

Guidelines for the sampling and analysis of dredged material intended for disposal at sea



INTERNATIONAL
MARITIME
ORGANIZATION
London, 2005

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Published by the
INTERNATIONAL MARITIME ORGANIZATION
4 Albert Embankment, London SE1 7SR

(ISBN 92-801-4192-9)

Electronic edition: 2005

IMO PUBLICATION
Sales number: E537E

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Executive summary

In the framework of the London Convention 1972 and the 1996 Protocol thereto generic "Waste Assessment Guidance" (WAG) were approved in 1997 for eight different types of waste allowed to be dumped at sea. One of them is dredged material.

Complementing the Waste Assessment Guidance, specific guidelines for assessment of the different wastes were developed and adopted in 2000, among them the *Specific Guidelines for Assessment of Dredged Material* (LC/SG 24/11, annex 3).

To further support practical implementation of this generic guidance, particularly for dredged material, the Scientific Group prepared an overview of information covering some of the main working steps:

- selection of substances of concern and effects to be determined;
- field sampling; and
- physical, chemical and biological analysis.

The guidance contained in this publication addresses the points above and is an amalgamation of two documents produced for the London Convention 1972 and the 1996 Protocol thereto:

- Guidelines for the sampling of sediment intended for disposal at sea (LC 24/17, addendum 1); and
- Selection and analysis of physical and chemical parameters for the assessment of dredged material quality (LC/SG 26/12, annex 2).

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1 Introduction

1.1 Background

This is an introductory guide for those with limited experience in sediment sampling and analysis.

It provides considerations and good practices for developing sampling plans in an effort to help users decide how to organize and prioritize their sampling activities to suit their particular goals, experience, budget and technical capabilities.



This publication also provides strategic guidance in making cost-effective, practical and environmentally-sound decisions on what to measure and how to measure it, and to assess sediments on a case-by-case basis. It reviews what to consider before analysis, selecting variables, conducting the analysis and important aspects of quality assurance and control.

The assessment of dredged material will include chemical and physical analysis and may also include chemical analysis of water and biological tissue. This report presents general guidance (figure 1) on sampling and on selecting and analysing physical, chemical, and biological parameters that are useful for the evaluation of sediments particularly dredged material.

This publication should provide generic guidance and make it clear that those who can implement the full guidance now should do so, while those that cannot should implement what they can as soon as practical and make it a national goal to develop the capability to progressively implement the remaining aspects in as few years as possible.

In areas where environmental evaluations of dredged material are not yet standard practice, it would be unnecessary and ill advised to attempt to begin with detailed implementation of all aspects of this guide. It would be equally ill advised to delay initiation of progressive implementation of a programme of environmental evaluation of dredged material. Such a programme could begin with general surveys and advance to routine implementation of more complex evaluations as environmental experience and sophistication are developed.

It is far better to conduct basic evaluations now than to do nothing because of a current inability to conduct sophisticated evaluations (after IADC 1997).

This guide is geared primarily to the Contracting Parties of the London Convention 1972 and its 1996 Protocol but it may be useful to anyone interested in best practices for sampling and analysing sediments. It is not broken down by players or stakeholders' roles (as this may vary according to project and area) but according to tasks to be done to make a plan and execute it. It is intended for:

- middle-managers and newcomers in the field of dredging having some scientific/technical knowledge;
- regulatory bodies that oversee and authorize ocean disposal of dredged material for which sediment sampling is required;
- programme managers who may have limited scientific background to make strategic or policy decisions on dredging and related activities;
- decision-makers such as port managers or licensing authorities, consultants and other persons or groups involved in dredging projects in areas where there is not an established expertise in environmental evaluations of dredged material;
- those planning the dredging of sediments; and
- those undertaking sediment sampling in the field.

This technical guidance has been developed in the form of a document that can be improved and updated in the future in the light of new technical developments and the results of scientific research. Suggestions from the readership for such improvements are welcome.

1.2 Structure of the Guidelines

There are several working steps towards obtaining representative information on sediments of interest (see figure 1):

- an initial assessment in which the goals and the existing information are analysed;
- a planning phase, in which the sampling plan and the analytical plan are prepared;

Step		Activity	Chapter	
A	Initial considerations	Check task/goal	2	
		Check stakeholder requirements		
		Collect project information		
		Decide on need for further data		
B	Selection of parameters for analysis	Establish parameter list	3	4
		Prepare an analytical plan		
		Check available personnel and equipment		
		Prepare a quality control plan		
C	Sampling plan	Prepare a sampling plan	5	
		Prepare a quality control plan		
		Prepare to sample		
D	Field sampling	Execute sampling	6	
		Store and prepare samples		
E	Physical, chemical and ecotoxicological analysis	Execute analyses	7	
		Assess data quality		
	Sediment quality assessment	Evaluate data	WAG*	
		Report results		

Figure 1 – Considerations for planning and execution of sediment sampling

* WAG = Waste Assessment Guidance of London Convention, *Specific Guidelines for Assessment of Dredged material*.

- an execution phase, in which the sampling is carried out;
- an analytical phase, in which the required analyses are carried out; and
- an evaluation phase, in which the information collected is used to help reach required decisions or actions.

The intent is to lead the reader through these various steps, recognizing that the process may be “iterative,” meaning that as questions are answered and data become available at one step, they may lead to further questions and require a reconsideration of previous steps. The steps are nonetheless numbered to move the reader towards the final outcome of producing information that will enable defensible and cost-effective decision-making.

Readers with limited experience should note that more information may be provided than is needed for simple sampling or analysis and that one body may not be responsible for all the steps. Readers are encouraged to use the sections that are relevant and adapt them to their own situation.

2 Initial considerations

For the assessment of the quality of dredged material it is desirable to obtain meaningful and sufficient information at minimum cost and effort.

2.1 Initial project assessment

In order to minimize cost and maximize efficiency it is helpful to carry out an “initial assessment”.

2.2 Initial assessment of existing data

Is there a need to sample or analyse additional data?

Sediment sampling and analysis can represent a substantial investment of time and money. Sometimes, a regulatory agency will provide instructions as to what it regards as necessary and sufficient sampling and analysis. Often, however, sampling and analysis are determined on a case-by-case basis. In addition, the development of the analytical and sampling plans involve co-operation among several different parties such as the dredging contractor, programme manager, regulator, laboratory etc. Each party may not be involved at all the stages but should be aware of the whole process.



Figure 2 – Field sampling underway

The following questions look at the need for and required extent of sampling and analysis.

1. *What is the intended dredging project and what is already known about the material?*

Volume, depth and basic particle size characteristics will be part of scoping the potential dredging work. The timing and methods to be used in the planned dredging project should also be considered as these can affect what components of the environment might need sampling and to what extent. If, for example, the project is to use equipment that increases sediment dispersion, it could raise additional concerns about conditions in the water column. This project information can also supply information on contaminants raising concern.

2. *Who will be involved and what are their roles?*

It is important to identify any laws and policies that control the intended project. In addition to the London Convention, other regional seas conventions may apply which have slightly different information requirements or criteria. See IADC, 1997 volumes 1 & 2a on “players” and legislation.

National, State (provincial, territorial, etc.) or local legislation and policy may exist that will also indicate the need for, and the level of, sampling and analysis. In some cases, these controls may be more stringent than international requirements. Identifying the relevant legislation also helps to identify who may be interested in the development of the plans, participation in the fieldwork and the use of the results. Those who grant funding or who are responsible for paying for the sampling project or laboratory work, will also set real constraints on what can be achieved.

Finally, the level of public or political interest in a given project can affect the need for sampling and analysis. Their involvement, in turn, could influence anything from the area to be examined to which questions need to be answered (IADC, 1997a).

3. What is wanted/needed?

Clear and achievable goals are essential. Goals can be framed with specific questions or with testable hypotheses. For example, if the goal is to decide whether sediment can be dredged from point A and deposited at sea at point B, then the main question might be: “*Does chemical contamination exceed set criteria?*”. The final questions must make it clear what information will need to be collected to reach a sound decision. These goals and questions form the basis of the study plan that includes both a sampling and analytical plan.

Definitions of goals are usually undertaken by management or a regulatory agency and can require modification or negotiation once faced with practical limitations of time, equipment, budget, etc. Further, the definitions of the goals should include an assignment of priorities if there is more than one goal. This will give direction to any adjustments or revisions that need to be done in the event that all the original goals cannot be met (Arctic Laboratories 1985).

Decision-making frameworks or criteria are beyond the scope of this publication but readers are encouraged to consult some of the many national and international decision-making framework documents (appendix 4, section 4.2)

4. *What data are already available?*

Historical information, information on current sources of pollution, related data from research or previous permits for those sites, etc., should be studied. This is an iterative step. Research on historical data is done first to help scope out the areas of concern and later to confirm any additional information that might be available on the chosen areas. Generally, if recent work shows contaminants are of little concern, additional information may not be needed.

5. *Is this enough to make a decision?*

Section 4.2 of the *Specific Guidelines for Assessment of Dredged Material* provides for the exemption of dredged material from detailed characterization under specific circumstances:

Specific Guidelines for Assessment of Dredged Material:

Dredged material may be exempted from the full characterization requested in paragraphs 4.3 to 4.9 if it meets one of the criteria listed below:

1. dredged material is excavated from a site away from existing and historical sources of appreciable pollution so as to provide reasonable assurance that the dredged material has not been contaminated, or
2. dredged material is composed predominantly of sand, gravel and/or rock, or
3. dredged material is composed of previously undisturbed geological materials.

Dredged material that does not meet one of these criteria will require a full characterization to assess its potential impact.

The “Guidance on Assessment of Sediment Quality” developed by Global Investigation of Pollution in the Marine Environment (GIPME 2000) further suggests the use of a weight of evidence approach to determine

additional sampling needs. In addition to particle size and obvious sources of pollution, it suggests looking into:

- uses of the site;
- ecological or economic value of the resources;
- obvious problems (lesions on fish, etc., contaminated seafood from the area); and
- comparison to reference sites.

“If the weight of evidence reveals no significant cause for concern, no further assessment of chemical contamination or associated biological effects is required” (GIPME 2000). This advice is useful and may be followed in the absence of legislation or policy requiring otherwise.

If a decision, based on available information can be made, sampling and analysis would only be undertaken to confirm the decision, if at all.

Working through the basic questions in a checklist should help the investigator to determine cases where sampling and analysis are not needed, or to lay out pre-determined limitations and requirements for a sampling and analytical programme. Within the initial assessment to be carried out, there needs to be due consideration of what can reasonably be obtained based on the realities, e.g., the following issues:

Issue	Questions to be answered, e.g.,
☐ Planned dredging project	☐ Details of the planned dredging project? ☐ Are there contaminant sources of concern? ☐ Is there available and useful historical information?
☐ Type of study and design	☐ Is it a pilot study, local investigation, monitoring, or other type?
☐ “Players in the field” involved	☐ Who are the “stakeholders” and other “players in the field”? ☐ What are their requirements?
☐ Legal requirements	☐ What is required by applicable laws? ☐ What is required by existing contracts?
☐ Analytical capacity	☐ What analytical capacity (equipment, expertise, personnel) is available?
☐ Budget available	☐ Who will commission, who will pay for the investigations? ☐ How much money is available?
☐ Time frame	☐ What are the deadlines for the investigations to reach decisions?

A plan to achieve the main goals can then be constructed. This will involve the development of analytical and sampling plans.

3 Selection of physical, chemical and biological parameters

A selection of variables and sampling methods should always be undertaken with good appreciation of the data analyses intended.

3.1 Existing data sources

Historical and project data need to be evaluated (see section 5.3).

Historical data on physical, chemical and biological consequences of previous dredging and disposal in the area may help indicate:

- (1) what has been demonstrated to be a problem,
- (2) what has been demonstrated **not** to be a problem, and
- (3) what is not yet known at the site.

Category 3 deserves the most investigative effort, 2 less, and 1 least, recognizing that more detailed information may be required to improve previous management strategies for identified issues of concern.

- What is available from physical analyses and engineering properties of sediments for preparation of a dredging bid (density, particle size distribution, etc.)?
- Adequacy of past sampling coverage as a model for the next sampling.
- Applicability and acceptability of past parameter list, detection limits, need for toxicity tests and appropriate species, etc.?
- Issues of interest to specific stakeholder groups.
- Potential examples for sampling and analysis plans, quality assurance and quality control plans, etc.
- Logistical constraints associated with sampling and analysis.
- Improvements for stakeholder participation and public relations.

3.2 Additional or new characterization

In general, the initial evaluation of a new-work/capital dredging project would require more comprehensive and less detailed analyses (in terms of both spatial coverage and the parameters addressed) than subsequent evaluations of the same project or evaluation for a maintenance dredging operation. It may not be helpful to devote great effort or resources to intensively sampling and analysing a small discrete area (either horizontally or vertically) if the logistics of the dredging programme mean that a larger area will be considered and dredged as a single unit.

3.3 Tiered approach for determining parameters

3.3.1 Potential sources of contamination

It may be helpful to construct a table of chemical, physical and biological parameters for consideration. They should be based on a “reason to believe” and on an evaluation of potential contaminant sources.

A pragmatic way to proceed may be:

- to start with a basic list of contaminants;
- to add/delete parameters based on ‘a reason to believe’ they are likely to be or not be important in the particular project being evaluated;
- to consider inclusion of degradation products in addition to parent compounds;
- to consider including contaminants commonly associated with some of the sources (e.g., particular types of industries) that may influence the status of the material to be dredged.

Such a list may assist in identifying why certain parameters should not be required for analysis, or why certain parameters that may otherwise not have been considered should be included. While some parameters may frequently be of concern, it is important to tailor the selected parameters for each project to the specific situation under evaluation where this is not contrary to local regulators’ requirements.

Unless required by regulators, a ‘standard list’ of parameters should not be selected by default for analysis for all projects.

It is counterproductive to include parameters for which reliable routine analytical methods, with environmentally relevant detection limits, do not exist or cannot be achieved. However, in areas that are still developing their expertise, it may be appropriate to use analytical requirements to encourage further refinements of analytical capabilities. It is also often counterproductive to include parameters for which no regulatory or interpretative guidance exists. However, lack of easily found guidance should not be used as a long-term excuse to avoid dealing with a legitimate contaminant of concern. Where chemical guidance does not exist, proponents may consider using alternative testing to determine effects, or lack of effects. Biological toxicity tests or appropriate models, combined with a good exposure analysis could be considered.

A biological toxicity test is an experimental procedure or method that makes use of a biological system (e.g., whole organisms, tissues, cells) to provide information about chemical exposure or effects. Numerous tests have been developed and used to study exposure and the effects of

chemicals in water and sediment (see appendix 4). In selecting biological tests it will be important to consider:

- The components of the environment (water, sediment, tissue);
- The endpoint or what effects are of concern (mortality, growth, reproduction); and
- The relevance and sensitivity of the species to be tested.

In order to minimise efforts and save money, it is recommended to make use of existing data to the maximum extent allowed by project goals and quality assurance/quality control considerations.

3.3.2 List of analytes to be considered

Appropriate analysis to assist in the environmental evaluation of a proposed dredging project may consider the dredged material and the disposal site. Based on the above considerations, parameters may be considered but not limited to:

- Physical parameters (if not yet investigated for dredging bids), e.g.,
 - grain size distribution (horizontal (within the area), vertical (to depth of dredge cut))
 - percent solids/water
 - density
- Chemical parameters, e.g.,
 - total organic carbon (TOC)
 - heavy metals, arsenic
 - polycyclic aromatic hydrocarbons (PAH)
 - biocides
 - chlorinated organics
- Biochemical and biological parameters, e.g.,
 - sediment toxicity:
 - Solid-phase toxicity tests
 - Water-column toxicity tests
 - Bioaccumulation tests
 - contaminant bioavailability
 - benthic communities (composition), e.g., sea grass or coral reef communities, kinds and abundances of fish and invertebrates in the area.

An annotated list of the chemical, physical and biological parameters to be analysed should be developed on a site-specific basis. The annotations should explain what each of the analysis demonstrate about the dredged material and how this information could be used in making management decisions. A PIANC Report (1998) discusses the relevance of these parameters in more detail.

For example:

- Why would TOC be measured, how could the results be used to standardize the results of other tests, and what is the advantage of doing so?
- What does the density of the sediment reveal about the sediment, and how could the results be used as part of the decision-making process?
- What standards, background data, action levels or other benchmarks are available to assess the contaminant status of the sediments to be disposed and how will these be used?

Justification of the list of variables:

In general, data should only be generated if a specific, concrete use can be identified, and the way the data will be interpreted can be described:

- An annotated list helps ensure that every analysis is carried out for a specific reason, so that the time, labour and money spent in sampling, analysis and evaluation of the data are justifiable.
- Such a list helps identify and prevent duplication, oversights or gaps in necessary information, or the generation of unnecessary information.

It also provides a justification for the analytical budget.

For example: Analysing compound X to 'characterize the sediment' is not sufficient; this objective is too vague and is not a basis for interpreting the data described. An adequate reason might be:

Compound X will be measured to determine its presence, distribution and concentration in the sediment to be dredged. Concentrations in the dredged material will be compared to concentrations in sediment from a reference area (or to applicable regulatory criteria, etc.). If concentrations in the dredged material substantially exceed concentrations in the reference material, sediment toxicity tests may be necessary or: If concentrations exceed the applicable regulatory criteria, the dredged material will be considered unacceptable for the use the criteria regulate.

3.4 Availability and reliability of laboratories

A wide variety of chemical, physical and biological parameters might be potentially relevant for the evaluation of a dredging and disposal project. The analytical methods for potentially relevant parameters range from simple, straightforward and reasonably available throughout much of the world, to complicated, difficult and practically not available in most countries.

It would be counterproductive to request analyses that cannot be reliably and reasonably performed in association with the project being evaluated.

For example, if a given chemical analysis could only be undertaken after several days of transport to get the samples from the dredging project to the laboratory at tropical temperatures, and in addition, the performance of that analyses was new to the laboratory (or they were untrained or unequipped to reach the required detection levels), the results may be of little value.

No matter how desirable sophisticated analyses may be, it is usually best to request analyses that are logistically practical and within the proven capabilities of the available laboratories. However, it may be appropriate to encourage the available laboratories to progressively expand their capabilities.

3.5 *Standardized methods*

Standardized analytical methods have been developed for many physical, chemical, biochemical and biological parameters, e.g., grain size, chemical, elutriate and toxicity testing. Judging the suitability of a particular test for an application should be based on the quality and quantity of scientific information available regarding the test itself and its application with field-collected sediments. For a list of standard methods, see appendix 4.

How to proceed:

- Direct contacts and queries to experienced colleagues and institutions world-wide may provide useful information on practical aspects of intended testing.
- An internet search may be of use in finding suitable methods.

Note that for many chemicals, a range of difficulties may be encountered in obtaining representative and reliable data due to sample heterogeneity, extraction, analysis and physical and chemical interferences.

The selection, adaptation or new development of a specific analytical method meeting given quality objectives is a difficult task requiring reliable expertise.

3.6 *Detection limits*

The detection limits required are usually set by the determining authority or a standardized method. They should be realistic, locally relevant and tied to action levels (cf. section 7.3) or similar benchmarks. Only if the detection limit for a contaminant is lower than the applicable regulatory level, is it possible to determine compliance with the regulatory requirement.

Not all laboratories will be able to meet the specified detection limits with their current equipment and methodologies. However, detection limits

need to be sufficiently low to determine background levels accurately, particularly where biological testing with reference levels are to be used, or to reliably detect organic substances that may have biological impacts at very low environmental concentrations. The laboratory used must be capable of achieving the recommended detection limits.

Detection of very low levels may be more affected by matrix interference in certain sediment samples. In this situation, the laboratory should strive to achieve the lowest detection limit possible in these samples and for this to be documented in the report.

3.7 Frequency of measurement

Generally this will depend on the quantity of material involved, sensitivity of the receiving environment, known inputs of contaminants and any problems revealed by previous investigations.

Where previous investigations have shown the sediment quality to be relatively consistent over time and there are no new pollution sources, it may be necessary to sample only every 3–5 years. However, where sediment chemistry is changing (due to a variable flux of pollutants, new pollution sources, spills, disturbance of contaminated sediments upstream or progressive removal of the contaminated layer), sampling should be carried out for each major dredging program.

Often, where chemical or toxicity data exists pertaining to the dredging site sediments and of a quality acceptable to the relevant authority, there will be an agreed time period for which such data remains valid.

4 Building an analytical plan – detailed considerations

4.1 Analytical plan

Within the initial assessment it is very helpful to set up an 'analytical plan' focusing particularly on the questions of selecting and analysing chemical parameters. Most of the considerations that make up the development of this 'analytical plan' will also be relevant to the sampling plan. Therefore, both sampling and analytical plans should be constructed concurrently.

A few notes are added to point out that a high degree of flexibility is necessary in developing an analytical plan:

- .1 Insistence on a defined level of quality assurance will pre-determine the sampling, sample storage and analytical methods that are appropriate. An analytical method, for example, that cannot be quantitatively standardized, will not satisfy certain QA requirements and should, therefore, be used with extreme caution.
- .2 The choice of an analytical method will dictate sample storage conditions, sub-sampling methods, sample size and laboratory equipment.
- .3 The quality assurance programme will influence the level of reporting and documentation required.
- .4 Limitations imposed by components of the plan may necessitate a revision to the budget, scope and/or assumptions of the working plan (conversely, changes in budget may necessitate revisions to the plan).
- .5 If a plan cannot be devised within the constraints described above then the goals may have to be revised.

4.2 Quality Assurance and Quality Control (QA/QC) for chemical parameters

Quality assurance and quality control are essential to ensure that the analysis and data collected meet the requirements for the assessment of dredged material.

QA/QC involves practices undertaken to assure the reliability of the data that result from the analyses. Good laboratory procedures include:

- *Written standard operating procedures (SOP)* for all work carried out on the project. A SOP is a detailed, laboratory-specific description of all actions taken to accomplish biological effect measurements or chemical analysis.

- *Documentation* that shows that all procedures of sample handling and analyses were followed successfully.
- Routine analytical *quality control protocols* should be used involving the analysis of blanks and reference materials with each batch of samples.
- The *reference materials* should include both Laboratory Reference Materials and independently produced Certified Reference Materials. In addition, the basis of certification must match the methods chosen for sample analysis.
- *Quality control charts* should be constructed allowing day-to-day analytical performance to be monitored. If the results obtained for the reference material in any batch of samples fall outside established acceptance limits, then the batch should be rejected and re-analysed.
- Participation in *laboratory proficiency schemes* is advised and involves the (regular) analysis of samples in which the analyte concentrations are known but undisclosed.
- Chain of custody documentation to ensure analytical data are traced to actual sample and sample location.

As with the QA/QC plan for sampling, an important consideration in planning for the analysis of dredged material is the type and number of quality control (QC) samples to analyse (see section 5.6). The sampling plan will indicate the number of QC samples and these should be factored into the cost of analysis.

4.3 *Quality Assurance and Quality Control (QA/QC) for biological parameters*

Where biological testing is required, there will also be particular QA/QC considerations to help ensure that the observations made are valid. For additional information see PIANC (2004, in prep.). As biological tests measure effects on living organisms, they are a relative rather than an absolute measure. The QA/QC programme should therefore be careful to take into account measurements that will assist the investigator in distinguishing between experimental error, natural variance, including variations in ambient conditions, and any effects due to anthropogenic contaminants in dredged materials.

The following are suggested elements of the biological QA/QC programme:

.1 *Data Quality Objectives (DQO)*

A process for establishing Data Quality Objectives (DQO), e.g., list the kind of variables that must be measured or monitored and the level of statistical significance that is required.

2 Documented Standard Operating Procedures (SOPs)

A number of protocols for biological tests have been published that can serve as the basis for SOPs that would be implemented by individual testing laboratories (appendix 4).

Test performance conditions:

Observations must be made during the conduct of a biological test to document any deviations from conditions that are specified within the SOPs. These observations generally include measurement of various water or sediment quality parameters, e.g., temperature within the test chamber, concentration of dissolved oxygen, pH, salinity, water hardness and other specified or relevant conditions. These measurements are performed to provide documented confidence that any effects observed at the conclusion of the test are not due to poor environmental conditions unrelated to the presence of contaminants.

3 Observation of positive control performance

A positive control test is performed as a means of documenting the sensitivity of the pool of test organisms to be used in a specific biological test of sediment. It is intended to identify batches of organisms that have an abnormal response to an exposure to contaminants, for instance, due to poor health.

4 Control charts

A control chart is generated within the testing laboratory using the same test organism and contaminant to gauge whether the organisms show signs of significant, unusual contaminant tolerance or sensitivity.

5 Observation of negative control performance

The negative control sediment is used to assess the good condition of the test organism under laboratory conditions when not exposed to contaminants. It is essentially free of contaminants and of any other characteristics that could adversely affect the test organism. Poor performance by test organisms in the negative control sediment measured at the conclusion of the test, may be caused by poor health in the test organisms or by deviations from experimental conditions specified in the protocol. In any event, failure to meet the negative performance control standard can justify the need to repeat the biological test.

6 Taxonomic verification of test organisms

Because biological tests make use of organisms rather than mechanical instruments to assess sediment, care must be taken to ensure that the species of organism called for in the protocol is what is actually used in the biological test.