European Mountain lake Ecosystems: Regionalisation, diaGnostics & socio-economic Evaluation

EMERGE

WP3: REGIONALISATION OF LAKE MICROPOLLUTANT DISTRIBUTION

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PROTOCOLS FOR THE ANALYSIS OF ORGANIC MICROPOLLUTANTS IN

1. **SEDIMENTS**
2. **SOILS**
3. **SEDIMENT TRAPS**
4. **BULK DEPOSITION**
5. **WATER**

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SEDIMENTS
WATER
AIR

Please, refer to MOLAR protocols for these compartments.

2. Soil cores.

4 Soil cores will be sampled in Lakes Redó (Pyrenees) and Ladove (Tatra), to determine ^{210}Pb, ^{137}Cs, $^{239/40}Pu$, metals, and organic micropollutants.

2.1 Compounds to be determined.

- Hexachlorobenzene
- DDT derivatives
- Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160, and 180).
- PAH: Parent compounds, methyl derivatives and sulphur derivatives.
- Total organic carbon.

2.2. Materials and reagents

- Cutting tools (non-plastic)
- Aluminium foil
- Distilled water
- Milli-Q water
- Acetone (for trace organic analysis, Merck)
- Soap (Extran-AP 13, alkaline, Merck).

2.3. Cleaning

The material to be used for soil sampling and sub-sectioning must be cleaned with 5% inorganic alkaline soap (Extran-AP 13) in distilled water (stirring 10 minutes). Rinsed with distilled water, Milli-Q water and acetone.
Protect of material with aluminium foil for drying, and until use.

2.4. Sampling procedure

Soil cores have to be collected using non-plastic tubes (teflon or stainless steel are preferred), if this is not possible, samples for trace organic analyses should be obtained from the central core area to avoid contamination from the walls. Tubes should be driven into the soil to a depth of at least 30 cm taking care to avoid compaction, dug out and the extruded into 1 cm sections for the top 10 cm and 2 cm sections thereafter.
Cores should be collected from undisturbed areas of lake catchment. Remove the living surface vegetation from the area where the soil is to be collected. For other site selection criteria, please refer to the sampling procedure for radionuclides analysis.

2.5. Sample preparation

The in situ volume of each core slice has to be recorded, and the dry weight fraction determined by drying overnight following the same procedure as for the lake sediment sample. Subsamples for organic micropollutant analysis have to be wrapped in aluminium foil. Two

aluminium sheets must be used: one in direct contact with the sample (previously rinsed), another to wrap the former. This last one should be labelled. A paper label than a water-resistant mark is preferred.
The sample corer should be cleaned and all soil removed before taken another sample.

2.6. Sample amount

1 g minimum of soil is needed

2.7. Sample Storage and Transportation

Soil sample should be stored frozen. If this is not feasible, they should be kept as cool as possible. These conditions should also be kept during transport, using boxes containing dry ice. Samples have to be sent to ICER-CSIC (Spain).

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2.8. Analyses

Soil analysis and extraction will be performed following the protocols describe for sediment samples (section 1).

3. Sediment traps.

Monthly sediment traps will be sampled in Lakes Redó (Pyrenees) and Ladove (Tatra), to determine radionuclides, metals, SCP, organic C, N, P, S, and organic micropollutants.

3.1. Organic micropollutants to be determined.

- Hexachlorobenzene
- DDT derivatives
- Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160, and 180).
- PAH: Parent compounds, methyl derivatives and sulphur derivatives.
- Weight of total particles.

3.2. Materials and reagents

- Cebtrifuge glass tubes
- Millipore 47 mm filtration apparatus
- 0.45 mm (GF/B) glass microfiber filters
- Tweezers
- Distilled water
- Milli-Q water
- Acetone (for trace organic analysis, Merck)
- Soap (Extran-AP 13, alkaline, Merck).

3.3. Cleaning

Plastic should be avoided in any instance. Clean all material filling them with a 5% inorganic alkaline solution of EXTRAN in distilled water. Let it stand for at least 12 hours. Empty and rinse the material with abundant tap water, distilled water, Milli-Q water and finally with acetone.
Wrap the cleaned material with aluminium foil and dry in an oven at 40C. Store wrapped in aluminium foil until use.

3.4. Sediment trap

Integrating (open) sediment traps with Teflon cups (or stainless steel) are preferred. Amount of trap material in monthly samples will be checked for POPs detection limits. If it is not enough, then samples integrating 3 months will be collected. Total trap material is preferred than aliquots from different traps. Collected samples have to be centrifuge in glass tubes, and store and transport frozen (see section 3.6.). Eventually, trap material can be recovered by filtration on glass microfiber filters (provided by Environmental Chemistry Department. ICER-CSIC. Spain)

3.5. Filtration on glass microfibre filters

1. Rinse the filtration apparatus with Milli-Q water

2. Record the filter label (preweighed glass microfiber filters wrapped in aluminium foil will be provided by the Environmental Chemistry Department).
3. Take the glass microfiber filter with cleaned tweezers and place it in the filtration apparatus.
4. Check whether the upper and lower parts of the apparatus are well assembled
5. Connect the apparatus to the vacuum
6. Pour the sediment trap sample in the upper part and connect the vacuum
7. Make sure that all trap material is transferred to the filtration apparatus. Rinse and stir adequately with some Milli-Q water three times in order to get that.
8. Take the glass microfiber filter with cleaned tweezers and BEND it. The two parts containing the trap material should face each other.
9. Wrap it in aluminium foil (that provided with the filter)

3.6. Sample storage and transportation

Filters or centrifuge tubes must be store frozen. If this is not feasible, then keep them as cool as possible. These conditions must to be kept also for transportation. Samples should be sent within boxes containing dry ice as soon as possible to the address and contact people indicated below.

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3.7. Analyses

Sediment trap analysis and extraction will be performed following the protocols describe for water particulate matter.

4. BULK DEPOSITION

4.1 Compounds to be determined.

* Hexachlorobenzene
* Hexachlorocyclohexanes (namely a and g isomers)
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

4.2. Other measurements.

* Weight of total particles
* Total organic particulate carbon (when sufficient material will be collected)

4.3. Materials and reagents.

- Dry and wet deposition sampler.
- Millipore 47 mm filtration apparatus.
- 0.45 mm (GF/B) glass microfibre filters.
- Glass bottles.
- Octadecylsilane membrane extraction disks (47 mm diameter, 0,5 mm thickness). Each disk contains about 500 mg C_{18} bonded silica.
- Measuring cylinders.
- Pasteur pipettes.
- Tweezers.
- Aluminium foil.
- Milli-Q water.
- Distilled water.
- Cyclohexane (for trace organic analysis, Merck).
- Dichloromethane (for trace organic analysis, Merck).
- Methanol (for trace organic analysis, Merck).
- Acetone (for trace organic analysis, Merck).
- Soap (Extran-AP 13, alkaline, Merck).

4.4. Cleaning.

- PLASTIC SHOULD BE AVOIDED IN ANY INSTANCE.

- THE CLEANING OF THE SAMPLING MATERIAL IS THE MOST IMPORTANT STEP OF THE SAMPLING WORK.

Bulk deposition reservoirs

CLEANING OF THE DEPOSITION RESERVOIRS MUST BE DONE BETWEEN EVERY SAMPLE

Bulk deposition reservoirs shoul be cleaned with 5% inorganic alkaline soap (EXTRAN AP13, Merck) in distilled water (stirring 10 minutes)

Rinse with distilled water, Milli-Q water and acetone.

Cleaning with ultrasonic bath.

- TWEEZERS AND EXTRACTION SYSTEM MUST BE CLEANED BETWEEN EVERY SAMPLE.

- Add 5% inorganic alkaline (e.g.EXTRAN AP 13, Merck) in distilled water into the ultrasounds reservoir.

- Put all the material inside the ultrasonic bath trying not to have bubbles of air into the material.

- Connect the ultrasonic for 15 minutes.

- Empty and rinse the material with abundant tap water.

- Rinse with distilled water, milli-Q water and finally with acetone.

- Wrap the cleaned material with aluminium foil and let it in the oven at 60 ?C for drying.

Cleaning without ultrasonic bath.

- Add 5% inorganic alkaline (e.g. EXTRAN MA 01, Merck) in distilled water and fill all the material that it has to be cleaned with.

- Let it stand for 24 hours.

- Rinse and dry the material as the last step.

Pasteur pipettes.

- Wrap the Pasteur pipettes with aluminium foil and let them remain into a oven at 400oC for 12 hours.

- Once it has been finished remain them always wrapped with aluminium foil, until used.

4.5. Collection of the sample.

- THE GLASS BOTTLE COLLECTOR OF THE SAMPLE MUST BE CLEANED

BETWEEN EVERY SAMPLE.

- Take a glass bottle and clean as the rest of the material.

- Put the atmospheric deposition sample inside and record the volume of the sample collected.

- Rinse and stir adequately with some milli-Q water three times in order to make sure that all the suspended particles and compounds in the wet reservoir are transferred to the glass bottle.

4.6. Filtration on glass microfibre filters.

- It is very important to **FILTRATE THE SAMPLE THE SAME DAY THAT YOU COLLECT IT**, 20% of the compounds analysed can be lost because of the adsortion in the bottle.

- Rinse the filtration apparatus with Milli-Q water.

- Record the filter label (glass microfibre filters are pre-weighted in the Department of Enviromental Chemistry).

- Take a glass microfibre filter with cleaned tweezers and place it in the filtration apparatus.

- Check whether the upper and lower parts of the apparatus are well assembled.

- Connect the apparatus to the vacuum.

- Pour the water sample in the upper part and connect the vacuum.

- Record the exact sample volume filtrated.

- Make sure that all the suspended particles in the glass bottle are transferred to the filtration apparatus. Rinse and stir adequately with some milli-Q water three times in order to make sure that all the suspended particles and compounds in the wet reservoir are transferred to the glass bottle.

- Take the glass microfibre filter with cleaned tweezers and store it as is indicated in the section 4.8.

4.7. Adsorption on membrane extraction disks.

- Transfer the filtered sample from the filtration apparatus into a cleaned glass bottle, in order to filter it through a membrane extraction disk (C_{18} empore disk).

- Take a membrane extraction disk with cleaned tweezers and place it in the filtration apparatus.

- Check whether the upper and lower parts of the apparatus are well assembled.

- Connect the apparatus to the vacuum.

- With the vacuum off, wash the membrane extraction disk with 10 ml of dichloromethane:cyclohexane (1:1) and allow to stand for 3 minutes.

- With the vacuum on, draw the remaining solvent through the disk. Let the vacuum for 1 minute until the disk become dry.

- With the vacuum off, add 10 ml of methanol to the membrane extraction disk and let stand for 3 minutes. **DO NOT ALLOW DISK TO RUN DRY.** If the disk remains dry in this step, replace the disk for a new one and start the process from the beginning.

- Pour the sample to the methanol that remains on the disk.

- Start the extraction. Water extraction should be slow. Adjust the vacuum trying to have a flow of 2 litres/hour.

- **NEVER ALLOW THE MEMBRANE EXTRACTION DISK GOES TO DRYNESS DURING EXTRACTION**. If it happens by accident, take out the membrane extraction disk and store it as it is indicated below. The rest of the water, must be filtered with a new membrane extraction disk, which should be cleaned as it has been previously described.

- When the extraction is finished, let the vacuum on for five minutes.

- Record the exact sample volume extracted.

- Store the membrane extraction disk wrapping it in aluminium foil as it is indicated in the section 4.8. **DO NOT BEND IT**.

4.8 Storage.

4.8.1. Glass microfibre filter.

- **BEND** the glass microfibre filter with the tweezers. The two parts containing the particulate material should face each other.

- Wrap it in aluminium foil.

4.8.2. Membrane extraction disk.

- **DO NOT BEND** the membrane extraction disk, just wrap it in aluminium foil.

- Indicate which is the upper part of the disk where the sample went through.

- Check the disk is completely extended/flat, for avoiding disk break when frozen.

- Record the filter label and give a sample identification to the filter and membrane. This information must be included together with the period of collection, date, site, temperature range during the collection and precipitation, collected volume, in a sample protocol form.

- Store the wrapped and labeled filters and membrane extraction disks at -20?C.

4.9 Blank of the bulk reservoirs.

- **THE BLANK OF THE BULK RESERVOIRS MUST TO BE DONE MONTHLY.**

- The bulk reservoirs must be cleaned with 5% inorganic alkaline (e.g.EXTRAN AP 13, Merck) in abundant distilled water (stirring 10 minutes). Rinse with distilled water and milli-Q water.

- Add 1L of milli-Q water into the bulk reservoirs. Cover it with aluminium foil. Let it stand inside the station (not outside) as much as possible (at least one day).

- Filter the blank of the reservoirs as a sample.

- Wrap, store and record the filter and membrane extraction disks as a sample.

- Clean the bulk reservoirs and put them in the sampling place.

4.10 Transport.

Samples should be sent within boxes containing dry ice to the address and contact people indicated below. Samples should be sent monthly.

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4.11. Analytical Methods

Levels of micropollutants in the particulate phase of deposition samples will be determined following the same procedure describe for particulate phase of the lake water (section 5).
Organic compounds present in the dissolved phase (membrane extraction disks) will be determined as describe elsewhere (Carrera et al., 1998[*]), including an additional clean up step by adsorption chromatography with alumina as describe for water particulate phase in section 5.7.

[*] Carrera G., Fernández P., Vilanova R.M., and Grimalt J.O. (1998). Analysis of trace polycyclic aromatic hydrocarbons and organochlorine compounds in atmospheric residues by solid-phase disk extraction. *Journal of Chromatography* A, **823**, 189-196.

5. WATER (DISSOLVED + COLLOID AND PARTICULATE MATTER)

5.1 Compounds to be determined.

* Hexachlorobenzene
* DDTs (namely, pp'-DDE and pp'-DDT)
* Polychlorobiphenyls (congeners Nos. 28+31, 52, 101+84, 118+149, 153, 138+163+160 and 180)
* PAH (63 individual compounds)

5.2 Other measurements.

* Weight of total particles

5.3 Materials and reagents.

-Tweezers
-Glass bottles
-Test tubes
-Pasteur pipettes
-Aluminum foil
-Infiltrex 2 Pump
-1 mm GF/B glass fiber filters (14.2 cm diameter)
-XAD-2 resin columns
-Sodium sulphate (analysis grade)
-Milli-Q water
-Dichloromethane (for trace organic analysis, Merck)
-Methanol (for trace organic analysis, Merck)
-Acetone (for trace organic analysis, Merck)
-Soap (Extran-AP 13, alkaline, Merck)
-d_{10}-pyrene
-d_{12}-benzo[*ghi*]perylene
-PCB 30
-octachloronaphthalene

5.4 Cleaning.

Plastic should be avoided in any instance.

1. Tweezers and the dry and wet+dry reservoirs should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-rinsed with distilled water, Milli-Q water and acetone
-wrapped in aluminum foil and left in the oven at 40ºC for drying.

2. Glass bottles should be
-cleaned with 5% inorganic alkaline soap (e.g. EXTRAN-AP 13, Merck) in distilled water (stirring 10 minutes)
-emptied, cleaned with tap water, Milli-Q water and acetone.
-left to dry and the open parts covered with aluminum foil.

3. The glass fiber filters and the Pasteur pipettes are kiln-fired at 400ºC.
4. Prior to sampling the XAD-2 columns are cleaned with 200 ml of methanol and 200 ml of dichloromethane. Then, further 200 ml of dichloromethane are collected to determine blank levels. If blanks are acceptable the columns are left moist with methanol not being allowed to dry out to prevent cracking of the extraction material. Columns should be stored in the refrigerator. Cleaning must be continued until low blank levels are achieved.

5.5 Sampling

The pump is introduced in the water column. The water is impelled through the filter and XAD2 column. Flow rate should be between 300-400 ml/min and the volume sampled about 100 liters.

5.6 Storage

The filters should be wrapped in aluminum foil and frozen (-20ºC) until analysis. The XAD-2 columns should be stored in the refrigerator but never freeze. These columns should be extracted within one week after sampling.

5.7 Analysis of particulate organic matter

The filter is freeze dried in an oil-free freeze-drier and weighed. Then, it is cut in small pieces and spiked with PCB 30, octachloronaphtalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The mixture is extracted by sonication with (2:1) dichloromethane:methanol (3 x 20 ml, 20 ml). The extract is vacuum evaporated to 2 ml and hydrolyzed overnight with 20 ml of (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigorous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

5.8 Analysis of dissolved+colloidal organic matter

The columns are eluted in the inverted direction to sampling with 200 ml of methanol and 200 ml of dichloromethane. The methanol fraction is extracted with 3 x 30 ml of *n*-hexane. This *n*-hexane extract is combined with the dichloromethane eluate and spiked with PCB 30, octachloronaphthalene, d_{10}-pyrene and d_{12}-benzo[*ghi*]perylene. The combined extracts are

hydrolyzed with 20 ml (w/w) 6% KOH in methanol. The neutral fraction is recovered with *n*-hexane (3 x 10 ml), vacuum evaporated until dryness and fractionated with a column containing 2 g of alumina. The aliphatic and organochlorinated fractions are recovered by elution of 4 ml of 10% dichloromethane in *n*-hexane. The aromatic fraction is collected by elution with 10 ml of 50% dichloromethane in *n*-hexane. Then, the solvent is concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

The organochlorinated fraction is purified additionally with agitation with sulphuric acid. After vigorous stirring in a Vortex (2 min) the two layers are decanted for removal of the sulphuric acid. This step is repeated another times renewing the sulphuric acid to get a clean and transparent *n*-hexane solution. The *n*-hexane concentrated under vacuum to a small volume, *e.g.* 50 ml of *iso*-octane, for instrumental analysis.

5.9 Instrumental analysis of polycyclic aromatic hydrocarbons.

A Carlo Erba GC8000 Series coupled to a mass spectrometer Fisons MD800 is used. This instrument is equiped with a 30 m HP-5 column coated with 5% phenyl methyl silicone. The oven temperature program is from 90 to 310ºC at 4ºC/min, held for 10 min. Injection and transfer line temperatures are 280 and 300ºC, respectively. Helium is the carrier gas (50 cm/s). Data are acquired in the electron impact mode with an electron energy of 70 eV and the operation is in selected ion monitoring. The injection is in the splitless mode (1 ml injected; hot needle technique), the split valve being closed for 48 s.

5.10 Instrumental analysis of organochlorinated compounds.

A gas chromatograph Hewlett-Packard Model 5890 (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and a split/splitless injector is used. This equipment is provided with a CPSIL-8 column (5% phenyl-95% methylpolysiloxane, 50 m length, 0.25 mm i.d., 0.25 mm film thickness; Chrompack, Middelburg, The Netherlands). Helium is the carrier gas (30 cm/s). The samples (2 ml) are introduced with an automatic injector Hewlett-Packard Model 7673A in the splitless mode. Injection and detector temperatures are 270 and 310ºC, respectively. Oven temperature is programmed from 80 to 150ºC at 10ºC/min and to 300ºC at 4ºC/min with a final holding time of 15 min. The make up gas is nitrogen (60 cm/s).

5.11 Quantitation.

Authentic standards of hexachlorobenzene, op'-DDE, pp'DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT and the polychlorobiphenyl congeners Nos. 28, 52, 101, 118, 138, 153 and 180 are used. Calibration curves (detector response *vs* amount injected) are performed for each compound. The range of linearity of the detector is evaluated from the curves generated by representation of detector signal/amount injected *vs* amount injected. All measurements are performed in the ranges of linearity found for each compound. In some cases, re-dilution and re-injection are performed to fit within the linear requirements.

5.12 Compound identification.

Structural identification is confirmed by analysis of selected samples by gas chromatography coupled to mass spectrometry in the chemical ionization mode and record of the negative ions. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL is used for the analyses. The chromatographic conditions are the same as described above. A DB-5 column was used.

Transfer line and ion source temperatures are 300 and 120ºC, respectively. The reagent gas is methane. Data are acquired by scanning from 50 to 500 mass

units at 1 s per decade. The samples selected for GC-MS analysis must allow to elucidate the composition of all the major peaks present in the gas chromatograms obtained with the ECD.