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Full Length Research Article

Assessment of the Effect of Industrial Brewery Effluent use in Irrigation, on the Heavy Metal Concentrations and Phytochemicals of *Bryophyllum Pinnatum* (Lam.) Oken

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Abstract

This study assessed the effects of Brewery effluent on the properties of *Bryophyllum pinnatum.* The experiment was conducted inside a screen house in the Botanical garden of the University of Nigeria, Nsukka. The plantlets were watered with six concentrations of Brewery effluent concentrations (0%, 20%, 40%, 60%, 80% and 100%) at two days intervals for 12 weeks. Data collected were subjected to analysis of variance and significant means separated using Least Significant Difference Test. The result of the physicochemical parameters showed that the pH (7.71±0.05), Total Dissolved Solid (2.55±0.50), Total Solid (29.31±0.22), Biological Oxygen Demand (1.97±0.12), Chemical Oxygen Demand (49.97±6.00) and Dissolved Solids (3.00±0.15) are within WHO and NESREA permissible standard limit while Total Suspended Solids (26.76±0.26) and Turbidity (38.80±11.70) were above the standard limits. The soil chemical properties after the experiment showed a significant difference in the pH (6.53), N (1.30), P (18.60), K (2.23), and Ca (0.23) levels across the different treatments. The mean values for the accumulation of the heavy metals revealed that copper (5.53±0.04), zinc (16.65±0.30), nickel (13.19±0.026), lead (0.77±0.003), and iron (68.00±0.00) except cadmium (33.32±0.57) were below WHO/ FAO permissible standard limits. The phytochemical analysis revealed the presence of tannins, alkaloids, flavonoids, saponins, and phenolic compounds in both treated and control plants. It is therefore recommended that Brewery effluent should not be discharged directly into the water bodies and their surroundings, but used as an alternative to mineral fertilizers.

Keywords: Industrial Brewery Effluent. Phytochemicals, heavy metal, *Bryophyllum Pinnatum*

Introduction

Breweries are a major consumer of fresh water and producers of nutrient-rich wastewater (Braeken *et al.*, 2014). Pollution in soil and water is closely associated with activities like manufacturing, agriculture, mining, industries, burning of waste and fossil fuels, and domestic waste disposal. All types of wastes or effluents and most of the bi-products from such industries pose serious threat to the water bodies, land, plants, animals and humans (Ramana *et al*., 2002; Sajid, 2013, Arjun *et al*., 2013).

Growing water scarcity threatens economic development, sustainable human livelihoods, environmental quality, and a host of other societal goals in the countries and regions around the world. Urban population growth particularly in developing countries, places immense pressure on water and land resources; it also results in the release of growing volumes of waste water, most of which are untreated. Wastewater is increasingly being used for irrigation in urban and peri-urban agriculture, and even in distant rural areas downstream of the very large cities. It drives significant economic activity, supports countless livelihoods particularly those of poor farmers, and very sustainable changes in the hydrology and water quality of natural water bodies.

Wastes from brewery industries, especially the liquid wastes which are normally considered as the main industrial pollutants containing organic and inorganic compounds, suspended solids, and toxic chemicals are discharged into the nearby water bodies making them toxic. The waste water becomes unstable for drinking, irrigation or other uses. It has been found that growth and yield of crops and soil health get reduced when the farmers use the effluents for irrigation of the cultivated land (Nandy and Kaul, 1994). Brewery processes generate large amounts of liquid waste. When the waste waters are diluted, the agricultural reuse of the treated effluents serve goals, such as promoting sustainable agriculture and conservation of the scarce water resources, while untreated effluents cause many plants growth related problems. (Arjun *et al*., 2013).

The current treatment process is costly and energy expensive (Power 2014, Rodda *et al.*, 2011). However, alternative technologies exist that could make the water and nutrients in the Brewery Effluent available for re-use or for use in other downstream applications. There is a need to identify potential alternative water, nutrient and energy resources and to develop the techniques needed to exploit their value (Rodda *et al.*, 2011, Power, 2014). The irrigation of agricultural crops is a potential downstream use for water and nutrients that have been recovered from Brewery Effluent.

Brewery effluent is an organic effluent that contain nitrogen and phosphorous (Braeken *et al.,*2014), essential for good plant growth (Freeman, 2005). Farmers spend money buying inorganic nitrogen and phosphorus fertilizers. Brewery effluent could be used as a source of water and nutrients in crop production (Kumar *et al*., 2010, Jones *et al*.,2013). Lettuce and tomatoes have been grown hydroponically on partially treated Brewery Effluent (Jones *et al*., 2013, Power, 2014). However, no research has been done on the possible use of brewery effluent as a source of irrigation water in agricultural crop production and the effect that this will have on crop production rates and soil characteristics. This research may provide a way in which breweries can find an end use for their effluent, meaning they would have zero waste.

Water is one of the key challenges that will determine the future of humanity on our planet (Arnell, 2004). It has even been suggested that water will soon be a bigger

crisis than oil for humanity (Power, 2013). It is vital that we develop technologies to ensure adequate supply of water to all users while minimizing environmental degradation and water resource pollution. Africa is faced with water, food and energy shortages (Rodda *et al*., 2011). This project has the potential to address these issues, since a "waste" product, which is currently a liability to industry, might be used to produce crops and create jobs when it is used as an irrigation source in crop production.

B pinnatum is also known as cathedral bells, life plant, miracle leaf and air plant in English, Oda opue in Igbo and Abamoda in Yoruba (CABI, 2017). They are phylloclades which are remarkable for their ability to produce bulblets, at their margin, between the teeth, adventitious buds appear which produce roots, stems, and leaves. When the plantlets fall to the ground, they root and can become larger plants. The fruits are follicles (10-15mm) which are found in the persistent calyx and corolla (Akachukwu and Farusi, 1995).

B pinnatum leaves are actually leaf - stem combinations called phylloclades. They are thick, fleshy, elliptical in shape, curved, with a crenate or serrated margin, often reddish as noted (Fontem and Schippers,2004). They are simple at the base of the stem; the phylloclades are imparipinnate at the top, 10-30 cm long, with three to five pairs of fleshy limb lobes. The *B pinnatum* has been recorded in Trinidad and Tobago as being used as a traditional for hypertension. In traditional medicine, the juice of the leaves is also used for kidney stones. *B pinnata* is used in local application against headache for the people of Amazon, it has multiple uses; the creoles use it roasted against inflammations and cancer and as an infusion and as a popular remedy for fever.

The main medicinal use of *B pinnatum* in south- East Asia is in the treatment of boils, wounds, burns and scalds. In Indonesia, pounded leaves are used as a diuretic, and leaves are used externally to treat sores and pain in back and feet, a poultice is sometimes applied on sore eyes or to relieve headache, a decoction is used internally to treat fever and oedema; an extraction of dried pounded leaves in water is used against haemorrhoids (Udoh, 2015).

In Malaysia, the crushed leaves are applied to the forehead to treat headache and to the chest to treat coughs and pains. In Brunei, a leaf infusion is drunk as a febrifuge (Ezekwe *et al*., 2001; Aja *et al*., 2010). In the Philippines, the leaves are used as an astringent, antiseptic, and against insect bites. Fresh pounded leaves are applied to burns and as poultices on boils. It therefore becomes imperative to encourage the protection, conservation and propagation of the plant species owing to its numerous medicinal benefits. The society at large will benefit from the findings of the work because there will be constant food production even during the period of drought.

This study was therefore carried out to assess the effect of brewery effluent on some soil chemical properties, to ascertain the concentration of heavy metals in various parts of *B pinnatum* (leaves, stems and root), and to determine the phytochemical components of *B pinnatum* treated with brewery effluent and the control.

MATERIALS AND METHODS

Study Location

The study was carried out in the Botanical Garden of University of Nigeria, Nsukka in Enugu State, located between longitude 7.39^{o} E and latitude 6.86^{o} N with elevation of 456m above sea level (Chinago, 2015). The annual rainfall of Nsukka ranges from

1845 mm to 2000 mm. The climate of Enugu is tropical with two major seasons, wet (April - October) and dry (November - March) seasons. Nsukka is both commercial and agrarian town producing varieties of vegetable crops including *B pinnatum*.

Collection of the experimental Materials

Fresh leaves of *B. pinnatum* used for the study were obtained from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Brewery effluent was collected from Ama Brewery Industry located at 9th mile Enugu State (Figure 1). The top soil and sandy soil were collected from the Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The poultry droppings were collected from the poultry farm of the Department of Animal Science, University of Nigeria, Nsukka.

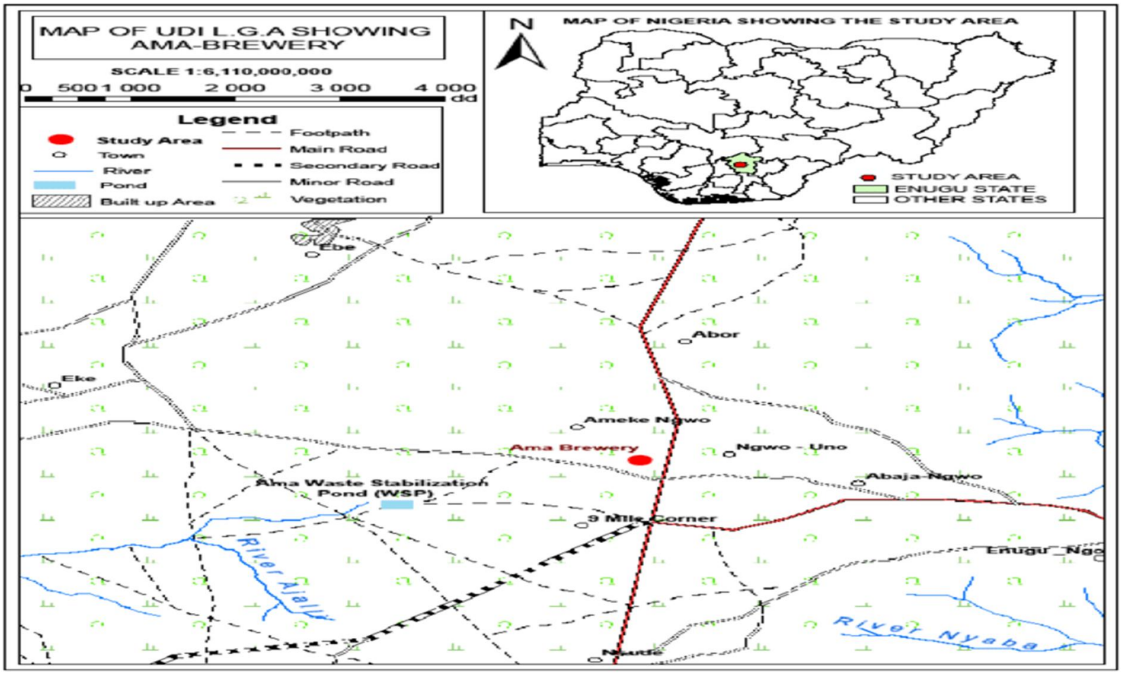


Figure 1: Map of Nigeria Showing the Study Area; Enugu State and Map of Udi L. G. A Showing Ama Brewery. Source: Nipost Postcode Map, 2020

Preparation of soil sample

The top soil, poultry manure and sandy soil were mixed in the ratio of 3:2:1 respectively. This is referred to as the amended soil. Two kilograms of amended garden soil were weighed and put into each of the 108 polythene bags.

Preparation of effluent concentrations

The brewery effluent applied was diluted as follows; 0% (100 ml tap water), 20% (20 ml effluent + 80 ml tap water), 40% (40 ml effluent + 60 ml tap water); 60% (60 ml effluent + 40 ml tap water); 80% (80 ml effluent + 20 ml tap water); and 100% (100 ml effluent).

Laboratory and field studies

Germination study

The germination study was done in the laboratory where six leaves of *B pinnatum* were placed in six petri dishes each and kept

moist by adding one milliliter each of effluent and tap water at intervals of two days. Five milliliters (5 ml) of effluent solution was added in different labeled petri dishes at 0%, 20%, 40%, 60%, 80%, and 100% concentrations. The experiment was replicated three times. They were left to stand for seven days and records were taken on the following:

Days of germination: the number of days it took to germinate was recorded. The treatments were monitored after to know which treatment that can retard or enhance germination.

Germination percentage: This was calculated respectively as:

Germination (%) $= \frac{no\ of\ regenerated\ plantlets}{no\ of\ buds}$ × 100 (Omosun *et al*., 2008)

Nursery bed preparation

Two baskets measuring 50cm in diameter and 20cm in height were filled with the amended soil to the brim. The leaves of *B. pinnatum* were planted through vegetative propagation into prepared medium. This was monitored and watered daily with watering can for three weeks.

Transplanting

The plantlets were transplanted four weeks after germination into 108 polythene bags each containing 2kg of the amended soil. The transplanting was done in the evening to avoid excessive effect of transpiration on the plantlets. The plants were watered with tap water at an interval of two days for two weeks for the plantlets to stabilize before the application of effluent. One hundred millilitres treatment was applied on the plantlets after 4 weeks of transplanting at the concentrations of 0%, 20%, 40%, 60%, 80%, and 100%. Irrigation was done at an interval of two days for 12weeks.

Experimental Design

The experiment was carried out in a Completely Randomized Design (CRD) comprising of six treatments. Each treatment was assigned six bags and replicated three times giving a total of 108 experimental units.

Data Collection

Three plants were randomly selected from each treatment and analyzed for minerals and heavy metal content (Omosun *et al.,*2008).

Chemical Analysis

Physicochemical properties of effluent and Tap water.

The Brewery effluent and tap water were analyzed in the Department of Biochemistry, University of Nigeria, Nsukka following the procedures of APHA (1998). It involves the measurement of the following: Dissolved Oxygen, Biological Oxygen Demand and Total Dissolved Solids. The heavy metals such as nickel, mercury, lead, arsenic, zinc, and cadmium were analyzed also, and calcium, potassium, magnesium, phosphate and nitrate were also analyzed.

pH

The pH of the effluent samples was measured with a pH meter (Metrohm, 827 Labo-mega symbol). The electrode of the pH meter was inserted into the tap water and effluent samples respectively, and the values on the screen were recorded (Jodeh *et al., 2012*).

Determination of Dissolved Oxygen (DO):

Dissolved oxygen was determined using Azide modification of Winklers's method as described by Hortwitz and Latimer (2006). Oxygen is fixed in the sample bottle by adding 2ml each of manganese sulphate and alkaline- iodide solution into the 250ml oxygen bottles filled with samples and mixed thoroughly by inverting the stopper bottle several times. Thereafter iodine was

released in the solution by adding 2ml concentrated sulphuric acid into the fixed samples. A measured200ml of each treated samples were pipette into a conical flask and titrated with standard 0.025N sodium thiosulphate until a yellow colour appeared. Then, 2ml of starch solution was added as an indicator and the titration continued to a colourless end point and the reading was taken.

$$\text{D.O.} = \frac{A \times N \times 8000}{V-4}$$

Where A = Volume of sodium thiosulphate

N = Normality of the titrant

V = Volume of the sample bottle. (APHA, 1998).

Determination of Biological Oxygen Demand (BOD).

This was determined by Winkler's method following the procedure described by APHA (1998). This was derived from the difference between the dissolved oxygen (DO) on Day 1 and on Day 5. The DO was estimated on both occasions by means of a dissolved oxygen meter, Hanna instrument (Model H19146). The DO- meter was calibrated once a week using a DO- probe filling solution. The DO- meter probe was first inserted into distilled water contained in a beaker to test the functionality of the instrument. Then the probe was rinsed with sample before insertion into the sample bottle. The sample bottle was covered properly and placed in a dark cupboard until the fifth day when the DO was measured again.

BOD = DO of day 1 minus DO of day 5. That is, BOD = $DO_1 – DO_5$ (APHA, 1998).

Determination of Chemical Oxygen Demand (COD)

This measures the oxygen equivalent consumed by organic matter in a sample during strong chemical oxidation. A measured 2.5 ml of sample was poured into a refluxing flask followed by 3.5 ml of sulphuric acid and 1.5g potassium dichromate ($K_2Cr_2O_7$) which was added to the solution. The mixture was digested for two hours, then cooled at room temperature and samples titrated with ferrous ammonium sulphate (FAS) using orthophenanthroline ferrous complex as indicator. The milliliters equivalent of O_2 is equal to the millilitres equivalent of FAS. The COD was determined as follows;

$$\text{COD} = (A - B \times 0.2 \times 20\ mg/l)$$

Where, A is sample titer and B is black titer. (APHA, 1998)

Determination of Total dissolved solids

This was estimated using a total dissolved solids/ conductivity meter (HACH Model Co 150). The probe was dipped into the bottle of thoroughly mixed sample and the readings taken after the meter indicates ready and results were recorded in mg/L.

Determination of Total organic carbon

The total organic carbon was determined using the wet oxidation method (APHA, 1998). Potassium dichromate (5 ml) was added to 0.5 g of the sample in a digester tube and the suspension swirled gently. Concentrated H_2SO_4 (7.5 ml) was added rapidly with an automatic pipette directly into the suspension and immediately swirled until properly mixed. The tube was placed in a pre-heated block at 145 -155^oC for 30 minutes, allowed to cool and made up to 50ml with deionized water. The digest was transferred to a 100 ml conical flask; 0.3 ml of acidic diphenylamine indicator was added and stirred properly with a magnetic stirrer. Ferrous ammonium sulphate (0.2 M) was titrated with the solution obtained. A blank sample was prepared in the same manner to serve as control

$$\%TOC = (0.003 \times 0.2\,Vb - Vs) \times 100$$

Where, TOC = Total oxygen Demand

V_b = Volume (ml) of 0.2 M ferrous ammonium sulphate used to titrate reagent blank solution and;

Vs = Volume (ml) of 0.2 M ferrous ammonium sulphate used to titrate sample solution.

Determination of Nutrients in the Effluent

Nitrate (N) determination

This was determined calorimetrically with phenol di-sulphonic acid using the methods of APHA (1998). Five millilitres of the sample solution were pipette into 50 ml conical flask. The sample was heated on water bath until it fumed, 0.2 ml of phenol di-sulfuric acid were added and diluted to 10 ml with distilled water. Absorbance was measured at 490 nm.

$$\text{Concentration (Mg/L)} = \frac{Abs \times D.F}{Slope}.$$

Where Abs = absorbance sample

D.F. = Dilution factor.

Phosphate(P)determination

Phosphate was determined using 721 VIS spectrophotometer following the procedures of APHA (1998).

Procedure: Five millilitres of the solution was pipette into a 50 ml of graduated flask. Molybdate mixture (10 ml ammonium molybdate plus 1ml of ascorbic acid) was added and made up to 50 ml with distilled water. The mixture was allowed to stand for 15 minutes for the colour to develop. Absorbance was measured at 400nm in a spectrophotometer (721 VIS).

Potassium (K) Determination

Potassium was determined using a flame photometer (Perkin - Elmer) following the procedure of AOAC (2002). Five percent potassium dichromate was added into 100 ml flask. This was dissolved with water and diluted to the marked level to make the stock solutions. From the stock solutions, 10 ml each was pipette and diluted to 100 ml with water. The flame photometer was calibrated using the standard solutions. The meter reading was set at 100% emission while the top concentration of standard was aspired. The standard solution was then aspirated and the emission reading recorded (% emission) with the blank and top standard (0 and 100% emission respectively). The sample solutions were aspirated into the instrument and the concentration of potassium was read from the standard curve.

The concentration of potassium was calculated using this formula;

$$\text{K (mg/l)} = \frac{\text{concentration reading in curve} \times \text{D}}{\text{Volume of sample}}$$

Where, D = Dilution factor.

Calcium Determination

Varsenate solution (EDTA) was prepared thus: 3.72 g of ethylene-diamine tetrachloride was dissolved in 800 ml of distilled water. The solution was made up to 1 liter with distilled water. Ten milliliters of the sample were pipette into 50 ml conical flask;25ml of distilled water and 25 ml of 19% potassium hydroxide was added, followed by a pinch of calcine indicator. The mixture was titrated with 0.01 N EDTA in a burette. The indicator changes colour when all calcium ions are complexed with EDTA showing a purple or blue colour.

Magnesium Determination

Ten milliliters of sample solution were pipette into 50 ml conical flask and 25 ml of distilled water added. Ammonia – ammonium buffer (25 ml) was added, followed by the addition of 2 drops of Erichrome – Black T indicator. The mixture

was titrated with 0.01 N EDTA (AOAC, 2002).

The concentration of magnesium was calculated using this formula;

$$\text{Mg (mg/l)} = \frac{T \times N \times Eqv.wt.ca \times 100 \times 50}{1000 \times wt.ofsample \times Alig}$$

Where, T =Sample titre, N = normality of EDTA; Eqv.wt..ca = equivalent weight of calcium; Wt= weight of sample, Alig. = aliquot.

Soil Chemical Properties Analysis

The soil was analyzed in the Department of Soil Science, University of Nigeria, Nsukka. Some selected soil chemical properties such as soil pH, nitrogen and exchangeable cations (K^+, Ca^{2+} and Mg^{2+}) and heavy metals (Zn, Pb and Cd) where determined according to the method of APHA, (1998).

Determination of soil pH

The pH of the soil samples was measured with a glass electrode in 1:2 sample water ratio suspension using Jenway pH, conductivity and temperature meter as follows: the pH meter was first calibrated with standard buffers of pH 4, 7 and 9. Ten grammes (10 g) of each sample was weighed into plastic containers and 20 ml of distilled water added. The mixture was stirred several times for 30 minutes and the suspension allowed to stand for 30 minutes undisturbed. The pH electrode meter was inserted into the settled suspension and the pH measured.

Determination of soil Total nitrogen

The total nitrogen (mg/g) was determined using the modified Kjeldahl digestion method as described by AOAC (2002). Ten grams (10 g) of the sample was weighed into a clean dry Kjeldahl flask and 20 g of catalyst mixture was added. The contents were thoroughly mixed and left to stand for 20 minutes, sulphuric acid (35 ml) was added to the flask, mixed well and left to stand for another 15 minutes. The contents were then digested over a Bunsen burner for about 2 hours and cooled. Distilled water (100 ml) was added to the mixture and distilled with 100 ml of sodium hydroxide solution and a few zinc granules in a distillation flask. Boric acid indicator solution (25 ml) was pipette out into a 500 ml Erlenmeyer flask and its delivery end kept below the condenser of distillation flask. The collection of distillate in the flask was confirmed by titrating it against 0.1 N Hydrochloric acid using an indicator. Color change from blue to light brown to pink indicated the end point. Total nitrogen was then calculated with the formula:

$$\text{Total nitrogen (mg/g)} = \frac{(V1 - V2) \times N \times 14}{S}$$

Where, V_1 = volume of titrant used against sample (ml)

V_2 = volume of titrant used against blank (ml)

N = Normality of titrant (0.1) and;

S = weight of sample used (g).

Determination of soil Available phosphorus

The available phosphorus (mg/kg) was determined using Bray method as described by AOAC (2002). A solution of 20 ml of 0.03 N Ammonium fluorides (NH_4F) was pipetted into 0.1 N hydrochloric acid in a test tube with 2.0 gram of sample. The solution was shaken for about 1 minute and allowed to settle after which it was filtered with Whatman No. 42 filter paper. One milliliter (1 ml) of the filtrate was added into test tube with 7 ml of distilled water; 1 ml of 2.5% ammonium molybdate and 1 ml of 2% ascorbic acid were added. These were thoroughly mixed and placed in a beaker of boiling water for 3 minutes and allowed to cool. A blank was also prepared alongside

the samples and the optical density at 620 nm was the read using Eel Calorimeter.

Determination of soil Exchangeable bases (K, Mg and Ca)

The exchangeable bases were extracted using the method described by AOAC (2002). The sample (2.5 g) was weighed out into a 125 ml Erlenmeyer flask. Twenty-five milliliters (25 ml) of 1 N NH_4OAc (ammonium acetate) displacing solution was added and buffered at pH 7. The sample was then placed in a shaker for 15 minutes and the resulting solution was filtered through a Whatman No. 42 filter paper into a 50 ml volumetric flask and diluted to 50 ml with extracting solution. From the solution, Ca and Mg were determined using Ethylene – diamine tetra acetic acid (EDTA) titration method while K was determined by flame emission spectrophotometer (721 VIS).

Analysis of Heavy Metals in Plant Parts

The three plants harvested at random from each treatment were analyzed for heavy metals (Pb, Cd, Zn, Ni and Cu) using Atomic Absorption Spectrophotometer (Analyst 200 Perkins Elmer) at Synergy Inspection Service, Olodi Apapa, Lagos.

The dried samples of different parts (leaf, stem and root) of *B. pinnatum* were ground into fine powder using a commercial blender (TSK – WestPoint, France). The digestion was carried out in triplicates for all the analysis. The procedure of AOAC (2002) and Siriangkhawut, *et al.* (2013) was used for the digestion of the plant samples. The plant samples (5 ml each) were then aspirated into Atomic Absorption Spectrophotometer and the absorbance recorded on the screen at different wavelength.

Preparation of *Bryophyllum pinnatum* leaf extracts

B pinnatum leaves were obtained from the Botanical garden of Plant Science and Biotechnology, University of Nigeria Nsukka. The samples were later taken to the Department of Crop Science, University of Nigeria Nsukka for identification. The leaves were air dried at room temperature (26 ^oC) for 8 weeks, after which it was milled into a uniform powder with aid of an electrical grinder. The ethanol extracts were prepared by soaking 100g of each dried plant powdered materials in 300 ml of 80% ethanol at room temperature for 48 h. The extract was filtered through a Whamann filter paper No.1 (QUALIGEN- Germany). The extracts were concentrated using a rotary evaporator at a maximum temperature of 45 ^oC. The weight of dried extracts was found to be 10.8% for *B. pinnatum*. The extracts were then dissolved in specified amount of distilled water to prepare the solutions into different concentrations (1.0, 2.0, 3.0 and 4.0%).

Table 1: FAO/WHO permissible standard for heavy metals.

Heavy metals	WHO/FAO
Copper	72.00
Lead	5.00
Zinc	100.00
Cadmium	0.12
Iron	415.00
Nickel	5.30

Source: FAO/WHO, 1996.

Preparation of 0.5 M HCl

First, the concentration of the concentrated HCl was determined using the formula:

$$\text{Molarity} = \frac{Concentration}{Molarmass\ (MM)} \quad (3.1)$$

$$\text{Concentration} = \text{Standard volume} \times \text{density } (\rho) \times \% \text{ purity} \quad (3.2)$$

The concentrated HCl acid has a density of 1.18 g/dm^3, a percentage purity of 37 % and molar mass of 36.5 g/mol. hence, for 1 liter (1000 cm^3) of standard HCl, the molarity is given by:

$$\text{Molarity} = \frac{Standard volume \times density\ (\rho) \times \%\ purity}{MM} \quad (3.3)$$

The volume of the concentrated acid to be diluted was calculated using the serial dilution formula

$$C_1V_1 = C_2V_2 \quad (3.4)$$

Where C_1 and C_2 are the molar concentrations of the concentrated and diluted HCl solutions respectively,
V_1 and V_2 are the volumes of the concentrated and diluted HCl solutions respectively. Therefore, 41.80 ml concentrated HCl was diluted in 1000 ml of distill water to produce the 0.5 M HCl solution.

Phyto-Chemical Analysis of the organic extract

Phytochemical analysis of the organic extract was carried out according to the general method of Tiwari *et al.* (2011). Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, steroids, phenols, and terpenoids in the sample. The extract contains many chemical constituents which are therapeutically active or inactive. The phyto-chemical screening for the extract was carried out in the Department of Pharmacy, University of Nigeria Nsukka, using the following standard methods (Tiwari *et al.,* 2011).

Test for Alkaloids

A 20 ml of 5 % sulfuric acid in 50 % ethanol was added to about 2 g of the extract and heated on a boiling water bath for 10 minutes then cooled and filtered. Thereafter, two drops of the following reagents: Mayers reagent (Potassium Mercuric iodide solution), Dragendorff's reagent (Bismuth Potassium Iodide solution) and 1 % picric acid solution were added to 2 ml of the filtrate.

The remaining filtrate was placed in 100 ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of dilute sulfuric acid. The extract was tested with a drop of Mayer's, Dragendor Wagners's and picric acid reagents. Alkaloids gave;
i. Cream precipitates with one drop of Mayer's reagent.
ii. Brick red precipitates with one drop of Dragendorff's reagent.
iii. Orange precipitates with one drop of Wagner's reagent.
iv. Yellow precipitates with one drop of picric acid reagent.

Test for saponins

Small quantity of the extract was dissolved in 10 ml of distilled water. This was shaken vigorously for 30 seconds and allowed to stand for 30 min. A honey comb was formed for more than 5min. The formation of honey comb indicates the presence of saponins.

Test for tannins

Exactly 1g of the extract was boiled with 50 ml of distilled water, filtered and the filtrate was used for the tests described below (Makkar and Goodchild, 1996);
A few drops of ferric chloride were added to 3 ml of the filtrate. A greenish black precipitate indicated the presence of tannins.

Test for flavonoids

A little quantity of the extract was boiled in 10 ml of ethyl acetate for 3 min, filtered and cooled. Then, 4 ml of the filtrate was shaken

with 1ml of dilute ammonia solution. An intense yellow colouration was absent indicating the absence of flavonoids (Boa *et al., 2005*).

Test for steroids

Exactly 5 ml of ethanol was added to 1g of the extract and the mixture was refluxed for a specified 15 minutes and then filtered. The filtrate was concentrated to 2.5 ml on boiling water bath and 5 ml of hot water was added. The mixture was allowed to stand for 1 h and waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform and 0.5 ml of the chloroform extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric acid was added. A reddish-brown colour at the interface indicated the presence of steroidal ring.

Test for phenols

Exactly 0.1 g of the extract was added to 10 ml of distilled water. The solution was heated in a boiling water bath for 3 min and filtered. A 2 ml aliquot of the filtrate was placed in each of 3 test tubes. The filtrate in one of the test tubes was diluted with distilled water in the ratio 1:4. A blue-greenish colour indicated the presence of phenols.

Statistical Analysis

The data from each treatment was subjected to analysis of variance (ANOVA) and means differences separated using Least Significant Difference Test (LSD) at $p< 0.05$ level of significance.

Results

Soil chemical properties

Soil chemical properties before and after the treatment with various concentrations of industrial brewery effluent are presented in Tables 2 to 6 and Figures 4 and 5, respectively. The result showed sharp increase in soil Ph, Nitrogen, Phosphorus, Potassium, Magnesium and Calcium in all treatments when compared with the 0% treatment (control). ANOVA showed that N, P, K, Mg and Ca were significantly increased after treatment ($P < 0.05$).

Table 2: Soil Chemical Properties before the experiment.

Soil Minerals	Values
pH (H_20)	6.5
Nitrogen (%)	1.30
Phosphorus (ppm)	18.60
Potassium (me / 100g)	2.23
Magnesium (me / 100g)	2.50
Calcium (me / 100g)	0.23

Soil chemical properties after the experiment

Effect of different concentration of Brewery effluent on the soil pH level cultivated with *B. pinnatum*

The soil pH in (H_20) ranged from 6.53 in the control (0%) to 7.50 in treatment (100%) as shown in Table 3. This indicated an alkaline condition. The soil sample had the highest pH (7.50) at 100% treatment which was significantly ($P < 0.05$) different from other treatments while the lowest pH values were observed in treatment 0% (control) with a value of 6.53. ANOVA showed that this effect was significant at $P < 0.05$.

Table 3: Effect of different concentration of Brewery effluent on the soil pH level cultivated with *B. pinnatum*

Conc.	Initial	Weeks					
		2	4	6	8	10	12
0%	6.53 ± 0.088	6.57 ± 0.067	6.53 ± 0.033	6.53 ± 0.033^{c}	6.50 ± 0.000^{b}	6.57 ± 0.033^{d}	6.53 ± 0.033^{d}
20%	6.53 ± 0.088	6.57 ± 0.067	6.50 ± 0.000	6.80 ± 0.000^{bc}	6.97 ± 0.033^{a}	7.07 ± 0.033^{c}	7.40 ± 0.000^{ab}
40%	6.53 ± 0.088	6.57 ± 0.067	6.50 ± 0.000	6.73 ± 0.033^{c}	6.90 ± 0.000^{b}	7.10 ± 0.000^{c}	7.33 ± 0.088^{bc}
60%	6.53 ± 0.088	6.60 ± 0.100	6.63 ± 0.067	6.87 ± 0.033^{ab}	7.00 ± 0.000^{a}	7.20 ± 0.000^{b}	7.30 ± 0.000^{a}
80%	6.53 ± 0.088	6.57 ± 0.067	6.63 ± 0.067	6.83 ± 0.033^{ab}	6.90 ± 0.000^{b}	7.13 ± 0.033^{bc}	7.50 ± 0.058^{cd}
100 %	6.53 ± 0.088	6.60 ± 0.100	6.60 ± 0.058	6.90 ± 0.000^{a}	7.00 ± 0.000^{a}	7.30 ± 0.000^{a}	7.50 ± 0.000^{ab}
LSD	NS	0.07	0.06	0.08	0.04	0.07	0.14

Data are presented as mean ± standard error. Means with different alphabets along each column are significantly different, separated using least significant difference test at P < 0.05

Effect of different concentration of Brewery effluent on the soil phosphorus level cultivated with *B. pinnatum*

The level of phosphorus also increased from treatment level 20% to 100%. Treatments level100% had the highest phosphorus with a mean value of 38.35 ± 0.84 mg/100g which was significantly different (P< 0.05) from other treatments while treatments level 0% had the lowest with a mean value of 18.60 ± 0.23 mg/100g.

Table 4: Effect of different concentration of Brewery effluent on the soil phosphorus level cultivated with *B. pinnatum*

Conc.	Initial	Weeks					
		2	4	6	8	10	12
0%	18.60 ± 0.23	18.68 ± 0.25	16.47 ± 1.04^{b}	16.95 ± 0.58^{c}	16.95 ± 0.58^{c}	16.95 ± 0.58^{c}	16.95 ± 0.58^{b}
20%	18.60 ± 0.23	18.60 ± 0.23	15.13 ± 0.14^{a}	22.47 ± 2.09^{ab}	22.47 ± 2.09^{ab}	22.47 ± 2.09^{ab}	35.42 ± 2.21^{a}
40%	18.60 ± 0.23	18.68 ± 0.25	12.71 ± 1.11^{b}	17.84 ± 0.60^{bc}	17.84 ± 0.60^{bc}	17.84 ± 0.60^{bc}	17.84 ± 0.60^{b}
60%	18.60 ± 0.23	18.60 ± 0.23	15.16 ± 0.17^{a}	24.33 ± 2.33^{a}	24.33 ± 2.33^{a}	24.33 ± 2.33^{a}	34.92 ± 2.81^{a}
80%	18.60 ± 0.23	18.69 ± 0.24	13.57 ± 0.48^{ab}	17.94 ± 0.58^{bc}	17.94 ± 0.58^{bc}	17.94 ± 0.58^{bc}	17.94 ± 0.58^{b}
100 %	18.60 ± 0.23	18.60 ± 0.23	15.15 ± 0.19^{a}	25.77 ± 1.92^{a}	25.77 ± 1.92^{a}	25.77 ± 1.92^{a}	38.35 ± 0.84^{a}
LSD	NS	0.08	2.04	4.8	4.8	4.8	4.79

Data are presented with mean ± standard error. Means with different alphabets along each column are significantly different, separated using least significant difference test at P < 0.05

Effect of different concentration of Brewery effluent on the soil magnesium level cultivated with *B. pinnatum*

In Table 5, Magnesium showed an increase in treated soils when compared with the 0% treatment (control). ANOVA showed that there was significant difference in the range of Magnesium (Mg) in all the treatments.

Table 5: Effect of different concentration of Brewery effluent on the soil magnesium level cultivated with *B. pinnatum*

Conc.	Initial	Weeks					
		2	4	6	8	10	12
0%	2.50 ± 0.01	2.03 ± 0.02^{b}	2.03 ± 0.02^{b}	2.03 ± 0.02^{b}	2.30 ± 0.01^{d}	2.30 ± 0.01^{d}	2.51 ± 0.01^{c}
20%	2.50 ± 0.01	2.18 ± 0.03^{a}	2.18 ± 0.03^{a}	2.18 ± 0.03^{a}	2.36 ± 0.01^{c}	2.36 ± 0.01^{c}	2.57 ± 0.01^{b}
40%	2.50 ± 0.01	2.04 ± 0.02^{b}	2.04 ± 0.02^{b}	2.04 ± 0.02^{b}	2.42 ± 0.01^{b}	2.42 ± 0.01^{b}	2.52 ± 0.01^{c}
60%	2.50 ± 0.01	2.20 ± 0.03^{a}	2.20 ± 0.03^{a}	2.20 ± 0.03^{a}	2.48 ± 0.01^{a}	2.48 ± 0.01^{a}	2.59 ± 0.01^{ab}
80%	2.50 ± 0.01	2.05 ± 0.03^{b}	2.05 ± 0.03^{b}	2.05 ± 0.03^{b}	2.42 ± 0.01^{b}	2.42 ± 0.01^{b}	2.52 ± 0.02^{c}
100%	2.50 ± 0.01	2.22 ± 0.03^{a}	2.22 ± 0.03^{a}	2.22 ± 0.03^{a}	2.48 ± 0.01a	2.48 ± 0.01^{a}	2.62 ± 0.01^{a}
LSD	NS	0.09	0.09	0.09	0.04	0.04	0.03

Data are presented with mean ± standard error. Means with different alphabets along each column are significantly different, separated using least significant difference test at $P < 0.05$

Effect of different concentration of Brewery effluent on the soil potassium level cultivated with *B. pinnatum*

The Potassium (K) increased progressively from treatment level 0% to 100%. Treatment level100% had the highest value of K with 5.28 ± 0.17which was significantly difference at P< 0.05, followed by treatments 80%, 60%, 40%, 20% and 0% which had the lowest value of Potassium with 0.24 ± 0.01. Multiple comparison using LSD at 2nd, 4th, and 6th Weeks after treatment (WAT) was not significantly different at $P < 0.05$.

Effect of different concentration of Brewery effluent on the soil calcium level cultivated with *B. pinnatum*

The Calcium increased with increasing level of industrial brewery effluent IBE. In Figure 4, Treatment level100% (100 ml IBE + 0 ml tap water) had the highest value of Calcium with 20.00 at 12th WAT which was significantly different at $P < 0.05$, followed by treatments levels80%, 60%, 40%, 20%. Treatment level 0% had the lowest value of Ca with 13.00 at 12th WAT. At 8th and 10th WAT, treatment levels20%, 60% and 100% gave the highest value of 4.00 while treatment levels0%, 40%, and 80% gave the lowest value of 3.00 which was significantly different at $P < 0.05$. At 2nd, 4th and 6th WAT, treatment levels 20%, 60% and 100% gave the highest value of 2.00 while treatment levels0%, 40%, and 80% gave the lowest value of 1.00 which was significantly different at $P < 0.05$.

Table 6: Effect of different concentration of Brewery effluent on the soil potassium level cultivated with *B. pinnatum*

	Initial	Weeks					
		2	4	6	8	10	12
0%	0.24 ± 0.01	0.42 ± 0.11	2.20 ± 0.16	2.20 ± 0.16	2.61 ± 0.17^{b}	2.61 ± 0.17^{b}	3.69 ± 0.16^{b}
20%	0.24 ± 0.01	1.45 ± 0.33	2.06 ± 0.37	2.06 ± 0.37	3.12 ± 0.07^{ab}	3.12 ± 0.07^{ab}	5.02 ± 0.22^{a}
40%	0.24 ± 0.01	0.69 ± 0.12	1.72 ± 0.03	1.72 ± 0.03	2.58 ± 0.25^{b}	2.58 ± 0.25^{b}	3.81 ± 0.20^{b}
60%	0.24 ± 0.01	1.36 ± 0.69	1.95 ± 0.03	1.95 ± 0.03	3.24 ± 0.18^{a}	3.24 ± 0.18^{a}	5.08 ± 0.18^{a}
80%	0.24 ± 0.01	0.65 ± 0.07	1.76 ± 0.06	1.76 ± 0.06	2.61 ± 0.20^{b}	2.61 ± 0.20^{b}	3.83 ± 0.19^{b}
100 %	0.24 ± 0.01	1.58 ± 0.49	1.96 ± 0.03	1.96 ± 0.03	3.22 ± 0.13^{a}	3.22 ± 0.13^{a}	5.28 ± 0.17^{a}
LSD	NS	NS	NS	NS	0.54	0.54	0.58

Data are presented with mean ± standard error. Means with different alphabets along each column are significantly different, separated using least significant difference test at P < 0.05

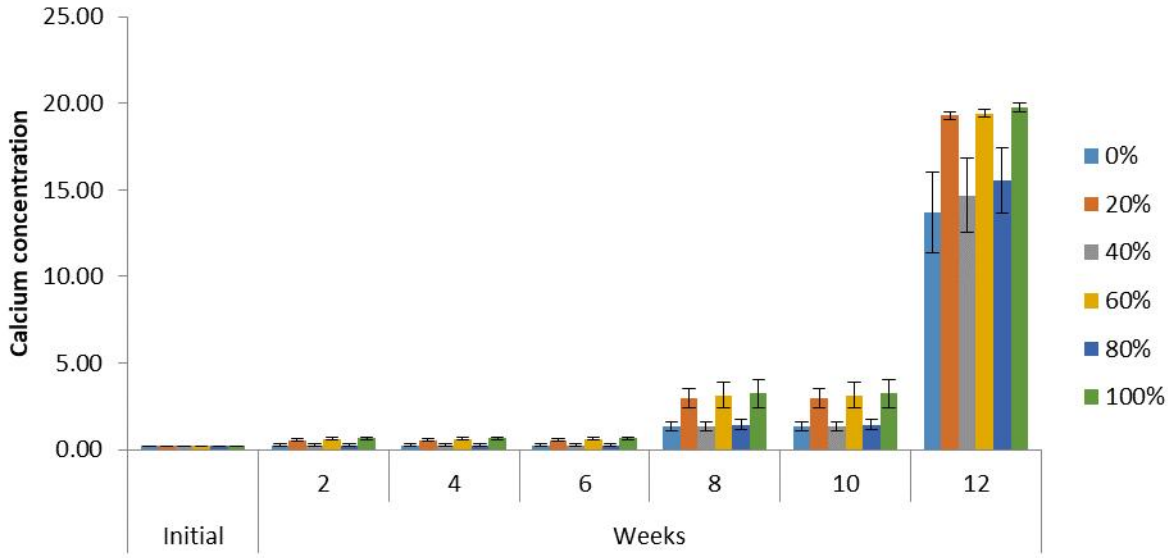


Figure 4: Effect of different concentration of Brewery effluent on the soil calcium level cultivated with *B. pinnatum*

Effect of different concentration of Brewery effluent on the soil nitrogen level cultivated with *B. pinnatum*

The total nitrogen of soil irrigated with IBE increased from treatment level20% to 100% (100 ml IBE + 0 ml topwater). In Figure 5, the highest (1.2000%) total nitrogen was obtained in treatment level100% which was significantly different (P < 0.05) from other treatments while treatment level0% control gave the lowest total nitrogen (0.2000%).

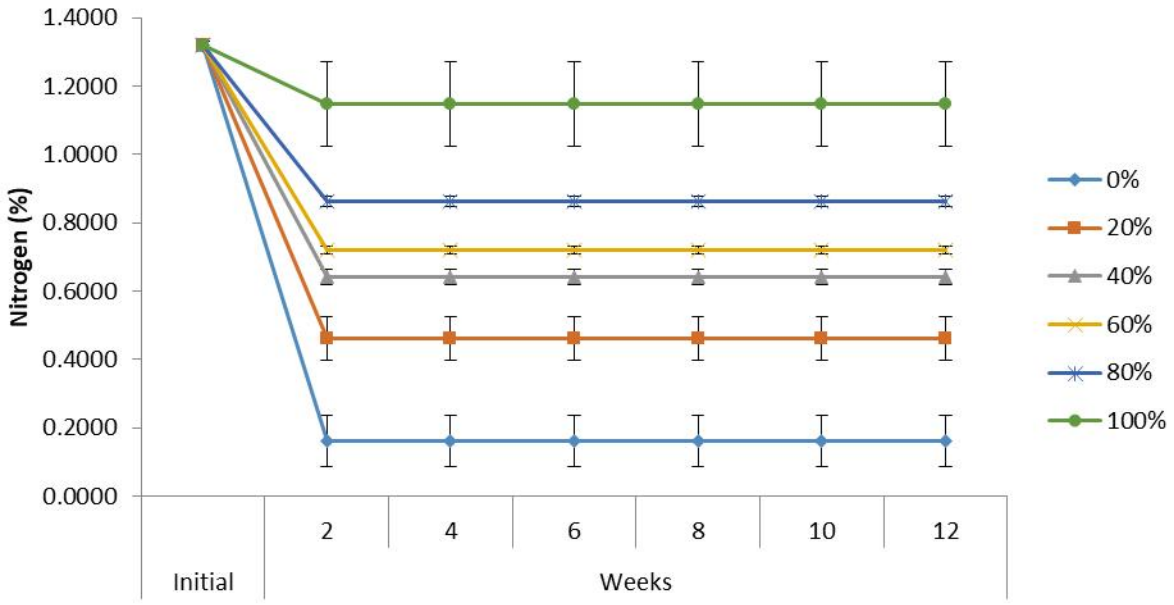


Figure 5: Effect of different concentration of Brewery effluent on the soil nitrogen level cultivated with *B. pinnatum*

Heavy Metal Accumulation in Plant Tissues (Leaf, Stem and Root).

The heavy metals (ppm) accumulation in the three plant parts (leaf, stem and root) of *Bryophyllum pinnatum* irrigated with different concentrations of IBE showed significant ($P < 0.05$) differences across the treatments and weeks. The heavy metal accumulation increased with the concentrations and time, with the highest value obtained at the end of the experiment as well as in the highest treatments.

Concentration of heavy metals and their deposition in leaf, stem and roots of Bryophyllum *pinnatum*

ANOVA showed highly significant increase in the Iron content of leaves, stems and roots ($P < 0.05$) from 2nd to 12th WAT (weeks after treatment) for all the concentrations. The highest concentration of Iron was obtained at 100% treatment level, and decreased progressively as the concentrations of effluent decreased (Table 7). The least concentration obtained in the leaf, stem and roots treatments at 2nd to 12th WAT fall within the WHO/FAO standard acceptable limit of 415.00ppm, for treatmentlevels20%, to 100%.

Table 7: Concentration of Iron (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

Plant part	Concentrations					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$=0.18)			
Leaf	1.03 ± 0.01^{n}	1.10 ± 0.01^{mn}	1.16 ± 0.01^{lmn}	1.21 ± 0.01^{klm}	1.28 ± 0.01^{jkl}	1.34 ± 0.01^{ijk}
Stem	1.40 ± 0.01^{ij}	1.49 ± 0.02^{i}	1.69 ± 0.02^{h}	2.10 ± 0.09^{g}	2.34 ± 0.01^{fg}	2.44 ± 0.03^{f}
Root	3.40 ± 0.01^{e}	3.82 ± 0.35^{d}	4.63 ± 0.02^{c}	5.17 ± 0.09^{b}	5.34 ± 0.01^{ab}	5.44 ± 0.03^{a}
			2 weeks ($LSD_{0.05}$=0.19)			
Leaf	48.60 ± 0.05^{j}	49.97 ± 0.04^{i}	50.28 ± 0.04^{i}	50.94 ± 0.07^{h}	51.68 ± 0.09^{g}	52.18 ± 0.12^{g}
Stem	61.00 ± 0.11^{f}	62.49 ± 0.01^{e}	63.00 ± 0.00^{d}	64.49 ± 0.02^{c}	65.98 ± 0.12^{b}	68.37 ± 0.20^{a}
Root	61.00 ± 0.11^{f}	62.49 ± 0.01^{e}	63.00 ± 0.00^{d}	64.49 ± 0.02^{c}	65.98 ± 0.12^{b}	68.37 ± 0.20^{a}
			4 weeks ($LSD_{0.05}$=0.30)			
Leaf	48.80 ± 0.11^{j}	49.12 ± 0.35^{i}	49.18 ± 0.41^{i}	50.54 ± 0.02^{h}	51.20 ± 0.05^{g}	51.81 ± 0.15^{f}
Stem	61.62 ± 0.13^{e}	61.80 ± 0.09^{e}	62.75 ± 0.04^{d}	64.69 ± 0.02^{c}	65.14 ± 0.03^{b}	67.65 ± 0.11^{a}
Root	61.62 ± 0.13^{e}	61.80 ± 0.09^{e}	62.75 ± 0.04^{d}	64.69 ± 0.02^{c}	65.14 ± 0.03^{b}	67.65 ± 0.11^{a}
			6 weeks ($LSD_{0.05}$=0.18)			
Leaf	48.76 ± 0.07^{k}	49.61 ± 0.11^{j}	49.65 ± 0.09^{j}	50.75 ± 0.10^{i}	51.73 ± 0.03^{h}	52.07 ± 0.14^{g}
Stem	61.41 ± 0.05^{f}	62.38 ± 0.07^{e}	63.18 ± 0.01^{d}	64.77 ± 0.11^{c}	65.68 ± 0.15^{b}	67.25 ± 0.01^{a}
Root	61.41 ± 0.05^{f}	62.38 ± 0.07^{e}	63.18 ± 0.01^{d}	64.77 ± 0.11^{c}	65.68 ± 0.15^{b}	67.25 ± 0.01^{a}
			8 weeks ($LSD_{0.05}$=0.14)			
Leaf	48.76 ± 0.06^{k}	50.09 ± 0.01^{j}	50.20 ± 0.01^{j}	50.82 ± 0.08^{i}	51.34 ± 0.06^{h}	51.83 ± 0.08^{g}
Stem	61.00 ± 0.15^{f}	62.52 ± 0.03^{e}	63.63 ± 0.09^{d}	64.00 ± 0.00^{c}	66.07 ± 0.02^{b}	67.24 ± 0.01^{a}
Root	61.00 ± 0.15^{f}	62.52 ± 0.03^{e}	63.63 ± 0.09^{d}	64.00 ± 0.00^{c}	66.07 ± 0.02^{b}	67.24 ± 0.01^{a}
			10 weeks ($LSD_{0.05}$=0.31)			
Leaf	49.00 ± 0.22^{j}	50.09 ± 0.04^{i}	33.45 ± 16.73^{k}	50.96 ± 0.05^{h}	51.36 ± 0.20^{g}	51.56 ± 0.41^{g}
Stem	60.91 ± 0.20^{f}	62.72 ± 0.13^{e}	63.82 ± 0.08^{d}	64.73 ± 0.10^{c}	65.86 ± 0.08^{b}	68.10 ± 0.03^{a}
Root	60.91 ± 0.20^{f}	62.72 ± 0.13^{e}	63.82 ± 0.08^{d}	64.73 ± 0.10^{c}	65.86 ± 0.08^{b}	68.10 ± 0.03^{a}
			12 weeks ($LSD_{0.05}$=0.29)			
Leaf	48.89 ± 0.11^{j}	50.10 ± 0.04^{i}	50.13 ± 0.02^{i}	51.10 ± 0.01^{h}	51.50 ± 0.08^{g}	51.78 ± 0.12^{g}
Stem	62.01 ± 0.01^{f}	62.60 ± 0.08^{e}	63.42 ± 0.26^{d}	65.29 ± 0.30^{c}	66.06 ± 0.02^{b}	68.00 ± 0.00^{a}
Root	62.01 ± 0.01^{f}	62.60 ± 0.08^{e}	63.42 ± 0.26^{d}	65.29 ± 0.30^{c}	66.06 ± 0.02^{b}	68.00 ± 0.00^{a}

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different, separated using least significant difference test at $P < 0.05$

Concentration of copper (mg kg^{-1}) deposition in leaf, stem and roots of *Bryophyllum pinnatum* irrigated with Brewery effluent

ANOVA showed highly significant increase in the Copper content of leaves, stems and roots ($P < 0.05$) from 2nd to 12th WAT for all the concentrations. The highest concentration of copper was obtained in 100% treatment level, and decreased progressively as the concentrations of effluent decreased (Table 8). The least concentration obtained in the leaf, stem and root treatments at 2nd to 12th WAT fall within the WHO/FAO standard acceptable limit of 72.00(mg kg^{-1}) from treatment levels 20%, to 100%.

Table 8: Concentration of copper (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

Plant part	Concentrations					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$= NS)			
Leaf	0.22 ± 0.01	0.32 ± 0.01	0.42 ± 0.01	0.50 ± 0.01	0.56 ± 0.01	0.88 ± 0.14
Stem	0.22 ± 0.01	0.32 ± 0.01	0.42 ± 0.01	0.50 ± 0.01	0.56 ± 0.01	0.88 ± 0.14
Root	1.22 ± 0.01	1.32 ± 0.01	1.42 ± 0.01	1.50 ± 0.01	1.56 ± 0.01	2.21 ± 0.19
			2 weeks ($LSD_{0.05}$= 0.08)			
Leaf	2.06 ± 0.01^{h}	2.76 ± 0.06^{g}	2.81 ± 0.08^{g}	3.02 ± 0.01^{f}	3.01 ± 0.01^{f}	3.03 ± 0.02^{f}
Stem	3.06 ± 0.01^{f}	3.76 ± 0.06^{e}	4.81 ± 0.08^{c}	4.02 ± 0.01^{d}	4.01 ± 0.01^{d}	4.03 ± 0.02^{d}
Root	5.06 ± 0.01^{b}	5.76 ± 0.06^{a}	5.81 ± 0.08^{a}	5.02 ± 0.01^{b}	5.01 ± 0.01^{b}	5.03 ± 0.02^{b}
			4 weeks ($LSD_{0.05}$= 0.16)			
Leaf	2.08 ± 0.01^{h}	2.62 ± 0.06^{g}	2.43 ± 0.18^{g}	3.04 ± 0.01^{f}	3.01 ± 0.01^{f}	3.01 ± 0.01^{f}
Stem	4.08 ± 0.01^{e}	4.62 ± 0.06^{c}	4.43 ± 0.18^{d}	4.04 ± 0.01^{e}	4.01 ± 0.01^{e}	4.01 ± 0.01^{e}
Root	5.08 ± 0.01^{b}	5.62 ± 0.06^{a}	5.43 ± 0.18^{a}	5.04 ± 0.01^{b}	5.01 ± 0.01^{b}	5.01 ± 0.01^{b}
			6 weeks ($LSD_{0.05}$= 0.12)			
Leaf	2.67 ± 0.04^{g}	2.69 ± 0.13^{g}	2.66 ± 0.06^{g}	3.12 ± 0.02^{f}	3.02 ± 0.01^{f}	3.02 ± 0.01^{f}
Stem	4.72 ± 0.04^{d}	4.69 ± 0.13^{d}	4.66 ± 0.06d	4.12 ± 0.02^{e}	4.02 ± 0.01^{e}	4.02 ± 0.01^{e}
Root	5.87 ± 0.04^{a}	5.69 ± 0.13^{b}	5.66 ± 0.06^{b}	5.12 ± 0.02^{c}	5.02 ± 0.01^{c}	5.02 ± 0.01^{c}
			8 weeks ($LSD_{0.05}$= 0.12)			
Leaf	2.55 ± 0.04^{i}	2.72 ± 0.14^{h}	2.79 ± 0.03^{h}	3.12 ± 0.02^{g}	3.02 ± 0.01^{g}	3.02 ± 0.01^{g}
Stem	4.43 ± 0.04^{e}	4.72 ± 0.14^{d}	4.79 ± 0.03^{d}	4.12 ± 0.02^{f}	4.02 ± 0.01^{f}	4.02 ± 0.01^{f}
Root	5.55 ± 0.04^{b}	5.72 ± 0.14^{a}	5.79 ± 0.03^{a}	5.12 ± 0.02^{c}	5.02 ± 0.01^{c}	5.02 ± 0.01^{c}
			10 weeks ($LSD_{0.05}$= 0.23)			
Leaf	2.78 ± 0.07^{h}	2.45 ± 0.21^{i}	2.83 ± 0.09^{h}	3.51 ± 0.16^{g}	3.59 ± 0.01^{g}	3.60 ± 0.01^{g}
Stem	4.78 ± 0.07^{ef}	4.65 ± 0.21^{ef}	4.83 ± 0.09^{e}	4.62 ± 0.16^{ef}	4.59 ± 0.01^{f}	4.60 ± 0.01^{ef}
Root	5.78 ± 0.07^{abc}	5.45 ± 0.21^{d}	5.83 ± 0.09^{a}	5.51 ± 0.16^{d}	5.59 ± 0.01^{bcd}	5.60 ± 0.01^{abcd}
			12 weeks ($LSD_{0.05}$= 0.14)			
Leaf	2.80 ± 0.09^{i}	2.50 ± 0.11^{j}	2.88 ± 0.06^{i}	3.15 ± 0.01^{h}	3.52 ± 0.04^{g}	3.53 ± 0.04^{g}
Stem	4.80 ± 0.09^{d}	4.50 ± 0.11^{e}	4.88 ± 0.06^{d}	4.15 ± 0.01^{f}	4.52 ± 0.04^{e}	4.53 ± 0.04^{e}
Root	5.80 ± 0.09^{a}	5.50 ± 0.11^{b}	5.88 ± 0.06^{a}	5.15 ± 0.01^{c}	5.52 ± 0.04^{b}	5.53 ± 0.04^{b}

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different, separated using least significant difference test at $P < 0.05$

Concentration of zinc (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

ANOVA showed highly significant increase in the Zinc content of leaves, stems and roots ($P < 0.05$) from 2nd to 12th WAT for all the concentrations. The highest concentration of Zinc was obtained at 100% treatment level, and decreased progressively as the concentrations of effluent decreased (Table 9). The least concentration obtained in the leaf, stem and root treatments at 2nd to 12th WAT fall within the WHO/FAO standard acceptable limit of 100.00(mg kg^{-1}) from treatment levels20%, to 100%.

Table 9: Concentration of zinc (mg kg^{-1}) deposition in leaf, stem and roots of *Bryophyllum pinnatum* irrigated with Brewery effluent

Plant part	Concentration					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$= 0.42)			
Leaf	3.00 ± 0.29^{h}	3.60 ± 0.31^{g}	4.47 ± 0.18^{f}	5.04 ± 0.03^{e}	5.30 ± 0.06^{de}	5.68 ± 0.04^{d}
Stem	3.00 ± 0.29^{h}	3.60 ± 0.31^{g}	4.47 ± 0.18^{f}	5.04 ± 0.03^{e}	5.30 ± 0.06^{de}	5.68 ± 0.04^{d}
Root	7.33 ± 0.17^{c}	7.27 ± 0.27^{c}	7.80 ± 0.50^{b}	8.04 ± 0.03^{b}	8.30 ± 0.06^{ab}	8.68 ± 0.04^{a}
			2 weeks ($LSD_{0.05}$= 0.12)			
Leaf	13.13 ± 0.07	14.29 ± 0.08	15.58 ± 0.16	17.60 ± 0.16	17.50 ± 0.04	17.93 ± 0.04
Stem	13.13 ± 0.07	14.29 ± 0.08	15.58 ± 0.16	17.60 ± 0.16	17.50 ± 0.04	17.93 ± 0.04
Root	13.20 ± 0.08	14.30 ± 0.08	15.60 ± 0.17	17.65 ± 0.17	17.54 ± 0.05	17.98 ± 0.06
			4 weeks ($LSD_{0.05}$= 0.09)			
Leaf	13.29 ± 0.04	15.03 ± 0.01	15.96 ± 0.04	17.44 ± 0.05	17.39 ± 0.05	18.37 ± 0.07
Stem	13.30 ± 0.04	15.15 ± 0.09	16.00 ± 0.05	17.44 ± 0.05	17.40 ± 0.05	18.38 ± 0.07
Root	13.29 ± 0.04	15.03 ± 0.01	15.96 ± 0.04	17.44 ± 0.05	17.39 ± 0.05	18.37 ± 0.07
			6 weeks ($LSD_{0.05}$= 0.20)			
Leaf	13.78 ± 0.06	15.53 ± 0.07	16.64 ± 0.13	17.67 ± 0.11	17.75 ± 0.14	18.79 ± 0.06
Stem	13.78 ± 0.06	15.53 ± 0.07	16.64 ± 0.13	17.67 ± 0.11	17.75 ± 0.14	18.79 ± 0.06
Root	13.80 ± 0.07	15.60 ± 0.07	16.65 ± 0.13	17.70 ± 0.11	17.79 ± 0.15	18.80 ± 0.06
			8 weeks ($LSD_{0.05}$= NS)			
Leaf	13.86 ± 0.15	15.54 ± 0.05	16.70 ± 0.07	17.12 ± 0.01	17.10 ± 0.01	18.40 ± 0.03
Stem	13.86 ± 0.15	15.54 ± 0.05	16.70 ± 0.07	17.12 ± 0.01	17.10 ± 0.01	18.40 ± 0.03
Root	13.86 ± 0.15	15.54 ± 0.05	16.70 ± 0.07	17.12 ± 0.01	17.10 ± 0.01	18.40 ± 0.03
			10 weeks ($LSD_{0.05}$= NS)			
Leaf	13.86 ± 0.07	15.54 ± 0.02	16.70 ± 0.03	17.12 ± 0.01	17.68 ± 0.07	16.30 ± 0.27
Stem	15.69 ± 0.11	15.96 ± 0.04	16.93 ± 0.49	17.12 ± 0.01	17.68 ± 0.07	16.30 ± 0.27
Root	15.69 ± 0.11	15.96 ± 0.04	16.93 ± 0.49	17.12 ± 0.01	17.68 ± 0.07	16.30 ± 0.27
			12 weeks ($LSD_{0.05}$= NS)			
Leaf	15.65 ± 0.10	17.08 ± 0.02	17.60 ± 0.04	17.53 ± 0.08	17.67 ± 0.11	16.64 ± 0.31
Stem	15.65 ± 0.10	17.08 ± 0.02	17.60 ± 0.04	17.53 ± 0.08	17.67 ± 0.11	16.65 ± 0.30
Root	15.65 ± 0.10	17.08 ± 0.02	17.60 ± 0.04	17.53 ± 0.08	17.67 ± 0.11	16.65 ± 0.30

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different, separated using least significant difference test at $P < 0.05$

Concentration of lead (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

ANOVA showed that were no significant differences in the Lead content of each plant part (leaves, stem and roots), from 2nd to 12th WAT for all the concentrations. The highest concentration of Lead was obtained at 100% treatment level in the root of *B pinnatum*, immediately after IBE application, and decreased progressively from 2nd to 12th WAT as the concentrations of the effluent decreased (Table 10). The least concentration was obtained in the control treatment for the leaf, stem and root, having the value of 0.51 ± 0.002; this was not significant at $P < 0.05$. The mean accumulation of Lead in the plant tissues increased across the weeks and treatments. The study revealed that in the plant parts, lead contents were within the WHO/FAO permissible limit (5.00 ppm) at 2nd to 12th WAT from treatment levels 0% to 100%.

Table 10: Concentration of lead (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

Plant part	Concentration					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$= 0.06)			
Leaf	2.34 ± 0.012^{h}	2.41 ± 0.018g	2.50 ± 0.012^{f}	2.56 ± 0.012^{ef}	2.62 ± 0.012^{de}	2.68 ± 0.012^{d}
Stem	2.34 ± 0.012^{h}	2.41 ± 0.018^{g}	2.50 ± 0.012^{f}	2.56 ± 0.012^{ef}	2.62 ± 0.012^{de}	2.68 ± 0.012^{d}
Root	2.34 ± 0.012^{h}	2.41 ± 0.018^{g}	3.50 ± 0.012^{c}	3.56 ± 0.012^{c}	4.62 ± 0.012^{b}	4.78 ± 0.11^{a}
			2 weeks ($LSD_{0.05}$= NS)			
Leaf	0.51 ± 0.002	0.56 ± 0.003	0.60 ± 0.003	0.66 ± 0.003	0.69 ± 0.003	0.71 ± 0.003
Stem	0.51 ± 0.002	0.56 ± 0.003	0.60 ± 0.003	0.66 ± 0.003	0.69 ± 0.003	0.71 ± 0.003
Root	0.51 ± 0.002	0.56 ± 0.003	0.60 ± 0.003	0.66 ± 0.003	0.69 ± 0.003	0.71 ± 0.003
			4 weeks ($LSD_{0.05}$= NS)			
Leaf	0.52 ± 0.003	0.57 ± 0.003	0.62 ± 0.003	0.67 ± 0.003	0.69 ± 0	0.72 ± 0.003
Stem	0.52 ± 0.003	0.57 ± 0.003	0.62 ± 0.003	0.67 ± 0.003	0.69 ± 0	0.72 ± 0.003
Root	0.52 ± 0.003	0.57 ± 0.003	0.62 ± 0.003	0.67 ± 0.003	0.69 ± 0	0.72 ± 0.003
			6 weeks ($LSD_{0.05}$= NS)			
Leaf	0.53 ± 0.003	0.57 ± 0.003	0.63 ± 0.003	0.67 ± 0.003	0.69 ± 0.003	0.73 ± 0.003
Stem	0.53 ± 0.003	0.57 ± 0.003	0.63 ± 0.003	0.67 ± 0.003	0.69 ± 0.003	0.73 ± 0.003
Root	0.53 ± 0.003	0.57 ± 0.003	0.63 ± 0.003	0.67 ± 0.003	0.69 ± 0.003	0.73 ± 0.003
			8 weeks ($LSD_{0.05}$= NS)			
Leaf	0.54 ± 0.003	0.56 ± 0.009	0.63 ± 0.003	0.68 ± 0.003	0.70 ± 0.003	0.74 ± 0.003
Stem	0.54 ± 0.003	0.56 ± 0.009	0.63 ± 0.003	0.68 ± 0.003	0.70 ± 0.003	0.74 ± 0.003
Root	0.54 ± 0.003	0.56 ± 0.009	0.63 ± 0.003	0.68 ± 0.003	0.70 ± 0.003	0.74 ± 0.003
			10 weeks ($LSD_{0.05}$= NS)			
Leaf	0.55 ± 0.003	0.59 ± 0.003	0.64 ± 0.003	0.68 ± 0.003	0.70 ± 0.003	0.75 ± 0.003
Stem	0.55 ± 0.003	0.59 ± 0.003	0.64 ± 0.003	0.68 ± 0.003	0.70 ± 0/003	0.75 ± 0.003
Root	0.55 ± 0.003	0.59 ± 0.003	0.64 ± 0.003	0.68 ± 0.003	0.70 ± 0/003	0.75 ± 0.003
			12 weeks ($LSD_{0.05}$= NS)			
Leaf	0.56 ± 0.003	0.59 ± 0.003	0.66 ± 0.003	0.68 ± 0.003	0.71 ± 0.003	0.77 ± 0.003
Stem	0.56 ± 0.003	0.59 ± 0.003	0.66 ± 0.003	0.68 ± 0.003	0.71 ± 0.003	0.77 ± 0.003
Root	0.56 ± 0.003	0.59 ± 0.003	0.66 ± 0.003	0.68 ± 0.003	0.71 ± 0.003	0.77 ± 0.003

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different separated using least significant difference test at $P < 0.05$

Concentration of nickel (mg kg^{-1}) deposition in leaf, stem and roots of *Bryophyllum pinnatum* irrigated with Brewery effluent

ANOVA showed that were no significant differences in the Nickel content of each plant part (leaves, stem and roots), from 2nd to 12th WAT for all the concentrations. The highest concentration of Nickel was obtained in 100% treatment levels for leaves, stems and roots, (Table 11). The least was obtained in the control (0% treatment level) at 2nd WAT, which was above the WHO/FAO standard permissible limit of 5.30(mg kg^{-1}). Both the leaves, stems and roots gave values above WHO/FAO standard limit from treatment levels 20%, to 100%.

Table 11: Concentration of nickel (mg kg^{-1}) deposition in leaf, stem and roots of Bryophyllum *pinnatum* irrigated with Brewery effluent

Plant part	Concentration					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$= 0.17)			
Leaf	7.75 ± 0.074^{h}	7.88 ± 0.012^{gh}	8.00 ± 0.003^{fg}	8.11 ± 0.006^{ef}	8.15 ± 0.006^{ef}	8.20 ± 0.015^{e}
Stem	7.75 ± 0.074^{h}	7.88 ± 0.012^{gh}	8.00 ± 0.003^{fg}	8.11 ± 0.006^{ef}	8.15 ± 0.006^{ef}	8.20 ± 0.015^{e}
Root	9.75 ± 0.074^{d}	9.88 ± 0.012^{d}	10.04 ± 0.032^{cd}	10.11 ± .006^{c}	10.48 ± 0.338^{b}	11.20 ± 0.015^{a}
			2 Weeks ($LSD_{0.05}$= NS)			
Leaf	5.93 ± 0.073	7.79 ± 0.006	7.90 ± 0.006	9.84 ± 0.023	8.96 ± 0.009	12.52 ± 0.040
Stem	5.93 ± 0.073	7.79 ± 0.006	7.90 ± 0.006	9.84 ± 0.023	8.96 ± 0.009	12.52 ± 0.040
Root	5.93 ± 0.073	7.79 ± 0.006	7.90 ± 0.006	9.84 ± 0.023	8.96 ± 0.009	12.52 ± 0.040
			4 Weeks ($LSD_{0.05}$= NS)			
Leaf	6.08 ± 0.015	7.58 ± 0.012	8.33 ± 0.138	10.21 ± 0.006	9.18 ± 0.015	12.72 ± 0.083
Stem	6.08 ± 0.015	7.58 ± 0.012	8.33 ± 0.138	10.21 ± 0.006	9.18 ± 0.015	12.72 ± 0.083
Root	6.08 ± 0.015	7.58 ± 0.012	8.33 ± 0.138	10.21 ± 0.006	9.18 ± 0.015	12.72 ± 0.083
			6 Weeks ($LSD_{0.05}$= NS)			
Leaf	6.06 ± 0.085	7.58 ± 0.053	8.38 ± 0.019	10.59 ± 0.098	9.55 ± 0.178	12.71 ± 0.135
Stem	6.06 ± 0.085	7.58 ± 0.053	8.38 ± 0.019	10.59 ± 0.098	9.55 ± 0.178	12.71 ± 0.135
Root	6.06 ± 0.085	7.58 ± 0.053	8.38 ± 0.019	10.59 ± 0.098	9.55 ± 0.178	12.71 ± 0.135
			8 Weeks ($LSD_{0.05}$= NS)			
Leaf	6.26 ± 0.035	7.50 ± 0.050	8.72 ± 0.062	10.71 ± 0.103	9.42 ± 0.213	12.93 ± 0.073
Stem	6.26 ± 0.035	7.50 ± 0.050	8.72 ± 0.062	10.71 ± 0.103	9.42 ± 0.213	12.93 ± 0.073
Root	6.26 ± 0.035	7.50 ± 0.050	8.72 ± 0.062	10.71 ± 0.103	9.42 ± 0.213	12.93 ± 0.073
			10 Weeks ($LSD_{0.05}$= NS)			
Leaf	6.17 ± 0.015	7.63 ± 0.132	8.78 ± 0.017	10.93 ± 0.073	9.68 ± 0.069	12.86 ± 0.100
Stem	6.17 ± 0.015	7.63 ± 0.132	8.78 ± 0.017	10.93 ± 0.073	9.68 ± 0.069	12.86 ± 0.100
Root	6.17 ± 0.015	7.63 ± 0.132	8.78 ± 0.017	10.93 ± 0.073	9.68 ± 0.069	12.86 ± 0.100
			12 Weeks ($LSD_{0.05}$= NS)			
Leaf	6.54 ± 0.042	7.71 ± 0.019	8.76 ± 0.113	11.11 ± 0.167	9.58 ± 0.191	13.17 ± 0.026
Stem	6.54 ± 0.042	7.71 ± 0.019	8.76 ± 0.113	11.11 ± 0.167	9.58 ± 0.191	13.17 ± 0.026
Root	6.54 ± 0.042	7.71 ± 0.019	8.76 ± 0.113	11.11 ± 0.167	9.58 ± 0.191	13.17 ± 0.026

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different separated using least significant difference test at $P < 0.05$

Concentration of cadmium (mg kg^{-1}) deposition in leaf, stem and roots of *Bryophyllum pinnatum* irrigated with Brewery effluent

ANOVA showed that increase in the Cadmium content of leaves, stems and roots was highly significant ($P < 0.05$) from 2nd to 12th WAT for all the concentrations. The highest concentration of Cd was obtained at 100% treatment level, and decreased progressively as the concentrations of effluent decreased (Table 12). The least concentration obtained in the leaf treatments at 2nd to 12th WAT, in 0% treatment level falls within the WHO/FAO standard permissible limit of 0.12ppm, but the stems and roots gave values above WHO/FAO standard limit from treatment levels 20%, to 100%.

Table 12: Concentration of cadmium (mg kg^{-1}) deposition in leaf, stem and roots of *Bryophyllum pinnatum* irrigated with Brewery effluent

Plant part	Concentration					
	0%	20%	40%	60%	80%	100%
			Initial ($LSD_{0.05}$= NS)			
Leaf	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.06 ± 0.01	0.10 ± 0.00	0.12 ± 0.01
Stem	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.06 ± 0.01	0.10 ± 0.00	0.12 ± 0.01
Root	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.06 ± 0.01	0.10 ± 0.00	0.12 ± 0.01
			2 weeks ($LSD_{0.05}$= 0.23)			
Leaf	0.08 ± 0.00^{m}	0.14 ± 0.00^{m}	0.20 ± 0.00^{m}	0.26 ± 0.00^{m}	0.29 ± 0.00^{m}	0.31 ± 0.00^{m}
Stem	1.08 ± 0.00^{l}	2.14 ± 0.00^{k}	3.20 ± 0.00^{j}	4.26 ± 0.00^{i}	5.29 ± 0.00^{h}	6.31 ± 0.00^{g}
Root	20.08 ± 0.00^{f}	21.80 ± 0.34^{e}	23.20 ± 0.00^{d}	24.26 ± 0.00^{c}	24.96 ± 0.33^{b}	25.31 ± 0.00^{a}
			4 weeks ($LSD_{0.05}$= 0.32)			
Leaf	0.09 ± 0.00^{m}	0.15 ± 0.00^{m}	0.21 ± 0.00^{m}	0.27 ± 0.00^{m}	0.30 ± 0.00^{m}	0.31 ± 0.00^{m}
Stem	1.09 ± 0.00^{l}	7.15 ± 0.00^{k}	8.21 ± 0.00^{j}	9.27 ± 0.00^{i}	10.30 ± 0.00^{h}	10.97 ± 0.33^{g}
Root	21.42 ± 0.34^{f}	22.15 ± 0.00^{e}	23.54 ± 0.33^{d}	24.27 ± 0.00^{c}	25.96 ± 0.33^{b}	26.31 ± 0.00^{a}
			6 weeks ($LSD_{0.05}$= 0.32)			
Leaf	0.10 ± 0.00^{m}	0.16 ± 0.00^{m}	0.22 ± 0.00^{m}	0.28 ± 0.00^{m}	0.31 ± 0.00^{m}	0.31 ± 0.00^{m}
Stem	1.10 ± 0.00^{l}	8.16 ± 0.00^{k}	9.22 ± 0.00^{j}	10.28 ± 0.00^{i}	11.31 ± 0.00^{h}	12.31 ± 0.00^{g}
Root	22.43 ± 0.34^{f}	23.16 ± 0.00^{e}	24.56 ± 0.34^{d}	25.61 ± 0.34^{c}	26.31 ± 0.00^{b}	27.64 ± 0.34^{a}
			8 weeks ($LSD_{0.05}$= 42)			
Leaf	0.11 ± 0.00^{m}	0.17 ± 0.00^{m}	0.23 ± 0.00^{m}	0.28 ± 0.00^{m}	0.32 ± 0.00^{m}	0.32 ± 0.00^{m}
Stem	1.11 ± 0.00^{l}	9.17 ± 0.00^{k}	10.23 ± 0.58^{j}	12.61 ± 0.33^{i}	13.98 ± 0.33^{h}	15.65 ± 0.33^{g}
Root	24.11 ± 0.00^{f}	25.50 ± 0.34^{e}	26.23 ± 0.00^{d}	27.28 ± 0.00^{c}	28.32 ± 0.00^{b}	29.32 ± 0.00^{a}
			10 weeks ($LSD_{0.05}$= 42)			
Leaf	0.12 ± 0.00^{m}	0.18 ± 0.00^{m}	0.27 ± 0.03^{m}	0.29 ± 0.00^{m}	0.32 ± 0.00^{m}	0.32 ± 0.00^{m}
Stem	1.12 ± 0.00^{l}	10.18 ± 0.00^{k}	11.27 ± 0.03^{j}	11.96 ± 0.34^{i}	14.32 ± 0.58^{h}	17.32 ± 0.57^{g}
Root	25.12 ± 0.00^{f}	26.18 ± 0.00^{e}	27.27 ± 0.03^{d}	28.29 ± 0.00^{c}	29.32 ± 0.00^{b}	30.32 ± 0.00^{a}
			12 weeks ($LSD_{0.05}$= 0.50)			
Leaf	0.12 ± 0.01^{m}	0.19 ± 0.00^{m}	0.25 ± 0.00^{m}	0.29 ± 0.00^{m}	0.32 ± 0.00^{m}	0.32 ± 0.00^{m}
Stem	1.12 ± 0.01^{l}	12.52 ± 0.34^{k}	14.91 ± 0.33^{j}	16.63 ± 0.34^{i}	18.65 ± 0.34^{h}	19.98 ± 0.33^{g}
Root	27.12 ± 0.01^{f}	28.19 ± 0.00^{e}	29.25 ± 0.00^{d}	29.96 ± 0.34^{c}	30.65 ± 0.34^{b}	33.32 ± 0.57^{a}

Data are presented with mean ± standard error. Means with different alphabets along each row are significantly different separated using least significant difference test at $P < 0.05$

Phytochemical Analysis.

The phytochemical analysis revealed the presence of tannins, alkaloids, terpenoids, flavonoids and saponins in the *B. pinnatum* extract. The phytochemicals screening of *Bryophyllum* showed that alkaloids, tannins, flavonoids and saponins were present in both treated and control plants (Table 14). These four phytochemicals are among those reported by majat *et al*., (2011) to be present in *Bryophyllum*; others being phenolic compounds; bryophyllol, bryophollone, bryphollenone and bufadienollides.

Table 14: The Phytochemical analysis of *B. pinnatum* leaves extracts subjected to brewery effluent irrigation:

Phytochemicals	*B. pinnatum* (control)	*B. pinnatum* treated with 100% effluent at 12 weeks
Alkaloids	+	++
Flavonoids	+	+
Saponins	+	+
Steroids	-	-
Tannins	+	++
Phlobatanins	+	+
Terpenoids	+	+
Phenols	+	+

Key: + = Slightly Present; - = Absent; ++ = moderately present.

Discussion

Naturally occurring freshwater have relatively pH of between 6 and 9, and most freshwater sources are more or less neutral. Thus, the Ph of wastewater needs to remain between 6 and 9 to protect and be beneficial to organisms (USEPA, 2004), and also 7 for drinking water (WHO, 1996). In this study, the pH values obtained were slightly higher than the neutral (7) pH of water. This could be due to detergents used for washing that constituted part of the brewery wastewater. The sources of water, process and skill of manpower are main factors that can vary the range of pH in brewery effluent (Lique, Bracho and Maier, 1990). The brewery effluent for crop production can affect the availability and toxicity level of heavy metals in soil.

The sources of water, process and skill of manpower are main factors that can vary the range of pH in brewery effluent (Lique, Bracho and Maier, 1990). The brewery effluent for crop production can affect the availability and toxicity level of heavy metals in soil.

However, the values of industrial brewery effluent were within the permissible standards of NESREA and WHO of 6 – 9 and 6.5 – 8.5, respectively. Hence the pH of both the fresh water and the industrial brewery effluent pose no threat to the organisms and the people using brewery effluent for other purposes. Similar results were recorded by Otokunefor and Obiukwu (2005) while evaluating the impact of refinery effluent on the physicochemical properties of a water body in the Niger Delta, Ekhaise and Anyasi (2005) on the influence of breweries effluent discharges on the microbiological and physicochemical quality of Ikpoba River Nigeria, Alao *et al*., (2010) on the impact of brewery effluent on water quality in Majawe River, Ibadan, Southwestern Nigeria, Egwuonwu *et al*. (2012) on the effects of Nigeria Breweries PLC, Enugu industrial wastewater discharge on surface water, Olorode and Fagade (2012) on Physico-chemical characteristics of Ama Brewery effluent and its receiving Ajali River .

In Table 1: All the temperature values obtained in the study were within the NESREA (2009) standards for effluent discharges but did not meet the WHO guideline values (12 – 25^{o}C).

The total suspended solids of tap water and the brewery effluent were above NESREA permissible limits. This could be as a result

of materials used for brewing like mash, spent grains, waste yeast, spent hops, grit and other inorganic substances. The values may have negative effect on plants found by decreasing light penetration, leading to a decrease in photosynthesis (Olorode and Fagade 2012).

Suspended solids may also interfere with feeding mechanisms of soil organisms such as certain benthic microbial organisms, and growth of plants. High suspended solids can create anaerobic conditions reducing ability to support a diversity of plant species (Breweries of Europe, 2002; USEPA, 2003).

Empirically, total dissolved solids concentrations are used to evaluate the quality of freshwater ecosystems. Total dissolved solids recorded in this study showed that the values are within the permissible standards of WHO and NESREA (WHO, 1996; NESREA, 2009). The result concurred with the findings of Egwuonwu *et al*., (2012) and Dimowo (2013). Furthermore, the physicochemical analysis of total solids is to ascertain dissolved solids in addition to suspended solids and settle-able solids in plants.

The result of this study showed that the total Solids of the brewery effluent and the tap water were within the permissible limits of WHO.

The five days biological oxygen demand (BOD5) is the most widely used parameter in evaluating of organic pollution waters. It is the amount of dissolved oxygen taken up by aerobic micro-organisms to degrade oxidizable organic matter which is measured over the period of 5 days. The values of biological oxygen demand of the brewery effluents and the tap water recorded in this study indicated that they were satisfactory and within WHO and NESREA permissible standards for effluent discharges to plants (WHO, 1996; NESREA, 2009). This suggested that the amount of biodegradable organic matter in the brewery effluent was low and the dilution by the tap water tend to reduce the dissolved oxygen demanding substances.

In relation to BOD, chemical oxygen demand is a measure of the equivalent amount of oxygen required to completely oxidize both biodegradable and non-degradable organic and inorganic matter. The chemical oxygen demand concentration was within the permissible limit of WHO and NESREA standards for effluent (WHO, 1996; NESREA, 2009). Similar results were recorded by Egwuonwu *et al*., (2012). The dissolved oxygen (DO) values were below WHO permissible standard of recommended levels of 5.0 mg/l (WHO, 1996). This could be as result of increase in total suspended solids and turbidity from the brewery effluent. The result of this study agreed with the finding of Ikpeaiyada and Onianwa (2009), Dimowo (2013) who reported lower values of DO in the various ecosystems studied. USEPA (2004) recommends that any water body that maintains aquatic life should contain 5 mg/l of dissolved oxygen for at least 16 hours of the day and the other 8 hours should not drop below 3 mg/l.

Importantly, the turbidity values obtained in the study at the effluent discharge showed higher values than the acceptable limits of WHO and NESREA (WHO, 1996; NESREA, 2009) while tap water is within the acceptable limits of WHO and NESREA. The reason for the murky appearance of the effluent discharge is attributed to the ingredients used in brewery industries such as cereal, adjuncts, hops and yeast. All these combines with water to form wastewater which is discharged as wastewater. This finding was in line with the reports of Ekhaise and Anyasi (2005); Olorode and Fagade (2012); and Egwuonwu *et al*. (2012) that all reported high turbidity values in

different rivers receiving brewery effluent which must have been influenced by brewery wastewater.

Finally, the odour of the brewery effluent was objectionable. This could be as a result of dissolved organic and inorganic compound such as nitrogen, sulphur and phosphorous which emanated from putrefaction of proteins and other organic materials (Ibekwe *et al*., 2004). Also, certain gases found in brewery wastewater can cause odour. Hydrogen sulfide and ammonia gases can be toxic and pose asphyxiation hazards. The observation in this study was in line with the reports of Breweries of Europe (2002) and Egwuonwu *et al*., (2012).

The study revealed progressive increase in soil pH as the concentration of the effluent increased. The soil nutrients (N, P, K, Mg and Ca) significantly increased as the concentrations of effluents increase. This could be probably as a result of constant applications of IBE

The pH values indicate that the experimental soil samples taken before brewery effluent application were slightly acidic starting from 6.53, and alkaline (7.4) in the brewery effluent used (Table 13).

Some selected properties of brewery effluent used for the experiment, and soils of the experimental site are given in Table 13: The soils of the experimental site are characterized by 1.30% of nitrogen, 18. 60 $mgkg^{-1}$ of available phosphorus, 2.23 $mgkg^{-1}$ of potassium, 2.50 $mgkg^{-1}$ of magnesium, 0.23 $mgkg^{-1}$ of calcium. Brewery effluent studied is characterized by 1.33% total nitrogen, 39.75$mgkg^{-1}$of phosphorus, 5.50 $mgkg^{-1}$ of potassium, 2.64 $mgkg^{-1}$ of magnesium and 20.00 $mgkg^{-1}$ of calcium.

Evident from the data presented in Table 13, the accumulation of heavy metal ions due to bio- liquid effluent were below the tolerable value for most of the metal ions. In general, the highest accumulation of zinc (17 $mgkg^{-1}$) at application of 100 % IBE, 12 WAT was below internationally tolerable standard of 100.00, iron (68.25 $mgkg^{-1}$) at application of 100% IBE,12 WAT below permissible standard of 415.00, cadmium (33.32 $mgkg^{-1}$) at application of 100% IBE, 12 WAT was above permissible standard of 0.12, nickel (13.17 $mgkg^{-1}$) at application of 100% IBE, 12 WAT was below permissible standard of 5.30, copper (5.53 $mgkg^{-1}$) at application of 100% IBE, 12 WAT was below internationally tolerable standard of 72.00, lead (0.77) at application of 100% IBE, 12 WAT, and lead was below the permissible standard of 5.00. It can then be generalized that the heavy metal accumulation was below allowable levels except cadmium.

The research result revealed that the concentrations of iron, cadmium and nickel are higher than the other heavy metals such as the copper and lead in *B pinnatum*.

Among the application of various levels of brewery effluent, 0% treatment level produced negative changes that was below the heavy metals in the soil before application. Lead and cadmium are considered as most hazardous metals that can be taken up by plants in high proportion (Egwuonwu *et al*., 2012).

Differences in Iron concentrations in the experimental site before and after the application of brewery effluent are significant ($P < 0.05$). The iron content of the plant before brewery effluent application was 1.03 for leaf, 1.40 for stem and 3.40 for root ($mgkg^{-1}$) with the range of iron revealed: 51.78 for leaf, 68.00 for stem and 68.00 for root ($mgkg^{-1}$) at 100% after application of brewery effluent. The application of 60% and 80% brewery effluent showed increase in the result of plant iron concentration. Even though, iron is an essential element

that is required in large amount for plant growth and development, the use of brewery effluent revealed non-significant difference with the plant iron concentration present before brewery effluent application. From 17 essential nutrients, iron is the necessary element that helps plants for the accomplishment of metabolism, photosynthesis and plant growth, and needed in abundance (Arjun and Nwachukwu,2013).

Copper is one of the micro nutrients that is required for plant growth without deviating from health manner and its optimum supply can be obtained from artificial and/or organic fertilizer including brewery effluent. Even though the function of copper is difficult to be known clearly, it can make a vital function in plant metabolism for the normal function of plant physiology (Awaleh and Soubaneh, 2014)

In this study, it was found that the concentration of iron in the plant before application and after application of 20% and 100% brewery effluent (Table 7) resulted to increase in the quantity of iron concentration in the plant. The contents of heavy metals in the *B pinnatum* cultivars revealed various results that ranges from 48.87 – 72.64 $mgkg^{-1}$ for iron, 3.07 -5.43 $mgkg^{-1}$ for copper, 13,80 – 18.89 mgkg-1 for zinc, 0.51 – 0.77 $mgkg^{-1}$ for lead, 2,02 – 3.55 $mgkg^{-1}$ for nickel and 0.08 – 0.32 $mgkg^{-1}$ for cadmium (Ozturk *et al*., 2011).Additionally, the authors reported the accumulation pattern for the heavy metals in the *B pinnatum* in order: Fe > Zn > Cu >Ni > Pb > Cd.

The phytochemical analysis revealed the presence of tannins, alkaloids, terpenoids, flavonoids and saponins in the *Bryophyllum pinnatum* extract. The phytochemicals screening of *Bryophyllum* showed that alkaloids, tannins, flavonoids and saponins were present in both treated and control plants (Table 14). These four phytochemicals are among those reported by Majaz *et al*., (2011) to be present in *Bryophyllum*; others being phenolic compounds, bryophyllol, bryophollone, bryphollenone and bufadienollides.

According to Cowan (1999), flavonoids and tannins are among several plant products utilized as antimicrobial agents, along with tepernoids. Egunjobi (1969) reported that medicinal plants with high concentration of tannins

Conclusion

The results obtained revealed that there was no significant effect of brewery effluent application on heavy metal concentration in the soil and plants after crop harvest, compared to an international standard tolerance level. The total growth rates of *B pinnatum* are highest at 100% treatment of brewery effluent rate and decreased in the directions below these treatments. In conclusion, *B pinnatum* responded well to the brewery effluent rate of 100% in terms of growth in the study area. Therefore, small holder farmers in this area could be advised to use a brewery effluent rate of 100% to optimize the vegetable yield. However, it is premature to come up with a conclusive recommendation since the experiment was conducted for one season only.

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