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Evaluation of Some Biochemical Parameters in Haemolymph of *Bulinus* specie in Ajiwa Reservoir Katsina State, Nigeria

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Abstract

Evaluation of some biochemical parameters was conducted in haemolymph of *Bulinus* sp in Ajiwa reservoir, Katsina state, Nigeria from September, 2014 to August, 2015. Four samples of *Bulinus* spwere collected at 5 different sampling locations every 3 months and were transferred into labelled plastic storage bottle and transported into laboratory for haemolymph extraction and analysis. Glucose, Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined using standard methods, procedures and instruments. The mean value of the biochemical parameters are Glucose (36.28±1.76mg/dl), AST (19.21±0.24U/L) and ALT (13.91±0.62U/L). The level of the parameters were slightly increasing in the following order of magnitude; station 3 > station 2 > station 4> station 1 and station 5 recorded the lowest value. Seasonal variation of the biochemical parameters were clearly observed in the *Bulinus* sp. Glucose, AST and ALT levels were higher during the wet season compared to dry season (45.83±1.83mg/dl to 27.49±0.93mg/dl), (24.61±0.16 to 13.80±0.13U/L) and (19.33±0.78 to 10.44±0.21U/L) respectively. This may be due to high influx of nutrients from the surrounding farm lands and other domestic activities like bush burning, washing clothes, bathing and cattle rearing within the vicinity of the reservoir. Alterations in these biochemical parameters (Glucose, AST and ALT) in the haemolymph, indicate disturbance of the oxidative carbohydrate and protein metabolism. Apart from evaluating energy content as biomarkers, this experiment shows how *Bulinus* sp undergoes metabolic alteration to overcome stress.

Keywords: Ajiwa reservoir, *Bulinus* sp, Haemolymph, AST, ALT, Glucose.

INTRODUCTION

Haemolymph is the circulatory fluid of molluscs which is slightly bluish in colour due to presence of respiratory pigment haemocyanine. Haemolymph transports nutrients, respiratory gases, enzymes, metabolic wastes and also toxicants throughout the body. Haemolymph with plasma and corpuscles can provide information pertinent to health assessment of animals or population. The class Gastropoda is of interest in pollution studies, as it comprises sedentary filter feeding invertebrates, which are likely to accumulate pollutants from the environment (Castro*et al.,* 2004). The ability of mollusc to concentrate high amount of chemicals without any apparent bad effects makes these animals very dangerous to their predators (Carpene, 1993). Many chemicals released into the environment are able to generate toxicity in aquatic organisms (Usman, 2016).

Biochemical and physiological indicators such as enzymes could be used as biomarkers (Barnhoorn, 1996), and to develop water quality indices (Mekkawy *et al.,* 2009).Such biochemical approach have been advocated to provide anearly warning of potentially damaging changes in the stress aquatic organisms (Anderson, 1993). Biochemical values measured in the blood of animals include Glucose, aspartate amino transferase and alanine amino transferase.

These values are useful in the assessment of the nutritional and health status of human beings and animals. Serum Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) in normal animals are low but after extensive tissue destruction, these enzymes are liberated into the serum (Liang, 2004; Lahogianni*et al.*, 2007).

ALT is also known as glutamic pyruvate transaminase (GPT), and AST as Glutamic oxaloacetate transaminase (GOT). ALT and AST serve as a strategic link between carbohydrate and protein metabolism, theyplayrole as an essential group of enzymes in the gluconeogenesis pathway (Castro*et al.,* 2004). Beyond this, the aminotransferases are good indicators of tissue lesions. They are known to be altered during various physiological and pathological conditions making it a possible biomarker. Transitory nature of changes in Glucose concentrations in haemolymph limits its usefulness as an indicator of stress, and therefore, is more promising as biomarkers of environmental pollution (Lagadic*et al.,* 1994).

Use of pesticides may endanger biological effects beyond those for which they were originally manufactured (Singh, 1990). An example of this is the agricultural insecticides which may interfere in the life, reproduction and infection of snail vectors of Schistosomiasis when they reach water bodies (Anderson, 1993). Organisms in response to carbohydrate depletion, use other substrates to obtain the energy needed for its maintenance through gluconeogenesis (Castro*et al.,* 2004). Alanine and aspartate are precursors for gluconeogenesis, among other amino acids the initial reaction is catalysed by ALT and AST. Amino transferases also called transaminases, constitute a group of enzymes that catalyses the inter conversion of amino acids in α-ketoacids by transferring amino groups (Bindya, 2008).

Transaminases and Glucose in molluscan haemolymph have been assessed by some researchers (Akande *et al.,* 2010; Kambale and Potdar, 2010; Bislimi *et al.,* 2013), but Glucose, AST and ASLT activity pattern in the haemolymph of *Bulinus* sp collected in a wild at different location in a reservoir is scanty. This present study focused on haematological analysis of ALT, Glucose and AST of *Bulinus* spacross different locations in Ajiwa reservoir Katsina State-Nigeria.

MATERIALS AND METHODS

Collection of *Bulinus* sp

Foursample species of gastropods (*Bulinus* sp) were collected from five different locations every 3 months, and were transferred into labelled plastic storage bottle and transported into laboratory for haemolymph extraction.

Haemolymph extraction

Collection of haemolymph from the gastropod snail *Bulinus* sp (0.34-2.01gm) was carried out according to the protocol of Kambale and Potdar, (2010) andAkande*et al.* (2010). Prior to bleeding the snails were cleaned with a filter paper to remove the adhering water. The haemolymph was collected with syringe inserted through a tiny hole made with a forcep on the shell above the pericardial region. The syringe was slowly moved deeper in to the pericardial regionuntil drops of pale blue haemolymph were aspirated. 3ml of the haemolymph collected was centrifuge at 1500rpm for 5 minutes in order to remove the haemocytes and cell debris. The resulting supernatant was used for the analysis.

Estimation of ALT, AST and Glucose level in the *Bulinus*sp haemolymph were carried out according to the method described by Mohun and Cook (1957) and Bindya(2008).

Estimation of Aspartate amino transferase (AST)

A 0.2ml sample of haemolymph was added to a test tube containing 1ml of phosphate buffer-substrate and 0.1Mphosphate, 0.1M L-aspartate, and 2mM 2-oxoglutarate. The solution was mixed well and incubated at 37ºC for 1hour. 0.5ml of 2, 4 DNPH and 2.5ml of0.4M NaOHwere added into the solution, and the red colour developed was measured in a HITACHI-U- 2001- UV-Vis

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Spectrophotometer at 510nm, and the enzyme activity was expressed in terms of U/L

Estimation of Alanine amino transferase (ALT)

A 0.2ml sample of haemolymph was added to a test tube containing 1ml of phosphate buffer-substrate, 0.1M phosphate, 0.2M L-alanine, and 2mM 2-Oxoglutarate. The solution was mixed well and incubated at 37ºC for 1hour. 0.5ml of 2, 4 DNPH and 2.5ml of 0.4M NaOHwere added to the solution, and the red colour developed was measured in a HITACHI-U-2001- UV-Vis spectrophotometer at 510nm, and the enzyme activity expressed in terms of U/L.

Estimation of Glucose

Glucose was estimated using Di-nitro salicylic acid test. A sample of approximately 0.1ml of haemolymph was added to 1ml of 5% TCA and centrifuged at 2500rpm for 5 minutes. 0.2ml of the supernatant sample and 2ml of DNS reagent were added and kept boiling for 10minutes. It was cooled to room temperature and absorbance was measured at 575nm using a HITACHI-U- 2001- UV-Vis Spectrophotometer. A reagent blank and a standard of Glucose solution were also maintained. Glucose content was expressed as mg/dl haemolymph.

RESULTS

Glucose

The mean quarterly variation of Glucose in thehaemolymph at five stations of Ajiwa reservoir are presented in Figure 1.The lowest mean Glucose level of 27.49±0.93mg/dl was recorded in March at station 5, while in September highest mean value of 45.83±1.83mg/dl was recorded. There was a progressive increase in glucose level from dry season to wet season (Table 1).

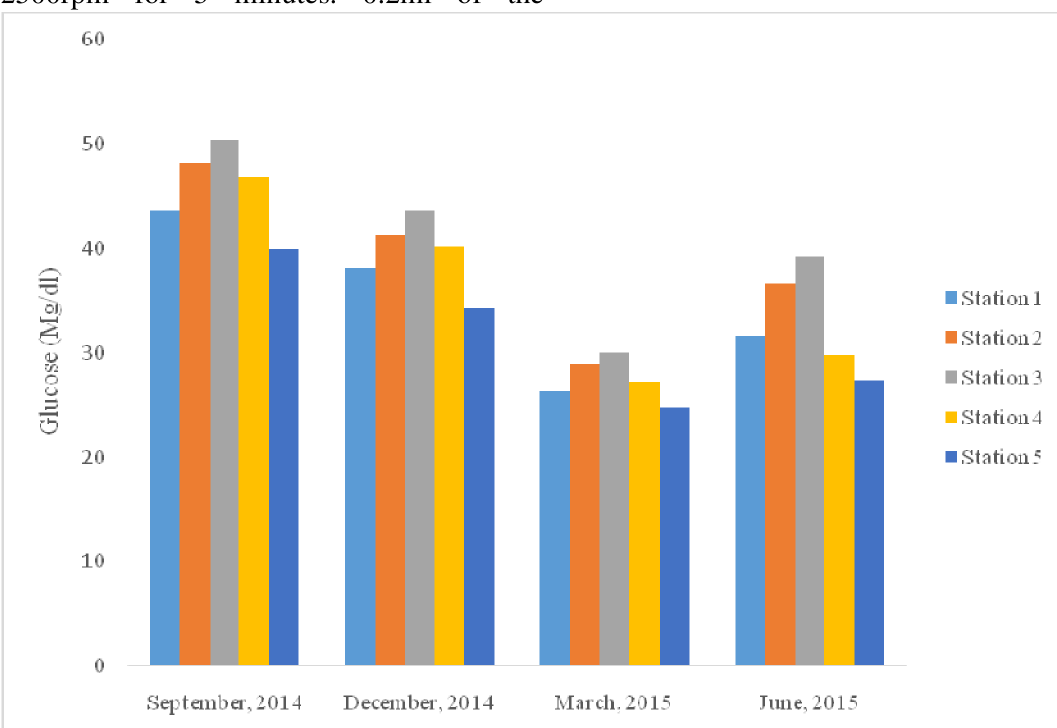

Figure 1: Mean quarterly variation of Glucose in haemolymph of *Bulinus* sp among sampled stations in Ajiwa reservoir.

Table 1: Mean quarterly variation of some Biochemical parameters in *Bulinus* sp (Haemolymph) in Ajiwa reservoir katsina, Nigeria

Months	Haemolymph Glucose	Haemolymph AST	Haemolymph ALT
September	45.83±1.83[a]	24.61±0.16[a]	19.33±0.78[a]
December	39.52±1.58[b]	21.44±0.19[b]	14.96±0.30[b]
March	27.49±0.93[d]	13.80±0.13[c]	10.44±0.21[d]
June	32.97±2.19[c]	16.99±0.15[b]	12.93±0.38[c]
Mean	**36.28±1.76**	**19.01±0.24**	**13.91±0.62**
P value	**0.000****	**0.000****	**0.000****

Note: means along the same column with the same alphabet are non-significantly different

** Highly significant at p<0.05

Aspartate amino transferase (AST)
The mean quarterly variations of AST in haemolymph of *Bulinus*sp in Ajiwa reservoir for all stations are presented in Figure 2. The AST mean value ranged between 13.80±0.13U/L to 24.61±0.16U/L during the study period. The result shows that there was a decrease of AST in March. The least AST mean value of 8.79±0.57 U/L was recorded in March at station 5, while the highest AST mean value of 11.65±0.59U/L was recorded in the September at station 3. Analysis of variance (Table 1) shows highly significant monthly variation ($p<0.01$). However, there were no significant variation between stations $p>0.05$ (Table 2).

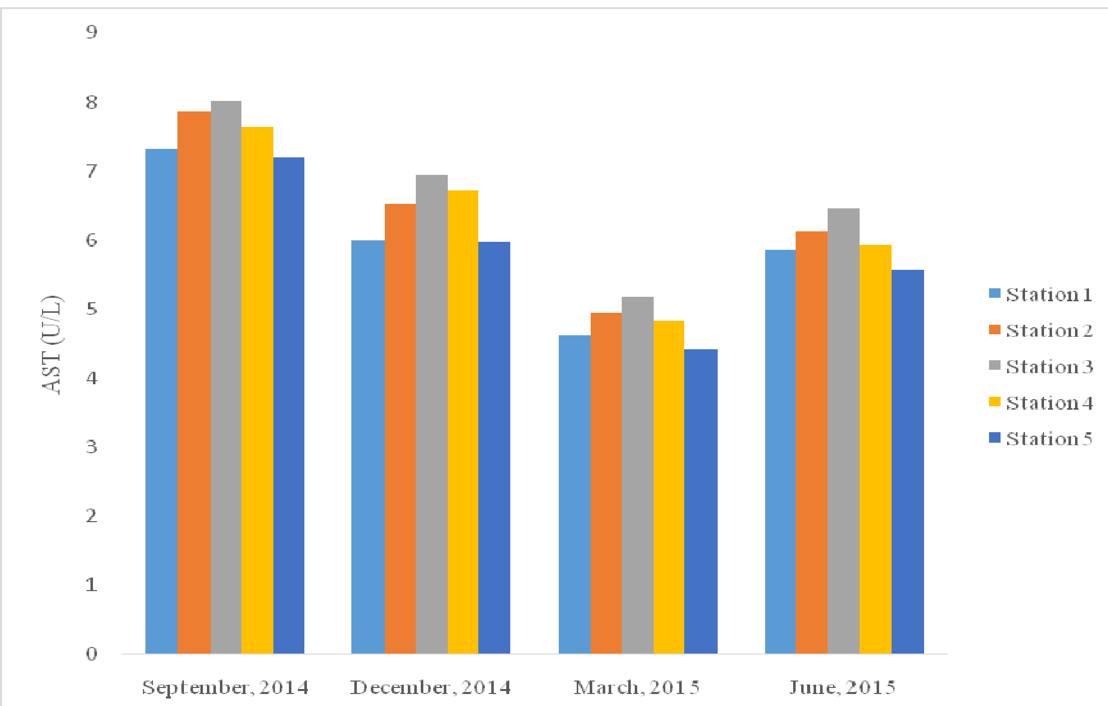

Figure 2: Mean quarterly variation of AST in haemolymph of *Bulinus*sp among sampled stations in Ajiwa reservoir.

Table 2: Mean value of some Biochemical parameters of *Bulinus* sp (haemolymph) across the stations in Ajiwa reservoir katsina, Nigeria

Stations	Haemolymph Glucose	Haemolymph AST	Haemolymph ALT
Station 1	34.94±3.77[a]	10.96±0.55[a]	13.42±1.38[a]
Station 2	38.83±4.04[a]	9.37±0.60[a]	14.24±1.34[a]
Station 3	40.84±4.28[a]	12.65±0.59[a]	15.29±1.95[a]
Station 4	36.04±4.55[a]	11.28±0.60[a]	13.64±1.39[a]
Station 5	31.61±3.44[a]	8.79±0.57[a]	12.98±1.31[a]
Mean	**34.45±1.76**	**11. 31±0.24**	**12.84±0.62**
P value	**0.555ns**	**0.846ns**	**0.835ns**

Note: means along the same column with the same alphabet are non- significantly different
ns: No significant at p>0.05

Alanine amino transferase (ALT)
The mean quarterly variation of ALT in haemolymph of *Bulinus* sp across the five stations of Ajiwa reservoir were presented in Figure 3. The mean value ranges from 10.44±0.21U/L to 19.33±0.78U/L. The lowest ALT mean value of 12.98±1.31U/L of was recorded in March at station 5, while in September highest mean value of 15.29±1.95U/L was recorded at station 3 .

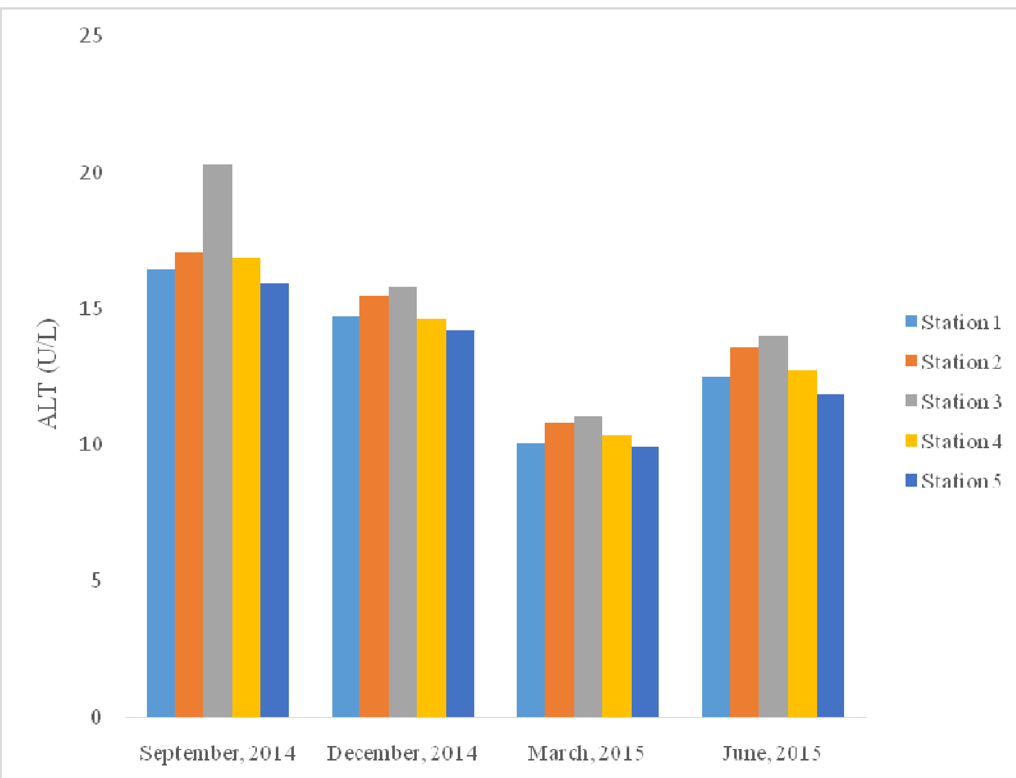

Figure 3: Mean quarterly variation of ALT in haemolymph of *Bulinus* sp among sampled stations in Ajiwa reservoir.

DISCUSSION

The results of this study recorded a remarkable alteration in the activity of the selected biochemical parameters in the haemolymph of *Bulinus* sp. These alterations goes in parallel with the elevation in the levels of water chemical parameters detected in Ajiwa reservoir as a result of contamination stress in the reservoir. The activity of glucose was slightly decreased in this order of magnitude; station 3 > station 2 > station 4 > station 1, station 5 recorded the lowest value.Glucose, AST and ALT levels were high during the wet season compare to dry season (45.83±1.83mg/dl to 27.49±0.93mg/dl), (24.61±0.16 to 13.80±0.13U/L) and (19.33±0.78 to 10.44±0.21U/L) respectively, this may be due to high influx of nutrients from the surrounding farm land and other domestic activities like washing clothes, bathing and cattle rearing within the surrounding of the reservoir. Such reduction in glucose activity in the *Bulinus* sp was also previously observed in some fishes exposed to toxicants (Bucher *et al.,* 1993; Korsguard, 2005; Osman *et al.,* 2010). Thus, the observed decrease in activity of glucose in the present work may reflect metabolic imbalance due to contamination stress of the aquatic environment. According the results of the present work altered metabolic enzymes activities can provide a tool to assess the geographical areas impacted by aquatic pollutions.

These overall changes in the metabolic key enzymes in the *Bulinus* sp collected from five different locations indicated that major changes occur in carbohydrate and protein metabolism. Glutathione (GSH) serves to protect the cell against oxidative damage as it conjugates with compounds of exogenous and endogenous origin (Singhal *et al.,* 1987). GSH production requires NADPH to be synthesized in the pentose phosphate metabolic pathway in which glucose participate. For this reason, glucose is considered as antioxidant enzymes (Mekkawy *et al.,* 2009).

Increase in the activity of serum amylase is an indication of increased carbohydrate degradation Reju*et al.,* (1993), while significant increase in the activity of transaminases in wet season might be an indication of increased protein catabolism through deamination which in turn may result in increase in glucogenic amino acids. This slight increase activity is either due to activation of enzyme as a direct effect of chemicals or due to higher availability of aspartate and alanine precursors because of the less utilisation of amino acids to counter the stress. Decrease in the Glucose level during dry season confirms earlier reports of Bindya (2008), that glucose being an essential metabolite must be supplied at a steady rate to the sensitive organs such as brain, heart kidney and skeletal muscles in order to maintain their vital functions in all situations. The depletion can therefore be attributed to the utilization of this molecule by those sensitive organs and muscles at least to maintain basal metabolic rate. Animals undertake adaptive responses to save nutritional reserves (Suseela*et al.,* 2007). In the present study with Bulinus sp, a reduction in filtration rate and minimal shell opening might be the adaptation to save energy.

CONCLUSIONS

Biochemical parameters (Glucose, AST and ALT) in haemolymph of *Bulinus* sp in Ajiwa reservoir katsina state, Nigeria were evaluated. Based on the obtained results, it is clear that there is a slight decreasedin the level of the abovementioned biochemical parameters in the haemolymph of *Bulinus* sp across the five different locations in the reservoir.

Thus, Alterations in these biochemical parameters in the haemolymph, indicated disturbance of the oxidative carbohydrate and protein metabolism. Apart from evaluating energy content as biomarkers, this experiment shows how *Bulinus* sp undergoes metabolic alteration to overcome stress.

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