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BIOCHEMICAL ALTERATIONS IN BAY SCALLOPS (*PLACOPECTEN MAGELLANICUS*) EXPOSED TO CRUDE OIL

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ABSTACT

This study was conducted to examine the biochemical alterations in the muscle and digestive system of the bay scallop (*Placopecten magellanicus*) exposed to 0.00, 2.50, 5.00 and 10.00 mL/L crude oil concentrations. The scallops were taken out of the toxicant media at intervals of 3, 6 and 24 hours for analysis. The parameters monitored were total protein (TP), total cholesterol (CHOL), creatinine (CREAT), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in the muscle and digestive systems of the organisms. The results showed that the metabolites (TP, CHOL and CREAT) fluctuated in the exposed concentrations and at the different times of examination. The trends of variation in both tissues of the animal were irregular and CHOL the lowest of the parameters recorded. However, the enzymes (AST, ALT and ALP) showed a general increase in activity in the various exposure concentrations, though such increase was not concentration and time dependent. The most notable increase in activity was observed in ALP in the digestive system at the 6 hours interval in 2.50 mL/L concentration which was 155.34 ± 3.58 IU/L. The results indicated that exposure of scallops to crude oil contamination or pollution caused some biochemical alterations that were tissue specific.

KEYWORDS: Bay scallops, crude oil, pollutants, muscle, Digestive system.

INTRODUCTION

Different chemical substances have been released into the aquatic environment and have been found to cause hazardous effects in marine and freshwater organisms. Most of these substances have the ability to accumulate in water dwelling organisms.[1] Crude oil or petroleum hydrocarbon exploration, exploitation, development and production activities produce environmental risks and impacts. Of most concern is the impact on fisheries and wildlife of not only accidental discharges resulting from blowouts or spills but also from permissible discharges of operational wastes such as drilling mud, cuttings and drilling water.[2]

Oil products are among the pollutants that are widely distributed in the marine habitat and the hydrocarbons are well known primary source of persistent toxicity in the aquatic environment.[3] Crude oil is regarded as a common contaminant throughout the marine environment the world over and at least about 20,000 tons per year enter the marine environment from offshore oil production alone.[4]

The physical nature of oil and the chemical components of oil enhance or influence the type and rate of the effect of oil on aquatic animals. Also, aquatic lives may be affected by clean-up operations or indirectly through physical damage to the habitats where plants and animals inhabit.[5] When oil spill occurs, it spreads immediately to cover the surface, while some of the components dissolve in water, others are degraded by bacteria or undergo oxidation and the degraded products eventually sink to the bottom sediment through gravitational force to pose threat to bottom dwelling organisms and bottom feeders such as the mollusk[6]

Crude oil or petroleum has been found in many studies to cause different biochemical alterations in aquatic organisms such as: the alteration of gill functions and ciliary activities of marine bivalve, *Venus verrucosa*[7], change in gill histopathology in *Chanos chanos*[8], reduced androgen levels in salmon and flounder[9], liver enlargement and depletion of reduced glutathione in rainbow trout[10] and oxidative stress in bivalves[11]

Bivalves and mollusks are sediment dwelling organisms and also bottom feeders, which move slowly or sluggishly. Due to their nature, moving away from a polluted environment is almost an impossible task. Thus they are the major targets of environmental pollution, especially during massive oil spill. Therefore, the environmentalist can use them as biomonitors and bioindicators of environmental pollution and toxicological studies.

This study therefore was carried out to examine the changes in the Biochemistry of the organs of scallops (*Placopecten magellanicus*), a bottom dwelling bivalve that belongs to the class of mollusks.

MATERIALS AND METHODS

Scallops (*Placopecten magellanicus*) of mean weight 20.79 ± 5.20 g were handpicked at low tide from the Kaa-Andoni axis of the Bonny river in Khana and Andoni Local Government areas of the Rivers State. They were transported in plastic buckets to the Chemistry Department Laboratory of the Rivers State University of Science and Technology Port Harcourt. One hundred apparently healthy scallops were acclimated to laboratory conditions in plastic tanks of thirty-liter capacity. The tanks were half filled with brackish water and sediments collected from same source. The acclimation was done for 24 h. The substrate was prepared by air-drying the sediment before being macerated in a mortar and sieved in a 2 mm mesh.

About 250 g of finely prepared sediment were put into each of the plastic tanks to serve as the substrate base. Completely randomized design (CRD) was used for the experiment. The experiment was divided into three treatment levels with three replicates and a control. The test media were prepared in the following concentrations: 2.50 mL/L, 5.00 mL/L, 10.00 mL/L and a control (0.00 mL/L) of crude oil. Ten of the test animals were introduced into each of the toxicant media.

Samples from the organs of the scallops were removed at the intervals of 3, 6 and 24 hours by separating or opening the valve into two and the organs of interest removed. About 0.5 g of each of the removed organs were finely ground and mixed with 5 mL 0.8 % perchloric acid for metabolites (total protein, cholesterol and creatinine) determination. The acid organ mixture was then centrifuged at the rate of 3000 rpm and then decanted into plain bottles. Another 0.5 g sample of the organs were removed and finely ground or homogenized and mixed with 5 mL physiological saline for enzyme (AST, ALT and ALP) assay.

The samples were then sent to Lively Stones Medical Diagnostic Laboratory Choba, Port Harcourt for the determination of total protein, cholesterol and creatinine. Total protein was estimated according to[12]) method, while cholesterol was estimated according to.[13] The estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity was done using the Reitman and Frannkel,[14] method for the quantitative *in-vitro*

determinations in serum using Randox laboratory test Kit (Antrim, UK). Alkaline phosphatase was determined by the method of Jahanbakhshi and Hedayati[15]

The data obtained were subjected to analysis of variance (ANOVA) to determine if significant differences existed between the means in the mortality at different levels of contamination. Where differences existed, Duncan's multiple range test (DMRT) was used to compare the means.[16]

RESULTS

The results of the total protein content in the muscle of the bay scallops were either slightly lower or equal to the value of the control (10.00 ± 0.00). However, the lower values were about 9.50 g/dl. In the digestive system of the bay scallops, the control value was 9.83 ± 0.53. Slight variations that were either higher or lower than the control value were observed. The higher values were about 10.00 g/dl and the lower values were about 9.50 g/dl (Table 1).

The total cholesterol levels in the muscle in the bay scallops were slightly higher in all the test concentration in the 24th hour and in the 3rd hour at 10.00 ml/L concentration. However, lower values were observed in 2.50 mL/L concentration in the 3rd and 6th hour and 10.00mL/L in the 6th hour. In the digestive system of the scallops, increase in values was observed only in the 3rd hour interval in 2.50 mL/L concentration and the 24th hour in the 5.00 and 10.00 mL/L respectively when compared to the control value, which was 0.93 ± 0.22 mmol/L (Table 2).

The creatinine levels in the muscle of the bay scallops were only higher than the control value in 2.50 ml/L and 10.00 mL/L in the 6th hour which were 13.50 ± 2.10 and 13.50 ± 1.98 g/dl and in the 24th hour in the 5.00 mL/L concentration which was 8.50 ± 1.23 g/dl as against the control value of 7.50 ± 1.87 g/dl. Lower values were obtained in all other concentrations at the various time intervals. In the digestive system of the bay scallops, higher values than those of the control (7.50 ± 0.28 g/dl) was observed in all the test concentrations in the 3rd hour interval. Other higher values than the control were observed in the 24th and 6th hour of the 2.50 and 5.00 mL/L concentrations respectively. All other observations were lower than the observed control value (Table 3).

The activity of AST in the muscle of the scallops were higher than that of the control in all the exposure concentrations, but were not time and concentration dependent. However, some

of the increases were not significant (P > 0.05). In the viscera, significant (P > 0.05) increase in activity was only observed at 2.5 mL/L (24th hour), 5.00 mL/L (3rd and 24th hours) and 10.00 mL/L in the 3rd hour (Table 4).

The activities of ALT in the muscle were all significantly (P > 0.05) higher than the control value in the 2.50 mL/L concentration at the different time intervals. In the 5.00 mL/L concentration, significant (P > 0.05) increases were observed at the 3rd and the 6th hours. In the 10 mL/L concentration, a significant (P > 0.05) increase was only observed in the 6th hour. In the digestive system, ALT activity was maintained as was in the control in the 3rd hour (2.5 mL/L) concentration and increased significantly (P > 0.05) in the 6th hour and then decreased (however, higher than the control) in the 24th hour. There were increases in activities in the 5 mL/L and 10mL/L in the 3rd hour of exposure. However, decreases to almost normal values were observed at the 6th and 24th hour intervals in both concentrations except 5.00 mL/L where a sharp increase was observed in the 24th hour (Table 5). The activities of ALP were all significantly (P>0.05) higher than the control value in all the exposure concentrations at all the time intervals in both the muscle and the digestive system of the scallop (Table 6).

Table 1: Total protein in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle protein (g/dl)			Digestive System protein (g/l)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00 (control)		10.00 ± 0.00^{a}			9.83 ± 0.53^{a}	
2.50	9.50 ± 1.03^{ab}	9.50 ± 0.53^{ab}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	9.50 ± 1.22^{ab}	10.00 ± 0.00^{a}
5.00	9.50 ± 0.93^{ab}	9.50 ± 0.66^{ab}	10.00 ± 0.00^{a}	9.50 ± 1.95^{ab}	9.50 ± 1.62^{ab}	10.00 ± 1.25^{a}
10.00	10.00 ± 0.00^{a}	9.50 ± 1.43^{ab}	9.50 ± 2.01^{ab}	9.50 ± 1.66^{ab}	9.50 ± 0.28^{ab}	10.00 ± 0.63^{a}

Figures with the same superscipt are not significantly different (P>0.005).

Table 2: Total cholesterol in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle cholesterol (mmol/L)			Digestive System cholesterol (mmol/l)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00 (control)		0.90 ± 0.01^{ab}			0.93 ± 0.22^{a}	
2.50	0.85 ± 0.11^{ab}	0.85 ± 0.03^{ab}	0.95 ± 0.10^{a}	1.00 ± 0.03^{a}	0.85 ± 0.02^{b}	0.90 ± 0.03^{b}
5.00	0.90 ± 0.03^{ab}	0.90 ± 0.07^{ab}	1.00 ± 0.00^{a}	0.85 ± 0.00^{b}	0.90 ± 0.04^{b}	1.00 ± 0.00^{a}
10.00	1.05 ± 0.20^{a}	0.85 ± 0.04^{ab}	0.95 ± 0.06^{a}	0.90 ± 0.16^{b}	0.80 ± 0.10^{bc}	1.00 ± 0.00^{a}

Figures with the same alphabet are not significantly different (P>0.005).

Table 3: Creatinine content in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle creatinine (g/l)			Digestive System creatinine (g/l)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00		7.50±1.87^{b}			7.50±0.23	
2.50	5.50 ±1.11^{bc}	13.50±2.10^{a}	5.50 ±0.51^{bc}	11.00±1.64^{a}	5.50±0.00^{b}	13.50±2.03^{a}
5.00	5.50±1.44^{bc}	5.50±0.29^{bc}	8.50±1.23^{b}	11.00±2.10^{a}	8.50±0.43^{ab}	5.50±0.00^{b}
10.00	5.50± 1.33^{bc}	13.50±1.98^{a}	5.50±1.07^{bc}	8.50±1.03^{ab}	5.50±0.04$_{b}$	5.50±0.11^{b}

Figures with the same alphabet are not significantly different (P>0.005).

Table 4: Aspartate transaminase (AST) in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle AST (IU/L)			Digestive System AST (IU/L)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00		35.00±0.03^{b}			35.00±0.14^{b}	
2.50	50.12 ±3.13^{a}	50.11±5.29^{a}	35.22 ±2.10^{b}	35.00±3.22^{b}	35.12±0.20^{b}	50.54±1.21^{a}
5.00	35.23±2.26^{b}	50.13±0.18^{a}	35.32±4.23^{b}	50.36±5.56^{a}	35.45±0.19^{b}	50.42±3.02^{a}
10.00	50.12± 1.09^{a}	35.44±3.22^{b}	50.16±3.11^{a}	50.55±7.45^{a}	35.30±2.61^{b}	35.31±1.70

Figures with the same alphabet are not significantly different (P>0.005).

Table 5: Alanine transaminase (ALT) in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle ALT (IU/L)			Digestive System ALT (IU/L)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00		20.02±0.03^{c}			20.05±1.80^{c}	
2.50	60.12 ±7.31^{a}	40.38±0.24^{b}	40.51 ±2.66^{b}	20.00±3.29^{c}	60.01±4.35^{a}	40.04±0.37^{b}
5.00	40.32±0.15^{b}	40.45±3.32^{b}	20.33±4.53^{c}	40.37±0.02^{b}	20.00±0.03^{c}	60.45±7.22^{a}
10.00	20.13± 0.54^{c}	40.66±1.06^{b}	20.00±3.43^{c}	40.12±6.05^{b}	20.00±0.32^{c}	20.00±1.55^{c}

Figures with the same alphabet are not significantly different (P>0.005).

Table 6: Alkaline phosphatase (ALP) in muscle and digestive system of scallops exposed to different concentrations of crude oil at different time intervals.

Concentration of crude oil (mL/L)	Muscle ALP (IU/L)			Digestive System ALP (IU/L)		
	3hrs	6hrs	24hrs	3hrs	6hrs	24hrs
0.00		36.67±1.11^{d}			41.50±4.89^{d}	
2.50	46.50 ±0.03^{cd}	65.30±8.21^{b}	55.33 ±6.43^{c}	65.18±6.44^{c}	155.34±3.58^{a}	95.01±6.90^{b}
5.00	46.50±0.34^{cd}	80.61±4.67^{ab}	85.00±4.55^{a}	55.43±3.76^{cd}	85.17±5.08$_{bc}$	65.12±6.81^{c}
10.00	55.21± 4.32^{c}	80.66±7.22^{ab}	85.60±8.07^{a}	46.50±6.13^{d}	70.53±5.20^{bc}	85.10±6.25^{bc}

Figures with the same alphabet are not significantly different (P>0.005).

DISCUSSION

The contamination of water bodies by crude oil and its associated products has been found to have toxic effects on organisms on contact.[17] Though the presence of these contaminants in

water may not lead to death or mortality of the organism in contact, yet may have significant effect on the organism later and consequently alter the physiological and biochemical functions of the organism. This alteration results from stress induced reactions.[18,19,20,21]

Proteins naturally is composed of globulins, fibrinogens and albumins and these are responsible for carrying vital functions such as the distribution of important materials from one part of the body to another during circulation. Protein also possess or exhibit transporting, nutritive, protective, buffering and energetic properties or functions in living organisms.[22] The assessment of protein is often used as part of laboratory diagnosis to determine the extent of wellness of an organism and also reveals varying underlying physiological conditions of cells and tissues especially in asymptomatic patients.[23,24] Reports of changes in protein content of organisms after exposure to toxicants have been documented.[22,24,25]

In this study, general decrease was observed in the protein content in the scallop (*Placopecten magellanicus*), which was not time and concentration dependent. However, slight increases were observed in some of the concentrations. Inhibition of protein synthesis observed in this study is similar to those observed in other studies with petroleum products[24,26,27] and other toxicants such as pesticides.[22,28]

According to [29], decrease in total protein content arises from the degradation and utilization of degraded products for metabolic processes with a resultant increase in free amino acids due to the decrease incorporation of amino acids in protein synthesis. High protein hydrolytic activity due to increased protease activity leads to decreased protein levels and a corresponding elevation in total free amino acids.[30] Protein reduction in the face of toxicant assault may be the blocking of protein synthesis, denaturing of protein, the inhibition of amino acid production or synthesis by the toxicant [31] which in this case is the crude oil. However, amino acids are the building blocks of proteins which when inhibited will result in decreased protein content of the organism. Reaction or interaction of the protein molecules with the components of the crude can also cause reduction in the protein content. Cell and tissue injury and the impairment of certain tissue functions can also be responsible for decreased protein content.[32] Pollutants such as petroleum products and pesticides can cause pollution induced apoptosis [33] which causes adverse effect on protein synthesis. Toxicant induced stress, overhydration, homeostatic balance and change in water equilibrium can

cause a change in the functions of vital cells responsible for protein synthesis [34,35,36] can also be responsible for the reduced total protein content observed in this study.

Proteins serve majorly in the building of cell structure and protection of the body against invaders to keep the immune system intact, therefore interference with the protein content as was observed in this study is consequentially detrimental to the organism.

There were both increase and decrease in the level of cholesterol in the tissues of the mollusk (*Placopecten magellanicus*) in this study. Cholesterol is a lipid and forms the building blocks for vitamin D and steroid hormones. It is found in progesterones, estrogens, testerones and their derivatives.[37,24] observed similar trend when they exposed *Tympanotonus fuscatus* to diesel contaminant.[27] observed both increase and decrease in cholesterol content in albino wistar rats fed *Ocimum gratissimum* after diesel induced hepatoxicity.[38] reported an increase in the levels of cholesterol in nile tilapia (*Oreochromis niloticus*) exposed to cypermethrin, lead and copper.

Increase in cholesterol level in the tissue as observed in some of the concentrations is a physiological response or adaptation to modulating fluidity and membrane packing.[24,39] The role of building and maintaining the structure of the organism can as well be sustained through the reduction of the permeation of tissue membrane to neutral solutes.[40] Accordingly, the increase in cholesterol will hinder its breakdown to the derivatives which may be less poisonous or other beneficial component to the organism thereby constituting toxicity.[24] Increase in tissue cholesterol can also arise from its absorption from haemopoietic (blood) tissues in the organism.

In humans, elevation of cholesterol is found to be a risk factor for coronary heart disease.[41] The increase is related to a situation known as lipolysis, which is controlled by norepinephrine, released which then interferes with the intracellular functions of Ca^{2+} in the cytoplasm.[42]

However, decline in cholesterol content may have resulted from the use of cholesterol and other lipids, the inhibition of its synthesis or injury in the tissues that have caused them to leak out.[24] According to[43], changes in cholesterol level can be caused by pesticides, which interfered with the permeability of hepatic (liver) cells and the resultant accumulation of the pesticide in the liver or other organs thereby disrupting their physiological functions.

Creatinine is a chemical generated from the metabolism of the muscle and is excreted by the kidneys.[44,45] When kidney functions are impaired, there is an expected rise in the level of creatinine. Creatinine is a metabolite of creatine. The concentration of creatinine in the plasma is an indication of skeletal muscle mass.[46] It is normally discharged or removed from the body after filtration of the glomeruli.[45] The exposure of the scallop or mollusk (*Placopecten magellanicus*) to crude oil produced fluctuations in the levels of creatinine in the muscle and digestive system of the mollusk (*Placopecten magellanicus*) studied. The increase and decrease in the levels of creatinine both constitute toxicity of crude oil to the organism.

According to[45], high levels of creatinine indicate substantial damages to the kidney and if the condition is allowed to continue, it will lead to end stage renal failure. They observed that after a long time of irreversible occurrence, the damaged cells will experience a diseased condition known as phynotypic transformation which stimulates fibroblasts and then convert to myofibroblasts. However, the organism in question do not possess well developed kidneys but may have other organs/ tissues responsible for such roles. Therefore, the rise in creatinine can be argued to affect such organs or cells responsible for the performance of such functions as the kidney in the animal. [47] carried out some investigation in Japanese men. The sick men were compared to normal men. They observed low creatinine in the sick ones and observed that severe cases of low creatinine are related to type 2 diabetes.

In this study, crude was found to have induced varying degree or changes in enzyme activities on the mollusk (*Placopecten magellanicus*) similar to those observed in similar studies with mollusk and other animals.[15,21,24,48,49] Though the activities of AST and ALT were irregular in comparison to the control, yet there was a general increase in the activities of these enzymes in the muscle and the digestive system of the mollusk. However, the activity of ALP was elevated above that of the control value in all the exposure concentrations and time intervals.

Generally, these enzymes (AST, ALP and ALP) are clinically important in the diagnosis of hepatic (hepatocellular) damage or disease.[50,51] In the event of damage in the organs, especially those of hepatic importance such as the liver, spleen or the kidney, these enzymes leak from the organs to the blood and this alters the permeability of the cell membrane to an appreciable degree.[15,50,52] also observed in their work with periwinkles (*T. fuscatus*) exposed to diesel, that the pathways of metabolic activities were changed as a result of alteration of

enzymatic activities. The alteration of enzyme (AST, ALT and ALP) in any organism reveals a disturbance in the general physiological structure of important organs or tissues and the membrane transport.[53] Another possible factor that might have played roles in the alteration in the activities of these enzymes (AST, ALT and ALP) was the direct interference of the toxicant with the tissues of the scallops (*Placopecten magellanicus*), which eventually resulted in the changes observed in the organism's biochemistry.[52]

However, the increase in the tissue enzyme activities (AST, ALT and ALP) indicates active cell transamination and effective use of the amino acids.[54] The increase could also result from overproduction of enzymes from the tissues[55] or absorption of the enzymes from haemopoeitic tissues. Elevation of AST and ALT gives the information on stressed induced augmentation imposed on the tissues by the toxicant[21], which in this case was the crude oil. Increase in AST and ALT was a response mechanism to achieving higher energy requirement in the face of toxicant effect.[56] Increase in AST, ALT and ALP indicates that the cell integrity was intact and thatthe structure of the cell membrane was alsoprotected.[57] It has however been observed that increase in these enzymes in the blood was an indication of tissue damage.[54,27,58] Increase in the activities of AST, ALT and ALP is a pathway of immune response at the initial stages of diseased condition in animals.[59] Increase and decrease in tissue enzymatic activities may be an adaptive response mechanism exhibited in the target tissue to counter or minimize the effect of the toxicant.[58]

ALP is a cytotoxic and genotoxic enzyme and is involved in adaptive cellular response to pollutants/ contaminants.[60] ALP is also involved in metabolic transport across membranes. The increase in the activities of ALP in the tissues examined may have resulted in active transport of metabolites in the scallops and effectively played the role management of toxicants and their metabolic wastes in the organism.[58] According to [61], ALP promotes the synthesis of glycogen through the deactivation of phosphorylase enzymes. It is also involved in the conversion of NADP to NAD[62] and also associated with the transport of phosphate bound compounds, nucleotides, carbohydrates and protein synthesis.[63] The increase in ALP activity in the tissues of this mollusk (*Placopecten magellanicus)*is indicative of the fact that the above mentioned roles were adequately perfomed in the organism to suppress the effect of the crude oil.

CONCLUSION

The alteration of these biochemical parameters is an indication that crude oil is toxic to scallops in particular and the environment at large. It also showed that the responses of the parameters were tissue specific and considering the shortness of the time involved in the study, the enzymes can be more reliable in examining crude oil toxicosis on bay scallops (*Placopecten magellanicus*). Therefore adequate environmental check be done consistently by government and relevant agencies to save the environment from the effect of crude oil since this organism is an essential and cheap protein source for coastline dwellers.

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