Causes of fish kill in a natural water purification system

Author's Details:
J. R. Ho[4], K. F. Chen[3],M. H. Wu[1] ,T. Y. Yeh[1]*, C. M. Kao[2],Y. H. Hsu[1]
[1]Department of Civil and Environmental Engineering, National University of Kaohsiung, Taiwan
[2]National Sun Yat-Sen University, Institute of Environmental Engineering,National Sun Yat-Sen University Kaohsiung, Taiwan [3]National Chi Nan University, of Civil Engineering, Taiwan [4]Environmental Protection Adminstration Correspondence to: Prof. T. Y. Yeh-National University of Kaohsiung-Department of Civil and Environmental –Engineering Kaohsiung 811, Taiwan

Abstract

An incident of fish kill occurred in the detention pond of a natural water purification system. The objective of this study is to reveal the cause of the incident. Novel measurements unprecedentedly employed in this study include: SOD to measure sediment oxygen decay; PCR to find sediment microbial activity. Algal bloom was the main reason for the serious fish kill. Particle size analysis demonstrated that large particles were settling in the upstream region. Chlorophyll A, COD, SS, phosphorus and E coli were following the stream direction. The end of the measuring point, the detention pond, demonstrated the serious eutrophication which leads to diurnal pH and DO fluctuations which induce anoxic condition at night, which induced the fish kill. PCR and microbial optical investigation demonstrated that blue green algae were the predominant species. The results of this study can serve as reference for other natural water purification systems worldwide, particular for high luminance and temperature climate, particularly in tropical regions.

***Keywords*:** Constructed wetland, algae

- **Introduction**

Constructed wetlands, natural water purification systems and novel green remediation approaches have been commonly employed in Taiwan and worldwide for water cleaning. Several constructed wetlands have been listed as national wetlands to be specially protected and managed. Constructed wetlands possess mitigation ability (Yeh et al., 2009; Kao et al., 2010) in regard to organic matter (BOD and COD), particulate matter (SS), nutrients (Nitrogen and Phosphorus), heavy metals (Cu and Zn), pathogen indicators (E. coli) and pharmaceutical personal care products (PPCPs). Constructed wetlands have long been a black box and pollution degradation was not revealed. Recently, numerous studies have been conducted to shed light on the black box.

A serious fish kill occurred in the University's detention pond which implied that the water quality was significantly reduced. The university suspected toxic algae as the cause of the fish kill. An investigation project was initiated to examine the fish kill and a proposal for water-related monitoring was accepted and conducted. The objective of this study was to examine algal blooms in the wetland system and the myth of such resulting fish kills. A literature review revealed that none conducted the following novel measurements unprecedentedly employed in constructed wetlands, including SOD to measure sediment oxygen decay; PCR to determine sediment microbial activity; SEM, EDX and FTIR to view the macrophyte rhizhere root surface in order to understand the pollutant adsorption. By virtue of the aforementioned results, the real cause of the fish kill could be revealed.

The objective of this study was mainly to focus on the cause of fish kill in natural water purification systems. Algal growth influential factors were also monitored for eutrophication of water bodies. This monitoring study can serve as reference for natural water purification systems in regard to fish kills and eutrophication.

2. Materials and methods

2.1 Study site

The constructed wetlands are situated in the University of Kaohsiung campus (22°73'N, 120°28'E). The length, width, average depth and flow velocity were 585m,5.2m, 0.5m and 67.5m/hr (1.88cm/s) respectively, while the detention pond at point has a depth of 1.2m.

2.2 Water quality analysis

Water parameters, including organic matter (BOD and COD), particulate matter (SS), nutrients (Nitrogen and Phosphorus), chlorophyll A, and pathogenic indicators (E. coli) were investigated.

2.3 Microbial PCR analysis

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction- denaturing gradient gel electrophoresis (PCR-DGGE) analysis were analyzed according to Boon et al. (2001). The Wizard DNA Clean-Up Kit (Promega, Madison, Wisconsin) was used to detect microbial community dynamics in the process of nitrogen transformation in the 1-g sediment samples

from surface-flow wetlands. Bacterial200-bp fragments of 16S rDNA V3 region for subsequent denaturing gradient gel electrophoresis (DGGE) analysis were obtained with the primer combination of 341 f with a gas chromatography clamp (40-nucleotide GC-rich sequence, 5-CCTACGGGAGGCAGCA G-3)and 534r (5-ATTACCGCGGCTGCTGG-3).

The PCR mixtures contained 10 ng of DNA extract, 4 pmol of each primer, and 5 U of Taq polymerase (Takara, Shiga, Japan) in the final concentration of 2.5 mM of Mg C12 and 0.12 mM ofdeoxyribonucleoside triphosphates in PCR buffer. The PCR amplification was conducted for 35 cycles: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min. The equal concentration of each amplified PCR product (2500 ng) was further performed with DGGE using a Bio-Rad DCode system (Bio-Rad, Hercules, California), as described by the manufacturer. The 10% Polyacrylamide gel with a 30 to 60% denaturant gradient was used, and electrophoresis was performed at 60°C and 70 V for 14 h. The gels were then stained with SybrGreenl and photographed. The relative intensity of amplified bands in the gels was analyzed with Phoreti ID software (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom). The PCR-amplified products were eletro-eluted from gel and then sequenced by provider (MdBio, Taipei, Taiwan).

Those sequences were evaluated using the basic local alignment search tool (BLAST) to determine the closest relatives in the GenBank databases (www.ncbi.nlm.nih.gov). Alignment of nucleotide sequences of PCR-amplified products generated a matrix of similarity coefficients with the neighbor-joining method (Saitouand Nei, 1987). The dendrogram based on the similarity coefficient was plotted with un-weighted pair-group method with arithmetic mean (UPGMA) method for clustering (Felsenstein, 1993).

2.4 Data and Statistical analysis

Data were evaluated relative to the control to understand their statistical variations. A triplicate of water and sediment samples were measured and recorded for statistical analyses. Statistical significance was assessed using a mean comparison test. Differences between treatment concentration means of parameters were determined by Student's t test. One-way ANOVA was also employed to show the variation among sample groups, level of $p < 0.05$ considered statistically significant was used in all comparisons. Means are reported mean ± standard deviation. All statistical analyses were performed with Microsoft Office EXCEL 2007.

3. Results and Discussion

3.1. Water parameter analysis

The results of these measurements are shown in Table 1. Algal growth-related water parameters chlorophyll A, COD, turbidity and SS were in increasing order from upstream to detention pond, which induced the fish kill. Serious algal bloom affected water pH and DO due to photosynthesis and the algal respiration effect. Diurnal pH and DO monitoring results are showed in Fig1. Algal photosynthesis produced CO2 and oxygen inducing pH and DO to rise in the day time. Increased DO was related to oxygen production and pH increase was due to algae using up CO_2, inducing dropping pH. In the night time, algal respiration enhanced CO_2 production to convert generation of carbonic acid leading to pH dropping.

3.2 Water quality and Algal species monitoring

Chlorophyll A concentration for A, B and C were: 192±71.8, 319.7±91.6,307.2±68.1µg/L. A, B and C turbidity were: 21.3±6.3, 27.5±6.3,27.0±11.5 NTU while SS were17.7±2.5, 30.7±7.8,34.3±10.4 mg/L. COD concentrations for A, B and C were: 19.1±6.7, 29.1±8.8,23.0±10.1 mg/L. Total poseurs concentration for A, B and C were: 192±71.8, 319.7±91.6,307.2±68.1µg/L.E coli concentrations for A, B and C were: 28,333 ± 7,505, 8,333 ± 8,082,2,333±,2309 CFU/100mL. The main processes for the removal of SS are sedimentation and filtration within systems. Phosphorus removal in wetlands might be induced by the plant uptake, accretion of wetland soils, microbial immobilization, retention by root bed media and precipitation in the water column.

Nitrogenous nutrient transformation in wetlands mainly occurs by biological processes including magnification, nitrification, gentrification, nitrogen fixation and nitrogen assimilation. For secondary treated sewage, the predominant forms of nitrogen might be ammonium and nitrate depending on aeration levels in secondary treatment processes. Nitrification and gentrification are generally indicated as the principal processes for nitrogen reduction (Reed et al. 1995). In this study total nitrogen levels fluctuated from upstream toward detention pond indicated nitrification and denitrification concurrently occurred in the sediment. Macrophytes may remove some nutrients through direct uptake and provide environments for more intense microbial activities. The vegetation in constructed wetlands also creates quiescent conditions for sedimentation as well as oxidation conditions for organic matter decomposition and nutrient transformation through bacterial activities from microbes suspended in the water column and attached around the root zones.

3.5 .1 Particle size measurement

The result of particle size measurement of three sampling points is shown in Table F. The sediment particle encompassed clay, silt and sand with the particle size in the descending sequence as <2 μm, 2-50 μm and 50-1000 μm. The larger particles of sand were expected to settle more easily than silt and clay. Five sampling points from upstream to detention pond showed that the total volume sand particles were in the decreasing order 74.08, 13.38, 3.1%, respectively, which indicated that most large particles settled in the upper stream sediment.The detention pond presented higher total amounts of clay which possessed higher cation exchange capacity (CEC) and pollutant adsorption in particular metal cation leading to higher metal mobility and bioavailability. This might be the cause that dissolved pollutants having increased and impacted the benthic ecology.

Microbial Identification Analysis. The PCR amplification of 16S rDNA and

DGGE analysis were performed to determine the dominant microorganisms in

nitrogen transformation in the surface-flow wetlands. Figure 3 shows the DGGE

profiles of thePCR-amplified 16S rDNA for sediments collected. The profile of

DGGE for detecting sediment samples showed that six strains of microorganisms

were predominant in nitrogen transformation. The nucleotide sequences of 16S

rDNA variable V3 regions obtained from PCR-amplified products of six specific microorganisms were compared with the database from GeneBank . Nitrosospira

and Nitrosovibrio, the possible species for strain 3, have been reported to

transform nitrogen through nitrification (Chan et al., 2008). Denitrificans, the

possible species for strain 1, and Pseudomonas, the possible species for strain

6,coexisted in the sediment and could have transformed nitrogen

through denitrification. Transformation of nitrogen has not been reported for some of the microorganisms. If some microbes have similar genetic background

and are closely related to some clusters, the microbes could transform nitrogen

compounds within surface-flow wetlands. Microbial identification results

indicated that nitrification/denitrification concurrently occur within the sediments

of surface-flow wetlands. This result might explain the significant total nitrogen

reduction in the surface-flow wetlands ($p < 0.05$).

4. Conclusion

Fish kill commonly occurs in the detention ponds and surface flow wetland due to algal bloom. Serious diurnal DO and pH were detected due to the photosynthesis and respiration elevating DO and pH in the daytime and decreasing DO and pH at night. Oxygen level fluctuation was the main cause of fish kill in this study site, and the possibility of toxic algae intrusion was left out. SOD and OPR revealed the black box toxic variation levels. Microbial PCR was conducted to illumine the species of microorganism in the sediment. The root of macrophyte was also investigated via SEM, EDX and FTIR. The results of this study can serve as reference for other wetlands or for the future research and solve the emergent fish kill to be substitute of algaecide copper sulfate, a toxicant of phytoplankton application.

Reference

APHA-AWWA-WEF. (1995). Standard methods for examination of water and wastewater, 19th ed. American Public Health Association, Washington, DC

Brix, H., Arias, C. A. (2005). The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. Ecological Engineering, 25, 491-500

Chen, T.Y., Kao, C.M., Yeh, T.Y., Chao, A.C., (2006). Application of a constructed wetland for industrial wastewater treatment. Chemosphere, 64, 497-502,

Chen, X.C., Kong, H.N., He, S.B., Wu, D.Y., Li, C.J., Huang, X.C., (2009a). Reducing harmful algae in raw water by light-shading. Process Biochemistry, 44, 357-360

Chen, X., He, S., Huang, Y., Kong, H., Lin, Y., Li, C., Zeng, G.., (2009b). Laboratory investigation of reducing two algae from eutrophic water treated with light-shading plus aeration. Chemosphere, 76, 1303-1307

Davies-Colley, R.J., Donnison, A.M., Speed, D.M., Ross, C.M., Nagels, J.M. (1999). Inactivation of fecal indicator micro-organism in waste stabilization ponds: interactions of environmental factors with sunlight. Water Research, 33, 1220-1230

Davis, L. C., Carias, C. C., Novais, J. M., Martins-Dias, S. (2005). Phytoremediation of textile effluents containing azo dye by using *Phragmites australis* in a vertical flow intermittent feeding constructed wetland. Ecological Engineering, 25, 594-605

Gottschall, N., Boutin, C., Crolla, A., Kinsley, C., Champagne, P. (2007). The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. Ecological Engineering, 29, 154-163

Gschlobl, T., Steinmann, C., Schleypen, P., Melzer, A. (1998). Constructed wetlands for effluent polishing of lagoons. Water Research, 32, 2639-2645

Herrera Melian, J.A., Araña ,J., González Díaz, O., Aguiar Bujalance, M.E., Doña Rodríguez, J.M. (2009). Effect of stone filters in a pond–wetland system treating raw wastewater from a university campus. Desalination, 237, 277-284

Jing, S.R., Lin, Y.F. (2004). Seasonal effect on ammonia nitrogen removal by constructed wetlands treating polluted river water in southern Taiwan. Environmental Pollution, 127, 291-301

Kadlec, R.H., Tanner, C.C., Hally, B.M., Gibbs, M.M. (2005). Nitrogen spiraling in subsurface-flow constructed wetlands: implications for treatment response. Ecological Engineering, 25, pp. 365-381

Kim, Y., Kim, W., (2000). Roles of water hyacinths and their roots for reducing algal concentration in the effluent form waste stabilization ponds. Water Research, 34, 3285-3294

Maiga, Y., Denyigba, K., Wethe, J., Ouattara, A.S. (2009). Sunlight inactivation of Escherichia coli in waste stabilization microcosms in a sahelian region (Ouagadougou, Burkina Faso). Journal of Photochemistry and Photobiology B: Biology, 94, 113-119

Mitsch, W. J., Day, J. W., Zhang, L., Lane, R. R. (2005). Nitrate-nitrogen retention in wetlands in the Mississippi River Basin. Ecological Engineering, 24, 267-278

Molle, P., Prost-Boucle, S., Lienard, A. (2008). Potential for total nitrogen removal by combining vertical flow and horizontal flow constructed wetlands: A full-scale experiment study. Ecological Engineering, 34, 23-29

Moreira, J.F., Cabral, A.R., Oliveira, R., Silva, S.A. (2009). Causal model to describe the variation of faecal coliform concentrations in a pilot-scale test consisting of ponds aligned in series. Ecological Engineering, 35, 791-799

Noorvee, A., Poldvere, E., Mander, U. (2007). The effect of pre-aeration o the purification processes in the long-term performance of a horizontal subsurface flow constructed wetland. Science of the Total Environment, 380, 229-236

Nurminen, L., Horppila, J. (2009). Life form dependent impacts of macrophyte vegetation on the ratio of resuspended nutrients. Water Research, 43, 3217-3226

Reed, S. C., Crites, R.W., Middlebrooks, E.J. (1995). Wetland System. Natural systems for waste management and treatment, 2nd, McGraw-Hill, New York

Sinistro, R., Sanchez, M.L., Marinone, M.C., Izaguirre, I. (2007). Experimental study of the zooplankton impact on the trophic structure of phytoplankton and the microbial assemblages in a temperate wetland (Argentina). Limnological, 37, 88-99

Shanthala, M., Shankar, P., Basaling, B. (2009). Diversity of phytoplankton in a waste stabilization pond at Shimoga Town, Karnataka State, India. Environ Monit. Assess, 151, 437-443

Song, A., Zheng, Z., Li, J., Sun, X., Han, X., Wang, W., Xu, M. (2006). Seasonal and annual performance of a full-scale constructed wetland system for sewage treatment in China. Ecological Engineering, 26, 272-282

Southichak, B., Nakano, K., Nomura, M., Chiba, N., Nishimura, N. (2006). *Phragmites australis*: A novel biosorbent for the removal of heavy metals from aqueous solution. Water Research, 40, 2295-2302

Steinmann, C.R., Weinhart, S., Melzer, A. (2003). A

combined system of lagoon and constructed wetland for an effective wastewater treatment. Water Research, 37, 2035-2042

Tang, X., Huang, S., Scholz, M., Li, J. (2009). Nutrient removal in pilot-scale constructed wetlands treating eutrophic river water: Assessment of plants, intermittent artificial aeration and polyhedron hollow polypropylene balls. Water Air Soil Pollut, 197, 61-73

Yeh, T.Y., Wu, C.H. (2009). Pollutants removal within hybrid constructed wetland system in tropical regions. Water Science and Technology, 59, 223-240

Yeh, T. Y., Pan, C. T., Ke, T. Y., Kuo, T. W. (2010). Organic matter and nitrogen removal within field-scale constructed wetlands: Reduction performance and microbial identification studies", Water Environment Research, 82, 27-33

Table Captions

Table 1: Water quality analysis results
Table 2: Water quality in detention pond
Table 3: Nutrient content levels in detention pond

Figure Captions

Fig. 1: Schematic diagram of pot experiment
Fig. 2: Diurnal water quality variation
Fig. 3: Fish kill events
Fig. 4: Microscopic observation results

Table 1: water quality analysis results

	A	B	C	D	E	F
Temp. (℃)	30.9±2.1	35.2±1.9	35.4±2.6	35.7±2.7	37.4±2.8	33.5±1.7
DO (mg/L)	6.23±2.7	8.48±3.1	17.93±3.9	18.65±3.5	17.15±3.4	14.71±4.2
pH	8.24±1.87	8.48±1.98	8.68±1.95	8.79±1.80	8.65±2.12	8.30±2.04

Table 2: water quality in detention pond

	A	B	C	D	E	F
Chorollphyl A(µg/L)	178.1±68.1	192.6±71.8	336.5±82.1	319.7±91.6	295.4±93.3	307.2±68.2
Turbidity (NTU)	10.0±3.6	21.3±6.3	32.3±7.5	27.5±6.3	25.1±4.9	27.0±11.5
COD (mg/L)	12.8±4.0	19.1±6.7	26.1±10.5	29.1±8.8	24.8±13.8	23.0±10.0
SS (mg/L)	9.7±1.5	17.7±2.5	36.1±1.5	30.7±7.8	28.8±6.4	34.3±10.4
TP (µg/L)	0.176±0.044	0.185±0.037	0.133±0.031	0.149±0.040	0.137±0.034	0.121±0.048

Table 3: Nutrient observation results (mg/L)

	A	B	C	D	E	F
Ammonia nitrogen	4.14±1.73	3.35±1.13	2.23±1.26	1.07±1.07	0.89±0.3	0.70±0.22
TKN	5.36±1.97	5.01±2.27	2.78±1.34	1.61±0.63	0.77±0.62	0.67±0.46
Nitrate nitrogen	1.27±0.57	1.47±0.79	0.87±0.49	1.00±0.42	0.27±0.31	0.31±0.43
Nitrite nitrogen	0.09±0.01	0.32±0.13	0.18±0.1	0.17±0.02	0.07±0.07	0.04±0.03
Total nitrogen	6.72±2.47	6.8±2.26	3.83±1.98	2.78±1.79	1.11±1.68	1.02±0.57

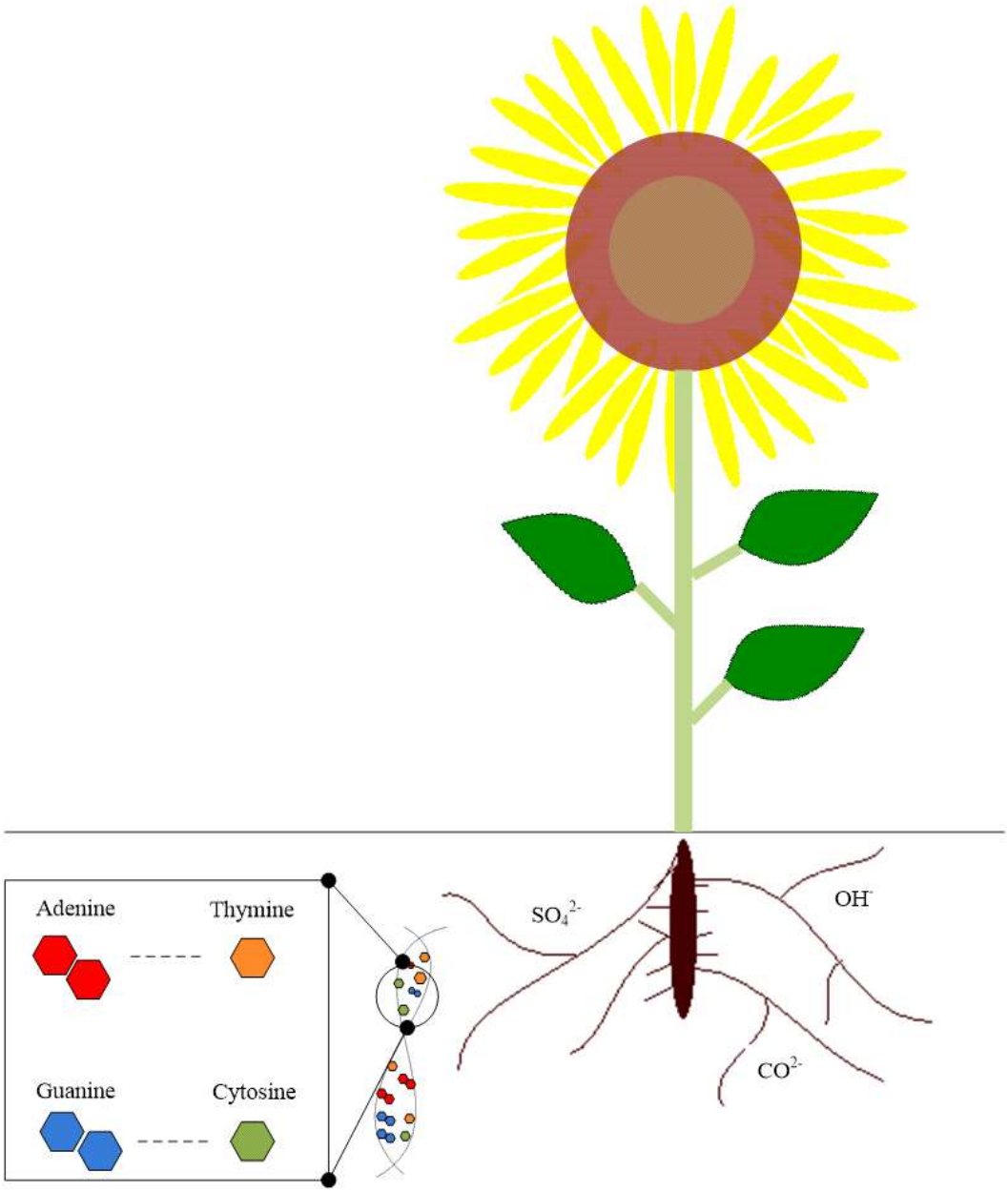

Fig. 1: Schematic diagram of pot experiment

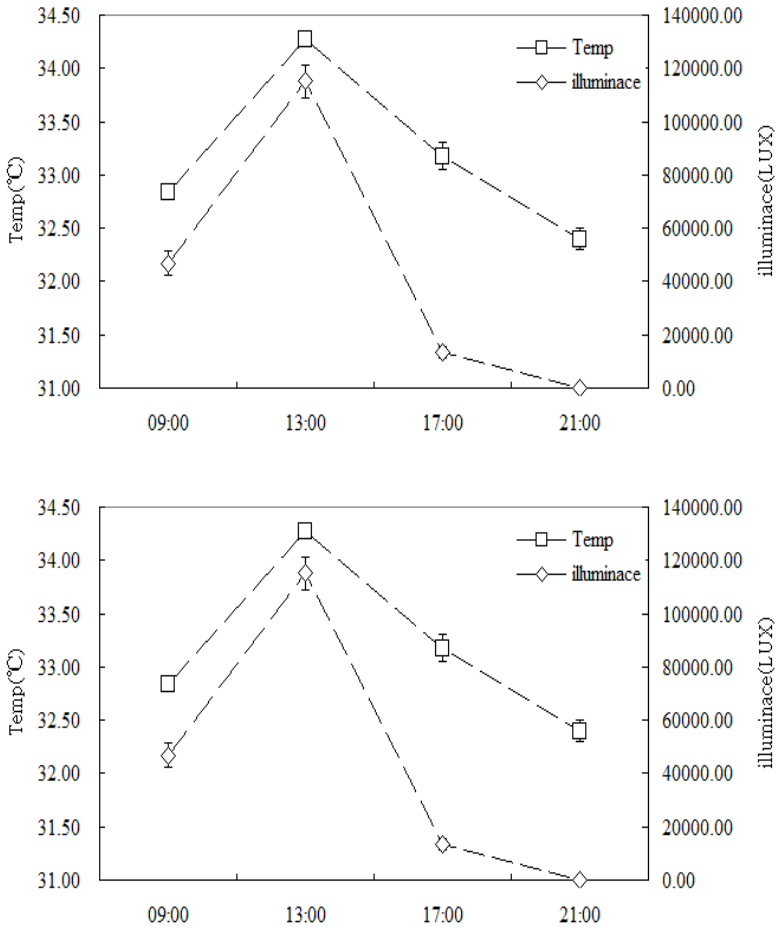

Fig. 2: Diurnal water quality variation

Fig. 3: Fish kill events

Oscillataria

Synechocystis

Scenedesmus

Navicula

Fig. 4: Microscopic observation results