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Impact of Sewage on Certain Biochemical Profiles of Indian Major Carp, *Labeo rohita* (HAMILTON)

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Abstract

The advance fingerlings of Labeo rohita (Hamilton) (65 ±2 g weight, 23 ±1.25cm length) were exposed to four sub lethal concentrations (25%, 50%, 75%, and 100%) of sewage effluent to determine its effect on the biochemical parameters. The trial was conducted for a period of 90 days from April, 2014 to June, 2014. Significant changes (P≤ 0.05 level) in the biochemical profile of the fish were observed in the experimental group compared with the control. Among the biochemical parameters, blood glucose, ALT, AST value showed an increasing trend whereas the total protein was decreased during the experimental period. Water quality parameters like ammonia, BOD, COD TDS, hardness and alkalinity exceed the permissible limits. The results of the study showed that the sewage water quality significantly impacted the status of the fish. It is suggested that these physiological parameters can be conveniently employed as health monitoring tools in sewage fed fish culture practices.

Keywords: Sewage, Biochemical parameters, freshwater fish, *Labeo rohita.*

Introduction

Indiscriminate discharge of wastes has aggravated the problem of aquatic pollution and contamination there by causing alterations in the natural condition of aquatic medium and consequently changes in the internal mechanism as well as morphological imbalance of aquatic organism[1]. Among different species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants[2]. Therefore they are very susceptible to physical and chemical changes which may be reflected in the value of one or more of the biochemical parameters[3]. Fish is one of the chief sources of food all over the world due to its nutritional values like essential protein, polyunsaturated fatty acids and liposoluble vitamins[4]. So farmers are mainly interested in general benefits like increased aquaculture production by use of low cost water source, source of nutrients and organic matters but are not well aware of its harmful effects like quality problems related to health. The impact of sewage effluent, both short and long term, on fish physiology plays an important role in establishment of water quality criteria and environmental health risk assessment[5]. The mutual action between a pollutant and a biological system can be calculated using some important biochemical markers[6]. The changes in biochemical (glucose, protein, ALT, AST and cortisol) biomarkers are greatly used to evaluate the toxic stress, integrity of the immune system and tissue damage[7]. The changes in these biomarkers are frequently susceptible to environmental or physiological changes and are simply quantifiable and supply an integrated measure of the physiological changes in an organism[8]. The present investigation has been taken up to elucidate the effects of sewage water on selected biochemical parameters of one of the important Indian major carp *Labeo rohita.*

Material and Methods

The experiment was carried out for a period of 90 days (from April 2014 to June 2014) in the wet laboratory of Department of Aquatic Environment Management division, Faculty of Fishery Sciences, West Bengal University of Animal & Fishery Sciences at Chakgaria, Panchasayar, Kolkata.700094, India (Lat. 22o 82'N; Long. 88o 20'E). The raw sewage was collected from nearby sewage canal situated at metropolitan city of East Kolkata Corporation and stored in rectangular fibre reinforced plastic tanks of 500L capacity. The physico-chemical characters of raw sewage was analysed just after collection following the method of APHA, 2002. Healthy advanced fingerlings of *Labeo rohita,* were collected from private fish farm at Naihati, North-24- Parganas with an average weight of 65 ±2 g and average length of 23 ±1.25cm and were transported in oxygen package to the wet laboratory of the department wet laboratory and were acclimatized for a minimum period of two weeks in 500L capacity FRP tanks. During acclimatization they were fed with commercially available pellet feed (Mohan feed and chemicals ltd.) containing 22% protein and 3% fat, daily once at the morning. Manual water exchange (10 to 15%) was done daily after siphoning out the left out feed and excreta.

Experimental Design: LC50 - 96 h test was carried out to determine the safe concentration level of sewage water. The final experiment was set in four treatment groups and one control group with triplicates following Completely Randomized Design (CRD). Selected sub lethal levels of 25%

(T_1), 50% (T_2), 75% (T_3), 100% (T_4) and a control group (T_0) were maintained in FRP tanks of 500liters capacity with a water volume of 250liters in each tank. Physico-chemical properties of all treatment waters and biochemical parameters of fishes were studied on initial day of experiment. Thereafter water quality parameters, viz. pH, DO (dissolved oxygen), temperature, alkalinity, hardness, BOD (biochemical oxygen demand), COD (chemical oxygen demand), phosphorus, ammonia, TS (total solids), TDS (total dissolved solids) were analysed on weekly basis following standard method and biochemical parameters like protein, glucose ALT and AST were analysed fortnightly following the methods as described below[9].

Serum collection: Blood samples were taken from five randomly sampled fish from each tank at an interval of fifteen days from the caudal vein of fish by using 2ml sterile disposable syringe then was stored in eppendorf tube and kept at room temperature for 30-40 minute. While the blood clots, giving a straw coloured supernatant at the top. Then it was centrifuged at 2500 rpm for 15 min to get the clear serum at the top. The supernatant was collected by micro centrifuge to another eppendorf tube and stored in deep freeze (-20^{0}C) for further analysis of biochemical parameters.

Biochemical analysis: The serum glucose was determined by using glucose test kit (Span Diagnostics Ltd.) following GOD-POD method and total serum protein was determined by using protein test kit (Autopak Diagnostics Ltd.) following Biruet method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done using ALT and AST test kits (Span Diagnostics Ltd.) following Modified UV (IFCC), and kinetic assay method.

Statistical analyses: All the physico-chemical parameters were compared by applying multivariate ANOVA using time and treatment as main factors followed by Duncan's multiple range test (DMRT) between the treatments. Similarly, the biochemical parameters like Serum glucose, total serum protein, ALT and AST over study period were analysed using multivariate ANOVA and DMRT. All the statistical analysis was performed by using SPSS 17. (Chicago, America).

Results and Discussion

Physico-chemical parameters: Average variations of water quality parameters (mean ± S.D) in different treatments during experimental period were given in table-1. From the result it indicated that, there is significant variation in some physico-chemical parameters like ammonia, BOD, COD, TDS, hardness, alkalinity among treatments and control group at $P \leq 0.05$. The minimum and maximum ammonia of water recorded from treatment groups was 0.04±0.001mg/l and 0.82±0.001mg/l. The values of Alkalinity, hardness and total dissolved solids varied between 277.81 to 345.35 mg/l, 708.49 to 915.26 mg/l and 883.51to 1197.56 mg/l respectively. A moderate variation in the biochemical oxygen demand and chemical oxygen demand value were visible which varied between 1.27 to 31.26 mg/l and 14.97 to157.32 mg/l respectively.

Table-1
Water quality in different treatments (mean ± standard deviation) during experimental period (90 days)

Water parameters	Treatments				
	T_0	T_1	T_2	T_3	T_4
pH	7.38±0.04^a	7.63±0.02^b	7.66±0.04^c	7.63±0.03^c	7.51±0.02^d
DO(mg/l)	4.81±0.06^a	5.17±0.04^b	5.29±0.03^b	5.34±0.04^c	5.14±0.05^c
Temperature(^{0}C)	29.14±0.12^a	29.26±0.10^a	29.23±0.21^a	29.17±0.17^a	29.22±0.12^a
Alkalinity(mg/l)	277.81±0.95^a	297.59±0.96^b	314±1.45^c	336.43±1.16^d	345.35±1.25^e
Hardness(mg/l)	708.49±1.41^a	772.56±1.91^b	835.34±2.47^c	879.17±1.62^d	915.26±3.69^e
BOD(mg/l)	1.27±0.06^a	14.98±0.15^b	21.36±0.88^c	28.55±0.62^d	31.26±0.45^e
COD(mg/l)	14.97±0.15^a	65.21±0.22^b	106.75±0.57^c	129.58±1.15^d	157.32±0.58^e
PO_4(mg/l)	0.129±0.015^a	0.145±0.008^b	0.146±0.001^b	0.149±0.005^c	0.149±0.002^c
Ammonia(mg/l)	0.04±0.001^a	0.13±0.002^b	0.34±0.001^c	0.63±0.001^d	0.82±0.002^e
TS(mg/l)	913.98±0.88^a	1036.75±0.61^b	1096.30±0.56^b	1129.11±1.24^c	1247.26±0.51^d
TDS(mg/l)	883.51±1.07^a	997.23±0.54^b	1058.40±0.16^c	1123.11±1.17^d	1197.56±0.62^e

Note: - T_0= Control T_1=25% sewage T_2= 50% sewage T_3=75% sewage T_4=100% sewage

High value of these parameters may be probably due to both organic and inorganic substance in the experimental water. Within the growing sewage fed aquaculture, it is accepted that good water quality is needed for maintaining viable aquaculture production. The present observations are comparable to findings of various researchers[10-12] in sewage fed aquaculture system. A change in the quality of sewage water by the presence of toxins/contaminants and higher load of organic and inorganic pollutants make it potentially harmful to aquatic life forms and may create stress to fish[11]. This may lead to physiological changes, which will be reflected in the value of one or more of the biochemical parameters.

Biochemical parameters: The fortnightly average fluctuations of biochemical parameters of *Labeo rohita* exposed in different concentrations of sewage are presented in table 2. Among the biochemical parameters the glucose, ALT and AST values showed a significant increase ($p \leq 0.05$) during 90th days of observation period figure-1, 3 and 4). The glucose, ALT and AST level of fish serum from experimental group varied from 60.54±0.36 to 78.23±0.78, 18.39±0.12 to 30.47±0.11 and 49.97±0.04 to 56.06±0.04 respectively. The total protein levels was found to be significantly declined ($p \leq 0.05$) compared to control fish in all the trials figure- 2. The protein level of fish found from control group was 7.57±0.04 g/dl and from treatment groups was 5.09±0.06 in T_1, 4.64±0.05 in T_2, 4.21±0.06 in T_3 and 3.71±0.50 in T_4 respectively.

In consensus with the present findings, increase in the blood glucose in sewage water have also been observed by one researcher in response to different parameters such as, increased NH_3 temperature, confinement, and handling[13]. Similar findings were also observed by various researchers who stated an increase in glucose a general response of fish to acute pollutant effects and stress due to presence of organic and inorganic pollutants in sewage water[14-15]. The rise in the glucose level is due to glycogenolysis in the earlier stages and gluconeogenesis in the later stages of stress in fishes[16]. The activity of AST and ALT enzymes in blood is used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress[17]. The pollution of aquatic media with domestic waste lead to an increase in the serum AST, ALT activities of fish[18-19]. An increase in the activities of blood transaminase has been attributed to tissue damage, particularly in the liver[20]. In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles[21-22]. It is generally accepted that an increase of these enzyme activities in the serum or plasma is a sensitive indicator of even minor cellular damage[5].

The investigation found that total protein level in experimental fish exhibited marked decrease than in the fish of control group. Similar type of observation has been reported by some researchers who stated that plasma protein levels decreased in fish samples from polluted waters in relation to that in control fish due to hyper protein anaemia[23]. The reduction in the protein level during the present study suggests disturbance in the physiological activity due to high BOD, COD and ammonia content in experimental waters. BOD is the most reliable parameter for judging the extent of pollution in the waste water[24]. Proteins, performing different biological functions are greatly influenced by environmental stressors. Measurement of proteins in blood can be used to determine the physiological phases of organism and mostly used as an indicator for general state of health[25-26] .The alteration of blood biochemical parameters might be indicative of unsuitable environmental conditions or the presence of excess of organic compounds[27].

Conclusion

The study outlined the effect of sewage effluent on selected biochemical parameters in *Labeo rohita*. The results of the present investigation indicate that exposure to sewage effluent even up to 25% induces significant changes in the biochemical profile of the Indian major carp *L. rohita.* Such changes in biochemical levels under the effect of sewage results in impairment of physiological processes, and hence give an idea about the health status and monitoring of the fish health during cultured in sewage water. More nutritional studies are warranted in this line, especially using other fresh water fishes towards attaining concordant results.

Table-2
Fluctuations in biochemical parameters (mean ± standard deviation) of *Labeo rohita* exposed to different concentration of sewage.

Biochemical parameters	Treatments				
	T_0	T_1	T_2	T_3	T_4
Glucose(mg/dl)	54.77±0.95^a	60.54±0.36^b	65.51±0.52^c	68.11±0.87^d	78.23±0.78^e
Total Protein(g/dl)	7.57±0.04^e	5.09±0.06^d	4.64±0.05^C	4.21±0.06^b	3.71±0.50^a
ALT(IU/L)	14.55±0.52^a	18.39±0.12^a	22.70±0.16^b	26.04±0.56^b	30.47±0.11^c
AST(IU/L)	44.59±0.10^a	49.97±0.04^b	53.14±0.29^c	54.02±0.10^d	56.06±0.04^e

Note: - T_0= control T_1=25% T_2= 50% T_3=75% T_4=100%
Mean with different superscript letters are significantly different ($P < 0.05$)

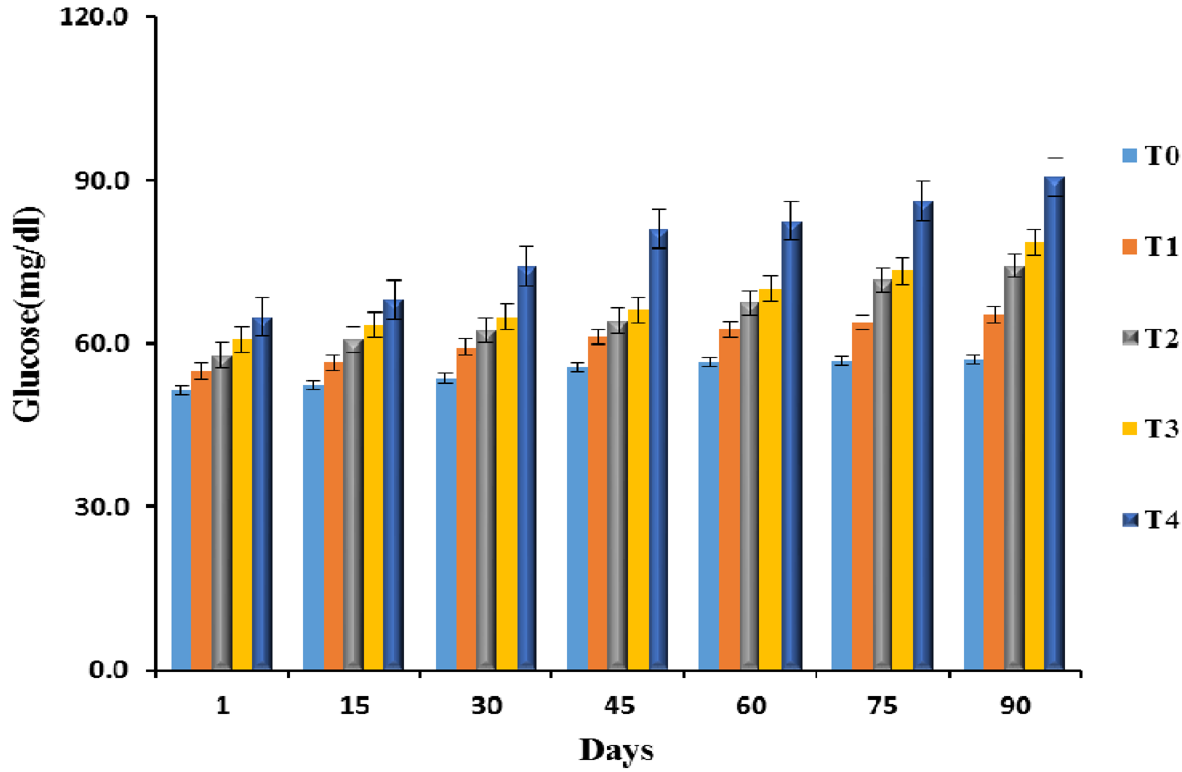

Figure-1
Fortnightly variations (mean ± standard deviation) of Glucose during the experimental period

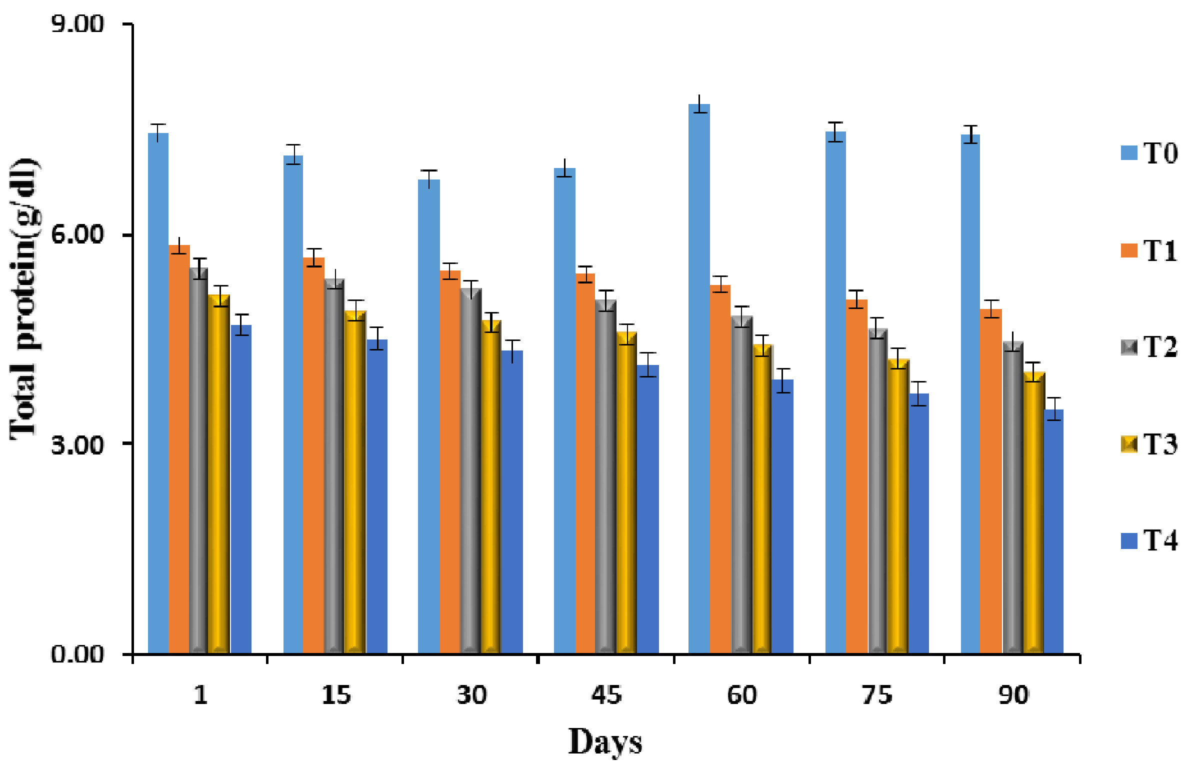

Figure-2
Fortnightly variations (mean ± standard deviation) of total protein during the experimental period

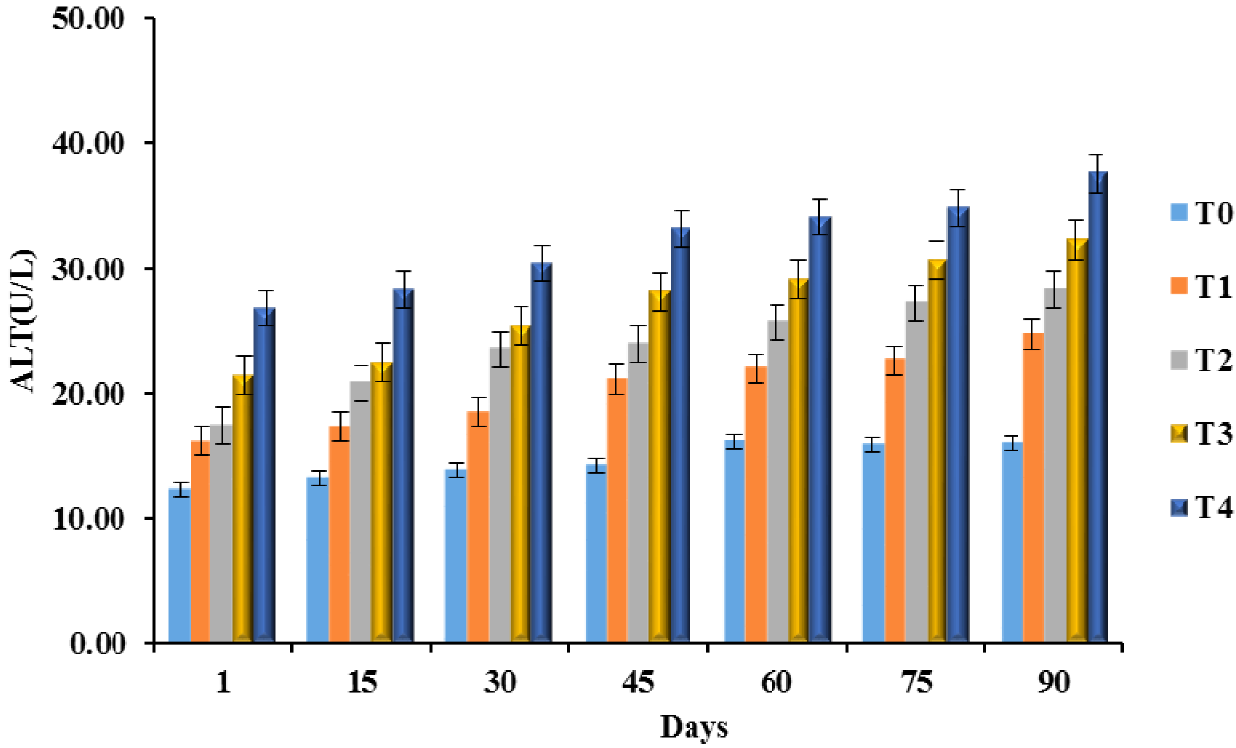

Figure-3
Fortnightly variations (mean ± standard deviation) of ALT during the experimental period

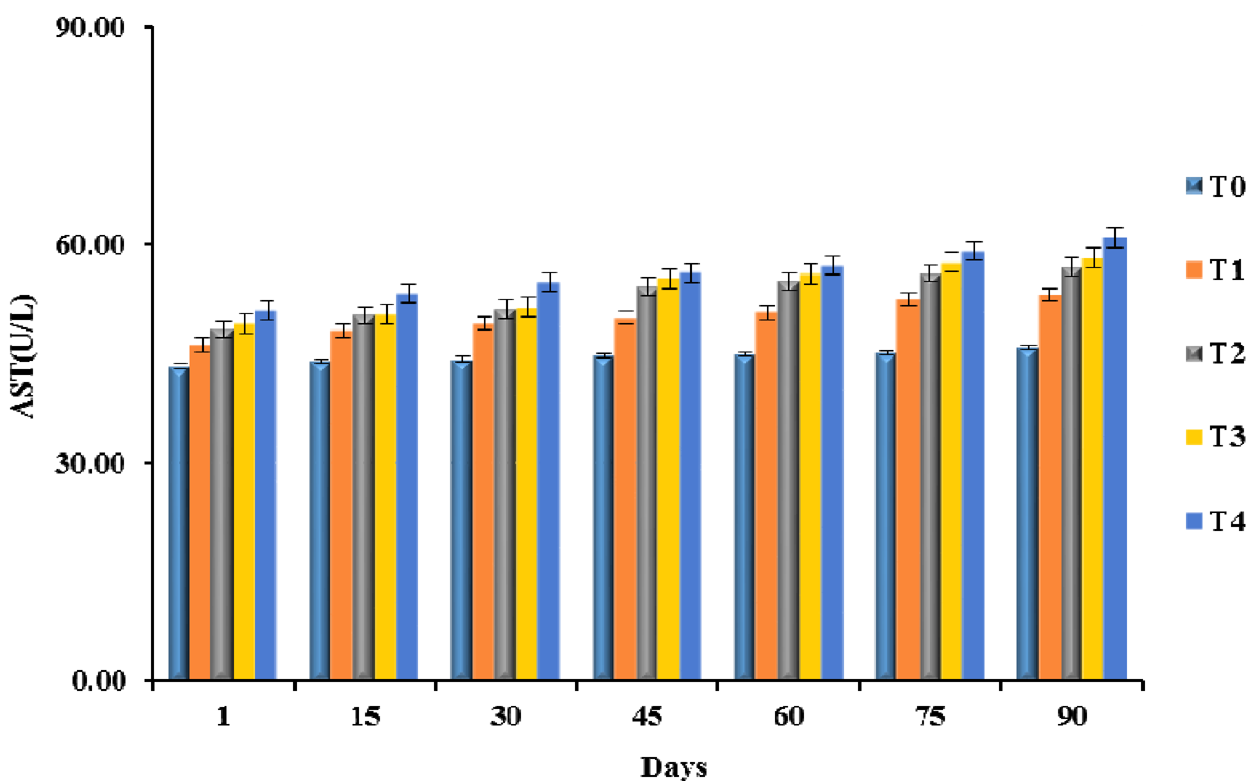

Figure-4
Fortnightly Variations (mean ± standard deviation) of AST during the experimental period

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