International Journal of Science and Advanced Innovative Research
ISSN: 2536-7315 (Print) 2536-7323 (Online)
Volume 3, Number 1, March 2018
http://www.casirmediapublishing.com

Toxicity of Copper Sulphate on Copepod species

Nwakanma, C., & Chimezie A. F.

Department of Environmental Management and Toxicology
College of Natural Resources and Environmental Management
Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
Email: dr.nwakanmac@gmail.com

ABSTRACT

The toxicity of copper sulphate on zooplankton was studied. Copepod, a tropical freshwater zooplankton was isolated from Qua Iboe river water and treated with copper sulphate solutions in concentration of 0,5,10,15 and 20 mg/l for a contact period of 48 hrs. Meanwhile physiochemical and microbiological characteristics of the river water was also studied. Results showed that the river water quality was not in conformity with regulations based on WHO permissible level. Most of the heavy metals failed as well as the BOD, DO and COD levels which recorded 7.03µg/l, 9.83µg/l and 11367mg/l respectively. The microbial loads were also higher than the permissible levels with total viable counts of bacteria load of 1.67×109cfu/ml and fungi load of 7.61×102cfu/g. Toxicity levels of Copper on live copepods was in the range of 23.83% to 57.1%. Toxicity was found to increase with increasing concentrations of $CuSO_4$ from 5µg/ml to 20µg/ml which represented active activity of pollutant against the organism. It was observed that discharge of $CuSO_4$ containing wastes into the environment constitute a level of pollution with potential adverse consequences to human health.

Keywords: algae, copepods, toxicity, food chain, ecology, copper sulphate

INTRODUCTION

Copper (Cu) is plentiful in the environment and essential for the normal growth and metabolism of all living organisms (Schroeder *et al.*, 2006). Abnormal levels of copper intake may range from levels as low as to induce a nutritional deficiency to levels as high as to acutely toxic (U.S Environmental Protection Agency, 2000). Copper is derived from Latin *curprum*, a corruption of Cyprium Cyprus being the source of Egyptian Roman copper. Copper is the most toxic of the heavy metals in fresh water and marine and often accumulates and causes irreversible harm to some species at concentrations just above levels required for growth and reproduction (Bopp *et al.*, 2008). Copper is essential for the successful growth and development of many species of aquatic organisms, but its rate and extent of accumulation and retention are modified by numerous biological and abiotic variables. Abiotic variables known to modify copper concentrations in tissues of aquatic biota include water temperature, pH, salinity, and depth. Biological variables affecting copper accumulations in marine organisms include the organism's size, age and developmental stage (Eisler, 2001).Copper is an important element that is needed in trace amount of around 5-20 µg / gm by living organisms which include humans, mammals, plants as well as aquatic organisms. It is needed for metabolism of carbohydrates and for active functioning of more than 30 enzymes. It also helps in haemoglobin and haemocyanin synthesis, which acts as the O_2 transporting pigments in blood of vertebrates and shellfish respectively. If the copper concentration exceeds more than 20 µg / gm it can prove to be toxic (Heike and Bradi, 2005).Copper has proved to be anti-fungal and anti-algal. It also kills molluscs and hence it proves that it is highly toxic to aquatic organisms and to the entire ecosystem at large. The most bioactive and toxic form of the copper is cupric ion Cu^{+2}. Aquatic life is 10–100 times more sensitive to the hazardous effects of copper than mammals. The main route of entry for any chemical is through the gills. From the gills, it is transported to various parts of the body via the blood stream. A fish gill gets frayed and loses their ability to regulate transport of salts such as NaCl and KCl into and out of the fish. These salts are necessary for proper functioning of cardiovascular and nervous system. When the salt balance is disrupted between body of copper exposed aquatic organism and environmental water, death of organism occurs. Copper will be more harmful to aquatic organisms if pH of water is acidic. Copper also adversely affects olfaction, meaning sense of smell in fish. Deflection of odour occurs when dissolved odorant molecules bind with olfactory receptor molecules when the olfactory tissues of the organism come in direct contact with the surrounding water which facilitates copper uptake. Copper can affect olfaction by competing with natural odorants for binding sites by affecting activation of the olfactory receptor neurons or by affecting intracellular signalling in the neurons (Baldwin, 2003). Blood provides an ideal medium for toxicity studies. Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition. The haematological parameters have been considered as diagnostic indices of pathological conditions in animals. Blood can serve as valuable tool in detecting physiological changes taking place in aquatic animals. Copepods are microscopic aquatic animals of the phylum Copepoda and are equally affected by the toxicity of copper. Copepods can be found in many freshwater environments and in moist soil, where they inhabit the thin films of water that are formed around soil particles. The habitat of copepods may include still water environments, such as lake bottoms, as well as flowing water environments, such as rivers or streams. Rotifers are also commonly found on mosses and lichens growing on tree trunks and rocks, in rain gutters and puddles, in soil or leaf litter, on mushrooms growing near dead trees, in tanks of sewage treatment plants, and even on freshwater crustaceans and aquatic insect larvae. (Dave, 1984). Copper is an essential element required by all living organisms, but it can be toxic to aquatic species when present at elevated concentrations. A wide range of contaminants are continuously introduced into the aquatic environment mostly associated with industrial, agricultural and domestic wastes run-off (Bopp *et al.,* 2008). Among these contaminants, heavy metals like copper constitute one of the main dangerous groups, because they are toxic, persistent and not easily biodegradable. The species and concentrations of metals in water are determined by geochemical processes and large scale releases into the aquatic environment by human activities (anthropogenic activities) (Wittmann, 2007). Rapid industrial developments as well as the use of metals in production processes have led to the increased discharges of heavy metals into the environment

(Koli*et al.*, 2003). According to Forstner and Prosi (2007) the harmful effects of heavy metals as pollutants result from incomplete biological degradation. Therefore, these metals tend to accumulate in the aquatic environment. Since heavy metals are non-biodegradable, they can be bio-accumulated by aquatic organism, either directly from the surrounding water or by ingestion of food (Patrick and Loutit, 2002). Copper is an essential compound for aquatic organisms in small quantities. However, copper becomes toxic when biological requirements are exceeded. The effects of copper on aquatic organisms can be directly or indirectly lethal. Different species, and even organisms within the same species, can exhibit different sensitivities to elevated copper levels in the water column. Organisms have different mechanisms by which they cope with and process copper. Some organism bio-accumulates and store copper, whereas others actively regulate its levels. In general, copper is actively regulated in fish, decapod crustaceans, and algae and stored in bivalves, barnacles, and aquatic insects than in rotifers (microscopic aquatic organisms). Therefore, to properly evaluate the copper-related effects on aquatic life, one must understand the factors that affect the biological fate of copper and the mechanisms by which it acts to produce its toxicity (Brix and Deforest, 2000). The aim of this study is to determine the toxicity of soluble copper on copepod species. Fish and crustaceans are 10 to 100 times more sensitive to the toxic effects of copper than are mammals. Algae, especially blue-green algae species, are 1,000 times more sensitive to the toxic effects of copper than are mammals, as several authors, including Forstner and Wittman (2007), Hodson *et al.*, (2007) and Brooks *et al.*, (2002), have demonstrated. Bioavailability and toxicity of copper to aquatic organisms depends on the total concentration of copper and its speciation (Araye *et al.*, 2003). In hard, moderately polluted waters, 43 to 88% of the copper is associated with suspended solids and not available to biota (Shah and Bharat 2014). Copper toxicity to aquatic biota is related primarily to the dissolved cupric ion (Cu^{+2}) and possibly to some hydroxyl complexes (USEPA, 2000; Hall *et al.* 2008). Soluble copper is largely complexed with carbonate, amino acids, or humic substances. Cupric copper, one of the most toxic forms constitutes 0.1 to 0.2% of this soluble material (Shah and Bharat, 2014). The toxicity of copper in its complexed, precipitated, or adsorbed form is less than that of the free ionic form (Bopp *et al.*, 2008). In aquatic invertebrates, copper causes gill damage at high concentrations, and in fishes it interferes with osmoregulation (Hodson *et al.*, 2009). In freshwater algae, movement of copper into cells occurs mainly by physical transport; the plasmalemma is the initial site of copper binding. Copper on the plasmalemma increases its permeability, as shown by the leakage of potassium and other ions from copper-treated cells and entry of copper into intracellular sites (Brige *et al.*, 2007). Marine prosobranch gastropods, like several other groups of mollusks and arthropods, normally accumulate and store copper and use it in the synthesis of hemocyanin, a blood pigment (Blanchard and Grosell, 2005). In gastropods, copper may elicit secretions of mucus by goblet cells; bind to hydrophilic regions of the external membranes of epithelial cells, altering their biochemical and biophysical properties; or disrupt the normal functioning of peroxidase and ferritin (Joshi, 2007). Peroxidation products, such as hydroperoxides and malondialdehyde, are toxic to vital functions of membranes and cells; bivalve mollusks challenged with ionic copper show significant increases in these products (Boulanger and Nikolaidis, 2003). Exposure of gastropods to high sub lethal concentrations of copper completely inhibits succinic dehydrogenase activity at whole body concentrations between 4.7 and 11.9 mg Cu/kg DW soft parts, causes a measurable decrease in heart beat rate, and adversely affects surface epithelia, especially those covering the head-foot and rectal ridge, disrupting osmoregulation and producing water accumulation in tissues (Wilde *et al.*, 2006). The primary lethal effect of copper in gastropod mollusks is caused by disruption of the transporting surface epithelium (Brooks *et al.*, 2007).

MATERIALS AND METHODS

Heavy metals in the water sample were determined instrumentally by atomic absorption spectrophotometer (AAS). The method described by Araye *et al.*, (2003) was said in which 50ml of the water sample was deposited in a mixed acid depositor. The 60ml portion of the water sample was treated with 10ml of concentrated Nitric acid solution and 5ml of peachlpric acid solution and heated under a cupboard until a clear solution was obtained (the digest). The digest was diluted to 100ml with distilled water and filtered through Whitman No 42 filter paper. The filtrate was used for the analysis. The instrument, Perking Elnor Atomic Absorption Spectrophotometer (AAS) was used according to the manufactures instructions. It was switched on and allowed to equilibrate for 15mns. It was fleshed with distilled water then the appropriate hollar-cathode tube for a particular amount (eg Cu) was put in place and the corresponding wavelength (322nm) was set. Following this, standard corner solution previously prepared and diluted to set concentrations in series, were in turns, aspirated into the machine.

This corresponding absorbance were recorded in the instrument and plotted into a curve which is used to extrapilate the level of the element in the test samples. Meanwhile the sample extract was aspirated into the instrument and its absorbance was recorded for the test element.

RESULTS AND DISCUSSION

Effect of copper on Copepod survival in water

Treatment	Total	Survivors of results	Dead no of results	Mortality vs Totality
Control a	7	7	-	0.00
(0) b	7	7	-	.00
c	7	7	-	.00
T_1 a	7	6	1	14.29%
(5mg/L) b	7	5	2	28.6
c	7	5	2	28.6
T_2 a	7	4	3	42.9
(10mg/) b	7	5	2	28.6

c	7	4	3	42.9
T_3 a	7	4	3	42.9
(15mg/L) b	7	3	4	57.1
c	7	4	3	42.9
T_4 a	7	3	4	57.1
(20mg/L) b	7	3	4	57.1
c	7	3	4	57.1

Table 1: Quality characteristics of Quality characteristics of water for Copepod isolation

Physical	Quality	WHO/EPA	Remarks
pH	5.87±0.56	6.0-8.0mg/L	Failed
Temp	30°C±0.00	0mg/L	Passed
TS	333.67±10.20	50mg/L	Passed
TDS	180.67±6.43	0mg/L	Failed
TSS	153.0±7.0	0mg/L	Passed
Chemical			
Hardness	22.03±0.40	50mg/L	Passed
Ca	16.66	75mg/L	Passed
Mg	6.03	50mg/L	Passed
NO_3	33.5	50mg/L	Passed
SO_4	283.4	500mg/L	Passed
PO_4	29.2	250mg/L	Passed
Cl	5.62	0mg/L	Passed
Metals			
Cu	0.14	2mg/L	Passed
Zu	5.72	3mg/L	Failed
Fe	3.94	5.0mg/L	Passed
Cd	0.02	0.003mg/L	Failed
Cr	0.04	0.05mg/L	Passed
Pb	0.16	0.01mg/L	Failed
As	0.02	0.02mg/L	
DO	9.83±0.23	4μg/L	Failed
BOD	7.03±0.12	5.0μg/L	Failed
COD	513.67±4.51	0mg/L	Failed
THC (cfu/ul)	$1.57 \times 10^9 \pm 0.08$	1×10^5cfu/ul	Failed
Fungi	$7.61 \times 10^2 \pm 1.16$		

Table 2: Toxicity of Copper sulphate solution on Copepod (zooplankton) at 48 contact period

Sample treatment	Total No	Survivors	No Dead	Toxicity (mortality %)
Control	7.0±0	$7.0^d \pm 0.0$	$0.0^a \pm 0$	$0.0^a \pm 0.0$
T_1 (5mg/L)	7.0±0	$5.33^c \pm 0.58$	$1.67^b \pm 0.58$	$23.83^b \pm 8.26$
T_2 (10mg/L)	7.0±0	$4.33^b \pm 0.58$	$2.67^c \pm 0.58$	$38.17^c \pm 7.43$
T_3 (15mg/L)	7.0±0	$3.67^a \pm 0.58$	$3.33^{cd} \pm$	$47.03^{cd} \pm 8.72$
T_4 (20mg/L)	7.0±0	$3.0^a \pm 0.0$	$4.00^d \pm 0.0$	$57.11^d \pm 0.00$

Values showed means of triplicate analysis ± standard deviation.

Results of quality characteristics of the river water is shown in table 1. From the results, it was observed that the quality characteristics of the river water did not differ significantly with those of other water bodies within the South Eastern Nigeria as reader by previous research repeats (Ibeneme *et al.*, 2014), Umunnakire and Johnson (2013) Ijeh *et al.*, (2013) Okeke and Adina (2013). The relief show a slightly acidic pH of 5.87 and total solid content of 333.67µg/l. The dissolved and suspended solids were 180.67µg/l and 153.0mg/l respectively. These values were found to conform to permissible limits for surface water (WHO, 2006). The anions nitrate, sulphates and phosphates had values of 33.5mg/l, 283.4µg/l and 29.4µg/l respectively and these values were also within the acceptable levels (APHA, 2005, WHO, 2004). The heavy metals in the water showed different levels. However, while most of the heavy metals were found to be at levels well above the permissible circuits, copper, the test metal was found to be well below the acceptable level. The biological indices indicated failure in BOD, Bacteriological load and fungi loads. The bacteria load was an average of 1.57×109cfu/ml which was well beyond the acceptable level. Also, the BOD was slightly above the thresholds. It was observed that the water quality was suitable for the growth and survival of most planktons.

Table 2 shows the toxicity effect of $CuSO_4$ solution on the test zooplankton scope pods Qom water. From the result, it was observed that the test pollutant, $CuSO_4$ decentrated toxicity on the planktons. Also, there are variations if significant difference ($p<0.05$) in the toxicity level of the different copper concentrations.

The survivor larvae decreased with increasing concentrations of the pollutant ($CuSO_4$). In all, after 48 hrs contact with the pollutant, the toxicity level was recorded - the range of 23.8% to 57.1% and was found to increase linearly with concentration. According to reports, the toxicity of copper depend on many factors and may be directly or indirectly (Zakeri and Shuib, 2003). The results obtained in this work supports that there was direct toxicity following contact with the test organisms. The mortality of the each organisms was probably due to the inability of the larvae to excrete infected copper in the water. WHO (2004) reported that the excretion of copper by animals was weaker than absorption and that such leads to accumulation of the toxic metal in the life of organisms. From the gathered information on this project, it was concluded that copper is toxic to the test zooplankton and possibly to others of different species. This imply that the discharge of the metal into the environment constitute an environmental issue that should be considered by stakeholders. It is recommended that this work be extended subsequently to accommodate other methods as well as to extend the contract time as only 5/40 mortality was rendered after 48 hrs.

REFERENCES

American Public Health Association (2005). Standard methods for examination of water and waste water. 20 edition.

Araya, M., Chen, B., Klevay, L. M., Strain, J. J., Johnson, L., Robson, P., Shi, W., Neilsen, F., Zhu, H., Olivares, M., Pizarro, F., and Haber, L. T. (2003). Confirmation of an acute no-observed-adverse-effect level (NOAEL) and low-observed-adverse-effect level (LOAEL) for copper in bottled drinking water in a multi-site international study. *Regul. Toxicol. Pharmacol.* 38:389–399.

Baldwin D. H.., Sandanl J. F., Labenia J. S. and Scholz N. L.(2003). Sublethal effects of copper on coho salmon : Impacts on non-overlapping receptor pathways in the peripheral olfactory nervous system, *Environ. Toxicol. Chem.,* **22**(10), 2266 - 2274.

Baldwin, D. H., J. F. Sandahl, J. S. Labenia and N.L. Scholz (2003). "Sublethal Effects of Copper on Coho Salmon: Impacts on Non-overlapping Receptor Pathways in the Peripheral Olfactory Nervous System," Environmental Toxicology and Chemistry 22(10): 2266-2274.

Birge, W. J., and Black, J.A. (2007). Effects of copper on embryonic and juvenile stages of aquatic animals. Pages 373-399 *in* J. O. Nriagu, editor. Copper in the environment. Part 2: health effects. John Wiley, New York.

Blanchard J, and Grosell, M. (2005). Effects of salinity on copper accumulation in the common killifish (*Fundulus heteroclitus*. *Environ Toxicol Chem* 24(6):1403–1413

Bopp, S.K., Abicht, H.K., Knauer, K. (2008). Copper induced oxidative stress in rainbow trout gill cells. *Aquat Toxicol* 86:197–204

Boulanger, B., and Nikolaidis, N.P. (2003). Mobility and aquatic toxicity of copper in an urban watershed. *JAM Water Resour Assoc* 39(2):325–336

Brix, K.V., and DeForest, D.K., (2000). Critical review of the use of bioconcentration factors for hazard classification of metals and metal compounds. Parametrix Inc., Washington, DC. Report No. 555–3690–001

Brooks, S.J., Bolam, T., Tolhurst, L., Bassett, J., Roche, J.L., Waldock, M., Barry, J., Thomas, K.V. (2007). Effects of dissolved organic carbon on the toxicity of copper to the developing embryos of the pacific oyster (*Crassostrea gigas*). *Environ Toxicol Chem* 26(8):1756–1763

Dave, G. (1984). Effects of copper on growth, reproduction and survival and haemoglobin in *Daphnia magna*. *Comp. Biochem. Physiol.,* 78C: 439–443.

Eisler, R. (2001) Trace metal concentrations in marine organisms. Pergamon Press, New York. 687 pp.

Forstner, U., and Witmann, G.T.W. (2007). Metal Pollution in the Aquatic Environment. Springer-Verlag, Berlin.

Heike and Bradi (2005). Heavy metals in the environment: Origin, interaction and remediation, *Elsevier/Academic* Press, London, 22-29.

Hodson, P.V., U. Borgmann and H. Shear (2009). "Toxicity of Copper to Aquatic Biota." In: Nriagu, J.O., ed., Biogeochemistry of Copper, Part II. *Health Effects*. John Wiley and Sons, New York, N.Y., pp. 307-372.

Ibeneme, S.I., Ofulume, R.N., Okechi, I.V., Haruna, L.N., Ukiwe, J.U., Udensi, J.C., Nwachukwu, and Adorah, H. U. (2014). Assessment of the quality of water resources of Acorba and Ovim areas, Isuikwuato southern Nigeria. *International journal of convent microbiology and applied Sciences.* Vol. 3, no.1:181-193.

Ijeh, B.I., and Onu, N N. (2013). Assessment of pollution level of groundwater in this part of IMO river basin, south eastern Nigeria. *International journal of water resources and environmental engineering*. Vol. 5(4) 194-202.

Joshi, B. H. (2014). Evaluation and characterization of heavy metal resistant fungi for their

Massie, H.R., Aiello, V.R., and Williams, T.R.C (1980). Changes in superoxide dismutase activity and copper during development and aging in the fruit fly *Drosophila melanogaster*. Mech. Ageing Dev., 12: 279–286.

Okeke, P.N. and Adinn, E.N. (2013). Water quality study of Otanine river in Owerri, Nigeria. *Universal journal of environmental research and technology*. Vol. 3, issue 6; 641-649.

Patrick F.M and Loutit M.W (2002). Passage of metals to freshwater fish from their food. *Water Res.* **12** 395-398.

Schroeder, S.R., LeBlanc, J.M., and Mayo, L. (2006). Brief report: A lifespan perspective on the development of individuals on the autism spectrum. *JADD, 26, 251- 254.*

Shah A., and Bharat, V. M. (2014). Studies on effect of cobalt sulphate on fish *Labeorohita, J. Environ. Res. Develop.,* **9**(1), 169-176.

U.S. Environmental Protection Agency (USEPA). (2000). Ambient water quality criteria for copper. U.S. Environmental Protection Agency Report 440/5-80-036. 162 pp.

Umunnakire, J.E. and Johnson, F.C. (2013). Preliminary investigation of some physiochemical parameters and water quality of IMO river, Nigeria. *Journal of environment and earth service.* Vol. 3 No. 8:55-60.

Wilde, K.L., Stauber, J.L., Markich, S.J., Franklin, N.M., Brown, P.L. (2006). The effects of pH on the uptake and toxicity of copper and zinc in a tropical freshwater alga (*Chlorella* sp).

Wittmann, G. T. W. (2007). Toxic metals. In: Förstner U and Wittmann GTW (eds.) *MetalPollution in the Aquatic Environment*. Springer- Verlag, Berlin, Germany. 3-68.

WHO.(2004). Guidelines for Drinking water Quality: (iii) Health, Criteria and Supporting Information, World Health Organization. Geneva.

Zakeri, H. A. and Shuib, N. S. (2013). Physiological and biochemical responses of a Malaysian red alga, *Gracilaria manilaensis* treated with copper, lead and mercury, *J. Environ. Res.*