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MANUAL ON SEDIMENT SAMPLING AND ANALYSIS

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1.   Introduction

Within the Regional Seas Program of UNEP, many scientists are concerned about sediment sampling and analysis and therefore there is an increasing demand for the reliable analysis of both organic and inorganic pollutants in sediments. On the other hand, the sampling strategy set prior to the monitoring activity is critically important and should be established with caution in order to represent the sampling site and achieve the statistical objectives of a trend monitoring programme.

The need for a revision of the trend monitoring programme in sediments was raised during the Second Review Meeting of MEDPOL Phase III Monitoring Activities (Saronida, 2003), after a first examination of the sediment monitoring data was made by an expert, and it was recommended by the meeting to revise the existing strategy (UNEP(DEC)/MED WG.243/4). Afterwards, an expert meeting to revise the strategy for trend monitoring of pollutants in coastal water sediments was organized in April 2005 (Athens) and the meeting report (UNEP(DEC)/MED WG.273/2) considered important recommendations for the revision.

Dr Jean-Pierre Villeneuve (IAEA/MESL) drafted the initial version of the manual aimed at presenting the state-of-the-art in sediment monitoring in coastal waters. It fully took into account the recommendations of the expert meeting on both sampling strategy and analysis. A detailed section on sampling instruments and sample handling is also included in the manual, because it was observed in the training courses organized by MED POL and IAEA/MEL that there is a lack of knowledge on different sampling instruments and the sampling/sample pretreatment techniques. The draft manual was discussed at the Third Review Meeting of MEDPOL Phase III Monitoring Activities (Palermo, December 2005) and further comments of the meeting were incorporated in the present text. The section on normalization procedures was revised by Dr Barak Herut (IOLR, Israel).

It is a considerable demand on resources to sample and analyze sediments, so, in order to facilitate the work of the laboratories in charge of monitoring, two different approaches (see the Conclusion) are indicated for sampling, sieving and analyzing the samples: the minimum requirement and the state-of-the-art, then laboratories could use the way that would correspond better to their needs and to their budgets.

2.   Sampling design

2.1   Objectives

Sediments have an important role to play in the monitoring of the environment as they are considered as the final sink of most contaminants. Marine sediments are closely inter-related to other compartments of the environment. Therefore, their use in monitoring should be part of an integrated monitoring programme.

By far the most important step in designing of the sampling strategy of the monitoring programmes is the strict definition of the objectives of the programme concerned where the objectives should be put as detailed, specific and quantifiable as possible. To this end, a number of important factors should be taken into account, including the nature of the control measure, the contaminant concerned, the nature and location of the inputs, statistical aspects of sampling and analysis etc.

In addition, a trend monitoring programme should permit statistical comparison of the concentration of contaminants between sites (spatial distribution), highlighting areas with high concentrations of contaminants that are of concern.   It is anticipated that a temporal

trend monitoring programme for trace metals will at a minimum have 90% power to detect a 5% per year change over a period of between 15 and 20 years.

2.2   Sampling sites

Within MED POL monitoring programmes basically two site typologies are considered:
Hot spots and coastal waters. As a matter of definition, coastal zone trend monitoring is done through a network of selected fixed coastal stations, with parameters that contribute to the assessment of trends and the overall quality status of the Mediterranean Sea. This type of monitoring is carried out on a regional basis. Trend monitoring of "hot spot" areas is done at intensively polluted areas and high risk areas where control measures have to be taken. These areas are designated by local authorities according to some common definitions provided by WHO-MED POL.

The definition of hot spots and coastal areas as regards sediment trend monitoring could be specified as follows:

- Hotspots are the most polluted sites as recorded using sediments and all such sites should be monitored (NB: these may not necessarily always be the same as the identified MED POL hot spots)
- Coastal sites are sites mainly located in the near shore coastal waters and a limited number of representative stations should be selected for state assessments.

Both hotspot and coastal areas are suitable for monitoring contaminants' content in sediments, however, only sedimentary basins with positive accumulation can be considered for monitoring. Coastal areas with sedimentation rates higher than ~5 mm/year are suitable for annual monitoring, whereas areas of lower accumulation rates should be monitored at a lower frequency. Sensitive areas for biological life and protected areas within the near shore coastal waters are also recommended to be included in the monitoring network
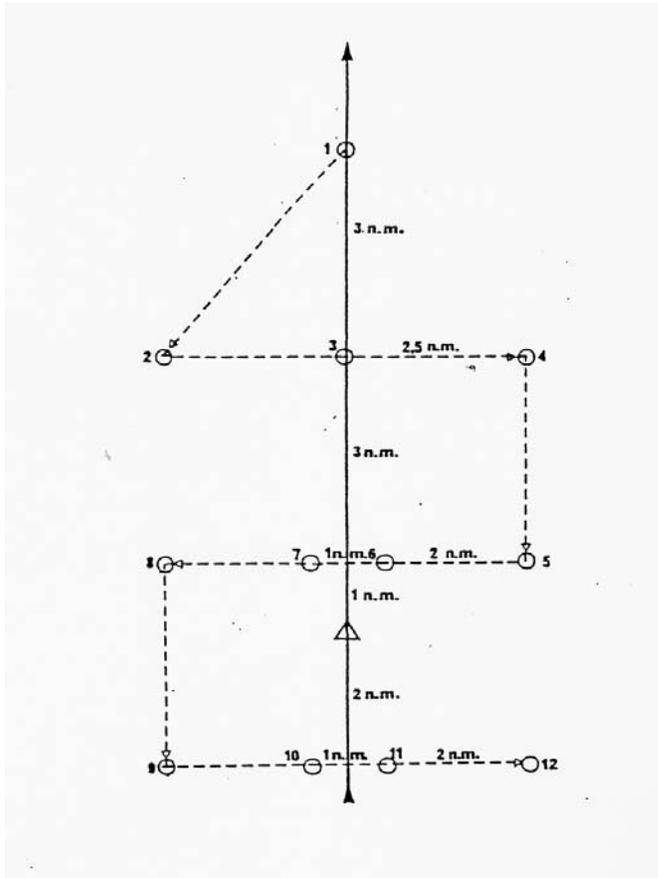

2.3   Sampling stations

Sample sites are normally chosen on a broad grid network or transects. It is recommended that at least **three** stations be chosen along the sediment distribution gradient of a selected site to include hot spot and the near-shore coastal area. While doing so, nearby sensitive areas for biological life should also be included in the network.

In an example case, "O" marks sampling stations in the grid below and "hot spot" station is marked by "Δ". The arrow is pointing in the direction of the residual current (distances are indicated in nautical miles).

It could be recommended to limit the number of stations for data quality assurance purpose, however, the selected station(s) should be representative for the hot spot and the other area of interest.

It is also recommended to examine the selected site for sedimentary purposes as an initial step of the work in order to identify the sediment structure of the whole area as well as the sedimentation rates. Fine and regular sedimentation sites are experienced as more favourable for monitoring purposes.

2.4 Number of samples

Multiple samples have to be collected at each station in order to achieve the statistical sensitivity of sampling. It was recommended to take at least **three** samples at each station area (ex: for an area with app. 10 m depth and 10 m radius). In the pilot phase of the programme (first five years) **five** samples for each station is recommended to better understand the sampling variability if it is not known from previous monitoring efforts. Pooling of individual samples is not recommended especially in the pilot phase in order to achieve the field variability, which is an essential parameter for power analysis and trend tests.

2.5 Sampling layer

For spatial trend monitoring at a distribution gradient, surface sediments (uppermost 5 mm) should be sampled both at hot spots and near-shore waters.

For temporal trends, it is recommended to either sample the upper 5 mm at coastal near-shore stations or use core sediments and sample a sediment-depth profile, provided the sedimentation rate is known. However, this will depend on the specific situation.

2.6 Sampling frequency

As a basis and general rule, it is recommended that the sampling frequency is adapted considering the sedimentation rate.

It is generally accepted that for monitoring temporal trends at hotspot stations with high sedimentation rates (>5 mm/year), the sampling frequency can be initially set as annual or more frequent. If the sedimentation conditions are very variable at selected hot spots other frequencies could be adopted. If sampling of deeper layers at near-shore coastal waters is adopted for temporal trends, then sampling frequency could also be reduced according to the accumulation rate at the site. Sampling frequency could also be reduced when parameters are close to or below the quality targets.

In monitoring programmes of seasonal sampling, special attention should be given to sites significantly affected by river sediment input, in which accumulation rates may change seasonally following flood events. Additional attention should be paid to local conditions such as compaction, bioturbation and re-suspension events.

3. Sampling instruments and sample handling

3.1 Sampling instruments

The type of sampling equipment required for sediment surveys is dependent upon the contaminants of interest and on the information requested. Samples of surface sediment taken from a grab can be used to provide an assessment of the present levels of contamination in an area. The use of a more sophisticated sampler, such as a box-corer, would add reliability to the sample, but also would increase the operating cost of the survey. The type of sampler should be chosen among the followings:

Sediment samplers could be divided roughly into 2 different techniques: grab sampling which collects surface and near surface sediments and coring which collects a column of the subsurface sediment and could be required to establish the historical pattern of the contamination. In all grab and core operations, a slow approach to the sea floor should be ensured to avoid the creation of "bow wave" that disturbs the sediment-water interface prior to sampling. In some circumstances, it would be, also, possible to have the samples collected by divers using either glass or Teflon beakers.

3.1.1 Grab sampler

Undisturbed surface sediment samples can provide an immediate assessment of the present levels of contamination in the area in relation to the textural and geo-chemical characteristics of the sediment. The sampler used must consistently collect relatively undisturbed samples to a required depth below the sediment surface and of sufficient volume to permit subsequent analyses.

The Van Veen grab is among the most commonly used grab samplers. With this bottom sampler, samples can be extracted from any desired depth. While it is being lowered, both levers are locked wide apart whereby the jaws are open. Upon making contact with the waterbed, the locking mechanism is released and when the rope is pulled out to raise the sampler, the jaws close.

The small model (Figure 1), with a surface of 250 cm^2, made of stainless steel has a weight of approximately 5 kg and could be hand-operated from a small vessel. It is not

recommended for greater water depth. The main problem with this sampler is that it is sometimes difficult to recover the surface layer of the sediment, so this type of sampler could be used only in case a coring device is not available.
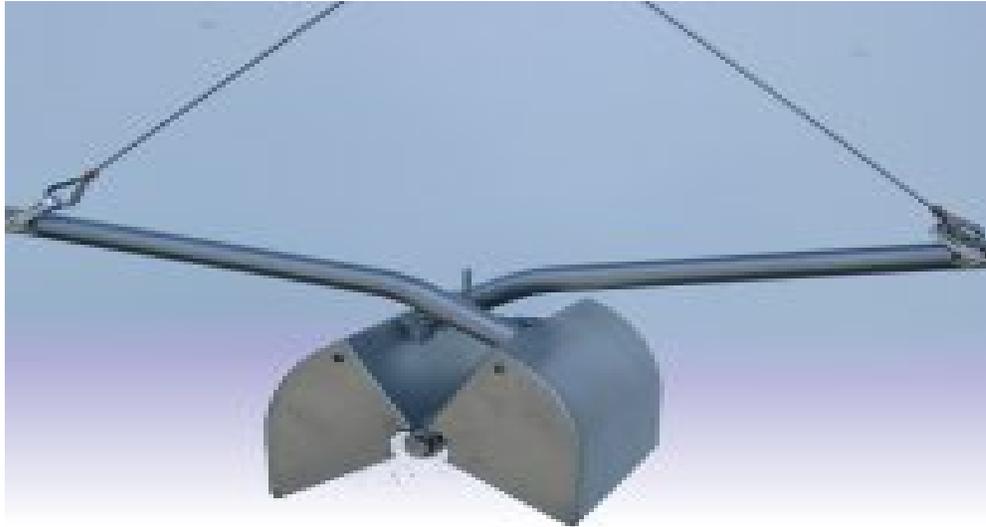
Figure 1: Van Veen grab operated manually (picture from Hydro-Bios, Germany).

There are other models of Van Veen grab, which are winch-operated, with a weight up to 80 kg. These models are presented in the **annex** to this document**.**

3.1.2   Corer

Sediment subsurface samples are often taken using barrel or box corers to determine the change in lithology and chemical composition with depth in order to assess environmental changes in metal fluxes with time. Cores are usually collected in areas of fine-grained sediments but specialized corers are available for coarse-grained sediments.

Figure 2: Gravity corer (picture from Hydro-Bios, Germany).

The main types of corers having cylindrical barrels are the gravity corer (Figure 2) which free-falls from the ship and penetrates the sea floor by gravity, and the piston corer which is released a set distance above the sea floor, penetrates the sediment by free fall, and sucks the sediment into the core barrel by an upward moving piston as the core is retrieved.

For trace metal analysis, plastic core liners are placed inside the core barrels to contain the sediment core sample and to avoid the problems of extrusion and contamination that occur in unlined barrels. When this kind of liner is used, care should be taken for collecting the sample for organic compounds determination, the sample should be collected at the inner part of the core at about a cm from the wall of the plastic liner. In general, the greater the diameter of the liner, the less will be the amount of distortion of the subsurface sediment by the corer penetrating the sediments. Core liners with internal diameters > 50 mm are usually satisfactory for obtaining samples for geochemical purposes.

After the corer is retrieved, the liners are capped at the bottom; the liner is removed from the barrel; the top is capped, and the core stored in a vertical position until all the water inside the liner has risen to the top. The liner is cut off at the sediment - water interface, capped and placed in a deep freezer or a cold room (4°C) for transport to the laboratory. Visual observations and measurements of sediment core samples should include information on the site number and location, depth, time, core length, lithology, stratigraphy, and any distortions in sediment layers.

In the laboratory, core sampling is best carried out by extruding the core upwards and slicing off layers (~ 1 cm) using a non-contaminating cutter (e.g. stainless steel, plexiglass or splitting the plastic core liners lengthwise, avoiding the smeared zone around the inside of the core liners and sampling the interior section of the core.

In order to check the repeatability of the sampling, more than one sediment sample can be collected within the same area. This can be done with the multi-core sampler (Figure 3). After analyzing the different samples, an estimation of the standard deviation due to sampling can be estimated.

Figure 3: Multi-core sampler.

3.1.3 Box corer

Rectangular sampling devices which obtain cores about 15-25 cm square and 15-60 cm deep are known as box corers (Figure 4) and can be recommended for detailed sampling at or below the sediment-water interface. The advantage of the various types of box or square corers is that they can recover the surface sediment and fauna virtually intact. They can be sub-sampled by inserting several 5 cm diameter tubes into them. However, when sub-sampling is used, the core material should be taken from the mid-part of the core to avoid any "edge effects". Such samples are treated in the same way as the core samples described above.

Figure 4: Box corer.

3.2 Sample handling

The procedure outlined below assumes that these samples will be collected from a vessel equipped with the basic collection facilities such as a winch, or other such lifting equipment and adequate refrigerated storage space.

Regardless of the equipment chosen for the sampling, it is useful to know the water depth at each station before starting the sampling. The purpose is to ensure adequate cable length for operation of the correct equipment and to control the speed of entry of the sampler into the sediment. The speed of deployment of the sampler can be critical to good operation and sample recovery. It is also useful to have some understanding of the currents at the sampling site. Strong near-bottom currents can lead to poor equipment deployment, deflect a grab sampler, or require a long cable/wire to be deployed. Care should be taken to ensure that the weight of the sampler is adequate for working at the particular current conditions.

On-board, the sediments contained in the grab sampler require attention to ensure that essential components are neither lost nor contaminated through improper handling. The most critical sampling and storage techniques relate to the avoidance of chemical

contamination and change in the physico-chemical characteristics of the sediments. Special steps should be taken to minimize contamination of the samples. For trace metal determinations, the use of a stainless steel grab sampler with Teflon coatings on all surfaces that come into contact with sediments, and polyethylene coated lowering cables are highly recommended. All samples should be collected into cleaned plastic (inorganic samples) or glass vials or aluminum containers (organic samples).

The actual collection procedure is quite simple:

i.   Prepare all sample containers for organic analysis by cleaning with solvent and heating in oven at 250 °C overnight.

ii.   Clean the sediment grab thoroughly with hot soapy water, rinse with tap water. Avoid placing the grab sampler on the open deck, keep in a large plastic or aluminum tub while not in use.

iii. Clean a large sized plastic or aluminum tub depending on the destination of the sample.

iv.   Cock the grab sampler.

v.   Haul sampler on-board.

vi.   Initially, a visual inspection should be made of the sample by means of the small trap doors on top of the grab to ensure that the sample has been collected in an undisturbed state and to determine if there is water on top of the sample. If water is present, it can be siphoned off with a glass tube or slowly drained so as not to wash the sample unduly.

> **Note :** Plastic bags or wide-mouth jars (polypropylene or borosilicate glass) should be used for temporary storage of sediments for trace metal analysis. Prior to their use, containers and glass or plastic parts associated with the sampling equipment should be cleaned with detergent and acid then rinsed with metal-free water. For trace organic analysis samples should be stored in cleaned wide-mouth borosilicate glass or aluminum containers. The samples should be stored frozen, or at a sufficiently low temperature (~ 4°C) to limit biological and chemical activity. It is recommended that a minimum sub-sample size be 50 grams.

vii. Once the top of the sediment is exposed, visual estimates of grain-size (coarse, medium, fine grained), color, and the relative proportions of the components should be made and recorded. By inserting the appropriate electrodes into the sample, *in situ* measurements can be made, such as pH.

viii. Most fine-grained sediments usually have a thin, dark yellowish brown surface layer resulting from the oxidization of iron compounds at the sediment-water interface. Since in most cases this layer represents the material being deposited at the present time, it should be sampled carefully with a non-contaminating utensil such as a plastic spatula for trace metals determination and a stainless steel one for organic compounds determination. About 10-30 g should be placed in a numbered polyethylene vial for trace metal analysis and in glass or aluminum container for organic analysis, sealed and frozen for transport to the laboratory.

ix.   After the surface layer has been sampled, the grab can be opened and an additional sample, representative of the subsurface, can be obtained. Observations of this material should include color and textural characteristics. To ensure a representative sample, about 100 to 200 grams (or even more) should be collected and placed in a numbered vial. The sample should be frozen quickly for return to the laboratory. Larger samples of about 1 kg are required for admixtures of gravel, sand and mud.

x.   Store all sediment samples deep-frozen or, at least, under refrigeration (4°C) until they are transported to the laboratory.

3.2.1   Taking part of the sample for analysis

Depending on the analysis required and on the material of the sampler (plastic liner for corer), the collection of sediment should follow an agreed protocol. The main idea being to avoid contact with plastic liner for organic compounds and contact with stainless steel for trace elements analysis.
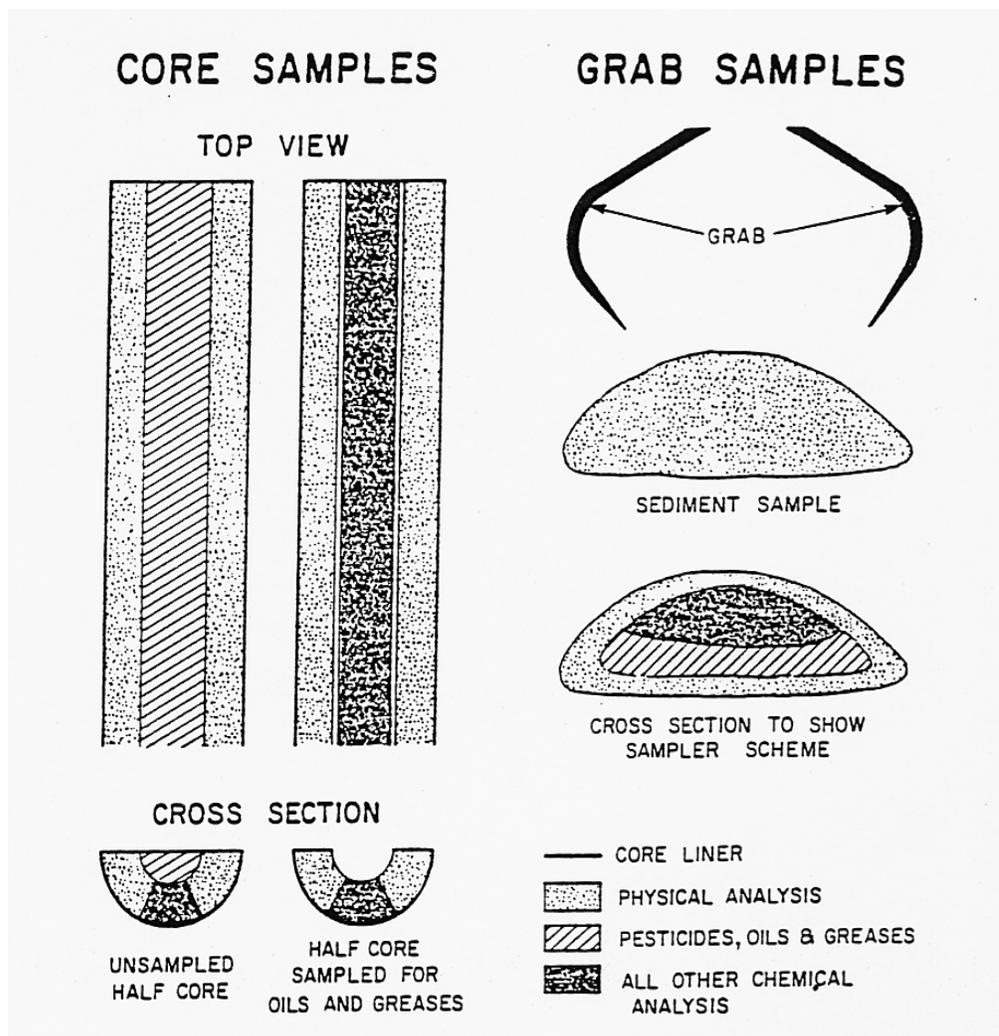

Figure 5: Collection of sediment according to analysis required.

The distribution of sediment depending on the analysis to be performed is indicated in Figure 5.

3.2.2   Pre-treatment of the sample

(i) Freeze-drying:

After collection, the sediment samples are transferred into pre-cleaned aluminum boxes or pre-cleaned aluminum paper for organic analysis or into plastic bags for trace element analysis and deep-frozen (or at least kept refrigerated at about 4°C during the transport to the laboratory in order to avoid the bacterial degradation in case of petroleum hydrocarbon analysis).

When in the laboratory, the sediment samples should be deep-frozen at -20°C and, when frozen, freeze-dried in a freeze-dryer. But it is always interesting to archive part of the sample in order to be able to re-analyze it in case of suspected contamination during the analytical process. So, before freeze-drying, one half of the sample should be stored, as such, in the deep-freezer for future reference (in this case it could be interesting to have a -80°C deep-freezer).

In order to proceed with minimal risk of contamination in the freeze-dryer, the samples should be covered with aluminum paper with some pins holes to let the water vapor evacuate and reduce the eventual cross-contamination.

Contamination from the freeze-dryer and from the vacuum pump should be monitored by freeze-drying, with all batch of samples, a portion of clean Florisil. By analyzing the Florisil it is, then, possible to check if the freeze-dryer does not contaminate the samples.

The samples could be weighed before and after freeze-drying in order to access the ratio of dry/wet weight for each sample.

Note: for frozen sample there is no storage limit in time, for freeze-dried samples, if the samples are kept in the **dark**, in a **cool place** (20°C) and with **Teflon tape** around the neck of the bottles to avoid the humidity to enter in the sample, the limit of conservation could be on the order of 10-15 years without deterioration of the sample.

(ii) Sieving:

After freeze-drying the sediment samples could be sieved in order to remove the small gravels, pieces of branches and shells. Before sieving, it is recommended to sort out, with stainless steel forceps (for organic analysis), or with plastic ones (for trace metal analysis), from the sediment sample the small pieces of shells, branches and leaves that could be present in the sample in order to avoid contamination by extra materials. To do that, the samples are transferred to the top sieve of a sieving machine and the machine is activated. Doing so, the sediment will be disaggregated and not crushed.

The question of sieving is very delicate, as many possibilities exist. Some may sieve at 1 or even 2 mm (pre-sieving), only to remove the small pieces of shells, leaves and branches while others may sieve at 250 μm. In most cases, sieving the sediments through a

63 µm sieve in order to separate the silt and clay from the sand and coarser material is both useful and practicable and it is a widely adopted procedure. However, sieving is not recommended for fine and homogeneous sediments, usually found in the zones with high sedimentation rates where the content of the contaminants will be highest because of their wealth of fine particles for which the contaminants have a particular affinity. Obviously, when it is not possible to find fine sediments, sieving can be recommended to extract the finest particles.

Ideally, the sample could be sieved at 63 µm and the two fractions (less than 63 µm and more than 63 µm) could be analyzed. Even in some cases, sieving at 20 µm is undertaken and 3 fractions are, then, analyzed: more than 63 µm, between 20 µm and 63 µm and less than 20 µm.

Since sieving may also cause contamination problems in the samples (basically for the organic contaminants), many steps of sieving should be avoided -if possible- and it may even be recommended to sieve only from 250 µm before organic contaminant analysis.

For spatial trend monitoring sieving is not a critical issue; however, sieving from <1 or <2 mm in the field is recommended to take place directly after sampling or after the freeze-drying step.

For temporal studies sieving is recommended over 63 µm. However, the important thing is to achieve programme consistency and therefore, it is not recommended to switch to any other fraction if all set criteria in terms of sufficient trend detection are met by a laboratory that is using a whole fraction (e.g. less than 1 or 2 mm) for temporal studies.

A preferable approach is to minimize pre-treatment procedures and unify them for all types of metal/organic analyses and monitoring programmes, both spatial and temporal. Accordingly, sieving to less than 63 µm should be avoided since dry sieving is not reproducible whereas wet sieving is complicated and may introduce the following faults: (i) metal release due to the use of water with different pH and salinity; (ii) mineral (carbonates) dissolution when distilled water is used; (iii) contamination during the sieving and the successive drying.

It is therefore recommended to use one-step dry sieving of the less-than-1 mm fraction in order to perform the analyses on total (bulk) sediment. The rationale for this recommendation is as follows:

(i) Better representation of all relevant size fractions; in some sites coarser fractions (generally, fine and medium sand) are dominant and may contain a significant portion of the total metal (or pollutant).
(ii) Simple to handle.
(iii) Applicable for a wide range of sedimentary provinces and suits a multi-national monitoring programme for the Mediterranean countries.
(iv) Facilitates the use of elemental normalizers (see below).
(v) Avoids potential contamination that might be introduced via wet sieving and successive drying.

(iii) Wet sieving:

Some laboratories use wet sieving techniques. One of the problems that occurs with this technique is the possibility of contamination for organic samples as the material used for this wet sieving method is plastic (silicone tubing and plastic tubes with nylon nets). Another

factor that has to be taken into consideration in using the wet sieving technique is the time consumed. The wet sieving method could, however, be used for trace metal work and in well-equipped and staffed laboratories.

> **Note:** If wet sieving is applied, it is recommended to perform it on board using in-situ seawater and thus avoid using in-lab fresh or distilled water in order to prevent metal release and mineral dissolution (see above). However, wet sieving should not be applied on board if there are technical limitations and potential contamination from vessel oils and metal corrosion or from local polluted seawater.

(iv) Archiving:

Archiving sediment (and biota) samples is a must in QA/QC procedures. All samples should be kept for the duration of the monitoring in order to be able to come back to any of them, or to all of them, in case of problems.

Archives should consist of different parts: the first one being the sample wet and deep-frozen as it has been collected. This archive will be used in case of contamination that can appear during the freeze-drying process. So, one part of the original sample can be extracted again, even wet and dried with sodium sulfate, if it appears that the freeze-dryer had contaminated the sample.

Then when the sample has been dried, and an aliquot has been analyzed, the remaining sediment sample should be kept in a glass bottle, with Teflon tape around the closing system (that should be aluminum for organic and plastic for trace metal) to protect against the moisture and then, stored in a cupboard in the dark and cool place. This way, the sample archived can be stored for 10-15 years, so, for the duration of the monitoring program.

4.   Normalization factors

4.1 Background

Pollutants tend to be associated with the fine particles of marine sediments due to the relatively higher surface area and the compositional characteristics of the fine particles. Both phyllosilicates and organic matter, which have a chemical affinity to trace elements and organic pollutants, are concentrated in the clay (less than 2 μm) and fine silt (2–20 μm) fractions. Most other minerals, including feldspars and heavy minerals, are found in the fine and coarse (20 - 63 μm) silt fractions, whereas the sand fraction (63 μm – 2 mm) mainly consists of carbonate (calcite, aragonite, dolomite) and/or silica (quartz, opal) minerals. Exceptions to this are coastal sediments of mafic and ultra-mafic terrains.

The metals of considerable environmental impact are As, Pb, Hg, Cd, Zn and Cu. Other metals, such as Mo, Ni, Cr and Co, may reflect anthropogenic input resulting from local quarrying and industrial activities. Anthropogenic Cd and Hg have a stronger affinity to organic matter than to clays, whereas natural Ni and Cr may be related to heavy minerals in certain sedimentological provinces. In order to detect anomalous concentrations of anthropogenic origin it is necessary to normalize the results by a physical or a chemical factor. Some elements may have background concentrations below or near the limit of detection for chemical analysis. Therefore, it has been shown that there is no single

normalizing factor that can cope with all pollutant metals in all types of coastal sediments, or even in a single type. Comparing the results to average crust, or upper crust, concentration has been shown to be of limited value for this purpose (Loring and Rantala, 1992; Covelli and Fontolan, 1997) and therefore it is not discussed here.

4.2 Review of normalization methods

4.2.1 Physical normalization

The carbonate and silica mineral groups naturally contain negligible amounts of trace metals and therefore serve as diluents of the marine sediments. Removal of much of those diluents should: a) enhance the analytical capability of detecting low-concentration pollutants; and b) enable comparison between samples on a compositional basis of improved homogeneity. Consequently, choosing the less-than-20 µm or less-than-63 µm fraction for analysis, as mentioned in document UNEP(DEC)MED WG.273/2 (Anavissos meeting report, May 2005), seems like an adequate solution for normalization. Several marine sediment studies of trace elements and their isotopic composition, especially of Nd and Sr, preferred to analyze the less-than-20 m fraction for geochemical purposes (e.g., Innocent et al., 2000; Krom et al., 2002). However, we are not aware of any such studies for environmental purposes. An essential difficulty in using this size fraction is that it excludes the contribution of trace elements in heavy minerals, and therefore the adequate evaluation of background values. Sieving the less-than-20 µm fraction is also technically problematic since it needs in-lab wet sieving with water of different pH and salinity, consumes more time and hence the process is more prone to both metal loss from the sample and contamination. Therefore, if physical normalization is adopted, the less-than-63 µm fraction is preferable to the less-than-20 µm fraction for environmental studies, as has been suggested in the Anavissos meeting report (May, 2005), though some of the difficulties remain.

Nevertheless, utilizing physical normalization by wet sieving might suffer from the following disadvantages: a) any sample manipulation is vulnerable to contamination; b) drying the sediment in an oven or freeze drier, a common practice (Loring and Rantala, 1992; Barbanti and Bothner, 1993), is an obstacle for sample desegregation before wet sieving. Ultrasonic treatment is needed in order to facilitate desegregation, which in turn may cause transfer of pollutants from solid to solution (Barbanti and Bothner, 1993); c) in cases of highly variable mineralogical composition, especially in the sand fraction, the normalization would not reflect this variability. Therefore, most environmental studies dealing with polluting metals use the total sample composition, where "total" generally means the less-than-2, or 1 mm fraction (Loring and Rantala, 1992).

An alternative approach for utilizing physical normalization may be applied in areas where preliminary data indicates that all, or almost all, trace elements and pollutants reside in the less-than-63 m fraction. In such cases the chemical analysis should be performed on a bulk sub-sample whereas grain-size analysis should be performed on another sub-sample. The chemical results are presented after normalization to the less-than-63 m fraction.

4.2.2 Chemical normalization by a representative element or elements

Chemical normalization has the following advantages: a) a single analytical procedure is practiced for the determination of all required elements, the pollutants and those used for normalization; b) minimal manipulation of the sample minimizes contamination; c) the chosen element, or elements, supposedly normalizes both the grain size and the composition variability.

The element most used for marine sediment normalization is aluminum (Al) since it represents aluminosilicates, the main group of minerals generally found in the fine sediment fractions. Aluminum supposedly: a) derives from detrital minerals, transported from the continent to the sea; b) has negligible anthropogenic input; c) behaves conservatively in normal marine environments. Therefore, Al is expected to normalize for grain-size and for mineralogical variability (Bertine and Goldberg, 1977; Din, 1992; Hanson et al., 1993; Daskalakis and O'Connor, 1995; Covelli and Fontolan, 1997, among others). Another advantage of Al is its easy, precise and accurate chemical determination.

Lithium (Li) has been shown to serve as a better normalizing element than Al in marine sediments enriched with 2:1 phyllosilicates, as in the North Sea where sediments derive from eroded glacier material (Loring, 1990). This element, which generally is not contributed by anthropogenic activity, has been recently found to be superior to Al in a Mediterranean study (Aloupi and Angelidis, 2001) but inferior to Al and to Fe in another Mediterranean study (Covelli and Fontolan, 1997). Loring and Rantala (1992) recommended determining at least Li and/or Al. Rubidium is similar to Li in its geochemical behaviour. As a trace substitute for K it may represent phyllosilicates, feldspars and some heavy minerals and it is not thought to result from anthropogenic activities. It has been used successfully in a few environmental studies in the UK (Allen and Rae, 1987; Grant and Middleton, 1990), but apparently not elsewhere.

Iron (Fe) has been successfully used for normalization in several studies (Rule, 1986; Sinex and Wright, 1988; Blomquist et al., 1992; Herut et al., 1993; Daskalakis and O'Connor, 1995; Schiff and Weissberg, 1999). However, it has been suggested that remobilization and precipitation can lead to changes in the pollutant/Fe ratio in anoxic sediments (Schiff and Weissberg, 1999). The latter are hardly expected to be found in Mediterranean sediments of open coasts.

A few studies used scandium (Grousset et al., 1995; Ackerman, 1980) and cesium (Ackerman, 1980), or also cerium, beryllium and europium (Herut et al., 1997), as the normalizing element. Since each of these elements may cause analytical difficulties, they are currently not recommended to be used on a routine basis.

4.2.3 Modes of chemical normalization

Chemical normalization by an element is to be performed by one of the following methods:

(i) By comparing the samples, suspected to be polluted, to nearby non-polluted samples of similar texture, mineralogical and major chemical composition. Background concentrations of the non-polluted samples can be established from surface sediments of other regions or from deep core samples of the same region, below the level of anthropogenic intervention. The potential pollutant concentrations should be compared with background averages in order to calculate the enrichment factor (EF) as follows:

$$(1) \quad EF = \frac{X(s)/N(s)}{X(b)/N(b)}$$

where X is the element and N the chemical normalizer (e.g. Al/Fe/Li) concentration; (s) is the sample; (b) is the background value. The evaluation of the EF value taken for estimating pollution should consider both natural variability and analytical errors (especially if the background concentrations were determined in/by another laboratory and/or analytical device).

(ii) By comparing the measured pollutant and chemical normalizers (or multi-element normalizers, Herut and Sandler, 2007) to their relationships in non-polluted (background) sediments, which have a linear relationship at the 95% confidence level, or better, and a high significance (P<0.001). The regression equation should follow either **y = ax** (x is the normalizing element) or **y = ax + b** (Loring and Rantala, 1992; Herut et al., 1995; Covelli and Fontolan, 1997; Roach, 2005). An estimate of the anthropogenic fraction and the ratio between the measured and predicted values (y) can be defined, where the predicted value is within the range of $1 \pm 2\sigma$.

(iii) By calculating the regression line between contaminant and normalizer through a pivot point, which is the concentration of both elements in a non-polluted sand fraction (Kersten and Smedes, 2002) of a selected standard sediment composition. This approach has been adopted by OSPAR (OSPAR/JAMP, 2002; OSPAR, 2005) and is presented in detail in Herut and Sandler (2007).

In summary - Aluminum (Al) and total organic carbon (TOC) determinations should be obligatory. If possible, the determination of Fe and Li as additional normalizers is recommended in order to better assess basin-wide spatial and temporal trends. The most practical normalization approach for the Mediterranean at this stage is the use of the linear regression equations.

The lack of standardized datasets for the Mediterranean prevents defining 'pivot values' and the use of the OSPAR chemical normalization approach. It is recommended that a standard analysis be performed for the areas to be monitored including: i) grain-size distribution in order to obtain the relations between physical and chemical normalizers; ii) heavy metal concentration in natural non-contaminated sand fraction; iii) mapping the chemical normalizers (Al, Fe, Li, TOC) range for selecting the proper standard sediment composition; iv) assessment of errors associated with the normalization approach.

4.3 Particle size analysis

Particle size analysis may be performed in order to better characterize the sediment nature and the sedimentological regime of the region monitored. The methods for fractionation into grain size can be found in UNEP/IOC/IAEA (1995) and in Loring and Rantala (1992).

The most time-efficient and robust way to obtain particle size analyses is by a laser-diffraction analyzer. There are many laser-diffraction analyzers on the market, such as the Malvern Mastersizer, the Coulter LS Particle Size Analyzer, or the Microtrac S3500 Analyzer. Laser diffraction is used to detect particle sizes in the range of ~ 0.1 to 2000 µm equivalent spherical diameter (depending on the instrument) using light scattering theory. The refractive and absorption indices for the material must be known for accurate measurements to be made.

Laboratories in which continuous grain-size counters (e.g. Mastersizer) are not available should follow Figure 6. The grain size distribution below 63 µm is determined by one of the sedimentation methods.

Sedimentation methods are based on the application of Stokes' Law, which describes the terminal velocity for an isolated sphere settling in a viscous liquid under the influence of an accelerating force such as gravity. Sedimentation techniques can be cumulative or incremental. In the cumulative method, the rate at which the particles settle is determined, typically, by weighing the mass of settled particles at a certain depth over time. In the incremental method, the change in concentration or density of the material with time is measured at known depths, typically using optical or X-ray sensing. Sedimentation methods

are best suited to particles in the range 2-50 µm and, therefore, may not be appropriate for bulk sediment. Temperature must be accurately controlled in order to keep viscosity constant. Incremental sedimentation techniques can be carried out using instruments such as the X-ray SediGraph, manufactured by Micrometrics.

Laboratories equipped with continuous grain-size counters should dry sieve a freeze-dried sub-sample according to the instrumental analytical range (old instruments – below 250 µm; new – below 1 or 2 mm).
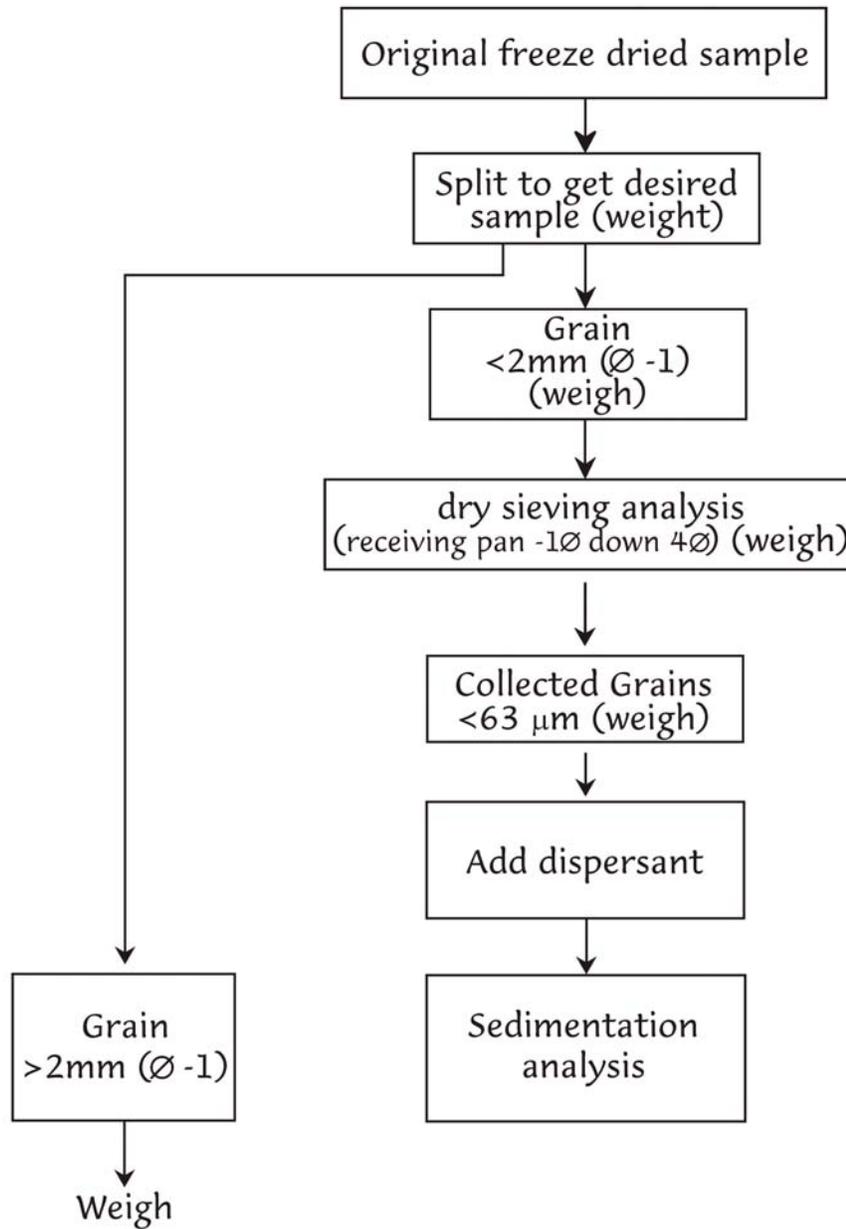
Figure 6: Sequence of steps for the grain size separation of a sediment sample (Please note that 2mm grain size stands also for one mm).

4.4   Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC)

Organic material interacts strongly with both organic and inorganic contaminants. The organic carbon is one of the measures of the organic material. Another parameter would be the determination of lipids, or lipid-like material. The measurement of the hexane extractable organic matter (or HEOM) is also a normalising variable.

The carbonate content (inorganic carbon) of the sediment is generally considered as a dilution factor of the main phases carrying the contaminants and should, also be determined.

Total inorganic carbon (or carbonates) are obtained by the difference of data:

$$TIC\ (\%) = TC\ (\%) - TOC\ (\%)$$

(i) Preparation of samples

Samples for TC analysis are weighed (mg) in tin boats and directly analysed. Samples for TOC analysis are weighed (mg) in tin capsules and acidified with H_2PO_4 1M until the inorganic carbon is removed (3 times in 8 hours intervals to the oven at 55°C). Tin boats and capsules are folded and pressed before the analysis.

(ii) Procedure

Analyses could be done with automatic analyser (such as Elementar "VARIO EL" Instrument) in CN mode. For the mass determination of C and N, an oxidation of the sample followed by the reduction of nitroxides is realized, coupled to chromatographic glass column separation and thermal conductivity detection for CO_2 and N_2.

> **Note**: In case a CHN analyser is available and used for the TC-TOC analysis, Total Nitrogen and Total Organic Nitrogen can be measured simultaneously which can provide a general insight of the lability of organic matter, simply based on the C/N ratio.

(iii) Quality control

Acetanilide standard (C_8H_9NO) is used as a correction factor for accurate and precise measurements (71.1 % C and 10.4 % N) and to control instrumental stability.

The precision of TOC and TC measurements in the samples depends in numerous random factors such as: weighing, use of an acidification step, sample structure (i.e. matrix), concentrations, as well as the instrumental noise. Coefficients of variation (% RSD) must be calculated for each pair of determination, specially, for TOC analysis, which includes an acidification step.

Alternative method to estimate Organic Material in case a CHN Analyser is not available:

The Organic Matter (OM) content in sediments can be measured with the following method:

a) Put the (wet) sediment sample in oven at 60°C for 24 hours (up to constant weight).
b) Weight approximately 1 g of dry sediment (precision 0.01 mg) in a small porcelain boat.
c) Put the sediment for ignition into a furnace at 450°C for 3 hours.
d) Weight the sediment after ignition (precision 0.01 mg).

The Organic Matter (OM) content is equivalent to the percentage of Loss of Weight (LOI %)

$$LOI\ \% = (W_{dry} - W_{ign}) \times 100 / W_{dry}$$

Where:

LOI % = Loss on Ignition (equivalent to the total Organic Matter)
W_{ign} = Weight after ignition
W_{dry} = Weight of dry sediment before ignition

5.   Analytical techniques for organic compounds

Before proceeding to the analysis, an aliquot will be taken from the bulk sample and in order to be sure that what is analyzed is representative of the collected sample, the sediment sample should be well homogenized. This could be done in a specialized laboratory homogenizer, but it could be done, more simply, with a spatula, taking care of mixing well the sediment sample before collecting the 10 g aliquot (for organic) or the 1-2 g aliquot (for trace metal) for the extraction.

The analytical part can be found in the Reference Methods for Marine Pollution Studies published by UNEP. All these Reference Methods are available, free of charge, from IAEA-MEL/MESL.

With a set (one for 10 samples, as a minimal requirement) of sediment samples extracted, sediment Reference Material should be extracted to check the quality of the data produced (UNEP/IOC/IAEA/FAO, 1990).

5.1   Chlorinated pesticides and PCBs.

The analytical method for chlorinated pesticides and PCBs in sediment samples, can be found in UNEP/IOC/IAEA, 1996.

5.2   Petroleum hydrocarbons.

The analytical method for petroleum hydrocarbons can be found in UNEP/IOC/IAEA, 1992.

5.3   Organophosphorus pesticides

The analytical method for organophosphorus pesticides in sediment samples can be found in UNEP/FAO/IOC/IAEA, 1997.

6.   Analytical techniques for trace metals

For trace elements, in general, the analytical methods can be found in UNEP/IOC/IAEA, 1995.
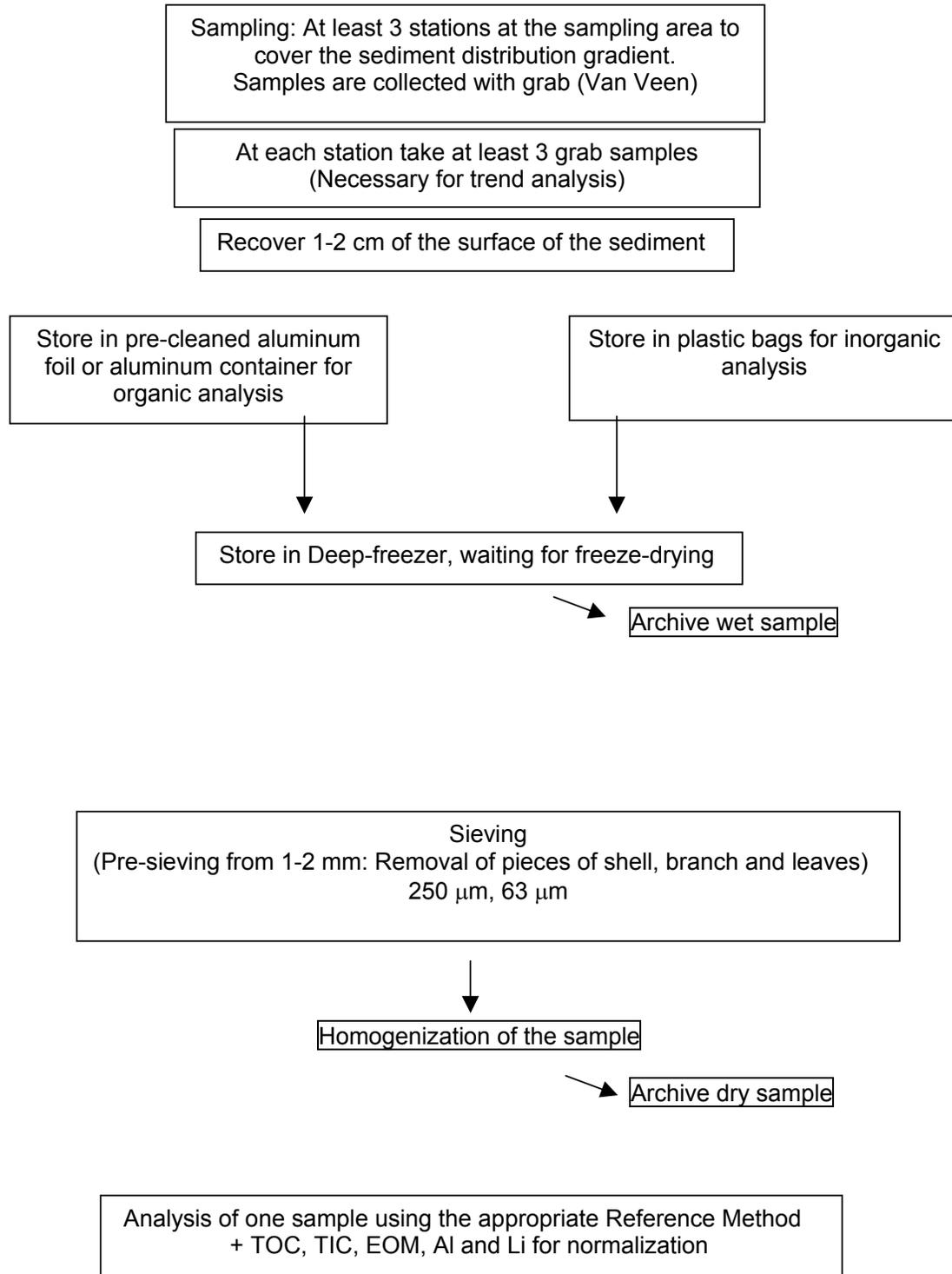
For mercury: in UNEP/IAEA, 1985 and UNEP/IOC/IAEA, 1985.

7.   Conclusions

We can consider two different approaches to the sediment sampling for monitoring projects. They follow the schematics below depending on the budget and the manpower of

the laboratories. One of the methods is a minimum requirement and the other would be the "state-of-the-art" methodology.

First approach (easiest and cheapest one):

Sampling: At least 3 stations at the sampling area to cover the sediment distribution gradient.
Samples are collected with grab (Van Veen)

At each station take at least 3 grab samples
(Necessary for trend analysis)

Recover 1-2 cm of the surface of the sediment

Store in pre-cleaned aluminum foil or aluminum container for organic analysis

Store in plastic bags for inorganic analysis

Store in Deep-freezer, waiting for freeze-drying

Archive wet sample

Sieving
(Pre-sieving from 1-2 mm: Removal of pieces of shell, branch and leaves)
250 μm, 63 μm

Homogenization of the sample

Archive dry sample

Analysis of one sample using the appropriate Reference Method
+ TOC, TIC, EOM, Al and Li for normalization

Second approach (complete procedure):

Sampling: A number of stations are selected on a grid
or transect to cover the sediment distribution gradient.
At least 5 stations in the studied area
Sample collected with corer or box - corer.

At each station take at least 3 cores.
(5 samples per station is recommended at the pilot
phase of trend monitoring)

Recover 1-2 cm of the surface of the cores

Store in pre-cleaned aluminum foil or aluminum container for organic analysis

Store in plastic bags for inorganic analysis

Store in Deep-freezer, waiting for freeze-drying

Archive wet sample

Freeze-dry

Homogenization of the sample

Archive dry sample

Analysis of samples using the appropriate Reference Method
+ TOC, TIC, EOM, Al and Li for normalization

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Annex

Pictures of some sediment sampling devices.

Large grab sampler
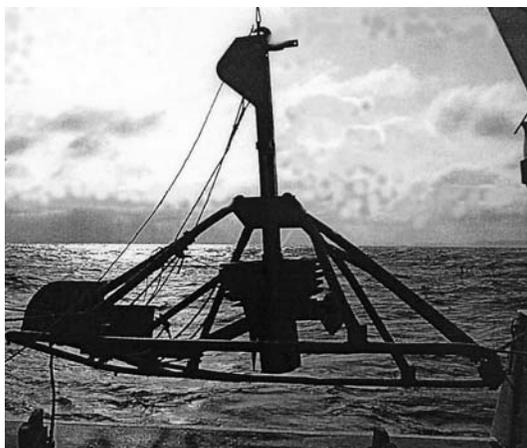(picture: S. de Mora)

Shipeck grab sampler.

Bottom sampler Ekman-Birge
(picture: Hydro-Bios, Germany).

Gravity core sampler
(picture: S. de Mora)

Reineck corer