Determination of the phosphorus content in sewage sludge: comparison of different aqua regia digestion methods and ICP-OES, ICP-MS, and photometric determination

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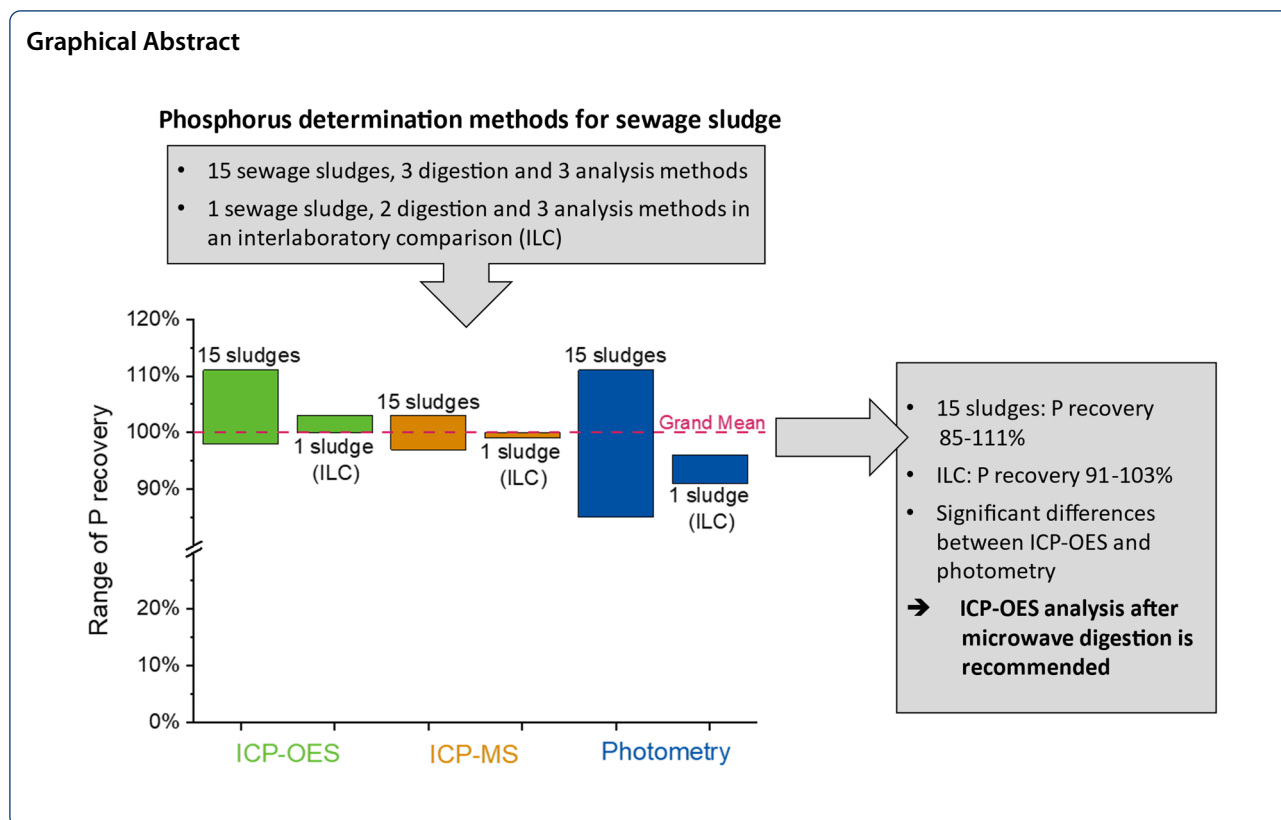
Abstract

Phosphorus recycling from sewage sludge will be obligatory in Germany from 2029. Phosphorus content determination in sewage sludge is crucial to assess the prescribed recycling rates. Currently, German law regards sample preparation using aqua regia digestion in a microwave or under reflux conditions as well as instrumental phosphorus determination by ICP-OES, ICP-MS, or photometric determination with ammonium molybdate as equivalent. However, it is questionable whether these methods are indeed equivalent regarding phosphorus quantification in sludges near the limit of 20 g/kg for mandatory recycling. To answer this question, 15 sewage sludges of 11 different wastewater treatment plants were investigated with all permitted method (digestion and measurement) combinations. Moreover, one sewage sludge was also examined in an interlaboratory comparison (ILC) with 28 participants. This study shows that the above-mentioned methods differ in some cases significantly but across all method combinations and sludges, phosphorus recovery was between 80 and 121% after normalization to the grand mean (average of 15 sludges between 85 and 111%). The ILC and the examination of 15 sludges produced largely similar results. There is a tendency to higher phosphorus recovery after microwave digestion compared to reflux digestion and ICP-OES measurements determine higher phosphorus contents than ICP-MS and photometric phosphorus determination. As a result, the authors recommend ICP-OES determination of phosphorus in sewage sludge after microwave digestion.

Keywords: Sewage sludge, Phosphorus recycling, Reproducibility, Repeatability, ICP-OES, ICP-MS, Photometry, Interlaboratory comparison

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Graphical Abstract

Phosphorus determination methods for sewage sludge

- 15 sewage sludges, 3 digestion and 3 analysis methods
- 1 sewage sludge, 2 digestion and 3 analysis methods in an interlaboratory comparison (ILC)

- 15 sludges: P recovery 85-111%
- ILC: P recovery 91-103%
- Significant differences between ICP-OES and photometry
- ➜ **ICP-OES analysis after microwave digestion is recommended**

Background

Wastewater and sewage sludge as phosphorus sources

Human excreta, wastewater, and sewage sludge are notable sources for the essential plant nutrient phosphorus. The total phosphorus potential in all human urine and feces accounts for about 13–22% of the global fertilizers demand according to different scenarios [15, 20, 22]. However, Kok et al. [15] expect only small shares like 4% of this discharged phosphorus potentially recoverable under the current economic conditions. In 2009, Cordell et al. [9] found that there was no global priority in politics to ensure sufficient phosphorus accessibility in future. They recommended national governments and international organizations to give greater value on phosphorus recycling to avoid future scarcity. So far, politics changed to a certain extend. In 2014, the European Union firstly listed phosphate rock as critical raw material [10].

As a result, there are tentative governmental efforts to stimulate phosphorus recycling from wastewater and sewage sludge in Europe. Switzerland was the first country with an obligatory phosphorus recycling[1] (from

2026) from wastewater or sewage sludge and other waste streams that are rich in phosphorus [25]. Austria aims to recycle phosphorus from 65 to 85% of the national sewage sludge by 2030; however, a binding regulation is still missing [7]. In Germany, phosphorus recycling from sewage sludge is mandatory from 2029 [4]. In 2019, the European fertilizers ordinance [3] was amended in order to harmonize national fertilizer regulations and to open the market for recycled fertilizer products.

German legislation

The German phosphorus recycling obligation applies for all wastewater treatment plants (WWTPs) with more than 20 g/kg phosphorus (related to the dry matter) in their sewage sludge. Apart from technical phosphorus recycling, agricultural sewage sludge utilization is also considered to be phosphorus recycling. However, agricultural sewage sludge utilization is not permitted for sludges from WWTPs with more than 100,000 population equivalents from 2029 and for sludges from WWTPs > 50,000 population equivalents from 2032.

Processes that recover phosphorus must fulfill a recycling rate of at least 80% in case of recycling from sewage sludge ash and 50% in case of recycling from sewage sludge. Otherwise, the recycling from sewage sludge is also sufficient if the remaining sludge after phosphorus

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[1] This phosphorus "recycling" is rather to be understood as phosphorus recovery (many technical recycling processes do not allow a full material recycling but a phosphorus extraction). However, to avoid confusions with the analytical phosphorus recovery, phosphorus "recycling" was chosen for terminology.

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Table 1 German EN (ISO) standards equivalent by ordinance [4] for phosphorus determination in sewage sludge

Standard	Volume	Description
EN 16174	2012	Sludge, treated biowaste and soil—Digestion of aqua regia soluble fractions of elements
EN 13346	2001	Characterization of sludges—Determination of trace elements and phosphorus—Aqua regia extraction methods
EN ISO 11885	2009	Water quality—Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)
EN 16170	2017	Sludge, treated biowaste and soil—Determination of elements using inductively coupled plasma optical emission spectrometry (ICP-OES)
EN ISO 17294–2	2017	Water quality—Application of inductively coupled plasma mass spectrometry (ICP-MS) – Part 2: Determination of selected elements including uranium isotopes
EN 16171	2017	Sludge, treated biowaste, and soil—Determination of elements using inductively coupled plasma mass spectrometry (ICP-MS)
EN ISO 6878	2004	Water quality—Determination of phosphorus—Ammonium molybdate spectrometric method

extraction has a phosphorus content below the recycling limit of 20 g/kg. Consequently, the determination of the recycling rate or the question whether a WWTP falls below the recycling obligation requires a reliable phosphorus quantification in the respective sewage sludge.

Analytical background

The German sewage sludge ordinance [4] mentions three different phosphorus determination methods and two digestion methods regarded equivalent for phosphorus quantification in sewage sludge (Table 1). These are inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), and photometric phosphorus determination with ammonium molybdate. Aqua regia digestion is the only permitted sample preparation and can be performed in a microwave or under reflux conditions.

This paper aims to clarify whether these extraction and determination methods are indeed equivalent (as stated by ordinance) regarding the phosphorus content results in municipal sewage sludges. It should be noted that none of the equivalent standards (Table 1) does list any process characteristics like precision values from inter-laboratory comparisons (ILCs) for phosphorus determination in sewage sludge matrix.

In their sewage sludge management review (also about analytical methods), Cieslik et al. [8] list ICP-OES, photometry, X-ray fluorescence (XRF), and nuclear magnetic resonance as applied technologies for phosphorus determination in recycling products from sewage sludge, whereas ICP-MS is mainly listed for several trace elements. As phosphorus does not display more than one stable isotope, ICP-MS measurements cannot be based on isotope dilution whereas several emission lines are available for ICP-OES measurements.

Photometric phosphorus determination with ammonium molybdate was originally developed for water samples with low phosphorus concentrations << 1 mg/l [27]. The calibration linearity is dependent on the potassium tartrate concentration [19] and limited to a maximum of 0.8 mg P/l [18, 23]. In terms of water quality monitoring, photometric phosphorus determination with ammonium molybdate offers opportunities for green analytic chemistry as devices can be portable, use of chemicals is low, and it represents a highly sensitive and robust method for water samples [23].

However, sewage sludge is an entirely different matrix compared to water especially regarding the high content of inorganic and organic solids. A digestion solution of sewage sludge with low phosphorus content (e. g. 15 g/kg) contains still 150 mg P/l (microwave digestion: 500 mg sludge in 50 ml) or 450 mg P/l (reflux digestion: 3000 mg sludge in 100 ml). Malý [19] also recommended this method for sewage sludge analysis. However, interferences by iron were detected and the digestion solutions were highly diluted to phosphorus concentrations of less than 0.1 mg/l.

Krüger and Adam [16] already found that organic matrices affect phosphorus digestion and recommended aqua regia ($HCl:HNO_3 = 3:1$) as suitable digestion reagent for total phosphorus detection by ICP-OES in sewage sludge. However, regarding studies on photometric phosphorus quantification, sewage sludge, wastewater, or sediment samples were not digested with aqua regia but with different ratios of H_2O_2, HCl, $HClO_4$, HF, and H_2SO_4 [18, 19, 23].

Moreover, ISO 6878 (photometric determination) required by AbfKlärV [4] does not include aqua regia digestion but digestion or oxidation with organic solvents, H_2SO_4, $K_2S_2O_8$, or HNO_3 and H_2SO_4. However, aqua regia digestion is the only permitted sample

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Table 2 List of investigated sewage sludges 1–15 with element contents in g/kg related to dry matter

Sludge no	WWTP		P	Ca	C	Fe
1		1	19	24	432	35
2	1		22	26	417	43
3		2	36	33	266	44
4		3	21	150	203	42
5	4		21	187	193	50
6	4		17	196	218	38
7	5		17	239	168	35
8	5		22	12	332	26
9		7	23	20	310	14
10	8		17	8	423	15
11	8		24	11	401	18
12	9		17	207	160	43
13	10		20	34	324	30
14		11	38	34	371	10
15	Sludge mixture		26	115	282	35

digestion by ordinance; thus, aqua regia digestions are analyzed according to "Conclusions" (detection of orthophosphate) of the standard. It is therefore questionable whether aqua regia digestion, which is unusual in this context, affects photometric phosphorus determination with ammonium molybdate.

Materials and methods
Characterization of sewage sludges
15 sewage sludges from 11 WWTPs were chosen for the investigation. Detailed information about the WWTPs and the sludge characterization is given in Additional file 1: Tables S1 and S2. Details on the sludge characterization are presented in Table 2. The carbon content was obtained with elemental analysis (Elementar Analysensysteme, Langenselbold, Germany); other elemental contents were determined using duplicate microwave aqua regia digestion (EN 16174) and ICP-OES measurement (ISO 11885). With a moisture measurement (Kilomatic, Hannover, Germany) of the dried sludges, elemental contents were related to the dry matter.

Sludge no. 15 was a mixture of different sewage sludges and investigated in an ILC. Phosphorus, calcium, and iron content were obtained by XRF (PANalytical, Almelo, Netherlands) investigations during the homogeneity study carried out for the ILC (Interlaboratory comparison). Based on the characterization details, three groups with special matrix features can be distinguished:

1. sludges rich in phosphorus (≥ 30 g/kg: 3, 14),
2. sludges rich in calcium (≥ 150 g/kg: 4, 5, 6, 7, 12), and
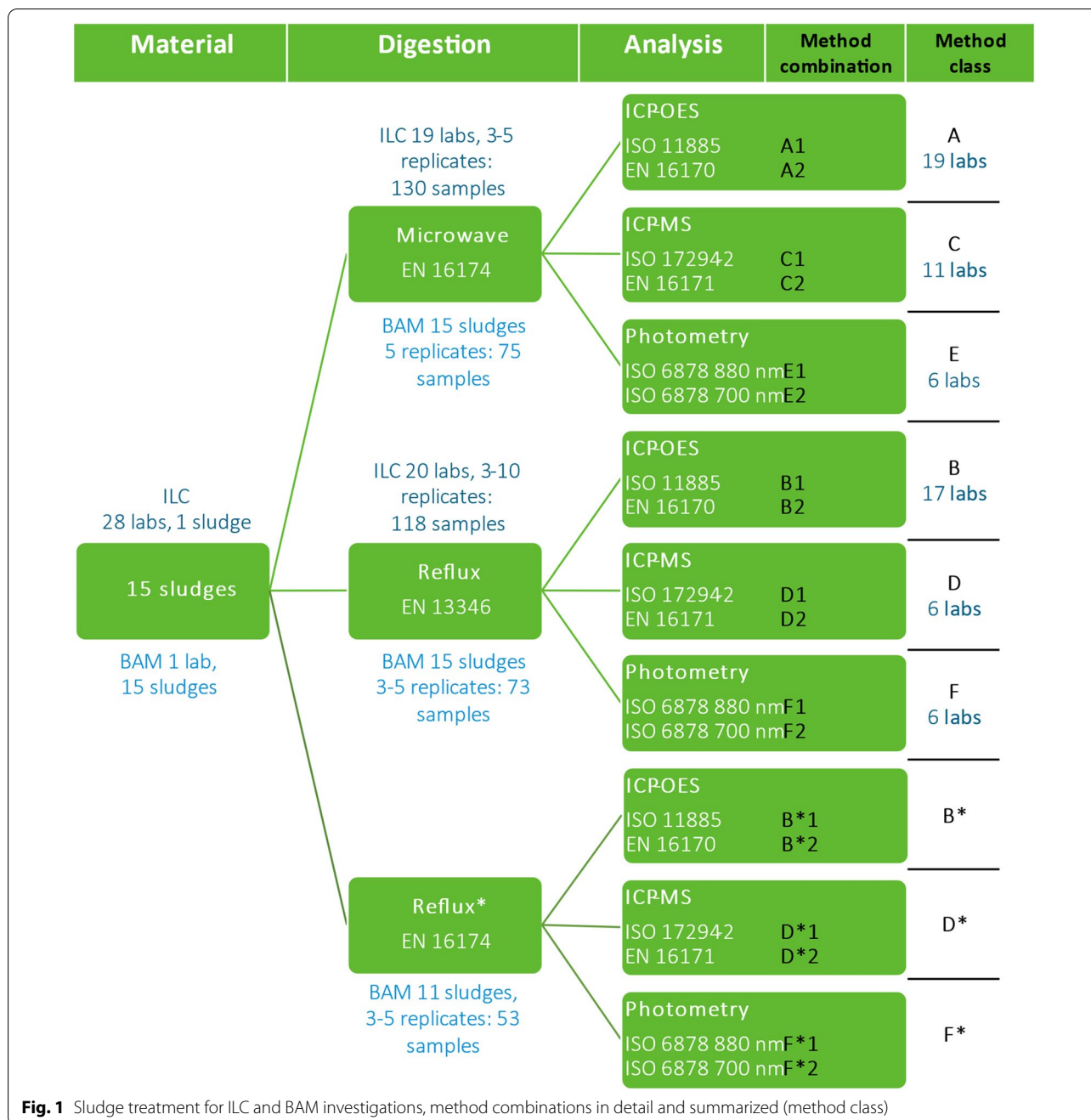3. sludges rich in carbon (≥ 400 g/kg: 1, 2, 10, 11).

Sewage sludge 15 was obtained by blending sludges from WWTP 9, 10, and 11 to receive a more representative sewage sludge test material which is not specially leaning to either one of the three categories. Details on the homogenization and bottling of the sewage sludge no. 15 are shown in Interlaboratory comparison.

Sewage sludge preparation and analytical procedures
The sewage sludges had been freeze-dried (STERIS plc, Derby, United Kingdom) and ground in a disk mill (Eaton Industries, Bonn, Germany) for former investigations and had been stored for at least several months at ambient temperature (20 °C). All sludges were digested with aqua regia in a microwave (MLS, Leutkirch, Germany) according to EN 16174 with respective sample intakes of 500 mg and five replicates per sludge (Fig. 1). BAM: Bundesanstalt für Materialforschung und -prüfung (author's investigations) ILC: Interlaboratory comparison *EN 13,346 prescribes 28 ml aqua regia for reflux digestion. EN 16,174 prescribes extra HNO_3 additions for reflux (= Reflux*) digestion in case of sludges with high organic carbon content (more than 500 mg of the 3000 mg sample intake: 1 ml HNO_3 for 100 mg additional organic carbon).

In addition, all sludges were digested under reflux conditions (EN 13346) using 3000 mg sample intake with five replicates per sludge. However, according to EN 16174, 28 ml aqua regia is only sufficient to digest 500 mg organic carbon. Therefore, 11 of 15 sludges (those with more than 500 mg organic carbon on 3000 mg sample intake) were also treated with additional HNO_3 depending on the carbon content during a further, expanded reflux digestion (EN 13346).

Microwave digestions were filtered (LABSOLUTE type 1005, 12–15 µm) and made up to 50 ml with distilled water. Reflux digestion solutions were filtered (LABSOLUTE type 2015, 5–8 µm) and made up to 100 ml with distilled water. Seven blank digestions were performed during microwave and reflux digestion. For the measurement, two sewage sludge certified reference materials (product IDs CRM029-50G and CRM031-40, Sigma-Aldrich, Laramie, USA) were digested as duplicates in the microwave and under reflux conditions. The phosphorus recovery rate from the reference materials was between 80 and 109%, on average at 101% (CRM029-50G) and 95% (CRM031-40G). Details are shown in Additional file 1: Table S3 and Figure S2. Blank values were below 0.3 mg/l (the phosphorus concentration in digestion solutions always > 100 mg/l). In total, there were 201 digestions of the 15 sludges (four digestions failed: sludge 15 reflux and expanded reflux), seven blank digestions, and four digestions of reference sewage sludges. The digestates were analyzed for phosphorus

Fig. 1 Sludge treatment for ILC and BAM investigations, method combinations in detail and summarized (method class)

with all methods considered equivalent by German regulations. The different combinations of analysis and digestion method were described as method combination A1–F*2 in detail and summarized as method classes A–F* (Fig. 1). Phosphorus contents were related to total sample intake substance instead of dry matter.

Photometric determination (Analytik Jena, Jena, Germany) with ammonium molybdate was done based on a 10-point external calibration (0.08–0.80 µg/l) which

was conducted twice. Analysis and calibration were performed and evaluated at 700 and 880 nm with a coefficient of determination R^2 of 99.94%. ICP-OES (Thermo Fisher, Waltham, USA) measurement was done based on a daily five-point external calibration (30–600 mg/l) at different emission lines axially (213.6 nm) and radially (277.5 and 178.2 nm) with R^2 of 99.92%. However, only the line with the best daily recovery rate (of reference materials, spiking and control standards) was

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evaluated (for most measurement days 213.6 nm). Microwave samples were not diluted before measurement. Reflux samples were diluted by a factor between 2 and 5. Each digested sample was analyzed in triplicate. ICP-MS (Thermo Fisher, Waltham, USA) measurement was done based on a daily five-point external calibration (0.02–1.00 mg/l) with a R^2 of 99.98%. Microwave samples were diluted by the factor 1000 and reflux samples were diluted by the factor 2000 before measurement. Each digestion solution was analyzed in three runs. An internal standard (100 µg/l In, Re, and Ru; elemental standards 1000 mg/l of Merck, Darmstadt, Germany) was used during the ICP-MS measurements. An overview of different calibrations is shown in Additional file 1: Figure S1.

ICP data were collected and processed with Qtegra (Thermo Fisher, Waltham, USA) software. Matrix effects at ICP-OES and ICP-MS were tested by spiking one replicate per sludge and digestion method and by 1:5 dilution according to EN 16170/16171. Elemental P standards (10,000 mg/l ICP-OES; 1,000 mg/l ICP-MS) of Merck (Darmstadt, Germany) were used to spike additional phosphorus between 40 and 130% (spiking factor 0.4–1.3) of the previously determined concentration (Additional file 1: Table S4). One calibration point (3, 4, and 5 depending on the measurement day) was used as control standard every 10–25 sample measurements to monitor the instrumental drift (deviations < 3%).

Interlaboratory comparison

One sewage sludge (no. 15) was also investigated in an ILC. This material was a mixture of different sewage sludges from three WWTPs (Characterization of sewage sludges). These sludges were sieved and the fractions below 200 µm were unified and homogenized for 12 h in a drum hoop mixer (Engelsmann, Ludwigshafen, Germany).

Afterward, the mixture was bottled by a spinning riffler (Retsch, Haan, Germany) with a cross-riffling step (Additional file 1: Figure S4) in 64 units of about 54 g. The homogeneity of the test sewage sludge was investigated by powder XRF based on a one-point calibration. The XRF device determined phosphorus, silicon, calcium, iron, and aluminum. Out of the 64 bottles, 10 bottled units in regular intervals from the line of bottling (at least one of each cross-riffling step) were chosen for the homogeneity study. They were not used in the ILC later. Triplicates (about 1,500 mg each) were taken from each bottle and measured by XRF. After that, the triplicates were shaken up to generate a new surface and measured again which appear reasonable because of the low effectively measured sample intake. Emergence depth of the characteristic radiation (max. 0.1 mm), sample holder diameter (27 mm), and bulk density give an effectively

analyzed sample intake of only 25 mg. To consider the drift of the device, sample 1–1 was measured six times in the beginning and after the shaking. Moreover, the bottle replicates (e. g. 1–1,1–2, 1–3) were not measured one after another but in the order 1–1, 2–1, 3–1, … 10–1, 1–2, 2–2, 3–2… etc., to reduce drift influences.

Participating laboratories were addressed by different distributors: Invitations were widely distributed, e. g., via German DIN standards committees, federal ministries, and the federal environmental agencies. Moreover, laboratories with corresponding accreditations were specifically searched for via the German accreditation agency. Each laboratory received a bottle containing 52–56 g of sludge no. 15, a description of tasks and an excel file for the documentation of analytical results, moisture content, device parameters, and instrumental settings.

The laboratories were asked to add no additional HNO_3 to their reflux digestion solutions dependent on the carbon content (explanation Fig. 1), so the resulting method classes were restricted to A-F and no "enhanced refluxes" (which are marked by a star in their abbreviation, Fig. 1). At least three independent digestions per method class were requested for the phosphorus determination. Results were related to the original substance and moisture content should be determined separately as duplicates. 27 German laboratories and one Austrian laboratory participated in the ILC. All digestion and analysis methods according to the standards in Fig. 1 plus ISO 54321:2021 (aqua regia digestion) were permitted. Performance of several method classes per laboratory was permitted and highly encouraged.

Data treatment

Processing of data obtained at the authors' laboratory included 1- and 2-way ANOVA as well as *t*-tests using Microsoft Excel version 2018. For a comparison of the phosphorus recovery of all sludges and statistical evaluation, results have been normalized to the grand mean and to the mean by digestion type. All laboratory data from ILC were treated anonymously. The ILC results were evaluated with the program PROLab Plus (QuoData, Dresden, Germany) according to Ref. [1].

Results and discussion

Phosphorus determination in 15 sewage sludges

The spiking and dilution during ICP-OES and ICP-MS measurement campaign for quality control do not indicate measurement faults caused by interference effects. A complete overview of the spiking and dilution recovery rates by sludge is given in Additional file 1: Table S4. Figure 2 shows all measured phosphorus contents in the 15 sludges by sample and method combination in detail
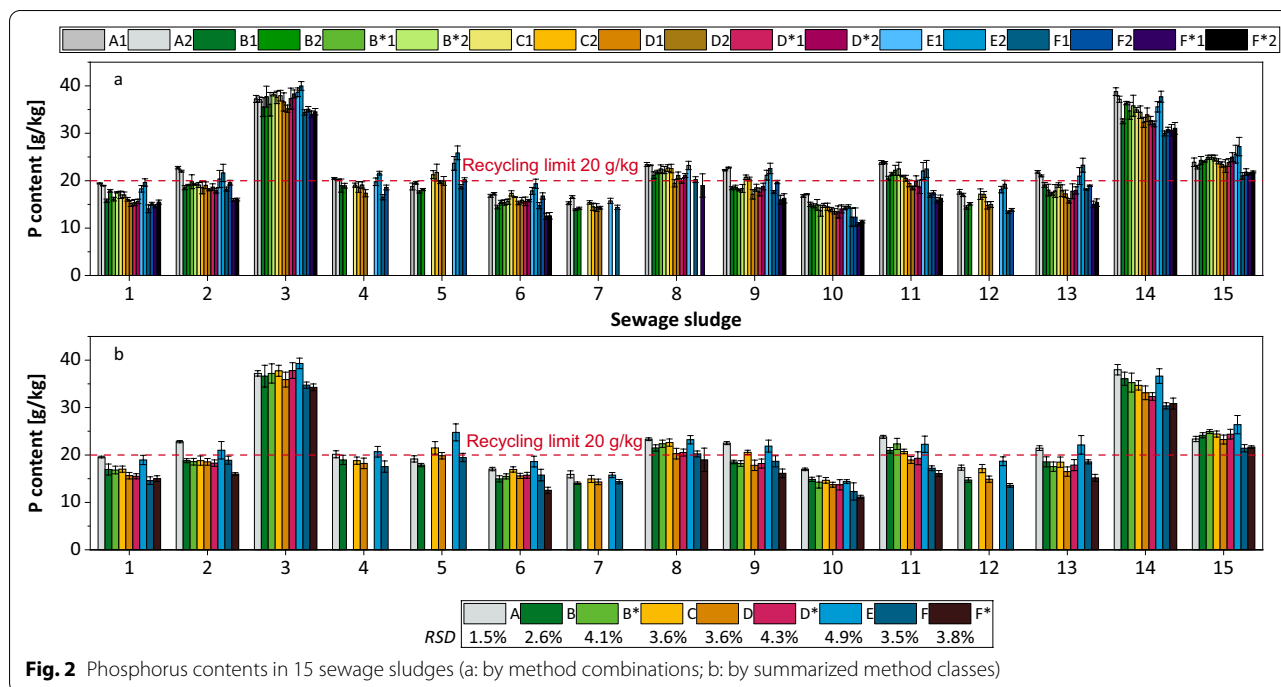
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Fig. 2 Phosphorus contents in 15 sewage sludges (a: by method combinations; b: by summarized method classes)

as well as summarized according to the method classes. Individual data of averages and standard deviations are shown in Additional file 1: Tables S5, S6.
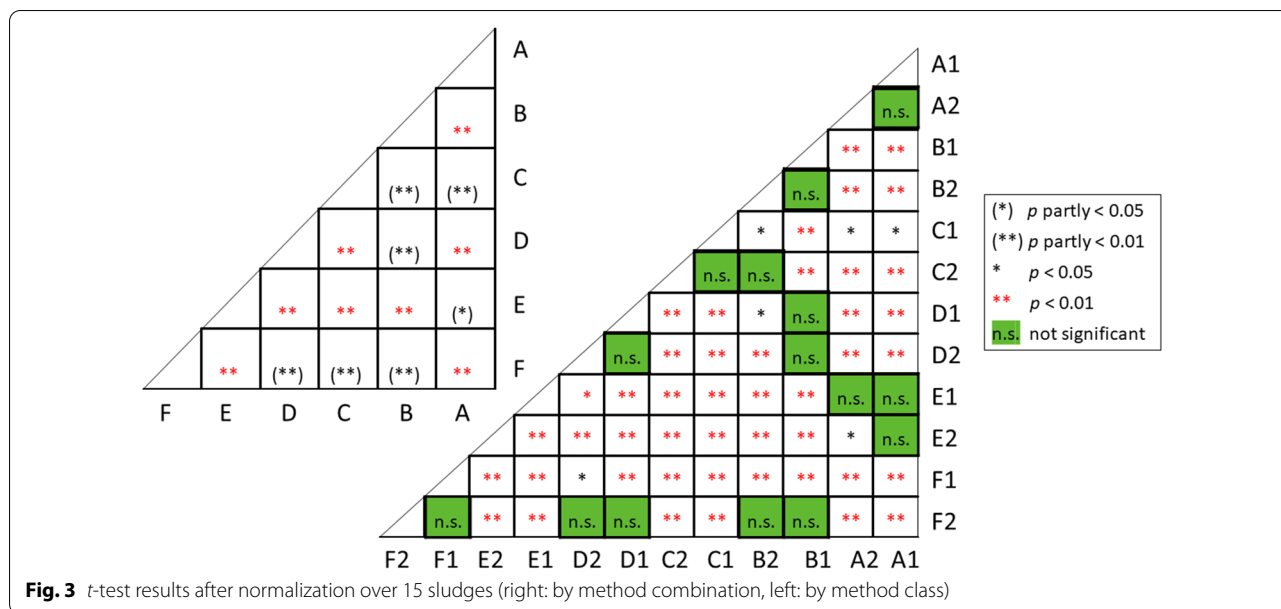
In many cases, ICP-OES determination after microwave digestion (method class A) provides the highest or second highest phosphorus recovery. This method class is additionally characterized by a low relative standard deviation[2] (*RSD*) of 1.5% on average. Other method classes tend to show higher *RSDs* between 2.6 and 4.9%.

ICP-MS precision can be significantly higher than ICP-OES regarding trace elements in lake water [5]. However, previous studies already found difficulties in phosphorus determination in wastewater by ICP-MS, such as high blank values [28] or background interferences with nitrogen in protein samples [6]. Moreover, there are indications that the lowest point of ICP-MS-calibration for phosphorus (20 µg/l) is close to the detection limit as reported in former studies [6, 11], whereas the highest points of calibration are close to 10,000 counts per second (device protection). Therefore, the dilution factors were chosen so that the concentration of the analyzed solution was between the third and fourth point of

calibration (200–500 µg/l). The ICP-MS advantage of isotope dilution is not possible for phosphorus. Literature on ICP-MS phosphorus determination in sewage sludge is scarce. Westerhoff et al. [26] determined phosphorus and 57 other elements in sewage sludge by ICP-MS and did not report similar problems; they give a lower detection limit for phosphorus at 6 µg/l in the digestion solution. However, standard deviations and detection limits were not described in this study or in the supporting data. Averages and standard deviations (n = 5). Below: average relative standard deviation (*RSD*) over all sludges (n = 15) by method class. Explanation of method abbreviation in Fig. 1.

Apart from instrumental phosphorus quantification, sample preparation seems to also affect the phosphorus recovery. However, comparisons of aqua regia microwave and reflux digestion of similar sample matrix are rare in literature. The best of the authors' knowledge is a tendency for higher yields after microwave than after reflux digestion according to Nieuwenhuize et al. [21] for Cd, Cu, Pb, Mn, Fe, and Zn in sediment and soil samples. Compared to other digestion procedures, such as heating block, microwave digestion shows higher precision for phosphorus and other elements despite lower sample intakes in plant leaves [11]. Generally, microwave digestion is currently highly accepted for the analysis of sewage sludge, sewage sludge ash, or recycled products from both [12, 16, 17, 24].

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[2] Relative standard deviation (*RSD*) and coefficient of variation (*CV*) describe the measurement variability within a given laboratory. CV_R and CV_r describe the reproducibility and the repeatability standard deviation among all laboratories participating in the ILC. To avoid confusions, *RSD* was chosen for the variability in the same laboratory and *CV* was chosen for the variability among different labs.

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Fig. 3 *t*-test results after normalization over 15 sludges (right: by method combination, left: by method class)

Photometric determination was developed for wastewater and water samples with low phosphorus concentrations $< < 1$ mg/l and digestions other than aqua regia. There was no literature found for photometric phosphorus determination with ammonium molybdate in sewage sludge after aqua regia digestion (Analytical background). Previous studies on phosphorus determination with ammonium molybdate found interfering effects of Fe^{2+} and Fe^{3+} ions [18, 19]. These potential redox partners interrupt the reduction of phosphomolybdic acid starting from concentrations of only 1.5 mmol/l or 0.08 mg/l [18]. The sewage sludges in this study contain about 10–50 g Fe/kg – this is equivalent to clearly higher iron concentrations in the samples of 0.10–0.75 mg/l (calculation based on digestion sample intakes and dilution factors). Although ascorbic acid can minimize these effects, high temperatures or longer reaction times are required for iron reduction [19] than those according to ISO 6878.

Statistical evaluation
To evaluate significant differences in the phosphorus recovery rates *t*-tests were performed for each pair of method combinations and of the method classes (Fig. 3). For this reason, phosphorus results are normalized to the grand mean by sludge (mean value of all different method combinations). The *t*-tests over 15 sludges (without expanded reflux: method classes B*, D*, and F*) show that results from method combinations of the same class are not significantly different with the except of E1 and E2: The photometric determination at 700 nm provides higher phosphorus results (average $+5\%$, maximum $+14\%$) than the most often

performed determination at 880 nm. For the microwave digestions (method combinations E1 and E2), these differences are significant ($p < 0.01$). Different *t*-test results for method combinations of the same method class were summarized as "partly < 0.05" or "partly < 0.01." Explanation of method abbreviations in Fig. 1

Apart from same method classes, hardly significant differences are found between method classes A and E (ICP-OES and photometric determination after microwave digestion). ICP-OES and ICP-MS determination also provide no significantly different results for the method combinations B1 and D1 or D2, and B2 and C2. Moreover, ICP-OES and ICP-MS determinations after reflux digestion (B1, B2, D1, D2) are similar to photometric determination at 700 nm after reflux digestion (F2). Other method combinations provide significantly different results ($p < 0.05$ or $p < 0.01$).

In summary it can be stated that method combinations of the same class show mostly no significant differences. Results from method class E are significantly different ($p < 0.01$) from B–F. Overall, the methods do not appear to be equivalent. With respect to the low overall variation of less than $\pm 21\%$ between minimum and maximum phosphorus recovery by method class (among all 15 sludges), however, they should nevertheless be regarded as sufficiently comparable.

The observed effects of digestion and instrumental determination are also found in a 2-way ANOVA of this normalized dataset. This two-way ANOVA (with the factors digestion method and analytical method) over the normalized phosphorus results in 15 sludges supports the found differences as both digestion and

Fig. 4 Phosphorus recovery by method class after normalization (a: grand mean, b: mean by digestion)
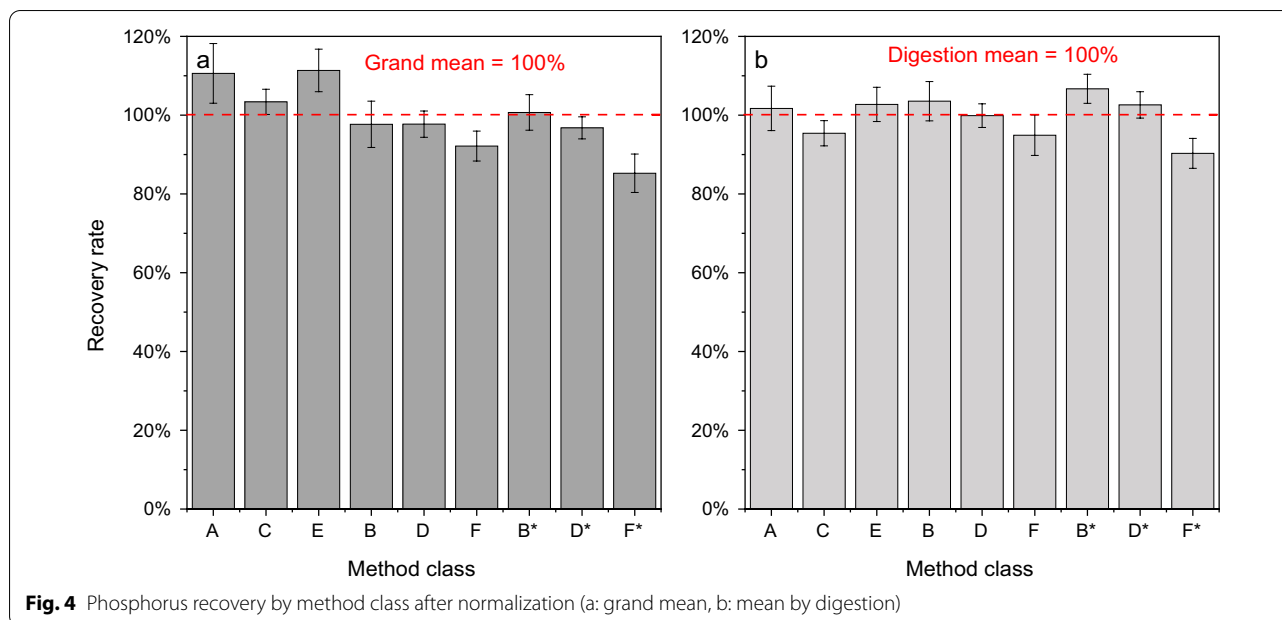
analytical methods have significant effects and an interaction was also found (Additional file 1: Table S9).

Comparison of phosphorus recovery across the sludges
Since sludges 1–15 display different phosphorus contents, the complete set of analytical data needs to be normalized to investigate the effect of digestion and instrumental phosphorus quantification procedures. As the "true" phosphorus contents are unknown, the recovery from all analytical procedures (grand mean) is used to normalize the data for each of the 15 sludges as performed for the *t*-tests and ANOVA (Statistical evaluation). Additionally, the phosphorus recovery data for each sludge are normalized (Fig. 4) to the respective averages of the digestion procedures, such as microwave (method classes A, C, and E), reflux (B, D, and F), and reflux with additional acid (B*, D*, and F*). Although normalization may involve risks of biases associated with differing matrix compositions, these influences should be limited due to the variety among the 15 different sewage sludges. A summary of the recovery rates from all 15 sludges for each method class is shown in Fig. 4. Some trends are visible.

Averages and standard deviations over all sludges (n = 15), explanation of method classes in Fig. 1 Compared to the grand mean, the analysis of 15 sewage sludges shows especially higher phosphorus content results for method classes A, C, and E, which are the microwave digestions. If the digestion factor is excluded by normalization, method classes A, E, B, and B* show the highest results. These method classes are all classes

with ICP-OES analysis or the photometric determination after microwave digestion.

The difference between the analytical methods is highest for the expanded reflux digestion with additional HNO_3 addition which was not tested by ANOVA as not all sludges have been treated this way (Interlaboratory comparison). A reason might be the amended acid which could affect the photometric determination. The acid is changed the most in case of sludges 1, 2, 10, and 11 due to their high carbon contents of about 400 g/kg (for the acid addition per sample see Additional file 1: Table S2). At the same time, carbon-rich sludges (1, 2, 10, and 11) show averagely higher differences between ICP-OES and photometry (F* and A normalized to the grand mean; F* and B* normalized to digestion). Two of these carbon-rich sludges show the highest difference in total with a recovery rate between 80% for method class F* and 118–121% for method class A. On the other hand, there is no general statement possible for the samples 4–7 and 12 that are rich in calcium (> 150 g/kg). Although sludges with high calcium contents show difficulties during reflux digestion as they high-foam when acid is added, the P recovery rate between reflux and microwave digestion is not clearly different to other sludges. More details on the recovery rate per sludge and method class are given in Additional file 1: Figure S3 and Tables S7, S8.

Finally, microwave digestion produces higher phosphorus results than reflux digestion in all 15 cases. ICP-OES measurement gives higher or similar (± 2% recovery rate) phosphorus results than ICP-MS and photometric determination in reflux and expanded reflux digestion with
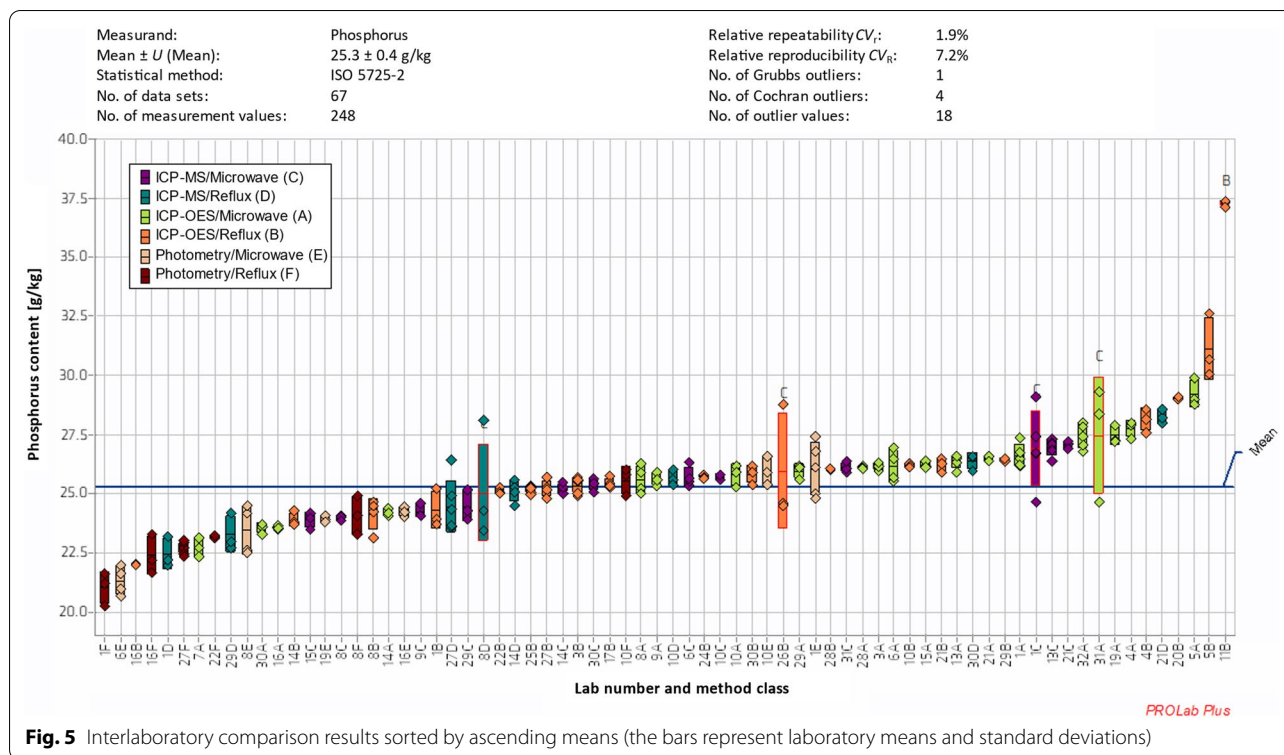
Fig. 5 Interlaboratory comparison results sorted by ascending means (the bars represent laboratory means and standard deviations)

more HNO_3 addition in 14 out of 15 cases. The samples from microwave digestion show high recovery rates for photometric determination, too.

Summarized over all sludges, ICP-OES determination provides higher phosphorus results than ICP-MS and photometric determination and microwave digestion provides higher results than reflux digestion. However, averaged over all 15 sludges, the recovery rate is between maximum 111% (A and E) and minimum 85% in method class F* (Fig. 4). If the digestion factor is excluded by normalization to the digestion mean, this variability is lower than ± 10% with a maximum recovery of 107% for method class B* and a minimum recovery of 90% for method class F*.

Interlaboratory comparison

The homogeneity study of the test material (sludge no. 15) shows no significant differences for phosphorus between the 10 bottles (Additional file 1: Table S11). However, after shaking, silicon values apparently drop by 6% on average and calcium values raise by + 11% on average. Density-dependent separation effects from shaking could be a reason for these results.[3] As a result, laboratories were told to change the sample before analysis in

a larger container and to shake it overhead before taking the replicates. The drift control of sample 1–1 resulted in higher values for all elements (2–12%). However, systematic effects on the samples are minimized by the order of the samples (Interlaboratory comparison). Moreover, phosphorus values stay constant after shaking with an *RSD* of 2.3%, and the inhomogeneity between the individual bottles u_{bb} was calculated at 0.6% according to the ISO-guide 35:2017 [2]. The XRF data are shown in Additional file 1: Figure S5 and Table S10.

Figure 5 shows the complete set of data provided by the participants. It is visually obvious that the methods tended to produce different results. Clearly, ICP-OES yields on average higher P recoveries than photometry.

Five outliers were found out of 72 different means of which four were Cochran outliers (higher *RSD*) and one was a Grubbs outlier (higher mean). Outliers were eliminated for further calculations. Due to the low reported CV_r, Cochran outliers should be carefully looked at – however, three of the four Cochran outliers show a clearly higher CV_r of more than 8%. A graphic presentation of all laboratory method class results sorted by analytical methods is shown in Additional file 1: Figure S6. The results were also evaluated by method class, instrumental analysis, and digestion method, which causes differing outlier test results (Table 3). The method class lab code is shown on the x-axis and represents the lab

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[3] XRF analyzes the sample top layer to a depth of 0.1 mm for the sludge matrix (arithmetical sample intake of 25 mg). Shaking can cause denser particles to sink to the bottom and less dense particles to accumulate at the top.

Table 3 ILC phosphorus results for different method classes (Fig. 1) and by digestion or analysis method

Method (class)	*I*	*n*	Outliers	$\bar{x}$ [g/kg]	*U* [g/kg]	CV_r [%]	CV_R [%]	HorRat	$\frac{CV_r}{CV_R}$ [%]
A	67	19	1	26.0	0.7	1.5	5.8	1.7	25
B	59	17	3	25.4	0.7	1.3	5.7	1.6	22
C	33	11	1	25.4	0.7	1.3	4.6	1.3	28
D	29	8		25.0	1.2	4.1	7.5	2.1	55
E	22	6		24.2	1.5	3.3	8.1	2.3	41
F	22	6		23.1	1.2	2.3	6.5	1.8	35
ICP-OES	138	36	4	25.7	0.5	1.4	5.8	1.7	24
ICP-MS	63	18	2	25.2	0.7	2.1	5.8	1.7	36
Photometry	44	12		23.6	1.0	2.9	7.5	2.1	39
Microwave	130	36	2	25.0	0.3	1.8	6.3	1.8	29
Reflux	118	31	3	24.2	0.5	2.1	8.0	2.3	27
Total	248	67	5	25.3	0.7	1.9	7.2	2.0	26

I: Number of measurement values: replicates included

n: Number of datasets: different lab averages by method class (several labs performed more than one method class), no outliers

$\bar{x}$: Mean phosphorus content

U: Expanded Uncertainty of $\bar{x}$ with factor $k = 2$: $U = 2\frac{s_R}{\sqrt{n}}$

CV_r: Relative repeatability standard deviation (s_r); variability of the replicate means per laboratory

CV_R: Relative reproducibility standard deviation (s_R); variability of the means of different labs

HorRat: Horwitz ratio or ratio of CV_R and predicted Horwitz coefficient of variation pCV_R; $pCV_R = 2^{(1-0.5log\bar{x})}$

$\frac{CV_r}{CV_R}$: Ratio of repeatability and reproducibility in %; 50–66% is common according to Horwitz [13]

number and the letter of the method class. There are four Cochran outliers (higher standard deviations of 8D, 26B, 1C, 19A) and one Grubbs outlier (deviating mean of 11B). They have been eliminated for the calculations. All values in Table 3 An overview of all outliers by type of evaluation (results separation by digestion method, analysis method or method class) is given in Additional file 1: Table S12. The outlier distribution obtained with a kernel density estimator (general view only) is shown in Additional file 1: Figure S8.

All 28 participating laboratories performed ICP-OES as analytical method. However, several labs performed several method classes. As a result, there are finally 72 different laboratory averages for the obtained method classes (Table 3). In terms of method classes (explanation Fig. 1), most labs performed A (n = 19) or B (n = 17) which means ICP-OES determination. On the other hand, only 6 labs performed method classes with photometric phosphorus determination (E and F) of which 3 labs are not accredited for this method. All laboratories with photometric determination performed only photometric measurements at 880 nm (for more details on accreditation status, employed standards, and instrumental settings see Additional file 1: Table S13). Therefore, results were also evaluated separated by digestion and analysis method (Table 3), rather than only by the combination of both.

An evaluation by method class, instrumental determination, and digestion method confirms the first impression of differing results. Microwave digestion and ICP-OES analysis provides the highest results. Generally, ICP-OES analysis provides higher results than ICP-MS which is higher than photometric analysis and microwave digestions shows higher results than reflux digestions. However, if the respective expanded uncertainties (*U*) are considered, the general means of ICP-OES and ICP-MS and of several method classes (Table 3) are not significantly different.

Generally, relative repeatability standard deviations (CV_r) and relative reproducibility standard deviations (CV_R) are higher for the photometric method classes (E, F) and for ICP-MS analysis after reflux digestion (D). The CV_R may be assessed on basis of the Horwitz model according to Horwitz and Albert [14] which suggests a prediction of CV_R only dependent on the measurand's concentration but regardless of the matrix or the analytical procedures and the analyte. The resulting Horwitz ratio (HorRat) is between 1.3 and 2.3 for all method classes (Table 3).

HorRat is close to the Horwitz model (≤ 2.0) for most combinations which is often observed for analytical procedures applied on a routine basis. On the other side, CV_r is tendentially lower than what might be expected from

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the Horwitz [13] model (50–66% compared to CV_R). A comparison of all means by method classes, digestion, or analytical methods shows values between 23.1 and 26.0 g/kg. Significant differences (*t*-tests) were found for method class F compared to method classes A–C ($p < 0.05$). However, for the photometric method classes E and F only six mean values are available.

Therefore, analytical methods were also compared by *t*-test. As a result, ICP-OES ($n = 36$) and photometry ($n = 12$) show also significantly different results ($p < 0.05$). An overview of the *t*-tests is provided in Additional file 1: Figure S9. Compared to the analysis of 15 sewage sludges, differences were found to be less significant due to the higher variances (the investigations in only one laboratory (Phosphorus determination in 15 sewage sludges) do not include the variability CV_R among laboratories).

Several laboratories varied the prescribed sample intakes and digestion parameters (acid ratios, aqua regia volume, digestion times, and temperatures). Photometry was based on calibration ranges beyond the maximum level (according to the permitted calibration range of ISO 6878) of 0.8 mg/l in three cases. Photometry was calibrated using 10 concentration levels on average, while ICP-OES and ICP-MS were based on 5 concentration levels on average. Six laboratories worked outside their calibration range which accounted for 44 values. An overview of the most important instrumental settings and measurement parameters and deviations from the prescribed sample intakes and standard procedures is provided in Additional file 1: Tables S14, S15. However, it is not possible to explain outliers with specific measurement parameters.

The determined moisture contents were between 0.1 and 9.3%, on average at 5.2%. Values below 3% moisture content should be doubted, as organic matter is hydroscopic, and samples were already sent with a moisture content of about 4%. Extreme values might be explained by sample handling as most laboratories reported moisture detection in a drying chamber (problems possible with hot samples, mass losses during transportation, incomplete dehydration, rehydration before weighing) and only four labs used a humidity scale. Detailed information on the reported moisture contents and the used determination methods is given in Additional file 1: Figure S7 and Table S12.

Conclusions

The investigation of 15 sewage sludges with highly different matrix compositions shows different phosphorus recoveries by method class. 2-way ANOVA and *t*-tests show that these differences are significant in many cases ($p < 0.01$). These tendencies were confirmed in an ILC with one sewage sludge (28 laboratories). However,

significant differences were found less often. In total, the different method classes tend to yield different phosphorus recoveries, but still can be considered sufficiently equivalent with a total reproducibility standard deviation of 7.2%. However, both investigations show the highest phosphorus recoveries and the lowest CV_r or RSD for method class A (ICP-OES determination after microwave digestion). Moreover, this method class represents various advantages (different wavelengths to avoid interferences, moderate dilution necessary, high routine, coverage of many further measurands, less time consuming).

Therefore, the authors recommend ICP-OES measurement after microwave digestion as the favorable method compared to ICP-MS or photometric determination in combination with reflux or microwave digestion. Nevertheless, based on the above-mentioned reasons, none of the method classes examined can be excluded on principle.

Supplementary Information

Additional file 1: Table S1 Origin of the 15 investigated sewage sludges and sludge treatment at respective wastewater treatment plant (WWTP). **Table S2** Characterization of the 15 investigated sewage sludges and $HNO3$ addition (for samples with more than 500 mg C on 3,000 mg according to EN 16174), contents related to dry matter. **Table S3** Phosphorus recovery of certified reference materials (sewage sludges, Sigma-Aldrich, Laramie, USA). Averages (n=2) of respective measurement day related to fresh matter (FM) and dry matter (DM). **Table S4** Recovery rates of spiked and diluted (1:5) samples by digestion (DG). Elemental P standards (10,000 mg/l for ICP-OES; 1,000 mg/l for ICP-MS diluted 1:10) of MERCK were used for spiking. **Table S5** Phosphorus contents by method combination in 15 sewage sludges in g/kg. **Table S6** Standard deviations of the phosphorus contents by method combination in 15 sewage sludges in g/kg. **Table S7** Phosphorus recovery rate of 15 investigated sewage sludges after normalization to the grand mean. **Table S8** Phosphorus recovery rate of 15 investigated sewage sludges after normalization to digestion mean. **Table S9** Two-way analysis of variance for the normalized (grand mean) 15 sewage sludges. **Table S10** XRF results in g/kg for homogeneity study of the sludge 15 for interlaboratory comparison. Powder analysis of 10 out of 64 bottles (regular intervals) from the line of bottling, three samples (x-1 to x-3) per bottle. After analysis sample shaking and remeasurement (x-4 to x-6). **Table S11** One-way analysis of variance of homogeneity study (XRF results for phosphorus content). **Table S12** ILC results by laboratory and method class (MC) and outliers (Grubbs or Cochran) according to ISO 5725-2

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(total summarized values main text, Table 3). **Table S13** Interlaboratory comparison, instrumental parameters by laboratory sorted by analytical method. **Table S14** Interlaboratory comparison, deviations of lab from the sample digestion prescribed in the sample instruction letter. **Table S15** Interlaboratory comparison, deviations by of lab of from the analytical method prescribed in the sample cover instruction letter. **Figure S1** Exemplary calibration curves for ICP-OES (a), ICP-MS (b), and photometric phosphorus determination with ammonium molybdate (c). **Figure S2** Phosphorus recovery rate from reference materials CRM029-50G (029) and CRM031-40G (031) by digestion and analysis. **Figure S3:** Phosphorus recovery from 15 sewage sludges as determined by method classes A–F* normalized to the grand mean (a) and normalized to the mean value by digestion (b). The error bars (a) represent the standard deviation of the five replicates (sludge 15 no. reflux digestions: three replicates) per sludge. For an explanation of the method class abbreviations see main text Figure 1. **Figure S4** Schematic representation of the cross-riffling to generate bottles for interlaboratory comparison (ILC). **Figure S5** XRF homogeneity results of 10 bottles (six replicates). Measurement of three samples per bottle, shaking and new measurements from measurement number 42. **Figure S7** Cumulative curve of the moisture content analyzed by the labs during the interlaboratory comparison. The error bars mark the standard deviation (n=2 to 6). **Figure S6** Interlaboratory comparison results sorted by analytical method (the bars represent laboratory means and standard deviations. The method class lab code is shown on the x-axis and represents the lab number and the letter of the method class. Outliers have been eliminated for the calculations. **Figure S8** Interlaboratory comparison, distribution obtained with a kernel density estimator. **Figure S9** Significant differences by method combination and method class (main text, Figure 1) from the interlaboratory comparison and investigation of 15 sewage sludges *t*-test.

Availability of data and materials
All evaluated data generated or analyzed during this study are included in this published article and its supporting information files. Raw measurement data can be handed out on request by the corresponding author. WWTP and laboratory data have been anonymized.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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