

4. Digestions

4.2 Subboiling digestions

In contrast to microwave digestions, subboiling digestions do not build up any pressure, which resulted in a lot of advantages. Such teflon vessels are cheaper than pressure digestion vessels (around 30 % of pressure digestion vessels) and there are no limited places such as in the microwave. Therefore, more vessels can be bought and processed simultaneously. Careful placement of blanks and empty vessels such as in (older) microwaves is not necessary. Digestions can be processed in normal drying ovens at 90 °C, which means a lower investment than for microwaves. However, the digestions time is, with 24 h, much longer than with a microwave. (Anderson 1976, Berthold et al. 2015).

4.2.1 Acidic persulfate solution: sediment ashes

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Suitability:

Phosphorus in sediments consists of numerous, very different available fractions. Phosphate in the interstitial water is easily available. However, this fraction is quantitatively of minor importance. Phosphorus is hardly available if it is organically bound, for example in biomass or in detritus. Some phosphate salts, which only can be dissolved under anoxic conditions, phosphate ions being adsorbed to clay minerals and other inorganic particles are difficultly available. (Berthold et al. 2018, Nausch 1981).

Large amounts of bound phosphates are removed under acidic conditions (HCl, HNO₃ or H₂SO₄) and by adding strong oxidising agents (H₂O₂ or persulfate). The usage of ashes instead of dry matter often improves the digestion yield. If higher iron concentrations are in the sediments, discolouration can complicate neutralisation. Other slightly soluble inorganics salts can complicate P determination as well. Phosphate ions react in acidic solutions with molybdate to molybdenum blue. Molybdenum blue is quantified photometrically. With phosphate relatively insoluble

compounds such as metal ions, iron, calcium and aluminium can be determined in the same sediment extract.

High phosphate concentrations strengthen eutrophication processes because in the often anoxic sediment a lot of phosphate is mobile and can be re-dissolved into the water column as well. TP is a basic parameter, demonstrating the total load with phosphorus. However, almost no conclusions can be drawn from TP concentrations for the phytoplankton availability of P.

Necessary sediment parameters:

- ▶ Determine water content and loss of ignition of the sediment sample!
- ▶ For a reference to area and volume the dry matter density is necessary. Alternatively, the dry matter density can be estimated from a correlation between water content and dry matter density (Berthold et al. 2018).

Protocol

Preparation of samples:

- ▶ Sediments and soils have to be sieved <2 mm. The fraction <2 mm (fine soil) is used for further analysis
- ▶ Grinding: The reproducibility is improved. However, grinding affects the availability of element in soils and sediments. Recommendation for mills, time of grinding and the energy input are extremely diverse and have to be adapted to the specific samples.
- ▶ Ashing: The advantage is the better availability of organically bound phosphate (organic substances are burned). However, inorganic compounds can be transformed. For example, normally yellow-brown goethite, a Fe(III)-oxid, can be transformed at 500 °C to reddish hematite changing the binding of phosphate (Derie et al. 1976, Prasad et al. 2006).. Poorly soluble salts such as $\text{Ca}_3(\text{PO}_4)_2$ remain mainly unavailable.

Procedure:

- ▶ Set 4 replicates per sediment sample. Store residual ashes until results are available.
- ▶ Weigh in ca. 50 (muddy sediment) to 100 mg (sandy or mineral sediment) of ashes (550 °C, 4 h) in test tubes with ground glass stopper
 - ▶ Weigh dish or small pieces of aluminium foil: Tara
 - ▶ Weigh and fill ca. 50 to 100 mg of ash into the test tube.

- ▶ Weigh out residual dust in the weighing dish. Subtract mass from weight in, note corrected weigh-in!
- ▶ Wipe out weighing dish. Tara.
- ▶ Add 10 ml of ultra-pure water, shake well,
- ▶ add 1 ml acidic persulfate reagent.
- ▶ Incubate closed test tubes at 90 °C for 24 h in the drying oven.



Fig. 4.2.1-1 Iron-containing sample after acidic persulfate digestion (red colour probably caused by iron oxides transformed to hematite)

Further procedure / neutralisation:

Samples do not have to be neutralised, if they will not be filtered (fine-grinded material), be measured manually immediately (not in Continuous Flow Analyser) and no retention samples have to be stored in PE-tubes (see below).

- ▶ Without Neutralisation:
 - ▶ cool down samples, postprocess latest after 2 hours. (e. g. for photometric P determination).
 - ▶ Transfer solution completely into a 50 ml volumetric flask and fill with ultra-pure water to the calibration mark.
- ▶ with Neutralisation (work under laboratory hood):
 - ▶ if samples have to be measured in the CFA Autoanalyser or be stored in plastic test tubes (also for a short time), the samples have to be pH-neutral. Such pH-neutral samples can be stored for longer time.
 - ▶ Cool down samples, postprocess latest after 2 hours.
 - ▶ Transfer solution completely into a 50 or 100 ml volumetric flask and rinse with some ml of ultra-pure water.

- ▶ Add 3 to 5 drops of nitrophenol solution, add dropwise as much 1 N NaOH (max. 4 ml), as the solution is slightly yellow and stays yellow after well shaking.

ATTENTION: At high Fe concentrations (samples discoloured red rot after ashing, Fig. 4.2.1-1) Fe can flocculate as yellowish flakes by addition of NaOH! Take care for re-dissolution of flakes by HCl addition!

- ▶ Titrate pH with 1 N HCl (with a burette) until colour changes to colourless.
- ▶ Fill to calibration mark.
- ▶ with Filtration:
 - ▶ For protection of the filtration apparatus only with neutralised samples!
 - ▶ If too many sediment particles remain in the suspension, turbidity blank values would be too high and variable. Filtrate sample via glass fibre filter. CAUTION: Solution is very corrosive!
 - ▶ Filtrate for CFA in any case!
 - ▶ Multiple filtration equipment is well suited to transfer filtrates in 50 ml PE-tubes (Fig. 4.2.1-2).

Samples with high iron concentration (visible as red discolouration after ashing, Fig. 4.2.1-1) can disturb titration by the development of iron flakes in the solution by addition of NaOH. Normally, iron flakes re-dissolve by addition of acid during neutralisation, but this has to be checked. Otherwise, turbidity by flakes and binding of P to the iron flakes can affect photometric P measurement (increase turbidity, removal of measurable P from solution).

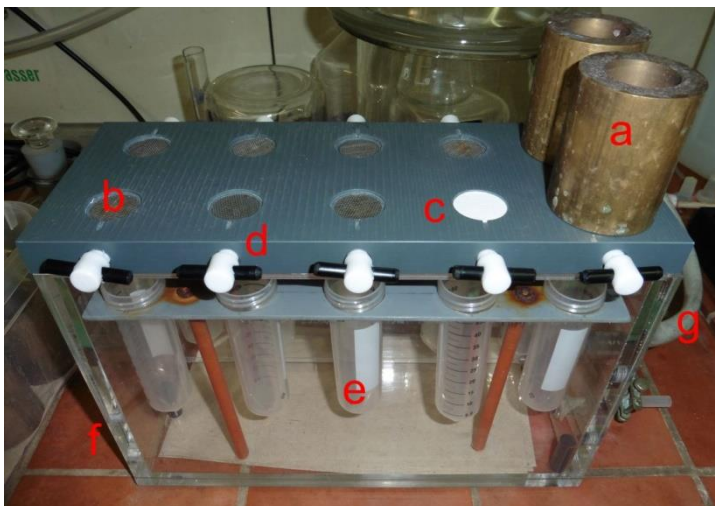


Fig. 4.2.1-2 Multiple filtration equipment for 50 ml tubes (do-it-yourself construction by University of Rostock) with a) enlarged filter attachment (from brass), b) filter support from steel, c) commercial glass fibre filter, d) Teflon tap, e) 50 ml PE-centrifuge tubes in PVC-rack, f) extra high evacuation vessel and g) hose to vacuum pump.

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Further processing:

- ▶ CFA: fill 2 x 20 ml vials and freeze. Discard residues.
- ▶ manual: fill in 50 ml plastic tubes.
- ▶ Subsequently, remove 15 ml (without shaking, particles sedimented), transfer into 25 ml Erlenmeyer flasks, process further such as for phosphate (+ 0.15 ml ascorbic acid + 0.3 ml molybdate mix reagent, see chapter 5.2.3).

If extinction is ≥ 0.8 (in a 5 cm cuvette), discard sample and begin anew with a diluted sample! empirical dilution for ashed sediment samples:

- ▶ Extinctions around 0.8) start new with dilution: 1 part sample (5 ml) and 2 parts (10 ml) ultra-pure water (5 ml Eppendorf pipette, 1:3)
- ▶ Extinctions ≥ 1 : 1 part sample (3 ml) and 4 parts water (4 ml 3 ml) (1:5)
- ▶ Extinctions > 1.5 can be diluted 1+9 (1.5 ml sample + 9 x 1.5 ml water, 1:10).

Calculations:

- ▶ correct results of photometric measurement by all dilutions, e. g. 10 ml water after neutralisation to 50 ml = x 5; and further dilution before measurement, e. g. 5 ml + 10 ml = x 3
- ▶ take calibration factor from phosphate determination

$n_{ML} = \frac{c_{ML} \cdot 50}{1000} = n_{NA}$	c_{ML} : concentration of measurement solution n_{ML} : amount of substance in 50 ml measurement solution is equal to n_{NA} : amount of substance in the extract, if no subsamples were taken and it was not diluted.
$E_{TM} = \frac{E_A(g) \cdot 100\%}{GR(\%)}$	E_{TM} : weight in (g dry matter E_A : weight in of ash (g) GR : residue on ignition (%)
$c_S = \frac{n_{NA}}{E_{TM}(g)}$	c_S : concentration in sediment dry matter

Hints for quality management for acidic persulfate digestion:

- ▶ A series of 50 tubes can be well processed with the neutralisation step
- ▶ Set 2 to 5 blank values per digestion series. These blanks also control effects of storage of especially acidic samples.

- ▶ Digest two 10 μM diphenyl phosphate standards (yield) and, if necessary, additionally glucose-6-phosphate standards (testing of dilution steps): fill 10 ml standard solution in test tubes without sediment. Add persulfate as well.
- ▶ If very high dilutions are necessary because of high P concentrations in the ashes and the weight in cannot be decreased (precision of the balance, transfer, ...), the standards should be diluted as well. In order to prevent dilution of standards lower than the limit of quantification, higher concentrated standards (20 or 50 μM) should be used.
- ▶ Use at least one sediment or soil standard (P-Campus, laboratory-own or certified, e.g. NIST).

Further hints for quality management see chapter 6!

Chemicals:

- ▶ Ultra-pure water should be deionised and silicate-free. Molybdate is reacting with silicate, which can be leached from some glasses and ion exchangers. Measurement of natural (low) silicate concentrations is suppressed by measurement conditions. Therefore, ultra-pure water from silicate-free ion exchangers should be used, e.g. from Rostocker Kraftwerk ("Kraftwerkswasser").
- ▶ **acidic persulfate solution:** dilute 5 ml 9 N H_2SO_4 (50 %) in 100 ml ultra-pure water. Dissolve 5 g potassium peroxide sulphate ($\text{K}_2\text{S}_2\text{O}_8$, N-poor) in the sulfuric acid solution. Can be stored at room temperature for around 1 week if light protected. If persulfate is precipitated the solution has to be prepared new.
- ▶ **1 N HCl:** add 83 ml of 37 % HCl to ca. 750 ml ultra-pure water in a 1 litre volumetric flask. Fill to 1 l after cooling down.

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