

# Phytoremediation potential of *Amaranthus hybridus* L. (Caryophyllales: Amaranthaceae) on soil amended with brewery effluent

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**Abstract.** Toxicity of heavy metals above the normal threshold constituted a threat to humanity and biodiversity. Phytoremediation has become a novel and emerging technology of cleaning polluted sites through the use of plants. A study was carried out at the screen house located besides the academic building of the Federal University of Technology, Akure, Nigeria, to evaluate the phytoremediation potential of *Amaranthus hybridus* L. (Caryophyllales: Amaranthaceae) on a brewery effluent. The parameters investigated include chlorophyll content, the concentration of the metals in the plants, Bioconcentration Factor (BCF) and Translocation Factor (TF) was studied. Three different concentrations of brewery effluent were used at 50, 100 and 150 mL/5 kg of soil, respectively. The results of this study under controlled conditions indicate that effluent application increased chlorophyll content, reduced plant height and stem girth. Three heavy metals (iron, cadmium, and chromium) were detected in the shoots and leave of both plants after the experimental period. The translocation factor (less than 1) and bioaccumulation factors (greater than 1) were below the permissible limits hence indicating a possible bio-accumulator for the heavy metals investigated. Brewery effluent reduce the plant height but increase the leaf area of *A. hybridus* under high concentrations which possibly suggest an adaptive mechanism developed by the plant under stress.

**Keywords:** Effluent; Bio-concentration factor; Permissible limit; Toxicity; Translocation.

## Introduction

There has been an increasing concern with regard to the accumulation of heavy metals in the environment as they pose a threat to both human health and the natural environment (Abioye et al., 2009). This is due to the fact that

unlike many substances, metals are not biodegradable and hence accumulate in the environment (Ghanbari-Bonjar and Leel, 2004).

Effluents are wastes produced from industries and they vary depending on the human activities that produce them. Production of these wastes is an

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integral part of industrial activities but unfortunately, our inability to anticipate the magnitude of undesirable consequences of uncoordinated release of effluents in our environment in connivance with industrial boom have resulted in wanton destruction of our ecosystem.

Higher plants can accumulate heavy metals in different concentrations, but significant differences in metal accumulation exist between and within plant populations. In this study *Amaranthus hybridus* L. (Caryophyllales: Amaranthaceae) was considered because it is a popular nutritious leafy vegetable crops, rich in proteins, vitamins and minerals, and consumed virtually in the whole continent of Africa, Asia and South America. Their quick growth and great biomass makes them some of the highest yielding leafy crops and therefore suitable for phytoremediation purpose.

The objective of this study therefore was to investigate the effect of brewery effluent on some soil chemical properties and growth of *A. hybridus* plant and to determine the phytoremediation potential of the plants using heavy metal uptake into roots and stem.

## Materials and methods

### Experimental site

The screen house experiment was carried out in the academic building of the Federal University of Technology, Akure (Latitude (7° 16' N and Longitude, 15° 12' E), Ondo State, Nigeria.

### Soil collection

Top soils (0-15 cm) were collected from Aba Oyo Community of the Federal University of Technology, Akure. The soils were thoroughly mixed by a mechanical mixer and passed through 4 mm sieve to remove fibre and non soil particulate in the sample. The chemical and physical properties of the soils were determined prior to planting.

### Seed and effluent collection

The seeds were obtained from International Institute of Tropical Agriculture, Ibadan. Brewery effluent was collected at the international brewery, Ilesha, Osun State, Nigeria.

### Experimental design

The experiment was laid out in a completely randomized design (CRD) with five replicates. The brewery effluent was applied at the following rates of 0, 50, 100 and 150 mL concentration per 5 kg soil and later left for 2 weeks to allow for mineralization planting the seeds. The plants were treated with effluent twice a week.

### Measurement of growth parameters

Growth parameters were observed and measured every 2 weeks after planting (WAP). This was done for 8 weeks after which the plants were harvested, dried in the oven at 105 °C for 48 h to a constant weight. The growth parameter measured were plant height, leaf area, stem girth, number of leaf and chlorophyll content of the fresh leaf. The chlorophyll content was determined according to the method of Arnon (1949).

### Soil analysis

Soil analysis was carried out before and after the experiment. The soil pH was determined at soil:water ratio of 1:1 using glass electrode pH meter. The particle size analysis of the soil was by the method of Bach et al. (1958) as modified by Day and Wong (1965) whereas the organic carbon content of both soil and effluent were analysed by chromic acid wet oxidation method of Fosu et al. (2004). The nitrogen was determined by microkjeldal procedure as described by Fosu et al. (2004). The available phosphorus was extracted with Bray and Kurtz solution 1 (Bray and Kurtz, 1945). Exchangeable acidity was determined by the KCl extraction and

titration methods of Maclean (1965). The total dissolved solids and suspended solids in the brewery effluent were analysed by the method of Ademoroti (1996). The alkalinity was determined by the method of Larson and Henley (1955). The electrical conductivity was determined using the model 230 HT CORNING conductivity meter. The Biological Oxygen Demand (BOD) and Chemical Oxygen demand (COD) were determined using the method of Ademoroti (1996).

#### Digestion of plant samples

The procedure according to Awofolu (2005) was used for digestion of plant sample. 0.5 g of sieved leaf samples were then weighed into 100 cm<sup>3</sup> beaker. A mixture of 5 cm<sup>3</sup> concentrated HNO<sub>3</sub> and 2 cm<sup>3</sup> HClO<sub>4</sub> were added to dissolve the sample. The beaker was heated at moderate temperature of 110 °C on a hot

plate for 1 h in a fume hood until the content was about 2 cm<sup>3</sup>. The digest was allowed to cool, filtered into 50 cm<sup>3</sup> standard volumetric flask and made up to the mark with distilled deionized water. A serial dilution method was used to prepared the working standards and the concentrations of the metals in each sample digest were determined using atomic absorption spectrophotometer (Buck Model 210 VGP) equipped with a digital readout system.

#### Determination of the Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the formula:

$$BCF = \frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (soil)}}$$

#### Determination of the movement of metals from roots to plants

To evaluate the potential of plants for phytoextraction, the

translocation factor (TF) was used. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant. It is represented by the ratio:

$$TF = \frac{\text{Metal concentration (stems + leaves)}}{\text{Metal concentration (roots)}}$$

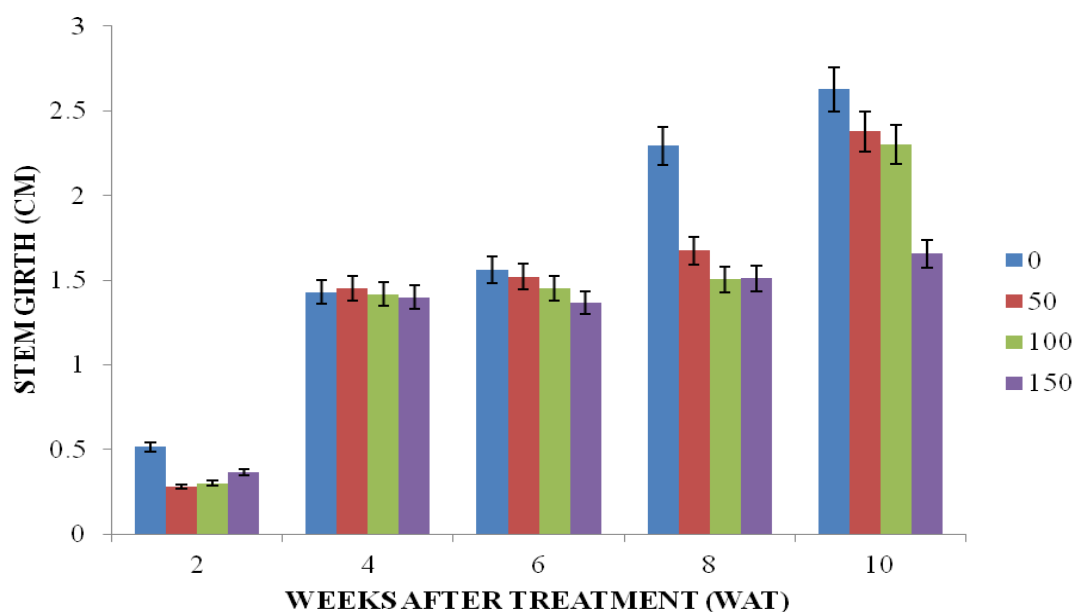
Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values < 1 with values > 1 indicating that the metals are stored in the stems and leaves.

#### Data analysis

Data obtained were subjected to analysis of variance and means were separated using Turkey's B test.

#### Results

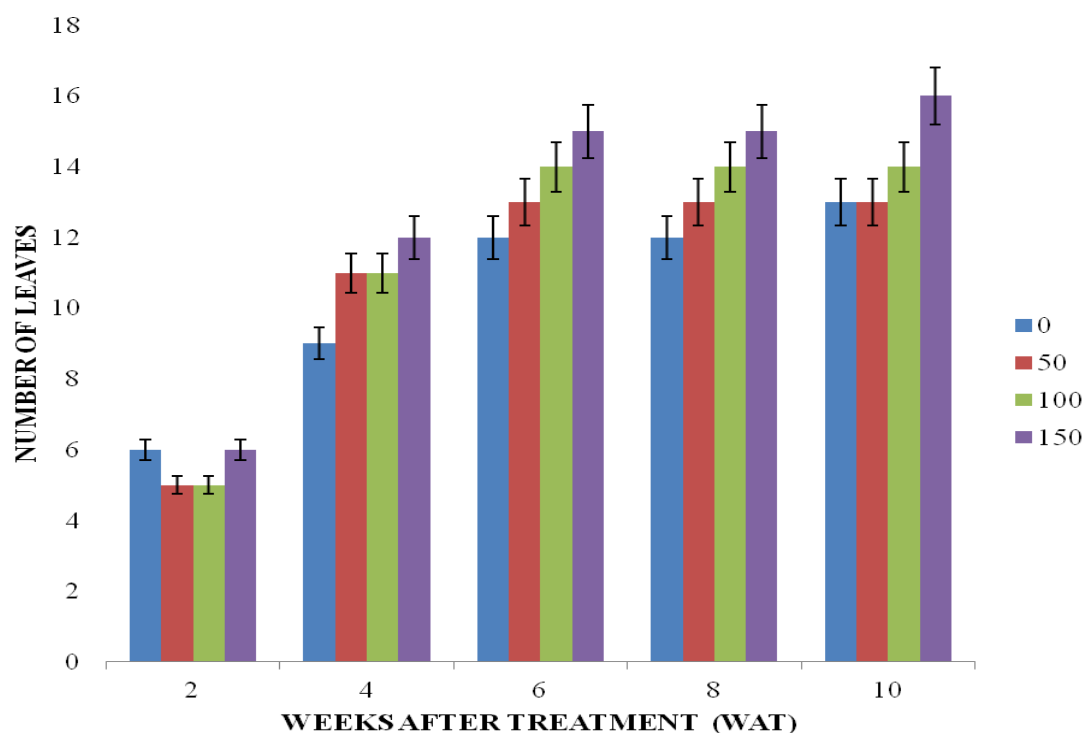
Effect of brewery effluent on the stem girth of *A. hybridus* is shown in Figure 1. There were significant differences on the effluent treated plants at 8 WAT and 10 WAT, respectively, under the control and 50 mL brewery effluent (BET). However, the BET does not have significant effect on the stem girth of *A. hybridus* at 4 WAT and 6 WAT, respectively.



**Figure 1.** Effect of brewery effluent (BET) on the stem girth of *A. hybridus*.

Figure 2 shows the effect of brewery effluent on the number of leaves of *A. hybridus*. It was observed that the BET had significant effect on the number of leaves of *A. hybridus* most especially at

the 6th, 8th, and 10th WAT, respectively, under the 150 mL BET. The highest number of leaves (16) was also recorded under the 150 mL BET relative to the control.



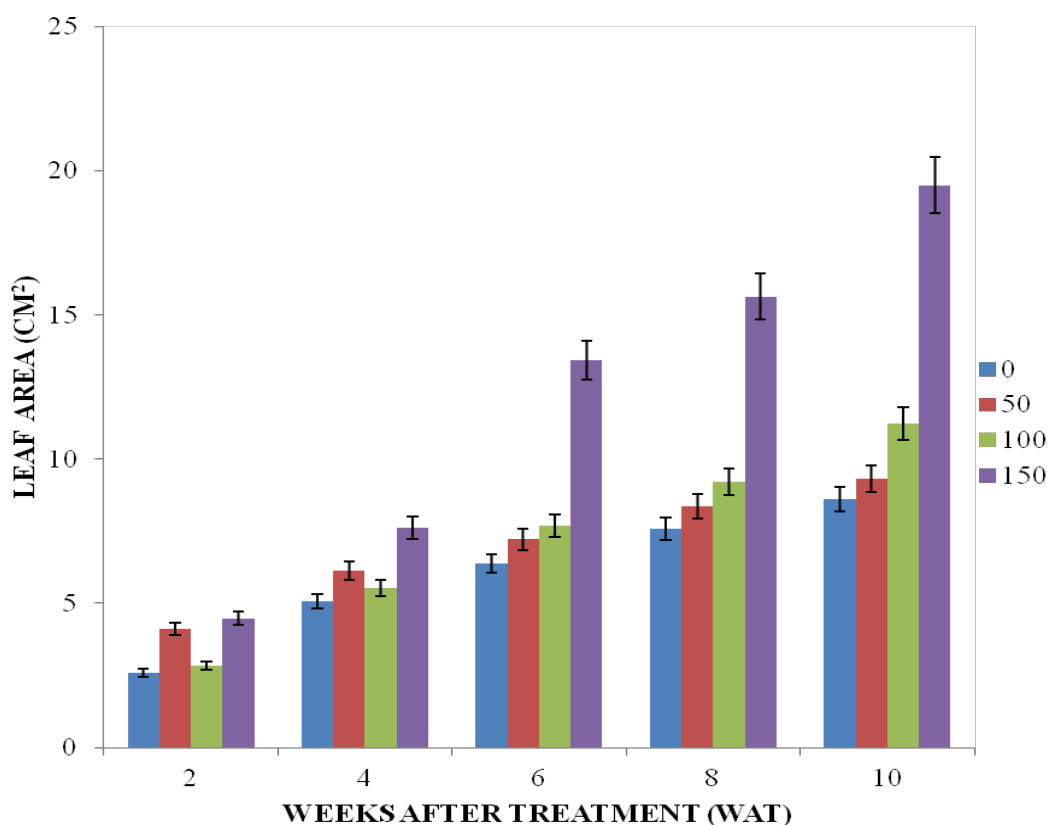
**Figure 2.** Effect of brewery effluent (BET) on the number of leaves of *A. hybridus*.

Effect of BET on the leaf area of *A. hybridus* is presented in Figure 3. There were significant rapid increases in the leaf area of *A. hybridus* under the 150 mL BET at 6th, 8th and 10th WAT.

Fresh and dry weight of *A. hybridus* under different concentrations of brewery effluent is shown in Table 1. The dry weight of *A. hybridus* was observed to decrease gradually as the effluent treatment increases.

Heavy metal accumulation of *A. hybridus* root ranged between 0.07 and

0.68 across the brewery effluent (Table 2). The highest heavy metal accumulation of 0.68 was recorded in iron at 150ml BET while the least was observed in copper (0.07) under the control treatment. *A. hybridus* shoot metal accumulation showed that the chromium accumulation ranged between 0.20 and 0.48 across the BET. Also the highest metal accumulation was observed in iron (0.55). However, the least was noticeable in copper (0.05) under the control (Table 3).



**Figure 3.** Effect of brewery effluent (BET) on the leaf area of *Amaranthus hybridus*.

**Table 1.** Effect of brewery effluent (BET) on the fresh weight and dry weight of *A. hybridus*

| Treatment | <i>Amaranthus hybridus</i> |                |
|-----------|----------------------------|----------------|
|           | Fresh weight (g)           | Dry weight (g) |
| 00        | 25.46                      | 3.59           |
| 50        | 28.10                      | 3.37           |
| 100       | 17.89                      | 2.39           |
| 150       | 15.18                      | 2.13           |

**Table 2.** Effect of brewery effluent (BET) on the heavy metal accumulation of *A. hybridus* root.

| Metal    | Treatment (mL)         |                         |                         |                        |
|----------|------------------------|-------------------------|-------------------------|------------------------|
|          | 0                      | 50                      | 100                     | 150                    |
| Iron     | 0.11±0.01 <sup>a</sup> | 0.40±0.03 <sup>ab</sup> | 0.48±0.08 <sup>ab</sup> | 0.68±0.19 <sup>b</sup> |
| Chromium | 0.10±0.03 <sup>a</sup> | 0.35±0.01 <sup>b</sup>  | 0.42±0.10 <sup>b</sup>  | 0.50±0.06 <sup>b</sup> |
| Copper   | 0.07±0.03 <sup>a</sup> | 0.15±0.06 <sup>b</sup>  | 0.17±0.10 <sup>b</sup>  | 0.21±0.02 <sup>b</sup> |

**Table 3.** Effect of brewery effluent (BET) on the heavy metal accumulation of *A. hybridus* shoot.

| Metal    | Treatment (mL)         |                        |                        |                        |
|----------|------------------------|------------------------|------------------------|------------------------|
|          | 0                      | 50                     | 100                    | 150                    |
| Iron     | 0.09±0.02 <sup>a</sup> | 0.52±0.08 <sup>b</sup> | 0.55±0.05 <sup>b</sup> | 0.55±0.08 <sup>b</sup> |
| Chromium | 0.20±0.04 <sup>a</sup> | 0.21±0.10 <sup>a</sup> | 0.35±0.01 <sup>a</sup> | 0.48±0.02 <sup>b</sup> |
| Copper   | 0.05±0.02 <sup>a</sup> | 0.41±0.09 <sup>b</sup> | 0.44±0.05 <sup>b</sup> | 0.40±0.02 <sup>b</sup> |

Chlorophyll content of *A. hybridus* under the BET is presented in Table 4. Chlorophylls a and b are present in the *Amaranth*. The highest chlorophyll

content of 8.85 was present in chlorophyll b at 100 mL treatment while chlorophyll a ranged between 3.43 and 4.85 with respect to the treatments.

**Table 4.** Effect of brewery effluent (BET) on the chlorophyll content of *A. hybridus*.

| Plant              | Treatment | Chl a (µg/mL) | Chl b (µg/mL) | Total Chl (µg/mL) |
|--------------------|-----------|---------------|---------------|-------------------|
| <i>A. hybridus</i> | 0         | 3.43          | 6.24          | 9.52              |
|                    | 50        | 3.88          | 6.42          | 10.03             |
|                    | 100       | 4.54          | 8.25          | 12.03             |
|                    | 150       | 4.85          | 8.85          | 13.99             |

Bioaccumulation factor (BF) of the heavy metals in *A. hybridus* under the BET is presented in Table 5. The BF of the heavy metals indicated that Fe ranged between 0.81 and 1.38 while Cu (1.45 and 1.59) and Cr (1.06 and 3.07). Cr accumulated more (1.45) at 150 mL BET the BF was less than 1 in Fe at 50 and 100 mL BET, respectively.

Translocation factor (TF) of heavy metals accumulated in *Amaranth* under BET is presented in Table 6. The TF of Cu is greater than 1 unlike Cr (0.50) and Fe (0.89) where their TF are less than 1 at 150 mL BET.

**Table 5.** Bioaccumulation factor of *A. hybridus* as influenced by brewery effluent.

| Metal | Treatment | Bioaccumulation factor ( <i>A. hybridus</i> ) |
|-------|-----------|---|
| Fe    | 50        | 0.98  |
|       | 100       | 0.81  |
|       | 150       | 1.38  |
| Cr    | 50        | 3.07  |
|       | 100       | 1.76  |
|       | 150       | 1.06  |
| Cu    | 50        | 1.59  |
|       | 100       | 1.63  |
|       | 150       | 1.45  |

**Table 6.** Translocation factor of *A. hybridus* as influenced by brewery effluent.

| Metal | Treatment | Translocation factor ( <i>A. hybridus</i> ) |
|-------|-----------|---|
| Fe    | 50        | 1.07  |
|       | 100       | 1.43  |
|       | 150       | 0.89  |
| Cr    | 50        | 1.31  |
|       | 100       | 1.01  |
|       | 150       | 0.50  |
| Cu    | 50        | 1.07  |
|       | 100       | 2.21  |
|       | 150       | 1.17  |

## Discussion

Increase in soil pH at the highest effluent treatment level in this research agrees with report of Orhue et al. (2005) using rubber effluent as an amendment in an ultisol. The Nitrogen decreased in this research indicates that the use of N by microorganisms for metabolic activities did not in any way reduce the C/N ratio below that of control. Ghanbari-Bonjar and Lee (2004) using cassava effluent reported an increase in soil nitrate. The reduced available Phosphorus at 50, 100 and 150 mL effluent concentration treatment may be due to higher P uptake resulting from favourable soil pH range. The brewery effluent reduces the leaf area of the plant significantly under the 50 mL treatment. Findings from this research are in conformity with Yilmaz et al. (2012), who reported that leaves from plants growing in polluted area were significantly reduced than leaves from an unpolluted area. The increase in leaf area recorded under 150 mL brewery effluents could possibly be due to some adaptive mechanisms employed by some characters in the plant in order to withstand the effluent concentrations. Morphological and physiological responses are integral components of plants under stressed conditions (Adu-

Gyamfi et al., 2012). The mechanisms of resistance to toxic chemicals have not been fully explored but could be suggestive of the ability of different plants to detoxify heavy metal or exclude them from the roots. Plant biomass (fresh and dry weight) of *Amaranthus hybridus* under brewery effluent decreased significantly (Khan et al., 2008). Heavy metals even at low concentrations may result in phytotoxicity by impairing a range of cellular activities and reducing the uptake of other essential nutrients (Khan et al., 2008). Plants respond to effluent stress and their uptake, translocation and accumulation of metabolites vary (McLaughlin et al., 2000) depending on their genetic make-up and eco-physiology. There is a likelihood that *Amaranthus* has a mechanism to detoxify excess Chromium in the vacuole, thus concentration of Chromium in the tissue were all below the WHO/FAO permissible limit recommended in vegetables (WHO, 2007). Transfer factor (TF) of Cu demonstrated greater affinity for the metal by *Amaranthus hybridus*. Copper, an essential micronutrient is an essential cofactor for several oxidative stress related enzymes (Yilmaz et al., 2012). The bioaccumulation factor represents the ability of *A. hybridus* to extract heavy metals from the soil. BCF values of zero indicate limited movement from the soil to the plant. According to Ismail et al., 2013, for a plant to be considered for phytoremediation. it should have a few of the following traits to make its use feasible: Ability to extract, degrade or stabilize the contaminant, Tolerance to high levels or concentrations of the contaminant, Rapid growth rate and high biomass production, Cosmopolitan growth and ease for harvesting.

The observation of the growth of the plant over a 10 weeks period indicates that *A. hybridus* can tolerate high brewery effluent concentrations as indicated by the high BCF index. Metals that are accumulated by plants and

largely stored in the roots of plants are indicated by translocation factor values less than 1. Values greater than one indicate translocation to the aerial parts of the plant. However, the ability of *A. hybridus* to be considered for phytoextraction has to be encouraged, as the Translocation Factor index indicates that only when the Cu is being translocated to the aerial parts of the plant. Findings by Hu et al., (2013) support Cu being concentrated in the roots and not translocated to the aerial parts of plants by determining the uptake and accumulation of Cu. *Amaranthus hybridus* clearly demonstrates that it can tolerate difficult soil conditions.

In this study, the morphological properties of plants show that brewery effluent application reduces plant height in *A. hybridus* but increased other morphological parameter such as number of leaves and leaf area. In agreement with the result of this research many researchers observed that at high concentration of numerous effluents such as textile, paper, marble, dairy and brewery along with high osmotic pressure, result in decreased germination (McLaughlin et al., 2000). This work contradicts the work of many researchers who documented that under different concentrations of brewery effluent, textile and paper effluents etc. there is improved seedling lengths of various crops (D'Amore et al., 2005). But it has been stated that high amounts of heavy metals in soils hinder plant growth, physiological, metabolic processes and nutrient uptake. (Khan et al., 2008). Lombi and Gerzabek (1998) also documented reduction in a variety of growth parameters of soybean with the application of textile dyeing effluent at various concentrations. Sposito and Page (1984) found growth promoting effect of treated textile waste when applied at low concentration.

There was a reduction in fresh and dry weight with increase in brewery wastewater concentration. The reduction in fresh and dry weight of *A. hybridus* is

in agreement with Kuo et al. (1983) who confirmed in their report of a reduction in total plant biomass in carbide waste polluted habitats. Similarly, John (2011) also reported the effect of carbide waste polluted soil on the biomass of tomato plants, in which the effects was attributed to reduction in cell expansion and photosynthesis which play a major role in reduction of fresh and dry weight. The result of reduction in fresh and dry weight obtained at relatively higher concentration of brewery wastewater can be explained by Lombi and Gerzabek (1998) that, it is possible that the toxicity of the brewery wastewater could have interfered with the plant metabolic activities leading to poor water absorbance and development.

This study revealed that *A. hybridus* is a hyperaccumulator of heavy metals (iron, chromium and copper). Three heavy metals were detected in the brewery waste water iron (Fe), chromium (Cr) and Copper (Co). These heavy metals were detected at relatively high concentration in root and shoot of *A. hybridus* which means that there was a translocation of heavy metal through root uptake which is supported by the work of Hiroaki (2013). These heavy metals were generally lower than suggested maximum tolerable limit (WHO, 1996), this is supported by the work of Basta et al. (2005) who performed an experiment with vegetables irrigated with waste water from different industries in Faisalabad and analyzed the presence of various heavy metals. They found that the concentration of Cadmium, Copper, Chromium, Lead and Zinc was less than the suggested maximum tolerable levels (0.01mg kg<sup>-1</sup>, 10.00 mg kg<sup>-1</sup>, 1.30 mg kg<sup>-1</sup>, 2mg kg<sup>-1</sup> and 5.00 mg kg<sup>-1</sup> respectively as reported by WHO, 1996). It is important to determine the metal contents of vegetables from health, food nutrition perspective and for crop yield technology point of view. Metal accumulation in edible portions of plants varies and depends on both soil



composition and rate of uptake by each plant. For good health and optimum human performance, adequate intake of essential elements and nutrients is crucial (Kapaj, 2006). Toxic effluents released from various industries contain heavy metals in addition to nutrients, which have an effect on plant and soil in a number of ways (Kuo et al., 1983). The excess of metal ions may induce a series of biochemical and physiological changes in plants (John, 2011). Accumulation of toxic heavy metals in plant living cells results in various deficiencies, reduction of cell activities and inhibition of plant growth (Basta et al., 2005). They also result in chlorosis, reduced water and nutrient uptake; affect enzymatic action by exchanging metals ions with metallo-enzymes, damage root tips and the enzymes (Khan et al., 2008).

The total chlorophyll content in each plant increased with increasing brewery effluent constituent. The consistency in chlorophyll content among the treatments indicates that the effluent is effective in promoting germination, growth, and chlorophyll and protein control. This observation collaborated with the views of Orhue et al. (2005) who reported that chlorophyll content of maize plant was positively enhanced by brewery effluent. This result indicates that brewery effluent stimulates the synthesis of chlorophyll and the synthesis is accelerated at low concentration of the effluent. Similar observations have been reported by Alexander (2006). The increase in chlorophyll content may be due to low level of heavy metals in the effluent and the availability of Fe/Mg for the synthesis of Chlorophyll. Keller et al. (2002) reported that excess concentration of some heavy metals such as Co, Cu, and Cr decrease chlorophyll concentration by inhibiting electron transport. While Cu has been reported by Sandmann et al. (1980) to decompose the chloroplast membrane of plant.

## Conclusion

This study concludes that brewery effluent reduce the plant height but increase the leaf area of *Amaranthus hybridus* under high concentrations which possibly suggest an adaptive mechanism developed by the plant under stress. The mechanism of detoxification of plants growing in polluted areas should be explored.

## Conflicts of interest

Authors declare that they have no conflicts of interests.

## References

- Abioye, P. O.; Abdul Aziz, A.; Agamuth, P. An Enhanced biodegradation of used engine oil in soil amended with organic waste. **Water, Air, & Soil Pollution**, v. 209, no. 1, p. 173-17, 2010. <https://doi.org/10.1007/s11270-009-0189-3>
- Ademoroti, C. M. O. **Standard methods for water and effluents analysis**. Ibadan: Foludex Press, 1996. v. 3.
- Adu-Gyamfi, R.; Dzomeku, I. K.; Lardi, J. Evaluation of growth and yield potential of genotypes of Kersting's groundnut (*Macrotyloma geocarpum* Harms) in Northern Ghana. **International Research of Agricultural Science and Soil Science**, v. 2, no. 2, p. 509-515, 2012.
- Alexander, G.V. Assessment of metal pollution in soil. In: Thornton, I. **Applied environmental geochemistry**. London: Academic Press, 2006. p. 355-394.
- Arnon, D. I. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. **Plant Physiology**, v. 24, no. 1, p. 1-15, 1949. <https://doi.org/10.1104/pp.24.1.1>
- Awofolu, O. R. A survey of trace metals in vegetation, soil and lower animal along some selected major roads in Lagos Metropolis. **Environmental Monitoring and Assessment**, v. 105, no. 1/3, p. 431-447, 2005. <https://doi.org/10.1007/s10661-005-4440-0>

- Bach, M. K.; Magee, W. E.; Burris, R. H. Translocation of photosynthetic products to soybean modules and their role in nitrogen fixation. **Plant Physiology**, v. 33, no. 2, p. 118-124, 1958. <https://doi.org/10.1104/pp.33.2.118>
- Basta, N. T.; Ryan, J. A.; Chaney, R. L. Trace element chemistry in residual-treated soil: Key concepts and metal bioavailability. **Journal of Environmental Quality**, v. 34, no. 1, p. 49-63, 2005. <https://doi.org/10.2134/jeq2005.0049dup>
- Bray, R. H.; Kurtz, L. T. Determination of total organic and available forms of phosphorus in soils. **Soil Science**, v. 59, p. 39-45, 1945. <https://doi.org/10.1097/00010694-194501000-00006>
- D'Amore, J. J.; Al-Abed, S. R.; Scheckel, K. G.; Ryan, J. A. Methods for speciation of metals in soils: A review. **Journal of Environmental Quality**, v. 34, no. 5, p. 1707-1745, 2011. <https://doi.org/10.2134/jeq2004.0014>
- Day, F. W.; Wong, A. S. Transfer of nitrogen from three pasture legumes under periodic defoliation in a field environment. **Australian Journal of Experimental Agriculture and Animal Husbandry**, v. 16, no. 83, p. 863-870, 1976.
- Fosu, M.; Kuhne, R. F.; Vlek, P. L. G. Improving maize yield in the Guinea Savannah zone of Ghana with leguminous cover crops and PK fertilization. **Journal of Agronomy**, v. 3, no. 2, p. 115-121, 2004.
- Ghanbari-Bonjar, A.; Lee, H. C. Intercropped wheat (*Triticum aestivum*) and bean (*Vicia faba*) as whole-crop forage: Effect of harvest time on forage yield and quality. **Grass and Forage Science**, v. 1, p. 28-36, 2004. <https://doi.org/10.1046/j.1365-2494.2003.00348.x>
- Hiroaki, R. M. Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. **Journal of Hazardous Substances Research**, v. 2, p. 11-25, 2013.
- Hu, W.; Ling, Q.; Shen, Y.; Gao, X. Use of bentonite to control the release of copper from contaminated soils. **Australian Journal of Soil Research**, v. 45, p. 618-623, 2013.
- Ismail, G.; Zhao, Q.; Kaluarachchi J. J. Risk assessment at hazardous waste contaminated sites with variability of population characteristics. **Environment International**, v. 28, p. 41-53, 2013.
- Kapaj, B. M. E. Beneficial use of effluents, wastes, and biosolids. **Communications in Soil Science and Plant Analysis**, v. 31, p. 1701-1715, 2006.
- Keller, C.; McGrath, S. P.; Dunham, S. J. Trace metal leaching through a soil-grassland system after sewage sludge application. **Journal of Environmental Quality**, v. 31, no. 5, p. 1550-1560, 2002.
- Khan, S.; Cao, Q.; Zheng, Y. M.; Huang, Y. Z.; Zhu, Y. G. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. **Environmental Pollution**, v. 152, no. 3, p. 686-692, 2008. <https://doi.org/10.1016/j.envpol.2007.06.056>
- Kuo, S.; Heilman, P. E.; Baker, A. S. Distribution and forms of copper, zinc, cadmium, iron, and manganese in soils near a copper smelter. **Soil Science**, v. 135, no. 2, p. 101-109, 1983.
- Lombi, E.; Gerzabek, M. H. Determination of mobile heavy metal fraction in soil: Results of a pot experiment with sewage sludge. **Communications in Soil Science and Plant Analysis**, v. 29, no. 16/17, p. 2545-2556, 1998. <https://doi.org/10.1080/00103629809370133>
- Maclean, R. Methods in cell biology. **Advances in Agronomy**, v. 45, p. 1-49, 1965.
- McLaughlin, M. J.; Hamon, R. E.; McLaren, R. G.; Speir, T. W.; Rogers, S. L. A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. **Soil Research**, v. 38, p. 1037-1086, 2000. <https://doi.org/10.1071/SR99128>
- McLaughlin, M. J.; Zarcinas, B. A.; Stevens, D. P.; Cook, N. Soil testing for heavy metals. **Communications in Soil Science and Plant Analysis**, v. 31, no. 11/14, p. 1661-1700, 2000. <https://doi.org/10.1080/00103620009370531>
- Orhue, O. R. Growth of maize and changes in some chemical properties of an ultisol amended with brewery effluents. **African Journal of Biotechnology**, v. 4, p. 118-125, 2005.

Sposito, G.; Page, A. L. Cycling of metal ions in the soil environment. In: Sigel, H. (Ed.). **Metal ions in biological systems**: Circulation of metals in the environment. New York: Marcel Dekker, 1984. p. 287-332.

Yilmaz, Ş.; Atak, A.; Erayman, M. Identification of advantages of maize-legume intercropping over solitary cropping through competition indices in the East Mediterranean Region. **Turkish Journal of Agriculture and Forestry**, v, 32, no. 2, p. 111-119, 2012.



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