Heliyon 10 (2024) e33284

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Assessment of secondary metabolites in *Pennisetum purpureum* planted into constructed wetlands using shale and laterite as substrate for wastewater treatment

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ARTICLE INFO

Keywords:
Constructed wetland
Pennisetum purpureum
Secondary metabolites
Substrates
Wastewater

ABSTRACT

Constructed wetlands (CWs) are systems designed to maximize pollutants removal by various mechanisms, most of which are associated with the presence of plants. However, the substances secreted by plants to defend themselves against external aggressions during their growth are very little studied in these systems. This study aimed to characterize the chemical constituents of *Pennisetum purpureum* extracts used in an experimental mesocosm filled with shale and laterite treating domestic wastewater. Above-ground biomass, strain diameter and secondary metabolites of *P. purpureum* plants grown on the different substrates (shale and laterite) were monitored, as were those grown on the experimental site (control). In addition, the removal performance of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total Kjedahl nitrogen (TKN) and Total Phosphorus (TP) was determined at the outlet of CWs. Plant biomass measured on the shale bed (13.7 ± 0.5 kg m^{-2}) was higher than on the laterite bed (12.5 ± 0.1 kg m^{-2}), both lower than the biomass obtained in the natural environment (14.9 ± 0.6 kg m^{-2}). Performances ranged from 83 ± 5.4 to 76.9 ± 7 % (COD), 84.7 ± 6.8 to 78 ± 8.1 % (BOD_5), 72.2 ± 10.7 to 55.5 ± 16.4 % (NTK) and 72.4 ± 4.9 to 58.4 ± 3.4 % (TP), with higher efficiencies in the shale-filled bed. Plant extracts from the experimental site were richer in secondary metabolites (total polyphenol [73.5 mgEAG/gMS], total flavonoids [18.1 mgEQ/gMS] and condensed tannin [13.3 mgEC/gMS]) than those from plants grown in CWs. However, plants in the shale-filled bed secreted more total polyphenol (57.7 mgEAG/gMS), total flavonoids (12.1 mgEQ/gMS) and condensed tannin (12 mgEC/gMS) than those in the laterite-filled bed. In short, wastewater and filtration materials have an influence on the secretion of secondary plant metabolites. However, of the two materials, shale seems to be better suited to CWs, as it promotes an environment close to the natural environment.

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https://doi.org/10.1016/j.heliyon.2024.e33284
Received 22 April 2024; Received in revised form 5 June 2024; Accepted 18 June 2024
Available online 18 June 2024

1. Introduction

In recent decades, artificial wetlands or constructed wetlands have been increasingly used as an alternative to traditional wastewater treatment processes (*i.e.* activated sludge, biological disks, etc.), due to their low installation and operating costs, ease of use and good integration into the landscape [1]. Indeed, the constructed wetland process has been used successfully around the world in the removal of organic pollutants [2,3], nutrients [4,5], pathogens [6,7] and emerging pollutants [8–10] in domestic, industrial and agricultural wastewater.

Constructed wetlands come in two basic variations depending on the direction of water flow (*i.e.* vertical-flow system and the horizontal-flow system). In horizontal-flow constructed wetlands, oxygen concentrations are limited [11], while unsaturated vertical-flow constructed wetlands operate under a predominantly aerobic regime. This condition promotes oxygen transfer [11,12], increasing nitrification rates as well as the oxidation capacity of organic matter [13,14]. In addition, several studies, including those by Pandey et al. [15], Ramesh et al. [16], Verma & Suthar [17] and Thalla et al. [18], have shown that vertical-flow constructed wetlands perform better than horizontal-flow wetlands in terms of both nutrients and organic matter in the purification of various wastewaters. It is probably for this reason that vertical-flow constructed wetlands are widely used in France [19–23]. Therefore, vertical-flow wetlands would be a suitable alternative for wastewater treatment.

Unlike wastewater treatment processes that focus on a single mechanism or type of pollutant, constructed wetlands use numerous interrelated symbiotic processes to simultaneously remove several pollutants (*i.e.* organic matter, nutrients, pathogens, etc.) [24,25]. These are physical, chemical and biological processes (*i.e.* sedimentation, precipitation, adsorption, assimilation by plants, biological transformations by micro-organisms, etc.). These mechanisms are more or less linked, via the main components of the wetland, in particular plants, substrates and microorganisms [12,26]. Thus, to better understand the functioning of constructed wetlands, several studies have been initiated on the role of each component of the process [19,27–30].

However, the purification performance of constructed wetlands is strongly depends on the choice of plant and substrate. Indeed, emergent herbaceous plants (Phragmites sp, Typha sp, Scirpus sp etc.), adapted to humid areas, are recommended for their high biomass production and their great capacity to capture atmospheric oxygen and solar energy, which prove favorable to the purifying activity of organisms [31]. Since then, several studies have been carried out with the aim of optimizing the efficiency of constructed wetlands by diversifying plants [12,32–35]. However, if most studies report on the role of plants in the degradation and assimilation of wastewater pollutants [29,31], very few studies to date address their adaptation mechanism to the environment of the constructed wetland system during effluent treatment. However, during wastewater treatment, pollutants very often present an external attack on plants. At the same time, macrophytes (plants) compete with microphytes (algae) in their growth pollutants [32,34]. Thus, to cope with attacks and inhibit the growth of algae, plants secrete secondary metabolites known to contain abundant phytochemicals, antimicrobials and pharmacologically active principles. These are anthraquinones, flavonoids, saponins, polyphenols, tannins and alkaloids which are extremely diverse chemical compounds with varied functions, each having well-defined biological activities [36–38]. One of the few works found in the literature is that of Ntakiyiruta et al. [37] who studied the variation in the composition of secondary metabolites secreted by two floating macrophytes, namely water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), to cope with external aggressions, in a free-water surface constructed wetland.

Relative to the plant species *Pennisetum purpureum*, it is a *Poaceae* having proven its influence in the treatment of wastewater in constructed wetland by the absorption of large quantities of nutrients, while producing abundant above-ground biomass [12,39–44]. In addition, *P. purpureum* is an excellent, highly productive forage, providing good quality silage and hay for feeding herbivores, cattle, goats, rabbits and sheep [45,46]. Thatches are used in the construction of huts, the covering of roofs, the making of fences and palisades as well as pipe stems. In addition, *P. purpureum* has very high productivity, as a biofuel crop [47,48]. Thus its use in constructed wetlands could generate income likely to ensure the maintenance of the process, especially since the quality of its biomass post wastewater treatment has been proven [7]. Furthermore, the study of the production of secondary metabolites of *P. purpureum* would constitute added value to scientific knowledge relating to the adaptation mechanisms of this plant in constructed wetlands treating wastewater.

As for the filtration material (substrate) it is of paramount importance in the operation of the constructed wetland [49]. It determines the reliability and purification performance of constructed wetlands. The choice of material is guided by the compromise between hydrodynamic constraints (adequate flow rates) which requiring a coarse particle size, and purification, which requires a fine particle size [50,51]. Sand is most often used because of its availability and the ability of its grains to resist shear forces. A thickness of between 20 and 80 cm is recommended to obtain excellent purification results [52]. However, referring to the review by Sanjrani et al. [53], very few studies address the use of geological materials such as shale and laterite in the implementation of artificial wetlands. However, these materials, which have no recognized mining interest, have shown a high capacity to absorb and adsorb pollutants from wastewater [54–56]. Consequently, the use of these materials in constructed wetlands could increase the purification efficiency of the process, especially in areas such as Côte d'Ivoire where they are found in abundance [54,57].

In Côte d'Ivoire, work on constructed wetlands has focused on plants of economic interest, including *Amaranthus hybridus* and *Corchorus olitorius*, which are food plants [58,59] and a range of forage plants: *Panicum maximum* [60,61], *Andropogon gayanus*, *Chrysopogon zizanioides*, *Echinochloa pyramidalis*, *Pennisetum purpureum* and *Tripsacum laxum* [12]. Overall, the results of this work were satisfactory. However, with regard to the plants tested, the opinion survey of a sample of the population showed a refusal to consume food plants because of their production origin. On the other hand, the survey showed that the above-ground plant biomass of forage plants could be used by livestock breeders and sellers. However, as the use of *P. maximum* resulted in a very marked enrichment of the filtrates from the constructed wetland in nitrogen, studies subsequent to the above-mentioned work are increasingly using the species *P. purpureum*, in view of the excellent results obtained with the latter.

This study aimed to characterize secondary metabolites in extracts of *P. purpureum* used in shale- and laterite-filled constructed wetlands treating domestic wastewater. This involved evaluating the growth response of *P. purpureum* and the purification performance of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Total Kjedahl Nitrogen (TKN) and Total Phosphorus (TP) as well as the secondary metabolites present in the plant extracts.

2. Material and methods

2.1. Experimental and feeding equipment

The study was carried out in 2023, over a period of six (6) months in the experimental pilot of the Biotechnology and Environmental Engineering Research Unit of NANGUI ABROGOUA University (Abidjan, Côte d'Ivoire, West Africa). This site is characterized by a humid tropical climate with an average temperature ranging from 25 to 33 °C and average rainfall from 23 to 525 mm. The experimental set-up consists of four (4) mesocosm beds in the shape of a square-based right block (0.4 m square and 0.6 m high) with a surface area of 0.16 m^2 made of masonry (Fig. 1), two (2) of which have been filled with the same geomaterial (*i.e.* shale or laterite) [substrate]. From bottom to top, the beds consisted of a 0.1 m layer of gravel (5/25 mm) and a 0.4 m layer of substrates consisting of shale or laterite with a grain size of between 1 and 3 mm. The layers of substrates and gravel making up the filtration bed and the treated water drainage bed respectively were separated by a geotextile to prevent the drainage bed from being blocked by the geomaterials. Each bed was fitted with an outlet nozzle ($\Phi = 32$ mm) to drain off the percolation water (treated water).

The beds were planted with *P. purpureum* seedlings (20 cm tall) from nurseries set up for this purpose on the experimental site. Three (3) plant seedlings of equal vigour were transplanted onto each bed, with a spacing of 30 cm between seedlings, relatively similar to that used by Zahui et al. [7]. The beds were fed for three days during the week (*i.e.* Monday, Wednesday and Friday) with no pre-treated domestic wastewater (*i.e.* 6.43 L d^{-1}, corresponding to 40.1 mm d^{-1}), collected from the NANGUI ABROGOUA University wastewater network because of its proximity to the experimental site, but above all to overcome any supply problems and to ensure that the water actually came from the right source. The water was stored in a cubitainer (1 m^3) placed 1.5 m above the ground on supports to supply the beds, as shown in Fig. 1.

2.2. Bed substrate preparation

The geomaterials (shale and laterite) used as substrate were taken from the Lomo-Nord site in central Côte d'Ivoire (6°39'0" N 4°58'60" W). These were crushed to a particle size of between 1 and 3 mm after sieving (Fig. 2). Mineralogical analysis showed that the geomaterials were rich in Al and Fe, with respectively 49.9 % Fe_2O_3 and 30 % Al_2O_3 for the shale and 31.40 % Fe_2O_3 and 66.01 % Al_2O_3 for the laterite.

2.3. Cultivation of P. purpureum in natural environment

To determine the evolution of *P. purpureum* in a natural environment, young stems of the plant were planted at the same date as the constructed wetland beds on the experimental site, with the same spacing of 30 cm between *P. purpureum* plants. The granulometric and physico-chemical characteristics of the soil at the experimental site are given in Table 1. This soil is assumed not to be subject to contamination at the University of NANGUI ABROGOUA.

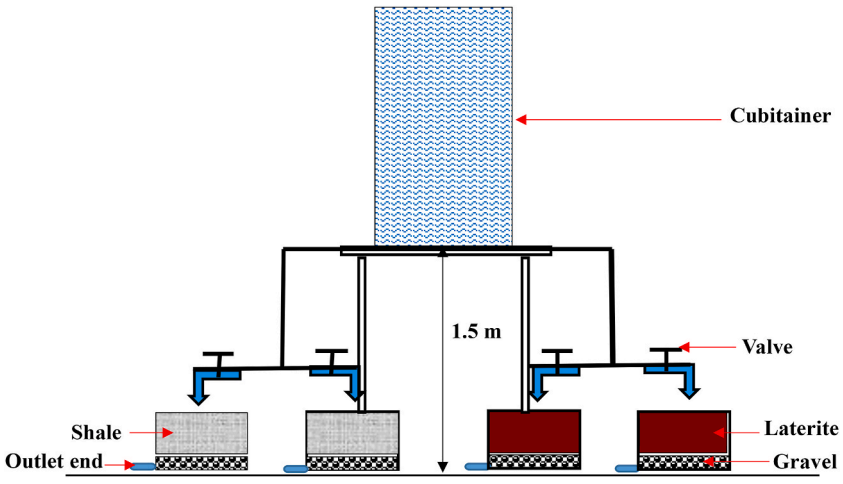

Fig. 1. The configuration of the pilot-scale.

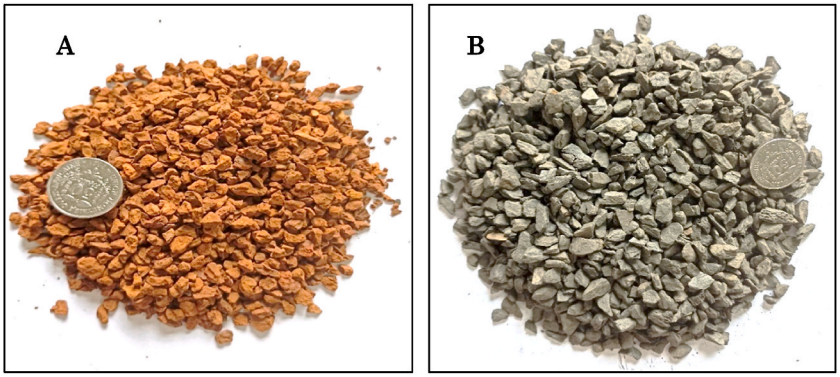

Fig. 2. Filter media used: laterite (A) and shale (B).

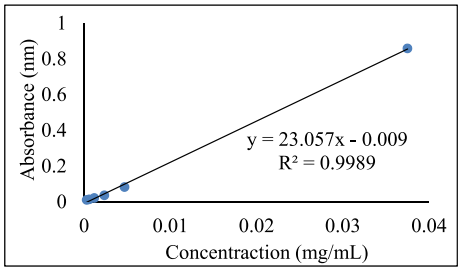

Fig. 3. Calibration curve for total polyphenols.

2.4. Growth response of *P. purpureum*

Plant growth response was monitored by measuring the length of three stems weekly (one from each strain (3) in the bed) using a measuring tape. At the end of the two-month growth cycle of the *P purpureum*, the plants were harvested and the diameter of the plant stumps measured to the nearest millimeter during the harvesting operation. A total of three harvesting sessions were carried out, during which plant above-ground biomass was determined by weighing the total fresh biomass. The production of plant biomass (P) was then calculated according to relationship (1).

$$P = \frac{FB}{S} \quad \text{Eq (1)}$$

With: P: plant biomass ($kg.d^{-1}.\ m^{-2}$); FB: fresh biomass (kg); S: bed surface (m^2).

2.5. Water sampling and analysis of physico-chemical parameters

During the experiment, water samples were taken at regular 15-day intervals at the inlet and outlet of each bed, and stored in polyethylene bottles (1 L) at 4 °C until analysis. A total of 12 samples were taken inlet and outlet of each bed.

pH and dissolved oxygen (DO) were determined in accordance with ISO 10523 [62] and ISO 5814 [63], respectively, while analyzes of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total phosphorus (TP) and total Kjedahl nitrogen (TKN) were determined according to ISO 6060/2 [64], ISO 5815/1 [65], ISO 6878/2 [66] and ISO 5663 [67], respectively.

Finally, purification performances were calculated according to Abissy and Mandi [68] for COD, BOD_5, NTK and TP, using Eq (2).

Table 1
Granulometric and physico-chemical characteristics of natural soil.

Granulometry		
Components	Clay (%)	10
	Silt (%)	18
	Sand (%)	72
physico-chemical		
Parameters	pH	5.6
	Organic matter (%)	10

$$Purification\ performance\ (\%) = \frac{Input\ load(mg) - Output\ load\ (mg)}{Input\ load\ (mg)}\ x\ 100 \quad \text{Eq (2)}$$

with:

Input load = Concentration (mg/L) x Volume of raw wastewater (L) supplied to the bed;

Output load = Concentration (mg/L) x Volume of water (L) returned to the bed outlet.

2.6. Analysis of secondary metabolites

2.6.1. Plant sampling

The above-ground biomass of *P. purpureum* grown on the experimental site and those in the constructed wetland beds were collected at the end of each two-month growth cycle of the plant during the experiment. The samples were then dried in the shade, in a room away from any ultraviolet radiation [69]. After drying, the samples were finely ground using a ball mill to prepare the plant extracts.

2.6.2. Preparation of plant extracts

The hydroalcoholic extract was obtained by macerating for 24 h a mixture of 10 g of plant biomass and 100 mL of 80 % ethanol. After each extraction operation, the mixture obtained was filtered and lyophilized after concentration in a rotary evaporator (rotavapor). The experiment was repeated three (3) times on the residue obtained with the same volume of extraction solvent. Finally, the yield was calculated using the following formula (Eq 3):

$$Extract\ yield(\%) = \frac{Extract\ mass}{Test\ drive} \times 100 \quad \text{Eq (3)}$$

2.6.3. Identification and characterization of chemical constituents

The chemical constituents of the extracts were characterized by tube staining and precipitation reactions. The main chemical groups were characterized using various reagents, in particular Dragendorff's reagent for alkaloids, ferric chloride solution for tannins and polyphenols, sulphuric acid reagent for sterols and terpenes, ammonia reagent for flavonoids, Fehling's reagent for reducing compounds and sodium hydroxide for coumarins [70].

The detection of persistent foam indicated the presence of saponins and the foam index was calculated as a positive reaction (Im = 1/N or N = number of tubes in which the foam height equals 1 cm). The results were interpreted as follows: + positive reaction; - negative reaction [70].

2.6.4. Determination of total polyphenols, flavonoids and tannins

- ***Determination of total polyphenols***

The total polyphenol content was determined using the Folin-Ciocalteu colorimetric method as described by Singleton et al. [71] and Heilerová et al. [72], as follows:

1.5 ml of Na_2CO_3 (17 %, w/v) and 0.5 ml of Folin-Ciocalteu reagent (0.5 N) were added to 1 ml of each extract. The whole set was then incubated at 37 °C for 30 min. A spectrophotometer reading was then taken at 760 nm against a blank without extract, taken as a reference.

Total polyphenols were quantified according to a linear calibration line ($y = 23.057x - 0.009$; $R^2 = 0.99$) [Fig. 3] using an extract of Gallic acid standard at different concentrations (between 0.292 and 37.5 µg/ml) under the same conditions as the sample.

The results were expressed in micrograms of Gallic acid equivalent per gram of dry matter (mg GAE/g DM) of the plant shred. The total polyphenol content (Q) was calculated according to the following relationship (Eq (4)):

$$Q = \frac{Cf \times d}{Ci} \quad \text{Eq (4)}$$

with:

Cf: extract concentration in Gallic acid equivalent (mgEAG/mL); Ci: Concentration of extract analysed (g/mL); d: Dilution factor; Q: Total polyphenol content (mgEAG/g dry matter).

- ***Flavonoids assay***

Total flavonoids were determined using the method of Arvouet-Grand et al. [73] and Doumbia et al. [70]. A mixture of 2 mL of 2 % aluminum chloride ($AlCl_3$) in methanol was added to an equal volume of extract. After 15 min incubation, the reading was taken using a spectrophotometer at 415 nm. Quercetin (0.292–75 µg/L), used as a standard, was used to establish the calibration curve ($y = 32.087 x + 0.0283$; $R^2 = 0.99$) [Fig. 4].

A mixture of 2 mL distilled water and 2 mL $AlCl_3$ (2 %) was used to zero the spectrophotometer. Flavonoid contents expressed as milligrams of Quercetin Equivalent (QE) per 100 mg of dry matter (mg QE/g) were determined by the following Eq (5):

$$Q = \frac{Cf \times d}{Ci} \times 100 \quad \text{Eq (5)}$$

with:

Q: Flavonoid content (mg EQ of 100 mg dry matter); Cf: Reading of the sample concentration (mg/mL EQ) on the standard curve; d: Dilution factor of the sample being measured; Ci: Initial concentration of the stock solution in (g/mL).

- ***Dosage of condensed tannins***

The determination of condensed tannins in the various extracts was carried out according to the method described by Heimler et al. [74]. For 400 µL of each sample, 3 ml of a 4 % methanolic vanillin solution and 1.5 ml of concentrated hydrochloric acid were added. The mixture was then incubated for 15 min and read on a spectrometer at 500 nm.

The concentrations of condensed tannins were deduced from the calibration ranges ($y = 1.8531x + 0.005$; $R^2 = 0.99$ [Fig. 5]) established with catechin (0.146–150 µg/ml), expressed as mg of catechin equivalent per mg of extract, using relationship 6.

$$Q = \frac{Cf \times d}{Ci} \quad \text{Eq (6)}$$

with:

Cf: Concentration of extract in catechin equivalent (µg/mL); Ci: Concentration of extract analysed (EC mg/mL); d: Dilution factor of the sample being measured; Q: Condensed tannin content (mgEC/g dry matter).

2.7. Data analysis

Data analysis was carried out using R studio 3.3. 2, including the Kruskal-Wallis, Mann Whitney, ANOVA and T-test variances after the Shapiro-Wilk test [75] The Shapiro-Wilk test was used to determine the normality of the data, followed by the ANOVA or Kruskal-Wallis test depending on whether or not the data followed a normal distribution. Then, in the event of a significant difference with the latter, the analyses were refined using the T-test and the Mann Whitney test respectively.

3. Results

3.1. Growth and plant production

The average stem heights, aboveground plant biomass and diameter of stumps developed by *Pennisetum purpureum* in the constructed wetlands and in the natural environment are recorded in Table 2. During the three harvest cycles, the average height of the plant in the natural environment (92.9 ± 26.5 cm) was significantly higher than those in the constructed wetlands which were respectively 77.6 ± 25.6 cm in the bed filled with shale and 50.9 ± 23.1 cm in that of laterite (ANOVA test: $p < 0.05$). Similarly, the height reached in the wetlands filled with shale was clearly distinguishable from that of the laterite bed ($p < 0.05$).

Plant biomass and stump diameter were respectively 16.9 ± 0.5 kg m^{-2} and 24.6 ± 2.2 cm in the natural environment, 14.7 ± 0.5 kg m^{-2} and 16.1 ± 1.3 cm in the bed filled with shale and 12.5 ± 0.1 kg m^{-2} and 12.7 ± 1.4 cm in the bed filled with laterite. Statistical analysis showed that the biomass and stump diameter of plants produced in natural environments were significantly greater than those in artificial wetlands ($p < 0.05$). Furthermore, in the wetlands, the biomass and diameter of the stumps were significantly higher in the shale-filled bed ($p < 0.05$).

3.2. Purification performance of artificial wetlands

3.2.1. Physical parameters

Minimum, maximum and average water volumes, as well as pH and dissolved oxygen (DO) values for raw water and filtrates from shale- and laterite-lined beds, are shown in Table 3. During the experiment, the values measured in the raw water varied between 6.8

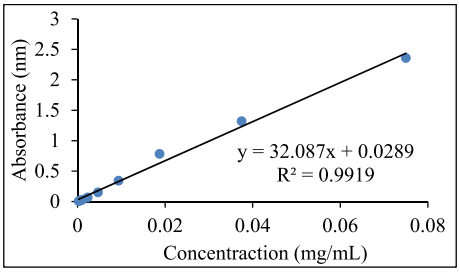

Fig. 4. Calibration curve for total flavonoids.

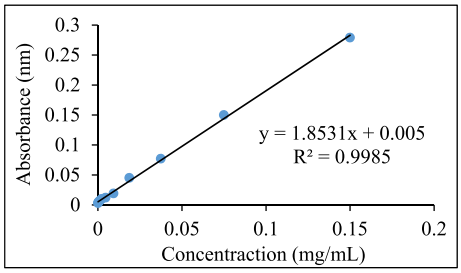

Fig. 5. Calibration curve for condensed tannin.

Table 2
Average stem length, biomass and diameter of stumps developed in constructed wetlands and natural environment.

Parameters	*Pennisetum purpureum* culture medium		
	Natural environment	Bed upholstered in shale	Bed lined with laterite
Stem length (cm)	92.9 ± 26.5^{a}	77.6 ± 25.6^{b}	50.9 ± 23.1^{c}
Above-ground biomass ($Kg.m^{-2}$)	16.9 ± 0.5^{a}	14.7 ± 0.5^{b}	12.5 ± 0.1^{c}
Stump diameter (cm)	24.6 ± 2.2^{a}	16.1 ± 1.3^{b}	12.7 ± 1.4^{c}

Values in the same line followed by the same superscript letter (*i.e.* a, b, c …) are not significantly different at $p > 0.05$.

and 9.5 (pH) and between 1.1 and 3 mg L^{-1} (OD), as shown in Table 3. These values decreased for pH, from 6.4 to 7.8 at the outlet of the shale-filled bed and from 6.8 to 7.8 at the outlet of the laterite-filled bed. However, the average pH value of the raw water was significantly higher than that of the constructed wetland filtrates (*t*-test: $p < 0.05$) and no significant difference was observed between the recorded pH values in the beds filtrates ($p > 0.05$).

Regarding DO, the values measured in filtrates were significantly higher than those of raw water (*t*-test: $p < 0.05$), varying between 4.8 and 9 mg L^{-1} in the filtrate of the shale-filled bed (SFB) and between 3.6 and 8.5 mg L^{-1} in that of the bed filled with laterite (LFB). However, the average DO values in the bed filtrates were not clearly distinguishable (*t*-test: $p > 0.05$).

Observation of the fate of the volumes of water applied showed that the volumes collected at the outlet of the constructed wetlands were well below the application volume (15 L). Furthermore, the volume collected at the outlet of the shale-filled bed (13 ± 0.5 L) was significantly lower than that of the laterite-filled bed (13.6 ± 0.5 L) [Mann Whitney test: $p < 0.05$].

3.2.2. Chemical parameters

Chemical oxygen demand (COD), biological oxygen demand (BOD_5), total Kjedahl nitrogen (TKN) and total phosphorus (TP) in the wastewater were significantly reduced in the filtrates from the constructed wetland beds (Mann Whitney test: $p < 0.05$) [Table 3]. In addition, the purification performance of these parameters was significantly higher in the shale-filled bed (SFB) than in the laterite-filled bed (LFB) [Mann Whitney test: $p < 0.05$].

COD concentrations decreased from 481.3 ± 89.4 mg L^{-1} in the raw water to 92.4 ± 29.5 mg L^{-1} and 118.7 ± 37.3 mg L^{-1}, respectively, in the filtrates from the shale and laterite beds, with purification efficiencies of 83 ± 5.4 % (SFB) and 76.9 ± 7 % (LFB). As for BOD_5, the average concentration measured was 288.6 ± 54.9 $mgO_2.L^{-1}$ in the raw water, compared to 48.3 ± 20.1 $mgO_2.L^{-1}$ at the SFB outlet and 65.4 ± 19.8 $mgO_2.L^{-1}$ at the LFB outlet. Equivalent purification performances for BDO_5 were 84.7 ± 6.8 % in the SFB and 78 ± 8.1 % in the LFB.

With regard to NTK, 143.6 ± 30.8 mg L^{-1} was recorded in raw water, compared to 46.5 ± 20.7 mg L^{-1} and 71.9 ± 32.2 mg L^{-1} in the filtrates from SFB and LFB, respectively. This corresponded to 72.2 ± 10.7 % NTK removal in the SFB and 55.5 ± 16.4 % in the LFB. As for total phosphorus, the concentrations analysed went from 11.8 ± 1.1 mg L^{-1} in the raw water, to 3.7 ± 0.1 mg L^{-1} and 5.4 ± 0.4 mg L^{-1} in the SFB and LFB filtrates, respectively. Total phosphorus purification efficiencies were 72.4 ± 4.9 % (SFB) and 58.4 ± 3.4 (LFB).

3.3. Secondary metabolites synthesised by Pennisetum purpureum

Table 4 shows the extract production rates obtained from plant samples from different *Pennisetum purpureum* growing media (shale bed, laterite bed and natural environment). As seen, the production of extracts with the plant grown in natural environment (20 %) was higher than that of plants grown in constructed wetlands. Additionally, samples of plants grown in laterite-filled beds produced more extract (13 %) than those grown in shale wetlands (11 %).

The phytochemicals revealed by the reactions highlighted the presence of polyphenols, flavonoids, tannins and saponins in all the samples (Table 5). On the other hand, coumarins, reducing compounds, alkaloids and proteins were absent in all samples. Sterols and polyterpenes were only present in the extract of *P. purpureum* grown in the natural environment.

Table 3
Minimum, maximum and average values of parameters and removal rates (R) determined in constructed wetlands.

Parameters		Volume	pH	DO	COD		BOD_5		NTK		TP	
		Value (L)	–	Value ($mg.L^{-1}$)	Value ($mg.L^{-1}$)	R (%)	Value ($mgO_2.L^{-1}$)	R (%)	Value ($mg.L^{-1}$)	R (%)	Value ($mg.L^{-1}$)	R (%)
Raw water	Avg	15^{a}	7.9 ± 0.4^{a}	2.0 ± 0.5^{a}	481.3 ± 89.4^{a}		288.6 ± 54.9^{a}		143.6 ± 30.8^{a}		11.8 ± 1.1^{a}	
	Max		9.5	3	644.8		389.3		194.7		13.8	
	Min		6.8	1.1	297.4		163.5		97.5		10.2	
SFB	Avg	13 ± 0.5^{b}	7.2 ± 0.3^{b}	7.2 ± 1.2^{b}	92.4 ± 29.5^{b}	83 ± 5.4^{a}	48.3 ± 20.1^{b}	84.7 ± 6.8^{a}	46.5 ± 20.7^{b}	72.2 ± 10.7^{a}	3.7 ± 0.1^{b}	72.4 ± 4.9^{a}
	Max	14	7.8	9	133.7	92,1	112.4	96,4	86.4	88,7	4.9	81,7
	Min	12	6.4	4.8	45.8	68,2	15.2	67	16.7	50	2.8	65,6
LFB	Avg	13.6 ± 0.5^{c}	7.3 ± 0.3^{b}	6.4 ± 1.4^{b}	118.7 ± 37.3^{b}	76.9 ± 7^{b}	65.4 ± 19.8^{a}	78 ± 8.1^{b}	71.9 ± 32.2^{b}	55.5 ± 16.4^{b}	5.4 ± 0.4^{c}	58.4 ± 3.2^{b}
	Max	14.5	7.8	8.5	208.1	89,7	105.4	90,4	143.4	80	6.2	65
	Min	13	6.8	3.6	69.6	52,3	38.7	58,2	34.6	30,4	4.4	49,3

Values in the same column followed by the same superscript letter (*i.e.* a, b, c …) are not significantly different at $p > 0.05$. SFB: shale-filled bed, LFB: laterite-filled bed.

Table 4
Production of *Pennisetum purpureum* extracts according to plant culture media.

Plant growing media	Extract production (%)
Shale-filled bed	11
Laterite-filled bed	13
Natural environment	20

3.4. Content of total polyphenols, total flavonoids and condensed tannins

The concentrations of total polyphenols, condensed tannins and total flavonoids in the extracts of plants grown in wetlands and in the natural environment are presented in Fig. 6. Overall, the extracts of *P. purpureum* plants from the natural environment concentrated the highest contents of secondary metabolites (polyphenol [73.5 mgEAG/gMS], total flavonoids [18.1 mgEQ/gMS] and condensed tannins [13.3 mgEC/gMS]).

In the constructed wetlands, extracts from plants grown in shale-filled beds had the highest levels of secondary metabolites. There

Table 5
Phytochemicals in *P. purpureum* grown in constructed wetlands and the natural environment.

Phytochemicals	Plant grown in beds filled with shale	Plant grown in beds filled with laterite	Plant grown in natural environment
Polyphenols	+	+	+
Flavonoides	+	+	+
Coumarins	–	–	–
Tanins	+	+	+
Sterols and Polyterpenes	–	–	+
Saponins	+	+	+
Reducing compound	–	–	–
Alkaloids	–	–	–
Proteins	–	–	–

+: Present; -: Absent.

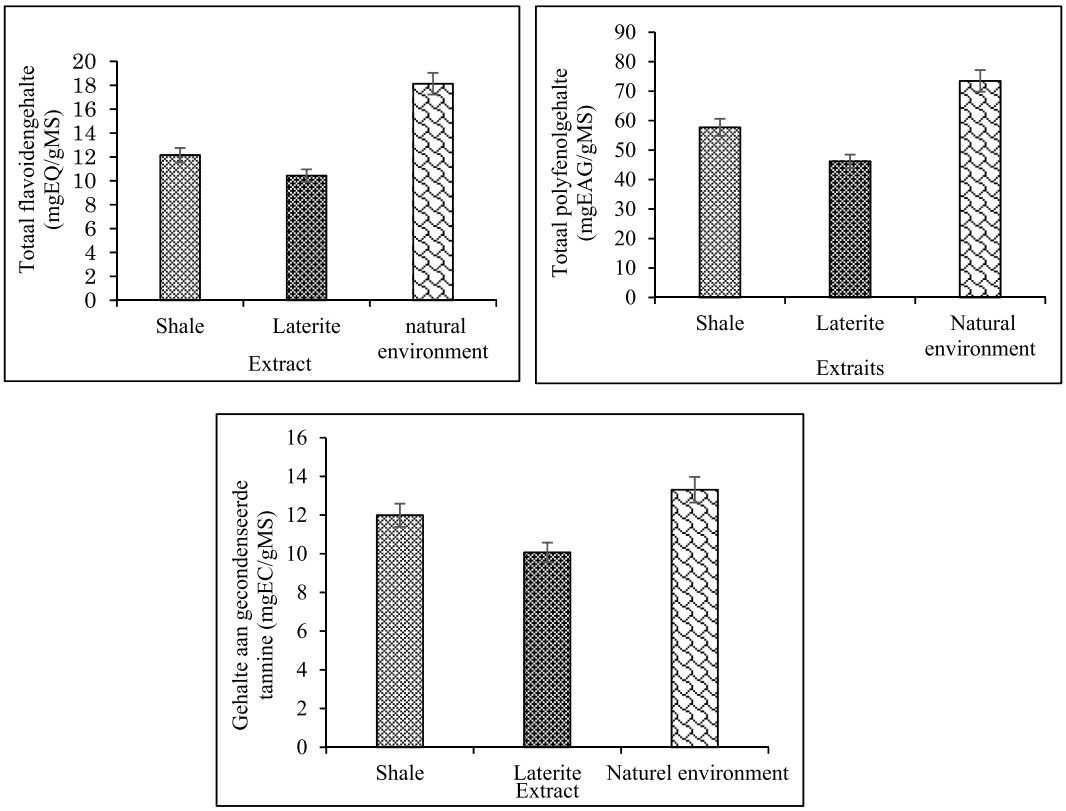

Fig. 6. Secondary metabolites in extracts of *Pennisetum purpureum* grown in the natural environment and wetland beds.

were 57.7 mgEAG/gMS of total polyphenol, 12.1 mgEQ/gMS of total flavonoid and 12 mgEC/gMS of condensed tannin in the plants of shale-filled beds, compared with 46.2 mgEAG/gMS of total polyphenol, 10.4 mgEQ/gMS of total flavonoid and 10.1 mgEC/gMS of condensed tannin respectively in extracts from plants grown in laterite-filled beds. However, quantitative analysis reveals a higher concentration of total polyphenols than tannins and total flavonoids in *P. purpureum* extracts, in both natural and artificial wetlands.

4. Discussion

This study examined the production of secondary metabolites in the extracts of *Pennisetum purpureum* used in constructed wetlands filled with shale and laterite treating domestic wastewater. The results showed that the plants were equally well adapted to the shale-filled and laterite-filled constructed wetlands, as evidenced by the heights achieved (77.6 and 50.9 cm in the shale- and laterite-lined beds respectively) and the above-ground biomass produced (14.7 kg m^{-2} in the shale lite and 12.5 kg m^{-2} in the laterite lite). This is linked to the used nature of the water supplied to the different beds during experimentation, as indicated by Zahui et al. [7]. However, *P. purpureum* grown in the natural environment produced larger stems and above-ground biomass compared with plants in constructed wetlands. This result is due to the more complete richness of the experimental site's soil in organic matter and other minerals essential for plant growth. In addition, the filtration materials used (*i.e.* shale and laterite) would have created competition with plants, due to their tendency to adsorb nutrients such as nitrogen and phosphorus compounds [76,77]. This would have reduced the amount of nutrients readily available to plants in the constructed wetlands [78].

Regarding the elimination of pollutants (COD, BOD_5, NTK and TP) in constructed wetlands, results showed efficiencies beyond 50 % both in beds filled with shale and laterite. These results were made possible on the one hand by the environmental conditions of the constructed wetlands, in particular pH values ranging from 6.8 to 7.8, which would have favored the development of purifying microorganisms. Indeed, the strong involvement of microorganisms in the pollutant degradation process in constructed wetlands has been demonstrated by several studies summarized in the review by Wang et al. [79]. According to the authors, all these studies, which show a general upward trend in the number of annual publications, converge on the idea that microorganisms present in constructed wetlands play an essential role in processes such as pollutant degradation and nutrient transformation. On the other hand, the elimination of COD, BOD_5, NTK and TP could be explained by the physical retention of certain wastewater compounds, observed during experimentation, on the surface of the filter media (*i.e.* schist and laterite) used in the present study. This observation has also been made in a number of studies, including those by Vymazal [80] and Ketema et al. [81], which point the finger at the organic compounds contained in the sludge retained in the filter beds. Also, the good aerial development of *Pennisetum purpureum* in developed wetland beds (between 12.5 $Kg.m^{-2}$ and 14.7 $Kg.m^{-2}$) is evidence of increased uptake of wastewater nutrients (nitrogen and phosphorus) by the plants. Finally, the mineralogical characteristics of shale and laterite, previously determined by Koua-Koffi et al. [54,82], suggest that the adsorption capacities of these materials have been put to use in constructed wetlands. Indeed, apart from the kaolinite present in both materials, the iron and alumina oxides and hydroxides contained in laterite and the other forms of clay (*i.e.* albite and dolomite) found in shale enable them to bind the nitrogen and phosphorus compounds contained in wastewater [54,56,82,83]. These analyses are in line with experiments carried out in batch and column mode by Arias et al. [78] and Volha et al. [84].

Comparing the performance of the constructed wetlands, we note that the shale-filled wetland performed better than the laterite-filled one, both for COD and BOD_5 removal, and NTK and TP. The differences observed could be explained by the physicochemical nature of the materials and their specific surface area [54]. Indeed, shale, in the presence of water becomes friable and breaks up into numerous sheets to transform into a highly plastic or swelling clay [85]. As the clay swells, it exerts additional mechanical pressure on the rock structure, resulting in cracking of varying degrees, which in turn creates space for plant root development. This root development would have been an asset for the shale-filled constructed wetland, especially as it would promote significant secretion of root exudates serving as a complementary energy source for the activity of purifying microorganisms [86,87]. In the laterite-filled bed, the rapid circulation of feed water observed during wastewater treatment would have resulted in a relatively low contact time between the wastewater and the material, as was the case in the work of Ama et al. [57]. On the other hand, because of their difficult penetrability, laterites would only allow the development of plants with weak root systems [88]; this remains a disadvantage for this material in terms of promoting good colonization of microorganisms that purify nutrients and organic matter contained in wastewater [87]. These observations are corroborated by the large above-ground plant biomass, stem length and diameter of *Pennisetum purpureum* recorded in shale beds compared with laterite-equipped beds. Above all, the high performance of the shale-lined constructed wetland compared with the laterite-lined wetland can be explained by the mineralogical composition of the shale, in particular its higher Fe content (49.9 % Fe2O3). Iron oxides, which are more abundant in the shale used, would have fed the iron reducing bacteria and increased the electrons available in the constructed wetland system and consequently favored phosphorus and nitrogen removal, as claimed by Hu et al. [89], for the limonite. These observations were also made by Shen et al. [90] and Zhang et al. [91] with sponge iron, an iron-rich substrate, which promoted the removal of nitrogen compounds and phosphorus in constructed wetlands.

The analysis of phytocompounds in *Pennisetum purpureum* plants grown in natural environments and in beds filled with shale and laterite revealed the absence of certain compounds such as coumarins, reducing compounds, alkaloids and proteins. This absence could be justified, on the one hand, by the chemical composition of the plant and, on the other, by the fact that the latter would not be used by the plants to combat aggressors during the treatment of wastewater [92,93]. Compounds such as polyphenols, flavonoids, tannins and saponins were present in *P. purpureum* plants regardless of the growing medium (natural environment and artificial wetland beds). The presence of these compounds is due to the ability of macrophytes to secrete these inhibitory substances during treatment to eliminate microphytes [37]. Their presence is beneficial for plants, enabling them to fight of aggressors and protect themselves against UV radiation. These compounds also promote stem elongation (flavonoids) and pollen germination (polyphenols) [36]. The observation of the presence of flavonoids, polyphenols and tannins and the absence of other secondary metabolites (*i.e.* alkaloids, sterols and

polyterpenes) in extracts of *P. purpureum* grown in the wetlands of the present study, was also carried out by Ntakiyiruta et al. [37] in their laboratory experiments carried out in both Côte d'Ivoire and Burundi in floating treatment wetlands with *Eichhornia crassipes* and *Pistia stratiotes*.

In terms of the three families of secondary metabolites studied, *P. purpureum* plants grown in natural environments were richer in phytocompounds than those transplanted to constructed wetland beds treating wastewater. This situation could be explained by the natural environmental conditions (temperature, precipitation) of the first medium (soil), and its more complete composition in trace elements necessary for good plant growth. Thus, we note that wastewater treatment tends to reduce secondary metabolite content in plants, which would have used them to combat aggressors and/or to eliminate microphytes (algae), in competition with macrophytes during treatment [37]. Furthermore, the lowest levels of flavonoids, polyphenols and tannins were observed in *P. purpureum* plants in the laterite bed. This low content could be explained by the plant's difficulty in developing on this medium, in view of the growth recorded, given that laterites only allow the development of plants with weak root systems [88].

Furthermore, quantitative analysis of phytocompounds revealed a high concentration of total polyphenol relative to tannins and total flavonoids, irrespective of the geomaterial used in the constructed wetland beds. The high concentration of total polyphenols in *P. purpureum* extracts could be explained by the fact that these are important phyto-micronutrient compounds capable of neutralizing free radicals [94]. Indeed, as fixed organisms in their immediate environment, plants are exposed to a multitude of stresses of both an abiotic (UV radiation, deficiencies and excesses in essential or toxic mineral elements) and biotic (micro-organisms) nature, depending on the nature of the soil and the type of microclimate in which the plants grow [94]. *P. purpureum* would have secreted more of these substances (*i.e.* total polyphenols) as it grew, in order to combat these stresses. These results are in agreement with those of Ayda et al. [95] and Enneb et al. [96], obtained in *Atractylis gummifera* and *Lawsonia inermis* extracts, respectively. Furthermore, the concentrations of total flavonoids obtained in the present study are relatively similar to those obtained in the work of Ntakiyiruta et al. [37], although these were more abundant than the other two compounds (*i.e.* tannins and total polyphenols) in their work. These results are justified by the fact that flavonoids are highly beneficial to plants, given their role in defense processes against bacterial and viral infections, and UV radiation [97]. Flavonoids also act as pigments or co-pigments and regulate stem elongation [98]. Indeed, flavonoids are biologically active molecules with a wide range of biological activities, particularly with regard to plant physiology, growth and development. They not only offer protection against harmful abiotic factors, but also facilitate interaction with other plants and micro-organisms thanks to their physical and biochemical properties. Moreover, in the face of harsh environmental conditions, flavonoids participate in the plant response by effectively regulating cell differentiation and growth [99,100].

5. Conclusions

The study enabled us to characterize the chemical constituents present in *Pennisetum purpureum* extracts used in constructed wetlands filled with shale and laterite. The use of geomaterials as substrate has resulted in good removal of COD, BOD_5, NTK and TP from wastewater. *P. purpureum* adapted well to the different beds and produced optimum plant biomass with the shale-filled reactor (16.9 kg m^{-2}). The highest wastewater pollutant removal efficiencies were achieved in the shale-filled constructed wetland (COD [83 %], BOD_5 [84.7 %], NTK [72.2 %] and PT [72.4 %]). Phytochemical screening showed that wastewater treatment tended to reduce the production of secondary plant metabolites used in constructed wetlands. However, compounds such as polyphenols, flavonoids, tannins and saponins were present in *P. purpureum* plants irrespective of the growing medium (natural environment and constructed wetland beds). These are total polyphenols (73.5 mgEAG/gMS for the natural environment, 57.7 mgEAG/gMS for the shale-filled bed and 46.2 mgEAG/gMS for the laterite-filled bed), total flavonoids (18.1 mgEQ/gMS for the natural environment, 12, 1 mgEQ/gMS for the shale-filled bed and 10.4 mgEQ/gMS for the laterite-filled bed) and condensed tannins (13.3 mgEC/gMS for the natural environment, 12 mgEC/gMS for the shale-filled bed and 10.1 mgEC/gMS for the laterite-filled bed). Thus, the metabolites found in these plants are largely in the form of total polyphenols.

Availability of data and materials

Data will be made available on request.

Funding

This research received no external funding.

CRediT authorship contribution statement

Nadège Fatim Traoré: Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jean-Marie Pétémanagnan Ouattara:** Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Franck Michaël Zahui:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amichalé Jean Cyrille Beda:** Writing – original draft, Software, Investigation, Formal analysis. **Aman Messou:** Writing – original draft, Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was carried out at the experimental site of the biotechnology and environmental engineering research unit of NANGUI ABROGOUA University (Abidjan, Côte d'Ivoire). We thank all the members of this unit for their criticism. We would also like to thank the Director of the central analysis laboratory at NANGUI ABROGOUA University (Abidjan) and at the University of Man, for carrying out all the analyses in this study.

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