An Appraisal of Copper and Zinc as Contaminants of Potential Ecological Concern (COPEC) on onions (*Allium Cepa L.*)

Ogeleka,[*] D. F. and Nwudu, A.

Department of Chemistry, Federal University of Petroleum Resources, Effurun, Delta State Nigeria

[*]Corresponding Author:E mail - dorysafam@yahoo.com; Tel: +234 8023243514

ABSTRACT

This research estimated the phyto-toxic effects of two heavy metals (copper and zinc) on onion (Allium cepa L).The effective concentration (EC_{50}) for copper and zinc following a 96 hour exposure was estimated at 1.04 mg/L ± 0.14 mg/L and 1.94 ± 0.17 mg/L respectively. The results indicated that at concentrations of 1.04 mg/L, copper may be considered as Contaminant of Potential Ecological Concern (COPEC) on the growth of onion (Allium cepa L) i.e. harmful effects of copper cannot be ruled out but may probably be a threat at concentrations ≤ 1.04 mg/L. However, zinc may not be considered as COPEC at concentration less or approximately < ≈ 2 mg/L (1.94 mg/L) i.e. zinc alone is not likely to cause ecological risk at concentrations lower than 2 mg/L. The lower the EC_{50} value, the more toxic the contaminant and vice versa. Similarly, if exposure to copper and zinc is likely to occur frequently in areas with heavy anthropogenic activities, then Allium cepa L would be able to absorb significant concentration of the toxicants and this could have multiplier effects on humans who daily consume significant quantity of the crop in their delicacies as a rich source of anti-oxidants.

Keywords: copper and zinc, contaminant of potential ecological concern (COPEC), growth inhibition, onions.

INTRODUCTION

The increasing presence of a myriad of pollutants like heavy metals, fertilizers, and pesticides in the biosphere (water, soil, and air) as a result of anthropogenic contribution to industrialization and urbanization has become an alarming phenomenon around the world. Major sources of metals in environmental media include; industrial activities, application of fertilizer, mining activities, indiscriminate dumping of hazardous waste, etc. Metals enter into the ecosystem intentionally and unintentionally and contaminate food materials, which could affect humans[1].

Although, some heavy metals are essential micronutrients for plant growth, constituents of enzymes, hormones and regulators of a variety of biochemical and physiological processes, they could become harmful if the required concentration is exceeded. Similarly, some heavy metals may destroy endocrine and chemoreceptors in organisms, cause gene alteration, affect sensitive enzymes, inhibit growth, impair reproduction and cause death[2,3,4].

The toxicity of heavy metals to plants could be affected by several factors, which include: plant species, specific metal, concentration, chemical form, soil composition and pH. Heavy metals are recalcitrant and do not readily biodegrade in the environment or are easily metabolized and as such bioaccumulate in organisms along the ecological food chain. Heavy metals are usually available in very large proportion in soil and aquatic ecosystems and exposure to high concentrations could induce oxidative stress in various species[5,6].

Soils used for agricultural activities in most regions of the world are contaminated with heavy metals as a result of excessive application of fertilizers, pesticides, herbicides, agrochemicals, sewage sludge (used as manure) and contaminated wastewater for irrigation practices. Consequently, phyto-toxicity results in weak plant growth and yield depression in addition to other toxic alterations at cell and molecular levels[7]. Heavy metals in environmental media render water, soils and sediment unsafe for aquatic, terrestrial and human lives.

Onion (*Allium cepaL.)* is cultivated in Nigeria and all over the world as a very important vegetable. Scientific investigations on onions revealed that it contains high quantity of bioactive compounds which are effective in the prevention of cancer, heart diseases and have lots of beneficial effects on human health[8,9]. *Allium cepa L* was chosen for this research because it is an important halophyte, with remarkable ability to survive under stress conditions. It is readily available all the year round and a viable vegetable consumed daily by humans[10]. This research therefore examined the phyto-toxic effects of copper and zinc on onions (*Allium Cepa. L*), since these metals play a vital role in the growth and developments of plant species and could be toxic at relatively high exposure levels.

MATERIALS AND METHODS

Materials

The test chemicals – copper (Cu) and zinc (Zn) metals of Analar grade were used for the bio-assessment. Stock solutions of 1000 mg/L Cu and Zn were prepared and serially diluted to obtain the concentrations required for the bioassay. The test species used was *A. cepa L* with mean radius of 6.91± 0.07 cm and mean weight of 75.70± 0.69 g.

Methods

The bioassay was assessed using the Organization for Economic Co-operation and Development, (OECD) protocol #208[11]. Onion bulbs (*A. cepa L*) that had been air-dried for 7 days were prepared for the experiment by shaving off the dried root at the base of the onions to expose the fresh meristematic tissues[12].

The bio-assessment for root growth inhibition started with a range-finding test in which the species were exposed to triplicate concentrations of 1, 10 and 100 mg/L of copper and zinc for 2 days. This was done to establish the concentrations that would be used for the screening (actual) test, which had serial dilutions of 4.0, 2.0, 1.0, 0.5 and 0.25 mg/L. The base of each onion was placed on the brim of the specimen containers such that the root system made contact with the extracts of the metals and control (water) in the dark for 96 h.

At the end of the exposure period, the roots of each onion bulb were removed with forceps and the length measured in cm. The results were used to determine the percentage root growth inhibition in relation to the control and the effective concentration (EC_{50}) which amounts to 50% effects of species exposed to the toxicants. The effect of the toxicants on the morphology of growing roots was also assessed.

Estimation of growth rate and toxicological risk assessment

Root growth inhibition

The root growth inhibition assessment was estimated using the effective concentration (EC_{50}). The percentage inhibition efficiency amounted to 50% of the controls for root inhibition and this was used to determine the responsiveness of onions to the test chemicals at 96 hours[11].The growth rate, percentage growth rate relative to control and the percentage growth inhibition efficiency of the metals were calculated using the equations (1) – (3).

Growth rate (cm/hr) = $\frac{mean\ length}{time}$ (1)

Percent growth rate (%) = $\left(\frac{GRs}{GRc}\right) \times 100$ (2)

Percent growth rate relative to control (%) = $\frac{GRc - GRs}{GRc} \times 100$ (3)

Where:

GRs = growth rate for sample

GRc = growth rate for control

Statistical analysis

Data analysis was performed using one way Analysis of Variance (ANOVA) in Statistical Package for Social Science (SPSS), IBM software (version 22). Concentration-response curves (EC_{50}) from the phyto-toxicity tests were analyzed with STATA software (version 8.2). The level of significance at P = 0.05 between the controls and the exposed species was also assessed. Other data were represented using different graph styles and patterns.

RESULTS AND DISCUSSIONS

The results of phyto-toxicological effects and metal analysis on onion (*A. cepa L*) exposed to different concentrations of copper and zinc are presented in Tables 1–5, Figures 1 – 2 and Plate 1.

Effective Concentration (EC$_{50}$) - growth retardation (inhibition)

The data from this study showed that there was root growth inhibition in the treatment tanks when compared to the control experiment which grew significantly (Tables 1 – 2 and Plate 1). The respective estimated effective concentrations (EC_{50}) for copper and zinc were 1.04 ± 0.14 mg/L and 1.94 ± 0.17 mg/L with a safe limit of 0.104 ± 0.05 mg/L and 0.194 ± 0.02 mg/L. The results indicated that at concentrations of 1.04 mg/L, copper may be considered as Contaminant of Potential Ecological Concern (COPEC) on the growth of onion (*Allium cepa L*) i.e. harmful effects of copper cannot be ruled out but may probably be a threat at concentrations ≤ 1.04 mg/L. However, zinc may not be considered as COPEC at concentration less or approximately < ≈ 2 mg/L (1.94 mg/L) i.e. zinc alone is not likely to cause ecological risk at concentrations lower than 2 mg/L. The lower the EC_{50} value, the more toxic the contaminant and vice versa[13].

The mean percentage growth rate relative to the control recorded for the respective concentrations of copper and zinc (0.25, 0.5, 1.0, 2.0, 4.0 mg/L) was 80%, 67%, 57%, 42%, 9% and 100%, 92%, 67%, 49%, 30% respectively. The mean percentage growth rate inhibition efficiency relative to the control recorded for copper and zinc were 20, 33, 43, 58, 91% and 0, 8, 33, 51, 70 respectively (Figures 1and 2). Phyto-toxicity impacted by the metals include decolouration of the test solutions and stunted growth especially in the higher concentrations of 2 mg/L and 4 mg/L. Other effects to the onions specie include; broken roots, twists, roots bent upwards, stunted growth, bulb deformation and tissue damage.

Table 1: Mean root length (RL) and growth rate (%) of *Allium cepa L* exposure to copper

Concentration, (mg/L)	Mean root length (RL) $\pm$ SD (cm)	Growth rate (cm/hr)	Percent growth rate relative to control (%)	Percent growth rate inhibition efficiency (%)
Control	7.9 ± 0.06	0.082	100 ± 0.0	0 ± 0.0
0.25	6.33 ± 0.09	0.066	80 ± 1.0	20 ± 0.0
0.50	5.3 ± 0.06	0.055	67 ± 0.5	33 ± 0.5
1.0	4.5 ± 0.04	0.047	57 ± 0.0	43 ± 0.5
2.0	3.3 ± 0.06	0.034	42 ± 0.5	58 ± 1.0
4.0	0.7± 0.02	0.007	9 ± 0.5	91 ± 1.0

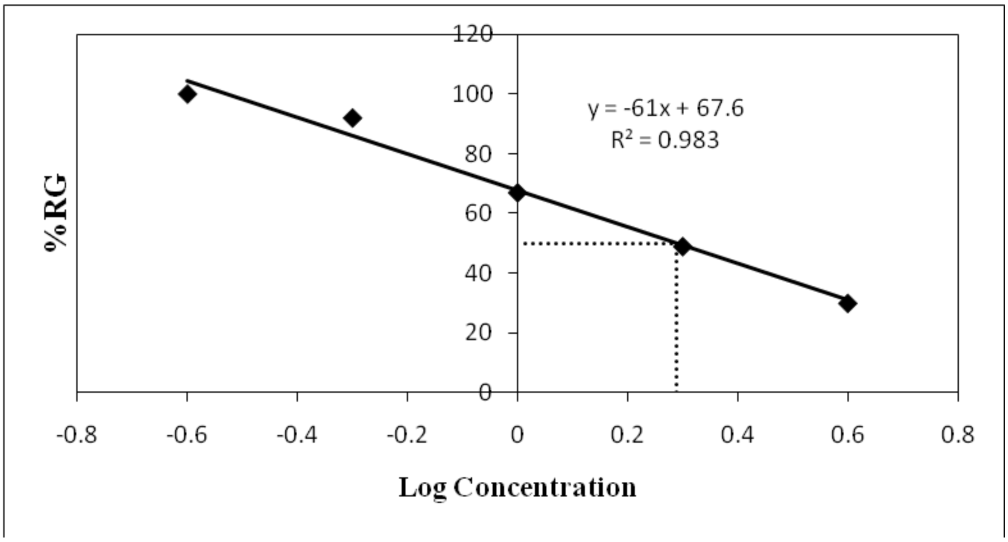

Figure 1: Mean (%) root growth (RG) of onions (*Allium Cepa L*) against log concentration of copper

Table 2: Mean root length (RL) and growth rate (%) of *Allium cepa L* exposure to zinc

Concentration, (mg/L)	Mean root length (RL) $\pm$ SD (cm)	Growth rate (cm/hr)	Percent growth rate relative to control (%)	Percent growth rate inhibition efficiency (%)
Control	7.9 ± 0.06	0.082	100 ± 0.0	0 ± 0.0
0.25	7.9 ± 0.06	0.082	100 ± 0.0	0 ± 0.0
0.50	7.3 ± 0.12	0.076	92± 1.0	8 ± 0.0
1.0	5.28 ± 0.04	0.055	67 ± 0.5	33 ± 1.0
2.0	3.84 ± 0.05	0.040	49 ± 0.5	51 ± 1.0
4.0	2.4 ± 0.01	0.025	30 ± 0.0	70 ± 2.0

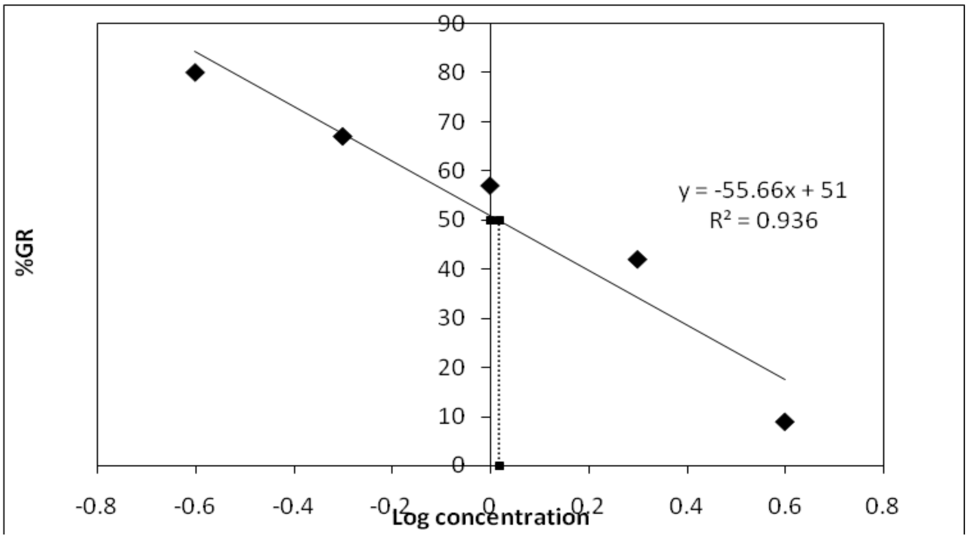

Figure 2: Mean (%) root growth (RG) of onions (*Allium Cepa L*) against log concentration of zinc

Plate 1: Plate showing the effects of exposure of copper to onions (*Allium cepa L*)

Ecological risk assessment of the heavy metals

On the Eco toxicological Risk Assessment Matrix (ERAM), risk levels can be classified as low, medium, or high (Table 3)[14,15]. If an environment is contaminated with heavy metals, animals (A), plants (P), environment (E) and community (C) may be affected and classification can be done based on exposure concentration, exposure duration and potency of the toxicant[14,15]. The risk levels are categorized in a numbered format. Each hazard is given a rating, and this was multiplied by the probability that these hazards would occur using the relationship:

Risk level = Hazard severity x likelihood of exposure (Table 4).

Hazard severity are rated as 1 (slight effect), 2 (minor effect), 3 (localized effect or damage). 4 [major effect (deaths)] and 5 [extensive effect (death of population)]. Similarly, the likelihood of occurrence or exposure are rated as 1 (seldom – A - yearly), 2 (frequent – B - quarterly), 3 (very likely – C - monthly). 4 (near certain – D - weekly) and 5 (certain – E – daily)[15,16]. The release of the chemicals - metals (hazard) into the environment could result in major damage or death, which could be given a rating of 4 (D). The next step is to consider how often each hazard is likely to occur due to exposure of the chemical or pollutant as indicated in Table 5. A daily uptake of the test metals by the plant species would be represented as D4 (P; E; C) or 16 (P; E; C) for copper (extremely toxic - $EC_{50} = 1.04 \pm 0.14$ mg/L) and zinc (extremely toxic - $EC_{50} = 1.94 \pm 0.17$ mg/L). Although Cu and Zn have the same eco toxicological risk assessment value, both were extremely toxic (EC_{50} - 1.0-10 mg/L)[17]. However, the data from this assessment showed that copper was more toxic to onion (*Allium cepa L*) than zinc i.e. the lower the EC_{50} concentration, the more toxic the contaminant[13].

Table 3: Ecotoxicological Risk Assessment Matrix (ERAM)

			CONSEQUENCE				INCREASING PROBABILITY				
SEVERITY			P	A	E	C	A	B	C	D	E
							Never experience the chemical in the area	Had been exposed / used in the area	Had been exposed / used in the area and other locations	Had been exposed / used several times in the area	Had been exposed / used several times in the area and other locations
0	Practically non-toxic	>1000	No injury	No effect	No effect	No impact	*Area 1				
1	Practically non-toxic	>1000	Slight injury	Slight effect	Slight effect	Slight impact					
2	Slightly toxic	100-1000	Minor injury	Minor effect	Minor effect	Limited impact					
3	Very toxic	10-100	Major injury	Localized effect	Localized effect	Considerable impact					
4	Extremely toxic	1.0-10	Single fatality	Major effect (deaths)	Major effect	National impact		Area 2		Area 3	
5	Super toxic	<1.0	Multiple fatality	Extensive effect (kills)	Massive effect	International impact					

Abbreviations: LC50 median lethal concentration in ppm. Data from (OECD[17], GESAMP[18])

Table 4: Ecotoxicological risk assessment for the study

Concentration of test metal	Frequency of exposure (a)	Hazard severity (b)	Risk level (a X b)	Hazard rating
Control	D	0	D 0	D 0 or 0 (P,E,C)
1.0 (Cu); 2.0 (Zn)	D	5	D 5	D 5 or 20 (P,E,C)

Table 5: Consequences of the effects of heavy metals using Ecotoxicological Risk Assessment Matrix (ERAM)

Concentration of test metals	Consequences				Toxic consequence
	Plant (P)	Animal (A)	Environment (E)	Community (C)	
Control	No injury	No effect	No effect	No impact	Practically non-toxic
1.0 (Cu); 2.0 (Zn)	Major effect (deaths)	Major effect	Major effect	Major effect	Extremely toxic

Discussion

Globalization and industrial technology / advancement had introduced toxicants including agrochemicals, production chemicals and electronic waste containing significant amount of heavy metals into environmental media. A large number of chemicals are being used by industries during production processes and the waste containing toxins are discharged into recipient systems, which could runoff into soils and as a result soils used for agricultural purposes are contaminated. Industrial wastes are among the major sources of heavy metal pollution in organisms, plants and the environment. Metal contaminants are mixed in the environmental system through human activities such as discharge of sewage, smelting processes, effluents and leaching of garbage[19].

Plant may accumulate significant amounts of heavy metals, decreasing the quality and biological value of products without physical symptoms of their damage. Similarly, since metals are used in the production of fertilizers, when such fertilizers are used excessively or indiscriminately, the metals rise to a toxic level in the receiving plant. The consumption of such plants could be toxic to man and other organisms[6,20].

Onion (*Allium cepa L.*) is a poor competitor and as a result of the inability to compete with weeds, herbicides and fertilizers are used on onion plantation. Some of the direct toxic effects caused by high concentration of heavy metals include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress. Some researches revealed that some substances could cause a decrease in the mitotic index in onion (*Alliumcepa L*) root tips[21,22]. This research showed that the release of copper and zinc into the environment through the use of compounds (substances) containing these heavy metals e.g. industrial wastewater and electronic wastes could have negative environmental impact on the growth of onion (*Allium cepa L.*). Although both copper and zinc affected the growth of onion in this appraisal, copper was more toxic than zinc to onion (*Allium cepa L*).

Copper is an essential micronutrient involved in a number of biological processes needed to sustain life, however, it can be toxic when present in excess. He *et. al.*,[23], reported that soil properties could influence absorption and accumulation of heavy metals in vegetables. Despite the role of copper as a micro nutrient, high concentrations can pose a threat to man when consumed[24]. Similarly, zinc is essential to all organisms and has an important role in metabolism, growth, development and general well-being. It is an essential co-factor for a large number of enzymes in the body. Zinc deficiency could lead to a myriad of metabolic disorders, while excess amount could result in many health related issues[25]. Zinc has its primary site of accumulation and may persist for many years without decomposition[26]. The bioavailability of these metals could disrupt different species' ability to fight disease and function.

The high mobility of these metals from the solution to the vegetable (onion), which could have resulted in the root growth inhibition amongst other effects, is an indication that in times of heavy water movement such as irrigation, uptake of these metals is bound to occur through waste water, pesticides or fertilizer that will be washed (runoffs) along farm lands. Thus it is imperative that these types of vegetables should not be cultivated in farms and fields close to heavy industrialized areas which have heavy vehicular movements or are irrigated with questionable water quality which could be sources of heavy metal contamination.

CONCLUSION

This appraisal revealed that copper and zinc were extremely toxic to the exposed test specie, *Allium cepa L*, with copper being slightly more toxic than zinc. The production, use and disposal of chemicals containing heavy metals should be properly regulated to minimize the adverse effect these metals and other harmful substances could cause to organisms in the environment and humans. It should be noted that high concentrations of Contaminants of Potential Ecological Concern (COPEC) (e.g. heavy metals) in environmental media could pose deleterious impact and ecological risk on species and this could have multiplier effects on humans, who daily consume significant quantity of the crop (*Allium cepa L*) in their delicacies as a rich source of anti-oxidants.

REFERENCES

1. Rai, P.K., Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: An eco-sustainable approach. *Int'l J Phytorem*, **10**(2) (2008) 133–160.

2. Nieboer, E. and Richardson, D.H.S., The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environmental Pollution B*.**1** (1980) 3-26.

3. Reeves, R.D. and Baker, A.J., Metal-accumulating plants, phytoremediation of toxic metals: Using plants to clean up the environment, p 193–229, New York, NY: Wiley, 2000.

4. Wintz, H., Fox, T. and Vulpe. C., Functional genomics and gene regulation in biometals research. Responses of plants to iron, zinc and

copper deficiencies.,*BiochemSoc Trans*, **30** (2002) 766–768.

5. Luoma, S.N. and Rainbow, P.S., Why is metal bioaccumulationso variable? Biodynamics as a unifying concept., *Environ SciTechnol*, **39** (2005) 1921–1931.

6. Maksimovic, I., Putnik-Delic, M., Ilin, Z. and Mirosavljevic, M., Essential (Cu and Zn) and trace (Pb and Cd) heavy metal loads in onion and potato.,*Agroznanje*, **13** (2012) 113-122.

7. Yadav, D.V., Jain, R. and Rai, R.K., Impact of heavy metals on sugar cane. In: Sherameti, I., Varma, A. (Eds.), Soil heavy metals. p. 339–367, Springer, Berlin Heidelberg, 2010.

8. Geetha, M., Ponmozhi, P., Saravan, A., Kumar, M. and Suganyadevi, P., Extraction of anthocyanin and analyzing its antioxidant properties from different onion (*Allium cepa*) varieties. *Journal Res Pharm Sci*, **2** (2011) 497-506.

9. Bernaert, N., De Paepe, D., Bouten, C.H., De Clercq, H., Stewart, D., Van Bockstaele, E., De Loose, M. and Van Droogenbroeck, B. Antioxidant capacity, total phenolic and ascorbatecontentas a function of the genetic diversity of leek (Allium ampeloprasum var. porrum). *Food Chem*, **134** (2012) 669-677.

10. Gevenez de Souza, L.F., Filho, A.B.C., Alberto de Tulio, F. andNowaki, R.H.D., Effect of sulphur dose on the productivity and quality of onions. *Australian J of Crop Sci*, **9**(8) (2015) 728 – 733.

11. Organization for Economic Co-operation and Development,(OECD)., Terrestrial Plants, Growth Test. OECD Guideline for testing of chemicals #208, p. 1-19, OECD, Paris. 2003a.

12. Rank, J. and Nielsen, M.H., A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures., *Hereditas*, **118** (1993) 49-53.

13. Landis, W.G and Yu, M.H, Introduction to Environmental toxicology. Impact of chemicals upon ecological systems 3rd Edition, p 1-279, Lewis Publishers. Boca Raton, FL. 2004.

14. Ogeleka D.F., Onwuemene C. and Okieimen F.E., Toxicity potential of Grassate® a non-selective herbicide on snail (*Achachatina marginata*) and earthworm (*Aporrectodea longa*). *Chemistry and Ecology*. **33** (5) (2017) 446 – 462.

15. Society of Environmental Toxicology and Chemistry, (SETAC)., Ecological risk assessment. A technical issue paper. P 1-4, 1010 North 12th Avenue Pensacola, FL 32501-3367. USA, 1997.

16. United State Environmental Protection Agency, (USEPA)., Ecological Risk Assessment Supplemental Guidance Interim Draft. Scientific Support Section Superfund Division EPA Region 4, 2015.

17. Organization for Economic Co-operation and Development, (OECD)., Environment, Health and Safety Publications Series on Pesticides Persistent, Bioaccumulative, and Toxic Pesticides in OECD Member Countries Results of Survey on Data Requirements and Risk Assessment Approaches.,No. 15: p 1 – 67, 2003b.

18. Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP), (IMO/FAO/UNESCO-OC/WMO/WHO/IAEA/ UN/UNEP). Towards safe and effective use of chemicals in coastal aquaculture. Reports and Studies, GESAMP. No. 65. Rome, FAO, 1997.

19. Ogeleka, D.F.,Edjere, O., Nmai, O., Ezeogu, P. and Okieimen, F.E., Consideration of contamination status of soils within the vicinity of automobile workshops in Warri, Delta State, Nigeria. *Sci J of Chem*, **6**(4) (2018) 56-65.

20. Al-Fraihat, A.H., Effect of different nitrogen and sulphur fertilizer levels on growth, yield and quality of onion (*Allium cepa, L.*). *Jordan J of Agric Sci*, **5**(2) (2009) 155-165.

21. Ghosheh,H.Z., Single herbicide treatments for control of broadleaved weeds in onion (*Allium cepa*).,*Crop Prot*, 23 (2004) 539-542.

22. Qasem, J.R., Chemical weed control in seedbed sown onion (*Allium cepa L.*).,*Crop Prot*, **25** (2006) 618-622.

23. He, Q., Ren, Y., Mohamed, I., Ali, M., Hassan, W. and Zeng, F.,Assessment of trace and heavy metal distribution by four sequential extraction procedures in a contaminated soil., *Soil & Water Res*, **8** (2013) 71–76.

24. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for copper. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, (2004).

25. Saraf, A. and Samant, A., Evaluation of some minerals and trace elements in *Achyranthes aspera Linn., Int. J. Pharma. Sci*, **3**(3) (2013) 229-233.

26. Murugan, S.S., Karuppasamy, R., Poongodi, K. and Puvaneswari, S.,Bioaccumulation pattern of zinc in freshwater fish *Channapunctatus* (Bloch.) after chronic exposure., *Turkish J of Fisheries and Aqua Sci*, **8**(1) (2008) 55-59.