J. Himalayan Ecol. Sustain. Dev. Vol 5. (2010) *ISSN 0973-7502*

NYMPHOIDES PELTATUM AS THE INDICATOR OF EXCESSIVE NUTRIENT LOADING IN THE ANTHROPOGENICALLY IMPACTED WATERS OF DAL LAKE – KASHMIR

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ABSTRACT

An unfavorable alteration in the condition and composition of Dal lake due to nutrient loading has made it less suitable in its natural state. Enhanced nutrient loading is most clearly reflected by increased macrophytic growth. Macrophytes form an important part of a Lake Ecosystem, but when these grow in excess the lake activities get hampered. The present study deals with *Nymphoides peltatum* as the indicator of excessive nutrient loading in the anthropogenically impacted waters of Dal lake – Kashmir. The entire study period was divided into two phases i.e. peak growth phase and senescence phase. The concentration of biomolecules was found maximum in the *Nymphoides peltatum* of site 1 which was most polluted or nutritionally rich, also the concentration decreased from peak growth phase to senescence phase. The study has shown a positive correlation between nutrient load in form of N and P and concentration of various biomolecules resulting in the increased biomass development.

Key words: Dal lake, Nutrient loading, macrophytes, *Nymphoides peltatum*, senescence, biomolecules, biomass

INTRODUCTION

Kashmir valley in India is famous for its mountains reaching to a height of 6,000 meters. Their elevations and depressions have created numerous lakes. Many of these lakes have been affected by the pollution due to domestic waste, agricultural waste, and sewage etc. leading to nutrient enrichment called eutrophication. Number of workers in past like Zutshi and Vass (1978), Trisal (1987) and Khan (1988) had worked on the physico-chemical parameters of water to determine the trophic status of water bodies. The wastes entering a water body incorporate nutrients like nitrogen and phosphorus into it, thus changing an oligotrophic lake into eutrophic. One molecule of phosphorus promotes the incorporation of 7 molecules of Nitrogen and 40 atoms of Carbon in aquatic algae (Wetzel and Manny, 1975 and Kasige and Takashi, 2008). These nutrient rich waters are infested with macrophytes (Best and Visser, 1987; Kroger *et al.*, 2007).

The water of Dal lake has deteriorated considerably in the last two decades due to opening up of catchment area and unplanned urbanization. The main environmental issues are excessive weed growth, reduction in water quality, enrichment of water and

high microbial activity. Watershed management has resulted in excessive Total phosphorus load (TP).

The present study is a step towards analyzing *Nymphoides peltatum* as the indicators of excessive nutrient loading in antropogenically impacted waters of Dal lake – Kashmir. *Nymphoides Peltatum* was found covering large area of Dal Lake. Its biochemical parameters showed a variation with respect to the sites and seasons.

MATERIAL AND METHODS

Sampling

The samples of water and experimental plant (*Nymphoides peltatum*) were collected from the Gagribal basin of Dal lake during the period of 5 months from July to November 2006 and were analyzed for their physico-chemical and biochemical parameters respectively. The three sites selected for sampling were House boat area as site 1(nutrient rich), along Boulevard side as site 2 (less nutrient present) and Kabotar khanna as site 3 (intermediate nutrient composition). The water samples were collected in plastic bottles of 1.5L capacity and were analyzed for various parameters using the method derived by APHA (1998).

A. Physical Parameter

1. Temperature

Water temperature was recorded with the help of a "standard centigrade thermometer" (0 - 50°).

B. Chemical Parameters

1. pH

pH of the water sample was measured with the help of pH meter (181- E).

2. Dissolved Oxygen (DO)

Modified Winkler's method was followed for the estimation of DO.

3. Biological Oxygen Demand(BOD)

It was determined by measuring the oxygen content of the sample by Winkler's method. The same sample was subjected to incubation for 5 days and again analyzed for DO using Winkler's method. The difference between the initial and the final concentration of oxygen gave the BOD of sample.

4. Free CO_2

It was determined by the titrimetric method, using Phenolphthalein as indicator.

5. Total Alkalinity

This parameter was determined by titrimetric method using Phenolphthalein and methyl orange as indicator.

6. Total Hardness

The total hardness of the water samples was estimated titrimetrically using EBT as indicator.

7. Chloride

Chloride content of the sample was determined titrimetrically using potassium chromate indicator.

8. Total Phosphorus

It was measured spectrophotometrically using SL – 150 spectrophotometer at 690 nm.

9. Nitrate Nitrogen

It was determined by Diphenyl sulphuric acid method. The color intensity was measured at 700 nm.

C. Biochemical Parameters

Preperation of Homogenate

The homogenate was prepared by adding 9ml of distilled water to a fine paste of 1gm leaves of *Nymphoides peltatum*, and then subjected to centrifugation. 2ml of this homogenate was mixed with 8ml of acetone in a 10 ml volumetric flask. The material was subjected to centrifugation at 10,000 rpm for 10 minutes at 4°centigrade. The color intensity was measured at different wavelengths for different parameters.

Pigment Estimation

Chlorophyll and carotenoids were extracted in 80% acetone and estimated photo metrically, according to the methods given by Strain *et al.* (1971) and Duxbury and Yentech (1956) respectively.

Carbohydrate (Free Sugars)

Free sugars were determined by Montgomery (1985).

Starch

Starch content of plant was determined by method of Agarwal *et al.* (1982).

Amino Acids

Amino acids were estimated by the method given by Lee and Takahashi (1960).

Proteins

The Protein content of plant material was determined by the method given by Lowry *et al.* (1951).

STATISTICAL ANALYSIS

Statistical analysis was performed by measuring standard deviation for determining uniformity of the observations (Banergee, 2006). A linear regression was used to correlate the effect of various physico-chemical parameters on the biomass of *Nymphoides peltatum.*

RESULTS

Tables 1, 2 and 3 showed the values of the physico – chemical parameters of waters of Dal lake analyzed from July to November 2006. Temperature showed a decrease from July to November. The pH values also showed a general decline from peak growth phase to senescence phase (Fig. 1). Also site 1, has shown greater pH compared to site 2. DO show a marked decrease in its values from July to November. DO of waters of site 2 was found to be higher than that of site 1. As compared to site 2 the water samples of site 1 had higher BOD. Free carbon dioxide and total alkalinity showed a marked decrease in its values from peak growth phase towards senescence phase. The parameters viz, total hardness, chloride content, nitrate nitrogen and total phosphorus has been found maximum in site 1, and these parameters have also shown a considerable decrease from peak growth phase towards senescence phase. The decrease has been more in site 2 as compared to site 1. Table 4, 5 and 6 showed the values of Biochemical parameters of *Nymphoides peltatum* growing in Dal Lake. Maximum concentration of all the

biochemical parameters was found in the *Nymphoides* of site 1 as compared to other two sites (Fig. 2). Also there was a marked decrease from peak growth phase to senescence phase. Besides it, the relationship between the physico-chemical and biomolecule parameters was also determined for amino acids, carbohydrates and chlorophyll, the results of which are depicted in Figs. 3-5.

Table 1. Physicochemical parameters of Dal lake waters (Site 1)

Parameter	July Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Temp (°C)	26.0 ± 0.94	26.0 ± 1.22	18.0 ± 0.71	18.0 ± 0.94	4.0 ± 0.35	3.0± 0.18
pH	8.9±0.16	8.8±0.48	8.4±0.21	7.5±0.18	7.4±0.12	7.4±0.12
DO (mg/L)	7.0±0.38	7.1±0.32	8.0±0.14	8.9±0.9	9.0±0.15	9.1±0.38
BOD (mg/L)	164.1±4.6	158.5±6.6	138.1±5.8	118.2±4.7	104.2±2.9	102.9±2.2
CO_2 (mg/L)	20.1±1.13	19.8±1.30	19.4±1.00	19.2±1.30	18.6±2.0	18.4±1.12
Total Alkalinity (mg/L)	96.9±3.40	95.3±4.00	95.2±3.70	93.1±3.30	86.8±3.2	76.9±3.10
Total Hardness (mg/L)	143.3±2.7	132.3±4.4	128.4±2.9	94.1±3.60	84.9±4.3	82.5±3.30
Chloride (mg/L)	43.9±3.10	42.6±2.60	41.1±3.50	40.3±2.10	39.3±1.7	39.1±1.50
Nitrate Nitrogen (µg/L)	425.2±11.18	394.3±11.7	380±15.8	264.1±6.2	232.1±9.4	230.3±7.9
Total Phosphorus (µg/L)	220.1±15.81	218.6±13.99	214.3±10.38	211.6±12.66	207.1±13.0	206.9±12.45

Table 2. Physiochemical parameters of Dal waters (Site 2)

Parameter	July Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Temp(°C)	26.0±0.35	26.0±0.79	19.0±0.79	18.0±1.00	5.0±0.50	4.0±0.18
pH	7.7±0.15	7.5±0.17	7.4±0.28	7.2±0.24	7.2±0.24	7.0±0.15
DO (mg/L)	8.3±0.41	8.9±0.15	9.4±0.15	9.7±0.36	9.8±0.36	9.9±0.12
BOD (mg/L)	145.2±6.20	139.2±7.50	111.4±3.09	98.5±1.67	95.6±4.60	94.3±3.50
CO_2 (mg/L)	18.9±2.1	18.4±2.0	17.6±1.14	16.9±1.10	16.4±0.7	15.7±0.8
Total Alkalinity (mg/L)	91.8±2.4	90.3±3.1	90.2±3.0	88.2±3.5	79.1±3.8	74.5±2.7
Total Hardness (mg/L)	129.2±3.4	126.3±3.2	113.1±2.5	72.2±4.3	64.6±3.8	64.6±4.5
Chloride (mg/L)	42.2±1.60	41.8±2.3	40.2±2.8	39.6±1.1	39.1±1.1	38.4±2.9
Nitrate Nitrogen (µg/L)	390.6±8.01	242.4±9.91	228.2±10.2	216.2±12.09	118.5±9.15	112.5±3.04
Total Phosphorus (µg/L)	216.5±12.6	216.2±1.09	212.1±14.7	207.4±13.03	204.5±10.30	204.2±10.30

Table 3. Physicochemical parameters of Dal lake waters (Site 3)

Parameter	July Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Temperature (°C)	27.0±0.79	26.0±0.61	19.0±0.35	18.0±0.71	5.0±0.71	4.0±0.18
pH	8.2±0.15	8.1±0.16	8.0±0.16	7.5±0.15	7.4±0.3	7.4±0.3
DO (mg/L)	7.1±0.15	7.6±0.30	8.9±0.15	9.0±0.5	9.1±0.22	9.5±0.61
BOD (mg/L)	159.4±6.70	153.1±5.11	113.2±3.30	112.6±4.3	102.6±4	99.7±3.9
CO_2 (mg/L)	19.2±1.5	18.6±0.9	17.9±0.5	17.1±0.6	16.4±0.6	15.7±0.6
Total Alkalinity (mg/L)	94.2±3.20	92.1±2.1	93.6±2.7	88.4±3.4	84.1±3.5	75.5±2.0
Total Harndess (mg/L)	135.1±3.5	130.8±4.6	120.2±4.1	90.4±3.6	80.6±4.6	80.2±4.1a
Chloride (mg/L)	43.4±3	41.9±3.2	40.4±2.9	39.8±2.3	39.2±1.4	38.8±2.3
Nitrate Nitrogen (µg/L)	411.5±8.9	382.1±12.4	370.4±15.8	244.5±7.7	218.1±8.9	203.5±10.9
Total Phosphorus (µg/L)	218.6±13.9	217.4±13	213.3±10.8	2209.9±15	206.7±11.1	206.7±11.1

Biochemical parameters of *Nymphoides peltatum* of Dal lake (Table 4)

Parameter	July +Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Chlorophyll-a (µg/ml)	5.42±0.45	4.82±0.12	4.32±0.20	3.62±0.23	3.12±0.16	3.10±0.07
Chlorophyll – b (µg/ml)	1.40±0.079	0.80±0.06	0.31±0.014	0.10±0.0007	0.003±0.0007	0.003±0.0007
Total chlorophyll (µg/ml)	5.63±0.13	5.13±0.09	4.63±0.08	3.93±0.08	3.43±0.12	3.33±0.10
Carotenoids (µg/ml)	1.86±0.044	1.23±0.063	0.751±0.038	0.62±0.032	0.62±0.032	0.41±0.016
Amino Acids (mg/ml)	4.94±0.11	4.44±0.051	3.95±0.13	3.81±0.15	3.81±0.152	3.75±0.127
Proteins (mg/ml)	1.14±0.08	0.64±0.032	0.24±0.016	0.12±0.01	0.98±0.075	0.88±0.070
Free sugars (mg/ml)	14.42±0.71	13.61±0.71	13.12±0.71	12.42±0.77	12.03±0.75	12.01±0.7
Starch, (mg/ml)	13.15±0.71	10.21±0.26	9.71±0.15	8.45±0.13	7.34±0.11	7.05±0.11

Table 5. Biochemical parameters of *Nymphoides peltatum* of Dal lake

Parameter	July Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Chlorophyll-a (µg/ml)	2.38±0.095	1.78±0.09	1.28±0.00	0.58±0.07	0.08±0.007	0.08±0.007
Chlorophyll – b (µg/ml)	1.10±0.08	0.501±0.02	0.003±0.0007	0.002±0.00	0.001±0.00	0.001±0.00
Total chlorophyll (µg/ml)	2.12±0.075	1.52±0.091	1.02±0.048	1.01±0.048	1.01±0.048	1.01±0.048
Carotenoids (µg/ml)	1.21±0.032	0.61±0.029	0.30±0.016	0.098±0.01	0.093±0.001	0.009±0.00
Amino Acids (mg/ml)	2.88±0.153	2.38±0.091	1.87±0.108	1.55±0.105	1.32±0.032	1.31±0.125
Proteins (mg/ml)	1.08±0.085	0.58±0.058	0.098±0.007	0.085±0.007	0.082±0.007	0.068±0.007
Free sugars (mg/ml)	12.92±1.00	11.15±0.72	10.25±0.50	10.18±0.72	9.12±0.71	9.11±0.17
Starch (mg/ml)	10.24±0.19	6.43±0.09	6.35±0.33	5.95±0.16	5.34±0.25	5.22±0.26

Table 6. Biochemical parameters of *Nymphoides peltatum* of Dal lake

Parameter	July Peak	August Growth Phase	September Senescence	October Phase	Nov 1st Week	Nov 4th Week
Chlorophyll-a (µg/ml)	2.48±0.08	1.88±0.09	1.37±0.08	0.68±0.04	0.18±0.007	0.14±0.007
Chlorophyll – b (µg/ml)	1.121±0.07	0.591±0.03	0.091±0.02	0.070±0.02	0.002±0.0005	0.002±0.0005
Total chlorophyll (µg/ml)	2.67±0.049	1.97±0.054	1.47±0.049	1.33±0.031	1.21±0.041	1.18±0.025
Carotenoids (µg/ml)	1.43±0.025	0.73±0.034	0.33±0.022	0.13±0.016	0.12±0.016	0.10±0.009
Amino Acids (mg/ml)	3.72±0.33	3.42±0.12	2.88±0.10	1.62±0.10	1.46±0.08	1.41±0.096
Proteins (mg/ml)	1.124±0.060	0.942±0.063	0.082±0.008	0.079±0.007	0.069±0.00	0.053±0.00
Free sugars (mg/ml)	13.47±0.34	13.15±0.19	13.05±0.11	12.11±0.17	10.22±0.26	9.93±0.05
Starch (mg/ml)	10.95±0.97	7.22±0.26	6.75±0.18	6.24±0.19	5.92±0.15	5.82±0.16

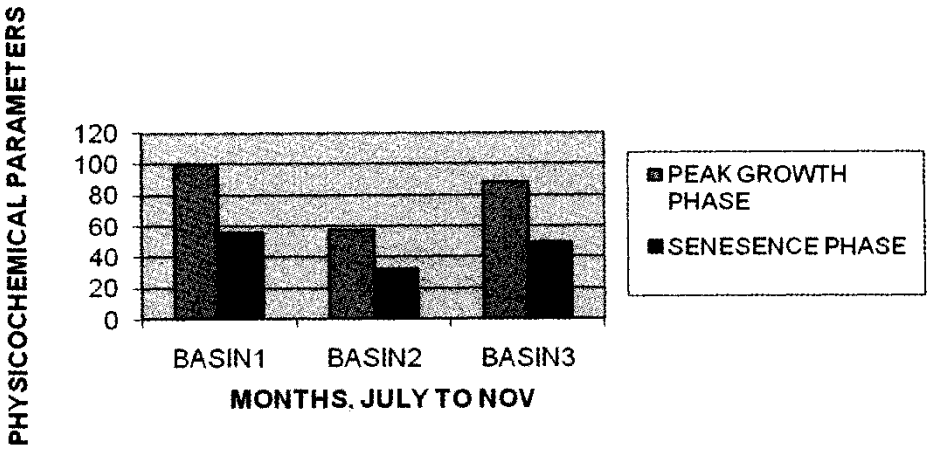

Fig. 1. Variation in physicochemical parameters of water of study area during peak growth phase and senescence phase

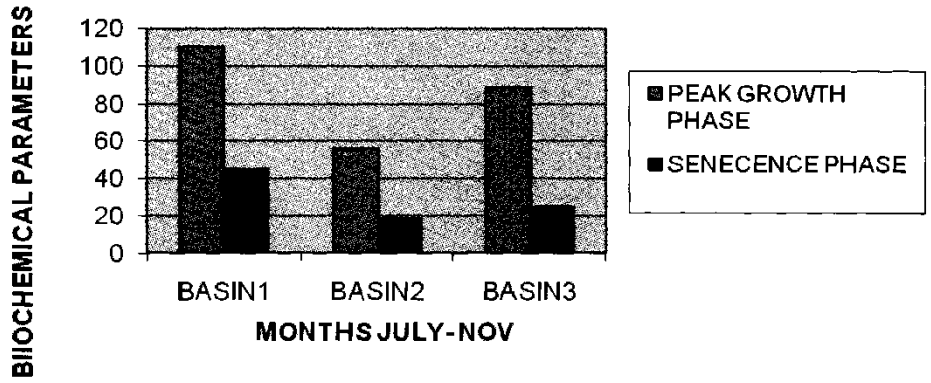

Fig. 2. Variation in biochemical parameters of *Nymphoides peltatum* during peak growth phase and senecense phase

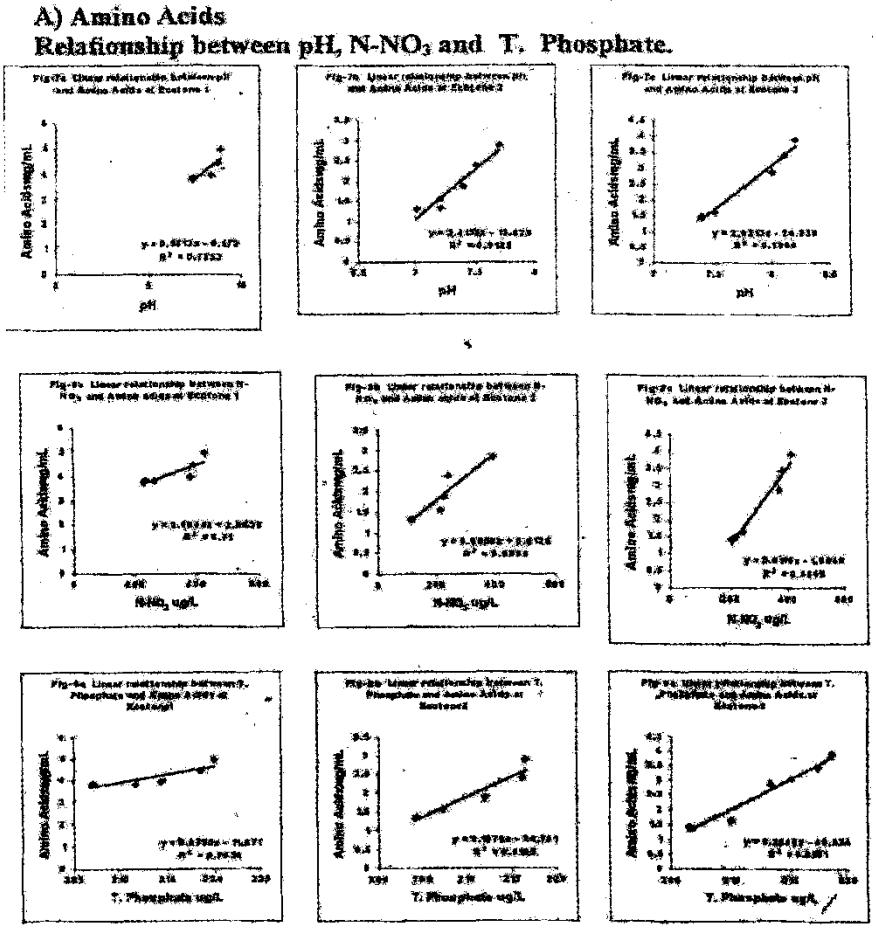

Fig. 3 . Linear relationships for biomolecules with basic physico-chemical parameters

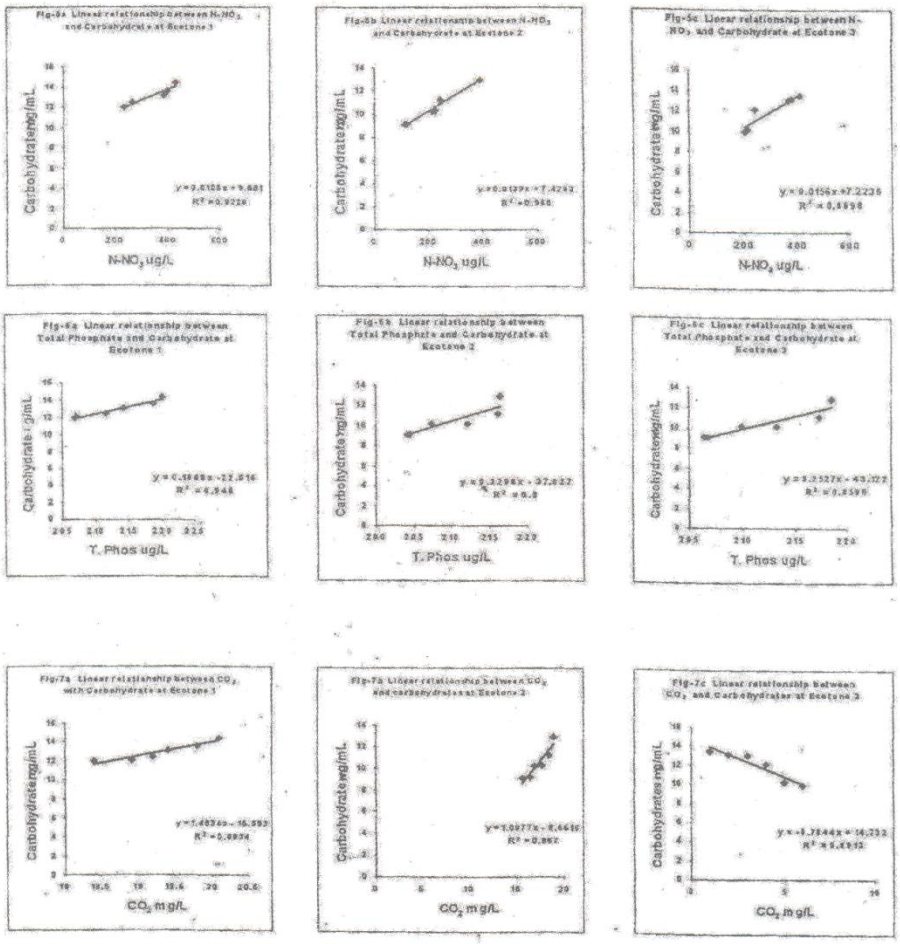

Fig. 4 . Carbohydrates relationship between N-NO_3, T. phosphate and CO_2

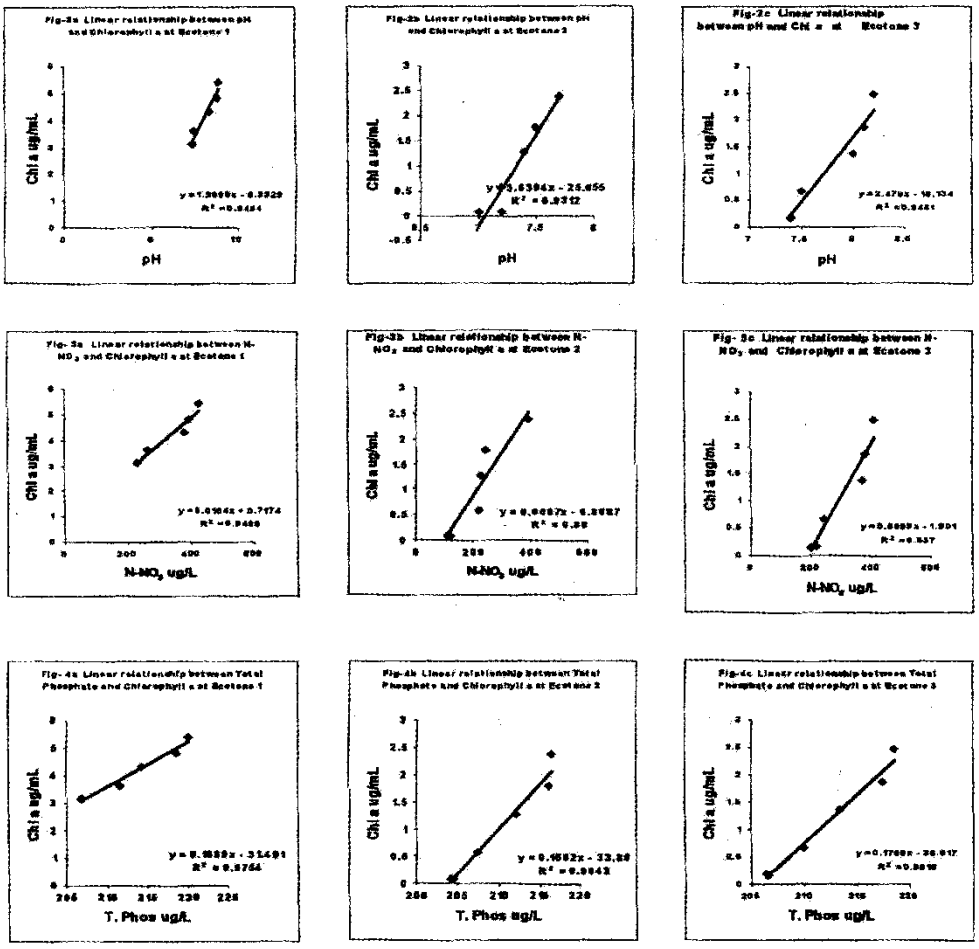

Fig. 5. Chlorophyll: Relationship between Temp, pH, N-NO_3, T. phosphate

DISCUSSION

The present investigation was carried out to analyze *Nymphoides peltatum* as the indicators of excessive nutrient loading in anthropogenically impacted waters of Dal Lake – Kashmir. It involved the analysis of physico – chemical parameters of macrophyte infested water and biochemical parameters of *Nymphoides peltatum*. The water temperature of the selected site varied from 27°C in July to 4°C in November. This might have been due to the fact that, the water and air temperature go more or less hand in hand as has been suggested by Macan (1958). The pH value of water showed a significant decrease from peak growth phase to senescence phase. On site wise analysis, comparatively low pH was recorded where human activities were less. This has earlier been reported by Vass (1979). The decrease in B.O.D from peak growth phase to senescence phase might be attributed to the decrease in the rate of decomposition in senescence phase and decrease in the input of organic wastes. The B.O.D of site 1 was found to be higher than site 2, due to high nutrient loading. Decrease in free carbon dioxide content might have been due to increase in oxygen content and decrease in rate of decomposition in senescence phase. The water of site 2 showed higher values of chloride than the waters of site 1, which may be due to the discharge of waste. The observed change in total alkalinity of the water of Dal Lake might be attributed to location, seasonal change, rainfall, waterman's activity and natural bottom deposits (Wanganoo *et al.*, 1984). Higher concentrations of Calcium and Magnesium in waters of site 1 might be attributed to the release of sewage, which can be supported by the findings of Dakshini and Soni (1979).

Decrease in the content of Nitrate nitrogen from peak growth phase to senescence phase might be supported by the findings of Ryther and Dustan (1971) who reported lowest values in winter and higher during summer in Kalinadi. This may also depend on latitude, basin, morphology, and drainage of the area. Site 2 showed comparatively lower values of Nitrate-nitrogen and total phosphorus which might be due to low input of sewage and domestic waste.

A decrease in pigments has been observed from peak growth phase to senescence phase, also the pigments of Nymphoides of site 1 have shown a higher value as compared to site 2, which can be attributed to the change in the intensity of light, as the rate of photosynthesis is directly proportional to the intensity of light (Singh and Srivastava, 1985). pH has been found to increase as the plants increase their photosynthetic activity (Chang, 1980) and has been found to be low where human activities were low. Nitrogen and Phosphorus level has been found to be higher in site 1 which has resulted in higher chlorophyll content. Nitrogen is an important component of the structure of chlorophyll, also the decrease in Mg^{2+} concentration has resulted in less formation of chlorophyll Magnesium phosphorus metal complex and in enzymatic transformation. The highest concentration of amino acids, proteins and carbohydrates in peak growth phase in *Nymphoides* of site 1 is probably related to increased

availability of N and P as these nutrients contribute to the biosynthesis of these biomolecules and are a part of enzymes as ATP and NADP involved in these processes (Salisbury and Ross, 1992). Decrease in the concentration of amino acids and proteins might be attributed to decrease in rate of photosynthesis, since $NADPH_2$ produced in light reaction reduces nitrate to amino acids. It can be further attributed to the decrease in enzymatic activity and low uptake. Barbieri and Esteves (1991) observed a positive relationship between nitrogen and phosphorus availability and protein concentration. Present analysis is in agreement with it. Phosphorus contributes to the biosynthesis of Proteins (Tahirruzaman and Kushari, 1989).

The decrease in carbohydrate (free sugar) and starch can be attributed to the decrease in temperature which influences the rate of decomposition (David and Hanlon, 1982).

ACKNOWLEDGEMENTS

I express my sincere gratitude to my Supervisor Dr. B. A. Ganai, Reader, Department of Biochemistry, University of Kashmir for guiding me through out this project. His knowledgeable supervision and evaluation is highly acknowledged, without whom this could not have been possible.

I am also thankful to the teaching staff of the Department of Environmental Sciences, S.P. College. My special thanks are due to Dr. Sabah – ul – Solim (Senior Scientist Lakes and Water ways) for all the help in statistics and computing. I would also like to thank the non teaching staff of the Department of Environmental Sciences and Biochemistry, S. P College for their assistance during preparation and data gathering.

I am highly indebted to my parents who have always believed in me and encouraged me to make my own choices.

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