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Heavy Metals Analysis of Breweries Effluent used in Soil Cultivated with *Glycine Max* (L.) Merr. Accessions with Biochar Augmentation

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Abstract

Beer production has promulgated the prevalence of heavy metal contamination in the environment (soil and water bodies). The magnitude of breweries wastewater-effluent effect discharged on soil and its effect on crop quality in terms of growth and physiology must therefore be investigated. This study aims to assess the level of seven heavy metals (HM) (lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn) and iron (Fe)) in breweries wastewater-effluent and its effect on the growth of *Glycine max* accessions (TGM-3990, TGM-1348, TGM-1732, TGM-2175 and TGM-928) as well as the influence of biochar soil amendment in the induction of HM tolerance in *G. max*. Effluent properties were analyzed using the A1 portable TDS/EC meter. HM in the wastewater-effluent was determined using Atomic Absorption Spectrometer (AAS). The experiment was set up in a complete block design. 3 seeds *G. max* accessions were planted in 7kg of sterilized soil (control, effluent and biochar treatments). Growth parameters were taken using standard methods, while the total photosynthetic pigments (TPP) were determined using the atLeaf chlorophyll meter. The wastewater-effluent recorded an electrical conductivity (EC) of 928µS/cm, total dissolved solids (TDS)

(464ppm), Temperature (31.9-32.6°C) and pH (5.37). Results for HM concentration showed; Pb (0.20mg/L), Ni (<0.001mg/L), Cr (0.03mg/L), Cu (<0.001mg/L), Cd (<0.001mg/L), Zn (<0.001mg/L) and Fe (8.74mg/L). The trend shows that Fe>Pb>Cr>Cd>Cu>Ni>Zn. Cr, Cd, Cu, Ni and Zn were significantly below the World Health Organization (WHO) and Waste Water Forum (WWF) permissible limit, while Fe and Pb significantly higher. *G. max* accessions TGM-928 (80%) and TGM-3990 (80%) had better germination percentage (GP), TGM-1348 (60%) had the lowest GP, while TGM-2175 recorded no germination in all treatments. At 6 weeks after planting (WAP), it was observed that irrigation of *G. max* accessions with breweries wastewater-effluent significantly (p=0.001) stimulated growth parameters such as shoot length for TGM-3990 which recorded; Treatment=19.53±0.94cm; Control=17.10±0.67cm; Biochar=40.83±1.01cm) while TGM-1732 had the highest shoot growth (T=38.57±1.53cm; C=25.00±0.00cm; B=82.33±5.70cm) when compared to their controls. TGM-3990 (T=32.20mg/kg, C=34.10mg/kg, 43.30mg/kg) recorded the least TPP contents, while TGM-928 (T=51.00mg/kg, C=45.60mg/kg, B=43.50mg/kg) recorded the highest TPP in all treatments. Amelioration of soil with biochar significantly (p=0.001) stimulated growth above the effluent treatment and control. Similar trend was observed for leaf area, petiole length, leaf number, stem girth and internode length. This study has shown that breweries effluent has the potential of growth enhancement in *G. max* cultivation and in combination biochar application reduces the need for additional fertilizer application.

Keywords: Beer; Biochar; Breweries effluent; *Glycine max;* Heavy metal; Lead.

1. Introduction

In the process of beer production, brewery wastewater effluent is produced by breweries industries while producing the different brands of beers. Lead (Pb) and mercury (Hg) has been reported to be one of the most predominant heavy metals present high concentrations which pose a great environmental concern having a negative impact on plants, soil microbial communities as well as aquatic organisms [1]. According to World Health Organization [2], heavy disease burden is strongly associated with the contamination of soil, water and air as a result of waste water effluents discharged by industries. These discharged waste water effluents includes; Fe, Ag, Hg, Ni, Pb, Cd, Cu, Zn, Cr and As which are some of the most toxic environmental pollutants once discharged [3]. These metals have detrimental effects once released into the environments in hazardous quantities because of its persistent nature and non-biodegradability as we as toxicity, bio-concentration and bioaccumulation in living organisms. Most breweries are usually situated in cities globally and their wastewater effluents (treated and untreated) are released directly into the environment [4].

In recent times, especially in urban centres and environments where water availability is of serious concern; industrial effluents (treated and untreated) usage for irrigational purposes is gaining momentum for the cultivation of vegetables and fruits. According to recent reports, the usage of breweries wastewater effluent (especially treated wastewater effluent) for irrigation has presented some advantages such as improved plant growth and quality as well as the improvement of soil properties that cannot be overlooked [4, 5].

Agricultural productivity is globally threatened due to environmental challenges which could be either biotic or abiotic. Issues like declining organic matter, poor soil water holding capacity and fertility is becoming predominant and farmers usually resort to the usage of inorganic fertilizers which in turn have negative environmental implications. The usage of biochar as soil amendment has numerous benefits in agriculture by improving soil properties which includes; lessening soil nutrient loss, improving nutrient and water retention capacity ultimately resulting in the improvement of plant growth and yield [6].

This research was designed to assess the level of seven heavy metals (HM) (lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn) and iron (Fe)) in breweries wastewater-effluent and its effect on the growth of *Glycine max* accessions (TGM-3990, TGM-1348, TGM-1732, TGM-2175 and TGM-928) as well as the influence of biochar soil amendment in the induction of HM tolerance in *G. max.*

2. Materials and Methods

2.1. Study Area and Source of Materials

The experiment was carried out in the Department of Botany, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria. Breweries wastewater effluent was obtained from Champion breweries, Uyo. Soil used was gotten from farms around the University and sterilized. Biochar was obtained from carbon-rich solid plant materials produced by the pyrolysis of biomass. Five (5) accessions of *G. max* were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo state.

2.2. Heavy Metal Analysis and Properties of the Effluent

Heavy metals concentration in the breweries effluent was analyzed Atomic Absorption Spectrometer (AAS) according to the methods of American Society for Testing and Materials (ASTM) (2010). Electrical conductivity (EC), total dissolved solids (TDS), Temperature and pH were determined using the A1 portable meter for TDS/EC.

2.3. Planting

About five (5) seeds of *G. max* were sown in their respectively planting bucket filled with sterilized sandy-loamy soil. Three replicates were made for each accession, with labels and laid out using Completely Block Design (CBD). After two weeks, 200ml wastewater was applied on each replicate of all accessions every 3 days for 6 weeks.

2.4. Growth Morphological Parameters

The shoot, petiole and internode length, leaf area and number of nodes of healthy leaves from the experimental plants were taken from 2 weeks after sprouting (WAS) using standard methods.

2.5. Determination of Total Photosynthetic Pigments

The atLeaf handheld chlorophyll meter was used for non-destructive estimation of the total photosynthetic pigments (TPP) estimation.

2.6. Determination of Turgid Weight (TW)

To determine the turgid weight (TW), leaves were soaked in water inside petri dishes kept in the laboratory. Leaf samples were weighed periodically by gently taking them out of the water and placed on the weighing balance until a steady weight was attained and recorded.

2.7. Determination of Biomass Yield

Values of fresh weight, leaf fresh weight, root length, total fresh weight and total dry weight were all determined using standard methods.

2.8. Statistical Analysis

All data in the present study were subjected to analysis of variance (ANOVA) using GraphPad Prism and data are presented as standard error of mean (± S.E.M.) of triplicate experiments. However, a probability level of $p<0.0001$ was considered statistically significant.

3. Results and Discussion

The quality of irrigation water and its control is dependent of the properties of the effluent e.g. EC and pH; as this will aid the identification and quantification of pollutants in the wastewater effluent [7]. Effluent properties in this study are shown in Table 1. The EC of the effluent in this study was 928 µS/cm, this is in line with the work of Tessema, *et al.* [8], who reported an effluent EC range of 3101 µS/cm and the maximum was 4630 µS/cm. Conductivity is measured to establish the pollution zone around an effluent discharge [9].

Table-1. Properties of the breweries wastewater effluent

Parameters	EC (µS/cm)	TDS (ppm)	Temperature (°C)	pH
	928	464	31.9-32.6	5.37

Results for HM concentration showed; Pb (0.20mg/L), Ni (<0.001mg/L), Cr (0.03mg/L), Cu (<0.001mg/L), Cd (<0.001mg/L), Zn (<0.001mg/L) and Fe (8.74mg/L). The trend shows that Fe>Pb>Cr>Cd>Cu>Ni>Zn. Cr, Cd, Cu, Ni and Zn were significantly below the World Health Organization (WHO) and Waste Water Forum (WWF) permissible limit, while Fe and Pb significantly higher (Table 2). [9] also reported high Fe and Pb in wastewater effluent. Pb toxicity causes the inhibition of root elongation, seedling development, seed germination, transpiration, plant growth, chlorophyll production, lipid peroxidation, ATP production as well as DNA damage via the overproduction of ROS. Though Fe in excess concentration can result in soil acidification and loss of availability of phosphorus and molybdenum when applied, Fe is an indispensable micronutrient essential for the synthesis of chlorophyll, also a key constituent of ferredoxin which plays an important role in biological nitrogen fixation and primary photochemical reaction in photosynthesis. This could account for the increased growth in *G. max* observed in this study when irrigated with breweries effluent wastewater.

Table-2. Heavy metals concentration of the breweries wastewater effluent

S/N	Metals	Effluent	WHO*	WWF*	SON*
1	Lead (Pb)	0.20	0.01	0.10	0.01
2	Nickel (Ni)	<0.001	0.07	0.20	0.02
3	Chromium (Cr)	0.03	0.05	0.01	0.05
4	Copper (Cu)	<0.001	2.00	0.20	1.00
5	Cadmium (Cd)	<0.001	0.003	0.01	0.003
6	Zinc (Zn)	<0.001	0.30	NA	0.30
7	Iron (Fe)	8.74	0.30	NA	0.30
*Permissible limits of some heavy metals in water in Wastewater Forum (WWF, 2007), World Health Organization (WHO, 2006) and Standard Organization of Nigeria (SON, 2016). NA (Not available).					

Amendment of soil irrigated with breweries effluent wastewater with biochar significantly ($p<00.001$) improved the growth of all *G. max* accessions (Figure 1, 2, 3, 4, 5, 6, 7, 8) to. This is in line with the work of Bonanomi, *et al.* [10], who reported that biochar application singly or co-composted promoted the growth of lettuce. Kammann, *et al.* [11], also proposed that co-composted biochars have a remarkable plant growth promoting effect compared to biochars used pure. This may be as a result of the improvement of soil fertility, high carbon storage, promotion of water retention capacity, and adsorption of soil pollutants.

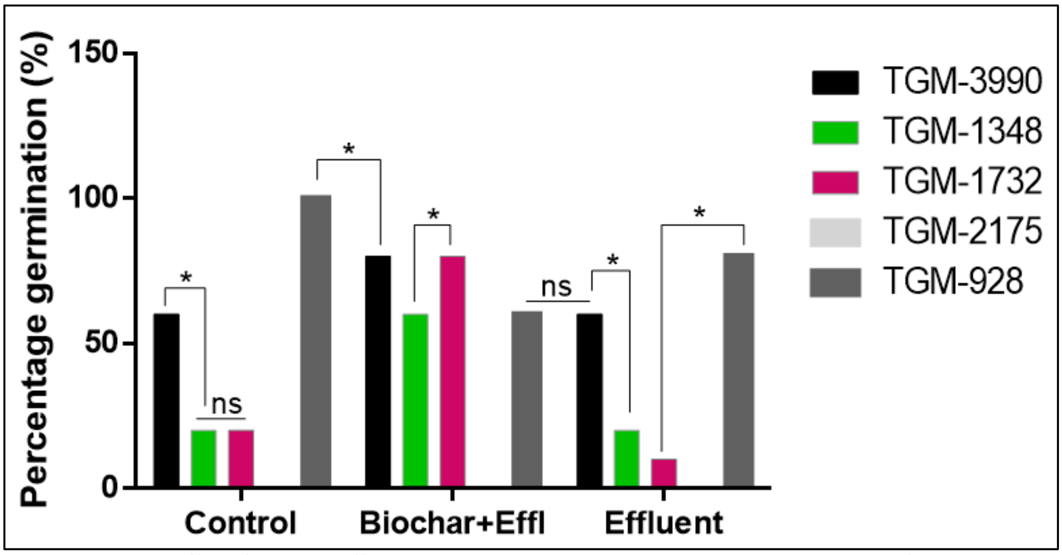

Figure-1. Effect of breweries effluent and biochar soil amendment on percentage germination of *G. max*

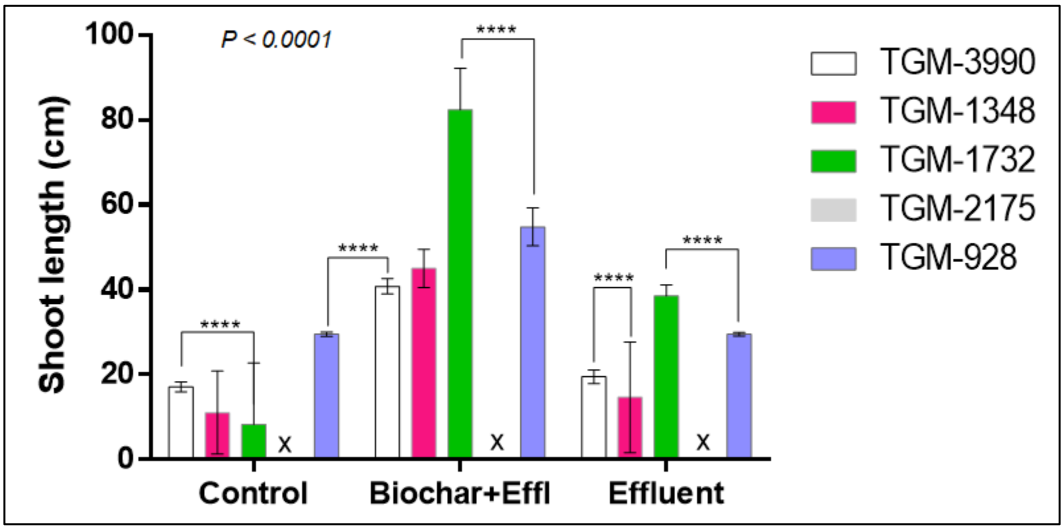

Figure-2. Effect of breweries effluent and biochar soil amendment on the shoot length of *G. max*

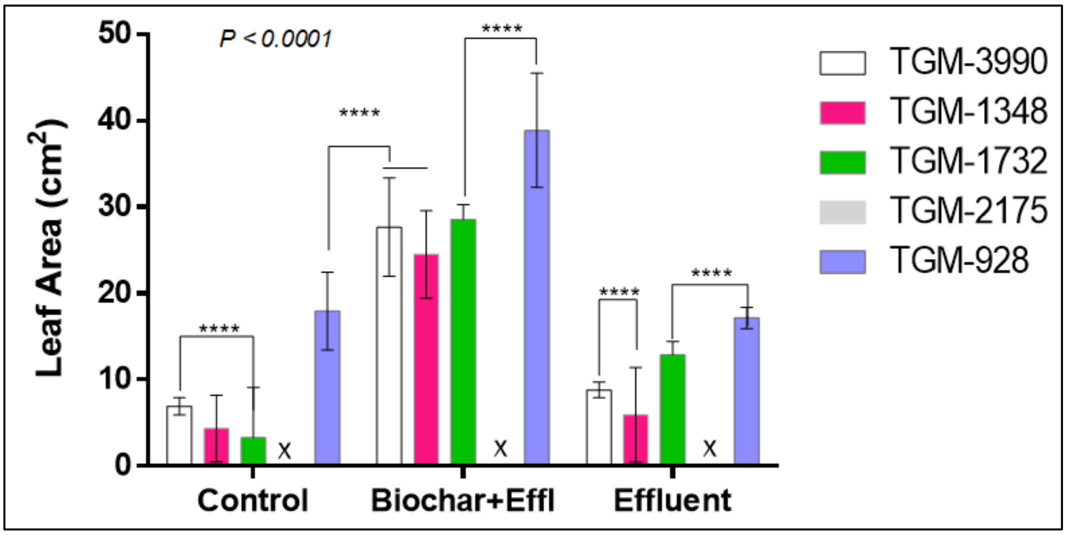

Figure-3. Effect of breweries effluent and biochar soil amendment on the leaf area of *G. max*

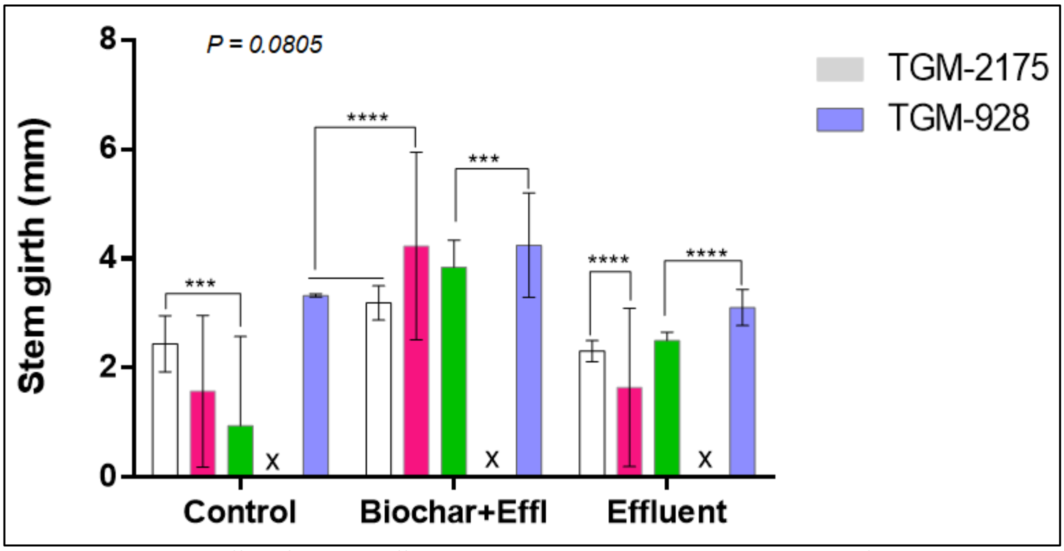

Figure-4. Effect of breweries effluent and biochar soil amendment on the stem girth of *G. max*

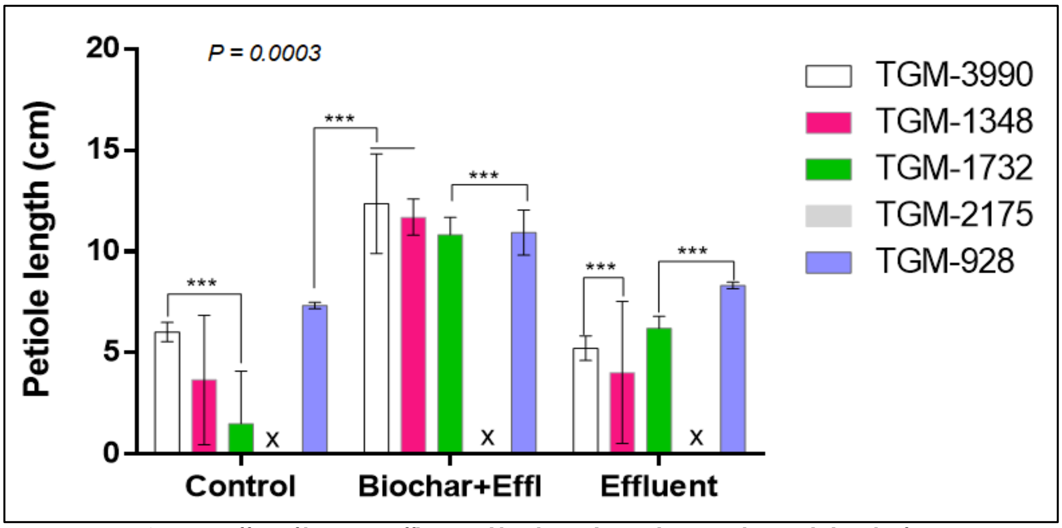

Figure-5. Effect of breweries effluent and biochar soil amendment on the petiole length of *G. max*

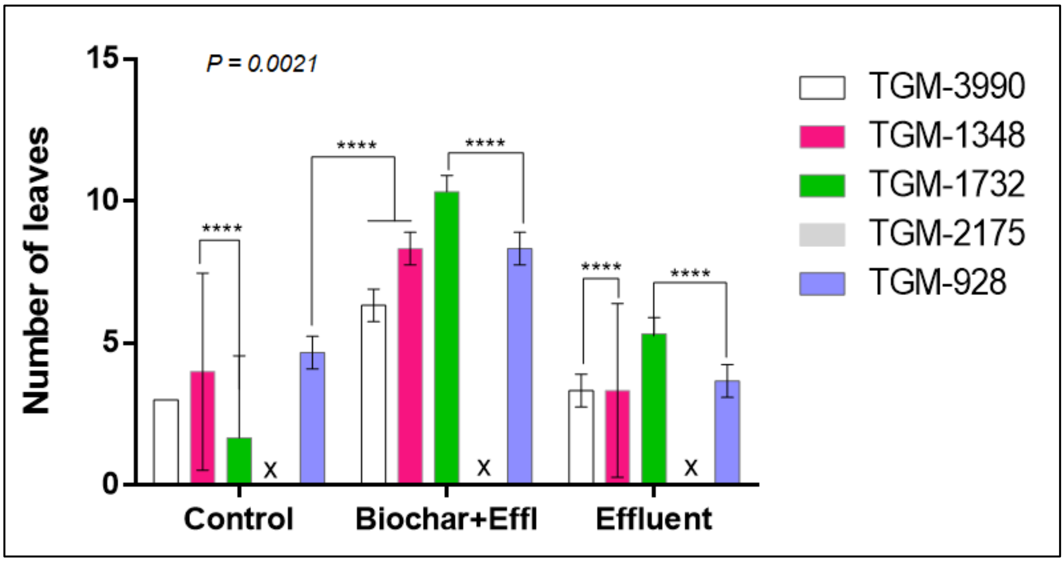

Figure-6. Effect of breweries effluent and biochar soil amendment on the number of leaves of *G. max*

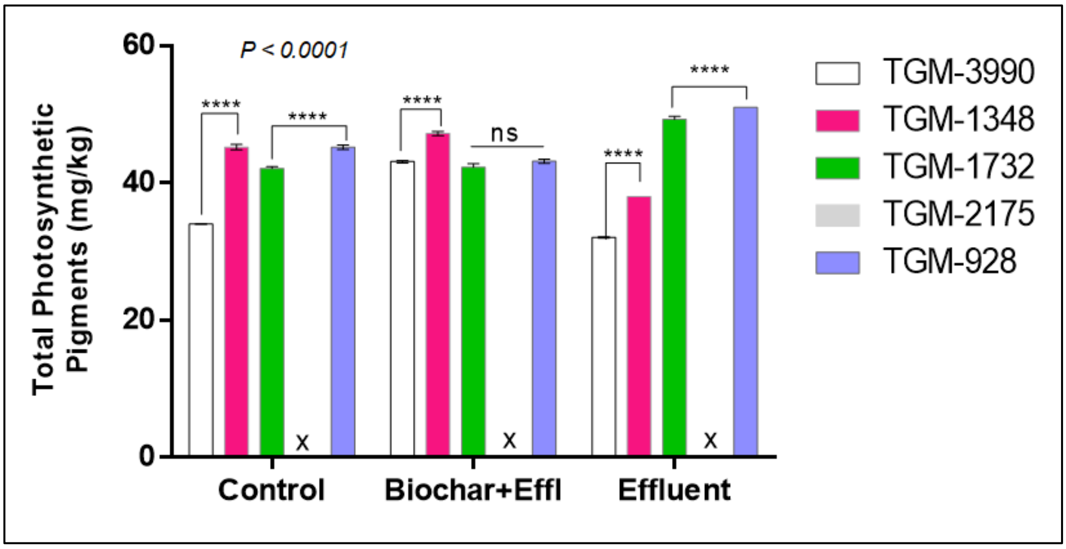

Figure-7. Effect of breweries effluent and biochar soil amendment on the total photosynthetic pigments of *G. max*

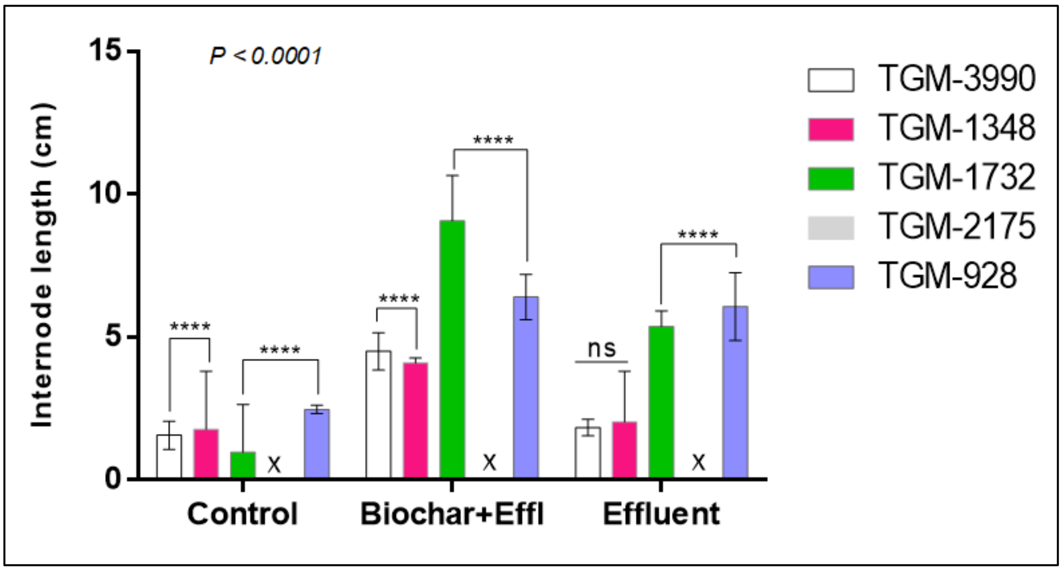

Figure-8. Effect of breweries effluent and biochar soil amendment on the internode length of *G. max*

The biomass yield of *G. max* planted on soil irrigated with breweries effluent and biochar soil amendment is shown on Table 3. From this study, it is observed that application of biochar with effluent significantly (p<0.0001) increased the total fresh weight, total dry weight and root length of all the accessions of *G. max* above the effluent treatment alone and their controls (Table 3).

Table-3. Biomass yield of *G. max* planted on soil irrigated with breweries effluent and biochar soil amendment

	TGM-3990	TGM-1348	TGM-1732	TGM-2175	TGM-928
Root Length (cm)					
Control	*28.00±1.15^b	58.66±0.88^a	40.00±1.15^b	0.00±0.00^a	48.00±1.52^a
Biochar + Effluent	54.00±4.50^a	40.00±1.52^b	49.66±10.83^a	0.00±0.00^a	40.00±1.52^b
Effluent	32.00±1.52^b	42.00±1.15^b	52.00±1.15^a	0.00±0.00^a	36.66±0.88^c
Total fresh weight (g)					
Control	2.36±0.44^b	3.60±0.68^b	1.90±0.05^b	0.00±0.00^a	19.60±0.37^b
Biochar + Effluent	42.83±0.69^a	61.60±1.51^a	47.13±2.48^a	0.00±0.00^a	54.56±2.42^a
Effluent	4.33±0.37^b	6.16±0.18^b	5.13±0.08^b	0.00±0.00^a	23.46±0.33^b
Total dry weight (g)					
Control	1.50±0.11^b	2.56±0.08^b	0.30±0.15^b	0.00±0.00^a	9.43±0.88^b
Biochar + Effluent	9.83±0.29^a	15.00±0.30^a	13.93±0.56^a	0.00±0.00^a	15.93±0.66^a
Effluent	2.13±0.08^b	2.66±0.03^b	3.90±0.00^b	0.00±0.00^a	5.36±0.20^b
*Mean of three replicates ± SEM. [a]Values of each column followed by the same letter are not significantly different at p<0.0001 level.					

4. Conclusion

This study has provided insights on the potential beneficial aspect of breweries wastewater effluent. The results showed that breweries wastewater effluent applied in moderate quantity can promoted the growth of *G. max*.

However, the application of untreated wastewater may pose a threat to plant/human health because of the presence of pollutants like Pb. The application of biochar as soil amendment improves soil physicochemical properties and promotes plant growth. Thus, further research is required to investigate the effects of long term biochar application on soil properties, microbial communities and nutrient mobilization.

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