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# Effectiveness of biochar filters vegetated with *Echinochloa pyramidalis* in domestic wastewater treatment

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## ABSTRACT

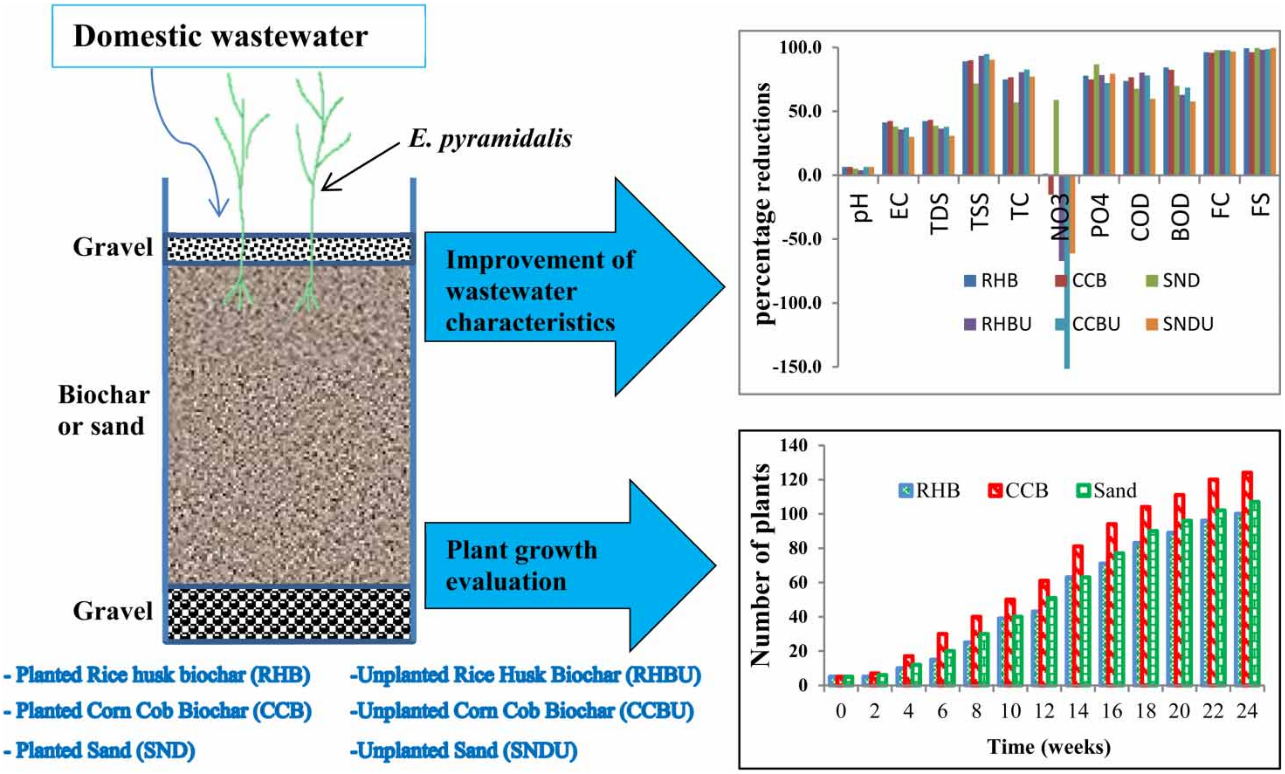
The use of biochar in constructed wetlands for domestic wastewater treatment is gradually being acclaimed by environmentalists due to its high specific surface area and porosity. In this study, the effectiveness of corn cob biochar (CCB) and rice husk biochar (RHB) in vertical flow constructed wetlands vegetated with *Echinochloa pyramidalis* was studied with sand as common reference material. The filters were fed with primarily treated domestic wastewater at a hydraulic loading rate of about 350 L/m$^2$/day for 6 months. Water samples were collected monthly for physicochemical and bacteriological analysis and plant growth assessed every two weeks throughout the study. Biochar filters were highly performant in wastewater improvement with no significant differences between the biochar types. Both biochars were more efficient than sand in the removal of chemical oxygen demand (COD), biochemical oxygen demand (BOD), true colour, total suspended solids (TSS) and total dissolved solids (TDS). However, sand filters performed better in the reduction of nutrients. All wetlands showed positive plant growth though the plants did not significantly affect the performance of the different filters for most parameters. However, a better plant growth was observed in the CCB filters. The study shows that CCB and RHB can effectively replace sand as substrates in constructed wetlands for wastewater treatment.

**Key words:** biochar, constructed wetlands, *Echinochloa pyramidalis*, filtration, vertical flow, wastewater treatment

## HIGHLIGHTS

- Valorisation of crop wastes through biochar production.
- Biochar filters are less effective in the removal of nutrients compared to sand.
- Significant organic matter removal in vegetated constructed wetlands.
- Significant reduction of bacteriological parameters regardless of substrate type.
- Biochar substrates promote the growth of *Echinochloa pyramidalis*.

## GRAPHICAL ABSTRACT




## INTRODUCTION

Continuous increase in the world's population results in an increase in the consumption of water and discharge of wastewater which causes heavy environmental pollution. The situation is made worse because nearly half of the world's population lack safely managed sanitation services. In 2020, 3.6 billion people lacked safely managed sanitation services, including 1.9 billion people with basic services, 580 million with limited services, 616 million using unimproved facilities, and 494 million practising open defecation (WHO/UNICEF 2020). The situation is accentuated in developing countries with about 90% of untreated sewage discharged into surface water bodies (Jakub *et al.* 2019) leading to pollution. A majority of homes in the developing countries use onsite sanitation facilities notably pit latrines and septic tanks which are in most cases unlined and inadequately desludged. As a consequence, wastewater is poorly treated leading to contamination of wells and boreholes used as sources of drinking water. Identifying and implementing suitable urban sanitation systems has been very daunting from time immemorial. In recent years, the problem has become more acute because there has been an ever increasing decline in water resources. Increasing the percentage of water being treated will go a long way to reduce health risks as well as promote its reuse for instance in enhancing agricultural productivity.

The absence of operational wastewater treatment systems in most developing countries have led to a growing interest in constructed wetlands technology. Constructed wetlands (CWs) are engineered systems exploiting natural processes involving wetland vegetation, filters' substrates, and the associated microbial assemblages in wastewater treatment (Vymazal 2010). Several studies suggest its use particularly in developing countries due to the lower operational and maintenance costs (Lekeufack *et al.* 2012; Dires *et al.* 2019). The efficiency of CWs is highly dependent on the filter medium as it allows the growth of microorganisms which aid in organic matter degradation and promotes the sedimentation and filtration of contaminants. The conventional filter material in CWs is sand which most often is associated to clogging problems. Moreover, there is scarcity of well-graded sand in some regions and high transportation costs due to its bulky nature (Dalahmeh 2016). In addition, the removal efficiency of nutrients in CWs with sand as media remains low (Vymazal 2014). The problems associated with the use of sand as media has led scientists to investigate alternative wastewater treatment technologies.

With its high specific surface area and porosity, biochar which is a carbon rich solid product from the pyrolysis of bio organic material is suggested as substrate in wastewater treatment using CWs (Gupta *et al.* 2015; Dalahmeh 2016; De Rozari *et al.* 2016; Kaetzl *et al.* 2018; Perez-Mercado *et al.* 2018). The utilization of biochar in wastewater treatment is governed by the mechanisms of adsorption, buffering, and immobilization of microbial cells (Pokharel *et al.* 2020). The high water-holding capacity and high porosity of biochar filters often results in a longer hydraulic retention time and hence improved purification capacity (Perez-Mercado *et al.* 2018). However, the physical, chemical and structural properties of biochar are expected to vary depending on the feedstock used and the biochar production conditions, suggesting a need for focus on the optimal design of biochar filters with respect to the most efficient feedstock.

Crop wastes and other organic matter are usually underused resources due to low heating value and slow decomposition rates. The wastes end up being burned in the field, dumped in waterways or piled to rot around processing units leading to pollution and soil degradation (Billa *et al.* 2019). These wastes could therefore be valorized by converting them into biochar. Furthermore, studies by Zhao *et al.* (2018) showed that crop waste biochars had a very high sorption to nitrate and hence potential adsorbents for wastewater filtration. Wetland vegetation equally plays a vital role in the purification of wastewater. Plants used in constructed wetlands should be locally available and capable of withstanding hypoxic stress conditions. Studies by Fonkou *et al.* 2005a report a great diversity of macrophytes growing in polluted wetlands in Cameroon and which can be used for phytopurification of wastewater. One of these plants is *Echinochloa pyramidalis* which has also been found to have the capacities of bio-accumulating nutrients and heavy metals (Fonkou *et al.* 2005b; Lekeufack *et al.* 2012). There are a few reports on the use of crop waste in CWs. However, some treatment parameters such as the removal of bacteriological parameters have not been thoroughly investigated. Moreover, the use of the plant *E. pyramidalis* has not been investigated on CWs with biochar as substrate. This paper assesses the performance of biochar filters produced from rice husk and corn cobs in the purification of domestic wastewater using a vertical flow constructed wetland (VFCW) vegetated with *E. pyramidalis*.

## MATERIALS AND METHODS

### Substrate preparation and characterization

Three substrates were tested in this study; rice husk biochar (RHB), corn cob biochar (CCB) and sand (SND). Rice husk was obtained from AFRIFOOD; a rice processing company located in Bafoussam, Cameroon while corn cobs were obtained from the Dschang station of the Institute for Agricultural Research for Development, Cameroon. The different biomasses were pyrolysed using a batch type custom built metal kiln at the University of Dschang, Cameroon. Each pyrolysis lasted for a period of approximately 4 h with temperature measurements taken every 30 min using a type K thermocouple. The pyrolysis time of 4 h was established from experimentation. Shorter periods did not permit complete pyrolysis of the feedstock especially for the rice husk biochar. Temperatures varied from 395 °C to 618 °C with an average of about 500 °C. The CCB was crushed and sieved to produce two grain size distributions; fine grained biochar (0.25–2 mm) and coarse grain (2–4 mm). RHB on the other hand did not require grinding. It was, however, sieved to the size distribution of 0.25–2 mm. Sand was purchased from the sand distribution arena in Dschang. The sand was properly washed using tap water in order to remove clay particles and then sieved to produce grain size distributions as those obtained for CCB. The biochar yield was calculated as the proportion of the weight of pyrolysis product to the original material. Three sub samples were collected from each of the seven batches of produced biochar and a resultant composite sample analysed in the laboratory of soil science and environmental chemistry of the University of Dschang following standard methods (EBC/IBI 2014). Sand properties were determined according to the methods described in Pauwels *et al.* (1992). The CCB obtained was a class 2 biochar and the RHB a class 3 biochar according to the norms of the International Biochar Initiative (EBC/IBI 2014) and comparable to results obtained by other researchers (Djousse *et al.* 2018; Billa *et al.* 2019). Table 1 shows the physical and chemical properties of the three substrates used.

### Filter design and experimental setup

Twelve microcosm VFCWs of 85 cm height and 30 cm diameter were set up and operated next to the wastewater treatment plant of the University of Dschang receiving domestic wastewater from the students' residence. The outlet pipe was raised to a height of 65 cm from the ground level in order to increase the hydraulic retention time. The interior of all the filters were lined with plastic to prevent them from rusting. In addition, a PVC pipe of 86 cm in height and 25 mm in diameter was inserted vertically into the system for the supply of oxygen. Each filter consisted of a bottom gravel layer, the principal substrate

**Table 1** | Characteristics of substrates tested in the study

| Parameters | CCB | RHB | Sand |
|---|---|---|---|
| Pyrolysis yield (%) | 27 | 33.3 | / |
| Particle size (mm) | 0.25–2 and 2–4 | 0.25–2 | 0.25–2 and 2–4 |
| Bulk density (g/cm$^3$) | 0.243 | 0.255 | 1.468 |
| Total porosity (%) | 91 | 90 | 45 |
| pH-water | 7.8 | 8.5 | 6.9 |
| Calcium (mg/100 g) | 2,160 | 160 | 72.14 |
| Potassium (mg/100 g) | 1,271.45 | 600.72 | 11.73 |
| Magnesium (mg/100 g) | 1,458 | 534.6 | 46.18 |
| Sodium (mg/100 g) | 270.27 | 185.65 | 22.99 |
| CEC – pH7 (mg/100 g) | 34 | 30 | 9 |
| Organic carbon (%) | 48.05 | 28.55 | 0.63 |
| Organic matter (%) | 96.10 | 57.10 | 0.37 |
| Total nitrogen (%) | 0.98 | 0.70 | 0.02 |
| Total phosphorus (mg/kg) | 625.617 | 98.181 | 18 |
| C/N | 49 | 41 | 16 |
| Ash content (%) | 4 | 43 | / |

and a top gravel layer. The arrangement of substrate in the filters was the same for CCB and sand since they had the same grain size distribution (Figure 1(a)). However, there was a slight variation for RHB due to the absence of coarse grains (Figure 1(b)). The top gravel layer was included for proper distribution of water on the filter surface while the bottom gravel layers served as a medium of drainage. Each layer was separated from the other using a fine net to avoid particles from moving downwards into other layers.

Nine out of 12 filters were vegetated with young shoots of *E. pyramidalis* collected from a natural wetland in Dschang with each filter receiving five plants. The nine vegetated beds consisted of the three substrates (CCB, RHB and SND) replicated 3 times. For each substrate type, one filter was left unplanted to serve as the control making up the three remaining filters. These were denoted RHB unplanted (RHBU), CCB unplanted (CCBU) and SND unplanted (SNDU) based on the substrate type.

Primary treated wastewater from the treatment plant was used in the study. The wastewater was allowed to flow directly on the experimental wetlands at a hydraulic loading rate of about 350 L/m$^2$/day in a vertical flow configuration. However, the system was fed with water from a nearby lake for a period of 1 week to allow the plants to establish in the wetlands. Three reservoirs of 50 L each were used for water supply with one reservoir supplying four filters. The reservoirs were refilled every morning and evening to ensure continuity of flow. Each reservoir had four different outlets provided with a tap for regulating the water entering the filters. These outlets were connected to a flexible pipe of diameter 10 mm perforated along its length to provide an even distribution of water to the surface of the filter. The filters were distributed along a table which served as stand for the wastewater reservoirs using a completely randomised design. The spacing between filters was set at 65 cm to avoid overcrowding when the plants begin to grow.

## Assessing the performance of biochar filters in wastewater treatment

The performance of biochar filters in wastewater treatment was assessed by comparing the efficiencies of the different biochar filters with sand in the improvement of physicochemical and microbiological characteristics of wastewater. In addition, the plant growth parameters were determined in order to elucidate the effect of substrate type on plant growth.

## Water sample collection and analysis

Wastewater samples were collected from the inlet and outlet of each wetland and analysed monthly in the laboratory of Applied Botany of the University of Dschang from May to October 2021 using standard methods (APHA *et al.* 2017). The

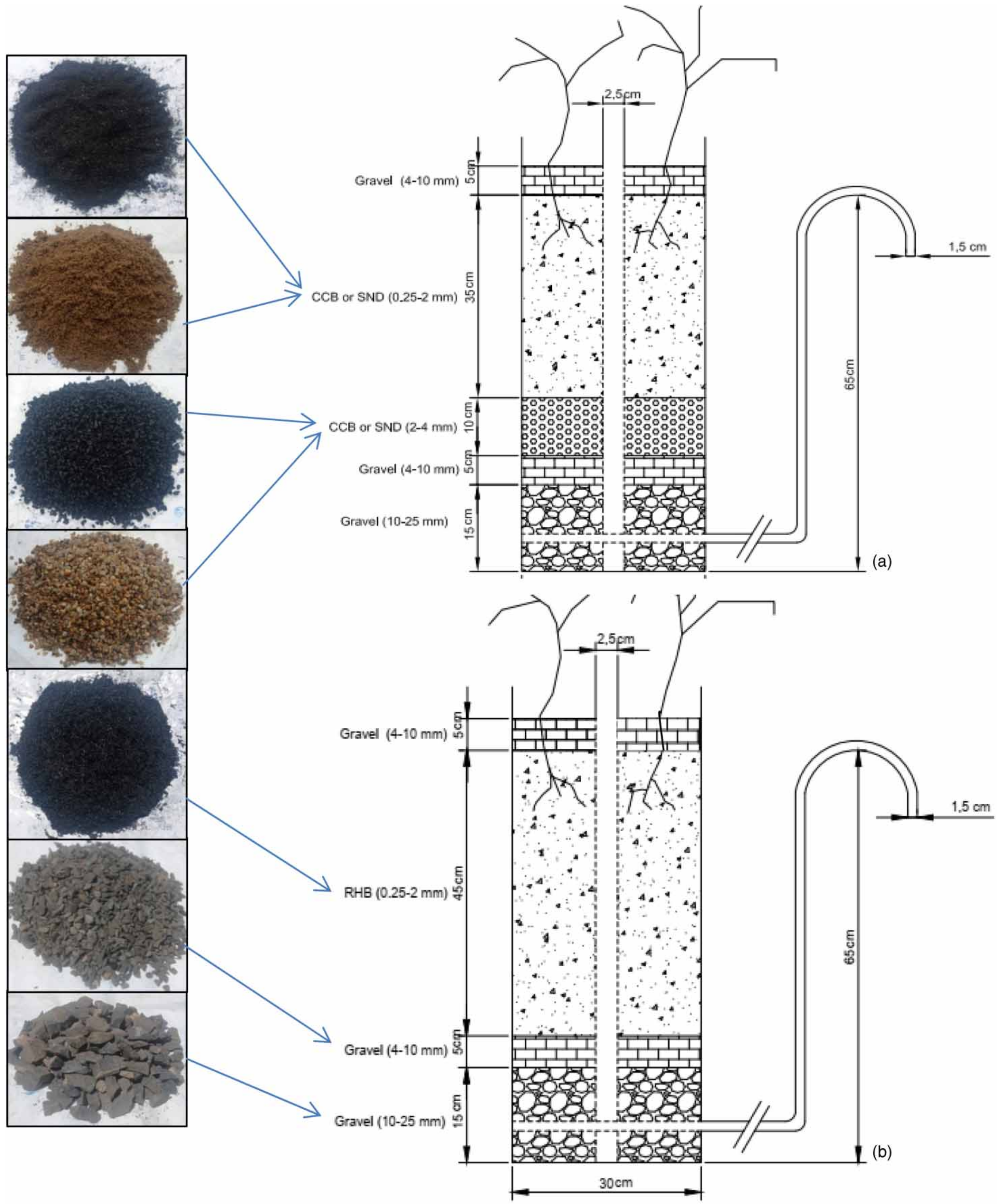



**Figure 1** | Longitudinal section of filters showing arrangement of substrates. (a) CCB and SND filters, (b) RHB.

parameters determined included the pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), true colour (TC), nitrates ($NO_3$-N), phosphates ($PO_4^{3-}$), chemical oxygen demand (COD), biochemical oxygen demand ($BOD_5$), faecal coliforms (FC) and faecal streptococci (FS).

The pH was measured using Eutech Instruments CyberScan 1,500 pH meter. The TDS and EC were measured using the Vintage Hach 44600 Conductivity/TDS meter. TSS, true colour, nitrates, phosphates and COD were all measured with a Hach DR/2500 spectrophotometer. TSS was measured using photometric method; true colour using the platinum-cobalt method; nitrates using the cadmium/reduction method with NitraVer5 powder pillows, phosphates using the ascorbic acid method with PhosVer3 powder pillows and COD using the dichromate reactor digestion method. The biochemical oxygen demand ($BOD_5$) value was measured by respirometric method using the BOD Trak in an incubator at 20 °C for 5 days. FC and FS were determined by the membrane filtration method using AC cellulose membranes filters with pore-size 0.45 $\mu$m. After incubation, colony-forming units (CFU) were enumerated using a colony counter.

### Plant growth parameters' measurement

Plant growth parameters were measured at intervals of 2 weeks throughout the experimental period. These included: the plant height, stem diameter, number of leaves and the number of plants per filter. Three healthy plants were chosen per filter for measurement of growth parameters. The chosen plants were marked with rubber bands of different colours to ensure continuity.

### Statistical and data analysis

All statistical analyses were carried out with the software SPSS 23.0. One-way ANOVA was used to compare mean values from different experimental units with Tukey's test used for post hoc comparisons. However, the independent student's t test was performed to compare mean values of the vegetated and non-vegetated units. All differences were tested using the least significance difference test ($P<0.05$). Percentage reduction rates were calculated using the formula $\%R = Ci - Ce/Ci \times 100$ where $C_i$ and $C_e$ are respectively the influent and effluent values of the parameter.

## RESULTS AND DISCUSSIONS

### Performance of wetlands in the improvement of physicochemical parameters

The characteristics of the influent and effluent in all wetlands are presented in Table 2 and the average percentage reductions in Table 3. The influent concentrations were significantly higher than the effluent concentrations for all parameters except for nitrates in which effluent values were considerably higher for the biochar filters in particular. The characteristics of the influent wastewater varied throughout the study with relatively lower concentrations recorded during the last 2 months. The concentration of the influent wastewater was dependent on the amount of rainfall and on the availability of students in

**Table 2** | Overall statistics of influent and effluent concentrations (mean ± standard deviation)

| | | Effluent concentrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Vegetated beds | | | Non-vegetated beds | | |
| Parameters | Influent concentrations | Rice husk biochar | Corn cob biochar | Sand | Rice husk biochar | Corn cob biochar | Sand |
| pH | 7.8 ± 0.2a | 7.3 ± 0.3a | 7.3 ± 0.4a | 7.4 ± 0.4a | 7.5 ± 0.6a | 7.3 ± 0.5a | 7.3 ± 0.5a |
| EC (µs/cm) | 2,653 ± 1,311a | 1,557 ± 1,134a | 1,527 ± 1,133a | 1,643 ± 1,030a | 1,705 ± 1,107a | 1,663 ± 1,001a | 1,858 ± 1,010a |
| TDS (mg/L) | 1,347 ± 689a | 777 ± 563a | 764 ± 578a | 825 ± 517a | 857 ± 558a | 838 ± 508a | 932 ± 506a |
| TSS (mg/L) | 210 ± 156c | 22.9 ± 11ab | 21.1 ± 3.9ab | 59.4 ± 55b | 13.8 ± 7.1a | 10.8 ± 10.9a | 20.3 ± 8.1ab |
| TC (Pt/Co) | 2,203 ± 618c | 553 ± 241ab | 515 ± 185a | 951 ± 315b | 428 ± 225a | 382 ± 286a | 503 ± 175a |
| $NO_3^-$ (mg/L) | 8.5 ± 9.3a | 8.4 ± 11.4a | 9.8 ± 12.3a | 3.5 ± 3.6a | 14.2 ± 11.3a | 23.7 ± 22.4a | 13.7 ± 16.1a |
| $PO_4^{3-}$ (mg/L) | 36.3 ± 21.3c | 8.0 ± 1.8ab | 9.1 ± 2.8b | 4.8 ± 1.7a | 7.9 ± 1.9ab | 10.1 ± 2b | 7.5 ± 2.2ab |
| COD (mg/L) | 540 ± 509b | 141.9 ± 120.9a | 126.1 ± 106.5a | 174.4 ± 189.8a | 106 ± 73.5a | 118.8 ± 92.6a | 217.5 ± 221a |
| $BOD_5$ (mg/L) | 124.8 ± 54c | 19.6 ± 16.8a | 21.9 ± 7.7a | 37.7 ± 10.7ab | 46.5 ± 13.5ab | 39.3 ± 19ab | 52.9 ± 16.4b |
| FC *$10^5$ (CFU/100 ml) | 263.3 ± 118b | 9.7 ± 13.7a | 10.8 ± 18.5a | 5.8 ± 5.6a | 5.8 ± 5.5a | 5.5 ± 5.1a | 8.5 ± 10.9a |
| FS *$10^5$ (CFU/100 ml) | 91.7 ± 34b | 0.7 ± 0.8a | 3.6 ± 4.1a | 0.4 ± 0.9a | 1.7 ± 2.3a | 1.2 ± 2a | 0.3 ± 0.5a |

Means followed by the same letter in the row are statistically similar at the 5% probability level.

**Table 3** | Average percentage removal of wastewater parameters in each wetland

| Parameters (%) | RHB | CCB | SND | RHBU | CCBU | SNDU |
|---|---|---|---|---|---|---|
| pH | 6.4 | 6.4 | 5.1 | 3.8 | 6.4 | 6.4 |
| EC | 41.3 | 42.4 | 38.1 | 35.7 | 37.3 | 30.0 |
| TDS | 42.3 | 43.3 | 38.8 | 36.4 | 37.8 | 30.8 |
| TSS | 89.1 | 90.0 | 71.7 | 93.4 | 94.9 | 90.3 |
| TC | 74.9 | 76.6 | 56.8 | 80.6 | 82.7 | 77.2 |
| $NO_3^-$ | 1.2 | −15.3 | 58.8 | −67.1 | −178.8 | −61.2 |
| $PO_4^{3-}$ | 78.0 | 74.9 | 86.8 | 78.2 | 72.2 | 79.3 |
| COD | 73.7 | 76.6 | 67.7 | 80.4 | 78.0 | 59.7 |
| $BOD_5$ | 84.3 | 82.5 | 69.8 | 62.7 | 68.5 | 57.6 |
| FC | 96.3 | 95.9 | 97.8 | 97.8 | 97.9 | 96.8 |
| FS | 99.2 | 96.2 | 99.6 | 98.1 | 98.7 | 99.7 |

the residence. During these last 2 months, most of the students had gone for the holidays thereby leading to a reduction in the influent concentration.

## Change in pH

The average influent pH was 7.8 with the average effluent pH being: 7.3 for RHB, CCB, CCBU and SNDU; 7.4 for SND and 7.5 for RHBU (Table 2). Biochar addition did not have a significant effect on pH though the biochar substrates had a higher pH than sand (Table 1). However, some studies have observed an increase in effluent pH when biochar is used as substrate in CWs (Gupta *et al.* 2015; Deng *et al.* 2019). The increase in effluent pH in these studies was attributed to the alkaline nature and high ash content of the biochar media. The result obtained in this study could be explained by the fact that during the 1 week acclimatization period, the filter was flushed out with water which could have washed away some of the ash present in the biochars. There was equally no significant difference between the vegetated and non-vegetated beds. The pH of wastewater is an important factor that may affect the performance of wetlands, mainly in terms of nitrogen and organic matter removal. The observed pH ranges in the wetlands were within the recommended range ($4.0<pH<9.5$) for the existence of many treatment bacteria (Gupta *et al.* 2015).

## Reduction of electrical conductivity and total dissolved solids

The removal efficiencies of EC and TDS was in the order of CCB>RHB>SND though no significant differences were observed within the wetlands. The average EC at the inlet was 2,653 μs/cm with effluent values being; 1,527 μs/cm for CCB, 1,557 μs/cm for RHB and 1,643 μs/cm for SND (Table 2). This led to an average reduction of 42.4%, 41.3%, and 38.1% respectively (Table 3). The average influent TDS was 1,347 mg/L with average effluent concentrations of 764 mg/L, 777 mg/L and 825 mg/L for CCB, RHB and SND respectively giving average reduction rates of 43.3% for CCB, 42.3% for RHB and 38.8 for SND. The higher performance of biochar filters can be attributed to its higher adsorptive capacity. The removal efficiency of these parameters was relatively low compared to others. The vegetated beds performed better (41.5% for TDS and 40.6 for EC) than the non-vegetated ones (35% for TDS and 34.3% for EC) but the results were insignificant at the 5% probability level. However, some studies have demonstrated better performance of planted beds compared to unplanted ones (Labed *et al.* 2014) with respect to dissolved solids. The vegetated sand filters performed better than the non-vegetated biochar filters showing that the role of plants could be more important than that of biochar in the removal of dissolved solids.

## Reduction of total suspended solids

The average suspended solid removal was in the order of CCB (90%)>RHB (89.1%)>SND (71.7%). In addition, the non-vegetated filters were significantly (92.9%) more effective than the vegetated ones (83.6%) in the reduction of TSS. The high performance of biochar filters could be due to their large specific surface area which increases their ability to retain solids. The lower reduction rates observed in planted beds could be due to the fragmentation of detritus from plants or the colloidal particles formed in the systems. The dense fibrous root system of *E. pyramidalis* probably promoted a high aeration in the substrate which could favour the infiltration of TSS. Moreover, the efficiency of the planted beds decreased slightly with plant growth compared to the non-vegetated beds with a negative reduction rate observed in the sand filters during the 6th month. TSS reduction rates for all unplanted beds stood at over 80% throughout the study. These results are consistent with those obtained by Labed *et al.* (2014) using the plant *Juncus effuses*. However, some studies have reported no significant differences between the planted and unplanted beds (Gupta *et al.* 2015; Dires *et al.* 2019; Nguyen *et al.* 2021) which could probably have been influenced by the system design since most of these studies were carried out on horizontal flow CWs. Throughout the study, the effluent of all biochar filters met the requirement (<50 mg/L) for the discharge of wastewater in Cameroon (MINEPDED 2005). However, values of up to 184 mg/L were obtained in SND filters.

## Reduction of true colour

The removal of colour was in the order of CCB>RHB>SND. The biochar filters were significantly more effective in the removal of water colour ($p<0.05$). The average reduction rates for the biochar filters were 76.6% for CCB and 74.9% for RHB. SND filters on the other hand presented a relatively lower removal rate of 56.8%. In addition, the non-vegetated beds were significantly more efficient (80.2%) in the removal of colour compared to the vegetated ones (69.4%). The high colour in the effluent of vegetated beds was probably due to the high concentration of TSS in these beds. This high colour could make the effluent unsuitable for some industrial processes. This parameter is rarely ever tested in treatment systems. However, colour in water has to be removed for aesthetics reasons and to make it suitable for industrial applications. Colour in domestic water results primarily from the presence of organic matter. Suspended particles especially colloidal size particles such as clay, algae, iron and manganese oxides, may also contribute to the colour of water.

## Nutrient removal

Nutrient removal in this study was examined through the determination of nitrates and orthophosphates. All wetlands showed an appreciable reduction in phosphates (>50%) but an increase in nitrate concentrations was observed in all filters with biochar filters registering the highest concentrations. Nutrient removal rate was in the order of SND>RHB>CCB.

The average nitrate concentration at the inlet was 8.5 mg/L with effluent values being 9.8 mg/L for CCB, 8.4 mg/L for RHB and 3.5 mg/L for SND. Moreover, higher nitrate concentrations were observed in the effluent of the non-vegetated beds: 23.7 mg/L for CCBU, 14.2 mg/L for RHBU and 13.7 mg/L for SNDU. The CCB filters were the least efficient both in the planted and unplanted beds. Overall, the wastewater used in this study had a very low nitrate concentration with a value as low as 0.3 mg/L registered in the inlet during the 6th month showing that nitrification in the primary treatment system was very low. This led to an overall increase in nitrate concentration in the effluent of the filters as organic nitrogen found in the raw wastewater was being converted to nitrates. This was more pronounced in the biochar filters showing that biochar favours the mineralization of $NH_4$-N into $NO_3$-N. This could be due to the presence of macrospores in the biochar which promote the development of microbial population. These microbes favour the nitrification of adsorbed ammonia in aerobic conditions leading to the release of the produced nitrate into wastewater. A longer hydraulic retention time may be necessary to promote the further breakdown of nitrates. In addition, the determination of total nitrogen concentration is necessary in order to elucidate the performance of such filters in nitrogen removal. The high nitrate concentrations in the effluent of biochar filters could also be due to the leaching of nitrogen from the substrate. Overall, the biochar filters contained higher concentrations of nitrogen with CCB containing the highest perhaps a reason for the higher nitrate concentrations in the effluent of CCB filters. The increase in nitrate concentration is supported by other studies (Kasak *et al.* 2018; Nguyen *et al.* 2021). Nevertheless, Gupta *et al.* (2015) observed a significant reduction in nitrates using wood biochar.

Phosphate reduction rates ranged from 57.7% to 83.1% for CCB with an average of 74.9% while those for RHB ranged from 50.3% to 88% with an average of 78%. Reduction rates for SND were slightly higher ranging from 61.1% to 94.5% with an average of 86.8% (Table 3). However, there were no significant differences between the wetlands. The high phosphate concentrations in the effluent of biochar filters compared to sand could also be attributed to leaching of nutrients from the biochar surface as in the case of nitrates. The biochars contained very high phosphate concentrations compared to the sand with CCB possessing the highest amounts (Table 1). The amount of phosphate present in the biochar could prevent the further adsoption of phosphorus and a subsequent release of the adsorbed phosphorus into the wastewater. Previous studies (Dalahmeh 2016; De Rozari *et al.* 2016) equally reported better phosphate removal in sand filters compared to biochar amended filters. De Rozari *et al.* (2016) observed very high concentrations of phosphates in effluent after a heavy rainfall supporting the fact that nutrients could leach from the biochar surface. Moreover, biochar possesses a negative surface charge which could result in low sorption ability for anionic species like nitrate and phosphate. Nonetheless, activated biochar filters have been observed to perform better than sand filters (Dalahmeh 2016). The vegetated bed in this study did not perform significantly better than the non-vegetated beds in the removal of nutrients. However, some other studies confirmed the effect of vegetation on the removal of nutrients (Labed *et al.* 2014; Gupta *et al.* 2015; Kasak *et al.* 2018).

## Organic matter removal

Organic matter reduction rates were evaluated using COD and $BOD_5$. Average COD reduction rates of 76.6%, 74.9% and 67.7% were observed for CCB, RHB and SND respectively. BOD removal rates followed a similar trend to COD. However, RHB filters were more performant than the CCB filters in the removal of $BOD_5$. Average $BOD_5$ reduction rates stood at 82.5%, 84.3% and 69.8% for CCB, RHB and SND respectively. Biochar filters were significantly more efficient than the sand filters in the removal of $BOD_5$ ($p<0.05$). In addition, the vegetated beds were statistically more efficient (78.9%) than the non-vegetated beds (62.9%) in the removal of $BOD_5$, demonstrating that plants play a vital role in organic matter degradation. Regarding the reduction of COD, there were no significant differences between the wetlands and vegetation did not significantly affect the treatments. However, the unplanted biochar beds performed slightly better (79.2%) than the planted beds (75.15%).

In this study, biochar amendment significantly promoted organic matter removal which is consistent with other studies (Gupta *et al.* 2015; Kaetzl *et al.* 2018; Perez-Mercado *et al.* 2018; Nguyen *et al.* 2021). This high organic matter removal in biochar filters is associated with the high adsorptive capacity of biochar for organic molecules (Kaetzl *et al.* 2018). Moreover, the macropores of biochar serve as a suitable medium for the growth of microorganisms which intend to promote the degradation of organic matter (Zhang *et al.* 2021). Increasing levels of microbial population with biochar addition is also reported by Deng *et al.* (2021). With regard to organic matter removal in CWs, several studies show a difference in the activity between the planted or unplanted wetlands (Lekeufack *et al.* 2012) which is explained by the fact that plants increase more oxygen into the system for the degradation of organic matter by microbes.


**Figure 2** | Aspects of the vegetation in the wetlands: (a) plants at one month, (b) plants at 4 months, (c) plants at 6 months.

### Performance of wetlands in the improvement of microbiological parameters

Pathogen removal was assessed through the determination of two indicator bacteria: faecal coliforms (FC) and faecal streptococci (FS). Microbes were the most removed parameter in all wetlands regardless of substrate or vegetation condition. Removal rates in all wetlands ranged from 90% to 100%. The average influent concentration was 263.3*$10^5$ CFU/100 mL for FC and 91.7*$10^5$ CFU/100 ml for FS (Table 2). FC removal rates of 95.9%, 96.3% and 97.8% were obtained for CCB, RHB and SND respectively while FS removal rates stood at 96.2%, 99.2% and 99.6% respectively (Table 3). SND filters were significantly more efficient than the CCB filters in the removal of FS but statistically similar to the RHB filters. The different filters did not show any significant difference in the reduction of faecal coliforms. This demonstrates that pathogen removal in CWs is mainly a physical process which is not greatly affected by substrate composition. Moreover, studies by Kaetzl *et al.* (2018), found no significant difference between biochar and gravel filters in the reduction of *E. coli* supporting the fact that biochar addition may have an insignificant effect on pathogen removal. There was equally no significant difference between the vegetated and the non-vegetated wetlands regardless of substrate type demonstrating that *E. pyramidalis* has very little to play in pathogen removal. These results corroborates the findings of Labed *et al.* (2014).

### Growth and productivity of plants in the different wetlands

All the vegetated wetlands showed positive growth without any obvious symptoms of toxicity or nutrient deficiency regardless of substrate type. Figure 2 shows the variation in the growth of plants over the experimental period. All transplanted plants had a mean height of about 35 cm at the start of the experiment. In the CCB filters, the plants grew to a mean height of 242 cm

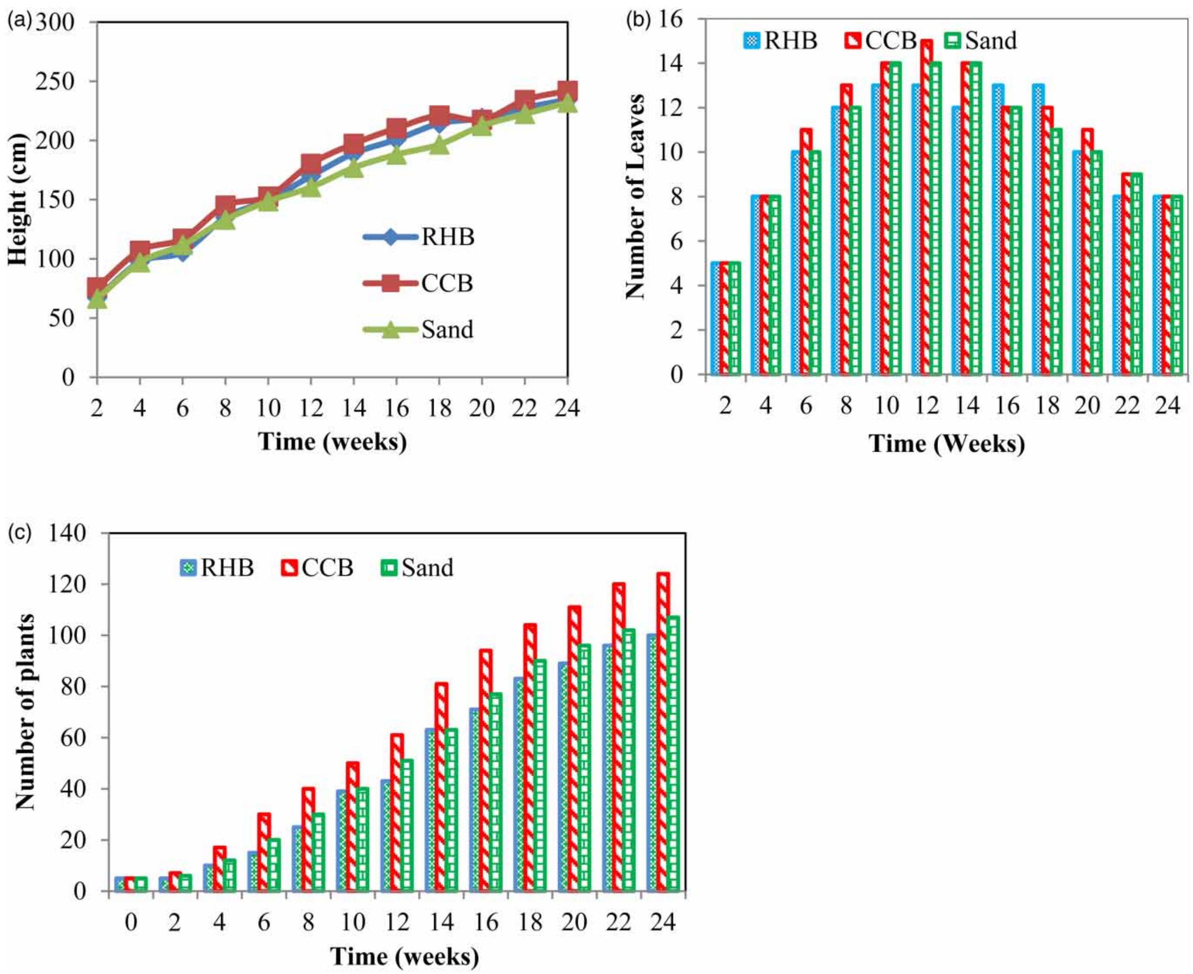



**Figure 3** | Variation of plant parameters in each wetland throughout the experimental period; (a) plant height, (b) number of leaves, (c) number of plants.

after 6 months with the tallest plant measuring 265 cm while those in RHB filters grew to an average of 234.5 cm with the tallest plant being 280 cm. Plants in SND filters grew from an average height of about 35–232 cm with the tallest plant measuring 260 cm. Figure 3(a) presents the variation in plant height for the different substrates. The number of leaves increased up to the 12th week for all filters with an average of 13 leaves per plant but then began to decline as the leaves at the bottom continued to dry off (Figure 3(b)). Plants densities increased rapidly throughout the study period from five plants per filter to an average of 124 plants per filter for CCB, 107 for SND and 100 for RHB (Figure 3(c)).

The diameter of the transplanted plants remained fairly constant at an average of 6 mm throughout the study period. However, the new plants had relatively larger sizes of up to 12 mm. Overall, biomass growth varied slightly among the mesocosm units with CCB filters showing a better plant growth but no significant differences were observed at the 5% probability level for all parameters indicating that biochar addition did not bring about any significant effect on plant growth. The rapid plant growth could be attributed to the high nutrient content of the domestic wastewater. The rapid growth of this plant in constructed wetlands corroborates the works of Lekeufack *et al.* (2012) and Kengne *et al.* (2008). This demonstrates the ability of *E. pyramidalis* to withstand oxygen depleted conditions and hence a good candidate for use in CWs and wastewater sludge dewatering beds despite the insignificant difference between the planted and unplanted beds in this study. The plant is also a good fodder crop, hence serving a dual purpose in wastewater treatment and animal nutrition.

## CONCLUSIONS

Two biochar types were tested as substrates in vertical flow constructed wetlands for the treatment of domestic wastewater. Both biochars were highly efficient in the removal of most of the parameters with average reduction rates of >90% for faecal indicator bacteria, >80% for TSS, >70% for phosphates and >60% for organic matter. However, biochar filters were only significantly better than the sand filters in the reduction of true colour and organic matter. The vegetated beds performed better than the non-vegetated ones in the removal of most parameters but a significant effect was only observed for organic matter. However, the vegetative beds were significantly less performant in the reduction of TSS and true colour. There was no significant difference in the performance of both biochars even though the corn cob biochar had better removal efficiencies. Corn cob biochars are however recommended given the ease of production and the availability of feedstock worldwide. The study confirms that biochar can be used for the enhancement of vegetated beds in the treatment of wastewater especially given the low bulk density of biochars and the possibility of reuse in agricultural systems. However, the mechanism involved in the removal of wastewater contaminants using biochar substrates should be further explored and elucidated. Moreover, higher pyrolysis temperatures could further enhance the purification capacity of these crop waste biochars.

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## DATA AVAILABILITY STATEMENT

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

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