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Proximate Composition of Mangrove Periwinkle; *Pachymelania aurita* Exposed to Aqueous and Ethanolic Bark Extracts of Mahogany; *Khaya grandifoliola*

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Abstract: The proximate composition to determine total protein, total carbohydrate, total lipid and moisture content of Mangrove Periwinkle; Pachymelania aurita exposed to Aqueous and Ethanolic Bark Extracts of Mahogany; Khaya grandifoliola were assessed. Samples were handpicked from the coastal waters in the shelly sand sediments during low tides. In the sublethal toxicities, the organisms were exposed to 1/10th, 1/100th and 1/1000th of the 96h LC50 of the extracts. Results reveal that, P. aurita on exposure to the bark extracts at sublethal dosage showed an increase ($P<0.05$) in ash content and total protein as the moisture content decreased. ANOVA showed significant differences (where $P<0.05$) in the proximate composition of the exposed organisms when compared with the control organisms. The Duncan multiple range test (DMRT) also showed significant differences between each extract concentration during the exposure period. The result implied that, Toxicological response of Mangrove Periwinkle; Pachymelania aurita to toxicants can be assessed by analyzing possible alterations of the proximate composition of the exposed organism and that any alteration to their composition may be as a result of the impact of a given toxicant to the physiology of the bio-indicator. It was concluded that P. aurita is able to tolerate sublethal doses of extracts of K. grandifoliola without major alterations to its proximate composition.

Keywords: Edible Periwinkle, Mahogany Extracts, Proximate Composition, *Khaya grandifoliola*

1. Introduction

In man's efforts to produce more food and raise the standard of living, agricultural as well as industrial activities are significantly increased. These activities often release agricultural chemicals, industrial wastes and energy into various ecosystems which may result in the disturbance of its delicate balance threatening the health and existence of living organisms including man. [1, 4, 13].

Wood waste can have a variety of physical and chemical adverse impacts on aquatic life depending on its form. Timber logs suspended on water bodies can slow down the water current, occasionally trap aquatic weeds and provide important attachment surface for algae and aquatic fungi populations. These large mases of wood may also provide an inappropriate substrate for benthic colonization which may smother aquatic plants and benthic organisms but aid the growth or germination of some fungal spores [10, 6].

The Lagos lagoon which serves as a waste disposal point for the entire communities in the Lagos metropolis also serves to provide food in form of fish and fisheries resources which are important nutritionally in the supply of protein, lipids, omega-3 fatty acids, carbohydrates, fat soluble vitamins and dietary minerals [9, 14].

In studies concerned with possible effects of pollutants on aquatic biota and their ecosystems, it is of great importance to consider the benthic organisms [3, 8]. *Pachymelania aurita*, an edible periwinkle, is a key organism in the pachymelania community in the southern Lagos lagoon. This study aims to examine if there are alterations to the proximate composition

and or organoleptic properties of *P. aurita* when exposed to sublethal doses of aqueous and ethanolic bark extracts of Mahogany (*Khaya grandifoliola).*

2. Materials and Methods

2.1. Test Animals

Edible periwinkles (*P. aurita*) were handpicked from the coastal waters in the shelly sand sediments of the southern part of the Lagos lagoon bordering the University of Lagos during low tides. 300 organisms were kept for 14 days in plastic holdings containing lagoon aerated water and sediments from the site of collection of the animals to stimulate a typical brackish water medium.

2.2. Test Compounds and Aqueous Extracts

The aqueous solution extracted was prepared according to standard method [4]. 200g of ground bark of *K. grandifoliola* was sieved and soaked in 1L of distilled water for 72hrs. it was filtered through a muslin cloth and stored at room temperature.

2.3. Ethanolic Extracts

1500g of ground *K. grandifoliola* was put in a Soxhlet extractor with 2.5L of 98% absolute ethanol reagent. It was heated for 3 – 4 hrs. The extracted solvent was collected and stored in a silver flask. The ethanol was recovered by distillation while the residue that was collected into a 100ml beaker was concentrated by heating at 78°C in a hot air oven, cooled in a condenser, weighed and stored in a dark glass bottle.

2.4. Sub-lethal Tests

Sub-lethal test procedures followed methods for fixed point discharge and static non-renewal tests. The test was conducted to determine the effects of sub-lethal dosages of the extracts that would alter the proximate composition and organoleptic properties of *P. aurita* when exposed for 21 days. 100 organisms were exposed per tank to $1/10^{th}$, $1/100^{th}$ and $1/1000^{th}$ of 96h LC_{50} values of aqueous and ethanolic extracts respectively. The toxicants were renewed every 48hrs.

2.5. Proximate Composition Assessment

The condition index of 30 animals per tank was measured every 7 days and their tissues were analyzed for proximate composition to determine total protein, total carbohydrate, total lipid and moisture content.

Total Protein: the following steps were used to determine crude protein

Digestion: sample + H_2SO_4 (conc). Using Hg as catalyst, Nitrogen is converted to NH_4.

Distillation: NH_4 is converted to NH_3 in the presence of NaOH, it is distilled off and collected in boric acid.

Titration: back titration using standard acid (HCL) and screened Methyl blue as the indicator.

Total Carbohydrate: this was analyzed using the following calculation;

Total carbohydrate = 100 – (crude protein + lipid + moisture +ash)

Total Lipid: this was achieved using the Soxhlet method. A clean dry flask was heated in an oven at 100°C, cooled in a desiccator and weighed. 50g of the sample was put in a filter paper and placed in the extractor. A weighed flask containing 100ml of petroleum spirit was connected to a reflux condenser extract was 4hrs. The residue from the filter paper was ground and returned for a further 1hr extraction. The filter paper was then removed while the petroleum spirit from the flask was distilled. The flask was dried for 2hrs at a 100°C, cooled and weighed. Total lipid was calculated as follows;

$$\%\text{Lipid} = \frac{weight\ of\ extracted\ oil}{weight\ of\ sample\ taken}\ X\ \frac{100}{1}$$

Moisture Content: the oven air method was used to determine the percentage moisture. 2g of the sample was places in a weighed dish and reweighed. The dish was then placed in a muffle furnace and dried for 6hrs at 100°C. The dish was cooled in a desiccator and weighed. The sample was dried for a further 1hr, cooled and reweighed to achieve a constant weight. Moisture content was calculated as follows;

$$\%\text{Moisture} = \frac{diff\ in\ wt\ before\ and\ after\ drying}{wt\ of\ sample\ taken}\ X\ \frac{100}{1}$$

Ash Content: 2g of the sample was heated at 560°C in a muffle furnace for 6hrs until a whitish grey ash remained. The dish was cooled in a desiccator and weighed. Ash content was calculated as follows;

$$\%\text{Ash} = \frac{weight\ of\ ash}{weight\ of\ sample}\ X\ \frac{100}{1}$$

Comparison of means by Duncan's Multiple Range Test (DMRT) and ANOVA were used to test for significance in results obtained from the proximate composition. All analysis was performed using computer statistical package SPSS 10.0.

3. Results

3.1. Sub-lethal Effects of Aqueous and Ethanolic Extracts of *K. grandifoliola* on Proximate Composition of *P. aurita*

The proximate composition analysis of *P. aurita* exposed to sublethal doses of *K. grandifoliola* for 21 days are shown in figures 1 and 2. The results show a high percentage of moisture, low percentage of ash, minimal percentage of protein and almost no carbohydrate and lipid.

3.2. Effects of Aqueous and Ethanolic Extracts of Sub-lethal Doses of *K. grandifoliola* on Total Protein (%) of *P. aurita*

The mean yield of protein (as shown in table 1) produced an increase in percentage in no particular order. In the

samples exposed to 1/1000th (0.00174ml/L, 3.48 x 10^{-5}g/L) aqueous extracts, the percentage yield of protein increased in 14 days and reduced on further exposure. In other aqueous concentrations, the percentage yield increased on further exposure.

In samples exposed to ethanolic extracts, the percentage yield of protein for 1/10th (0.024ml/L, 4.8×10^{-3}g/L) concentration increased on further exposure while the 1/100th (0.0024ml/L, 4.8×10^{-4}g/L) and 1/1000th (0.00024ml/L, 4.8 ×10^{-5}g/L) concentrations showed an increase in protein in 14 days and a decrease on further exposure (as shown in table 2). The one-way analysis of variance, ANOVA, showed a significant difference (P<0.05) in percentage protein of *P. aurita* exposed to sublethal concentrations of the extracts (during 7, 14 and 21 days of exposure). Further analysis using Duncan Multiple Range Test (DMRT) at P=0.05 showed that *P. aurita* exposed to aqueous extract for 7 days had significant difference between the 1/10th, 1/100th and 1/1000th concentrations. On exposure for 14 days, there was a significant difference between all concentrations (table 2). DMRT (P=0.05) for *P. aurita* on exposure for 7 days to the ethanolic extract showed significant difference between the control and other concentrations. In turn, the 1/10th and 1/100th were significantly different from the 1/1000th concentrations. When further exposed from 14 – 21 days, DMRT analysis showed a significant difference between all concentrations (table 3).

3.3. Effects of Aqueous and Ethanolic Extracts of Sub-lethal Doses of *K. grandifoliola* on Total Carbohydrate (%) of *P. aurita*

The mean percentage carbohydrate of *P. aurita* showed no stable increase or decrease in the aqueous extracts (table 3) and ethanolic extracts (table 4) of the sublethal doses of *K. grandifoliola*. In the 1/1000th concentration of the aqueous extract, there was a decrease in the percentage of carbohydrate within 21 days, while there was an increase in the 1/100th concentration. In the 1/10th concentration of the ethanolic extract, there was an increase in percentage carbohydrate for the first 14 days. There was also a decrease in the 1/10th concentration of aqueous extract for the first 14 days but there was no significant difference on further exposure for 21days. ANOVA for the ethanolic extracts of *K. grandifoliola* showed no significant difference (P>0.05) in carbohydrate (%) of *P. aurita* when exposed for 7 days while it showed a significant difference on further exposure from 14 – 21 days. DMRT (P=0.05) showed no significant difference in the first 14 days of exposure to aqueous extracts and a significant difference when further exposed for 21 days. DMRT of the carbohydrate (%) on exposure for 7 days showed a significant difference but no significance on further exposure as seen on table 4.

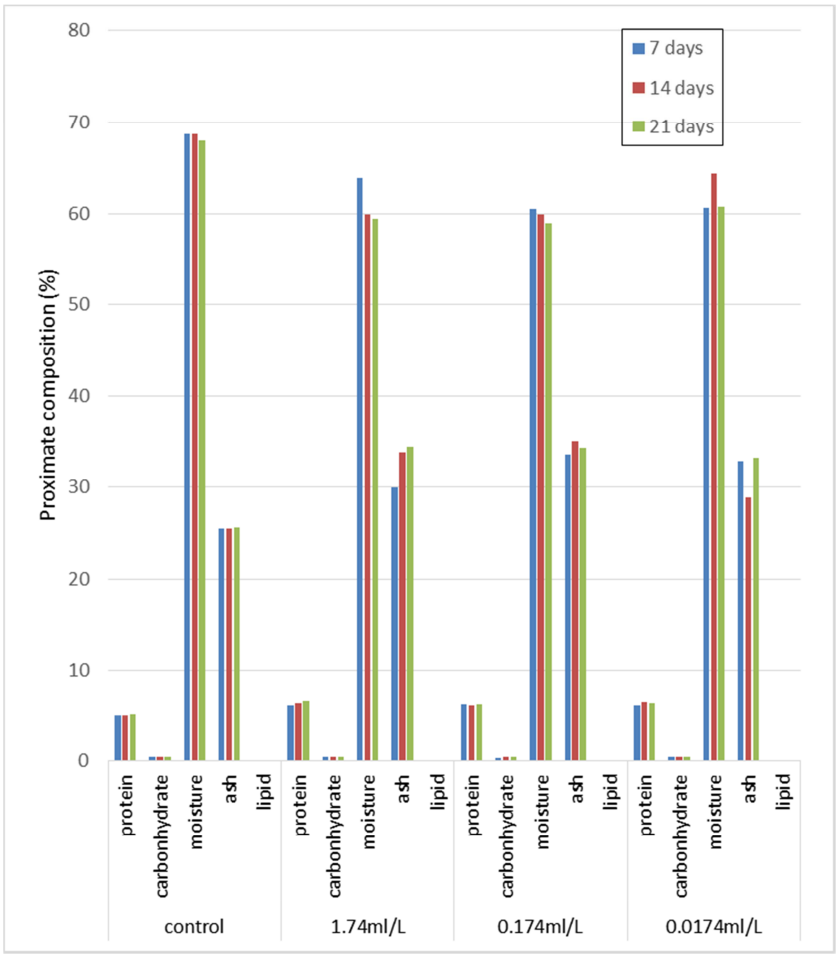

Figure 1. *Proximate composition of Pacymelania aurita exposed to sublethal doses of aqueous extract of Khaya grandifoliola.*

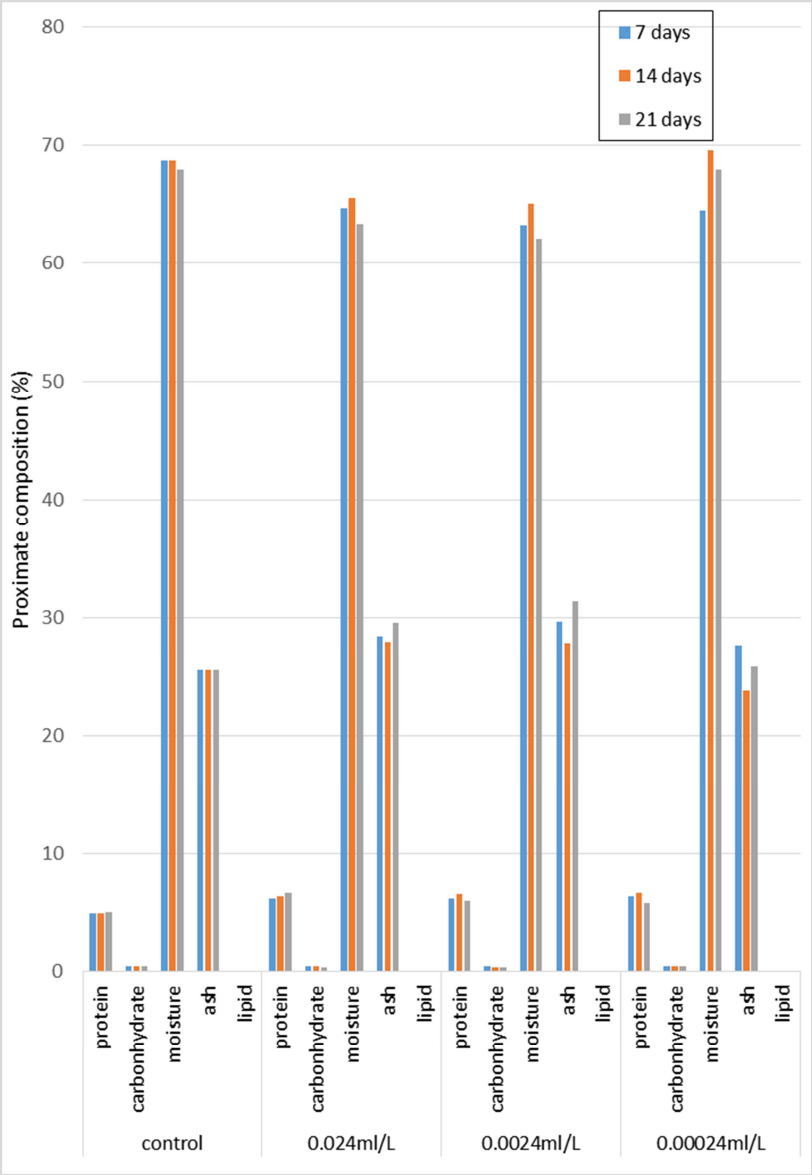

Figure 2. *Proximate composition of Pachymelania aurita exposed to sublethal doses of ethanolic extract of Khaya grandifoliola.*

Table 1. *Percentage mean proximate composition (Total Protein) of Pachymelania aurita exposed to sublethal dose of aqueous extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Protein		
		7	14	21
Control	3	5.0400^{a}	6.0033^{a}	6.3067^{b}
1.74ml/L ($1/10^{th}$)	3	6.1067^{b}	6.4333^{c}	6.5767^{d}
0.174ml/L ($1/100^{th}$)	3	6.2200^{b}	6.1767^{b}	6.2400^{a}
0.00174ml/L ($1/1000^{th}$)	3	6.1133^{b}	6.5333^{d}	6.3500^{c}

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 2. *Percentage mean proximate composition (Total Protein) of Pachymelania aurita exposed to sublethal dose of ethanolic extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Protein		
		7	14	21
Control	3	5.0400^{a}	6.0033^{a}	6.3067^{b}
0.024ml/L ($1/10^{th}$)	3	6.2533^{b}	6.4033^{b}	6.7200^{d}
0.0024ml/L ($1/100^{th}$)	3	6.2233^{b}	6.5667^{c}	5.9867^{b}
0.00024ml/L ($1/1000^{th}$)	3	6.3800^{b}	6.4100^{d}	5.8467^{a}

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 3. *Percentage mean proximate composition (Total Carbohydrate) of Pachymelania aurita exposed to sublethal dose of aqueous extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Carbohydrate		
		7	14	21
Control	3	0.4467[a]	0.4233[a]	0.4400[b]
1.74ml/L (1/10[th])	3	0.3933[a]	0.4033[a]	0.4100[a]
0.174ml/L (1/100[th])	3	0.3467[a]	0.4067[a]	0.4300[b]
0.00174ml/L (1/1000[th])	3	0.4367[a]	0.4200[a]	0.4033[a]

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 4. *Percentage mean proximate composition (Total Carbohydrate) of Pachymelania aurita exposed to sublethal dose of ethanolic extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Carbohydrate		
		7	14	21
Control	3	0.4467[a]	0.4233[b]	0.4400[c]
0.024ml/L (1/10[th])	3	0.4200[a]	0.4367[c]	0.4000[a]
0.0024ml/L (1/100[th])	3	0.4433[a]	0.4067[a]	0.4000[a]
0.00024ml/L (1/1000[th])	3	0.4400[a]	0.4200[n]	0.4133[b]

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

3.4. Effects of Aqueous and Ethanolic Extracts of Sublethal Doses of *K. grandifoliola* on Total Moisture (%) of *P. aurita*

The mean percentage of moisture of *P. aurita* showed an almost stable decrease on exposure to aqueous extracts of *K. grandifoliola* for 21 days (1/10[th] and 1/100[th] concentrations) therefore there was a decrease on prolonged exposure. However, the 1/1000[th] concentration showed a fluctuation in water content as exposure increased (as shown in table 5). on exposure to ethanolic extracts, the water content of *P. aurita* fluctuated (table 6). ANOVA for exposure to sublethal doses of aqueous as well as ethanolic extracts showed a significant difference (P>0.05) while DMRT showed a significant difference within the first 7 days, between the 1/100[th] and 1/1000[th] as well as between the control and 1/10[th] concentrations. After 14 days of exposure, there was a significant difference between 1/10[th], 1/100[th] and 1/1000[th] concentrations while after 21 days of exposure all concentrations were significantly different. DMRT for the ethanolic extracts showed significant difference between each concentration on exposure for 21 days.

3.5. Effects of Aqueous and Ethanolic Extracts of Sublethal Doses of *K. grandifoliola* on Total Ash (%) of *P. aurita*

On exposure to aqueous extracts of *K. grandifoliola*, there was an increase in the ash content of *P. aurita* as the moisture content decreased. There was an increase in ash content on prolonged exposure to the 1/10[th] and 1/100[th] concentrations while there was a fluctuation in ash content on prolonged exposure to the 1/1000[th] concentration (as shown in table 7). ANOVA analysis showed a significant difference (P< 0.05) in ash content on exposure to aqueous and ethanolic extracts for 7, 14 and 21 days. DMRT analysis also showed significant difference between each concentration of and ethanolic extracts for 7, 14 and 21 days of exposure (table 8).

Table 5. *Percentage mean proximate composition (Total Moisture) of Pachymelania aurita exposed to sublethal dose of aqueous extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Moisture		
		7	14	21
Control	3	68.4933[c]	66.8000[c]	66.0033[d]
1.74ml/L (1/10[th])	3	63.8667[b]	59.8667[a]	59.4300[b]
0.174ml/L (1/100[th])	3	60.5267[a]	59.8733[a]	58.9567[a]
0.00174ml/L (1/1000[th])	3	60.5700[a]	64.3600[b]	60.7733[c]

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 6. *Percentage mean proximate composition (Total Moisture) of Pachymelania aurita exposed to sublethal dose of ethanolic extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Moisture		
		7	14	21
Control	3	68.9933[c]	66.8000[c]	66.0033[c]
0.024ml/L (1/10[th])	3	64.7733[b]	65.5367[b]	63.3200[b]
0.0024ml/L (1/100[th])	3	63.2367[a]	65.1067[a]	62.1467[a]
0.00024ml/L (1/1000[th])	3	64.5033[b]	69.5967[d]	68.0133[d]

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 7. *Percentage mean proximate composition (Total Ash) of Pachymelania aurita exposed to sublethal dose of aqueous extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Ash		
		7	14	21
Control	3	25.5567^{a}	26.7800^{a}	27.7233^{a}
1.74ml/L ($1/10^{th}$)	3	29.9933^{b}	33.9167^{c}	34.4567^{d}
0.174ml/L ($1/100^{th}$)	3	34.5700^{d}	35.0433^{d}	34.3767^{c}
0.00174ml/L ($1/1000^{th}$)	3	32.7233^{b}	28.8967^{b}	33.3000^{b}

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 8. *Percentage mean proximate composition (Total Ash) of Pachymelania aurita exposed to sublethal dose of ethanolic extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Ash		
		7	14	21
Control	3	25.5267^{a}	26.7800^{b}	27.7233^{b}
0.024ml/L ($1/10^{th}$)	3	28.5467^{c}	27.9200^{c}	29.5867^{c}
0.0024ml/L ($1/100^{th}$)	3	29.6633^{d}	27.9033^{c}	31.4700^{d}
0.00024ml/L ($1/1000^{th}$)	3	27.7200^{b}	23.8300^{a}	25.8800^{a}

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 9. *Percentage mean proximate composition (Total Lipid) of Pachymelania aurita exposed to sublethal dose of aqueous extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Lipid		
		7	14	21
Control	3	0	0	0
1.74ml/L ($1/10^{th}$)	3	0	0	0
0.174ml/L ($1/100^{th}$)	3	0	0	0
0.00174ml/L ($1/1000^{th}$)	3	0	0	0

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

Table 10. *Percentage mean proximate composition (Total Lipid) of Pachymelania aurita exposed to sublethal dose of ethanolic extracts of Khaya grandifoliola.*

Concentrations	No. of tests	Total Lipid		
		7	14	21
Control	3	0	0	0
0.024ml/L ($1/10^{th}$)	3	0	0	0
0.0024ml/L ($1/100^{th}$)	3	0	0	0
0.00024ml/L ($1/1000^{th}$)	3	0	0	0

Means with the same subscript letter in a column are not significantly different in the DMRT (P=0.05)

3.6. Effects of Aqueous and Ethanolic Extracts of Sublethal Doses of *K. grandifoliola* on Total Lipid (%) of *P. aurita*

P. aurita, before exposure to sublethal doses of aqueous and ethanolic extracts of *K. grandifoliola,* contained no lipid content (as shown on tables 9 and 10). after exposure to the extracts for 21days, *P. aurita* still contained no lipid content.

4. Discussion

The proximate analysis of the sampled *P. aurita* showed it had a high moisture content (± 65%) <25% protein content, <1% carbohydrate, 23% - 35% ash and no lipid content. This agrees with the work of other researchers [5, 7, 12]. They reported that the lipid and carbohydrate contained in sea foods are generally low. The proximate composition of *P. aurita* on exposure for 21 days to aqueous and ethanolic extracts of *K. grandifoliola* showed some uncertain alterations. The (%) total protein which increased within the first 7 days could as a function of stress proteins – also referred to as heat-shock proteins or hsp - these are the primary defense mechanisms that are activated by the occurrence of denatured proteins. Differences in response of species and the wide variation in constitutive stress proteins levels among and within species are due to physiological and environmental factors surrounding that species [2]. The level to which (%) protein increases in any individual species of *P. aurita* is dependent to a large extent on its tolerance level and its ability to trigger its defense mechanism.

In mollusks, the open circulation system permits the body water to be mixed with ions, colloids, food substances, metabolites and heat resulting in the hemolymph. The concentration of body water is determined by factors such as the formation of waste products (which would in turn cause a loss of water), metabolism of food substances (which would cause gain of metabolic water) and gain or loss of water

through permeable surfaces [11]. As animals remove toxic wastes form their bodies, the soluble forms are dissolved in the body fluid and brought to the blind end of the excretory tubule. When *P. aurita* was exposed to sublethal doses of *K. grandifoliola,* the moisture content reduced from 68.49% (unexposed) to as low as 57% within 7 days and eventually rose to 58.96% on further exposure for 21 days. As the soluble portions of the toxicants where dissolved in the body fluid and excreted, it reduced the moisture content of the organism.

The carbohydrate content, the store of excess energy, was also altered as *P. aurita* was stressed. The organism had to expend energy and heat as it excreted its toxic wastes.

There was no recorded lipid content in *P. aurita* before, during and after exposure to sublethal doses of the toxicants.

5. Conclusion

The toxicological response of estuarine benthic macroinvertebrate (as bio-indicators) to toxicants can be assessed by analyzing possible alterations of the proximate composition of the exposed organism. Any alteration to the proximate composition may be as a result of the impact of a given toxicant to the physiology of the bio-indicator. This study has revealed that *P. aurita* is able to tolerate sublethal doses of extracts of *K. grandifoliola* without major alterations to its proximate composition.

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