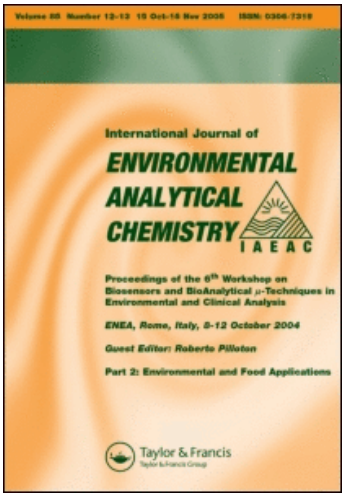

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Antarctic Environmental Specimen Bank-First 5 Years of Experience

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To cite this Article Soggia, Francesco , Abelmoschi, Maria Luisa , Riva, Simona Dalla , De Pellegrini, Rodolfo and Frache, Roberto(2001) 'Antarctic Environmental Specimen Bank-First 5 Years of Experience', International Journal of Environmental Analytical Chemistry, 79: 4, 367 — 378

To link to this Article: DOI: 10.1080/03067310108044395

URL: http://dx.doi.org/10.1080/03067310108044395

Intern. J. Environ. Anal. Chem., Vol. 79(4), pp. 367-378
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ANTARCTIC ENVIRONMENTAL SPECIMEN BANK – FIRST 5 YEARS OF EXPERIENCE

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(Received 10 January 2000; In final form 20 July 2000)

The Project on an Antarctic Environmental Specimen Bank (Banca Campioni Ambientali Antartici – BCAA) began in 1994 in order to collect and classify samples from the Antarctic ecosystem to be used for future studies.

The objectives of the project are similar to the general aims of the Environmental Specimen Banks, but they specifically focus on the chemical aspects concerning the research activities of the Italian Project on the "Micropollutants Chemistry" (Sector "Chemical Contamination" of the Italian Antarctic Research Programme – PNRA).

During these first years the facilities suitable for storing a significant number of specimens (now over 2,000) at different temperatures (from −30°C to −150°C) and a database system, specifically designed for managing and consulting information concerning both the storage of samples and data on their chemical characterisation, have been developed.

In addition, a programme for validating the procedures of Antarctic organisms storage by checking the stability of some chemical parameters both in fresh and freeze-dried specimens has been developed.

Keywords: Environmental Specimen Bank; Antarctica; Environmental monitoring; long-term storage; storage experiment; retrospective analysis

INTRODUCTION

Major aims of Environmental Specimen Banks[1] consists in the long-term storage of selected environmental specimens in order to study the presence and the evolution of noxious substances by retrospective identification and quantification of contaminants including those not known at the time of storage.

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The project on the BCAA was established in 1994 and has been developed within the Italian Antarctic Research Programme (PNRA). It is now an integral part of the Project on the "Micropollutants Chemistry" (Sector "Chemical Contamination")[2] and plays an important role both from an analytical point of view and as collection and storage of samples from Antarctica, a remote area now internationally considered as a privileged observatory on global changes in the environment.

The presence of the BCAA highlights the need for checking and monitoring, even in this geographic area, potentially noxious substances; in fact pollutants are generally present in Antarctica because of the atmospheric and oceanic circulation that carries them from the more anthropic areas to the remotest ones.

The initial phase, developed from a study on the facilities and programmes of the Environmental Specimen Banks[3–9], enabled to fix the procedures regarding collecting and storing at suitable temperatures and to manage the information concerning samples. During these first years the activity was concentrated on the systematic collection of environmental samples for long-term storage. A large number of samples were stored and consequently the pilot phase can be considered as fully completed.

BCAA FACILITIES

The BCAA laboratory is housed at the Department of Chemistry and Industrial Chemistry (University of Genoa – Italy).

The facilities used in BCAA are shown in Figure 1. The major space is used for low temperature rooms at −30°C with a total capacity of 40 m^3. In addition, in an air-conditioned room, there are three deep freezers at −80°C with a total capacity of 1.1 m^3 and a cryogenic freezer at −150°C with a total capacity of 0.2 m^3. Another room is equipped with laminar flow hood, biohazard hood, freeze dryer, homogeneizer, ultrapure water unit, vacuum packaging machine and a computer for on-line data management.

A 15 kw auxiliary generator unit has been installed in an external area in order to feed freezers automatically in case of prolonged black-outs on the supply mains.

SAMPLES COLLECTION

The Antarctic Continent is a privileged observatory for research on global changes and it can be considered as the largest environmental and climatological memory of the Earth.

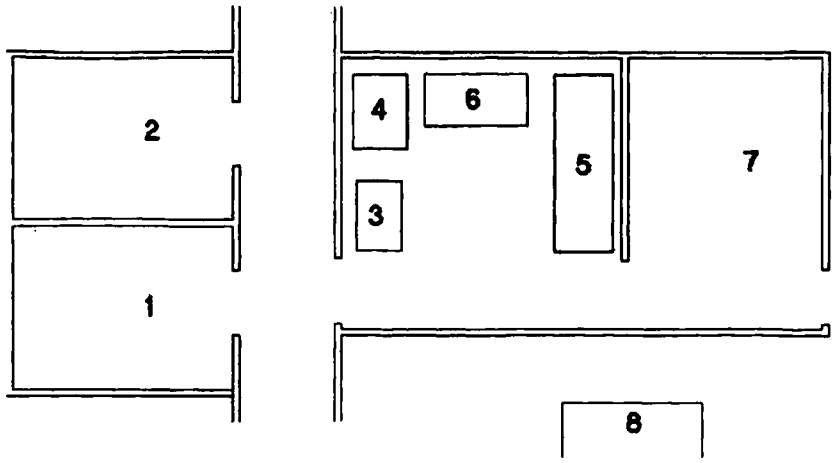

FIGURE 1 BCAA facilities 1, 2: rooms at −30°C (total capacity 40 m^3) 3,4,5: three deep freezer at −80°C (total capacity 1.1 m^3) 6: cryogenic freezer at −150°C (capacity 0.2 m^3) 7: laboratory room 8: auxiliary generator unit

The long distance from densely populated areas, the isolation due to its geographical polar position, the presence of the Antarctic Circumpolar Current which prevents the direct mixing of waters and the nearly total ice covering (that represents the largest fresh water reservoir in the world and regulates the thermal balance of the Earth by constituting the cold source for the global thermodynamic system) are characteristics which make Antarctica a very important area for collecting environmental samples to be stored and used for future studies concerning the transport and accumulation of potentially dangerous substances.

Criteria for selection of samples

The sampling techniques depend on environmental matrices (liquid or solid and abiotic or biotic) and on the concentration level of analytes (major, minor, trace and ultra-trace for total and speciation analysis) that will be studied. The samples collected were chosen for their significance and usefulness as environmental indicators.

The sampling procedures, already described in a previous paper[10], were developed following the ESB indications relevant to the Standard Operative Procedures (SOPs)[11,18].

Sampling area

For logistic reasons due to the presence of the Italian summer base at Terra Nova Bay in Victoria Land, samples were collected between the 70th and the 80th parallel south and the 150th and the 180th meridian east, as shown in Figure 2.

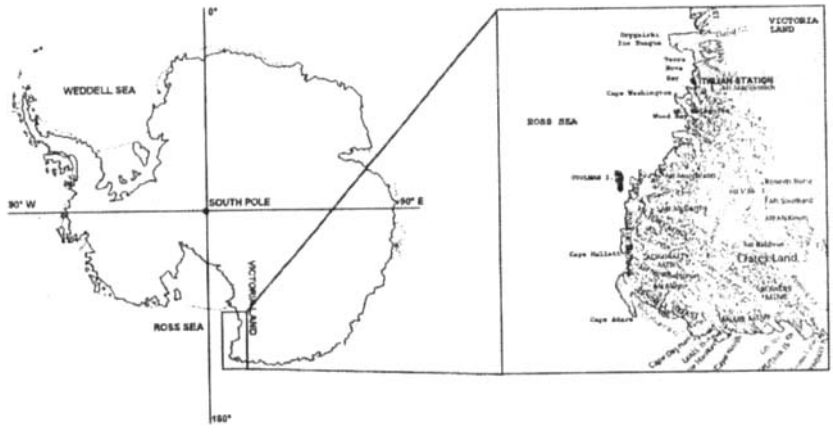

FIGURE 2 Sampling area

Collection and storage of samples

The collection and storage of abiotic and biotic samples from different Antarctic environments (terrestrial, freshwater, marine and atmosphere ecosystems) began systematically during the austral summer in 1994/1995 and continued until today.

The terrestrial and freshwater environments are characterised by a very limited biological activity; in fact the sampling regards only a few biotic matrices (moss, lichen and lacustrine algae). As regards the abiotic samples the following matrices have been sampled: soil, snow, firn, ice, lake sediment and lake water near Terra Nova Bay.

The marine environment has a much greater diversity that enables collection of a more specimens of organisms (fish, molluscs, crustaceans). The abiotic samples collected are mainly sediments, suspended particulate matter and water.

The atmospheric sampling regarding aerosol and particulate was carried out in a site on the coast 3 km south the Italian base at Terra Nova Bay.

The total number of samples managed by the BCAA exceeds 2,000. Table I shows the type of samples. The quantity of each sample varies from less than 0.1g (suspended particulate matter) to 0.5–2 kg (sediment, soil).

TABLE I Environmental samples stored in BCAA

Matrices	%
Atmospheric Particulate	1
Snow	6
Soil	16
Terrestrial Vegetable organisms	3
Lake sediments	7
Marine Sediments	25
Marine Animal organisms	32
Sea Water	4
Marine suspended Particulate matter	5
Pack-Ice	1

STABILITY OF ANALYTES DURING SAMPLE STORAGE

The different problems to deal with during the long-term storage of biological samples are depending on the condition of the material (e.g.: fresh or freeze-dried tissue), the type of container used (more or less permeable to gases) and the storage temperature (only below −135°C the enzymatic activity is completely stopped). One of the check activities performed by ESBs consists in storage experiments[5,12] in order to validate chemical stability.

After considering the different environmental matrices stored in the BCAA, the first check activity concerned a biotic matrix, as it is more subject to degradation with time. The muscular tissue, both fresh and freeze-dried, of an Antarctic fish (*Trematomus bernacchii*) was used to perform the experiment; it was collected during the 13th Italian Antarctic Expedition (1997/1998) in Terra Nova Bay (74° 42,730′ S; 164° 08,000′ E) at a depth of about 70 m.

The fresh material was put in different plastic containers (polyethylene and polycarbonate) of different thickness (bags, jars, vials) to check the effect on gases permeability. The freeze-dried material was put only in glass vials.

The parameters to be tested in this first phase were total and organic mercury contents, because of their great scientific interest due to the effects that mercury contamination can have on the whole ecosystem. Moreover, the mercury concentration level in the muscular tissue is high enough, because the accumulation depends on the fish size and on their position in the food-chain (bioaccumulation). The analytical methods, used also for many years in our laboratories for speciation (organic mercury), are quite reliable (see Table III). We can also refer and compare results with recent studies done in the same area and with the same species[13].

Sample preparation

A composite and homogenised sample was prepared in order to eliminate the remarkable differences of mercury concentration in different fishes. It consisted of muscular tissue of 39 female specimens with weights ranging from 101 to 336 g (average length: 27.5 cm; average weight: 297 g) frozen at −30°C when being collected and stored until the experiment was carried out.

After the homogenisation 2.4 kg of muscular tissue were obtained; 400 g were freeze-dried and separated into 30 sub-samples of about 2–3 g each and the rest of the fresh sample was divided into 120 sub-samples of 10–30 g each. Table II shows the storage conditions of the sub-samples, fresh or freeze-dried, stored at different temperatures after being packed into different types of containers.

TABLE II Storage conditions used for stability test

Homogenized fresh sample (2.4 kg of muscular tissue)			
120 Fresh sub-samples		*30 Freeze-dried sub-samples*	
Containers	*Temperatures*	*Containers*	*Temperatures*
Glass vials Polyethylene vials Polyethylene bags Polycarbonate jars	−30°C, −80°C, −150°C	Glass vials	+25°C, +4°C, −30°C

Analytical methodology

The sub-samples homogeneity was verified within and between bottles. Eleven sub-samples for between-bottle homogeneity and one sub-sample for within-bottle homogeneity were chosen[14]among the freeze-dried samples.

The subsamples homogeneity was verified by determination of As, Zn, Mg, in addition to total mercury (Hg-tot) and organic mercury (Hg-org). All the determinations were carried out at the same time in two laboratories.

The determination of As, Zn, Mg was performed by inductively coupled plasma (ICP-AES mod. JY24) equipped with an ultrasonic nebulizer after the mineralization of an aliquot of freeze-dried muscular tissue in a microwave oven with nitric acid.

As regards Hg-tot, the sample mineralised as described above, was analysed by cold vapour – atomic absorption spectrometry, both after preconcentration over gold (Au-CV-AAS)[15], and with a continuous flow system to reduce Hg using $NaBH_4$.

Hg-org was determined using the same techniques above described, after extraction in toluene and back-extraction in a cysteine solution[15].

The accuracy and precision of analytical methods were checked by using three Certified Reference Materials TORT-2, DORM-2 and LUTS-1 (National Research Council of Canada). Table III shows our results compared with the certified ones.

TABLE III Precision and accuracy of analytical methods. All data are reported in mg kg^{-1} in dry weight

CRMs	*TORT-2*		*DORM-2*		*LUTS-1*	
	Certified values	*This work*	*Certified values*	*This work*	*Certified values*	*This work*
Hg-tot	0.27±0.06	0.26±0.05	4.64±0.26	4.68±0.30	–	n.d.[a]
Hg-org	0.152±0.013	0.150±0.015	–	n.d.[a]	–	n.d.[a]
Mg	n.c.[b]	–	n.c.[b]	–	601±28	550±35
Zn	180±6	172±9	26.5±2.3	23.6±2.0	82.9±5.4	91.7±7.2
As	21.6±1.8	20.2±1.5	18.0±1.1	16.5±1.3	19.0±0.9	17.9±1.3

a. n.d. = not determined
b. n.c. = not certified

Results

The concentration values (dry weight), are shown in Table IV, represent the means of the determinations of the two laboratories.

TABLE IV Between and within sub-samples homogeneity

	Between-bottle homogeneity (four determinations on the content of each of 11 subsamples)			*Within-bottle homogeneity (9 replicate determinations on the content of one subsample)*		
	average	*s.d.*	*c.v.%*	*average*	*s.d.*	*c.v.%*
Hg-org (μg/g)	0.891	0.060	6.7	0.868	0.070	8.1
Hg-tot (μg/g)	0.97	0.07	7.2	0.99	0.09	9.1
Mg (mg/g)	1.24	0.09	7.3	1.31	0.05	3.8
As (μg/g)	42.6	1.5	3.5	43.8	2.4	5.5
Zn (μg/g)	24.4	1.4	5.7	25.2	1.0	4.0

The between and within sub-samples homogeneity was verified by applying t-test at the 95% confidence level, which did not show any significant differences.

Control chart

The control chart[16] is a way to verify certain conditions (parameters, concentration levels, etc.) under statistical control. We selected a sequential chart that enables to check the progression, depending on time related factors, of the possible change of concentration levels, both absolutely and according to the distribution between the inorganic and organic form of mercury.

Previous studies[5] found that analysis of total mercury in mussel homogenate tissue samples show no significant difference during a two-year-storage at −20°C, −80°C and −196°C in different containers (polyethylene bottle, ampoule and bottle glass). Our experiment also aimed at verifying if there is an increase (possible contamination due to mercury vapours) or a decrease of total mercury for a longer period of years, and if there are changes in the distribution between the organic and inorganic form (possible degradation of organism tissues and of chemical forms under which the metal is present at the beginning).

After one year the results obtained are in accordance with the mentioned studies[5], that is in all the different types of storage the are no significant differences respect to initial concentration of total and organic mercury (see Figure 3).

DATABASE MANAGEMENT SYSTEM

The large number of samples stored and the amounts of related data, makes the data management computerisation a must for recording and search into the database.

After a first stage of experimentation consisting in developing a software (BCAA.EXE ver. 1.0 it)[10] suitable for the data management, the database has been expanded and developed with Microsoft® ACCESS 97 and it is available on internet by a server Windows® NT 4.0 (**http://*www.bcaa.unige.it***), where it is possible to obtain information on sampling, classification, storage and possible chemical characterisation of samples.

The new database is interfaced by a link to the Data Centre CHI (Italian System for the Antarctic Chemical Data Exchange of the PNRA)[17].

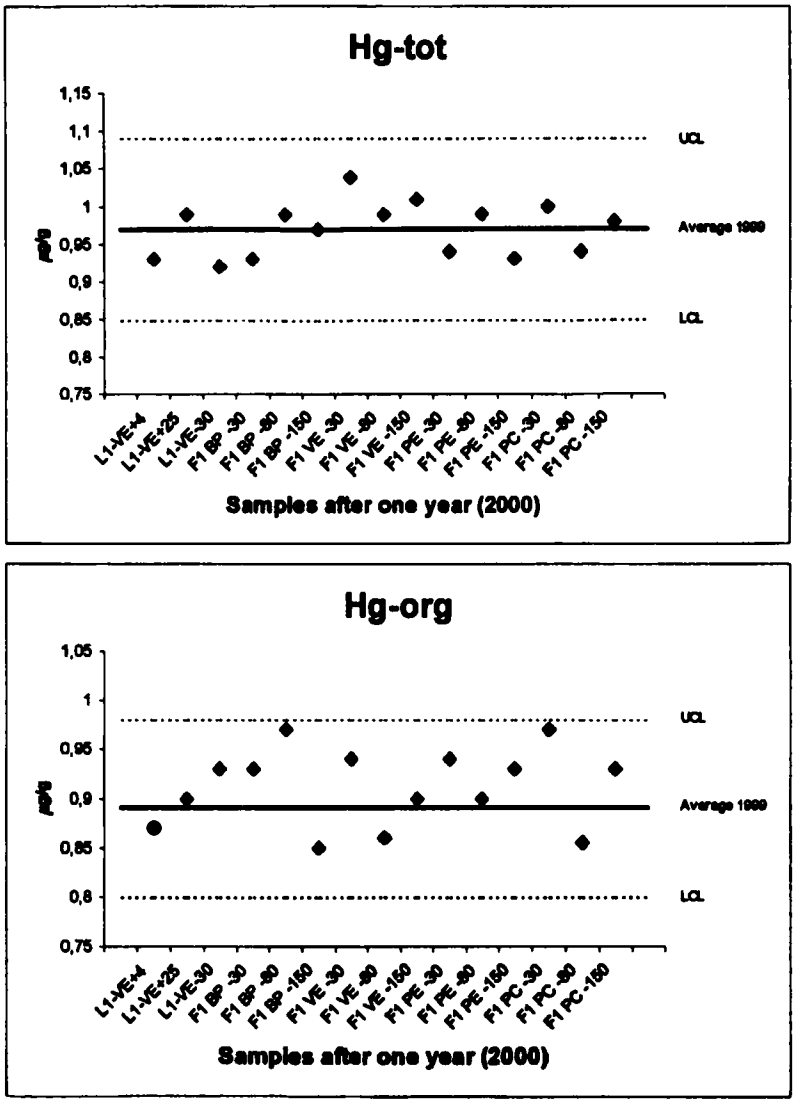

FIGURE 3 Control chart after one year. L1: freeze-dried muscular tissue, F1: fresh muscular tissue, VE: glass vials, BP: polyethylene bags, PE: polyethylene vials, PC: polycarbonate jars, Storage temperatures: +4°C, +25°C, −30°C, −80°C and -150°C

Data storage and display

Inside the BCAA site (see Figure 4), data are divided into 3 environments: atmosphere, terrestrial and freshwater, and marine environment.

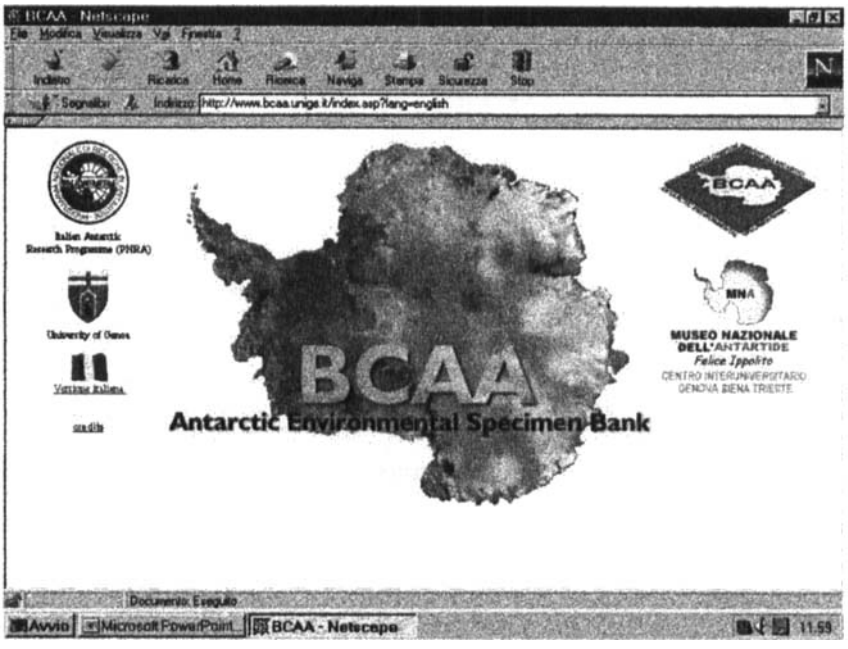

FIGURE 4 Homepage of BCAA

Each environment is divided into a series of matrices as shown in Table V. There are two possible choices in using the database:

- quick search procedure: to enter keywords;
- step by step procedure: to choose the various options according to the sample of interest.

TABLE V Antarctic environmental matrices

Code	*Name*
Atmosphere	
AI	Air
AP	Atmospheric Particulate
FO	Fall Out

Code	*Name*
Terrestrial and Freshwater Environments	
SN	Snow
FR	Firn
IC	Ice
SL	Soil
TV	Terrestrial Vegetable organisms
TA	Terrestrial Animal organisms
LI	Lake Ice
LW	Lake Water
LP	Lake suspended Particulate matter
LS	Lake Sediment
LV	Lake Vegetable organisms
LA	Lake Animal organisms
IW	Ice melting Water
Marine Environment	
SW	Sea Water
MP	Marine suspended Particulate matter
MS	Marine Sediment
MV	Marine Vegetable organisms
MA	Marine Animal organisms
PI	Pack Ice
PP	Pack ice suspended Particulate matter

FUTURE PROSPECTS

During the next three years (1999–2001) the BCAA, will continue to collect samples following a statistical design with its own programme of research complementary to the general guidelines of the New Project "Micropollutants Chemistry", in order to manage some thousands of samples.

In the next future an automatic system for storage with liquid nitrogen by installing a 1500–3000 litres tank and three or four 500–1000 litres containers for samples will be developed.

Another important aspect of the Project is the interdisciplinary collaboration of the specimen bank within the PNRA. Consequently, the specimen bank will collaborate with other Sectors that collect systematically samples in areas of general

interest and carry out the chemical characterisation of some environmental parameters to be followed during a certain period of time.

The programme concerning the validation procedure on the storage of samples will continue and will be developed.

Acknowledgements

This work is financially supported by Italian Antarctic Research Programme (PNRA).

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