ISSN No: 2565-5132 (Print)

Original Research Article

Occurrence of Red-Bloom in Fish Pond in Chitwan District, Nepal

Ram Bhajan Mandal[1], Sunila Rai[1], Madhav Kumar Shrestha[1], Dilip Kumar Jha[1], Narayan Prasad Pandit[1] and Shiva Kumar Rai[2]*

[1]Agriculture and Forestry University, Rampur, Chitwan, Nepal
[2]Department of Botany, Post Graduate Campus, Biratnagar, Nepal

****Corresponding Author:*** Ram Bhajan Mandal, Agriculture and Forestry University, Rampur, Chitwan, Nepal
E-mail: rbmandal2008@gmail.com

Abstract

Red-bloom in fish pond has become a common problem in Nepal. In order to assess causative agents and seasonal variations of red bloom in fish pond, a study was conducted in the eastern, western and southern sites of Chitwan district from September, 2013 to June 2014. Preliminary data on occurrence and effect of red bloom were collected by visual observation and household survey of 30 randomly selected households using semi-structured questionnaire. Water quality and phytoplankton population were monitored in those ponds for four seasons, viz., autumn, winter, spring and summer. Among phytoplanktons, *Euglena sanguinea* was abundant in the pond water indicating the reason of red color in the pond. *E. Sanguinea* exhibited seasonal variation corresponding with the occurrence of red bloom in the pond. Highest density of *E. Sanguinea* was in summer (1628.4±70.4 number/L) which can be attributed to higher temperature and total nitrogen content of the water. Greater red bloom was observed in the southern site compared to eastern and western sites, where water was more stagnant, un-drainable and came from under-ground source.

Key words: red-bloom, seasonal variation, *Euglena sanguine*

Introduction

Red-bloom in fish ponds is a common occurrence in Chitwan, Nepal. Red bloom fish ponds are frequently covered with brick-red colored thin film of fine dust. However, red-bloom does not occur forever in fish ponds, it keeps changing diurnally and seasonally and its dynamism is still unknown. Red bloom appears in the late morning and disappears in the evening. Scum over the surface of water is red during bright sunshine and the scum becomes green whenever sunshine ceases (Kol, 1929; Heidt, 1934; Johnson, 1939). The red blooms are mostly seen in the marshy land and in ponds with high organic load. In fish ponds, eutrophication due to sedimentation of nutrients from feed and fertilizers is said to induce phytoplankton bloom including euglenophytes which causes red blooming in pond water (Ohio EPA, 2013). Red bloom gives unpleasant look, shades the lower waters, inhibits photosynthesis and causes water quality problems such as depletion of dissolved oxygen (Zimba et al., 2004; Zimba et al., 2010). This problem has been raised many times from farmers and extension officials, and has received wide attention.

Materials and Methods

The study was carried out in three sites of Chitwan, viz., eastern, western and southern. The eastern site included Kathar, Bhandara and Piple Village Development Committee (VDC), western site included Saradanagar, Jagatpur, Gunjnagar VDC and southern site included Ayodhyapuri, Madi-Kalayanpur and Baghauda VDC. The study included field observation, household survey and laboratory analysis of water quality and phytoplankton. For household survey, a semi-structured questionnaire was developed for interview of 30 randomly selected households with 10 in each site of the district to gather preliminary data on pond status, occurrence and effect of red bloom. Water quality and phytoplankton were monitored in 9 ponds, with 3 ponds in each site. Water samples were collected seasonally from red ponds by using water column sampler and phytoplankton by using plankton net from September 2013 to June 2014 in the morning (7:00 to 8:00 am). Water quality parameters such as dissolved oxygen (DO), pH, and temperature were recorded in situ whereas total phosphorus, total nitrogen and chlorophyll-a were analyzed at laboratory of Aquaculture and Fisheries Department of Agriculture and Forestry University by using standard methods (APHA, 1985). For phytoplankton sampling, five liters of pond water (up to 50 cm depth) was collected using graduated bucket and filtered with plankton net (mesh size 5 µm) and then preserved in 5% formaldehyde solution. Phytoplanktons were identified following Prescott (1951) and

ISSN No: 2565-5132 (Print)

Rai and Rai (2007) and classified according to Guiry and Guiry (2016). Phytoplanktons were counted using Sedgwick-Rafter (S-R) cells and quantified with APHA (1976). Data was analyzed by one way ANOVA using SPSS (version 16) followed by Duncan Multiple Range Test (DMRT) and significant level was considered at the level of 5% ($P<0.05$). All means were given with ± 1 S.E.

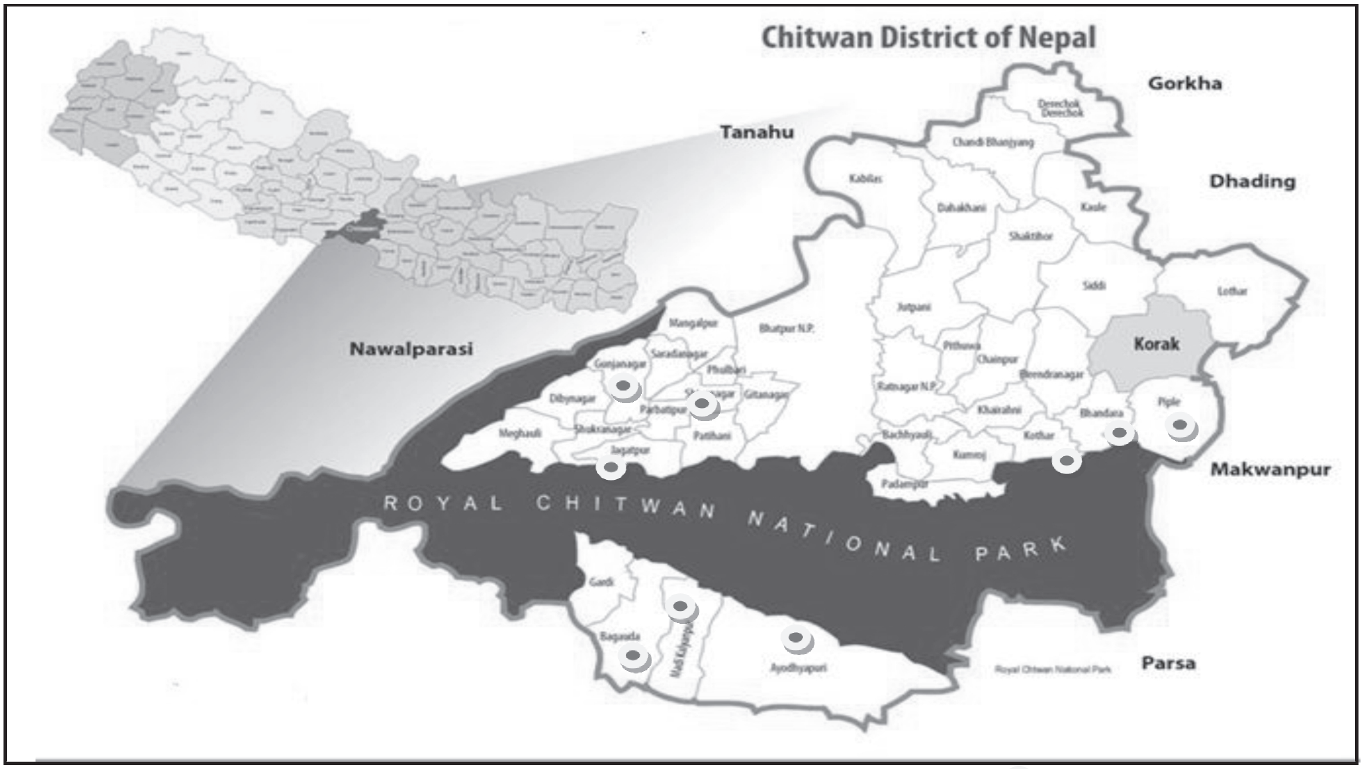

Figure 1 Map of Chitwan district including sampling sites indicated by sign .

Results and Discussion

Household survey

Water sources of most of the ponds were ground water recharge (48%), followed by river (35%) and boring (17%). There were 43% red bloom pond, 33% green and 23% slightly red pond during survey. Red bloom ponds were more in southern site (55%), moderate in western (40%) and less in eastern (35%) site (Table 1). Red bloom occurrence was higher in old, undrainable ponds using organic fertilizer compared to new, drainable ponds using inorganic fertilizer. In aggregate, around 52% respondents did not experience negative effect of red bloom on fish production while 22% had experienced low production due to red bloom. Around 25% respondents observed surface gulping of fish in the morning. Red bloom occurred maximum in winter (35%), minimum in summer (10%), moderate in autumn (20%) and in spring (17%) and 18% year round. Year round red bloom was reported highest in the southern site (45%) followed by western site (10%) while no year round red bloom was reported in the eastern site.

Water quality and phytoplankton abundance

Field observation and water quality monitoring in three different sites of Chitwan district showed that water quality parameters such as temperature, Dissolved Oxygen (DO), total alkalinity, total nitrogen and total phosphorus varied significantly ($P<0.05$) with season (Table 2). Temperature was higher in summer, autumn and spring than in winter in the eastern site. DO was higher in spring, summer and winter than in autumn, while DO in the winter was on par with summer and spring in eastern sites. Total alkalinity was higher in summer than in rest seasons in the eastern site. Total phosphorus was higher in winter than in rest seasons in the eastern site. In the western site, total nitrogen was higher in summer than in spring, although total nitrogen in autumn and winter did not differ with those of summer and spring. Combined mean DO was higher in spring season (7.0±0.2 mg/L) than autumn (5.8±0.2 mg/L) but was on par with those of winter and summer. Combined mean of total nitrogen was higher in summer (3.3±0.09 mg/L) than those in spring, autumn and winter seasons.

DO content was highest in summer and spring in the eastern and southern sites with red bloom fish ponds being more in southern and western region than in eastern region. Site wise seasonal variation of water quality parameters is shown in Table 2 and that of phytoplankton density is shown in Table 3. Results showed that water quality parameter such as pH, chlorophyll-a, total alkalinity and total phosphorus were not significantly different ($P>0.05$) among the red bloom fish ponds.

ISSN No: 2565-5132 (Print)

Table 1. Findings of household survey of fish ponds in three different sites of Chitwan district

Particular	Respondents (%)			
	Eastern	Western	Southern	
Water source				
River	70	15	20	35
Ground recharge	5	70	70	48
Boring	25	15	10	17
Pond water color				
Green	40	30	30	33
Slightly red	25	30	15	23
Red	35	40	55	43
Occurrence of red bloom				
New pond (< than three years pond)	10	10	10	10
Old pond (> than three years pond)	30	20	20	23
Drainable pond	5	5	0	3
Un-drainable pond	30	30	30	30
Organic fertilizing pond	20	30	25	25
In-organicfertilizing pond	5	5	15	8
Problems in red pond				
Surface gulping of fish	30	25	20	25
Low fish production	20	25	20	22
High fish production	5	0	0	2
No change in fish production	45	50	60	52
Season of red - bloom				
Autumn (Sept.- Nov,)	10	30	20	20
Winter (Dec.- Feb.)	40	40	25	35
Spring (March- May)	20	20	10	17
Summer (Jun.- Aug.)	30	0	0	10
All Season	0	10	45	18

Table 2. Season wise water quality parameters (mean±S.E.) in the eastern, western and southern sites of Chitwan districts

Region/Parameter	Autumn	Winter	Spring	Summer
Eastern Chitwan				
Temperature (^{0}C)	30.4 ± 0.8^{b}	20.1 ± 0.8^{d}	25.0 ± 0.1^{c}	34.4 ± 0.6^{a}
pH	5.6	6.4	7.7	7.7
DO (mg/L)	5.2 ± 0.1^{b}	6.1 ± 0.0^{a}	7.35 ± 0.8^{a}	6.3 ± 0.2^{a}
Chlorophyll-a (mg/L)	45.85 ± 1.7^{a}	46.35 ± 0.8^{a}	46.55 ± 0.02^{a}	45.15 ± 0.31^{a}
Total alkalinity (mg/L)	85.65 ± 2.3^{b}	88.15 ± 0.37^{b}	82.25 ± 3.0^{b}	101.2 ± 1.4^{a}
Total phosphorus (mg/L)	0.18 ± 0.0^{b}	0.23 ± 0.0^{a}	0.20 ± 0.0^{b}	0.19 ± 0.01^{b}
Total nitrogen (mg/L)	2.7 ± 0.2^{a}	2.8 ± 0.1^{a}	2.7 ± 0.1^{a}	3.1 ± 0.2^{a}
Western Chitwan				
Temperature (^{0}C)	31.1 ± 0.5^{b}	21.2 ± 0.1^{d}	23.8 ± 0.1^{c}	35.4 ± 0.2^{a}
pH	7.5	7.6	8.3	8.1
DO (mg/L)	6.8 ± 0.1^{a}	6.7 ± 0.0^{a}	7.2 ± 0.1^{a}	6.7 ± 0.5^{a}
Chlorophyll-a (mg/L)	112.25 ± 44.7^{a}	106.35 ± 40.7^{a}	101.80 ± 36.9^{a}	96.45 ± 34.2^{a}
Total alkalinity (mg/L)	84.60 ± 8.9^{a}	101.40 ± 12.9^{a}	129.85 ± 26.5^{a}	126.30 ± 19.4^{a}
Total phosphorus (mg/L)	0.40 ± 0.1^{a}	0.38 ± 0.08^{a}	0.38 ± 0.09^{a}	0.37 ± 0.08^{a}
Total nitrogen (mg/L)	2.4 ± 0.2^{ab}	3.0 ± 0.1^{ab}	2.3 ± 0.3^{b}	3.3 ± 0.1^{a}

ISSN No: 2565-5132 (Print)

Table 2. Continued . . .

Southern Chitwan				
Temperature (^{0}C)	30.7±0.2^b	21.7±0.1^d	23.9±0.1^c	36.1±0.1^a
pH	7.4	7.7	7.8	8.4
DO (mg/L)	5.4±0.1^b	6.2±0.1^{ab}	7.2±0.2^a	6.6±0.4^a
Chlorophyll-a (mg/L)	50.75±12.3^a	48.45±9.9^a	47.15±6.7^a	43.55±6.3^a
Total alkalinity (mg/L)	112.25±16.2^a	116.90±18.0^a	118.0±15.4^a	121.70±15.0^a
Total phosphorus (mg/L)	0.26±0.7^a	0.28±0.02^a	0.26±0.02^a	0.25±0.02^a
Total nitrogen (mg/L)	2.5±0.5^a	3.4±0.5^a	2.2±0.1^a	3.4±0.1^a
Combined mean				
Temperature (^{0}C)	30.7±0.1^b	21.0±0.2^d	24.3±0.2^c	35.3±0.3^a
pH	6.8	7.2	7.9	8.1
DO (mg/L)	5.8±0.2^b	6.5±0.2^{ab}	7.0±0.2^a	6.4±0.2^{ab}
Chlorophyll-a (mg/L)	69.6±17.1^a	67.1±15.5^a	65.2±14.1^a	61.7±13.2^a
Total alkalinity (mg/L)	94.1±5.8^a	102.1±19.1^a	110.0±8.8^a	116.4±5.6^a
Total phosphorus (mg/L)	0.28±0.5^a	0.29±0.03^a	0.28±0.03^a	0.27±0.03^a
Total nitrogen (mg/L)	2.5±0.2^b	3.1±0.1^{ab}	2.4±0.2^b	3.3±0.09^a

Phytoplankton analysis showed that *Euglena sanguine* was dominant among phytoplanktons (Table 3). Except *Euglena*, density of other phytoplanktons did not differ significantly (P>0.05) in different seasons. Density of *E. sanguinea* was higher in summer than in spring in three sampling sites. Density of *E. proxima* was higher in summer than in spring in the eastern site, while it was on par with autumn and winter. In the southern site, density of *E. proxima* was higher in summer and winter than in autumn but was not different than that of spring. Combined density of *E. sanguinea* was highest in summer, lowest in spring and intermediate in autumn and that of winter was on par with summer and autumn. Combined density of *E. proxima* was higher in summer than in autumn, winter and spring.

Red surface scum in fish ponds was probably due to *E. sanguinea* because its population density was higher than other phytoplanktons. *E. sanguinea* are red coloured algae. They contain haematochrome, a red coloured pigment (Gerber and Hader, 1994). Although the actual causes of red-blooming is not clearly understood, red pond water was found to be dominated by *Euglena* species such as *E.sanguinea* (Benham, 1913; Dendy, 1913; Lind, 1938; Pringsheim, 1956; ICLARM, 1995) and *E. shafiqii* (Rehman, 1998). According to Pringsheim (1956) *Euglena* sp. are typically found in hypereutrophic of total nitrogen and total phosphorous. Present finding agrees fairly well with finding of Mishra and Saksena (1993) and Hosmani (1988) who reported that the percentage of euglenophytes (*Euglena* species) was greater in red ponds compared to other planktons due to nutrient richness of water bodies (Costa and Garrido, 2004; Lopez *et al*., 2008; Costa, 2014).

Population of *E. sanguinea* varied seasonally along with temperature and total nitrogen. High temperature and total nitrogen might have favored growth and multiplication of *E. sanguinea.* Its density was higher in summer and lower in spring which did not correspond with findings of household survey. Household survey revealed that occurrence of red bloom was greater in winter followed by autumn, spring and summer. The differences could be attributed to human error to memorize and express the event of red bloom. Questionnaire interview of farmers was based on their past experience and might be difficult them to recall. On the other hand, red bloom might not be critical to them as per survey findings majority of farmers (52%) did not experience reduced fish production due to red bloom.

Although red-blooms in fish ponds are reported to be harmful but no such effect was reported by farmers in the present study. However, surface gulping by fish had been observed by farmers probably due to low dissolved oxygen caused by phytoplankton bloom mainly in summer. Prolonged low DO might cause mass fish mortality. Zimba et al. (2004) and Rodgers (2008) reported that *Euglena* sp. produce ichthyotoxin which is fatal to fish.

Conclusions

Red-bloom in fish ponds is probably due to *E. sanguinea*, which is versatile in shape and covers the whole pond, leading to a low DO and hence fish mortality. Although farmers are not pretty much sure on its effect, detail study is needed on dynamics of red bloom and its effect on fish production. Present study identified the causative agents of red bloom and its seasonal variation but it could not clear the causes of *Euglena sanguinea* bloom. Increased temperature and total nitrogen accompanied with higher *E. sanguinea* density during summer indicate that the temperature and total nitrogen could be responsible for *E. sanguine* bloom.

ISSN No: 2565-5132 (Print)

Table 3. Seasonal abundance of Phytoplankton (number/L) in red-bloom fish pond of the eastern, western and southern sites of Chitwan district.

Phytoplankton	Autumn	Winter	Spring	Summer
Eastern site				
Euglena sanguinea	1113.3±114.6[a]	1243.3±113.5[a]	600.7±32.3[b]	1412±103.7[a]
Euglena proxima	168.7±5.0[ab]	154.3±32.8[ab]	96.7±12[b]	263.7±38[a]
Glenoidinumquadridens	115.0±5.3	120.0±2.2	118.0±0.2	75.0±1.2
Chroococcus	110.0±1.6	112.0±1.3	25.0±0.4	30.0±1.1
Ankistrodemusfalcatus	60.0±0.5	75.0±1.2	60.0±1.1	65.0±1.2
Crucigeneatetrapedia	20.0±0.1	18.0±0.1	15.0±0.1	10.0±0.1
Oscillatoriasps.	18.0±0.2	12.0±0.1	155.0±0.2	5.0±0.01
Pandorinamorum	25.0±0.1	126.0±1.2	6.0±0.0	110.0±0.8
Western site				
Euglena sanguinea	1320±43.6[ab]	1452±63.5[a]	1046±127.3[b]	1645.7±72.1[a]
Euglena proxima	175±18.9[a]	150±43[a]	215.3±3.4[a]	243.3±49[a]
Euglena rostrifera	28.0±3.2	111.0±1.2	45.0±0.5	23.0±3.2
Glenoidinumquadridens	77.0±.3.1	50.0±1.2	14.0±.6	12.0±.3
Desmodesmids	85.0±.6	90.0±0.4	10.0±0.1	14.0±0.3
Crucigeneatetrapedia	17.0±1.1	18.0±0.2	12.0±0.1	9.0±0.1
Oscillatoriasps.	28.0±0.2	15.0±0.1	145.0±3.6	43.0±2.4
Naviculasps.	90.0±1.8	43.0±1.2	12.0±0.2	11.0±0.2
Sencillaestrella	12.0±0.1	15.0±1.1	20.0±0.1	43.0±1.3
Scenedesmusdimorphus	29.0±0.8	40.0±1.2	15.0±0.2	80.0±3.3
Merisopedia glance	34.0±0.5	14.0±0.2	19.0±0.3	21.0±0.1
Chlamydomonussps.	12.0±0..2	13.0±0.1	10.0±0.1	7.0±0.1
Southern site				
Euglena sanguinea	1436.7±89.5[ab]	1669.4±78.0[ab]	1238.7±68.1[b]	1926.7±17.6[a]
Euglena proxima	215.7±3.4[b]	222.3±1.0[a]	240±40.4[ab]	233±6.6[a]
Glenoidinumquadridens	130.0±1.0	124.0±0.6	128.0±0.8	80.0±0.2
Chroococccus	67.0±0.4	60.0±0.6	45.0±0.2	15.0±0.1
Ankistrodemusfacatus	12.0±0.1	18.0±0.2	20.0±0.1	12.0±0.1
Desmodesmids	22.0±0.1	15.0±0.1	18.0±0.1	30.0±0.2
Crucigeneatetrapedia	32.0±0.2	32.0±0.6	22.0±0.2	11.0±0.01
Oscillatoriasps.	-	11.0±0.02	67.0±0.6	12.0±0.02
Selenastrumgracile	12.0±0.1	23.0±0.2	12.0±0.1	55.0±0.8
Sencillaestrella	34.0±1.1	12.0±0.2	13.0±0.1	40.0±0.3
Scenedesmusdimorphus	60.0±1.2	40.0±0.1	34.0±0.2	21.0±0.2
Coelastrum	80.0±0.2	16.0±0.1	40.0±0.1	12.0±0.1
Combined mean of *Euglena*				
Euglena sanguinea	1290.0±64.4[b]	1454.9±75.5[ab]	962.0 ±1.03[c]	1628.4±70.4[a]
Euglena proxima	186.4±9.3[b]	209.1±32.3[b]	184.0±25.2[b]	280.0±22.5[a]

Acknowledgements

Authors are thankful to the farmers of Eastern, Western and Southern sites of Chitwan, Nepal for their kind cooperation and help during the field study. We would also like to thank Department of Aquaculture and Fisheries, Agriculture and Forestry University, and Department of Botany, Postgraduate Campus, Biratnagar for providing lab facilities. We are grateful to Directorate of Research and Extension, Agriculture and Forestry University, Rampur Chitwan, Nepal for financial support.

References

APHA. Standard methods for examination of water and waste water. American Public Health Association Inc., New York, 1976.

APHA Standard methods for examination of water and waste water (16[th]Ed.). American Public Health Association, American Water Works Association 1015, Fifteenth street NW, Washington D.C. ,1985.

APHA. Standard methods for examination of water and waste water (22[nd] ed.), American Public Health Association, Washington, 2012.

Benham, C.E. 19. A red-water phenomenon due to Euglena. Nature, 1913, 91, No. 2285, p. 607.

ISSN No: 2565-5132 (Print)

Costa, P.R. Impact and effects of paralytic shellfish poisoning toxins derived from harmful algal blooms to marine fish. John Wiley and Sons Ltd, 2014.

Costa, P.R. and S. Garrido. Domoic acid accumulation in the sardine *Sardinapilchardus* and its relationship to *Pseudonitzschia* diatom ingestion. Marine Ecology Progress Series, 2004, 284, 261-268.

Dendy, A. A red-water phenomenon due to Euglena. Nature, 1913, No. 2284, Vol. 91, p. 582.

DoFD. Annual report 2012-2013. Directorate of Fisheries Development (DoFD), Central Fisheries Building, Balaju, Kathmandu, Nepal, 2013.

Guiry, M.D. and G.M. Guiry. *Algae Base*. World-wide electronic publication, National. University of Ireland, Galway, 2016, Cited from: http://www.algaebase.org.

Heidt, K. Hamatochromwanderungbei *Euglena sanguinea* Ehbg. Ber. dtsck. bot. Ges., 1934, 52,607-13.

Hosmani, SP. Seasonal changes in phytoplankton communities in a freshwater pond at Dharwar, Karnatak State, India., Phykos ,1988, 27, 82-7.

ICLARM. Red coloring from Euglena sanguinea. Naga, the ICLARM Quarterly, 1995, 18 (4), p. 28.

Johnson, LP. A study of Euglena rubra Hardy. Trans. Amer. mnicr. Soc., 1939, 58, 42-48.

Kol, E. "Wasserblute" der Sodateiche auf der Nagy Magyar Alfold (GrossenUngarischenTiefebene). Arch. Protistenk., 1929, 66, 515-22.

Landsberg J. The effects of harmful algal blooms on aquatic organisms. Reviews in Fishery Science, 2002, 10, 113–390.

Pringsheim, EG. Contributions towards a monograph of the genus *Euglena*. Nova ActaLepoldinaLind, E.M. 1938. Studies in the Periodicity of the Algae in Beauchief Ponds, Sheffield. Journal of Ecology, 1956, 26 (2),257-74.

Lopez, CB, Jewett EB, Dortch Q,Walton BT, Hudnell HK. Scientific assessment of freshwater harmful algal blooms. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology. Washington, DC, 2008, 65p.

Lopez, CB, Jewett EB, Dortch, Q, Walton BT, Hudnell, HK. Scientific Assessment of Freshwater Harmful Algal Blooms. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology. Washington, DC, 2008, 65p.

Mishra, S.R. and D.N. Saksena. Photo planktonic composition of sewage polluted Morar (Kalpi) River in Gwalior, Madhya Pradesh. Environ. Ecol, 1993,11, 625-9.

Ohio EPA. Seeing red: Emerging harmful algal blooms. Newsletter for Ohio's Public Drinking Water Systems. Spigot News ~ summer 2013~ Page 2.

Prescott, GW. Algae of the western great lakes area. WM.C. Brown Publishers, Dubuque, Iowa, 1951, 125, 27-34.

Rai SK and Rai RK. Some euglenophycean algae from Biratnagar, Nepal. Our Nature , 2007, 5, 60-6.

Rehman, SU. A red bloom of *Euglena shafiqii*, a new species, in Dal Lake, Srinagar, Kashmir. Water Air and Soil Pollution, 1998, 108, 69-82.

Rodgers, J.H. 2008. Algal toxins in pond aquaculture. SRAC Publication No. 4605, 1-8.

Zimba PV, Rowan M and Trimer R. Identification of euglenoid algae that produce ichthyotoxin(s). Journal of Fish Diseases, *2004,* 27,115-117.

Zimba PV, Moeller PD, Beauchesne K, Lane HE, Triemer RE. Identification of euglenophycin- A toxin found in certain euglenoids. Toxicon, 2010, 55(1), 100-4.