

High Strength Wastewater Reclamation Capacity of *Vetiver Grass* in Tropics: The Case of Ethiopia

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ABSTRACT: It is generally accepted that industrial wastewater like tannery effluent is high strength wastewater. The aim of this study was to examine the capacity of *Vetiver grass* for the treatment of high strength wastewater in a constructed wetland. Two constructed wetland beds were designed and one of them was not planted used as a control group. The grass was planted with 20 cm by 20 cm distance from each seedling. The biometric characteristics of *Vetiver grass* was evaluated by taking randomly selected clusters of the grass. The concentration of chromium in the extract of parts of the grass was determined by atomic absorption spectrophotometer. The Chromium bioaccumulation and Translocation factor was estimated. Composite samples before and after treatment of 4 different hydraulic retention time was collected. The physiochemical analysis of the wastewater has been carried out. The constructed wetland bed with *Vetiver grass* performed that, BOD, COD, NH₄-N, NO₃-N, TN, PO₄-P, and TP were reduced at the retention time of 9 days by 91.9%, 96.3%, 62%, 86%, 88.7%, 96.3%, and 92.2% respectively. Chromium was also reduced by 97% at retention time of both 7 and 9 days over the planted bed. The bed with plant performs significantly better than without plant at *P*-value <.01. Therefore, *Vetiver grass* has a capacity to reclaim high strength industrial wastewater in tropical areas.

KEYWORDS: Wastewater, constructed wetland, *Vetiver grass*, retention time, physiochemical analysis

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Introduction

Industrial wastewater is the most common source of environmental pollution in the world. It is discharged commonly with organic matters, hydrocarbons, nutrients, heavy metals, toxins, and salts which may pollute receiving waters rendering them unsuitable as a community water supply or pose a threat to aquatic life and surrounding environment at large United Nations World Water Assessment Programme (WWAP).¹

Industrial wastewaters like tannery effluents are high strength wastewater than domestic sewage, and relatively more difficult to treat. The tannery effluents apart from the most toxic heavy metals like Chromium (Cr), it contains chemical impurities mostly dissolved substances such as inorganic salt cations (Fe, Zn, Cu, Ca, Na, etc.); anions such as SO₄²⁻, NO₃⁻, PO₄³⁻ and also physicochemical parameters such as, COD, TSS, TDS, etc. with high concentration.²

In the world, much wastewater is being generated and most of them, especially in developing countries are not treated before discharging into the environment. An estimated of 90% wastewater in developing countries like Ethiopia is still discharged directly to rivers and streams without any waste processing treatment or after retention period of some hours in stabilization pond.³ Aquatic ecosystems are used either directly or indirectly as recipients of potentially toxic liquids from domestic, agricultural, and industrial wastes.⁴

A study finding in Ethiopia showed that industrial wastewater discharged without any treatment had an extreme harmful effect to aquatic flora, fauna, and to human beings through

food chains. In this study again, the authors concluded that the effluent treatment ponds of the textile factory do not efficiently reduce the chemicals in the discharge. The observed effects of the “treated effluent” on phytoplankton biomass and fish fry indicate the undesirable effects the effluent will have on Lake Hawassa. This finding is also an evident for most of industrial wastewater discharges to the nearby environment either not treated or partially treated which indicates that, the need for efficient and affordable industrial wastewater treatment technology in the country.⁵

The wastewater treatment technologies can be grouped into 2 main systems: Conventional systems such as activated sludge and trickling filters and the second systems are non-conventional such as waste stabilization ponds (WSP) and constructed wetlands (CW). Conventional methods are mostly used in developed nations while non-conventional methods are increasingly used in developing ones because of cost implication. Non-conventional methods (ie, WSP and CW) have been used worldwide to treat wastewater with good performance.⁶ The systems are generally inexpensive to construct, operate, and maintain, they are of low energy consumption, have high pollutant removal efficiencies and have ability to treat different types of wastewater from various sources.⁷

Pollutants are removed in constructed wetlands by several complex physical, chemical, and biological processes. However, constructed wetland is not complete and perfect to treat especially high strength wastewater since the contribution and potential of substrates is not considered, for example the removal of



total phosphorus varied between only 40% and 60% in all types of constructed wetlands using the sand and gravel as a substrates.⁸

As Belmont et al⁹ and Chen et al¹⁰ pointed out, constructed wetland used as a wastewater treatment technologies have a good potential to effectively remove nutrients, heavy metals, organic pollutants, suspended solids, and pathogenic organisms. This effective pollutant removal efficiency is directly related to several mechanisms in the constructed wetland systems. Mainly sedimentation, filtration, volatilization, adsorption, plant uptake, and bacterial activity are involved.¹¹⁻¹³ In designing the good constructed wetland, the main biological component in the system is aquatic plants (Vetiver grass in this case). However, it is important in determining the appropriate plant species that can survive in the wastewater environment, especially if the intended wastewater is high strength like tannery wastewater, because only suitable plant species can treat a highly concentrated pollutant in the wastewater.¹⁴

On the other hand, the Vetiver is a unique tropical plant that has been proven and used in some 100 countries for soil and water conservation, land rehabilitation, pollution control, water quality improvement and many other environmental applications.¹⁵ It is characterized by its large biomass and having a dense root system extending up to 3 m in depth. Vetiver system is based on the use of Vetiver grass, which was first recognized for having "super absorbent" characteristics suitable for the treatment of wastewater and leachate generated from landfill.¹⁶

Taking into account the growth of industrialization in Ethiopia and the expected demand for wastewater treatment, low-cost, locally available, appropriate and ecofriendly approaches play an important role in the development of high strength wastewater treatment technology in the country. In this practical approach, this study investigated the principle and design of subsurface flow constructed wetlands for the efficient treatment of high strength wastewater focusing on tannery wastewater by using Vetiver grass as constructed wetland plant.

Method and Materials

Description of the study site

The study was conducted at Modjo town found in Oromiya regional state, east Shewa zone, Ethiopia (Figure 1).

Since most of the tanning industries in Ethiopia located at Modjo town, Modjo tannery was selected for this study purposely which is located at Modjo town 70 km far south east direction from the capital city Addis Ababa, Ethiopia. The daily wastewater discharge of the tannery during this study was around 400 m³/day. The wastewater generation has 3 separate streams; these are general wastewater, chromium containing wastewater, and sulfide containing wastewater.

Experimental design and setup

Experimental design. Comparative experimental study design was carried out to evaluate high strength wastewater reclamation capacity of *Vetiver grass* (*Chrysopogon zizanioides*) with

gravel substrate in the horizontal subsurface flow constructed wetland (Figure 2).

Experimental setup. Two pilot-scale horizontal subsurface flow constructed wetland beds were established with 4 m length, 0.8 m width, and 0.8 m depth. Each cell was plastered with cement and covered with plastic materials to protect the ground water contamination. Each constructed wetland bed was connected with the equalization tank by pipe and fixed 3/4 inch flow control valve (Figure 2).

Each cell filled with gravel substrate in which one of them were planted with the Vetiver grass (*Chrysopogon zizanioides*) and the other was not planted used as a control. The effective size of the substrates were determined, it was 5 to 17 mm this is in line with study done by Alemu et al¹⁷ which was ranged 6 to 20 mm.

Operation of the pilot-scale constructed wetland beds

The grass was collected from Holeta agricultural research institute west Shewa, Oromiya regional state Ethiopia and transported to the study site. Prior to the real experiment, the plant specimen was taken and species identification was carried out in Addis Ababa University national herbarium unit and the plant was confirmed as *Chrysopogon zizanioides* (Vetiver grass).

The grass was planted in one of the bed with 20 cm by 20 cm distance from each seedling. It was grown for the first 1 month with tap water and then 75:25 tap water and wastewater ratio for the next 1 month and then 50:50 ratio for the other 3 months finally after 5 months nursery and adaptation period the experiment was started using tannery wastewater only without dilution.

Wastewater parameters: The analyzed industrial wastewater parameters were DO, BOD₅, COD, TSS, TDS, NH₄-N, NO₂-N, NO₃-N, Total nitrogen (TN), PO₄-P, Total phosphorus (TP), Chloride, Sulfide, Sulfate, Total chromium, pH, salinity, and EC.

Sample collection methods: The wastewater from different section of Modjo tannery were collected and directed to the sedimentation tank by passing through screening chamber. After 24 hour the wastewater pumped from the sedimentation tank to the equalization tank then based on the 4 different hydraulic retention time, the flow rate was adjusted and fed to the constructed wetland beds. Composite samples before and after treatment of 3, 5, 7, and 9 days hydraulic retention times were collected. The samples were preserved and transported to the laboratory by acidification with concentrated sulfuric acid in glass bottles for COD test and by freezing in Polyethylene containers for other parameters. Samples have been acidified at the time of collection with concentrated HNO₃ for acid digestion of the sample to heavy metals analysis.

Wastewater sample analysis: Onsite measurement of the wastewater like temperature, pH, and DO were carried out at the site in the tannery environmental quality control laboratory using portable pH meter (Wagtech International N374, M128/03IM, USA) and DO meter (Hach P/N HQ30d, Loveland, CO, USA) for Dissolved oxygen and temperature.

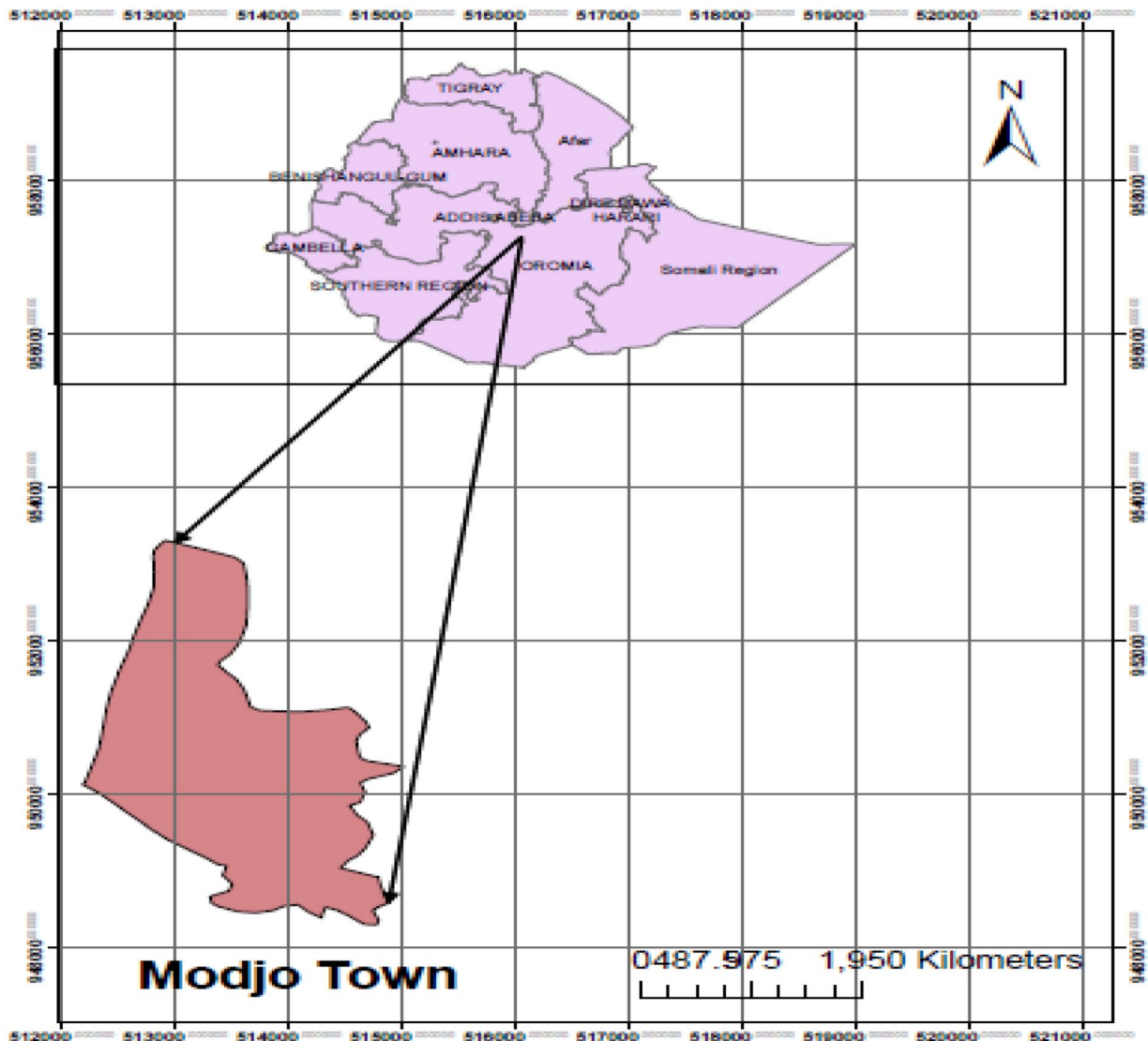


Figure 1. Map of study area.

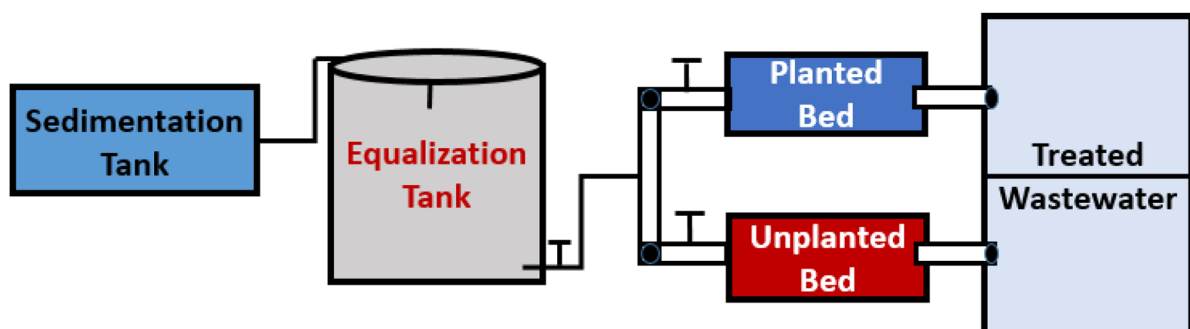


Figure 2. Schematic diagram of the horizontal subsurface constructed wetland.

The BOD sensor and inductive stirring system AQUA LYTIC model type ET618-4 and Open Reflux Titrimetric Method were used to determine BOD and COD respectively. For NH_4-N , NO_2-N , NO_3-N , TN, PO_4-P , and TP, TSS, and TDS, Chloride, Sulfide, and Sulfate analysis, Phenate method, Diazotization, Phenoldisulfonic Acid, Persulfate method, Stannous Chloride, Vanadomolybdo, and acid digestion method, Gravimetric method, Argentometric Method, Methylene blue

method, and Gravimetric with Ignition of residual, used respectively by using Spectrophotometer (Hach model DR/3900 portable spectrophotometer, Loveland, USA). EC and Salinity also determined using laboratory method with platinum-electrode type and electrical conductivity method. Chromium was analyzed by acid digestion for wastewater sample and ash method for plant and substrate samples using Flame Atomic Absorption Spectrophotometer (AAS), (model AAS NOUA-400, Germany).

Table 1. Input wastewater characteristics (concentrations are in mg/L, except pH, salinity, and EC).

S. NO	PARAMETERS	MEAN	RANGE
1	pH	10.166 ± 2.02	8-12
2	BOD ₅	1641 ± 373.55	1243-1984
3	COD	6953.333 ± 339.41	6593-7267
4	NH ₄ -N	253.3333 ± 11.6	240-261
5	NO ₃ -N	116.6667 ± 26.63	94-146
6	TN	650.3333 ± 93.62	546-727
7	PO ₄ -P	88.06667 ± 40.77	46.5-128
8	TP	144.5333 ± 20.75	128.6-168
9	Sulfide	241.3333 ± 101.16	137-339
10	Sulfate	1072.817 ± 352.74	668.86-1320
11	Chloride	1919 ± 1271.6	657-3200
12	TSS	1868 ± 863.1	1217-2847
13	TDS	5877.3 ± 2294.77	3250-7489
14	EC (μs/cm)	8550 ± 2089	6540-10710
15	Salinity (%)	0.69 ± 0.22	0.44-0.88
16	[Cr] _T	18.33 ± 6.66	14-26

These analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater.¹⁸ The raw tannery wastewater was examined using the above procedures used as an input to evaluate the efficiency of the designed horizontal subsurface flow constructed wetland (Table 1).

Plant sample analysis

The biometric characteristics of *Chrysopogon zizanioides* in the Horizontal subsurface flow constructed wetland (HSSFCW)



Figure 3. Vetiver grass (*Chrysopogon zizanioides*): Photo Mekonnen Birhanie Aregu.

was evaluated by taking randomly selected clusters of the grass from near to the wastewater inlet point, central, and outlet point (Figure 3). The plants were manually dug, washed properly with tap water, followed by distilled water to remove adsorbed soil particulates, trimmed carefully to separate root and shoot part of the plant, dry in a direct sunlight for more than 1 month first (Figure 3) and finally an oven dry was done at 65°C until constant weight is obtained then the dry biomass was measured on digital weight scale.

The chromium accumulation capacity of the grass was determined by separating dried plant parts into above and below the ground levels of the substrate. The concentration of chromium in the extract was determined by atomic absorption spectrophotometer (AAS) using standard methods.

Based on the result found from the biometry data of the plant, The Cr bioaccumulation factor (*BAF*) and Translocation factor (*TF*) of *Chrysopogon zizanioides* plant species was estimated (as a method described by Baker et al¹⁹ and Shanker et al²⁰):

$$BAF = \frac{mg\ Cr / kg\ dw\ plant}{mg\ Cr / L\ Wastewater}$$

Translocation factor of Cr was also calculated as:

$$TF = \frac{mg\ Cr / kg\ dw\ above\ ground}{mg\ Cr / kg\ dw\ below\ ground}$$

Data analysis: Mean and standard deviations were calculated to estimate the concentration of each parameter of the samples. The pollutant removal efficiency of constructed wetland beds were determined by the formula:

$$Removal\% = \frac{(C_0 - C_t)}{C_0} \times 100$$

Where C_0 is the parameter concentration in the untreated wastewater and C_t is the parameter concentration in the treated wastewater at the hydraulic retention time t .

Statistical analysis

The hypothesis was tested by 2 sample *t*-test, using R statistical software and Originlab: R version 3.2.2 (2015-08-14), Platform: x86_64-w64-mingw32/x64 (64-bit) to determine whether an observed difference between the means of the groups is statistically significant or not.

Result and Discussion

Wastewater treatment efficiency of constructed wetland bed with and without Vetiver grass

Two equal sized beds were prepared; one of the beds was randomly selected and planted with Vetiver grass. The treatment potential of this plant was assessed using both control unite (bed without plant) and the study unite (bed planted with the grass).

After 6 months, the harvested root and shoot length measurement showed that, the mean root length below the substrate top surface was 43.33 cm and the height of the shoot above the top level of the substrate was 58.67 cm, which indicates the root-shoot ratio of 0.74. The growth of the plant in this bed was limited compared to grown in a normal fertile soil because, the main characteristics of this plant is having the vertical root growth which is difficult to pass through the gravel bed.¹⁶

Based on the result the dry wet of the root was 80 g and the shoot was 77 g. The dry mass ratio of the root to shoot was 1.04 in this constructed wetland bed. The reason for this may be the growth limitation of below the substrate (root) vertically may be allowed to the formation of abundant root network branch and making tufted which was weighed greater than the shoot (above the top substrate level).^{16,21}

BOD₅, COD, and TSS removal efficiency of constructed wetland bed with and without plant

Based on the finding, the concentration of BOD₅, COD, and TSS of the treated tannery wastewater in gravel bed without plant were (319 ± 35.8, 639.33 ± 46.7, and 105 ± 14.18) and (310.33 ± 35.64, 607 ± 15.87, and 86.33 ± 5.51) at hydraulic retention time (HRT) 3 and 5 days respectively. Which is all of the parameters are out of the standard limit value in both hydraulic retention times. Whereas at HRT 7 and 9 days it was observed that at 7 days (296.67 ± 20.81, 576 ± 40.95, and 84 ± 7.21 mg/L) and 9 days (272.67 ± 20.5, 548 ± 124.77, and 80.67 ± 5.69 mg/L).

On the other hand the treated wastewater in a constructed wetland bed with the same substrate and planted with Vetiver grass (*Chrysopogon zizanioides*) showed that the concentration of BOD₅, COD, and TSS at 3 days HRT (179.66 ± 13, 430 ± 55.67, and 85.66 ± 10.6), at 5 days (171 ± 8.54, 291.66 ± 23.7, and 76 ± 8.54 mg/L), at 7 days (148.33 ± 12.58, 282 ± 15.1, and 73 ± 2.64 mg/L), and at 9 days (133.67 ± 18.22, 255.33 ± 37.16, and 70 ± 8.19 mg/L). In this case the BOD₅, and COD concentration of the treated tannery wastewater with gravel bed and the plant Vetiver grass was within the permissible discharge limit value by WHO²² and Ethiopian

Environmental Protection Authority (EEPA)²³ but still TSS was not under the allowable value (Table 2).

The respective percentage removal of those 3 variables in this planted bed was (89.05%, 93.82%, and 95.41%), (89.58%, 95.81%, and 95.93%), (90.96%, 95.94%, and 96.09%), and (91.85%, 96.33%, and 96.25%) after 3, 5, 7, and 9 days retention time respectively (Figure 4).

This result is similar with study done in Kenya, the Vetiver planted subsurface constructed wetland achieved 82.4% COD removal and 94.6% TSS removal.²⁴ Another author also obtain 84%, 92.4%, and 95.3% COD removal at 3, 5, 7 days retention time respectively, which indicates increasing efficiency with retention time that attributed better contact time for microbial degradation of organic matters.²⁵

As this finding showed that, the selected plant (Vetiver grass) was very effective to remove both BOD₅ and COD. In this case this result is proven by previous studies such as a study done in India, observed that the wastewater treatment using Vetiver system had significant potential to reclaim the wastewater. The Vetiver system was able to remove 80% to 85% of BOD₅, 85% to 90% of COD.²⁶

Nutrient removal efficiency of constructed wetland bed with and without plant

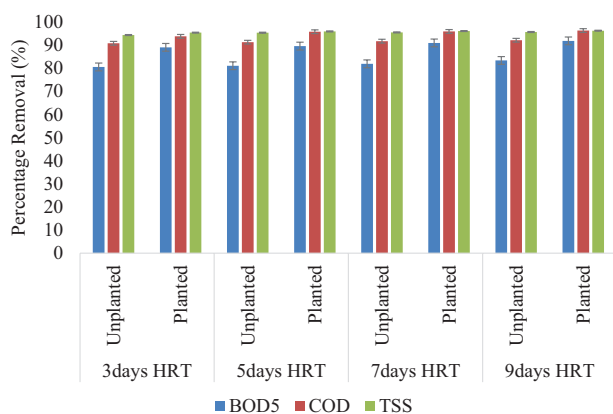
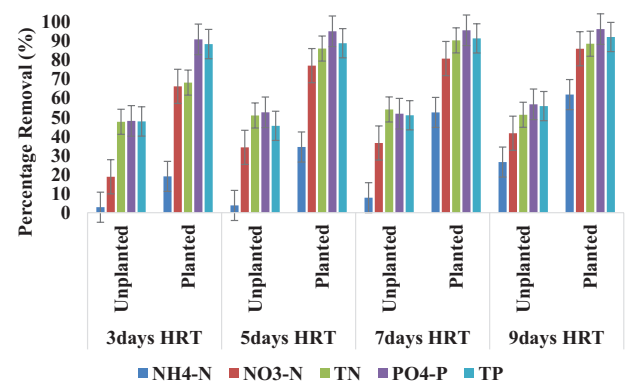
The nutrient concentration of the treated effluent through gravel bed without plant also measured and found to be NH₄-N, NO₃-N, and PO₄-P were at HRT 3 days (246 ± 10.15, 94.66 ± 7.37, and 45.66 ± 2.51 mg/L), 5 days (243.66 ± 50.8, 76.66 ± 15.27, and 41.66 ± 7.1 mg/L), 7 days (233.33 ± 30.55, 74 ± 8.18, and 42.33 ± 11.37 mg/L), and 9 days (186 ± 10, 68 ± 3.6, and 38 ± 5.3 mg/L). This result indicated that all the nutrient parameters were 6 to 8 fold greater than the maximum allowable treated effluent value.

The better results were shown from treated effluent by gravel substrate bed planted with Vetiver grass. In this case the concentration of NH₄-N, NO₃-N, and PO₄-P of the effluent were after 3 days (205 ± 51.9, 39.33 ± 5.51, and 8 ± 4 mg/L), after 5 days (166 ± 11.53, 26.66 ± 2.1, and 4.23 ± 0.49 mg/L), after 7 days (120 ± 6, 22.33 ± 3.51, and 3.8 ± 0.8 mg/L), and after 9 days (96.33 ± 3.81, 16.33 ± 1.51, and 3.23 ± 0.25 mg/L). This effluent from gravel bed planted with the grass showed better quality in terms of nutrient concentration reduction than the bed without plant. For example NO₃-N and PO₄-P were reduced by 86% and 96.33% respectively over 9 days retention time (Figure 5). The better nutrient removal efficiency achieved in the planted bed than unplanted may be due to the phosphorus and nitrogen uptake capacity of Vetiver grass this is supported by the study done by Otieno et al.²⁴

Another recent study also evidenced that, both the absorption capacity of *P. Australis* in the aerial and root parts and the adsorption capacity of substrates (gravel and sand) were analyzed. Results showed that the concentrations of TP decreased in the wastewater at 3 days HRT, showing removal efficiency values of 78%.²⁷

Table 2. Characteristics of treated wastewater in constructed wetland bed with Vetiver grass at different HRT.

PARAMETERS	INFLUENT (MG/L, EXCEPT PH, EC, AND SALINITY)	EFFLUENT CONCENTRATION AT DIFFERENT HRT (MG/L, EXCEPT PH, EC, AND SALINITY)				EEPA AND WHO IRRIGATION WQS
		3D	5D	7D	9D	
pH	10.16 ± 2.02	6.66 ± 0.76	6.85 ± 0.57	7.17 ± 1.04	7.67 ± 1.53	6-9
BOD ₅	1641 ± 373.55	179.66 ± 13	171 ± 8.54	148.33 ± 12.58	133.67 ± 18.22	200
COD	6953.33 ± 339.41	430 ± 55.67	291.66 ± 23.7	282 ± 15.1	255.33 ± 37.16	500
NH ₄ -N	253.33 ± 11.6	205 ± 51.9	166 ± 11.53	120 ± 6	96.33 ± 3.81	30
NO ₃ -N	116.66 ± 26.63	39.33 ± 5.51	26.66 ± 2.1	22.33 ± 3.51	16.33 ± 1.51	10
TN	650.33 ± 93.62	206.33 ± 10	90.33 ± 6.66	62.33 ± 8.02	73.67 ± 8.48	60
PO ₄ -P	88.06 ± 40.77	8 ± 4	4.23 ± 0.49	3.8 ± 0.8	3.23 ± 0.25	5
TP	144.53 ± 20.75	16.66 ± 1.15	16 ± 1	12.33 ± 2.08	11.33 ± 2.11	10
Sulfide	241.33 ± 101.16	9.4 ± 0.7	3.86 ± 1.71	3.63 ± 0.35	7.2 ± 1.71	1
Sulfate	1072.82 ± 352.74	370.55 ± 12	115.66 ± 8.14	103.67 ± 7.37	114.13 ± 31.22	—
Chloride	1919 ± 1271.6	595.66 ± 53.35	530 ± 45.82	533.33 ± 45.1	686.67 ± 20.81	1000
TSS	1868 ± 863.1	85.66 ± 10.6	76 ± 8.54	73 ± 2.64	70 ± 8.19	50/600
TDS	5877.3 ± 2294.77	1177.66 ± 1006.6	1178.33 ± 17.1	1060 ± 52.85	1103.33 ± 23.10	2100
EC (μs/cm)	8550 ± 2089	2307.66 ± 2080.6	1982 ± 9.16	1785 ± 170.88	1820.33 ± 10.80	1200
Salinity (%)	0.69 ± 0.22	0.25 ± 0.05	0.25 ± 0.01	0.24 ± 0.03	0.25 ± 0.03	—
TCr	18.33 ± 6.66	0.66 ± 0.32	0.5 ± 0.10	0.47 ± 0.15	0.5 ± 0.1	2

**Figure 4.** BOD₅, COD, and TSS removal efficiency of planted and unplanted bed.**Figure 5.** Nutrient removal efficiency of planted and unplanted constructed wetland bed.

In this case the hydraulic retention time has an impact in the reduction of nutrients from the wastewater in the horizontal subsurface flow constructed wetland similar trend shown on the study of hydraulic retention time and loading rates.²⁵ However, the concentration of phosphate was under the standard value except 3 days hydraulic retention time and total phosphorus also at the borderline at 9 days HRT.

Sulfur and chloride removal efficiency of gravel bed with and without the grass

The sulfide, sulfate, and chloride concentration of the effluent from gravel bed without plant were at 3 days HRT (22.66 ± 9.3 , 321.28 ± 61.77 , and 1251.33 ± 257.59 mg/L), at 5 days (20 ± 2.64 , 297.66 ± 38.55 , and 1264.33 ± 31.10 mg/L), at 7 days (20.33 ± 3.5 , 313 ± 13.11 , and 1292.67 ± 56.95 mg/L), and at 9 days

(30.33 ± 6.78 , 307 ± 10.81 , and 1214.33 ± 102.50 mg/L). These values didn't meet the standards discharge limit set by both EEPA²³ and WHO.²²

Similar measurement was done on the sample taken from the gravel bed planted with the grass, the effluent concentration of those parameters (sulfide, sulfate, and chloride) were at 3 days (9.4 ± 0.7 , 370.55 ± 12 , and 595.66 ± 53.35), at 5 days (3.86 ± 1.71 , 115.66 ± 8.14 , and 530 ± 45.82 mg/L), at 7 days (3.63 ± 0.35 , 103.67 ± 7.37 , and 533.33 ± 45.10 mg/L), and at 9 days (7.2 ± 1.71 , 114.13 ± 31.22 , and 686.67 ± 20.81 mg/L).

The result of this treatment wetland showed that chloride reduction achieved from (64.22% at 9 days HRT to 72.38% at 5 days HRT) but in all hydraulic retention time the concentration of chloride were under the standard discharge limit value given by both EEPA²³ and WHO.²² A study finding also showed that 82.6% chloride removal efficiency of constructed wetland planted with aquatic macrophytes *Eichhornia crassipes* from textile wastewater.²⁸

The concentration of sulfide after 7 days retention time was reached to 3.63 ± 0.35 mg/L resulted in reduction of 98.49% which is nearest to the standard value of 1 mg/L. But the highest reduction of sulfate at 7 days retention time and was 90.34%. A Study done in Ethiopia and reported on the removal efficiency of sulfate and sulfide, by the constructed wetland planted with *phragmites karka* were 71.8% and 88.7% respectively that yields sulfate and sulfide in the final treated effluent were 88 ± 120 and 0.4 ± 0.44 mg/L respectively.¹⁷ This efficiency difference may be due to the plant type and the strength of the wastewater enter to the constructed wetland beds.

EC, TDS, and salinity reduction efficiency of constructed wetland with and without *Vetiver grass*

TDS (mg/L), EC ($\mu\text{s}/\text{cm}$), and Salinity (%) of the effluent from gravel bed without plant were also determined and found to be at 3 days (2149.33 ± 106.59 , 3955.66 ± 521.38 , and 0.28 ± 0.06), at 5 days (2152.66 ± 110.82 , 3668.33 ± 293.48 , and 0.30 ± 0.06), at 7 days (2047.67 ± 52.24 , 3484.67 ± 54.24 , and 0.3 ± 0.04), and at 9 days (2028.33 ± 10.42 , 3547.67 ± 95 , and 0.32 ± 0.07). TDS after 7 and 9 days retention time reduced by 65.16% and 65.49% respectively, which was under the standard but both electric conductivity and salinity were out of it at all hydraulic retention time, however the maximum reduction of EC and salinity were (59.24% and 59.42% at HRT of 7 and 3 days).

The concentration of these wastewater parameters from gravel bed with the plant effluent were at 3 days HRT (1177.66 ± 1006.6 , 2307.66 ± 2080.6 , and 0.25 ± 0.05), at 5 days (1178.33 ± 17.10 , 1982 ± 9.16 , and 0.25 ± 0.01), at 7 days (1060 ± 52.85 , 1785 ± 170.88 , and 0.24 ± 0.03), and at 9 days (1103.33 ± 23.10 , 1820.33 ± 10.80 , and 0.25 ± 0.03). TDS was much lower than the standard value of 2100 mg/L at all retention time. The maximum TDS, EC, and salinity reduction potential were seen at retention time of 7 days 86.96%, 79.12%,

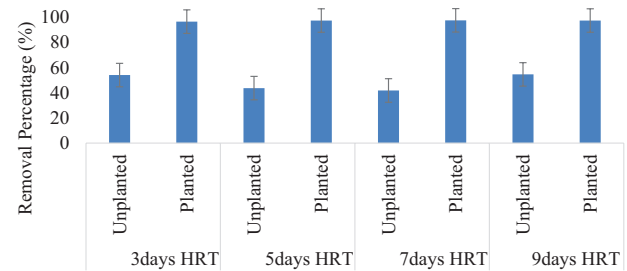


Figure 6. Chromium removal efficiency of planted and unplanted constructed wetland bed.

and 65.22% respectively. This result is supported with the findings obtained from constructed wetland treatment study using aquatic macrophytes which shows 90.2% and 87.2% reduction of TDS and EC respectively from textile wastewater.²⁸

Chromium removal efficiency of constructed wetland bed with and without plant

Phytoremediation is considered an innovative, economical, and environmentally compatible solution for remediating some of heavy metal contaminated sites. It is the use of green plants to clean-up contaminated soils, sediments, or water. In this study the expected heavy metal (chromium) from treated tannery wastewater was evaluated and based on the result, the effluent concentration of total chromium from gravel bed without plant at different hydraulic retention time 3, 5, 7, and 9 days were (8.43 ± 1.1 , 10.33 ± 3.21 , 10.67 ± 2.1 , and 8.33 ± 3.2 mg/L) respectively, which accounts from 41.79% at 7 days HRT to 54.56% at 9 days HRT. Whereas its effluent concentration of gravel bed with plant at 3, 5, 7, and 9 days found to be (0.66 ± 0.32 , 0.5 ± 0.1 , 0.47 ± 0.15 , and 0.5 ± 0.1 mg/L) respectively (Table 2).

The heavy metals like chromium removal mechanisms in a horizontal subsurface flow constructed wetland include interactions between several wetland elements, such as the plant biomass (roots, stems, and leaves), biofilm, substrates, and water.²⁹ In this study the maximum total chromium reduction was seen by 97.44% at 7 days retention time and the minimum was 96.40% at the first 3 days retention time by the planted bed (Figure 6) but all are in-line with the standard limit value. This result clearly showed that in all hydraulic retention time the concentration of total chromium from the bed without plant were beyond the standard allowable value (2 mg/L) and the reverse was true from the bed planted with *Vetiver grass*.

The better efficiency of the planted bed was supported by the previous research findings done by Amenu³⁰ in the tannery wastewater analysis showed that, the total chromium was reduced up to 99.3% for an inlet average total chromium loading rate of 40 mg/L. This result difference between the planted and unplanted bed might be depend on the metal uptake capacity of the *Vetiver grass*.

A study conducted in Greece on 2 horizontal subsurface flow pilot scale units and 2 vertical flow pilot-scale units each one of them was used as a control filled with gravel substrate

and the other one as a study unit filled same gravel size and planted with *cattail*. The applied hydraulic retention times (HRTs) were 6, 8, and 20 days. According to the findings, horizontal subsurface flow constructed wetland demonstrated higher removal capacities in comparison to the vertical one while in both types the planted units indicated better performance compared to the unplanted ones.³¹

The efficiency of horizontal subsurface flow constructed wetland for the treatment of tannery wastewater by considering different parameters were determined and compared between unplanted as a control unit and planted bed with *Vetiver grass* as a study unit. The result showed quite different findings for different variables at different hydraulic retention time but the overall treatment efficiency considering all the selected wastewater parameters for this study from all 4 hydraulic retention time was tested statistically for their significance difference using 2 sample t-test statistics. Based on the test statistics, the constructed wetland bed planted with *Vetiver grass* perform better than the unplanted bed significantly at P -value $< .01$.

Study done previously on the potential of vetiver system (VS) for the treatment of wastewater showed that the wastewater treatment using vetiver system has significant potential to reclaim the wastewater. In that study for example, the system was able to remove 80% to 85% of BOD and 85% to 90% of COD.³²

Effect of seasonal variation on the pollutant removal efficiency of constructed wetland bed planted with Vetiver grass

In this section the horizontal subsurface flow constructed wetland with *vetiver grass* was evaluated on the difference in pollutant removal efficiency based on the seasonal variation focus on dry and rainy season. In this study there was a clear difference in removal efficiency of the horizontal subsurface flow constructed wetland for many of the wastewater pollutants in the 2 seasons. For most pollutant parameters, better result was registered in rainy seasons. Even though there was a difference in the organics, nutrient, and chromium removal efficiency of this horizontal subsurface constructed wetland between dry and rainy seasons (Table 2), the difference was not statistically significant at the confidence interval of 95% (P -value = .3).

Many researchers concluded that, the pollutant removal efficiency of horizontal subsurface constructed wetlands was better in rainy and high temperature season. For example Zhai et al³³ evaluated seasonal variation on the nutrient removal efficiency of full scale hybrid constructed wetland and remarked that, there was a significant positive correlation on seasonal variation and nutrient removal was observed.

Seasonal variation of organics removal in the bed with Vetiver grass

The BOD₅ and COD removal efficiency of horizontal subsurface flow constructed wetland with *vetiver grass* at 3 and 5 days

retention time in dry and rainy seasons were BOD₅ (89.05% and 89.59%) in dry and (91.54% and 87.66%) in rainy season while COD (93.82% and 95.81%) in dry and (95.97% and 95.70%) in rainy season respectively (Table 3).

Many researchers reported that seasonal variation had an effect on the organics removal efficiency of horizontal subsurface flow constructed wetland one of the factor may be the dilution of the wastewater and another factor also optimum temperature may facilitate the organic matter degradation by the microorganisms for example a study done by Steer et al³⁴ reported that, the analysis of variance for subsurface constructed wetland systems indicated that biochemical oxygen demand reduction was around 10% less efficiently reduced during winter and summer season than the other seasons.

Seasonal variation of nutrient removal in constructed wetland bed with Vetiver grass

Seasonal variation of nutrients removal efficiency of subsurface constructed wetland with gravel substrate and *Vetiver grass* was evaluated in this study. The result showed that (NO₃-N and TN concentration in the effluent were reduced by (66.29%, 77.15%) and (68.27%, 86.11%) at 3 and 5 days retention time respectively in dry season and (78.43%, 77.51%) and (76.81%, 84.51%) at 3 and 5 days retention time in rainy season respectively (Table 2). In addition to the microbial nitrification and denitrification, ammonia volatilization, the plant uptake may contribute for the nitrogen removal. All these nitrogen removal mechanisms of horizontal subsurface constructed wetland affected by seasonal variations. In this study more nitrogen was removed during rainy season. The 2 most important factors were dilution of the wastewater by the rain water makes lower concentrations of the pollutants and the tropical temperature with diluted wastewater could be suitable environment for the growth and multiplication of microorganisms to degrade the pollutants including nutrients. Similar study was done previously and concluded that; season had significant effect on removal efficiency of TN, NH₄-N, NO_x-n, TP, and dissolved P with higher values of spring-summer period than autumn-winter.³⁵

Phosphate (PO₄-P) and total phosphorus (TP) removal efficiency of this constructed wetland also evaluated in both dry and rainy seasons. The result revealed that (90.92%, 95.20%) and (88.47%, 88.93%) were removed at 3 and 5 days retention time respectively during dry season and (91.64%, 93.26%) and (92.53%, 89.03%) were removed at 3 and 5 days respectively during the rainy season. This indicates that with similar fashion the efficiency of this constructed wetland showed better removal efficiency during rainy season than dry one which is presented in Figure 7. In the same manner like that of nitrogen, Mesquita et al³⁵ also indicate that its removal efficiencies were significantly higher in summer periods than those in winter periods.

Table 3. Seasonal variation on the wastewater treatment efficiency of constructed wetland bed with *Vetiver grass*.

PARAMETERS	DRY SEASON			RAINY SEASON				
	3D		5D	3D		5D		
	EFFLUENT MEAN CONCENTRATION	% REMOVAL	EFFLUENT MEAN CONCENTRATION	EFFLUENT MEAN CONCENTRATION	% REMOVAL	EFFLUENT MEAN CONCENTRATION		
BOD ₅	179.66 ± 13	89.05	171 ± 8.54	89.58	113.33 ± 3.05	91.54	165.38 ± 10.78	87.66
COD	430 ± 55.67	93.82	291.66 ± 23.7	95.81	270.33 ± 20.5	95.97	288.56 ± 21.88	95.70
NO ₃ -N	39.33 ± 5.51	66.29	26.66 ± 2.1	77.15	24.73 ± 1.97	78.43	25.79 ± 2.29	77.51
TN	206.33 ± 10	68.27	90.33 ± 6.66	86.11	130.8 ± 15.14	76.81	87.38 ± 7.62	84.51
PO ₄ -P	8 ± 4	90.92	4.23 ± 0.49	95.20	5.07 ± 2.44	91.64	4.09 ± 0.41	93.26
TP	16.66 ± 1.15	88.47	16 ± 1	88.93	10.53 ± 1.89	92.53	15.47 ± 1.13	89.03
Sulfide	9.4 ± 0.7	96.10	3.86 ± 1.71	98.40	6.14 ± 0.15	97.68	2.90 ± 1.51	98.90
Sulfate	370.55 ± 12	65.46	115.66 ± 8.14	89.22	234.6 ± 21.14	58.91	85.13 ± 12.89	85.09
Chloride	595.66 ± 53.35	68.96	530 ± 45.82	72.38	376.07 ± 28.59	81.75	315 ± 26.62	84.71
TSS	85.66 ± 10.6	95.41	76 ± 8.54	95.93	84 ± 9.64	96.05	77.67 ± 3.21	96.35
TDS	1177.66 ± 1006.6	79.96	1178.33 ± 17.10	79.95	1040.23 ± 923.66	83.26	705.97 ± 109	88.64
EC (µs/cm)	2307.66 ± 2080.6	73.01	1982 ± 9.16	76.82	2076.9 ± 1872.58	74.12	1387.4 ± 6.41	82.71
Salinity (%)	0.25 ± 0.05	63.77	0.25 ± 0.01	63.77	0.19 ± 0.05	71.64	0.15 ± 0.03	77.61
TCr	0.66 ± 0.32	96.40	0.5 ± 0.1	97.27	0.63 ± 0.31	95.98	0.4 ± 0.08	97.45

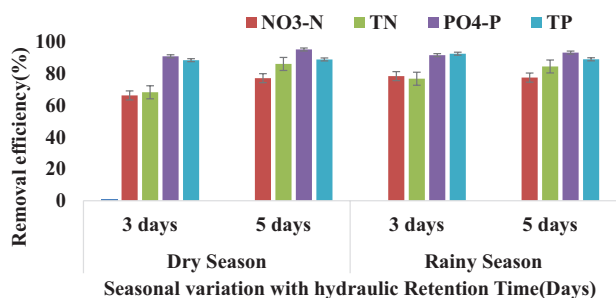


Figure 7. Seasonal variation of nutrients removal efficiency.

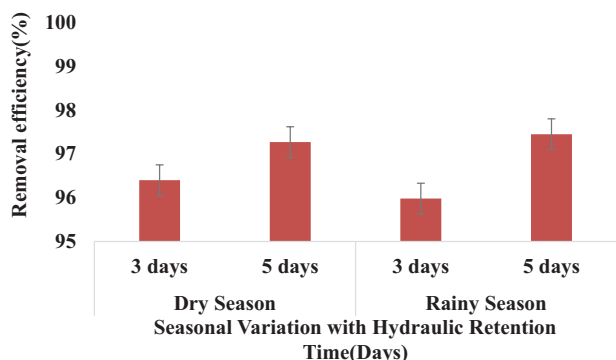


Figure 8. Seasonal variation of total chromium removal efficiency.

Seasonal variation of chromium removal

This research results also illustrated that in the planted horizontal subsurface flow constructed wetland, the maximum chromium removal efficiency was 97.45% during the rainy season and 97.27% in dry season at 5 days retention time (Figure 8). In both season chromium was removed effectively and the final effluent concentration was below the WHO²² and EEPA²³ standard guideline. This little efficiency difference might be due to the dilution effect of the rain water. Another reason for the removal variation might be the temperature difference between the seasons. Seasons with high temperature favors for the growth of the plant to have high biomass and enhances the microbial activities.

Similar result was shown in the study done on the wastewater treatment efficiency of constructed wetland in Greece in which temperature proved to affect Cr (VI) removal in both planted and unplanted units. In the planted unit, maximum Cr (VI) removal efficiencies of 100% were recorded at HRT's of 1 day. The planted units showed higher Cr (VI) removal efficiencies in the same period (95% for temperatures greater than

15°C and 80% for temperatures less than 15°C) the efficiency variation was significant due to the seasonal variation). the author also conclude that, the effect of temperature on planted constructed wetland unit performance was mainly caused by the reeds' annual growth cycle, as in low temperatures common reeds limit their growth and usually decay.³⁶

The plant biometry and chromium uptake capacity

Biometry data of the Vetiver grass was analyzed. The basic biometry data included were growth rate, dry weight, and chromium up take capacity above and below the ground. The result showed that the mean growth of the grass at the sixth month above the ground was 58.66 cm and below the ground was 43.33 cm. The grass dry weights also indicate that 77 g above ground and 80 g below the ground (Table 4).

The chromium uptake capacity of the grass in this constructed wetland bed was evaluated and revealed that, the grass below the ground uptake more chromium than above the ground that was (8.88, 36.62 mg/kg) (Table 3).

Based on the result found from the biometry data of the grass, The Cr bioaccumulation factor (BAF) and Translocation factor (TF) of *vetiver grass* was estimated, (as a method described by Baker et al¹⁹ and Shanker et al²⁰). Therefore BAF of this plant were above the ground (0.48 L/kg) and below the ground (1.99 L/kg) whereas TF was (0.24).

Plants with more BAF and TF values can remove metals like chromium from the wastewater that might be due to harvesting the areal part of the plant removes chromium from the bed. Plant species with high capacity to translocate chromium from below the ground to above the ground enhances continuous absorption of chromium from the constructed wetland bed.^{37,38} Translocation can reduce again chromium concentration and hence that reduce toxicity potential of the metal ions to the root.

In general we can say that Vetiver grass (*Chrysopogon zizanioides*) has an exceptional gift to absorb and to tolerate extreme levels of nutrients, salinity, as well as a range of heavy metals and capable of consuming large quantities of water to produce a massive growth.

Conclusion

The present investigation has achieved good removal efficiency for the removal of various physicochemical wastewater parameters. Using gravel as a substrate, at different HRTs (3, 5, 7, and 9 days) it was effective in reducing almost all the physicochemical parameters. The performance of planted

Table 4. Biometry data of Vetiver grass in constructed wetland bed.

SUBSTRATE BED	ABOVE GROUND				BELOW GROUND				TF
	LENGTH (CM)	DRY WEIGHT (G)	CR UPTAKE (MG/KG)	BAF (L/KG)	LENGTH (CM)	DRY WEIGHT (G)	CR UPTAKE (MG/KG)	BAF (L/KG)	
Gravel	58.66	77.00	8.88	0.48	43.33	80.00	36.62	1.99	0.24

constructed wetland bed was found better in comparison to the unplanted bed at a significant level, P -value $<.01$. The removal of various parameters increased with increasing HRTs. This pilot scale study demonstrates that, *Vetiver grass* (*Chrysopogon zizanioides*) has a capacity to reclaim high strength industrial wastewater with different pollutants including heavy metals and nutrients efficiently. This study also showed that, seasonal variation affects the removal efficiency of horizontal subsurface flow constructed wetland with gravel substrate and the plant *Vetiver grass*.

On the other hand, constructed wetlands are affordable and reliable green technologies for the treatment of various types of wastewater. Compared to conventional treatment systems, constructed wetlands offer an environmentally friendly approach, low-cost, fewer operational, and maintenance requirements, and have a high potential for application in developing countries, particularly in tropical countries. Therefore the use of horizontal subsurface flow constructed wetland with *Vetiver grass* for the treatment of industrial wastewater in tropical countries like Ethiopia is recommended.

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Author Contributions

Mekonnen Birhane Aregu designed the study, conducted the experiments, Collected, analyzed, and interpreted the data and wrote the manuscript. Negasa Eshete Soboksa and Girum Geberemeskel Kanno involved in the data collection, analysis, and manuscript writing of this research.

Availability of Data and Material

The dataset and materials used for this manuscript is available from the corresponding author and can be shared whenever necessary.

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REFERENCES

- WWAP (United Nations World Water Assessment Programme). *The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource*. UNESCO; 2017.
- Md Kawser A, Monika D, Md Monirul I, Mosammat Salma A, Shahidul I, Abdullah Al-Mansur M. Physicochemical properties of tannery and textile effluents and surface water of river Buriganga and Karnatoli, Bangladesh. *World Appl Sci J*. 2011;12:152-159.
- Shu L, Waite TD, Bliss PJ, Fane A, Jegatheesan V. Nanofiltration for the possible reuse of water and recovery of sodium chloride salt from textile effluent. *Desalination*. 2005;172:235-243.
- Demirezen D, Aksoy A, Uruç K. Effect of population density on growth, heavy metal by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton* biomass and nickel accumulation capacity of Lemna gibba (Lemnaceae). *Chemosphere*. 2007;66:553-557.
- Gebre Mariam Z, Desta Z. The chemical composition of the effluent from Awassa textile factory and its effects on aquatic biota. *Sinet Ethiop J Sci*. 2002;25:263-274.
- Marks S. *Quantifying Transverse Dispersion in a Subsurface Flow Constructed Wetland*. MSc thesis. Department of Chemical Engineering, University of Queensland; 1999.
- Njau KN, Renalda M. Performance of horizontal subsurface flow constructed wetland in the removal of tannins. *Can J Civ Eng*. 2010;37:496-501.
- Vymazal J. Removal of nutrients in various types of constructed wetlands, science direct. *Sci Total Environ*. 2007;380:48-65.
- Belmont MA, Zurita F, De Anda J. Treatment of domestic wastewater and production of commercial flowers in vertical and horizontal subsurface-flow constructed wetlands. *Ecol Eng*. 2009;35:861-869.
- Chen Z, Wu S, Braeckvelt M, et al. Effect of vegetation in pilot-scale horizontal subsurface flow constructed wetlands treating sulphate rich groundwater contaminated with a low and high chlorinated hydrocarbon. *Chemosphere*. 2012;89:724-731.
- Chazarenc F, Gagnon V, Comeau Y, Brisson J. Effect of plant and artificial aeration on solids accumulation and biological activities in constructed wetlands. *Ecol Eng*. 2009;35:1005-1010.
- Ballesteros F, Vuong TH, Secondes MF, Tuan PD. Removal efficiencies of constructed wetland and efficacy of plant on treating benzene. *Sustain Environ Res*. 2016;26:93-96.
- Langergraber G. Are constructed treatment wetlands sustainable sanitation solutions? *Water Sci Technol*. 2013;67:2133-2140.
- Prasad R, Rangari PJ, Jasutkar D. Performance evaluation of constructed wetland in treating domestic wastewater. *Int J Latest Res Eng Technol*. 2016;2:28-33.
- Calderon IM. (2010) *Green Movement Against Green Water*. Honors thesis. College of Agriculture and Life Sciences, Landscape Architecture of Cornell University, USA
- Gupta P, Roy S, Mahindrakar AB. Treatment of water using water hyacinth, water lettuce and vetiver grass – a review. *Resour Environ*. 2012;2:202-215.
- Alemu T, Lemma E, Mekonnen A, Leta S. Performance of pilot scale anaerobic-SBR system integrated with constructed wetlands for the treatment of tannery wastewater. *Environ Process*. 2016;3:815-827.
- APHA. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. American Public Health Association; 2005.
- Baker AJM, Mcgrath RRD, Smith JAC. Metal hyper accumulator plants: a review of the ecology and physiology of biochemical resource for phytoremediation of metal-polluted soils. In: *Phytoremediation of Contaminated Soil and Water*. University of Oxford; 2000:85-107.
- Shanker AK, Djanaguiraman M, Sudhagar R, Chandrashekar CN, Pathmanabhan G. Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO₄) roots. *Plant Sci*. 2004;166:1035-1043.
- Truong PN. *Vetiver Grass Technology for mine tailings rehabilitation*. Paper presented at: Proceedings of Ground and Water Bioengineering for Erosion Control and Slope Stabilisation; 1999; Manila. Accessed May 22, 2021.
- WHO. *Guidelines for Drinking Water Quality*. 4th ed. WHO; 2011:678.
- EPA (Ethiopian Environmental Protection Authority). Standards for Industrial Pollution Control in Ethiopia, Part Three: Standards for Industrial Effluents. ISIS Project-US/ETH/99/068/ETHIOPIA, EPA/UNIDO; 2003.
- Otieno AO, Karuku GN, Raude JM, Koeh O. Effectiveness of the horizontal, vertical and hybrid subsurface flow constructed wetland systems in polishing municipal wastewater. *Environ Manag Sustain Dev*. 2017;6:158-173.
- Ewemoje OE, Sangodoyin AY, Adegoke AT. On the effect of hydraulic retention time and loading rates on pollutant removal in a pilot scale wetland. *J Sustain Dev Stud*. 2015;8:342-355.
- Mathew M, Rosary SC, Sebastian M, Cherian SM. Effectiveness of vetiver system for the treatment of wastewater from an institutional kitchen. *Procedia Technol*. 2016;24:203-209.
- García-Valero A, Martínez-Martínez S, Faz Á, et al. Treatment of wastewater from the tannery industry in a constructed wetland planted with *Phragmites australis*. *Agronomy*. 2020;10:176.
- Sivakumar D, Shankar D, Vijaya PA, Valarmathi M. Constructed wetland treatment of textile industry wastewater using aquatic macrophytes. *Int J Environ Res*. 2013;3:1223-1232.
- Kadlec RH, Wallace S. *Treatment Wetlands*. 2nd ed. CRC Press; 2009.
- Amenu D. Evaluation of selected wetland plants for the removal efficiency of pollutants from wastewater. *Int J Adv Multidiscip Res*. 2015;2:63-66.
- Papaeangelou V, Gikas GD, Tsihrintzis VA. Effect of operational and design parameters on performance of pilot-scale vertical flow constructed

- wetlands treating university campus wastewater. *Water Resour Manag.* 2016;30: 5875-5899.
32. Mini M, Claramma R, Mathukutty S, Sandra MC. Effectiveness of vetiver system for the treatment of wastewater from an institutional kitchen, international conference on emerging trends in engineering, science and technology (ICE-TEST-2015). *Procedia Technol.* 2016;24:203-209.
 33. Zhai J, Xiao J, Rahaman M, John Y, Xiao J. Seasonal variation of nutrient removal in a full-scale artificial aerated hybrid constructed wetland. *Water.* 2016;8:551.
 34. Steer D, Fraser L, Boddy J, Seibert B. Efficiency of small constructed wetlands for subsurface treatment of single-family domestic effluent. *Ecol Eng.* 2002;18:429-440.
 35. Mesquita MC, Albuquerque A, Amaral L, Nogueira R. Seasonal variation of nutrient removal in a full-scale horizontal constructed wetland. *Energy Proc.* 2017;136:225-232.
 36. Sultana MY. 2014) *Treatment of Industrial and Agro-Industrial Wastewater Using Constructed Wetlands.* PhD dissertation. Laboratory of Environmental Systems, Department of Environmental and Natural Resources Management, School of Engineering, University of Patras, Agrinio, Greece.
 37. Skeffington RA, Shewry PR, Peterson PJ. Chromium uptake and transport in barley seedlings (*Hordeum vulgare* L.). *Planta.* 1976;132:209-214.
 38. Perk MV. *Soil and Water Contamination: From Molecular to Catchments Scale.* Routledge; 2006.