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Research Article
Deleterious Effects of Cadmium Solutions on Onion (*Allium cepa*) Growth and the Plant's Potential as Bioindicator of Cd Exposure

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Abstract

Background and Objective: Anthropogenic activities are constant cause of increased chemical contamination with varied possible adverse effects on ecosystem and human health. Bioaccumulation and trophic transfer of pollutants and their cumulative effects on biological systems have been reported. This study investigated the variable action of cadmium on *Allium cepa* L. (*A. cepa*) and its potential in usage as bio-indicator of cadmium toxicity under laboratory conditions. **Materials and Methods:** Twenty four onion plants were exposed to increasing concentrations of cadmium solutions (0.1×10^{-4}, $5\times10\times^{-4}$, 1×10^{-3}, 5×10^{-3} and 1×10^{-2} M) for 14 days. Using the roots and leaves developments as biological endpoint, bio accumulation was evaluated in roots, bulbs and leaves. **Results:** Cadmium caused inhibition of root and leaves elongation at above 1.0×10^{-3} M. The accumulation of Cd in the bulbs occurred at treatment with higher molar concentrations. Total phenolic content in the onion bulbs was decreased by almost 60% by increased concentration of cadmium solutions from 1×10^{-4} to 1×10^{-1} M. **Conclusion:** The root growth of *A. cepa* could serve as a tool for characterising the bio-indication of cadmium exposure in wastes and effluents condition.

Key words: *Allium cepa*, ecotoxicology, effluents, bio-indicator, cadmium toxicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anthropogenic activities are constant cause of increased chemical contamination with varied possible adverse effects on ecosystem and human health. Bio-accumulation and trophic transfer of pollutants and their cumulative effects on biological systems have been reported[1]. Cadmium, a heavy metal, is a member of group IIb in the periodic table of elements present in soils, sediments, air and water. Anthropogenic sources of cadmium to the environment were: Refining and use; copper and nickel smelting and fossil fuel combustion. Natural sources of cadmium to the atmosphere were: Volcanic activity, forest fires and windblown transport of soil particles. It is important to note that the anthropogenic sources of cadmium add 3-10 times more cadmium to the atmosphere than natural sources[2]. Other sources of concern were phosphate fertilizers, which may contain high concentrations of cadmium depending on the origin of the rock and the application of contaminated sewage sludge as a soil amendment[1]. Major occupational exposure occurs in non-ferrous metal smelters, in the production and processing of cadmium, its alloys and compounds and, increasingly, in the recycling of electronic waste. Non-occupational exposure was mainly from cigarette smoke which contains relatively high concentrations of this element; for non-smokers who are not occupationally exposed, diet was the main route of exposure to cadmium[3]. Cadmium was listed by the US Environmental Protection Agency as one of 126 priority pollutants. In most studies, the half-life in humans was estimated to be between 15 and 20 years. It can cause osteoporosis, non-hypertrophic emphysema, irreversible renal tubular injury, anemia, eosinophilia, anosmia and chronic rhinitis. The classical example of the importance of cadmium as an environmental contaminant was the outbreak at the Jinzu River in Japan of a severe disease (Itai-Itai disease) characterized by severe pain, bone fractures, proteinuria and severe osteomalacia-, which appeared mainly among women[4] . It seems that the disease was caused by the ingestion of rice and water contaminated with cadmium originating in a mine slag, in combination with nutritional factors. Cadmium was a potent human carcinogen and occupational exposure to it has been associated with cancers of the lung, the prostate, the pancreas and the kidney[4]. There was a fast growing problem concerning cadmium contamination of cultivated land, resulting into contamination of crops especially vegetables, leading to various health challenges including cancer in particular.

Several investigations successfully used higher plants as sensitive and rapid bio-tools for screening the chemical contamination of the atmosphere, soils, surface and ground waters, landfill leaches and wastewater/sludge[5]. Such monitoring systems have been shown to be ecologically relevant and cost-effective. The common onion (*Allium cepa*) is found across a wide range of latitudes and altitudes in Europe, Asia, America and Africa, reaching a world annual production of several million ton. The use of *A. cepa* as a biological test system was first introduced in 1938 to evaluate the cytogenetic effects of colchicines[6,7] and since then this species has been used as a bio-indicator for different classes of pollutants, such as trace metals, polycyclic aromatic hydrocarbons and halogenated pesticides. Bioaccumulation of chemicals in specific tissues, inhibition of root and leaf elongation, cytogenetic or mutagenic effects and oxidative stress responses have been characterized in various toxicological studies with *A. cepa.* Practical advantages in the use of plants as bio-indicator of pollutants include sensibility, reproducibility and rapidity of results, as well as the need of small volumes of samples and low cost.

The aim of this study was to determine the physiological and morphological responses of *Allium cepa L*. towards increased cadmium toxicity, evaluate its potential to accumulate the metal and its associated environmental consequences.

MATERIALS AND METHODS

Sample collection: Samples of onion bulbs were purchased from onion vendors in Okitipupa Central market, Okitipupa, Ondo State, Nigeria.

Preparation of cadmium solution: The standard solution of Cd was prepared using cadmium chloride (analytical grade). The solution was prepared into1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3} and 1×10^{-2} M using standard procedure and the pH was made slightly acidic using a phosphate buffer.

Sample preparation: The samples were air dried for 7 days. All samples external sheath were then peeled to expose the fresh part of the bulb; the dried root was cut off using a razor blade to expose the fresh part of the root. The onions were placed in a bowl of distilled water to avoid the drying up of the fresh part. The excess water was then wiped off using a tissue paper.

Sample treatment: The samples were placed on the top of an open containers with sliced out to accommodate almost 50% of the bulb and filled with 100 mL of various cadmium solutions (1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3} and 1×10^{-2} M). The calculated Cd concentrations in ppm units were 1.24, 6.21, 12.41, 62.05 and 124.1 ppm, respectively. The onion samples were ensured to be partly (>60%) immersed into the solutions. The range of nominal cadmium concentrations were environmentally realistic and similar to those measured in waste waters, industrial effluents and acid mine drainage. The exposure was carried out for 14 days in the absence of light. After this period, the growth of both roots and leaves were measured in centimetres.

Determination of cadmium accumulation: Roots, bulbs and leaves of *A. cepa* were dried at 60°C until it reached a constant weight. Approximately 0.2 g of dried samples was digested under pressure with 5 mL of aqua regia. Cadmium concentration was determined using atomic absorption spectrophotometer.

Determination of total phenolic content: Exposed samples were dissected using a knife, separating inner and outer parts of the sample. The samples were then blended using a mortar and pestle to 0.5 g of extracted sample, 10 mL 0f 90% methanol was added and stirred on a magnetic stirrer for 20 min. To 0.5 mL of the extracted sample,1.5 mL (1:10 v/v diluted with distilled water). Folin Ciocalteu reagent was added and allowed to stand for 5 min at room temperature. After 5 min, 2.0 mL of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation, development of blue colour was observed. Absorbances of the resulting solution in the different samples were measured at 660 nm using spectrophotometer.

Statistical analysis: Statistical analyses of biological and chemical parameters were performed using one way ANOVA to estimate the degree of variance, 95% of confidence interval and superscript letters were not significantly different at $p<0.05$ by the software package SPSS version 16.0.

RESULTS

Cadmium caused inhibition of roots and leaves elongation particularly with increasing effects at higher exposure doses (Fig. 1a-c). The results of accumulation of

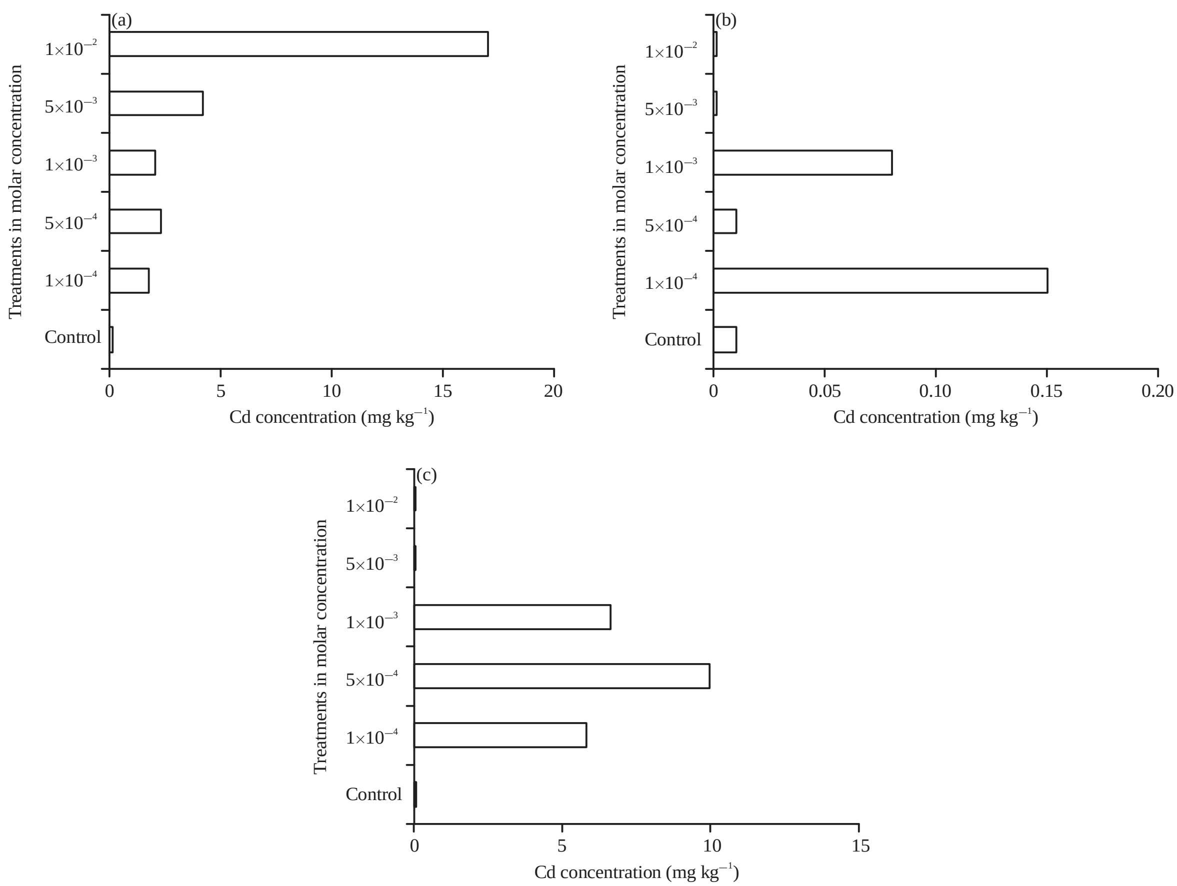

Fig. 1(a-c): Mean concentration of Cd determined in (a) Bulbs, (b) Leaves and (c) Roots of onion after 14 days period

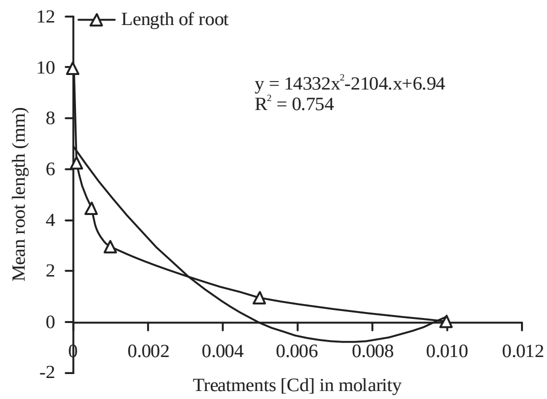

Fig. 2: Variation of root length (m) with increased dosage of Cd

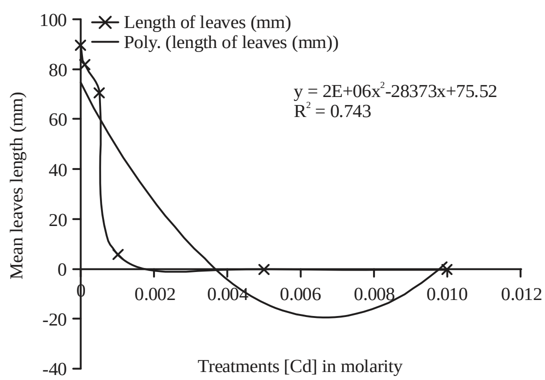

Fig. 3: Variation of leaves length (mm) with increased dosage of Cd after 14 days of exposure

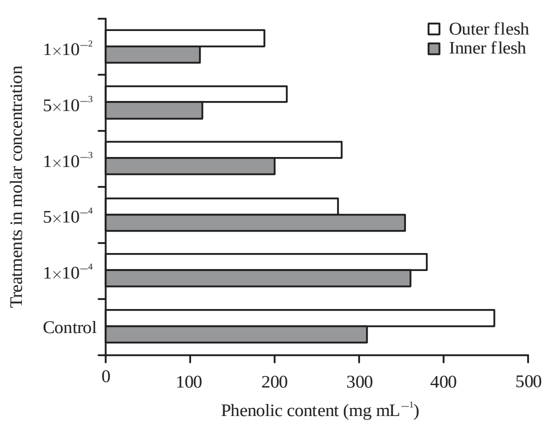

Fig. 4: Variation of phenolic contents determined in bulb with Cd treatments after 14 days of exposure

Cd in bulbs, leaves and roots with values range of 1.40-17.75 mg kg^{-1} for bulbs, 0.11- 0.23 mg kg^{-1} for leaves and 5.75- 7.00 mg kg^{-1} for roots, were presented in Fig. 1. Results on the bio-accumulations of cadmium were found to

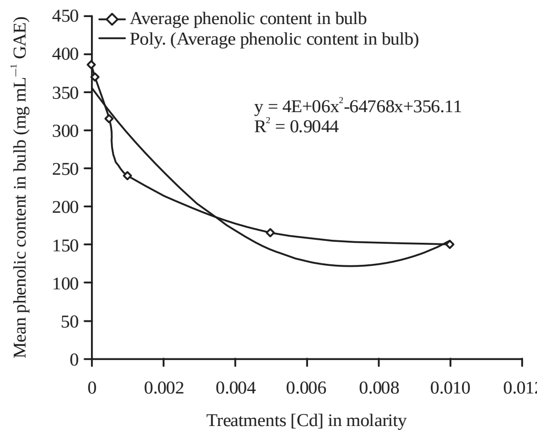

Fig. 5: Mean phenolic content in bulbs of onion after 14 days of treatment

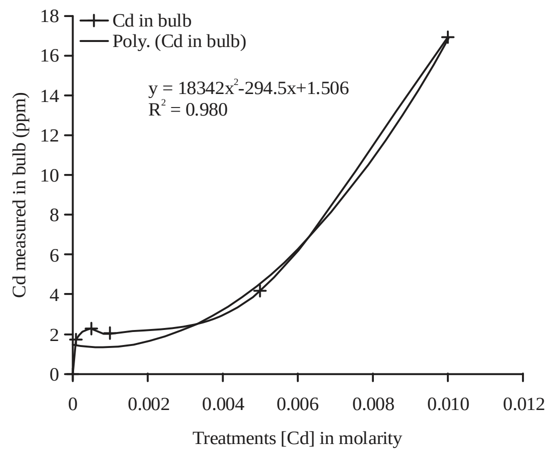

Fig. 6: Mean Cd determined in bulbs of onion after 14 days of treatment

be the highest (17.5 mg kg^{-1}) in the bulb, when the concentration of cadmium in the solution was 1×10^{-2} M and lowest (0.11 mg kg^{-1}) in the leaves when the concentration was 1×10^{-3} M.

The results of changes in the lengths (mm) of roots and leaves after exposure were depicted with Fig. 2 and 3, respectively. Roots values ranged from 10-63 mm and leaves values ranged from 58-82 mm. Figure 4 presented the results of total phenolic content expressed as mg mL^{-1} GAE with the values ranging from182.18-391.27 mg mL^{-1} GAE for inner flesh and 182.55-445.8 mg mL^{-1} GAE for outer flesh. The results show that cadmium particularly displayed inhibition of root elongation with higher effects at the higher exposure doses. The effect was more severe on the growth of leaves, which were inhibited at both concentrations of 5×10^{-3} and 1×10^{-2} M. Figure 5 presented the phenolic acid contents in bulbs, Fig. 6 showed

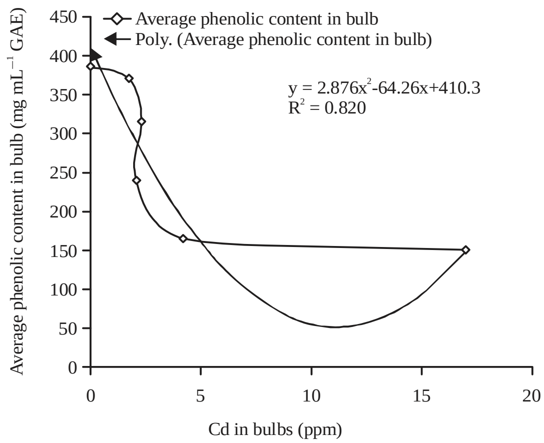

Fig. 7: Relationship between the measure Cd and mean phenolic acid content in onion bulbs

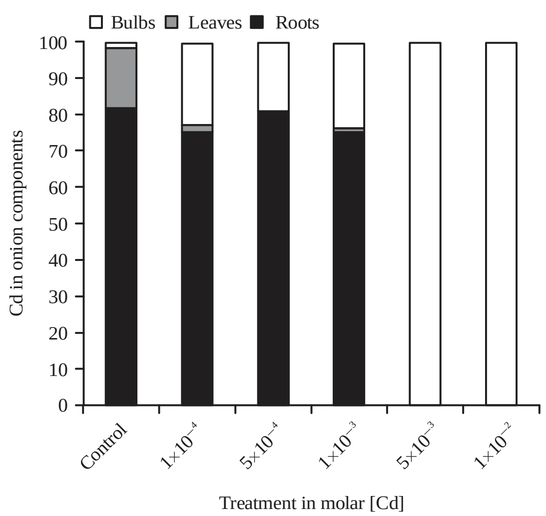

Fig. 8: Partitioning of Cd in the different onion parts after 14 days of treatments

the mean [Cd] in bulbs and Fig. 7 showed the relationship between measured [Cd] and mean phenolic acid contents in onions bulbs.

DISCUSSION

From Fig.1, it could be observed that the concentrations of Cd in roots, leaves and bulbs increased as the strength of Cd concentration increased. However, the increments do not follow a regular known pattern. The highest cadmium accumulation was obtained in the bulbs followed by roots and then the leaves. This may imply that onions have the ability for storage in its bulbs. The standard[8] permissible limit of Cd in vegetables was 0.1 mg kg^{-1}. Onion bulb was the edible portion mostly assessed by man and used majorly as food. With the highest bio-accumulation potential of 17.75 mg kg^{-1} in this study without any symptom of deterioration even at the exposure maxima of cadmium concentration of 0.01 M, it connotes the bulbs cadmium bio-accumulation potential. This means that, onions planted on cadmium polluted soils can accumulate cadmium above the[8] permissible limits. Similarly, onions plant may serve as good phyto-remediation alternative for cadmium contaminated soils but not without an associated high deleterious health implication if mistakenly consumed afterwards.

Cd is a non-essential element that negatively affects plants growth and development. Cadmium can alter the uptake of minerals by plants through its effects on the bio availability of other minerals from the soil or through a reduction in the population of soil microbes[9]. Stomata opening, transpiration and photosynthesis have been reported to be affected by cadmium in nutrient solutions, but the metal was taken up into plants more readily from nutrients solutions than from soil[10]. It reduced the absorption of nitrates and its transport from roots to shoot by inhibiting the nitrate reductase activity in the shoot[11]. In general, cadmium has been shown to interfere with the uptake, transport and use of several elements and water by plants[12]. Chlorosis and leaves stunting are the main easily feasible symptoms of cadmium toxicity in plant leaves.

From Fig. 1, it can be seen that at higher concentrations (5×10^{-3} and 1×10^{-2} M), there was shrinkage of the onion bulb and no forms of new tissues (i.e. leaves/roots) were not regenerated. Cadmium toxicity may have affected the plasma membrane permeability causing a reduction in its water content[6].

The partitioning of the Cd into the three major components of onions is shown in Fig. 8. Cadmium was more accumulated in the bulbs, followed by roots and then leaves but the highest concentration was observed in the bulb. Normally cadmium was mainly retained in the roots and only small amount were transported to the shoot[13]. The roots and leaves of onions have little or no economic values compared to the bulbs which serve as spices and medicine for man. Accumulation of Cd concentration in leaves of onion vegetables was lower than that of bulbs and roots. There was no growth of leaf at 5×10^{-3} and 1×10^{-2} M cadmium concentrations. From Fig. 2 and 3, it was found that the percentage root length 'decrements' calculated by subtracting the actual measured length from the control length reading and expressed as percentage of the control for concentrations of 1×10^{-4}, 5×10^{-4} and 1×10^{-3} M are 37, 55 and 70%, respectively while the leaves length decrements are 8.9, 21.1 and 93.5%, respectively. The higher the percentage decrement the greater the toxic effects of the Cd metal on the plant organ measured. Results from previous researches[14,15,10] corroborates very well with the observations recorded in the present study[14] showed that there is correlation between microscopic (genotoxicity) and macroscopic (morphological) parameters of *Allium cepa* as the heavy metal investigated in that study induced cytotoxicity, mutagenicity and genotoxicity. Heavy metals such as Cd and Hg in man was said to be able to initiate production of active oxygen radicals and induce formation of pulmonary fibrosis and lung cancer[16]. Another study revealed that *A. cepa* had significant effects even at the lower cadmium concentrations in terms of growth inhibition. The leaves showed a significant accumulation at the dose of 0.5 $\mu g\ mL^{-1}$ and a lower growth[16].

From Fig. 4, 5 and 7 showed that the total phenolic content expressed as mg mL^{-1} of gallic acid decreases as the concentration of cadmium treatments increases. The reduction in phenolic content changes the colour of the samples from reddish purple to a lighter shade of purple. The earlier reports on the effect of cadmium showed that it affected the enzyme activity in the treated organisms. Cadmium has no known physiological function in plants and it is a highly toxic metal due to its reactivity with S and N atoms in amino acids[9]. It was also known to be able to affect cellular structures and produce membrane damage, disruption of the electron transport, inhibition/activation of enzymes and alteration of DNA[17]. Although cadmium does not belong to the Fenton-type class of metals, it was known to trigger lipid peroxidation in tissues at early exposure times and several studies have shown the activation of anti-oxidative defences against cadmium toxicity[18]. Phenolic compounds have been described as electron donating agents and therefore, they can act as anti-oxidants[11], acting as reducing agents, hydrogen donors and singlet oxygen quenchers and preventing the evolution of oxidant free radical and reactive species derived from metal catalysis by Fenton-like reactions[12,13].

It was reported that ribonuclease activity in cadmium treated rice and also the nucleoli synthesis (RNA protein synthesis) in onion root tip cells exposed to cadmium were inhibited [4]. Cadmium also inhibited nitrate reductase activity by reducing the nitrate absorption and transport in the plant. Cadmium, unlike other heavy metals was unable to generate free radicals by itself, however, reports have indicated superoxide radical, hydroxyl radical and nitric oxide could be generated indirectly[19] showed the generation of non-radical hydrogen peroxide which by itself became a significant source of free radicals via the Fenton chemistry. Cadmium could replace iron, zinc and copper from a number of cytoplasmic and membrane proteins like ferritin, which in turn would release and increase the concentration of unbound iron or copper ions. These free ions participate in causing oxidative stress via Fenton chemistry[20].

CONCLUSION AND FUTURE RECOMMENDATIONS

It was observed that onion can accumulate cadmium metal and the secondary metabolites -phenolic acid content responded negatively to cadmium increased concentrations. Since the roots and leaves were easily affected by increased concentrations of the heavy metals. A systematic grading and assessments of the rate of cessation of the roots and leaves per treatment concentration may serves as a bio-indicating tool when thinking of the usage of *A. cepa* for bio-monitor of Cd toxicity.

Lands, especially for agricultural purposes should be tested for cadmium contamination before plant cultivation to reduce the risk of accumulating cadmium. Cadmium various uses in pigments, batteries and electroplating should be reduced to minimal level and or be replaced with less toxic metals with almost same properties. On the same note, the use of cadmium in phosphate fertilizers should be reduced to the best minima level or at most be eradicated. Finally, mining and smelting which are the major anthropogenic sources of cadmium, should be done in a controlled environments and non-residential areas.

The state of health and healthy living of human populace depend largely on the health status of the environment. This, therefore, places the responsibility on man to continuously and consistently monitor the environment for heavy metal pollutants.

SIGNIFICANCE STATEMENT

This study discovered the deleterious effects of cadmium on the *Allium cepa*. This study will help researchers to unfold the mechanism and mode of action of cadmium on living tissues especially at the molecular level.

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