietnam's technical regulation on industry wastewater discharge quality (QCVN 40:2011/BTNMT).

[b] Total concentration of Cr (III) and Cr (VI).

In this study, the concentrations of Cr did not significantly decrease after treating the wastewaters by HCW and MHCWs (Table 3). However, Cr concentrations of INLET wastewater and OUTLET samples (HCW and MHCWs) were lower than 1.0 mg L^{-1}, which did not exceed the permissible limit (1.1 mg L^{-1}) set for industrial wastewaters in Vietnam. The results showed that both HCW and MHCWs had less effective removal of Cr, ranging from 24.6 to 34.6% (0.02–0.03 mg m^{-3} day^{-1}) for both HCW and MHCWs (Figure 8). It is unclear whether HCW and MHCWs can efficiently remove Cr when INLET wastewaters have a low concentration or not. It is because previous studies have proved that CW has effectively removed the Cr (over 80%) from wastewater (Hadad *et al.* 2006; Khan *et al.* 2009). Moreover, the input loading of wastewaters is a critical factor for achieving higher removal rates employing wetland systems (Saeed & Sun 2017). In the next study, an assessment of Cr removal efficiency with high concentration of INLET should be conducted to confirm the Cr removal efficiencies of HCW and MHCWs.

Overall, it has been hypothesized that more substrate layers and a synergistic association of the plants and earthworms would enhance the removal of heavy metals. However, more substrate layers (seven layers) showed higher removal efficiency of Cu and Pb in comparison to fewer substrate layers (four layers). In contrast, earthworm addition into MHCW did not significantly affect the removal efficiency of heavy metals. Chen & Hu (2019) indicated that earthworms had an insignificant effect on heavy metal accumulation in the plants and a significant effect on heavy metals removal from wastewater in unplanted wetland systems.

CONCLUSION

The present study focused on the efficiencies of the arrangement of three substrates and organisms that were used in HCWs for treatment of mixed domestic-industrial wastewater of an academic campus (VAST) before discharging into the urban water bodies. The removals of NO_3^--N, TKN, TP, BOD, Cu and Pb were more efficient in the wetlands that were arranged in seven substrate layers (MHCW-1 and MHCW-2) than in those with an arrangement of four substrate layers (HCW). Particularly, MHCW-2 (seven substrate layers and earthworm addition) showed higher removals of BOD and Cu than MHCW-1 (seven substrate layers and without earthworms) and HCW (four substrate layers).

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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