2. Selection of Method

2.2 Influence of the Matrix

Dana Zimmer, Rhena Schumann

Important properties affecting the selection of the method of digestion or the matrix of measurement solution are water content, organic substance, carbonate, aluminium and iron, because they can affect solubility of P significantly. This affects in turn steps of digestion and the selection of P determination methods.

The matrix is also important for aqueous samples. For example, arsenate, silicate and nitrate interfere with the molybdenum blue reaction (Grasshoff et al. 1999, chapter 5.2.3). Nitrate causes problems in millimolar concentrations, also in solutions with nitric acid or *aqua regia*. Furthermore, a salt matrix effect exists; e.g. salt concentrations deepen colour intensity of molybdenum blue. The pH value is important mostly for acidic extracts. A strong self-coloration (e.g. by plant pigments) also interferes with photometric P determination.

2.2.1 Mineral Matrices: Soils, Sediments, Muds

Soils, sediments and muds are mostly classified by their concentration of organic matter (table 2.2-1). Most mineral soils in Central Europe/ Germany have soil organic matter (SOM) concentrations of less than 2 %. Exceptions are Tschernosems/Chernozems (rare in Central Europe) with around 6 % of organic matter (Wikipedia: Chernozem, WRB classification). All soils with concentrations of SOM of less than 15 % are classified as minerals soils. According to Blume et al. (2010) and Ad-hoc-AG Boden (2005), soils with SOM concentrations between 15 and 30 % are defined as (in German) "Anmoor" and with SOM concentration > 30 % they are defined as peat.
Mineral soils normally can be digested with aqua regia (or hydrofluoric acid and/or perchloric acid) in a microwave without previous ashing. Previous ashing of soil should be preferred at 10 % or more of SOM. “Anmoor” soils and peat are not digested such as mineral soils but as organic material. That means that they are ashed before digestion with *aqua regia* or HNO_3 + H_2O_2.

Before the digestion of mineral soils or mineral sediments (especially calcareous mud) by *aqua regia* carbonate concentrations have to be

tested. Carbonate concentration up to 2 % should not be problematic. If carbonate concentrations are higher than 2 %, adding of concentrated HCl (for *aqua regia*) would decompose carbonate to CO_2, the sample could bubble out the vessel, and part of the HCl would have been consumed. In such calcareous soils and sediments carbonate should be eliminated previously to digestion. This can be done by HCl or by heating up to 900 °C. If carbonate concentrations are higher than 30 %, it is not possible and appropriate to digest samples with a*qua regia*.

Analogous to mineral soils, muds are classified by their soil texture according to their grain size distribution in sand-, silt- and clay-muds. Sand is defined as separate particle < 2 mm to > 63 µm, silt as < 63 µm to > 2 µm and clay as < 2 µm (Ad-hoc-AG Boden 2005). The percentage of grain size classes determines the soil texture (Ad-hoc-AG Boden 2005, p. 142).

Table 2.2-1 Definitions of different soils (Blume et al. 2010) and sediments (e.g. Schlungbaum et al. 1979) according to their soil organic matter concentration (SOM)

Material	SOM (%)
mineral soil	< 15
"Anmoor"	15-30
moor/peat	> 30
mineral sediments	< 5
organic sediments	> 5

In soil science the term "mud" for sediments with more than 5 % of SOM is classified in far greater detail (tables 2.2-2 and 2.2-3). However, such sediments have not been widespread in lakes. Therefore, less data about phosphorus concentrations are available; more data exist for ponds and moors (cf. chapter 1.6).

Table 2.2-2 Classification of muds according to KA5 (Ad-hoc-AG Boden 2005) with symbols

Type of mud	Texture of mud	Material composition		
		SOM (%)	Carbonate ($CaCO_3$) %	Silicate (%)
organo-mineral mud (Fm)	Sand mud (Fms) Silt mud (Fmu) Clay mud (Fmt) Diatom mud (Fmi) Calcareous mud (Fmk)	5…< 30	no information	mainly
organic mud (Fh)	Algae mud (German: Lebermudde) (Fhl) Peat mud (Fhh) Detritus mud (Fhg)	≥ 30		no information

Table 2.2-3 Description of muds, according to KA 5, p. 164 and Meier-Uhlherr et al. (2015)

Texture of mud	Description
Sand mud	with clearly visible parts of organic substances, thin horizon
Silt mud	with clearly visible parts of organic substances, especially in old moraine areas and "Thüringer Becken"
Clay mud	with clearly visible parts of organic substances, plastic, soapy or greasy consistence, mostly thin horizon
Diatom mud	from Diatom residues, can be distinguished from calcareous mud by HCl addition and from clay mud only by microscope
Calcareous mud	in fresh condition plastic or elastic, disaggregates not completely by HCl addition, a plenty of undissolved material (> 20 mass-%), from sedimented calcareous particles or calcareous material formed from dead organisms (e.g. stoneworts, snails)
Algae mud	homogeneous from elastic (liver-like), gelatinous consistency, conchoidal cracking, formed by dead, decomposed algae residues (phytoplankton) and characterizes deep, calm areas rich of algae, but poor in higher aquatic plants lakes
Peat mud	with clearly visible peat residues, brown-black
Detritus mud	most common lake sediment, often with seeds and visible residues of aquatic plants, homogeneous dense, plastic to a bit elastic material, from very fine decomposed organic substances

The typical reference value for P concentrations is dry matter mass of the material (chapter 3.2). For samples with similar water content and similar density this value is well-suited to compare different samples. When only fertilizer effects have to be evaluated the dry matter mass works well. However, soil volume, wet and dry matter and ash mass have to be determined, if available P in area or volume (of soil) have to be evaluated.

If muds or sediments have to be digested, the dry matter density (of the undisturbed sample) and the water content have to be determined, because different pore volumes and water content can limit comparison of different samples. Samples with high organic matter (OM) concentrations often have high water content. For digestions of ashes the loss of ignition has to be determined. In calcareous samples carbonate should be destroyed by heating to 900 °C.

The water content can affect dry matter density significantly (figure 2.2-1). Therefore, P concentrations should refer to volume or in the case of sediments to the ecosystem effecting area (sediment – water – interface). Interpretations of P concentration in relation to dry matter mass can result in misunderstanding/miscalculation of P availability or interrelation between P und OM concentrations (cf. chapter 1.7). Furthermore, water content and OM concentrations are correlated. That means that high P concentrations in muddy sediments cannot be interpreted as "organically bound" (figure 2.2-2). This is especially the case, if the dry matter mass is the reference value.

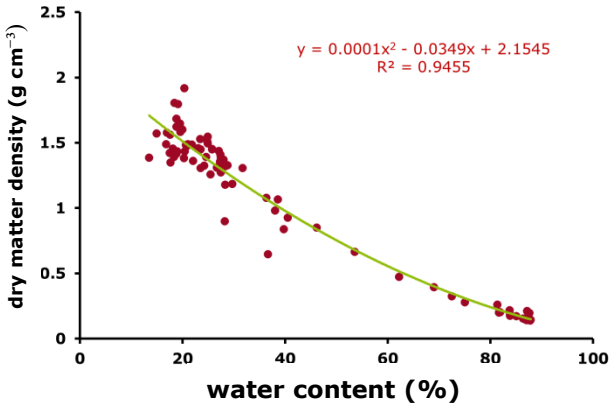

Figure 2.2-1 Interrelation between water content (%) and dry matter density (g dry matter cm^{-3}) in sediment samples from the Darss-Zingster Bodden chain and along the southern Baltic Sea coast up to ca. 4 m Water depth. $N_{replicates}$= 4

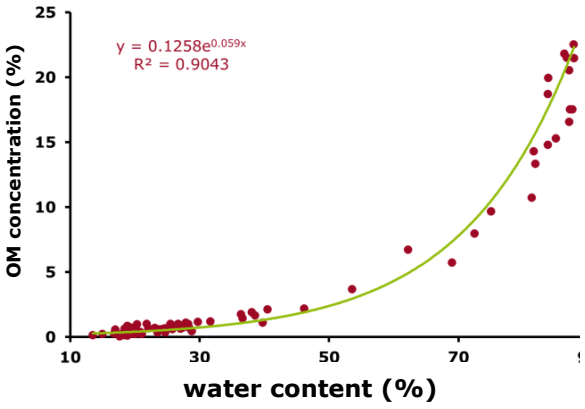

Figure 2.2-2 Interrelation between water content (%) and OM concentration (loss of ignition in %) of 84 samples in figure 2.2-1

Generally, it is possible to digest samples with high OM concentrations (or even biomass) such as minerals samples. However, ashing before digestion is recommended (table 2.2-4).

Table 2.2-4 Overview of sample preparation of P-poor matrices

Preparation	
Material	**Working steps**
Soils	air-drying or drying at 40 °C/60 °C in drying oven
	< 2 mm sieving
	if necessary, homogenisation and/or ashing
	if necessary, destroying of carbonate
Sediments	drying at 60 °C in drying oven
	if necessary, homogenisation and/or ashing
	if necessary, destroying of carbonate

In soil science only the fine soil (< 2 mm) is analysed. All material > 2 mm are called soil skeleton. It is analysed separately only in exceptional cases!

2.2.2 Organic matrices (biomass) with low water content: algae, plants, litter, peat, organic-rich muds, manure, compost, bone char, bone chips, other biochar, mounting resin

If samples with high OM concentrations are digested directly, density and especially water content have to be determined (exception charcoal). Bone chars, bone chips and other bio chars are water poor (perhaps hydrochar is not) and do not need to be dried. Bone char can be digested finely crushed or as discrete particles of 1 to 4 mm. If single particles of char shall be digested, long digestion times concerning the microwave (45 min) are necessary to ensure a high energy input. Bone char is, in contrast to other biochar, P-rich. That means that such bone char extracts must be diluted very strongly.

Organically bound phosphates are hard to extract (e.g. Svendsen et al. 1993). Therefore, extraction methods are very strict and include high energy input (pressure and temperature), strong acids and if necessary high concentrations of an oxidizing agent. If ashes are extracted, the organic matrix is destroyed, and method of P quantification can be selected more freely.

Table 2.2-5 Overview of sample preparation and phosphorus detection in P-rich matrices (best as ashes)

Preparation	
Material	**Working steps**
peat and muds	perhaps sieving <2 mm after drying
	perhaps ashing
	destroying carbonate if necessary
plants and litter	ground to dust fineness after drying (woody plants have to be ground in each case
	and/or ashing
potato tuber, liquid manure and the like	better freezing and Lyophilisation
	ashing if necessary
bones	ground finely after drying
bone char and the like	perhaps crush finely, no other preparation necessary
Preparation of extracts	
some extracts	dilution

2.2.3 Water rich matrices: animal tissue and tissue fluid, liquid manure, digestate

Animal biomass is mostly analysed as dry matter or ash. Most materials are P-rich, because most economically interesting organisms have P-rich endoskeleton or exoskeleton (vertebrates with bones, mussels with shells). For evaluation of results it is very important to know the scientific question: if total P amount removed from ecosystem (and therefore with bones) or if only the P amount in the used biomass/product is interesting. This also has to be considered for literature studies.

During preparation of fatty tissues, a strong odour is released. That means that such tissues should be at least lyophilized before ashing. A fat solvent extraction can be tried with hexane or a similar solvent to extract fat from bones (e.g. Lamoureux et al. 2011, Murden et al. 2017). High concentrations of OM are correlated with strong soot formation. For this reason, biomass could be incinerated outdoors. However, some losses of small ash particles are possible, which is why this method is not suited for the determination of loss of ignition. Either, very large masses are ashed and the loss is low or very small samples are ashed to decrease the formation of soot. Alternatively, a new method for ashing in a microwave system is available (Phönix, Fa. CEM).

For the analysis of animal tissues, the dilution of extracts is essentially before measurement, since most analytical methods work in the µmol

range. Therefore, secondary errors for multiple dilutions have to be calculated (table 2.2-6).

Table 2.2-6 Overview of sample preparation and Phosphorus determination in animal tissue (best in ashes)

Preparation	
Material	**Working steps**
all materials	drying and ashing
soft tissues	perhaps lyophilisation and fragmentation
	only measure without ashing, if special digestion protocols exist
Preparation of extracts	
all extract	dilution

2.2.4 Water-rich matrices: seston, precipitation (rainfall or snow), aerosols, sludge, waste water, liquid manure, slurry, digestates

Such matrices are water samples (seston), rainfall with or without dust and aerosols. Because of the mixed liquid and solid phases aliquoting for measurement replication is problematic. Either aliquots are stored separately (frozen) and are digested completely or suspension is accurately mixed after thawing and before digestion (Table 2.2-7).

Digestates are water- and organic-rich materials, such as liquid manure, silage and sewage sludge. According to their amount such samples should be prepared as samples in table 2.2-2 or 2.2-3.

Table 2.2-7 Overview of sample preparation of P-poor matrices

Preparation	
Material	**Working steps**
Seston	Freezing water samples at -20 °C according to the number of replicates and digestion method 50-100 ml
	Thawing quickly before measurement (in warm water)
	shake intensively before aliquoting

References

Ad-hoc-AG Boden, Bodenkundliche Kartieranleitung, 5th ed., 438 pages, Hannover 2005, Ed. Federal Institute for Geosciences and Natural Resources in cooperation with the State Geological Surveys of Germany, ISBN: 9783510959204

bgr.bund.de, KA 5 Geländeformblatt

Blume H-P, Brümmer GW, Horn R, Kandeler E, Kögel-Knabner I, Krezschmar R, Stahr K, Wilke B-M, Thiele-Bruhn S, Welp G (2010) Scheffer/Schachtschabel. Lehrbuch der Bodenkunde. Spektrum Akademischer Verlag, 16th ed., ISBN: 9783827414441

Meier-Uhlherr R, Schulz C, Luthardt V (2015) Steckbriefe Moorsubstrate. 2nd unchanged ed. HNE Eberswalde (ed.), Berlin

Phönix, CEM https://cem.de/applikation/veraschung-/-muffelofen, last access: 16.09.2024

Schlungbaum G, Nausch G, Stolle S (1979) Sedimentchemische Untersuchungen in Küstengewässern der DDR. VIII. Spezielle Untersuchungen zur Verteilung von Phosphaten und Eisenverbindungen in der Sedimentoberflächenschicht des Barther Boddens. Meer Beitr Sekt Biol Univ Rostock 7: 499-505

Svendsen LM, A Rebsdorf P Nornberg (1993) Comparison of methods for analysis of organic and inorganic phosphorus in river sediment. Wat Res 27: 77-83, DOI: 10.1016/0043-1354(93)90197-P

Wikipedia https://de.wikipedia.org/wiki/Schwarzerde

For citation: Zimmer D, Schumann R (*year of download*) Chapter 2.2 Influence of the Matrix (Version 1.1) in Zimmer D, Baumann K, Berthold M, Schumann R: Handbook on the Selection of Methods for Digestion and Determination of Total Phosphorus in Environmental Samples. DOI: 10.12754/misc-2020-0001