

RESEARCH ON EVALUATION OF TRACE CHEMICALS AT WASTEWATER TREATMENT PLANTS

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Project period: FY 2000-2005

Previous studies have pointed out the occurrence of many trace chemicals such as endocrine disrupting chemicals (EDCs) in sewage. Therefore, the method to estimate their fate and risk should be established.

In FY2004, improvement of EDCs measurement with ELISA method was studied, and behavior of free estrogen, their conjugates and synthetic estrogen (oral contraceptive pill) in wastewater treatment plants was investigated.

Main results are as follows;

- 1) Three pre-treatment procedures for removing cross-reacting and/or disturbing substances in measuring 17β -estradiol (E2) and estrone (E1) with ELISA method were compared. The result indicates that it is effective to remove humic-acid fraction in improving the accuracy of measuring E1 in influent.
- 2) Behavior of free estrogens and estrogen sulfates in a wastewater treatment process was studied under aerobic condition ($MLDO > 5\text{mg/L}$) and long SRT (60 days) using a membrane bioreactor. Free estrogens were completely removed, while estrogen sulfates were decreased to some extent and remained in the secondary effluent. This result indicates that estrogen sulfates are much more persistent than free estrogens.
- 3) The occurrence of ethinyl estradiol (EE2), which is main component of an oral contraceptive pill, in influent and secondary effluent in 10 municipal wastewater treatment plants were studied. EE2 was detected by both analytical methods (HR-GC/MS and LC/MS/MS) and ELISA method. Concentrations of EE2 in influent were under detection limit and those in secondary effluent ranged from n.d. to 0.28ng/L .

Key words: endocrine disrupting chemicals, estrogen, nonylphenol, ELISA, wastewater treatment

EFFECTS ON WATER ENVIRONMENT AFFECTED BY SEWERAGE SYSTEMS

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Project period: FY 2001-2005

OBJECTIVES

Limited studies have been conducted so far for quantitative evaluation of changes in water and water-related material cycles due to change in the watershed, particularly development of sewerage system. Furthermore quite few studies are available on its influence on aquatic ecosystem. The aims of this study are, therefore, to clarify the influence of the change in watersheds, especially the development of sewerage system, on the change in water flows and pollutant loads of the rivers, and to examine its impacts on river water quality and aquatic ecosystems..

Results

The summary of this study conducted in FY2004 is as follows:

- 1) Development of analytical model of pollutant discharge for Teganuma Lake and Ohtsugawa River system was attempted by applying survey data in this field.
- 2) We conducted a exposure test using Medaka fish (*Oryzias latipes*) for secondary effluent and sand filter effluent to examine the influence of estrogen-like activity, and we could observe vitellogenin production in 20% of Medaka exposed to the secondary effluent.
- 3) We applied the Algal Growth Potential Test using a micro-plate to evaluate the influence of water quality of a river on aquatic ecosystem, and almost the same results were obtained as the existing AGP test procedure.

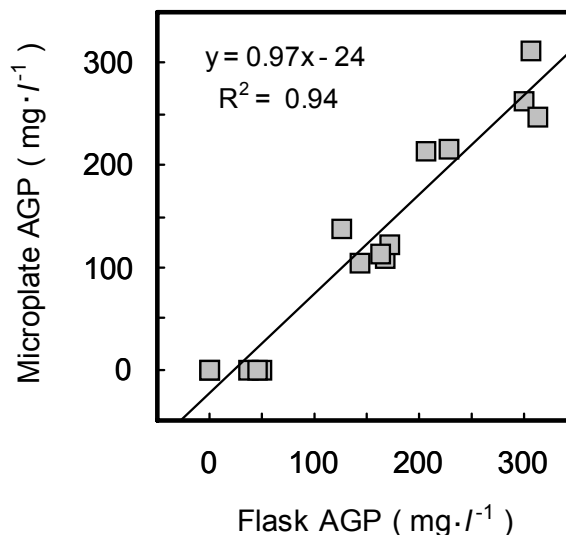


Figure Relation between Flask AGP and Microplate AGP

Evaluation of River Water Quality from the Viewpoint of Aquatic Ecosystem

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Project period: FY 2000-2005

OBJECTIVES

In recent years, sewerage systems have widely spread in urban areas. At the same time, the volumetric percentage of water derived from sewage treatment plants (STPs) in rivers has been increasing. As a result, the quality of river water is greatly influenced by the treated wastewater. It is, therefore, assumed that the treated wastewater may exert positive or negative impacts on natural ecosystems. However, studies on impacts of STP discharge upon organisms and receiving rivers are very limited.

The objective of this study is to investigate the relationship between water quality and living organisms. In FY 2004, we investigated the fate of organic and inorganic compounds originating from sewerage effluent and the bioaccumulation of estrogenic substances and metals in periphytons and benthos in Tama River.

Results

The summary of this study conducted in FY2004 is as follows:

- (1) The fate of sewage related compounds in the river was investigated in summer. As a result, inorganic nitrogen and phosphorus, estrogen and nonylphenol related compounds were not decreased in the section of 1000m to 3000m lower stream from the STP, which was contrary to that significant decrease in concentration of estrogen along river stream was observed in previous study in summer.
- (2) River water, periphyton and benthic invertebrates were collected at the upper site of the Tama River (St.1 and St.2) and the site downstream of STP discharge (St.,3) and it was shown that the content of Mn, Zn, Pb, Cu and Mo among heavy metals in periphyton were raised after inflows of STP effluent (Fig. 1). However, biomagnification of heavy metals through the food web was not observed.

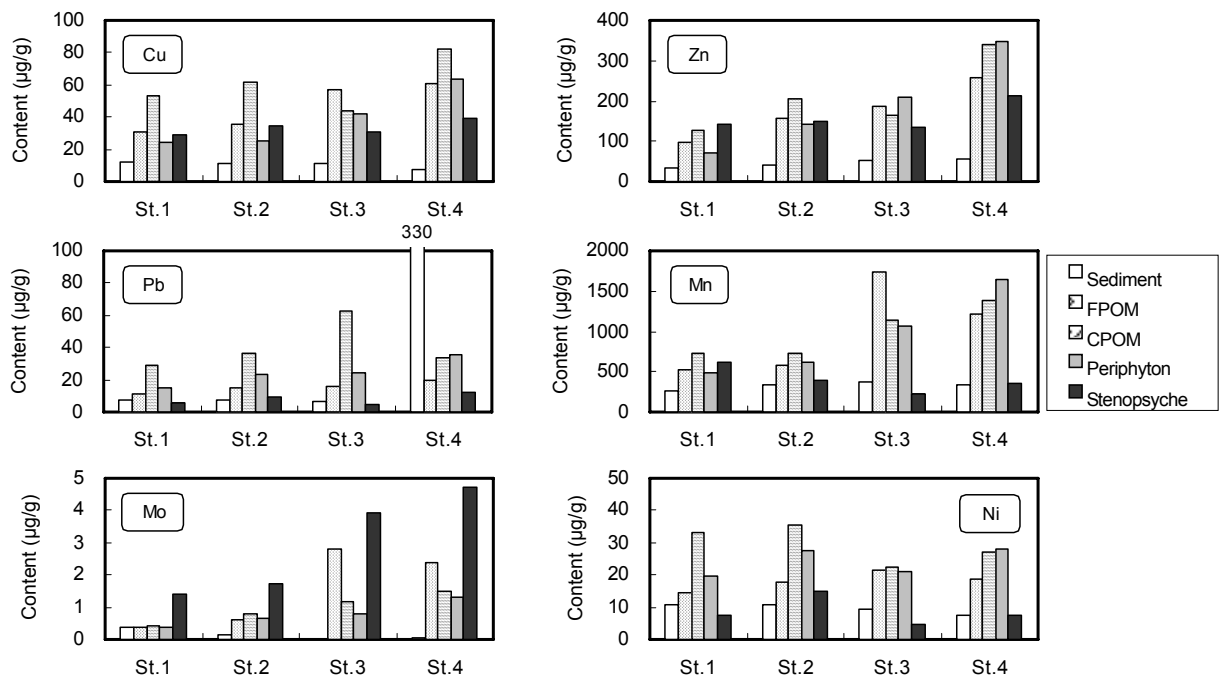


Figure 1. Concentrations of metals in sediment, particulate organic matter, periphyton and stenopsysche collected from Tama River.

TRANSPORT OF CONTAMINANTS AT SEDIMENT-WATER INTERFACE

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Project period: FY 2000-2005

The aim of this study is to clarify release mechanisms of nutrients from lake and reservoir sediments from the viewpoint of eutrophication countermeasure.

In FY 2003, we conducted a field investigation in Ushikubiri reservoir, an experiment of oxygen supply in bottom layer, and a laboratory experiment using sediment samples. The following results were obtained.

- 1) Oxygen supply experiment in the reservoir has been conducted since FY 2002. Temperature, DO and ORP in water column were continuously monitored by the sensor during experimental period. Then, we observed the continuous change of oxidation-reduction potential and nutrients concentrations in water column with or without oxygen supply. As a result, increase of phosphorus concentrations in water column that were not found in aerobic conditions was observed after the stop of oxygen supply. However, the phenomena was not as clear as that of last year.
- 2) Release tests with anoxic condition using core sediment samples collected before and after oxygen supply experiments in the reservoir were conducted. The result was that release rates of nitrogen were not different between before and after the oxygen supply. In addition, dissolved oxygen concentration in the test column increased of nitrogen purge.
- 3) Batch stirring experiments were conducted using the surface, middle and bottom layers of a core sediment samples, and the rates and potentials of nutrient release from sediment mud under varied temperatures, aerobic and anoxic conditions were quantitatively assessed. From the experiments, following results were obtained; the difference of dissolution amount of phosphorus among sediment samples was small under aerobic conditions; both release concentrations and rates were promoted under anoxic conditions and high temperature (20 degree C) compared with those under aerobic conditions and low temperature (5 degree C); the nitrification rate of surface layer sediments under aerobic conditions was significantly bigger than those of other layer sediments.

Key words: sediment, nutrients, elution, DO, ORP, monitoring.

BEHAVIOR OF CHEMICALS FROM URBAN DISCHARGE IN WATER ENVIRONMENT

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Project period: 2001 - 2005

OBJECTIVES

The target substances of this study are endocrine disruptors (EDs) such as decomposition by-product of surface active chemicals and human and animal related hormones originating from urban wastewater, and the aims of this study are development of efficient investigation and analytical techniques of EDs in river water and sediment samples and the clarification of fate of EDs in water environment.

RESULTS

In FY 2004, we conducted development of analytical techniques of the conjugated forms of natural estrogens in wastewater treatment plants, surveys of concentrations of nonylphenol and its derivatives (NPs) in water and sediment samples in a lake, development of simulation model of NPs based on the surveys, and evaluation of estrogen decomposition rates of water and sediment samples in a reservoir. Then, following results were obtained.

1) We modified the analytical method of the conjugated forms of estrogens that we reported last year. The recovery efficiencies of conjugated estrogens (i.e., E1-S, E2-S, E3-S, E1-G, E2-G, E3-G and E2-S&G) were improved, showing 87 - 120% for secondary effluent and 60-110% for wastewater influent (See Figure 1).

2) NPs concentration of the water samples in the lake decreased along flow direction, but that of the sediment samples showed opposite tendency. A simulation model was developed incorporating decomposition rates and adsorption constants obtained from experiments, and the model could calculate the characteristics of NPs composition and distribution of the water samples.

3) Decomposition rates of E2 were larger in the surface layer sediments or under aerobic condition than in the middle and bottom layer sediments or under anaerobic condition. If the decomposition rates were evaluated per unit mass of SS, the rates were high in the water samples than in the sediment samples (See Figure 2).

Research Paper

1) F. Li, J. Tsumori, N. Yamashita, H. Tanaka and Y. Suzuki, Biodegradation and biotransformation pathways of 17 β -estradiol by reservoir sediment under aerobic and anaerobic conditions, Environmental engineering research, Vol.41, pp.447-458 (2004)

2) T. Ohiwa, M. Sueoka, K. Tanabe, K. Komori and Y. Suzuki, Determination of conjugated estrogens in sewage by LC-MS/MS, The 7th annual meeting of Japan society of endocrine disrupters research, p.145 (2004)

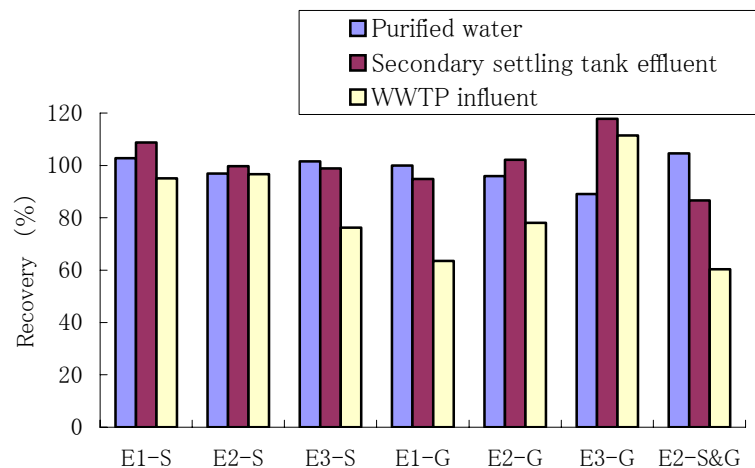


Figure 1 Recoveries (%) of estrogens from purified water samples through the modified analytical method

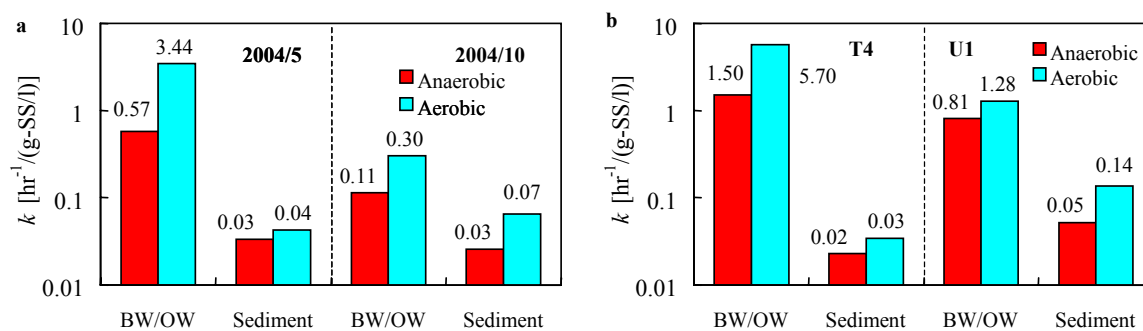


Figure 2 Comparison of E2 degradation rate between water (BW/OW) and sediment

Evaluation of Estrogen-like Substances using Bioassay

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Project period: FY 2001-2005

OBJECTIVES

In recent years, a new environmental issue occurred, namely the appearance of endocrine disruptors (EDs), in our water environment. In this study, in order to evaluate the multiple effects of the EDs, the estrogen-like activities in water samples were measured by *in vivo* bioassay using Japanese Medaka and by *in vitro* bioassay using DNA recombinant yeasts involving human estrogen receptor gene. In FY2003, we clarified that natural estrogen, especially estrone (E1), is the most contributor to estrogen-like activity detected in the wastewater with recombinant yeast screening. Therefore, surveys were conducted in a wastewater treatment plant (WWTP) in order to elucidate the fate of natural estrogen, included 17 β -estradiol (E2) and E1, and estrogenic activity along the processes.

Results

The summary of this study conducted in FY2004 is as follows:

- (1) Relatively higher removal of E2, E1 and estrogenicity in WWTP were observed in summer survey than in winter survey. Though effective removal of SS, BOD and COD were demonstrated in the both surveys, low removal efficiency of nitrogen was observed in the winter surveys (16% for winter survey and 41% for summer survey)
- (2) Effective removal of E2 (70% in winter and 87% in summer) was observed in the both surveys. However, concentration of E1 was increased by 740% in winter and 50% in summer along the treatment process.

Estrogenicity was also increased by 97% in winter and 39% in summer along the process. The estrogen-like activity of the samples measured by YES was compared to theoretical estrogenic activity calculated from concentrations and relative estrogenic propensities of E1, E2 and nonylphenol. Although there were some differences between the measured and calculated activities, the detected estrogen-like activities were mainly caused by the natural estrogens. These results suggest that appropriate treatment and control of natural estrogens in the WWTP are necessary to reduce the estrogen-like activity of treated wastewater.

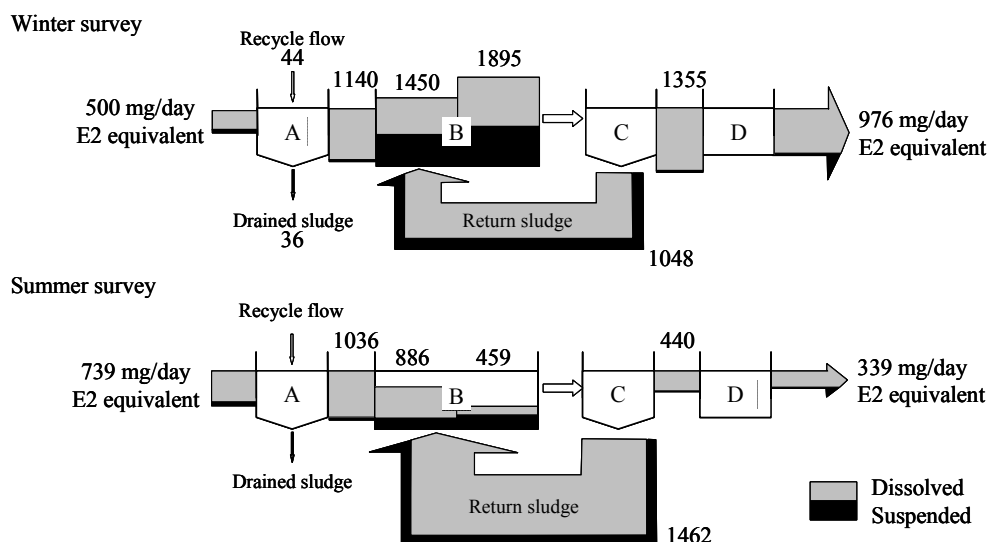


Figure 1. Mass balance of estrogenic activity (in mg/day, E2 equivalent) through physicochemical treatment (A and C), biological treatment (B), and chlorination process (D) during winter and summer in a municipal sewage treatment plant in Japan.
A: Primary settling tank; B: Aeration tank; C: Final sedimentation tank; D: Chlorination tank

EVALUATION OF THE WATER ENVIRONMENT IN KANTO REGION

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OBJECTIVES

The aims of this study are to evaluate current water quality from the view point of the water environment, and to collect useful data of watersheds and water quality to preserve the water environment in Kanto region. For these purposes, we investigated water quantity and water quality of the rivers, their relation to their watershed conditions, the influence of estrogen-like substances on fish by using exposure tests, and the influence of nutrients on eutrophication of rivers by using algal growth potential test.

Results

The summary of this study conducted in FY2004 is as follows:

- (1) Some river samples showed toxicity in algal growth inhibition test from the end of April to the middle of May, but pesticides could not be detected in those samples.
- (2) AGP map in Tone River was obtained during the period without rainfall in summer and winter.
- (3) The on-site exposure system of Medaka fish (*Oryzias latipes*) with water temperature control was improved, which enabled experiments in various sites under the same exposure condition.

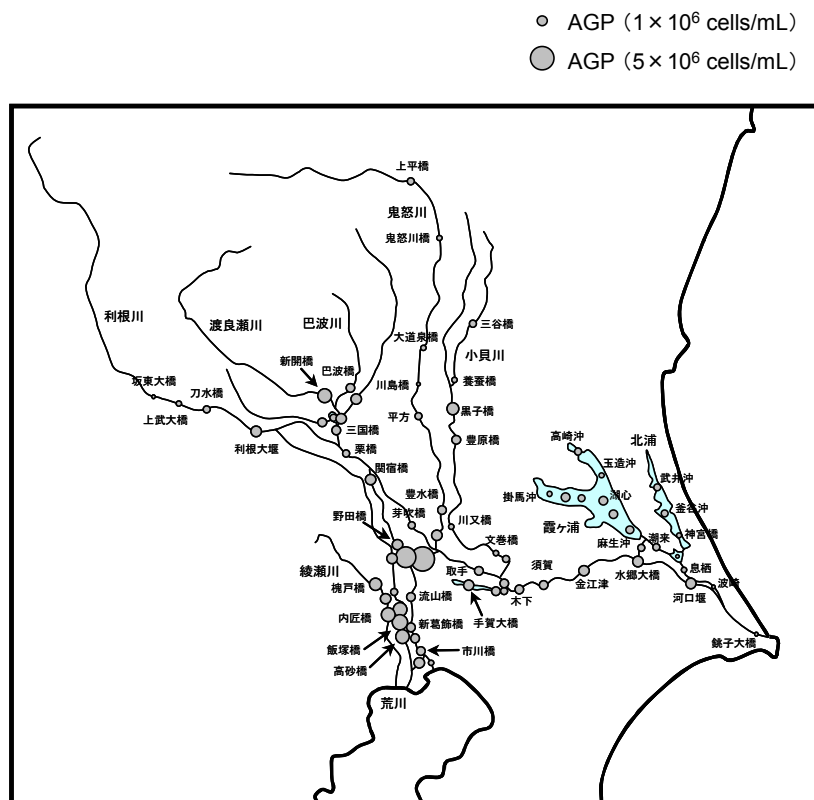


Figure AGP map in Tone River in August of 2004

Effects of Estrogen-Like Substances in Urban Wastewater on Fish and Their Indication

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Project period: FY 2002-2005

OBJECTIVES

Estrogen-like substances have been suspected to cause the feminization of wild fish in some rivers in Japan. To elucidate the influence of estrogen-like substances on fish in the rivers, we have developed an on-site fish exposure system using medaka *Oryzias latipes*.

Results

The summary of this study conducted in FY2004 is as follows:

- (1) At the water quality monitoring station that is located the most downstream in this study field and where eight sewage treatment plant discharges are received, all the male medaka were found to produce vitellogenin.
- (2) Estrone (E1), that was mainly detected in downstream river water, was thought to cause the induction of vitellogenin of the male medaka.
- (3) The occurrence of vitellogenesis seems to coincide with the occurrence of estrogenic activity of the river water.

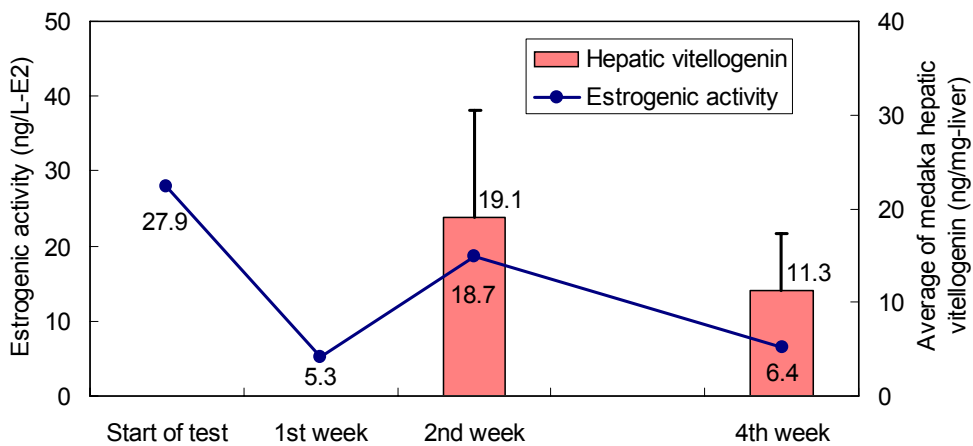


Figure Hepatic vitellogenin productions of the male medaka and changes of estrogenic activity at Ishihara WQMS in the spring of 2004

Detection of Environmental Stresses on Aquatic Organisms Using Gene Analysis Technology

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Project period : FY 2002-2006

OBJECTIVES

Some stresses, which are caused by chemical substances, may not be detected with traditional bioassay methods. Nowadays, a method that can be used to analyze and evaluate the influences of such chemicals in gene levels has been developed. However, this method is mainly used in the medical field. In this study, we attempted to apply this method to the environmental field for evaluation of the chemical impacts on aquatic organisms present in rivers or streams.

RESULTS

IntelliGene Cyano CHIP Ver.2.0 (Takara Bio Inc.), in which genes of cyanobacteria *Synechocystis sp.* (PCC6803) was fixed, was used to evaluate the chemical impacts on the aquatic organism. Effects of chemicals such as herbicide, pharmaceutical, surfactant and heavy metal on the organism were evaluated with the Cyano CHIP. The major results obtained in FY 2004 are as follow:

(1) RNA extracted from *Synechocystis sp.* cells was analyzed to check the quality of RNA samples by capillary electrophoresis. As a result, the two clear peaks of 16s and 23s ribosomal RNA of prokaryote were detected. Then, it is confirmed that RNA required for the DNA microarray analysis was sufficiently extracted in this experiment.

(2) Cyanobacterium *Synechocystis sp.*, which was cultivating with BG-11 medium, was exposed to herbicide DCMU, antibacterial agent clarithromycin, surfactant LAS, heavy metal zinc and STP (Sewage Treatment Plant) effluent sample. Gene expression by exposure to the chemicals was analyzed by DNA microarray using the the Cyano CHIP Ver2.0. As a result, variation of gene expression was not observed for the samples exposed to the DCMU, LAS, Zn and STP effluent compared with unexposed control sample. Meanwhile, significant variation of gene expression pattern was observed for the sample exposed to the antibacterial agent clarithromycin, suggesting that gene expression of *Synechocystis sp.* will be affected by exposure to the antibacterial agent.

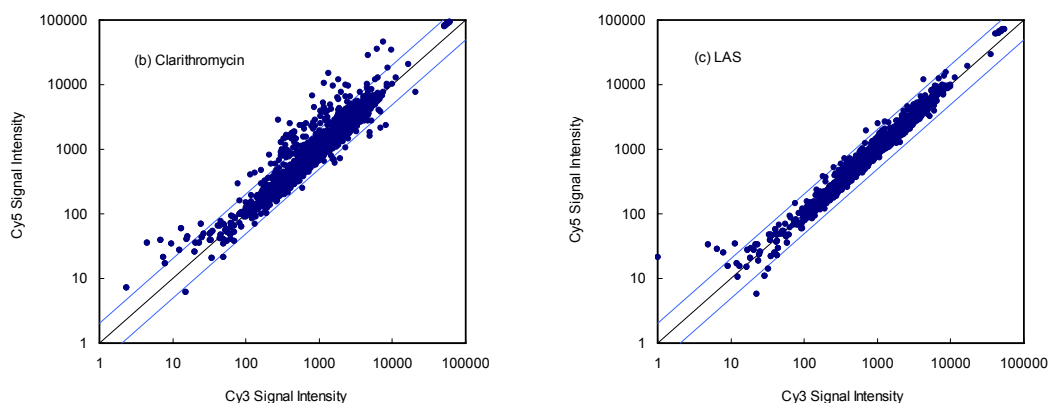


Figure 1. Gene expression analysis of *Synechocystis sp.* by DNA microarray

Evaluation of the effects and improvement of performance of river purification works

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Water Quality Division

Project period: 2003 - 2005

OBJECTIVES

River purification works including many river water purification facilities (RWPFs) have been carried out in order to improve water quality of many rivers. However, information is quite limited on what effects can be expected by the works and on whether expected performance of RWPPs is actually maintained. The governments and agencies relevant to the RWPPs hope researchers to clarify the above information.

Therefore, PWRI leads the collaborative research on evaluation of the effects and improvement of performance of river purification works among regional bureaus in Ministry of Land, Infrastructure and Transport. This study conducted by PWRI focuses on the removal performance of the RWPPs from the viewpoint of micro pollutant, particularly estrogen-like substances and ammonia, tries to understand their removal mechanisms, and will finally find the ways to improve their performance in design and operation.

RESULTS

In FY 2004, we examined the effectiveness of RWPFs for the removal of estrogen-like activity by Yeast Estrogen Screen assay (YES). The ammonia removal experiment was conducted using the experiment model of a RWPF. The following results were obtained.

1) We investigated the removal property of YES in the RWPF of aerated string contact oxidation process (ASCOP). The removal efficiency of estrogen-like activity measured by YES (55%) was greater than those of BOD (10%) and TOC (6%). Although the main purpose of RWPFs is to remove organic substances, ASCOP could remove not only organic substances (e.g., BOD) but also estrogen-like activity.

2) The ammonia removal experiment was conducted using the gravel contact oxidation process (GCOP) of the experiment model. Ammonia was oxidized well in GCOP with aeration but not oxidized well in GCOP without aeration (See Table 1).

Research Paper

1) Norihide Nakada, Koya Komori, Yutaka Suzuki, Hiroyuki Kawano, Yukio Nagato, Hiroaki Tanaka : Evaluation of the River Water Treatment Facilities for Reduction of Estrogenic Compounds, Proceedings of China-Japan Joint Symposium on Environmental Chemistry, pp.319-320 (2004.10)

Table 1 Concentration of NH₄-N and NO_x-N in water samples

Item	Sample	n	min	25%	median	75%	max
NH ₄ -N (mg/l)	Influent	116	0.9	6.5	7.5	8.6	12.7
	Effluent I		0.1	0.4	0.6	1.3	6.6
	Effluent II		1	6.4	7.4	8.4	17.4
NO _x -N (mg/l)	Influent	116	0.1	0.1	0.2	0.6	1.3
	Effluent I		1.6	6.5	7.4	8.1	13.7
	Effluent II		0.1	0.1	0.1	0.4	2.3

Utilization research of the monitoring technology in the measure against dioxin of river sediment

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Project period: 2003 - 2005

OBJECTIVES

In recent years, the pollution by dioxines (DXNs) including tetra- through octa-chlorodibenzo-p-dioxins (PCDDs), tetra-through octa-chlorodibenzofurans (PCDFs), and coplanar polychlorobiphenyls (Co-PCBs) has become a major nationwide problem, as dioxins have high toxicity even in trace concentrations. It is necessary to take suitable countermeasures to dioxin with establishment of the Law Concerning Special Measures Against Dioxins (Law No. 105 of 1999). Based on the Dioxins Law, the sediment quality standard (150pg-TEQ/g) was stipulated on July 22, 2002. When sediment exceeding the standard is found, the contaminated area will be identified through surveys around there, and then remedial measures such as dredging will be conducted. It is expected that the number of samples for investigating contaminated area increases. It is necessary to develop a quicker analysis method and continuous monitoring technology.

In this research, development of simpler detection technologies which use such as the enzyme-linked immunosorbent assay method and a continuous monitoring technology is attempted.

RESULTS

In FY 2004, we applied a simple detection technology to the dioxin pollution spot and evaluated its applicability. The following result was obtained.

1) The method tested was quadropole gas chromatography mass spectrometry (Q-pole GC/MS) that was focusing on 2,3,7,8,-TeCDF. The Q-pole GC/MS method was applied to a survey at a small river. The obtained values of Q-pole GC/MS method differed from those measured by the standard method for samples below 150 pg-TEQ/g, but, both values became closer for samples over 300 pg-TEQ/g (See Figure 1 and 2). Therefore, a simple detection technology such as Q-pole GC/MS method is applicable to such surveys as to know the high concentration pollution area.

Research Paper

- 1) K. Komori, H. Tanaka, M. Yasojima, M. Minamiyama, Y. Suzuki, Y. Miyake, M. Kato, K. Urano, Applicability of inexpensive and rapid analytical methods for dioxins in river sediment, The Society of Environmental Instrumentation Control and Automation, Vol.9, No.2, pp.133-140, 2004 (in Japanese)
- 2) K. Komori, H. Tanaka, M. Yasojima, Y. Suzuki, Simple analytical method for dioxins in river sediments by Q-pole GC/MS, Proceedings of the 41st Environmental Engineering Forum, pp.107-109, 2004 (in Japanese)

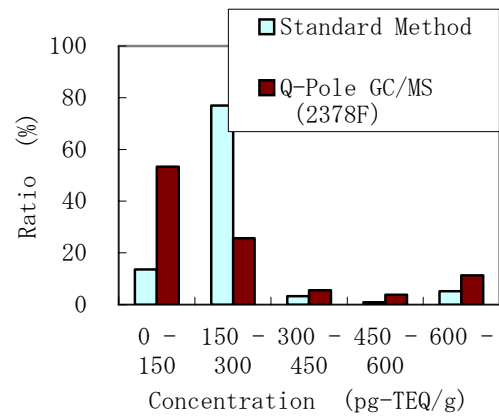
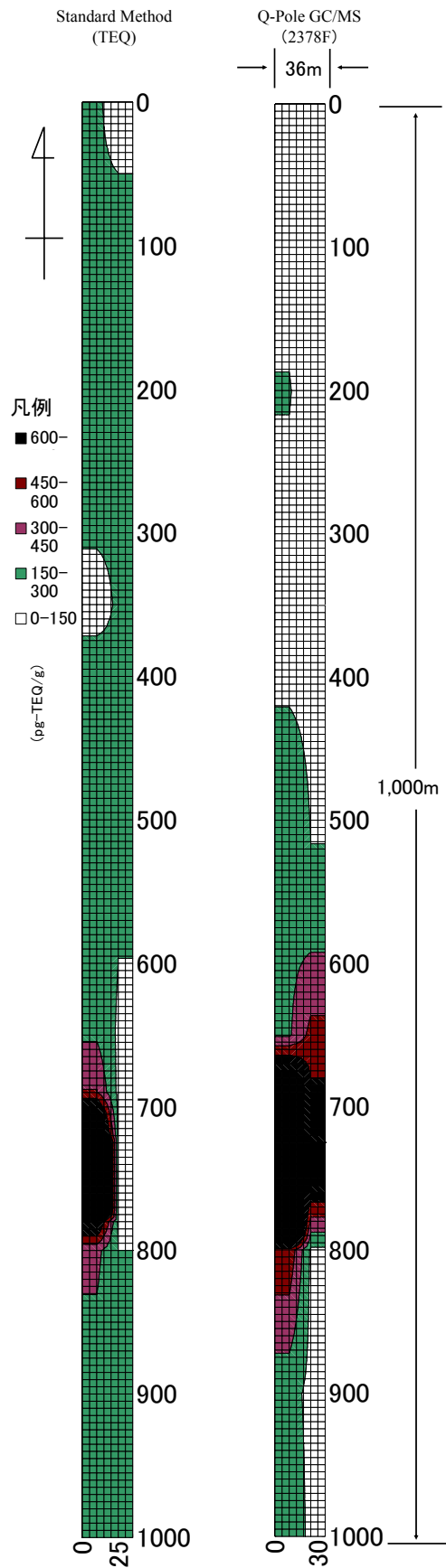


Figure 2 Distribution of DNXs concentration at a small river

Figure 1 Results of DNXs surveys at a small river

Evaluation of Ecotoxicological Effects of Chemicals in Aquatic Environment

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Water Quality Team

Project period : FY 2003-2007

OBJECTIVES

In recent years, various kinds of chemicals are used widely, according to the progress of industry. It has been reported that various chemicals such as pesticide and endocrine disrupter exist in waste, sewage treatment and river water. From these backgrounds, the purpose of this study is to investigate the effects of chemicals to organisms in the aquatic environment.

RESULTS

The summary of this study conducted in FY2004 is as follows.

(1) Bioassays were conducted to evaluate the ecotoxicological effects of pharmaceuticals discharged into water environment on aquatic organisms. Antibacterial agent, Levofloxacin (LVFX) and Clarithromycin (CAM), which were popular antibiotics in Japan, were selected as pharmaceuticals tested in this research. Algal growth inhibition test using green alga *Pseudokirchneriella subcapitata* (NIES-35) was conducted. As a result, EC50, LOEC and NOEC were 1,200 µg/L, 630 µg/L, 310 µg/L for LVFX and were 11 µg/L, 6.3 µg/L, 3.1 µg/L for CAM, respectively. Toxicity of CAM was considered to be about 100 times higher than that of LVFX.

(2) Ecological risk assessment of LVFX and CAM was conducted with comparison between predicted no-effect concentration (PNEC) and predicted environmental concentration (PEC). As a result, PEC for the LVFX was always to be lower than PNEC. But PEC for the CAM was to be higher than PNEC when dilution ratio of CAM in waters is less than 20 times. Considering the ratio of PEC/PNEC, it was evaluated that LVFX had no risk and CAM had risk depending on the dilution ratio. Therefore, it will be necessary to pay attention to the impact of CAM discharged into rivers and streams on the aquatic ecosystem.

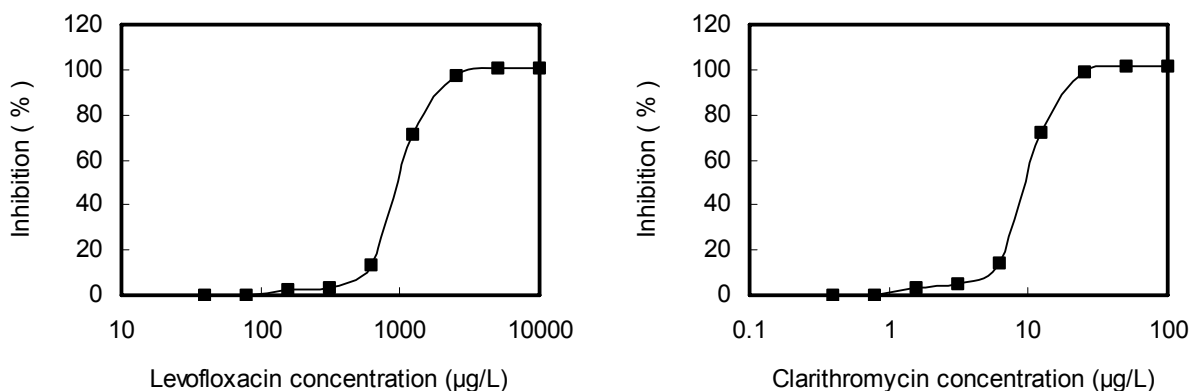


Figure 1. Results of algal growth inhibition assay for Levofloxacin (LVFX) and Clarithromycin (CAM)

Evaluation of effect of nutrients load from urban area on coastal sea environment

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Project period: FY2004-2006

OBJECTIVES

Changes of environment and ecosystem in coastal sea are public concern, and a hypothesis is proposed which relates those changes to the change of nutrients load caused by recent forest management or construction of infrastructure such as river management facilities and sewerage systems.

In this research, nutrients load through sewerage systems and the effect of the treated wastewater on seaweed growth were evaluated.

Results

The summary of this study conducted in FY2004 is as follows:

- (1) Nitrogen load from sewerage system occupies a major portion of the total nitrogen load, but as for phosphorus, particulate form discharged from watershed through rivers during rain events is the major source. Silicate is mainly supplied through rivers in dissolved form, and the concentration of treated wastewater is a little higher than that of river water.
- (2) Treated wastewater contributed to the growth and color development of seaweed by its nutrients. However, additional research is needed to clarify the effect of chlorine and other elements.

Table Experimental conditions

No.	Treated wastewater	Sea Water
A-1	Plant A	Artificial
A-2	Plant A	Local (area A)
A-cont1	-	Artificial
A-cont2	-	Local (area A)
B-1	Plant B	Artificial
B-2	Plant B	Local (area B)
B-3	Plant B *disinfected with ozone	Artificial
B-cont1	-	Artificial
B-cont2	-	Local (area B)

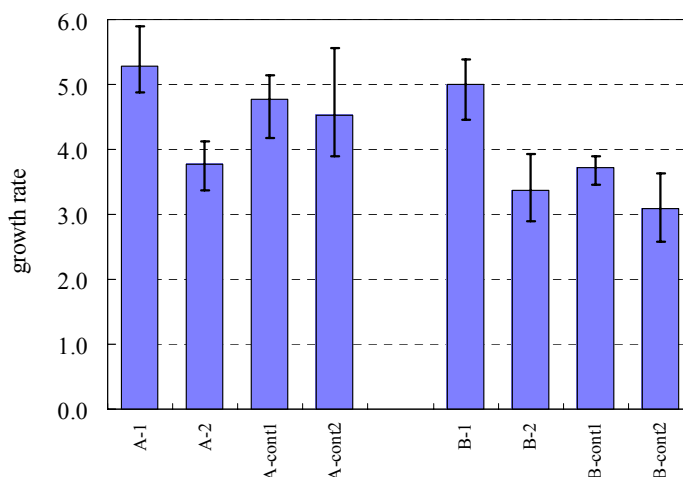


Figure Growth rate of seaweed (cultured for 8 days)