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Acetylcholinesterase activities in the Nervous system of snail *Lymnaea acuminata* as Biomarkers of Water pollutants in Ramgarh Lake, Gorakhpur, UP, India

Singh Nitu
Department of Applied Science and Humanities, Buddha Institute of Technology, Gida, Gorakhpur, UP, INDIA

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Abstract

The effect of different environmental factors in different season and their relative effect on acetylcholinesterase (AChE) enzyme in the nervous tissue of snail Lymnaea acuminate were determined during various months of the year (2007-2008). Temperature, pH, dissolved oxygen (DO), carbon dioxide (CO_2), conductivity and turbidity were measured in control (tap water) as well as polluted water (Ramgarh lake) was measured simultaneously, that such ,the nervous tissue of the snail was assayed for the activity of acetylcholinesterase at 24h and 96h, physical and chemical parameters of the twelve months of the same year. Highest inhibition of AChE was observed in summer season and minimum in winter season. In summer season AChE inhibition was high because temperature, carbondioxide (CO_2) and turbidity was high and pH, dissolved oxygen (DO), conductivity was low. There was a significant product positive and negative correlation between AChE and pH Temperature, dissolved oxygen (DO), carbondioxide (CO_2), conductivity and turbidity of water in corresponding months. Analysis of variance (ANOVA) was used to analyze differences in biomarkers activity and each site.

Keywords: Acetylcholinesterase, nervous system, snail, biomarkers, water pollutants, Ramgarh lake.

Introduction

The measurement AChE activity is used worldwide as biomarkers of environmental contamination. Kinetics of acetylcholinesterase inhibition in *Lymnaea acuminata*[1] by carbamate and organophosphorus pesticides has been extensively worked out. Since, *Lymnaea acuminate* is an aquatic snail and these pesticides and other pollutant are present in water as agricultural pollutants. The present study will be based on the use of this aquatic snail *L. acuminata* as bioindicator. The water quality and the effect of pollutant in Ramgarh Lake will be measured with biomarkers such as acetylcholinesterase. Biochemical biomarkers are increasly used in ecological risk assessments of aquatic ecosystem to identify the incidence of exposure to and effects caused by xenobiotics, AChE is inhibited by phosphate and carbamate esters that are commonly used as insecticides[2]. In the present study the different parameter such as Temperature, pH, dissolved oxygen (DO) and carbondioxide (CO_2) corresponding to acetylcholinesterase activity in the nervous tissue of exposed snails will be studied round the year (2007-2008) from May to April. A correlation between the pollutant containing water and corresponding changes in AChE activity in the nervous tissue will be established to predict the level of water pollution.

Material and Methods

Test material: Different parameters such as Temperature, pH and suspended particles were measured by thermometer, digital pH meter and titration method. Dissolved oxygen and CO_2 was measured according to the standard methods for the examination of water and waste water[3].

Bioassays: Adults *L. acuminata* (2.6±0.3 cm) were collected locally and used as test animals. The experiments were set up with four different region polluted water {RKBK (A), Railway (B), Nowkavihar (C) and Jharkhandi (D)} and dechlorinated tap water as control. Snails were kept in glass aquarium containing dechlorinated tap water and were allowed to acclimatize to the laboratory condition for 72h. Dead animal were removed immediately to prevent contamination to other live snails. Laboratory condition acclimatized snails were used for the experimental purpose. The nervous tissue was dissected from the buccal mass of the snail of control as well as polluted water and then used for measurement of enzyme activity to observed any differences in inhibition between control and polluted snail. Nearly 12 to 15 snails were dissected to get 50 mg of nervous tissue.

Enzyme assays: Acetylcholinesterase: Acetylcholinesterase activity was measured according to the method of Ellman et al[4] as modified by Singh et al[5]. Fifty milligrams of nervous tissue was homogrnized in 1.0 ml of 0.1 M phosphate buffer pH 8.0 for 5 min in an ice bath and centrifuged at 1000 g for 30 min at 4^0C. The supernatant was used as an enzyme source. The change in optical density at 412 nm was monitored for 3 min at 25^0C. Enzyme activity has been expressed as µ mole 'SH' hydrolysed/min/mg protein.

Protein estimation: Protein estimated by the method of Lowry et al[6].

Statistical analysis: Results have been expressed as mean ±SE of six replicates. Product correlation and analysis variance (ANOVA) method was applied in between different physical and chemical parameter to corresponding changes in the enzyme activity was used in different months.

Results and Discussion

There was a significant time dependent variation (P<0.05) in the physical and chemical parameters (2007-2008) against *L. acuminata* to different sites of water viz. A (RKBK), B (Kurnagat), C (Nowkavihar) and D (Railway). In control group of animals kept in dechlorinated tap water. There was observed a significant effect of different parameters throughout the year. Highest inhibition of acetylcholinesterase (AChE) was observed in months of May to August and lowest inhibition of AChE was observed in month of December to February at different exposure period. In control group, significant positive and negative correlation (P<0.05) between AChE and corresponding pH, DO, CO_2 , conductivity and turbidity of each months at 24h and 96h exposure period (table 1 to 4).

Table-1
Effect of pH, dissolved oxygen, carbon dioxide, conductivity, turbidity, and tem. On the snail *Lymnaea acuminata* with different water of May 2007 to April 2008 at 24h exposure periods

Month	Parameter	pH	D.O (mg/l)	CO_2 (mg/l	Conductivity (µ mhos/cm)	Turbidity (NTU's/ feet)	Tem. (0˚C)	AChE (µ mol 'SH'hydrolysed/min/mg protein)
May	Control	7.8	1.5	15	7.2	3	35.5	0.82 ± 0.008 (100%)
	A	7.9	2.4	55	5.0	12	35.5	0.51 ± 0.032 (53.7%)
	B	7.6	2.2	46	7.0	7	35.5	0.37 ± 0.006 (46.2%)
	C	7.8	2.0	45	8.0	9	35.5	0.26 ±0.004 (32.5%)
	D	7.7	1.9	39	10.0	8	35.5	0.24 ± 0.004 (25.3%)
June	Control	7.6	1.4	18	19.0	7	36.0	0.83 ± 0.005 (100%)
	A	7.8	2.3	68	28.0	15	36.0	0.55 ± 0.005 (64.7%)
	B	7.7	2.2	59	27.0	14	36.0	0.38 ± 0.004 (44.0%)
	C	7.8	2.1	55	26.0	13	36.0	0.26 ± 0.004 (32.5%)
	D	7.7	1.5	45	28.0	13	36.0	0.17 ± 0.002 (21.2%)
July	Control	7.9	1.9	16	25.0	8	36.0	0.80 ± 0.037 (100%)
	A	7.7	2.5	78	27.0	12	36.0	0.51 ± 0.032 (53.7%)
	B	7.4	2.4	74	29.0	15	36.0	0.38 ± 0.004 (44.0%)
	C	8.2	2.2	72	29.0	10	36.0	0.32 ± 0.004 (33.7%)
	D	7.8	2.1	66	35.0	14	36.0	0.17 ± 0.002 (21.2%)
Aug.	Control	8.5	1.0	18	4.0	8	36.0	0.80 ± 0.037 (100%)
	A	8.9	2.7	67	6.0	7	36.0	0.51 ± 0.032 (53.7%)
	B	8.6	2.5	62	8.0	21	36.0	0.37 ± 0.006 (46.2%)
	C	8.1	2.3	60	5.0	20	36.0	0.26 ±0.004 (32.5%)
	D	8.4	1.6	50	9.0	29	36.0	0.24 ± 0.004 (25.3%)
Sep.	Control	8.4	1.5	14	35.0	16	35.0	0.85 ± 0.010 (100%)
	A	8.8	4.2	55	20.6	10	35.0	0.61 ± 0.009 (73.5%)
	B	9.8	3.9	42	21.3	16	35.0	0.47 ± 0.056 (56.6%)
	C	8.8	3.5	42	21.5	15	35.0	0.33 ± 0.008 (40.2%)
	D	9.3	3.3	32	30.0	12	35.0	0.24 ± 0.004 (25.3%)
Oct.	Control	9.5	1.0	10	32.3	12	27.0	0.83 ± 0.005 (100%)
	A	9.4	4.9	28	28.0	6	27.0	0.64 ± 0.004 (75.3%)
	B	7.2	4.5	26	27.7	12	27.0	0.53 ± 0.086 (63.8%)
	C	9.6	4.0	25	20.4	15	27.0	0.34 ± 0.008 (41.5%)
	D	9.5	3.9	20	34.0	12	27.0	0.33 ± 0.008 40.2%
Nov.	Control	9.1	1.2	11	28.4	11	27.0	0.85 ± 0.010 (100%)
	A	9.5	7.2	35	31.9	3	27.0	0.67 ± 0.008 (80.7%)
	B	10.2	6.5	29	25.9	5	27.0	0.53 ± 0.086 (63.8%)
	C	9.6	4.8	27	25.5	9	27.0	0.47 ± 0.056 (55.3%)
	D	7.9	4.7	22	38.5	8	27.0	0.34 ± 0.008 (40.0%)
Dec.	Control	9.8	1.3	0.8	54.8	10	25.0	0.80 ± 0.037 (100%)
	A	9.6	10.0	20	63.5	6	25.0	0.67 ± 0.008 (80.7%)
	B	9.9	9.2	18	39.8	5	25.0	0.53 ± 0.086 (63.8%)

	C	10.1	8.9	16	37.6	8	25.0	0.52 ±0.032 (62.6%)
	D	10.0	8.5	14	45.0	8	25.0	0.51 ± 0.032 (53.7%)
Jan.	Control	9.6	1.8	0.5	59.9	8	25.0	0.85 ± 0.010 (100%)
	A	10.0	12.0	15	60.7	1	25.0	0.74 ± 0.008 (90.2%)
	B	9.6	10.5	12	47.4	12	25.0	0.64 ±0.004 (75.3%)
	C	9.9	9.3	11	46.0	5	25.0	0.54 ± 0.035 (63.5%)
	D	9.8	7.5	0.9	48.5	4	25.0	0.51 ± 0.032 (53.7%)
Feb.	Control	9.7	1.5	0.8	58.9	8	26.0	0.85 ± 0.010 (100%)
	A	9.9	9.8	20	60.6	18	26.0	0.74 ± 0.008 (90.2%)
	B	8.2	7.2	19	62.9	7	26.0	0.64 ±0.004 (75.3%)
	C	8.1	4.5	18	62.8	1	26.0	0.53 ± 0.086 (63.8%)
	D	9.8	3.8	11	47.0	7	26.0	0.47 ± 0.056 (55.3%)
March	Control	8.6	1.0	14	25.4	8	35.0	0.85 ± 0.010 (100%)
	A	8.8	8.2	35	21.4	6	35.0	0.61 ± 0.009 (73.5%)
	B	9.1	6.5	30	30.8	8	35.0	0.55 ± 0.004 (57.9%)
	C	7.9	4.5	28	34.1	2	35.0	0.33 ± 0.008 (40.2%)
	D	9.1	3.5	22	40.7	7	35.0	0.26 ±0.004 (32.5%)
April	Control	8.5	1.2	17	37.0	9	34.0	0.85 ± 0.007 (100%)
	A	8.9	6.8	30	33.3	28	34.0	0.55 ± 0.005 (64.7%)
	B	8.8	5.5	25	38.3	15	34.0	0.47 ± 0.056 (55.3%)
	C	9.5	4.1	22	32.4	30	34.0	0.34 ± 0.008 (40.0%)
	D	8.9	4.0	19	40.8	28	34.0	0.28 ± 0.008 (32.9%)

Each experiment was replicated 6 times and the value of pH, DO, CO_2, Conductivity, Turbidity and Temperature is the mean of two replicates and AChE is the mean ± SE were measured after every 24h period. ANOVA and product moment correlation coefficient in between the AChE and different parameters indicate significant (P<0.05) (+) (*) negative correlation. A. RKBK, B. Kurnaghat, C. Nowkavihar, D. Railway

Table-2

Effect of pH, dissolved oxygen, CO_2 and tem. On the snail *Lymnaea acuminata* with different water of May 2007 to April 2008 at 96h exposure periods

Month	Parameter	pH	D.O (mg/l)	CO_2 (mg/l)	Conductivity (µ mhos/cm)	Turbidity (NTU's/ feet)	Tem. (0˚C)	AChE (µ mol 'SH'hydrolysed/min/mg protein)
May	Control	8.3	0.5	10	4	3	35.5	0.80 ±0.037 (100%)
	A	8.2	1.5	45	6	2	35.5	0.44 ± 0.004 (51.8%)
	B	8.3	1.2	40	5	4	35.5	0.26 ± 0.006 (30.6%)
	C	8.4	1.0	32	5	5	35.5	0.17 ± 0.002 (17.8%)
	D	8.0	0.8	28	6	2	35.5	0.14 ± 0.002 (16.7%)
June	Control	7.0	0.7	12	29	5	36.0	0.82 ± 0.008 (100%)
	A	8.5	2.4	48	35	4	36.0	0.44 ± 0.004 (51.8%)
	B	8.2	2.1	36	38	3	36.0	0.27 ± 0.007 (31.8%)
	C	8.5	1.9	30	30	6	36.0	0.17 ± 0.002 (17.8%)
	D	8.5	2.0	25	33	5	36.0	0.14 ± 0.002 (16.7%)
July	Control	8.5	0.5	10	28	3	36.0	0.80 ±0.037 (100%)
	A	7.6	1.7	42	32	5	36.0	0.51 ± 0.032 (61.4%)
	B	7.5	1.6	31	35	8	36.0	0.28 ± 0.008 (32.9%)
	C	7.2	1.3	25	39	7	36.0	0.26 ± 0.006 (30.6%)
	D	7.2	1.0	20	30	5	36.0	0.17 ± 0.002 (17.8%)
Aug.	Control	7.7	0.4	11	7	2	36.0	0.80 ±0.037 (100%)
	A	7.8	1.9	38	9	5	36.0	0.44 ± 0.004 (51.8%)
	B	7.8	1.6	33	8	4	36.0	0.28 ± 0.008 (32.9%)
	C	8.0	1.2	26	7	2	36.0	0.17 ± 0.002 (17.8%)
	D	8.1	1.1	22	8	2	36.0	0.14 ± 0.002 (16.7%)
Sep.	Control	8.4	0.8	14	31	11	35.0	0.83 ± 0.005 (100%)
	A	9.5	3.5	32	24.8	10	35.0	0.51 ± 0.032 (61.4%)
	B	9.3	2.4	27	25	10	35.0	0.37 ± 0.008 (38.9%)
	C	9.0	2.3	24	29	9	35.0	0.32 ± 0.013 (38.5%)
	D	9.1	1.8	19	30.5	8	35.0	0.28 ± 0.008 (32.9%)
Oct.	Control	8.3	1.0	10	33.5	12	26.0	0.84 ± 0.003 (100%)
	A	7.8	2.1	28	29.2	10	26.0	0.58 ± 0.007 (69.9%)

	B	7.8	2.5	23	28.5	11	26.0	0.45 ± 0.011(54.2%)
	C	7.9	2.8	19	29.2	12	26.0	0.34 ± 0.085 (40.0%)
	D	7.7	2.4	15	28.7	25	26.0	0.27 ± 0.007 (31.8%)
Nov.	Control	6.8	1.0	10	21.7	8	28.0	0.84 ± 0.003 (100%)
	A	8.0	3.8	25	22.5	2	28.0	0.55 ± 0.004 (64.7%)
	B	7.9	2.5	23	22	3	28.0	0.43 ± 0.049 (51.8%)
	C	7.8	2.2	20	21.1	3	28.0	0.39 ± 0.013 (46.9%)
	D	7.6	2.0	16	21.4	2	28.0	0.33 ± 0.042 (39.8%)
Dec.	Control	8.6	0.9	08	58.4	9	24.0	0.85 ± 0.007 (100%)
	A	8.0	4.1	19	67.1	4	24.0	0.64 ± 0.004 (75.3%)
	B	8.1	3.5	16	47.8	3	24.0	0.58 ± 0.007 (68.2%)
	C	8.2	3.0	11	43.4	5	24.0	0.43 ± 0.049 (51.8%)
	D	8.1	2.8	18	45.4	4	24.0	0.34 ± 0.085 (40.5%)
Jan.	Control	8.4	1.1	10	60.9	9	23.0	0.85 ± 0.007 (100%)
	A	8.2	5.0	18	62.4	9	23.0	0.77 ± 0.008 (91.7%)
	B	8.3	4.3	16	50.7	4	23.0	0.64 ± 0.004 (76.2%)
	C	8.3	3.8	14	49.7	4	23.0	0.46 ± 0.123 (54.1%)
	D	8.2	2.8	11	51.9	6	23.0	0.44 ± 0.004 (52.4%)
Feb.	Control	8.4	1.0	07	59.2	9	25.0	0.84 ± 0.003 (100%)
	A	8.3	3.5	29	64.6	6	25.0	0.58 ± 0.007 (69.9%)
	B	8.2	3.0	25	63.3	7	25.0	0.52 ± 0.004 (61.9%)
	C	8.3	2.4	21	64.9	7	25.0	0.34 ± 0.085 (40.0%)
	D	8.1	2.0	19	53.6	6	25.0	0.33 ± 0.023 (38.8%)
March	Control	8.5	0.7	09	69.7	9	35.0	0.83 ± 0.005 (100%)
	A	8.3	3.1	28	75.9	9	35.0	0.54 ± 0.004 (56.8%)
	B	8.3	2.6	25	68.6	8	35.0	0.37 ± 0.008 (38.9%)
	C	8.5	2.4	21	74.7	9	35.0	0.27 ± 0.007 (31.8%)
	D	8.3	1.8	15	68.2	9	35.0	0.26 ± 0.006 (30.6%)
April	Control	8.2	0.8	08	65.1	9	33.0	0.85 ± 0.007 (100%)
	A	8.1	2.2	30	100.6	13	33.0	0.53 ± 0.086 (62.3%)
	B	8.3	2.0	25	97.4	11	33.0	0.44 ± 0.004 (51.8%)
	C	8.3	1.6	21	88.3	14	33.0	0.28 ± 0.008 (33.3%)
	D	8.3	1.5	18	96.7	9	33.0	0.27 ± 0.007 (31.8%)

Each experiment was replicated 6 times and the value of pH, DO, CO_2, Conductivity, Turbidity, Temperature is the mean of two replicates and AChE is the mean ± SE were measured after every 96h period. ANOVA and product moment correlation coefficient in between the AChE and different parameters indicate significant ($P<0.05$) (+) (*) negative correlation. A. RKBK, B. Kurnaghat, C. Nowkavihar, D. Railway

RKBK (Site A), there was significant negative correlation between AChE activity in different month and corresponding temperature, pH/ DO/ CO_2/ conductivity/turbidity, when snails were kept of 24h in water (table 1 and 3). When snails were kept for 96h, negative correlation in between AChE activity in nervous tissue and temperature/ pH/ DO/free CO_2/turbidity and there was significant positive correlation in between AChE activity and dissolved O_2/conductivity (table 2 and 4).

Kurnaghat (Site B), There was significant negative correlation between AChE activity in different month and corresponding temperature/ pH/free CO_2/ turbidity and positive correlation between AChE activity in different month and corresponding DO/conductivity, when snails were kept of 24h in water (table 1 and 3). When snails were kept for 96h, there was significant negative correlation in between AChE activity in nervous tissue and temperature/ DO/free CO_2/turbidity and positive correlation in between AChE activity and free CO_2/conductivity (table 2 and 4).

Nowkavihar (Site C), There was significant negative correlation between AChE activity in different month and corresponding temperature/ pH/free CO_2/ turbidity and positive correlation between AChE activity in different month and corresponding DO/conductivity when snails were kept of 24h in water (table 1 and 3). When snails were kept for 96h, there was significant negative correlation in between AChE activity in nervous tissue and pH/free CO_2/turbidity and positive correlation in between AChE activity and temperature/ DO /conductivity (table 2 and 4). Railway colony (Site D), There was significant negative correlation between AChE activity in different month and corresponding temperature/ pH/free CO_2/ turbidity and positive correlation between AChE activity in different month and corresponding DO/conductivity when snails were kept of 24h in water (table 1 and 3). When snails were kept for 96h, there was significant negative correlation in between AChE activity in nervous tissue and temperature /pH/free CO_2/turbidity and positive correlation in between AChE activity and DO /conductivity (table 2 and 4). There was significant product correlation between parameters in different months and corresponding AChE activity in the nervous tissue of snail *L. acuminata*. Significant positive and negative product correlation was observed between AChE activity of control group and different sites.

Table-3
Inter-relation between different physicochemical parameter and Acetyl cholinesterase (AChE) in Ramgarh Lake at 24h exposure period 'r' value 'p' value

Correlation	T.W (Control)	Site(A)	Site(B)	Site(C)	Site(D)	T.W (Control)	Site (A)	Site(B)	Site(C)	Site(D)
Temperature × AChE	+0.45	-41.8	-0.48	-0.75	-0.62	IS	<0.01	IS	<0.01	<0.05
pH × AChE	-0.51	-1.43	-0.68	-0.59	-0.70	IS	<0.01	<0.02	<0.05	<0.02
Dissolved oxygen× AChE	-160.8	-2.03	+0.68	+0.82	+0.92	<0.01	<0.01	<0.02	<0.01	<0.001
Carbon dioxide × AChE	-2.79	-4.3	-0.50	-0.78	-0.74	<0.01	<0.01	IS	<0.01	<0.01
Conductivity × AChE	-0.41	-0.18	+0.61	+0.74	+0.72	IS	IS	<0.05	<0.01	<0.01
Turbidity × AChE	-0.25	-0.15	-0.76	-0.68	-0.76	IS	IS	<0.01	<0.02	<0.01

* 'IS' means insignificant

Table-4
Inter-relation between different physicochemical parameter and Acetyl cholinesterase (AChE) in Ramgarh Lake at 96h exposure period

	'r' value					'p' value				
Correlation	**T.W (Control)**	**Site(A)**	**Site(B)**	**Site(C)**	**Site(D)**	**T.W (Control)**	**Site (A)**	**Site(B)**	**Site(C)**	**Site(D)**
Temperature × AChE	-3.65	-0.54	-0.89	+1.91	-0.62	<0.01	IS	<0.001	<0.01	<0.05
pH × AChE	-0.81	-0.65	+7.75	-0.73	-0.59	<0.01	<0.05	<0.01	<0.01	<0.05
Dissolved oxygen× AChE	+0.57	+0.79	-3.98	+1.89	+1.60	IS	<0.01	<0.01	<0.01	<0.01
Carbon dioxide × AChE	-0.54	-14.1	-7.11	-0.88	-0.68	IS	<0.01	<0.01	<0.001	<0.02
Conductivity × AChE	+0.44	+5.85	+0.53	+0.44	+0.34	IS	<0.01	IS	IS	IS
Turbidity × AChE	-0.72	-0.57	-3.71	-0.65	-2.28	<0.01	IS	<0.01	<0.05	<0.01

* 'IS' means insignificant.

Figure-1
Map of Ramghar Tal

Figure-2
Ramgarh Lake

It is clear from result section, that the AChE activity is affected by abiotic factor. The temperature of water is a significant factor which alters the activity of AChE in each month of year. When water temperature is high in summer season (May-July)[7, 8], the AChE activity is high. Contrarily, when water temperature is low in winter season activity of AChE is less. High temperature caused more mortality of snails. At higher temperature degradation of organic and inorganic nitrogenous waste releases more energy[9]. High temperature of water during summer season accelerates biodegradation of organic matter both in bottom deposit and overlying layer of water by bacteria and other microorganisms. Low temperature is beneficial to aquatic organisms and reduces excessive growth of aquatic vegetation[7-9]. The pH value of water in Ramgarh lake is decreases in winter season because in winter season the temperature is low (17^0C-18^0C). In summer season the pH is maximum (7.6-7.9) as the temperature is high (35^0C -37^0C)[10-12]. In summer month pH might be due to increased chemicals in reservoir/ increased photosynthetic activities/low dilution capacity of water. The minimum pH was recorded in winter season which may be due to decreased chemical reaction/ photosynthetic activities[12,13]. The pH of water is also a significant factor and also affects the organism as well as pollutant[8]. The pH of water (different sites and control) increases after summer season and become highest in the month of winter season (January) and lowest in the August. AChE values are highest in winter season and lowest in summer season in different site and control water[11]. Conductivity of water was high in winter season (December to February). Singh and Singh[11] reported less conductivity and high temperature in summer season. High conductivity show less mortality. The highest values of conductivity were found at site C, both in summer and winter. Conductivity above 100 µ mhos/cm has been associated with impaired water bodies and may reflect a decrease in water quality[2]. Turbidity was generally low which represents clear water. Extremely clear water is often an indicator of acidic conditions. Turbidity of water was maximum in summer season causes high temperature and high mortality[7]. Dissolved oxygen is also a factor that inhibits the AChE activity. In winter season the dissolved oxygen is high (December to February) and in summer season the concentration of dissolved oxygen is low (May to July). In winter water holds more oxygen[14] and as a result less mortality of snails occurs during this period. At higher temperature the dissolved oxygen concentration decreases, which is reflected by higher mortality of snails[10]. Animal's mortality due to low oxygen is most common during hot, dry spells when algae grow and then die quickly[12]. Dissolved oxygen significantly varies in different months of the year. Water in winter season holds more oxygen and as a result, less mortality occurs during this period. At higher temperature, dissolved oxygen concentration decreases which is reflected by higher mortality of the snails. Dissolved oxygen is one of the major components, which are used by snails during metabolic activity[15,16]. Consequently at higher temperature increasing rate of metabolism in snail body may release more CO_2 which affect the pH of water[17,18], as the time duration increases, concentration of CO_2 (reduced by snails) increases in the water which affects the pH of water. This was evident by elevated concentration of CO_2 decrease in pH of water during summer[19]. The biomarker analysis showed that the bioindicator responded to neurotoxic chemicals (i.e. inhibit AChE activity) in the lake at all four sites, with the degree of activity inhibiting from site A to site D. Low AChE activity indicates a large degree of pollution of neurotoxic compounds at all the four sites in Ramgarh lake. According to Bouquene and Galgani[20] AChE activity may be inhibited by phytotoxins released into the water column during phytoplankton's blooms[21]. The result clearly indicates that a significant ($P < 0.05$) positive and negative correlation between environmental factors and AChE activity in the nervous tissue of *L. acuminata*. The high AChE activity of *Lymnaea acuminata* was also observed in the month of summer; this indicates that the AChE

activity of *Lymnaea acuminata* was also affected by abiotic factors found during the month of summer. Although in control group the activity of enzymes is significantly altered throughout year, yet AChE activity in nervous tissue of snails kept in water sample of Ramgarh lake are significantly lower than the corresponding control in that month.

Conclusion

From the obtained results, shows that variant environmental factors viz., temperature, pH, DO, CO_2, Conductivity, and turbidity can significantly alter the activity of acetylcholinesterase against L. acuminata. The changes in the abiotic factors in different month of the year 2007-2008 by the pollutant present in Ramgarh lake have significant role, as it significantly alter the acetylcholinesterase/sensitive enzyme is snail L. acuminata for monitoring of pollutant. It clearly indicates that by comparing the activity of these enzymes in snail nervous tissue, residing in water body with respect to control one can predict the presence of pollutant. The above study clearly demonstrates that the snail *L. acuminata* and the activity of AChE in nervous tissue of snail can be used as bioindicator and biomarker for the predication of water quality.

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