The National Environmental Specimen Bank in Korea: Establishment and Standard Operating Procedures

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Abstract—The National Environmental Specimen Bank (NESB) was designed by the National Institute of Environmental Research (NIER) in 2007 as an archive to record and assess the quality of the Korean environment. The total floor space of the two-story NESB building constructed in 2009 is 2,340 m² including the basement. Its cryogenic storage room has the capacity for 74 liquid nitrogen vapor-phase freezers.

Upon completion of the facility, a pilot study was launched to test Standard Operating Procedures (SOPs) for collection, storing, and evaluating samples of species representative of the major Korean ecosystems. These procedures will be applied over an extended period to improve the reliability of environmental monitoring and the effectiveness of ecosystem management.

The first five specimens selected were shoots of red pines and Korean pines, leaves of Mongolian oaks, eggs of domestic pigeons, and muscles and livers of common carps.

Keywords: National Environmental Specimen Bank, Standard Operating Procedures

INTRODUCTION

For the past 30 years, Environmental Specimen Banks (ESBs) around the world have been serving as essential instruments for supporting environmental policy and management. They not only provide documentary evidence of the current state of the environment but also establish a baseline for gauging the effectiveness of policy measures and for investigating unanticipated developments (Kim, 2007; Kim and Yoo, 2007).

Encouraged by the successful operations and applications of well-known ESBs, especially in German, Japan, and the United States, Korean decision makers and scientists recommended an ESB project to the Korean government that would address not only current environmental issues but also emergent future issues (Kim, 2007).

In 2005, the Ministry of Environment’s “Research on Enlargement Strategy of Environmental Budget Investment” ranked the NESB project as third of nine priority projects identified. Consequently, in 2007 NIER granted seed money to
start the project with construction of a NESB building on its campus (Kim, 2007). This paper introduces the newly established NESB in Korea with a detailed description of the facility and its operational plan for the future.

**NESB ESTABLISHMENT**

**NESB budget**

The total budget from 2007 to 2010 is approximately 7.6 billion won including construction of the NESB building and equipment such as cryogenic freezers, electric freezers, vibrating cryomill and vertical liquid nitrogen storage tank.

Instruments such as liquid chromatographs (LC) and inductively coupled plasma mass spectrometers (ICP-MS) for organic and inorganic analysis will be installed in 2010. The NESB facilities will be enlarged with initiation of human specimen sampling after 2015. The breakdown of the budget for the preparation of facilities and SOPs is shown in Table 1.

**NESB design and construction**

The NESB is part of the Ecosystem Assessment Division in NIER’s Ecology
Research Department. In February 2007, a committee for site selection chose a location on the campus of NIER with sufficient space for future expansion (Kim, 2007).

The NESB occupies a two-story building with basement occupying 1,600 m² of ground space. This building includes offices, storage rooms, an International Organization for Standardization (ISO) Class 5 clean-air space for processing samples, ISO Class 7 clean-air space for the production of analytical materials and a freezer room (Kim, 2007).

Key facilities of NESB are located on the 1st floor. The cryogenic liquid nitrogen (LN) freezer room area is capable of storing 74 LN vapor-phase freezers of which 10 have already been installed. Five or seven more will be added on annual base. The electric freezer room will be used for temporary storage of specimens and the storage of raw samples. Currently, there are four electric freezers, all connected to a liquid nitrogen in-line piping system as a part of a back-up system with an 11,495L vertical LN storage tank. The LN piping system ensures that the entire pipeline is kept at cryogenic temperatures (see Fig. 1).

The whole operation will be monitored by a control system located next to the cryogenic freezer room. Tank pressure must remain relatively low for freezer operations. Length of the transfer line is minimized to reduce liquid nitrogen losses. The 2nd floor will be designed for analysis and future storage. Instruments for organic and inorganic analysis will be located in the analysis laboratory.
Operation of the NESB

In 2009, the NESB began pilot studies to formulate and test the feasibility of SOPs for conducting NESB operations. After reviewing their results, the SOPs will be revised accordingly before the systematic banking of environmental specimens begins. The re-allocation of budget and personnel will also be reviewed as the NESB evolves into a fully functional institution (Kim, 2007). The NESB’s operational flowchart is shown in Fig. 2.

DEVELOPMENT OF STANDARD OPERATING PROCEDURES

Standard Operating Procedures (SOPs) prepared by the NESB specify detailed instructions for collecting, handling, and storing environmental specimens.

Types of specimens

The main factor in the collection of specimen types is the identification of representative species at each level of the food chain in different major ecosystems. Therefore, specimens are selected according to the following criteria (Federal Ministry for the Environment, 2000):

- Wide distribution, enabling comparisons between different sampling areas.
- Suitable indicators represent the main levels of the food chain.
- Readily identifiable in the field.
- Unendangered species enabling long-term sustainable sampling.
- Species relatively immune to migratory movements.
- Species with high tolerance to urban polluted environment.

Sampling SOPs will specify the frequency and quantity of specimen collections. Most sampling will take place annually, at the same time of year. Representative species will be collected over a long period of time so that comparisons among the samples reflect the different environmental impacts on

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sampling categories</th>
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<th>Sampling purpose</th>
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</thead>
<tbody>
<tr>
<td>Red Pine</td>
<td>One year old shoot</td>
<td>Mar.–May</td>
<td>15 trees</td>
<td>Air pollution monitoring</td>
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<td></td>
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<td>150 g per tree</td>
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<tr>
<td>Korean Pine</td>
<td>One year old shoot</td>
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<td>15 trees</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>150 g per tree</td>
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<tr>
<td>Mongolian Oak</td>
<td>Leaves</td>
<td>Aug.–Sep.</td>
<td>15 trees</td>
<td>Air pollution monitoring</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>150 g per tree</td>
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<tr>
<td>Pigeon</td>
<td>Egg</td>
<td>Mar.–Aug.</td>
<td>25 eggs</td>
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</tr>
<tr>
<td>Common Carp</td>
<td>Muscle and liver</td>
<td>Aug.–Nov.</td>
<td>8–10 carps</td>
<td>Water pollution monitoring</td>
</tr>
</tbody>
</table>

Table 2. Sampling guidelines for five different environmental specimens.
Establishment of National Environmental Specimen Bank (NESB) in Korea

The first five selected specimens are red and Korean pine shoots, Mongolian oak leaves, domestic pigeon eggs, common carp muscles and livers. Other specimens being sampled are the zelkova tree and freshwater mussel. In addition, soil and earthworms in terrestrial ecosystems; sediment in riverine ecosystems; blue mussel, mullet, black-tailed gull, and sea lettuce in marine ecosystems are being considered for possible sampling (Kim et al., 2009). Preliminary sampling guidelines for the first five specimens are shown in Table 2. SOPs for specimen types will be continuously revised throughout the pilot project in 2010.

Samples representing terrestrial ecosystems will be collected from urban, agricultural, forest, and preservation areas; three sampling sites will be designed in each sampling area. Hence the number of terrestrial samples will be 84. Riverine ecosystem samples will be collected from three sites along the middle and lower reaches of the four main rivers in Korea: Han River, Nakdong River, Geum River, and Youngsan River, for a total of 36. A total of 36 specimens will likewise be collected from marine ecosystems. Thus, a total of 156 types of environmental specimens will be collected annually. The NESB will develop cooperative programs with regional agencies and universities to conduct the sampling (Kim et al., 2008b).

Fig. 3. Distribution map of red pine (>30 years) (a) and Korean pine (>20 years) (b).
Site selection

Selection of sampling locations within the sites was carried out by the stratified random sampling method to insure representativeness (Umwelt Bundes Amt (UBA), 2003). Existing monitoring stations will also be utilized for sampling purposes. The sampling frame for specimen collection is focused on geographical distribution and ecological significance. In addition, specimen site locations should demonstrate varying degrees of disturbance by human activities.

Sampling sites and the age of the trees sampled for red pine, Korean pine, and Mongolian oak are based on the national forestry physiognomy. Fourth age-class red pine (more than 31 years old), 41% of total red pine, is distributed mostly in the east and south of Korea. Third age-class Korean pine (more than 21 years old), 26.2% of the total, is distributed mostly in the interior; and fourth age-class Mongolian oak (more than 31 years old), 39.5% of the total, is distributed mostly in the east (Kim et al., 2008b; see Fig. 3).

Difficulty was encountered in selecting sampling sites for domestic pigeon

Fig. 4. Diagram of five selected specimens with Korea map.
EGGs due to lack of either natural or artificial nesting areas. After the pigeon was designated as “nuisance” to public health by Korean authorities, some bird nests were removed. Although some of the candidate sites (Han River, Guem River, Sachun Nosan, and Hampyung Gisan parks) have been selected based on a field survey, further investigation will be needed to identify more suitable sites. The diagram of five selected specimens with Korea map is shown as Fig. 4.

Specimen handling

The samples will be handled and packaged using materials such as stainless steel and Teflon to prevent contamination during and after sampling. Preparation of samples usually takes place under strict hygienic conditions (e.g. in a mobile laboratory brought to the sampling site). The specimens are deep-frozen using liquid nitrogen to below \(-150^\circ C\) throughout the process of transportation, homogenization, and transfer to long-term storage or directly to chemical analysis (Kim, 2007).

In the case of red pine, Korean pine, and Mongolian oak, biomass and dry mass of the leaves are measured. Lengths of lateral branches are measured for red and Korean pine. Fresh and dry weights of domestic pigeon egg and shell thickness are also measured. Common carp will be dissected to record the weights of organs such as muscle, heart, spleen, and liver.

Specimen storage

Each of the 156 type specimens will contain 1,000 g of material, or 10,000 g in the case of soil specimens. Of these, 144 types will be divided into 100 sub-specimens of 10 g for most specimens. In the case of soil specimens, the 12 types will be divided into 100 sub-specimens of 100 g. Thus, the overall annual NESB acquisition capacity of environmental specimens will yield 15,600 sub-specimens.

Most biological specimens are stored in 10 g per vial and 100 g per vial in the case of soil specimens. One 1400L cryogenic freezer can store either 1600 (100 g vial) or 8,000 (10 g vial) sub-specimens. After 2015, the six types of human specimen, including blood, saliva, will further add to the storage inventory. In all, NESB will require five additional cryogenic freezers per year. Hence, by the year 2030, the NESB will reach its present cryogenic freezer capacity (Kim et al., 2008a). The storage capacity for the NESB will be reviewed and readjusted according to future SOP and storage requirements.

CONCLUSIONS AND DISCUSSION

The NESB in Korea was established in 2009 with the cooperation of German, Japanese and USA ESBs. In 2009 a pilot study was launched to formulate and test SOPs for environmental specimen collection, handling, and storage. As a result, the NESB will begin full operation this year with the pilot banking of representative environmental specimens.

The NESB will enhance the quality of environmental assessment based on real-time and retrospective analysis, and will enlarge the scope of its efforts
through international as well as domestic cooperation. We hope that such efforts will strengthen the foundations of future Korean environmental policy and will bring new insight to effective environmental management.

Although some SOPs regarding site selection, sampling, storage, and physical analysis have been studied since 2007, with the support of other ESBs around the world, we have now reached a point where we can offer a fully operational facility for the analysis and improvement of environmental conditions in the country and region. The wide application of the NESB findings will further improve the reliability of environmental monitoring, health impact assessment, and risk assessment in Korea.

REFERENCES

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