CHAPTER V

OBSERVATIONS AND RESULTS

5. Observations and Results

5.1. Physico- chemical parameters

Site 1 (Baidya Fish Farm)

Results of the air temperature and physico-chemical parameters of water of Site 1 (Baidya Fish Farm) are shown in Table 5.1 and Table 5.2. Table 5.1 shows the result of air temperature and physico-chemical parameters of water of the first year (Nov. 2008 to Oct. 2009) study period. Table 5.2 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009 to Oct. 2010) study period. Table 5.3 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site1. Fig.5.1 shows the monthly variations in air temperature at Site 1 in the first year and the second year study periods. The Figs.5.1 to 5.9 show histograms and Figs. 5.10-5.13 show line graphs of the monthly variations of different physico-chemical parameters of water at Site 1 in the first year (Nov. 2008 to Oct. 2009) and the second year (Nov. 2009 to Oct. 2010) study periods.

Air temperature

The minimum air temperature was recorded as 20.01±0.132ºC during the month of December and the maximum air temperature was 33.02±0.325°C in the month of April during the first year study period (Table 5.1). The minimum air temperature was 19.2±0.452°C in the month of January and maximum air temperature was 32.5±0.497°C in the month of March during the second year study period (Table 5.2).

During year 1, the air temperature showed a decreasing trend during the months of November and December and thereafter it increased January onwards up to April. The air temperature during the year 2 showed decreasing trends from November to January. Decreasing trend was also observed during the months of September to October in both years (Tables 5.1, 5.2; Figs. 5.1, 5.10).

The air temperature had positive and significant correlation with water temperature ($r = 0.933$, $P<0.01$), pH ($r = 0.603$, $P<0.05$) and biological oxygen demand ($r = 0.645$, $P<0.05$) but inverse and significant correlation with DO ($r = -.535$, $p<0.10$), TA ($r = -0.537$ $p < 0.10$) and free carbon dioxide ($r = -0.652$, $P<0.05$) (Table 5.3).

Water temperature

The lowest surface water temperature was 17.05±0.550°C in the month of December and highest was 29±0.320°C was in the month of September during the first year (Table 5.1). The minimum temperature was 17.3±0.526 °C in the month of January and the highest was 31.4 ±0.327 °C was in the month of September during second year study period (Table 5.2).

During year 1, the water temperature showed a decreasing trend during the months of November and December, thereafter the temperature increased (Table 5.1; Figs. 5.2, 5.11). During year 2 also it showed decreasing trend from November to January. Decreasing trend was also observed during the months of September to October in both years (Table 5.1; Figs. 5.2, 5.11).

The water temperature had positive and significant correlation with air temperature ($r = 0.933$, $P<0.01$) and pH ($r = 0.688$, $P< 0.05$) but inverse and significant correlation with free CO_2 ($r = -0.729$, $P<0.01$) and DO ($r =-0.710$, $P <0.01$) (Table 5.3).

pH

The minimum pH was recorded 6.22± 0.309 in the month of April and maximum 8.3± 0.17 was in the month of February during the first year (Table 5.1, Fig.5.3) and minimum pH was 7.8± 0.221 in March and maximum 9.2± 0.32 was in May during the second year (Table 5.2, Fig. 5.3). pH had positive and significant correlation with total hardness ($r = 0.681$, $P<0.05$), air temperature ($r = 0.603$, $P<0.05$) and water temperature ($r = 0.688$, $P<0.05$) but inverse and significant correlation with DO ($r= - 0.496$, $p<0.1$) and total alkalinity ($r= - 0.487$, $P<0.1$) (Table 5.3).

Free carbon dioxide

The maximum free carbon dioxide was 174.15± 0.326 mg/L in the month of June and minimum 18.48 ± 0.287 mg/L was in the month of October during the first year study period (Table 5.1; Fig 5.4) In the second year study period maximum CO_2 was 71.28± 0.326 mg/L in January and minimum 2.24± 0.645 mg/L was in May (Table 5.2; Fig.5.4). Free carbon dioxide showed positive and significant correlation with BOD ($r =0.679$, $P<0.01$), chloride ($r = 0.781$, $P<0.01$), total alkalinity ($r=0.497$, $P <0.10$) and phosphate ($r =0.523$, $P<0.10$) but inverse and significant correlation with air temperature ($r = -0.652$, $P<0.05$) and water temperature ($r=-0.729$, $P <0.05$) (Table 5.3).

Dissolved oxygen

The minimum dissolved oxygen 4.80 ± 0.335 mg/L was found in the month of November and maximum dissolved oxygen was 7.83 ± 0.297 mg/L in April during the first year study period (Table 5.1; Fig.5.5). During the second year study period, the maximum dissolved oxygen was 10.73±0.258 mg/L in the month of October and minimum was 2.7± 0.248 mg/L in the month of April (Table 5.2; Fig.5.5). The dissolved oxygen showed positive and significant correlation with air temperature (r = 0.535, P<0.10) and chloride (r = 0.553, P< 0.10) but inverse and significant correlation with pH (r=-0.496, p <0.10), water temperature (r=- 0.710, p< 0.01) and BOD (r=-0.634, p <0.05) (Table 5.3).

Biological oxygen demand

The maximum biological oxygen demand was 3.54±0.038 mg/L in the month of September and minimum 0.35±0.33 mg/L in the month of February during the first year study period (Table 5.1; Fig.5.6). It was maximum 9.28 ±0.063 mg/L in November and minimum 0.27±0.032 mg/L in August in the second year study period (Table 5.2; Fig.5.6). It had positive and significant correlation with CO_2 (r = 0.679, P<0.01) and water temperature (r = 0.685, P<0.05) but inverse and significant correlation with air temperature (r = -0.645, P<0.05), DO (r = - 0.634, P< 0.05), chloride (r= -0.599, P<0.05) and total alkalinity (r= -0.624, P<0.05) (Table 5.3).

Chloride

The maximum chloride was 29.84 ±0.260 mg/L in the month of January and minimum 2.13±0.216 mg/L was in the month of December during the first year (Table 5.1; Fig.5.7) and maximum 10.0 ±0.261 mg/L in June and minimum 1.1 ±0.260 mg/L was in April of second year study period (Table 5.2; Fig.5.7). It had positive and significant correlation with free carbon dioxide (r=0.781, P<0.01) and total alkalinity (r= 0.665, P<0.05), DO (r=0.553, P<0.10) and inverse and significant correlation with BOD (r= -0.599, P<0.05) (Table 5.3).

Total alkalinity

The maximum total alkalinity was 208±0.452 mg/L in the month of May and minimum was 97.76± 0.721 mg/L in the month of December during the first year study period (Table 5.1; Fig.5.8). During the second year, maximum T.A. was 243.6± 0.521 mg/L in February and minimum 83.6± 0.325 mg/L was in October (Table 5.2; Fig.5.8). It had positive and significant correlation with total hardness (r = 0.799, P<0.01), DO (r= 0.696, P< 0.05), air temperature (r= 0.637, P< 0.05) and chloride (r = 0.665, P<0.05) but inverse and significant correlation with BOD (r= -0.624, P< 0.05) (Table 5.3).

The total alkalinity showed a decreasing trend from the month of June, 2009 to October, 2009. The value of total alkalinity of June (166.25 ± 8.957 mg/L) showed significant decrease (P<0.01) compared to the value of total alkalinity of May (208.0±0.452 mg/L) in year 1 (Table 5.1; Figs.5.8, 5.12). The value of total alkalinity of June (121.9±0.645 mg/L) showed significant decrease (P<0.01) compared to the value of total alkalinity of May (154.0 ±1.062 mg/L) in year 2 with slight increase during the month of August, 2010 (101.2 ± 0.443 mg/L) but it was lower than that of May (154.0 ±1.062 mg/L). The total alkalinity remained low from June to October for five months in both years (Table 5.2; Figs.5.8, 5.12).

Total hardness

The maximum total hardness was 144.6 ± 0.463 mg/L in the month of March and minimum was 82.19 ± 0.679 mg/L in the month of August during the first year study period (Table 5.1). It was maximum132.66 ±0.463 mg/L in March and minimum 49.5 ±0.463 mg/L in December in second year study period (Table 5.2). It had positive and significant correlation with total alkalinity (r= 0.799, P<0.01) and pH (r= 0.681, P< 0.05) (Table 5.3).

Total hardness showed a decreasing trend from April to October in year 1. The values of May (118.3 ±1.25 mg/L) showed significant decrease (P<0.01) compared to April (123.6±0.657 mg/L) in the first year (Table 5.2; Figs.5.9, 5.13). It also showed a decreasing trend from June to October in year 2. The values of June (81.18 ±0.844 mg/L) showed significant decrease (p<0.05) as compared to May (126.72±0.095 mg/L) in second year (Table 5.2; Figs.5.9, 5.13). Total hardness remained low from May to October for six months in year 1 and from June to October for five months in year 2.

Table 5.1 shows air temperature, water temperature and the physico-chemical parameters of water at Site 1 (Baidya Fish farm, Tankisinwari) from Nov. 2008- October 2009**(Mean ± S.D., N=5).**

Parameters	**Months**											
Site1 – I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Air Temp. (ºC)	25.07 ±0.095	20.01 ±0.132	22.17 ±0.275	25.07 ±0.095	29.95 ±0.310	33.02 ±0.325	31.20 ±0.081	29.05 ±0.129	26.07 ±0.170	29.50 ±0.081	30.50 ±0.170	28.17 ±0.150
Water Temp. (ºC)	21.12 ±0.095	17.05 ±0.550	18.20 ±0.216	22.15 ±0.173	24.35 ±0.506	28.99 ±0.216	28.52 ±0.170	28.17 ±0.150	25.07 ±0.170	28.17 ±0.150	29.0 ±0.320	26.07 ±0.170
pH	7.62 ±0.05	8.17 ±0.150	8.12 ±0.120	8.30 ±0.170	8.20 ±0.170	6.22 ±0.309	6.50 ±0.081	7.32 ±0.095	6.37 ±0.309	6.72 ±0.095	7.25 ±0.129	7.82 ±0.098
Free CO2 mg/L	20.68 ±0.090	37.45 ±0.057	79.65 ±0.114	101.84 ±0.028	120.25 ±0.645	79.11 ±0.095	70.90 ±0.294	174.15 ±0.326	147.31 ±0.358	48.05 ±0.129	55.49 ±0.082	18.48 ±0.287
DO mg/L	4.80 ±0.335	5.88 ±0.078	6.27 ±0.170	7.28 ±0.022	7.16 ±0.035	7.83 ±0.297	7.04 ±0.009	7.47 ±.032	7.04 ±0.009	5.52 ±0.083	6.25 ±0.127	6.52 ±0.090
BOD mg/L	1.94 ±0.046	1.02 ±0.028	2.32 ±0.095	0.35 ±0.33	0.67 ±0.049	1.17 ±0.017	1.18 ±0.012	0.62 ±0.051	0.79 ±0.012	2.98 ±0.310	3.54 ±0.038	3.06 ±0.033
Chloride mg/L	5.12 ±0.095	2.13 ±0.216	29.84 ±0.260	25.56 ±0.079	22.72 ±0.137	23.14 ±0.026	21.3 ±0.045	25.56 ±0.017	25.56 ±0.017	12.15 ±0.129	4.10 ±0.083	6.13 ±0.124
T. Alk mg/L	137.25 ±0.208	97.76 ±0.721	133.12 ±0.095	156.0 ±1.173	187.2 ±1.676	198.2 ±0.559	208.0 ±0.452	166.25 ±8.957*	158.18 ±0.843	110.75 ±0.208	101.22 ±0.543	128.52 ±0.368
T. Hard mg/L	118.37 ±1.25	122.4 ±0.573	105.2 ±0.08	107.6 ±0.660	144.6 ±0.463	123.6 ±0.657	118.3 ±1.25*	90.2 ±0.095	90.8 ±0.028	82.19 ±0.679	101.52 ±0.164	106.08 ±0.121

***** Significant differences at 1% level, ** Significant differences at 5% level

Table 5.2 shows air temperature, water temperature and the physico-chemical parameters of water at Site 1 (Baidya Fish farm, Tankisinwari) from Nov. 2009- October 2010**(Mean ± S.D., N=5).**

Parameters	Months											
Site1- II	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	24.50 ±0.415	21.50 ±0.416	19.20 ±0.452	25.10 ±0.81	32.50 ±0.497	31.30 ±0.359	29.40 ±0.359	29.10 ±0.374	28.20 ±0.432	31.30 ±0.359	29.20 ±0.432	31.10 ±0.371
Water Temp. (ºC)	25.50 ±0.535	19.10 ±0.273	17.30 ±0.526	22.20 ±0.216	28.50 ±0.415	29.50 ±0.082	28.50 ±0.415	29.50 ±0.082	30.20 ±0.216	30.30 ±0.051	31.40 ±0.327	29.20 ±0.216
pH	8.30 ±0.170	8.90 ±0.097	8.20 ±0.095	8.20 ±0.095	7.80 ±0.221	8.30 ±0.095	9.20 ±0.320	8.80 ±0.096	8.50 ±0.081	8.90 ±0.097	9.10 ±0.150	8.80 ±0.096
Free CO_2 (mg/L)	2.98 ±0.235	5.02 ±0.134	71.28 ±0.326	47.52 ±0.082	5.10 ±0.095	2.24 ±0.0645	2.24 ±0.645	2.29 ±0.231	4.05 ±0.258	8.80 ±0.207	4.58 ±0.257	2.24 ±0.225
DO (mg/L)	10.17 ±0.221	8.83 ±0.521	7.34 ±0.231	6.67 ±0.452	6.71 ±0.145	2.70 ±0.248	8.64 ±0.215	6.67 ±0.046	6.69 ±0.118	6.61 ±0.340	9.31 ±0.561	10.73 ± 0.258
BOD (mg/L)	9.28 ±0.063	5.39 ±0.165	7.34 ±0.355	6.67 ±0.065	1.37 ±0.034	1.75 ±0.062	1.75 ±0.055	3.81 ±0.311	5.51 ±0.067	0.27 ±0.032	7.77 ±0.048	3.83 ±0.117
Chloride (mg/L)	2.0 ±0.124	2.0 ±0.091	9.0 ±0.075	6.0 ±0.134	5.0 ±0.077	1.1 ±0.260	1.0 ±0.241	10.0 ±0.261	5.0 ±0.087	4.0 ±0.135	2.0 ±0.155	7.0 ±0.240
Total Alk (mg/L)	109.89 ±0.891	104.0 ±0.865	150.0 ±1.02	243.6 ±0.521	162.5 ±0.756	154.0 ±0.884	154.0 ±1.062	121.9 ±0.645*	92.0 ±0.766	101.2 ±0.443	99.0 ±0.355	83.6 ±0.325
Total hard (mg/L)	91.02 ±1.035	49.5 ±0.463	130.56 ±0.647	130.68 ±0.751	132.66 ±0.463	126.72 ±0.458	126.72 ±0.095	81.18 ±0.844**	75.24 ±0.363	77.22 ±0.537	79.2 ±0.237	73.26 ±0.572

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.3 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 1 (average of the corresponding month values) during Nov. 2008 – Oct. 2010; N=12; d.f. =11.

S1- I +II		Water Temp. (ºC)	pH	Free CO_2 (mg/L)	DO (mg/L)	BOD (mg/L)	Chloride (mg/L)	Total alkal (mg/L)	Total hard (mg/L)
Air Temp. (ºC)	P cor.	.933*	.603**	-.652**	-.535	.645**	.136	-.637**	.028
	Sig. (2-t)	.000	.038	.022	.073	.024	.674	.050	.931
Water Temp. (ºC)	P cor.	1	.688**	-.729*	-.710**	.685**	.060	.353	-.278
	Sig. (2-t)		.013	.007	.00	.049	.853	.260	.381
pH	P cor.		1	-.336	-.496	.091	-.293	-.487	.681**
	Sig. (2-t)			.285	.101	.779	.355	.108	.015
Free CO_2 (mg/L)	P cor.			1	.500	.679*	.781*	.497*	-.165
	Sig. (2-t)				.098	.017	.003	.100	.608
DO (mg/L)	P cor.				1	-.634**	.653**	.696**	.153
	Sig. (2-t)					.029	.049	.012	.635
BOD (mg/L)	P cor.					1	-.599**	-.624**	-.348
	Sig. (2-t)						.039	.030	.268
Chloride (mg/L)	P cor.						1	.665**	-.048
	Sig. (2-t)							.018	.882
Total alkalinity (mg/L)	P cor.							1	.799*
	Sig. (2-t)								.002
Total hard (mg/L)	P cor.								1
	Sig.(2-t)								

*** Significant at 1% level (P<0. 01), ** significant at 5% level (P<0.05) and Values not marked denote non-significant correlation.**

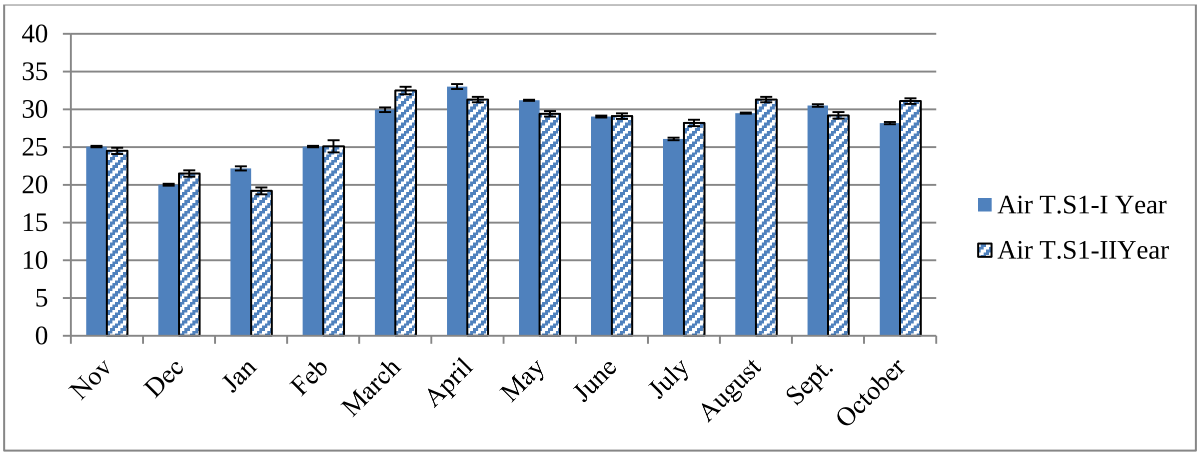

Fig.5.1. Monthly variations in air temperature at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

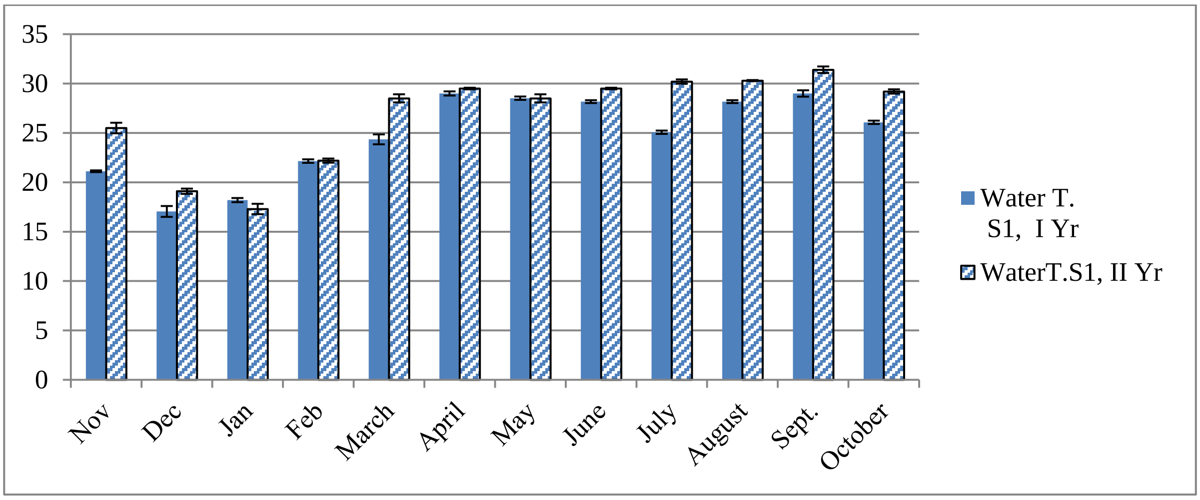

Fig.5.2. Monthly variations in water temperature at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

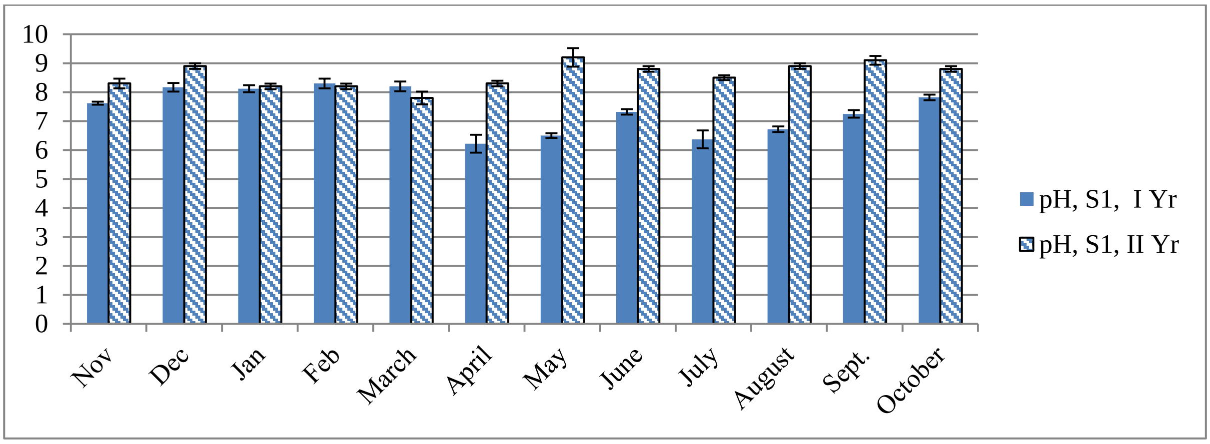

Fig.5.3. Monthly variations in pH at Site1 during the first and second year study periods (Nov. 2008- Oct. 2010).

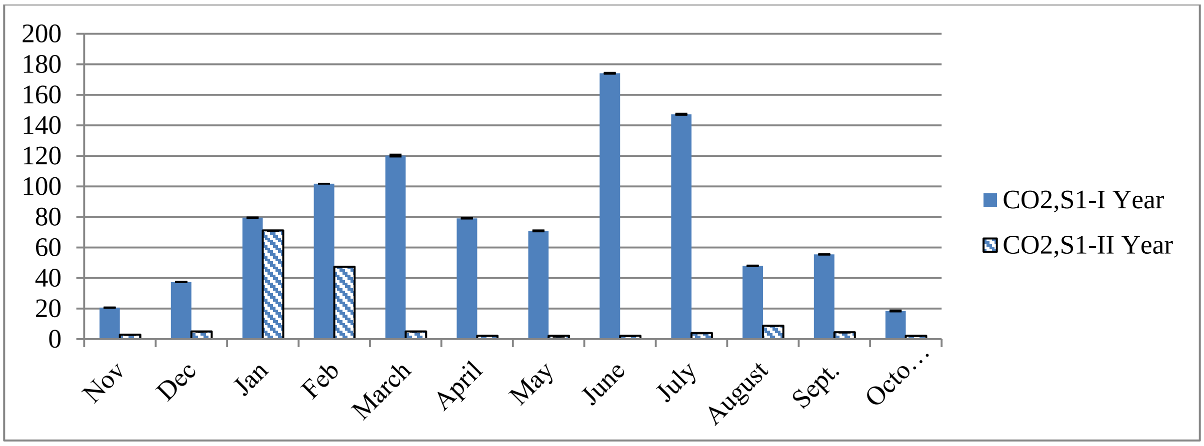

Fig.5.4. Monthly variations in free CO_2 at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

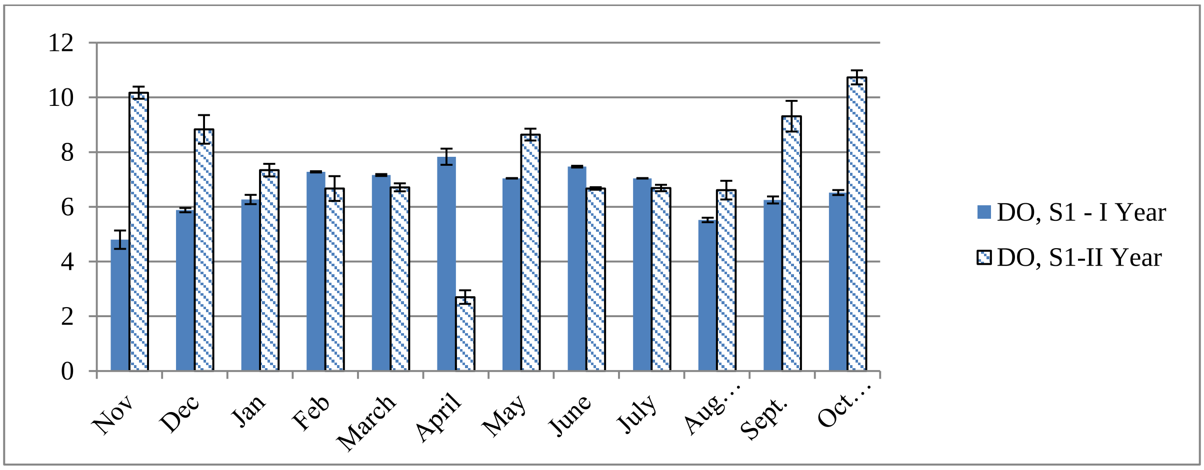

Fig.5.5. Monthly variations in DO at Site 1 during the first and second year study periods (Nov.2008- Oct.2010).

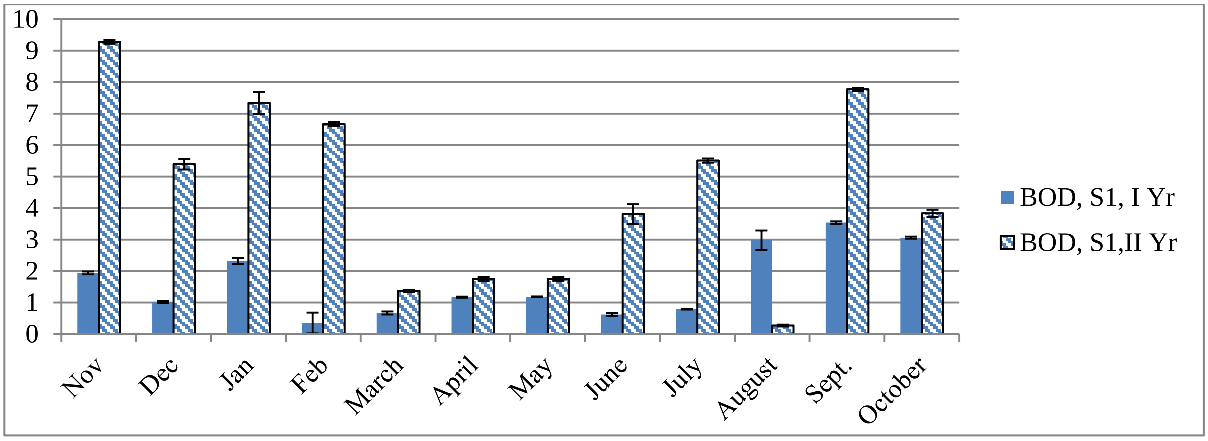

Fig.5.6. Monthly variations in BOD at Site 1 during the first and second year study periods (Nov.2008- Oct.2010).

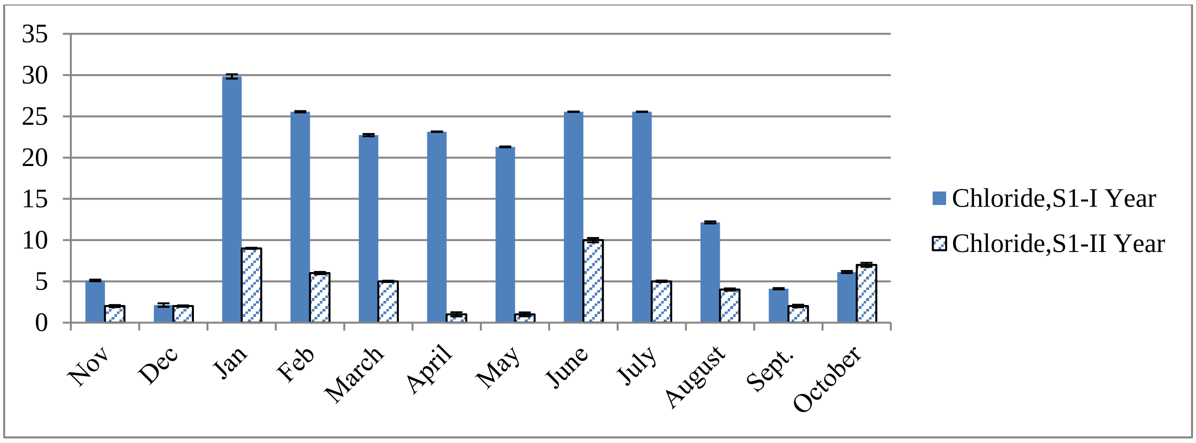

Fig.5.7. Monthly variations in chloride at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

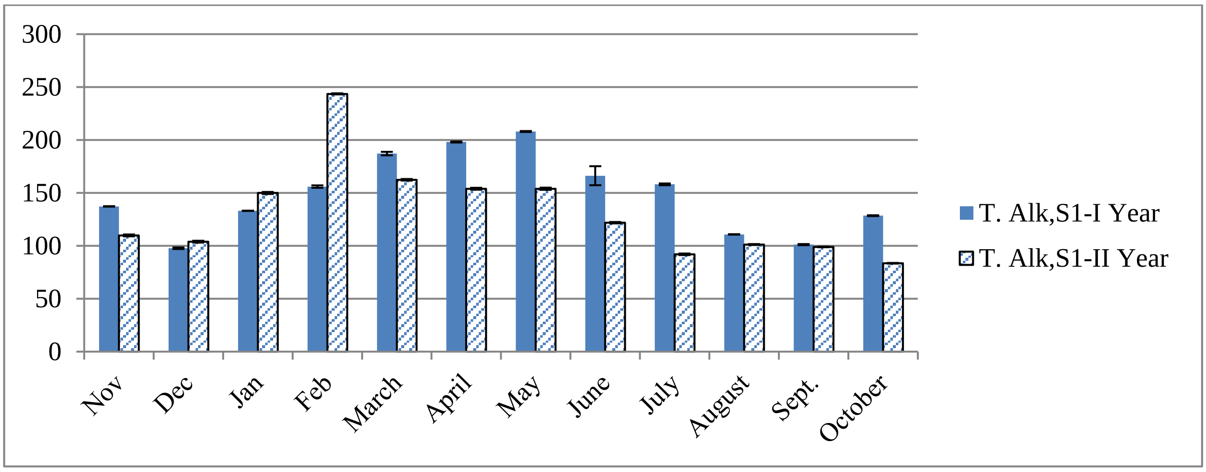

Fig.5.8. Monthly variations in total alkalinity at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

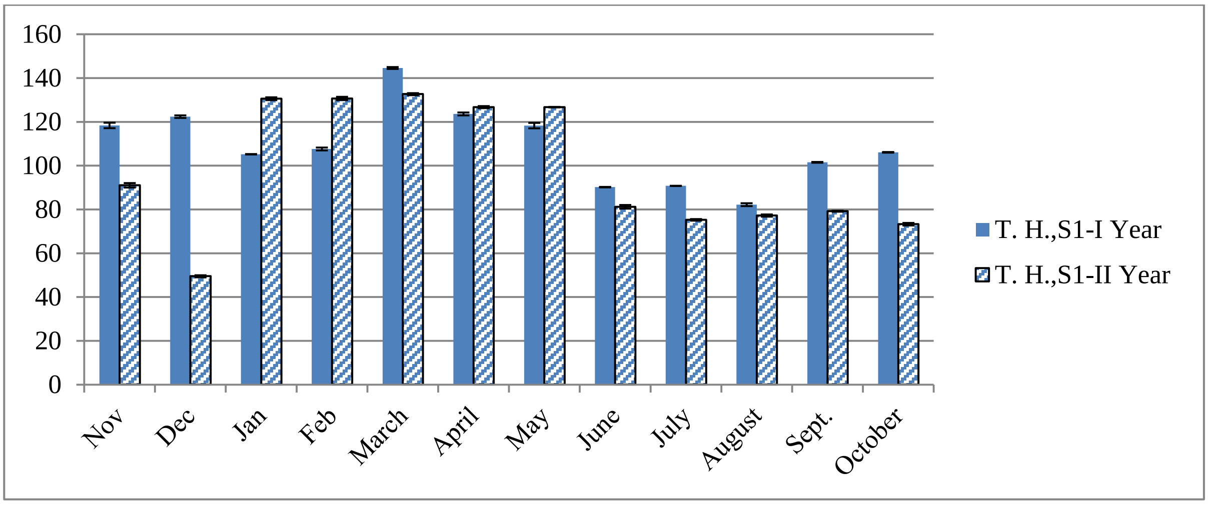

Fig. 5.9. Monthly variations in total hardness at Site 1 during the first and second year study periods (Nov. 2008- Oct. 2010).

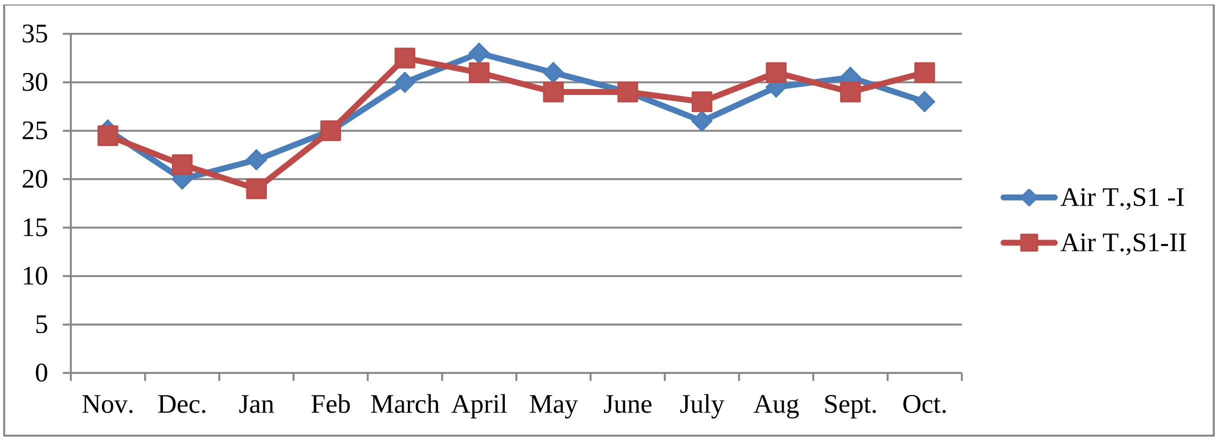

Fig.5.10. Line graph of monthly variations in air temperature at site 1 during the first and second year study periods (Nov.2008 - Oct.2010).

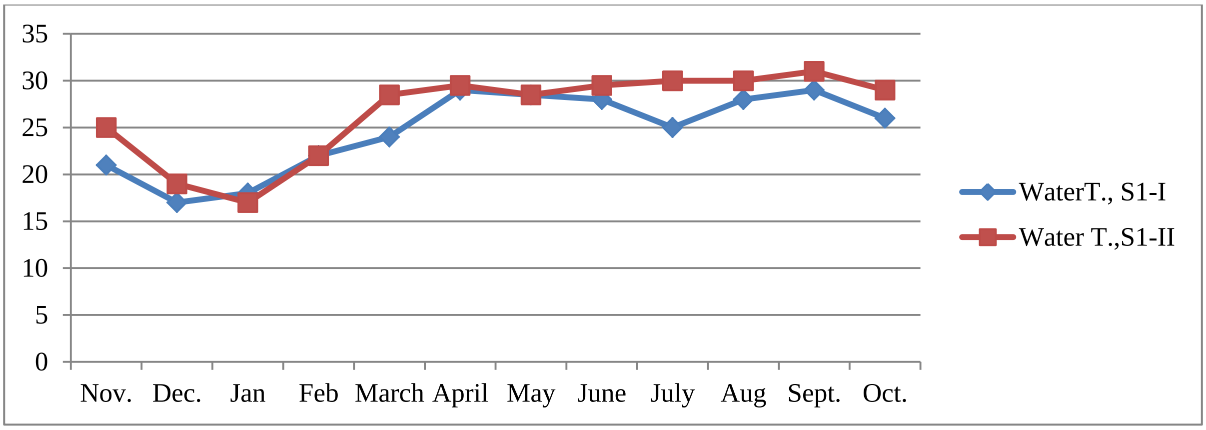

Fig.5.11. Line graph of monthly variations in water temperature at site 1 during the first and second year study periods (Nov. 2008 - Oct.2010).

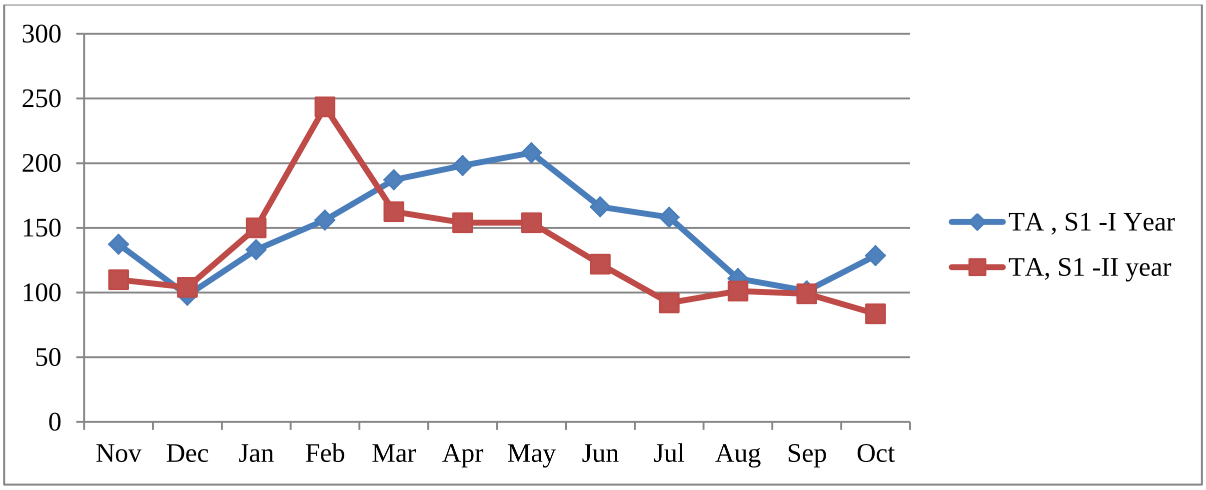

Fig.5.12. Line graph of monthly variations in total alkalinity at site 1 during the first and second year study periods (Nov. 2008 - Oct.2010)

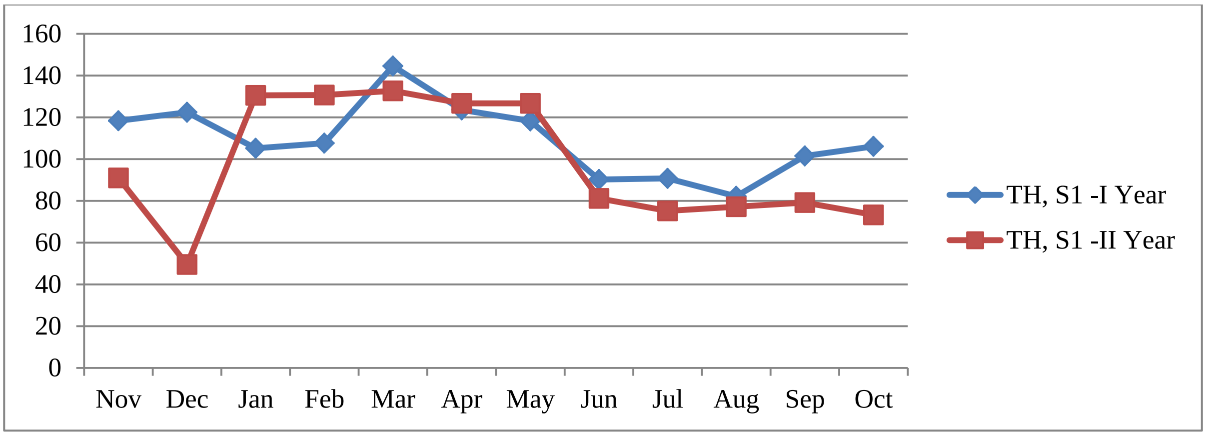

Fig.5.13. Line graph of monthly variations in total hardness at site 1 during the first and second year study periods (Nov. 2008 - Oct.2010).

Site 2 (Babiya Birta Fish Farm)

Results of the air temperature and physico-chemical parameters of water of Site 2 (Baibia Birta Fish Farm) are shown in Table 5.4 and Table 5.5. Table 5.4 shows the result of air temperature and physico-chemical parameters of water of the first year (Nov. 2008 to Oct. 2009) study period. Table 5.5 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009 to Oct. 2010) study period. Table 5.6 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site 2. Fig.5.14 shows the monthly variations in air temperature at Site 2 in the first year and the second year study periods. The Figs.5.14 to 5.22 shows histogram and Figs. 5.23- 5.26 show line graph of the monthly variations of different physico-chemical parameters of water at Site 2 in the first year (Nov. 2008 to Oct. 2009) and the second year (Nov. 2009 to Oct. 2010) study periods.

Air temperature

The minimum air temperature was 19.5±0.236°C in the month of December and maximum was 33±0.145°C in the month of April during the first year study period (Table 5.4).The minimum air temperature was 18.5±0.439°C in January and maximum was observed in March (30 ±0.633°C), April (30 ±0.356°C) and September (30 ±0.214°C) in the second year study period (Table 5.5).

The air temperature showed a decreasing trend from November to January and September to October during year 1 and year 2 both (Tables 5.4, 5.5; Figs. 5.14, 5.23). The air temperature had positive and significant correlation with water temperature (r=0.818, P<0.01) and total alkalinity (r=0.616, p<0.05) but inverse and significant correlation with free carbon dioxide (r = - 0.759, P<0.01) and dissolved oxygen (r = -0.647, P<0.05) (Table 5.6).

Water temperature

The lowest surface water temperature was 17.0±0.452°C in December and maximum was 30.0±0.526°C in April during the first year (Table 5.4) and the minimum water temperature was 17.5±0.315°C in January and maximum was 31.0±0.342°C in September during second year study period (Table 5.5).

The water temperature showed decreasing trend during the winter months of November to January in both year 1 and year 2. Decreasing trend was also observed during the months of September to October in both years (Tables 5.4, 5.5; Figs.5.15, 5.24).

The water temperature had positive and significant correlation with air temperature (r=0.818, P<0.01) and phosphate (r=0.609, P<0.05) but inverse and significant correlation with CO_2 (r =-0.741, P<0.01) pH (r=-0.539, P<0.10) and DO (r = -0.747, P<0.01) (Table 5.6).

pH

The minimum pH was 6.6± 0.315 in the month of April and maximum was recorded 8.8±0.24 in November during the first year (Table 5.4; Fig. 5.16) and minimum 7.3 ± 0.231 was in the month of April and maximum 8.7± 0.211 was in February in the second year (Table 5.5; Fig.5.16). pH had positive and significant correlation with dissolved oxygen (r =0.828, P<0.01), total alkalinity (r = 0.629, P<0.05), biological oxygen demand (r =0.728, P<0.01) but inverse and significant correlation with total hardness (r= - 0.681, p<0.05) and free carbon dioxide (r = -0.513, P<0.10) (Table 5.6).

Free Carbon Dioxide

The minimum free CO_2 was 1.909 ± 0.536 mg/L in the month of November and maximum free carbon dioxide was 179.59± 0.332 mg/L in the month of June during the first year (Table 5.4; Fig. 5.17). The minimum free CO_2 was 2.24 ± 0.105 mg/L in the month of May and maximum was 23.76± 0.544 mg/L in the month of January in the second year study period (Table 5.5; Fig.5.17). Free carbon dioxide showed positive and significant correlation with chloride (r =0.648, P<0.05), total alkalinity (r =0.688, P<0.05) and phosphate (r =0.748, P<0.01) but inverse and significant correlation with air temperature (r=-0.759, P<0.01) and water temperature (r=-0.741, P <0.01) (Table 5.6).

Dissolved Oxygen

The minimum dissolved oxygen was 4.96±0.089 mg/L in the month of December and maximum was 7.83±0.325 mg/L in the month of March during the first year (Table 5.4; Fig.5.18). The minimum dissolved oxygen was 3.8±0.321 mg/L in the month of April and maximum was 9.71± 0.257 mg/L in the month of February during the second year study period (Table 5.5; Fig.5.18). The dissolved oxygen showed positive and significant correlation with pH (r =0.828, P<0.01) and free carbon dioxide (r = - 0.647, P<0.05) but inverse and significant correlation with air temperature (r=-0.647, p <0.05), phosphate (r=-0.600, P< 0.05) and water temperature (r= -0.747, P < 0.01) (Table 5.6).

Biological oxygen Demand

The maximum biological oxygen demand was 4.53±0.162 mg/L in the month of September and minimum was 0.23±0.134 mg/L in the month of July during the first year (Table 5.4; Fig.5.19). Maximum BOD was 5.78 ± 0.063 mg/L in January and minimum was 0.75± 0.416 mg/L in August during the second year study period (Table 5.5; Fig.5.19). It had positive and significant correlation with pH (r = 0.728, p<0.01) and chloride (r= 0.627, P<0.05). Inverse and significant correlation with total alkalinity (r= -0.648, P<0.05) (Table 5.6).

Chloride

The maximum chloride was 44.87 ± 0.235 mg/L in the month of April and minimum was 13.0± 0.116 mg/L in the month of December during the first year (Table 5.4 ; Fig.5.20) and maximum 25.99 ± 0.606 mg/L was seen in June and minimum 4.0±0.224 mg/L in December of the second year study period (Table 5.5; Fig.5.20). It had positive and significant correlation with free carbon dioxide (r=0.648, P<0.05) and total alkalinity (r=0.834, P<0.01) and phosphate (r=0.592, P<0.05) (Table 5.6).

Total Alkalinity

The maximum total alkalinity was 135.3 ± 0.453 mg/L in the month of May and minimum 67.68± 0.32 mg/L in the month of December during the first year study period (Table 5.4). During the second year study period, maximum total alkalinity was 176± 0.532 mg/L in May and minimum 82.5± 0.486 mg/L in March (Table 5.5). It had positive and significant correlation with free CO_2 (r=0.688, p <0.05), pH (r=0.629, P<0.05), phosphate (r = 0.642, P<0.05) and biological oxygen demand (r = 0.693, P<0.05) (Table 5.6).

Total Alkalinity showed decreasing trend from July to October 2009. The values of total alkalinity in July (114.4±0.667 mg/L) showed significant decrease (p<0.01) compared to June (135.2±0.351 mg/L) in the first year (Table 5.4; Figs.5.21, 5.25). The values of total alkalinity in the month of June (108.1±0.459 mg/L) showed significant decrease (P<0.05) as compared to May (176.0 ±0.875 mg/L) in the second year. The values increased slightly during the months of September and October 2010 but values were lower in comparison to that of the month of May (176.0 ±0.875 mg/L) (Table 5.5; Figs. 5.21, 5.25). Total alkalinity remained low for four months from July to October in the year 1 and for five months from June to October in the year 2.

Total Hardness

The maximum total hardness was 94.0 ±0.932 mg/L in the month of July and minimum was 69.36 ±0.736 mg/L in the month of October during the first year (Table 5.4) ; maximum was 116.82 ±0.996 mg/L in November and minimum was 63.36 ±0.765 mg/L in December during the second year study period (Table 5.5). It had inverse and significant correlation with BOD (r= -0.643, P<0.05) and pH (r= -0.681, P<0.05) (Table 5.6).

The values of total hardness in August (86.4±0.655 mg/L) showed significant decrease (p< 0.01) as compared to July (94.0 ±0.932 mg/L) in the first year. It remained low for three months from August to October (Table 5.4; Figs.5.22, 5.26). Likewise in the second year it showed a decreasing trend from June to August and increased slightly during September and October. The values in June (99.0 ±0.330 mg/L) were significantly decreased (P<0.01) as compared to May (102.86 ±0.431 mg/L) in the second year (Table 5.5; Figs. 5.22, 5.26). It remained low for five months from June to October in the second year.

Table 5.4 shows air temperature, water temperature and physico-chemical parameters of water at Site 2 (Babiya Birta fish pond, Morang) from Nov. 2008- October 2009. **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 2- I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (°C)	23.0 ±0.325	19.5 ±0.236	23.0 ±0.214	26.0 ±0.245	29.0 ±0.134	33.0 ±0.145	30.0 ±0.221	29.5 ±0.095	25.5 ±0.437	28.0 ±0.342	30.0 ±0.332	27.0 ±0.165
Water Temp. (°C)	20.0 ±0.374	17.0 ±0.452	18.0 ±0.215	21.0 ±0.336	23.0 ±0.223	30.0 ±0.526	28.0 ±0.456	29.0 ±0.126	29.0 ±0.456	29.0 ±0.371	29.5 ±0.217	25.0 ±0.275
pH	8.8 ±0.24	8.1 ±0.212	8.7 ±0.325	7.4 ±0.216	6.8 ±0.332	6.6 ±0.315	7.3 ±0.168	7.2 ±0.256	6.6 ±0.122	7.4 ±0.345	8.3 ±0.470	8.7 ±0.335
Free CO_2(mg/L)	1.909 ±0.536	56.1 ±0.573	65.47 ±0.657	87.29 ±0.634	60.01 ±0.731	78.2 ±0.315	76.38 ±0.553	179.59 ±0.332	136.4 ±0.675	16.02 ±0.132	18.48 ±0.408	36.96 ±0.560
DO (mg/L)	7.67 ±0.223	4.96 ±0.089	7.67 ±0.342	7.44 ±0.421	7.83 ±0.325	6.65 ±0.210	6.26 ±0.167	6.65 ±0.208	6.65 ±0.097	6.88 ±0.275	6.16 ±0.551	7.66 ±0.345
BOD (mg/L)	2.63 ±0.035	1.95 ±0.057	3.84 ±0.076	2.74 ±0.015	0.39 ±0.041	0.78 ±0.063	0.7 ±0.076	0.85 ±0.035	0.23 ±0.134	1.8 ±0.087	4.53 ±0.162	3.81 ±0.112
Chloride (mg/L)	16.99 ±0.216	13 ±0.116	32.09 ±0.217	31.38 ±0.237	31.24 ±0.216	44.87 ±0.235	42.6 ±0.257	32.66 ±0.218	44.02 ±0.275	14 ±0.120	14 ±0.139	15 ±0.431
Total Alkalinity (mg/L)	80.36 ±0.563	67.68 ±0.320	108.16 ±0.336	105.04 ±0.345	124.8 ±0.442	115.4 ±0.642	135.3 ±0.453	135.2 ±0.351	114.4 ±0.667 **	95.94 ±0.655	69.3 ±0.671	79.8 ±0.539
Total hardness (mg/L)	77.52 ±0.661	91.8 ±0.546	82.0 ±0.711	80.2 ±0.534	90.66 ±0.477	80.6 ±0.576	76.0 ±0.635	92.0 ±0.895	94.0 ±0.932	86.4 ±0.655 **	84.24 ±0.563	69.36 ±0.736

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.5 shows air temperature, water temperature and physico-chemical parameters of water at Site 2 (Babiya Birta fish pond, Morang) from Nov. 2009- October 2010. **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 2 - II.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	23.0 ±0.227	20.5 ±0.234	18.5 ±0.439	22.0 ±0.492	30.0 ±0.633	30.0 ±0.356	27.0 ±0.312	25.5 ±0.336	28.0 ±0.215	29.5 ±0.423	30.0 ±0.214	29.0 ±0.415
Water Temp. (ºC)	24.0 ±0.219	19.0 ±0.231	17.5 ±0.315	20.0 ±0.355	27.0 ±0.218	29.0 ±0.332	29.0 ±0.273	29.0 ±0.344	30.0 ±0.265	30.0 ±0.556	31.0 ±0.342	28.0 ±0.213
pH	7.5 ±0.231	8.3 ±0.175	7.8 ±0.114	8.7 ±0.211	8.5 ±0.253	7.3 ±0.231	8.1 ±0.223	7.6 ±0.098	8.5 ±0.347	7.9 ±0.216	8.2 ±0.310	7.5 ±0.128
Free CO_2 (mg/L)	9.90 ±0.452	6.69 ±0.225	23.76 ±0.544	16.02 ±0.365	2.40 ±0.247	4.49 ±0.132	2.24 ±0.105	4.58 ±0.545	6.07 ±0.634	8.80 ±0.551	4.58 ±0.322	3.78 ±0.163
DO (mg/L)	5.56 ±0.164	7.14 ±0.344	7.86 ±0.231	9.71 ±0.257	5.94 ±0.221	3.80 ±0.321	5.37 ±0.211	4.94 ±0.225	5.82 ±0.097	6.17 ±0.203	6.20 ±0.242	6.30 ±0.313
BOD (mg/L)	1.47 ±0.067	1.67 ±0.055	5.78 ±0.063	2.43 ±0.052	2.27 ±0.043	2.39 ±0.079	3.54 ±0.088	3.87 ±0.097	2.59 ±0.065	0.75 ±0.416	4.22 ±0.025	0.83 ±0.045
Chloride (mg/L)	9.0 ±0.302	4.0 ±0.224	18.99 ±0.442	17.99 ±0.345	17.99 ±0.341	21.99 ±0.433	23.99 ±0.552	25.99 ±0.606	13.0 ±0.350	14.0 ±0.403	5.0 ±0.203	15.0 ±0.476
Total Alkalinity (mg/L)	141.64 ±0.655	128.0 ±0.438	100.0 ±0.677	151.2 ±0.757	82.5 ±0.486	110.0 ±0.539	176.0 ±0.875	108.1 ±0.459**	101.2 ±0.443	99.0 ±0.376	112.2 ±0.445	112.2 ±0.558
Total hardnes (mg/L)	116.82 ±0.996	63.36 ±0.765	99.96 ±0.457	87.12 ±0.540	81.18 ±0.412	104.94 ±0.345	102.86 ±0.431	99.0 ±0.330**	85.15 ±0.243	83.16 ±0.289	93.06 ±0.376	99.0 ±0.435

***** Significant differences of t-test at 1% level, ** Significant differences of t-test at 5% level.

Table 5.6 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 2 (average of the corresponding month values) during Nov. 2008 – Oct. 2010; N=12; d. f. =11.

S2-I+II		**Water Temp. (ºC)**	**pH**	**Free CO_2 (mg/L)**	**D.O. (mg/L)**	**BOD (mg/L)**	**Chloride (mg/L)**	**Total alkal(mg/L)**	**Total hardn(mg/L)**
Air Temp. (ºC)	P cor.	.818*	.571	-.759*	-.647**	-.272	.442	.616**	-.103
	Sig. (2-t)	.001	.052	.004	.023	.393	.150	.046	.751
Water Temp. (ºC)	P cor.	1	-.539	-.741*	-.747*	-.251	.277	.330	.071
	Sig. (2-t)		.071	.006	.005	.432	.383	.296	.826
pH	P cor.		1	-.513	.828*	.728*	-.541	.629**	-681**
	Sig. (2-t)			.088	.001	.007	.069	.029	.102
Free CO_2 (mg/L)	P cor.			1	.647**	-.549	.648**	.688**	.475
	Sig. (2-t)				.023	.064	.023	.013	.119
DO (mg/L)	P cor.				1	.058	.091	.211	-.301
	Sig. (2-t)					.858	.778	.510	.341
BOD (mg/)	P cor.					1	.627**	.693**	-.643**
	Sig. (2-t)						.029	.012	.052
Chloride (mg/L)	P cor.						1	.834*	.135
	Sig. (2-t)							.001	.675
Total alk. (mg/L)	P cor.							1	.199
	Sig. (2-t)								.536
Total hardness (mg/L)	P cor.								1
	Sig. (2-t)								

*** Significant at 1% level (P<0. 01), ** significant at 5% level (P<0. 05) and Values not marked denote non-significant correlation.**

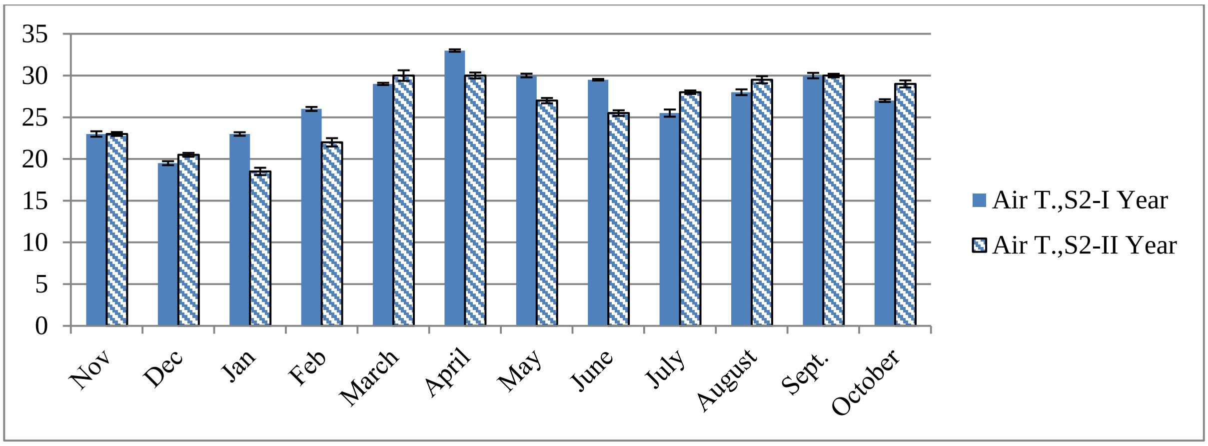

Fig.5.14. Monthly variations in air temperature at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

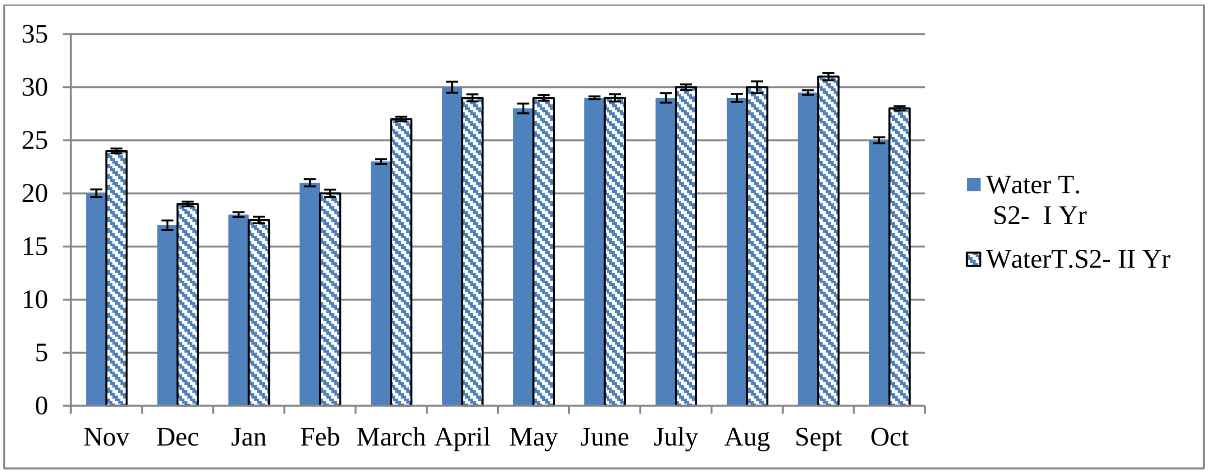

Fig.5.15. Monthly variations in water temperature at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

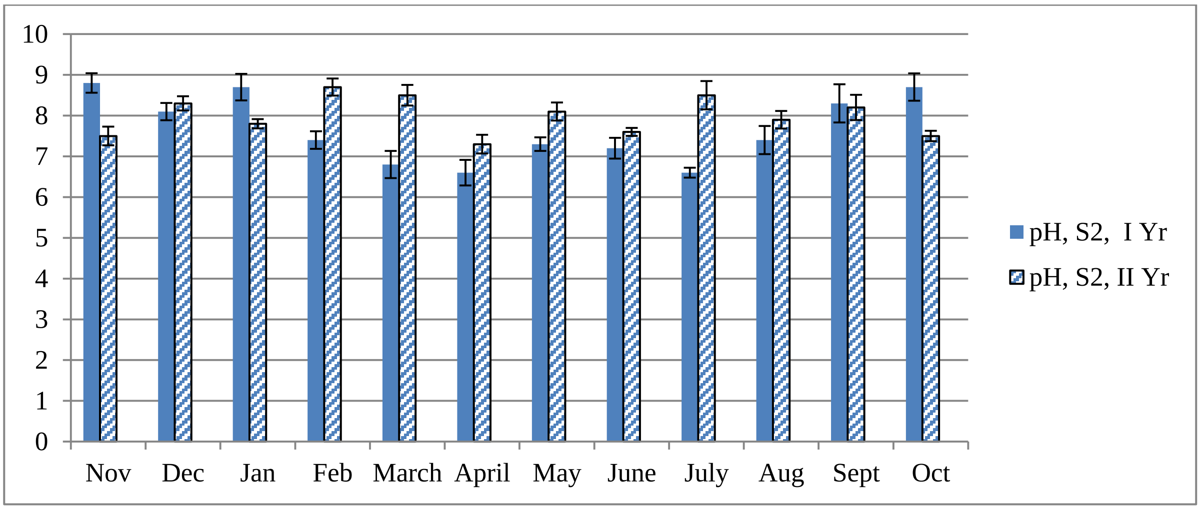

Fig.5.16. Monthly variations in pH at Site 2 during the first and second year study periods (Nov.2008- Oct.2010).

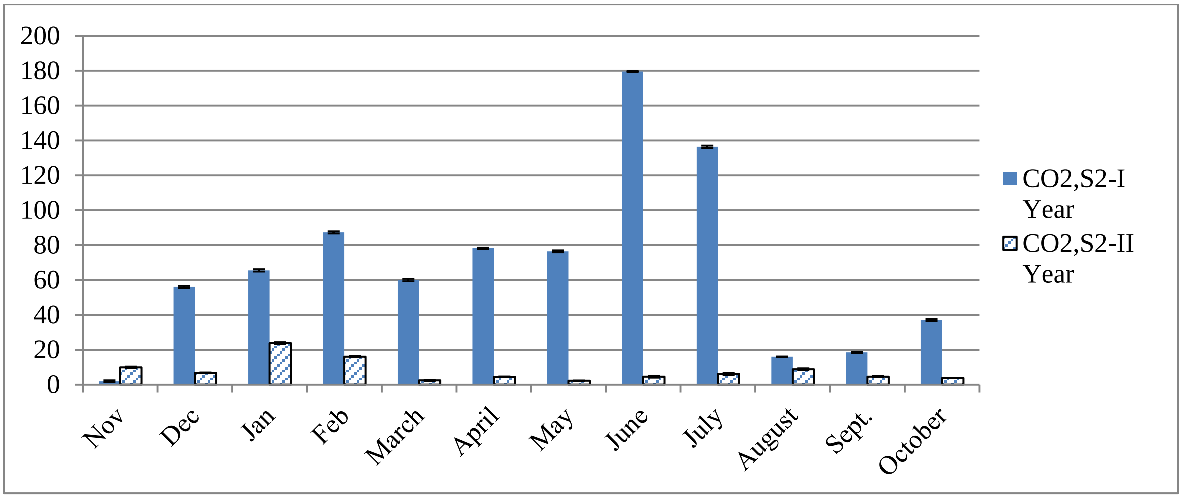

Fig.5.17. Monthly variations in CO_2 at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

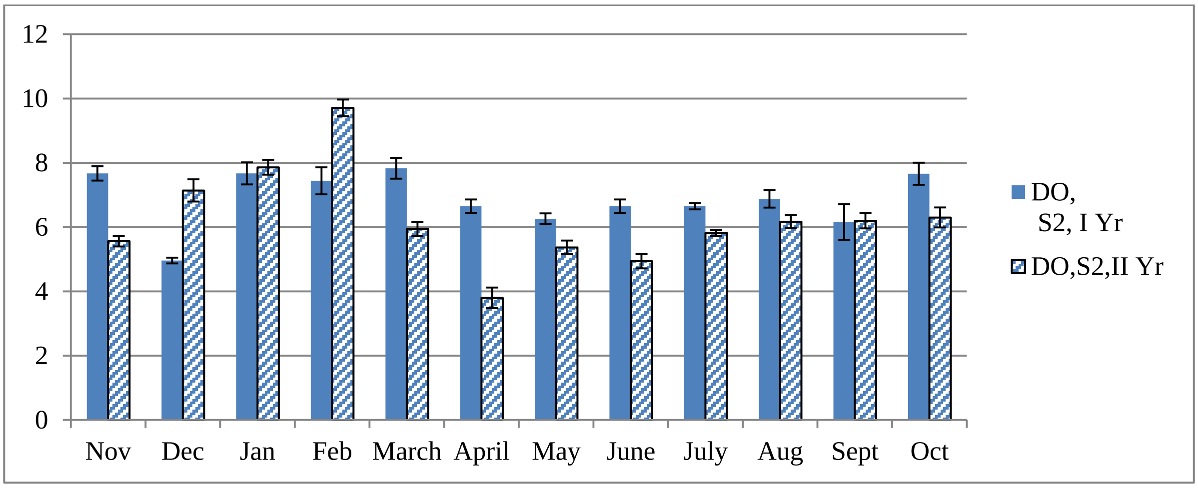

Fig. 5.18. Monthly variations in DO at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

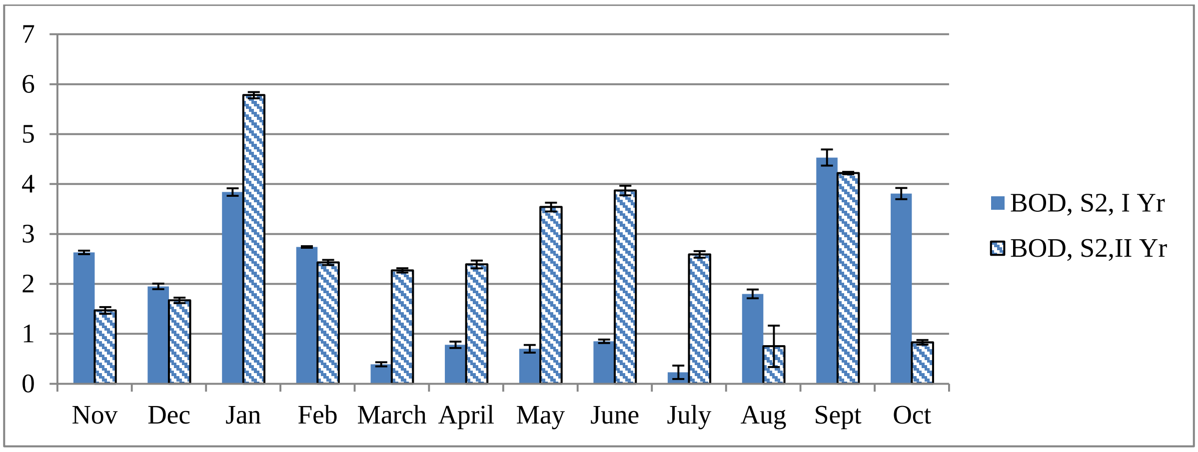

Fig.5.19. Monthly variations in BOD at Site 2 during the first and second year study periods (Nov.2008-Oct.2010).

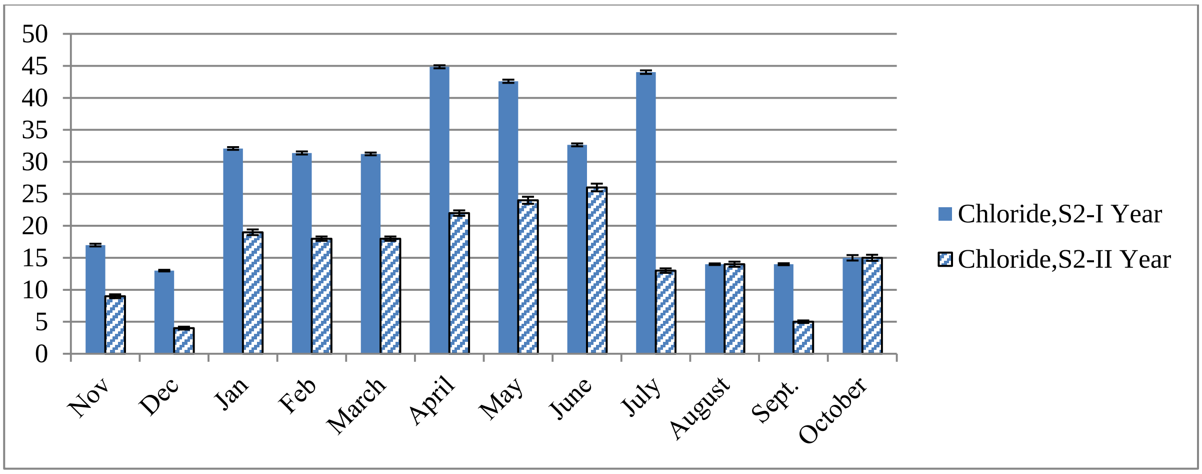

Fig.5.20. Monthly variations in chloride at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

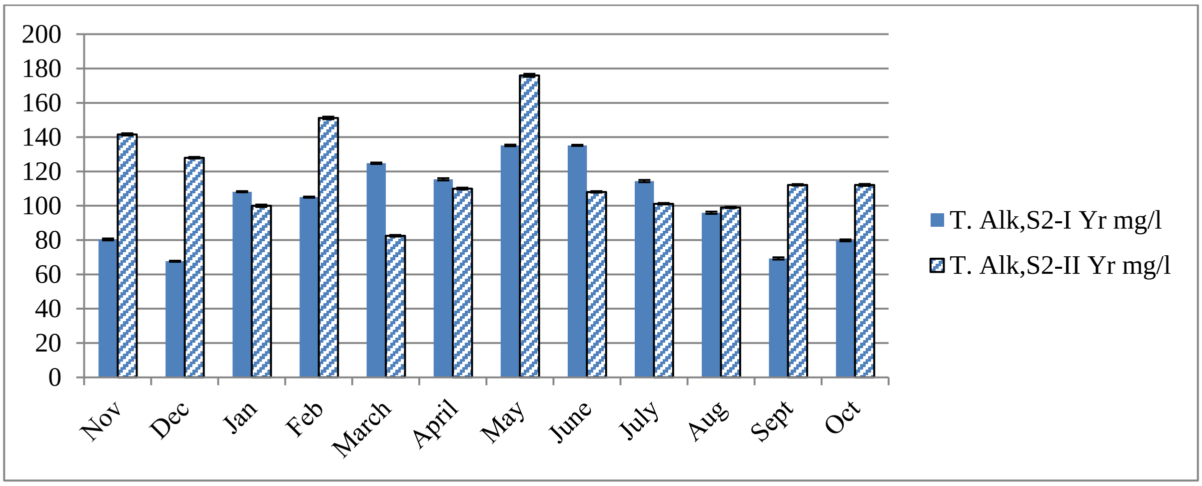

Fig.5.21. Monthly variations in total alkalinity at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

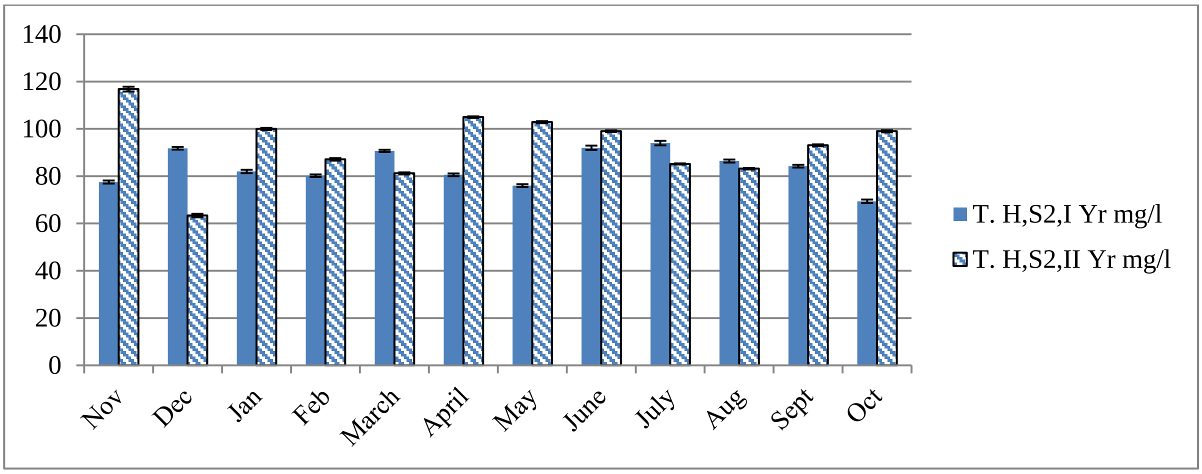

Fig.5.22. Monthly variations in total hardness at Site 2 during the first and second year study periods (Nov. 2008- Oct. 2010).

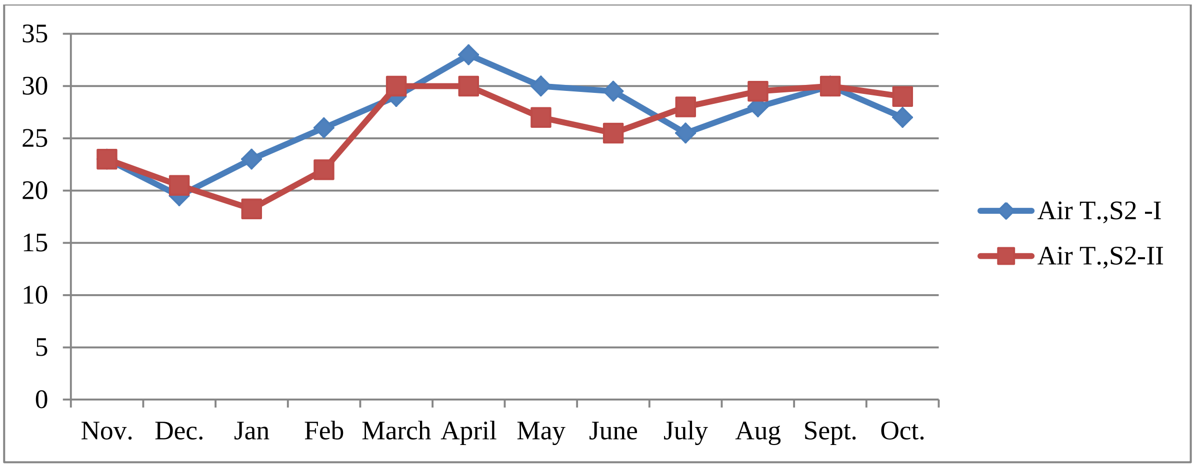

Fig.5.23. Line graph of monthly variations in air temperature during the first and second year study periods (Nov. 2008 - Oct.2010).

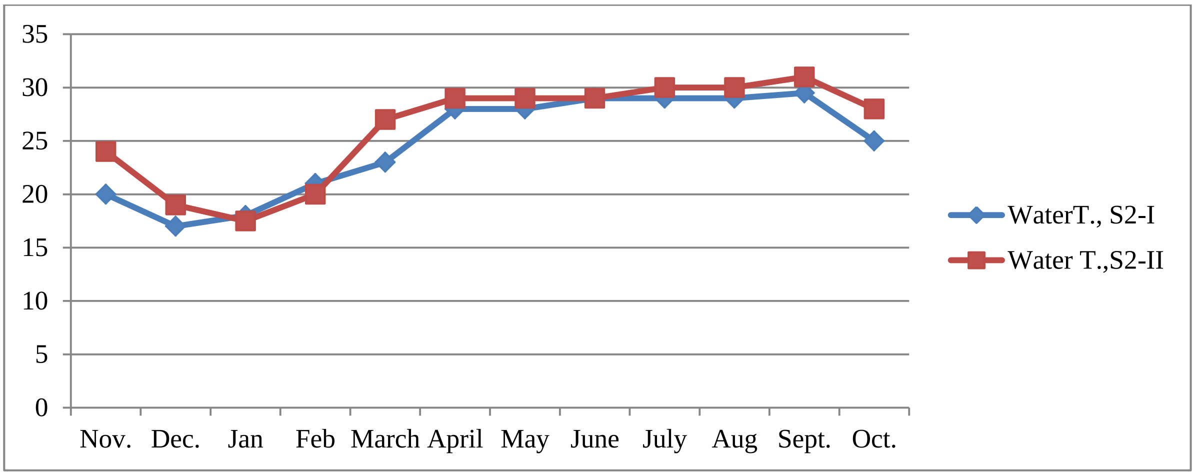

Fig.5.24. Line graph of monthly variations in water temperature during the first and second year study periods (Nov. 2008 - Oct.2010).

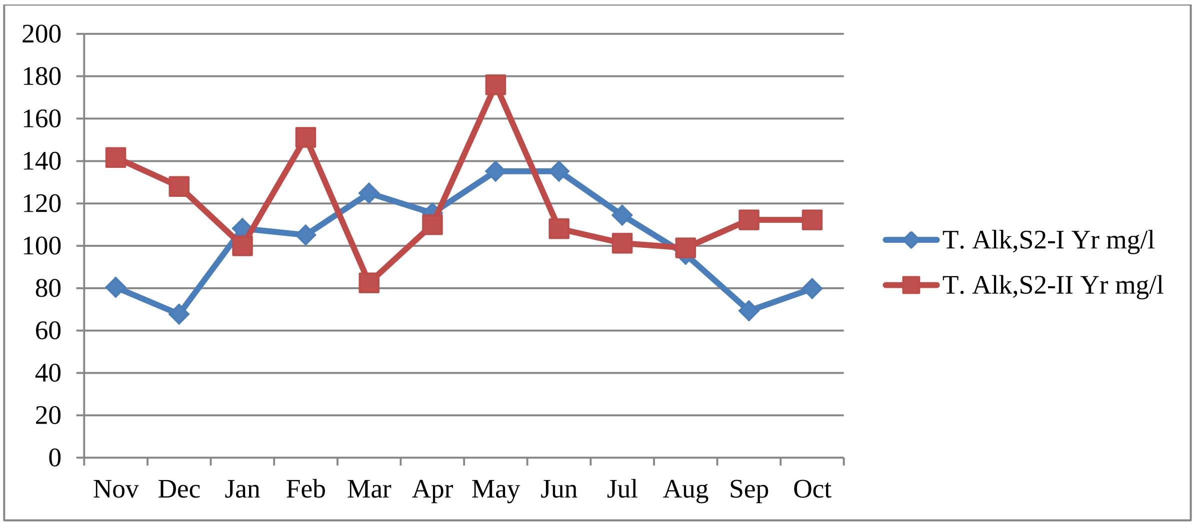

Fig.5.25. Line graph of monthly variations in total alkalinity at site 2 during the first and second year study periods (Nov. 2008 - Oct.2010).

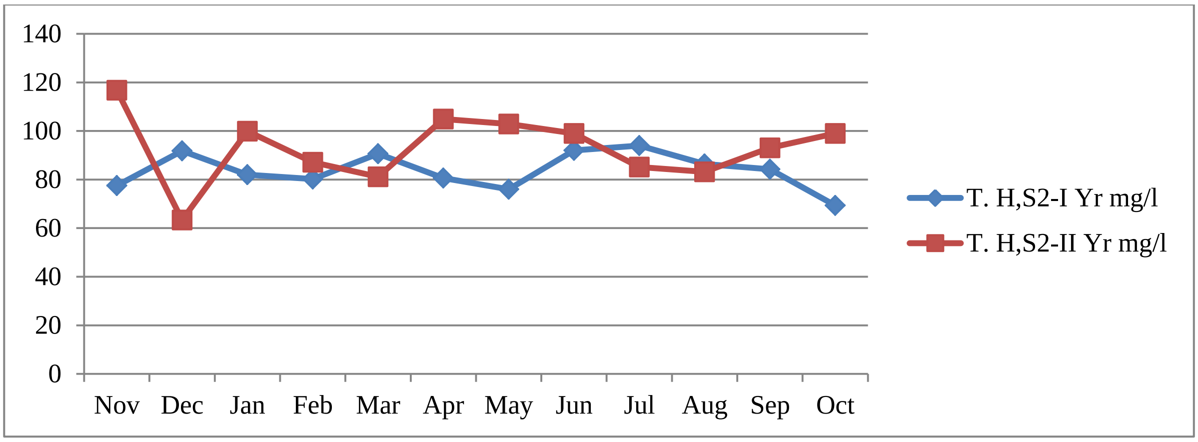

Fig.5.26. Line graph of monthly variations in total hardness at site 2 during the first and second year study periods (Nov. 2008 - Oct.2010).

Site 3 (Tarahara Fish Farm)

Results of the air temperature and physico-chemical parameters of water of Site 3 (Tarahara Fish Farm) are shown in Table 5.7 and Table 5.8. Table 5.7 shows the result of air temperature and physico-chemical parameters of water of the first year (Nov. 2008 to Oct. 2009) study period. Table 5.8 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009 to Oct. 2010) study period. Table 5.9 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site 3. Fig.5.27 shows the monthly variations in air temperature at Site 3 in the first year and the second year study periods. The Figs.5.27 to 5.35 show histograms and Figs. 5.36 to 5.39 show line graphs of the monthly variations of different physico-chemical parameters of water at Site 3 in the first year (Nov. 2008 to Oct. 2009) and the second year (Nov. 2009 to Oct. 2010) study periods.

Air temperature

The minimum air temperature was 19.75 ±0.645°C in the month of December and maximum was 31.62 ±0.478 °C in April during the first year study period (Table 5.7). The minimum air temperature was 17.52 ± 0.445°C in the December and maximum was 30.5 ± 0.386°C in September during the second year study period (Table 5.8).

The temperature showed a declining trend during the winter months of November to January in both the year1 and year 2. Decreasing trend was also observed during the months of September to October in both years (Tables 5.7, 5.8; Figs.5.27, 5.36). The air temperature had positive and significant correlation with water temperature (r=0.893, P<0.01) but inverse and significant correlation with dissolved oxygen (r = - 0.669 P<0.05) and total hardness (r = -0.673, P<0.05) (Table 5.9).

Water temperature

The lowest surface water temperature was 15.3±0.489°C in the month of December and highest 29.12± 0.275°C in the month of April during the first year study period (Table 5.7). The maximum water temperature was 30.25 ± 0.347°C in the month of September and the minimum 17.31±0.459°C in the month of December during the second year study period.

The temperature showed a decreasing trend during the winter months of November to January in both the years. Decreasing trend was also observed during the months of September to October in both years (Tables 5.7, 5.8; Figs.5.28, 5.37). The water temperature had positive and significant correlation with air temperature (r = 0.893, P<0.01) but inverse and significant correlation with dissolved oxygen (r = -0.704, P<0.05) and total hardness (r= -0.909, P<0.01) (Table 5.9).

pH

The minimum pH was 6.67 ± 0.125 in the month of April and maximum 8.62 ± 0.095 in January, during the first year study period (Table 5.7; Fig.5.29). The minimum pH was 7.08± 0.058 in October and maximum 10.02 ± 0.276 was in February during the second year study period (Table 5.8; Fig.5.29). pH had positive and significant correlation with dissolved oxygen (r =0.660, P<0.05), BOD (r =0.846, P<0.05) but inverse and significant correlation with temperature of air (r = -0.523, P<0.10) and temperature of water (r =-0.671, P<0.05) (Table 5.9).

Free carbon dioxide

The maximum free carbon dioxide was 135.6±1.356 mg/L in the month of June and minimum was 16.75 ± 0.952 mg/L in the month of September during the first year (Table 5.7; Fig.5.30). During the second year, the maximum free CO_2 was 114.58 ± 1.356 mg/L in the month of June and minimum was 12.24 ± 0.584 mg/L in May (Table 5.8; Fig.5.30). Free CO_2 showed positive and significant correlation with DO (r=0.854, P<0.01), chloride (r=0.648, P<0.05), total alkalinity and (r=0.616, P<0.05) but had an inverse and significant with BOD (r=-0.627, P<0.05) (Table 5.9).

Dissolved oxygen

The maximum dissolved oxygen was 8.92±0.221 mg/L in the month of January and the minimum was 4.86±0.079 mg/L in the month of August during the first year study period (Table 5.7, Fig.5.31). In the second year, the maximum dissolved oxygen was 10.16 ±0.215 mg/L in February and minimum 2.94 ± 0.305 mg/L was recorded in September (Table 5.8; Fig. 5.31). The dissolved oxygen showed positive and significant correlation with total alkalinity (r =0.715, P<0.01), CO_2 (r =0.854, P<0.01), chloride (r =0.625, P<0.05) and pH (r = 0.660, P<0.05) but inverse and significant correlation with air temperature (r = - 0.669, P<0.05), water temperature (r = - 0.704, P<0.05) and biological oxygen demand (r = -0.810, P <0.01) (Table 5.9).

Biological oxygen demand

The maximum biological oxygen demand was 5.31±0.082 mg/L in January and minimum was 0.47 ±0.145 mg/L in May during the first year study period (Table 5.7; Fig. 5.32). During the second year, the maximum biological oxygen demand was 7.14±0.263 mg/L in December and minimum was 0.45 ± 0.075 mg/L in November (Table 5.8; Fig. 5.32). It had positive and significant correlation with pH (r =0.846, P<0.01) but inverse and significant correlation with dissolved oxygen (r = -0.810, P<0.01) (Table 5.9).

Chloride

The maximum chloride was 12.98 ± 0.416 mg/L in January and minimum was 5.2± 0.288 mg/L in October during the first year study period (Table 5.7; Fig.5.33). During the second year, the maximum chloride was 9.02± 0.525mg/L in the month of June and minimum was 1.06± 0.035 mg/L in April (Table 5.8; Fig.5.33). It had a positive and significant correlation with DO (r = 0.625, P<0.05) and CO_2 (r= 0.648, P<0.05) (Table 5.9).

Total alkalinity

The maximum total alkalinity was 202.50± 5.802 mg/L in the month of January and minimum was 103.40 ± 0.469 mg/L in the month of September during the first year study period (Table 5.7; Fig.5.29). During the second year, the maximum total alkalinity was 215.03 ±1.089 mg/L in the month of March and minimum was 72.74 ± 1.092 mg/L in the month of December (Table 5.8, Fig.5.34). It had positive and significant correlation with DO (r = 0.715, P<0.01), CO_2 (r = 0.616, P<0.05) and TH (r = 0.592, P<0.05) (Table 5.9).

Total alkalinity showed decreasing trend from June to September. The values in the month of June (125.62 ±0.805 mg/L) was significantly decreased ($P<0.01$) as compared to May (167.12 ±0.689 mg/L) in the first year study (Table 5.7; Figs.5.34, 5.38). In second year, decreasing trend was seen from June to October. The value of June (124.22 ±0.995 mg/L) was significantly decreased ($P< 0.01$) as compared to May (136.40 ±1.642 mg/L) (Table 5.8; Figs. 5.34, 5.38). It remained low for five months from June to October in both years.

Total hardness

The maximum total hardness was 164.4 ± 1.478 mg/L in January and minimum was 83.6 ± 0.585 mg/L in the month of July during the first year study period (Table 5.7; Fig.5.35). During the second year, the maximum total hardness was recorded 163.26±1.023 mg/L in February and minimum 35.64±1.578 mg/L in the month of January (Table 5.8; Fig.5.35). It had positive and significant correlation with total alkalinity ($r= 0.592$, $P<0.05$) but inverse and significant correlation with air temperature ($r= -0.673$, $P<0.05$) and water temperature ($r= -0.909$, $P<0.01$) (Table 5.9).

The hardness showed a decreasing trend from the months of April to August and increased slightly during the months of September and October but the values were less than that of during the month of April. The values in April (101.2 ±0.776 mg/L) showed significant decrease ($p< 0.01$) as compared to March (146.14±0.985 mg/L) in the first year (Table 5.7; Figs. 5.35, 5.39). It also showed decreasing trend from March 2010. The values in March (156.420±0.675 mg/L) was significantly lower ($P<0.01$) as compared to February (163.26 ±1.023 mg/L) in the second year (Table 5.8; Figs. 5.35, 5.39). It remained low for seven months from April to October in the first year and for eight months from March to October in the second year.

Table 5.7 shows air temperature, water temperature and physico-chemical parameters of water at Site 3 (Tarahara fish pond, Sunsari) from Nov. 2008- October 2009. **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 3- I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	21.25 ±0.645	19.75 ±0.645	20.87 ±1.108	24.75 ±0.645	29.25 ±0.645	31.62 ±0.478	29.12 ±0.629	29.25 ±0.288	25.75 ±0.645	29.37 ±0.478	29.25 ±0.288	27.45 ±0.420
Water Temp. (ºC)	18.75 ±0.228	15.3 ±0.489	18.75 ±0.288	21.5 ±0.408	23.57 ±0.434	29.12 ±0.275	27.07 ±0.25	27.45 ±0.42	27.07 ±0.25	27.12 ±0.275	27.25 ±0.645	25.27 ±0.499
pH	7.9 ±0.089	8.05 ±0.129	8.62 ±0.095	8.12 0.095±	7.325 ±0.095	6.67 ±0.125	8.12 ±0.629	8.2 ±0.081	7.05 ±0.057	7.12 ±0.275	8.2 ±0.216	7.62 ±0.478
Free CO_2 (mg/L)	21.63 ±1.203	55.02 ±1.275	91.05 ±1.078	126.35 ±0.864	135.12 ±0.853	101.96 ±0.416	93.15 ±0.580	135.6 ±1.356	113.35 ±0.850	49.13 ±1.3	16.75 ±0.952	38.16 ±0.623
DO (mg/L)	5.71 ±0.335	5.84 ±0.079	8.92 ±0.221	8.61 ±0.115	7.86 ±0.354	8.1 ±0.127	7.04 ±0.225	7.83 ±0.009	8.90 ±0.553	4.86 ±0.079	5.45 ±0.245	5.75 ±0.365
BOD (mg/L)	2.91 ±0.145	2.30 ±0.067	5.31 ±0.082	3.67 ±0.238	0.65 ±0.253	1.74 ±0.057	0.47 ±0.145	0.54 ±0.235	2.35 ±0.082	2.78 ±0.365	3.5 ±0.325	3.35 ±0.346
Chloride (mg/L)	8.2 ±0.332	5.3 ±0.082	12.98 ±0.416	9.88 ±0.334	11.32 ±0.221	12.06 ±0.132	8.46 ±0.129	12.2 ±0.629	8.41 ±0.145	9.96 ±0.546	6.21 ±0.223	5.2 ±0.288
Total Alkalinit (mg/L)	147.96 ±1.860	128.72 ±1.112	202.5 ±5.802	194.95 ±1.962	176.82 ±1.189	157.7 ±0.877	167.12 ±0.689	125.62 ±0.805 *	135.8 ±0.585	118.07 ±0.449	103.4 ±0.469	133.02 ±0.694
Total Hardness (mg/L)	138.72 ±2.125	157.08 ±1.325	164.4 ±1.478	148.6 ±1.036	146.14 ±0.985	101.2 ±0.776 *	96.32 ±1.745	91.2 ±1.558	83.6 ±0.998	92.88 ±0.756	108.25 ±0.955	118.23 ±0.779

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.8 shows air temperature, water temperature and physico-chemical parameters of water at Site 3 (Tarahara fish pond, Sunsari) from Nov. 2009 - October 2010. **(Mean ± S.D., N=5).**

Site 3-II Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	19.12 ±0.345	17.52 ±0.445	18.37 ±.608	21.35 ±0.545	28.25 ±0.745	28.02 ±0.478	24.13 ±0.229	25.15 ±0.278	29.5 ±0.635	29.17 ±0.378	30.5 ±0.386	27.85 ±0.620
W Temp. (ºC)	21.26 ±0.325	17.31 ±0.459	17.45 ±0.246	19.20 ±0.218	25.26 ±0.335	27.12 ±0.275	26.57 ±0.251	28.05 ±0.42	28.65 ±0.254	30.12 ±0.235	30.25 ±0.347	25.87 ±0.578
pH	7.33 ±0.185	8.68 ±0.426	7.82 ±0.565	10.02 ±0.276	7.72 ±0.076	7.76 ±0.325	7.51 ±0.427	7.62 ±0.281	8.05 ±0.068	7.81 ±0.078	7.64 ±0.216	7.08 ±0.058
Free CO_2 (mg/L)	19.52 ±1.325	15.12 ±1.205	47.52 ±1.078	16.03 ±0.965	15.12 ±0.853	14.56 ±0.817	12.24 ±0.584	114.58 ±1.356	18.35 ±0.915	16.14 ±1.325	16.75 ±0.652	15.68 ±0.323
DO (mg/L)	4.48 ±0.215	8.48 ±0.067	8.81 ±0.229	10.16 ±0.215	4.64 ±0.308	7.71 ±0.125	3.04 ±0.232	3.31 ±0.058	4.81 ±0.373	4.65 ±0.079	2.94 ±0.305	4.22 ±0.265
BOD (mg/L)	0.45 ±0.075	7.14 ±0.263	3.38 ±0.172	7.01 ±0.241	2.68 ±0.158	5.02 ±0.089	2.04 ±0.165	1.75 ±0.245	2.85 ±0.064	0.82 ±0.325	1.03 ±0.227	0.51 ±0.243
Chloride (mg/L)	2.70 ±0.092	2.58 ±0.184	4.21 ±0.317	4.02 ±0.314	3.12 ±0.322	1.06 ±0.035	4.14 ±0.132	9.02 ±0.525	5.11 ±0.097	4.01 ±0.374	6.10 ±0.152	5.03 ±0.238
Total Alkalin (mg/L)	144.08 ±1.663	72.74 ±1.092	180.25 ±4.532	117.55 ±1.876	215.03 ±1.089	195.57 ±1.877	136.40 ±1.642	124.22 ±0.995*	119.7 ±0.887	101.23 ±0.849	118.75 ±0.559	117.86 ±0.893
Total Hardn (mg/L)	138.72 ±2.125	116.82 ±1.721	35.64 ±1.578	163.26 ±1.023	156.42 ±0.675*	152.32 ±1.445	97.02 ±1.342	102.95 ±0.906	93.06 ±1.097	83.16 ±0.356	93.01 ±0.978	110.85 ±0.719

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.9 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 3 (average of the corresponding month values) during Nov.2008 – Oct. 2010; N=12; d. f. =11.

S3-I +II		**Water Temp. (°C)**	**pH**	**Free CO_2 (mg/L)**	**DO (mg/L)**	**BOD (mg/L)**	**Chloride (mg/L)**	**Total alkalin(mg/L)**	**Total hardn (mg/L)**
Air Temp(°C)	P corr.	.893*	-.523	.241	-.669**	-.373	.308	-.199	-.673**
	Sig(2-t)	.000	.081	.450	.048	.232	.331	.535	.017
Temp.of water (°C)	P corr.	1	-.571**	.148	-.704**	-.299	.148	-.429	-.909*
	Sig.(2-t)		.051	.647	.011	.346	.647	.165	.000
pH	P corr.		1	-.219	.660**	.846*	-.053	.315	.515
	Sig.(2-t)			.495	.019	.001	.870	.318	.086
Free CO_2 (mg/L)	P corr.			1	.854*	-.627**	.648**	.616**	-.049
	Sig.(2-t)				.000	.051	.023	.039	.880
DO (mg/L)	P corr.					.810*	.625**	.715*	.155
	Sig.(2-t)					.001	.030	.009	.631
BOD (mg/L)	P corr.					1	-.044	.028	.316
	Sig.(2-t)						.892	.930	.317
Chloride (mg/L)	P corr.						-.624**	.555	.026
	Sig.(2-t)						.046	.061	.935
Total alkal(mg/L)	P corr.							1	.592**
	Sig.(2-t)								.043
Total hard(mg/L)	P corr.								1
	Sig. 2-t)								

*** Significant at 1% level (P<0.01), ** Significant at 5% level (P<0.05) and Values not marked denote non-significant correlation.**

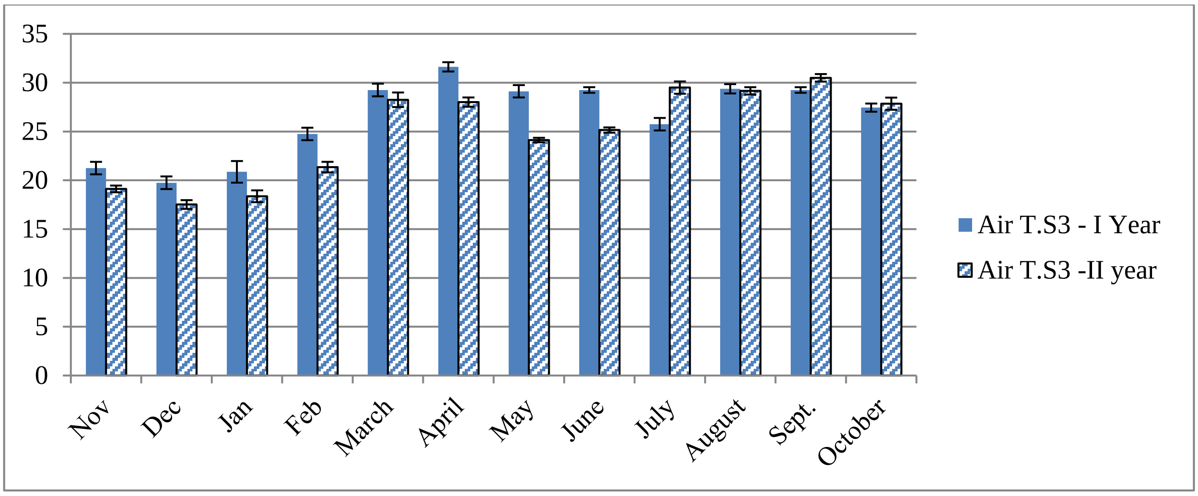

Fig.5.27. Monthly variations in air temperature at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

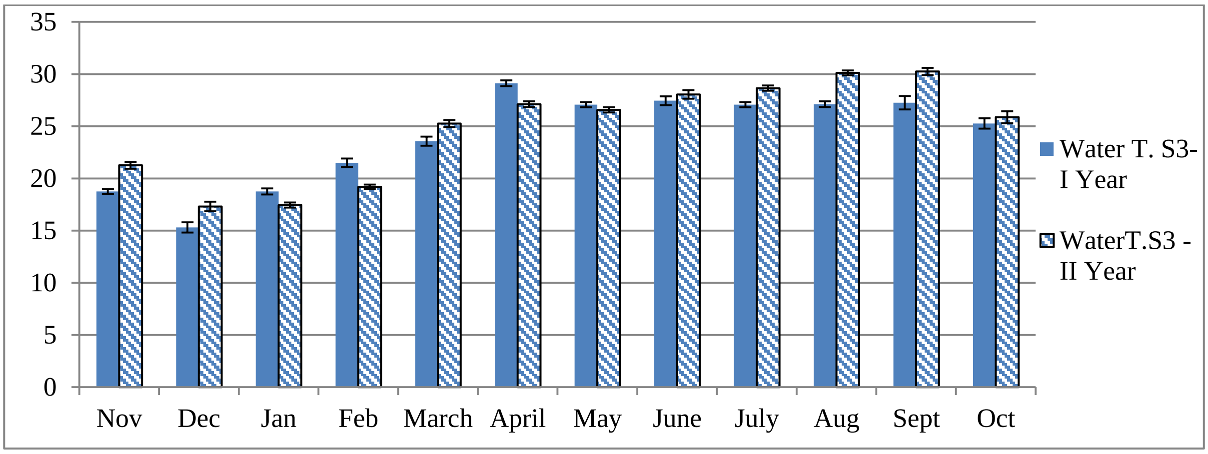

Fig.5.28. Monthly variations in water temperature at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

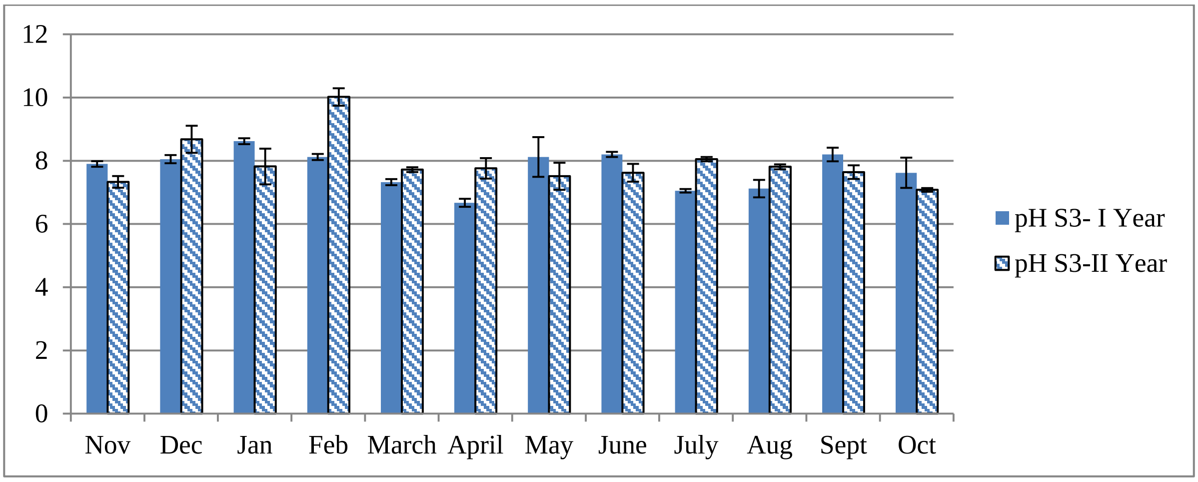

Fig.5.29. Monthly variations in pH at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

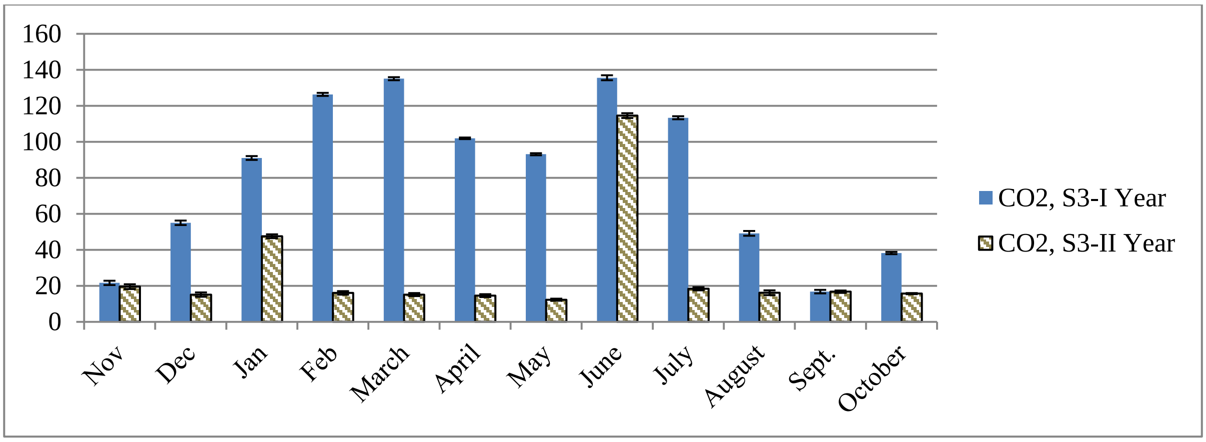

Fig.5.30. Monthly variations in Free CO_2 at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

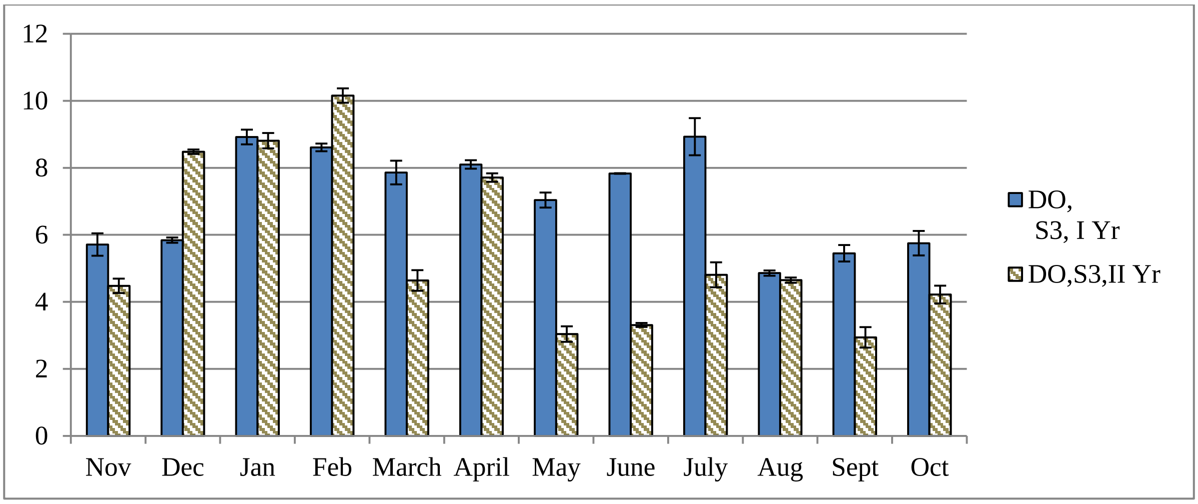

Fig.5.31. Monthly variations in DO at Site 3 during the first and second year study periods (Nov.2008- Oct.2010)

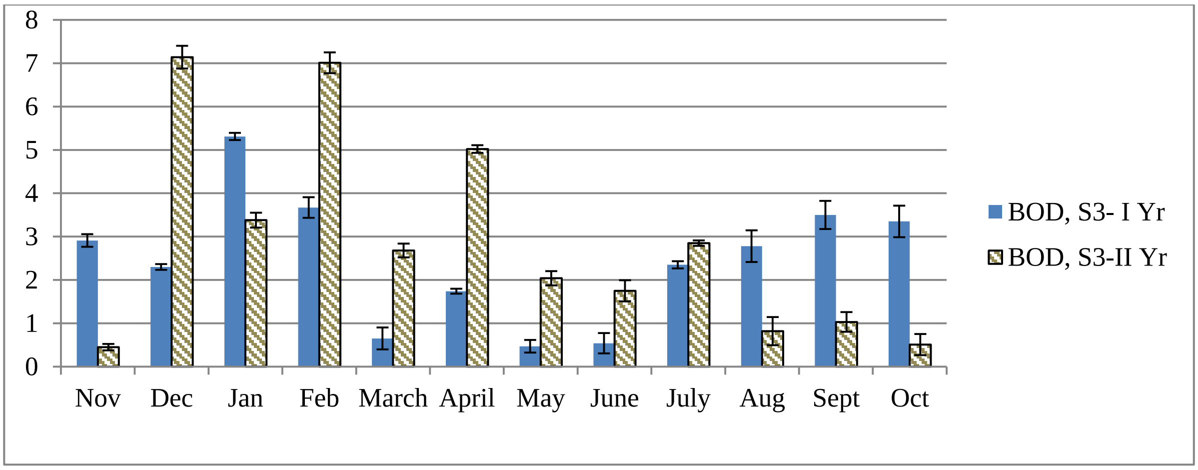

Fig.5.32. Monthly variations in BOD at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

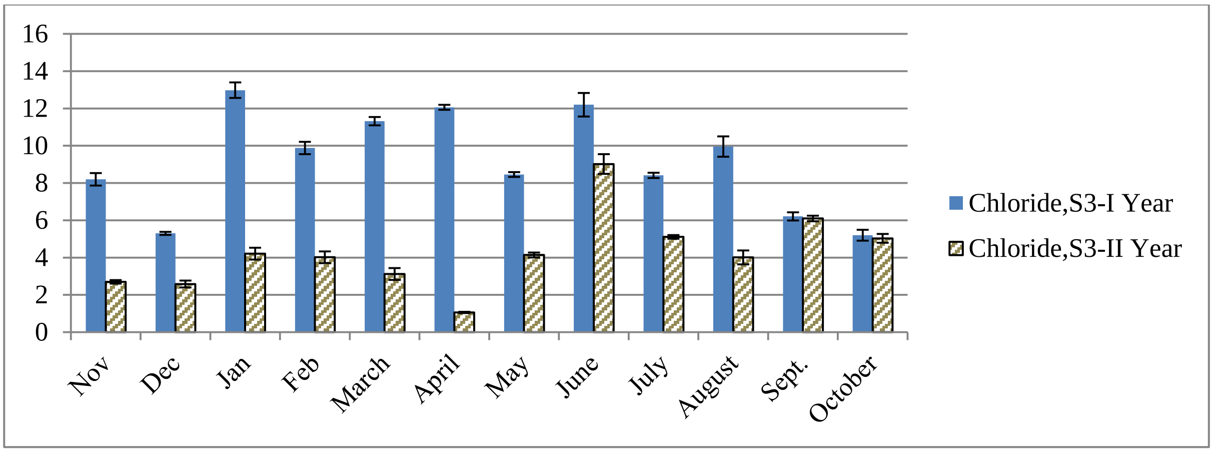

Fig.5.33. Monthly variations in Chloride at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

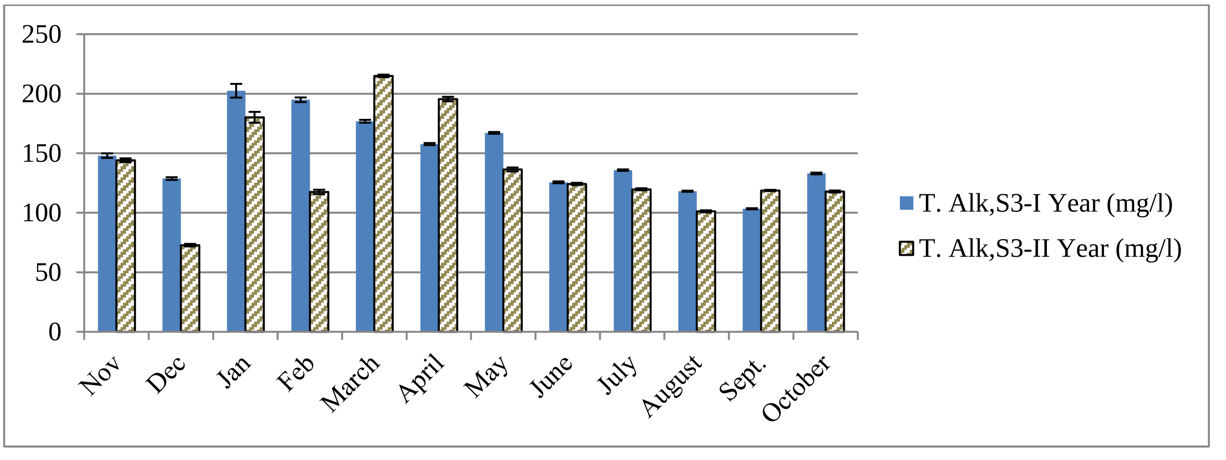

Fig.5.34. Monthly variations in TA at Site 3 during the first and second year study periods (Nov. 2008- Oct. 2010).

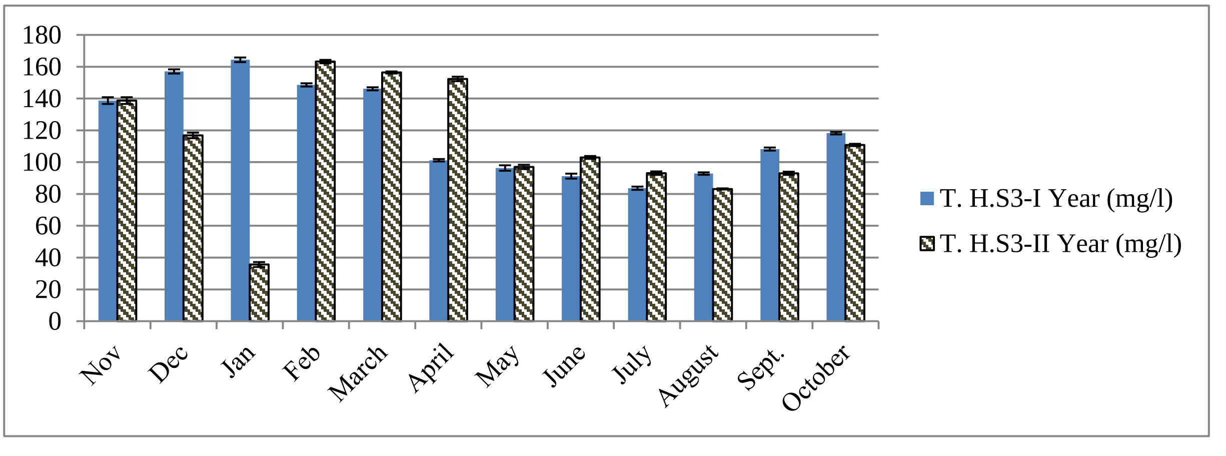

Fig.5.35. Monthly variations in T.H. at Site 3 during the first and second year study periods (Nov.2008- Oct. 2010).

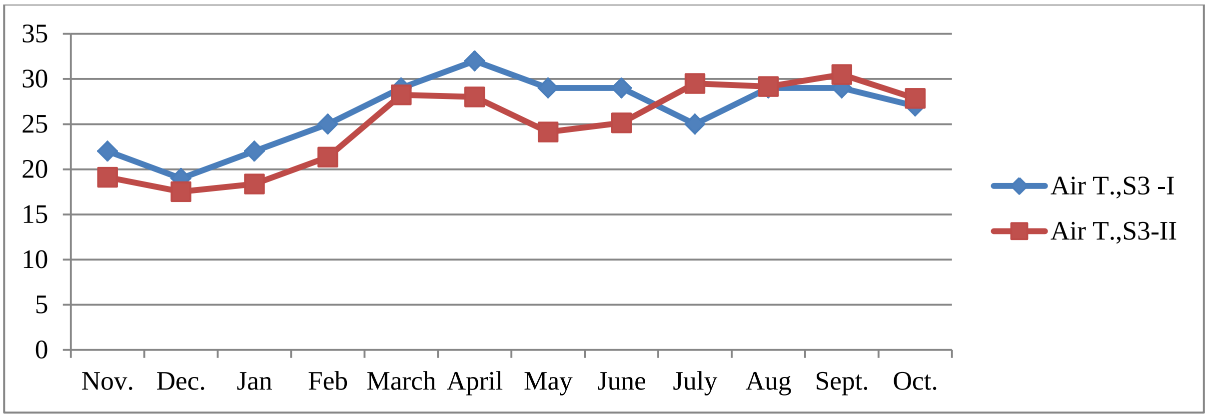

Fig.5.36. Line graph of monthly variations in air temperature at site 3 during the first and second year study periods (Nov. 2008 - Oct.2010).

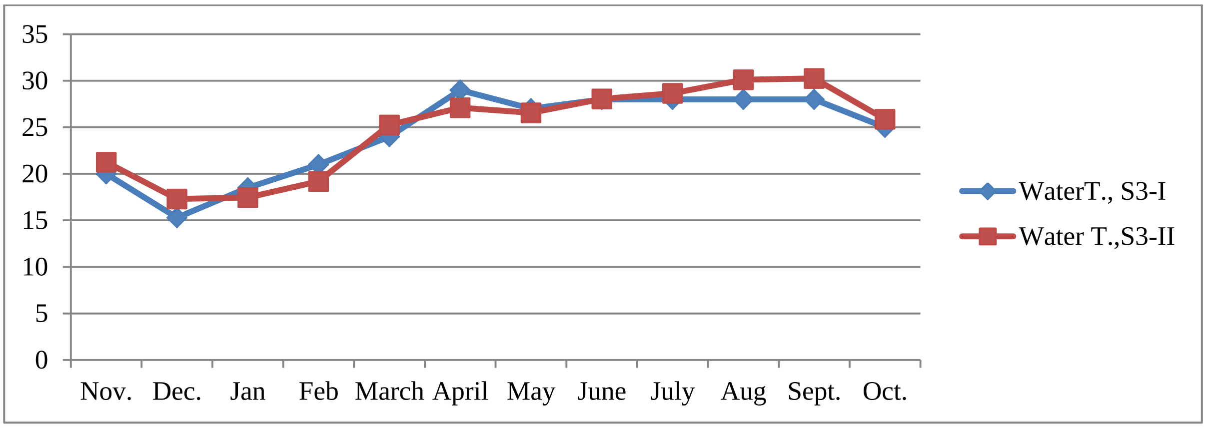

Fig.5.37. Line graph of monthly variations in water temperature at site 3 during the first and second year study periods (Nov. 2008 - Oct.2010).

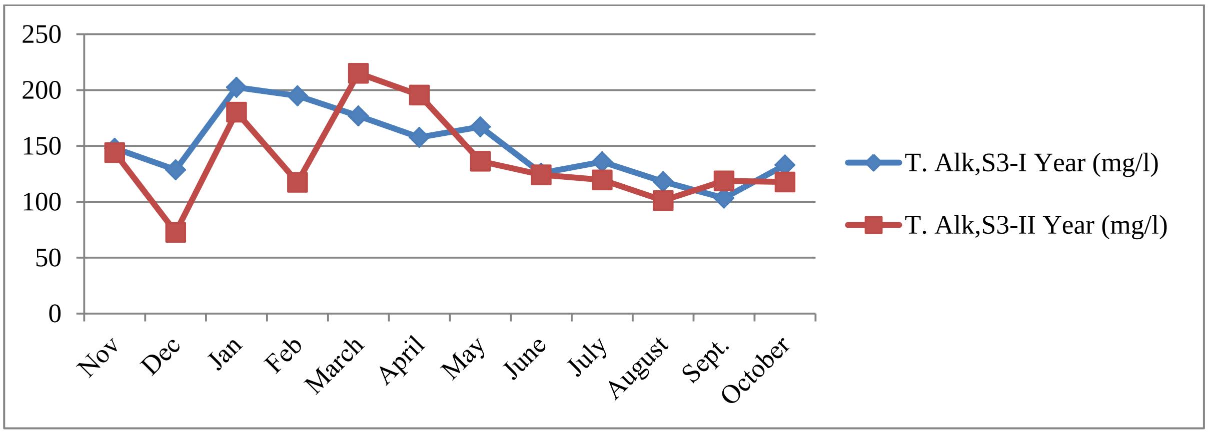

Fig.5.38. Line graph of monthly variations in total alkalinity at site 3 during the first and second year study periods (Nov. 2008 - Oct.2010).

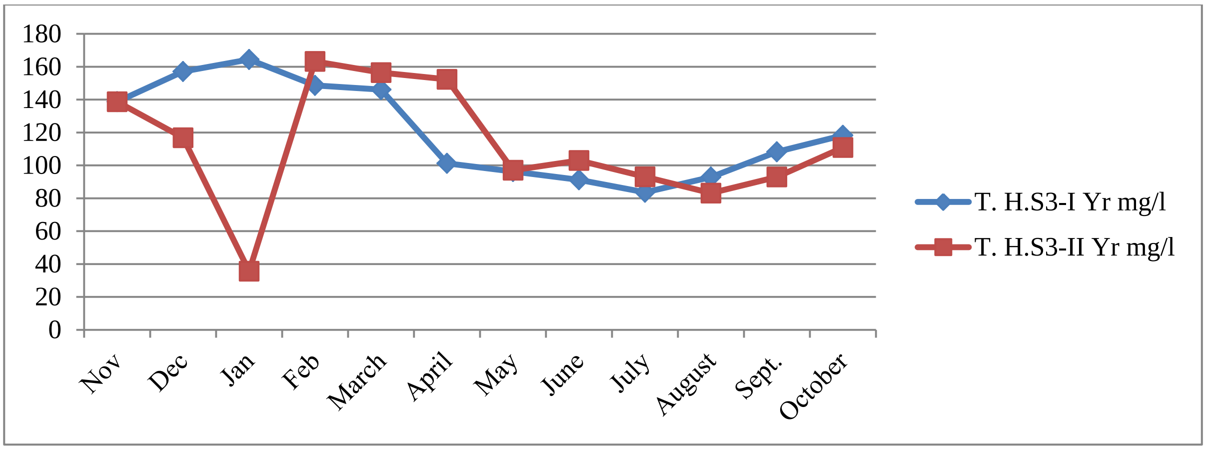

Fig.5.39. Line graph of monthly variations in total hardness at site 3 during the first and second year study periods (Nov. 2008 - Oct.2010).

Site 4 (Betana wetland)

Betana wetland consists of an ox-bow lake with an area of 5.5 ha. It is surrounded by sal forests (Charkoshe Jhaadi) in east, north and west sides and Mahendra highway in the south. It is flooded during rainy season.

Results of the air temperature and physico-chemical parameters of water of Site 4 are shown in Table 5.10 and Table 5.11. Table 5.10 shows the results of air temperature and physico-chemical parameters of water of the first year (Nov.2008 – Oct.2009) study period. Table 5.11 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009- June 2010). Table 5.12 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site 4. The Fig.5.40 shows the monthly variations in air temperature at site 4 in the first year and the second year study periods. The Figs.5.40 to 5.48 show histograms and Figs. 5.49 to 5.52 show line graphs of the monthly variations of different physico-chemical parameters of water at Site 4 during the first year (Nov. 2008 – Oct. 2009) and the second year (Nov. 2008 – Oct. 2010) study periods.

Air temperature

The minimum air temperature was 18.03 ± 0.347°C in December and maximum was 31.01± 0.274°C in August during the first year study period (Table 5.10 and Fig.5.40). The maximum air temperature was 29.1± 0.285°C in the month of March and minimum 17.10 ±0.237°C in the month of January during the second year study period (Table 5.11 and

Fig.5.40). Air temperature showed positive and significant correlation with water temperature (r =0.947, $P<0.01$) but it had inverse and significant correlation with free CO_2 (r= - 0.685, $P<0.05$), pH (r = -0 .653, $P<0.05$) and dissolved oxygen (r = - 0.582, $P<0.05$) (Table 5.12).

During year 1, the air temperature showed declining trend during the month of November. In the month of December 2008, it was lowest (18.03 ± 0.347°C) and it increased slightly (18.10 ±0.523) in the month of January, 2009. Thereafter it increased February onwards up to March (Table 5.10; Figs.5.46, 5.57). The air temperature during the year 2 showed decreasing trends from November to January (Table 5.11; Figs.5.40, 5.49). Decreasing trend was also observed during the months of August to October in both years.

Water temperature

The maximum water temperature was 29.12±0.235°C in August and minimum 17.14 ± 0.316°C in the month of January during the first year (Table 5.10 and Fig.5.41). During the second year study period, the maximum water temperature was 28.12 ± 0.523°C in August and minimum 18.04 ± 0.365°C in the January (Table 5.11 and Fig.5.41). The water temperature showed positive and significant correlation with air temperature (r =0.947, $P<0.01$) and phosphate (r=0.635, $P<0.05$) but it showed inverse and significant correlation with pH (r = - 0.692, $P<0.05$), dissolved oxygen (r=-0.576, $P<0.05$) and free CO_2 (r=-0.798, $P<0.01$) (Table 5.12).

The water temperature showed decreasing trend during the winter months of November to January in both year 1 and year 2. Decreasing trend was also observed during the months of August to October in both years. It remained low during winter months (Tables 5.11, 5.12; Figs.5.41, 5.50).

pH

The maximum pH was 8.15±0.365 in the month of January and minimum 6.64 ± 0.271 in September during the first year study period (Table 5.10, Fig.5.42). The maximum pH was 7.60 ± 0.327 in December and minimum was 6.61±0.229 in February during second year (Table 5.11 Fig.5.48). pH showed inverse and significant correlation with air temperature (r=-0.653, $P<0.05$), water temperature (r=-0.692, $P< 0.05$) and biological oxygen demand (r=-0.613, $P<0.05$) (Table 5.12).

Free carbon dioxide

The maximum free carbon dioxide was recorded 73.92 ± 1.552 mg/L in September and minimum 3.37 ± 0.638 mg/L in May during the first year study period (Table 5.10 and Fig. 5.43). The maximum free carbon dioxide was 23.75 ± 0.874 mg/L in January and minimum 2.24 ±0.557mg/L in April during the second year study period (Table 5.11 and Fig. 5.43). Free carbon dioxide showed inverse and significant correlation with chloride (r = -0.596, P<0.05), water temperature (r = -0.798, P<0.01), air temperature (r = -0.685, P<0.05) (Table 5.12).

Dissolved oxygen

The maximum dissolved oxygen was 7.31 ±0.185 mg/L in January and minimum 3.19 ± 0.379 mg/L in August during the first year study period (Table 5.10 and Fig.5.44). The maximum dissolved oxygen was 9.74 ± 0.235 mg/L in April and minimum 3.19 ± 0.254 mg/L in June (Table 5.11 and Fig.5.44). The dissolved oxygen showed inverse and significant correlation with water temperature (r =-0.596, P<0.05), air temperature (r = -0.582, P<0.05) (Table 5.12).

Biological oxygen demand

The maximum biological oxygen demand was 4.62 ± 0.254 mg/L in the month of September and minimum was 0.84± 0.014 mg/L in the month of February during the first year study period (Table 5.10 and Fig.5.45). During the second year, the maximum biological oxygen demand 6.22 ±0.048 mg/L was seen in the month of April and minimum 0.26 ± 0.076 mg/L in the month of December (Table 5.11 and Fig. 5.45). BOD showed no significant positive correlation but it had inverse and significant correlation with pH (r = - 0.613, P< 0.05) (Table 5.12).

Chloride

The maximum chloride was 5.02 ±0.531 mg/L in June and minimum was 2.02 ± 0.095 mg/L in September during the first year study period (Table 5.10 and Fig.5.46). During the second year, the maximum chloride was 7.05 ±0.324 mg/L in January and minimum 1.01 ± 0.093 mg/L in March (Table 5.11 and Fig.5.46). Chloride showed inverse and significant correlation with free CO_2 (r = -0.596, P< 0.05) (Table 5.12).

Total alkalinity

The maximum total alkalinity was recorded 195.33 ± 1.776 mg/L in February and minimum 69.56 ±1.152 mg/L in December during the first year study period (Table 5.10 and Fig.5.47). During the second year, the maximum total alkalinity was recorded 197.43 ± 2.756 mg/L in February and minimum 103.23±0.867 mg/L in September (Table 5.11 and Fig. 5.47). The total alkalinity showed positive and significant correlation with total hardness ($r = 0.580$, $P<0.05$) (Table 5.12).

Total alkalinity remained low during August, September and October in the first year study period. Total alkalinity in the month of June (116.62 ±0.956 mg/L) significantly ($p<0.01$) decreased in comparison to that of May (132.01±1.742 mg/L) in the first year (Table 5.10; Figs.5.47, 5.51). There were fluctuations in the values of total alkalinity during March, April, May and June, 2009. Similar patterns in total alkalinity were noticed during second year study period (Table 5.11; Figs. 5.47 and 5.51).

Total hardness

The maximum hardness was 130.43±1.623 mg/L in February and minimum 97.02±0.754 mg/L in August during the first year study period (Table 5.10 and Fig.5.48). During the second year, the maximum total hardness was 118.84± 1.623 mg/L in February and minimum was 89.13± 0.659 mg/L in September (Table 5.11 and Fig.5.48). Total hardness showed positive and significant correlation with total alkalinity ($r = 0.580$, $P<0.05$) but inverse and significant correlation with water temperature ($r =-0.623$, $P<0.05$) (Table 5.12).

The values of total hardness in March (108.91±0.745 mg/L) showed significant decrease ($p< 0.01$) as compared to February (130.43 ±1.623 mg/L) in the first year. It remained low for six months from March to August (Table 5.10; Figs.5.48, 5.52). Likewise in the second year it showed a decreasing trend from March to September for seven months with slight fluctuation. The value in May (106.92 ±1.563mg/L) was significantly decreased ($P<0.05$) as compared to April (110.78 ±1.544 mg/L) in the second year (Table 5.11; Figs. 5.48, 5.52). It remained low for six months from May to October in the second year.

Table 5.10 shows air temperature, water temperature and physico-chemical parameters of water at Site 4 (Betana wetland, Belbari, Morang) from November 2008- October 2009 **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site4 – I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Air Temp. (ºC)	21.18 ±0.259	18.03 ±0.347	18.10 ±.523	24.85 ±0.369	29.99 ±0.628	27.78 ±0.775	27.12 ±0.322	26.05 ±0.731	29.86 ±0.657	31.01 ±0.274	29.15 ±0.362	26.03 ±0.557
Water Temp. (ºC)	19.0 ±0.125	19.01 ±0.217	17.14 ±0.316	22.12 ±0.335	27.06 ±0.523	27.85 ±0.475	26.07 ±0.351	27.13 ±0.328	28.95 ±0.272	29.12 ±0.235	27.3 ±0.534	25.07 ±0.476
pH	7.82 ±0.534	7.66 ±0.327	8.15 ±0.365	7.13 ±0.229	7.61 ±0.576	6.83 ±0.317	7.51 ±0.733	7.34 ±0.256	7.5 ±0.075	6.93 ±0.174	6.64 ±0.271	7.31 ±0.073
Free CO2 (mg/L)	41.36 ±1.476	37.42 ±1.235	12.15 ±0.675	24.96 ±0.887	6.23 ±0.353	4.58 ±0.567	3.37 ±0.638	5.09 ±0.056	8.03 ±0.926	12.54 ±1.323	73.92 ±1.552	55.448 ±0.826
DO (mg/L)	7.08 ±0.356	5.84 ±0.067	7.31 ±0.185	5.89 ±0.124	5.14 ±0.068	6.88 ±0.235	7.17 ±0.342	4.92 ±0.254	4.82 ±0.473	3.19 ±0.379	5.41 ±0.362	7.16 ±0.231
BOD (mg/L)	2.61 ±0.045	2.25 ±0.026	1.35 ±0.029	0.84 ±0.014	1.22 ±0.056	4.32 ±0.067	3.55 ±0.115	2.81 ±0.149	1.83 ±0.057	1.02 ±0.065	4.62 ±0.254	2.11 ±0.056
Chloride (mg/L)	4.10 ±0.063	2.03 ±0.059	4.5 ±0.226	3.61 ±0.342	3.01 ±0.192	4.0 ±0.237	4.01 ±0.135	5.02 ±0.531	5.01 ±0.109	4.03 ±0.275	2.02 ±0.095	3.84 ±0.082
Total Alkalin. (mg/L)	115.64 ±1.253	69.56 ±1.152	122.05 ±2.634	195.33 ±1.776	132.03 ±1.187	117.21 ±1.953	132.01 ±1.742	116.62 ±0.956 **	130.02 ±0.987	118.83 ±1.745	109.27 ±0.857	119.73 ±0.995
Total Hard (mg/L)	116.28 ±2.227	112.2 ±1.523	110.03 ±1.378	130.43 ±1.623	108.91 ±0.745 **	106.92 ±1.544	110.82 ±1.563	108.90 ±0.976	104.94 ±1.065	97.02 ±0.754	112.32 ±0.957	110.16 ±0.817

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.11 shows air temperature, water temperature and physico-chemical parameters of water at Site 4 (Betana wetland, Belbari, Morang) from November 2009- October 2010 **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 4- II Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Air Temp. (ºC)	22.03 ±0.359	20.01 ±0.475	17.10 ±.237	24.05 ±0.691	29.1 ±0.285	27.02 ±0.475	26.12 ±0.229	25.05 ±0.318	29.01 ±0.537	29.02 ±0.742	26.15 ±0.624	28.03 ±0.355
Water Temp. (ºC)	21.81 ±0.225	19.01 ±0.317	18.04 ±0.365	21.13 ±0.357	26.06 ±0.523	28.05 ±0.745	25.02 ±0.351	27.51 ±0.432	27.03 ±0.372	28.12 ±0.523	27.13 ±0.343	25.01 ±0.273
pH	7.12 ±0.534	7.60 ±0.327	7.15 ±0.365	6.61 ±0.229	7.11 ±0.576	6.82 ±0.317	6.95 ±0.733	7.23 ±0.256	7.5 ±0.075	7.01 ±0.174	7.14 ±0.271	7.11 ±0.073
Free CO_2 (mg/L)	17.92 ±0.976	15.05 ±0.735	23.75 ±0.874	23.54 ±0.887	5.12 ±0.325	2.24 ±0.557	3.37 ±0.623	4.59 ±0.076	8.1 ±0.928	13.2 ±0.526	9.15 ±0.755	9.46 ±0.523
DO (mg/L)	5.52 ±0.257	7.43 ±0.067	7.99 ±0.085	5.84 ±0.224	4.82 ±0.068	9.74 ±0.235	4.92 ±0.342	3.19 ±0.254	5.47 ±0.473	5.16 ±0.359	6.88 ±0.462	5.91 ±0.235
BOD (mg/L)	0.85 ±0.055	0.26 ±0.076	3.72 ±0.053	0.84 ±0.026	1.35 ±0.059	6.22 ±0.048	3.61 ±0.107	1.82 ±0.049	1.03 ±0.066	0.44 ±0.073	0.71 ±0.145	0.28 ±0.045
Chloride (mg/L)	2.01 ±0.037	5.02 ±0.065	7.05 ±0.324	4.1 ±0.352	1.01 ±0.093	2.0 ±0.257	5.21 ±0.135	6.02 ±0.537	5.01 ±0.809	5.03 ±0.372	2.02 ±0.065	5.13 ±0.084
Total Alkalin (mg/L)	117.22 ±1.156	114.06 ±1.654	110.05 ±1.563	197.43 ±2.756	130.03 ±1.187	118.81 ±1.753	132.01 ±1.342	115.02 ±0.953 *	126.50 ±0.977	116.63 ±1.785	103.23 ±0.867	107.81 ±0.985
Total Hardness (mg/L)	95.04 ±1.325	108.95 ±1.563	114.23 ±1.375	118.84 ±1.623	110.88 ±0.645	110.78 ±1.544	106.92 ±1.563 *	104.94 ±0.976	105.10 ±1.067	95.04 ±0.854	89.13 ±0.659	104.94 ±0.816

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.12 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 4 (average of the corresponding month values) during Nov. 2008 – Oct. 2010; N=12; d. f. =11.

S4-I +II		**Water Temp (ºC)**	**pH**	**Free CO_2 (mg/L)**	**DO (mg/L)**	**BOD (mg/L)**	**Chloride (mg/L)**	**Total alk (mg/L)**	**Total hard (mg/L)**
AirTemp. (ºC)	P Cor.	.947*	-.653**	-.685**	-.582**	.106	.114	.290	-.398
	Sig.(2-t)	.000	.021	.014	.047	.742	.725	.360	.200
Water Temp.(ºC)	P Cor.	1	-.692**	-.798*	-.596**	.260	.145	.082	-.623**
	Sig.(2-t)		.013	.002	.050	.415	.653	.800	.051
pH	P Cor.		1	-.185	.312	-.513	.243	-.143	.092
	Sig.(2-t)			.564	.323	.088	.447	.657	.777
Free CO_2 (mg/L)	P Cor.			1	.174	.285	-.596**	-.241	.301
	Sig.(2-t)				.589	.369	.041	.451	.342
DO (mg/L)	P Cor.				1	.316	.038	.008	.431
	Sig.(2-t)					.317	.908	.981	.162
BOD (mg/L)	P Cor.					1	-.225	-.379	-.081
	Sig. (2-t)						.481	.224	.802
Chloride (mg/L)	P Cor.						1	.319	-.238
	Sig.(2-t)							.312	.456
Total alkalinity (mg/L)	P Cor.							1	.580**
	Sig.(2-t)								.048
Total hardness (mg/L)	P Cor.								1
	Sig.(2-t)								

*** Significant at 1% level (P<0. 01), ** Significant at 5% level (P<0. 05) and Values not marked denote non-significant correlation.**

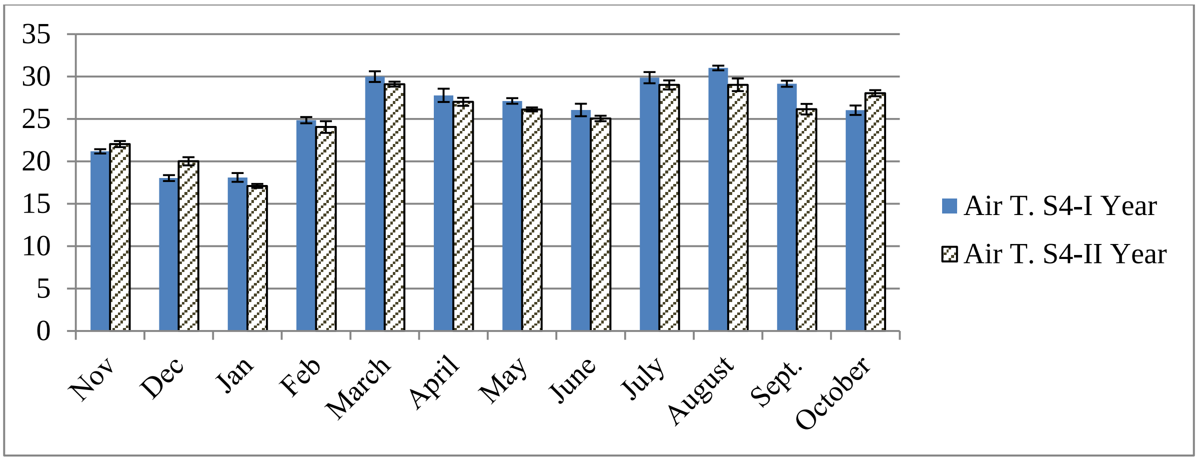

Fig.5.40. Monthly variations in air temperature at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

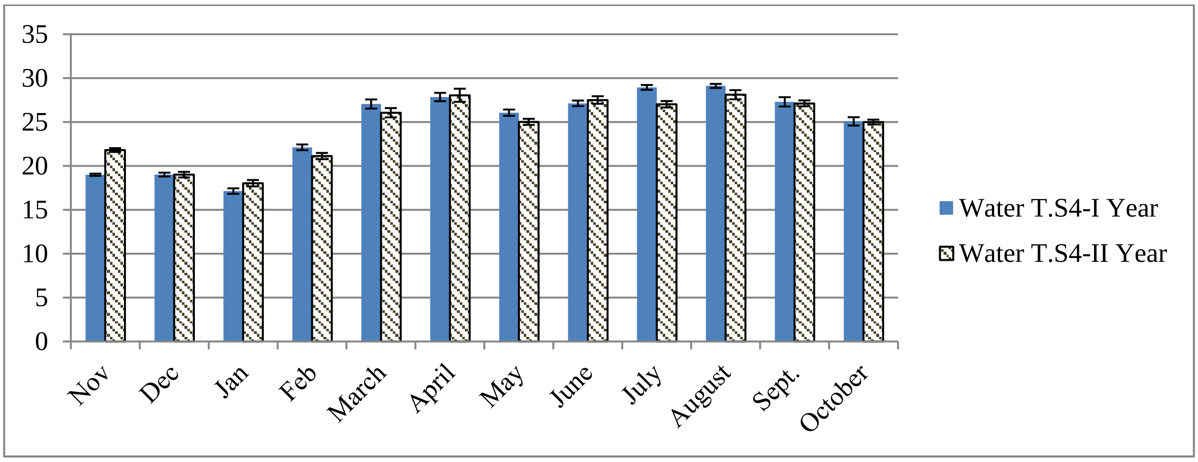

Fig.5.41. Monthly variations in water temperature at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

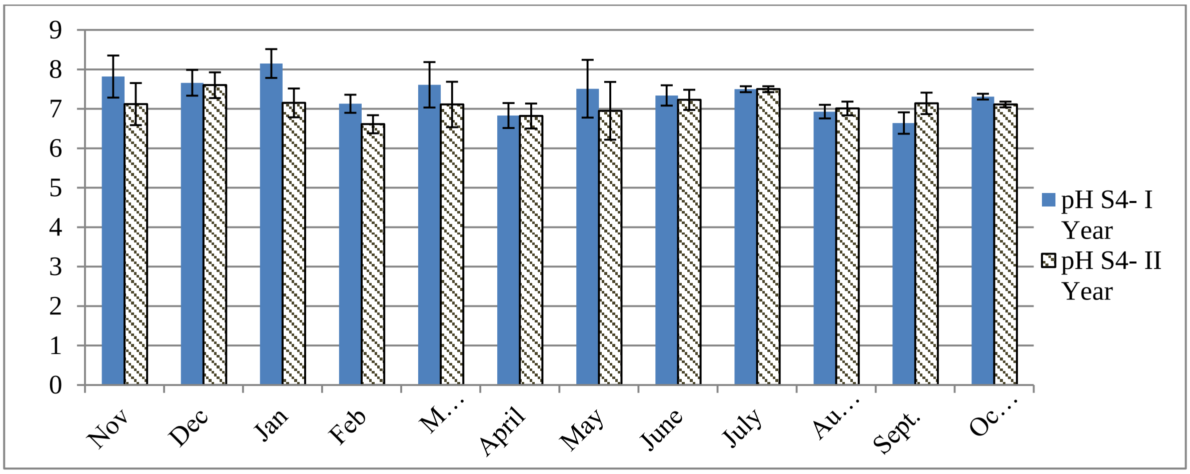

Fig.5.42. Monthly variations in pH at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

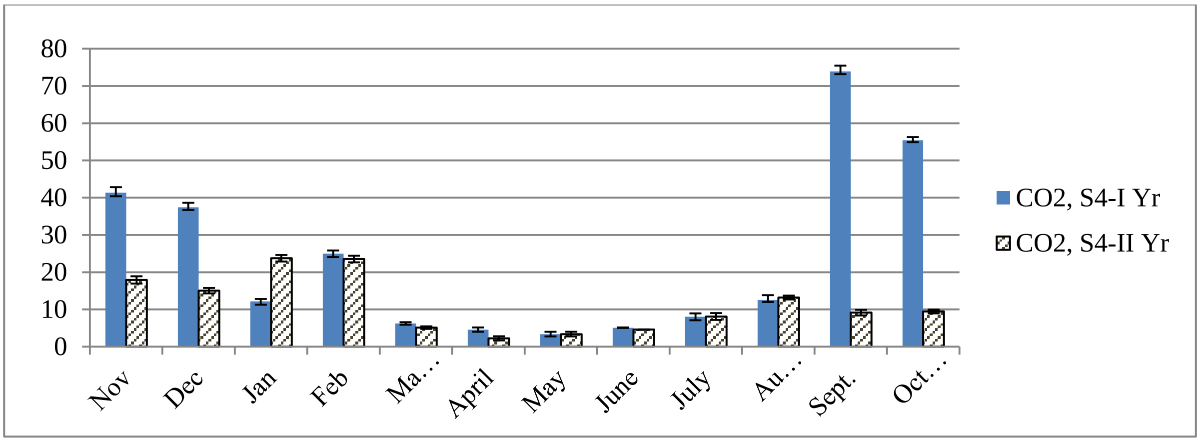

Fig.5.43. Monthly variations in CO_2 at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

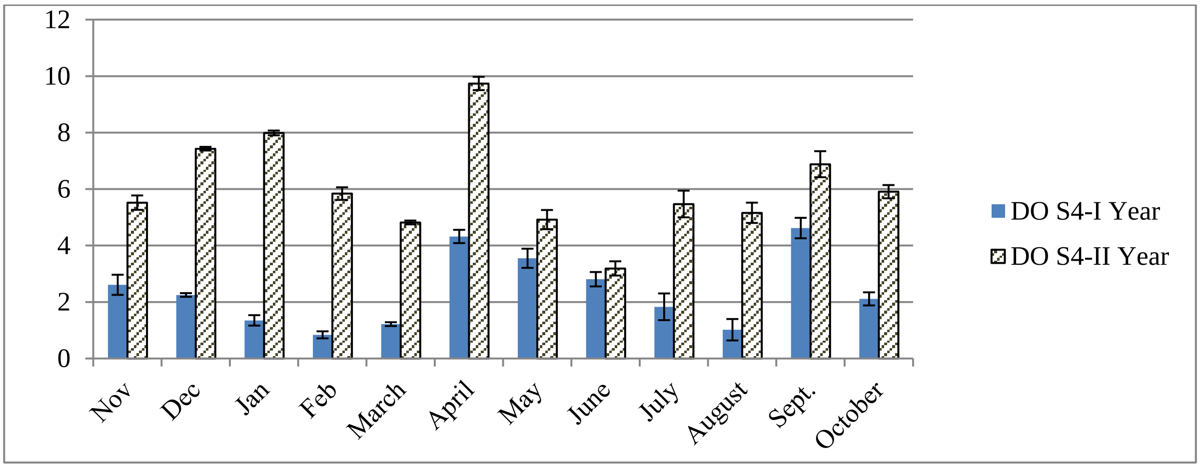

Fig.5.44. Monthly variations in DO at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

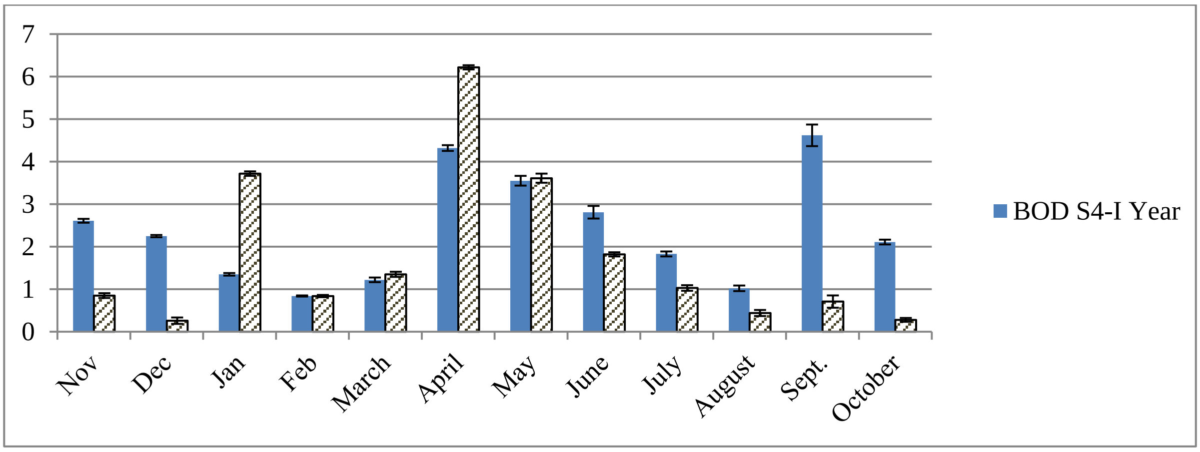

Fig.5.45. Monthly variations in BOD at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

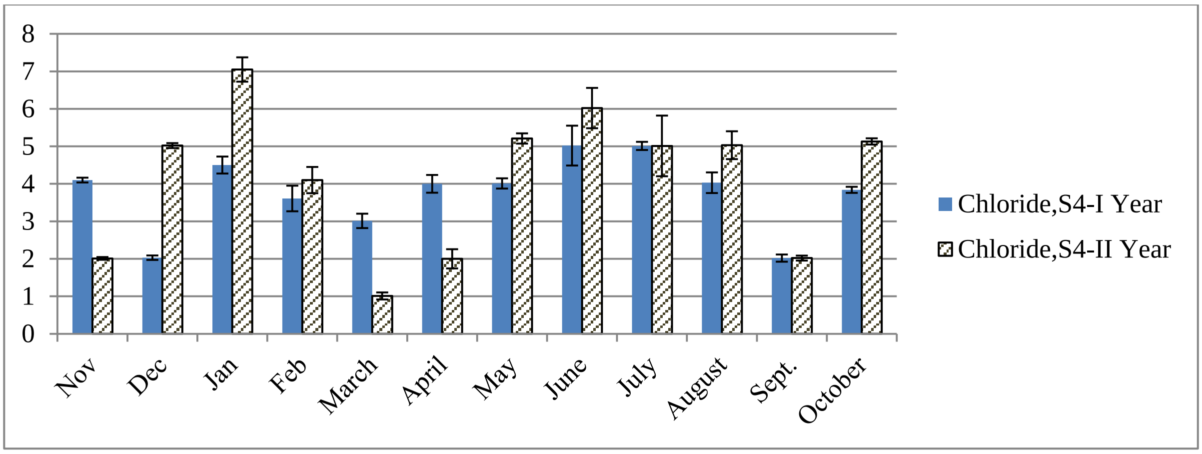

Fig.5.46. Monthly variations in chloride at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

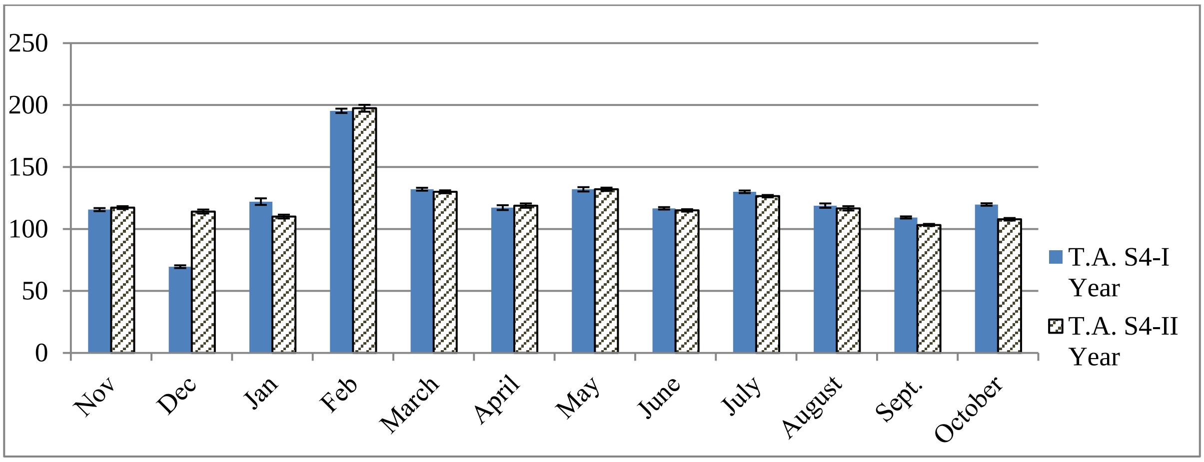

Fig.5.47. Monthly variations in total alkalinity at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

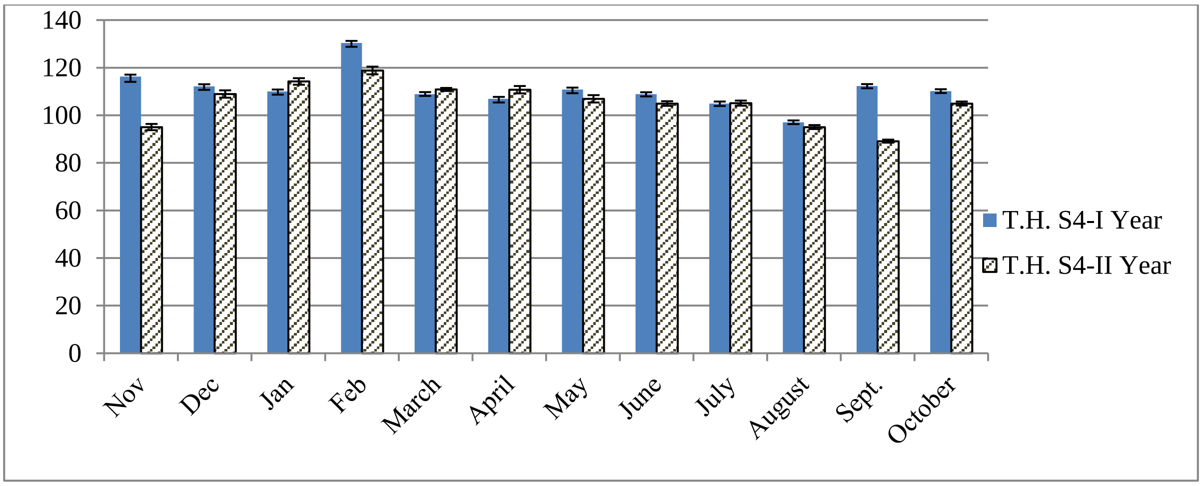

Fig.5.48. Monthly variations in total hardness at Site 4 during the first and second year study periods (Nov. 2008- Oct. 2010).

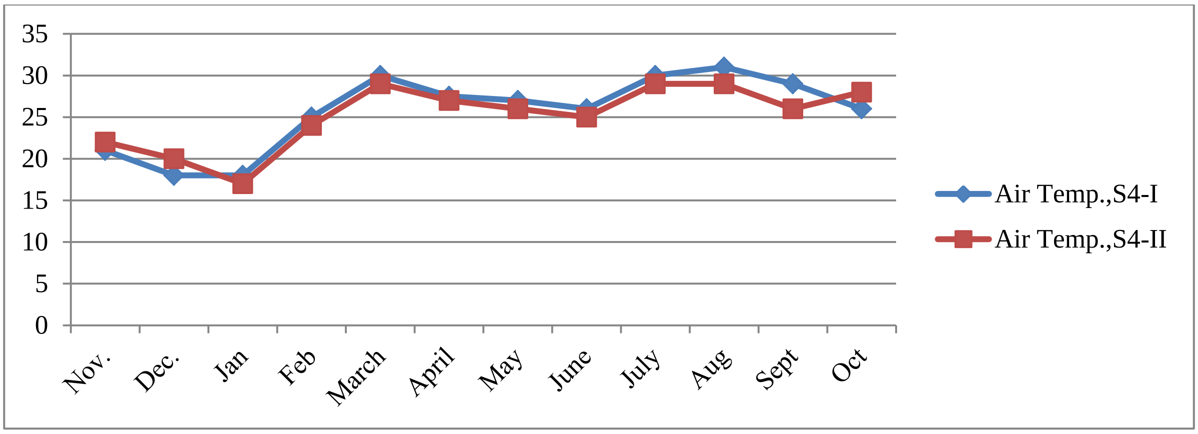

Fig.5.49. Line graph of monthly variations in air temperature at site 4 during the first and second year study periods (Nov. 2008 - Oct.2010).

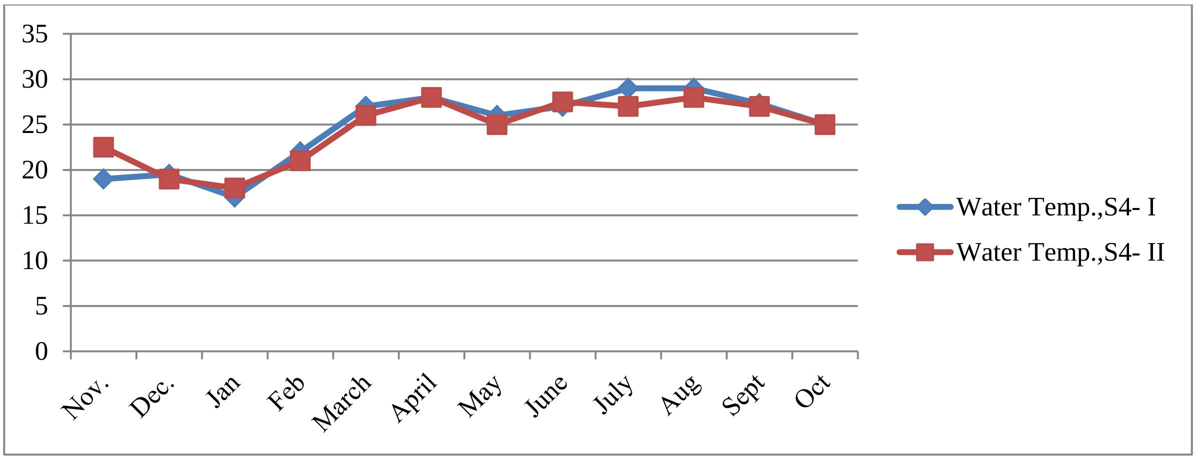

Fig.5.50. Line graph of monthly variations in water temperature at site 4 during the first and second year study periods (Nov. 2008 - Oct.2010).

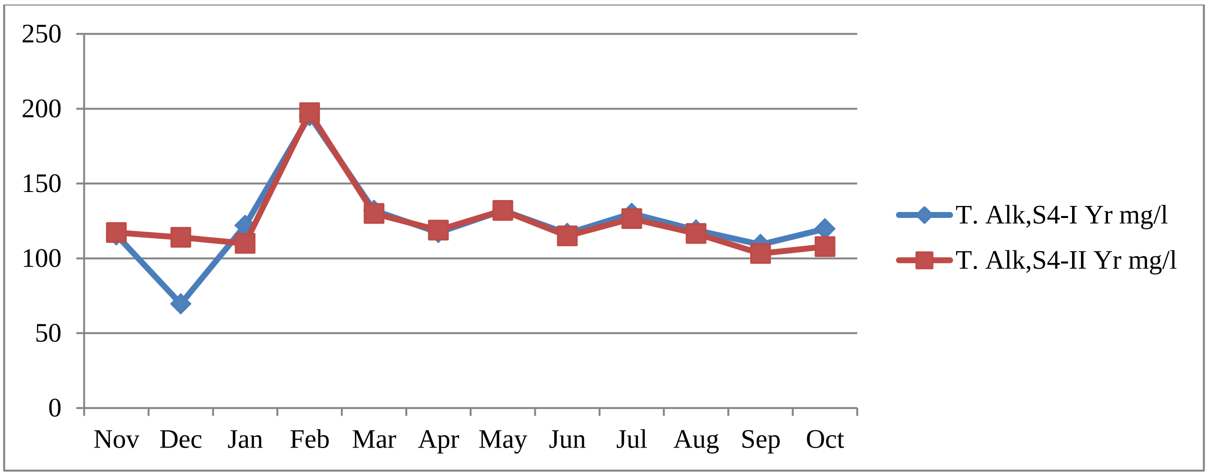

Fig.5.51. Line graph of monthly variations in total alkalinity at site 4 during the first and second year study periods (Nov. 2008 - Oct.2010).

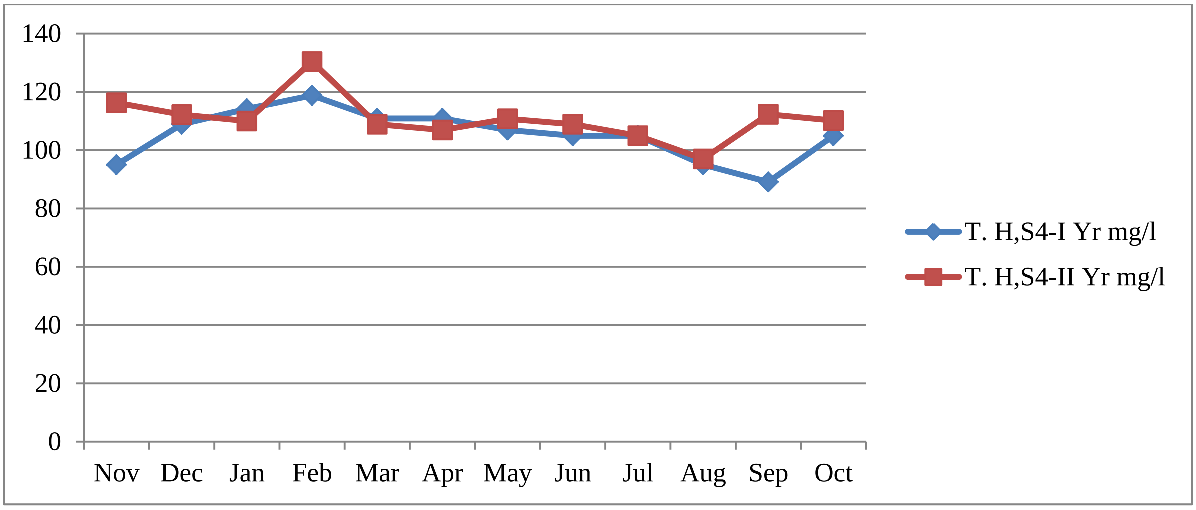

Fig.5.52. Line graph of monthly variations in total hardness at site 4 during the first and second year study periods (Nov. 2008 - Oct.2010).

Site 5 (Singhia River)

Results of the air temperature and physico-chemical parameters of water of Site 5 are shown in Table 5.13 and Table 5.14. Table 5.13 shows the results of air temperature and physico-chemical parameters of water of the first year (Nov. 2008 – Oct. 2009) study period. Table 5.14 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009- June 2010). Table 5.15 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site 5. The Fig.5.53 shows the monthly variations in air temperature at Site 5 in the first year and the second year study periods. The Figs.5.53 to 5.62 show histograms and Figs. 5.63 to 5.66 show line graphs of the monthly variations of different physico-chemical parameters of water at Site 5 in the first year (Nov. 2008 - Oct. 2009) and the second year (Nov. 2008 – Oct. 2010) study periods.

Air temperature

The minimum air temperature was 19.24±0.359°C in the month of February and maximum 31.13±0.521°C was in the month of September during the first year study period (Table 5.13, Fig.5.53). The minimum air temperature was 19.05±0.293°C in the month of January and maximum air temperature was 32.03 ±0.615°C in the month of April during the second year (Table 5.14, Fig.5.53). Air temperature showed positive and significant correlation with water temperature (r = 0.964, P<0.01), chloride (r = 0.639, P<0.05) but it had inverse and significant correlation with pH (r = -0.656, P<0.05) and DO (r = -0.608, P<0.05) (Table 5.15).

During year 1, the surface air temperature remained low during winter months (November 2008 to February 2009) thereafter it increased March onwards up to April (Table 5.13, Figs.5.53, 5.63). It also remained low during winter months (November 2009 to February 2010) in the second year study period (Table 5.14; Figs.5.53, 5.63).

Water temperature

The lowest surface water temperature was 18.25±0.335°C in the month of February and highest 29.1±0.436°C in the month of April during the first year (Table 5.13, Fig.5.54) and the minimum water temperature was 17.21±0.376°C in the month of January and the highest was 30.02±0.657 °C in the month of May during second year study period (Table 5.14, Fig. 5.54). The water temperature had positive and significant correlation with air temperature ($r = 0.964$, $P<0.01$), chloride ($r = 0.637$, $P<0.05$) but inverse and significant correlation with pH ($r =-0.639$, $P<0.05$) (Table 5.15).

The water temperature remained low during the winter months of November to February in both year 1 and year 2 (Tables 5.13, 5.14; Figs.5.54, 5.64). Decreasing trend was also observed during the months of August to October in year 1 and September to October in the year 2.

pH

The minimum pH was 6.46± 0.254 in the month of September and maximum 8.33± 0.529 in the month of February in first year (Table 5.13, Fig.5.55) and minimum pH was 6.82± 0.275 in July and maximum 8.60 ± 0.529 in February in the second year (Table 5.14, Fig.5.55). pH had inverse and significant correlation with air temperature ($r= -0.656$, $P<0.05$) and water temperature ($r= - 0.639$, $P<0.05$) (Table 5.15).

Turbidity

The turbidity was lowest 15.57±1.304 NTU in January and highest 395.05± 0.3.377 in July in the first year (Table 5.13, Fig.5.56).Turbidity was lowest 45.03±0.064 NTU in November and the highest was 345.05± 3.579 NTU in July during second year (Table 5.14, Fig. 5.56). It showed positive and significant correlation with water temperature ($r =0.604$, $P<0.05$) and phosphate ($r =0.675$, $P<0.05$) but inverse and significant correlation with free carbon dioxide ($r = -0.605$, $P<0.05$) (Table 5.15).

Free carbon dioxide

The maximum free carbon dioxide was 39.12± 0.945 mg/L in the month of January and minimum 8.36± 0.923 mg/L in the month of July during the first year study period (Table 5.13; Fig.5.57). In the second year study period maximum free CO_2 was 21.55± 0.569 mg/L in March and minimum 6.58± 0.652 mg/L in September (Table 5.14, Fig.5.57). Free carbon dioxide showed positive and significant correlation with total alkalinity (r =0.654, P<0.05) but inverse and significant correlation with turbidity (r =-0.605, P<0.05), DO (r=-0.721, P <0.01) and phosphate (r=-0.670, P <0.05) (Table 5.15).

Dissolved oxygen

The minimum dissolved oxygen was 4.35 ± 0.185 mg/L in the month of January and maximum dissolved oxygen was 7.72 ± 0.085 mg/L in the November during the first year study period (Table 5.13; Fig.5.58). In the second year study period, the maximum dissolved oxygen was 9.29±0.099 mg/L in the month of December and minimum 5.11±0.068 mg/L in the month of March (Table 5.14; Fig.5.58). The dissolved oxygen showed inverse and significant correlation with free carbon dioxide (r = -0.721, P<0.01), biological oxygen demand (r = - 0.634, P <0.05) and temperature of air (r=-0.608, P<0.05) (Table 5.15).

Biological oxygen demand

The maximum biological oxygen demand was 5.77 ±0.065 mg/L in the month of August and minimum 1.36±0.075 mg/L in the month of October during the first (Table 5.13; Fig. 5.59) and maximum biological oxygen demand was 3.72 ±0.054 mg/L in May and minimum was 0.06 ±0.062 mg/L in December during the second year study period (Table 5.14; Fig.5.59). It had positive and significant correlation with total alkalinity (r = 0.729, P <0.01) but inverse and significant correlation with DO (r = -0.634, P<0.05) and total hardness (r= -0.688, P<0.05) (Table 5.15).

Chloride

The maximum chloride was 11.11±0.135 mg/L in the month of May and minimum 3.01±0.069 mg/L was in the month of December during the first year (Table 5.13; Fig. 5.60). Maximum chloride was 15.1±0. 093 mg/L in November and minimum was 4.05 ± 0.069 mg/L in December of second year study period (Table 5.14; Fig. 5.60). It had positive and significant correlation with air temperature (r= 0.639, P<0.05), water temperature (r=0.637, P<0.05) (Table 5.15).

Total alkalinity

The maximum total alkalinity was 243.52±2.534 mg/L in the month of January and minimum 107.16± 2.453 mg/L in the month of December during the first year study period (Table 5.13; Fig.5.61). In second year maximum TA was 191.11± 1.742 mg/L in May and minimum was 134.26± 2.857 mg/L in September (Table 5.14; Fig.5.61). It had positive and significant correlation with free CO_2 (r=0.654, P<0.05) and BOD (r =0.729, P<0.01) (Table 5.15).

Total alkalinity of the month of July 2008 (127.92±0.987 mg/L) significantly (P<0.01) lower than that of the month of June, 2009 (164.10±2.856mg/L) and it remained low in the month of August, 2009 (110.71±1.745 mg/L) during the first year study period (Table 5.13; Figs.5.61, 5.65). During second year study period, total alkalinity was found low in the month of June (156.40 ± 2.856 mg/L) to September, 2010 (134.26 ± 2.857 mg/L) for four months (Table 5.14; Figs. 5.61, 5.65).

Total hardness

The maximum total hardness was 173.22±1.795 mg/L in the month of January and minimum was 95.05±0.899 mg/L in the month of August during the first year (Table 5.13, Fig.5.62). Maximum total hardness was 173.22±1.795 mg/L in January and minimum was 85.14±1.967 mg/L in December during the second year study period (Table 5.14, Fig.5.62). It had positive and significant correlation with total alkalinity (r= 0.539, P<0.10) but inverse and significant correlation with BOD (r = - 0.688, P<0.05) (Table 5.15).

Total hardness was found significantly (p< 0.01) lower in the month of July, 2009 (116.62 ±1.247 mg/L) in comparison to that of the month of June, 2009 (162.36±1.976 mg/L) during first year. It remained low in July and August in the first year (Table 5.13; Figs.5.62, 5.66). During second year total hardness was significantly (P< 0.01) lower in May (126.5±1.716 mg/L) than that of April (151.61±1.485 mg/L). It remained low in the month of May, June, July and September, 2010 (108.93 ±0.875 mg/L). Prior to September, 2010 fluctuations in the values of total hardness were observed (Table 5.14, Figs. 5.70, 5.66).

Table 5.13 shows air temperature, water temperature and physico-chemical parameters of water at Site 5 (Singhia river, Morang) from Nov. 2008- October 2009. **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 5 - I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	21.01 ±0.356	20.00 ±0.442	20.53 ±.293	19.24 ±0.359	30.05 ±0.347	31.01 ±0.615	29.10 ±0.432	27.05 ±0.276	30.02 ±0.357	27.54 ±0.524	31.13 ±0.521	28.02 ±0.576
Water Temp. (ºC)	20.03 ±0.325	19.31 ±0.217	19.01 ±0.316	18.25 ±0.335	25.03 ±0.523	29.10 ±0.436	28.02 ±0.354	26.10 ±0.327	29.03 ±0.572	26.82 ±0.435	28.01 ±0.345	26.04 ±0.347
pH	7.5 ±0.254	7.87 ±0.377	8.31 ±0.395	8.33 ±0.529	8.11 ±0.446	8.23 ±0.357	7.81 ±0.433	7.19 ±0.356	7.32 ±0.275	6.81 ±0.278	6.46 ±0.254	6.92 ±0.178
Turbidity (NTU)	74.05 ±0.075	25.91 ±0.089	15.57 ±1.304	67.03 ±0.926	215.04 ±3.578	55.12 ±0.865	58.12 ±0.935	225 ±1.763	395.05 ±3.377	256.0 ±0.465	98.45 ±0.335	76.55 ±0.815
Free CO_2 (mg/L)	15.42 ±1.645	37.45 ±1.265	39.12 ±0.945	21.53 ±0.687	20.28 ±0.569	23.23 ±0.765	25.53 ±0.839	28.13 ±0.456	8.36 ±0.923	16.54 ±1.357	14.20 ±1.452	38.11 ±0.628
DO (mg/L)	7.72 ±0.085	4.91 ±0.087	4.35 ±0.185	7.4 ±0.224	7.18 ±0.068	6.65 ±0.125	6.23 ±0.078	5.75 ±0.095	5.77 ±0.273	6.92 ±0.097	5.11 ±0.086	6.61 ±0.237
BOD mg/L	2.69 ±0.067	3.53 ±0.029	3.12 ±0.037	2.86 ±0.025	2.42 ±0.065	2.05 ±0.061	2.42 ±0.015	3.21 ±0.069	3.07 ±0.057	5.77 ±0.065	1.58 ±0.054	1.36 ±0.075
Chloride (mg/L)	6.07 ±0.093	3.01 ±0.069	4.04 ±0.096	6.01 ±0.142	9.0 ±0.192	8.02 ±0.127	11.11 ±0.135	8.08 ±0.031	6.05 ±0.459	9.10 ±0.645	9.02 ±0.075	8.06 ±0.084
Total Alkalin. (mg/L)	192.16 ±2.175	107.16 ±2.453	243.52 ±2.534	232.03 ±1.857	185.50 ±1.887	162.02 ±2.956	156.05 ±1.742	164.10 ±2.856	127.92 ±0.987 *	110.71 ±1.745	186.96 ±2.857	188.07 ±1.995
Total Hardness (mg/L)	168.05 ±2.267	144.84 ±1.967	173.22 ±1.795	126.54 ±1.623	162.36 ±1.845	157.61 ±1.485	151.66 ±1.716	162.36 ±1.976	116.62 ±1.247 *	95.05 ±0.899	159.84 ±0.875	160.45 ±0.583

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.14 shows air temperature, water temperature and physico-chemical parameters of water at Site 5 (Singhia River, Morang) from Nov. 2009- October 2010. **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 5- II Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (ºC)	22.0 ±0.275	21.20 ±0.442	19.05 ±.293	20.06 ±0.359	31.10 ±0.347	32.03 ±0.615	31.5 ±0.432	29.05 ±0.276	30.02 ±0.357	30.5 ±0.524	30.12 ±0.521	27.04 ±0.576
Water Temp. (ºC)	22.5 ±0.523	19.01 ±0.437	17.21 ±0.376	18.52 ±0.435	29.03 ±0.546	29.5 ±0.439	30.02 ±0.657	28.50 ±0.427	29.03 ±0.672	29.12 ±0.635	29.01 ±0.634	25.56 ±0.534
pH	7.68 ±0.254	8.02 ±0.377	8.4 ±0.395	8.6 ±0.529	8.5 ±0.446	7.23 ±0.357	7.12 ±0.433	8.02 ±0.356	6.82 ±0.275	7.41 ±0.278	7.53 ±0.254	7.31 ±0.178
Turbidity (NTU)	45.03 ±0.064	53.11 ±0.068	56.00 ±1.967	49.02 ±0.926	213.053±0.578	47.52 ±0.865	47.15 ±0.735	227 ±1.864	345.05 ±3.579	332.05 ±3.465	330.01 ±3.335	49.23 ±0.578
Free CO_2(mg/L)	16.72 ±0.645	18.36 ±0.265	19.81 ±0.945	19.53 ±0.687	21.55 ±0.569	19.82 ±0.765	21.15 ±0.839	12.29 ±0.456	13.5 ±0.923	8.84 ±0.557	6.58 ±0.652	7.53 ±0.427
DO (mg/L)	8.26 ±0.095	9.29 ±0.099	7.15 ±0.265	6.68 ±0.424	5.11 ±0.068	5.75 ±0.165	6.81 ±0.178	6.77 ±0.105	5.55 ±0.289	6.81 ±0.115	6.77 ±0.124	7.35 ±0.342
BOD (mg/L)	1.9 ±0.078	0.06 ±0.062	1.5 ±0.037	1.75 ±0.045	3.41 ±0.065	3.10 ±0.061	3.72 ±0.054	2.31 ±0.053	2.30 ±0.059	1.73 ±0.057	1.42 ±0.044	1.8 ±0.065
Chloride (mg/L)	15.10 ±0.093	4.05 ±0.069	8.00 ±0.096	6.01 ±0.142	10.0 ±0.192	9.82 ±0.127	10.01 ±0.135	11.06 ±0.031	10.55 ±0.459	10.45 ±0.645	10.42 ±0.075	14.54 ±0.084
Total Alkali (mg/L)	167.20 ±2.175	164.03 ±2.453	171.15 ±2.534	173.53 ±1.857	187.5 ±1.887	190.50 ±2.956	191.11 ±1.742	156.40 ±2.856*	166.12 ±0.987	169.91 ±1.745	134.26 ±2.857	178.2 ±2.857
Total Hardness (mg/L)	169.23 ±2.267	85.14 ±1.967	173.22 ±1.795	165.24 ±1.623	121.34 ±1.845	151.61 ±1.485	126.5 ±1.716*	146.56 ±1.976	144.42 ±1.247	156.75 ±0.899	108.93 ±0.875	165.25 ±0.583

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.15 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 5 (average of the corresponding month values) during Nov.2008 – Oct. 2010; N=12; d. f. =11.

S5 – I +II		Water T. (ºC)	pH	Turbidity (NTU)	Free CO_2 (mg/L)	DO (mg/L)	BOD (mg/L)	Chloride (mg/L)	Total alkalin. (mg/L)	Total hard (mg/L)
Air Temp. (ºC)	P cor.	.964*	-.656**	.484	-.249	-.608**	-.314	.639**	-.360	-.076
	Sig.(2-t)	.000	.020	.111	.434	.036	.321	.025	.250	.816
Water Temp. (ºC)	P cor.	1	-.639**	.604**	-.257	.024	-.214	.637**	-.446	-.201
	Sig.(2-t)		.025	.046	.420	.940	.505	.026	.147	.531
pH	P cor.		1	-.427	.079	.242	0.000	-.247	.200	.201
	Sig.(2-t)			.166	.806	.449	1.000	.439	.533	.532
Turbidity (NTU)	P cor.			1	-.605**	.228	.294	.261	-.452	-.557
	Sig.(2-t)				.037	.475	.353	.412	.140	.060
Free CO_2 (mg/L)	P cor.				1	-.721*	-.127	-.205	.654**	-.022
	Sig.(2-t)					.008	.694	.522	.021	.947
DO (mg/L)	P cor.					1	-.634**	.362	-.095	-.244
	Sig.(2-t)						.027	.247	.769	.445
BOD (mg/L)	P cor.						1	-.206	.729*	-.688**
	Sig.(2-t)							.520	.007	.013
Chloride (mg/L)	P cor.							1	.034	-.343
	Sig.(2-t)								.917	.276
Total alkalinity (mg/L)	P cor.								1	.539
	Sig.(2-t)									.071
Total hard (mg/L)	P cor.									1
	Sig.(2-t)									

*** Significant at 1% level (P<0. 01), ** significant at 5% level (P<0. 05) and Values not marked denote non-significant correlation.**

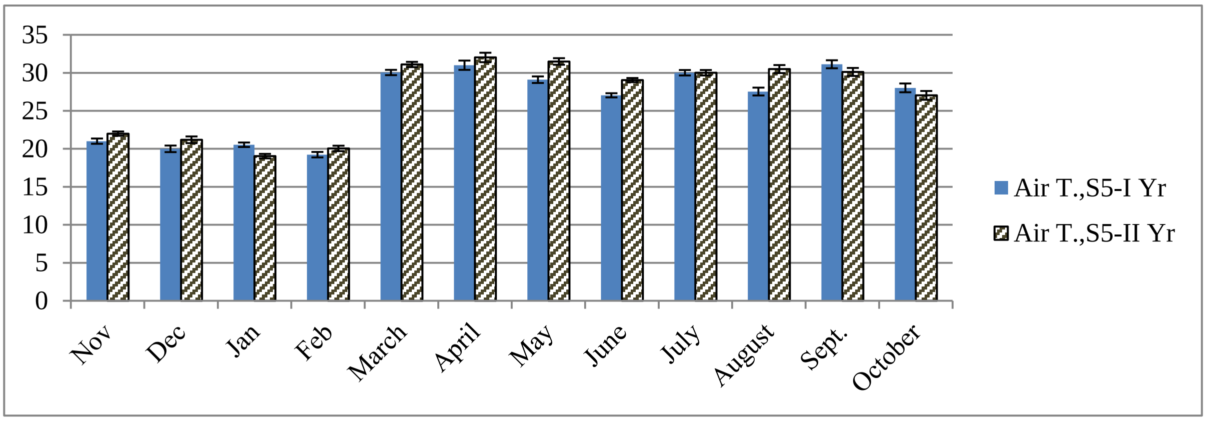

Fig.5.53. Monthly variations in air temperature at Site 5 during the first and second year study Periods (Nov. 2008- Oct. 2010).

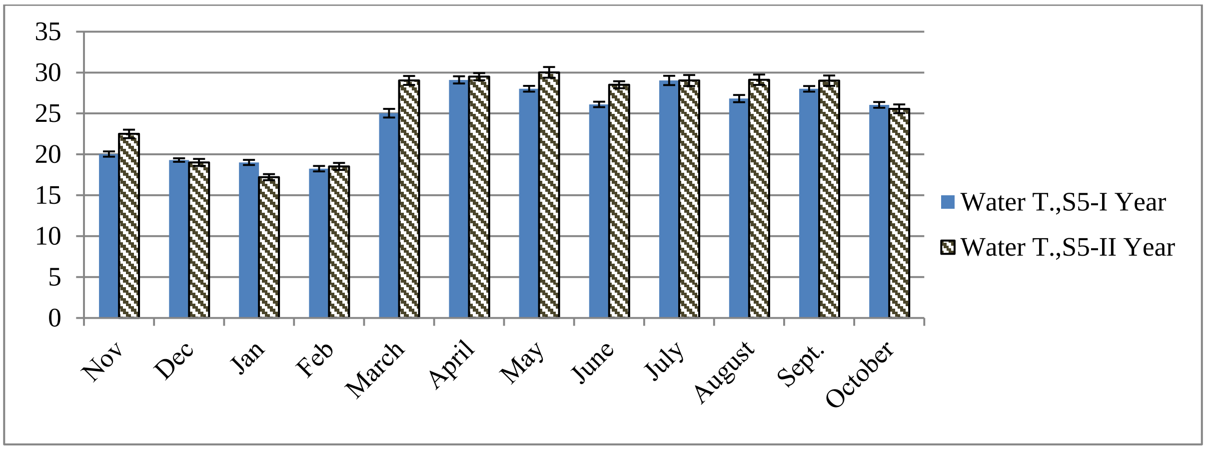

Fig.5.54. Monthly variations in water temperature at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

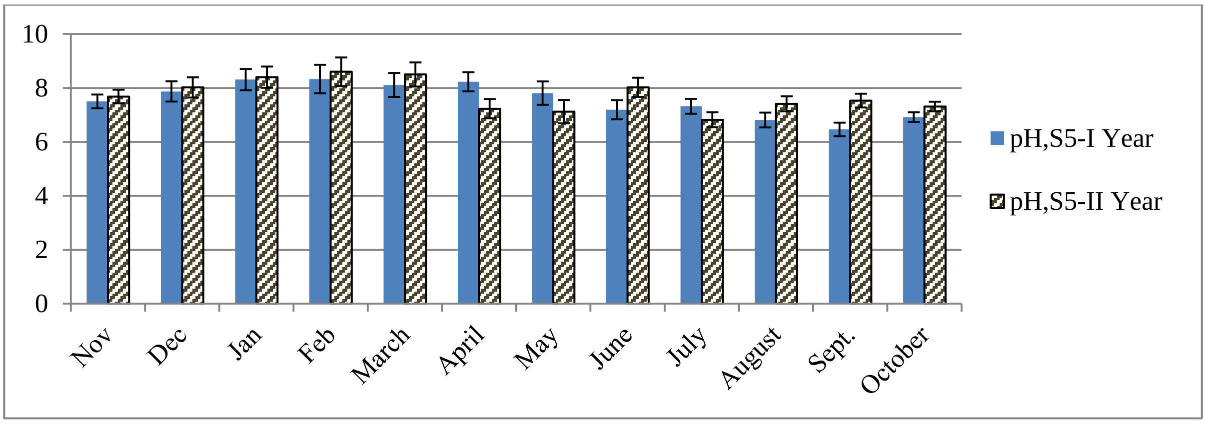

Fig.5.55. Monthly variations in pH at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

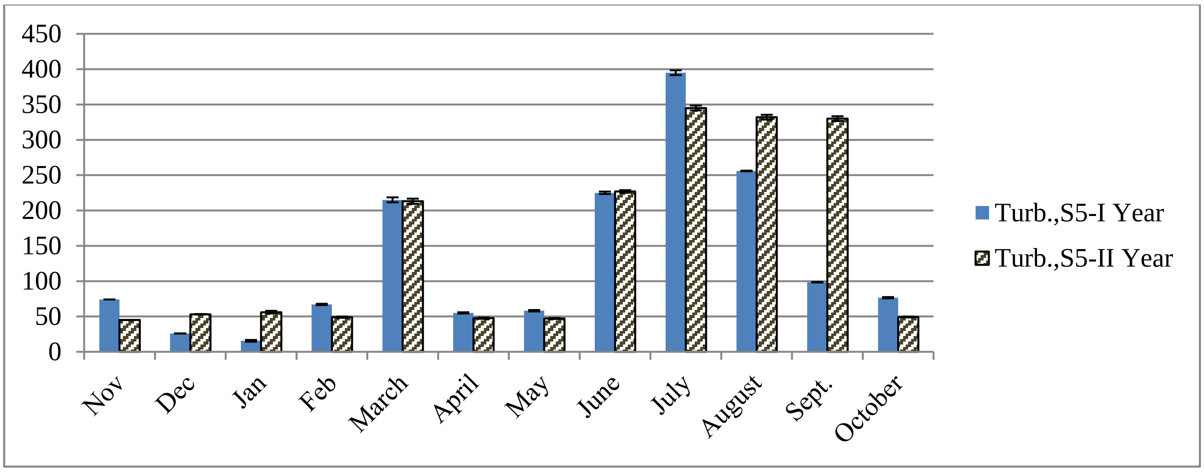

Fig.5.56. Monthly variations in turbidity at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

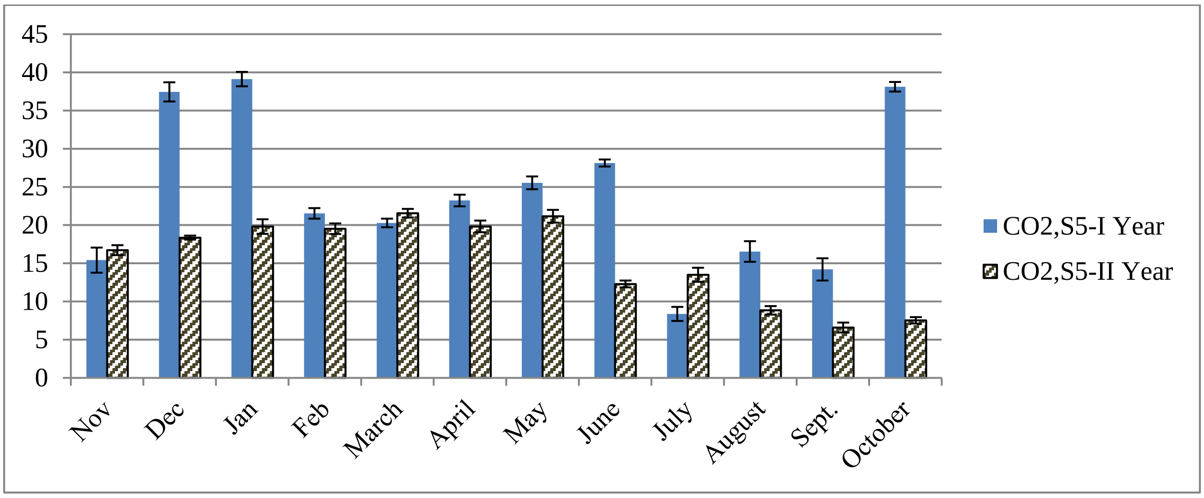

Fig.5.57. Monthly variations in CO_2 at Site 5 during the first and second year study periods (Nov.2008- Oct.2010).

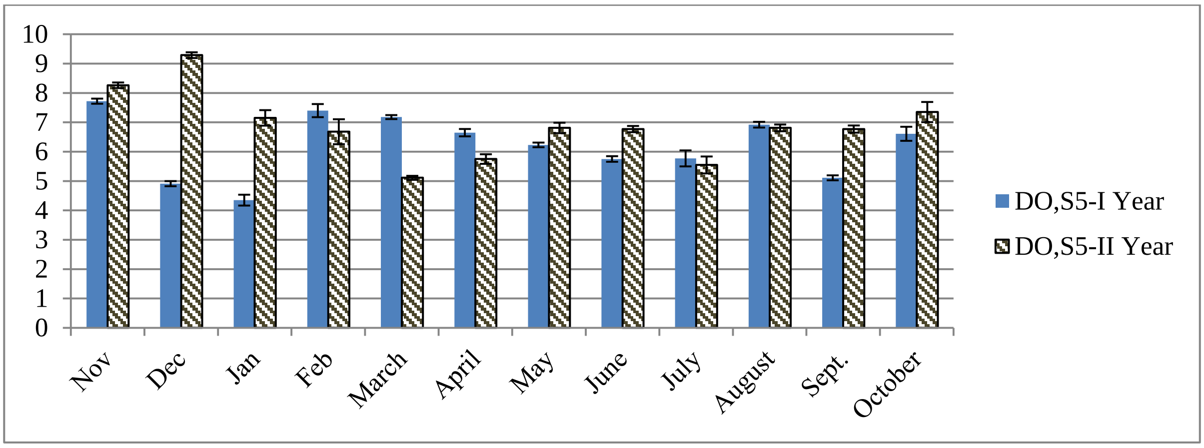

Fig.5.58. Monthly variations in DO at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

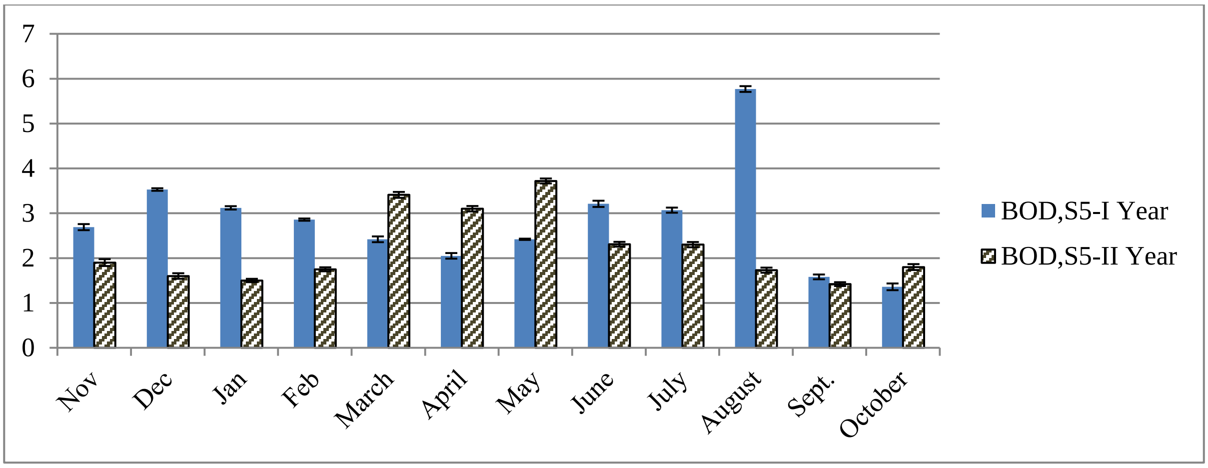

Fig.5.59. Monthly variations in BOD at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

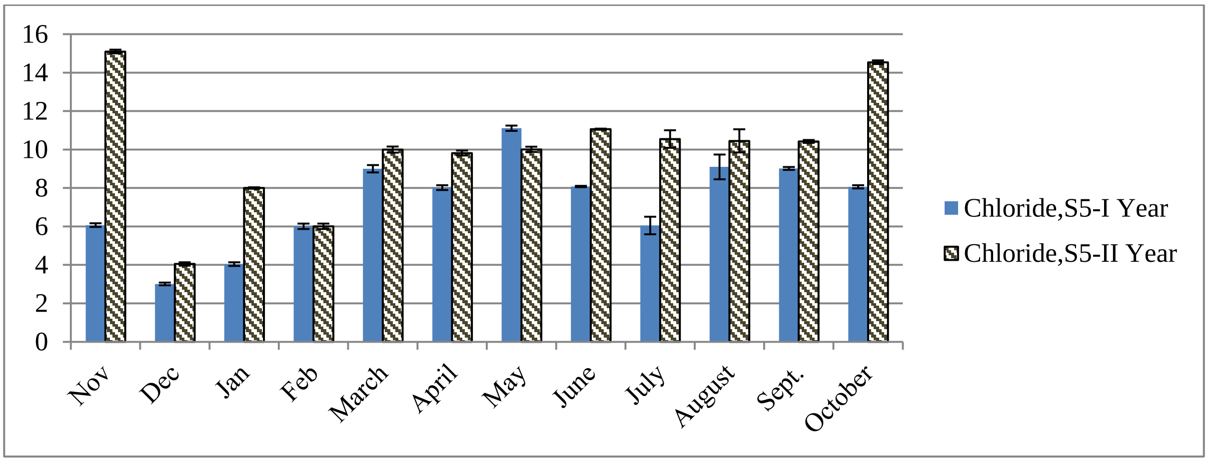

Fig.5.60. Monthly variations in chloride at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

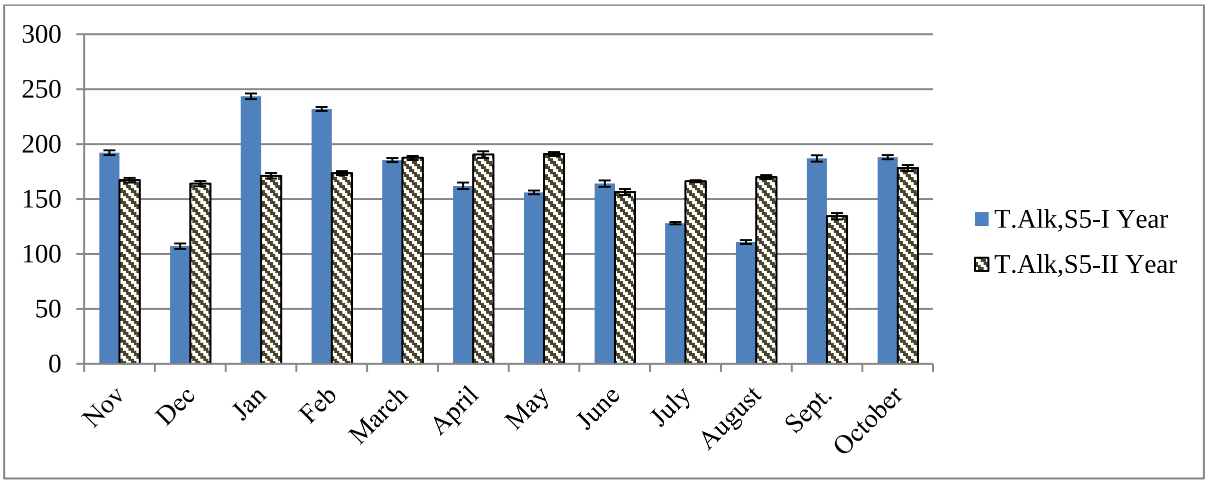

Fig.5.61. Monthly variations in total alkalinity at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

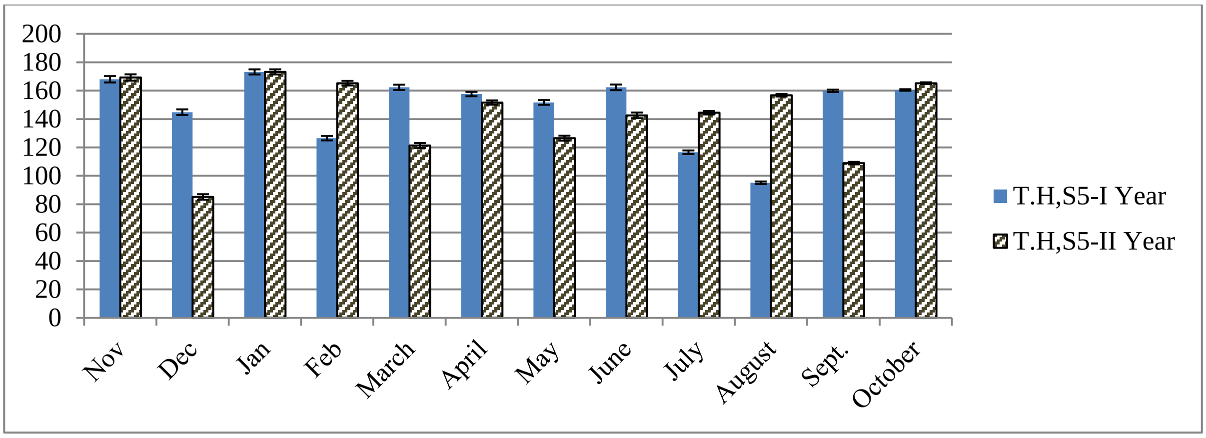

Fig.5.62. Monthly variations in total hardness at Site 5 during the first and second year study periods (Nov. 2008- Oct. 2010).

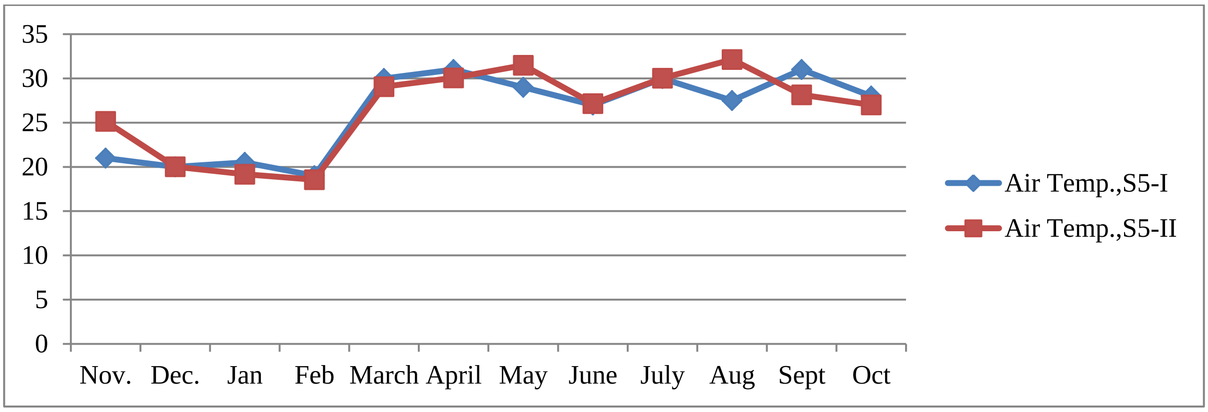

Fig.5.63. Line graph of monthly variations in air temperature at site 5 during the first and second year study periods (Nov. 2008 - Oct. 2010).

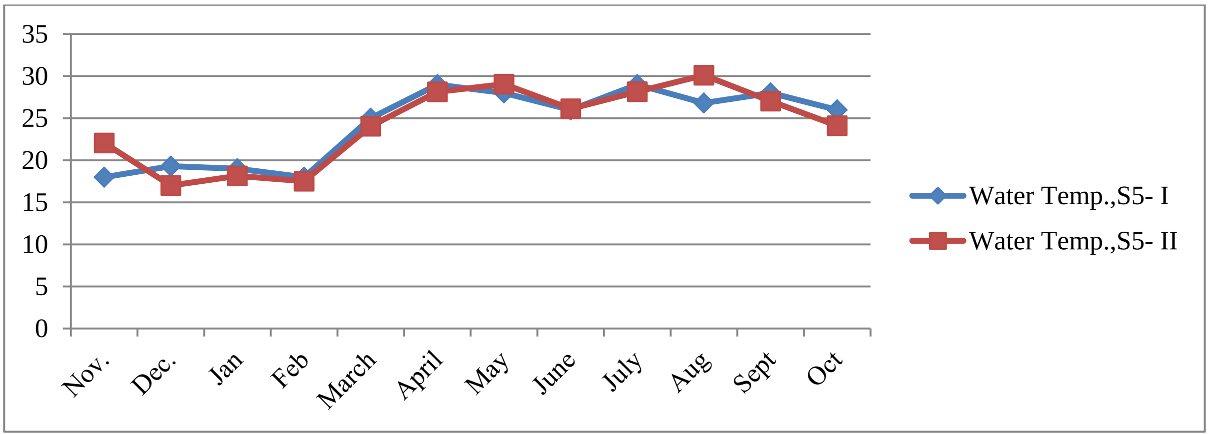

Fig.5.64. Line graph of monthly variations in water temperature at site 5 during the first and second year study periods (Nov. 2008 - Oct.2010).

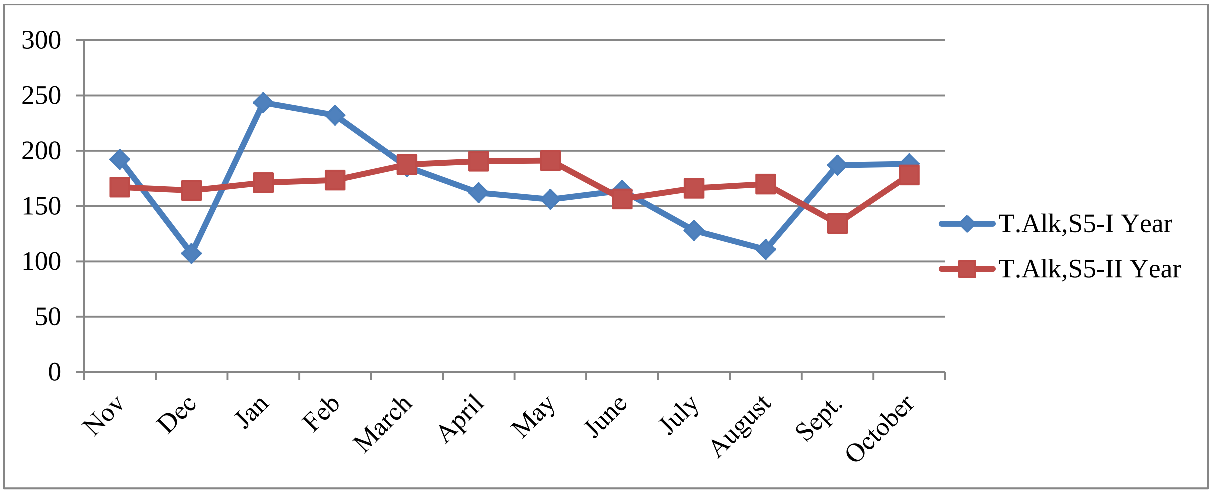

Fig.5.65. Line graph of monthly variations in total alkalinity at site 5 during the first and second year study periods (Nov. 2008 - Oct.2010).

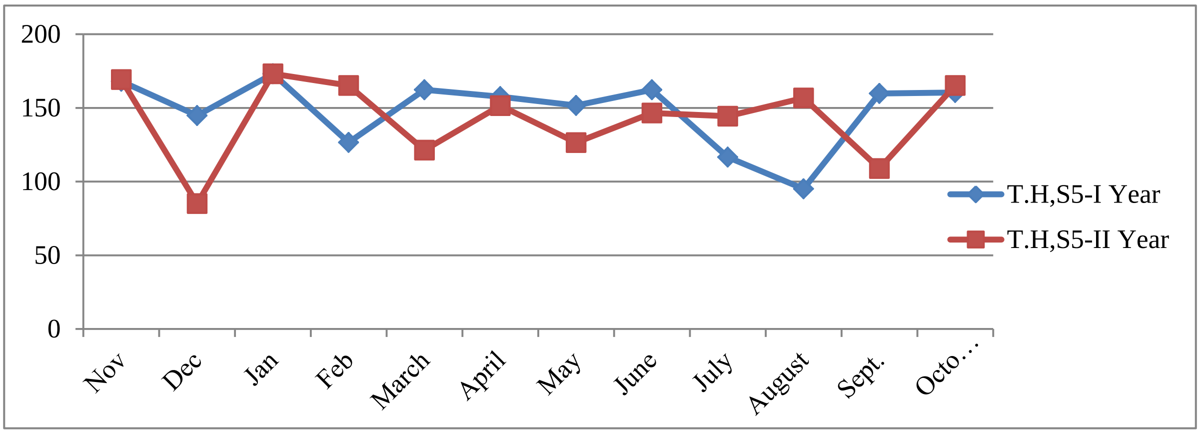

Fig.5.66. Line graph of monthly variations in total hardness at site 5 during the first and second year study periods (Nov. 2008 - Oct.2010).

Site 6 (Budhi River)

Results of the air temperature and physico-chemical parameters of water of Site 6 are shown in Table 5.16 and Table 5.17. Table 5.16 shows the results of air temperature and physico-chemical parameters of water of the first year (Nov. 2008 – Oct. 2009) study period. Table 5.17 shows the results of air temperature and physico-chemical parameters of water of the second year (Nov. 2009- June 2010). Table 5.18 shows the correlation coefficient (r) of air temperature and different physico-chemical parameters of water at Site 6. The Fig.5.67 shows the monthly variations in air temperature at Site 6 in the first year and the second year study periods. The Figs.5.67 to 5.76 show histograms and Figs. 5.77 to 5.80 show line graphs of the monthly variations of different physico-chemical parameters of water at Site 6 in the first year (Nov. 2008 - Oct. 2009) and the second year (Nov. 2008 – Oct. 2010) study periods.

Air temperature

The minimum air temperature was 18.55 ±0.469 °C in the month of February and maximum 32.14±0.524 °C was in the month of August during the first year study period (Table 5.16; Fig.5.67). The minimum air temperature was 18.14±0.287°C in the month of January and maximum air temperature was 32.13 ±0.448 °C in the month of May during the second year (Table 5.17; Fig.5.67). Air temperature showed positive and significant correlation with water temperature (r =0.982, P<0.01) and it had inverse and significant correlation with dissolved oxygen (r = - 0.893, P<0.01) (Table 5.18).

The surface temperature remained low during winter (December to February) in both the years (Table 5.16, 5.17; Figs.5.67, 5.77).

Water temperature

The lowest surface water temperature was 17.01±0.217°C in the month of December and highest 30.12 ± 0.235 °C in the month of August during the first year (Table 5.16, Fig.5.68) and the minimum temperature was 17.15±0.335°C in the month of January and the highest 29.12±0.635 °C in the month of August during second year study period (Table 5.17, Fig.5.68). The water temperature had positive and significant correlation with air temperature (r = 0.982, P<0.01) but inverse and significant correlation with dissolved oxygen (r = -0.869, P<0.01) (Table 5.18).

The water temperature remained low during winter months (December to February in both the years. Decreasing trend was also observed during the months of September to October in both years (Tables 5.16, 5.17; Figs.5.68, 5.78).

pH

The minimum pH was 6.67± 0.271 in the month of September and maximum 8.5± 0.365 in the month of January in first year (Table 5.16; Fig.5.79) and minimum pH was 6.78± 0.271 in September and maximum 8.3± 0.236 in January in the second year (Table 5.17; Fig. 5.69). pH had no significant positive correlation inverse and significant correlation with turbidity (r= -0.924, p<0.01) (Table 5.18).

Turbidity

The turbidity was lowest 42.30 ±0.565 NTU in December and highest 1065.0±3.335 NTU in September in the first year (Table 5.16; Fig.5.70). Turbidity was lowest 48.01±1.435 NTU in January and was highest 1071.0± 2.359 NTU in September during second year (Table 5.17; Fig.5.80).The turbidity had positive and significant correlation with CO_2 (r =0.700, P<0.05) and phosphate (r =0.615, P<0.05) but inverse and significant correlation with pH (r = -0.924, P<0.01) (Table 5.18).

Free carbon dioxide

The maximum free CO_2 was 80.08± 1.352 mg/L in month of September and minimum was 14.56 ± 0.359 mg/L in the month of March during the first year study period (Table 5.16; Fig.5.71). In the second year study period, maximum free CO_2 was 17.5± 0.687 mg/L in February and minimum was 10.45± 0.625 mg/L in July (Table 5.17; Fig.5.71). Free carbon dioxide showed positive and significant correlation with turbidity (r = 0.700, P<0.05) (Table 5.18).

Dissolved oxygen

Minimum dissolved oxygen was measured 5.16±0.095 mg/L in the month of June and maximum was 8.26 ± 0.185 mg/L in January during the first year study period (Table 5.16; Fig.5.72). In the second year study period, the maximum dissolved oxygen was 8.4±0.285 mg/L in the month of January and minimum 4.59± 0.097 mg/L in the month of August (Table 5.17 and Fig.5.72). The dissolved oxygen showed inverse and significant correlation with air temperature (r=-0.893, p <0.01), water temperature (r = -0.869, P <0.01) (Table 5.18).

Biological oxygen demand

The maximum biological oxygen demand was 4.95 ±0.061 mg/L in the month of April and minimum 2.34 ±0.025 mg/L in the month of February during the first (Table 5.16 and Fig. 5.73). It was maximum 4.15 ±0.045 mg/L in May and minimum 0.26 ±0.087 mg/L in December in the second year study period (Table 5.17 and Fig.5.73). It had positive and significant correlation with air temperature (r = 0.768, P <0.01), water temperature (r = 0.496, P< 0.05) and inverse and significant correlation with DO (r = -0.469, P < 0.05) (Table 5.18).

Chloride

The maximum chloride was 10.2±0.086 mg/L in the month of October and minimum was 3.01±0.069 mg/L in the month of December during the first (Table 5.16 and Fig.5.74); maximum chloride was 13. 35± 0.097 mg/L in August and minimum was 2.5 ± 0.069 mg/L in December of second year study period (Table 5.17 and Fig.5.74). It had inverse and significant correlation with CO_2 (r=-0.656, P<0.05) (Table 5.18).

Total alkalinity

The maximum total alkalinity was 240.03±2.74 mg/L in the month of January and minimum 111.6± 0.815 mg/L in the month of July during the first year study period (Table 5.16 and Fig. 5.75). In second year, maximum total alkalinity was 238.6±2.534 mg/L in January and minimum 127.92± 0.987 mg/L in July (Table 5.17 and Fig.5.75). It had positive and significant correlation with BOD (r = 0.805, P<0.05) (Table 5.18).

Total alkalinity was significantly (p<0.01) lower in the month of July, 2009 (111.6.62 ±0.815 mg/L) as compared to the month of June (192.4±2.735mg/L) during first year (Table 5.16; Figs.5.75, 5.79). The values of total alkalinity were found significantly (P<0.05) lower in July (127.92±0.987mg/L) than that of June (211.60±2.856mg/L)during second year study period. It was slightly increased in August and remained low in September and October (Table 5.17; Figs. 5.75, 5.79).

Total hardness

The maximum total hardness was 190.0±1.845 mg/L in the month of March and minimum 89.01±0.875 mg/L in the month of August during the first year (Table 5.16 and Fig.5.76) and in the second year study period, maximum 196.02±1.976 mg/L was seen in June and minimum 85.14±1.956 mg/L in December (Table 5.17 and Fig.5.76). It had positive and significant correlation with chloride (r= 0.644, P<0.05) (Table 5.18).

The values of total hardness were significantly (P<0.01) lower in the month of July (150.04 ±1.206 mg/L) than that of June (180.12 ±1.976 mg/L) during first year study period (Table 5.16; Figs.5.76, 5.80). During second year total hardness was found to be significantly (P<0.05) lower in the month of July, 2010 (132.6 ±1.206 mg/L) compared to that of the month of June, 2010 (196.02±1.976 mg/L) and it remained low in August,September and October (Table 5.17; Figs. 5.76, 5.80).

Table 5.16 shows air temperature, water temperature and physico-chemical parameters of water at Site 6 (Budhi River, Sunsari) from Nov. 2008- October 2009 **(Mean ± S.D., N=5).**

Parameters	**Months**											
Site 6-I Yr.	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (°C)	25.15 ±0.158	20.02 ±0.342	19.17 ±.293	18.55 ±0.469	29.08 ±0.328	30.07 ±0.517	31.5 ±0.432	27.15 ±0.373	30.02 ±0.457	32.14 ±0.524	28.15 ±0.621	27.01 ±0.577
Water Temp. (°C)	22.05 ±0.125	17.01 ±0.217	18.14 ±0.316	17.52 ±0.335	24.06 ±0.523	28.13 ±0.475	29.05 ±0.351	26.13 ±0.328	28.15 ±0.272	30.12 ±0.235	27.03 ±0.534	24.11 ±0.476
pH	7.9 ±0.234	8.17 ±0.327	8.5 ±0.365	8.32 ±0.229	7.9 ±0.576	7.65 ±0.317	7.77 ±0.733	7.47 ±0.256	7.72 ±0.075	6.91 ±0.174	6.67 ±0.271	7.83 ±0.073
Turbidity (NTU)	80.15 ±0.615	42.30 ±0.565	45.21 ±1.245	99.03 ±0.623	135.04 3±0.398	83.20 ±0.667	85.14 ±0.735	140.00 ±1.566	235.15 ±1.275	800.00 ±2.465	1065.00 ±3.335	125.00 ±0.518
Free CO2 (mg/L)	29.80 ±1.477	37.42 ±1.365	27.5 ±0.745	25.84 ±0.687	14.56 ±0.359	22.33 ±0.567	28.72 ±0.836	28.13 ±0.156	29.85 ±0.926	27.46 ±1.327	80.08 ±1.352	30.91 ±0.526
DO (mg/L)	7.43 ±0.265	6.42 ±0.087	8.26 ±0.185	7.33 ±0.224	5.72 ±0.068	6.65 ±0.125	5.52 ±0.078	5.16 ±0.095	5.35 ±0.273	5.84 ±0.097	5.63 ±0.086	7.22 ±0.237
BOD (mg/L)	4.10 ±0.067	2.66 ±0.029	3.77 ±0.037	2.34 ±0.025	2.45 ±0.065	4.95 ±0.061	2.62 ±0.015	3.38 ±0.069	3.45 ±0.057	3.12 ±0.065	2.72 ±0.054	4.15 ±0.075
Chloride (mg/L)	9.07 ±0.093	3.01 ±0.069	6.04 ±0.096	6.51 ±0.142	6.00 ±0.192	7.70 ±0.127	6.16 ±0.135	5.02 ±0.031	8.50 ±0.109	9.45 ±0.175	10.02 ±0.075	10.20 ±0.086
Total Alkalini (mg/L)	163.56 ±2.345	144.76 ±2.384	240.03 ±2.74	192 ±1.747	220.53 ±2.656	222.01 ±2.476	197.01 ±1.561	192.4 ±2.735	111.6 ±0.815**	129.63 ±1.475	216.48 ±2.752	197.17 ±1.892
Total Hardness (mg/L)	176.55 ±2.347	159.12 ±1.925	142.03 ±1.798	166.53 ±1.623	190 ±1.845	164.34 ±1.485	176.02 ±1.716	180.12 ±1.976	150.04 ±1.206**	89.01 ±0.875	151.22 ±0.975	105.01 ±0.587

* Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.17 shows air temperature, water temperature and physico-chemical parameters of water at Site 6 (Budhi River, Sunsari) from Nov. 2009- October 2010 **(Mean ± S.D., N=5).**

Parameter Site 6-II Yr.	**Months**											
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**
Air Temp. (°C)	24.12 ±0.256	21.02 ±0.362	18.14 ±.287	19.25 ±0.396	30.08 ±0.354	31.07 ±0.567	32.13 ±0.448	28.15 ±0.573	30.02 ±0.657	31.04 ±0.588	29.05 ±0.532	28.21 ±0.498
Water Temp. (°C)	21.52 ±0.237	19.03 ±0.521	17.15 ±0.335	17.51 ±0.354	27.03 ±0.632	28.01 ±0.445	29.02 ±0.537	27.15 ±0.524	28.5 ±0.473	29.12 ±0.635	29.03 ±0.565	26.51 ±0.476
pH	7.98 ±0.324	8.26 ±0.287	8.3 ±0.236	8.1 ±0.245	7.95 ±0.375	7.77 ±0.314	7.8 ±0.347	8.11 ±0.653	7.9 ±0.275	7.08 ±0.174	6.78 ±0.271	7.98 ±0.275
Turbidity (NTU)	215.2 ±1.354	81.05 ±0.059	48.01 ±1.435	97.03 ±0.562	129.01 ±0.579	94.05 ±0.467	98.04 ±0.753	131.05 ±1.256	225.12 ±1.375	782 ±2.765	1078 ±2.359	455 ±0.717
Free CO_2 (mg/L)	12.55 0.085	16.69 ±0.568	14.5 ±0.749	17.5 ±0.687	12.55 ±0.563	11.6 ±0.656	16.32 ±0.736	14.58 ±0.516	10.45 ±0.625	13.24 ±0.736	16.86 ±0.755	15.96 ±0.529
DO (mg/L)	6.35 ±0.335	8.31 ±0.076	8.4 ±0.285	6.65 ±0.207	5.16 ±0.079	5.84 ±0.096	4.71 ±0.075	6.36 ±0.098	5.05 ±0.073	4.59 ±0.097	5.72 ±0.078	6.20 ±0.086
BOD (mg/L)	3.74 ±0.056	0.26 ±0.087	2.35 ±0.074	3.75 ±0.025	4.12 ±0.056	2.45 ±0.063	4.15 ±0.045	2.27 ±0.069	2.13 ±0.077	3.23 ±0.068	0.72 ±0.078	3.66 ±0.097
Chloride (mg/L)	11.63 ±0.993	2.5 ±0.069	9.01 ±0.096	6.32 ±0.142	6.1 ±0.192	11.10 ±0.127	10.32 ±0.135	11.2 ±0.231	9.14 ±0.109	13.35 ±0.097	5.24 ±0.075	8. 06 ±0.087
Total Alkalinity (mg/L)	208.01 ±2.175	196.12 ±2.453	238.60 ±2.534	194.04 ±1.857	227.53 ±2.887	208.01 ±2.956	198.05 ±1.742	211.60 ±2.856	127.92 ±0.987 *	221.02 ±1.745	202.4 ±2.857	219.53 ±1.892
Total Hardne (mg/L)	188.05 ±2.645	85.141 ±1.956	157.62 ±1.579	151.61 ±1.862	140.58 ±1.845	144.04 ±1.587	179.5 ±1.786	196.02 ±1.976	132.60 ±1.206 *	116.61 ±0.975	178.2 ±0.975	162.05 ±0.879

***** Significant differences at 1% level, ** Significant differences at 5% level.

Table 5.18 shows Pearson's correlation coefficient (r) for air temperature and physico-chemical parameters of water at Site 6 (average of the corresponding month values) during Nov. 2008 – Oct. 2010**; N=12; d.f. =11.**

S6- I+II		**Water Temp. (°C)**	**pH**	**Turbidity (NTU)**	**Free CO_2 (mg/L)**	**DO (mg/L)**	**BOD (mg/L)**	**Chloride (mg/L)**	**T.Alkalinity (mg/L)**	**T.Hardness (mg/L)**
Air Temp. (°C)	P Corr.	.982*	-.306	.356	-.410	-.893*	.768*	.394	-.185	-.167
	Sig.(2-t)	.000	.333	.256	.186	.000	.001	.230	.564	.623
Water Temp. (°C)	P Corr.	1	-.403	.484	-.336	-.869**	.496**	.375	-.183	-.107
	Sig.(2-t)		.194	.111	.285	.000	.023	.256	.569	.754
pH	P Corr.		1	-.924**	-.098	.251	.227	-.066	-.118	.231
	Sig.(2-t)			.000	.763	.430	.478	.846	.714	.494
Turbidity (NTU)	P Corr.			1	.700**	-.300	-.155	.010	.045	-.099
	Sig.(2-t)				0.011	.343	.632	.976	.890	.772
Free CO_2 (mg/L)	P Corr.				1	.473	-.055	-.656**	.283	.478
	Sig.(2-t)					.120	.864	.049	.373	.137
DO (mg/L)	P Corr.					1	-.469**	-.482	.256	.241
	Sig.(2-t)						.014	.133	.421	.475
BOD (mg/L)	P Corr.						1	.447	.809**	-.018
	Sig.(2-t)							.168	.025	.958
Chloride (mg/L)	P Corr.							1	.119	.644**
	Sig.(2-t)								.727	.026
Total Alkalinity (mg/L)	P Corr.								1	.155
	Sig.(2-t)									.649
Total Hardness (mg/L)	P Corr.									1
	Sig.(2-t)									

*** Significant at 1% level (P<0. 01), ** significant at 5% level (P<0. 05)**

Values not marked denote non-significant correlation.

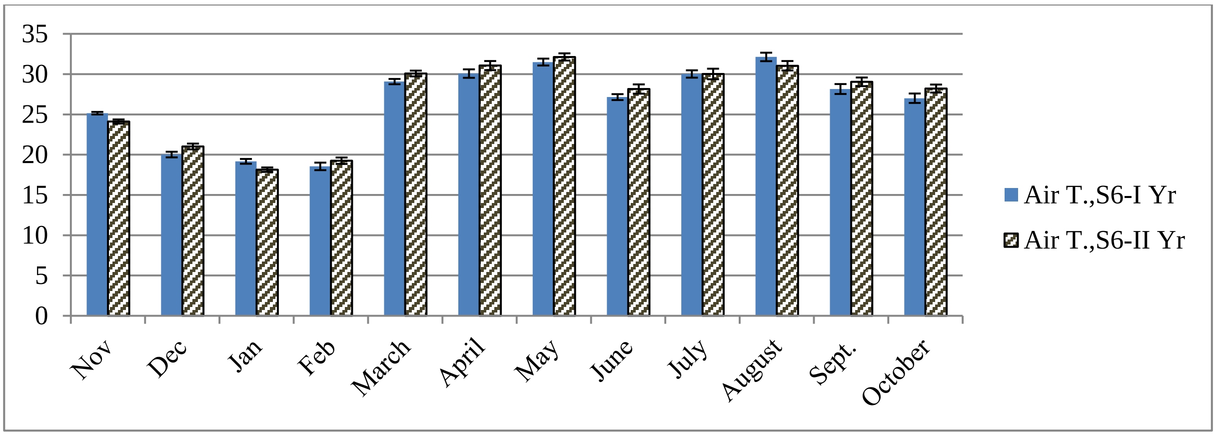

Fig.5.67. Monthly variations in air temperature at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

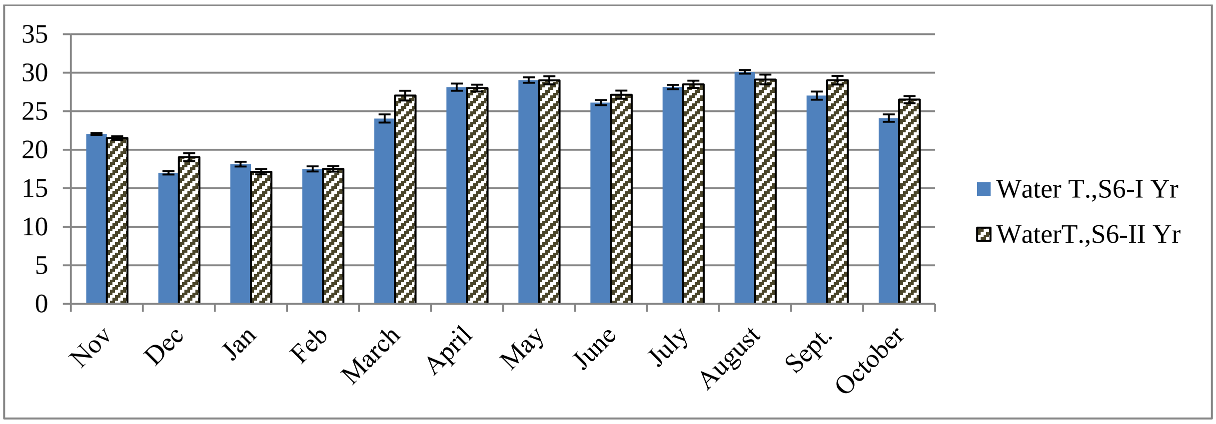

Fig.5.68. Monthly variations in water temperature at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

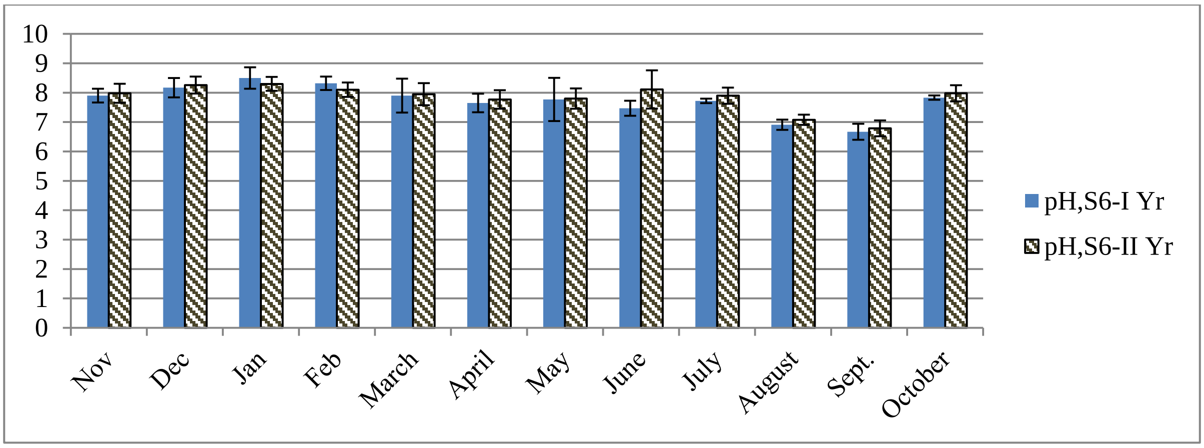

Fig.5.69. Monthly variations in pH at Site 6 during the first and second year study periods (Nov.2008- Oct.2010).

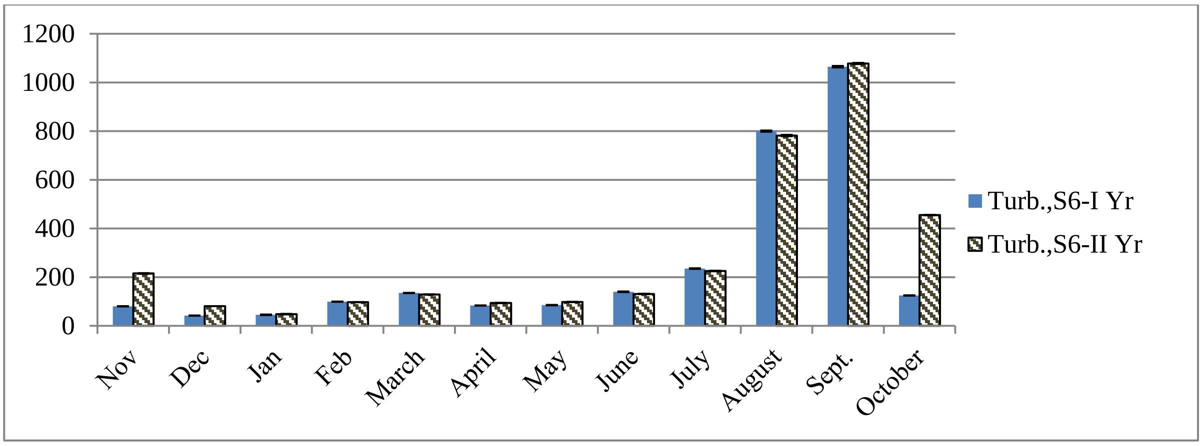

Fig.5.70. Monthly variations in turbidity at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

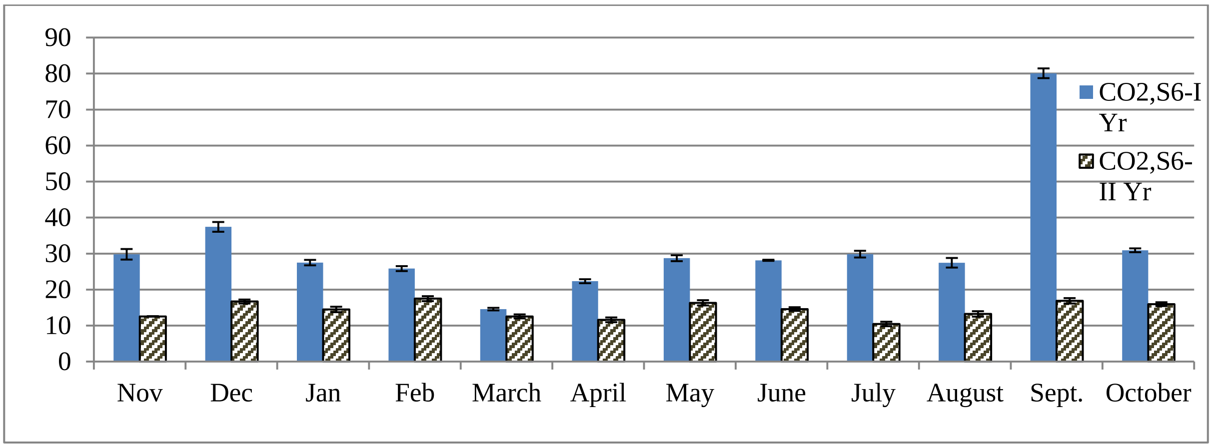

Fig.5.71. Monthly variations in free carbon dioxide at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

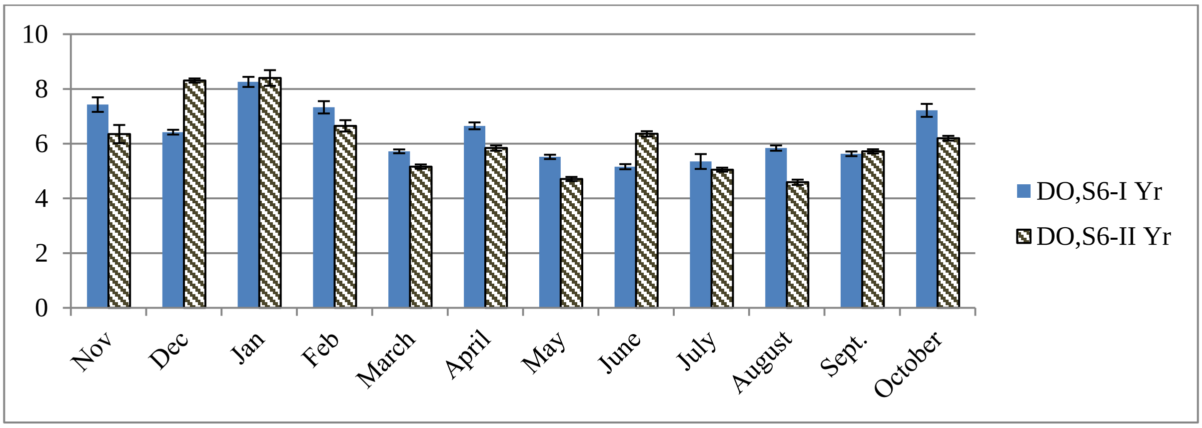

Fig.5.72. Monthly variations in dissolved oxygen at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010)

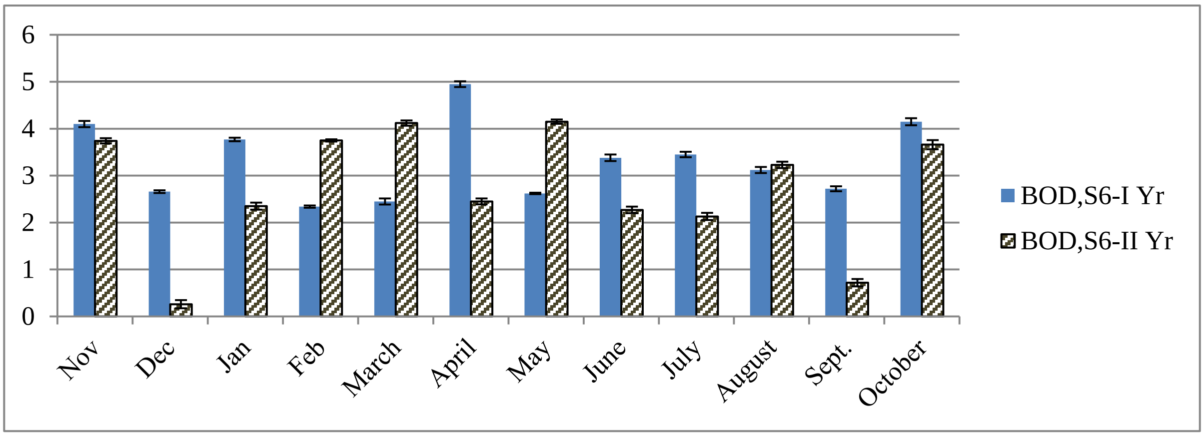

Fig.5.73. Monthly variations in Biological oxygen demand at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

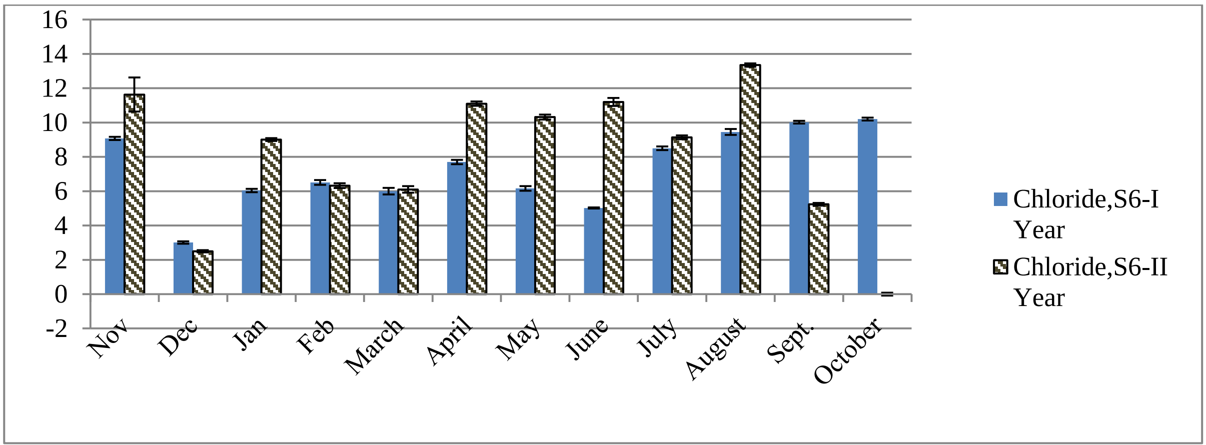

Fig.5.74. Monthly variations in chloride at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

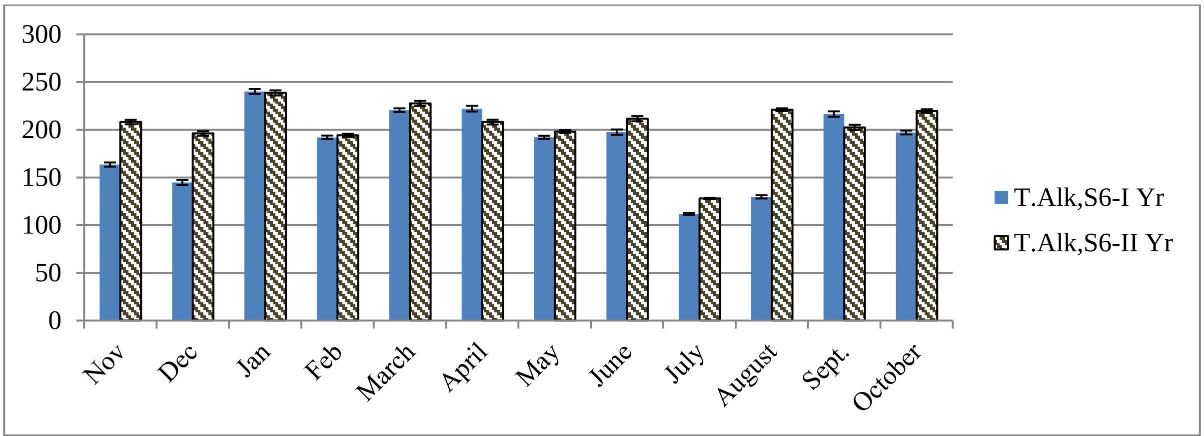

Fig.5.75. Monthly variations in total alkalinity at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

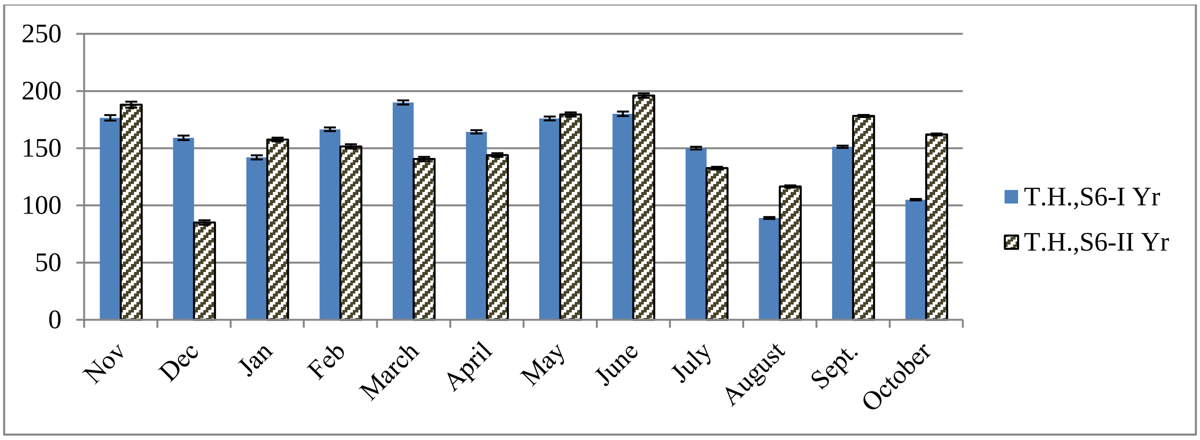

Fig.5.76. Monthly variations in total hardness at Site 6 during the first and second year study periods (Nov. 2008- Oct. 2010).

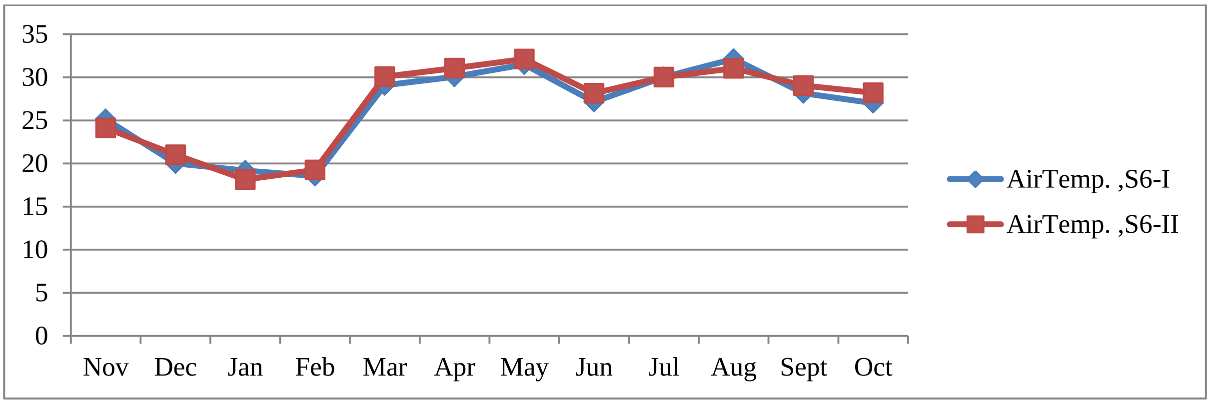

Fig.5.77. Line graph of monthly variations in air temperature at site 6 during the first and second year study periods (Nov. 2008 - Oct.2010).

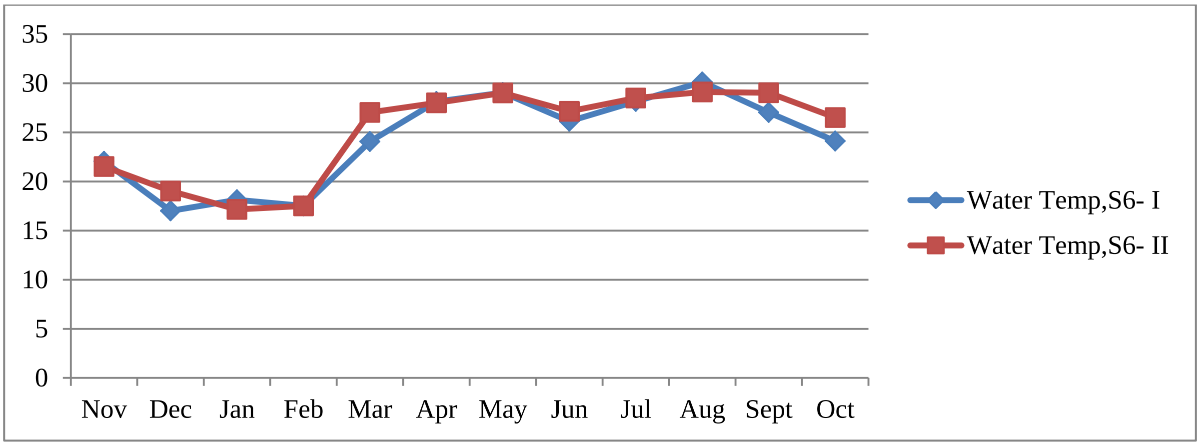

Fig. 5.78. Line graph of monthly variations in water temperature at site 6 during the first and second year study periods (Nov. 2008 - Oct.2010).

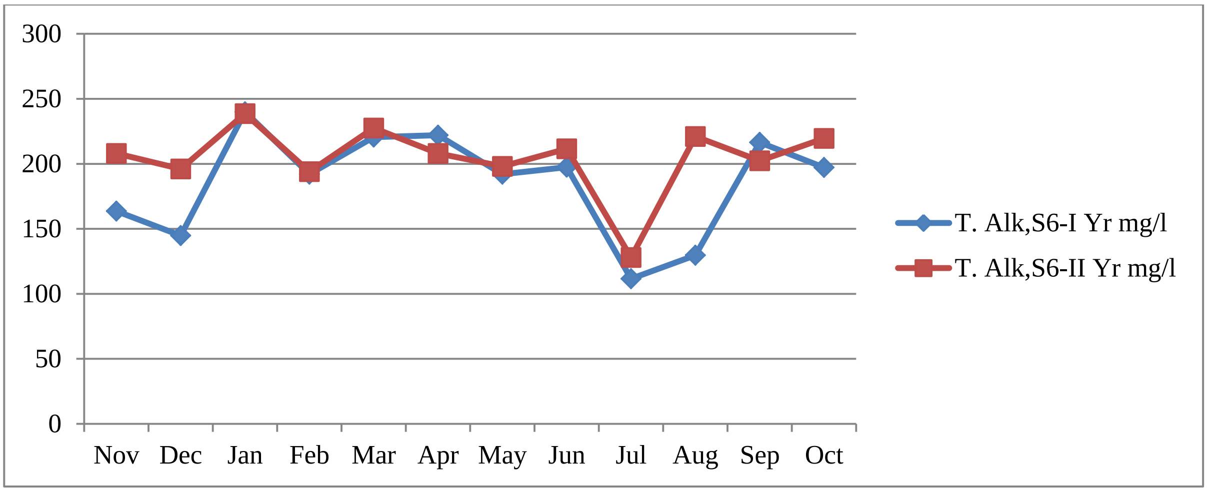

Fig.5.79. Line graph of monthly variations in total alkalinity at site 6 during the first and second year study periods (Nov. 2008 - Oct.2010).

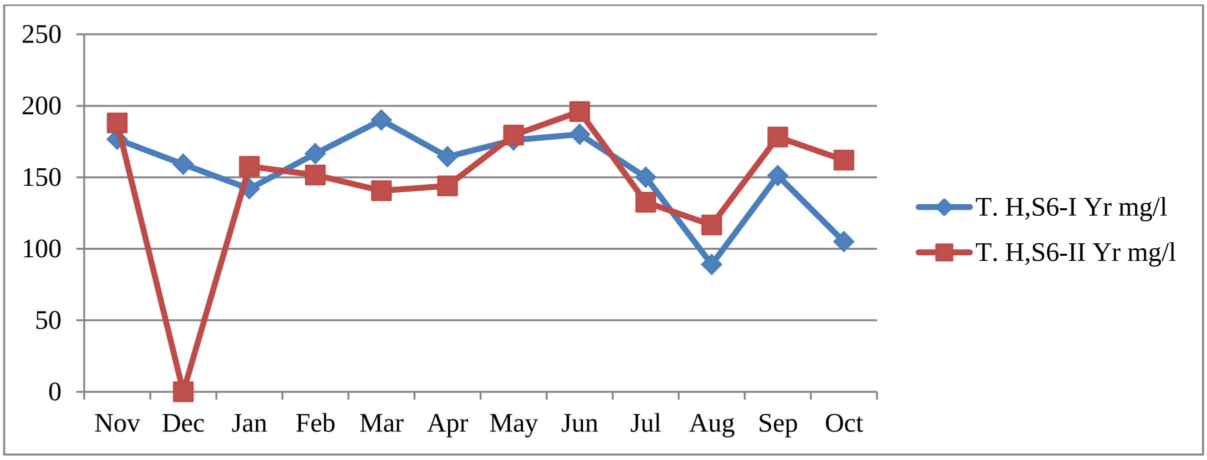

Fig.5.80. Line graph of monthly variations in total hardness at site 6 during the first and second year study periods (Nov.2008 - Oct.2010).

Seasonal variations of air temperature and physico-chemical parameters of Site 1 (Baidya Fish Farm)

The seasonal variation in air temperature and physicochemical parameters of Site 1 is shown in table 5.19.

The air temperature was higher in summer than that of in rainy season in the first and second year study periods and the lowest temperature was recorded in winter of both the years. The water temperature of Site 1 was highest in summer in the first year and in rainy season during the second year. In the first year, the highest pH was recorded in winter whereas the lowest was in rainy season. In second year, pH was highest in rainy season and lowest was in winter season. The CO_2 was highest in summer in the first year and in winter in the second year. DO was maximum in summer in the first year and in rainy season in the second year. BOD was highest in rainy season during first year and lowest in summer in the first year. Total hardness was maximum in summer in both the years. Total alkalinity was highest in summer in the first year but it was highest in winter in the second year. Chloride content was recorded maximum in summer in the first year and in winter in the second year (Table 5.19).

Table 5.19 Seasonal variations in air temperature and physico-chemical parameters of water at Site 1 during the whole study period (Nov.2008 - Oct.2010).

Parameters of Site 1.	**Year I**			**Year II**		
	Winter	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**
Air Temp. (°C)	23 ±2.449	30.75 ±1.707	28.5 ±1.957	22.35 ±2.688	30.37 ±1.701	29.75 ±1.5
Water Temp. (°C)	19.5 ±2.380	27.37 ±2.286	27 ±1.825	20.75 ±3.50	29 ±0.577	30 ±0.816
pH	8.07 ±0.330	7.07 ±0.865	7 ±0.648	8.4 ±0.336	8.52 ±0.607	8.82 ±0.02
Free CO_2(mg/L)	59.89 ±37.387	111.15 ±47.427	67.32 ±55.666	31.45 ±33.722	2.97 ±1.420	4.91 ±2.775
DO (mg/L)	6.04 ±1.012	7.36 ±0.537	6.32 ±0.635	8.25 ±1.564	6.18 ±2.495	8.33 ±2.030
BOD (mg/L)	1.4 ±0.900	0.91 ±0.306	2.58 ±1.217	4.87 ±1.686	1.655 ±0.19	4.34 ±3.160
Chloride (mg/L)	15.59 ±14.127	23.18 ±1.771	11.89 ±9.726	4.75 ±3.403	4.25 ±4.272	4.5 ±2.081
Total alkalinity (mg/L)	131.02 ±24.309	189.95 ±18.090	124.42 ±25.117	151.87 ±64.476	148.1 ±17.920	93.95 ±17.937
Total hardness (mg/L)	113.39 ±8.298	119.05 ±22.498	95.12 ±10.797	100.44 ±38.752	128.5 ±3	76.23 ±2.556

Seasonal variations of air temperature and physico-chemical parameters of Site 2 (Babiya Birta Fish Farm)

The seasonal variation in air temperature and physicochemical parameters of Site 2 is shown in table 5.20.

The air temperature of Site 2 was highest in summer in the first year but in the rainy season in the second year. The lowest temperature was recorded in winter in the second year. The water temperature was highest in rainy season in both the years. The highest pH was recorded in winter and lowest in the summer in both years. The free CO_2 was highest in summer in the first year whereas in winter during the second year. The DO was recorded higher in winter in both years. The BOD was highest in winter in the first year and in summer during the second year.

Total hardness was highest in summer in both the years. Total alkalinity was highest in summer in the first year and in winter during the second year. Chloride was maximum during summer in both the years (Table 5.20).

Table 5.20 Seasonal variations in air temperature and physico-chemical parameters of water at Site 2 during the whole study period (Nov. 2008 - Oct.2010).

Parameters of Site 2	Year- I			Year- II		
	Winter	Summer	Rainy	Winter	Summer	Rainy
Air temperature (°C)	23.87 ±2.954	30.37 ±1.796	27.625 ±1.887	21.0 ±1.957	28.12 ±2.250	29.12 ±0.853
Water temperature (°C)	19.0 ±1.825	27.5 ±1.732	28.12 ±2.096	20.12 ±2.780	28.5 ±1	29.75 ±1.258
pH	8.25 ±0.645	6.97 ±0.330	7.77 ±0.944	8.07 ±0.548	7.87 ±0.507	8.04 ±0.405
Free CO_2 (mg/L)	56.98 ±28.442	101.38 ±60.272	51.96 ±57.060	14.09 ±7.517	3.45 ±1.255	5.8 ±2.209
DO (mg/L)	6.93 ±1.321	6.847 ±0.680	6.83 ±0.625	7.567 ±1.721	5.01 ±0.906	6.12 ±0.209

BOD (mg/L)	3.29 ±1.735	0.68 ±0.202	2.59 ±1.953	2.83 ±2.004	3.017 ±0.806	2.09 ±1.650
Chloride (mg/L)	23.36 ±4.805	37.84 ±6.891	21.75 ±14.850	12.49 ±7.228	22.49 ±3.415	11.75 ±4.573
Total alkalinity (mg/L)	90.31 ±19.550	127.65 ±9.525	89.86 ±19.690	130.21 ±22.276	119.15 ±39.920	106.15 ±7.043
Total hardness (mg/L)	82.88 ±6.225	84.81 ±7.773	83.5 ±10.314	91.81 ±22.533	96.995 ±10.826	90.09 ±7.317

Seasonal variations in air temperature and physico-chemical parameters of Site 3 (Tarahara Fish Farm)

The seasonal variation in air temperature and physicochemical parameters of Site 3 is shown in table 5.21.

The air temperature was minimum in winter in both the years but it was maximum in summer of the first year and in rainy season during second year. Water temperature was minimum in winter and maximum in rainy season in both the years. pH was lowest in rainy season and was highest in winter in both the years. DO was lowest in the rainy season of both the years, whereas it was the highest in summer of first year and in winter of second year. Lowest BOD was recorded in summer of first year and in rainy season of the second year.

In both years, alkalinity was found to be lowest in the rainy season; but it was maximum in the winter of first year and in summer of the second year. The total hardness was lowest in the rainy season of second year and the highest in the winter season of first year. Free CO_2 level was highest in the summer of first year and lowest in the rainy season of second year. Chloride content was highest in the summer of first year and lowest in the winter of second year (Table 5.21).

Table 5.21 Seasonal variations in air temperature and physico-chemical parameters of water at site 3 during the whole study period (Nov.2008 - Oct.2010).

Parameters of Site 3	Year- I			Year- II		
	Winter	Summer	Rainy	Winter	Summer	Rainy
Air temperature (°C)	21.717 ±2.159	29.81 ±1.208	27.955 ±1.712	19.09 ±1.642	26.387 ±2.062	29.255 ±1.094
Water temperature (°C)	18.805 ±2.539	26.75 ±2.331	28.723 ±.941	18.805 ±5.965	26.75 ±8.298	28.722 ±9.409
pH	8.1725 ±0.312	7.578 ±0.723	7.497 ±0.532	8.462 ±1.178	7.652 ±0.112	7.645 ±0.412
Free CO_2(mg/L)	73.512 ±45.214	116.457 ±22.121	54.347 ±41.569	24.547 ±15.431	39.125 ±50.381	16.73 ±1.165
DO (mg/L)	7.27 ±1.731	7.707 ±0.461	6.247 ±1.826	7.982 ±2.445	4.675 ±2.140	4.155 ±0.847
BOD (mg/L)	3.547 ±1.301	0.85 ±0.597	2.995 ±0.53	4.495 ±3.21	2.872 ±1.483	1.302 ±1.053
Chloride (mg/L)	9.09 ±3.21	11.01 ±1.743	7.445 ±2.146	3.377 ±.856	4.335 ±3.375	5.062 ±0.853
Total Alkalinity(mg/L)	168.532 ±35.869	156.815 ±22.213	122.572 ±14.966	128.655 ±45.276	167.805 ±44.298	114.382 ±8.802
Total Hardness (mg/L)	152.2 ±11.065	108.715 ±25.281	100.74 ±15.468	113.61 ±55.333	127.177 ±31.536	95.02 ±11.534

Seasonal variation in air temperature and physico-chemical parameters of Site 4 (Betana Wetland)

The seasonal variation in air temperature and physicochemical parameters of Site 4 is shown in table 5.22.

Air temperature as well as water temperature was highest in rainy season and lowest during winter in both years of study. pH was lowest in rainy season and highest in summer of the first year and was minimum in summer and maximum in rainy season of second year. DO was maximum in winter season of both the years, but minimum in rainy season of the first year and in summer of second year. BOD was maximum in summer of both the years, but minimum in winter of first year and rainy season of second year. Free CO_2 level was lowest in summers of both the years, but highest in rainy season of first and in winter of second year. Maximum chloride was recorded in winter season of second year and minimum in winter of first year as well as summer of second year during the entire study period (Table 5.22).

Table 5.22 Seasonal variations in air temperature and physico-chemical parameters of water at Site 4 during the whole study period (Nov. 2008 **-** Oct.2010).

Parameters of Site 4	**Year- I**			**Year-II**		
	Winter	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**
Air Temperature (°C)	20.54 ±3.226	27.735 ±1.663	28.346 ±2.130	20.797 ±2.965	26.822 ±1.718	28.052 ±1.350
Water temperature (°C)	19.317 ±2.064	27.027 ±0.7314	27.61 ±1.8817	19.997 ±1.767	26.66 ±1.378	26.822 ±1.304
pH	7.13 ±0.425	7.322 ±0.346	7.095 ±0.384	7.12 ±0.404	7.027 ±0.179	7.19 ±0.214
Free carbon dioxide (mg/L)	28.972 ±13.241	4.817 ±1.186	37.484 ±32.352	20.065 ±4.297	3.83 ±1.288	9.977 ±2.225
Dissolved Oxygen (mg/L)	6.53 ±0.773	6.027 ±1.161	5.145 ±1.638	6.695 ±1.201	5.667 ±2.828	5.855 ±0.749
BOD (mg/L)	1.762 ±.811	2.976 ±1.322	2.395 ±1.553	1.417 ±1.559	3.25 ±2.206	0.615 ±0.328
Chloride (mg/L)	3.56 ±1.082	4.01 ±0.820	3.725 ±1.246	4.545 ±2.091	3.56 ±2.429	4.297 ±1.519
Total alkalinity (mg/L)	125.645 ±52.008	124.467 ±8.724	119.462 ±8.481	134.69 ±41.929	123.967 ±8.328	113.542 ±10.273
Total hardness (mg/L)	117.235 ±9.17	108.887 ±1.592	106.11 ±6.805	109.265 ±10.308	108.38 ±2.942	98.552 ±7.848

Seasonal variations in air temperature and physico-chemical parameters of Site 5 (Singhia River)

The seasonal variation in air temperature and physicochemical parameters of Site 5 is shown in table 5.23.

The air temperature of Site 5 was highest in summer of both the years. The lowest temperature was recorded in winter in both the years. The water temperature was higher in rainy season in the first year and in summer in the second year. Lowest temperature was recorded in winter of both the year. Turbidity was highest in rainy season and lowest in winter of both the years. The highest pH was recorded in winter in the first as well as during the second year. Lowest pH was found in rainy season of first year and summer in second year. The CO_2 was highest in winter season in the first year and in summer in the second year. Lowest CO_2 was recorded in rainy season of both years. DO was highest in winter in both the years and lowest in rainy season of both years.

Total hardness was highest in summer in the first year but in winter in the second year. It was lowest in rainy season in first year and in summer in second year. Chloride content was recorded maximum in summer in the first year and in rainy season in the second year, but minimum in winter of both the years. Total alkalinity was highest in winter season in the first year but in summer in the second year and lowest in rainy season. BOD was highest in winter during first year and in summer in the second year (Table 5.23).

Table 5.23 Seasonal variations in air temperature and physico-chemical parameters of water at Site 5 during the whole study period (Nov.2008 - Oct.2010).

Parameters of Site 5	**Year I (2008-09)**			**Year II (2009-10)**		
	Winter	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**
Air Temp. (°C)	20.1 ±0.758	29.30 ±1.692	29.22 ±1.719	20.57 ±1.292	30.92 ±1.303	29.42 ±1.600
Water temp. (°C)	18.65 ±0.608	27.05 ±1.853	27.47 ±1.315	19.56 ±2.734	29.26 ±0.649	28.18 ±1.747
pH	8.002 ±0.396	7.83 ±0.464	6.87 ±0.354	8.17 ±0.408	7.71 ±0.657	7.26 ±0.311

Turbidity (NTU)	45.64 ±29.201	138.32 ±94.434	206.51 ±148.95	50.79 ±4.790	133.68 ±99.836	264.08 ±143.39
Free CO_2 (mg/L)	28.38 ±11.725	24.29 ± 3.341	19.30 ±13.001	18.60 ±1.404	18.70 ±4.338	9.11 ±3.070
DO (mg/L)	6.095 ±1.712	6.04 ± 0.608	6.02 ±0.820	7.84 ±1.169	6.11 ±0.827	6.62 ±0.760
BOD (mg/L)	3.05 ± 0.365	2.23 ± 0.231	2.945 ±2.030	1.302 ±0.844	3.13 ±0.605	1.81 ±0.364
Chloride (mg/L)	4.94 ±1.710	9.05 ± 1.443	8.05 ±1.447	8.29 ±4.817	10.22 ±0.565	11.49 ±2.034
Total alkalinity (mg/L)	193.71 ±61.759	166.91 ±12.849	153.15 ±39.999	168.97 ±4.206	181.37 ±16.726	162.12 ±19.247
Total hardness (mg/L)	153.16 ±21.617	158.49 ±5.078	132.99 ±32.569	148.20 ±42.171	135.50 ±14.034	143.83 ±24.799

Seasonal variations in air temperature and physico-chemical parameters of Site 6 (Budhi River)

The seasonal variation in air temperature and physicochemical parameters of Site 6 is shown in table 5.24.

The air temperature of Site 6 was highest in rainy season in the first year but in summer season in the second year. The lowest temperature was recorded in winter in both the years. The water temperature was highest in rainy season and lowest was in winter in the first as well as the second year. The highest pH was recorded in winter and lowest in the rainy season in both years. The free CO_2 was highest in rainy season in the first year but in winter in the second year. Lowest free CO_2 was in summer season in both the years.

The study revealed that turbidity was highest in rainy season and lowest in winter. The DO was recorded highest in winter in both years. Lowest DO was found in summer in both years. BOD was highest in winter during first year and during summer in the second year. Lowest BOD was in summer season in the first year and rainy in second year. Chloride content was maximum in rainy season in the first year and during summer in the second year and lowest value was recorded in winter of both years. Total alkalinity was highest in summer in both the years and lowest was in rainy season of both years. Total hardness had higher value in summer in both the years and lowest was rainy season in the first but in winter of second year (Table 5.24).

Table 5.24 Seasonal variations in air temperature and physico-chemical parameters of water at Site 6 during the whole study period (Nov.2008 - Oct.2010).

Parameters	**Year- I**			**Year –II**		
Site 6, Budhi	**Winter**	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**
Air Temp. (°C)	20.72 ±3.012	27.95 ±4.64	29.33 ±2.34	20.63 ±2.61	30.35 ±1.693	29.58 ±1.222
Water Temp. (°C)	18.68 ±2.293	26.84 ±2.21	27.35 ±2.51	18.8 ±1.986	27.80 ±0.921	28.29 ±1.217
pH	8.22 ±0.253	7.69 ±0.182	7.28 ±0.578	8.16 ±0.147	7.90 ±0.156	7.71 ±0.422
Turbidity (NTU)	66.67 ±27.58	110.8 ±30.93	556.28 ±449.93	110.32 ±72.83	113.06 ±1.73	635.03 ±373.389
Free CO_2(mg/L)	30.14 ±5.117	23.43 ± 6.581	42.07 ± 9.37	15.31 ±2.234	13.762 ±2.109	14.12 ±2.894
DO (mg/L)	7.36 ±0.752	5.76 ± 0.635	6.01 ±0.831	7.42 ±1.078	5.51 ±0.728	5.39 ±0.711
BOD (mg/L)	3.967 ± 2.176	3.35 ± 1.140	3.36 ±0.605	2.525 ±1.644	3.24 ±1.027	2.43 ±1.312
Chloride (mg/L)	6.157 ±2.485	6.22 ± 1.107	9.49 ±0.717	7.36 ±3.901	9.68 ±2.418	8.94 ±3.364
Total alkalini (mg/L)	185.08 ±41.457	207.98 ±15.506	163.72 ±50.928	209.19 ±20.548	211.29 ±12.245	192.71 ±44.016
Total hardness (mg/L)	161.05 ±14.563	177.62 ±10.621	123.82 ±31.642	145.60 ±43.351	165.03 ±27.130	147.36 ±27.870

Seasonal variations in air temperature and physico-chemical parameters of water at six sites during the whole study period (Nov. 2008 – Oct. 2010).

Monthly data on air temperature and physico-chemical parameters of water of six sites of the whole study period (Nov.2008–Oct. 2010) were interpolated as seasonal values and were shown in Table 5.25. The maximum air temperature was recorded in summer followed by rainy season and winter at the Sites 1, 2, 3, 4, 5 and 6. The maximum air temperature was recorded 30.56°C at Site 1 in summer and minimum was 20.38°C at Site 5 in winter. The maximum water temperature was recorded in rainy season followed by summer and winter at most of the sites. The maximum water temperature was recorded 28.935 °C at Site 2 and minimum was 18.74°C at Site 6.

The maximum turbidity was recorded 595.655 NTU at Site 6 and minimum 48.215 NTU at Site 5. The maximum pH was recorded in winter followed by rainy season and summer at sites 1-6. The maximum pH was recorded 8.317 at Site 3 and minimum 7.065 was at Site 5. The maximum dissolved oxygen was recorded in winter season followed by summer and rainy season at all sites except Site 2. The maximum dissolved oxygen occurred in winter followed by rainy season and summer at Site 2 .The maximum dissolved oxygen was recorded 7.626 mg/L at Site 3 and minimum 5.201 mg/L at Site 3 in rainy season.

The maximum free carbon dioxide was recorded in summer season followed by rainy season and winter at Site 1, Site 2 and Site 3, the maximum free carbon dioxide was recorded in winter followed by rainy season and summer at Site 4 and Site 6 but at Site 5, maximum free carbon dioxide was found winter followed by summer and rainy season. The maximum free carbon dioxide was recorded 77.791 mg/L at Site 3 and minimum 4.3235 mg/L at Site 4. The biological oxygen demand was recorded maximum in summer season followed by rainy and winter seasons at Site 4, Site 5 and Site 6 but at Site 1, Site 2 and Site 3 maximum values of BOD were in winter followed by rainy season and summer. The maximum biological oxygen demand was recorded 4.87 mg/L and minimum was 1.282 mg/L at Site 1.

The total alkalinity was recorded maximum in winter season followed by summer and rainy seasons at almost all the sites. It was recorded maximum 209.635 mg/L in summer at Site 6 and minimum 98.005 mg/L at Site 2 in rainy season. The maximum total hardness was recorded in winter season followed by summer and rainy season at all the sites and it was recorded maximum 153.325 mg/L at Site 6 and minimum 85.675 mg/L at Site 1. The maximum chloride was found in summer season followed by winter and rainy season at Site

1, Site 2 and Site 3 but maximum chloride was found in rainy season followed by summer and winter season at Site 5 and Site 6 but at Site 4 maximum was in winter, followed by rainy season and summer. The maximum chloride was recorded 30.165 mg/L at Site 2 and minimum 3.785 mg/L was at Site 4.

Table 5.25 Seasonal variations in air temperature and physico-chemical parameters of water at all sites during the whole study period (Nov. 2008 - Oct. 2010).

Parameters	Site 1 Average			Site 2 Average			Site 3 Average			Site 4 Average			Site 5 Average			Site 6 Average		
	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy
Air Temp. (°C)	22.675	30.56	29.125	22.435	29.245	28.372	20.403	28.098	28.605	20.668	27.278	28.199	20.38	30.11	29.32	20.675	29.15	29.455
Water Temp.(°C)	20.125	28.185	28.5	19.56	28.00	28.935	18.805	26.75	28.722	19.657	26.843	27.216	19.105	28.155	27.825	18.74	27.32	28.125
pH	8.235	7.795	7.91	8.16	7.42	7.905	8.317	7.615	7.571	7.125	7.174	7.142	8.086	7.77	7.065	8.19	7.795	7.495
Turbidity (NTU)	-	-	-	-	-	-	-	-	-	-	-	-	48.215	136	235.295	88.495	111.93	595.655
Free CO2 (mg/L)	45.67	57.06	36.115	35.535	52.415	28.88	49.029	77.791	35.538	24.518	4.323	23.730	23.49	21.495	14.205	22.725	18.596	28.095
DO (mg/L)	7.145	6.77	7.325	7.248	5.928	6.475	7.626	6.191	5.201	6.612	5.847	5.50	6.967	6.28	6.361	7.39	5.635	5.70
BOD (mg/L)	4.135	1.2825	3.46	3.06	1.848	2.34	4.021	1.861	2.148	1.589	3.113	1.505	2.176	2.68	2.377	3.246	3.295	2.895
Chloride (mg/L)	10.17	13.715	8.195	17.925	30.165	16.75	6.233	7.672	6.253	4.052	3.785	4.011	6.615	9.635	9.77	6.758	7.95	9.215
Total alkali (mg/L)	141.445	169.025	109.185	110.26	123.4	98.005	148.594	162.31	118.477	130.167	124.217	116.502	181.34	174.14	157.635	197.135	209.635	178.215
Total hardn (mg/L)	106.915	123.775	85.675	87.345	90.902	86.795	132.905	117.946	97.88	113.25	108.633	102.331	150.68	146.995	138.41	153.325	121.325	135.59

Test for significant and insignificant differences in air temperature and physico-chemical parameters of water among sites and seasons.

Tables 5.26 to 5.38 show the significant and insignificant differences in air temperature and physico-chemical parameters of water among sites and seasons.

Table 5.26 shows air temperature is significantly different at 1% level among seasons since F–value (calculated value) is greater than F critical (tabulated value) but differences in air temperature were insignificant among sites since F- value is less than F -critical.

Table 5.26 Variations in air temperature in different sites and seasons.

Seasons (A.T.)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
W	22.675	22.43	20.4	20.66	20.38	20.67
S	30.56	29.24	28.09	27.27	30.11	29.15
R	29.13	28.37	28.6	28.19	29.32	29.45
ANOVA						
	Source of Variation	*F*	*P-value*	*F crit*		
	Among Seasons	195.1408399*	9.73131E-09	4.102 (α= 0.05)	7.559 (α= 0.01)	
	Among Sites	2.702376281	0.084951348	3.325 (α= 0.05)	5.636 (α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05).**

Table 5.27 shows differences of water temperature are significant at 1% level among seasons since F -value (calculated value) is greater than F critical (tabulated value) but insignificant among sites since F- value is less than F -critical.

Table 5.27 Variations in water temperature in different sites and seasons.

Seasons (W.T.)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
W	20.125	19.56	18.805	19.65	19.1	18.74
S	28.185	28	26.75	26.84	28.155	27.57
R	28.5	28.935	28.723	27.21	27.82	27.82
ANOVA	*Source of Variation*	*F*	*P-value*	*F crit*		
	Among Seasons	501.0990881*	9.41179E-11	4.102 (α= 0.05)	7.559 (α= 0.01)	
	Among Sites	1.924866646	0.176897322	3.325 (α= 0.05)	5.636 (α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.28 shows pH is significantly different at 1% level among seasons since F -value (calculated) is greater than F critical (tabulated value) but differences of pH are insignificant among sites since F- value is less than F -crit.

Table 5.28 Variations in pH in different sites and seasons.

Seasons (pH)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
W	8.235	8.15	8.32	7.125	8.08	8.19
S	7.79	7.42	7.61	7.17	7.77	7.79
R	7.19	7.9	7.56	7.14	7.06	7.49
ANOVA	*Source of Variation*	*F*	*P-value*	*F crit*		
	Among Seasons	8.15135781*	0.007943228	4.102 (α= 0.05)	7.559 (α= 0.01)	
	Among Sites	2.819258626	0.076625552	3.325 (α= 0.05)	5.636 (α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.29 shows CO_2 has significant differences at 5 % level among sites since F-value is greater than F critical but insignificant differences among seasons since F- value is less than F - critical.

Table 5.29 Variations in free carbon dioxide in different sites and seasons.

Seasons (Free CO_2)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
W	45.67	35.54	48.87	24.51	23.49	22.73
S	57.06	52.41	77.78	4.32	21.49	18.6
R	36.11	28.88	35.53	23.72	14.2	28.09
ANOVA	*Source of Variation*	*F*	*P-value*	*F crit*		
	Among Seasons	1.221780957	0.335178718	4.102 (α= 0.05)	7.559 (α= 0.01)	
	Among Sites	4.83937653**	0.016516315	3.325 (α= 0.05)	5.636 (α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.30 shows DO has significant differences at 1% level among seasons since F -value is greater than F critical but insignificant differences among sites since F- value is less than F - critical.

Table 5.30 Variations in dissolved oxygen in different sites and seasons.

Seasons (DO)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
W	7.145	7.248	7.625	6.61	6.965	7.39
S	6.77	5.928	6.185	5.84	6.28	5.635
R	7.325	6.475	5.197	5.495	6.361	5.7
ANOVA						
	Source of Variation	*F*	*P-value*	*F crit*		
	Among Seasons	9.446575087*	0.004966215	4.102(α= 0.05)	7.559(α= 0.01)	
	Among Sites	1.725048788	0.216454949	3.325(α= 0.05)	5.636(α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.31 shows insignificant differences of BOD among seasons and sites since F- value is less than F-critical.

Table 5.31 Variations in biological oxygen demand in different sites and seasons.

Seasons (BOD)	**S1**	**S2**	**S3**	**S4**		**S5**	**S6**
W	4.14	3.06	4.02	1.58		2.18	3.24
S	1.28	1.755	1.86	3.11		2.68	3.29
R	3.46	2.34	2.146	1.6		2.38	2.89
ANOVA							
	Source of Variation	*F*	*P-value*	*F crit*			
	Among Seasons	1.033159789	0.3909542	4.102(α= 0.05)	7.559(α= 0.01)		
	Among Sites	0.559821389	0.729000902	3.325(α= 0.05)	5.636(α= 0.01)		

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.32 shows TA has significant difference at 1% level among sites and seasons since F -value is greater than F critical.

Table 5.32 Variations in total alkalinity in different sites and seasons.

Seasons (TA)	**S1**	**S2**	**S3**	**S4**		**S5**	**S6**
W	141.45	110.26	168.53	130.17		181.34	197.14
S	169.03	123.4	156.82	124.22		174.14	209.64
R	109.19	98.005	122.58	116.5		157.63	178.22
ANOVA							
	Source of Variation	*F*	*P-value*	*F crit*			
	Among Seasons	12.2537379*	0.00204378	4.102(α= 0.05)	7.559(α= 0.01)		
	Among Sites	23.97949889	2.87171E-05	3.325(α= 0.05)	5.636(α= 0.01)		

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05)**

Table 5.33 shows TH has significant difference at 1% level among sites and 5% level among seasons since F -value is greater than F- critical.

Table 5.33 Variations in total hardness in different sites and seasons.

Seasons (TH)	S1	S2	S3	S4	S5	S6
W	106.92	87.35	132.905	113.25	150.68	153.32
S	123.78	90.9	117.95	108.64	146.99	171.32
R	85.68	86.79	97.88	102.34	138.41	135.59
ANOVA						
	Source of Variation	*F*	*P-value*	*F crit*		
	Among Seasons	7.08781108**	0.012109095	4.102(α= 0.05)	7.559(α= 0.01)	
	Among Sites	21.25500753*	4.93702E-05	3.325(α= 0.05)	5.636(α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05).**

Table 5.34 shows chloride has significant difference at 1% level among sites since F -value is greater than F critical but insignificant among seasons since F- value is less than F -critical.

Table 5.34 Variations in chloride in different sites and seasons.

Seasons Chloride	S1	S2	S3	S4	S5	S6
W	10.17	17.925	6.24	4.05	6.62	6.76
S	13.715	30.165	7.68	3.79	9.64	7.95
R	8.195	16.75	6.26	4.02	9.77	9.22
ANOVA						
	Source of Variation	*F*	*P-value*	*F crit*		
	Among Seasons	2.428096035	0.138185499	4.102(α= 0.05)	7.559(α= 0.01)	
	Among Sites	12.27768635*	0.000525286	3.325(α= 0.05)	5.636(α= 0.01)	

***indicates significance at 1% level (P<0. 01), ** indicates significance at 5% level (P<0.05).**

5.2. Studies on the fish affected with epizootic ulcerative syndrome

A total 444 naturally infected fishes (Table 5.35)showing lesions on the body; 60% (262) *Cirrhinus mrigala*, 30% (130) *Labeo rohita* and *Labeo bata*, 8 % (36) *Catla catla*, *Channa* spp., *Puntius* spp., *Clarias batrachus, Heteropneustes fossilis, Mystus tengara* and *Lepidocephalichthys guntea* (rarely) (Figs. 5.81a and b to 5.88a and b, 5.89,5.90, 5.91 and 5.92) were collected during winter months of the year 2008-2015 from different affected ponds in various locations of the Sunsari and Morang districts of eastern Nepal and were used for the isolation of fungi. The infected fish were brought to the laboratory alive for further detailed observations.

Table 5.35 shows EUS affected fishes collected during study period (Dec. 2008- Feb. 2015)

S.No.	Fish species	Collection date	No. of fish collected				
			S1	S2	S3	Total	
1.	*Cirrhinus mrigala*	25.12.2008	5	20	5	30	262
		18.2.2009	7	23	5	35	
		15.1.2010	5	25	5	35	
		9.3.2011	5	27	3	35	
		18.2.2012	5	30	5	40	
		23.3.2013	5	30	5	40	
		25.2.2014	2	25	3	30	
		26.2.2015	2	15	-	17	
2.	*Labeo rohita*	18.2.2009	1	3	1	5	43
		15.1.2010	2	3	-	5	
		23.3.2011	3	5		8	
		26.2.2012	3	6		9	
		14.12.2013	2	4		6	
		25.1.2014	2	3		5	
		10.3.2015	1	4		5	
3.	*Catla catla*	18.2.2009	-	2	-	2	17
		15.1.2010	1	1	-	2	
		23.3.2011	-	2	-	2	
		26.2.2012	1	3		4	
		23.3.2013		2		2	
		25.1.2014	1	2		3	
		20.2.2015		2		2	

4.	*Labeo bata*	18.2.2009	1	8	1	10	87
		15.1.2010	2	8	-	10	
		23.3.2011	1	9	-	10	
		26.2.2012	3	15	-	18	
		23.3.2013	2	12	-	14	
		25.1.2014	3	12	-	15	
		20.2.2015	2	8	-	10	
5.	*Channa striatus*	18.2.2009	1	1		2	9
		15.1.2010	1	1		2	
		23.3.2011	1	1		2	
		26.2.2012	-	2	-	2	
		23.3.2013		1		1	
6.	*Puntius* sp.	18.2.2009	1	1		2	10
		15.3.2010	1	1		2	
		23.3.2011	1	1		2	
		26.2.2012		2		2	
		15.2.2014	1	1		2	
7.	*Mystus tengara*	15.3.2010		1		1	4
		26.2.2012		2		2	
		15.2.2014	1			1	
8.	*Clarias batrachus*	25.2.2009			1	1	4
		15.3.2010		1		1	
		15.3.2012	1	1		2	
9.	*Heteropneustes fossilis*	25.2.2009	1			1	5
		26.2.2012	1	1		2	
		15.3.2013		1	1	2	
10.	*Lepidocephalichthys guntea*	26.2.2012	1			1	3
		9.6.2015	1	1		2	
Grand Total			80	329	35		444

In the early stage of lesion the fish showed single or multiple red spots on the body surface (Fig. 5.90). Some fishes showed moderate type of ulcer with erosion of the epidermis (Figs.5.83, 5.89). In the advanced stage ulcer became deep and necrotic with occasional haemorrhages (Figs. 5.81a and b, 5.85a and b).

(a) (b)

Fig.5.81 a and b naturally EUS infected *Cirrhinus mrigala*

(a) (b)

Fig.5.82 a and b naturally EUS infected *Labeo rohita*

Fig.5.83. Naturally EUS infected *Catla catla*

(a)

(b)

Fig 5.84 a and b naturally EUS infected *Labeo bata*

(a)

(b)

Fig.5.85 a and b naturally EUS infected *Channa striata*

(a)

(b)

Fig.5.86a and b naturally EUS infected *Puntius* sp.

(a)

(b)

Fig.5.87a and b naturally EUS infected *Mystus tengara*

(a)

(b)

Fig.5.88 a and b naturally EUS infected *Clarias batrachus*

Heteropneustes fossilis, Lepidocephalichthys guntea and affected fish in group were as follows:

Fig.5.89. Naturally infected *H. fossilis*

Fig.5.90. Naturally infected *Lepidocephalichthys guntea*

Fig.5.91. Naturally EUS affected fish (in group) *C. mrigala, C. striatus, L.bata, C. catla* and *M. tengara*

Fig.5.92. Naturally EUS affected *C. mrigala* and *Labeo bata* (in group).

5.3. Other fish diseases

Infection in tilapia

Some tilapia fishes weighing 150-200 gm were seen affected and ultimately died in a cement tank at Tarahara (Figs.5.93 and 5.94). Fishes were swimming slowly near the surface of the water .Affected fish didn’t feed at all. Eyes were protruded out with unusual red auses. Abdomen was swelled and after dissection, black ascitic fluid came out. Liver was pale in colour.

Fig. 5.93. Infected tilapia

Fig.5.94. Infected tilapia in concrete tank

Fig. 5.95. Infected *Cyprinus carpio* at Site 3

Fig.5.96. Infected *Cyprinus carpio*

Haemorrhagic septicaemia of carps

In the month of August 2010, five years old female common carp was affected (Fig.5.95). Scales on the sides of the body were slightly raised and hemorrhages were noticed on the body surface. The fish ultimately died. Some other carps were also affected (Fig. 5.96).

Abdominal Dropsy

It was found more commonly in *Labeo rohita*, *Cirrhinus mrigala* and *Oreochromis mossambica*.The infected fishes showed swollen abdomen (Fig. 5.97). After dissection, it was noticed that in one tilapia, intestine was filled with gas bubbles (Fig. 5.98).

Fig. 5.97. Dropsy in *Labeo rohita*

Fig. 5.98. Gas bubble filled in intestine of naturally dropsy infected tilapia.

Fin rot

Fraying and marked reduction of fins until destruction in tilapia was found in Tarahara and Baidya fish farms (Figs. 5.93 and 5.99). Leison on the body surface along with fin rot was observed in case of a *Cirrhinus mrigala* (Fig. 5.100).

Fig.5.99. Tilapia fin rot

Fig.5.100.Body lesion with fin rot in *C. mrigala*

5.4. Histopathological observation of EUS affected fishes

1. *Cirrhinus mrigala*

Ulcer

Initial stages of ulcer changed the normal architecture of the epidermis (Fig.5.81a and b). Histological section of advance lesions showed the complete loss of epidermis and the underlying musculature were replaced by granulomatous and inflammatory tissues. In some areas, myonecrosis and fungal hyphae, black stained with GMS, were often found. H-E stained section also showed presence of fungus (Fig. 5.101).

Liver

The histological section of liver showed degenerative changes and infiltration of blood capillaries.Necrotic changes, chord like arrangement with enlarged sinusoids and severely vacuolated hepatic cells were observed in some areas whereas no fungi were detected (Fig. 5.103).

Kidney

In histological section of kidney, necrotic changes and hemorrhages were seen in some areas of kidney. Tubular degeneration and vacuolation of tubular cells were seen but no evidence of the presence of fungi in the section of kidney was found (Fig. 5.105).

2. *Labeo rohita*

Ulcer

In the section of the early stages of lesions, deterioration of the normal structure of epidermis was observed. Advanced lesions showed complete loss of epidermis and the underlying musculature were replaced by granulomatous and inflammatory tissues. In some regions myonecrosis was also observed. Fungal hyphae were seen in section stained with H-E and GMS (Fig. 5.82a and b; Fig.5.102).

Liver

The stained section showed degenerative changes and infiltration of blood capillaries. Necrotic changes, chord like arrangement with enlarged sinusoids and severely vacuolated hepatic cells were observed in some areas. There was no evidence of presence of fungi (Fig. 5.104).

Kidney

Tubular breakage, tubular necrosis, vacuolation of tubular cells and haemorrhages in some areas of the section of the kidney of naturally infected *Labeo rohita* were observed. Fungi were not found in the section (Fig. 5.106).

3. *Catla catla*

Ulcer

In the section of early skin lesions epithelial necrosis with haemorrhage from the underlying dermis were observed. The epidermis at the margins of the ulcer was hyperplastic and thickened. In some regions myonecrosis was also developed. Some aseptate invasive fungal hyphae were distinctly visible in section stained with H-E and GMS (Fig.5.83; Figs.5.108, 5.118).

Liver

Fungal invasion was not observed in the liver tissues stained with Haematoxylene – Eosin and Grocott stain. Degenerative changes and infiltration of blood capillaries of liver were observed. Chord like arrangement with enlarged sinusoids and highly vacuolated hepatic cells were also observed (Fig.5.110).

Kidney

Renal tissues showed tubular and haematopoetic tissues degeneration along with the haemorrhages in some areas of the section (Fig.5.112).

4. *Labeo bata*

Ulcer

The section of deep ulcerated area displayed the complete loss of epidermis and the dermal layer lost its normal structural design and developed granulomas. Several non septate hyphae were observed in the dermis (Fig 5.84a and b; Fig. 5.107).

Liver

Section of liver showed vacuolation, enlarged sinusoids, arrangement of hepatocytes in chord like fashion and infiltration of blood capillaries in some areas in naturally infected *Labeo bata* (Fig. 5.109).

Kidney

Tubular breakage, tubular necrosis, vacuolation of tubular cells and haemorrhages in some areas of the section of the kidney of naturally infected *Labeo bata* were observed but no fungus (Fig. 5.111).

5. ***Channa striata***

Ulcer tissue

The initial lesions in epidermis of naturally infected *Channa striata* showed loss of its normal structure. In case of advanced lesions, non-septate fungal hyphae were frequently observed in dermis and musculature. The noticeable important changes were formation of granuloma and myonecrosis (Fig.5.85a and b; Fig. 5.113 and 5.114).

Liver

In the section of liver of naturally infected *Channa striata*, mild focal degenerative changes of hepatic cells occurred. There were several haemorrhagic spots in the sections of the liver. Vacuolation of hepatocytes with necrotic changes in some areas and infiltration of blood capillaries were spotted. Fungi were not detected in the section of the liver (Fig. 5.115).

Kidney

Necrotic changes in specific haemopoetic areas, haemorrhages and tubular vacuolation in the section of kidney of naturally infected *Channa striata* were observed (Fig. 5.116).

6. ***Puntius* sp.**

Ulcer

The section of ulcerated area showed a complete loss of epidermis.The normal structure of the dermal layer was lost and replaced by granulomas. Several non septate fungal hyphae were observed in the dermis (Fig.5.86a and b; 5.117).

Liver

The section of liver of the naturally infected *Puntius* sp. showed vacuolation in the hepatocytes. Infiltration of blood capillaries were also seen in some regions (Fig.5.119).

Kidney

Haemorrhages were observed in some areas of the sections of the kidney of naturally infected *Puntius* sp. and no fungal hyphae was detected.Tubular breakage, tubular necrosis and vacuolation of tubular cells were observed in the section of the kidney (Fig.5.120).

7. ***Clarias batrachus***

Ulcer

The section of early stages of lesions showed loss of the normal architechture of the epidermis and advanced lesions showed complete loss of epidermis and the underlying musculature were replaced by granulomatous and inflammatory tissues. In some regions, myonecrosis was also developed. Fungal hyphae were seen in section stained with H-E and GMS (Figs. 5.87a and b; 5.121).

Liver

The stained section showed degenerative changes and infiltration of blood capillaries. In some areas, hepatic cells were found to have necrotic changes, chord like arrangement with enlarged sinusoids and severe vacuolation. There was no evidence of presence of fungi (Fig.5.123).

Kidney

Tubular breakage, tubular necrosis, vacuolation of tubular cells and haemorrhages were observed in some areas of the section of the kidney of naturally infected *Clarias batrachus*. Besides these, haemopoietic tissue degeneration was also observed. Fungi were not found in the section (Fig. 5.125).

8. ***Mystus tengara***

Ulcer

The section of ulcerated area showed a complete loss of epidermis.The normal structure of the dermal layer was lost and replaced by granulomas. Several non septate fungal hyphae were observed in the dermis.Granuloma formation and myonecrosis were prominent in the centre of the ulcer (Figs.5.88a and b; 5.122).

Liver

The section of liver of the naturally infected *Mystus tengara* showed vacuolation in the hepatocytes and in some regions the hepatocytes were arranged in a chord like arrangement with enlarged sinusoids. Infiltration of blood capillaries were also seen in some regions (Fig. 5.124).

Kidney

Haemorrhages were observed in some areas of the sections of the kidney of naturally infected *Mystus tengara* and no evidence of fungal hyphae. Tubular breakage, tubular necrosis and vacuolation of tubular cells were observed in the section of the kidney (Fig. 5.126).

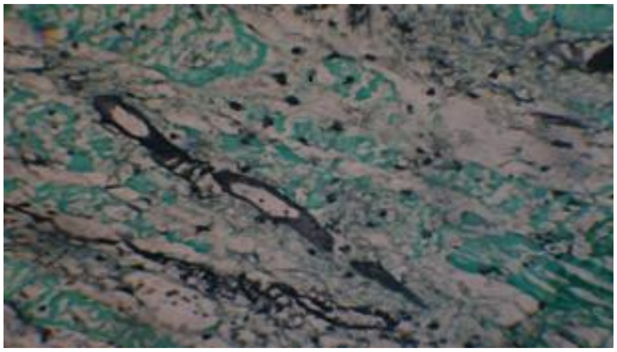

Fig.5.101. Section of ulcer of naturally infected *Cirrhinus mrigala* showing *Aphanomyces* sp. (GMS, x 400).

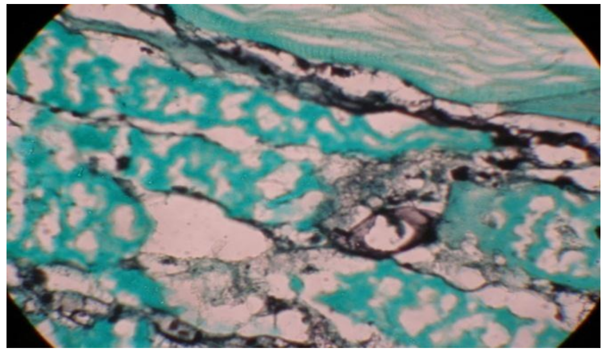

Fig. 5.102. Section of ulcer of naturally infected *Labeo rohita* showing Aphanomyces sp. (GMS x400).

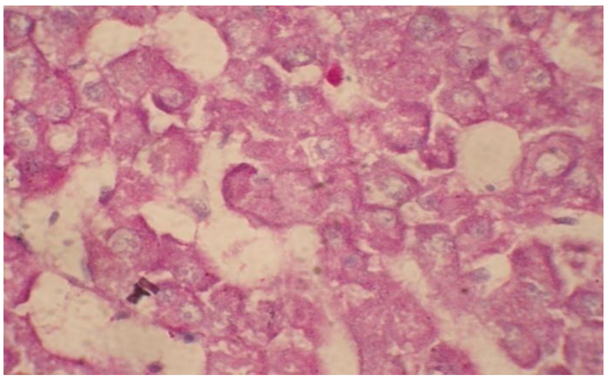

Fig.5.103. Section of liver of naturally infected *Cirrhinus mrigala* (H-E x 400).

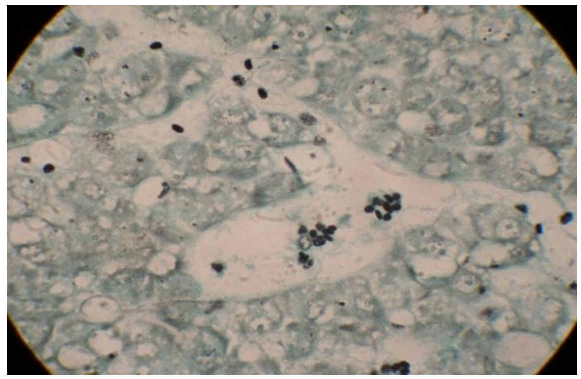

Fig.5.104. Section of liver of naturally infected *Labeo rohita* (PAS x 400).

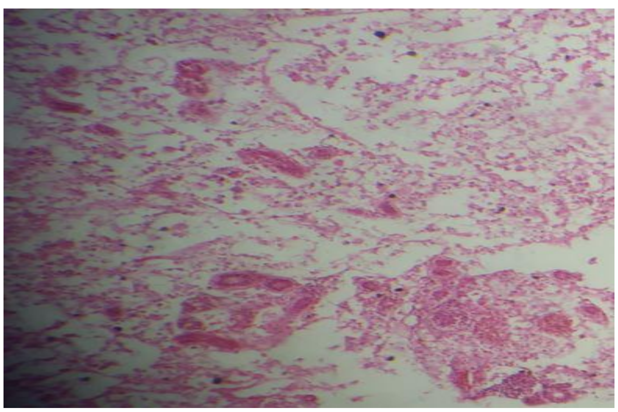

Fig.5.105. Section of kidney of naturally infected *Cirrhinus mrigala* (H-E x 400)

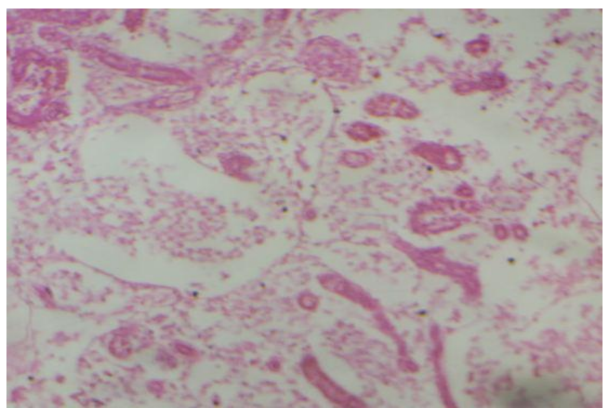

Fig.5.106. Section of kidney of naturally infected *Labeo rohita* (H-E, x 400).

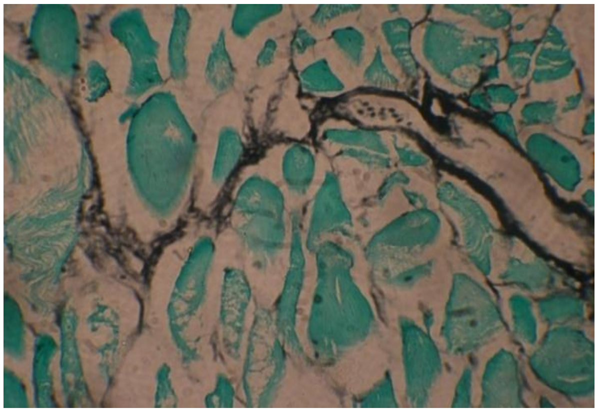

Fig.5.107.Section of ulcer of naturally infected *Labeo bata* showing Aphanomyces (GMS, x 400).

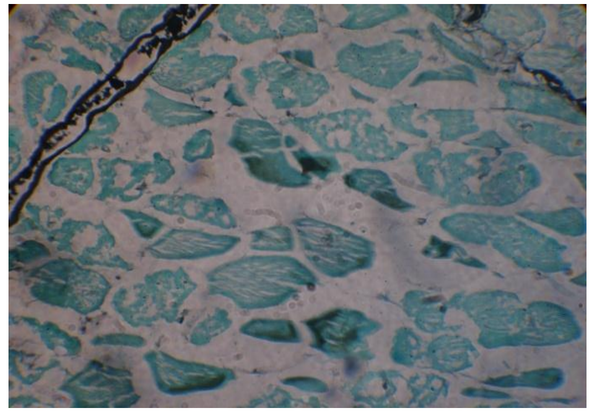

Fig.5.108. Section of ulcer of naturally infected *Catla catla* showing fungus (*Aphanomyces invadans*) hyphae (GMS, x400).

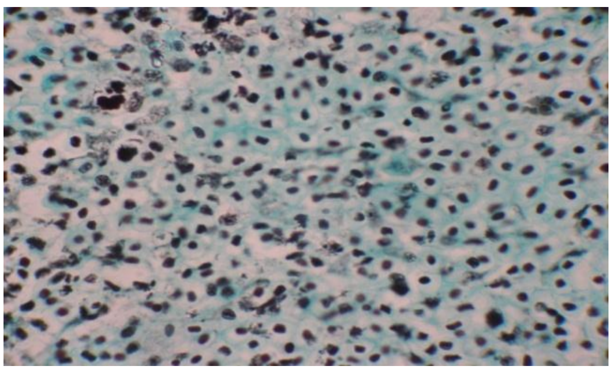

Fig.5.109. Section of liver of naturally infected *Labeo bata* showing necrosis and vacuolation (GMS,x400).

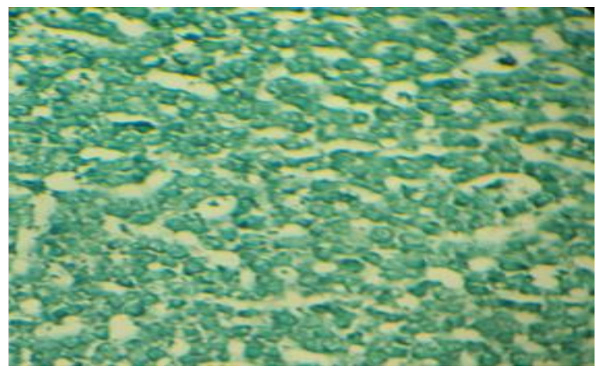

Fig.5.110. Section of liver of naturally infected *Catla catla* showing vacuolation (GMS,x400).

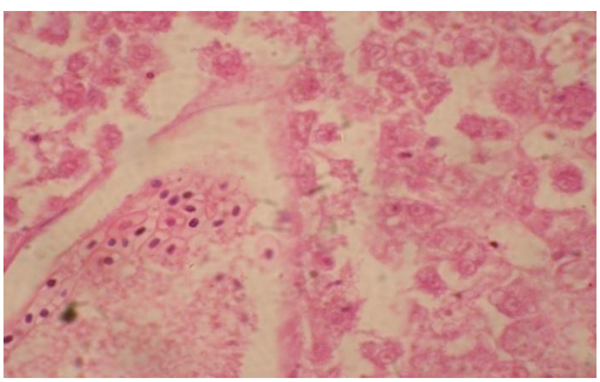

Fig.5.111. Section of kidney of naturally infected *Labeo bata* showing necrotic changes, haemorrhages and tubular vaculation (H-E,x400)

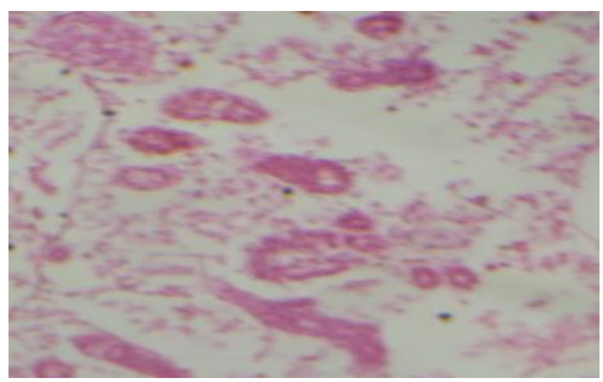

Fig.5.112. Section of kidney of naturally infected *Catla catla* showing necrotic changes, haemorrhages and tubular vaculation (H-E,x400

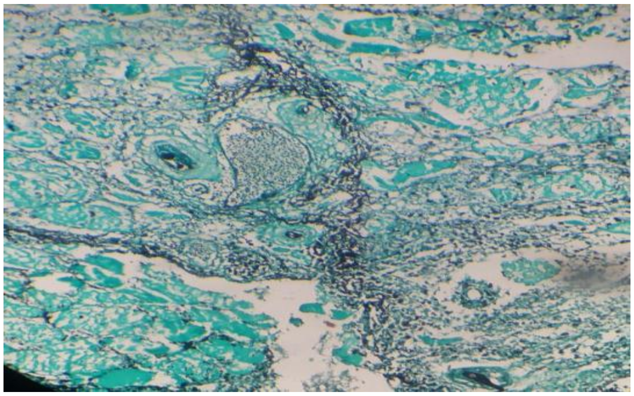

Fig.5.113. Section of the ulcer of naturally infected *Channa striatus* showing the presence of fungal hyphae (GMS, x 400).

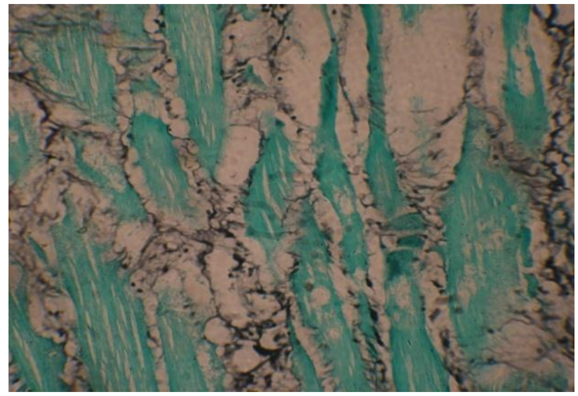

Fig.5.114. Section of muscle of heavily infected *Channa striatus* (GMS, x 400)

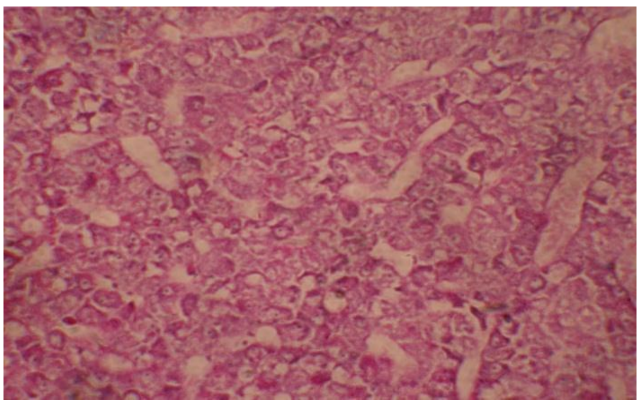

Fig.5.115.Section of liver of *Channa striatus* (H-E, x 400)

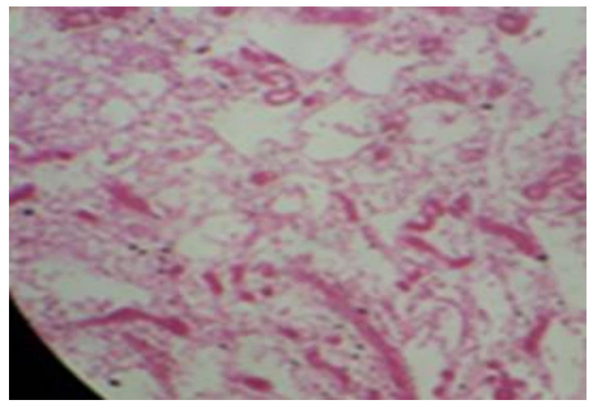

Fig. 5.116. Section of kidney of *Channa striatus* (H-E, x 400)

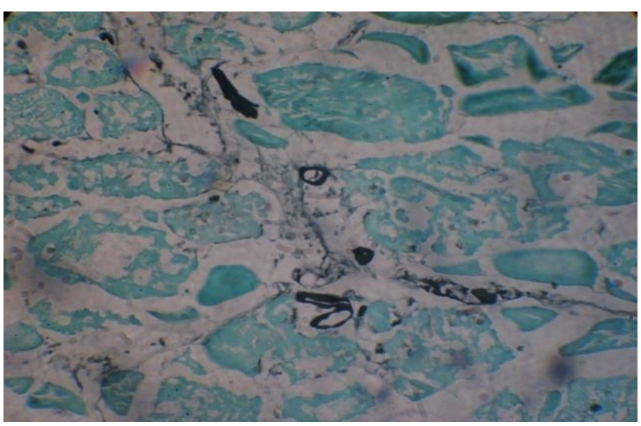

Fig.5.117. Section of muscle of infected *Puntius* sp. with *Aphanomyces* sp.(GMS, x 400)

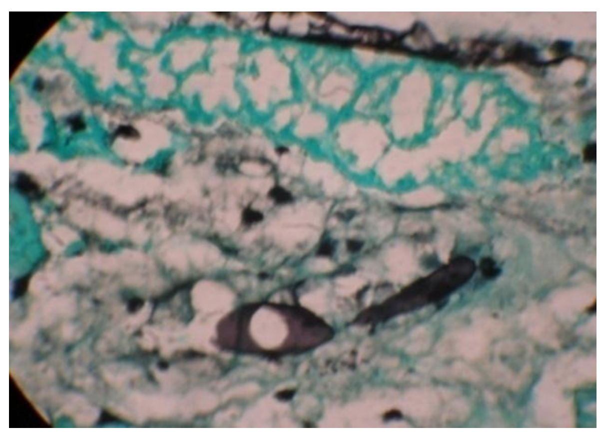

Fig.5.118. Section of ulcer of naturally infected *Catla catla* (PAS,x 400)

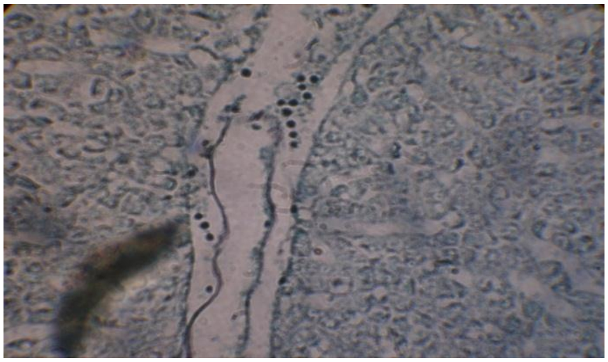

Fig.5.119. Section of liver of *Puntius* sp. (PAS, x400)

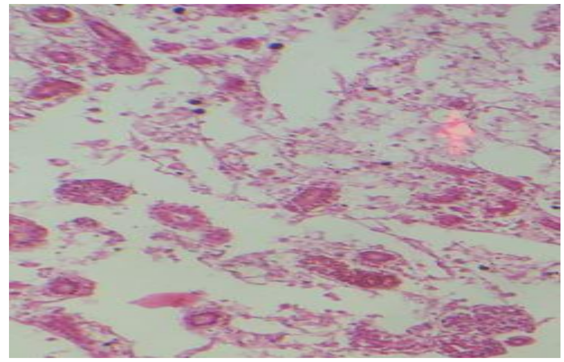

Fig.5.120. Section of kidney of infected *Puntius* sp. (H-E,x400)

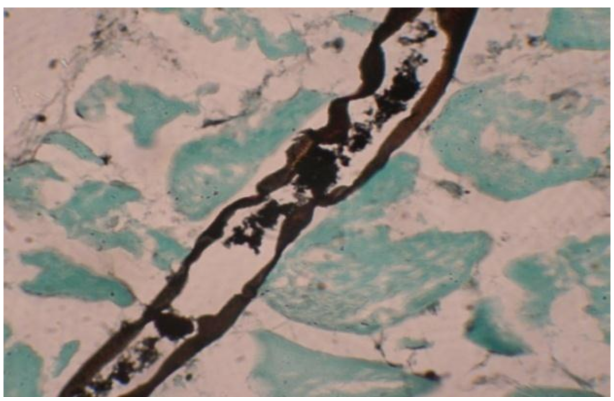

Fig. 5.121. Section of muscle of *Clarias batrachus* showing *Aphanomyces* hyphae(GMS, x 400)

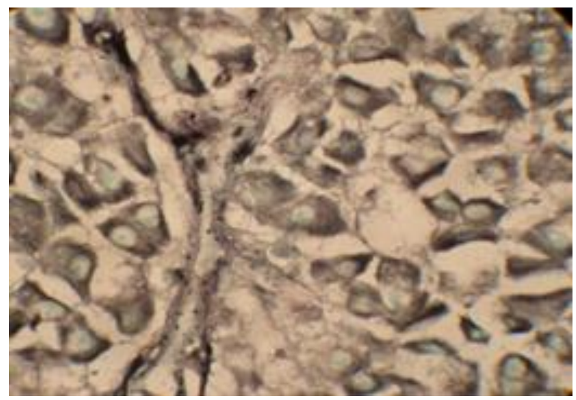

Fig. 5.122. Section of muscle of naturally infected M*ystus tengara* showing granulomatous changes (PAS,x 400)

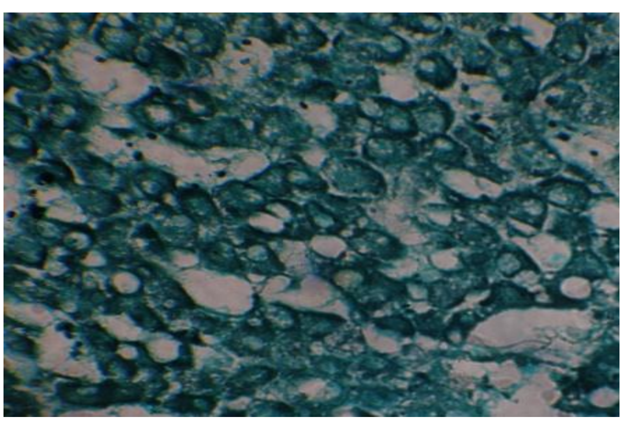

Fig.5.123. Section of liver of *Clarias batrachus* (GMS,x 400)

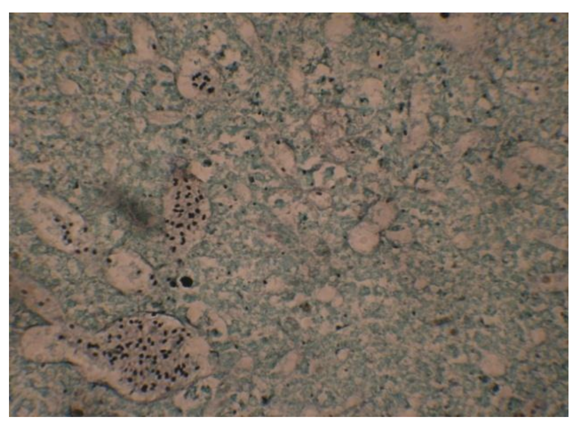

Fig.5.124. Section of liver of naturally infected *Mystus tengara* (PAS,x 400)

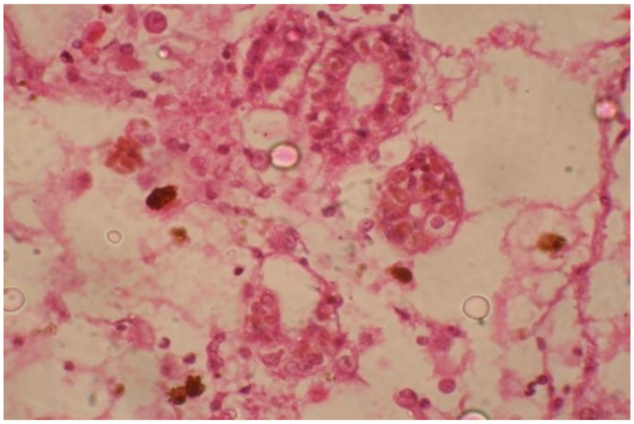

Fig. 5.125. Section of kidney of *Clarias batrachus* (H-E, x 400)

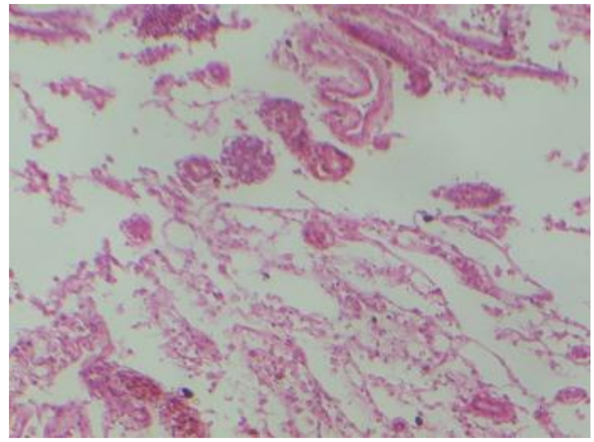

Fig.5.126.Section of kidney of *Mystus tengara* (H-E, x 400)

5.5. Isolation of Bacteria and their characterization

Four types of bacteria were isolated from ulcers of *Cirrhinus mrigala* (Table 5.36). Four types of bacteria were isolated from ulcers of *Catla catla* (Table 5.37). Three types of bacteria were isolated from ulcers of *Channa striatus* (Table 5.38). Four types of bacteria were isolated from ulcers of *Puntius* sp. (Table 5.39). Four types of bacteria were isolated from ulcers of *Mystus tengara* (Table 5.40). Four types of bacteria were isolated from ulcers of *Labeo bata* (Table 5.41).

Results of the morphological observations (Figs. 5.127, 5.128, 5.129, 5.130, 5.131, 5.132, 5.133 and 5.134) and biochemical test of the bacterial isolates from ulcers of different fishes are given in Tables 5.36, 5.37, 5.38, 5.39, 5.40 and 5.41.

Altogether twenty three bacteria were isolated from the ulcers of six infected fishes, out of which fourteen were *Aeromonas hydrophila*, three were *A. caviae*, one was *A. veroni biovar sobria*, two were *Pseudomonas* sp., two were *Micrococcus* sp. and one was *Moraxella* sp..

Out of fourteen *A. hydrophila*, two $Cm_1$and Cm_3 from *Cirrhinus mrigala*, three (Cc_1, Cc_2 and Cc_3) from *Catla catla*, one Cs_1 from *Channa striata*, two (P_1 and P_3) from *Puntius* sp., four (Mt_1,Mt_2,Mt_3 and Mt_4) from *Mystus tengara* and two (Lb_2 and Lb_3) from *Labeo bata* were isolated. Out of three *Aeromonas caviae*, one (Cm_4) from *C. mrigala* and two (Cs_2 and Cs_3) from

C. striata were isolated. *A. veroni biovar sobria,* was isolated only from *Labeo bata*. Two *Pseudomonas* sp. (Cc_4 and Lb_1) were isolated one each from *Catla catla* and *Labeo bata*. Two *Micrococcus* sp. were isolated one each from *Cirrhinus mrigala* (Cm_2) and *Puntius* sp. (P_4). One *Moraxella* sp. was isolated from *Puntius* sp. (P_2) (Table 5.42).

Table 5.36 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Cirrhinus mrigala.*

	Bacteria Isolates			
	Cm_1	**Cm_2**	**Cm_3**	**Cm_4**
Shape	rod	sphere	rod	rod
Occurance	single	single	single	single
	pairs	pairs		
		tetrads		
Size	2.8-3.2x0.75-0.8 µm	1.2-1.6µm diameter	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8µm
Spores	–	–	–	–
Agar Colonies	circular	circular	circular	circular
	smooth	smooth	smooth	smooth
	convex	convex	convex	convex
Gram reaction	–	+	-	-
Motility	+	-	+	+
Growth at:				
25°C	g	m	g	g
30	g	g	g	g
37	m	g	m	m
42	n	n	n	n
Growth at 6% NaCl	-	+	-	-
Indole Production	+	-	+	+
Resistance to Ch	-	-	-	+
VP	+	-	+	-
Nitrate	+	w	+	+
Gas from glucose	+	-	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	0	F	F
Acid from:				
Glucose	+	+	+	-
L-arabinose	+	-	+	+

Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	-	+	-
ODC	-	-	-	-
ADH	+	-	+	+
Pigment production	-	Bright yellow	-	-

+, positive; -, negative;0, neutral, g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation - Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; w, weak.

Table 5.37 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Catla catla.*

	Bacterial isolates			
	Cc_1	**Cc_2**	**Cc_3**	**Cc_4**
Shape	rod	rod	rod	rod
Occurance	single	single	single	single
				pairs
	chains	chains	chains	or chains
Size	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8 µm	2.8-3.2x0.75-0.8 µm	2.2-0.3x0.7-0.8 µm
Spores	-	-	-	-
Agar Colonies	circular	circular	circular	circular
	smooth	smooth	smooth	smooth
	convex	convex	convex	slightly convex /flat
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at:				
25°C	g	g	g	m
30°	g	g	g	g
37°	m	m	m	g
42°	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	+	+	-

Resistance to Ch	-	-	-	-
VP	+	+	+	-
Itrate	+	+	+	+
Gas from glucose	+	+	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	F	F	0
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	-
LDC	+	+	+	-
ODC	-	-	-	-
ADH	+	+	+	+
Pigment production	-	-	-	Yellowish green in King's B medium

+, positive; -, negative; g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation - Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase.

Table 5.38 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Channa striata*.

	Bacterial isolates		
	Cs_1	**Cs_2**	**Cs_3**
Shape	rod	rod	rod
Occurance	single	single	single
Size	2.8-3.3x0.7-0.75µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm
Spores	-	-	-
Agar Colonies	circular	circular	circular
	smooth	smooth	smooth
	convex	convex	convex
Gram reaction	-	-	-
Motility	+	+	+
growth at:			

25°C	g	g	g
30°	g	g	g
37°	m	m	m
42°	n	n	n
Growth at 6% NaCl	-	-	-
Indole Production	+	+	+
Resistance to Ch	-	+	+
VP	+	-	-
Nitrate	+	+	+
Gas from glucose	+	-	-
Oxidase	+	+	+
Catalase	+	+	+
O-F test	+	+	+
Acid from:			
Glucose	+	+	+
L-arabinose	+	+	+
Sucrose	+	+	+
Mannitol	+	+	+
Esculin hydrolysis	+	+	+
LDC	+	-	-
ODC	-	-	-
ADH	+	+	+
Pigment production	-	-	-

+, positive; -, negative;0, neutral; g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation -Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase.

Table 5.39 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Puntius* sp.

	Bacterial isolates			
	P_1	P_2	P_3	P_4
Shape	rod	rod	rod	sphere
Occurance	single	single	single	single
	pairs		pairs	pairs
	chains		chains	tetrads or irregular clusters
Size	2.8-3.2x0.75-0.8µm	1.5-1.7x0.9-1.9 µm	2.8-3.2x0.75-0.8µm	1.2-1.6 µm diameter

Spores	-	-	-	-
Agar Colonies	circular smooth convex	circular smooth convex	circular smooth convex	circular smooth convex
Gram reaction	-	-	-	+
Motility	+	+	+	-
Growth at:				
25°C	g	m	g	m
30°	g	g	g	g
37°	m	g	m	g
42°	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	-	+	-
Resistance to Ch	-	-	-	-
VP	+	-	+	-
Nitrate	+	-	+	W
Gas from glucose	+	-	+	-
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	0	F	0
Acid from:				
Glucose	+	-	+	+
L-arabinose	+	-	+	-
Sucrose	+	-	+	+
Mannitol	+	-	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	-	+	-
ODC	-	-	-	-
ADH	+	-	+	-
Pigment production	-	-	-	Bright yellow colonies

+, positive; -, negative; g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation -Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; w, weak.

Table 5.40 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Mystus tengara.*

	Bacterial isolates			
	Mt_1	**Mt_2**	**Mt_3**	**Mt_4**
Shape	rod	rod	rod	rod
Occurance	single	single	single	single
	pairs	pairs	pairs	pairs
	chains		chains	chains
Size	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm
Spores	-	-	-	-
Agar Colonies	circular	circular	circular	circular
	smooth	smooth	smooth	smooth
	convex	convex	convex	convex
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at:				
25°C	g	g	g	g
30° C	g	g	g	g
37°C	m	m	m	m
42°C	n	n	n	n
Growth at 6% NaCl	-	-	-	-
Indole Production	+	+	+	+
Resistance to Ch	-	-	-	-
VP	+	+	+	+
Nitrate	+	+	+	+
Gas from glucose	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	F	F	F	F
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	+
LDC	+	+	+	+
ODC	-	-	-	-

ADH	+	+	+	+
Pigment production	-	-	-	-

+, positive; -, negative; g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation - Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase.

Table 5.41 Morphological and biochemical characteristics of bacteria isolated from the ulcers of *Labeo bata.*

	Bacterial isolates			
	Lb_1	**Lb_2**	**Lb_3**	**Lb_4**
Shape	rod	rod	rod	rod
Occurance	single	single	single	single
	pairs		pairs	pairs
	chains		chains	chains
Size	2.2-0.3x0.7-0.8 µm	2.8-3.2x0.75-0.8µm	2.8-3.2x0.75-0.8µm	2.5-3.0x0.7-0.8µm
Spores	-	-	-	-
Agar Colonies	circular	circular	circular	circular
	smooth	smooth	smooth	smooth
	convex	convex	convex	convex
Gram reaction	-	-	-	-
Motility	+	+	+	+
Growth at:				
25°c	m	g	g	g
30°	g	g	g	g
37°	g	m	m	m
42°	n	n	n	n
Growth at 6% NaCl	-	-	-	
Indole Production	-	+	+	+
Resistance to Ch	-	-	-	+
VP	-	+	+	+
Nitrate	+	+	+	+
Gas from glucose	-	+	+	+

Oxidase	+	+	+	+
Catalase	+	+	+	+
O-F test	0	F	F	F
Acid from:				
Glucose	+	+	+	+
L-arabinose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Esculin hydrolysis	+	+	+	-
LDC	-	+	+	+
ODC	-	-	-	-
ADH	+	+	+	+
Pigment production	Yellowish green in King's B medium	-	-	-

+, positive; -, negative; g, good growth; m, moderate growth; n, no growth; Ch, cephalothin; VP, Voges-Proskauer reaction; O-F, Oxidation - Fermentation; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase.

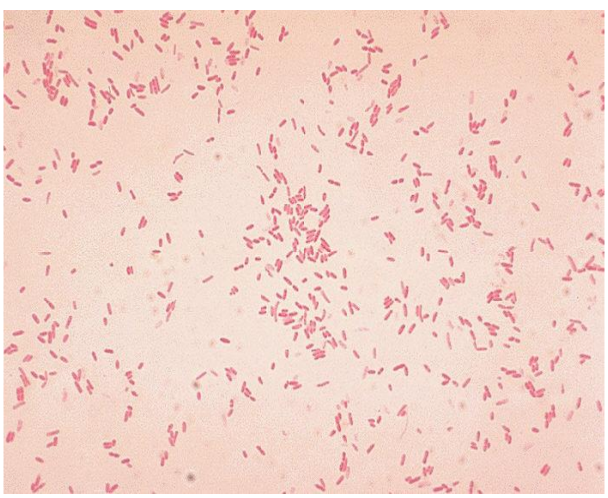

Fig.5.127. *Aeromonas caviae*, Cs_2(X400)

Fig.5.128. *Micrococcus* sp., P_4(x400)

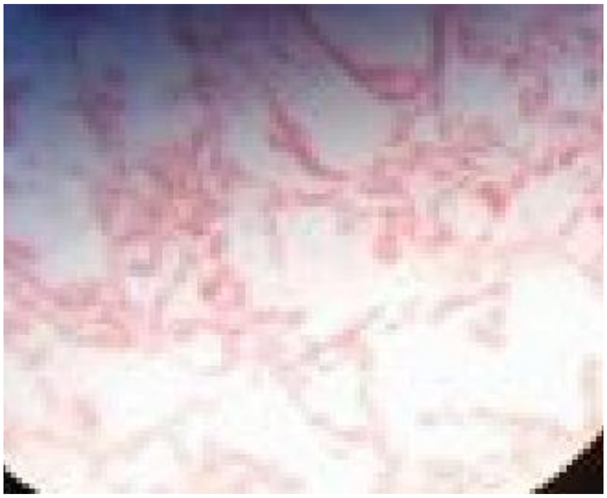

Fig.5.129. *Pseudomonas* sp. ,Cc_4 (X1000)

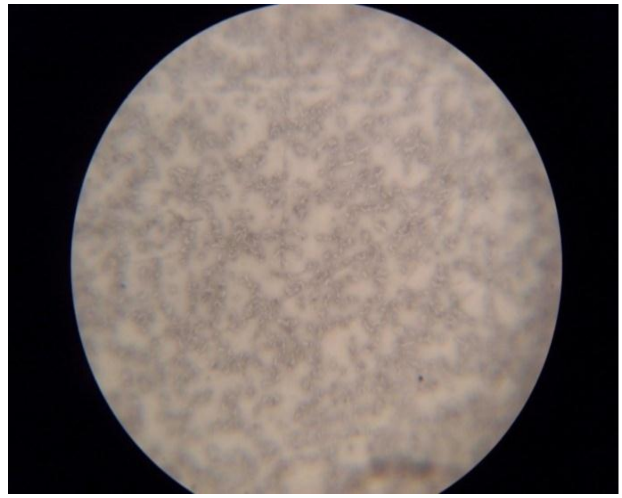

Fig.5.130. *Aeromonas hydrophila* Cm_1, (x400)

Fig.5.131. Pure culture of bacteria in agar slant

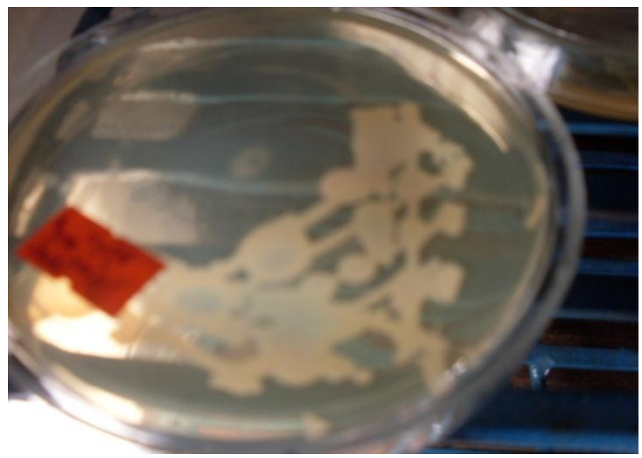

Fig.5.132. Bacterial culture after 48 hrs of incubation

Fig.5.133. Bacterial culture after 48 hrs of incubation

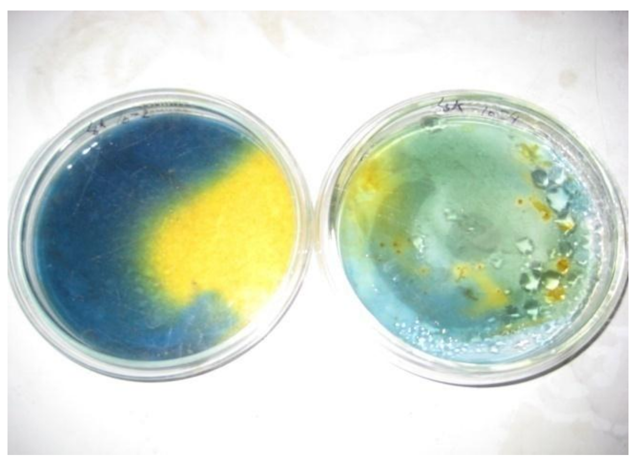

Fig.5.134. *Aeromonas* sp. Confirmatory test

5.6. Pathogenicity test of the isolated bacteria

Among 23 bacterial isolates (Table 5.42), 20 were found to be pathogenic (86.95%) after intramuscular administration of these isolates to the healthy *Heteropneustes fossilis* fish. Two *Micrococcus* spp. (P_4 and Cm_2) and one *Moraxella* sp. could not induce any ulcer at the site of injection in healthy fish. Methodology for inoculation of bacteria in healthy fish is discussed in details under materials and methods.

Moderate to severe ulcers were found at the injection site. Initially red patches appeared at the site of injection, it swelled gradually and after 72 hrs, the skin and underlying muscle layer eroded and it developed into ulcer (Figs.5.135, 5.136 and 5.137). In control set, the fish received only saline suspension. No disease sign was noticed. All fish, in which ulcers developed, however did not die. The moderate ulcers were healed in some fish. No notable change of the swimming behabiour was also observed.

Fig. 5.135. *Heteropneutes fossilis* showing manifestation of ulcer after 24 hrs of intramuscular injection with the culture of *A. hydrophila*, Cm_1.

Fig.5.136. *H. fossilis s*howing manifestation of ulcer after 48 hrs of intramuscular injection with *A. hydrophila*,Cc_4

Fig. 5.137. *H. fossilis* showing manifestation of ulcer after 96 hrs of intramuscular injection with *A. hydrophila*, P2.

Table 5.42 Pathogenic and non-pathogenic bacteria isolated from EUS affected fish.

Bacteria	**No. of isolates**	**Pathogenic**	**Non-Pathogenic**
Aeromonas hydrophila (Cm_1,Cm_3,Cc_1,Cc_2,Cc_3,Cs_1,P_1,P_3,Mt_1,Mt_2,Mt_3, Mt_4, Lb_2 and Lb_3)	14	14	0
Aeromonas caviae (Cm_4, Cs_2, Cs_3)	3	3	0
A. veronii biovar sobria (Lb_4)	1	1	0
Pseudomonas sp. (Cc_4, Lb_1)	2	2	0
Micrococcus sp. (Cm_2, P_4)	2	0	2
Moraxella sp. (P_2)	1	0	1
Total	**23**	**20**	**3**

5.7. Fungus isolation and characterization

In the culture, newly formed hyphae were appeared after 6 hours of incubation at 23-25ºC examined under inverted phase contrast microscope (CKII, Olympus). The growth of the hyphal tips was monitored routinely and next transfer was done after 24 hours. The pure culture was obtained after repeated transfer and finally transferred to GPA and GPYA for routine maintenance. The cotton blue stained ulcer tissue revealed the presence of branched, aseptate fungus mycelium observed through microscope in all samples. The mycelium of fungal isolate grown on GPA and GPYA were also branched, aseptate but narrower than those found in ulcer tissue. It also showed the presence of terminal zoosporangia having a single row of zoospores.

Identification of fungi was done by examining the asexual characteristics and particular characteristics of zoosporangia which were not wider than the hyphae. A single row of primary zoospores was found within the zoosporangia (Figs. 5.138, 5.139, 5.140 and 5.141 of A_1, A_2, A_3 and A_4 respectively).

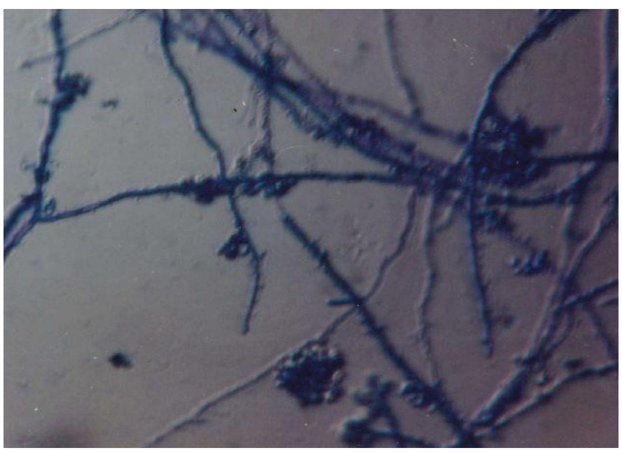

Fig.5.138.Zoosporangia of *Aphanomyces* sp. from ulcer of naturally infected *Cirrhinus mrigala*. (A_1)

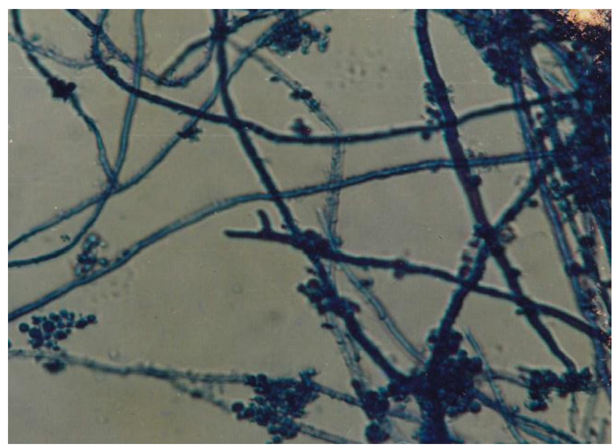

Fig.5.139. Zoosporangia of *Aphanomyces* sp. from ulcer of naturally infected *Catla catla*(A_2)

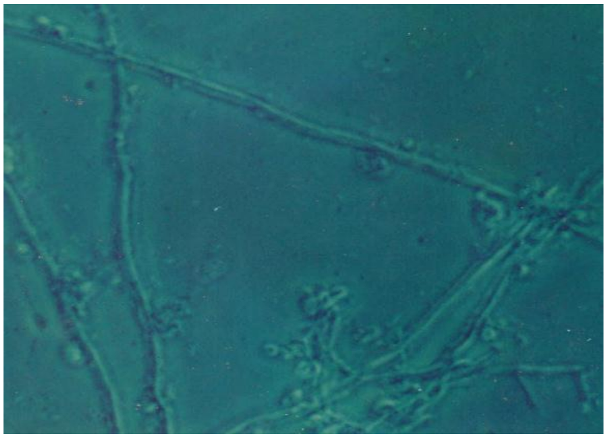

Fig.5.140. Zoosporangia of *Aphanomyces* sp. from naturally infected *Labeo bata* (A_3)

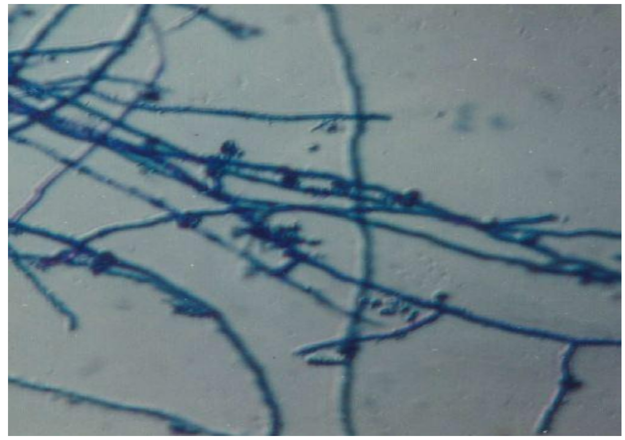

Fig.5.141. Zoosporangia of *Aphanomyces* sp. from naturally infected *Puntius* sp. (A_4)

The fungal isolates grew slowly in culture media from 25-30ºC but did not grow at 37ºC. *Aphanomyces* spp.were isolated from ulcer tissues of *Cirrhinus mrigala, Channa striatus, Labeo rohita, Labeo bata, Catla catla, Mystus* sp.*, Puntius* sp. and *Clarias batrachus.*

5.8. Pathogenicity test of isolated fungus *Aphanomyces* sp. in *Heteropneustes fossilis*.

Healthy fish showed the red spot at the site of injection after 48 hrs of inoculation.Then the red spot increased in size and ulcer developed after 72 hrs. Among treated fishes 43.33% mortality were recorded during 15 days observation. In control set of fish no ulcer formation and mortality were observed.

Fig.5.142. *H. fossilis s*howing manifestation of ulcer after 48 hrs of intramuscular injection with *Aphanomyces* sp. zoospores.

Fig.5.143. *H. fossilis s*howing manifestation of ulcer after 72 hrs of intramuscular injection with A*phanomyces* sp. zoospores.

Table 5.43 shows percentage mortality and nature of ulcer formation in *Heteropneustes fossilis* injected intramuscularly with saline suspensions of *Aphanomyces* sp. zoospores from *Cirrhinus mrigala* (A_1).

	No. of fishes	No. of fishes dead	Nature of ulcer		Mortality
			Moderate (erosion in epidermis)	Advanced (necrotic)	
Control	30	0	0	0	0
Saline suspension of *Aphanomyces* sp. zoospores (A_1)	30	13	6	11	43.33%

5.9. Histopathology of experimentally infected fish *Heteropneustes fossilis* with isolated Zoospores of *Aphanomyces* sp. (A_1).

Ulcer

The epidermis and dermis of skin tissues of the ulcerated area were lost but severe myonecrosis and granuloma were seen when dermis was present.In some cases haemorrhages were observed. Aseptate fungal hyphae were stained black with Grocott metenamine stain in the dermis and underlying musculature (Fig.5.144).

Liver

Some areas of the liver hepatic cells, vacuolation and chord like arrangement with enlarged sinusoids were observed. No fungus was detected but haemorrhages were also observed in some areas (Fig.5.145).

Kidney

Necrotic changes were observed in some haematopoietic areas but no fungal hyphae were detected in kidney tissues (Fig.5.146).

The sections of muscle, liver and kidney of control fish (*Heteropneustes fossilis*) are shown in figs. 5.147, 5.148 and 5.149).

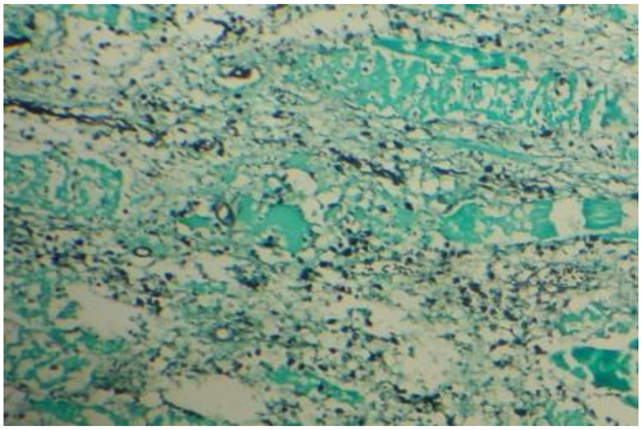

Fig.5.144. Section of ulcer of experimentally infected *H. fossilis* with *Aphanomyces* sp. zoospores (GMS, x 400)

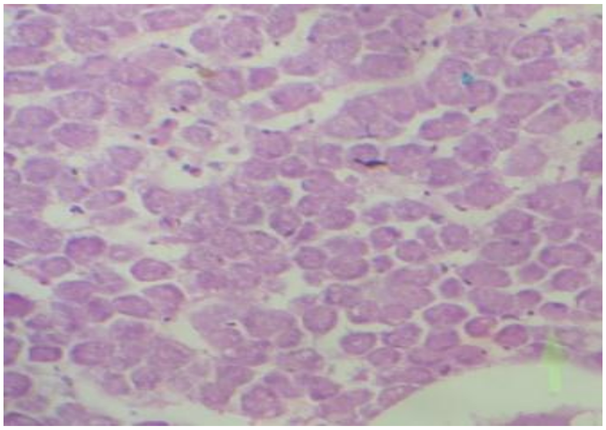

Fig.5.145. Section of liver of experimentally infected *H. fossilis* (H-E,x 400)

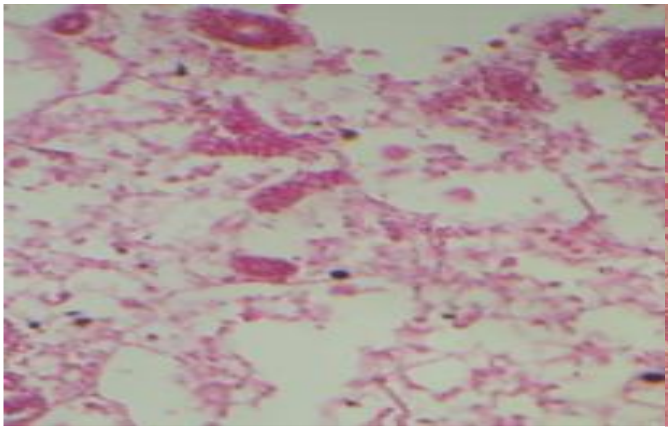

Fig.5.146. Section of kidney of experimentally infected (*H. fossilis*)(H-E,x400)

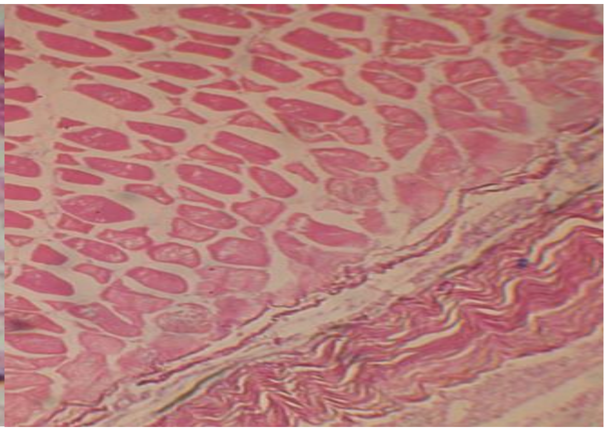

Fig.5.147. Section of normal muscle of *H. fossilis* (control)

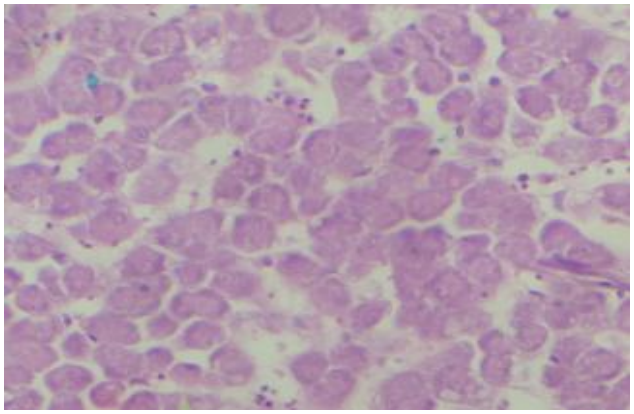

Fig.5.148. Section of normal liver of *H. fossilis* (control)

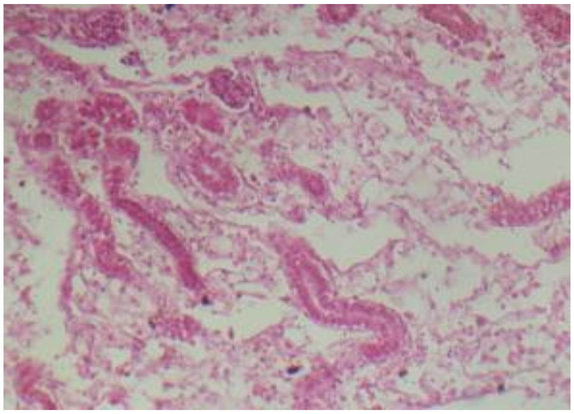

Fig.5.149. Section of normal kidney of *H. fossilis* (control)