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A simplified method for total phosphorus digestion with potassium persulphate at sub-boiling temperatures in different environmental samples

Abstract

An alternative digestion method for total phosphorus determination at sub-boiling temperatures (90 °C) was tested successfully for seston samples and adapted also to sediment and plant ashes. The digestion reagent consists of alkaline and acid potassium persulphate. Only rather simple laboratory equipment is necessary: muffle furnace for dry matter, oven and a photometer. The comparably small reaction vessels (30 ml PFA tubes for seston and 20 ml glass tubes for ashes) allow treating many samples at once. Some seston samples were better digested under long sub-boiling condition. There were no significant differences between HCl and acid persulphate digestion for sediments. P concentrations found in e.g. *Chara* spp. ranged between 1.3 and 2.0 mg g dry mass $^{-1}$, which is comparable with concentrations in the literature. New determination limits were equal or better than the high temperature digestions. Best data sets amounted to 0.12 and 0.19 µmol l^{-1} for alkaline and acid persulphate, as determination limits respectively. Combined standard uncertainties were 8.7 and 12 % for both digestion agents under sub-boiling conditions.

Keywords: phosphorus, digestion, persulphate oxidation, molybdenum blue, sediment, macrophytes

1 Introduction

There are many different methods for total phosphorus (TP) digestion from sediments, soil, water and organisms tissue (e.g. ANDERSEN 1976; HANSEN & KOROLEFF 1999; SCHIEFERSTEIN 1999). Phosphorus compounds must be converted into phosphate, which is determined photometrically. Alternatively, a digestion into dissolved phosphorus compounds is sufficient, if they are detected by inductively coupled plasma (ICP, e.g. MUNTER 1990). ICP is mainly used in soil (e.g. JONES 1998) and plant sciences. Marine biologists, biogeochemists and limnologists prefer photometry (HANSEN & KOROLEFF 1999; JARVIE et al. 2002; GIMBERT et al. 2007). Most disciplines have developed their own digestion methods due to different needs in measurement ranges, operability and laboratory equipment. Consequently, reproducibility and determination limits are different or remain to be compared.

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The chemical wet digestion is the most commonly applied method and uses always a concentrated acid and often an oxidation agent. Acids are hydrochloric acid (ANDERSEN 1976; ASPILA et al. 1976) or sulphuric acid (HEDLEY et al. 1982) for soil and marine sediments, nitric acid for plants and macrophytes (SCHIEFERSTEIN 1999; BRAGATO et al. 2006; GONZÁLES-ALCARAZ et al. 2012), sulphuric acid (HANSEN & KOROLEFF 1999) and perchlorate acid (STRICKLAND & PARSON 1972) for water samples. Nitric acid is problematic, because high concentrations of nitrate may interfere with the phosphate determination by molybdenum blue (HANSEN & KOROLEFF 1999). Perchloric acid is a very strong acid with strong oxidising properties. Therefore, high laboratory safety standards are necessary for this procedure. Most often, high-temperature digestion is assisted by high pressure in a microwave or autoclave (e.g. ASPILA et al. 1976).

Additional oxidation agents support digestion and remove background colour, e.g. extracted chlorophyll or phaeopigments. Such agents are hydrogen peroxide (HEDLEY et al. 1982; BRAGATO et al. 2006) or potassium persulphate (MENZEL & CORWIN 1965; ASPILA et al. 1976; HANSEN & KOROLEFF 1999). High temperature and / or a high pressure promote a good and quick phosphate recovery. A laboratory microwave is a suitable apparatus for these requirements, although it is very expensive in acquisition. Sometimes, a UV-degradation is used to meet these requirements (ARMSTRONG et al. 1966; MCKELVIE et al. 1989).

Alternatively, digestions function at sub-boiling temperatures, when the reaction time is long enough. HUANG & ZHANG (2009) introduced a new digestion method at sub-boiling temperatures (90 °C) with neutral persulphate for total phosphorus in water samples. Only low budget equipment is needed: an oven and reaction tubes, which do not need to hold high pressure (and are not so costly). Moreover, the batch can be as large as can be handled for neutralisation the next day. This is under our conditions ca. 70 digestions. Most laboratory microwaves treat at once 10-40 samples. Therefore, we tested if this method is good enough for water samples of highly turbid waters.

We adopted the acid digestion and persulphate digestion for sediment ashes from sediment fractionation (HUPFER et al. 1995 and references cited therein) and tested the sub-boiling treatment. Additionally, other materials were tested, e.g. plant and algal material. Different reaction times and temperatures were applied to sediments, water and plant samples. The digestion of dried (and not combusted) material is common in plant and soil science. Therefore, dried mass was compared to ashes. Digestion efficiency, reproducibility and determination limits were measured for all methods. Finally, all samples were neutralised after digestion, which is often not done (*cf.* Tab. 2). Especially the acid persulphate digestion samples may be too acidic, which hinders the molybdenum blue reaction (GOING & EISENREICH 1974; PAI et al. 1990).

The aim was to find a harmonised method for as many sample types as possible, which works without specialised equipment, is low cost and robust as well as rather easy to learn. This paper shows the first results of the effort to include many different laboratories and disciplines.

2 Material and Methods

2.1 Study area

The Darß-Zingst Bodden chain (DZBC) is a shallow estuary at the Southern Baltic coast. The DZBC consists of four basins called “Bodden”. The mean water depth is around 2 m (SCHLUNGBAUM 1994). The Zingster Strom is the deepest part, which connects the two innermost boddens. The Grabow is the outermost water body (sampling place Dabitz) with the strongest influence of Baltic Sea water. The Bodstedter Bodden (place Michaelsdorf) is one of the inner water basins, which have the highest seston and phytoplankton biomass (SCHUMANN et al. 2001). Some samples were taken at various regions at the outer Baltic coast (from Flensburg in the west to Lubmin in the east) and had seston contents compared to the Baltic Sea samples. All those sites are much less eutrophicated compared to the rather closed DZBC. Sediments were taken only at shallow sites (< 2 m depth), where macrophytes can grow or were present. All nearshore sites had fine sandy sediments with LOI <2%, which seems rather unchanged since the 1970s (e.g. NAUSCH & SCHLUNGBAUM 1993).

Data for quality management originated from monitoring 2012 – 2014 of the Biological Station Zingst and from the project BACOSA in 2013 and 2014 (*cf.* Figure legends). The influence of pure water quality on DL and blanks cannot be excluded over the three years.

Water samples were taken at three sites in the DZBC (the two mentioned above and the Zingster Strom) and the six sites at the outer Baltic coast. Sediment cores were sampled in the Bodstedter Bodden and Salzhaff. Macrophytes originated from the DZBC only (Table 1).

Water samples of 50 ml were stored in polypropylene reaction tubes at -20 °C for at least 24 h. Sediments were taken with sediment corers (36 or 60 mm diameter). At least, five cores were separated into two horizons (0 – 2 cm and 2 – 5 cm) per sampling site (= 15 samples). Sediment was weighed wet and after drying at 105 °C for 24 h. The mass loss is the water content. Aliquots of the dried samples were combusted at 550 °C (muffel furnace, Heraeus) for 24 h. This mass loss (LOI, loss on ignition) corresponds to organic matter.

Submersed macrophytes (*Potamogeton pectinatus* (L.)*, Myriophyllum* spp. (Ponted. ex L.), *Ruppia* spp. L., *Najas marina (*L.)*, Chara tomentosa* (L. 1753) and *Chara* spp. (Gray)) were sampled in the Grabow (Tab. 1) at 0 – 1 m water depth. Macrophyte species or genera were determined and weighed separately. Mostly, above-ground parts were analysed. Samples were dried at 105 °C for 24 h. Parts of the dried samples were combusted at 550 °C (muffel furnace, Heraeus) for 4 h. Ashes and dry mass were used for all digestions in 4 replicates.

Tab. 1: Sampling sites, their coordinates and the salinity range (PSU) during sampling (2013-2014).

Sampling site	Coordinates	PSU
Darß-Zingst Bodden Chain		
Bodstedter Bodden	54° 22.30' N 012° 34.15' E	4 – 7
Zingster Strom	54° 25.77' N 012° 41.35' E	4 – 8
Grabow	54° 22.02' N 012° 48.36' E	6 – 10
Outer coast		
Geltinger Bucht	54° 45.14' N 009° 51.95' E	10
Orther Bucht	54° 32.68' N 011° 05.62' E	10
Salzhaff	54° 01.00' N 011° 32.00' E	11
Vitter Bucht	54° 33.48' N 013° 07.08' E	8 – 9
Griebener Bucht	54° 35.58' N 013° 08.04' E	8 – 9
Spandowhagener Wiek	54° 08.88' N 013° 42.18' E	3

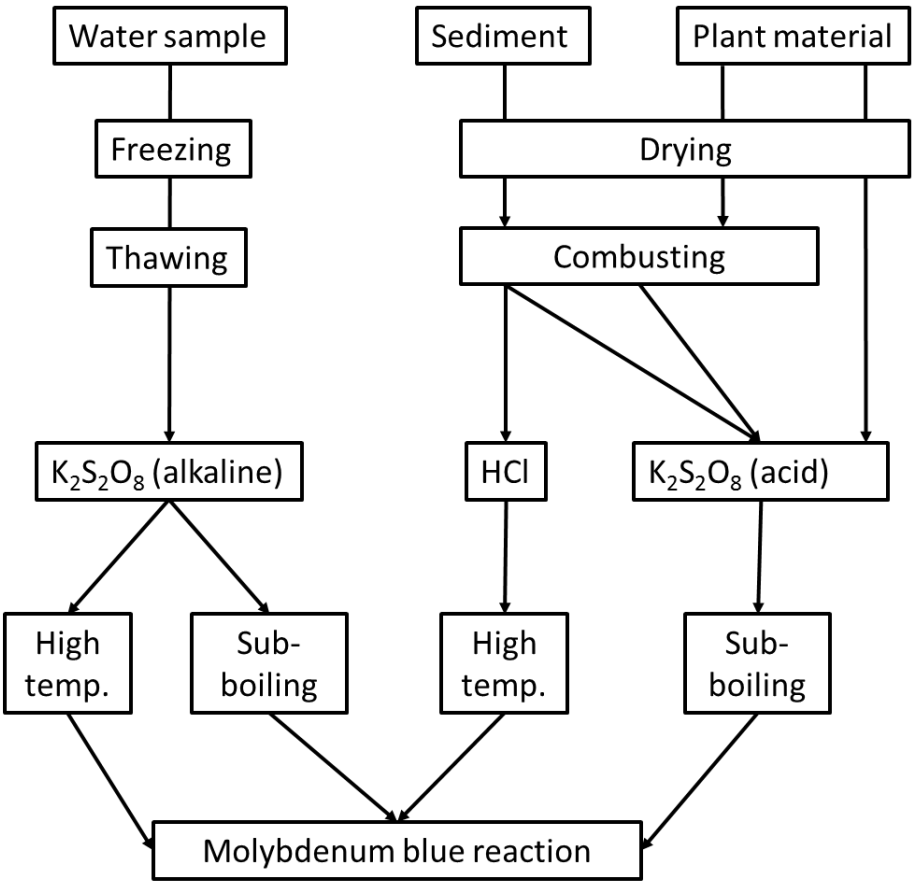

Fig. 1: Work flow of sample treatments, digestion procedures and phosphorus determination. "High temperature" is digestion in a microwave (high pressure and temperature, 40 min.) or on a heating plate (sediment) with 120 °C to 220 °C for 15 - 20 min. "Sub-boiling" is digestion in an oven at 90 °C for 24 h at normal pressure (ca. 1 bar). $K_2S_2O_8$: Persulphate

2.2 Chemical analyses

Ultrapure water was used for P-determination of DM or ashes and for blanks. A biofilter, two activated carbon-filters, a cation filter, a percolator, an anion filter and a mixed bed filter containing anionic and cationic filter purified the water (Power Station Rostock). Final conductivity was < 0.05 µS cm^{-1}.

Tab. 2: Original methods and adaptations performed here.

	Original method	pH	Source	Adaptation
alkaline persulphate	120 °C for 40 min, no neutralisation	12.5 (start) to 2.0 (end)	HANSEN & KOROLEFF (1999), HUANG & Zhang (2009)	90 °C for 24 h, neutralisation
acid persulphate	120 °C for 40 min, no neutralisation, 4,5 N H_2SO_4	ca. 2	HANSEN & KOROLEFF (1999)	90 °C for 24 h, neutralisation, 9 N H_2SO_4
HCl	220 °C for 20 min, no neutralisation	ca. 1	ANDERSEN (1976)	cooking for 20 min, with neutralisation

An alkaline persulphate solution digested water samples in tubes made of perfluoralkoxy-polymere (PFA). Initially, Oxisolv® (Merck) was the digesting agent (KÖTHE & BITSCH 1992). One spoon (delivered with the reagent powder, ca. 0.16 g) Oxisolv® was added to 15 ml sample (HANSEN & KOROLEFF 1999). Later, a 100 ml persulphate solution with 5 g (ca. 0.2 mM) $K_2S_2O_8$, 3 g (ca. 0.5 mM) H_3BO_3 and 1.5 g (0.375 mM) NaOH was used instead of Oxisolv®. The digestion solution (1.5 ml) was added to 15 ml digestion solution (HANSEN & KOROLEFF 1999). The mixtures were digested in a laboratory microwave (Lavis-1000). Suspensions were heated up for 2 min at 120 °C and afterwards kept inside the microwave for 18 min followed again by 2 min heating and 18 min waiting. Afterwards, the vessels were rather hot and under pressure depending on the initial vessel temperature for another 5 min or longer. Finally, suspensions were treated in 20 ml PFA reaction vessels (AHF, Germany) for 24 h at 90 °C (HUANG & ZHANG 2009). Replicates were not measured regularly in earlier times. In this study, up to five replicates were investigated and are presented in range control charts.

Sediments and plant matter were digested in acid persulphate or the most common respective procedure (Fig. 1, Tab. 2). About 5 mg of plant ashes or 30 - 100 mg sediment ashes were weighed and placed into 20 ml glass tubes filled with 15 ml ultrapure water. DM was similar for sediment, but 10-20 more for biomass, because of its high LOI. Acid persulphate solution (1.5 ml) was added, which contained 5 g (0.2 mM) $K_2S_2O_8$ and 5 ml 9 N (50 %) H_2SO_4 in 100 ml ultrapure water. This solution contained a two times higher H_2SO_4 concentration than in HANSEN & KOROLEFF (1999), which leads to a more efficient digestion of acid-labile P (not shown). Samples digested for 24 h at 90 °C. Corresponding sediment samples digested with 15 ml 1 N HCl for 20 min at 220 °C (Andersen, 1976). All these digestions were quadruplicated. The comparison of original or common digestion procedures for plant material (Tab. 2) is not complete yet.

All samples cooled down to room temperature. They were neutralised with nitrophenol as an indicator after alkalisation with ammonia (1 part concentrated ammonia plus three parts ultrapure water) for water samples or 1 M NaOH for all others and titration with 1 M HCl. Finally, water samples were brought to 25 ml and suspended material to 100 ml by adding ultrapure water.

Phosphate was determined as soluble reactive phosphorus (SRP) photometrically using the molybdenum blue reaction (HANSEN & KOROLEFF, 1999). A continuous flow analyser with a 5 cm cuvette (CFA, Alliance Instruments, MALCOLM-LAWES & WONG, 1990) measured SRP and digestion solutions of seston. SRP was measured in filtrates without scattering and the persulphate bleached the seston particles considerably. Additionally, the CFA is equipped with a so-called matrix photometer, which corrects for scattered light. All other samples were measured in a 5 cm cuvette (OG optical glass) at 885 nm with a spectral photometer (Hach-Lange, DR 3900). Dry matter (DM) or ash particles were not filtered (Tab. 2), because the few small sediment particles were mostly retained in the vessels upon neutralisation or sedimented rapidly in the cuvette and did not influence the result.

Each digestion batch included several blanks and two different P solutions (potassium dihydrogen phosphate, diphenlyphosphate or glucose-6-phosphate, each 10 µmol·l^{-1}). Standards and blanks were treated like normal samples like the respective samples. The phosphate standard was introduced years ago, as it did not always work to get more TP than SRP especially in anoxic samples of high concentrations (>10 µmol l^{-1}). Nevertheless, we kept the routine, because this standard helps to find dilution mistakes (which happens often during training courses). Glucose-6-phosphate is an often-used standard, but it is very expensive. Diphenylphosphate worked as well. So far, we kept both for all tests, but will turn to diphenylphosphate in the future. Results were drawn into control charts.

2.3 Statistical and data evaluation

Determination limits (DL, also limit of quantification or LOQ, German: Bestimmungsgrenze) were calculated out of 10 blanks for each method (German: Leerwertmethode after DIN 32645; WELLMITZ & GLUSCHKE 2005). Detection limit (also limit of detection or LOD, German: Nachweisgrenze) is always 1/3 of DL under these conditions and will not be discussed separately in this work.

$$DL\ (\mu mol\ l^{-1}) = 9 \cdot S_B\ (\mu mol\ l^{-1}) \qquad (A)$$

S_B = standard deviation of blanks (µmol l^{-1})

$9 = 3 \cdot \Phi_{0,01}$ (Φ for 10 blanks = 3)

Three types of control charts were drawn: blank charts with blanks of accompanying the digestion batches (not those of DL calculation), reference charts for all sorts of standards and range charts or standard deviation charts in % of arithmetic mean for reproducibility of samples (equation B or C). We decided to set blanks as sufficient, which were < DL, standards must stay within ± 15% tolerance limit and ranges as met, when within 15 % of range (compared to LUNG, State Agency for the Environment, Nature Conservation and Geology of Mecklenburg-Western Pomerania). The advantage of this documentation as control charts is that readers can interpret the results with the given or their own ranges.

$$\text{Range}(\%) = \frac{|\text{Maximum} - \text{Minimum}|}{\bar{x}} \cdot 100\% \quad \text{(B)}$$

Range = (German: Spannweite) as the difference between the highest and lowest value for n=2

$\bar{x}$ = arithmetic mean

$$s_x = \sqrt{\frac{\sum_{n=1}^{i}(x_i - \bar{x})^2}{n-1}} \quad \text{(C)}$$

s_x = standard deviation for n > 2 random samples out of a population (stabw in Excel 2007 or older, stabw.s from Excel 2010 on)

n = sample number

i = number of samples

x_i = individual measurement / sample value

Reproducibility is characterised by the combined standard deviation of standards and samples (D).

$$s_c = \sqrt{s_{sa}^{\ 2} + s_{st}^{\ 2}} \quad \text{(D)}$$

s_c = combined standard deviation

s_{sa} = sample standard deviation

s_{st} = standard deviation of standards

IBM SPSS Statistics 20 was used for statistical analysis. Shapiro-Wilk-test tested all the data on normal distribution. Man-Whitney-U-Test analysed all nonparametric, t-test all parametric data of sediment and plant material for significant differences. All results stated here as significant had a p-value of ≤ 0.05.

3 Results

3.1 Quality management

The DL for SRP was 0.10 µmol l^{-1} under best conditions measured manually and reached 0.04 µmol l^{-1} in CFA. Values in the upper range were from 2012 an improved over the next two years. In contrast, DL was about 5-times higher, when only a 1 cm cuvette was used. The DL of this measurement in a 1 cm cuvette was from aliquots of the best reading in 5 cm. The DL of the alkaline persulphate digestion (water samples) improved by an order of magnitude from Oxisolv® and persulphate in the microwave and was best after 24 h digestion at 90°C in PFA (Tab. 3). In glass vessels, the DL values were slightly higher.

Tab. 3: Determination limit (DL in µmol l^{-1}) for soluble reactive phosphorus (SRP), sub-boiling (90 °C) and high temperature (120 °C for Oxisolv® and alkaline persulphate, 220 °C for HCl) digestions for total phosphorus. PFA: perfluoralkoxy-polymere, n = number of DL determinations

Digestion	Method	Reaction vessels	DL	n
without (SRP)	Photometer (5 cm cuvette)	glass or polyethylene tubes	0.10 - 0.55	11
	Photometer (1 cm cuvette)		0.70	1
	Continuous flow analyser (5 cm cuvette)	glass capillary	0.04 – 0.29	15
High temperature	Oxisolv®	PFA	1.06 - 1.17	2
	alkaline persulphate	PFA	0.29 - 0.68	4
	HCl	glass flasks	0.22	1
Sub-boiling	alkaline persulphate	PFA	0.12 - 0.33	6
	acid persulphate	glass tubes	0.19 - 0.21	2

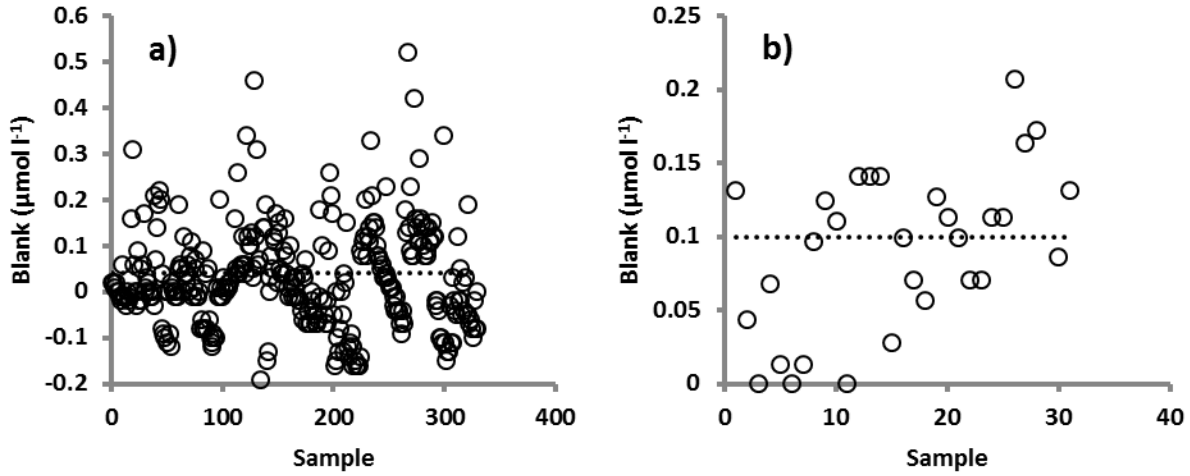

Fig. 2: Blank Control charts of SRP-measurement in ultrapure water measured a) in a continuous flow analyser (all data 2014, n = 344, median = 0.02 µmol l^{-1}) and b) manually (n = 30, median = 0.1 µmol l^{-1}). Dotted line: best determination limits.

Blanks of SRP were often < DL (Fig. 2). However, many high blank values occurred with the CFA especially when the blank followed directly immediately high concentrations. The many negative values from the CFA could not be eliminated yet.

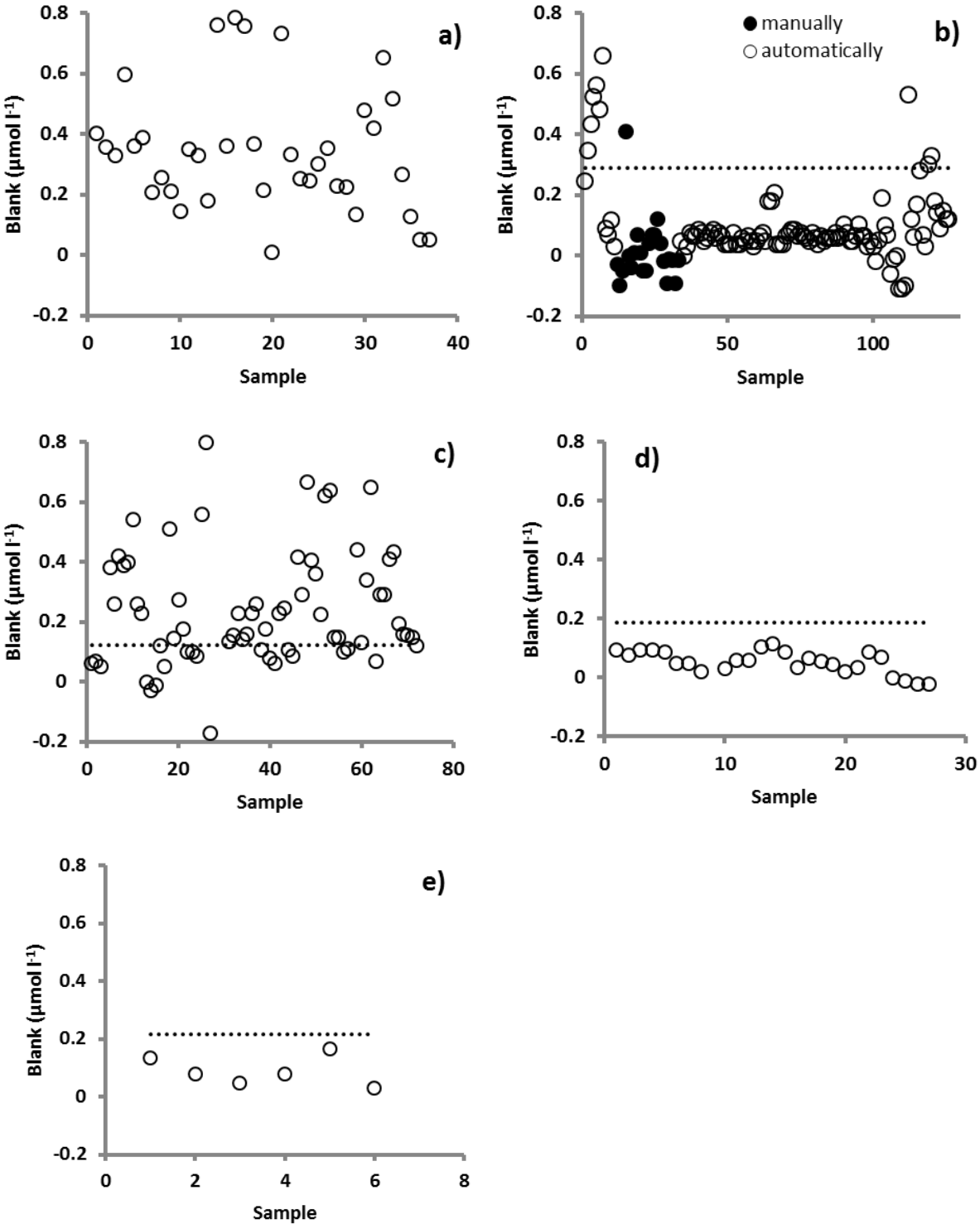

Fig. 3: Blank control charts (ultrapure water) during all digestions a) with Oxisolv® (data from monitoring 2012 and testing 2013, n = 37, median = 0.33 µmol l^{-1}) and b) with alkaline persulphate in a microwave (data from monitoring and testing late 2013, n = 126, median = 0.07 µmol l^{-1}), c) sub-boiling digestion with alkaline persulphate (data from monitoring and project BACOSA 2014, n = 75, median = 0.21 µmol l^{-1}) and d) with acid persulphate (data project BACOSA 2014, n = 26, median = 0.06 µmol l^{-1}), e) 1N HCl digestion on a heating plate (data project BACOSA 2014, n = 6, median = 0.08 µmol l^{-1}). a) to c) measured in the analyser and d) to e) determined manually. Dotted line: best determination limits.

The blanks for all samples digested in PFA vessels averaged at 0.33 µmol l^{-1} with Oxisolv®, 0.07 µmol l^{-1} with alkaline persulphate in the microwave and 0.21 µmol l^{-1} in the sub-boiling treatment (Fig. 3 a-c). The declining noise of these blanks is reflected in the decreased DL. Unfortunately, there was another influence factor in this comparison. The blank control charts accompanied (logically) the respective measuring batches, which were conducted in 2012, 2013 and 2014, respectively. There were separate reaction tubes for blanks or samples in the microwave. This was not continued for the tubes of sub-boiling methods, because a contamination from previous samples is better observed with randomly used tubes. Negative blanks occurred again only in the CFA measurements. The high blanks may not only reflect DL of the methods, but eventually pure water quality. The manufacturer of persulphate was not changed. All acid digestions (so far) had lower DL and all blanks were measured in late 2013 and through 2014 manually (control charts, Fig. 3 d-e).

Oxisolv® often over- or underestimated standards (Fig. 4 a). Standards digested with alkaline persulphate fitted into the tolerance limits after the first batches (Fig. 4 b and c). Acid persulphate digestions were often at or above the upper part of the 15 % percentile (Figure 4 d).

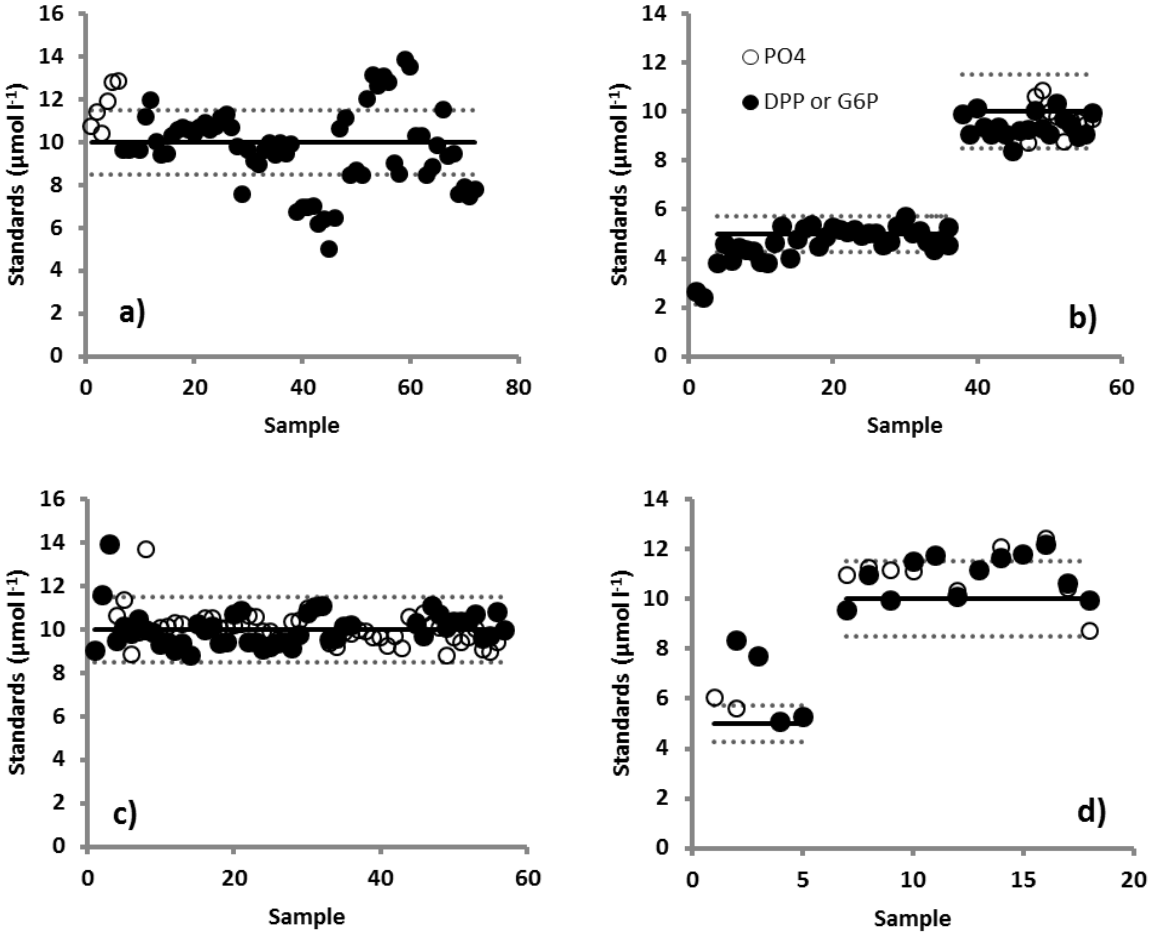

Fig. 4: Standard control charts (5 or 10 µmol·l^{-1}) for all digestions with a) Oxisolv® (data from monitoring 2012, n = 66, median = 9.7 µmol l^{-1}) and b) alkaline persulphate in a microwave (monitoring 2013, n = 19, median = 9.3 µmol l^{-1}), c) with alkaline (monitoring and BACOSA 2014, n = 49, median = 9.9 µmol l^{-1}) and d) acid persulphate under sub-boiling conditions (BACOSA 2014, n = 12, median = 11.1 µmol l^{-1}). Dotted line: ± 15 % as tolerance limits, solid line: reference values.

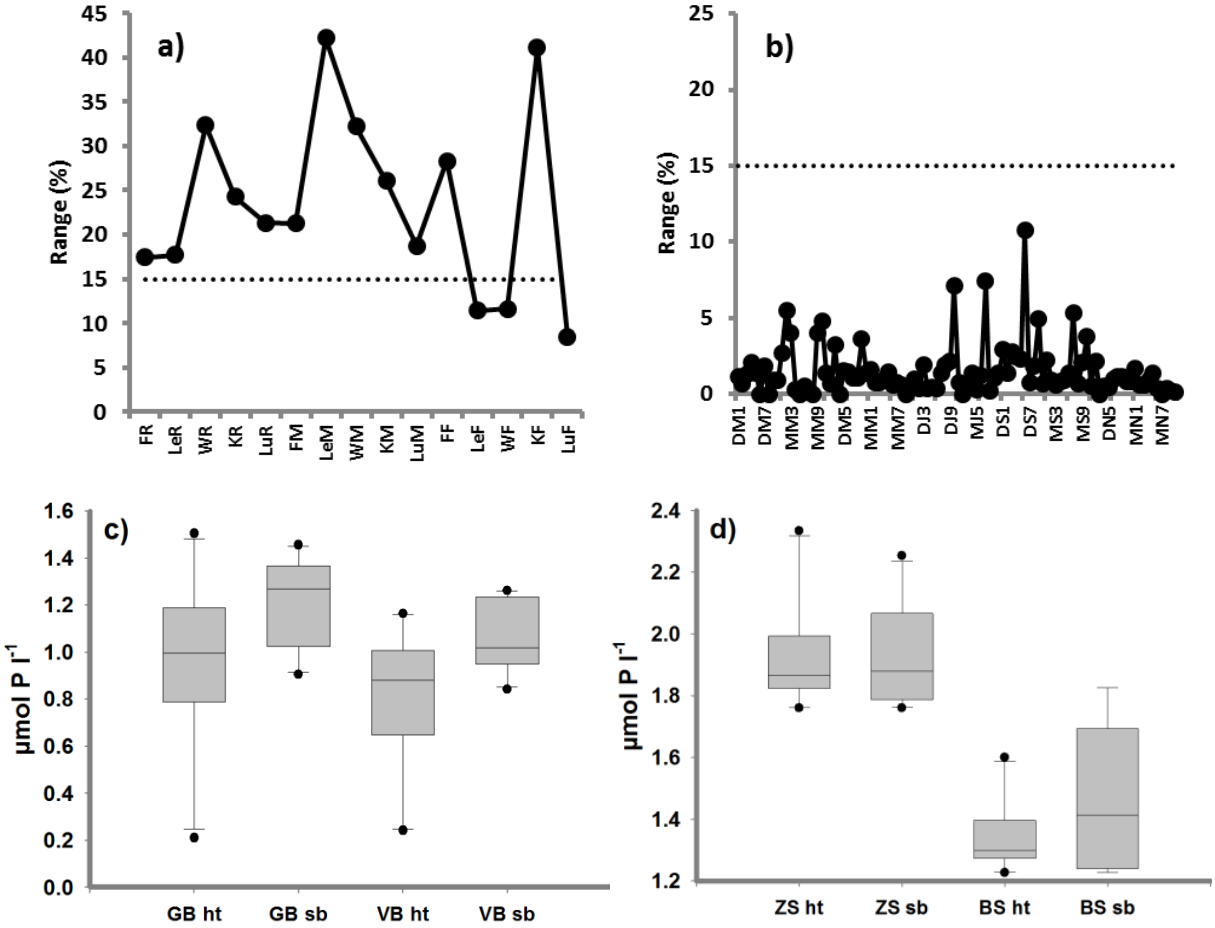

Fig. 5: Range control charts a) of samples from the outer Baltic coast from June and July 2013 in 4 replicates each (first letters F, Le, W, K, Lu are the places of sampling Flensburg, Lemkenhafen, Werder, Kloster and Lubmin, last letters R, M, F stand for sites of reed, macrophytes and free of growth and b) of 100 samples from the Darß-Zingst bodden chain measured in duplicates (first letter M, D are the places Michaelsdorf and Dabitz, followed by sampling month and number of sites), c) comparison of high temperature and sub-boiling persulphate digestion of water sample replicates (GB = Griebener Bucht 11th Jun 2013, VB = Vitter Bodden 4th Dec 2013, $n_{samples}$ = 10) and d) of measuring replicates (ZS = Zingster Strom, BS = Baltic Sea 4th Dec 2014, ht = high temperature / microwave, sb = sub-boing, $n_{replicates}$ = 10). Lines in boxes: median, boxes: interquartile distances, Whisker: 10% and 90% percentile, closed circles: outliers.

3.2 Comparison of natural samples

Seston samples were not compared to Oxisolv® digestions, because DL was high and several standards were not digested completely (Fig. 3 a and 4 a). Additionally, we observed often Oxisolv® crystals after the high temperature treatments. Therefore, Oxisolv® digestion was terminated anyway. Samples digested at high temperature (microwave) had a very poor reproducibility (Fig. 5 a). Duplicates from sub-boiling treatments (Fig. 5 b) were much better and never exceeded a range of 15 %, which was our quality limit. True sample replicates digested at high temperature had so many low values that the median was lower compared to the sub-boiling treatment of the same samples (Fig. 5 c). Another test with measuring replicates from one sample each lacked the lower outliers and had same results with both methods (Fig. 5 d).

Seston sample reproducibility was much better after the sub-boiling treatment (Tab. 4). For sediments, sample standard deviation was similar in the common high temperature HCl and sub-boiling digestion in acid persulphate. However, a chemically defined standard was not found, which could be digested in HCl.

Tab. 4: Combined standard deviation (s_c) for each digestion procedure calculated from standard deviations of standards (s_{st}) and samples (s_{sa}) in %. Data basis: Fig. 4 -7.

	s_{st}	s_{sa}	s_c
Oxisolv®, high temperature	19.2		
alkaline persulphate, high temperature	5.2	21.3	23.6
alkaline persulphate, sub-boiling	8.6	1.0	8.7
HCl, high temperature		9.1	
acid persulphate, sub-boiling	8.8	sediments 10.8	13.9
		tissue 8.2	12.0

There were no significant differences (p = 0.7) between TP concentrations for HCl and acid persulphate digestions at sub-boiling conditions of sediment ashes so far (Fig. 6).

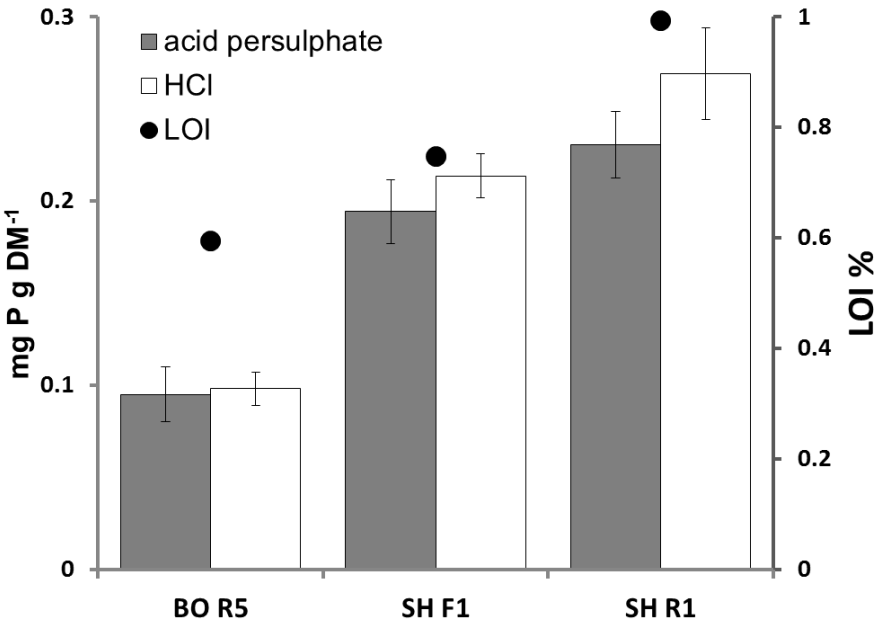

Fig. 6: HCl and acid persulphate digestions of sediment ashes (BO R5: Bodstedter Bodden / Michaelsdorf reed 5, Salzhaff / Werder SH F1: free sediment 1 as well as SH R1: reed 1). LOI: loss on ignition, horizons 2-5 cm, digestions per sample n = 4, error bars: standard deviation.

Moreover, the acid persulphate digestion extracted the same amount of TP in dried sediments and ashes (Fig. 7). There were no significant differences (p = 0.357), so that the oxidation conditions were strong enough to digest sediments of at least < 2% LOI as well as DM.

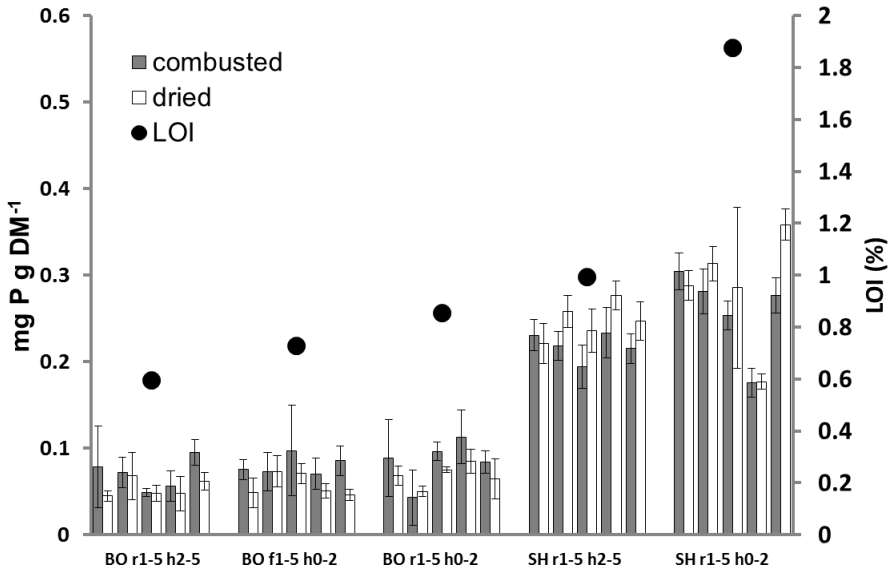

Fig. 7: Total phosphorus (mg g dry mass^{-1}) in combusted and dried sediment samples digested by acid persulphate at 90 °C for 24 h. BO: Bodstedter Bodden, SH: Salzhaff, r = reed. f = without any macrophytes, numbers are replicate cores, horizons labelled are 0 – 2 cm and 2 – 5 cm. Loss on ignition (LOI) in percent was determined in a separate core once each sample. Digestions per sample n=4, error bars: standard deviation.

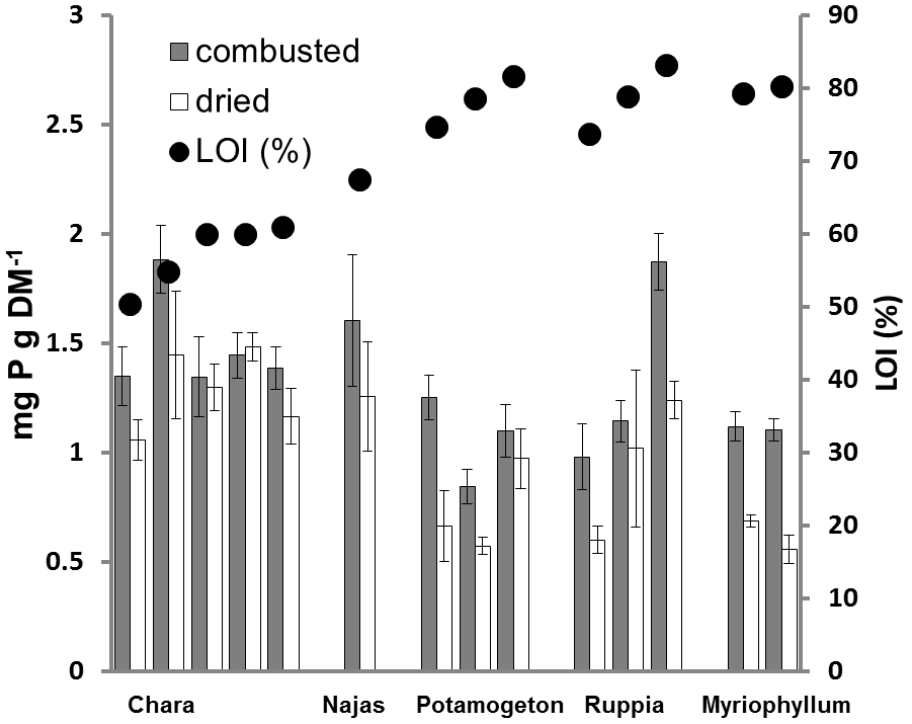

Fig. 8: Total phosphorus concentrations of different submersed macrophytes measured in dried and combusted plant material. Samples were from different plots in the DZBC out of 0.5 – 1.5 m water depth. C: *Chara* spp., N: *Najas marina*, P: *Potamogeton pectinatus*, R: *Ruppia* spp., M: *Myriophyllum* spp. Digestions per sample n=4, error bars: standard deviation.

The acid persulphate digestion extracted phosphorus better from combusted plants (Fig. 8). There was a significant difference between the extracted amount of P in dried and combusted plant samples ($p = 0.015$). The amount of P found in the extracts did not correlate with LOI.

For sediments, sample standard deviation was similar in the common high temperature HCl and sub-boiling digestion in acid persulphate (Tab. 4). However, a chemically defined standard was not found, which could be digested in HCl.

4. Discussion

One of the most used determination methods for phosphate is the molybdenum blue reaction. The measuring range is between 10 nmol l^{-1} to 10 µmol l^{-1} (PATEY et al. 2008). However, the DL was strongly dependent on the optical path length, so that the nanomolar range is only reached with cuvettes of > 10 cm. Only 24 out of 170 screened publications gave their DL (e.g. MURPHY & RILEY 1962; NAUSCH & NAUSCH 2004, 2006; TANAKA et al. 2006; VAHTERA et al. 2010). Some of those cited other authors for DLs (e.g. BALWIN et al. 2003; PASTUSZAK et al. 2003). DL should be evaluated regularly, at least once with changing equipment or personnel. The manual SRP determination was susceptible for contamination possibly by the reaction tubes. Additionally, there are interferences with silicate, arsenate and nitrate with the molybdenum blue reactions that might increase (arsenate, silicate) or decrease (nitrate) the colour intensity (e.g. JOHNSON 1971; MOTOMIZU et al. 1989; LINGE & OLDHAM 2001). The impact of nitrate is important for all phosphorus digestion procedures, which use concentrated nitric acid or *aqua regia*. Plants and other agricultural material are often digested that way (e.g. ARAÚJO NÓBREGA et al. 2007), so that the phosphorus content in the extract has to be measured with another method, e.g. by ICP.

Alkaline persulphate digested seston as well as Oxisolv®, but had best reproducibility and throughput with the sub-boiling method. The laboratory microwave used in this work has only 10 digestion places (can be as large as 40 places, but they are very expensive). A better microwave with temperature or pressure control provides most likely better results than we gained, but is not necessary for the samples and methods assembled here. For the sub-boiling treatment, ca. 100 PFA tubes can be incubated simultaneously in one medium sized oven. These tubes are pressure resistant < 3 bar so that the digestion does not need technical precautions or supervision at 90 °C. High pressure and or high temperature support only a faster digestion. The crucial point is, then, ensuring temperature or pressure control in the microwaves. If the necessary conditions can be kept, microwave digestions may be as good (and are quicker) than the sub-boiling method. The burst discs of the Teflon® reaction vessels often break, may cause harm and the samples are lost. Standards may be digested and the next sample not, so that a nominal value within the tolerance limits does not prove a good sample digestion and *vice versa*.

Reaction vessels made of plastics must be chosen very carefully. Polycarbonate tubes are resistant and were already used successfully (MAHER et al. 2002). Polyethylene or -propylene cannot be recommended, because they are not resistant enough to strong oxidizing agents. Moreover, blank extracts in centrifugation tubes made of polypropylene increased the signal strongly (BERTHOLD 2013). The slightly higher blanks in seston extracts compared to SRP blanks indicate some difficulties in

the PFA reaction vessels. The frequency of cleaning baths (1 N HCl) for reaction vessels must be increased.

Seston of turbid waters was also completely digested under low-pressure conditions as reported by MAHER et al. (2002). HUANG & ZHANG (2009) used the here described sub-boiling treatment completely without a microwave. Our results support those authors in that both methods are equally successful using alkaline persulphate digestion. However, all other problems, like storage, standards, pH and neutralisation procedures as reviewed in WORSFOLD et al. (2005), remain to be considered carefully for both digestion treatments. The accumulation of seston on filter may be wise for low concentrations, but the filters lead to elevated blanks (own observations and SUZUMURA 2008).

HCl digestion is the accepted method for sediments (ANDERSEN 1976; OSTROFSKY 2012) and was even applied to plant ashes (SIONG et al. 2006). However, the HCl digestion of sediment ashes is a tedious, dirty and hazardous procedure (open heating plate, boiling HCl, HCl vapor, hot bulb condensers, nevertheless the threat of a dried and burned sample and a low throughput). The whole procedure must be supervised continuously and hot acid material must be handled. Acid persulphate digestion had almost the same digestion strength than the common HCl digestion (ANDERSEN 1976), who found that an oxidation with persulphate recovered less (60 to 69 %) than HCl. It was possible to increase this rate up to 85 – 98 % with an acid persulphate digestion at sub-boiling temperatures, when the reaction time was long enough (24 h). ASPILA et al. (1976) found similar results, but with a high-temperature Teflon® bomb method (high pressure).

Acid persulphate digested dried and combusted sediment material just as well (not shown). It remains to be tested if that will hold true for LOI > 2 %. Therefore, all sediments still have to be combusted before digestion (e.g. DANCER et al. 1998). MALÁ & LAGOVÁ (2014) recommended also testing at least some digestion procedures for the material to be investigated. We further recommend determining water content, dry bulk density and LOI for each sample (sediment core and slice) separately. The samples have to be packed for drying and combustion anyway, so that only some (more) masses have to be noted down. The sediments of the DZBC are very heterogeneous in respect to water content and, therefore vary in density. In respect to other questions, a different strategy may be preferred, which consists of small cores (some replicates) sliced into 2-3 layers for element analyses and one large core sliced into many very small layers for water content and LOI. However, this strategy brings additional uncertainties for element contents.

Plants and algae are digested most often in concentrated acids under high temperature and or pressure (e.g. ZHELJAZKOV & MCNEIL 2008). The authors evaluated the procedures in respect to the elements that should be determined (more than phosphorus) and found that not all measures (combustion, digestion) lead to an ultimate result for all target elements. Moreover, the measuring solution had often a yellow-green colour, when DM was digested in pure acid (e.g. ARAÚJO NÓBREGA et al. 2007). The plant pigments can interfere with the photometrical measurements as well as high nitrate concentrations (from nitric acid, see introduction). The coloured extract is not a problem for ICP detection. The addition of an oxidation agent helps overcoming this problem (e.g. KELLEY et al. 1946). Combusted plant material was easier to handle (ash instead of large and heterogeneous pieces of biomass) and was often better to digest (also e.g. SOON & KALRA 1995). Most often, there is enough biomass for combustion. DM can, however, be digested, when biomass is too low. In that case, acid persulphate or another strong oxidising agent, like perchloric acid (e.g. BLINDOW

1992), shall be used. So far, the phosphorus concentrations found here are comparable to values reported in literature. Phosphorus in *Chara* spec. ranged between 1 and 2.5 mg g DM^{-1} (KUFFEL & KUFFEL 2002) and was in this study for different *Chara* spp. and thallus sizes 1.3 - 2.0 mg g DM^{-1}.

The acid persulphate digestion can also be verified with a dissolved organic standard, but the HCl digestion failed. There are an estuarine sediment and a soil standard available from the National Institute of Standards and Technology (NIST estuarine sediment). As far as we know, there is only one commercial reference material for algae, a NIST Chlorella powder. Agriculturally used plant standards, e.g. spinach, apple or citrus leaves, can be bought in a greater variety, but all are DM only. However, it is very expensive to keep an assortment of NIST standards or use them in method development. Therefore, we produced larges batches of different biomasses and ashes in addition to sediment DM and ashes with different LOI.

Neutralisation procedures are most often not mentioned. That is not necessary for ICP measurements, because this method is corrected for all kinds of matrix effects. Photometric methods require very acid conditions, but not too low pH. Moreover, very acid samples corrode autoanalysers. Therefore, we recommend a neutralisation procedure for the molybdenum blue method.

All digestion methods need their own DL determination, which are higher than those for SRP. DL for acid persulphate digestions (0.2 µmol l^{-1}) were in the range of 0.15 to 0.3 µmol l^{-1} (ROWLAND & HAYGARTH 1997; POTE & DANIEL 2000). The DL of the sub-boiling alkaline persulphate digestion (0.2 µmol l^{-1}) was also similar to literature (0.2 µmol l^{-1}, PATTON & KRYSKALLA 2003). However, DL are rarely presented in method papers and almost never in research papers. Most of the papers use certified standard material to test correctness. Many other (e.g. HUANG et al. 2004) give standard deviation of replicates, but do not combine sample and standard material variation. Sample variability shall be low, of course, but may not be minimised due to material heterogeneity, which was large for soil and sediments.

Obviously, there are many digestion and determination methods for phosphorus in different materials. Many of them work and can be used according to the aim of the study and the available equipment. We presented here a low budget method for total phosphorus digestion, which is easy to establish in most laboratories with high investments. Moreover, this method allows digesting many samples simultaneously.

Zusammenfassung

Für die Gesamtphosphorbestimmung wurde eine alternative Aufschlussmethode bei Niedrigtemperaturen (90 °C) für Seston erfolgreich getestet und für Sedimente sowie Pflanzenbiomasse angepasst. Die Aufschlussreagenzien bestanden aus basischer oder saurer Kaliumperoxodisulphatlösung. Nur einfache Laborausstattung, wie Muffelofen, Trockenschrank und Photometer, ist notwendig. Die relativ kleinen Reaktionsgefäße (30 ml PFA Röhrchen und 20 ml Reagenzgläser) ermöglichen einen hohen Probendurchsatz. Einige Sestonproben wurden im Trockenschrank besser aufgeschlossen als in der Mikrowelle. Bei den Sedimentaschen gab es keine signifikanten Unterschiede zwischen kochendem HCl- und saurem Persulphataufschluss. Die z. B. in *Chara* spp. gefundenen Phosphorkonzentrationen betrugen 1.3 - 2.0 mg g Trockenmasse^{-1} und sind mit anderen Literaturquellen vergleichbar. Die neuen Bestimmungsgrenzen waren ähnlich oder besser als die der Mikrowellenaufschlüsse. Die besten Bestimmungsgrenzen betrugen 0.12 und 0.19

µmol l^{-1} für basisches bzw. saures Persulphat. Die kombinierten Standard-unsicherheiten betrugen für beide Reagenzien 8.7 % bzw. 12%.

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