Asian Journal of Basic Science & Research (AJBSR)
Volume 1, Issue 1, Pages 08-14, October-December 2019

Toxic Effects of Produced Formation Water on the Growth Performance of *Skeletonema costatum*

Research Article

Received: 09 October 2019 Accepted: 21 November 2019 Published: 11 December 2019

Ogbonne, Fabian Chinedu; Salami, Moses Adelakun & Nwokwu, Peter Chidi.

Nigerian Institute for Oceanography and Marine Research, P.M.B. 12729, Victoria Island, Lagos.

Corresponding Author
Author Email:
ogbonnefebian@yahoo.com

Abstract: Discharges of produced formation water (PFW) from offshore oil and gas production is a continuous source of pollutants to the marine aquatic ecosystems. Produced formation water contains traces of hydrocarbon which are relatively resistant to bio-degradation and have been reported to cause adverse effect on marine organism. Therefore, the acute toxicity of produced formation water was assessed by evaluating their effects on growth performance of *Skeletonema costatum.* The test design employed includes eight concentrations (1.0, 1.8, 3.2, 5.6, 10, 18, 32 and 56%) in triplicate of the test toxicant and six control. Cell density of Skeletonema *costatum* was determined in each of the culture vessels from every 24 hour of exposure to 72 hours, Cell density growth rate in every treatment, percentage growth inhibition by various concentrations as well as the IC50 of PFW on the *S. costatum* were determined following standard methods. The control cultures recorded exponential growth within 72 hours. All the PFW concentrations resulted in inhibition of cell growth of S. *costatum* within 72 hours. The IC50 values for PFW ranged from 1.55 ml/l to 1.99 ml/l indicating high acute toxicity. Cell growth inhibition of *Skeletonema costatum* increased significantly ($P<0.05$) from 3.2% to 56% exposure concentrations indicating that direct discharge of these effluents into the aquatic ecosystem could poses considerable hazard to the indigenous aquatic biota. Consequently, more efforts should be put in place by the regulatory agency of oil industry in ensuring that the operators adhere strictly to effective guidelines of waste water treatment.

Keywords: *Skeletonema costatum,* Produced formation water, Growth Inhibition.

INTRODUCTION

Environmental pollution has become one of the major problems of global concern, as countless number of toxic compounds, emanating typically from oil and gas industry activities are being released into our environment incessantly (Sameeh *et al*., 2015). In many scenarios, harmful chemicals induce strong acute toxic effects to exposed aquatic organisms when released to the environment, but frequently the consequences are delayed due to the effects of bioaccumulation and bio magnification (Sameeh *et al*., 2015). Early detection of toxic substances in the environment and their biological effects on living organisms has become increasingly significant. Consequently, toxicity measurement of produced water effluent in aquatic ecosystem is a very important part of environmental monitoring.

The negative biological effects of pollutants present in all kinds of environmental samples can be assessed using different living organisms such as *Skeletonema costatum* (Aidar *et al*., 1999). The biological response following exposure of living organisms to environmental sample usually gives an information on toxicity, estrogenicity and genotoxicity of the whole mixture of chemical compounds present in that particular sample (Sameeh *et al*., 2015). Apart from being sensitive only to the bioavailable fraction of pollutants, bioassays equally have the power to assess the integrated effects of interacting chemical compounds and to detect the compounds, which are toxic only due to bioactivation (Aidar *et al*., 1999). Conventionally, marine microalgae (Skeletonema costatum) are used for aquatic toxicity measurement especially as sensitive indicators of toxicity of produced waters in the Oil industry.

Generally, treated crude effluent from oil operations contain several constituents namely hydrocarbons, organic and inorganic chemicals, dissolved salts, metals, drilling mud, production chemicals, bacteria and some radioactive materials and these are often associated with environmental impacts (Neff, 2002). Produced water (PW) is water from the formation produced along with oil or gas (Stromgren et al., 1995). It may sometimes also contain injection water and condensation water. The produced water originates mainly from the oil-bearing formation, the reservoir, where it occurs more or less intermixed with the oil and usually appears in the production stream from the start (Stromgren et al., 1995).

Despite the Perceived adverse effects of produced water generated during oil and gas exploration activities in the Niger Delta region of Nigeria on the marine aquatic environment, there is dearth of information on the effects of produced water on growth performance of marine algae (*Skeletonema costatum*) as test organism. However, this study was undertaken to investigate the toxicity effect of oil industry produced formation water on growth performance of *Skeletonema costatum*.

MATERIALS AND METHODS

Sample Collection

The phytoplankton samples were collected from the five Cowry Creek locations (N 06^{o} 26’ 46.3’’, E 003^{o} 24’ 63.1’’) and Lagos Harbour (N 06^{o} 24’ 46.6’’, E 003^{o} 24’ 0.78’’), with 20µm mesh size plankton net, towed on a motorized boat. Samples were kept in an ice box and transported to the Microalgae laboratory of Nigerian Institute for Oceanography and Marine Research, Lagos for analysis and isolation.

Isolation and Identification of microalgae

Samples were examined microscopically for the presence of the target specie (*Skeletonema costatum*) in the laboratory using standard identification method (Newell and Newell, 1963; Hasle and Syvertsen, 1996). Micropipette washing technique was used in the isolation of the alga as described by Phang and Chu (1999). The tips of glass Pasteur pipettes were heated in a flame and stretched when it became soft. The tips of the pipettes were then broken using a pair of fine forceps to form micropipettes. The micropipettes were plugged with some cotton, sterilized with oven at 126°C for 15 minutes and attached to soft rubber tubing. These were used to pick up single cells from the collected sample under a microscope. The target organism was transferred to a drop of sterile water on a glass slide and the procedure was repeated several times to wash it and to make it free from contamination (Hoshaw and Rosowski, 1973; James, 1978; Guillard, 1995). Thereafter, the isolated microalgae species were allowed to grow in Guillard (f/2) medium and maintained in the laboratory.

Preparation of Growth Algal Media and concentrated growth medium

All Algal media and concentrated growth media were prepared according to ISO 10253 (ISO 2006).

Preparation of dilution series of test media

Experiments with produced water were carried out in triplicates for each assayed concentrations (1, 1.8, 3.2, 5.6, 10, 18, 32, 56 and control) according to the standard recommended by ISO 10253 (ISO 2006).

Toxicity Tests

Skeletonema costatum was selected for the tests since it is an ecologically important component of the phytoplankton in coastal waters. Cells were inoculated into experimental flasks containing the range of produced water concentrations and initial cell densities of about 2 x 10^4 cells /ml were added. All the test vessels and control were incubated at a nominal temperature of 25 ± 2^0C under continuous white light. The photon fluorescence rate was uniform and in the range of 60 µmol/m^2.S to 120 µmol/m^2.S by using between four to seven fluorescent lamps (power rating 30 watts) of the universal white according to IEC 60081 at a distance of approximately 0.35 meter from the algal culture medium. The cultures were continuously and gently shaken in order to keep the cells in free suspension and to facilitate CO_2 mass transfer from air to water and in turn reduce pH shift. Cell counting were performed by using hemocytometer after every 24±2 hours for 3 days. The test lasted for 72 ± 2 hours. At the end of the test, the pH of the samples of each concentration of the test substance and the controls were determined, the appearance of the cells and the identity of the test organism were confirmed by microscopy. IC50 was determined by using Probit analysis tool (Finney, 1952; Hahn and Soyer, 2008).

Determination of Physico-chemical parameters and Heavy metals of Produced Formation Water

The physico-chemical parameters of produced formation water were analyzed before they were used for the bioassay experiments and the following parameters were analyzed: Biological Oxygen Demand (BOD), Total Hydrocarbon Content (THC), Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Electrical Conductivity, Salinity, Hydrogen Ion Concentration (pH), Temperature, Chemical Oxygen Demand and Heavy metals.

Statistical Analysis

The results obtained were subjected to statistical analyses using the Statistical Packages for Social Sciences (SPSS) software, version 20 (IBM). Significant differences between obtained measurements were determined using analysis of variance (ANOVA) at $p<0.05$.

RESULTS AND DISCUSSION

Physico-chemical Properties of Produced Formation Water

The physico-chemical characteristics of the test produced formation water shows that the color of the produced water was colorless. The Temperature was above the recommended limit (20 ± 2^0C). Other parameters were either below or within the recommended limits that will support the experiment. However, of specific importance is the Total Dissolved Solid (TDS) content of the produced water (11375) which was far above recommended standard regulatory limits (500ppm).

Table 1: Physico-chemical Analysis of Produced Formation Water used in the Bioassays

PARAMETER	Produced Water Sample	NESREA Limit	US EPA Limit
Total Dissolve Solid (TDS) (ppm).	11375	500	500

Dissolve Oxygen (DO) (ppm).	3.2	-	-
Colour.	Colourless	-	-
Biological Oxygen Demand (BOD) (ppm).	36	50	250
Chemical Oxygen Demand (COD) (ppm).	73	3 – 900	3 – 900
Total Hydrocarbon Content (THC) (ppm).	86.5	50 – 1000	50 – 1000
Cadmium (Cd) (µg/l).	ND	0.005	0.005
Copper (Cu) (µg/l).	0.092	2	2
pH	7.22	6 – 9	6.5 – 8.5
Salinity (PPT)	20	-	-
Temperature (^{0}C)	27	20 ± 2	20 ± 2
Conductivity (µs/cm)	22750	-	-

ND = Not Detected

Percentage of growth inhibition (acute toxicity test) of produced formation water against *S. costatum* within 48 and 72 hours shows that Produced formation water sample caused significant inhibition of the growth of the *Skeletonema costatum* at concentrations above 3.2% for both 48 and 72hours exposure time. 100% inhibition of the growth of the *Skeletonema costatum* by the toxicant were observed from 10% to 56% concentrations of the toxicant.

Table 2: Percentage of growth inhibition of *Skeletonema costatum* by produced formation water.

Concentration of Produced water (%)	Percentage (%) of growth inhibition caused by Produced formation water	
	48 hours	72 hours
1.0	17.40	52.055
1.8	12.23	48.385
3.2	42.4	93.041
5.6	66.002	91.55
10	100	100
18	100	100
32	100	100
56	100	100
Control	0	0

However, the relationship between concentration and effects (% inhibition on probit and concentration of logarithmic-scaled) of produced water against *Skeletonema costatum* within 48 and 72 hours was used to determine

the IC_{50} and IC_{10} effects of produced formation water (PFW) on the growth of *Skeletonema costatum* within 48 and 72hours exposure period. The results of IC_{50} and IC_{10} values from produced formation water against *Skeletonema costatum* within the exposed periods revealed that the 72-hours IC_{50} values was 1.55 ml/l while 72-hours IC_{10} value was 0.34 ml/l indicating high acute toxicity. However, the IC_{50} values for Produced Formation Water for 48 hours was 1.99 ml/l, while 48-hours IC_{10} value was 1.02 ml/l and does not contrast with the report of Aidar *et al*. (1999), who study the acute toxicological effects of produce water from an oil maritime terminal on *Skeletonema costatum.* The lower the IC_{50} value the higher the acute toxicity effects. The IC_{50} value is the maximal inhibitory concentration of the test substances (Produced formation water) that inhibit 50% growths of the tests organisms (*Skeletonema costatum*) within a defined period of time.

Produced Formation water growth inhibition curve (Cell density of *Skeletonema costatum* against time in 48 and 72 hours) reveals that low concentrations of the toxicant can stimulate phytoplankton growth, even at effluent concentrations above the IC_{50} value, after an initial inhibition of *Skeletonema costatum* growth for up to 72 hours, cell division was stimulated at concentration of 1.0, 1.8 and 3.2, but the maximum yield did not surpass that of controls. However, there is apparent difference in growth response of *Skeletonema costatum* in various concentrations of toxicants tested in this study and the patterns of growth show that the produced formation water samples were acutely toxic to the *S. costatum.*

The toxicity of the produced water was found to vary significantly, depending on the concentration and time exposure of *Skeletonema costatum* and the cell growth inhibition increases with increase the in the toxicant concentration and its subsequently in agreement with the study of Stromgren *et al*. (1995) who reported that actual impacts related to marine algae growth inhibition depend on the concentrations that exist over the exposure time found in the environment. The produced formation water (PFW) sample caused significant inhibition of the growth of *Skeletonema costatum* at concentrations above 3.2% for both 48 and 72hours exposure time. 100% inhibition of the growth of the *Skeletonema costatum* by produced formation water were observed from 10% to 56%. Consequently, the lowest observed effect concentration (LOEC) estimates for the exposure times were 1.8%. However, at 48-hours exposure period, the IC_{50} values for *Skeletonema costatum* was 2.69 ml/l, while 48-hours IC_{10} value was 1.07 ml/l and this results are in total agreement with OECD (2000). The lower the IC_{50} value is, the higher the acute toxicity effects (ISO, 2006). Toxicity is attributable to toxic components of produced waters such as alky-phenols, PAHs, MAHs, low molecular weight organic acids, Barium, Mercury (Neff *et al.*, 2011). Toxic effects of the produced formation waters on marine microalgae will unquestionably cause distress in fish abundance and other aquatic resources. Therefore, more efforts should be put in place by oil industry regulatory agency to ensure effective and adequate treatment of produced formation water prior to their discharge into natural water bodies by oil and gas industry operators.

REFERENCES

Aidar, E., Sigaud-Kutner, T.C.S., Bicego, M.C., Schinke, K.P., Gianesella, S.M.F. and Braga, E.S. (1999). Evaluation of produced water toxicity from an oil maritime terminal through Skeletonema costatum toxicity tests. Rev. Bras. Oceanogr. 47(2):137-144.

Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenleib, R. and Giger, W. (2001). Arsenic contamination of groundwater and drinking water in Vietnam: a human health threat. Environmental Science and Technology, 35 (13): 2621-2626.

Finney, D. J., Ed. (1952). Probit Analysis. Cambridge, England, Cambridge University Press.

Frost, T.K, Johnsen, S and Utvik, T.I (1998). Environmental Effects of Produce Water Discharges to the Marine Environment. OLF, Norway. http:/www.olf.no/static/en/rapporter/producewater/ summary.html.

Guillard, R.R.L. (1995). Culture Methods. In: Manual on Harmful Marine Microalgae (eds. G.M. Hellegraeff, D.M. Anderson and A.D. Cembella). IOC Manuals and Guides No. 33. UNESCO. Pp. 45-62.

Hahn, E. D. and R. Soyer. (2008). "Probit and Logit Models: Differences in a Multivariate Realm." http://home.gwu.edu/~soyer/mv1h.pdf

Hamilton, M.A., Russo, R.C. and Thurston, R.V. (1978). Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science Technology 12(4):417-422.

Hasle, G. R. and Syvertsen, E. E. (1996). Marine diatoms. In: Tomas, C. R. (ed) Identifying marine diatoms and dinoflagellates. Academic Press, Inc., San Diego, 385 pp.

Hoshaw, R.W. and J.R. Rosowski. (1973). Methods for microscopic algae. In: Handbook of Phycological Methods: Culture Methods and Growth Measurements. (ed. J.R. Stein). Cambridge University press. London, UK. pp 53-68.

ISO 2006 (International Organization for Standardization) (2006). Water quality—Determination of acute lethal toxicity to marine algae. Draft International Standard. Geneva, Switzerland.

Johnson, K.G., Ghose, A., Epstein, E., Lincecum, J., O'Connor, M.B. and Van Vactor, D. (2004). Axonal heparan sulfate proteoglycans regulate the distribution and efficiency of the repellent slit during midline axon. Journal of Experimental Biology 207(5): 743-74.

Kaur, J., Jain, M., Kaur, T. and Jain, R. (2009). Differential roles played by the native cysteine residues of the yeast glutathione transporter, Hgt1p. FEMS Yeast Research 9 (6):849-66.

Kuehn, R. L., Berlin, K. D., Hawkins, W. E. and Ostrander, G. K. (1995). Relationships among petroleum refining, water and sediment contamination and fish health. Journal of Toxicology and Environmental Health, Part A Current Issues 46 (1), 101-116.

Neff, J. M. (2002). Bioaccumulation in marine organisms: effect of contaminants from oil well produced water. Elsevier pp. 251 - 468.

Neff, J., Lee, K. and DeBlois, E.M. (2011). Produced water: Overview of composition, fates,

and effects. In: Lee, K., Neff, J. (Eds.), Produced Water. Springer, New York.

Newell, G. E. and R. C. Newell (1963). Marine Plankton – A Practical Guide. Hutchison Educational, London, pp. 1-221.

OECD (2010). Review for Organization for Economic Cooperation and Development

Phang, S.M. and Chu, W.L. (1999). Algae culture Collection, Catalogue of Strains. Institute of Post Graduate Studies and Research, University of Malaya, Kuala Lumpur, Malaysia. Pp.77.

Prosser, C.L. (1991). Oxygen: respiration and metabolism. In: Prosser, C.L.(Ed.), Comparative Animal Physiology,4theditionWiley-Liss, New-York, pp.109–165.

Sameeh, A. M., Alia, A. A., Azza, W. I., Neeven, H. M. and Walaa, A. M. (2015). Toxicity of some pesticides, heavy metals and their mixtures to Vibrio fischeri bacteria and Daphina magna comparative study. Journal of Biology and Life Science 6 (2): 2157 – 6076.

Stromgren, T., Sorstrom, S. E., Schou, L., Kaarstad, I., Aunaas, T., 0. G. Brakstad, O.G. and Johansend, O. (1995). Acute Toxic Effects of Produced Water in Relation to Chemical Composition and Dispersion. Marine Environmental Research 40(2):147-169.