ABSTRACT: The discharge of untreated wastewater causes serious public and environmental health problems. Hence, the present study aimed to evaluate the combined adsorption potential of the two substrates (Pumice and Scoria) in a horizontal subsurface flow constructed wetland. The substrates were collected from the Ethiopian rift belt. Composite samples from tannery wastewater before and after treatment of four different retention times (RT) were collected and analyzed. *Chrysopogon zizanioides* was planted in one of the mixed substrate beds and grown for 5 months before running wastewater for the treatment. The maximum removal efficiency of the planted bed revealed that BOD$_5$ at RT 7 days effluent concentration of 59.33 mg/L (96.38% removal), COD at RT 7 days 129.33 mg/L (98.14% removal), NO$_3$-N at RT 7 days 0.28 mg/L (99.76% removal), Sulfate at RT 7 days 0.27 mg/L (99.9% removal), PO$_4$-P RT 9 days 0.01 mg/L (99.9% removal), TP at RT 7 days 6 mg/L (95% removal), Sulfide at RT 7 days 0.27 mg/L (99.9% removal), sulfate at RT 9 days 87.9 mg/L (91.8% removal), and total Chromium at RT 7 days 0.1 mg/L (99.45% removal) respectively. The efficiency of the study and control beds was tested by a Two-Sample t-Test. The result showed that there was a significant difference at a 95% confidence interval, p-value = .002. Hence, the mixed substrate with plants performs better than the unplanted one, which means it can be effective for the treatment of high-strength industrial wastewater using horizontal subsurface flow constructed wetland.

KEYWORDS: Wastewater, pumice, scoria, substrate, *Chrysopogon zizanioides*

Introduction

Industrial wastewater is the most important source of environmental pollution and contains several pollutants such as toxic heavy metals like chromium, cadmium, nickel, lead, and mercury, and also different forms of plant nutrients (Collivignarelli et al., 2019; Oruko et al., 2019). On the other hand, industrialization is crucial for the economic development of developing countries like Ethiopia. However, rapid industrialization is believed to be one of the main causes of environmental pollution (Meyer et al., 2019).

According to studies, a very small proportion of wastewater, around 8%, is treated in developing countries, including the study area (Van Rooijen et al., 2010; WWAP, 2017). Another study found that 90% of wastewater in developing countries is still discharged directly into rivers and streams with no wastewater treatment (Shu et al., 2005).

Rather than the conventional wastewater treatment methods, phytoremediation is one of the biological wastewater treatment methods used in different parts of the world by different techniques (Roongtanakiat et al., 2007). This is the concept of a plant-based treatment system and microbiological processes to reduce hazardous pollutants from the wastewater. One of the techniques that could be used could be constructed wetlands. For example, a horizontal subsurface constructed wetland (Cunningham et al., 1995).

In the horizontal subsurface flow constructed wetland system, the removal of wastewater pollutants was attained by different mechanisms like sedimentation, filtration, precipitation, ion exchange, adsorption, microbial interactions, and plant uptake (Choudhary et al., 2011). In this case, both the plant's pollutant removal capacity and the substrate adsorption potential should be considered, if constructed wetland is an option for wastewater treatment.

In our case, many industries discharge into receiving water bodies like rivers, streams, and lakes without treatment, which causes ecological imbalance and a public health risk to downstream communities. Therefore, the aim of this study is to develop efficient, appropriate and innovative constructed wetland technology for high strength wastewater treatment using locally available and low-cost substrates by integrating scoria and pumice with *Chrysopogon zizanioides*.

Method and Materials

Description of study area

The study was conducted in the eastern part of Ethiopia, in Oromiya regional state, East Showa zone, Modjo town. The study area is found entirely inside the rift valley belts (Figure 1).

Study design

A comparative experimental study design was carried out to determine the efficiency of a mixed substrate (Pumice and Scoria) with and without the plant *Chrysopogon zizanioides* using a horizontal subsurface flow constructed wetland.

Experimental setup

The substrates (Pumice and Scoria) were collected from the rift valley belts of Ethiopia (Figure 1). The physicochemical characteristics of the substrates were determined using XRF...
analysis and found suitable for adsorption (Aregu et al., 2018). The substrates were crushed and graded. The effective size and uniformity coefficient were determined by using a standard sieve before filling in the constructed wetland beds.

The two independent horizontal subsurface flow constructed wetland (HSSFCW) beds were installed, which have equal dimensions of 4 m length, 0.8 m width, and 0.6 m effective depth and are filled with the prepared mixed substrates (Figure 2). We have used sedimentation and equalization tanks before the wastewater enters the constructed wetland bed to prevent clogging.

The untreated wastewater discharged from the tannery industry was entered into a settling tank and waited for 24 hours before being pumped to the equalization tank. The CWs are fed from the equalization tank through a 3/4 inch pipe with a gate valve for each bed. The efficiency of each bed is then recorded according to the retention time of 3, 5, 7, and 9 days.
The plant (Chrysopogon zizanioides) was transported to the study area from one of the agricultural research institutes found west of Addis Ababa, the capital city of Ethiopia. It was grown for 5 months in various proportions of tap water and wastewater before running fully the industrial wastewater for treatment. The proportion of tap water to tannery wastewater was 75:25, 50:50, and 25:75 respectively.

Sampling and laboratory analysis
The wastewater sample was done before and after the treatment with the predetermined retention time (RT) using standard methods (APHA, 2005).

The efficiency of each bed for different wastewater parameters were calculated (1).

\[
\text{Removal\%} = \left( \frac{C_0 - C_t}{C_0} \right) \times 100
\]

(1)

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 \( t \).

The major wastewater parameters analyzed in this study were BOD\(_5\), COD, NH\(_4\)-N, NO\(_3\)-N, Total-N, PO\(_4\)-P, Total-P, Sulfide, Sulfate, Chloride, TSS, TDS, EC, Salinity, and total Chromium.

The chromium bioaccumulation factor (BAF) (2) and translocation factor (TF) (3) of the plant were determined using a method described by two scholars (Baker et al., 2000; Shanker et al., 2004):

\[
\text{BAF} = \frac{\text{mg Cr/kg dw plant}}{\text{mg Cr/L wastewater}}
\]

(2)

\[
\text{TF} = \frac{\text{mg Cr/kg dw above ground}}{\text{mg Cr/kg dw below ground}}
\]

(3)

Statistical analysis
R-software and Originlab pro 2017 were used for data analysis and graphing.

Result and Discussion
Wastewater treatment efficiency of mixed substrate (pumice and scoria) in a horizontal subsurface flow constructed wetland

We have used high-strength wastewater from the tannery industry for this study. The characteristics of the untreated raw wastewater were determined by the major physicochemical wastewater parameters. The result showed that BOD\(_5\), COD and TSS were 1641 ± 375.6, 6953 ± 339.4, and 1868 ± 863.1 mg/L respectively. The concentrations of orthophosphate, ammonium, and nitrate were also determined to be 88 ± 40.8, 253.3 ± 11.6, and 116.7 ± 26.6 mg/L respectively. The total chromium concentration was also determined and found 18.33 ± 6.7 mg/L of the sample wastewater. This result is in-line with different studies done previously that indicated the characteristics of tannery wastewater were very high-strength wastewater for example, Birhanu (2007) and Alemu et al. (2016) point out this fact in their independent study at different time.

The most important factors that determine this efficiency are proper design, selection of potential plant species, and efficient substrates. Due to this fact, this investigation focuses on the efficiency of horizontal subsurface flow constructed wetland using mixed substrate with the plant type Chrysopogon zizanioides (vetiver grass). To come up with the best efficiency of the constructed wetland for the permissible discharge limit of organic and inorganic pollutants set by national and international standards, selecting the most efficient substrates was essential. Hence, in the present study, the combined adsorption potential of the two substrates (Pumice and Scoria) was evaluated in both planted and unplanted constructed wetland cells.

BOD\(_5\), COD, and TSS removal efficiency of mixed substrate bed with and without plant

The result of this investigation revealed that BOD\(_5\), COD, and TSS efficiency of HSSFCW using mixed substrate without plant were the effluent concentration of BOD\(_5\) (348 mg/L (84.55% removal), 245 mg/L (85.07% removal), and 224.33 mg/L (86.33% removal)), COD (780 mg/L (88.78% removal), 532.87 mg/L (92.34% removal), 316.67 mg/L (95.45% removal) and 300.67 mg/L (95.68% removal)) and TSS (88.17 mg/L (96.75% removal), 55.67 mg/L (97.02% removal), and 54.33 mg/L (97.09% removal) at 3, 5, 7, and 9 days retention time respectively (Table 1).

Even though the removal efficiency of this constructed wetland cell was above 75%, the concentration of these organic pollutants did not meet the discharge limit of both national and international standards, especially in the first 3 and 5 days of retention time.

However, all three parameters in the treated wastewater in this constructed wetland with mixed substrate and the plant Chrysopogon zizanioides met the standard guideline at all retention times by BOD\(_5\) (100 mg/L (93.91% removal), 70 mg/L (95.73% removal), 59.33 mg/L (96.38% removal) and 61.67 mg/L (96.24% removal)), COD (201 mg/L (97.11% removal), 136.88 mg/L (98.03% removal), 129.33 mg/L (98.14% removal), 146 mg/L (97.90% removal)) and TSS(74 mg/L (96.04% removal), 48.83 mg/L (97.39% removal), 46.67 mg/L (97.51% removal) and 44 mg/L (97.64%)) at 3, 5, 7, and 9 days retention time (RT) respectively (Table 1).

Organic matter is decomposed in constructed wetlands with horizontal subsurface flow by both aerobic and anaerobic
Table 1. Industrial Wastewater Treatment Efficiency of Mixed Substrate Bed with and without Plant at Different RT.

| PARAMETERS | INFLUENT CONC. (MG/L) | 3 DAYS RT | 5 DAYS RT | 7 DAYS RT | 9 DAYS RT |
|------------|------------------------|-----------|-----------|-----------|-----------|
|            |                        | UNPLANTED (%) | PLANTED (%) | UNPLANTED (%) | PLANTED (%) | UNPLANTED (%) | PLANTED (%) | UNPLANTED (%) | PLANTED (%) |
| BOD₅       | 1641 ± 373.55          | 78.79     | 93.91     | 84.55     | 95.73     | 85.07     | 96.38     | 86.33     | 96.24     |
| COD        | 6953.33 ± 339.41       | 88.78     | 97.11     | 92.34     | 98.03     | 95.45     | 98.14     | 95.68     | 97.90     |
| NH₄-N      | 253.33 ± 11.6          | 38.29     | 79.08     | 80.78     | 98.63     | 80.39     | 98.83     | 84.08     | 98.29     |
| NO₃-N      | 116.66 ± 26.63         | 70.28     | 93.14     | 94.57     | 100       | 94.57     | 99.76     | 95.14     | 99.66     |
| TN         | 650.33 ± 93.62         | 68.79     | 82.16     | 88.38     | 94.24     | 90.82     | 95.80     | 87.39     | 94.21     |
| PO₄-P      | 88.06 ± 40.77          | 92.69     | 95.23     | 92.81     | 96.45     | 95.08     | 96.79     | 95.83     | 99.99     |
| TP         | 144.53 ± 20.75         | 86.85     | 92.58     | 87.08     | 94.46     | 89.39     | 95.85     | 90.54     | 91.14     |
| Sulfide    | 241.33 ± 101.16        | 97.44     | 98.93     | 99.75     | 99.89     | 99.76     | 99.89     | 99.39     | 99.71     |
| Sulfate    | 1072.82 ± 352.74       | 74.15     | 73.61     | 91.02     | 90.71     | 91.30     | 91.64     | 91.48     | 91.81     |
| Chloride   | 1919 ± 1271.6          | 30.35     | 54.51     | 81.93     | 85.81     | 82.21     | 85.63     | 77.24     | 80.16     |
| TSS        | 1868 ± 863.1           | 95.28     | 96.04     | 96.75     | 97.39     | 97.02     | 97.51     | 97.09     | 97.64     |
| TDS        | 5877.3 ± 2294.77       | 75.33     | 82.79     | 79.09     | 85.38     | 80.51     | 91.23     | 79.89     | 90.89     |
| EC (µs/cm) | 8550 ± 2089            | 67.04     | 77.42     | 71.65     | 80.65     | 82.99     | 88.50     | 82.64     | 88.27     |
| Salinity (%)| 0.69 ± 0.22            | 60.87     | 76.81     | 69.57     | 79.71     | 72.46     | 79.71     | 72.46     | 79.71     |
| TCr        | 18.33 ± 6.66           | 97.44     | 97.65     | 98.75     | 99.24     | 98.91     | 99.45     | 97.98     | 99.02     |
microbial processes as well as by sedimentation and filtration of suspended and organic matters. Because of the high organic loading rate and continuous saturation of the constructed wetland cell with substrate, anoxic, and/or anaerobic processes are overcome while aerobic processes are restricted. The better results in the removal of BOD₅, COD, and TSS obtained in the planted constructed wetland cell were due to the combined effect of substrates, plants, and microbes’ potential to reduce organic pollutants and suspended solids.

On the other hand, the spongy and networked nature of the plant root enhances the filtration of solid matter and microbial activities. For example, macrophytes provide a large surface area for attached microbial growth. A study done by Birhanu (2007) reported that BOD₅ (98%), COD (90%), and TSS (83.9%) removal were achieved from tannery wastewater using horizontal subsurface constructed wetland planted with Cyperus alternifolia and Cyperus papyrus.

Another study that evidenced this finding was a review result of Vymazal and Kröpfelová (2009). They summarized the results of more than 400 horizontal subsurface flow constructed wetlands from 36 countries around the world. The survey revealed that the high removal efficiencies for BOD₅ and COD were achieved. The survey also revealed that this type of constructed wetland is successfully used for both secondary and tertiary treatment.

**Nutrient removal efficiency of mixed substrate bed with and without the plant**

The concentration of ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N) and total nitrogen (TN) were reduced after the industrial wastewater filtered out through the constructed wetland cell with mixed substrate but without plant at different retention times. The final discharge concentration from this cell were NH₄-N (156.33 mg/L, 38.29% removal), (48.67 mg/L, 80.78% removal), (49.67 mg/L, 80.39% removal) and (40.33 mg/L, 84.08% removal), NO₃-N (34.67 mg/L, 70.28% removal), (6.33 mg/L, 94.57% removal), (6.33 mg/L, 94.57% removal) and (5.67 mg/L, 95.14% removal) and TN (202.93 mg/L, 68.79% removal), (75.60 mg/L, 88.38% removal), (59.67 mg/L, 90.82% removal) and (82 mg/L, 87.39% removal) at 3, 5, 7, and 9 days retention time respectively (Table 1).

While the concentration of ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N), and total nitrogen (TN) were reduced after the industrial wastewater passed through the constructed wetland cell with mixed substrate and the plant Chrysopogon zizanioides at four different hydraulic retention time, the final discharge concentration from this cell were NH₄-N (53 mg/L, 79.08% removal), (3.48 mg/L, 98.63% removal), (2.97 mg/L, 98.83% removal) and (4.33 mg/L, 98.29% removal), NO₃-N (8 mg/L, 93.14% removal), (BDL, 99.99% removal), (0.28 mg/L, 99.76% removal) and (0.40 mg/L, 99.66% removal), and TN (116 mg/L, 82.16% removal, 37.43 mg/L, 94.24% removal), (27.33 mg/L, 95.80% removal) and (37.67 mg/L, 94.21 % removal) at 3, 5, 7, and 9 days retention time respectively(Table 1).

Tadesse Alemu et al. (2016) reported that the efficiency of horizontal subsurface flow constructed wetland systems with gravel substrate and the plant Phragmites karka for NH₄-N, NO₃-N, and TN removal at 3-day HRT was 82.5 %, 84.2%, and 77.7 %, and at 5 days RT, 81%, 82%, and 82.81% respectively.

Good removal efficiency was seen in the constructed wetland with planted cell. In fact, in horizontal subsurface flow constructed wetlands, nitrogen removal is subject to a wide range of transformations that include physical, chemical, and biological processes involving various mechanisms such as ammonification, nitrification, with further denitrification, microbial immobilization, and matrix adsorption (Dzakpasu et al., 2015).

The removal of PO₄-P and TP in the unplanted constructed wetland cell revealed that at 3 days RT (92.69% and 86.85%), 5 days RT (92.81% and 87.08%), 7 days RT (95.08% and 89.39%), and 9 days RT (95.83% and 90.54%) respectively. But the removal of PO₄-P and TP in the planted constructed wetland cell with Chrysopogon zizanioides revealed that at 3 days RT (95.23% and 92.58%), 5 days RT (96.45% and 94.46%), 7 days RT (96.79% and 95.85%), and 9 days RT (99.99% and 91.14%) respectively (Figure 3). This finding is evidenced by the result of a laboratory batch experiment study that showed coarse-grained ochre with high iron (III) oxides removes 90% of all phosphorus forms from sewage effluent (Heal et al., 2005).

Phosphorous removal efficiency of horizontal subsurface flow constructed wetlands varies significantly due to the complex combination of physical, chemical, and biological processes involving mainly adsorption, precipitation, sedimentation in pores of the substrate media, peat accretion and burial, and to a lesser extent biomass uptake (Dzakpasu et al., 2015). The phosphorus removal efficiency of HSSFCWs with the commonly used gravel substrate is limited because the gravel substrate was poor in iron and aluminum hydrous oxide minerals as well as in calcium and magnesium concentrations, elements essential for adsorption and precipitation of insoluble forms in wetlands, the most important mechanisms for removing phosphorus in those systems (Dzakpasu et al., 2015). But in this study, the two substrates (Pumice and Scoria) are rich in such mineral content that they resulted in significant removal of phosphate and total phosphorus from the tannery wastewater (Aregu et al., 2018). As Brix et al. (2001) pointed out, adsorption of phosphorus into the substrate medium is a major removal mechanism for phosphorus in subsurface flow constructed wetlands. Selecting a sand medium with a high phosphorus sorption capacity is therefore important to obtain sustained phosphorus removal.

In general, the results revealed that the nutrient removal efficiency of planted cell was better than the unplanted ones. Tanner (2001) reported that most studies comparing planted
versus unplanted subsurface flow flow-constructed wetland system for wastewater treatment (HSSFCW) show a significant and positive effect of macrophytes on pollutant removal.

**Sulfur and chloride removal efficiency of mixed substrate bed with and without the plant**

The results obtained from the unplanted wetland bed revealed that sulfide, sulfate, and chloride were reduced to 6.19 mg/L about 97.44% removal, 277.36 mg/L about 74.15% removal, 1336.5 mg/L about 30.35% removal after 3 days treatment, respectively.

This result is also improved by the addition of the treatment days without plant and becomes 0.60 mg/L about 99.75% removal, 96.39 mg/L about 91.02% removal, 346.67 mg/L about 81.93% removal after 5 days of treatment, respectively. But there was no significant change in the efficiency by allowing 2 and 4 days of additional treatment time in the same substrate bed without plant from 5 days. In the next 7 and 9 days of treatment time, the results revealed that 99.76%, 91.30%, and 82.21% removal and 99.39%, 91.48%, and 77.24% removal of sulfide, sulfate and chloride respectively. This result is supported by the study done recently which indicated that more than 50% of chloride was removed from synthetic brine solution using non-planted constructed wetland (Chairawiwut, 2015). The result from the planted bed showed that sulfide, sulfate and chloride were reduced to 2.58 mg/L about 98.93% removal, 283 mg/L about 73.61% removal, and 873 mg/L about 54.51% removal after 3 days treatment respectively.

This result is also improved by the addition of the treatment days and becomes 0.27 mg/L about 99.89% removals, 99.67 mg/L about 90.75% removals, and 272.4 mg/L about 85.81% removals after 5 days treatment, respectively. However, there was no significant difference in efficiency when 2 and 4 days of additional treatment time were allowed instead of 5 days. In the next 7 and 9 days of treatment time, the results revealed that 99.89%, 91.64%, and 85.63% removal and 99.71%, 91.81%, and 80.16% removal of sulfide, sulfate, and chloride respectively.

In general, both planted and unplanted constructed wetland beds with mixed substrate perform efficiently in the removal of sulfur and chloride pollutants from tannery wastewater. The constructed wetland beds remove sulfur and chloride efficiently, especially at 5 and 7 days of retention time, but there was no significant difference between the results of the planted and unplanted beds. This indicates that even though there was a great value and better results for this plant in removing other pollutants from the tannery wastewater, the value of the substrates to remove sulfur and chloride is greater than the plant *Chrysopogon zizanioides*.

The sulfur and chloride removal results of this study are supported by the result reported by different scholars. For example, David and Kola (2013) reported that sulfate was reduced by 94% while chloride was reduced only by 37.5% using reed beds for the treatment of wastewater. Another study also revealed that 53.44% to 55.27% of chloride was removed from wastewater by subsurface constructed wetland (Dhanya & Jaya, 2013).

**EC, TDS and salinity reduction efficiency of mixed substrate bed with and without plant**

Based on this study’s finding, EC, TDS and salinity removal efficiency of unplanted constructed wetland beds used for a control subject for the assessment of the potential of plants with mixed substrate to remove those pollutant parameters revealed that EC 2817.83, 2424.33, 1454.67, and 1484.33 mg/L (67.04%, 71.65%, 82.99%, and 82.64%), TDS 1450, 1228.67,
1145.33, and 1182 mg/L (75.33%, 79.09%, 80.51%, and 79.89%) and salinity 0.27, 0.21, 0.19, and 0.19 mg/L (60.87%, 69.57%, 72.46% and 72.46%) at 3, 5, 7, and 9 days respectively. The indicated amount of total dissolved solid and salinity were reduced due to the adsorption and ion-exchange removal capacity of the mixed substrates.

Unlike the unplanted constructed wetland beds (control subject), the EC, TDS and salinity removal efficiency of the planted beds (study subject) with Chrysopogon zizanioides on the same mixed substrate showed that EC 1930.33, 1654.20, 983.33, and 1002.67 mg/L (77.42%, 80.65%, 88.50%, and 88.27%), TDS 1011.70, 859.43, 515.33, and 535.67 mg/L (82.79%, 85.38%, 91.23%, and 90.89%) and salinity 0.16, 0.14, 0.14, and 0.14 mg/L (76.81%, 79.71%, 79.71%, and 79.71%) at 3, 5, 7, and 9 days retention time respectively. Dhanya and Jaya (2013) reported almost identical results when they examined three different wastewater treatment efficiency of constructed wetland, revealing that the percentage reduction in TDS content was recorded as 97.44, 81.51%, and 80.0% in wastewater one, wastewater two, and wastewater three, respectively.

Another study, also done by Shelef et al. (2012), evaluates the capability of Bassia indica plants to remove salinity from a wastewater in three constructed wetland systems and reduced the salinity by 20% to 60% in comparison with unplanted systems. Farzi et al. (2017) also concluded that, the halophyte plants tolerated a broad range of salinity and reduced the salinity of solutions by different percentages in their salt phytoremediation research.

Wastewaters with high amounts of EC, TDS, and salinity discharged from industries like tanning industries affect both aquatic and terrestrial ecosystems adversely. Most of the time, these pollutants are removed using conventional methods (e.g., using physicochemical equipment, biological reactors, or a combination thereof) are feasible for treating most of this type of wastewaters (Wu et al., 2013). However, the high cost of these methods limits their application in many areas, especially in developing countries like Ethiopia. For this reason, horizontal subsurface flow constructed wetlands should be successfully used for treating tannery wastewater because they are eco-friendly and low-cost, and they provide a potential alternative technology option for this type of wastewater treatment.

**Chromium removal efficiency of mixed substrate bed with and without plant**

The result of this finding indicates that both the planted and unplanted beds remove more than 97% of chromium. However, the total chromium removal efficiency of the unplanted constructed wetland bed showed 0.47, 0.23, 0.2, and 0.37 mg/L (97.44%, 98.75%, 98.91%, and 97.98%) at 3, 5, 7, and 9 days retention time respectively (Figure 3). This successful efficiency was achieved due to the cumulative adsorption potential effect of the two substrates resulted from the best physicochemical characteristics of both pumice and scoria substrate for heavy metal adsorption (Aregu et al., 2018; Alemayehu & Lennartz, 2009; Alemayehu et al., 2011). The performance of this constructed wetland, however, decreased slightly and became basically stable as time passed. This was because of the saturation of the substrate to adsorb metallic ions. However, the final effluent concentration of chromium at all retention times was under the standard limit value of WHO (2011) and Ethiopian Environmental Protection Authority (EEPA, 2003)

Whereas the planted bed with Chrysopogon zizanioides removed 0.43, 0.14, 0.10, and 0.18 mg/L (97.65%, 99.24%, 99.45%, and 99.02%) at 3, 5, 7, and 9 days retention time respectively (Figure 4). This result is greater than the result obtained from an unplanted bed. This is because the selected plant has a great role in up-taking chromium from the wastewater, in addition to the chrome adsorption potential of the mixed substrate. The chromium removal performance of planted constructed wetland bed was better than unplanted bed. This result was evidenced by Sultana (2014). The final effluent concentration of chromium in this study was under the standard limit value of WHO (2011) and EEPA (2003)

In one study previously done, pilot-scale horizontal subsurface flow constructed wetland units were built and operated. The units contained fine gravel as a substrate in both units. One unit was planted with *common reeds* and another one was kept unplanted as a control unit. The performance of the planted units was very effective as the mean Cr (VI) removal efficiency was 85% and the efficiency maximum reached 100%, but the unplanted one showed less performance efficiency (Michailides et al., 2013).

Many researchers have shown that the removal efficiency of constructed wetlands could be significantly improved by optimizing the substrates, especially for nutrients like phosphorus and heavy metals like chromium. In this study, two potential substrates were identified and selected for tannery wastewater treatment in a horizontal subsurface flow constructed wetland and again integrated with potent plants to resist toxicity, shock, and drought with high metal and nutrient uptake capacity. Both the nature and characteristics of the substrates and the plant Chrysopogon zizanioides make the constructed wetland...
effective for more than 97% removal of chromium from tannery wastewater at all four hydraulic retention times (Figure 3).

Almost similar results were found in the a study finding reported by Tadesse and Seyoum (2015), which revealed that the horizontal subsurface flow constructed wetland cells with different plant species and gravel substrate were tested and found that the minimum chromium removal efficiency was 97.7% using the P. karka and the maximum was 99.3% using the plant B. aethiopium.

Considering the removal efficiency of 15 wastewater pollutant parameters within the four different retention times, the efficiency between the planted and unplanted bed was tested using a Two-Sample t-Test. The result revealed that, there was a significant difference at 95% confidence interval, p-value = .002. Hence, the mixed substrate with the plant Chrysopogon zizanioides performs better than the unplanted bed.

**Seasonal variation of efficiency of mixed substrate bed**

In this study, the effect of combining two different substrates for the removal of various industrial wastewater pollutants in a horizontal subsurface flow constructed wetland was explored including the effect of seasonal variation on its efficiency (Table 2). Seasonal and temperature variations, as various scholars have pointed out, have a significant effect on the treatment performance of horizontal subsurface flow constructed wetland (Zhai et al., 2016).

According to this study’s finding, the concentration of many industrial wastewater parameters in final treated wastewater during the dry season was slightly more concentrated than during the rainy seasons, but the efficiency difference was not statistically significant at 95% confidence interval, p-value = .3 considering the two retention times and fifteen wastewater parameters.

**Seasonal variation of organics removal in mixed substrate with Chrysopogon zizanioides**

The removal efficiency of BOD$_5$ was (93.91% and 95.73%) and COD (97.11% and 98.03%) at 3 and 5 days of hydraulic retention time in the dry season, but it was (95.25% and 94.95%) and (98.09% and 98.03%) in the rainy season at 3 and 5 days of retention time respectively (Table 2).

As various scholars concluded, seasonal and temperature variations were the two important factors that affected both BOD$_5$ and COD removal efficiency of horizontal subsurface flow constructed wetlands. One reason may be the dilution of the wastewater and optimum temperature may facilitate degradation of the organic matter by the microorganisms, in addition to the plant type. For example, Steer et al. (2002) reported that, BOD$_5$ reduction was around 10% less efficiently reduced during the winter and summer seasons than in spring and autumn.

**Seasonal variation of nutrients removal in mixed substrate bed with the plant**

The nutrient removal efficiency of the understudied constructed wetland with mixed substrate and the plant Chrysopogon zizanioides was varied between the two seasons of dry and rainy. NH$_4$-N, NO$_3$-N, TN, PO$_4$-P, and TP were reduced by (79.08% and 98.63%), (93.14% and 100%), (82.16% and 94.24%), (95.23% and 96.45%), and (92.58% and 94.46%) at 3 and 5 days retention time in dry season respectively but slightly better efficiency was seen in rainy season which accounts (86.49% and 98.64%), (95.64% and 99.36%), (87% and 93.59%), (95.47% and 95.02%), and (95.27% and 94.43%) with similar retention time of 3 and 5 days respectively which is illustrated at Figure 5.

This study is supported by the study done by Steer et al. (2002). In that study, ammonia was reduced by 20% more efficiently in the wet season when compared with the other seasons. Phosphorus reductions display complex seasonal variations that imply that the least efficient phosphorus reduction occurs in winter.

Another study investigated seasonal variations in the removal of NH$_4$-N and NO$_3$-N from a full-scale horizontal subsurface flow constructed wetland located in Beijing, China, together with the plant species and number of associated bacteria. The results indicated that, the removal of NH$_4$-N and NO$_3$-N varied seasonally, with higher efficiency values in summer and autumn. The most important reason was that the bacterial numbers changed seasonally accordingly. With higher numbers in the warm seasons (summer and autumn) and lower numbers in the cold seasons (spring and winter) (Xie et al., 2016).

**Seasonal variation of chromium removal in mixed substrate bed**

The removal mechanism of heavy metals in CWs is a complex combination of physicochemical and biological processes including sedimentation, binding to substrate, plant uptake, and precipitation as insoluble forms (mainly sulfides and (oxy-)) hydroxides (Kadlec & Knight, 1996). Normally, plants receive elements in their ionic forms. This may be one of the reasons for the high amount of chromium removal efficiency seen in this study.

Considering all the aforementioned facts about heavy metal removal in constructed wetland, there was no significant performance difference between the seasonal variations. In this study, chromium was reduced by 97.65% and 99.24% in the dry season and 97.38% and 99.49% in the rainy season at 3 and 5 days of hydraulic retention time, respectively (Figure 6).
Table 2. Seasonal Variation on the Industrial Wastewater Treatment Efficiency of Mixed Substrate Bed with Chrysopogon zizanioides.

| PARAMETERS  | DRY SEASON |          |          | RAINY SEASON |          |          |
|-------------|------------|----------|----------|-------------|----------|----------|
|             | 3 DAYS     | 5 DAYS   | 3 DAYS   | 5 DAYS      | 3 DAYS   | 5 DAYS   |
| Effluent Mean Conc. | % REMOV | Effluent Mean Conc. | % REMOV | Effluent Mean Conc. | % REMOV | Effluent Mean Conc. | % REMOV |
| BOD₅        | 100 ± 5.3  | 70 ± 3   | 63.67 ± 8.96 | 95.25 | 67.69 ± 3.66 | 94.95 |
| COD         | 201 ± 19.52 | 136.88 ± 5 | 128 ± 24 | 98.03 | 132.27 ± 2.78 | 98.03 |
| NH₄-N       | 53 ± 17.35 | 3.48 ± 0.63 | 33.33 ± 10.01 | 86.49 | 3.36 ± 0.56 | 98.64 |
| NO₃-N       | 8 ± 3.6    | 0 ± 0    | 5 ± 2    | 95.64 | 0.73 ± 0.21 | 99.36 |
| TN          | 116 ± 9.16 | 37.43 ± 2.2 | 73.33 ± 9.24 | 87 | 36.18 ± 1.92 | 93.59 |
| PO₄-P       | 4.2 ± 1.06 | 3.13 ± 0.65 | 2.75 ± 0.75 | 95.47 | 3.02 ± 0.58 | 95.02 |
| TP          | 10.73 ± 3.61 | 8.01 ± 4.72 | 6.67 ± 1.81 | 95.27 | 7.85 ± 4.65 | 94.43 |
| Sulfide     | 2.58 ± 1.51 | 0.27 ± 0.03 | 1.72 ± 1.05 | 99.35 | 0.2 ± 0.03 | 99.92 |
| Sulfate     | 283.10 ± 273.95 | 99.67 ± 32.7 | 174.88 ± 160.88 | 69.41 | 73.23 ± 24.35 | 87.18 |
| Chloride    | 873 ± 97   | 272.40 ± 228.7 | 552.9 ± 76.99 | 73.16 | 174.4 ± 144.91 | 91.54 |
| TSS         | 74 ± 22.34 | 48.83 ± 7.2 | 46.27 ± 11.38 | 97.82 | 28.93 ± 3.07 | 98.64 |
| TDS         | 1011.70 ± 864.61 | 859.43 ± 50.35 | 893.78 ± 793.33 | 85.62 | 519.02 ± 116 | 91.65 |
| EC (μs/cm)  | 1930.33 ± 1740.53 | 1654.20 ± 97.3 | 1605.97 ± 1339 | 80 | 1157.67 ± 68.15 | 85.57 |
| Salinity (%)| 0.16 ± 0.05 | 0.14 ± 0.01 | 0.13 ± 0.03 | 80.6 | 0.08 ± 0.018 | 88.06 |
| TCr         | 0.43 ± 0.32 | 0.14 ± 0.07 | 0.41 ± 0.305 | 97.38 | 0.08 ± 0.04 | 99.49 |
This investigation is evidenced by the study done on the wastewater treatment efficiency of constructed wetland in Greece, in which temperature was 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 at $p$ value = .004. The author also concluded 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 (Sultana, 2014).

The plant biometry and chromium uptake capacity

In this study, the biometry data analysis was done by collecting *Chrysopogon zizanioides* from a constructed wetland bed filled with mixed substrate (Pumice and Scoria). The plant’s growth rate, its dried weight, and its potential for total chrome up-take capacity were recorded by separating the plant parts into two, which are above and below the ground level of the substrate.

This plant biometry analysis result revealed that, the plant had grown up to 99 and 26 cm. on average at the end of 6 months above and below ground level, respectively. Its dried weight was also 147.60 and 105.73 gm above and below the ground in the indicated period, respectively (Table 3).

The chromium up-take capacity of *Chrysopogon zizanioides* in the mixed substrate bed was also examined and the result showed that, more chromium concentration was found in the root than in the stem per kilogram of its parts, 37.51 and 9.32 mg/kg, respectively (Table 3).

The higher the BAF and TF values, the more they indicated the high capacity of the plant in the removal of toxic heavy metals from the wastewater. The possible reason might be due to harvesting the stem part of the plant removes chromium from the bed. The high translocation potential from root to stem enhances continuous up-take of metals from the bed (Perk, 2006; Skeffington et al., 1976). High translocation,
Table 3. Biometry of Chrysopogon zizanioides.

| PARAMETERS            | ABOVE GROUND (STEM) | BELOW GROUND (ROOT) |
|-----------------------|---------------------|---------------------|
| Length (cm)           | 99.00               | 26.00               |
| Dry weight (gm)       | 147.60              | 105.73              |
| Cr Uptake (mg/kg)     | 9.32                | 37.51               |
| BAF (Lkg⁻¹)           | 0.51                | 2.05                |
| TF                    | 0.25                | 0.25                |

Note. The BAF (2) of the stem part (0.51 Lkg⁻¹) and root (2.05 Lkg⁻¹) whereas TF (3) were (0.25) (Table 3).

therefore, decreases the metal concentration below the ground and hence reduces the toxicity of the metal ions to the root.

Conclusion
The wastewater used for this study was characterized as a very high strength wastewater with different pollutants, including nutrients and heavy metals. The bioaccumulation and translocation factor of Chrysopogon zizanioides were high, which means that this plant can remove chromium from the wastewater effectively.

The mixed substrate has a potential capacity to reduce hazardous pollutants like nutrients and heavy metals from high strength industrial wastewater. Even though the substrates have a good pollutant reduction potential via different mechanisms, mainly adsorption and ion exchange, constructed wetland beds with the plant Chrysopogon zizanioides showed better efficiency at removing those hazardous pollutants from high strength industrial wastewater than the unplanted ones. It is because of the cumulative effect of substrate adsorption and plant uptake capacity.

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Authors’ Contributions
The Author designed the study, conducted the experiments, Collected, analyzed and interpreted the data and wrote the manuscript.

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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