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Impact of lead on survival and biophysiological parameters of Horn snail, *Indoplanorbis exustus* Deshayes, 1834

Prabhakaran P.* **and Rajan M.K.**
Department of Zoology, Ayya Nadar Janaki Ammal College, Virudhunagar District, Sivakasi-626124, India
prabhakarentl@gmail.com

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Abstract

The toxicity of lead on the biophysiological parameters of fresh water horn snail, Indoplanorbis exustus was evaluated under laboratory condition. Mortality rate was assessed and lethal concentration LD_{50} was calculated as 9.886 ppm at 96 h. To determine the toxic properties of lead on metabolism the oxygen consumption rate, biochemical content and bioaccumulation rate were observed in adult snail on exposing them to acute and chronic exposure of lead. The oxygen consumption rate and biochemical content showed decrease in increasing exposure time and concentration. The biomagnifications after chronic exposure to lead showed an accumulation rate of 32 ppm in the snail body at higher concentration. The results showed that the snail I. exustus was sensitive to lead on comparing with other tolerant gastropods.

Keywords: Horn snail, Lead, Mortality, Oxygen consumption rate, Bioaccumulation.

Introduction

Humans are facing one of the most horrible ecological crises in the form of pollution. Among the various kinds of pollutants, heavy metals form one of the toxic pollutants released into the environment by anthropogenic activities, which can be bio-magnified in the food chain. Among 104 elements existing in the nature, there are only 80 metals, among which 18 of them are toxic[1]. Heavy metals are those groups of metals and non-metals which have density above 6gm/cm3. Several toxic elements, such as arsenic, cadmium, lead, mercury, uranium, chromium and bismuth are often included in this group[2]. Water sediments and biota are generally metal reservoirs in aquatic environment[3].

The extent of this wide spread but diffused contamination has raised concern about their hazards on plants, animals and humans. Lead is one of the naturally occurring metals in small amount in the earth's crust. Metallic lead is usually insoluble in water, but it combines with other chemicals in water to form lead compounds, that depends on the acidity and temperature of the water[4].

Lead has been used in various processes like manufacturing of storage batteries, piping, cable sheeting, solders for electronics, in ammunition and as a shielding material for X-rays and other radiations[5]. As chemical form, lead is used as gasoline additives, in PVC stabilizers (0.7-2% Pb), pigments (lead chromate) in plastics, paint and ceramics, as rust-inhibitive primers (red lead), lubricants (lead naphthenate), in glasses (lead silicate), fluorescent tubes, for glazing in table ware ($PbSiO_3$) and in small electric motors as permanent magnets (lead-ferrite)[6]. In addition to pollutant sources, lead bearing limestone and galena (PbS) contribute lead to natural waters[7]. It causes a wide range of biological effects that depends on the level and duration of exposure. Its principal biochemical effect in humans and animals is defective haemoglobin formation and also suppresses the activity of enzymes like, Na-K ATPase of red cell membranes, alkaline phosphatase, aldolase and monoamine oxidase[2].

Snails are one of the important components of the aquatic ecosystem. These are tiny creatures sprout in the polluted water like weeds. They can sense accurately the very low levels of metals[8]. Aquatic snails are able to use chemical cues to sense the pollution. Since, toxicants in polluted environments are often patchily distributed, they have developed the ability to defeat and avoid toxicants[9]. Heavy metals directly or indirectly affect the distributions and abundance of snails in freshwater by affecting their growth and reproduction.

The present study deals with aquatic fresh water horn snail, *Indoplanorbis exustus*. It is one of the freshwater aquatic snail and an important vector, involved in the spread of schistosomiasis, fasciolasis and amphistomiasis in domestic animals and sometimes in humans in various South-East Asian countries[10]. The planorbid group of snails is extremely adaptable to any type of polluted water and in water with too little oxygen. They avoid simply flowing water, since they cannot get a foothold on the moving substrate, because they have a high sinistral shell. On the basis of respiratory equipment, they are well adapted to unfavorable conditions[11]. Fewer data is available on the toxicity of lead on horn snail *I. exustus.* Hence the present study has been undertaken and focused to bring out the impact of lead on the biophysiological parameters of *I. exustus*.

Materials and methods

Collection of animal: The freshwater aquatic horn snails, *Indoplanorbis exustus* Deshayes, 1834 (Fam: Planorbidae, Class: Gastropoda) were collected from the college pond in Sivakasi (9.45°N 77.8167°E 101 MSL, Tamil Nadu, India). Identified based on Panha and Burch. They have well-vascularized erectile skin flap in the respiratory cavity, which serves as a "gill". The animal has planispiral shell, which wound in a flat plane, with small aperture. They have filiform tentacles, tubular pneumostome and separate gonopores at left side, with pseudobranch[12]. Prior to analysis the snails were acclimatized for three days under lab condition with ambient temperature (25- 32 ^{0}C) and 16:8 light: dark cycle in 5 L capacity plastic tub using dechlorinated water and fed with leaf detritus *ad libitum* Snails of 0.6 - 0.8 mm in diameter and with a weight varying between 0.199 – 0.225g were selected for the study.

Preparation of toxicant: The stock solution of lead 10 g/l was prepared from lead acetate A.R. grade purchased from Fischer Scientific chemicals using deionized water in a volumetric flask.

Determination of LC_{50} value: From the stock solution, one to ten ppm concentrations of lead were prepared in dechlorinated tap water taken in 500 ml bottles. A group of 10 snails were introduced into each bottles (specific concentration) and three replicates were maintained and an appropriate control was maintained using water alone. The test solutions were replaced daily and the number of snails survived under different concentrations was recorded at 24 h interval. The experiment was carried out under static conditions. The no. of adults died in each treatment was corrected with the control using Abbott's formula[13]. The percentage kill was converted to probit kill by using the conversion table given by Finney[14] and the LD_{50} value was determined by XLSTAT software package for probit analysis.

Effect on physiology: To evaluate the effect of lead on the physiology of snail, the test animals were divided into two groups. The first group was exposed to acute dose of lead (LC_{50} = 4.8 mg/l). The second group was exposed to sub-lethal doses of lead such as 1, 2, 3, 4 and 5 ppm respectively. 20 acclimatized animals were introduced into each concentration for determining its oxygen consumption rate. The experiment was carried out in 1 L bottles under static conditions. Triplicates were maintained for each treatment along with a control containing dechlorinated tap water.

Determination of dissolved oxygen: The amount of dissolved oxygen in the samples was determined by following Winkler's method[15]. The bottles were kept closed, in order to avoid entry of atmospheric air. The amount of oxygen was determined at an interval of 24 h up to 96 h exposure for acute dose and once in three days up to 30 days for chronic exposure. They were compared with control. The water was replaced once in every three days and the initial value of dissolved oxygen was calculated every time before replacement of water. The mg of oxygen consumed per snail was calculated for different concentration from the data recorded. During the chronic exposure period the snails were fed with detritus.

Biochemical analysis: The biochemical analysis such as, total carbohydrate content (Anthrone method)[16], proteins[17] and lipids[18,19] were determined in acute and chronic exposed snails. The foot tissues were dissected out using dissection knife and weighed every 24 h upto 96 h for acute exposure. In chronic exposed snails the biochemical changes were analysed once in 10 days upto 30 days. The biochemical contents were then compared with control.

Bioaccumulation: After 30 days of exposure to toxicant lead, at sub-lethal doses from 1 to 5 ppm, the animal was taken out. The whole body was removed carefully from the shell and placed in a watch glass of 10 mm dia. It was then dried in an oven at 80°C for 2 days. The dried powder of snails was then kept in an airtight specimen bottle. The dried sample was digested in 10ml mixture of nitric acid and perchloric acid of 9:1 ratio at 100^{0}C till dryness and cooled down to room temperature. Then it was diluted to 25ml using distilled water. The solution was filtered and lead content was estimated using Atomic Absorption Spectrophotometer[20], AA-6300 Shimadzu.

Results and discussion

Molluscans serve as a pertinent species for monitoring the effect of pollution. Therefore, in the present work the effect of heavy metal lead on mortality rate, physiology, biochemical characteristics and on rate of bioaccumulation in the horn snail, *I. exustus* has been carried out to establish the relationship between them. The results of this investigation are presented and discussed below.

Mortality rate: The rate of mortality in horn snails was significantly increased with increase in concentration of lead and period of exposure. 50% mortality of snail occurred at a concentration of 10ppm after 96 h of exposure and at 120 h of exposure 50% of mortality occured at 9ppm when compared to control. The probit analysis showed LD_{50} as 9.886 ppm at 96 h. whereas, after 120 h of exposure LD_{50} was 8.628 ppm ± 0.05, 144 h of exposure 50% mortality was observed at 8 ppm concentration. Further for 7 ppm 168 h of exposure showed 50 % mortality (Table1). The probit kill occurs at 9.886± 0.19 ppm after 96 h of exposure.

Similarly significant correlation between mortality and increasing doses of lead and length of exposure in snail *Limicolaria flammea*[21]. At high concentrations of lead and cadmium, horn snail *I. exustus* showed specific behaviour in their movement. They slowly become inactive, protrude out from shell and were unable to attach their feet, became motionless at high concentrations and eventually die. Similar behavioral changes in *Taphius glabratus*, which was unable to attach their feet to the container surface on exposure to cadmium and copper at 0.05 - 0.1ppm was reported[22]. The mortality of snail was due to toxicant lead in water. The physiological and biochemical features also played major role in causing mortality of *I.exustus.* The mortality due to heavy metals was due to deterioration of cell dynamics, cell membrane damage and by more diffusion of heavy metals into cells which results in cell necrosis and death[23].

Oxygen consumption: The oxygen consumption is one of the most important energetic physiological phenomenon, which controls the metabolic activities. It is an indicator of metabolic rate and status of stress condition of animals[24].

During acute exposure to lead (LD_{50} value), the snail showed continuous decrease in oxygen consumption rate. The rate of oxygen consumption was found to reduce with the increasing days of exposure from 0.106 ± 0.01 (24 h) to 0.0422 ± 0.011 (96 h) which was lower than the control (0.139 ± 0.006) (Table-2). However, in the control the rate of oxygen consumption was found to be same with the increasing hours. It was found to decrease slightly for first 15 days of exposure of lead at sub lethal doses from 1ppm to 5ppm (Table-3). But in the remaining 15 days of exposure, there was a drastic reduction of oxygen consumption. At higher concentrations, the oxygen consumption rate was observed to drastically vary from 0.133 ± 0.003 (3^{rd} day) to 0.044 ± 0.004 (30^{th} day) in 4ppm and 0.133 ± 0.001 (3^{rd} day) to 0.04 ± 0.0032 (30^{th} day) in 5ppm when compared to control. Similar result was observed in rate of oxygen consumption of *Corbicula striatella* after acute and chronic exposure of lead[25]. They observed a strong decrease in the rate of consumption when compared to control. Decline in oxygen consumption was observed in *Viviparus bengalensis* after prolonged exposure i.e 48, 72 and 96 h to copper, zinc and mercury[26].

Metabolic processes like respiration in mussels have reported to be adversely affected by the presence of certain heavy metals like copper, cadmium and zinc[27]. The sub lethal concentration of copper, mercury and cadmium depressed the respiratory rate in marine mussel, *Perna viridis*[28] and in *Lamellidens marginalis* when exposed to mercury[29].

Table-1: Mortality rate of horn snail *Indoplanorbis exustus* on exposure to lead.

Dose	Mortality rate of horn snail (%)*						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
1 ppm	0.00	0.00	0.00	0.00	10.0 ±0.3	13.3 ± 0.4	13.3 ± 0.33
2 ppm	0.00	0.00	13.3 ±0.33	10.0 ±0.0	10.0 ± 0.4	13.3 ± 0.5	20.0 ± 0.0
3 ppm	0.00	0.00	0.00	0.00	6.7 ± 0.0	10.0 ± 0.0	10.0 ± 0.3
4 ppm	0.00	13.3±0.3	16.7± 0.4	16.7 ±0.3	20.0 ± 0.58	23.3 ± 0.3	30.0 ± 0.4
5 ppm	0.00	0.00	10.00 ± 0.0	20.0 ± 0.0	23.3 ± 0.3	30.0 ± 0.5	30.0 ± 0.3
6 ppm	0.00	13.4 ± 0.5	20.00 ±0.3	20.0 ± 0.0	23.3 ± 0.3	30.0 ±0.33	33.3 ± 0.3
7 ppm	0.00	0.00	10.00 ±0.0	30.0 ± 0.58	43.3± 0.4	50.0 ± 0.3	60.0 ± 0.3
8 ppm	0.00	13.3± 0.5	30.00± 0.3	40.0± 0.0	40.0± 0.0	43.3 ± 0.4	46.7 ± 0.3
9 ppm	0.00	0.00	20.00± 0.0	40.0 ±0.3	50.0 ±0.0	60.0 ± 0.3	73.3 ± 0.5
10 ppm	0.00	10.0 ± 0.0	33.3 ± 0.3	56.7 ± 0.3	66.7± 0.3	76.7 ± 0.3	90.0 ± 0.57
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LD_{50} (ppm)	-	-	-	9.886 ± 0.19	8.628 ±0.05	7.902 ±0.07	6.27 ± 0.17

*Values are mentioned in Mean ± S.E; ppm-parts per million.

Table-2: The rate of oxygen consumption of horn snail, *Indoplanorbis exustus* after acute exposure to lead.

	Average O_2 consumption mg/day*			
	24 h	48 h	72 h	96 h
Control	0.139 ± 0.006	0.139 ± 0.005	0.137 ± 0.002	0.135 ± 0.005
Lead (9.88ppm)	0.1016 ±0.001	0.084 ± 0.012	0.0575 ± 0.025	0.0355 ± 0.021

*Values are mentioned in Mean ± S.E.

Table-3: The rate of oxygen consumption in horn snail, *Indoplanorbis exustus* after chronic exposure to lead.

Days	Control	Concentrations of lead mg/day*				
		1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
3	0.126 ± 0.002	0.106 ± 0.004	0.106 ± 0.012	0.12±0.001	0.133 ± 0.003	0.133 ± 0.001
6	0.146 ± 0.004	0.136 ± 0.001	0.136 ± 0.003	0.126±0.005	0.123 ± 0.045	0.126 ± 0.0023
9	0.147 ± 0.003	0.140 ± 0.002	0.140 ± 0.031	0.133±0.0021	0.129 ± 0.032	0.119 ± 0.004
12	0.160 ± 0.005	0.13 ± 0.006	0.12 ± 0.005	0.123±0.003	0.102 ± 0.02	0.102 ± 0.002
15	0.150 ± 0.001	0.120 ± 0.001	0.140 ± 0.031	0.933±0.035	0.08 ± 0.001	0.093 ± 0.001
18	0.160 ± 0.007	0.093 ± 0.003	0.091 ± 0.021	0.08±0.0032	0.0733 ± 0.003	0.079 ± 0.007
21	0.166 ± 0.002	0.113 ± 0.007	0.105 ± 0.01	0.086±0.0041	0.066 ± 0.045	0.06 ± 0.002
24	0.165 ± 0.012	0.106 ± 0.042	0.094 ± 0.02	0.074±0.003	0.065 ± 0.0041	0.04 ± 0.031
27	0.166 ± 0.04	0.116 ± 0.031	0.081 ± 0.012	0.069±0.003	0.051 ± 0.008	0.042 ± 0.0051
30	0.168 ± 0.003	0.098 ± 0.005	0.080 ± 0.032	0.061±0.004	0.044 ± 0.004	0.04 ± 0.0032

*Values are mentioned in Mean ± S.E.

In case of aquatic animals, there is no way to escape from the toxicants and majority of its body part is continuously exposed to it is the respiratory surface i.e. gills. In the present investigation, a decline in oxygen consumption was observed in subsequent exposure to lead at various doses. It may be due to the onset of poisoning, which causes gill damage and formation of mucus film over the gills. These are the possible reasons for the reduction in the efficiency of oxygen uptake and show a reduction or change in metabolic activities.

Biochemical changes: The reduction in oxygen consumption also affects the metabolism, which can be indicated in biochemical content of tissues. The biochemical alterations in the body are the indication of stress in the organism. They are involved in unfolding the adaptive protective mechanism of the body to combat the toxic effect[30]. In the present study, the biochemical characteristics, such as, total carbohydrate, protein and lipid content in the tissue showed variations on acute and chronic exposure of lead.

Lead caused slight increase in carbohydrate and lipid content at 24 h of exposure. But then, they caused significant reduction in carbohydrate, protein and lipid content after 48, 72 and 96 h of exposure (Table-4). The carbohydrate content was found to reduce from 207.62 ± 0.43 mg/g (24 h) to 153.21 ± 0.56 mg/g (96 h) when compared with control. The protein content in the control was observed to increase from 139.16 ± 0.54 mg/g (24 h) to 140.26 ± 0.34 mg/g (96 h). But in experimental snails, it decreased with increasing hours of exposure as 128.93 + 10.56 mg/g (24 h), 120.41 ± 16.7 mg/g (48 h), 110.03 ± 0.38 mg/g (72 h) and 94.72 ± 10.34 (96 h). The lipid content was found to decrease from 78.79 ± 3.91 mg/g (24 hrs) to 60.24 ± 0.45 mg/g (96 hrs) in the foot tissue on acute exposure of lead, when compared to control.

On chronic exposure, carbohydrate content was found to increase with increasing concentrations of lead in the tissue after 10 days (Table-5). It causes decrease in carbohydrate content in each concentrations with increase in days of exposure from 209.16 ± 0.12 mg/g to 190.61 ± 0.16 mg/g in 1ppm, 213.12 ± 1.25 mg/g to 184.56 ± 0.02 mg/g in 2 ppm, 217.51 ± 0.15 mg/g to 209.76 ± 0.31 mg/g in 3 ppm, 230.71 ± 0.11 to 204.31 ± 0.031mg/g (4 ppm) and 229.11 ± 0.042 mg/g to 190.96 ± 24 mg/g in 5 ppm, when compared to control. But in experimental snails, the protein was drastically reduced with increasing concentrations of lead and increase in days of exposure. While, the lipid content increased in level than the control on increase in concentration and days of exposure.

Table-4: The effect on total carbohydrate, protein and lipid content (mg/g) in the foot of horn snail, *Indoplanorbis exustus* after acute exposure to lead.

Treatments		Acute exposure of lead *			
		24h	48h	72h	96h
Control	Carbohydrate	203.21±1.22	210.16±0.32	204.32±0.42	203.21 ± 0.02
	Protein	139.16± 0.54	139.2± 0.45	137.8 ±0.42	140.26± 0.34
	Lipid	62.43± 0.763	63.23± 0.04	60.96± 0.02	67.28± 0.005
Lead (9.88 ppm)	Carbohydrate	207.62± 0.43	210.45±0.32	163.42±0.41	153.21± 0.56
	Protein	128.93±1.56	120.41±1.72	110.03±0.38	94.72± 10.34
	Lipid	78.79 ± 3.9	89.12± 0.46	64.47± 1.43	60.24± 0.45

*Values are mentioned in Mean ± S.E.

Table-5: Effect on total carbohydrate, protein and lipid content (mg/g) in the foot of horn snail, *Indoplanorbis exustus* after chronic exposure to lead.

Days		Control	Concentrations of lead *				
			1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
10	Carbohydrate	204.51 ± 0.12	209.16 ± 0.12	213.12 ± 1.25	217.51 ± 0.15	230.71± 0.11	229.71±0.042
	Protein	139.16 ± 0.91	130.54 ± 0.012	131.01± 0.015	125.71 ± 0.007	119.71± 0.001	117.84 ± 0.12
	Lipid	62.4 ± 0.311	64.97 ± 0.003	65.61 ± 0.126	68.10 ± 0.003	71.14 ± 0.007	73.78 ± 0.084
20	Carbohydrate	212.12 ± 0.141	201.43 ± 0.001	209.45 ± 0.007	213.64 ± 0.003	221.31±0.005	217.14±0.031
	Protein	151.61 ± 1.20	129.01 ± 0.042	125.76± 0.003	120.51 ± 0.007	115.71± 0.015	104.3 ± 0.130
	Lipid	62.1 ± 0.120	70.14 ± 0.003	75.61 ± 0.005	79.31 ± 0.121	83.31 ± 0.008	86.56 ± 0.002
30	Carbohydrate	230.71 ± 0.04	190.61 ± 0.16	184.56 ± 0.02	209.76 ± 0.31	204.31± 0.03	190.96 ± 2.41
	Protein	140.17 ± 0.57	124.12 ± 0.072	129.01 ± 0.15	111.93 ± 0.02	106.76± 0.014	98.45 ± 0.002
	Lipid	70.19 ± 0.012	79.17 ± 0.054	82.51 ± 0.013	85.31 ± 0.002	89.82 ± 0.314	92.31 ± 0.007

*Values are mentioned in Mean ± S.E.

In estuarine clam, *Villorita cyprinoids* decrease in glycogen level was reported early due to heavy metal intoxication[31]. Hence, the decrease in protein content may be due to anaerobic metabolism, which leads to increase in the utilization of organic reserves. Further, the reduced state of protein is from the inhibition of enzymes involved in protein synthesis. The heavy metals inhibit the activity of various enzymes through formation of metal salt complexes with a number of legends such as SH-PO_2, NH_2-COOH and histidine[32]. It caused an increase in lipid content, by anoxic endogenous oxidation process for survival under cadmium stress[30] by inhibition of lipase activity due to heavy metal treatment. The increase in lipid in the animal may be due to detoxifying the metal stress and to overcome from it. Thus, the metal such as, cadmium and lead cause a significant change in metabolism of the animal, which was indicated in the variations of biochemical characteristics.

Bioaccumulation: The absorption and accumulation of heavy metals may affect the life in various processes at different stages. In the present investigation, the rate of accumulation of lead increases with increase in concentrations of metal (Table-6; Figure-1). The accumulation was found to be as 4.80 (1 ppm) and 32.41 mg/g (5 ppm). Similar result was reported that on exposure of mercury to *Parreysia cylindrica*, the rate of accumulation was increased with increasing days of exposure[33].

Table-6: Bioaccumulation rate of lead mg/g in snail *I.exustus* on chronic exposure of lead.

Control	Concentration of lead *				
	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
0.29± 0.003	4.80± 0.06	14.68 ± 0.08	16.33 ± 0.15	18.19 ± 0.11	32.41 ± 0.15

*Values in mean ± S.E.

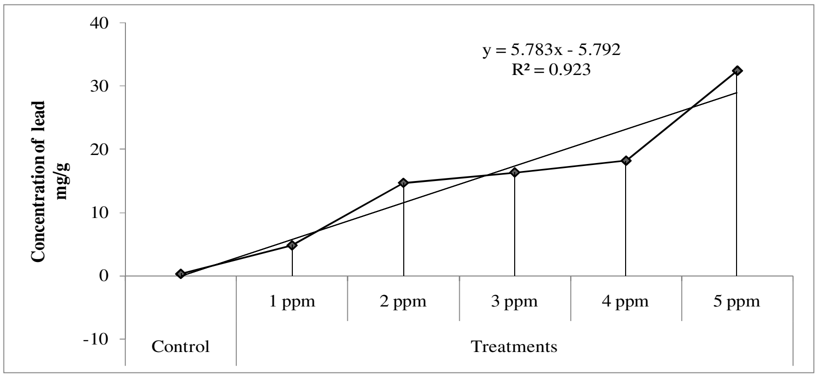

Figure-1: Concentration of lead accumulated in snail *I.exustus* on chronic exposure of lead.

In the snail *Helix aspera,* collected from highly traffic site showed a marked increase in the accumulation of lead and DNA damage[34]. In the spiny rock oyster, *Spondylus spinosus*, from coastal waters of Iskenderun Bay, Turkey the level of heavy metals, showed their range as 4.63 – 352mg/kg in respect to lead[35]. The extent of accumulation in biota is dependent on the chemical effects of the metal, its tendency to bind to particular substance and on the lipid content and composition of the biological tissue[36]. The accumulation of metal was based on the expression of metal binding protein in the tissues. Lead showed higher accumulation in the present study.

Conclusion

The study of bio-indicator organism can reveal the biological impact of pollution over a geographical and temporal scale. The toxic impact brings physiological, biochemical or pathological alterations in an organism. In the biochemical characteristics, the level of carbohydrate, protein and lipid were found to be decreasing on acute exposure of lead; while on chronic exposure, the level of lipid showed increasing trend during the experimental period but the level of carbohydrate and protein was found to be decreasing with increasing days of exposure. In conclusion, it has been surmised that lead have a significant impact on various characteristics of horn snail represented as a pollution indicator in fresh water. Moreover, their population was reducing due to the release of various industrial pollutants around the source of fresh water which can be monitored to protect the community of horn snail.

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