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 2002, 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

1. Development of ELISA method for measuring estrogens in wastewater

Pretreatment procedures of ELISA kit for measuring estrone (E1) were studied. Following two procedures were compared about influent and secondary effluent of 20 WWTPs.

Procedure-I (P-I) :Sampled, Refrigerated, Solid Phase Extraction with C18, Eluted with dichloromethane

Procedure-II (P-II):Sampled, Added ascorbic acid, Refrigerated, Solid Phase Extraction with C18, florisil and aminopropyl.

In both procedures, almost all estrone added to water samples was recovered in final extraction. About P-I influent, values measured by ELISA were much overestimated compared with those by LC/MS/MS. But about P-II influent, P-I secondary effluent and P-II secondary effluent, values measured by ELISA were within 1.5 times as much as those by LC/MS/MS. Therefore, using florisil and/or aminopropyl were effective for reducing overestimation.

2. Understanding of behavior of trace chemicals in wastewater treatment process

A bench-scale conventional activated sludge treatment process accepting actual municipal wastewater was studied. Concentrations of 17β-estradiol (E2) and estrone (E1) of the influent were the same as those of the

primary effluent, and so it is thought that degradation of E2 and E1 did not occur in primary sedimentation process. Concentrations of E2 were gradually decreased in the aerobic process and finally became lower than the detection limit (0.5ng/L) in the middle of the aerobic process. On the other hand, concentrations of E1 in the aerobic process temporarily increased and reached to 1.8 times as much as those of the primary effluent and then gradually decreased. E2 seemed to be easily decomposed by activated sludge and changes to E1 as some previous surveys reported. Degradation rate of E1 was smaller than that of E2, so that E1 remained in the aeration tank.

Concentrations of NP, nonlyphnol ethoxylates (NPnEOs) and nonylphenoxy acetic acids (NPnECs) drastically changed in the aerobic process. NP and NPnEOs decreased and became from one tenth to one hundredth as much as those of the influent, while NPnECs became more than twice as much as those of the influent. In the influent and the primary effluent, various NPnEOs (n=1-12) existed almost equally, while NPnEOs (n=5-15) disappeared and there remained from NP1EO to NP4EO in the final effluent. On the other hand, in terms of NPnEC, there existed a little high NP2EC and NP3EC and almost equal NP1EC and NP4EC～NP10EC in the influent and the primary effluent. In the aeration tank, NPnECs (n=5-15) declined and NPnECs (n=1-4) increased. About 96% of NPnECs in the final effluent were NPnECs (n=1-4). These results indicated that NPnEOs were drastically decomposed in aerobic process. And it is considered that there are two major degradation pathways. One degradation pathway is that NPnEO changes to shorter EO-chain NPnEO. The other degradation pathway is that NPnEO changes to NPnEC and further to shorter EO-chain NPnEC. In the aerobic process, NPnECs accumulated because of difference between degradation rate of NPnEOs and that of NPnECs. Moreover, it is likely that NPnEOs (n=1-4) changed to NPnECs because obvious increase of NPnECs (n=5-15) were not observed. The result indicated that NPnEOs are decomposed to not NP but NPnEO (n=1-4) and NPnEC(n=1-4) in aerobic wastewater treatment process.

Finally, a method for measuring estrogen conjugates in wastewater with LC/MS/MS was developed and applied to actual wastewater. Target substances were followings; estrone-3-sulfate (E1-S), β-estradiol 3-sulfate (E2-S), estriol 3-sulfate (E3-S), estrone β-D-glucuronide (E1-G), β-estradiol 17-(β-D)-glucuronide (E2-G), estriol 3-(β-D-glucuronide) (E3-G), β-estradiol 3-sulfate 17-glucuronide (E2-S&G) and estradiol 3,17-disulfate (E2-diS). Further improvement of pretreatment procedure were needed because only less than 50 per cent of sulfates and glucuronides added to water sample were recovered after pretreatment. We applied the method and found that removal efficiency of free estrogens (E2 and E1) in wastewater treatment was 89-99%, while removal efficiency of a conjugated estrogen (E2-G), which is said to be easily decomposed by activated sludge and changes to E2, was 58-93%. And it was observed that concentrations of estrogen conjugates in the influent and the final effluent were 2-100 times as much as those of free estrogens. Observed concentrations of E1-S and E2-S in the influent were as much as values, E1-S (6.4ng/l), E2-S (2.7-62.9ng/l), which is previously reported. But, observed concentrations of E3-S and E2-G in the influent were about 30 times as much as previously reported values, E3-S (4.5ng/l), E2-G (<0.8-12.7ng/l).

EFFECTS ON WATER ENVIRONMENT AFFECTED BY SEWERAGE SYSTEMS

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Water Quality Team

Project period: FY2001-2005

OBJECTIVES

In recent years, sewerage systems are being constructed steadily in Japan. Therefore, the water discharged through sewerage systems is increasing and has occupied the major part in water circulation in the watershed, partially in urban areas. A previous report has shown that in certain river areas, the reuse ratios for wastewater after treatment have exceeded 90%.

The major objective of the present research was to examine the impacts of consolidation of sewerage works upon the river water quality and aquatic organism ecosystems. Besides this, the influences of chemical compounds in sewage effluent, especially endocrine disrupting chemicals performing like feminine hormones, on aquatic organisms such as fishes were also studied.

RESULTS

In 2002, an observatory study on the pollution discharges of the Ohtsu River, an urban river flows into the Lake Teganuma, was performed. Then, the impacts of associated river basin variances on the stream conditions and the discharged pollution loads were examined. Moreover, to evaluate the estrogenic effects of sewage discharges on aquatic organisms, a risk assessment experiment was conducted by exposing a fish species to the wastewater using an experimental unit installed in a wastewater treatment plant.

The research of this year led to the following results. The changes in the mechanisms of water circulation were assessed. The needs for further investigations of the accumulation and decomposition of organic substances and nutrient salts in river channel were clearly demonstrated in order to better understand the sewage impacts on natural water environment systems.

To investigate the sewage impacts on fishes, two series of exposure tests using male carp and mixed male and female carp, respectively, were performed. No matter which test series was concerned, the VTG formation was not recognized for the male carp used. Similarly, for the parallel control test conducted using tap water after the removal of residual chlorine, the VTG formation for the male carp was not found either. The reason for the observed result that VTG was not induced in the male carp was probably because the male carp's physiological activities depressed in the period of April when the test was conducted, taking into account the understanding that

male carp is physiologically most active in early spring.

By introducing the measurement of EROD activity, tests related to the P4501A1 formation of carp became feasible. An increasing tendency of EROD activity was observed in the test series of exposure to treated wastewater samples. It was thus conceived that drug-metabolic enzyme was probably induced with the treated wastewater.

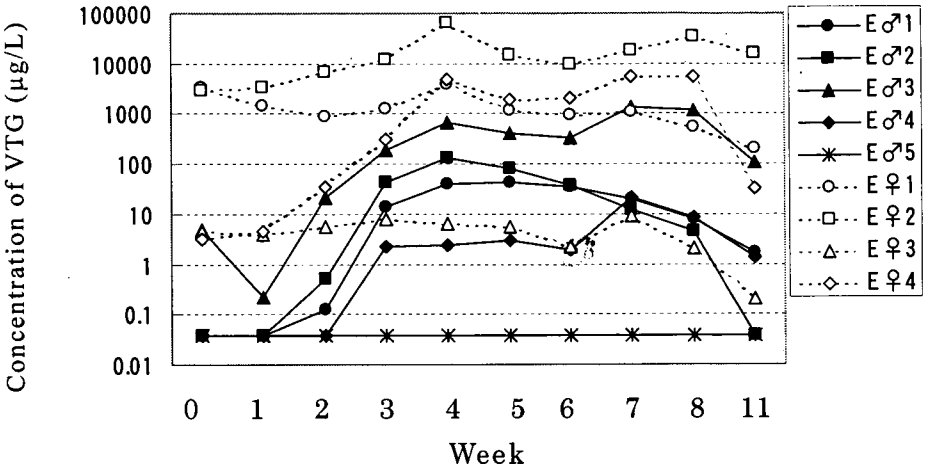

Fig.1 Concentration of VTG (μg/L)

SYSTEMATIC WAY FOR SURVEYING WATER QUALITY OF RIVERS

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Project period: 2000 - 2002

OBJECTIVES

Water quality surveys are now performed in conformity with the River Erosion Control Technical Standard, however, individual river administrative officials are allowed to independently decide the needed survey locations and survey frequencies. It is necessary to clarify the significance of the measurements and set rational survey conditions for implementation of more efficient water surveys. The present situation is that river water quality surveys are not necessarily systematized.

This study, has been therefore, carried out in cooperation with a couple of technical offices in order to systematize water quality surveys and finalize related regulations. The Public Works Research Institute will study the basic theory of water quality surveys performed by river administrative officials and in particular, the bottom sediment survey method that is one specific category of survey method that has not been sufficiently systematized.

RESULTS

In 2002, an analysis based on existent manuals and the data of executed questionnaire investigations towards regional bureaus of the Ministry of Land, Infrastructure and Transport was conducted. Then, present situations in regard of the sediment release tests and the oxygen consumption rate tests were evaluated. Based on these, the sediment release tests and the oxygen consumption rate tests were proposed in consideration of the present technological levels.

And, future subjects and work needed for systematic sediment investigations were put in order as follows.

Major Subjects and Measures for Standardization of Sediment Releasing Experiments

1. Sampling Method

The representative capability of supplied samples to targeted water bodies is examined based on case studies and statistical analyses.

2. Comparison of Experimental Methods

The results obtained by employing different methods for an identical sample should be compared.

3. Cautions to Be Paid

1) The run time needed for indoor batch experiments was analyzed based on observed results. A run time of about 10 days, which is shorter than 15-20 days practiced so far, was confirmed suitable for the releasing trend to be stable.

2) The volumetric ratio of water to the sedimented mud played an important role when the releasing of nutrients from disturbed sedimentate mud was assessed. Also, from the viewpoint of the phenomenon evaluation, analytical results for nutrients in both dissolved and particulate forms were confirmed necessary.

3) Cautions that had to be noticed for manipulation of the experiments were summarized. An examination of each individual item is found indispensable from now on.

4. The Releasing and Oxygen Consumption Mechanisms of Sediments

By characterizing the phosphorus species in sedimented particulates, these mechanisms should be elucidated by examining the phenomena involved in respective processes.

5. Assessment of Experimental Results:

1) Considerations relate to the calculation of nutrients' releasing rates were presented based on the results obtained from batch and sequential indoor experiments.

2) Examinations were needed as related to the assessment of results obtained using in-situ experimental facilities.

Besides this, an action draft of "River Water Quality Investigation Outlines (draft)" was summarized. The subject which became clear is as the following.

1. Basic considerations related to monitoring indices, locations and frequencies were summarized when an investigation plan was to be prepared. All these were systematically summarized as a draft version of 'River Water Quality Investigation Outlines (draft)'.

2) Existent experimental methods and releasing data obtained through experiments performed by the Ministry of Land, Infrastructure and Transport were analyzed and assessed, for releasing experimental methods with few descriptions on detailed technical criteria. As a result, the test methodology for monitoring river sediments was summarized in details.

3) As a future topic relates to sediment investigations, furthered evaluations and modifications are considered necessary for test methods that have not been thoroughly compared.

Investigations should also be made on such aspects as the subjects of the outlines, the technological development information of foreign countries. Based on the results, efforts that could lead to furthered improvement of practical technologies, involving planning, monitoring, and method modification and development, are expected.

DEVELOPMENT OF TECHNOLOGY FOR DIOXIN CONTAMINATED SOIL COUNTERMEASURES

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Project period: 2000 - 2002

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.

The content of dioxins in the bottom sediment may greatly change with collection position. In order to carry out appropriate construction management and countermeasure evaluation, it is necessary to estimate the distribution of dioxins quickly and simply. The official method which is high resolution GC/high resolution MS (HRGC/HRMS) method of measuring dioxins in bottom sediment and in water samples, from sampling to getting analysis results, is time consuming, so it is necessary to develop a quicker analysis method.

In this research, it aims at development of the simple detection technology which used the enzyme-linked immunosorbent assay ($ELISA_{ECO}$ and $ELISA_{DT2}$) method, Ah receptor binding assay (Ah-IA) and high resolution GC/low resolution MS (HRGC/LRMS) method. Moreover, a judgment is difficult if the accuracy management technique of the official method is complicated like analysis and it is not a specialist. Technique which can be easily judged also by whom is proposed.

RESULTS

In this research, the following results were obtained about the simple analysis method and the accuracy management technique.

1) We carried out joint research with private sector about $ELISA_{ECO}$, $ELISA_{DT2}$ and Ah-IA. We investigated the feature of $ELISA_{ECO}$, $ELISA_{DT2}$ and Ah-IA, and it were compared with the actual sample. The value of $ELISA_{ECO}$ and Ah-IA showed the larger value than the concentration of DXNs. The value of $ELISA_{DT2}$ showed the large value or the small value than the concentration of DXNs.

2) Comparison examination was carried out using the sample which performed the pretreatment of the same dryness, extraction, and refining about the simple detection technique by $ELISA_{ECO}$, $ELISA_{DT2}$, Ah-IA and HRGC/LRMS. Though there were few samples (20 samples), comparatively good correlation was obtained (Figure 1).

3) We patternized and systematized the check work of measurement data and the reported measured value created the software which checks whether it is obtained in the state of satisfying the quality control specification defined by the official method.

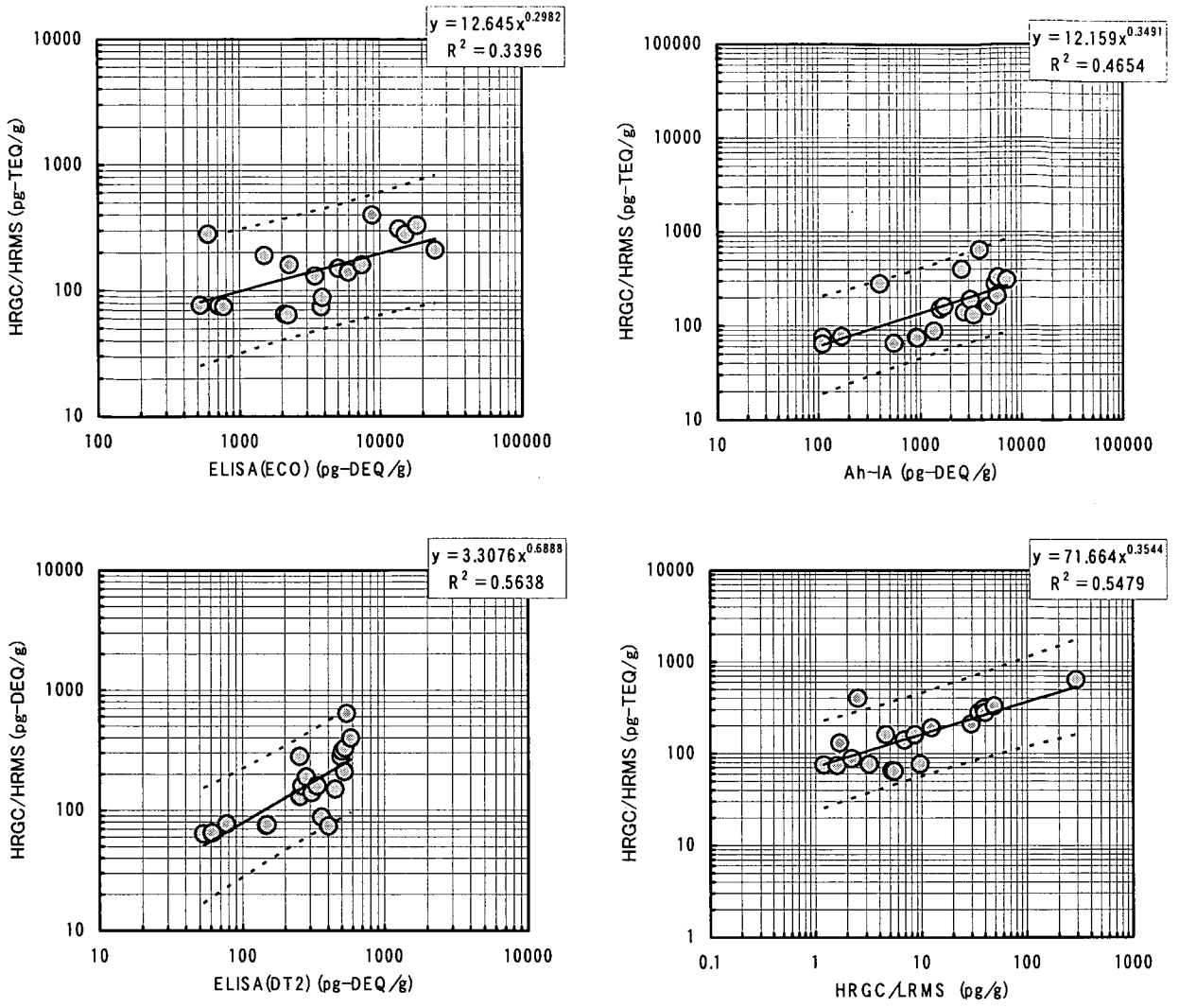

Figure 1 Relationship between the simple detection technique and the official method

(----: 95% confidence interval)

Evaluation of River Water Quality from the Viewpoint of Aquatic Ecosystem

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Water Quality Team
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 in river water sources quantity derived from sewage treatment plants (STPs) has been increasing. As a 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, studies on STP discharge's impacts upon organisms and receiving rivers are very limited.

The final objective of this study is investigated the relationship between water quality and living organisms. Tama River was selected as the research site.
In FY 2002, we investigated the mixing characteristics of treated wastewater and river water. Furthermore, we investigated the diversities and the quantities of benthos.

RESULTS

The summary of the study conducted in FY 2002 is as follows.

(1)The treated wastewater was not mixed to river water at 100m downstream from the discharge outlet (St.2). Figure-1 shows the changes of the electric conductivity changed in the cross direction of the stream at St.2. Treated wastewater was nearly blended at downstream position 1400m away from the discharge outlet (St.3). The blended behavior in summer differed greatly from that in winter. This difference was considered to be attributed to temperature differences between the river water and the treated wastewater.

(2) The quantity of benthos differed in the cross direction at the St.2 where treated wastewater was not mixed sufficiently with the river water as shown figure-2. There were declining tendencies for the amounts of benthos and also for the diversity index towards the left bank of the river where the rate of treated wastewater was high.

< This study is done as a part of the research of River Ecology Research Group.>

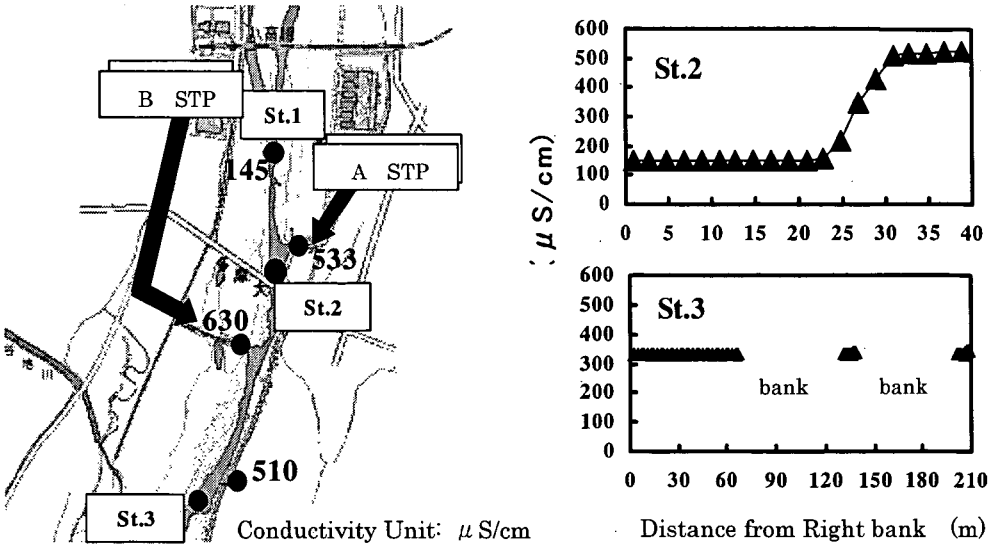

Fig.-1 Conductivities of river water in Tama Ohashi area

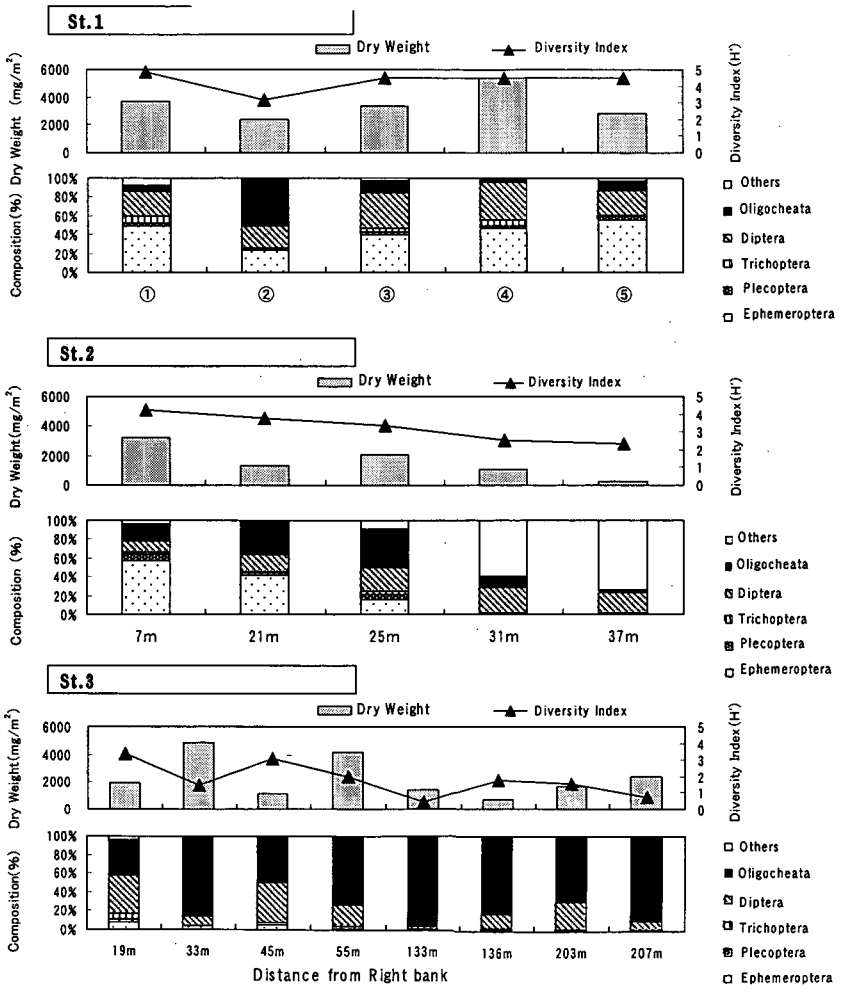

Figure-2 Dry weight, Diversity Index and Composition of Benthos

TRANSPORT OF CONTAMINANTS AT SEDIMENT-WATER INTERFACE

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Project period: FY2001-2005

OBJECTIVES

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. For such purposes, the changing mechanisms of pollutants (including nutrient salts) in sediments should be clarified, and effective approaches for assessment of the sediment impacts upon water quality are urgently needed.

The aims of this investigation were to elucidate the releasing mechanisms of nutrients form sediments, and to propose a test methodology for quantification of the released nutrients, and an estimation method for assessing the impacts of sediments on water qualities.

RESULTS

An indoor experiment was performed in 2002 for investigating the exchanges of nutrients between sediment particulates and water, accompanied by such processes as settlement, sorption and releasing. And, investigations were also made for the purpose of building an *in-situ* monitoring system for water column and surface layers of the sedimented mud in the spot of lake and dam reservoirs.

The relations of the released amounts of nutrients with such continuous *in-situ* monitoring results as water temperature, DO (Dissolved oxygen) and ORP (Oxidation reduction potential) were examined. The *in-situ* experiment was conducted in the Ushikubiri Reservoir, a pre-reservoir of the Miharu Dam, from July to September of 2003. This experiment involved supplying oxygen water with a higher oxygen concentration in the reservoir. During the experimental period, water temperatures, DO and ORP were consistently monitored, and data relate to these indices were also collected both before and after the experiment.

Water quality results collected nine days after the introduction of the oxygen water were compared with those collected one day prior to the oxygen introduction. A decrease in the NH4-N concentration was observed as a result of the rise in the DO concentration of the water at the depth of 5-6m. And, a rise in the NO3-N concentration was also confirmed. The result implied that nitrification in the deep part of the water column was promoted. On the other hand, the remarkable decrease in the NO3-N concentration observed in the water depth of 3-4m was conceived to be a result of enhanced denitrification. The decreases in the content of TN, DTN and DOC observed at the same water depth could be also explained with the hypothesis raised above. Nitrification and denitrification were also confirmed in the water column of the Point B. It was conceivable that the quantity of ammonia released from the sedimented mud was efficiently removed.

Besides all these, field investigations and indoor experiments were also conducted for quantification of nutrients

released from sediments and for assessment of related releasing mechanisms.

From now on, an ORP sensor will be used in spot observations, and the oxidation-reduction conditions in deep water layer and bottom mud will be confirmed. Based on this approach, efficient *in-situ* observatory methodologies needed for monitoring and estimation of water quality and released quantities of nutrients will be developed. In regard of indoor experiments, a turbidity monitoring system is decided to be combined in addition to DO and ORP. An experimental approach for estimating the released quantities of nutrients from sediments, based on indoor static and suspended releasing experimental modes, will be established. All finding obtained will benefit the systematization of nutrient releasing test approaches.

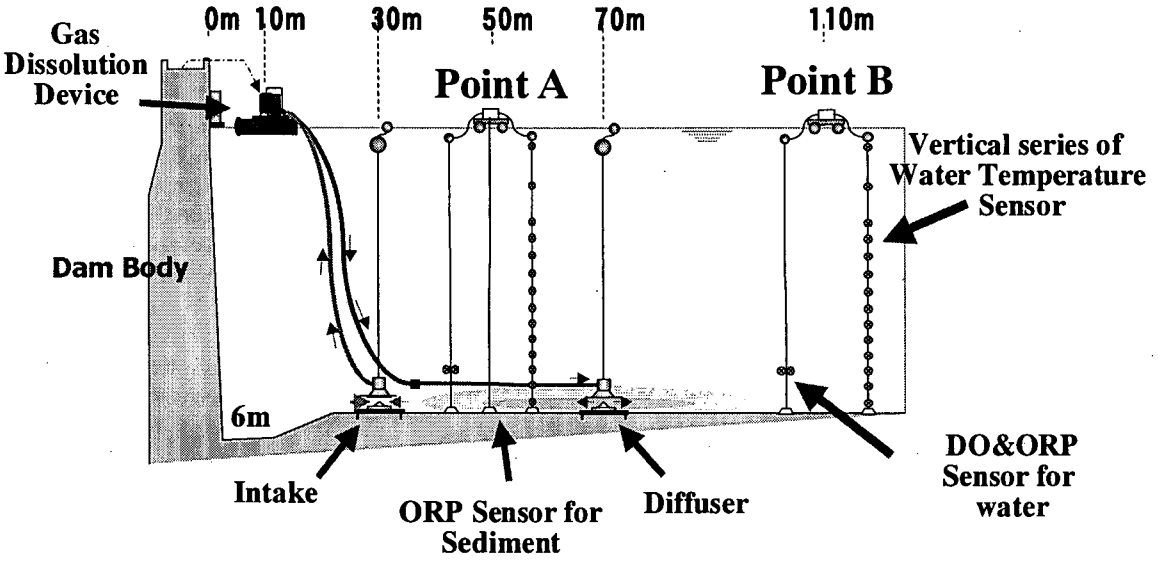

Fig.1 High Oxygen Supply System to the Deep Layers in Reservoir and Water Quality Monitoring System

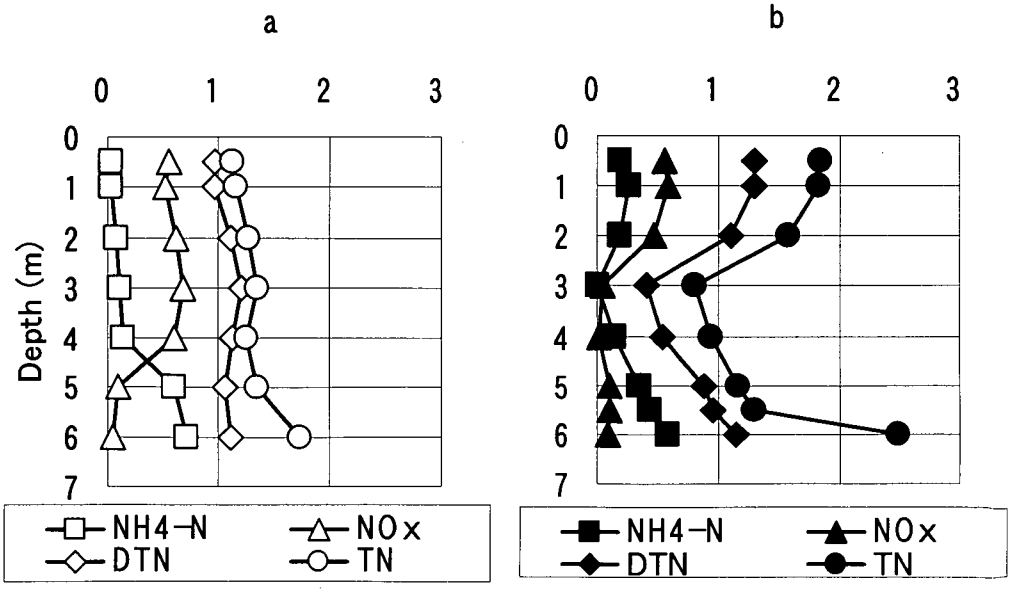

Fig.2 Concentration of Nitrogen compounds in water column at Point A (a: one day before and b: nine days after the supply of oxygen water)

BEHAVIOR OF CHEMICALS FROM URBAN DISCHARGE IN WATER ENVIRONMENT

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Water Quality Team

Project period: FY 2001-2005

OBJECTIVES

Recently, it is reported that the internal secretion disturbance of the wild creature may occur due to decomposition by products of surface active chemicals medicine in water environment, and humans and animals related hormones, and so on. Efficient risk reduction countermeasures are thus required by eliminating there sources and presence levels in discharges. To achieve such purposes, the behaviors of the risky chemicals should be grasped, and the influences of such chemicals exerted onto water systems should be examined.

This project aims at the development of efficient investigation and analytical techniques in urban discharge in water and sediments of river water systems for endocrine disrupting chemicals. The changing behaviors relate to decomposition and formation in the water environment are investigated.

RESULTS

In 2002, an examination on analytical approaches for nonylphenoxyacetic acid in sedimented mud samples was made. By modifying conventional GC/MS based analytical techniques, the recovery ratios ware enhanced. Using the modified method, the behaviors of endocrine disrupting chemicals in water environment were studied.

Observatory studies on lakes and rivers were performed, and the inflow conditions of city drainages, changes along the river channel and accumulation characteristics in the bottom mud were clarified.

1) The possibility relates to estrogens and NPs' sorption onto the bottom mud, and their changes and decomposition within the pathway of rivers was demonstrated.
2) The accumulative conditions of estrogens (NP, BPA, and E2) onto attached algae and benthos were investigated. It was found that E2 and NP were concentrated at higher levels in attached algae and benthos than in the water phase. As illustrated in figure 1, the contents of estrogens and NPs in the bottom mud of the lake polluted by the city drainage is comparatively high. And, it was also found that these compounds existed in higher concentrations in the bottom mud for a long time, even if certain proportions of them were consistently decomposed under aerobic conditions.
3) The changes of estrogens and NPs in the basin greatly affect the vertical distributions of E1 and NPs in the bottom mud. The mole-based NP concentrations resembled those of NPEO.
4) The quantity of NPEC was one-order lower, and the existence ratios of these compounds (NP, NPEO, NPEC) were comparatively stable. The NP ratio was higher in the sediments than in the river water, and NPEC showed a tendency of being smaller. It was found that the bottom mud and the bottom organisms contained higher contents of estrogens and NPs than the water phase did.
5) The results suggest that the food-chains of organisms in water are probably an important pathway for water pollution by endocrine disrupting chemicals oriented from city drainages.

In coming studies, the transform of estrogens into organisms is to be investigated, in addition to their decomposition and changing behaviors in rivers and, pretreatment approaches that could further enhance the recovery

ratios of NPEC for quantitative analysis of their presence in sediment samples are expected.

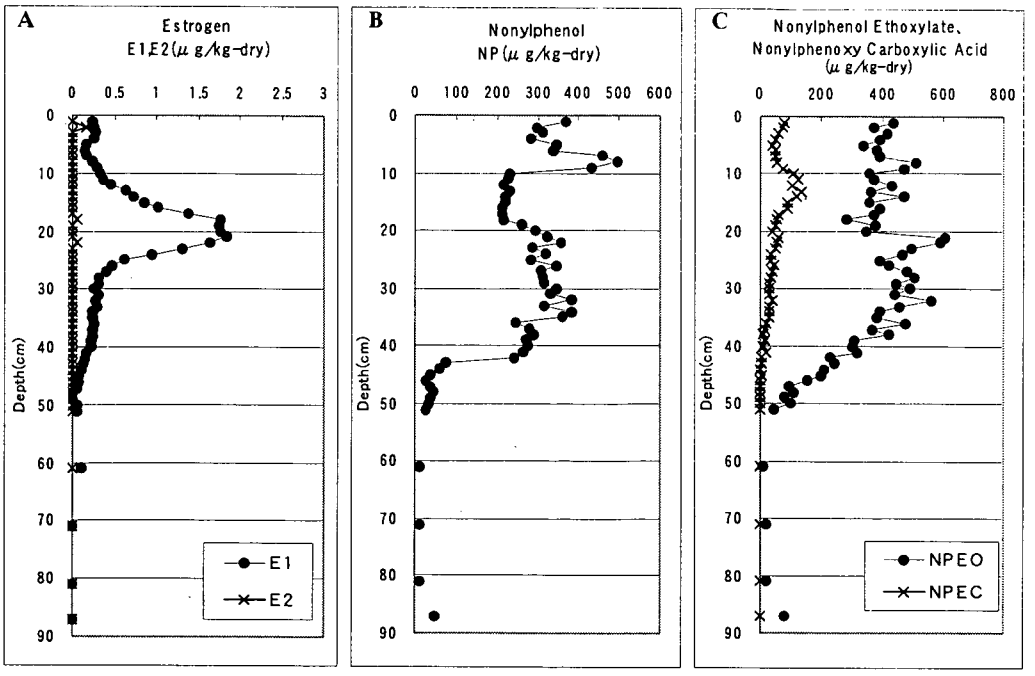

Fig.1 Vertical concentration profiles for E1, E2, NP, NPnEO and NPnEC in sediments of Lake Teganuma

Evaluation of Estrogen-like Substances using Bioassay

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Water Quality Team
Project period : FY 2001-2005

OBJECTIVES

In recent years, a new environmental issue has emerged, 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 using DNA recombinant yeasts involving human estrogen receptor gene. Besides this, using fractionation techniques and recombinant yeast assay, the water samples to characterize and estimate major origins of the estrogenicity were fractionated.

RESULTS

The summary of this study conducted in FY2002 is as follows.

(1) As shown in Figure 1, the estrogen-like activity in sewage could be effectively removed in a sewage treatment plant. The reduction ratio relative to the median values of estrogen-like activity measurements was about 76%.

(2) We compared the observed estrogen-like activity to the estimated estrogen-like activity of estrogen-like substances measured by the instrumental analysis. The results shown in Figure 2 indicated that E1 was the primary contributor to the theoretical estrogen-like activity in the treated wastewater, and both E1 and E2 are contributors in the untreated wastewater. However, the estrogen-like activity was not perfectly explained by estrogen and estrogen-like substances.

(3) We fractionated water samples with a solid phase extraction column and several different polarized solvents. The estrogen-like activities of the treated wastewater samples were mainly found in a single fraction, and those of the untreated wastewater were in a couple of fractions.

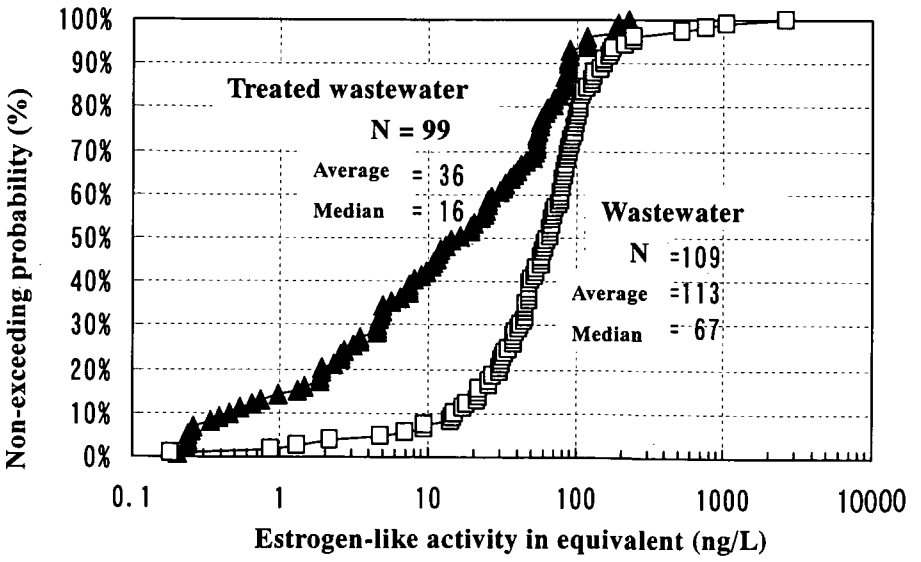

Fig.1 Estrogen-like activities of wastewater before and after treatment

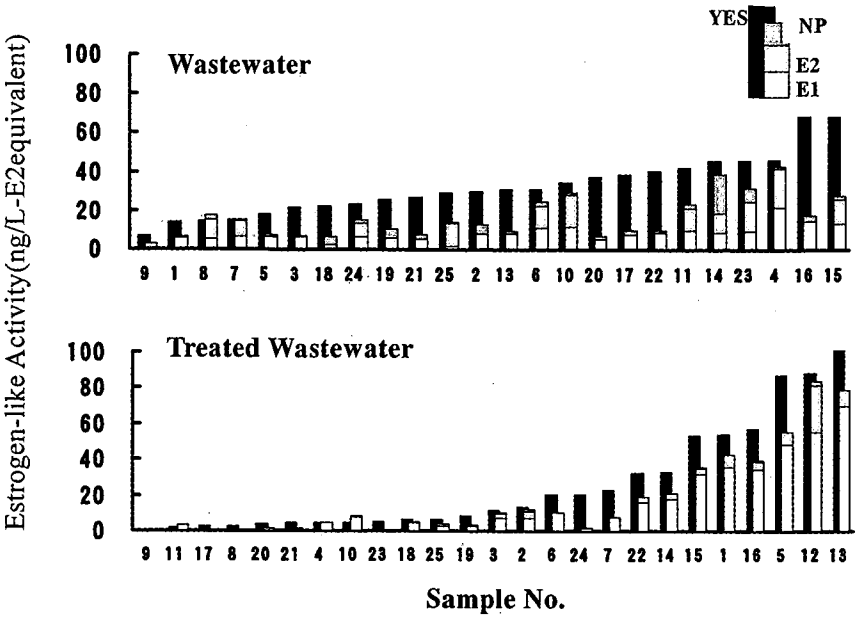

Fig.2 Comparison between the observed estrogen-like activity and the theoretical estrogen-like activity estimated the results of chemical analysis

Detection of Environmental Stresses on Aquatic Organisms Using Gene Analysis Technology

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Water Quality Team

Project period : FY 2002-2006

OBJECTIVES

Human genome research has been progressed on worldwide levels in recent years. It is expected that medical chemicals, which are effective on individual gene levels, will be manufactured and used in the near future. These kinds of medicines taken by people will move into sewers. If probably not treated enough in sewage treatment plants, they will merge into rivers through the effluent from the plants, which may bring about stresses onto aquatic organisms stress and may also affect human health.

Some stresses caused by such new chemicals 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.

RESULTS

In FY2002, our major attention was paid to the gene diagnosis using DNA chips and studies on whether this method could be actually applied to the evaluation method of chemicals influence in the environmental field were performed.

A lot of genes can be treated with DNA chips at a time. DNA chips were spotted with genes like cDNA fragments. Then, mRNA extracted from a given species of organism was labeled with fluorescence. And then, the labeled mRNA was hybridized with genes spotted on the chip. If the difference in gene expressions is clear between the sample given a sort of stress and the control sample, this means that it is possible to utilize a method for the evaluation of water environment.

The summary of the study is as follows.

1. When the DNA chip is used to evaluate water environment, it is necessary to make an exclusive DNA chip having hundreds of spots. Each spot is used to derive a single gene. If we are to study all genes in a given organism, we need a lot of time to analyze data about the genes and a lot of money to make the DNA chip. So, we considered that it was effective to select genes obtained through screening. We concluded that, for practical use, the screening procedure was necessary.

2. We selected a screening method using microbeads. This method was advantageous because it did not require the DNA sequence data. In this screening process, only mRNA derived from the tissues was used. Microbeads were constructed using the mRNA derived from the tissues being treated and not with a stress substance. Fluorescence labeled probes were then prepared using the mRNA derived from the tissues treated with a stress substance. Using the mRNA derived from the control tissues, colored fluorescence labeled probes were also prepared. Microbeads and probes were hybridized, and genes confirmed to have expression changes were selected.

It was clear that the extract and preservation conditions of the pre-treatment process should be controlled more strictly than those of the general electrophoresis method and the PCR method. This pre-treatment process was applied to Japanese medaka, and its suitability was confirmed by a microchip-type electrophoresis test.