Histological Impact Of Calcium Carbonate On The Juveniles Of The Brackish River Prawn

Okogbue Blessing C., Hart A. Ibitoru., Ansa Ebinimi J., Ikpi Gabriel

Abstract: Most agro-based inorganic chemical are heavily employed today in lots of environmental remedial activities apart from their core use in agriculture. The dangers of over use of these products have necessitated toxicological investigations using bio-indicators to assess potential impacts on aquatic life. Calcium carbonate was tested on the brackish river prawn, *Macrobrachium macrobrachion* in a completely randomized design to determine its long term effect on the prawns. The experiment was conducted for two weeks and afterwards the body tissues were extracted and prepared on glass slides for photomicrography. The results showed that the toxicant did not negatively impact the muscles and carapace as there were no degenerations of the cells and tissues. This revealed that the agricultural lime is non-toxic to the juvenile brackish river prawns and also showed that it is safe for use in prawn culture as source of calcium for proper growth and development. Also the correlation was significant at P< 0.05 confidence level. The water quality parameters occurred at permissible ranges for prawns and the relationships between parameters did not influence destructive impact on the test organisms. It has also created an avenue for further research on this product use as nutrient supplement in prawn culture

Index Terms: Biodiversity, calcium carbonate, environmental remediation, M. macrobrachion, pollution, **prawn** culture, toxicity.

1 INTRODUCTION

The increasing demand on some inorganic chemicals has made research on their potential impacts on the environment very important. These compounds are most times used as nutrient sources, electron acceptors in oil spill remediation and other environmental remedial activities apart from their core applications in agriculture [14]. Such compounds of calcium origin and other fertilizers are massively used today for related activities. Calcium hydroxide has been employed in metal polluted soil as precipitation agents when used with EDTA to ensure recycling [13]. Calcium carbonate was also used as sealing agent to increase the density of drilling fluid in oil explorations [12]. Technological innovation has evolved the input of these substances outside their known use as pH buffers in pond water quality management. The need for investigations on these compounds is paramount to maintain healthy environment. Inorganic chemicals as the name implies, are bound to be destructive when abused. Chemicals or rather, the toxicity of a chemical could be determined by the quantity used or the concentration administered. The "dose makes the poison" as Feliciano [7] established, therefore, in order to come up with safe concentrations, toxicity tests in laboratory assays and field assessments using bio-indicators is expedient, in that they help to draw lines for thresholds.

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This research revolves around calcium carbonate. This lime has intoxicating potentials being an inorganic chemical. It is widely used in pond sediment and soil garden liming. As the interest to adopt the use in other environmental activities rises, toxicity investigations is very important in order to establish the thresholds and also look at the long term potential impact on the aquatic systems. Pollution pressure on biodiversity and water quality status of aquatic ecosystems needs to be minimized to the barest minimum, if the ecosystem goods and services will benefit the stakeholders. This study therefore, was carried out under laboratory conditions to establish the long term effect of calcium carbonate when juveniles of brackish river prawns (*Macrobrachium macrobrachion)* were exposed to different concentrations. The body tissues were analyzed on histological basis to establish toxic impacts by the toxicant.

2. EXPERIMENTAL PROCEDURE

2.1 MATERIALS

Juveniles of *Macrobrachium macrobrachion*, with the common name, brackish river prawn was used for this experiment. Agricultural lime, *Calcium carbonate* ($CaCO_3$) was used as the toxicant. Other materials are, plastic bowls, netting covering, scoop net, weighing balance, meter rule, measuring cylinders, pipette, test tubes, spatula, etc.

2.2 METHODS

Collection: Non-return valve traps (Fig. 3) were used to collect the prawns from the canals. The test prawns were collected in cages submerged in the canals located in the African Regional Aquaculture Centre (ARAC), Port Harcourt in Rivers State of Nigeria. They were afterwards transferred to the laboratory to acclimatize and for the bioassays.

Selection: Juvenile prawns were randomly selected for the experiment. Selected prawns weighed between 1.83-4.22 g with total body length of 5.0-6.6 cm, carapace length ranging from 2.0 – 2.5 cm and post orbital length from 4.5 - 5.0 cm. The weight and lengths were obtained using mettler weighing balance and meter rule on measuring

board respectively.

Stock preparation: One gramme (1g) of $CaCO_3$ was dissolved in distilled water and made up to 1000 ml, this gave one gramme per liter (1g/l). From 1g/l solution which is equivalent to 1000mg/l, 1ml of stock solution was made up to 1 litre to give 1mg/l. $C_1V_1 = C_2V_2$ was used to realize the desired concentrations as stated above for chronic concentrations [5], [6].

Where C_1 = concentration of stock solution (1000mg)
V_1 = volume of stock solution needed for each concentration
C_2 = desired concentration of test solution (in mg/l)
V_2 = final volume of water needed in tanks (e.g. litres)

Bioassay procedures: The sub-lethal toxicity test was carried out using chronic concentrations of 20mg/l, 40mg/l and 80mg/l and 0mg/l (as control). They were stocked at one prawn per two (2) litres of water and six (6) prawns were tested for each concentration.

Water quality analysis: The water quality parameter of the test media was determined for:

I. Temperature (^oC) using mercury in glass thermometer. The glass rod was dipped into the bioassay tanks and held for 3-5minutes and the value pointed by the thread, gave the temperature value of the test media.

II. ii) pH values were obtained using the fresh water aquaculture test kit model AQ-Z, Code 3633-03 LaMotte. A test tube was filled with the sample water up to the 5ml level mark. 10 drops of wide range pH indicator was added, the test tube was then capped and mixed. It was inserted into the Octa-slide viewer, the pH octa-slide bar was also inserted, and then the value recorded gave the pH reading.

III. Dissolved oxygen (mg/l) was also analyzed by titrimetric method with the aquaculture test kit using sodium thiosulphate as the titrant. The sample bottle was submerged to collect water sample. The side of the bottle was tapped to release any air bubbles, the cap was replaced afterwards and the bottle retrieved from water. 8 drops of manganese sulphate ($MnSO_4$) was added followed by 8 drops of alkaline iodide (KI) immediately. It was shaken and left to stand for 2 minutes for the precipitate to settle, this resulted in oxygen fixation. 8 drops of dilute sulphuric acid (H_2SO_4) was added to dissolve the precipitate. Another test tube was filed with 20ml of the fixed sample water and titrated with sodium thiosulphate (0.025N) with continuous swirling until the yellow colour of the sample became faint. 8 drops of starch indicator solution was added to get a blue black colour. Titration continued until the blue colour disappeared. The value of the titrant gave the concentration of dissolved oxygen in mg/l.

IV. Total alkalinity (mg/l) and total hardness (mg/l) were both analyzed using the same titrimetric method. For total alkalinity, 5ml of water sample was taken into a test tube. 4 drops of BCG-MR indicator was added. This was mixed and the sample turned blue-green. A direct reading titrator was filled with the alkalinity titration reagent (H_2SO_4) and mixed at intervals. Titration was continued until the blue-green colour turned pink. The quantity of the reagent used up in the titration gave the value of the total alkalinity in mg/l.

V. To determine total hardness, the 12.9ml test tube was filled with the sample water. 5 drops of hardness reagent 5 (a solution of sodium salts) was added, covered and mixed. 5 drops of hardness reagent 6 (a mixture of ethanol, methanol & calcium) was also added and covered. On mixing, the sample water turned red. The direct reading titrator was filled with hardness reagent 7 (EDTA di-sodium salt solution). On titration, the water sample changed from red to clear blue and at the point of colour change, the value of the EDTA was read off and that gave the value of the total harness ($CaCO_3$) in mg/l. The parameters were all monitored throughout the duration of the experiment following the methods of APHA [3].

2.3. HISTOLOGICAL STUDIES (PHOTOMICROGRAPHY):

Body tissues (carapace and muscle) samples were collected from each concentration including the control and were analyzed for histological studies. The tissue samples of the test organisms were extracted and fixed in 10% formalin for 24 hours [2]. These samples were cut in 3cm cubes using microtome. These sectioned organs were washed under running tap for 2 hours and then dehydrated using 95% ethanol. They were infiltrated and embedded in paraffin wax in an embedding chamber and also stained using haematoxylin and eosin. Smears and tissue imprints were made on glass slides for analysis under an Olympus binocular light microscope at the magnifications of X400 and X1000. Lesions and degenerations were parameters used to assess the impact of the toxicant.

Data analysis: Correlation was used in the statistical analysis at $P < 0.05$ for testing the significance and relationships between parameters.

3. RESULTS AND DISCUSSION

TABLE 1
Values of the physico-chemical parameters of the test media for *M. macrobrachion* exposed to calcium carbonate in the sub-lethal toxicity test

Conc. (mg/l)	pH	DO (mg/l)	Temp. (mg/l)	TA (mg/l)	TH (mg/l)
Control – 0	6.40±0.0	3.60±1.0	27.80±0.2	71.20±0.0	84.55±55.0
A – 20	6.50±0.0	4.00±0.4	27.93±0.2	75.65±0.0	84.55±55.0
B – 40	6.75±0.0	4.40±0.8	27.93±0.1	57.85±0.0	120.15±0.0
C – 80	7.00±0.0	4.30±1.0	27.80±0.2	75.65±35.6	89.00±0.0

The water quality parameters determined for the toxicity test media fell within the tolerable ranges for tropical fish species [1]. Correlation is significant at the 0.05 confidence level (Table 2). Temperature range was between 27.80 and 27.93 as the lowest and highest values recorded in the tests. While pH ranged from 6.40 to 7.00, dissolved oxygen (DO) ranged from 3.60 to 4.40, total alkalinity (TA) ranged from 57.85 to 75.65 and total hardness (TH) from 84.55 to 120.15 (all in mg/l) throughout the test period as the lowest and highest values respectively. The prawns were more energized as concentration increased although prawns were sluggish when compared to the ones exposed to high concentrations in acute bioassays [10]. This implies that the test media was conducive instead. This therefore suggests that calcium carbonate will be very beneficial for proper growth and development of juvenile prawns at these concentrations. This was confirmed by Spotts [11] who also emphasized that young prawns molt much more frequently once or twice weekly, hence will need lots of calcium. Moreso, high concentrations of 1280mg/l to 5120mg/l resulted to depositing of chalk on rostrum of prawns when exposed in acute toxicity tests [10]. This also implies that high concentrations of lime can clog gills of prawns despite the fact that it was not toxic to the prawns, hence death could also result from asphyxiation at very high concentrations of the calcium compound. Also, LD_{50} of 6.45g/kg (6,450mg/kg) for calcium carbonate was reported by Lewis [8] for rats. This confirms low toxicity of this compound.

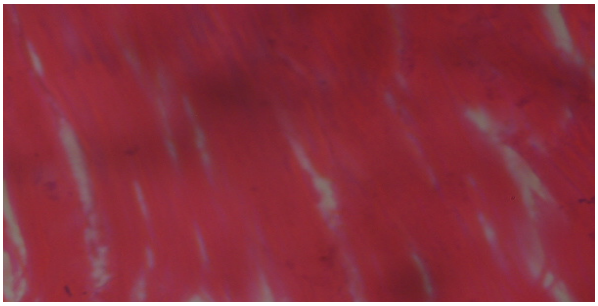
TABLE 2
Correlations for the physico-chemical parameters in the $CaCO_3$ sub-lethal toxicity test.

Correlations

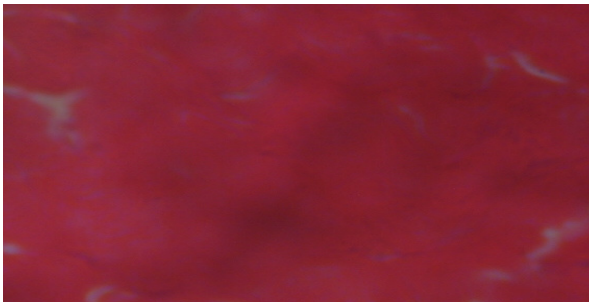
		pH	DO (mg/l)	Temp. (°c)	TA (mg/l)	TH (mg/l)
pH	Pearson Correlation	1	.579	-.498	-.638	-.461
	Sig. (2-tailed)		.042	.502	.362	.539
	N	4	4	4	4	4
DO (mg/l)	Pearson Correlation	.579	1	-.698	-.975*	.379
	Sig. (2-tailed)	.042		.302	.025	.621
	N	4	4	4	4	4
Temp. (°C)	Pearson Correlation	-.498	-.698	1	.834	-.451
	Sig. (2-tailed)	.502	.302		.017	.549
	N	4	4	4	4	4
TA (mg/l)	Pearson Correlation	-.638	-.975*	.834	1	-.376
	Sig. (2-tailed)	.362	.025	.017		.624
	N	4	4	4	4	4
TH (mg/L)	Pearson Correlation	-.461	.379	-.451	-.376	1
	Sig. (2-tailed)	.539	.621	.549	.624	
	N	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

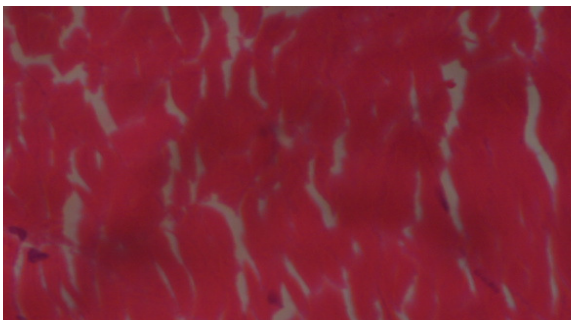
In this sub-lethal toxicity test, positive correlations occurred between pH and DO (r=0.579), TA and temperature (r=0.834) and also between DO and TH (r=0.379). This showed increase of one parameter as the other increased. Negative correlation occurred in temperature with pH (r=-0.451), TA and pH (r=-0.638), DO and temperature (r=-0.698), DO and TA (r=-0.975), pH and temperature (r=-0.498), pH and TH (r=-0.461) and also between TH and TA (r=-0.0376).



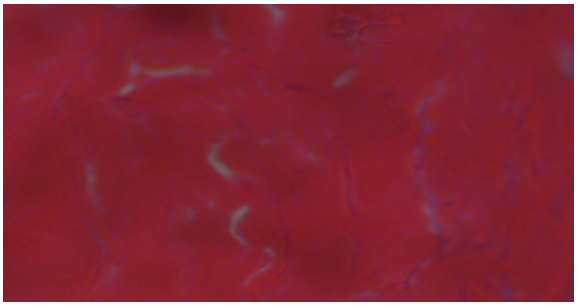
Control - 0mg/l



A - 20mg/l

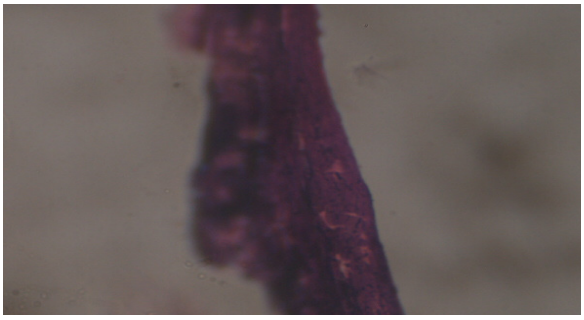


B - 40mg/l

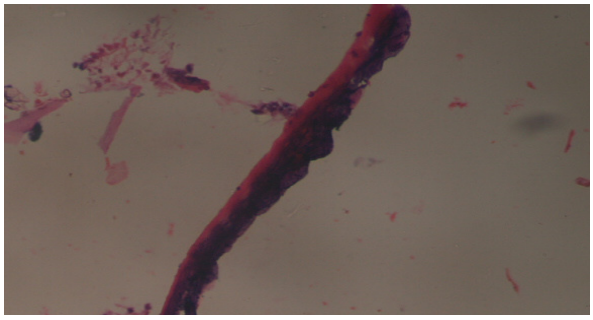


C - 80mg/l

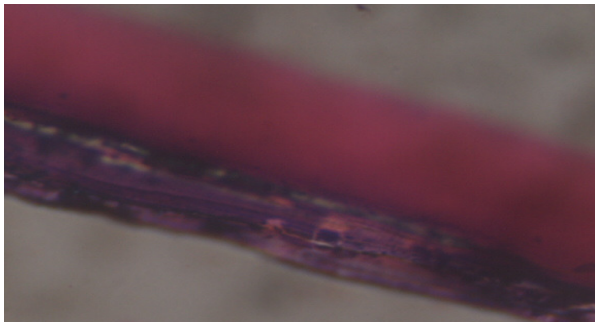
Figure 1. Muscle tissues of *M. macrobrachion* after 14 days exposure to different concentrations of $CaCO_3$



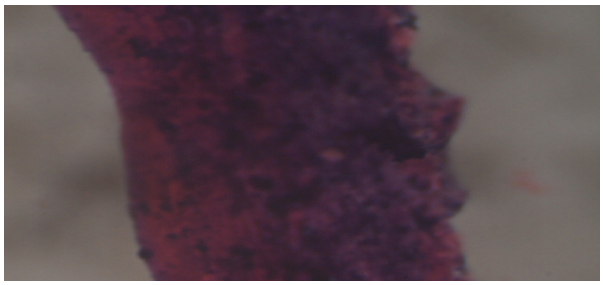
Control - 0mg/l



A - 20mg/l



B - 40mg/l



C - 80mg/l

Figure 2. Carapace tissues of *M. macrobrachion* after 14 days exposure to different concentrations of $CaCO_3$

These values showed the changes had no impact on the water quality since the parameters occurred at tolerable ranges for the test organisms. This could be attributed to reasons why there was no destructive impact on the body tissues (Fig. 1 and 2). The results of the histology (Fig. 1 and 2) revealed that calcium carbonate had no negative impact on the body tissues of the prawns tested both on the muscle and carapace. This

non-toxicity of calcium carbonate on juvenile prawns could also be attributed to the high requirement of calcium for calcification of shells (carapace). The choice of body tissues for histology and assessment of lime toxicity is based on the fact that there is constant contact of these organisms with water which house them and so applies in even times of pollution. According to Black [4] as is established in general toxicology, poison impacts a victim either by inhalation (via gills & lungs), by ingestion (through food or drink) or by absorption (via skin surfaces). This makes the body tissue a good workable tool in aquatic toxicology. Therefore, the absence of degenerated cells and body tissues simply indicated the safe use of calcium carbonate at exposed concentrations.

Figure 3. Non-return valve traps for catching prawns

CONCLUSION

This study has revealed the potential use of calcium carbonate in prawn culture techniques. It also buttressed the point that calcium carbonate is more or less non-toxic although as an inorganic chemical, it could be toxic to some other organism and so could only be refered to as being slightly toxic or of low toxicity. Calcium carbonate is therefore recommended in prawn culture for proper growth and development of juveniles especially during molting. Nutritional requirement report on calcium intake where daily allowable intake of 1200mg/kg has been recommended for children/aged people, 1000mg/kg for pregnant and lactating mothers and less doses for other categories [9] for proper development and good health in human. This supports the inference of this study.

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