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Evaluation of Cytotoxic and Phytotoxic Potentials of "Chi Limited" Industrial Effluent on *Allium Cepa* and *Vigna unguiculata*

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Abstract

Cytotoxicity and phytotoxicity of Chi limited industrial effluent were investigated using *Allium cepa and Vigna unguiculata.* Physicochemical properties, heavy metals and pH of the industrial effluent were determined in accordance with standard method. Onions root growth inhibition test was used to assess the cytotoxicity of the effluent. Onion bulbs were exposed to 0%(control), 4%, 8%, 12% and 16% concentrations of the effluent samples in the dark for 72 hours before measuring the root lengths of the onion bulbs. Also, the seeds of *Vigna unguiculata* were planted in Petri dishes in the laboratory at concentrations of 0%(control), 5%, 10%, 15% and 20% for more than six days before measuring the root length, shoot length and determining the number of germinating seeds in each concentration of the effluent. *A. cepa* root tips exposed to effluent concentrations ranging from 4% to 16% v/v showed a significant reduction in mitotic index (MI) from 39% to 33% compared to control which is 77%, indicating effluent induced cytotoxicity. Statistical analysis using analysis of variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the mean root lengths of *A. cepa* exposed to different concentrations of Chi limited industrial effluent at both day six and twelve. Also, the effects of concentrations and varieties on the shoot lengths of *Vigna unguiculata* are statistically different compared to root lengths which are not significantly different at $P< 0.05$.

Keywords: Cytogenotoxicity; Phytotoxicity; *Allium cepa; Vigna unguiculata*; Mitotic Index

Abbreviations: MI: Mitotic Index HNO3: Nitric Acid; HCl: Hydrochloric Acid; FLP: Formic Latic Propanoic Acid; PAHs: Polycyclic Aromatic Hydrocarbons; IITA: International Institute of Tropical Agriculture.

Introduction

Nigeria as a developing country is exposed to three major problems which are population, poverty and pollution. Pollution has been in existence for a long time, and it became a global problem due to the development of industries and rapid urbanization around the 19th century. It occurs when the environment cannot convert the pollutant into harmless form Odutayo, et al. [1], which has negative effect on both plants and animals. Water pollution caused by industrial effluents has been one of the major issues of global environmental concern [2]. Many of these pollutants enter into the environment and water bodies from industries, agricultural farmlands and domestic wastes [3]. The excessive levels of these pollutants in the environment are causing a lot of damages to human and animal health, plants and trees including tropical rainforest locations.

Industrial effluents are the main source of direct and often continuous input of pollutants/toxicants into aquatic ecosystems which has long-term implications on functionality of the ecosystem [4], with the terrestrial ecosystem not left out. Indiscriminate discharge of untreated wastewater directly or indirectly into water or land may render water resources and the environment unsafe to man and other living organisms [5-8]. Heavy metals are one of the most persistent pollutants in wastewater, as they cannot be degraded, but have the ability to accumulate in successive levels, thus, producing potential human health risks in subsequent additions to the food chain. The accumulation of these heavy metals in polluted water bodies depends on type of industries in the region, way of life and awareness of the impact of pollutant on the environment by indiscriminate disposal of wastes [9].

Because industrialization is considered a key indicator to development, as earlier stated, there are no regulatory measures or disciplinary actions taken against pollution and hazardous wastes released into the environment by these industries.

Chi Limited has diverse interest in food, healthcare, agriculture, engineering, and other industries; it is a fast-moving consumer goods company that provides consumer products in the diary, beverages, and snacks sectors. The production processes end up to generate wastewater which are discharged out of the company into the drainage system. Industrial effluents pollute the environment by releasing various types of contaminants such as; heavy metals, Polycyclic Aromatic Hydrocarbons (PAHs), etc especially the aquatic systems [10]. Several researches Babatunde & Oladele [6,11,12] have showed the possible genotoxic, cytotoxic, phytotoxic and overall growth reduction effects of industrial effluents on biological systems using biological tests which have given positive results.

Cowpea [*Vigna unguiculata (L) Walp*.] is a dicotyledonous crop in the order Fabales, and family, Fabaceae. It is an annual herbaceous legume and a member of the genus, *Vigna* (Peas and Beans). All cultivated cowpeas are found within the universally accepted *V. unguiculata* subspecies *unguiculata* classification, which is then commonly divided into four cultivar groups: Unguiculata, Biflora, Sesquipedalis, and Textilis Ibrahima, 2012. It is one of the most popular grain legumes in Africa as well as in some regions of America and India. Cowpea is native to central Africa. It is widespread throughout the tropics and most tropical areas between 4oN to 30oS and below an altitude of 2000m. Rich in potassium with good amount of calcium, magnesium, phosphorous, Vitamin A and C, thiamine, riboflavin, niacin, Vitamin B6, and panthothenic acid.

The sensitivity of plants to different compounds can be used in toxicity tests to identify toxicants. Plant bioassays are well-established systems used for screening and monitoring of environmental pollutants Izharul, et al. 2016. Phytotoxicity is the impact of various compounds or pollutants on seed germination and subsequent growth, while plant cytotoxicity tests detect a wide range of cellular damage and alteration in mitotic activities.

This research was set up to ascertain what effects untreated wastewater from this company will have on the plants. In this study, *A. cepa* root-tip assay was used to evaluate the cytotoxic effects of untreated effluent obtained from Chi Limited at Ajao Estate in Lagos metropolis in that plant roots are very useful in testing because the root tips are often the first to be exposed to chemicals in the soil and water and most importantly onion root tips are very sensitive to genetical damage by chemicals and *Vigna unguiculata* assay was used to evaluate the phytotoxic potentials of the effluent. The presence of metacentric chromosome in *Allium cepa* cells makes microscopic assessment easier. This study investigated the activities of the wastewater effluent on seed germination, root and growth inhibiting tendencies.

The major objectives of this research were:

a) To determine the cytotoxic potentials of different concentrations of the effluent on the mitotic indices of *Allium cepa*.
b) To evaluate the phytotoxic potentials of different concentrations of the industrial effluent on the root length and shoot length of *Vigna unguiculata*.
c) To evaluate the phytotoxic effect of different concentration of Chi industrial effluent on the growth of *Vigna unguiculata*.

Materials and Methods

a. Collection of Effluent

Industrial effluent sample was collected from the discharge point of Chi Limited located in Ajao Estate area of Lagos State, Nigeria. The treated effluent was collected in a 5-Litre plastic container and stored in a refrigerator pending its usage. The effluent was diluted with distilled water to produce the series of dilutions for investigation.

b. Analysis of Chi Limited Effluent

The Chi Limited effluent used for this research was analysed for physico-chemical parameters according to standard analytical method USEPA, 1996 [13]. 50ml of the effluent sample was transferred into a 250ml conical flask and 20ml of concentrated Nitric acid (HNO3) was added. Using a heater, low heat was applied to the solution for about 15-20 minutes, the temperature was increased to medium heat for 30 minutes and finally the temperature was increased until complete digestion was achieved. The flask was rotated at intervals until the digest was clear (white fumes), heating was continued for few minutes to ascertain complete digestion (i.e a clear solution). The resulting solution was allowed to cool, and then filtered into a 50ml volumetric flask. The beaker was rinsed into the filter paper with little quantity of distilled water and the solution was made up to mark. The filtrate was then transferred into a plastic vial for instrumental analysis. The heavy metals determined in the effluent; Lead (Pb), Zinc (Zn), Copper (Cu), Manganese (Mn), Cadmium (Cd), Magnesium (Mg), Iron (Fe), Nickel (Ni) and Chromium (Cr) were analysed using Atomic Absorption Spectrophotometer (Buck Scientific Model 210 VGP) while flame photometer (FP 902 PG model) was used for the determination of Sodium (Na), Potassium (K) and Phosphorus (P) were also determined by the use of a Spectrophotometer. A reagent blank was prepared and analyzed following the same procedure with distilled water in the stead of sample. Determination of the pH of the effluent was done by pouring 80ml of the effluent into a 100ml glass beaker. Then, the electrode of the pH meter was inserted into the effluent in the beaker and the pH reading was taken.

c. Biological Materials

The biological materials used for this study includes; onions (*Allium cepa*) and cowpea seeds (*Vigna unguiculata*). The common purple onion bulbs of average size were commercially purchased from Oye Local Market, Ekiti, Nigeria. They were sun dried for 2 weeks. Five varieties of cowpea seeds (A1: TVU-16065, A2: TVU-420, A3: TVU-15660, A4: TVU-15038, and A5: TVU-15081) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

d. Cytogenotoxicity Assay

The dried roots present at the base of the onion bulbs were carefully shaved off with a surgical blade to expose the ring of primordial roots prior to the test. The onion bulbs were exposed to graded concentrations of the effluent (4%, 8%, 12%, and 16%) in transparent plastic containers away from direct sunlight, onion roots exposed to distilled water served as the control. Each concentration was done in triplicates. The exposure time was for 72 h under a laboratory temperature of 28 ± 1°C. After the exposure time, the roots of each bulb were randomly selected and root tip was cut with sharp blade between the hours of 9-11 am prior fixation in ethanol: Glacial acetic acid (3:1, v/v) for 24hrs after which was stored in the refrigerator prior microscopy study.

e. *A. Cepa* Phytotoxicity Test

For growth inhibition study, five onion bulbs were exposed to the effluent in different concentrations of 0%, 4%, 8%, 12%, and 16%. Readings were taken 2 times consecutively with an interval of six days, the roots of three onion bulbs with the best growth at each concentration were removed with sharp scissors and their lengths measured (in cm) with a metre rule and the number of roots on each bulb was counted and recorded.

i. Microscopy Study

Two root tips were selected for each slide, they were removed from the fixative with forceps and were hydrolyzed in 18% Hydrochloric acid (HCl) for 8 minutes, rinsed with the fixative. Slides were prepared using squashing and staining techniques and stained with a drop of Formic Latic Propanoic acid (FLP)-orcein for 8-10 minutes, as described by Adegbite & Olorode [14]. Microscopic observation at 100X was done with CELESTRON Microscope (Model HNB-107BN) fitted with AMScope 990 Digital Camera–3.34 megapixels).The slides were examined under the microscope to determine the cytotoxic effect in terms of mitotic index (MI). The mitotic index was calculated as the number of dividing cells per 100 observed cells Fiskesjo [15].

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

f. *Vigna unguiculata* Phytotoxicity Assay

The viability of the seeds was determined by recasting the seeds in water to determine the floaters and the sinkers [1]. The sinkers represent the viable seeds while the floaters represent seeds that might not grow. Surface sterilization of the final sinkers was then performed by immersing the seeds into 10% sodium hypochlorite or commercial bleach (i.e.10ml bleach to 90ml water) for 5 minutes to reduce contamination [1]. For each variety, six cowpea seeds were placed in petri dishes lined with filter paper and cotton wool containing 30ml of the effluent in different concentrations (5%, 10%, 15%, and 20%), while the petri dishes lined with filter paper and cotton wool containing 30 ml of distilled water served as the control. The experimentation of each concentration including the control was carried out in triplicates. The seeds were planted under room temperature for seven (7) days, after which the seed germination (%), shoot length (cm) and root length (cm) were measured. The shoot length measurement was taken from the base to the apical leaf of the plant using a transparent plastic ruler; while the root length was also measured by the same procedure after it was harvested and carefully washed with distilled water [1].

Statistical Analysis

The results obtained from *Vigna unquiculata* test and *Allium cepa* root growth inhibition and mitotic index were presented as means and standard deviation using the one way ANOVA to test for significance between the control and the different concentrations at 95% confidence interval. Statistical significant differences between the control and the different concentrations of the effluent were determined using Turkey post-hoc test at $p<0.05$ degree of freedom.

Results and Discussion

g. Physicochemical Properties of the Effluent

The results of the physicochemical parameters of the effluent samples and ISI standard for tolerance limits of industrial wastewaters discharge into inland surface waters are given in Table 1. The effluent pH was measured to be pH = 6.09 which is slightly acidic, which was within the tolerance limits. Nitrite was found to be absent while nitrate, chloride, sulphate, sodium, potassium, phosphorus, calcium, magnesium, etc; were present in different concentrations. Heavy metals such as nickel, cadmium, copper, zinc, iron, chromium and lead were present but all in trace or negligible amount (less than 0.1), except zinc which has a value of 0.2, and this did not affect root elongation because it is highly negligible. All these heavy metals when in trace amount are needed for plant growth as trace elements. The presence of chloride in the wastewater functions as trace element needed by plants for photosynthesis, cation balance and transport within the plant. Other elements present in the wastewater such as calcium, sodium, magnesium, etc are also needed for plant growth. Magnesium is absorbed by plants to make chlorophyll. Chlorophyll absorbs light used for photosynthesis. Nitrate Ions provides nitrogen for the construction of vital molecules e.g. amino acids. Amino acids are used to make proteins. Proteins are required for growth.

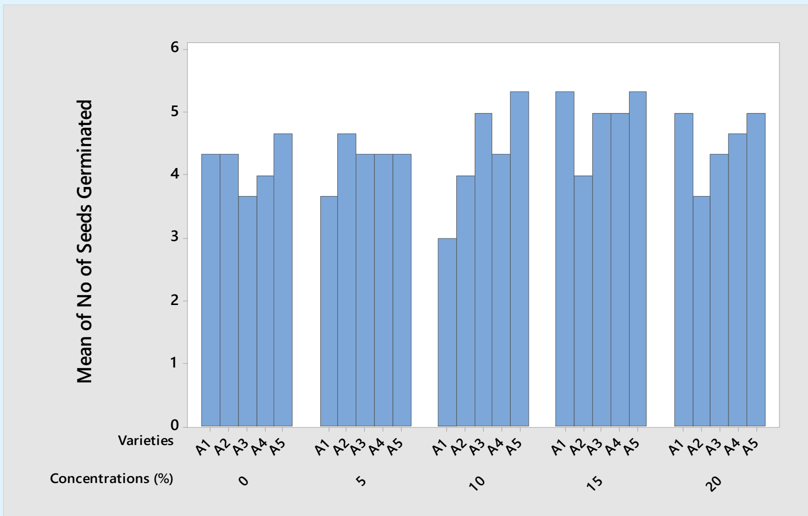
Parameters	Effluent Samples
pH	6.09
Chloride (Cl^-) (mg/L)	12.76
Nitrate (NO_3^-) (mg/L)	1.62
Nitrite (NO_2^-) (mg/L)	ND
Sulphate (SO_4^{2-}) (mg/L)	1969.7
Sodium (Na) (ppm)	116
Potassium (K) (ppm)	13.6
Phosphorus (P) (ppm)	88.2
Calcium (Ca) (ppm)	31.7
Magnesium (Mg) (ppm)	31.7
Nickel (Ni) (ppm)	0.077
Cadmium (Cd) (ppm)	0.059
Zinc (Zn) (ppm)	0.236
Copper (Cu) (ppm)	0.006
Cromium (Cr) (ppm)	0.001
Lead (Pb) (ppm)	0.027

Table1: Physicochemical properties of Chi Limited Industrial Effluent.

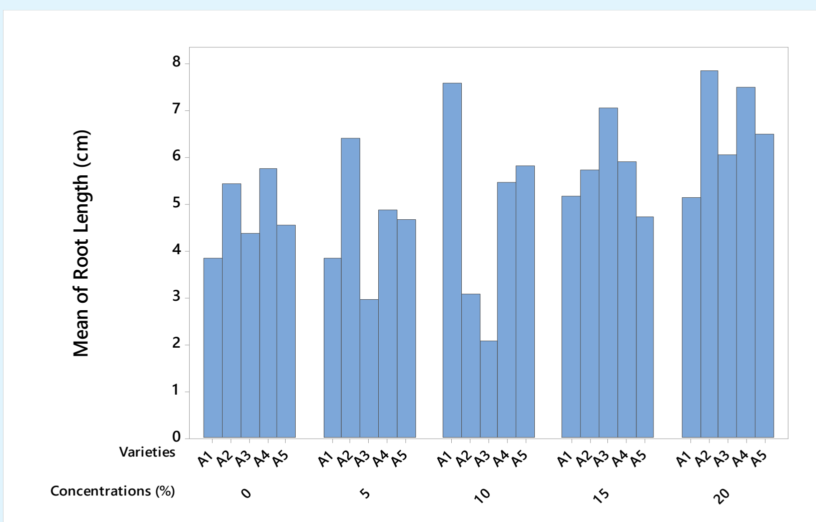
h. Phytotoxicity Evaluation

In the present study, different accessions of *Vigna unguiculata* seeds were germinated in different concentration of Chi-limited effluent and the numbers of seeds germinated in different concentration of the effluent were observed at the sixth day of planting. The result indicates Figure 1 that no seed inhibition was observed for all the accessions at higher concentration, especially accession A1: (TVU-16065) and A5: (TVU-15081) have the highest seed germination (83%) at 15% concentration compared to control (66%). It shows that moderate concentration of Chi limited industrial effluent promote root growth of *Vigna unguiculata,* which is in accordance with the findings of Odutayo, et al. [1], which stated that the different concentrations of the industrial effluent had little or no effect on the germination of Zea mays seeds as most of the concentrations had 100%growth. Also, Izharul, et al. stated that no seed germination inhibition of Vignaradiata was observed up to 50% (v/v) effluent concentration of pulp and paper mill effluents and that only 10 and 30% seed germination inhibition was observed at 75 and 100% (v/v) effluent concentrations respectively, which shows that diluted effluents did not affect *V. radiata* seed germination significantly.

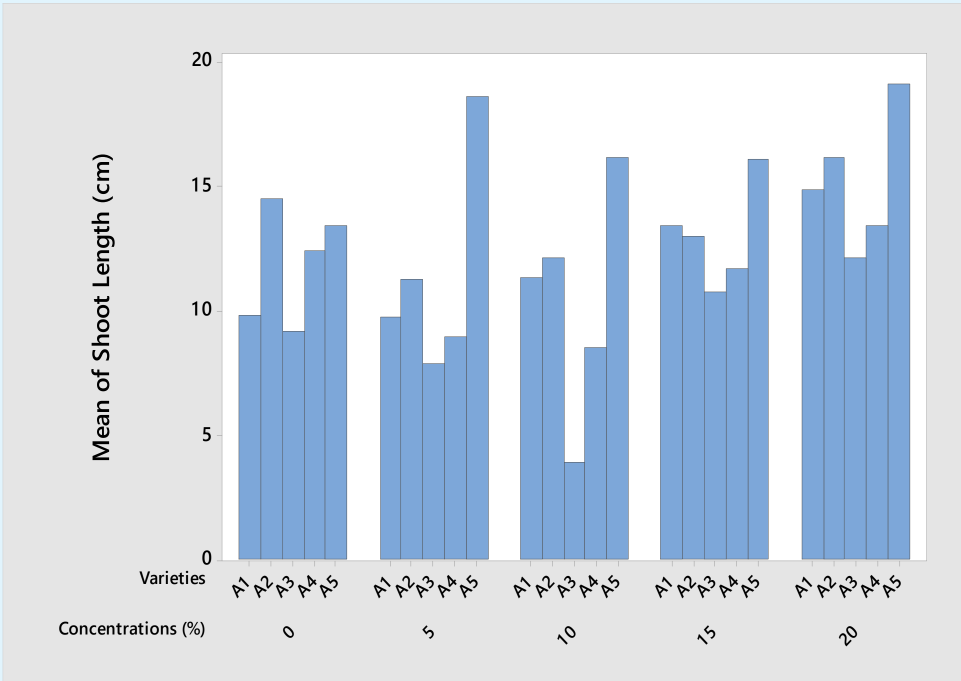
The effect of different concentration of effluent on seedling growth of the five different accession of *Vigna unguiculata* is shown in Figure 2. Root lengths of 6-day-old seedling of accessions A2 and A4 were highest at 20% (v/v) effluent concentration, even more than control. Also, the shoot lengths Figure 3 of 6-day-old seedling of accession A5 were found to be high as the concentration of the effluent increases and highest at 20% (v/v) effluent concentration compared to the control, which was found to be the lowest. This may be due to the presence of nitrates and other elements in the effluent which encourage root and shoot proliferation. Root growth inhibition of *A. cepa* root is as a result of toxicity induced from inhibition of the cell division [15,16]. The effects of different concentrations of Chi limited industrial effluent on the root lengths and root number of *A. cepa* are shown in Figures 4 & 5. Onions grown in Chi limited industrial effluent showed decreased root length when compared with the control, while it showed increased in root number as the concentration of the effluent increases.



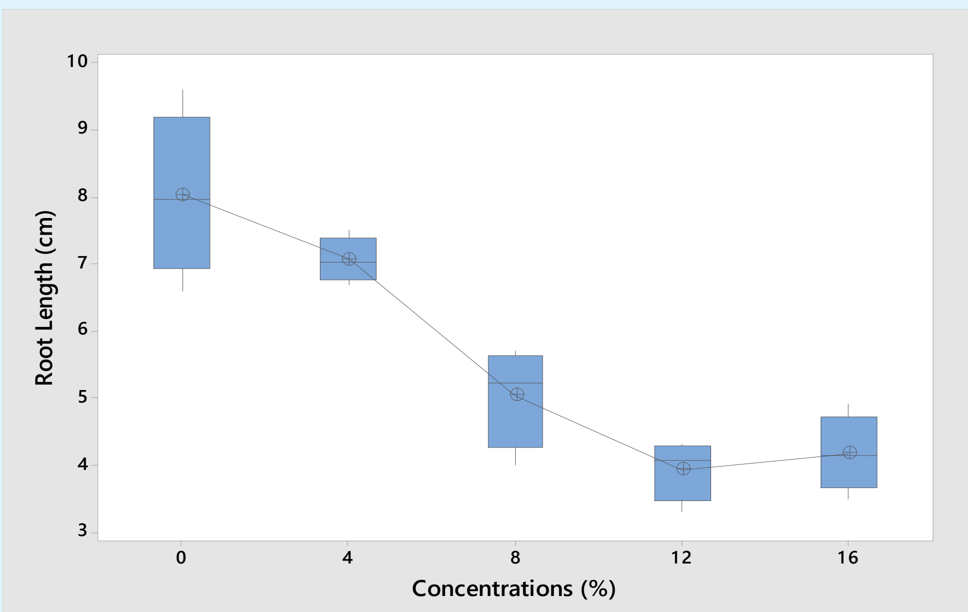
Results are the means of three determinations ±standard error.
Figure 1: Seed germination at day 6.



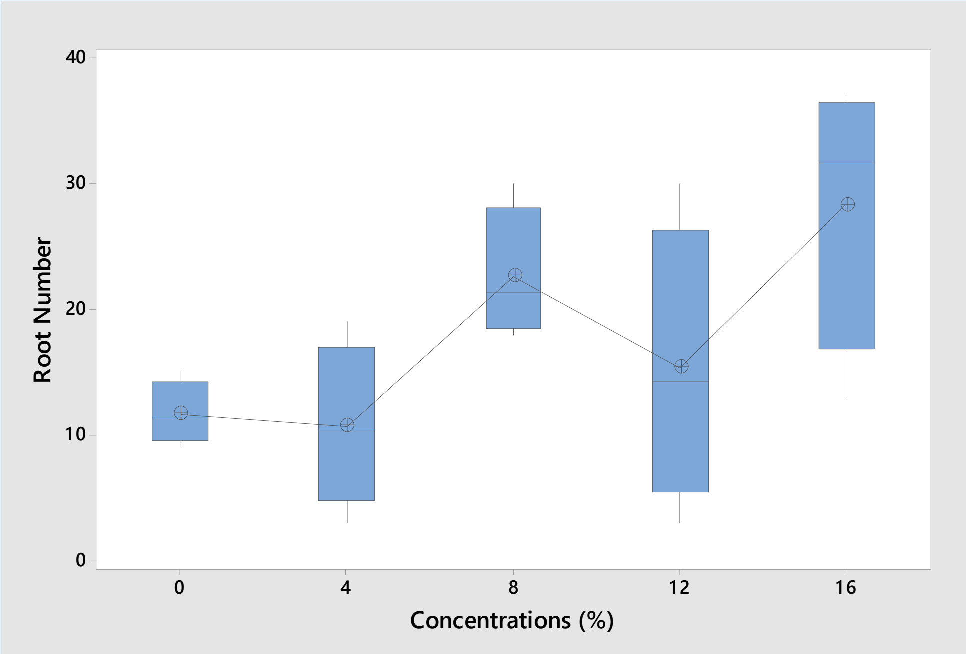
Results are the means of three determinations ±standard error.
Figure 2: Showing mean root length of *Vigna unguiculata*.



Results are the means of three determinations ±standard error.
Figure 3: Showing mean shoot length of *Vigna unguiculata* at day 6.



Results are the means of three determinations ±standard error.
Figure 4: Root length of *A. cepa* after treatment with different concentrations of effluent.



Results are the means of three determinations ± standard error.
Figure 5: Root number of *A. cepa* after treatment with different concentrations of effluent.

i. Mitotic Index

Mitotic index is a good parameter to measure cellular proliferation and to determine the percentage of cells undergoing mitosis. According to Leme & Marin-Morales [17], mitotic index (MI) is a good method in biomonitoring to assess the effect of number of pollutants on cell division. MI measures the proportion of the cells in the mitotic phase of the cell cycle and its inhibition could be interpreted as cellular death [18]. The effect of Chi "limited" industrial effluent on percentage MI in *A. cepa* is shown in Table 2. The percentage of mitotic index was lower than the control at all tested effluent concentrations and it decreased progressively with increasing effluent concentrations. As shown in Figure 6, the mitotic index decreases as the concentration of the effluent increases. The mitotic index ranges from 77, 39, 35, 33 and 33 in 0%, 4%, 8%, 12% and 18% respectively of the effluent. This is in accordance with the findings of Babatunde, et al. [19] which states that mitotic index showing proportion of diving cells decreased with increase concentration of UTH effluent samples. This showed that the UTHeffluent samples were capable of inhibiting mitosis during cell division and this can have far reaching consequences on overall growth and development of the organism.

Concentration (%)	Number of cells counted	Dividing cells	Mitotic Index
0	90	69	77
4	100	39	39
8	121	42	35
12	120	40	33
16	112	37	33

Table 2: Showing MI (%) in root tip cells of *A.cepa* following treatment with different concentrations of Chi Limited Industrial Effluent.

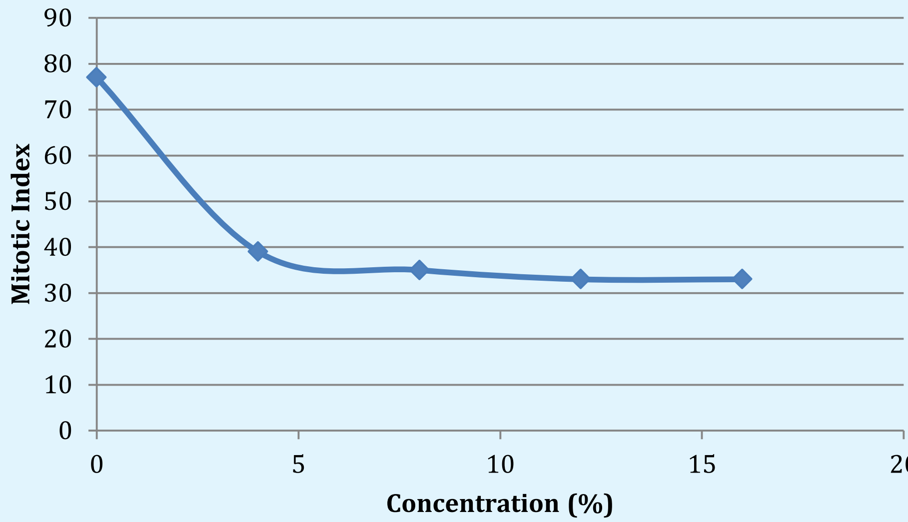


Figure 6: The effect of Chi industrial effluent on percentage MI in *A. cepa.*

Conclusion and Recommendation

From this study, the effluent promotes germination, root and shoot growth of *V. unguiculata* at moderate concentration but inhibits root length and mitotic index of *A. cepa.* This shows that at low concentration, Chi industrial effluent could encourage the growth of some plants while it could also inhibit growth and performance of others at the same concentration [20-22]. Thus, there is need for the adoption of proper treatment and bioremediation strategies to alleviate the pollution hazards caused by this wastewater for the good performance of those plants.

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