

RESEARCH ON EVALUATION OF TRACE CHEMICALS AT WASTEWATER TREATMENT PLANTS

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Project period: FY2001 – 2005

OBJECTIVES

Sewage works accept domestic and industrial wastewater, treat them and discharge treated water into the receiving water. Wastewater contains various chemicals and it has been pointed out that a very small amount of chemicals (trace chemicals) still remain in final effluent from wastewater treatment plant. Therefore, at present, it is required to reduce trace chemicals from wastewater treatment plant and to ensure safety of final effluent. In this survey, endocrine disrupting chemicals were mainly focused on out of various trace chemicals. Objective of this survey is to develop methods for estimating suspected endocrine disrupting chemicals in wastewater. In FY 2003, we conducted 1) development of ELISA method for roughly and rapidly measuring free estrogens in wastewater and 2) development of analytical methods for correctly measuring free/conjugated estrogens, nonylphenol (NP) and its related substances and understanding of behavior of these trace chemicals in wastewater treatment process.

RESULT

Many previous studies have pointed out the occurrence of many trace chemicals such as endocrine disrupting chemicals in sewage. Therefore, the way to estimate their fate and risk should be established. Application of ELISA method to measurement of trace chemicals and behavior of estrogen and nonylphenol in sewage treatment were studied in FY 2003,

Main results are as follows

- 1) Two pretreatment procedures for removing cross-reacting and/or disturbing substances in measuring 17β -estradiol in sewage samples with the ELISA method were compared, but there were no obvious difference between the procedures.
- 2) The occurrence of free and conjugated estrogens in influent and secondary effluent in 20 municipal wastewater treatment plants was surveyed. Result shows that 17β -estradiol (E2) and estriol (E3) were effectively removed in wastewater treatment, while removal efficiency of estrone (E1) was about 50%. Moreover, significant levels of conjugated estrogens exist even in treated sewage as well as sewage, which has not been reported so far. Further, increase in conjugated estrogens during wastewater treatment suggests the possibility of transformation of conjugated estrogens from unidentified form of estrogens in this study although analytical method requires further improvement because of their low recovery ratio.

3) Activated sludge treatment apparatus was operated fed with nonylphenol ethoxylates (NPnEOs) as a sole organic substance. In aerobic process ($DO \approx 1.0\text{mg/L}$), nonylphenol (NP) was produced in activated sludge, which indicates that formation of NP was strongly affected by dissolved oxygen concentration in aerobic process.

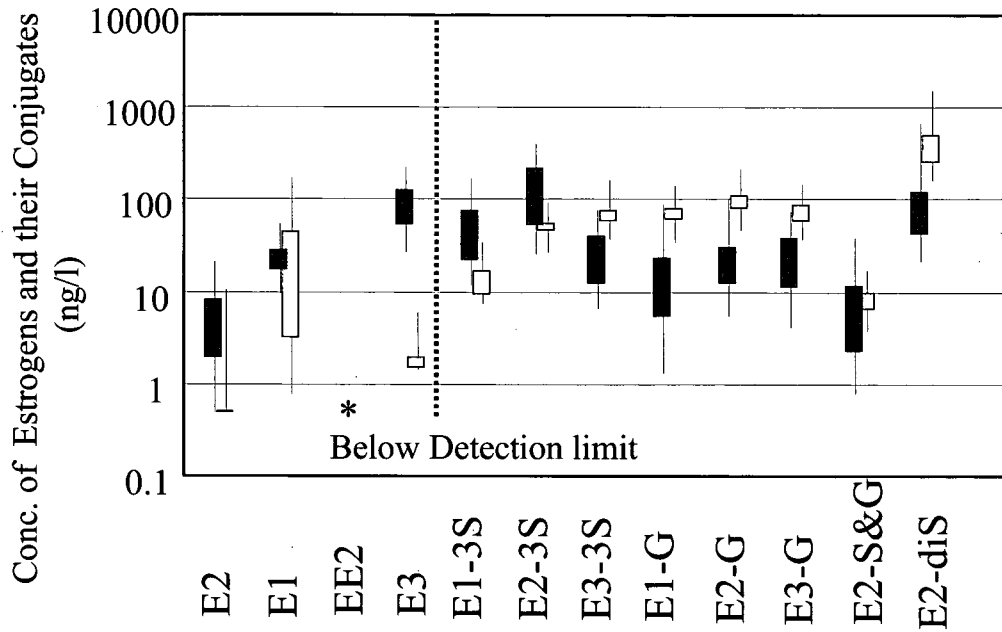


Figure 1 Concentrations of Selected Estrogens and their Conjugates in Influent / Secondary Effluent from 20 WWTPs

REFERENCES

1. K. Komori, *et al* : Analysis and Occurrence of Estrogen in Wastewater in Japan, Proceedings of IWA ECOHAZARD 2003 Conference, 45/1-45/8, 2003.9.
2. Y. Okayasu, *et al* : Degradation of nonylphenol ethoxylate in aerobic wastewater treatment process, Proceedings of IWA ECOHAZARD 2003 Conference, 13/1-13/7, 2003.9.

Effects on Water Environment Affected by Sewerage Systems

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Project period: 2001 - 2005

Limited studies have been conducted so far for quantitative evaluation on change 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 of sewerage works upon river water quality and aquatic ecosystems.

In FY 2003, the following results were obtained:

- 1) To develop an analytical model for Teganuma Lake and Ohtsugawa River system , we selected suitable one among several candidates and collected frame data on the watershed, which are necessary to operate the model.
- 2) We conducted an exposure examination using Medaka fish (*Oryzias latipes*) in a tank installed in a sewage treatment plant to examine the influence of estrogen-like substances in treated wastewater (Fig.1).
- 3) We applied the Algal Growth Potential Test using a micro-plate to evaluate the influence of water quality of the river receiving treated wastewater on the aquatic ecosystem.

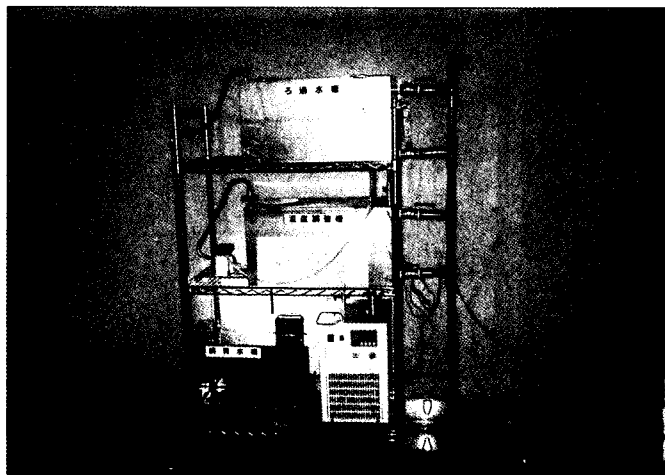


Fig.1 Medaka Fish Exposure System

Evaluation of River Water Quality from the Viewpoint of Aquatic Ecosystem

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Project period : FY 2001-2005

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) to the receiving river water has been increasing. As the result, the quality of river water is greatly influenced by the quality and the quantity of treated wastewater. It is, therefore, assumed that the treated wastewater may exert positive or negative impacts on natural ecosystems. However, STP discharge's impacts upon organisms and receiving rivers are very limitedly studied.

RESULTS

The final objective of this study is investigation of the relationship between water quality and living organisms. In FY 2003, we investigated the fate of organic and inorganic compounds originated from sewerage effluent in the Tama River. Furthermore, we evaluated the bioaccumulation of estrogen and estrogen-like substances in periphytons and benthos in a river.

The summary of this study conducted in FY2003 is as follows:

- 1) The fate of compounds discharged from STPs in river water was investigated. As the results, inorganic nitrogen and phosphorus were not decreased in the section of 1000m to 3000m down stream from the STP. On the other hand, estrogenic organic compounds were slightly decreased in the section, especially in summer.
- 2) River waters, STP effluents, periphytons and benthic invertebrates were collected around STP discharge point in the Tama River, and estrogen and estrogen-like substances in these samples were analyzed. As the results, the bioaccumulation was not significantly high in higher ones. Additionally, the biomagnifications of estrogen and estrogen-like substances via periphytons to benthic invertebrates contributed little to the overall bioaccumulation of these substances.

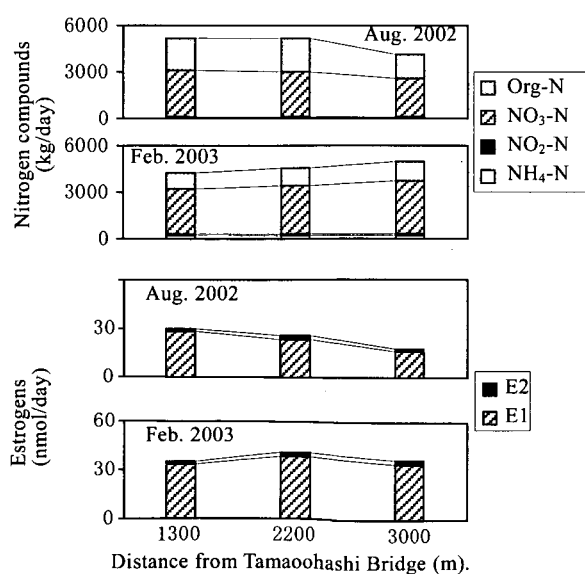


Figure 1. Flux of nitrogen compounds and estrogens

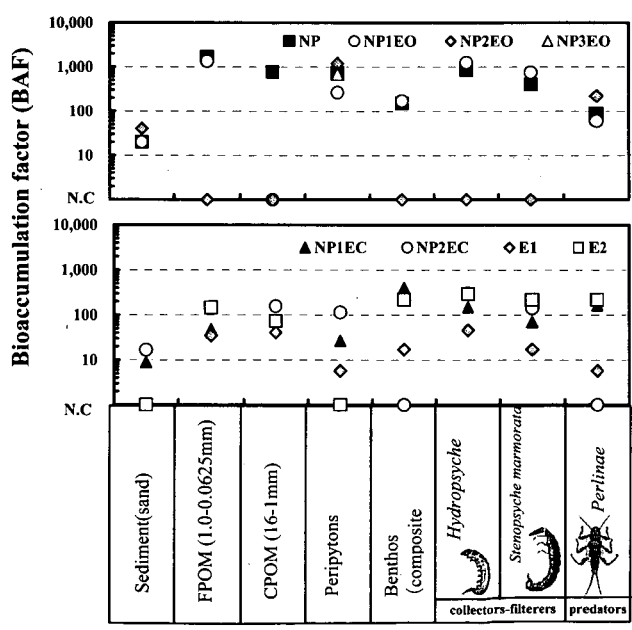


Figure 2. Bioaccumulation factor of estrogens and nonylphenol-related compounds

TRANSPORT OF CONTAMINANTS AT SEDIMENT-WATER INTERFACE

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Project period : FY 2000-2005

OBJECTIVES

The aim of this study is to clarify release mechanisms of nutrients from lake and reservoir sediments from the viewpoint of eutrophication countermeasure. The release of pollutants from the sediment mud in water systems is a reason that causes delays for the water quality improvement. Besides their impacts on water quality, the delays may also influence the environment of aquatic organisms. It is thus necessary to establish some effective countermeasures relate to the bottom mud and to predict the future water quality.

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.

RESULTS

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) 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). (Fig. 1)

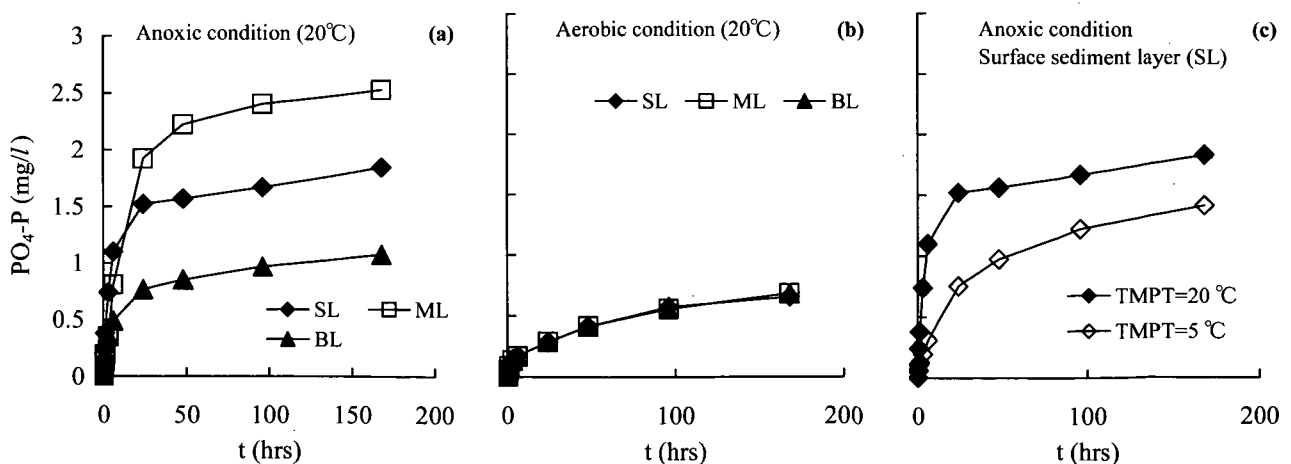


Fig. 1 Concentration profiles of PO₄-P under aerobic and anaerobic conditions

BEHAVIOR OF CHEMICALS FROM URBAN DISCHARGE IN WATER ENVIRONMENT

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Project period : FY 2001-2005

OBJECTIVE

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 the 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.

In FY 2003, we conducted the development of analytical techniques of the conjugated forms of natural estrogens in the sewerage treatment plant, the surveys of concentrations of estrogens, nonylphenol and its derivatives in water and sediment samples in river and lake, and laboratory experiment of estrogens' fate in sediment samples.

RESULTS

The following results were obtained.

- 1) We developed the analytical method of the conjugated forms of estrogens by using LC/MS/MS. Though a good recovery rate of 94-120% could be obtained for free estrogens, the recovery rate of the conjugated forms of estrogens were low, which were obtained from the addition and recovery examination for wastewater and treated wastewater.
- 2) To determine horizontal distribution of EDs in water and surface sediments in Lake Teganuma, field investigation was conducted. Nonylphenol (NP) concentrations showed a tendency of increase towards the downstream. On the other hand, the longitudinal distributions of estrone (E1), nonylphenol ethoxylates (NPnEO) and nonylphenoxy acetic acids (NPnEC) in the surface sediments showed that the concentrations are the highest at the inlet, and are fairly constant at lower levels towards the downstream. The obtained results indicate that mechanisms of adsorption and sedimentation associated with suspended solids such as algae of internal product. (Fig. 1)
- 3) Batch biodegradation experiments of 17β -Estradiol (E2) were conducted using the surface, middle and bottom layers of a core sediment sample from a drinking water reservoir; the impacts of temperatures, sediment mud position, aerobic and anoxic conditions upon the degradability of spiked E2 were assessed. The coefficient of decomposition rate of surface position sediment was significantly higher than middle and bottom position one. (Fig. 2)

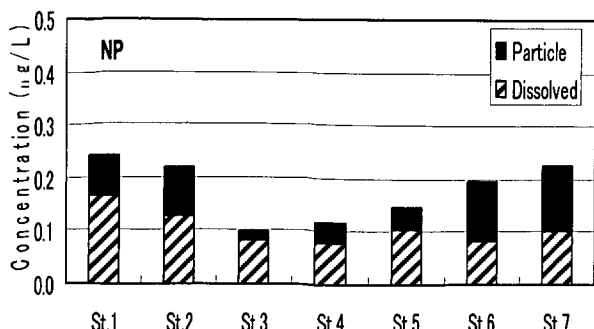


Fig. 1 Concentrations of NP in the longitudinal direction with the flow path in Lake Teganuma.

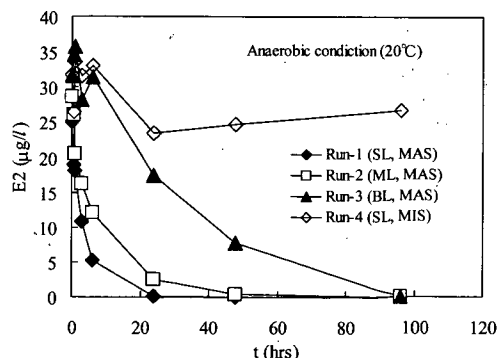


Fig. 2 Concentration profiles of spiked E2.

Evaluation of Estrogen-like Substances using Bioassay

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Project period : FY 2001-2005

OBJECTIVES

In recent years, a new environmental issue has 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. Besides this, fractionation techniques combined with each bioassay were used to characterize and estimate major origins of the estrogenicity. Furthermore, the estrogenicity detected in river samples were compared with population and development of wastewater treatment facilities in the watershed where each river sample was collected.

RESULTS

The summary of this study conducted in FY2003 is as follows:

- (1) Environmental sample was fractionated not into aggregated estrogens but into each estrogen using high performance liquid chromatography. As a result of the fractionation technique combined with the yeast estrogen screen assay, the contribution of estrone was larger than that of 17β -estradiol in treated wastewater in sewage treatment plants.
- (2) A comparison between the estrogen-like activity of major river waters in Japan and the population density in the river basins indicates that the population density was a large contributing factor to the estrogen-like activity in major rivers in Japan. No clear relationship between wastewater treatment facilities provision ratio and estrogen-like activity of the river water was found.

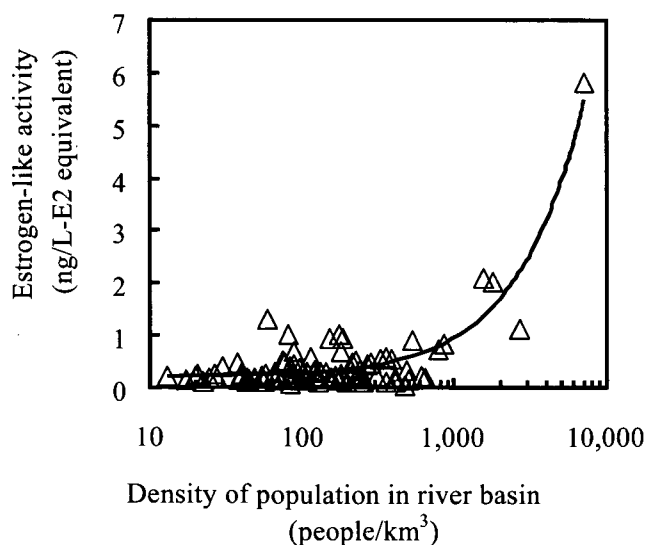


Fig. 1 Relationship of estrogen-like activity and density of population in river basin

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

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Project period: 2002 - 2005

The purpose of this study is to evaluate the influence of estrogen-like substances contained in river water receiving effluent of urban wastewater treatment plant on the feminization of male fish by keeping the fish in the river water; to develop a simple indicator of fish feminization by combining estrogenic activity measured by DNA recombinant yeast and water fractionation method; to clarify the source of the estrogen-like substances in urban wastewater for the control of the fish feminization. Research results of FY2003 are as follows; 1) When adult male medaka were exposed for 14 day to 17 β -estradiol (E2) solution , Lowest-observed adverse effect level (LOAEL) was 5ng/L, which concentration could be termed high level among those in urban wastewater. On the other hand, when exposure periods were 2 or 7 days, no observed adverse effect level (NOAEL) of E2 was 5ng/L. Therefore, when male fish is exposed to low estrogen concentration, longer period is effective to detect the estrogenic activity. 2) The potential of estrogenic activity of estrone (E1) to produce vitellogenin in male medaka was 0.18-0.26 times of that of E2. 3) Adult male medaka were exposed to river water receiving treated urban wastewater at the site of automatic water quality monitoring stations, and if the estrogenic activity exceeded the LOAEL, hepatic vitellogenin was detected from 10% as hepatic vitellogenin induction for endpoint. 4) As a result of optimizing the fractionation of the estrogen-like substances in the effluents of wastewater treatment plants, estrogens and their conjugates were effectively separated, and it was shown that E1 greatly contributed to the estrogenic activity of the effluents.

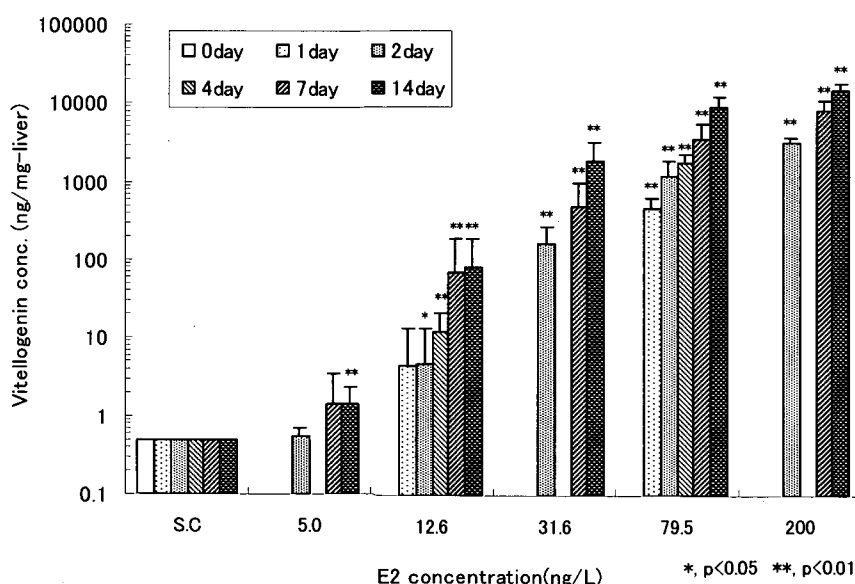


Fig.1 Hepatic vitellogenin concentration of male medaka exposed to 5 class E2 solutions.

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 chemicals like medicines, may not be detected with traditional bioassay methods. Nowadays, a method that can be used to analyze and evaluate the influences of such chemicals from 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 treated wastewater. More specifically, we are aiming at development of a DNA chip to identify the cause of fish-kill.

RESULTS

The major results obtained in FY 2003 are as follow:

- (1) After some medaka were exposed to cyanide solution, their genes were extracted. As a control, the genes of medaka that were not exposed to cyanide were also extracted.
- (2) The difference in gene expression between the above two medaka groups was investigated (Figure 1), which resulted in that expression of 889 genes was promoted but 2859 genes was suppressed by cyanide.
- (3) 189 genes were finally determined based on the above selected known genes and were put on our prototype medaka DNA chip that aims at identification of the cause of fish-kill due to spill accident of cyanide in rivers.

Further study may include applicability of the prototype to identification of cyanide and other environmental stresses and improvement of the prototype chip that can detect more environmental stresses.

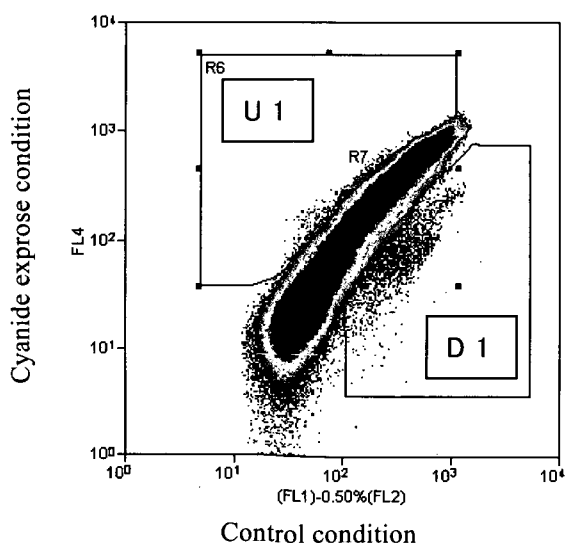


Figure 1. Gene expression under the control and cyanide expose conditions

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

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Project period: 2003 - 2005

OBJECTIVES

River purification works including many river water purification facilities(RWPPs) have been carried out in order to improve water quality of many rivers. The information is quite limited on what effects are expected in improvement of river water quality 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 2003, the following results were obtained.

1) The aerated gravel contact oxidation process (AGCOP) was selected because the process is one the most commonly applied to Japanese rivers. The removal efficiency of estrogen-like activity measured by DNA recombinant yeast assay (67%) is equal or greater than those of SS (66%) and BOD (40%). Therefore, the RWPP aiming at removal of BOD and ammonia is expected to reduce estrogen-like activity of river water to some extent.

2) The experiment model of the AGCOP was created in bench scale that can change hydraulic retention time. The diffusion property of the reactor in the AGCOP process was obtained by tracer test.

Research Paper

1) KOMORI, K., MIYAMOTO, N., TANAKA, H., SATO, C., HAYASHI, K. and HORIUCHI, S.: Reduction of the Estrogen-like Substances and Estrogen-like Activity in the River Water Treatment Facilities, ABSTRACTS -International Association for Great Lakes Research 46th Annual Conference & International Lake Environment Committee 10th World Lakes Conference-, p.81, 2003.6

Table-1 Concentrations of Estrogen-like activity, SS and BOD

	Estrogen-like activity (ng/l)		SS (mg/l)		BOD (mg/l)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
1	1.3	0.3	5	0.5	3.9	2.8
2	1.4	0.6	1	0.5	3.1	2.7
3	2.1	0.1	3	0.5	3.6	2.7
4	2.2	0.8	19	2.6	7.5	2.1
5	3.1	0.8	7	3	5.7	2.8
6	2.3	1.4	8	7	4.4	3.7
Max.	3.1	1.4	19	7	7.5	3.7
Min.	1.3	0.1	1	0.5	3.1	2.1
Av.	2.1	0.7	7	2.4	4.7	2.8

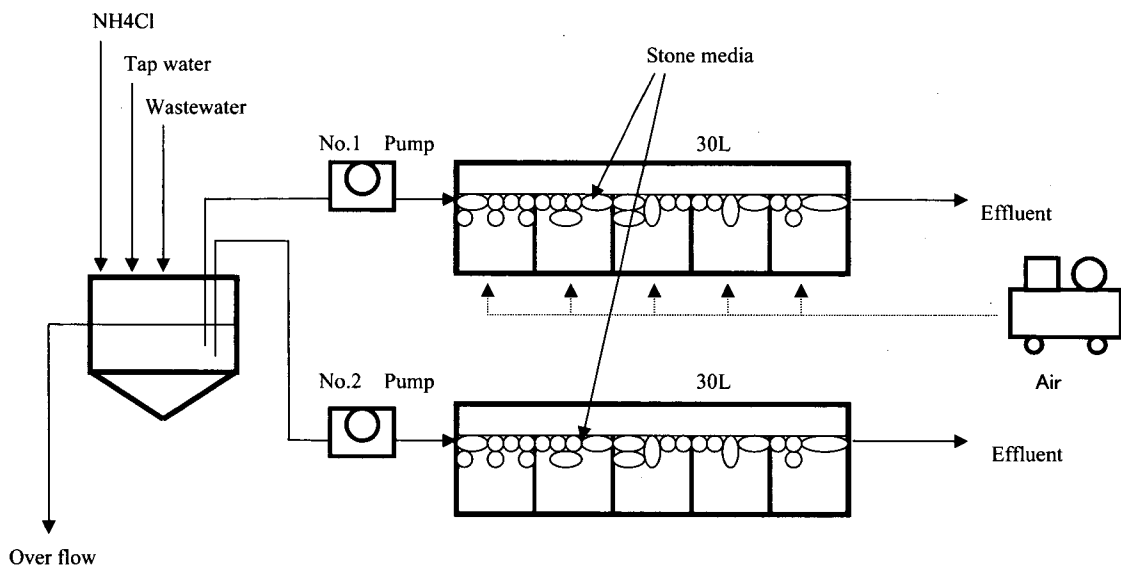


Figure 1 Experimental apparatus

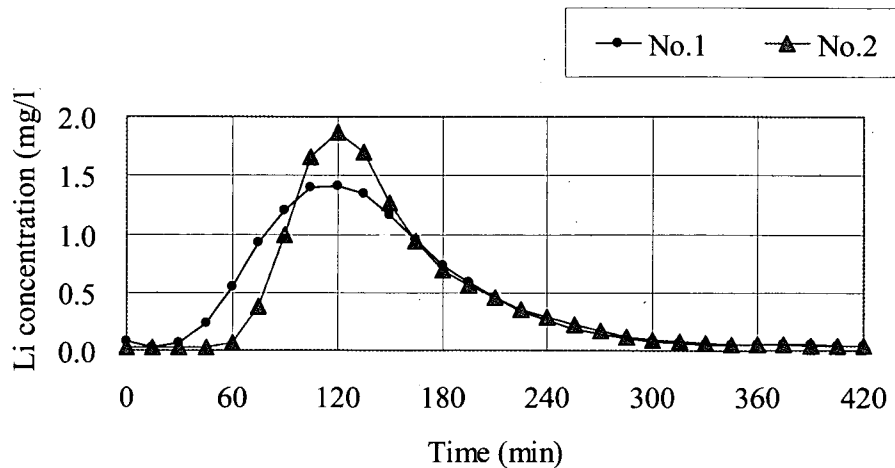


Figure 2 Tracer test

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 correspondence to dioxin with establishment of the Law Concerning Special Measures Against Dioxins (Law No. 105 of 1999). Based on the Dioxins Law, stipulated the sediment quality standard (150pg-TEQ/g) for dioxins on July 22, 2002. When the sediment exceeding the standard is found, the contaminated area will be identified through the survey around there, and then remedial measures such as dredging will be conducted. It is expected that the number of samples for investigation on contaminated area survey increases. It is necessary to develop a quicker analysis method and continuity monitoring technology.

In this research, it aims at development of the simple detection technology which used the enzyme-linked immunosorbent assay method so on and a continuity monitoring technology.

RESULTS

In FY 2003, the following results were obtained.

- 1) The examination about the combination of the simple detection technology and the pretreatment was carried out using 20 samples that were obtained from four rivers where contamination of DXNs in the sediments were identified. It became clear that the combination of a Q pole GC/MS and pressurized fluid extraction, silica gel, activated carbon has the high correlation with the regulating method.
- 2) The field study of contentious monitoring of turbidity was conducted due to the secondary pollution, i.e. dredging by the novel turbidity meter, which proved the applicability of the turbidity meter to the contentious monitoring of turbidity in actual field for a long period.

Research Paper

- 1) Shusuke Ishibashi, Takashi Suzuki, Koya Komori, Hiroaki Tanaka, Continuous Monitoring of the Turbidity Changed by Dredge a River Bed, The Society of Environmental Instrumentation Control and Automation, Vol.8, No.2, pp.23-26, 2003 (in Japanese)
- 2) Y. Miyake, M. Kato, K. Urano, K. Komori, M. Minamiyama, Development of an Efficient Method for Investigating Dioxins in Sediments by Dioxin-like SNVOxs, Proceedings of the 38th Annual Workshop of the Japan Society on Water Environment, p.315, 2003 (in Japanese)

Figure 1 Flow diagram of analytical methods. Combination of extraction, clean-up and detection.

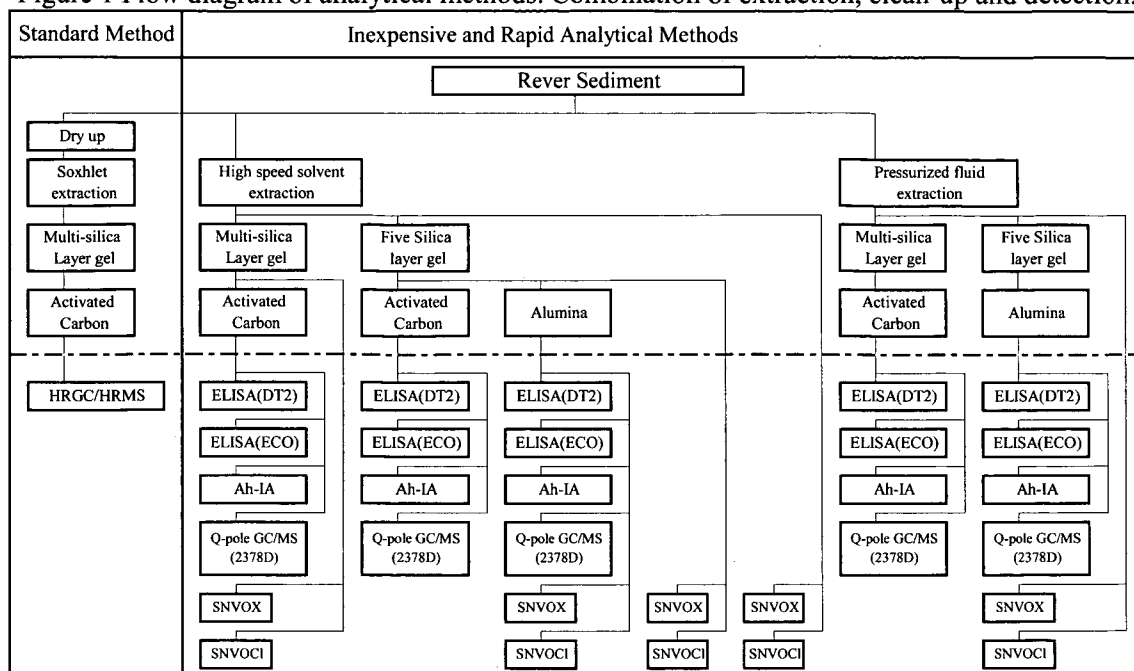


Table 2 Relationship between the standard method and the simple analytical techniques

Detection	Extraction	Clean-up	$y=ax^b$ (n=20)		
			a	b	r
ELISA(DT2)	HSSE	M AC	15.5	0.46	0.54
		F AC	5.30	0.56	0.59
		F AL	15.4	0.39	0.36
	PFE	M AC	12.8	0.47	0.59
		F AL	11.6	0.46	0.57
		F AL	11.6	0.46	0.57
ELISA(ECO)	HSSE	M AC	19.6	0.32	0.37
		F AC	1.26	0.66	0.61
		F AL	2.51	0.61	0.71
	PFE	M AC	6.74	0.41	0.67
		F AL	0.65	0.75	0.38
		F AL	0.65	0.75	0.38
Ah-IA	HSSE	M AC	5.61	0.56	0.61
		F AC	447	-0.14	0.22
		F AL	35.7	0.21	0.30
	PFE	M AC	11.6	0.38	0.61
		F AL	202	-0.03	0.04
		F AL	202	-0.03	0.04
Q-pole GC/MS (2378D)	HSSE	M AC	100	0.30	0.71
		F AC	105	0.27	0.77
		F AL	119	0.25	0.61
	PFE	M AC	79.7	0.37	0.89
		F AL	93.0	0.29	0.79
		F AL	93.0	0.29	0.79
SNVOX	HSSE	M	249	0.43	0.61
		F AL	239	0.63	0.65
		F	140	0.57	0.43
	PFE	None	32.5	0.50	0.59
		None	22.3	0.58	0.63
		None	22.3	0.58	0.63
SNVOCl	HSSE	M	254	0.44	0.62
		F AL	239	0.63	0.64
		F	165	0.72	0.60
	PFE	None	32.9	0.51	0.60
		None	24.2	0.56	0.63
		None	24.2	0.56	0.63

PFE: Pressurized Fluid Extraction method, HSSE: High-Speed Solvent Extraction method

M: Multi-layer silica, F: Five-layer silica, AC: Activated carbon, AL: Alumina

Evaluation of Ecotoxicological Effects of Chemicals in Aquatic Environment

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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 FY2003 is as follows.

(1) Water samples were collected at the Tama River area. As the results of the experiments using algal growth inhibition test, toxic effect to algal growth was not detected as for the sewage treatment and the Tama River water samples. Meanwhile, toxic effect was observed for the sample from wastewater channel discharged into the Tama River. The inhibition ratio of algal growth was 20% compared with algal growth of the control. Although the toxicity was not strong, this indicated that toxic substance responsible for the algal growth inhibition would be contained in that sample. But toxic effect was not observed as for the samples of the Tama River after mixing of water from wastewater channel. Substances caused the toxic effect must be investigated in the next study.

(2) Concentration by solid phase extraction cartridge (PLS-2) was conducted for the Tama River and sewage treatment water samples. Figure 1 shows relationship between concentration ratio and algal growth inhibition. Toxic effect was detected when the values of concentration ratio become more than 3.1. Inhibition ratios were almost 100% in the case of the values of concentration ratio become more than 25. The value of EC50 was 6.6, which was calculated from the relationship between concentration ratio and algal growth inhibition. Table 1 shows the values of EC50 calculated for the other samples in the same manner. The values were 14-17 for river water samples and 5.4-9.1 for sewage treatment water samples. The EC50 values for sewage treatment water tend to be lower compared with river water. Table 1 shows the results of toxicity unit (TU) that is a reciprocal number of EC50 value ($TU=1/EC50$). The values of TU were 0.06-0.07 for river water and 0.11-0.18 for sewage treatment water. The TU values for sewage treatment water tend to be higher compared with river water. Since high TU value means high toxicity, the toxicity of sewage treatment water was higher than that of river water.

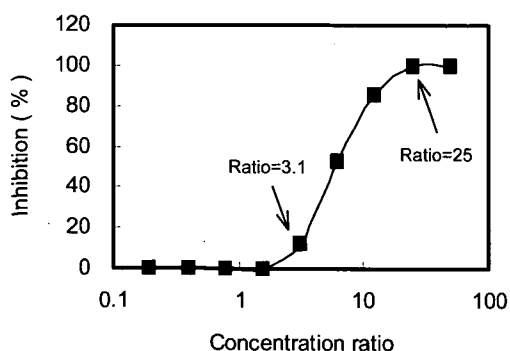


Figure 1. Relationship between concentration ratio and algal growth inhibition (%)

Table 1. EC50 and Toxicity Unit (TU) values at the Tama River area

Station	EC50	TU
St.3	14.0	0.07
St.4	16.5	0.06
St.5	17.0	0.06
STP-1	6.6	0.15
STP-2	5.4	0.18
STP-3	9.1	0.11

St.3-St.5: River water sample

STP1-STP3: Sewage treatment water sample