NATURAL DISASTER AND THE ENVIRONMENTAL SPECIMEN BANKING - REPORT FROM "ENVIRONMENTAL TIME CAPSULE PROGRAM" AT THE NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES, JAPAN -

Atsushi TANAKA[1], Akinori TAKEUCHI[1], Yoshikatsu TAKAZAWA[1], Zinichi KARUBE[1], Mai TAKAGI[1], Mitsuha YOSHIKANE[1,2], Haruhiko SEYAMA[1], Yasuyuki SHIBATA[1,*]

[1]Center for Environmental Measurement and Analysis, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan; [2]Present address; Graduate School of Frontier Sciences, University of Tokyo, Kashiwanoha, Kashiwa, Chiba 277-8561, Japan

Introduction

Environmental Specimen Banking (ESB) has been playing important and complementary roles to the environmental monitoring by keeping part of the environmental samples collected for monitoring without change so that retrospective analysis in future will reveal past unattended / undetected environmental changes / human impacts clearly. ESBs have been conducted in several European countries, such as Sweden and Germany, United States, Canada, Japan etc., and new activities have started / been prepared in France, Australia, Korea, China etc[1]. The indispensable role of ESB has been recognized internationally. Several international meetings on ESB have been organized recently[1,2]. ESB is now being incorporated into the global monitoring plan of the Stockholm Convention, and will play pivotal role in its effectiveness evaluation[3].

At the National Institute for Environmental Studies (NIES), Japan, ESB started in 1980 with three large -20 C cold rooms and several freezers, and collected various environmental samples, including wildlife samples and sediments etc[4]. In addition to our own activities, samples from other organizations were stored, including the environmental monitoring samples by the Ministry of the Environment, Japan, from 1978 until present. In 2002, a new program called "Environmental Time Capsule Program" started, in which a systematic collection of bivalves around Japan island and fishes and sediments in 20 locations of Tokyo Bay were conducted[5,6]. A new storage facility, Environmental Time Capsule building, constructed in 2004 in which insulated tanks cooled by liquid nitrogen and two -60 C cold rooms as well as freezers were equipped for long-term storage of various environmental samples for future. In addition to the collection of samples, environmental monitoring of several priority chemicals, such as PFOS and other fluorosurfactants, in coastal environment, and a development of new terrestrial biomonitoring method by using dragonfly as indicator organisms have been conducted[7,8].

At 11 March 2011, the Great East Japan Earthquake occurred followed by attack of huge Tsunami along the Pacific coastline of East Japan. The earthquake and the tsunami as well as the nuclear power plant accident at Fukushima caused tremendous damage to the Japanese society and the environment, and also threatened the continuous operation of ESB at NIES. Here we report our experiences and efforts to tackle with this huge natural disaster.

Materials and Methods

The Time Capsule Facility consists of 19 insulated tanks (550 L each) cooled by liquid N_2, insulated transfer tubes and an outer liquid N_2 storage tank (5000 L), two -60 C cold rooms, several freezers and refrigerators, sample dissecting room, cryo-homogenization room, sample preparation room for the analysis, and analytical instrument room. The rooms housing insulated tanks, cryo-homogenization system, and sample preparation facility have charcoal and HEPA filters to minimize risk of contaminating the samples.

Bivalve samples, including *Mytilus galloprovincialis* (blue mussel), *Septifer virgatus*, and *Saccostea mordax* (oyster), were collected and partly de-shelled on site (typically 50 to 200 g as soft tissue). The soft tissues were weighed and frozen by liquid nitrogen, and sent to the institute in a dry-shipper cooled by liquid nitrogen. The samples were coarse-fractured and then fine powdered by cryo-homogenization systems made of titanium, and poured into clean glass bottles and kept in insulating tanks cooled by liquid nitrogen under -160 C. Other portions of the bivalves were kept at-60 C as a whole. Typically in every season a portion of ice made of purified water (Milli Q; Millipore Co. Ltd.) was cryo-homogenized in the same system in order to check contamination levels of priority chemicals, which have been mainly used in commercial products, including

perfluorochemicals, alkylphenols and phthalate esters as well as heavy metals, during sample processing for storage. Also part of the homogenized ice was kept in the same tank for enabling us checking contamination levels by other chemicals in future.
Chemicals analysis was performed either by GCMS (Agilent 6890GC + 5973MSD, or JEOL JMS700 HRGC/HRMS) or LCMSMS (AB Sciex 4000 QTRAP) after proper pretreatment procedure. Element analysis was conducted with an ICPMS (Agilent 7500 series) after decomposition with nitric acid by microwave digestion system (Milestone).

Effects of the Earthquake to ESB at NIES

The earthquake occurred at 14:46 on 11 March (Friday), 2011. Its magnitude was 9.0 and seismic intensity scale at Tsukuba was reported to be 6 lower (Japanese original standard, from 0 to 7). The earthquake was characterized by the long duration (2 to 3 minutes duration of strong quake) with prevailing long (around 7 sec) and short (0.1 to 1 sec) frequency shakes.
Fortunately the building, the storage facilities, stored samples and staffs had no critical damage by the earthquake. Insulated tanks and liquid N_2 transfer lines are fixed to the floor and the walls or ceilings, respectively, and were apparently not affected by the earthquake. Only a connection in the transfer line was found to be loosen and tighten again a few months later. So far no damaged glass bottle has been found in the insulated tanks. Bulk samples have been kept in plastic cases on the shelves in -60 C cold rooms. All the shelves were fixed to the floor and also tightly connected each other to prevent large shake by the earthquake. Also plastic nets and metal rods were set to all the shelves to prevent drop-off of the cases by the strong earthquake. These systems were proven to work efficiently and prevented damage of the stored samples. Freezers and refrigerators, on the other hand, showed some weakness against the earthquake. Although they had stoppers in the bottom, many of them moved from the original positions by the earthquake. In one case, the refrigerator almost hit and destroyed the connector of the electricity supply at the wall. Also they stopped operation by the electricity shutdown, which will be described later.
Many of the instruments and PCs on the table also moved around, and we were lucky that no instruments dropped off from the table except for an evaporator and PC monitors. Many of the organic solvents as well as acids were kept in stockers without damage. However, concentrated acid spilled out to the floor from the plastic containers, which were used to wash glassware for elemental analysis. Major problems were found on gas cylinders to supply helium or other gases to the analytical instruments. Although all the cylinders were fixed to the stands by chain, some of the cylinders were fallen down by the earthquake either by breaking connectors of the chains or apparently jumping over the chains ! It was really fortunate that no damage occurred to the cylinders themselves and no leaks of gases occurred.
ESB operations were threatened by the electricity shutdown after the earthquake. Supply of electricity to the facilities within the institute was automatically shut down in order to prevent fires / other problems. Headquarters of the institute put priority to secure human lives, and asked all the staffs in the institute to go back to their home immediately and stay with their families. Also considering possible occurrence of large aftershock, the headquarters decided to restart the electricity on next Monday (14 March), for all the staffs will be needed to check damages to the buildings and facilities before restarting electricity. Insulated tanks were expected to last at least two weeks at low temperature without additional supply of liquid N_2. Also large cold rooms at -60 C were expected to keep freezing temperature for several days due to refrigerant activity of stored samples themselves as well as thick concrete floors. In fact the temperature of the room was found to be at -47 C on 14 March when the electricity was recovered. Major problems were anticipated to the samples in freezers. As the facility did not have emergency power supply, we telephoned many gas companies and got 270 kg of dry ice to keep samples in freezing condition during weekend until recovery of electricity.
After the earthquake, the institute decided to set up several emergency power generators to keep operation of ESB and other key facilities. The efforts of fixing cylinder stands and instruments etc. have been being designed and conducted, too, in order to minimize possible damage to the facility and human life by the earthquake. We experienced, however, severe shortage of oil supply to the gas stations in wide areas in East Japan after the earthquake for several weeks. If long term stop of electricity and oil supplies occur simultaneously due, for example, to a strong earthquake underneath the area, our ESB operation will be threatened again. We have to consider wider aspects and continue to up-grade safety levels of the operation.

Response of NIES ESB Activity against the Earthquake

Our facility was among the earliest buildings in which electricity was recovered. After the recovery on 14 March, we set up high volume air sampler on the roof of the building and started ambient air sampling from the afternoon of 15 March. We had a sampler for organic pollutants, not for radioisotopes, and added activated carbon fiber felt as a back-up to trap volatile iodine to the quartz fiber filter for particles. We collaborated with Prof. K. Masumoto and colleagues at High Energy Accelerator Research Organization (KEK) to monitor radioactivities on the filters, and reported the data on the homepage of KEK[9]. As shown in the homepage, several radioisotopes, including cesium-134, cesium-137, iodine-131, iodine-133 and tellurium-132, were detected on the filters. Their levels showed general decline during half a year monitoring period with considerable variations, which may reflect differences in wind directions and other transport processes, chemical properties etc. A detailed analysis of the data is now underway[10]. In addition to these gamma-ray emitting isotopes, we also started measurement of iodine-129, a long-lived radioisotope, in order to reveal total emission and deposition pattern of iodine-131, which has been thought to be a key isotope because of its potential health effects. Iodine-129 is a beta-ray emitting nucleus with half life of 16 million years, and is analyzed by an accelerator mass spectrometry technique. We heated part of filters with electric furnace and volatilized iodine, trapped in alkali solution, precipitated with silver, and analyzed them with AMS at Scottish Universities Environmental Research Center in collaboration with Dr. S. Xu and Prof. S. Freeman[11]. The preliminary data was promising and we will continue the analysis to determine iodine-129/iodine-131 ratios and to estimate iodine-131 distributions quantitatively.

Before the earthquake, we collected and kept bivalve samples in several locations along the Pacific coastline of East Japan[12]. We re-visited these places after the earthquake and collected samples in order to know the levels and their changes of radioisotopes and other chemicals. Although huge Tsunami destroyed ports, breakwaters and houses along the coastline and changed the landscape drastically, bivalves survived in many places. We measured cesium-134, cesium-137 and iodine-131 in soft tissues of bivalves as well as in sea water. We also measured several PAHs in order to check possible effects of oil pollution. We plan to continue sampling and analysis of bivalves, and will expand target chemicals, including POPs, as well as heavy metals.

Other ESB Activity at NIES

In addition to the emergency response to the Great East Japan Earthquake, bivalve sampling along the coastline of Japan continues. We plan to cover major coastline of Japan in five years interval and repeat this process to continuously monitor the environment and its changes. This year we collected samples in the western part, Chugoku area.

In addition to them, we continue researches on the accumulation properties of dragonflies against PFOS and other chemicals in order to develop and establish efficient bio-monitoring method of terrestrial environment by using dragonfly as bioindicator organism. We have examined time-dependent accumulation of PFOS and other perfluorochemicals in the three common dragonflies in Japan after emergence, and found that their levels in male generally increase with their body weights during immature stage and reach maximum and plateau after maturation. Also the levels and compositions in mature male of several different species were found to be comparable each other when caught at the same location. On the other hand, females showed similar trends but showed clear decline after maturation, possibly due to egg-laying process. The results generally support the idea that use of mature male dragonflies of these species will be suitable for terrestrial monitoring[8]. This year we selected another popular and common dragonfly in Japan, *Orthetrum albistylum speciosum* (SHIOKARA TONBO in Japanese) in order to accumulate further supporting information on its applicability to the monitoring and expanding target compounds. We got a large number of its juveniles from a swimming pool of primary school nearby the institute. The juveniles are kept in an experimental pond in our institute covered by net, and dragonflies after emergence will be collected, marked, released, and re-captured in order to reveal time-trend of PFOS and other chemicals levels in their body.

Conclusions

During the environmental specimen banking program for three decades, we have experienced several environmental topics related to pollution, including oil pollution by a Russian tanker sunken in Japan Sea (1997), Great Hanshin earthquake and the resultant pollution (1995), large mortality of a marine bird, Rhinoceros Auklet, at western Hokkaido (1999), and sudden death of an endangered bird, Japanese crane (2002). The natural disaster experienced last year, the Great East Japan Earthquake, however, is by far the largest one we have ever experienced. Also this is our first experience that the disaster directly affected and threatened the ESB program itself. We hope our experience and actions will contribute to better and more efficient operation of ESB in the world.

Acknowledgements
Part of the program have been conducted as special research and other research programs at the National Institute for Environmental Studies, while other part was conducted as a Time Capsule Program supported by the Ministry of the Environment (FY2002 – FY2010). Part of the dragonfly program was supported by the research fund from the Ministry of the Environment. We acknowledge Ministry of the Environment for partly supporting radioisotope analysis after the earthquake. We also thank Ms. Y. Kanda, M. Moto, S. Komori, M. Kobayashi, M. Yanai, S. Sueki, and Zhao Qin for their help and support to the program.

References:
1. Becker P., Gunter EW, Schlueter C, Shibata Y, Wise SA (2006) *J Environ Monit*. 8(8): 776-778, and the following articles in the issue, the special issue on Environmental Specimen Banking
2. ESB session in Dioxin 2007 (Tokyo); ESB session in SETAC 5th World Congress (Sydney, 2008); ESB symposium at Ehime, Japan (2009); International ESB Symposium at Berlin (2010)
3. Decision SC-4/31 of the Conference of the Parties of the Stockholm Convention at its 4th meeting (2009)
4. Morita M, Yoshinaga J, Mukai H, Ambe Y, Tanaka A, Shibata Y. (1997) *Chemosphere* 34 : 1907-1919.
5. Shibata Y, Tanaka A, Horiguchi T, Hashimoto S, Takazawa Y, Mukai H, Yoshikane M, Hirota M, Kanda Y, Komori S, Kobayashi M, Uehiro T (2007) *Organohalogen Comp*. 69 : 1725-1728.
6. Takeuchi A, Shibata Y, Tanaka A. (2012) *Biopreservation Biobanking* 10(2) : 174-177.
7. Yoshikane M, Takazawa Y., Tanaka A., Komori S., Kobayashi M., Kanda Y., Shibata Y. (2007) *Organohalogen Comp.* 69 : 1717-1720
8. Yoshikane M, Komori S, Kobayashi M, Yanai M, Ueda T, Shibata Y. (2009) *Organohalogen Comp*.71: 1946-1951
9. http://www.kek.jp/ja/Research/ARL/RSC/Radmonitor/ (in Japanese)
10. Doi T, et al., manuscript in preparation.
11. Shibata Y, Tanaka A, Doi T, Toyoda A, Masumoto K, Xu S, Freeman S. (2012) presented at the 1st Meeting of Society for Remediation of Environmental Radioactivity, Fukushima
12. see homepage of Time Capsule Program ; http://www.nies.go.jp/timecaps1/summary/objectiveE.htm