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Thirty Years of Progress in Environmental Specimen Banking

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Abstract—The long-term preservation of well-documented representative specimens for retrospective studies is an important complement to environmental research and monitoring. From a small international group of interested university scientists, government officials and researchers who in the late 1970s proposed formalizing environmental specimen banking, the community continues to grow. Environmental specimen banks (ESBs) are being used for a variety of purposes, such as investigating temporal trends in ecosystems, evaluating the results of governmental environmental policies and regulations, and providing a resource for evaluating animal health. ESBs also have a role in quality assurance, as specimens banked from ongoing contaminant monitoring programs are readily available for reanalysis and verification of previous analytical results. The National Institute of Standards and Technology (NIST) began environmental specimen banking in 1979 in collaboration with the U.S. Environmental Protection Agency to establish a bank of human liver specimens for contaminant trend monitoring and future retrospective analyses for "new pollutants." Over the last 30 years the NIST ESB has expanded and diversified, incorporating a variety of specimen types as part of U.S. government environmental research and monitoring programs. This diversification is similar to what is happening with many other ESBs, responding to the trend in environmental research to incorporate new biomolecular disciplines and to become more integrated in nature.

Keywords: environmental specimen banking, ESB, retrospective study, contaminants, time trends

INTRODUCTION

Formal environmental specimen banking is the systematic long-term preservation of well-documented, representative environmental specimens that are available for deferred (i.e., retrospective) analysis and evaluation. Such specimens preserved over long periods of time (i.e., decades) represent a valuable resource that can be used for future retrospective investigations and for verifying the results of previous studies through re-analysis of banked materials. Banked specimens may include cells, tissues and fluids of biota, soils, sediments, ice cores, etc. Specimens

collected for formal banking must follow strict protocols designed specifically for banking purposes. Procedures required for preservation over decades or centuries are used. For the majority of environmental specimen banking programs, cryogenic storage has been the preferred manner of preservation.

Formal environmental specimen banking programs and the infrastructure to support such programs on a national scale were developed in the 1970s by several countries, most prominently Germany, Canada, Sweden, and the U.S.A. (Becker *et al*., 2006). In most cases, these banking programs were established as part of ongoing research and monitoring programs. The idea behind incorporating specimen banking into these programs was to establish resources of materials that could be analyzed in the future for accumulative chemical contaminants that had not yet been recognized as contaminants when the original collections were made, and therefore were not being monitored or measured. Through use of such a banking system, one can determine when these contaminants appeared in the ecosystem and whether they were increasing or decreasing in concentrations. Banking a portion of specimens collected for immediate analysis (i.e., monitoring) can also provide a quality assurance function by allowing for future re-analysis to verify the original results, or by allowing for future analyses using newly-developed and more sensitive and accurate analytical methods. This capability can expand and improve the database generated by a monitoring program.

NIST'S ENVIRONMENTAL SPECIMEN BANK (ESB) SYSTEM

In 1979, the National Institute of Standards and Technology (NIST) in collaboration with the U.S. Environmental Protection Agency began a program of banking human liver specimens for contaminant trend monitoring. The protocols, procedures, and specialized equipment developed for this program have provided the basis for all subsequent banking components that NIST has instituted for other research and monitoring over the last 30 years (see Table 1).

Several factors must be considered when establishing an environmental specimen banking program. These factors include standard collection and banking protocols (or Standard Operating Procedures; SOPs), long-term storage requirements, specimen handling and processing requirements, specimen inventory tracking and data management needs, specimen access policy, specimen security, and verification of specimen stability.

The infrastructure required to support an environmental specimen banking program includes both a facility and equipment for specimen handling, long-term preservation, specimen tracking and inventory database creation and support, and the staff required to conduct the work. This infrastructure is termed the environmental specimen bank (ESB). The NIST ESB System consists of two facilities: one facility, the National Biomonitoring Specimen Bank, is located at the NIST main campus in Gaithersburg, Maryland, and the other, the Marine Environmental Specimen Bank, is located at the Hollings Marine Laboratory in Charleston, South Carolina (Becker and Wise, 2006). The NIST ESB System is characterized by having well developed banking protocols and SOPs, computerized sample tracking (chain-of-custody) systems, maintenance of many forms of data

Table 1. Research and monitoring programs provided banking services by the NIST ESB System.

Program	Specimens	Dates
Human Liver Specimen Bank	Livers	1979–1994
Human Blood Serum Collection	Blood serum	1986
Nutrients in Human Diet Program	Food specimens	1986
Human Blood Spots	Blood spots	1994
EPA Mussel Watch Program	Mussels and oysters	1976–1978
National Status and Trends Program	Mussels, oysters, fish livers and muscle, marine sediments	1983–present
EXXON VALDEZ Damage Assessment Program	Mussels, oysters, fish livers and muscle, marine sediments	1989
Super Fund Site	Whole fish	1994
Alaska Marine Mammal Tissue Archival Project	Blubber and fat, liver, kidney, muscle	1987–present
Marine Mammal Health and Stranding Response Program	Blubber, liver, kidney	1990–present
Bottlenose Dolphin Health Assessment Program	Skin/blubber biopsies, plasma, whole blood, urine, milk	2005–present
Seabird Tissue Archival and Monitoring Project	Egg contents	1999–present
Peregrine Falcon Monitoring Plan	Egg contents and feathers	2003–present
Regional Monitoring Program for Water Quality in the San Francisco Estuary	Seabird egg contents, fish muscle, bivalves, sediments	2009–present

associated with original specimens, cryogenic storage using ultra-cold (–80°C) electric freezers and liquid nitrogen vapor-phase (–150°C) storage freezers, electronic security systems, electronic monitoring of storage conditions 24 hours a day, 365 days a year, and classified air clean room laboratories for preparing banked specimens for analysis. A variety of human, environmental, and wildlife specimens are presently banked by NIST (see Table 1).

The statistical design of sampling procedures for any research or monitoring program and the banking component of such programs must be given careful thought. Design requirements will vary based on the nature of the parent program providing specimens to the bank. The many programs providing specimens to the NIST ESB System have a wide variety of individual goals and objectives. For example, the National Oceanic and Atmospheric Administration's National Status and Trends (NS&T) Program was established in the early 1980s to monitoring spatial and temporal trends in anthropogenic contaminants in the coastal marine environment throughout the entire U.S.A. The program included collecting specimens for immediate analyses and collecting a subset of these specimens for long-term banking for retrospective analyses. Careful consideration was given to the type of matrices to be collected, the analytes to be measured, the spatial distribution of the collection sites, and the frequency of collections necessary for observing changes at a predetermined level of confidence.

Banking for the EXXON VALDEZ Damage Assessment Program was quite different. A subset of fish, shellfish, and sediment samples that were collected following the 1989 crude oil tanker spill in Prince William Sound, Alaska, were banked for future chemical analysis for verification of analytical measurements made during the damage assessment studies, thus providing a quality assurance function.

Banking for the NS&T Program was a major milestone in the expansion of the NIST ESB System. Based on the success of banking for this program, NIST was able to expand its effort to support other marine-related research and monitoring programs (see Table 1).

THE INTERNATIONAL GROWTH OF ENVIRONMENTAL SPECIMEN BANKING

Historically, the primary reason for environmental specimen banking was to provide materials that could be used for analyzing trends in exposure to previously unrecognized pollutants or for pollutants for which analytical techniques were inadequate at the time of collection (Luepke, 1979). Since the 1970's, ESBs have been established in several countries. Table 2 lists 19 ESBs located in 13 countries representing five continents (similar to the NIST ESB system, the German ESB consists of two facilities, one at the University of Münster and one at Frauenhofer IME).

Reasons for establishing banking programs vary and have included such things as evaluating the results of governmental policies and regulations (Rüdel and Schröter-Kermani, 2007; Kim, 2007), evaluating changes in health of animal populations (Bartels and Kotze, 2006; Becker and Wise, 2006), and investigating

Table 2. Formal Environmental Specimen Banks throughout the world.

Country	Bank and Institution
Brazil	*Marine Mammals Specimen Bank (BAMM)*, University of Rio Grande
Canada	*Canadian Wildlife Service Specimen Bank*, National Wildlife Research Center
	National Aquatic Biological Specimen Bank, Environment Canada
Denmark	*Tissue and Data Bank for Greenland*, National Environmental Research Institute
Finland	*Paljakka Environmental Specimen Bank*, Finnish Forest Research Institute
France	*Observatoire de Recherche sur l'Envronnement (ORQUE)*, University of Pau
	ANDRE Observatoire Pérenne de l'Environnement (OPE), University of Pau
Germany	*The German Environmental Specimen Bank* (*Environmental Specimen Bank*, Frauenhofer IME, &
	Environmental Specimen Bank for Human Organ Specimens, University of Muenster)
Italy	*Mediterranean Marine Mammal Tissue Bank*, University of Padua
	The Antarctic Environmental Specimen Bank (BCAA), Genoa
Japan	*ESB for Global Monitoring (es-BANK)*, Ehime University
	Time Capsule for Environment and Endangered Wildlife, National Institute of Environmental Studies
South Africa	*Biological Resource Bank*, National Zoological Gardens SA
Spain	*Environmental Specimen Bank of Galicia*, University of Santiago De Compostela
Sweden	*Environmental Specimen Bank*, Swedish Museum of Natural History
U.K.	*National Fish Tissue Archive*, Centre for Ecology and Hydrology
U.S.A.	*NIST ESB System (National Biomonitoring Specimen Bank*, NIST Gaithersburg, &
	Marine Environmental Specimen Bank, Hollings Marine Laboratory)
	CDC and ASTDR Specimen Packaging, Inventory and Repository, Centers for Disease Control and Prevention
	Alaska Frozen Tissue Collection, Museum of the North, University of Alaska

temporal trends in ecosystems (Becker and Wise, 2006; Tanabe, 2006; Odsjö, 2006; Uehiro *et al*., 2007). For some ESBs the reason is simply to retain materials of scientific worth for future, presently undefined, research purposes.

Other factors determine a banking program's design. Some programs are *ecosystem based* (e.g., the banked specimens represent important ecosystem compartments and may represent more than one ecosystem) as illustrated by the German ESB, Korean ESB, and ORQUE (France). Other programs are *contaminants of interest based* (e.g., using a specific matrix to determine trends in environmental inputs of specific contaminants) as represented by the Mussel Watch Program and Seabird Tissue Archival and Monitoring Project of the U.S.A. Programs such as banking for the Peregrine Falcon Monitoring Plan and the National Marine Mammal Tissue Bank in the U.S.A., the Mediterranean Marine Mammal Tissue Bank in Italy, and the Biological Resource Bank SA in South Africa are *species of interest based*. Banking for the EXXON VALDEZ Damage Assessment Program in the U.S.A. is *based on quality assurance needs*.

The value of environmental specimen banking is becoming more broadly recognized by the scientific and management community. The "newly-recognized" contaminants that planners were thinking about when justifying the development of the early specimen banks are now at the forefront of contemporary environmental concern. More scientific papers using banked specimens are appearing in the scientific literature, thus demonstrating the value of banking. Issues regarding climate change and its potential role in changing historical patterns of contaminant transport and cycling through the ecosystem have clearly underscored the value of banking for retrospective research (Noyes *et al*., 2009). Finally there is a successful history, particularly in Germany, of using environmental specimen banking for evaluating environmental regulations and policy (Rüdel *et al*., 2009).

THE FUTURE OF ENVIRONMENTAL SPECIMEN BANKING

Long-term environmental specimen banking is at the point of showing real value to the scientific community and to society in general. There are presently three trends in banking that one can project to continue into the future.

The first trend is global expansion of ESBs. Early development of ESBs occurred in a few countries in Western Europe, North America, and Asia, which was represented by Japan. Interest is now world-wide. Within the last five years, three new banking programs and supporting ESB facilities have been developed, two in France and one in Korea. ESB facilities have been upgraded in the U.S. and Japan, and efforts have been made to develop formal ESB programs in other countries, such as Norway, Spain, Portugal, Brazil, and Australia. In fact the most recent symposium, the *International Symposium on Environmental Specimen Bank (IESB Symposium 2009)—Exploring Possibility of Setting-Up ESBs in Developing Countries* involved representatives from established and developing ESBs and individuals from developing countries with interest in environmental specimen banking and suggests that interest in geographic expansion of environmental specimen banking will continue.

As mentioned earlier, cryogenic storage has been the preferred manner of

preservation for the majority of environmental specimen banking programs. Cryogenic facilities require relatively large capital investments that may deter banking in developing countries. Thus, there is a need to evaluate alternate storage methods, such as frozen but not cryogenic storage, air dried and storage at room temperature, and freeze-dried and storage at room temperature. However one should be cautious when considering alternate storage conditions and take into consideration both the stability of the analytes of interest and the stability of the matrix being stored under such conditions.

The second trend is increasing international cooperation among ESBs which is directly related to the world-wide expansion of environmental specimen banking. This cooperation is illustrated by exchange of information through international workshops and conferences, exchange of banking protocols and SOPs among ESBs, and the recent web-based clearing house for communication and information exchange among ESBs established by the German Federal Environment Agency (https://inter-esb.org/). The first international workshop on ESB was held in Luxembourg in 1977 (Berlin *et al*., 1979). The main objective of this workshop was to assess the types of environmental pollutants and human specimens most suitable for "biological monitoring" and to evaluate the probable usefulness of biological specimen banking. There have been 18 international ESB workshops and conferences since that first workshop in 1977, culminating in the *IESB Symposium 2009* recently held at Ehime University, Matsuyama, Japan.

International cooperation among ESBs is also demonstrated by ongoing efforts by established ESBs to provide advice and support for developing banks and, in some instances, exchange of personnel among banks. Most recently, plans for exchanging samples among ESBs as part of a cooperative analytical exercise are being developed. These activities of interaction and cooperation among the ESBs are being stimulated by the realization that many if not most environmental issues presently faced go beyond national borders and are international in nature. Future international monitoring and research efforts must include environmental specimen banking as an important tool for attacking these problems.

The third trend in banking is diversification of ESBs to address a variety of scientific disciplines. For many ESBs there is a trend of expanding beyond only providing a resource for contaminant measurements. This expansion includes banking blood serum and cell lines for wildlife disease studies, banking for genetics and genomics research, banking for biotoxin monitoring, and banking for basic ecological research (e.g., biodiversity, ecosystem structure and function, etc.). Monitoring anthropogenic contaminants in the environment is still the primary application of ESBs, and individual banks for cell lines, serum, and genetic materials already exist. However, environmental monitoring and research programs are now becoming broader and more sophisticated in their scope and collections, incorporating biological response investigations at the molecular level and modeling changes in ecological structure and function. Such highly integrated investigations will require more sophisticated ESBs that can provide the greater variety of related and integrated specimens required for a variety of analytical work within such programs.

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