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Assessing Heavy Metal Pollution of Groundwater Using Root Growth Kinetics of *Allium Cepa* as a Bio-Indicator

Franklin C. Uzodinma[1], Ijeoma I. Nwajuaku[2], Ethelbert O. Mezie[3], Ifeanyi K. Omaliko[4]

[1,2,3,4]Department of Civil Engineering. Nnamdi Azikiwe University, Awka. Anambra State,Nigeria.
Email[1,2,3,4] : fc.Uzodinma@unizik.edu.ng, ii.nwajuaku@unizik.edu.ng, eo.mezie@unizik.edu.ng, ik.omaliko@unizik.edu.ng.

***Corresponding Author Email:** eo.mezie@unizik.edu.ng

Abstract: *Groundwater, one of the major sources of potable water in rural areas may contain heavy metals with potential cytotoxic and genotoxic effects. These rural areas are often characterized by the unavailability of advanced testing methods. This study investigates the use of Allium cepa as an economical short-term alternative in bioassay for the assessment of heavy metals in rural groundwater. Two species of onions; Allium cepa L. and Allium cepa b. were first sprouted for 24 hrs in a control groundwater sample and then subjected to different concentrations of heavy metals (cadmium, iron, chromium and lead) induced in the groundwater sample. Consequently, the mean daily root growths were measured after 24 hrs, 48 hrs, 72 hrs and 96 hrs while the percentage root growth inhibition was calculated with respect to the control. Other physical changes to the roots of both Allium cepa species were noted and recorded. Results obtained indicate that increase in concentration of the heavy metals in the groundwater sample and duration of exposure led to increase in growth inhibition of Allium cepa L roots. The Allium cepa b. specie did not give a similar response; although there was a general root growth inhibition, but no trend was observed in Allium cepa. b. However, the roots of the Allium cepa b. species subjected to different metal concentrations exhibited physical changes that were peculiar to the different metals. Thus, this study recommends the use of both species to assess the presence, and determine the extent of contamination by heavy metals in rural groundwater as a short-term alternative in rural areas where advanced testing techniques are not available or not within reach area.*
Keywords:

KEYWORDS*: *Alluim cepa, Bioassay, Potable water, Pollutants, Water treatment .

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I INTRODUCTION

Over two third of the Earth's surface is occupied by water, while less than a third is covered by land [1]. With continuous increase in the Earth's population, there is a consequent of increasing pressure on the Earth's resources with water at the forefront of human activities including industrialization, agriculture, mining, and channel-alteration amidst others which have led to reduced quality of available water resources. Poorer and mediocre water quality leads to pollution of water which has become a global issue. Consequently, the need for reforms, evaluation and continuous revision of water policies to prevent contamination of water body altered by human activities [2][3].

Rural areas are mostly associated with large populations and with most of their surface water polluted with agricultural runoff, sewage and wastewater, oil pollution and radioactive substances [4]; they depend on groundwater as a cleaner source of potable water. The intake of contaminated ground water directly or indirectly adds to the health challenge of the population.

Heavy metal pollution is one of the serious problems of fresh water bodies which are highly vulnerable to natural and anthropogenic interferences [5]. Due to some properties like high solubility, long half-life period, non-biodegradable nature and tendency of bioaccumulation and bio-magnification, they cause deleterious effects on aquatic flora and fauna as well as directly and indirectly affect human health [6]. Heavy metals like chromium, cadmium, nickel, iron and lead are carcinogenic in humans and epidemiological studies on genotoxic effects of these heavy metals have been reported in various biological systems [7][8]. Toxicity through heavy metals including arsenic, cadmium, lead, mercury, chromium, nickel, manganese and iron, is a widespread problem in developing countries [9]. The consumption of drinking water with these heavy metals leads to diarrhea, kidney damage, nervous problems, bone problems, liver damage, cancer and reproductive disorders [10].

In a continuous bid to prevent and control water pollution, bioassay tests were developed to be utilized as a tool in evaluating possible damaging effects of effluents that are discharged into water bodies [11]. Just as structural health monitoring target physical or mechanical changes in a building [12], bioassays target biological changes in an organism. These biological changes could be observed on the cellular levels [13]. Higher plants are considered good genetic models to monitor the environment since the primary target of toxins is the DNA, which all organisms possess. According to [14], Allium cepa bioassay is commonly used for monitoring surface water and groundwater quality. The Allium cepa bioassay is a cheap and easy test unlike other tests that require prerequisite preparations of samples that are tested. The use of Allium cepa in bioassay enhances testing various toxicity terminals viz. inhibition of root growth, and mitotic alterations [13]. In the Allium test, root growth inhibition and the appearance of stunted roots indicate retardation of growth and genotoxicty, while root wilting indicates toxicity [15]

Although cytotoxic and genotoxic effects on Allium cepa have been reported following exposure to a number of pollutants including heavy metals [2][17][18][19], pesticides [20], complex mixtures such as industrial and municipal wastes [21][22] and emerging contaminants [23]. However, there is little or no consideration for those in rural areas where there is no available advanced testing methods or technical education to obtain, study and interpret the microscopic appearance of normal interphase cells and dividing cells in the Allium cepa root tip meristem which most of these researches consider as a means to evaluate toxicity. Similarly, other species of Allium cepa other than Allium cepa L. have not been considered to determine their suitability as bio-indicators. Consequently, as an economical short-term alternative, this study aims to investigate root the kinetics of two Allium cepa species as a bio-indicator of heavy metals in rural groundwater.

II. MATERIALS AND METHODS

Water samples were obtained from a groundwater source at kwata, and medium sized onion bulbs (*Allium cepa L.* and *Allium cepa b.*) of 15-20 mm diameter were commercially obtained from Eke Awka market, both locations are in Awka, Anambra State, Nigeria. (6^o12’22.92” N, 7^o4’5.36” E). The test materials were kept under dry conditions and mould attacked test onions were discarded. Care was taken to avoid poorly grown onions, to account for a number of bulbs in the population that would be poor growing; seven replicate bulbs were used for each test sample and control. The best five were chosen for examination. This is similar to the process carried out by [24] where the samples were assayed with the use of *Allium* chromosome aberration assay and *Allium* MNC assay without prior treatment with growth inhibitors.

The test specimens were prepared by removing the outer scales and the bottom plate. However, the rings of the root primordial were left intact. The base of the peeled bulbs was put into control water during the continued cleansing procedure to protect the primordial from drying. These series of cleaned medium sized bulbs of onions, (*Allium cepa L.* and *Allium cepa* b.*)* were first sprouted in control water as described by [15] by immersion. When the roots have reached lengths of 1 – 2 cm, test are performed at intervals of 24 hrs going forward.

The base specimens after sprouting in control water were then exposed directly to the different concentrations of the heavy metal contained in transparent containers of 310 ml each. Ground water without any heavy metal induced was used as the control. Initial concentrations of the heavy metals to be tested present in the sample are shown in Table 1.

Table 1: Initial concentration of heavy metals in groundwater sample

Heavy Metal	Concentration (ppm)
Chromium	0.007 ppm
Iron	0.056 ppm
Lead	0.0651 ppm
Cadmium	undetected

Seven samples each for the different metal concentration (0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l) were used for this experiment. The set up for the experiment was carried out at room temperature of 20^{o}C and a relative humidity of 55/75%. Samples were arranged and tagged according to the name and concentration of heavy metals contained as used in the experiment.

A. Root Lengths Measurement

Root lengths from the seven samples for each concentration of the different metals were measured at 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs with a meter rule from the base of the specimen to the root tip and the mean root lengths obtained. The percentage root growth inhibition of each concentration was calculated thus;

$$\frac{[\text{Root length of control (cm)} - \text{Root length at concentration(cm)}]\times 100}{\text{Root length of control(cm)}} \qquad (1)$$

B. Analysis

Percentages, Excel sheets and line graphs were used in this study to represent the root growth inhibition in the two species of onions. Photographs were taken of macroscopic changes on the roots and root tips. Regression analysis was used to show the interactive relationship between the root lengths of the specimens used in this study and the different concentrations of each metal while the significant difference between the concentration of the different metals and root length of the specimens were analyzed using analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

The results of the effect of different heavy metals that comprise cadmium, iron, lead and chromium on the roots length of *Allium cepa L.* and *Allium cepa b.* are show in sections A to D.

A. Effect of Cadmium Metal

The effect of the different concentration of cadmium metal with time on the roots length of *Allium cepa L.* and *Allium cepa b.* are shown in Fig.1 and Fig.2 respectively. It is observed that for the *Allium cepa L.* species, there was inhibition in root length growth as the duration of treatment and concentration of the metal increased. At the highest concentration of 0.9 mg/l used in this study, the highest root growth inhibition, 58.7% with respect to the control specimen was observed for the cadmium induced samples. This result is consistent with [2] and [25] who concluded that root growth inhibition increased with increasing cadmium concentration and duration of treatment.

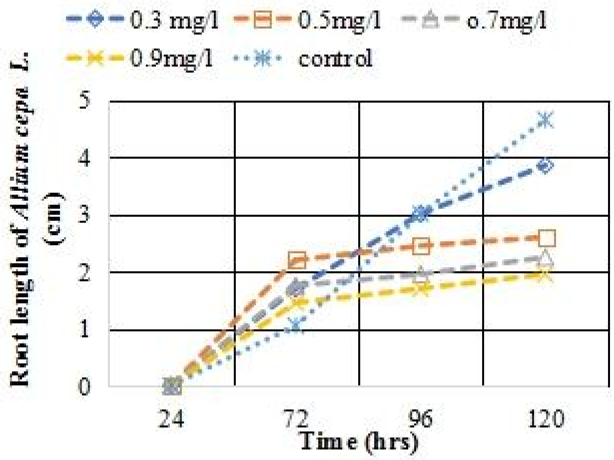

Fig.1: Variation of root length of *Allium cepa L.* with time after treatment with cadmium metal

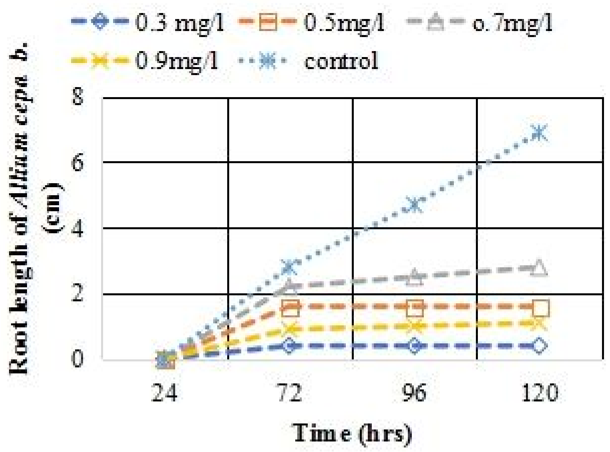

Fig. 2: Variation of root length of *Allium cepa b.* with time after treatment with cadmium metal

However, the same trend was not observed for the *Allium cepa b.* species, from the results obtained, of cadmium metal inhibited the root growth with respect to the control generally but the order in which this inhibition occurred is non – linear and non – sequential. The percentage root growth inhibition at 0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l of cadmium metal is 94.2%, 76.8%, 59.4% and 84.06% respectively. In fact, it is observed that as the concentration of cadmium metal in the water sample increased, the root growth inhibition decreased except at 0.9 mg/l concentration where the inhibition increased abruptly to 84.06%.

In addition, comparing the daily growth rate of both species, *Allium cepa L.* species showed linearity with respect to the concentration of the cadmium metal and duration of exposure while *Allium cepa b.* did not. The mean daily growth rate of both species of onion is presented in Fig.3.

Allium cepa b. *Allium cepa L.*

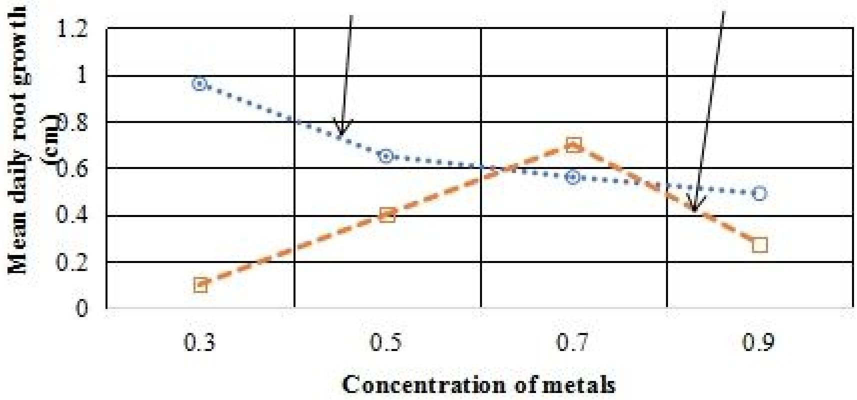

Fig. 3: Mean daily growth rate of roots for both onion species in different concentrations of cadmium metal

During the experiment, other macroscopic effects other than the root growth inhibition were noted; it was observed that roots of all the *Allium cepa b.* species treated with cadmium metal all bent inwards towards the primordial, while those of *Allium cepa L.* remained straight outwards of the primordial (see Plate 1).

Plate 1: Roots of the white onion subjected to cadmium treatment bending inwards towards the primordial

B. Effect of Iron Metal

It was observed that Iron metal generally inhibits root growth in both species of the onions used in this study. The effect of the different concentration of iron metal with time on the root's length of *Allium cepa L.* and *Allium cepa b.* is shown in Figs. 4 and 5 respectively.

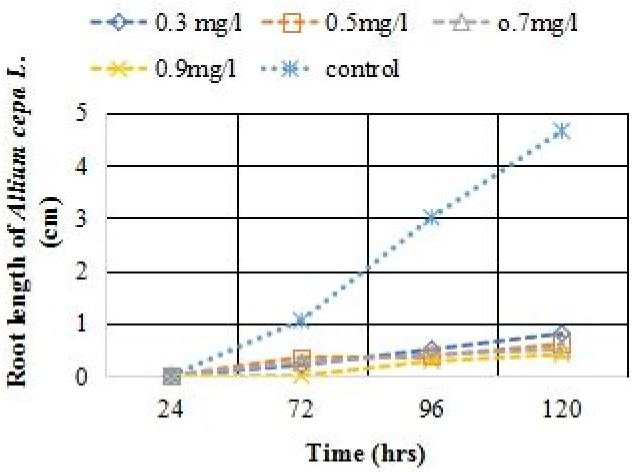

Fig. 4: Variation of root length of *Allium cepa L.* with time after treatment with iron metal

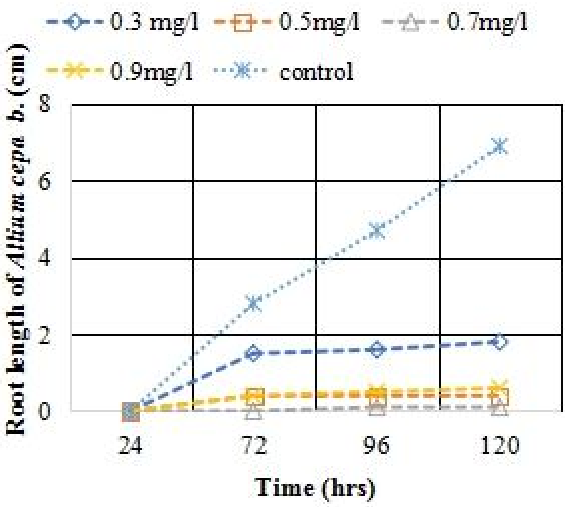

Fig.5: Variation of root length of *Allium cepa b.* with time after treatment with iron metal

Both species of onions treated to iron metal exhibited highest root growth inhibition overall amongst metals considered in this study. Roots of *Allium cepa L.* species were inhibited linearly with increase in iron metal content reaching a root growth inhibition of 91.4% at 0.9 mg/l concentration. Although root growth inhibition was high in the *Allium cepa b.* species, the root inhibition was nonlinear. The percentage inhibition in *Allium cepa L.* at 0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l is 82.80%, 87.10%, 89.25% and 91.40% respectively while the percentage inhibition at 0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l is 73.91%, 94.2%, 98.55% and 91.3% respectively in *Allium cepa b.* species.

Allium cepa L. *Allium cepa b.*

Daily growth rate for both species of onions treated with different concentrations of iron metal is shown in Fig.6. The mean daily growth rate for the *Allium cepa L.* reduces as the concentration and duration of exposure increases. The mean daily growth rate of the *Allium cepa b.* species increased with increase in concentration and duration of exposure up to 0.9 mg/l where an inhibition in the mean daily growth rate was observed.

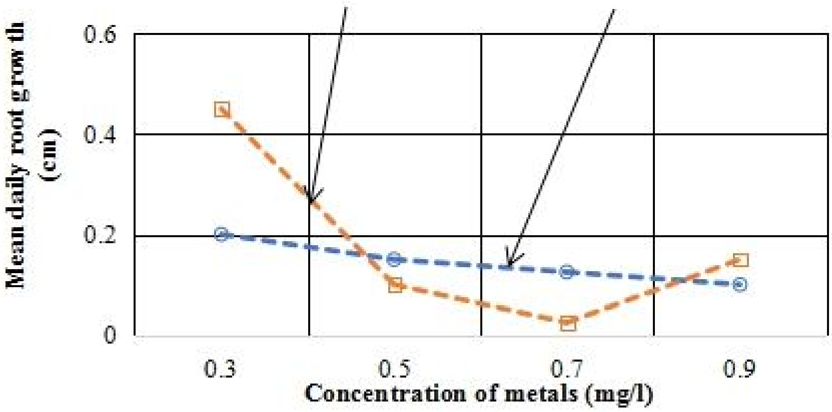

Fig. 6: Mean daily growth rate of roots for both onion species in different concentrations of iron metal

In addition, onion specimens subjected to treatment with iron metal had brown roots especially in the *Allium cepa b.* species (see Plate 2). This may be as a result of insoluble ferric form of iron where small oxidized iron particles become suspended in water and cling to the roots.

Plate 2: Brown roots of specimens subjected to iron concentration

C. Effect of Chromium Metal

The effect of the different concentration of cadmium metal with duration of exposure on the root's length of *Allium cepa L.* and *Allium cepa b.* are shown in Fig. 7 and Fig. 8 respectively. The root growth inhibition of *Allium cepa L.* increased with increase in time of exposure and concentration of chromium metal present in the ground water sample. This is in agreement with Singh [18] who concluded that the roots of *Allium cepa L.* exposed to different concentration of chromium in different time interval showed significant inhibition in root length as concentration increased. However, this was not same for the *Allium cepa b.* species. The percentage growth inhibition at 0.3 mg/l, 0.5 mg/l, 0.7 mg/l and 0.9 mg/l is 73.91%, 43.48%, 62.31% and 62.31% respectively for the *Allium cepa b.* species. It can be observed that although the presence of chromium metal in the water generally inhibits the root growth of the *Allium cepa b.* species, the relationship is nonlinear with no trend.

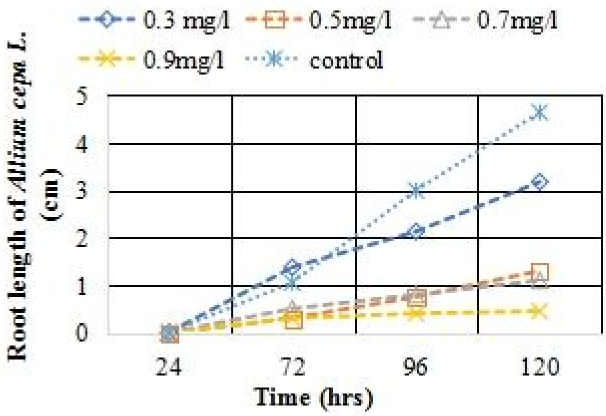

Fig.7: Variation of root length of *Allium cepa L.* with time after treatment with chromium metal

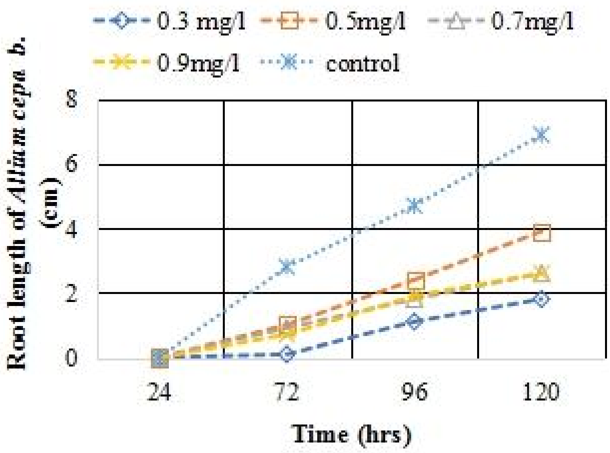

Fig.8: Variation of root length of *Allium cepa b.* with time after treatment with chromium metal

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The roots of *Allium cepa L.* showed linear relationship with respect to mean daily growth rate on exposure to different concentration of chromium metal in the water sample. Increase in concentration and time of exposure led to inhibition in daily growth rate while the roots of *Allium cepa b.* species did not. The mean daily growth rate for both species of onions is shown in Fig.9.

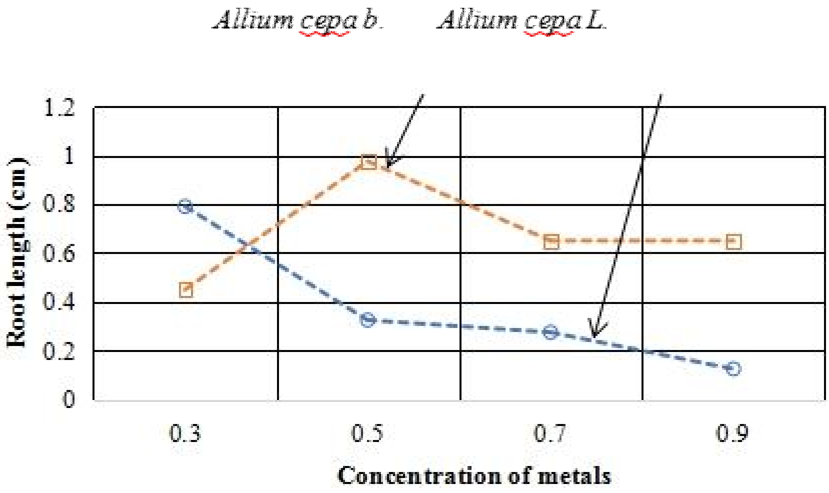

Fig. 9: Mean daily growth rate of roots for both onion species in different concentrations of chromium metal

In addition, it was observed that all roots of both species of onion treated with chromium metal had red ring-like tips, and this remained throughout the duration of the experiment (see Plate 3).

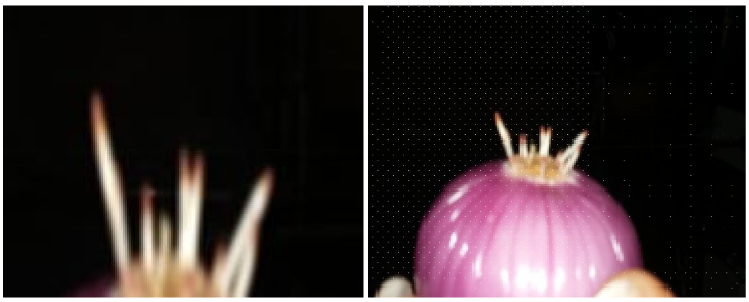

Plate 3: Red ring-like tips of *Allium cepa* roots treated to concentrations of chromium metal

D. Effect of Lead Metal

The introduction of lead metal in the groundwater sample inhibited root growth for both species of onions considered in this study. The effect of the different concentrations of lead metal with time on the root's length of *Allium cepa L.* and *Allium cepa b.* is shown in Figs.10 and 11 respectively. It is observed that for the *Allium cepa L.* species, the root length was inhibited as the duration of time and concentration of the metal increased. This result is consistent with those of Palacio et al [26] who concluded that lead metal reduces root growth of *Allium cepa L.* and causes mitotic irregularities. However, this is not same for the *Allium cepa b.* species which displayed a non-linear relationship.

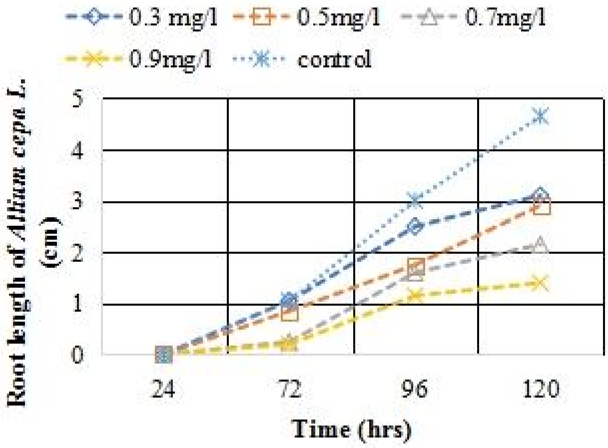

Fig. 10: Variation of root length of *Allium cepa L.* with time after treatment with lead metal

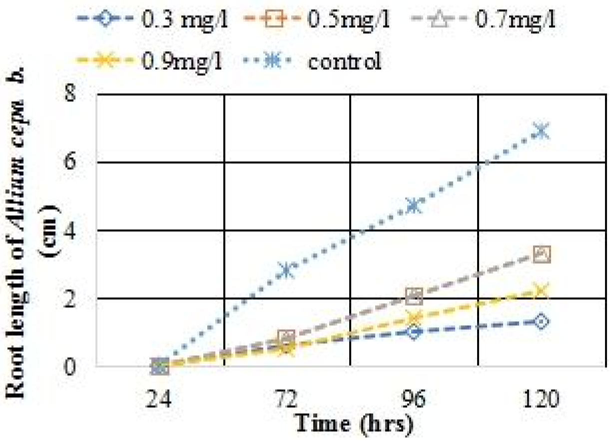

Fig. 11: Variation of root length of *Allium cepa b.* with time after treatment with lead metal

The roots of *Allium cepa L.* showed linearity with respect to mean daily growth rate on exposure to different concentration of lead metal in groundwater, that is, increase in concentration and time of exposure led to inhibition in daily growth rate while the roots of *Allium cepa b.* species did not. The mean daily growth rate for both species of onions is shown in Fig.12.

Allium cepa L. *Allium cepa b.*

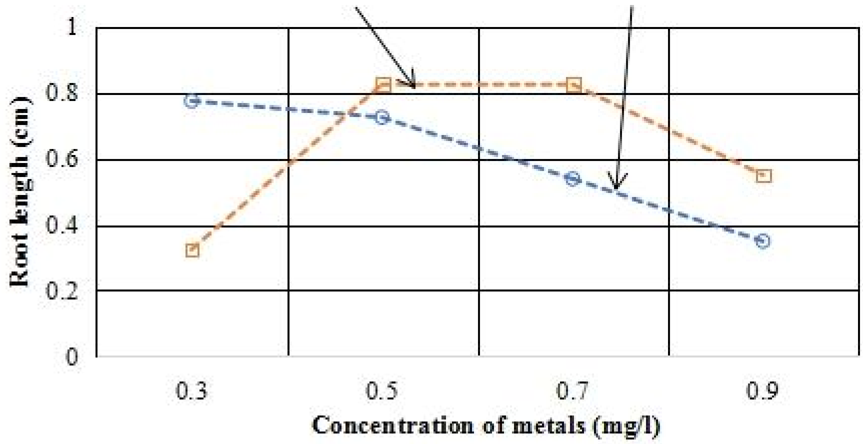

Fig. 12: Mean daily growth rate of roots for both onion species in different concentrations of lead metal

The percentage inhibition at different concentrations of the heavy metals used in this study for *Allium cepa L.* and *Allium cepa b.* species are shown in Figs. 13 and 14 respectively.

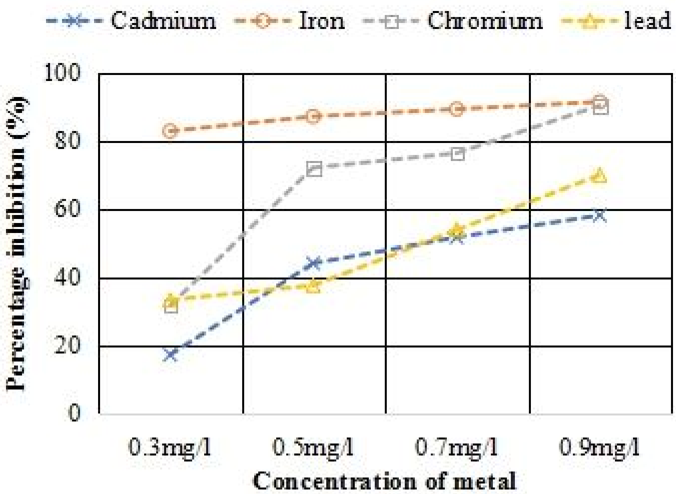

Fig. 13: Percentage growth inhibition on *Allium cepa L.* root growth at different concentrations of heavy metals

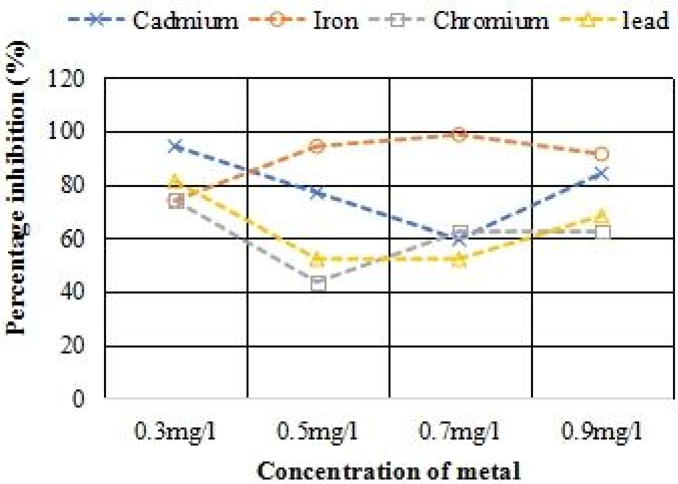

Fig. 14: Percentage growth inhibition on *Allium cepa b.* root growth at different concentration of metals

E. Summary of Statistical Analysis

ANOVA tests were carried out to determine if there exists significant difference within the different concentrations of the heavy metals used in this study with respect to the daily root length of *Allium cepa L.*. Results obtained are shown in Table 2.

Table 2: ANOVA table for the different concentrations of heavy metals and *Allium cepa L.* root length

Metals	DOF	Calculated F- value	P - value	Critical F- value
Cadmium	15	0.976833	0.435891	3.49025
Iron	15	0.993939	0.428729	3.49025
Chromium	15	5.409112	0.013790	3.49025
lead	15	0.664383	0.589763	3.49025

From Table 2 and based on inferences from statistical analysis, there is no significant difference between different concentrations of cadmium, iron, and lead with time on the root lengths of *Allium cepa L.* since the critical F- value is greater than the calculated F – value. However, this is not same for chromium metal where the calculated F- value is greater than the critical F- value. In the later, Scheffes test (QI macros 2017 software) was also applied to determine the source of the significant difference. Results obtained indicated that 0.3 mg/l concentration was the source of the significant difference with F^1 value of 1.339997.
Results of the mean root lengths from the different concentration of heavy metals were also evaluated by Regression analysis. R^2 values (which describes to what percentage the concentration explains the variance in the root lengths) obtained were; 87.4%, 96.5%, 86.4% and 94.9% in *Allium cepa L.* species for cadmium, iron, chromium and lead respectively, and 11.9%, 45.6%, 3.6% and 28.9% in *Allium cepa b.* species for cadmium, iron, chromium, and lead respectively.

F. Implication and Application of Results obtained in Rural Groundwater Quality Control

Results obtained indicate that *Allium cepa L.* species can be considered as an efficient short-term bio-indicator for heavy metals present in groundwater and to an extent indicate severity of contamination. Hence, its use in bioassay assessment for rural groundwater is a cheap, easy to use and effective means to monitor rural ground water quality. This technique provides a short-term alternative when other concise methods are not available. One of its numerous advantages is the fact that the test specimen (*Allium cepa L.*) is readily available and does not need prerequisite preparations. In addition, reading off observations and interpretation of results is quite easy. The use of *Allium cepa L.* in monitoring heavy metals can provide valuable information on the presence and concentration of these metals and thus can be used as a tool to complement chemical analysis.
Allium cepa b. an alternative, cannot be considered a good bio-indicator to be used independently in bioassay. This is because, it does not show profound trend of relationship between concentration of heavy metals, growth rate inhibition and time of exposure as observed when using *Allium cepa L.* despite certain observations such as bending of roots inwards towards the primordial, change in colouration of the roots, stunted growth as compared to the control and appearance of red rings on the root tip which similar to *Allium cepa L.*, indicate the presence of heavy metals.

This study provides an alternative that is cheap, easy to handle, dependable and safe biological technique in the bioassay of heavy metals present in groundwater. It is essential for rural areas where certain technological advancement in the area of groundwater testing is not within reach. This research also presents a cheap prerequisite testing technique to chemical analysis in determining possible heavy metals present in a water sample before full scale analysis is carried out in order to save cost. A combination of bioassay and chemical analysis of heavy metals present in groundwater will provide a powerful approach and a comprehensive assessment of groundwater quality.

IV. CONCLUSION

This study provides additional and valuable information on the use of *Allium cepa* in bioassay for rural ground water assessment. The presence of heavy metals considered in this study in groundwater is deleterious to biological systems. Consequent to results obtained, the following conclusions can be drawn.

Firstly, *Allium cepa L.* species of onion is a good, easy to handle and dependable bio-indicator of heavy metals that may be present in groundwater. *Allium cepa* are plants and properties amongst different obtainable samples may differ. Hence, the use of *Allium cepa L.* in monitoring heavy metals can be used as a tool to complement chemical analysis in order to obtain a comprehensive assessment of ground water quality. To this effect, bioassay using *Allium cepa L.* as an independent technique is not to be used as an ultimate tool in groundwater assessment.

Secondly, heavy metals in groundwater inhibit root growth of *Allium cepa b.* hence; it can be used to indicate the presence of contamination by heavy metals. Although, certain physical observations such as bending of roots inwards towards the primordial, change in colouration of the roots, stunted growth as compared to the control and appearance of red rings on the root tip indicate the presence of certain heavy metals, it cannot however be used independently to determine the extent of contamination. Consequently, it should not be considered a good bio-indicator for heavy metals present in groundwater.

Thirdly, the use of root growth inhibition of *Allium cepa L.* in combination with other observations on the roots of *Allium cepa b.* species can be used to assess the presence, and determine the extent of contamination by heavy metals in rural groundwater as a short term alternative in rural areas where advanced testing techniques are not available or not within reach.
Fourthly, the technique presented in this study is a cheap, easy to handle, easy to interpret, require no prerequisite preparation and readily available method that can be used by households in rural areas.

Conflict of Interest: There is no conflict of interest associated with the work.

References

[1] Water Science School (2019). How much water is on the earth?. Retrieved on 10th May, 2023. URL: https://www.usgs.gov/special-topics/water-science-school/science/how-much-water-there-earth
[2] Bakare A.A., O.A. Alabi, A.M. Gbadebo, O.I. Ogunsuyi, C.G. Alimba. 2017. "In vivo cytogenotoxicity and oxidative stress induced by electronic waste leachate and contaminated well water" Challenges, 4: 169-187.
[3] Anacleto L.R., M.M. Roberto, M.A. Marin-Morales. 2017. "Toxicological effects of the waste of the sugarcane industry, used as agricultural fertilizer, on the test system Allium cepa" Chemosphere. 173: 31-42.
[4] AtlansScientific (2022). Surfacewater Pollution – a complete overview. Retrieved on 11th May, 2023. URL: https://atlas-scientific.com/blog/surface-water-pollution/
[5] Sanders, M.J. 1997. "A field evaluation of the fresh water river crab,Potamonantes warren as a bioaccumulation indicator of metal pollution". Thesis; Rand Afrikaans University, South Africa.
[6] Chopra, A.K., C. Pathak, G. Prasad. 2009. "Scenario of heavy metal contamination in agricultural soil and its management". J. Applied & Natural Sciences,1(1):99-108.
[7] Olorunfemi, D.I. U.M. Ogieseri, A. Akinboro. 2011. "Genotoxicity Screening of industrial effluents using onion bulbs (Allium cepa.)" Journal of Applied Science and Environmental Management, 15 (1): 211 – 216.
[8] Sharma, S., and A.P. Vig. 2012. "Genotoxicity of Atrazine, avanoxan, diuron, quisolofor-p-ethyl-herbicides using Allium cepa root chromosomal aberration assay". Terrestrial and Aquatic Enviromental Toxicology, 6(2):90-95.
[9] Ganesan A. and N. Panneerselvam. 2013. "Analysis of Nickel Induced Genotoxicity in Root Meristem of Allium cepa" Int. J. Biological Technology, Vol. No. 4(2):19-22.
[10] Gode F. and E. Pehlivan. 2006. "Removal of Chromium (III) from Aqueous Solutions using Lewatit S 100: The effect of pH, time, metal concentration and temperature". Journal of Hazardous Materials,136: 330-337.
[11] Garg S. and D. Kansal. 2014. "Use of Bioassay Test as a Performance Indicator in Effluent Treatment Systems including Role in encouraging shift towards use of Green Chemicals" International Journal of Multidisciplinary Research and Development. 1(2).
[12] Uzodinma F.C., P.D. Onodagu, and H.C. Aginam 2022. ''Structural health monitoring in Nigeria: Bridging the gap between literature and practical application''. FUOYE Journal of Engineering and Technology (FUOYEJET), in press.
[13] USEPA 2018. "Bioassays for Evaluating Water Quality Screening for total bioactivity to assess water safety" EPA/600/F-18/048 March.
[14] Leme D.M. M.A. Marin-Morales. 2009. "Allium cepa test in environmental monitoring: a review on its

application”. Mutation Research 682: 71 – 81.

[15] Fiskesjo G. 1985. “The Allium test as a standard in environmental monitoring”. Hereditas 102(1): 99 – 112.

[16] Odeigah P.G., O. Nurudeen and O. Amund. 1997. “Genotoxicity of oil field waste water in Nigeria” Hereditas. 126: 161-167.

[17] Barbosa J.S. T.M. Cabral, D.N. Ferreira, L.F. Agnez-Lima, S.R. Batistuzzo de Medeiros. 2010. “Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals”. Ecotoxicology and Environmental Safety 73: 320 – 325.

[18] Singh P. 2015. “Toxic effect of chromium genotoxicity and cytotoxicity by use of Allium cepa L.” International Journal of Research in Engineering and Applied Sciences, 5: 1-10

[19] Hemachandra C.K.and A. Pathiratne. 2015. “Assessing toxicity of copper, cadmium and chromium levels relevant to discharge limits of industrial effluents into surface waters using common onion, Allium cepa bioassay”. Bulletin of Environmental Contamination and Toxicology 94(2): 199 – 203.

[20] Liman R.., I.H. Cigerci, D. Akyil, Y. Eren, and M. Konuk. 2011. “Determination of genotoxicity of fenaminosulf by Allium and comet tests”. Pesticide Biochemistry and Physiology 99: 61 – 64.

[21] Radic S., D. Stipanicev, V. Vujcic, M.M. Rajcic, S. Sirac, and B. Pevalek-Kozlina. 2010. “The evaluation of surface and wastewater genotoxicity using the Allium cepa test”. Science of the Total Environment 408: 1228 – 1233.

[22] Siddiqui A.H., S. Tabrez, and M. Ahmad. 2011. “Validation of plant-based bioassays for the toxicity testing of Indian waters”. Environment Monitoring and Assessment 179: 241 – 253.

[23] Herrero O., J.M. Martin, P.F. Freire, L.C. Lopez, A. Peropadre, M.J. and Hazen. 2012. “Toxicological evaluation of three contaminants of emerging concern by use of the Allium cepa test”. Mutation Research 743: 20 – 24.

[24] Rank J., and M.H. Nielsen. 1993. “A modified Allium test as a tool in the screening of genotoxicity of complex mixtures”. Hereditas 118: 49 – 53.

[25] Wang, Q. L., L. T. Zhang, J. H. Zou, D. H. Liu and J. H. Yue. 2014. ‘‘Effects of cadmium on root growth, cell division and micronuclei formation in root tip cells of Allium cepa var. agrogarum L.,’’. PYTON 83: 291-298.

[26] Palacio, S.M., F. R. Espinoza-Quiñones, R.M. Galante, D. C. Zenatti, A. A. Seolatto, E. K. Lorenz, C.E. Zacarkim, N. Rossi, M. A. Rizzutto, and, M. H. Tabacniks. 2005. “CorrelationbetweenHeavy Metal Íons (Copper, Zinc, Lead)Concentrationsand Root Length ofAllium Cepa L. In Polluted River” Water. Brazilian Archives of Biology and Technology, 48: 191-196.