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Growth Response of *Abelmoscus esculentus* (L.) Monench Planted in Lead Contaminated Soil.

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ABSTRACT: This study was carried out in the undergraduate project plot of the department of Plant Biology and Biotechnology, University of Benin from March to July 2020, to determine the impact of lead contamination on okra plant. A field experiment was conducted using 4 levels of Pb pollution namely 25, 50, 75, 100 mg/kg and control. Each bag containing 5 kg of air dried sandy loam soil was arranged in a completely randomized design (CRD) with three replicates. Five (05) seeds were sown in each bag. All bags were watered regularly. Plants were thinned down to one per bag after a week of emergence. Data on morphological parameters such as seedling emergence (%), plant height (cm), number of leaves, number of leaves lost, leaf area (cm^2), stem girth (cm), root length (cm), chlorophyll content, flowering and number of fruits per plant were taken. Chemical analysis of soil was carried out before contamination and after the experiment. Protein content (%) was determined using the micro - Kjeldahl method. The results showed that at ($P<0.05$), there was no significant difference between the control and the treated plants in emergence percentage with control having the 60.00±10.69 percent emergence and the 50mg/kg treatment having the lowest (57.14±8.08) emergence percentage). Similar results were obtained for other morphological parameters. There was however significant adverse effect on chlorophyll and protein content.

Keywords: Contamination, *Abelmoschus esculentus*, Heavy metal, Growth

Introduction

The term heavy metal is used to describe more than a dozen elements that are metals or metalloids and are considered important environmental pollutants e.g. Chromium, Arsenic, Cadmium, Lead, Mercury, Nickel, Zinc, Manganese, Thallium, Silver, Beryllium, Copper, Stibium, and Selenium (Adriano *et al*., 2005; Ali and Khan, 2017). They are defined as any metallic elements that have relatively high density and high atomic weight greater than $4g/cm^3$ or 5 times or more, greater than water and is toxic at low concentration. Heavy metals are natural components of the Earth's crust which cannot be degraded or destroyed hence their persistence in all parts of the environment.

The effects of toxic heavy metals have been intensively studied at biochemical and physiological levels such as photosynthesis (Kupper *et al*, 2000; Abdullahi *et al*, 2021), transpiration (Pandey and Sharma, 2002), enzyme activity (Astolfi e*t al*., 2005) and metal accumulation in tissue (Palmieri *et al*., 2005). Prasad (1997), Linger *et al.* (2005) and Morkunas *et al* (2018), reported that metal toxicity reduces plants growth and vigour, causes death in extreme cases, interferes with photosynthesis, water relation, respiration, reproduction and causes changes in certain organelles and the disruption of membrane structure and functions of different plant species.

According to Ashraf and Ali (2007) and Rizvi *et al* (2020) heavy metal exert toxic effects on soil microorganisms which results in the change of diversity, population size and overall activity of the soil microbial communities and consequently affect the growth of the plants and finally cause the plants to die. The effect of heavy metal toxicity on the growth and development of plants differs according to the particular heavy metal for that process. Adverse effects have been recorded at very low concentrations of metals such as Lead

(Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (As) which do not play any beneficial role in plant growth in the growth medium. Kibra (2008) noticed significant reduction in height of rice plants growing on the soil contaminated with 1 mg Hg/kg with reduction in tiller and panicle formation.

The processes of plant uptake of metals differ between metals. For example, Pb uptake is by passive process and that of Cu and Zn is by active or a combination of both (Alloway, 1995; Hogan and Monosson, 2010).

Okra [*Abelmoschus esculentus* (L.) Monench] is an economically important vegetable crop cultivated in many countries such as India, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Myanmar, Pakistan, Thailand, Malaysia, Brazil, Ethiopia, Cyprus, Ghana and the Southern United States (FAOSTAT, 2008). In Nigeria, it is widely grown, distributed and consumed either in fresh or in dried form (Fatokun and Chedda, 1983; Fatin *et al*, 2021). Its fruits are used as vegetable, boiled, sliced or fried. It is mainly grown by many farmers because of its tender texture which is highly mucilaginous and useful in soup thickening. Okra seed is rich in proteins up to 20% and 20% oil (Rao *et al*., 1991; Fatin *et al*, 2021). The okra seed flour could also be used to fortify cereal flour (Adelakun, 2008).

The aim of the study was to investigate the effects of lead on growth and development of *Abelmoschus esculentus* as well as the chlorophyll and protein content of the okra plant. This study will also provide data on lead bioaccumulation in okra which has implications on the safety of its consumption.

Materials and methods

Study area: The experiment was carried out behind the shopping complex building of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, which lies within the tropical rain forest zone, during the period of March to July, 2020.

Source of seeds: Seeds of okra used in this research were purchased from the Oba market in Benin, Benin City Edo state.

Soil: Soil used in the present study was collected from an area measuring 50 m x 50 m marked plot at Capitol in University of Benin, Benin City, Edo state, Nigeria. Top soil (0-10 cm) was collected randomly from the designated plot.

Heavy metal: Lead (Pb) was used in this study. It was obtained from its soluble salt, lead nitrate [$Pb(NO_3)_2$].

Experimental design: The experimental design used for this research was the completely randomized design (CRD). The concentrations were 25 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg and control (0 mg/kg).

Heavy metal preparation: The quantity of the heavy metal corresponding to the various treatments was calculated. Calculation was done by relating the molecular weights of the individual elements and that of the compound.

Pollution of soil by heavy metal: The soil-filled planting bags were polluted with Pb of varying concentrations (25, 50, 75 and 100 mg/kg) with the control left untreated.

Methodology: *Abelmoscus esculentus* seeds were used for this experiment. There were a total of 35 planting bags containing mixture of sandy/ clay/ loam soil in nature with a pH of 6.23; each bag contained 5kg of air dried soil. Five (05) *A. esculentus* seeds were sown in each bag containing untreated soil (Control) and soil mixed with various levels of lead (viz., 25, 50, 75 and 100 mg/kg). All bags were watered daily. Each treatment including the control was replicated three times. Plants were thinned to a maximum of one per bag after a week of emergence. The entire set up was left in the open for 4 months, without mechanically disturbing the soil. Residual heavy metal concentration was determined in soil and plant at Faculty of Agriculture Laboratory, University of Benin. Chemical analysis on uptake of the heavy metal was conducted with the use of an Atomic Absorption Spectrophotometer.

Field data collection: The seeding emergence was recorded at 4 to the 7^{th} day of sowing and expressed as percentage of total seeds sown. The height of the plant was measured using a tape rule in (cm) from the soil level to the tip of the plant. This measurement was taken weekly for a period of nine (9) weeks. The total number of leaves per plant in the various treatments and the control were determined by visual counting. This was carried out weekly for ten (10) weeks. Leaf loss measured by counting the number of leaves that had dropped off per plant in the various treatments and the control. This was carried out weekly for ten (10) weeks. Leaf Area (cm^2) determined using the graph sheet method. The stem girth was measured by tying a thread round the stem of the plant 5cm above the ground level and the thread then traced on well calibrated tape rule in cm.

Chlorophyll content determination: A portable chlorophyll meter was used to obtain relative chlorophyll concentrations on the plants. This meter can quickly and nondestructively assess the chlorophyll status of plants by simply clamping the meter over foliar tissue, then receiving an indexed chlorophyll content reading in less than 2 seconds. These values were measured as chlorophyll content index (CCI). For the accuracy of measurement, three (3) leaves per plant sample were randomly selected for their chlorophyll content

determination and then average into one value. The chlorophyll content of the plant was determined on the fourth (4) and eight (8) week before the experiment was terminated.

The root length of the plant was measured using a tape rule in (cm). Number of days taken from the day of sowing to the day first flower was recorded as days to flowering. The number of fruits per plant was determined by counting using visual observation. This was done for 4 weeks.

Soil chemical analysis: Soil sample used for the experiment were subjected to chemical and microbial analysis. The chemical and microbial analysis was done before and after the experiment, in accordance to the methods of Ibitoye (2008). Measurement was performed in triplicate.

Soil sample preparation: Soil samples were dried at temperature 22-25^{o}C, crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried 2 mm samples were stored in polythene bags for subsequent analysis. The 2 mm fraction was used for the determination of selected soil metal analyses.

Total Nitrogen determination (using Kjeldahl method)

Digestion: 1 g of air-dried soil of fine tilt was weighed into a 250 ml Kjeldahl flask. One catalyst tablet (comprising of $CuSO_4$, K_2SO_4 and a pinch of selenium) was added. The mixture was heated till it became clear (light green colour). This was allowed to cool after it had been removed from heat. About 10 ml of water was added and the content was filtered using Whatman 45 filter paper into 100 ml volumetric flask, it was then filled up to mark and shaken together for proper mixing.

Distillation: 10ml aliquot was transferred into 500 ml Kjeldahl flask. 30 ml of water was added. 15 ml of NaOH (excess base) was added and the mixture was heated. 25 ml distillate was collected in 5 ml boric acid indicator; the NH_4-Nitrogen was determined by titrating distillate with 0.01 M standard HCl. Colour changed from green to pink and % N was calculated as follows:

$$T \times M \times \frac{14}{100} \times \frac{v1}{v2} \times \frac{100}{w}$$

where: T = Titre value of sample
M = Concentration of HCl used for titration in molarity
1000 = constant
V1 = Final volume
V2 = Volume of aliquot used for distillation
W = Weight of sample used

Determination of available phosphorus (Bray-1 Method): 5 g of soil sample was weighed into extractions cup. 30 ml of Bray-1 solution was added and stirred in a mechanical shaker for 5 mins. It was then filtered into a reagent bottle. 1 ml of extract (aliquot) was pipetted into a 50 ml volumetric flask, 6 ml of distilling water was added. Two (2) ml of colour developing reagent was added and properly mixed. 1 ml of ascorbic acid solution was added. It was left for about 10 minutes for the colour to change and the solution was determined at 650 nm in visible-range spectrophotometer.

Calculation

$$P \text{ (ppm) } (\mu g \text{ P/kg of soil}) = R \times \frac{30}{5} = R \times 6$$

Organic carbon (using the Walkley-Black wet-oxidation method): 1 g of soil was weighed into 250 ml conical flask. 10 ml of potassium dichromate was added. 20 ml of concentrated H_2SO_4 was added vigorously. It was allowed to stay for 30 minutes. 100 ml of water was added. 5 drops of ferrion indicator was added. The mixture was titrated with 0.5 N ferrous sulphate ($FeSO_4.7H_2O$). A blank titre was also taken but without soil sample.

% Organic carbon was calculated as follows:

$$\% \text{ Organic carbon} = \frac{(B-T) \times N \times 0.003F \times 100}{W}$$

where: B = Blank titre value
T = Sample titre value
N = concentration of FeSO4 in normality
F = Correction factor (1.33)

******* % Organic Carbon was converted to % organic matter by multiplying it by 1.724 and answers were reported in g/kg by multiplying % organic carbon or % organic matter by 10.

Exchangeable Bases (K, Ca, Mg, Na)

Ammonium saturation method: 10 g of soil was weighed into a 250 ml soil shaking bottle. 100 ml of 1 N ammoniun acetate was added and was shaken in a mechanical shaker for 1 h. The soil was filtered using a Whatman No. 45 filter paper into a 100 ml volumetric flask and made up to mark with ammoniun acetate solution and stored in a 100 ml plastic reagent bottle. K and Na were read in a "Jennway model" flame photometer and Ca and Mg were read in a *Unicam* series 969 model Atomic Absorption Spectrophotometer (AAS).

pH Determination

Soil pH in H_2O 1:1: 10 g of soil was weighed into 50 ml beaker. 10 ml of distilled water was added. The mixture was stirred for 30 minutes intermittently, and pH was read in a standardized pH meter.

Confirmation with $CaCl_2$: Two drops of 1 M $CaCl_2$ solution was added into the solution and stirred for 10 minutes intermittently and the pH was read using a standardized pH meter.

Exchange acidity (E.A-titration method): 5 g of air-dried soil was weighed into a soil shaking bottle. 50 ml of 1M KCl was added and the mixture shaken for 1 hour in a mechanical shaker. The soil was filtered into a 100 ml volumetric flask and was made up to 100 ml mark with 1M KCl solution. 25 ml of soil extract was measured into a 250 ml conical flask 5 drops of phenolphthalein indicator was added.

Mixture was titrated with 0.05N NaOH to pink endpoint. The exchangeable acidity was calculated as follows:

$$\frac{V \times 0.05 \times 100}{W} \text{ (Mg equivalent/100g)}$$

where: W = weight of sample used
V = Titre value

Particle size determination (hydrometer method): Fifty-one (51) g of air dry soil (<2 mm) was transferred into 250 ml beaker. 50 ml of calgon (sodium hexamatoxphosphate plus sodium carbonate), 100 ml of distilled water was added and stirred vigorously for 1 min using glass stirring rod and allowed to stand for 30 min. The suspension was transferred into a mixer and mixed for 15 min at a medium speed and thereafter, transferred into a sedimentation cylinder and made up to 1 litre mark using distilled water.

Measurements: The suspension was mixed in the cylinder by several vertical movement of the plunger. The cylinder was then placed on a flat surface and then timed. The first reading was taken after 40seconds by sliding the hydrometer slowly into the suspension, and thereafter temperature reading was taken. The second reading was taken 2hours later and then calculated to determine percentage sand, silt and clay.

CEC (cation exchangeable capacity) = Ca + Mg + Na + K + H

Micronutrient determination {available nutrient} using DPTA-TEA extract method): Ten (10) g of soil was weighed into a soil shaking bottle, 50 ml of DPTA-TEA solution was added and suspended, and it was shaken for 2 h in mechanical shaker. The soil was filtered using Whatman No 1filter paper into a 100 ml volumetric flask ready for micro-nutrient determination (Fe, Mn, Zn, Cu, Pb) using an Atomic Absorption Spectrophotometer (AAS).

Determination of microbial population: The microbial population counts were determined by the serial dilution, using 1 × 10 dilution factors. A total of 5 dilutions were done per sample and plating was done from the 4^{th} dilution, and calculated accordingly. The agar (nutrient agar and potato dextrose agar) used were incubated after inoculation. The counting was done with the aid of hand lens dialing. Counting was done for 5 days before it became impossible to count because counting was physically done.

Plant chemical analysis

Plants samples preparation: Freshly harvested plants were brought into the laboratory and washed in running water followed by distilled water to remove soil particles and any air-borne pollutants. The okra plants were divided into shoot (stems and leaves), roots and fruits and properly labeled. Fresh weight of each plant part was determined immediately using electronic weighing balance machine before being sliced to uniform thickness with stainless steel knife for quick drying and easier grinding. The sliced plant samples were arranged on foil paper and oven dried at the temperature of 70 °C for 24 h until a constant weight was attained and weighed once again. The temperature of 70 °C was used to avoid excess burning of the plant samples and to avoid destroying heavy metals found in them. The dried plants parts were ground separately into fine powder using a pestle and mortar until it was able to pass through a 0.425 mm sieve mesh size, and finally packed into airtight cellophane bags to minimize heat build-up and stored in the desiccators until required for analysis.

Determination of crude protein content: The crude protein of the sample was determined using the micro – Kjeldahl method described by AOAC (1990). The principle of this method is based on the transformation of protein and that of the other nitrogen containing organic compounds, other than nitriles and nitrates into ammonium sulphate by acid digestion.

$$\text{Sample nitrogen} + H_2SO_4\,(aq) \rightarrow \text{Catalyst } (NH_4)_2SO_4\,(aq)$$
$$(NH_4)_2\,SO_4\,(aq) + 2NaOH\,(aq) \rightarrow 2NH_3\,(aq) + 2H_2O + Na_2SO_4\,(aq)$$
$$NH_3\,(aq) + H_3BO_3\,(aq) \rightarrow NH^{+4}\,(aq) + H_2BO^{-3}\,(aq)$$
$$H^+\,(aq) + H_2BO^{-3}(aq) \rightarrow H_3BO_3\,(aq)$$

The sample (0.5g) was weighed into a micro – Kjeldahl digestion flask of Foss automatic digester block system, it was shaken and allowed to stand for some time. One tablet of selenium catalyst with a mixture of 2:1 copper sulphate and potassium sulphate was added followed by the addition of 20 cm^3 concentrated sulphuric acid. The flask was heated on the digestion block at 450 °C for 1 h until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 100 cm^3 volumetric flask and diluted to the mark with water.

An aliquot of the digest (10 cm^3) was transferred into another micro-Kjeldahl flask along with 20 cm^3 of distilled water, and placed in the distilling outlet of the micro–Kjeldahl distillation unit. A conical flask containing 20cm^3 of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (20 cm^3, 40 %) was added to the content in the Kjeldahl flask by opening the funnel stopcock. The distillation start and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in 5cm^3 of boric acid mixed indicator, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.1N of hydrochloric acid; the end point was obtained when the colour of the distillate changed from green to pink. A reagent blank was also digested, distilled and titrated.

Crude protein is a measure of nitrogen in the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.25. This is based on the assumption that, proteins contain about 16 %N which includes both true protein and non – protein N and does not make a distinction between available or unavailable protein (Udo and Ogunwele, 1986). The crude protein was calculated using

% crude protein = %N x 6.25

The nitrogen content of the sample is given by the formula below.

$$N_2\ (\%) = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \times T.B$$

where: TV = Titre value of acid (cm^3)
Na = Concentration or normality of acid (0.1N HCl)
V1 = Volume of distilled water used for distilling the digest (100cm^3).
V2 = Volume of aliquot used for distillation (10cm^3)
G = Original weight of sample used, g
0.014 = milliequivalent of Nitrogen
100 = percentage

Statistical analysis: Statistical package for social sciences (SPSS), version 17.0 was used for all statistical analysis. To explain the effect of heavy metal lead (Pb) on the growth parameters of *A. esculentus,* One Way analysis of variance (ANOVA) was performed, lead being used as the factor. This was then followed by Duncan's Multiple Range Tests. Statistical significance was set at $P< 0.05$. Descriptive statistics were also used to explain the action.

Results

Table 1 shows the effect of lead on percentage (%) seedling emergence of *A. esculentus*. From the data at day 4 and day 5 of planting, lead appeared to have a stimulating effect on the seedling emergence percentage compared to the control. From day 6 to day 7, the lowest seedling emergence was observed in 50 mg/kg with values of 48.57 ± 11.43 % and 57.14 ± 8.08 % respectively. The highest percentage emergence of 68.57±19.52 % was recorded at day 7 in the 75 mg/kg treated plants compared to control with 60.00±10.69 %. There was no significant difference at 5% level of probability between control and the treated plants in the present study.

Table 1: Effect of lead on seedling emergence (%) of *A. esculentus.*

Concentration (mg/kg)	**Time (days)**			
	4	**5**	**6**	**7**
0	11.43±5.95[a]	11.43±5.95[a]	54.29±12.12[a]	60.00±10.69[a]
25	17.14±8.08[a]	20.00±8.73[a]	60.00±9.76[a]	60.00±9.76[a]
50	22.86±8.08[a]	22.86±8.08[a]	48.57±11.43[a]	57.14±8.08[a]
75	14.29±5.71[a]	28.57±7.38[a]	65.71±7.19[a]	68.57±7.38[a]
100	20.00±6.17[a]	20.00±6.17[a]	57.14±26.90[a]	62.86±10.17[a]

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$

Table 2 shows the effect of lead on the height of *A. esculentus*. Data for the control (0 mg/kg) and the various treatments (25, 50, 75 and 100 mg/kg) did not follow a particular pattern consistent with the concentration gradient. The 100mg/kg treated plants had the highest mean value of 38.00 ± 5.13 cm followed by 37.67 ± 3.71 cm for 25mg/kg treatment. At this time control mean height was 36.67±5.55 cm. There was no significant difference ($p<0.05$) between the heights of control and the lead treated plants.

Table 2: Effect of lead on plant height (cm) of *A. esculentus*

Concen-tration (mg/kg)	Weeks After Planting						
	3	**4**	**5**	**6**	**7**	**8**	**9**
0	15.47±0.93[a]	16.70±0.70[a]	22.33±1.76[a]	24.50±1.89[a]	26.00±2.00[a]	29.33±2.91[a]	36.67±5.55[a]
25	16.07±0.20[a]	17.90±0.78[a]	22.67±0.67[a]	25.17±0.73[a]	27.70±0.54[a]	30.00±1.53[a]	37.67±3.71[a]
50	14.80±1.06[a]	17.27±0.96[a]	19.67±2.03[a]	21.70±1.82[a]	24.30±2.36[a]	28.33±2.19[a]	36.00±3.21[a]
75	15.47±1.35[a]	17.33±1.01[a]	24.33±2.60[a]	27.17±3.03[a]	19.43±10.11[a]	21.40±10.83[a]	26.00±13.05[a]
100	17.23±0.66[a]	18.77±0.79[a]	22.00±1.53[a]	24.50±2.75[a]	28.33±3.84[a]	32.27±5.40[a]	38.00±5.13[a]

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$.

The effect of lead treatment on number of leaves of *A. esculentus* is shown in Table 3. At the third week after planting, the highest number of leaves was recorded for the 75 mg/kg treatment with a mean value of 4.67±0.33. At the end of the experiment (10 WAP) however, values of 7.00±0.00, 7.00±0.58, 6.67±0.67, 7.33±0.67 and 6.67±0.33 were documented for control and the 25, 50, 75 and 100 mg/kg treatments respectively. There was no significant difference at $p<0.05$ between all the treatment.

Table 3: Effect of lead on number of leaves of *A. esculentus*

Concen-tration (mg/kg)	Weeks After Planting							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0	4.33±0.33[a]	5.33±0.33[ab]	5.67±0.33[ab]	7.00±0.00[a]	7.00±0.00[a]	7.00±0.00[a]	7.00±0.00[a]	7.00±0.00[a]
25	4.33±0.33[a]	5.33±0.33[ab]	5.33±0.33[ab]	6.33±0.33[a]	6.67±0.33[a]	6.67±0.33[a]	6.67±0.33[a]	7.00±0.58[a]
50	4.00±0.00[a]	4.67±0.33[a]	5.00±0.00[a]	6.67±0.67[a]	6.67±0.67[a]	6.67±0.67[a]	6.67±0.67[a]	6.67±0.67[a]
75	4.67±0.33[a]	6.00±0.00[b]	6.00±0.00[b]	7.00±0.58[a]	7.00±0.58[a]	7.00±0.58[a]	7.00±0.58[a]	7.33±0.67[a]
100	4.33±0.33[a]	5.33±0.33[ab]	6.00±0.00[b]	6.33±0.33[a]	6.33±0.33[a]	6.33±0.33[a]	6.67±0.33[a]	6.67±0.33[a]

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$

Table 4 shows the effect of Pb treatment on number of leaves lost by *A. esculentus*. At 5 WAP, control and the 50 mg/kg treatment had all their leaves intact with the 25mg/kg losing the highest amount of leaves. At week 7, the 50 mg/kg treatment still had not lost any leaf though the 25mg/kg and 75mg/kg treatments had the same number of leaves lost (1.00±0.58 and 1.00±0.58 respectively), followed by 100mg/kg treatment with 0.67±0.53. At the 10 WAP, plants in 25mg/kg lost the most number of leaves with mean value of 3.00±1.16. There was no significantly difference at $p< 0.05$ between control and the treated plants throughout the experiment.

Table 4: Effect of lead on number of leaves lost *A. esculentus*

Concentration (mg/kg)	Weeks After Planting				
	6	**7**	**8**	**9**	**10**
0	0.00±0.0[a]	0.33±0.33[a]	0.33±0.33[a]	1.33±0.33 [a]	1.67±0.33[a]
25	1.00±0.5[a]	1.00±0.58[a]	2.00±1.16[a]	2.33±0.88[a]	3.00±1.16[a]
50	0.00±0.0[a]	0.00±0.00[a]	1.00±0.58[a]	1.67±0.88[a]	2.00±1.16[a]
75	0.67±0.3[a]	1.00±0.58[a]	1.00±0.58[a]	1.67±0.33[a]	2.00±1.58[a]
100	0.33±0.33[a]	0.67±0.33[a]	0.67±0.33[a]	1.67±0.33[a]	1.67±0.33[a]

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$

The effect of lead on the leaf area of *A. esculentus* is presented in Table 5. The leaf area values were highest in control plants throughout the entire experiment. The smallest leaf area was recorded in the 100 mg/kg treatment with the mean value of 63.21±5.45, 66.89±4.43 and 79.16±2.81 cm^2 respectively from 5WAP to 7WAP. At 9WAP, the lowest value was recorded in 25mg/kg treatment with mean value of 80.39±27.27 cm^2. There was no significant difference between control and all the treatments at $p< 0.05$, at the end of the experiment.

Table 5: Effect of lead on leaf area (cm^2) of *A. esculentus*

Concentration (mg/kg)	Weeks After Planting				
	5	6	7	8	9
0	112.91±18.72^{b}	120.27±22.15^{b}	129.48±10.75^{b}	101.86±11.71^{a}	131.93±17.28^{a}
25	101.25±14.61^{ab}	108.00±14.15^{ab}	103.70±18.72^{ab}	89.59±18.30^{a}	80.39±27.27^{a}
50	72.41±11.16^{ab}	83.45±13.83^{ab}	95.73±1.06^{ab}	81.00±7.36^{a}	101.87±7.23^{a}
75	68.11±7.44^{a}	74.25±6.23^{ab}	90.82±11.71^{a}	84.07±9.64^{a}	100.02±13.87^{a}
100	63.21±5.45^{a}	66.89±4.43^{a}	79.16±2.81^{a}	76.09±8.66^{a}	90.82±8.66^{a}

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$.

Table 6 shows the effect of lead on the stem girth (cm) of *A. esculentus.* The stem girth increased as the number of weeks increased. The stem girth was higher in control plants throughout the experiment with a mean value of 3.20±0.12 cm at the end of the experiment. The lowest mean value of 2.20±1.10 was found in 75mg/kg treated plants at the same time. There was however no significant difference between control stem girth and the stem girth values recorded for the lead treated plants.

Table 6: Effect of lead on stem girth (cm) [mean ± standard error] of *A. esculentus*

Concentration (mg/kg)	Weeks After Planting		
	6	8	9
0	2.20±0.06^{a}	2.80±0.10^{a}	3.20±0.12^{a}
25	2.17±0.12^{a}	2.50±0.17^{a}	2.80±0.15^{a}
50	2.40±0.26^{a}	2.50±0.26^{a}	2.73±0.29^{a}
75	1.90±0.95^{a}	1.96±0.98^{a}	2.20±1.10^{a}
100	2.37±0.29^{a}	2.53±0.24^{a}	2.73±0.28^{a}

Mean values with similar superscripts within the same row are not significantly different from each other at $p<0.05$.

Table 7 indicates the result on the effect of Lead on chlorophyll contents in the leaves of okra plant, determined during the vegetative and reproductive stage of the plant. At the vegetative stage, the highest chlorophyll content of 11.7±0.96 was observed in the control plant while the lowest was recorded in 50mg/kg treatment with mean value of 7.17±0.52. The chlorophyll contents of the treated plant (25, 50, 75 and 100mg/kg) had lower values compared to control. At the reproductive stage, the concentration of lead in soil at 25 and 50mg/kg had a stimulating effect on chlorophyll content compare to the control. The lowest chlorophyll content of 5.30±0.22 was recorded at 75mg/kg. Higher lead concentration in the soil reduced the chlorophyll content of the plant. However, there was significant difference in all the treatment at $p<0.05$.

Table 7: Effect of lead on chlorophyll content index (CCI) of *A. esculentus*

Concentration (mg/kg)	Chlorophyll content index (CCI)	
	Vegetative stage	Reproductive stage
0	11.77±0.96^{b}	11.38±0.26^{bc}
25	8.87±0.82^{ab}	12.90±0.69^{c}
50	7.17±0.52^{a}	12.40±1.01^{c}
75	7.87±0.24^{a}	5.30±0.22^{a}
100	8.90±2.05^{ab}	9.90±0.64^{b}

Mean with similar superscripts within the same column are not significantly different from each other at $p<0.05$.

Table 8 shows the results for root length. At 5 weeks after planting, the concentration of lead appeared to have a stimulating effect on root length compare to the control. The highest value of 7.50±0.31 cm was recorded in 75 mg/kg of soil. However, there was no significant difference at ($p<0.05$) between 25 and 100 mg/kg of soil, but there was significant difference at ($p<0.05$) between the control and the Pb treated plants. At the end of the experiment (15WAP), lower concentration of Pb (25 mg/kg) was observed to enhance the plant root length with mean value 24.67±7.45 cm compared to control value 20.93±11.57 cm. High concentration of 75 mg/kg was observed to reduce the root length value 8.00±0.23. However, there is no significant different among the treatment at 5% level of probability.

Concentration (mg/kg)	Weeks After Planting	
	5	15
0	4.00±0.17^{ab}	20.93±11.57^{a}
25	4.50±0.46^{b}	24.67±7.45^{a}
50	7.50±0.31^{c}	15.27±5.50^{a}
75	3.40±0.31^{a}	8.00±0.23^{a}
100	4.40±0.12^{b}	13.33±0.88^{a}

Table 9 shows the effect of lead on flowering. The control plant flowered earlier (66.7±6.06) days after planting before 25, 50, 75 and 100mg/kg of Pb in soil. The initiation of first flowering was delayed by lead treatment (25, 50, 75 and 100mg/kg) with number of days 79.00±3.00, 84.67±4.48, 81±27.01 and 75.67±14 respectively. The data collected showed that there was no significant difference at ($P<0.05$) in the number of days to first flower between the treated plants and the control plants.

The number of fruits produce by okra plant in this experiment is presented in Table 9. There is no significant difference at ($p<0.05$) in the number of fruits produced by the plant samples in all the treatments groups. However, the lowest number of fruits produced by okra plant was recorded in the 75mg/kg treatment group with a mean value of 0.67±0.33.

Table 9: Effect of lead on flowering and number of fruits per plant of *A. esculentus*

Concentration (mg/kg)	Days to first flowering	Number of fruits/plant
0	66.67±6.06^{a}	1.33±0.33^{a}
25	79.00±3.00^{a}	1.00±0.00^{a}
50	84.67±4.48^{a}	1.33±0.33^{a}
75	81.00±27.01^{a}	0.67±0.33^{a}
100	75.67±14.81^{a}	1.00±0.00^{a}

Mean values with similar superscripts within the same column are not significantly different from each other at $p<0.05$

The result from Table 10 indicates that heavy metal treatment under different Pb concentrations resulted in decreased protein content of the fruits. Protein contents were significantly lower in metal treated seeds compared with the control with the values 30.92±0.58, 12.83±0.58, 18.67±0.58, 20.42±0.58 and 18.08±0.58 respectively. The reduction was not linear. There was significant difference at 5% level of probability between the metal treated plant and the control.

Table 10: Effect of lead treatment on the Protein content (%) of okra fruit

Treatment (mg/kg)	Protein content (%)
0	30.92±0.58^{d}
25	12.83±0.58^{a}
50	18.67±0.58^{bc}
75	20.42±0.58^{c}
100	18.08±0.58^{b}

Mean with similar superscripts within the same column are not significantly different from each other at $p<0.05$.

Table 11 shows the properties of soil before and after the experiment. Before planting, the soil was acidic and texturally sandy-clay-loam. The soil contained organic carbon, organic matter, nitrogen, potassium, magnesium, calcium, sodium, iron, manganese, zinc as well as lead components and microorganisms (fungi and bacteria). In the initial soil (before amendment of soil with lead) the pH of the soil was 6.23. At the end of the experiment, all the lead treated soils and the control showed an increase in pH where the highest pH of (7.13) was recorded in 100mg/kg soil. However, there is significant difference at ($P< 0.05$) in pH among the various Pb concentrations in soil. Also the soil microbial load (Fungi and bacteria) increased in all the lead treated soil than the control compared to the initial soil microbial load. From the final soil analysis, the concentration of lead in soil decrease the microbial load compared to the control.

From Table 11, after planting, the organic carbon, organic matter, available phosphorus, sodium, iron, manganese, calcium, total nitrogen, potassium, cation exchange capacity, pH, lead, fungi and bacteria components of the soil increased at various residual Pb concentrations compare to control while the soil Zinc, Copper, Magnesium, Manganese, however declined at various levels of Pb concentrations compared to control.

When compared with the initial soil elements, the soil pH, calcium, potassium, Pb, fungi and bacteria increased in both the control and lead treated soil but the soil organic matter, organic carbon, total nitrogen Magnesium,

Available phosphorus, Iron, Manganese, Zinc, Copper and Chromium decreased. However, there was a significant difference at (P< 0.05) among the various residual Pb concentrations and control

Table 11a: Analysis of soil before and after the experiment

Treatment	pH	Sand (%)	Silt (%)	Clay (%)	Org. cab (g/kg)	Org. mat (g/kg)	T.N (g/kg)	Ca (cMol/kg)	Mg (cMol/kg)	K (cMol/kg)
Control Pre-Planting	6.23	888.33	68.33	43.33	18.19	33	1.43	2.25	1.04	0.22
Control Post – Planting	6.60^{ab}	890.00^{c}	49.33^{a}	60.67^{a}	12.35^{b}	21.30^{b}	0.67^{b}	2.24^{b}	0.93^{b}	0.34^{b}
25 mg/kg	6.77^{b}	885.00^{b}	51.00^{b}	64.00^{b}	13.04^{b}	22.47^{b}	0.73^{c}	2.35^{c}	0.90^{b}	0.33^{b}
50 mg/kg	6.43^{a}	884.00^{b}	54.33^{d}	61.67^{a}	9.88^{a}	17.03^{a}	0.52^{a}	2.21^{a}	0.83^{a}	0.26^{a}
75 mg/kg	7.10^{c}	880.67^{a}	51.00^{b}	68.33^{c}	15.15^{c}	26.13^{c}	0.73^{c}	3.64^{e}	1.33^{d}	0.52^{d}
100 mg/kg	7.13^{c}	881.33^{a}	52.67^{c}	67.00^{c}	16.55^{d}	28.53^{d}	0.82^{d}	3.02^{d}	1.23^{c}	0.46^{c}

Mean values with similar superscripts within the same column are not significantly different from each other at p<0.05

Table 11b: Analysis of soil before and after the experiment contd

Treatment	Na (cMol /kg)	Av. P (mg/kg)	CEC (cMol /kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Pb (mg/kg)	Fungi cfu
Control Pre-Planting	0.65	7.39	4.19	296.31	14.85	22.03	34.4	0.14	0.09	2
Control Post – Planting	0.14^{a}	6.34^{a}	3.65^{b}	215.10^{a}	12.77^{c}	18.30^{bc}	29.67^{c}	0.06^{a}	0.10^{a}	8.00^{b}
25mg/kg	0.17^{b}	7.23^{d}	3.75^{c}	220.00^{a}	11.43^{c}	17.23^{ab}	24.67^{b}	0.09^{b}	4.08^{b}	2.67^{a}
50mg/kg	0.12^{a}	6.72^{b}	3.42^{a}	259.37^{ab}	9.02^{b}	15.43^{a}	21.77^{a}	0.11^{c}	11.30^{c}	3.00^{a}
75mg/kg	0.25^{d}	7.02^{c}	5.74^{e}	288.37^{b}	7.08^{a}	19.67^{c}	24.53^{b}	0.13^{cd}	19.23^{d}	3.00^{a}
100mg/kg	0.21^{c}	6.83^{b}	4.89^{d}	303.57^{a}	11.83^{c}	17.20^{ab}	30.40^{c}	0.14^{d}	35.23^{e}	3.33^{a}

Mean values with similar superscripts within the same column are not significantly different from each other at p<0.05

Discussion

Lead is a widely distributed heavy metal which is also the most difficult pollutant to control. It produces structural and biochemical changes in plants (Kosobrukhov *et al.,* 2000; Mahdi *et al* 2021). This study revealed the effect of Pb on the growth, development and yield of okra plant under different levels of lead treatment (25, 50, 75, 100mg/kg and control). From the result, there was no significant difference in all the treatments at 5% level of probability for seedling emergence. Data collected also show that plant height, number of leaves, number of leaves lost, leaf area, stem girth, root length, flowering and number of fruits per plant were not significantly affected by lead. Anoliefo and Umweni (2004), observed that growth of *Launae taraxcifolia* plants in lead treated soils compared favourably with control. Edegbai and Victor (2019) reported that there was no significant difference between control and other treated plants for the growth parameters when Okra was grown in soil contaminated with cadmium and lead.

Height is one of the important indicators to evaluate the growth of okra plants in Pb at different concentration in the soil. The impact of Pb on the height was not linearly related with the dose. This was contrary to the report of Sana Choudhary *et al.,* (2012) and Edegbai & Anoliefo (2016), that higher concentrations of Pb nitrate significantly reduced plant height in fenugreek (*Trigonella foenum-graecum*) plant.

Various researchers while working with various plants and heavy metals have reported adverse effect of heavy metals on growth. Sandalio *et al.* (2001) in pea plant and John *et al*., (2009) in *Brassica juncea* reported reduction of stem girth along the heavy metal pollution gradient. This could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja *et al.,* 1990; Morkunas *et al* 2018). Azmat and Haider (2007) observed a decrease in leaf area, stem and root elongation in *Phaseolus mungo* (black gram) due to high concentration of heavy metal in the soil; Reduction in leaf area due to high cadmium toxicity was also reported by Schutzendubel and Polle (2002) in *Populus canescens* (grey poplar), Zhou and Qiu (2005) in *Sedum alfredii,* Skorzynska-Polit and Baszynski (1997) in runners bean plant due to decrease activities of many enzymes involved in the fixation of CO_2, changes in thylakoid organization, reduce chlorophyll contents and

inhibition of photosynthetic activities and disturbing the interaction of chlorophyll molecules into the stable complex. Orhue and Uzu (2011) also reported reduction in leaf area, number of leaf and stem girth of Fluted pumpkin under Chromium stress. John *et al*., (2000) also observed a reduction in root length of *Brassica juncea* L. (Brown mustard) by Cd and Pb. Reduction in root length has also been observed in different crops by different workers who worked on different heavy metals. Reduction in root length by Nickel has been reported by Peralta *et al*. (2000) in Alfalfa, Ezhilvannan *et al*., (2011) in groundnut and Parida *et al*. (2003) in fenugreek. Reduction in root length of wheat by Cd was also reported by Oncel *et al.* (2000) and Xu *et al*. (2008) in garlic. The reduction in the growth of these plants' roots may be as a result of suppression of the elongation of growth rate of cells, because of an irreversible inhibition exerted by the heavy metal on the proton pump responsible for the root length elongation process (Aidid and Okamote, 1993), affecting the ultra-cellular structure of meristematic cells and inhibition of the size of plant cells and intercellular spaces (Kukier and Chaney, 2004).

In this study, growth measurements were at variance in the response of the okra to the heavy metals. From the results obtained *A. esculentus* was more resilient to heavy metal pollution. The ability of living matter to take up potentially toxic species depends upon a number of factors including the nature of the polluting species and metabolism of the organism. Absorption mechanisms and quantity absorbed are influenced by plant species (and cultivar), growth stage, physiological state of the plant, and the presence of other elements. Once in the plant, a metal can be sequestered in the roots in vacuoles or in association with cell walls and organelles or translocated to above ground parts in xylem as organic or inorganic complexes.

In the natural setting, certain plants have been identified which have the potential to take up heavy metals. At least 45 families have been identified to have hyperaccumulation potentials; some of the families are *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae,* and *Scrophulctriaceae* (Salt *et al.*,1998). Among the best-known hyperaccumulators is *Thlaspi caerulescens* commonly known as alpine pennycress (Kochian, 1996). Without showing injury, it accumulated up to 26,000 mg/kg of Zn; and up to 22 % of soil exchangeable Cd from contaminated site (Brown *et al.*, 1995; Gerard *et al.,* 2000). *Brassica juncea,* commonly called Indian mustard, has been found to have a good ability to transport lead from the roots to the shoots. The phytoextraction coefficient for *Brassica juncea* is 1.7 and it has been found that a lead concentration of 500 mg/1 is not phytotoxic to *Brassica* species (Henry, 2000).

Variability in response to "toxic" levels of metals by different plants is due to a number of defenses. These include exclusion from the root, translocation in nontoxic form, sequestering in nontoxic form in the root or other plant parts, and formation of unusable complexes containing metals that may otherwise be inserted into biomolecules instead of the proper element (e.g., As replacing P) (Peterson, 1983).

Robinson *et al*. (1998) found *T. caerulescens* as hyperaccumulator for Cd and Zn and could remove as high as 60 kg Zn/ha and 8.4 kg Cd/ha. It can accumulate as high as 2600×10^{-6} Zn without showing any injury (Brown *et al*., 1995) and extract up to 22 % of soil exchangeable Cd from the contaminated site. It also showed remarkable Cd tolerance (Sneller *et al*., 2000; Escarré*et al*., 2000; Lombi *et al*., 2000). *T. caerulescens* has higher uptake of Cd due to specific rooting strategy and a high uptake rate resulting from the existence in this population of Cd-specific transport channels or carriers in the root membrane (Schwartz *et al*., 2003).

It becomes clear that different mechanisms of metal accumulation, exclusion and compartmentation exist in various plant species. In *T. caerulescens*, Zn is sequestered preferentially in vacuoles of epidermal cells in a soluble form (Frey *et al*., 2000). In *A. halleri* leaves, Zn was found to be accumulated in the mesophyll cells (Kupper *et al*., 2000; Zhao *et al*., 2000; Sarret *et al*., 2002). Cosio *et al*. (2004) investigated the mechanisms of Zn and Cd accumulation in three different plant species through ion compartmentation by measuring the short term Cd and Zn uptake in mesophyll protoplast of *T. caerulescens* "Ganges" and *A. halleri*. Their study suggests the existence of regulation mechanism on the plasma membrane of leaf mesophyll protoplast.

Chlorosis was associated with reduced leaf chlorophyll contents. In the present study, in the early stage (5WAP), the chlorophyll contents were significantly lowered across the Pb treatment when compared to the control (Table 7). After the reproductive stage however, lower concentration of 25 and 50mg/kg was found to stimulate the chlorophyll contents. Previous authors showed that lead stress resulted in large reduction of chlorophyll, owing to both chloroplast disorganization and reduction in the amount of thylakoid and grana, and direct inhibition of chlorophyll structure owing to the replacement of key nutrients such as Mg, Fe, and Cu by lead (Sengar and Pandey, 1996 and Akinci *et al.,* 2010). Earlier study also observed similar results in which Pb stress decreased the amount of photosynthetic pigments of wheat leaves (Dogan and Colak, 2009). Heavy metal stress such as lead caused reduction in photosynthetic pigments either by reducing their synthesis or enhanced biodegradation (Somashekaraiah *et al.* 1992). Azmat *et al*. (2009) reported that *Phaseolus mungo* and *Lens culinaris* (Lentils) plants undertake adaptive mechanisms aimed to protect photosynthesis against the damaging effects of lead; foliar morphological modifications were induced by exposure to 1.2 mM Pb, which resulted in an increased number of trichomes and stomata, thus allowing these species to maintain photosystem II efficiency and reduced water evaporation from the leaves during stress. There was also delay in days to flowering in the present study. Wani *et al.* (2007) reported delay in flowering by Lead in chickpea.

Lead also affected the biomass production of okra plant. There was a decrease in fruit production as seen in Table 9. The impact of Pb in the number of fruits formed was not linearly related with the dose. Babich *et al*. (1982) reported that plants have their own threshold level to different types and concentration of heavy metal.
Lead treatment shows a diphasic effect on soluble protein concentration. A significant decrease in the fruit protein content was observed with heavy metal treated plants in this study, this is in accordance with the findings of Salgare and Acharekar (1992) who reported that growth performance as well as pigment, carbohydrates and protein content showed a decreasing trend with increase in the level of industrial pollution. Decreased levels of protein content in heavy metal exposed tissues have been reported by many workers. Relatively strong affinities of heavy metal ions for side chain ligands of protein indicate that enzyme and other functional proteins are one of the primary targets of metal toxicity (Smilde, 1981; Balali-Mood *et al* 2021).
There was presence of heavy metal content in the initial soil (Table 11). There is no doubt that heavy metals are present in the soil naturally and are non – degradable (Adeyeye, 2005). The organic carbon, organic matter, available P, Na, Fe, Mn, Zn, Cu, Cr components of the soil declined at various residual Pb concentrations. The decrease of these nutrients was not consistent and may be attributed to plant uptake at various levels of Pb treatment. The soil pH, Ca, K, Mg, Pb, Fungi, Bacteria and Cation Exchange Capacity however increased at various levels of Pb concentrations. There was significant difference between the various residual Pb concentrations and in all the elements.
The soil pH is also significant part of the important external environmental parameters that can affect plant growth as well as control the solubility and availability of plant nutrients in the soil. All treatments showed an increase in soil pH. Generally, most plants are able to grow normally within a certain range of soil pH, although the rate of plant survival will decline when plants are cultivated in extreme acidic and alkaline conditions. From the present results, the pH range for okra growth is in the slightly acidic pH range. Moyin-Jesu (2007) reported that a higher soil pH would probably be more suitable to increase the growth and yields of vegetables especially okra.

Conclusion

Contrary to the findings of many researchers who observed that lead contamination produced toxic impact on plant height, number of leaf per plant, stem girth, root length, leaf area, flowering, number of fruit produced per plant and leaf loss, this study has shown that there was no significant difference between the control and the treated plants. There was however significant effect on chlorophyll and protein content of the plant. Consequently, cultivation of okra on metal polluted soils should be avoided or appropriate control measures should be adopted to maintain the heavy metal content of the soil below the damage threshold level.

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