A Projection into the Quality of Phytoplankton Consumed by Primary and Secondary Consumers in Areas Exposed to Water Soluble Fractions (Wsf) of Petroleum Products using Protein and Carbohydrate Biomolecules.

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ABSTRACT

***Background:** Plants and algae (primary producers) manufacture their own food which other animals (primary and secondary consumers) now depend on to survive. However, the environment where these primary producers manufacture their food is important in the quality of nutrients supplied along the food chain.*

***Objective:** To ascertain if the phytoplankton in polluted aquatic environments produce quality nutrients for the primary and secondary consumers.*

***Methods:** Pandorinamorum, was used in the study. The ratio of 1:9 of oil to water was used to get the water soluble fraction (WSF) of diesel and kerosene fuel oil respectively. The species was exposed to various concentrations of WSF of diesel and kerosene fuel oils in an experiment that lasted for 14 days. The effects of these WSFs were also tested on the species protein and carbohydrates biomolecules. Absorbance readings were taken using a Jenway Spectrophotometer at 2 days intervals.*

***Results:** Pandorinamorum was inhibited at all concentrations of WSF of diesel oil while WSF of kerosene inhibited growth at higher concentrations of 75% and 100% but stimulated growth at lower concentrations. The biomolecules assessed showed signs of being suppressed by the fluctuations observed throughout the experiment.*

***Conclusion:** Primary and secondary consumers that feed on phytoplankton algae in areas exposed to WSF of petroleum products may not have the best of nutrients from their primary producers in that environment due to poor synthesis of algal biomolecules.*

Keywords: Phytoplankton, Diesel, Kerosene, Biomolecules, Petroleum

INTRODUCTION

Oleivera, *et al.*, defined microalgae (phytoplankton) as plant organisms found in water as well as different wet surfaces and possess no roots, stems or leaves but have chlorophyll as their main photosynthetic pigment (1). They are a reliable source of nutrient as a result of the rich protein content and other substances they possess (2). This has made them gain relevance in animal nutrition and aquaculture. However, increase in water pollution arising from oil spill and other hydrocarbon laden anthropogenic activities are beginning to threaten the quality of biomolecules produced by these primary producers and by extension, the quality of what is consumed by fishes and other biological components of the aquatic environment which depend on them for food. Santos *et al.,* has reported that the toxicity of hydrocarbons can cause damage in DNA of several organisms (3) while Fabregas *et al.,* observed that crude oil affected the chlorophyll *a* content and growth of *Tetraselmissuecica* (4). Kadiri and Eboigbodin (11), observed that *Eudorinaelegans,* was inhibited in all the fuels used at all concentrations (5). Parab*et al.,* also found out signs of toxicity of high concentrations

of crude oil and water soluble fractions (WSF) on *Thalassiosira*sp, with a consequent reduction in the DNA and RNA (6).

Petroleum products are a serious anthropogenic contaminant in the aquatic ecosystem and have affected the phytoplankton community composition (7) and biosynthesis of algal biomolecules (6). Microalgae are rich in biomolecules. These include; protein, lipids, vitamin, carbohydrate, pigments and minerals as well as other small molecules (8).Theses components give the algae the characteristic of being used as a supplement for human and animal feed of which these nutrient contents are rapidly replacing conventional ingredients in animal feeds and aquaculture (9). These nutritional compositions (biomolecules) give energy and strength to the organism (10). The contents of each of these nutrients however vary depending on the physiological responses and strain of the algae (11).

In areas of oil spill in water bodies, inhabitants lament severe loss of aquatic biodiversity, loss of aesthetic value of the water and water pollution. As a result of this pollution, the ability of primary producers to produce food (through photosynthesis) for other species is affected because of the physiological damage the pollutants have done to them. When this happens, adequate food is not supplied in the right measure through the food chain and this is reflected in the quality of what is harvested from the aquatic environment. This is because consumption of microalgae by primary and secondary consumers means consuming the nutrients produced in them.

Since algae are the primary producers of the aquatic ecosystem and are at the base of the food chain, the quality of what they produce has a direct effect on their consumers. This study is therefore vital as it uses the Chlorophyte, *Pandorinamorum* to evaluate the quality of food substances (protein and carbohydrate) produced in algae found in petroleum polluted areas which other biological components of the water body will be consuming.

Materials and methods

Pandorinamorum, which belongs to the family of Volvocaceae, was used in the study. Water sample containing the phytoplankton species was collected using a one liter plastic container, from a fish pond in Ambrose Alli University, Ekpoma. The sample was centrifuged and the water decanted. The phytoplankton concentrate was then washed with distilled water three times by adding distilled water to the test tubes and centrifuging along with the decanting of the water in other to wash of nutrients acquired from the pond water. A ratio of 1:9 of oil to water was used to get the water soluble fraction (WSF) of diesel and kerosene fuel oil respectively following the methods of Anderson *et al.,* (12). 100ml of the petroleum product was added to 900ml of distilled water and stirred with an electric magnetic stirrer for 2hours at 800rpm and allowed to stand in a separating funnel from where the water soluble fraction (WSF) was collected. Concentrations of 0, 10, 25,50,75 and 100% of WSF was made with Chu No 10 algal growth medium (which was sterilized at 120°C for 15mins) and used for the experiment which was set up in triplicate. 5ml of the washed alga (*Pandorinamorum*) was inoculated into the mixture of various concentrations of the two fuel oils in a 200ml glass container and placed under sunlight for 14 days. Growth absorbance reading was taken using a Jenway spectrophotometer at 750nm every two days. Phenol-sulphuric method of Dubois *et al.,* was used in the determination of total carbohydrate (13). Estimation of protein content was done by biuret method of Mahesha, (14).Absorbance readings were taken at 620nm and 540nm for carbohydrate and protein determination and the final concentrations were calculated from their various standard curves.

Toxicity assessment was calculated as follow:

$$\% \text{ Inhibition} = 100 - \frac{\text{Measured biomass} \times 100}{\text{Theoretical biomass}}$$

$$\text{Growth rate: } \mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

N_1 = initial cell density/No

N_2 = final (measured) cell density/No

t_2 = time at end of experiment

t_1 = time at start of experiment(15)

Results

The growth rate of *Pandorinamorum*in WSF of diesel oil is represented in Figure 1. Negative growth rate was observed in 50%, 75% and 100% respectively, a reflection of toxicity of the oil at higher concentrations. Lower concentrations (0%, 10%, and 25%) however showed signs of negative growth rate at different days of the experiment.

Generally, the protein and carbohydrate content did not show any discernible trend of increase in content throughout the study .Rather there are

fluctuations in the content throughout the study. Signs are that there are possibilities of suppression of synthesis of biomolecules in the species by the test oils.

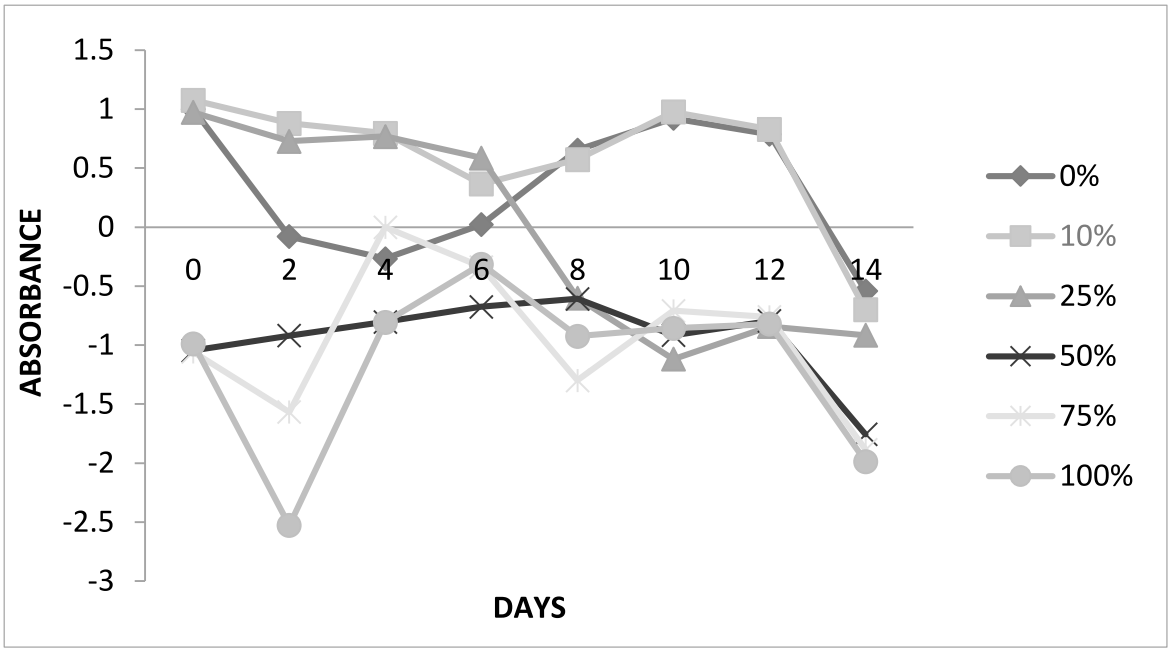

Figure 1: Growth rate *Pandorinamorum* in WSF of diesel

Figure 2 showed reduced growth rate at higher concentrations (75% and 100%) and growth stimulation at lower concentrations (10%, 25% and 50%) including that of the control (0%).

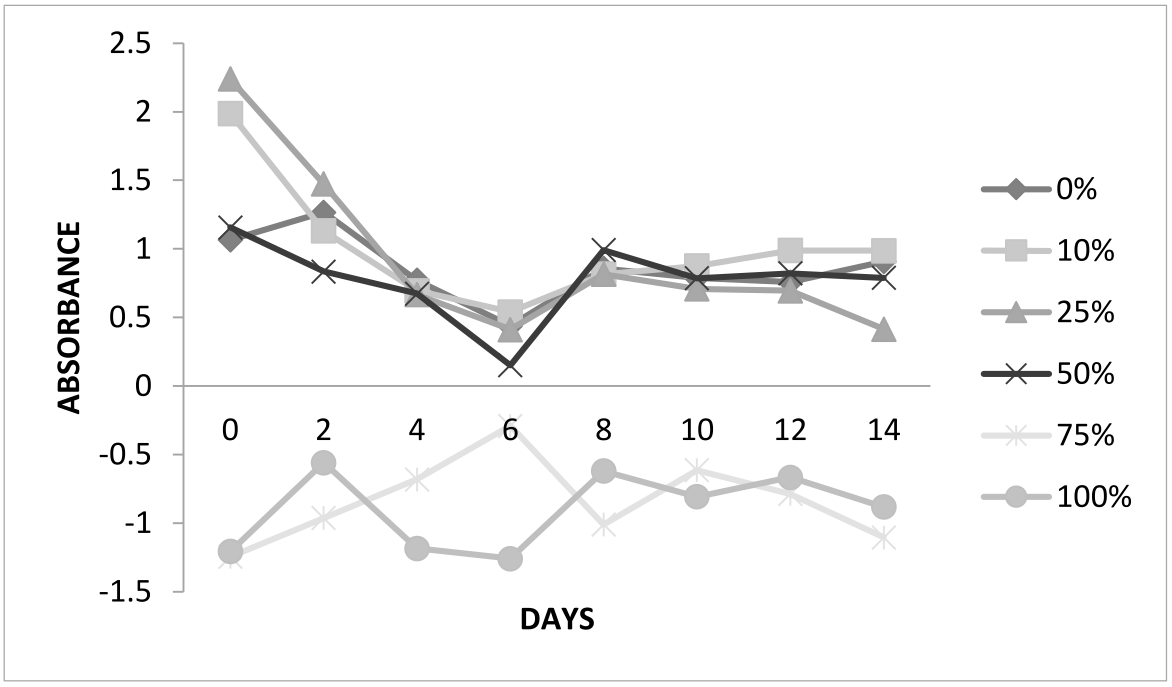

Figure 2: Growth rate of *Pandorinamorum* in WSF Kerosene

Growth was inhibited with increasing concentrations in WSF of diesel oil in figure 3 while growth was stimulated at lower concentrations of WSF of kerosene oil and inhibited at higher concentrations.

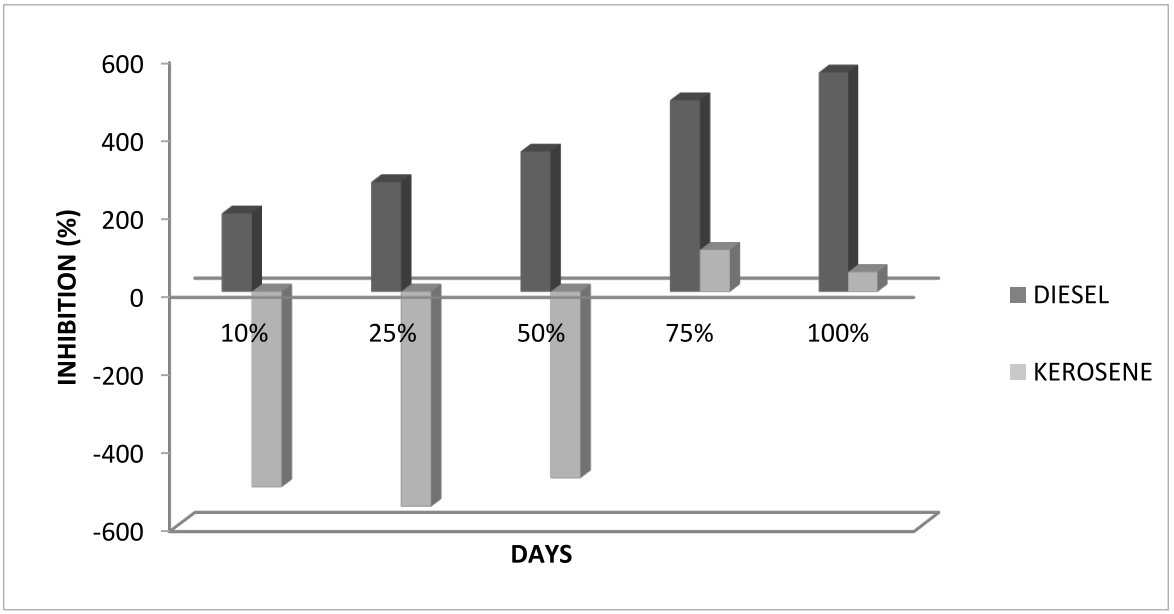

Figure 3: Growth inhibition and stimulation

In figure 4, there was a reduction in carbohydrate content in all concentrations of WSF of diesel oil at day 14 except for an increase in the control.

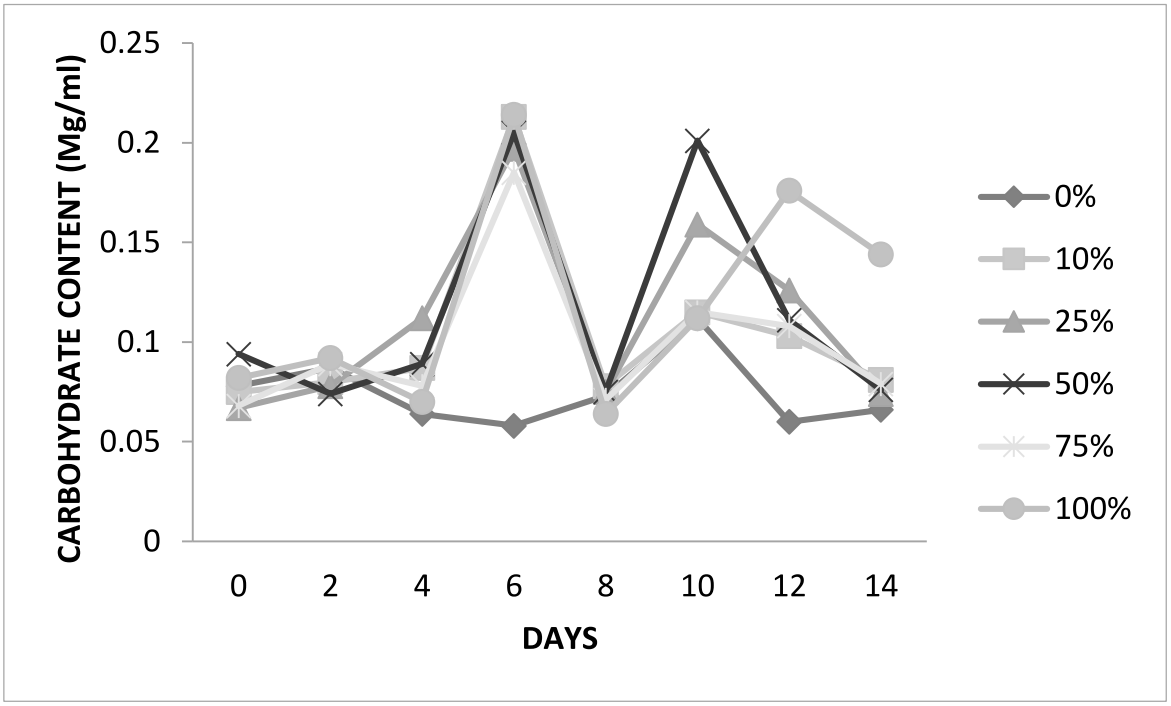

Figure 4: Carbohydrate content in WSF of diesel oil

In figure 5,protein content in WSF of carbohydrate showed a decrease in content from day 2 till day 6.There was a slight increase in day 8 followed by a decrease in all concentrations at day 14 except for control and 10% concentration.

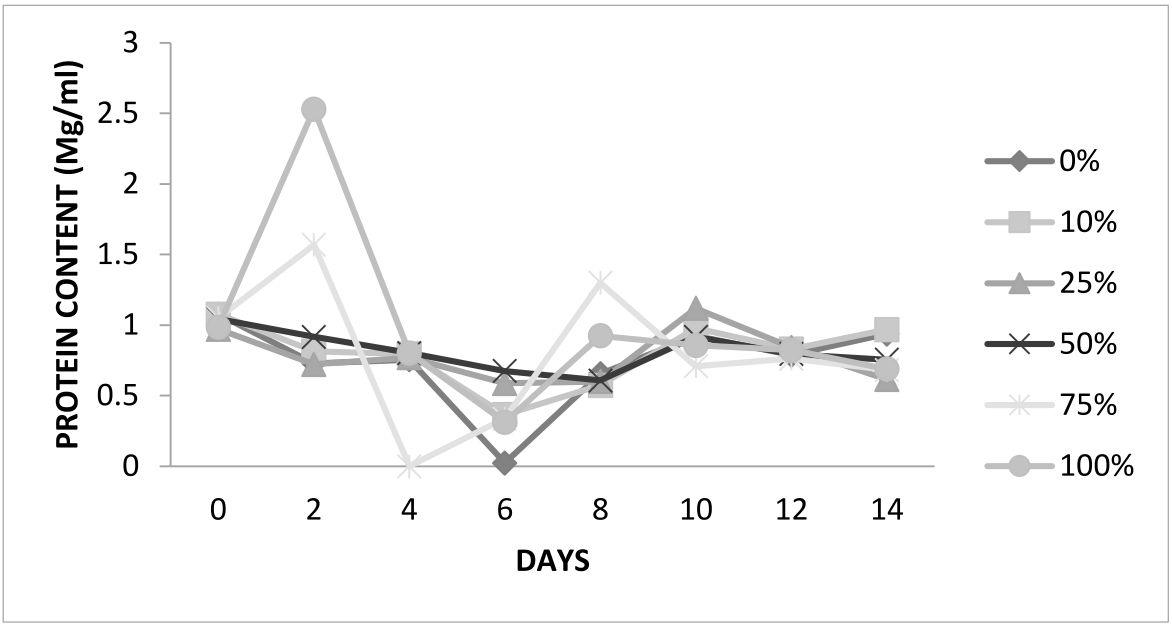

Figure 5: Protein content in WSF of diesel oil

In figure 6, there were variations in concentrations of carbohydrate in WSF of kerosene oil with a decrease in day 14 except for the control (0%) and 75% which showed a slight increase.

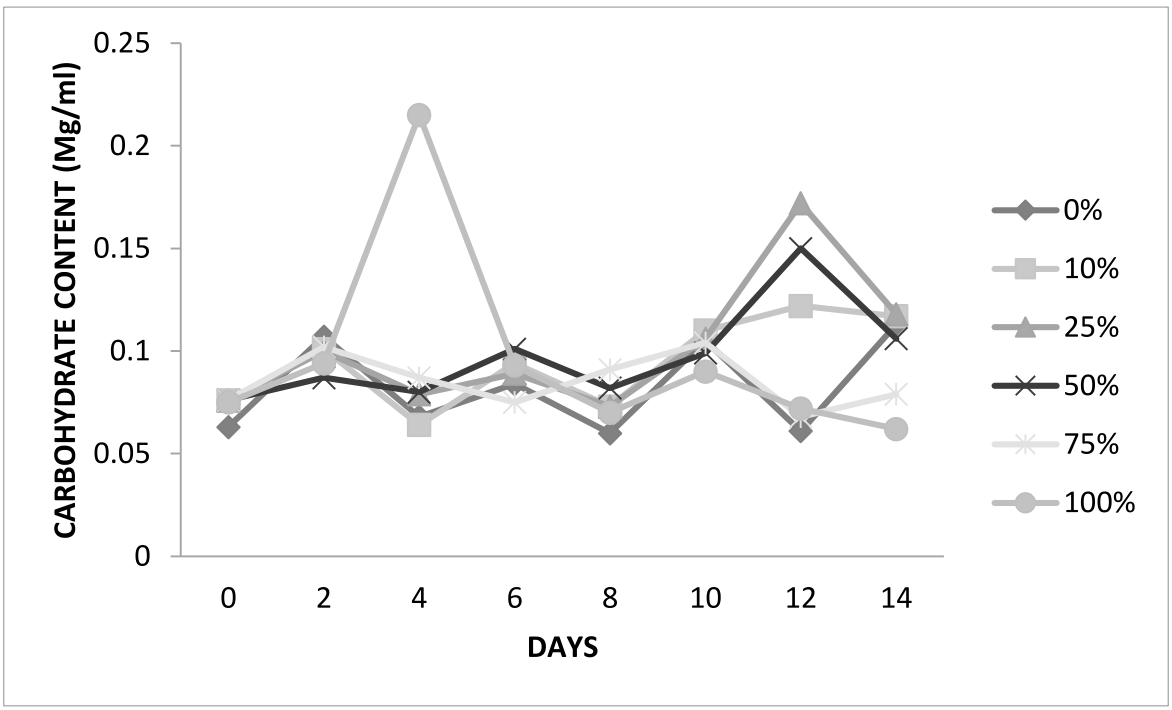

Figure 6: Carbohydrate content in WSF of kerosene oil

In figure 7,all concentrations including the control showed increase in protein content in WSF of kerosene at day 14 after an initial decrease from day 0 to day 4 followed by fluctuations.

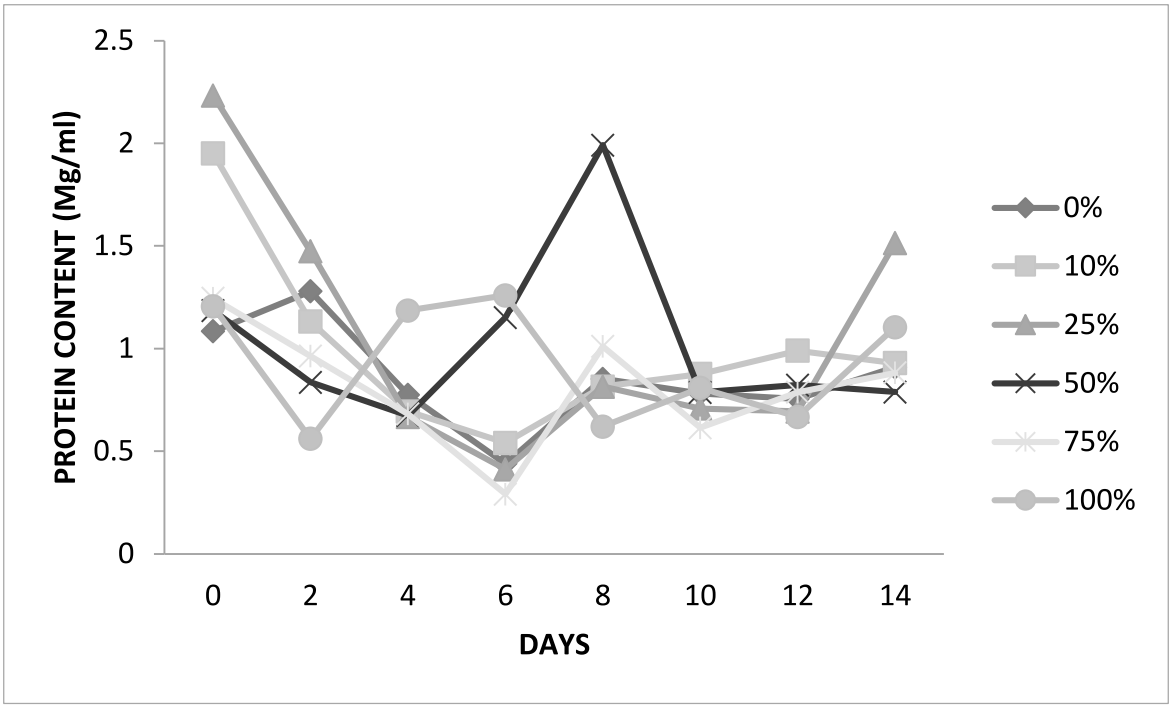

Figure 7: Protein content in WSF of kerosene oil

Discussion

*Pandorinamorum*exhibited different growth responses to both fuel oils. While there was growth inhibition with increasing concentration of the WSF of diesel oil, there was growth stimulation at 10%, 25% and 50% concentrations of WSF of kerosene oil with growth inhibition only observed at 75% and 100% higher concentrations. Such different responses in one fuel oil have been documented by Bhattaecharjee and Fernando, with *Chaetoceroscalcitrans* on diesel WSF (16). Observed inhibition of the WSF of diesel fuel oil on the Chlorophyte*Eudorinaelegans* has been reported by Kadiri and Eboigbodin (5). According to Bretherton, many Chlorophytes are sensitive to oil and many may not survive till the end of the study or even recover during the period of the study (17). Al Obaidy and Lami, corroborated this observation when they stated in their study that diesel fuel and crude oil disrupt membrane of phytoplankton species and thereby affect membrane fluidity (7). El-Sheekh*et al.,* also highlighted the possibility of growth inhibition at high concentrations (18).Growth stimulation which was observed at low concentrations of kerosene is in agreement with the findings of Kadiri and Eboigbodin and Al Obaidy and Lami, that microalgae are able to utilize WSF as a carbon source for photosynthesis(5) and (7). Inhibition of *Pandorinamorum* at all concentrations of diesel oil and higher concentrations of kerosene could have been as a result of free radicals and oxidative stress as a result of its exposure to oils (19). Along this same line, Barkia*et al.,* stated that when reactive oxygen species (ROS) produced during normal metabolism are not neutralized by cell constituents, they can bring about death or damage of cells by stimulating free radical chain reactions (11).

Observations of the biomolecules (protein and carbohydrate) studied did not follow the trend of growth rate of the species in both fuel oils where negative growth rates were recorded in higher concentrations for the species. Instead there were fluctuations in protein and carbohydrate content with no discernible increase in the content of the biomolecules till the end of the experiment; an indication that apart from inhibition of the algal growth at high concentrations, the fuels also have the tendency to suppress biosynthesis of biomolecules. The effects of toxicity of WSF of petroleum oils and acute toxicity of hydrocarbons on biomolecules of phytoplankton have also been reported by other authors; Santos *et al.,*

reported that the acute toxicity of hydrocarbons can cause serious damage to DNA of several organisms (3). Al Obaidy and Lami, discovered that protein content of *Chlymadomonasangulosa* reduced when exposed to aqueous extract in batch culture (7). Winter *et al.,* pointed out that the rate of photosynthesis of *Trichodesmium*sp. was depressed when exposed to WSF from a mix of four Exxon fuel oils in their investigation (20). Bott and Rogenmuser, went further to reveal in their findings that chlorophyll concentrations of attached algal community were reduced when exposed to extract of no.2 fuel oil (21). This they opined will have implications on food web as the effect on primary producers will affect productivity of species of commercial importance. Parab*et al.,* supported this by stating that oils at high concentration could alter food webs and energy pyramids (6). According to Al Obaidy and Lami, there was inhibition of DNA and protein synthesis as well as starch synthesis reduction in *Scenedesmusarmatus*when exposed to different concentrations of dispersant (DP-105) and their mixtures (7). Morales-Loo and Goutxand O'Brien and Dixon reported that WSF can disrupt chlorophyll-*a*, lipid pigment and glycolipids formation to bring about accumulation of sterol (22) and (23). The possibility of hydrocarbon contamination from anthropogenic sources to accumulate in aquatic species and produce deleterious consequences in the natural ecosystem have also been pointed out by Kadiri and Eboigbodin (5). Ruess and Muller-Navarra, underscored the importance of biomolecules when they revealed the essential role biomolecules play in trophic interaction across taxa in the aquatic environment (24). They stated that the availability of biomolecules at the base of the food web determines the mode of trophic transfer and the structure of the food web. Therefore, the quality of biomolecules synthesized by phytoplankton is important to the wellbeing of the biodiversity in the aquatic and terrestrial ecosystems. The role of phytoplankton (primary producers) in the food web is to supply protein, carbohydrate and mineral salts to primary consumers like zooplankton which are in turn consumed by fishes (secondary consumers) like *Clarias* and *Alestes*. In this process, energy is transferred from one level to the other. This flow could be devoid of its energy and nutrient or disrupted if the synthesis of biomolecules is suppressed by WSF of petroleum products or other toxic pollutants. The economic importance of algae in the aquatic environment cannot be over emphasized. The flow of energy could be stalled by their absence and if still present but exposed to pollutants, the availability of quality protein and carbohydrate they produce as primary producers to sustain aquatic biodiversity of commercial interest may no longer be guaranteed.

Conclusion

From the study, it shows that firstly,*Pandorinamorum* cannot be employed as a species for bioremediation in fuel oil polluted areas. Secondly, primary and secondary consumers that feed on phytoplankton algae in areas exposed to WSF of petroleum products may not have the best of nutrients from their primary producers in that environment due to poor synthesis of algal biomolecules. This then puts to question the quality of what is consumed in the entire food chain of that habitat. Phytoplankton (microalgae) are rich sources of protein, carbohydrates, lipids, vitamins and other molecules. The presence of these bioactive compounds makes them relevant in feeds, food and industries. It is therefore projected that if more oil pollution or WSF of petroleum products are on the rise in the aquatic environment, the algae in the environment will be affected and consequently, the production of their biomolecules. This will have an adverse effect on the primary and secondary consumers which depend on phytoplankton for nutrient and energy.

Recommendation

It is recommended that water bodies affected by WSF of petroleum products should begin to undergo remediation and laws should be enacted and enforced to ensure that petroleum products and other toxic pollutants don't find their way into the water body or if possible, reduced drastically. More so, more research should be carried out using other organic and inorganic pollutants in other to compare their effects on other biomolecules to ascertain if other pollutants can slow down their synthesis.

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References

1. Oleivera, O.,Gianesella, S., Silva, V., Mata, T, Caetano, N. (2017). Lipid and carbohydrate profile of a microalga isolated from wastewater Energy. Procedia, 136: 468-473

2. Barka, A. and Blecker, C. (2016). Microalgae as a potential source of single-cell proteins. A review. Biotechnol. Agron.Soc. Environ, 20 (3):427-436

3. Santos, C.A., Lenz, D., Brandao, G.P., Chippari-Gomes, A.R. and Gomes, L.C. (2013). Acute toxicity of the water-soluble fraction of diesel in *Prochilodusvimboides*Kner (Characiformes:Prochilodontidae). Neotropical Ichthyology, 11(1):193-198

4. Fabregas,J., Herrero, C. and Veiga, M.(1984). Effect of oil and dispersant on growth and Chlorophyll *a* content of the marine microalga *Tetraselmissuecica*. Applied and Environmental Microbiology, 47 (2):445-447

5. Kadiri, M.O. and Eboigbodin, A.O. (2012).Phytotoxicity assessment of water soluble fractions of refined petroleum products using microalgae. ActaBotanicaHungarica,54 (3-4): 301-311

6. Parab,S.R., Pandit, R.A., Kadam, A.N., Indap, M.M. (2008). Effect of Bombay high crude oil and its water-soluble fraction on growth and metabolism of diatom *Thalassiosira* sp. Indian Journal of Marine Sciences, 37(3):251-255

7. Al Obaidy, A.H.M.J. and Lami, M.H.M. (2014). The toxic effects of crude oil in some Freshwater Cyanobacteria. Journal of Environmental Protection, 5:359-367

8. Rajvanshi, M., Sagaram, U.S., Subhash, G.V., Kuma, G.R.K., Kumar, C., Gorvindachary, S. and Dasgupta, S. (2019).Biomolecules from microalgae for commercial applications. Taylor and Francis.38p

9. Yaakob, Z., Ali, E., Zainal, A., Mohamade, M. and Takriff, M.S. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. Journal of Biological Research- Thessaloniki, 21 (6)

10. Mondal, S. (2018). Biomolecules. Lecture notes. Biochemistry-II (BP203T). B.Pharm-II Se. GITAM

11. Barkia, I.,Nazamid, S. and Manning, S.R.(2019). Microalgae for high-value products towards humanhealth and nutrition. Mar. Drugs, 17:304

12. Anderson, L.W.,Neff.J.M.,Cox, B.A.,Tatem, H.E.and Hightower, G.M.(1974). Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to Estuarine crustaceans and fish. Marine Biol, 27:75-88

13. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.J, (1956). Colorimetric method for determination of sugar and related substances. Analytical Chemistry. 28(3): 350-356.

14. Mahesha, H.B. (2012). Estimation of protein by biuret method. Yuvaraja's College, University of Mysore, Mysore. 2p

15. Opute, F.I., Nwankwo, D.I., Kadiri, M.O., Ogbeibu, A.E. and Chia, M.A. (2015). Third annual training workshop on algal biotechnology manual. Benin City, 72p

16. Bhattaecharjee, D. and Fernando, O.T.(2008). Short-term studies on the effect of water soluble fraction of diesel on growth of *Chaetoceroscalcitrans*,Paulsen. Res. J.Environ.Toxicol. 2:17-22

17. Bretherton, L., Williams, A., Genzer,J., Hillhouse, J.,Kamalanathan, M., Finkel, Z.V.and Quigg, A. (2018). Physiological response of 10 phytoplankton species exposed to Macondo oil and thedispersant, corexit..J.Phycol. 54:317-328

18. El-Sheekh, M.M., El-Naggar, A.H., Osman, M.E.H. and Haieder, A. (2000). Comparative studies on thegreen alga Chlorella homospaera and Chlorella vulgaris with respect to oil pollution in the River Nile.Water, Air and Soil Pollution,124:187-204

19. Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D. and Show, P. (2019). Microalgae: A potential alternative to health supplementation for humans. Food Science and Human Wellness,8:16-24

20. Winter, K..Baalen, C.V. and Nicol, J.A.C. (1977). Water soluble extractives from petroleum oils: chemicalcharacterization and effects on microalgae and marine animals. Reun. Cons. Int. Explor. Mer. 171:166-174\

21. Bott, T.L. and Rogenmuser, K. (1978). Effects of No.2. fuel oil, Nigeria crude oil and used crankcase oil onattached algal communities: acute and chronic toxicity of water-soluble constituents.Appl.Environ. Microbiol, 36 (5):673

22. Morales-Loo, M.R. and Goutx, M. (1990). Effects of water soluble fraction of the Mexican crude oil"Isthmus Cactus" on the growth, cellular content of chlorophyll-a and lipid composition ofplanktonic microalgae. Marine Biol., 104:503-509

23. O'Brien, P.Y. and Dixon, P.S. (2016). The effects of oils and oil components on algae: A review.British Phycological Journal, 11(2):115-142

24. Ruess, L. and Muller-Navarra, D. C. (2019). Essential biomolecules in food webs. Frontiers in Ecology and Evolution, 9:1-18