Phytoremediation of Soil Contaminated with Brewery and Beverage Effluents using *Cynodon dactylon*

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ABSTRACT
Phytoremediation is a natural treatment process that involves the use of green plants and microorganisms to degrade, accumulate, or eliminate various types of contaminants and pollutants in soil and water through physical, chemical and biological processes. Thus, this study evaluated the effects of green plant (*Cynodon dactylon* L.) in phytoremediation of soil contaminated with brewery and beverage wastewater (BBW). Four microcosm plots were used to conduct this experiment, the plots were irrigated with BBW and tap water (control). The results indicated that BBW is characterized with high organic contents [BOD_5 (270.25 mg/L), COD (591.50 mg/L)], suspended solids [total suspended solids (372.75mg/L), total solids (74.75 mg/L)], and nutrients [phosphate (20.83 mg/L)], no significant change in the soil texture was observed, however, there was increase in pH, electrical conductivity and chlorine content of the soil. The phytoremediation of the polluted soil decreased the concentrations of Cu by 25% after 49 days of the experimentation with about 75% of the heavy metal retained in the soil. However, future studies should focus on decrease of the build-up of salts (electrical conductivity) in the soil which could negatively affect crop yield as well as the determination of sodium adsorption ratio of the brewery and beverage wastewater to ascertain its suitability for irrigation purpose.

Keywords: *Beverage wastewater, Contaminated soils, Cynodon dactylon, Phytoremediation, Threshold limits.*

1 INTRODUCTION

Brewery and beverage industries are both water consuming and waste generation industries. According to Fillaudeau et al. (2006), beer is the fifth most consumed beverage in the world behind tea, carbonates, milk and coffee with an average consumption of 23 litres/person per year. For instance, an average of 2 - 8 hectolitres (hl) of water/hl of beer is consumed in the production of beer (Fillaudeau et al., 2006; Simate, 2015; Jaiyeola & Bwapwa, 2016; Srivastava et al., 2016) consequently, large volumes of effluent are produced (Fillaudeau et al., 2006; Simate, 2015; Jaiyeola & Bwapwa, 2016), approximately 10 - 20 times the beer produced according to Fillaudeau et al. (2006) and Jaiyeola & Bwapwa (2016). These effluents are discharged into drains or waterways (Simate, 2015) or soils which can lead to death of crops or reduction in crop yields, cause death or reproductive failure in fish, shellfish and wildlife, contamination of drinking water supplies, as well as accumulation and dissemination of toxic chemicals in sediments, that may further endanger the ecosystems and threaten public health (Simate, 2015; Srivastava et al., 2016). Though, the effluent vary in their composition (Simate, 2015), it is required to treat the effluent before discharge into the environment or for reuse purposes.

1.1 CHARACTERISTICS OF BREWERY WASTEWATER

The composition of brewery wastewater is highly variable, it has a characteristic that differ from time to time and from location to location due to the numerous different processes in the production of beer (Simate, 2015; Srivastava et al., 2016). The effluent is characterized by high levels of organic matter [biological oxygen demand (BOD) and chemical oxygen demand (COD)], nutrients (nitrogen, ammonia content, phosphates), solids [total suspended solids (TSS), total dissolved solids (TDS) and total solids (TS)], chloride salts and heavy metals (Simate 2015; Jaiyeola & Bwapwa, 2016; Srivastava et al., 2016). However, organic materials are the main concern of brewery wastewater, as well as suspended solids and pathogenic (Adelegan & Agbede, 2011). Accordingly, Simate (2015) reported 1200 – 3600 mg BOD/L and 2000 – 6000 mg COD/L and 2901 – 3000 mg TSS/L as well as 25 – 80 mg N/L and 10 – 50 mgPO43--P/L in brewery wastewater. Jaiyeola & Bwapwa (2016) also reported the same quantity of BOD, COD, nitrogen and phosphorus though with lower range of TSS (200 – 1000 mg/L). Both Simate (2015) and Jaiyeola & Bwapwa (2016) reported low concentration of heavy metals in brewery wastewater.

1.2 PHYTOREMEDIATION OF POLLUTED SOILS AND WASTEWATER

Phytoremediation is a natural treatment process that involves the use of green plants and microorganisms to degrade, accumulate, or eliminate various types of contaminants and pollutants in soil and water through physical, chemical and biological processes (Jing et al., 2007; Peng et al., 2009; Samer, 2015; Mustapha & Lens, 2018). It processes include constructed wetlands, rhizofiltration, rhizodegradation, phytodegradation, phytoaccumulation, phytotransformation and hyperaccumulators (Jing et al., 2007; Singh et al., 2012; Oh et al., 2014; Samer, 2015).

Phytoremediation is used for the treatment of organic pollutants (Mustapha et al., 2015; Mustapha et al., 2018), heavy metals (Md. Nizam et al., 2016; Mustapha et al., 2018) and radionuclides (Shawai et al., 2017). This natural treatment system is a promising alternative in reducing sludge production, has low operation and maintenance cost as well as efficient and effective wastewater treatment system (Mudgal et al., 2010; Mustapha et al., 2015). Thus, this paper describes the phytoremediation of soil contaminated with brewery and beverage effluents using *Cynodon dactylon*.

2 METHODOLOGY

2.1 DESCRIPTION OT THE STUDY SITE

The study site is located at Gidan Kwano Campus, Federal University of Technology, Minna, between Lat. 09^{o} 32' 27.01''N and Long. 06^{o} 28'31.59''E (Galadima, 2014), in the sub-humid tropics with two distinct climatic seasons; wet/rainy and dry/harmattan seasons. The mean annual air temperature is about 27.2^{o}C with the highest and lowest occurring in the month of March and December respectively. The relative humidity falls between 50 to 70% annually with total annual mean value of about 65% (Mustapha et al., 2015).

2.2 EXPERIMENTAL PROCEDURE

Insert Four replicate plots of size of 30 cm x 25 cm x 20 cm (length x breadth x height) were used for the phytoremediation of brewery and beverage contaminated soils. Plot A (two duplicates) was irrigated with brewery and beverage wastewater and plot B (two duplicates) was irrigated with tap water, which served as the control plot to enable the assessment of the phytoremediation potential of C. dactylon. To maintain soil moisture, daily watering/irrigation of the plots were done twice daily morning and evening hours for four weeks with about 1L of water each to avoid leaching of the water from the bottom of the pots. The plots were then sown with ten seeds of C. dactylon each, raking it lightly to cover about 1/8 inch with soil material. To achieve even distribution of water and no disturbance of the sown seeds, fine spray was used and the soil was continuously kept moist. Weeding were also done as needed.

2.3 WASTEWATER ANALYSIS

The wastewater was collected from the effluent discharge point of the International Brewery and Beverage Industry (IBBI) Kaduna. The samples were analyzed onsite for pH and temperature (using a handheld instrument) and electrical conductivity (EC) using a portable HACH conductivity meter. The samples were conveyed to the laboratory for further analyses according to the procedures described in the Standard methods for the examination of Water and Wastewater (APHA, 2002); total dissolved solids (TDS) and total suspended solids (TSS) using gravimetric method, dissolved oxygen (DO) using Winkler's modification method, biological oxygen demand (BOD) determined by incubation method at 20 0C for 5 days, chemical oxygen demand (COD) using open reflux titration method, spectrophotometric analysis for phosphate and nitrate-N and for the metals using a Buck 210 Atomic Absorption Spectrophotometer.

2.4. SOIL ANALYSIS

The soils used were collected from the experimental site (Campus premises, Gidan Kwanu). There are two experimental plots replicated, A and B. Plot A was used for the contaminated soil and plot B for the reference (uncontaminated) soil. The soils were sieved through a 4.0 mm sieve and thoroughly missed to ensure homogeneity. The sieved soils were analyzed for textural class and physical and chemical characteristics (pH, EC, alkalinity) according to standard analytical methods for soil analysis. Post-harvest soil samples were collected from each plot for analyses. The pH and EC values of the soil samples were measured electrometrically in a 1:2.5 and 1:5 suspension of soil and water, respectively as described by Md. Nizam et al. (2016). The soil samples taken at different interval of time from each experimental plot were air dried, crushed and passed through a 0.2 mm sieve were analyzed for heavy metal using a Buck 210 Atomic Absorption Spectrophotometer.

2.5 PLANT ANALYSIS

The choice of plants for phytoremediation is very crucial, thus the selection criteria should include geographical distribution, climate and habitat conditions, wastewater composition, availability of the plants, long term maintenance and agronomic management costs, project aims (Leto et al., 2013) and as well as biomass production (Ebrahimi et al., 2013), thus, Cynodon dactylon is a good candidate species for the phytoremediation of brewery and beverage wastewater. Additionally, C. dactylon is also known for its high adaptability to stress environment and heavy metal accumulation (Mustapha et al., 2018b).

To determine the phytoremediation ability of C. dactylon, the plants were harvested at the end of the experiment from each plot. The plant samples were thoroughly washed under running tap water and then rinsed with deionized water to remove any soil particles attached to it. The plant height was measured using a metre rule. Plant samples were oven-dried and finely ground and digested with HNO3 and H2O2 following the procedure described by Md. Nizam et al. (2016). Zn, Mn, Fe, Mn, Cl and Cu concentrations were determined from the extract using a Buck 210 Atomic Absorption Spectrophotometer.

2.6 REMOVAL EFFICIENCY (%)

The ability of C. dactylon to reduce heavy metal concentrations in the experimental plots was estimated by calculating the removal efficiency of C. dactylon by subtracting the initial concentration of heavy metals in the polluted soil at the startup of the experiment from the concentration at the end of the experiment. This is expressed in Mustapha & Lens, 2017 as:

$$Removal\ efficiency\ (\%) = \frac{Concentrations_{Intial} - Concentrations_{final}}{Concentrations_{Initial}} \quad (2.1)$$

3 RESULTS AND DISCUSSION

All Figures and Tables inserted should be properly referenced in the discussion of the results. Results and discussion entails the use of words to describe the implication of the results expected/obtained. Often, Figures, Tables and Plates are powerful means for proper technical result reporting and discussion. Examples of Figures and Tables are given in Figure1 and Table 1.

The results of the analysis of brewery and beverage wastewater revealed a high concentrations of BOD5 and COD (270 and 592 mg/L), this high BOD5 and COD concentrations is similar to concentrations reported by Adelegan & Agbede (2011); Simate (2015); Srivastava et al. (2016) for brewery and beverage wastewater. Consequently, the discharge of wastewater with high levels of organic compounds can deplete dissolved oxygen for aquatic species survival (Simate, 2015). Wastewater with high concentrations of organic matter is synonymous to high amount of suspended solids (Simate, 2015). Accordingly, this present study contained large amount of suspended solids that ranged from 331 to 435 mg/L of TDS and 72 to 78 mg/L of TSS above the permissible limits for wastewater discharge (Table 4.1). These results are similar to results obtained in Simate (2015) and Jaiyeola & Bwapwa (2016). High suspended solids in discharged wastewater can result into low light accessibility for photosynthetic organisms (Simate, 2015). Simate (2015) and Jaiyeola & Bwapwa (2016) also reported low concentrations of heavy metals in discharged brewery and beverage wastewater as reported in this present study.

3.1 CHARACTERIZED BREWERY AND BEVERAGE WASTEWATER

The characteristics of brewery and beverage wastewater collected at the effluent discharged point of IBBI is presented in Table 4.1. The table is a descriptive statistics that includes values of mean, minimum, maximum, range, standard error, standard deviation and variance using the SPSS Statistics 16.0 for Windows (SPSS Inc., Chicago, IL, USA; version 16.0). The wastewater consists of concentrations higher than the Federal Ministry of Environment (FME), Nigeria threshold limit for the discharge into river bodies for food processing industry (Adelegan and Agbede, 2011). The wastewater had values ranging from 8.14 to 8.19 for pH, 28 to 340C for temperature, this relative high temperature may be due to discharge of hot liquor and steam condensates from the brewery and beverage operation as reported by Belay and Sahile, (2013), 2.0 to 2.5 mg DO/L, 253 to 295 mg BOD/L, 570 to 619 mg COD/L, with lower concentrations of heavy metals below the threshold limits (Table 4.1). The BBW contained low levels of dissolved oxygen (DO), due to high concentrations of BOD5 and COD in the BBW. This can make water unsuitable for drinking, irrigation or any other uses if this effluent is discharged into waterbodies or the environment (Olajumoke et al., 2010). However, the wastewater had mean suspended solids and organic contents concentration higher than the threshold limits according to standards given for food processing industry by FME (Nigeria).

3.2 SOIL QUALITY

The soils of the experimental plots was sandy clay loam soils (10% clay, 10 - 15% silt and 70 - 80% sand) classified according Md. Nizam et al. (2016). Soil pH was alkaline in nature. The soil chemistry were affected based on the sources of the water types used for irrigation, soils irrigated with BBW had a higher pH than soils irrigated with tap water (Table 4.2). Also, soils irrigated with BBW had higher conductivity (1581 $\mu s/cm^2$) than the tap water irrigated soils (Table 4.2). This is an indication of the accumulation of salts due to the irrigation with BBW wastewater.

Taylor et al. (2018) reported a similar observation in their study with higher pH and conductivity in the soils irrigated with brewery wastewater. Soil irrigated with BBW was characterized with high conductivity and pH, these qualities are known to negatively affect the growth and health of plants (Taylor et al., 2018),

TABLE 1: CHARACTERISTICS OF DISCHARGED BREWERY AND BEVERAGE WASTEWATER

Parameter	Mean	Minimum	Maximum	Range	Std. Error	Std. Deviation	Variance	*Threshold limit
pH	8.17	8.14	8.19	0.06	0.025	0.03536	0.001	6.5-7.5
Temp	30.50	28.00	34.00	6.00	1.3229	2.64575	7.000	30-36
Alkalinity	103.25	98.00	110.00	12.00	2.5617	5.12348	26.250	200[a]
Hardness	144.25	124.00	160.00	36.00	8.2298	16.45955	270.917	500[a]
EC	1097	990	1200	210	42.8836	85.76713	7356	1000
DO	2.20	2.00	2.50	0.50	0.1080	0.21602	0.047	<0.2
TDS	372.75	331.00	435.00	104.00	22.1524	44.30482	1963	2000
TSS	74.75	72.00	78.00	6.00	1.2500	2.50000	6.250	30
BOD	270.25	253.00	295.00	42.00	9.2320	18.46393	340.917	30
COD	591.50	570.00	619.00	49.00	10.3963	20.79263	432.333	80
Nitrate	2.65	2.26	3.00	0.74	0.1605	0.32104	0.103	20
Phosphate	20.83	18.80	22.50	3.70	0.7793	1.55858	2.429	5
Cl	19.83	17.80	22.30	4.50	1.0896	2.17926	4.749	600
Zn	0.55	0.47	0.63	0.16	0.0375	0.07500	0.006	<1
Mn	0.18	0.13	0.22	0.09	0.0193	0.03862	0.001	20
Cu	0.40	0.31	0.50	0.19	0.0417	0.08347	0.007	1
Fe	0.54	0.43	0.62	0.19	0.0413	0.08261	0.007	20

however, *C. dactylon* grew healthy. This may be related to *C. dactylon* being a salt tolerant plant.

TABLE 2. CHARACTERISTICS OF BREWERY AND BEVERAGE CONTAMINATED SOILS

Characteristics	Uncontaminated soil	Contaminated soil	Post Treated Soil
Sand	78.24	78.80	-
Silt	13.50	12.00	-
Clay	8.26	8.20	-
Textural Class	Sandy clay loam	Sandy clay loam	Sandy clay loam
pH	7.89	9.07	8.08
Electrical conductivity, µs/cm^2	890	1581	1022

3.3. PLANT: GROWTH AND DENSITY OF *C. DACTYLON*

In order to assess the phytoremediation potential of *C. dactylon* on soil contaminated with brewery and beverage wastewater, *C. dactylon* was planted in the contaminated soil, plant stem height (Fig. 1) and plant density (Fig. 2) were measured and recorded. Post hoc (Bonferroni) test was used to determine the effect of wastewater and tap water on the growth of *C. dactylon* (Table 4.3). The results indicate that from day 7 to day 14, there were no significant difference on the type of water that was used. However, with increased exposure of wastewater and tap water over time, plant growth (Fig. 1) and density (Fig. 2) was higher in the plot irrigated with wastewater than the tap water. Also, there was a higher positive effect of the wastewater on the growth of *C. dactylon* each week than tap water (Fig. 1 and Fig. 2). Indicating that the constituents in the water types (BBW and tap water) and soils may have affected the growth and health of *C. dactylon*.

Sufficient concentrations of macro- and micro-nutrients are required for healthy plant growth (Taylor et al., 2018). Meanwhile, tap water contain low concentration of nutrients, this is observed in Fig. 1, accordingly, higher plant growth and health in the plants irrigated with brewery and beverage wastewater was recorded. Thus, *C. dactylon* utilized the nutrients in the brewery and beverage wastewater that was used to irrigate it. In addition, the water pH and air temperature were appropriate for the growth of *C. dactylon*.

The salinity of wastewater is of utmost importance when it comes to irrigation, this is because sodic soils are unsuitable for the cultivation of most vegetable crops, it can lead to low yield, cause extreme flocculation and decrease in infiltration rates (Taylor et al., 2018). The BBW wastewater used in this present study contained low salinity with a concentration that ranged from 990-1200 µs/cm^2. Figs. 2a, b and c shows the growth and density of *C. dactylon* from the time of sowing the seeds to establishment. After 5 days of sowing, young *C. dactylon* began to sprout and by day 30, the seed bed was well established. *C. dactylon* grew and spread rapidly by rhizomes and stolons over the period of experimentation (Fig. 2). It indicate that *C. dactylon* has a good endurance to contaminants in brewery and beverage wastewater (Fig. 2).

The rapid growth of *C. dactylon* implies that it has high biomass productivity and uptake capacity which is an important feature for phytoremediation (Md. Nizam et al., 2016).

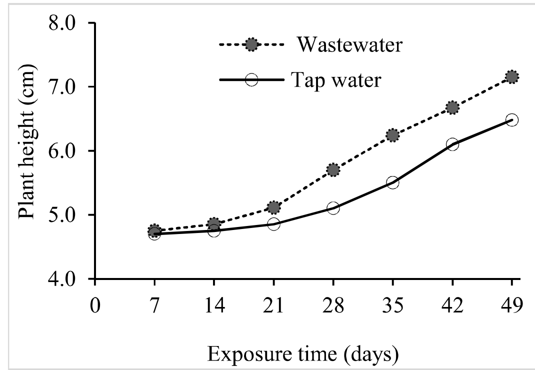

Figure 1: *C. dactylon* growth rate over time

TABLE 3: EFFECTS OF WASTEWATER AND TAP WATER ON PLANT GROWTH RATE (CM)

Exposure time	**Wastewater**			**Tap water**		
	Mean*	**Min**	**Max**	**Mean**	**Min**	**Max**
07	$4.74^{a}\pm0.01$	4.73	4.75	$4.71^{a}\pm0.01$	4.70	4.72
14	$4.83^{b}\pm0.04$	4.80	4.85	$4.78^{b}\pm0.04$	4.75	4.80
21	$5.11^{c}\pm0.01$	5.10	5.11	$4.84^{c}\pm0.01$	4.83	4.85
28	$5.69^{d}\pm0.02$	5.67	5.70	$5.09^{d}\pm0.02$	5.07	5.10
35	$6.22^{e}\pm0.03$	6.20	6.24	$5.54^{e}\pm0.02$	5.52	5.55
42	$6.69^{f}\pm0.02$	6.67	6.70	$6.09^{f}\pm0.01$	6.08	6.10
49	$7.17^{g}\pm0.02$	7.15	7.18	$6.27^{g}\pm0.02$	6.25	6.28

*Values are means of two duplicates (n = 2), mean±standard deviation. Values on the same column with different superscript are significantly different ($P\leq0.05$) while those with the same superscript are not significantly different ($P \geq 0.05$) as assessed by LSD, Tukey (HSD) and Duncan's Multiple Range Test.

Fig. 2. Plant density: (a). Emergence of *C. dactylon* 5 days after sowing; (b). 14 days after sowing and (c). 28 days after sowing

3.4. HEAVY METAL CONTENT OF SOIL AND *C. DACTYLON*

To determine the phytoremediation potential of *C. dactylon* for brewery and beverage contaminated soil, the pre-planting and post-harvest soils were analyzed for heavy metal concentration (Table 4.4). The heavy metal concentrations in the pre-planting soil ranged from 0.61 to 10.22 mg/L while post-harvest concentrations ranged from 0.60 to 10.18 mg/L. The results showed a lower concentrations in the post-harvest soil. This is an indication of the phytoremediation potential of *C. dactylon* (Mustapha et al., 2018b). The results revealed a low concentrations of heavy metals in the contaminated soils. This low metal concentrations is most likely due to the low concentrations of heavy metals in brewery and beverage wastewater. The observation is in agreement with reports of Simate (2015) and Jaiyeola & Bwapwa (2016). Although, metals are more soluble in acidic soil than alkaline soils, the heavy metal concentrations in the soil were found to be within acceptable levels.

TABLE 4. HEAVY METAL CONTENTS IN THE EXPERIMENTAL SOILS

Heavy metal (mg/g)	Initial soil content (before sowing the seeds)	Post-harvest content from wastewater plot	Post-harvest content from tap water plot
Zn	14.21	14.08	13.71
Mn	0.61	0.60	0.61
Fe	10.22	10.18	10.25
Cu	6.88	5.22	6.87
Pb	Bdl	Bdl	Bdl

Plants are known to take up heavy metals and other pollutants into their tissues as reported in literatures (Mudgal et al., 2010; Ali et al., 2012; Md. Nizam et al., 2016; Mustapha et al., 2018b). These values were within the threshold limits, nevertheless, the plants were able to take up the some heavy metals into their tissues.

C. dactylon is an ideal plant species for phyto extraction of heavy metals, from the results of this present study, the plant grew rapidly, produce high biomass and accumulated metals into its shoots. *C. dactylon* was observed to accumulate some quantity of heavy metals into it parts (Table 4.5) with Zn being the most accumulated and Mn as the least accumulated.

Zn is an essential element to plants that is easily taken up by roots (Mustapha et al., 2018b). In a study conducted by Mustapha et al. (2018b), demonstrating the bioaccumulation and translocation ability of *C. dactylon* of Zn from petroleum wastewater, *C. dactylon* accumulated 184 mg/kg and 407 mg/kg of Zn into its shoots and roots, respectively with a translocation factor of 0.5. This is an indication of the exclusion strategy of Zn in *C. dactylon* (Bragato et al., 2006).

TABLE 5. PLANT ANALYSIS BEFORE AND AFTER PLANTING FOR *C. DACTYLON* IRRIGATED WITH BREWERY AND BEVERAGE WASTEWATER

Parameter (ug/g)	Concentrations (initial) @ day 14 after sowing	Concentrations (final) wastewater @ day harvest (day 30)	Concentrations (final) tap water@ day harvest (day 30)
Zn	1.24	3.45	2.96
Mn	0.65	0.82	0.77
Fe	0.34	1.22	1.13
Cl	0.96	1.05	1.08
pb	bdl	bdl	bdl

3.5. PERFORMANCE EVALUATION: HEAVY METAL RETENTION

This study showed a low removal efficiency of the studied heavy metals. This may be attributed to many factors such as (1). The BBW was characterized with low levels of Zn, Mn, Fe and Cu; thus, the metals were non-available; (2). Substrate medium is the primary sink for heavy metals present in the aquatic environment (Mustapha & Lens, 2017). Fine textured substrate was used, they are known to accumulate metals if they contain high amount of organic matter, hence, a higher retention of the studied heavy metals were observed with a corresponding lower removal of the heavy metals (Fig. 3); and (3). The short experimental period (49 days), a longer experimentation may result into higher removal efficiencies as reported in literature (Mustapha et al., 2018b).

In addition, of the four (4) heavy metals studied, Cu had the highest removal and the least retained in the substrate medium while Fe was the most retained and lowest removal performance. Nutrient can enhance phytoremediation processes, however, the BBW contained low concentrations of nitrate and a higher concentrations of phosphate (Table 4.1), and nitrate are more easily assessable by plants and microorganisms. This may also have contributed to the low performance of this system.

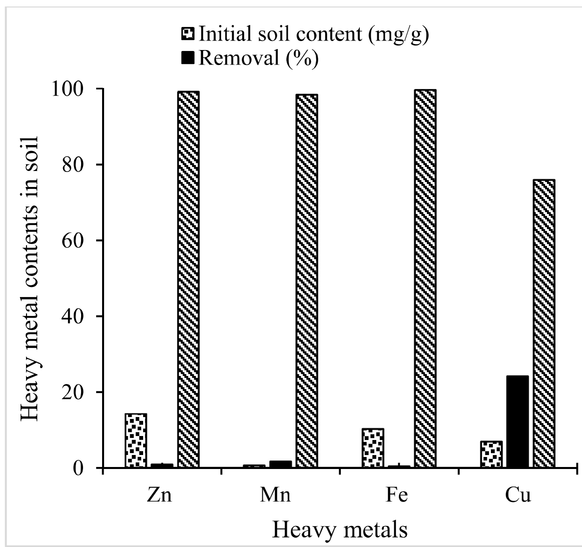

Fig. 3. Removal and retention of heavy metals

4 CONCLUSION

The characterized discharged brewery and beverage wastewater contained high levels of organic contents and suspended solids above the threshold limits for food processing industry. Thus, to prevent environmental pollution and protect public health, phytoremediation is

recommended as a tertiary/advance wastewater treatment system for brewery and beverage industry to thoroughly treat its effluent before its discharge into the environment.
The brewery and beverage wastewater was characterized with low concentrations of heavy metals that were within the threshold limits for wastewater discharge.
C. dactylon was observed to accumulate some quantity of heavy metals into it parts with Zn being the most accumulated and Mn as the least accumulated.
Also, further studies will be required to analyze the soil structural porosity and bulk density in order to make proper conclusion on the effectiveness of phytoremediation of *C. dactylon* for treatment of brewery and beverage contaminated soil. Furthermore, sodium adsorption ratio of the brewery and beverage wastewater should be determined to ascertain its suitability for irrigation purposes. It is also recommended that the experiment should be ran for longer period of time.

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