MONITOOL
new tools for water quality monitoring

Sample processing and analysis

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1. Introduction

The Monitool project aims to provide a robust database of dissolved and labile metal concentrations in transitional and coastal waters for adapting existing Environmental Quality Standards (EQS; 0.45 µm filtered) suitable for passive sampling devices (EQS-DGT) in order to evaluate the chemical status of the waters under the WFD. To this end, a survey programme consisting of co-deployment of passive sampling devices and spot sampling will be taken place by 8 Partners, covering the Atlantic region from Canary Islands to the Scottish Highlands & Islands, as well as the Mediterranean area.

2. Scope

The objective is to develop a protocol for sample processing and analysis that must be followed by all participating Partners. The protocol will define a series of methodologies that will guarantee the comparability and reproducibility of data obtained from each Partner region. This protocol describes the methodology for:

- Annex 1: Seawater filtration – using DigiFILTERs and DigiTUBES for analysis of trace metals by voltammetry and seaFAST coupled with ICP-MS
- Annex 2: Determination of trace metals in seawater by seaFAST coupled with ICP-MS
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- Annex 5: Determination of Suspended Particulate Matter (SPM)
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- Annex 8: Determination of Dissolved Organic Carbon (DOC)

3. Applications

Each participating Partner has their own list of analysis requirements.

It is the responsibility of the participating Partner to ensure that the protocol is adhered to. In any case of deviation, the participating Partner should notify WP5 and WP4 leaders of the changes so that the deviations could be taken into consideration at the time of data interpretation.

It is also the responsibility of the participating Partner to ensure that all the Health and Safety aspects are covered in their own laboratory prior to any commencement of the analysis.

Annex 1: Seawater filtration – using DigiFILTERs and DigiTUBES for analysis of trace metals by voltammetry and seaFAST coupled with ICP-MS

1. Materials and reagents

DigiFILTER 0.45 micron Teflon https://www.scpscience.com/en/products/details?id=010-500-070

DigiTUBES 50 ml Non RackLock w/caps https://www.scpscience.com/en/products/details?id=010-500-263&name=digitubes-50ml-non-racklock-wcaps-750

50 ml plastic syringes (to request SPS at the time of order)

Alternatively, if the DigiTUBES are available in 100 ml volume (availability is subject to supplier's stock) then the use of 100 ml DigiTUBES is preferred (https://www.scpscience.com/en/products/details?id=010-501-261 and https://www.scpscience.com/en/products/details?id=010-501-070&name=digifilter-045-micron-for-100ml-tubes)

SPS Science also offers a Field filtration kit which is composed of 25 DigiFILTERS, 30 of 50 ml DigiTUBES, foam rack and syringes (http://www.scpscience.com/ContentPages/PDF/MK-MKG003-DFFK-2.0-E.pdf)

Nitric acid, Aristar grade (similar specifications or better) https://uk.vwr.com/store/product/2345081/nitric-acid-69-aristar-for-trace-analysis

2. Procedure

All the DigiTUBES and DigiFILTERS must be thoroughly cleaned with 10% nitric acid before filtration and rinsed with deionised water.

2.1. For analysis of trace metals by voltammetry

This filtration will be carried out by all Partners on-site on-site or at a nearby site as soon as the sample is collected, and the acidified filtrate will be sent to IST to perform analysis of trace metals by voltammetry.
IST requires 100 ml of filtrate therefore each filtration will need one DigiFILTER, three 50 ml DigiTUBES (or two 100 ml DigiTUBES) and a syringe.
Gloves must be worn for the whole filtration process.

- Label the DigiTUBES clearly with sample information.
- Rinse the DigiTUBES with 10% HNO_3.
- Rinse DigiTUBE N° 1 with an aliquot of seawater and repeat one more time.
- Fill up DigiTUBE N° 1 with the collected seawater and screw the DigiFILTER (with the red insert on) onto it.
- Screw the DigiTUBE N° 2 onto the top of the open side of the DigiFILTER. Ensure that you have a firm seal on the entire assembly.
- Invert the DigiFILTER assembly so that the water sample is on the top.
- Connect the syringe into the hole of the DigiFILTER and remove the red insert from the DigiFILTER.
- Pull the piston to allow the filtration process. Discard the first 10 ml of filtrate.
  Tip: insert a piece of wooden stick between the two ends of the piston to keep it in place and leave the filtration to complete. Put the lid on the DigiTUBE once completed.
- If using 50 ml DigiTUBE, refill DigiTUBE N° 1 and repeat the filtration process by collecting the filtrate in the DigiTUBE N° 3. If filtration is too slow, then the DigiFILTER might need to be replaced.

- Acidify as soon as possible the filtrate by adding 0.035 ml of HNO_3 (69%, Aristar grade) to each 50 ml of filtered seawater sample (or 0.070 ml of HNO_3 to 100 ml of filtered seawater sample) to obtain pH 2.
- Send all the filtrates (2 x 50 ml DigiTUBES or 1 x 100 ml DiguTUBE) as soon as it is practicable (in a cooler box) to IST laboratory for analysis of trace metals by voltammetry.

2.2. For analysis of trace metals by seaFAST ICP-MS

This filtration process will be carried out on site or at a nearby site and will be performed as above, with 100 ml of sample.
The filtrate will be sent to IPMA for analysis of trace metals by seaFAST coupled with ICP-MS.

Annex 2: Determination of trace metals in seawater by seaFAST coupled with ICP-MS

The analytical determination of trace elements (Cd, Pb, Ni, Al, Cr, Co, Cu, Fe, Mn and Zn) in filtered estuarine and seawater will be performed by ICP-MS with an online pre-concentration system – seaFAST (Elemental Scientific). The seaFAST is an automated sample introduction system for analysis of seawater and other high matrix samples. It delivers excellent accuracy and repeatability, giving labs research-quality results with minimal effort.

All the chemicals used are of laboratory grade purity (suprapure or distilled) and the water is ultra-pure (18.2 MΩ.cm). All reagents, standards, samples, and blanks are prepared in acid (HCl and HNO_3) cleaned LDPE or Teflon flasks.

The SeaFast is initially cleaned by flushing several solutions (CH_3OH, HNO_3, HCl-acidified seawater, TMG-HCl, ultrapure water and HNO_3) recommended by the manufacturer through all tubing.
The quadrupole ICP-MS equipped with a Peltier Impact bead spray chamber and a concentric Meinhard nebulizer is used for metal determination. The equipment is set up by ensuring low variability of counts (RSD <1%), 140Ce16O/140Ce < 0.010 and 137Ba++/137Ba<0.010. The experimental parameters settled are forward power 1400 W; peak jumping mode; 150 sweeps per replicate; dwell time 10 ms; dead time 30 ns. The isotopes used for quantification are subject to minimum isobaric and polyatomic interferences (111Cd, 208Pb, 60Ni, 27Al, 52Cr, 59Co, 65Cu, 56Fe, 55Mn and 66Zn).
The isotope 115In is regularly used as online internal standard. The solution is prepared with ultrapure water, HNO_3 (2% v/v), a stock solution of In for ICPMS determinations obtaining 10 µg/L as final concentration.

Sample will be analysed undiluted.

Quality Control (QC) solutions containing the same elements of quantifications on an acid-based solution (HNO_3 2% v/v) are run every 10-20 samples depending on the total number.

A 7-point calibration curve ranging from 0.010 to 100 µg L^{-1} (varying with the natural occurrence of each metal) is used for quantification. Not all metals have the same range of concentrations in the calibration curve. Standard solutions will be prepared using stock solutions for ICPMS determinations diluted accordingly in ultra-pure water with 2% (v/v) HNO_3.

The precision and accuracy of the analytical procedures are controlled through repeated analysis of determined elements in certified reference materials (eg. NASS, CASS, and SLEW from the National Research Council of Canada). Each batch of samples will include a blank, a certified reference material and a QC solution.

Annex 3: Determination of lead and cadmium labile fraction (at pH=2) by Anodic Stripping Voltammetry and total dissolved Nickel by Cathodic Stripping Voltammetry.

1. Sample collection and preservation

Samples should be filtered using 0.45 µm membrane filter to remove suspended and particulate matter. The filtrate should be acidified to pH 2 by the addition of ultra-pure nitric acid (see Annex 1, section 2.1). Samples should be stored in pre-cleaned acid-soaked polyethylene, TFE bottles or DigiTUBES and once acidified, stored in refrigerator until analysis.

1. Equipment, Materials and Reagents

Voltammetric measurements should be performed using a potentiostat and a conventional three-electrode configuration: a Static Mercury Drop Electrode (SMDE) or Thin Mercury Film Electrode (TMFE), as the working electrode, a reference electrode (either saturated calomel or silver/silver chloride electrodes) and an auxiliary electrode (carbon rod or a platinum wire).

Glassware and electro analytical cells should be previously acid-cleaned, kept for 24 hours in 6 M HNO_3, after which should be rinsed with deionised water. TFE vessels can also be used as voltammetric cells.
All reagents should be of high-purity grade (preferentially supra-pure) namely HNO_3 and $Hg(NO_3)_2$ (for thin mercury film electrodes preparation), dimethylglyoxime (DMG), NH_3 and NH_4Cl (used for Ni determination). Solutions should be prepared with deionised water with at least 18 megohm-cm of resistivity.

Working standard solutions of 1 mgL^{-1} should be prepared from commercially available metal standards 1000 mg L^{-1}. Diluted standard solutions should be prepared daily in HNO_3 pH=2 to cover the concentration range desired.

For TMFE, a stock deposition solution should be prepared by dissolving 0.325 g of $Hg(NO_3)_2$ in 100 mL 0.01 M HNO_3. For Ni determination a stock aqueous solution $5x10^{-2}$ M DMG, in 0.1 M NaOH and an ammonium buffer (pH=9) 2 M NH_3 + 2 M NH_4Cl should be prepared.

1. Procedure

1.1. Cd and Pb by ASV

Depending on the sample concentration, Anodic Stripping Voltammetry (ASV) can be carried out in the stripping step in the direct current (dc) mode or using pulse variation, differential pulse (DP) (or square wave (SW)). The level of detection for metal determination also depends on the working electrode: the SMDE is best suited for analysis from approximately 1 µg/L, while a TMFE for detection below 1 µg/L.
All determinations should be done using the standard addition method. A sample volume of 10-20 mL should be pipetted to the voltammetric cell, and 4-5 additions of a standard solution containing both metals in a concentration adequate for the level of detection should be done. Adjust volume of standard addition and/or concentration of the standard solution to obtain a 30% to 100 % increase of the analytical signal and total volume variation $\leq$ 1%.

The solutions should be purged with nitrogen for 10-15 min while stirring. The nitrogen should continue to flow on the top of the solution during all experiments. Deoxygenation step should be shortening to 1 min after initial gas purge.
The instrument conditions to be used are those listed in Table 1.

Table 1 - Procedure conditions for Pb and Cd determination by SWASV

Instrumental Parameter	Value (units)
Deposition Potential	-0.9 (V)
Deposition Time	100-500 (s)
Quiescent time	10 (s)
Initial Potential	-0.9 (V)
Final Potential	-0.2 (V)
SW frequency	25 (Hz)
SW Amplitude	0.025 (V)
Step Potential	0.005 (V)

The drop size of the SMDE should be controlled and kept constant within each determination. The smallest size is advisable. The stirring rate should be controlled and kept constant within each determination. Rotation rate of 2000 rpm is recommended.
A blank (deionised water acidified to pH=2 with HNO_3) should be run in the beginning of each working session for best results. Blanks are critical because of the high sensitivity of the method.

All measurements should be performed at least in duplicate under clean and acclimatised conditions (25 ± 2 °C).

1.2. Ni by CSV

Cathodic Stripping Voltammetry (CSV) is carried out in the SMDE. Depending on the sample concentration, CSV can be carried out in the stripping step in the direct current (dc) mode or using pulse variation, differential pulse (DP) or square wave (SW).
Before analysis samples must be UV-digested for two hours in order to remove interferences of dissolved organic matter. This treatment provides a measure of the total Ni dissolved content.
All determinations should be done using the standard addition method. A sample volume of 10-20 mL should be pipetted to the voltammetric cell and appropriate volumes of the stock solutions of DMG and the buffer added so that the final concentrations in the cell are $5x10^{-4}$ M DMG and $2x10^{-2}$ M NH_3 + $2x10^{-2}$ M NH_4Cl. If necessary the pH should be adjusted to 9 before the determination by the standard addition method. The solutions should be purged with nitrogen for 10-15 min while stirring. The nitrogen should continue to flow on the top of the solution during all experiments. Deoxygenation step should be shortening to 1 min after initial gas purge.
The instrument conditions to be used are those listed in Table 2

Table 2 - Procedure conditions for Ni determination by SWCSV

Instrumental Parameter	Value (units)
Deposition Potential	-0.7 (V)
Deposition Time	90 (s)
Quiescent time	10 (s)
Initial Potential	-0.8 (V)
Final Potential	-1.3 (V)
SW frequency	25 (Hz)
SW Amplitude	0.025 (V)
Step Potential	0.005 (V)

The drop size of the SMDE should be controlled and kept constant within each determination.
The stirring rate should be controlled and kept constant within each determination. Rotation rate of 2000 rpm is recommended.
A blank (deionised water plus the appropriate volumes of the stock solutions of DMG and the buffer pH=9) should be run in the beginning of each working session for best results.
All measurements should be performed at least in duplicate under clean and acclimatised conditions (25 ± 2 °C).

QA/QC control: Throughout the entire procedure, blank reagents, quality control standards and certified reference material should be used. A blank (deionized water acidified to pH=2 with HNO_3) should be run in the beginning of each working session. Blanks are critical because of the high sensitivity of the method. Analytical variability should be calculated as standard deviation (SD) of duplicates and values below 10%-15% should be considered satisfactory.

Annex 4: Determination of trace metals in DGT by ICP-MS/ICP-OES

This analytical method is extracted from the protocol guide provided by DGT Research Ltd: "*DGT Research, 2003. DGT – for measurements in waters, soils and sediments*", http://dgtresearch.com/dgtresearch/dgtresearch.pdf

1. Reagents and materials

- Ultrapure grade nitric acid (69%)-Aristar grade
- Milli-Q water (>18 megaohm)
- Plastic micro-centrifuge tube (1.5 ml)
- Clean plastic tweezers/forceps

2. Sample Treatment and Analysis

This procedure is for all Partners (IFREMER for the main DGTs from campaigns).
Gloves are to be worn at all time, and procedure to be conducted in a positive pressure laminar flow hood to minimise cross contamination.
Follow this procedure for all deployed DGTs, DGT field blank and DGT lab blank.

- To retrieve the resin gel after deployment insert a screw driver (NOTE: use a plastic one, if that's not possible, then put the screwdriver inside of a glove to avoid the contamination of the samples) into the grove in the cap and twist it. The cap will be broken at the weak point. Remove the broken cap and then peel off the filter and diffusive gel layer to reveal the bottom resin-gel layer.
- Place the resin gel, with plastic tweezers, in a clean sample tube and add 1 ml of 1 M HNO_3 solution (if 1.5 ml centrifuge tubes are used). Make sure the resin gel is fully immersed in the HNO_3 solution. Leave it 24 hours at least before analysis.
- Pipette an aliquot from the sample tube and dilute it at least 5 times with Milli-Q prior to analysis by AAS, ICP-MS or ICP-OES.

Calculation of the DGT Measured Concentration

a. First calculate the mass of metal accumulated in the resin gel layer (M) using equation (1)

$$M = C_e * (V_{HNO3} + V_{gel}) / f_e \quad (1)$$

where

- C_e is the concentration of metals, in g/L, in the 1 M HNO_3 elution solution
- V_{HNO3} is the volume of HNO_3 added to the resin gel
- V_{gel} is the volume of the resin gel (typically 0.15 ml)
- f_e is the elution factor for each metal (typically 0.8)

b. The concentration of metal measured by DGT (C_{DGT}) can be calculated using equation (2).

$$C_{DGT} = (M * \Delta g) / (D * t * A) \quad (2)$$

where

- Δg is the thickness, in cm, of the diffusive gel (0.078 cm) plus the thickness of the filter membrane (0.014 cm)
- D is the diffusion coefficient of metal in the gel (see Table 1 for open pore gel. Please note the numbers need to time E-6 and the unit should be cm^2/sec)
- t is deployment time (in seconds)
- A is the exposure area, 3.14 cm^2

Table 1. Diffusion coefficients of metal ions in DGT gel (open pore) at different temperatures from 1 to 35°C
Unit of D: E^{-6} cm^2/s

°C	Ag	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
1	6.58	2.22	2.84	2.77	2.36	2.91	2.85	2.73	2.69	3.75	2.84
2	6.83	2.30	2.95	2.88	2.45	3.02	2.96	2.83	2.80	2.89	2.94
3	7.09	2.39	3.06	2.99	2.54	3.13	3.07	2.94	2.90	4.04	3.05
4	7.35	2.48	3.18	3.10	2.63	3.25	3.18	3.05	3.01	4.19	3.17
5	7.62	2.57	3.29	3.21	2.73	3.36	3.30	3.16	3.12	4.34	3.28
6	7.89	2.66	3.41	3.32	2.82	3.48	3.42	3.27	3.23	4.49	3.40
7	8.17	2.75	3.53	3.44	2.92	3.61	3.54	3.39	3.34	4.65	3.52
8	8.45	2.85	3.65	3.56	3.02	3.73	3.66	3.50	3.46	4.81	3.64
9	8.74	2.94	3.78	3.68	3.13	3.86	3.79	3.62	3.58	4.98	3.77
10	9.04	3.04	3.90	3.80	3.23	3.99	3.91	3.74	3.70	5.14	3.89
11	9.34	3.14	4.03	3.93	3.34	4.12	4.04	3.87	3.82	5.31	4.02
12	9.64	3.25	4.16	4.06	3.45	4.26	4.18	4.00	3.94	5.49	4.15
13	9.95	3.35	4.30	4.19	3.56	4.39	4.31	4.12	4.07	5.67	4.29
14	10.27	3.46	4.43	4.32	3.67	4.53	4.45	4.26	4.20	5.85	4.42
15	10.59	3.57	4.57	4.46	3.79	4.68	4.59	4.39	4.33	6.03	4.56
16	10.92	3.68	4.72	4.60	3.91	4.82	4.73	4.52	4.47	6.21	4.70
17	11.25	3.79	4.86	4.74	4.03	4.97	4.87	4.66	4.60	6.40	4.85
18	11.59	3.90	5.01	4.88	4.15	5.12	5.02	4.80	4.74	6.60	4.99
19	11.93	4.02	5.15	5.02	4.27	5.27	5.17	4.95	4.88	6.79	5.14
20	12.28	4.14	5.30	5.17	4.39	5.42	5.32	5.09	5.02	6.99	5.29
21	12.64	4.26	5.46	5.32	4.52	5.58	5.47	5.24	5.17	7.19	5.44
22	13.00	4.38	5.61	5.47	4.65	5.74	5.63	5.39	5.32	7.40	5.60
23	13.36	4.50	5.77	5.63	4.78	5.90	5.79	5.54	5.47	7.61	5.76
24	13.73	4.62	5.93	5.78	4.91	6.06	5.95	5.69	5.62	7.82	5.92
25	14.11	4.75	6.09	5.94	5.05	6.23	6.11	5.85	5.77	8.03	6.08
26	14.49	4.88	6.26	6.10	5.19	6.40	6.28	6.01	5.93	8.25	6.24
27	14.88	5.01	6.43	6.27	5.32	6.57	6.45	6.17	6.09	8.47	6.41
28	15.27	5.14	6.60	6.43	5.47	6.74	6.62	6.33	6.25	8.69	6.58
29	15.67	5.28	6.77	6.60	5.61	6.92	6.79	6.50	6.41	8.92	6.75
30	16.08	5.41	6.94	6.77	5.75	7.10	6.96	6.66	6.58	9.15	6.92
31	16.49	5.55	7.12	6.94	5.90	7.28	7.14	6.83	6.74	9.39	7.10
32	16.90	5.69	7.30	7.12	6.05	7.49	7.32	7.00	6.91	9.62	7.28
33	17.32	5.83	7.48	7.29	6.20	7.65	7.50	7.18	7.09	9.86	7.46
34	17.75	5.98	7.47	7.47	6.35	7.84	7.69	7.36	7.26	10.10	7.64
35	18.18	6.12	7.85	7.66	6.51	8.03	7.87	7.53	7.44	10.35	7.83

Annex 5: Determination of Suspended Particulate Matter (SPM)

1. Equipment, materials and reagents

- 500 ml of water sample in HDPE container
- Millipore flat tip tweezers or similar forceps
- Cyclopore™ Track Etched Membrane 0.4 µm Polycarbonate (hydrophilic) 47 mm diameter filter
- Filtration unit (e.g.: comprising a 300 ml funnel, round glass seal, lass base & tubulated cap, ground joint flask, 1 litre anodised aluminium spring clamp, clamp stand and clamps)
- Vacuum/Pressure Pump, 220 V, 50 Hz for filtration.
- 500 ml wash bottle of deionised water (18 megohm)
- 500 ml measuring cylinder
- Calibrated balance, to 5 decimal place
- Polonium-210 static eliminator disk (Static solutions 62.9 MBq, 1.70 mCi Activity, 1.7 MILLICURIES nominal) stored in radiation safe. *This is used to remove all the static that occurs during the weighing process, therefore making the weighing more accurate. An alternatvie alternative option could be used (i.e. anti-static built-in balance).*
- Desiccator, Silica gel, self-indicating, course (BDH prod 300627B) and Vacuum tubing Nalgene and Vacuum pump
- Plastic petri dish with lid

2. Procedure

- Dry the polycarbonate by placing it in the vacuumed desiccator for 24 h. Once dry, record its weight and store it in a petri dish, covered with a lid.
- Clean the filtration unit with deionised water and carefully using the forceps, place the pre-weighed polycarbonate filter with the shiny side of the filter up.
- After rinsing the measuring cylinder with a sample aliquot, pour an appropriate volume of sample into the cylinder and record the exact volume. The recommended volume to be filtered for coastal water is 500 ml for coastal and 250 ml for estuary site.
- Shake the sample bottle well then pour the required volume into the filtration unit.
- Start the filtration process and once all the sample has been filtered, rinse the empty measuring cylinder with 50 ml de-ionised water and pass it through the filter to remove any particles stuck to the sides of the cylinder.
- Just before the filter paper is about to be sucked dry, rinse the filter funnel walls with 50 ml of de-ionised water to wash any particulate matter that has stuck to the glass funnel onto the filter. Repeat the procedure with another 50 ml of deionised water to remove salt deposits as well as particulate matter still stuck to the glass funnel.
- Leave on filtration unit on for 5 minutes to air-dry the filter.
- Carefully remove the filter using the forceps and place it in its original petri dish holder, with its shiny side up, and cover it securely with a lid.
- Leave it to dry in a vacuumed desiccator for 7 days.
- After 7 days, carefully remove the filter with forceps and weigh it. Record the weight.
- Carefully return the filter, shiny side up, in its original petri dish and leave to desiccate for another 7 days before second weighing.
- Repeat the procedure for a 3rd weighing.
- When the papers have been weighed three times, calculate the 1.96 standard error.

For coastal water column samples, the acceptable error is ≤ 0.1 and for estuary samples the acceptable error is ≤ 0.25 for 3 consecutive weighing.
If the criteria is not met after 5 weighing, then this procedure should be repeated.

Calculate the SPM as below:

$$\text{SPM (mg/L)} = \frac{\text{Weigh of SPM and filter (mg)} - \text{ weight of clean filter mg)}}{\text{Volume filtered (in L)}}$$

Calculate the mean SPM with standard deviation.

Annex 6: Determination of Dissolved Oxygen

Dissolved oxygen (DO) determination measures the amount of dissolved (or free) oxygen present in water. The method below used to determine DO is a modification of the classical Winkler (1988) titration procedure. The seawater sample, contained in a glass, stoppered bottle, is treated with a strong manganese (II) solution (Manganous sulphate) and a concentrated reagent containing sodium iodide and sodium hydroxide (alkaline iodide). The bottle is then carefully stoppered so as to exclude bubbles of air and shaken. The dissolved oxygen reacts with the precipitated manganese (II) hydroxide, and under these highly alkaline conditions manganese is oxidised to manganese (III).

$$Mn^{2+} + 2OH^- \longrightarrow Mn(OH)_2\ (s)$$

$$½\ O_2 + 2Mn(OH)_2\ (s) + H_2O \longrightarrow 2Mn(OH)_3\ (s)$$

When the solution is acidified in the presence of the iodide, the oxidised manganese (III) is reduced to manganese (II) and iodine equivalent to half the original dissolved oxygen concentration of the water is liberated.

$$2Mn(OH)_3\ (s) + 6H^+ + 2I^- \longrightarrow 2Mn^{2+} + I_2 + 6H_2O$$

The liberated iodine and surplus iodide ions combine to generate the I_3^- complex.

$$I_2 + I^- \longrightarrow I_3^-$$

The iodine is titrated with a standard thiosulphate solution.

$$I_3^- + 2S_2O_3^- \longrightarrow 3I^- + S_4O_62^-$$

One mole of oxygen is equivalent to 4 moles of thiosulphate. Hence the concentration of oxygen in the seawater sample can be calculated. The thiosulphate solution is intermittently standardised with a standard iodate solution.

1. Materials and reagents

A Winkler bottle: this is a piece of laboratory glassware specifically made for carrying out the Winkler test. These bottles have conical tops and a close fitting stopper to aid in the exclusion of air bubbles when the top is sealed. This is important because oxygen in trapped air would be included in the measurement and would affect the accuracy of the test. Alternatively, a 120 ml bottle with glass stoppered can be used, but it must be calibrated as per section 1.

- Manganous sulphate (450 g in 1 L Milli-Q water)
- Alkaline Iodide (320 g sodium hydroxide and 600 g sodium iodide in 1 L Milli-Q water)
- Sodium Thiosulphate (0.2 N)
- Sulphuric acid (5 M)
- SiS DO auto-titrator

2. Calibrations of glass stoppered bottle.

This procedure must be carried out in a temperature controlled environment at 20˚C. Distilled water and bottles should be left in the room 24 hours prior to performing this procedure.

- Give each bottle and stopper a unique and matching identification number.
- Weigh bottle & stopper and record the weight.

- Using the distilled water carefully fill each bottle. Gently replace glass stopper and carefully dry the bottle and weight the full bottle & stopper.
- Empty bottles and allow to dry overnight
- Repeat the above steps till you have three weights of the bottle dry (dry weight) and filled with water (wet weight).

Calculate the volume of the bottle in the following way
Volume (ml) = Mean wet weight (g) - Mean dry weight (g)

3. Sample collection

Wear nitrile gloves for this procedure to avoid sample contamination and risk of chemical burns.

Sub-samples for dissolved oxygen determinations should be taken as soon as possible after sample collection. To prevent oxygenation of sample, it should be the FIRST aliquot sampled. Three calibrated bottles should be filled from each the water sampler.

- Make a note of the calibrated bottle number used.
- Attach the silicon rubber tubing to the tap of the Niskin bottle and gently flushing the water into the glass stoppered bottle (the flushed volume should be approx. 3 x of the bottle volume).
- Once flushed, the sample is introduced slowly into the bottom of the oxygen bottles through the silicon rubber tube. The seawater should be allowed to overflow the bottle. Seawater flow should be restricted by pinching the tubing between thumb and forefinger, when placing the tube into the bottle and when removing it. To avoid excessive agitation of sample (the water should remain flowing - as stopping the flow may cause air bubbles to be introduced when the flow is restarted).
- As soon as possible after the 3 replicate bottles are filled and immediately prior to fixing, determine the temperature of the water in each bottle using the electronic thermometer. Record the fixing temperature, and depth of sample on log sheet, next to bottle ID number. Obtain the *in-situ* sample temperature & salinity from CTD profile.

4. Sample fixing

Gently put the stopper onto the bottle to displace ≈ 2 ml of the volume out.
The sample is fixed by the addition of 1 ml of manganous sulphate solution followed by 1 ml of alkaline iodide solution. Wipe away drops from pipette tip with a clean tissue after use.
Re-stopper the bottle and invert it several times to mix the contents, taking care not to trap any air bubbles.
Store in a cool dark place.
The samples can now be analysed once the precipitate has settled as described below.
Alternatively, the samples can be stored in the dark up to 1 week before analysis. Ensure that the bottles are completely airtight.

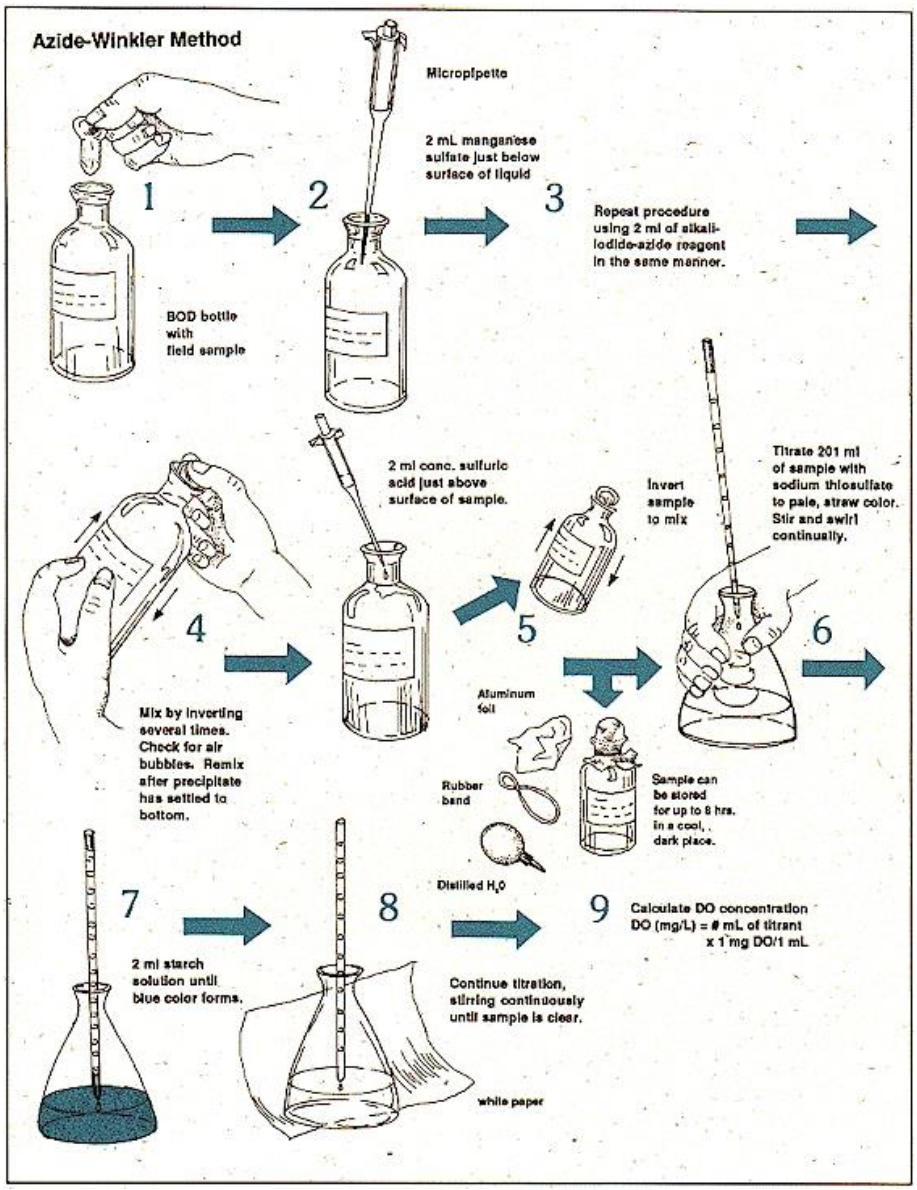
5. Analysis

- Wipe sample bottle to remove any moisture or smears. Bottle must be totally clean for titration to be successful.
- Gently remove the stopper from the first bottle and rinse the end of stopper into sample with Milli-Q water.
- Gently introduced the stirring bar into the bottle and add 1 ml of 5 M H_2SO_4, taking care not to disturb precipitate too much.

- Switch on stirrer, the speed should be such that no vortex is formed.
- Place bottle in photometer and start titration.
- Record the volume of the titrant and calculate the DO concentration.

6. Calculation

The results must take into account the temperature of fixing, salinity and the exact sample volume.



Annex 7: Determination of Water Turbidity via Nephelometry

The following method will provide the results in NTU, however other nephelometric method providing results in FNU is also acceptable as the difference between NTU and FNU appears to be negligible.

Introduction

i. This method covers the determination of turbidity via nephelometry in saline waters sampled as part of the Monitool project.
ii. The applicable range of this method is 0-40 nephelometric turbidity units (NTU). Samples with higher NTU values may be diluted to obtain results within this range.
iii. The method is based upon a comparison of the intensity of the light scattered by the sample under defined conditions with the intensity of the light scattered by a standard reference suspension. The higher the intensity of this scattered light, the higher the turbidity. Readings, in NTUs, are taken using a nephelometer, designed to the specifications given in Sections 2.ii and 2.iii. A standard suspension is used to calibrate the instrument.
iv. Formazin polymer is used as a primary turbidity suspension for water, prepared from the reaction of hydrazine sulphate and hexamethylenetetramine. Formazin turbidity standards can also be purchased as a commercially prepared concentrated stock solution.

2. Equipment and Supplies

i. Nephelometer: Used to measure the turbidity of sample, with a light source for illuminating the sample, and one or more photoelectric detectors with a readout device to indicate intensity of light scattered at right angles to the incident light. This system should be design so that little stray light reaches the detectors in the absence of turbidity and should be free from drift after a short warm-up period.
ii. Differences in physical design of nephelometers will cause differences in measured NTU values, even if the same suspension is used for calibration. To minimize such differences, the following design criteria should be observed:
    i. Light source: Tungsten lamp operated at a colour temperature between 2200-3000 ºK.
    ii. Distance traversed by the incident light and scattered light within the sample tube: Total not to exceed 10 cm.
    iii. Detector: To be centred at 90° to the incident light path and not to exceed ± 30° from 90°. The detector, and filter system if used, shall have a spectral peak response between 400 nm and 600 nm.
iii. The sensitivity of the instrument should permit detection of a turbidity difference of 0.02 NTU or less in waters having NTU values less than 1 unit. The instrument should measure from 0-40 NTU.
iv. Sample tubes: Must be made of clear, colourless glass or plastic. They should be kept clean, both inside and out, and be discarded when they become scratched or etched. A light coating of silicon oil may be used to mask minor imperfections in glass tubes. They should not be handled at points where the light strikes, but should be provided with sufficient extra length or a protective case so that they may be handled. They should be checked, indexed and read at the orientation producing the lowest background blank value.

3. Reagents and Standards

i. Turbidity-free reagent water: Deionised distilled water (passed through a 0.45 µm pore size membrane filter, if filtered deionised water shows a lower turbidity measurement than unfiltered water).
ii. Stock standard suspension (Formazin): To be purchased commercially prepared or prepared via the following method:

    i. Dissolve 1.00 g hydrazine sulphate, $H_6N_2O_4S$ (EC 233-110-4) in reagent water and dilute to 100 mL in a volumetric flask. Caution is required as hydrazine sulphate is carcinogenic.
    ii. Dissolve 10.00 g hexamethylenetetramine, $C_6H_{12}N_4$ (EC 202-905-8) in reagent water and dilute to 100 mL in a volumetric flask.
    iii. In a 100 mL volumetric flask, mix 5.0 mL of each solution (Sections 3.2.1 and 3.2.2). Allow to stand for 24 hours at 25 ± 3 ºC, then dilute to the mark with reagent water.

iii. Primary calibration standards: Mix and dilute 10.00 mL of formazin stock standard suspension to 100 mL with reagent water. The turbidity of this suspension is defined at 40 NTU. For other values, mix and dilute portions of the suspension as required.
iv. A new formazin stock standard suspension should be prepared monthly. Primary calibration standards should be prepared daily by dilution of the stock standard suspension.
v. If using a commercially prepared concentrated stock standard suspension, it may be diluted and used as required.

4. Sample Collection, Preservation and Storage

i. Samples should be collected and stored in prepared HDPE containers. Volume collected should be sufficient to ensure a representative sample, allow for replicate analysis (if required) and minimize waste disposal. Typically, 100 ml of sample is sufficient.
ii. No chemical preservation of samples required. Cool samples to 4 ºC.
iii. Samples should be analysed as soon as possible after collection, and should be stored at 4 ºC prior to analysis.

5. Calibration and Standardization:

i. Nephelometer calibration: Follow the manufacturer's instructions. Measure standards covering the range of interest. At least one standard should be run in each instrument range to be used. Some instruments should permit the adjustment of sensitivity so that scale values will correspond to turbidities. Solid standards such as lucite blocks should not be used due to potential calibration changes due to surface scratches.

6. Analysis Procedure:

i. For turbidities less than 40 NTU: Allow the samples to come to room temperature before analysis (if possible). Mix sample thoroughly to disperse solids, and wait until air bubbles disappear from samples. Pour into nephelometer sample tube. Read turbidity directly from instrument scale or pre-prepared calibration curve.
ii. For turbidities exceeding 40 NTU: Dilute sample with turbidity-free reagent water until the turbidity falls below 40 NTU, and the sample can then be analysed. The turbidity of the undiluted sample is then computed from the turbidity falls below 40 NTU.
iii. If nephelometer is equipped with several separate scales, the higher scales should only be used as indictors of required dilution volumes to less than 40 NTU.

Annex 8: Determination of Dissolved Organic Carbon (DOC)

The dissolved organic carbon content of seawater is defined as:
The concentration of carbon remaining in a seawater sample after all particulate carbon has been removed by filtration and all inorganic carbon has been removed by acidification and sparging.

In this method, the Total Carbon (TC) and Inorganic Carbon (IC) are measured then DOC will be the difference between TC and IC.

Reagents and materials:

- 250 ml clear glass bottles (DURAN type) containing the water sample
- 27 ml sample vials
- Autosampler
- PC LOW035 TOC-L and Shimadzu Total Organic Carbon analyser
- GF/F filters
- Polycarbonate inline filter holder
- Potassium hydrogen phthalate (reagent grade)
- Sodium carbonate (reagent grade)
- Sodium hydrogen carbonate (reagent grade)

Procedure

- All tubing and the polycarbonate inline filter holder should be acid washed (10% HCl) and rinsed with copious quantities of de-ionised water prior use. The washing is necessary to remove any residual carbon.
- GF/F filters should be combusted at 450 °C for at least 4 hours prior to use and stored in a sealed glass container.
- The filter holder, with filter in place, must be well flushed with sample prior to collection of filtrate for analysis.
- It is also important when filtering samples to filter slowly to avoid puncturing the filter.

Standard preparation

Stock standard

- Total Carbon, 1000 C_T mg/L: weight out 2.125 g of i potassium hydrogen phthalate and make up to 1 L with Milli-Q water.
- Inorganic Carbon, 1000 C_I mg/L: weight out 3.497 g of reagent grade sodium hydrogen carbonate and 4.412 g of and make up to 1 L with Milli-Q water.

Calibration standard solutions

Prepare the following standard solutions by serial dilution of the stock standard solution in Milli-Q water: 0.5, 5, 10, 20, 40 and 100 mg C/L.
Limits for acceptance of the cal curve are a standard deviation of 0.3 and a coefficient of variation limit of 2.0%.

After verifying the optimal operation of the TOC analyser, samples are placed on an auto sampler for analysis. The samples are run in triplicates, with Milli-Q water (used to dilute the standard solution) and the method blank.