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Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils

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Abstract

Phosphorus is an important macronutrient and the accurate determination of phosphorus species in environmental matrices such as natural waters and soils is essential for understanding the biogeochemical cycling of the element, studying its role in ecosystem health and monitoring compliance with legislation. This paper provides a critical review of sample collection, storage and treatment procedures for the determination of phosphorus species in environmental matrices. Issues such as phosphorus speciation, the molybdenum blue method, digestion procedures for organic phosphorus species, choice of model compounds for analytical studies, quality assurance and the availability of environmental CRMs for phosphate are also discussed in detail.

Keywords: Phosphorus; Natural waters; Soils; Sampling; Sample treatment; Sample digestion; Quality assurance

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1. Introduction

The determination of phosphorus species in environmental matrices provides essential data for assessing the health of ecosystems, investigating biogeochemical processes and monitoring compliance with legislation. At the catchment scale, for example, phosphorus export from both point and diffuse sources can result in increased primary production and eutrophication, with the potential for seasonal development of toxic algal blooms, which can have a major impact on global water quality [1]. For accurate measurements, knowledge of phosphorus speciation is required as environmental behaviour is often critically dependent on its physico-chemical form. In aquatic systems, for example, phosphorus species are found in "dissolved", "colloidal" and "particulate" fractions, as inorganic and organic compounds and in biotic and abiotic particles [2]. The common operationally defined aquatic forms of phosphorus and the various terms used to describe them are shown schematically in Fig. 1. The reliability and comparability of data for any of these fractions will depend on the operational protocols used and the accuracy of the method.

Most manual and automated methods of phosphorus determination are based on the reaction of phosphate with an acidified molybdate reagent to yield phosphomolybdate heteropolyacid, which is then reduced to an intensely coloured

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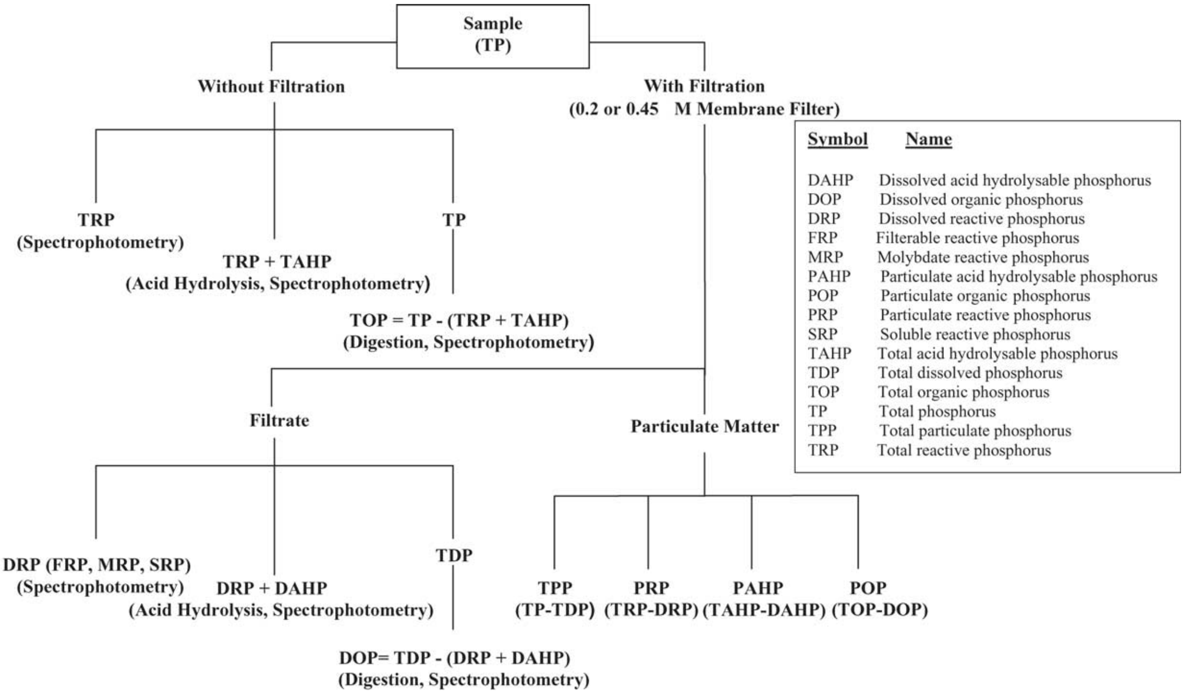


Fig. 1. Operationally defined aquatic P fractions (adapted from [2]).

blue compound and determined spectrophotometrically [3].

$$\mathrm{PO_4}^{3-} + 12\mathrm{MoO_4}^{2-} + 27\mathrm{H}^{+} \rightarrow \mathrm{H_3PO_4(MoO_3)_{12}} + 12\mathrm{H_2O}$$

$$\mathrm{H_3PO_4(MoO_3)_{12}} + \text{reducing agent} \rightarrow \text{phosphomolybdenum blue } [\mathrm{Mo(VI)} \rightarrow \mathrm{Mo(V)}]$$

There are many modifications of the original Murphy and Riley method [4], particularly the use of different reductants (e.g. ascorbic acid, tin(II) chloride) and acid strengths. As shown in the above reaction scheme, the phosphomolybdenum blue complex is formed in an acidic environment and its absorbance spectrum is dependent on the acidity, type of reductant and phosphate concentration. Under low acidity conditions, for example, non-linear colour development [5] and non-phosphate sensitized reduction (self-reduction of the molybdate) can occur. A variety of $[\mathrm{H}^{+}]/[\mathrm{MoO_4}^{2-}]$ ratios have been reported in the literature, with a ratio of 70 and a pH range of 0.57–0.88 suggested for optimum sensitivity (maximum rate of colour formation) [6].

Ascorbic acid and tin(II) chloride are the most commonly used reductants when determining phosphate concentrations in natural waters. Ascorbic acid acts as a 2-electron reductant [7] with the major advantages being that it is less salt sensitive and colour development is fairly independent of temperature [6]. Ascorbic acid on its own however has the major disadvantage of slow colour development [8], but the addition of antimony as a catalyst increases the rate of reduction of the complex [4]. Using tin(II) chloride generates a product with a wavelength maximum at 690–700 nm as compared with 882 nm for ascorbic acid [2]. This allows greater sensitivity when a solid state detector (using a red light emitting diode light source) is used [9]. However, disadvantages include unstable colour development, a considerable salt error, temperature dependence and unsatisfactory performance at high phosphorus concentrations [10].

Interferences in the formation of the phosphomolybdenum blue complex include arsenate, silicate, chromium, copper, nitrite, nitrate and sulphide [11]. However, arsenate interferences can be eliminated by reducing As(V) to As(III) prior to measurement [6], e.g. by the addition of sodium thiosulphate [12]. The acid/molybdate ratio can be altered to enhance the selectivity for phosphate relative to silicate [4]. In addition, use of an appropriate extraction solvent, e.g. *n*-butanol, is an efficient way of eliminating interference from silicate [13].

The phosphorus determined in the filtered fraction using the above reaction is defined as “molybdate reactive” phosphorus (MRP) or dissolved reactive phosphorus (DRP). It has also been called soluble reactive phosphorus (SRP) and filterable reactive phosphorus (FRP). However, this method also determines acid labile phosphorus containing compounds (organic and condensed phosphorus species) which can lead to overestimation of free phosphate [3,6]. Similar problems have been reported in the determination of total reactive (unfiltered) phosphorus (TRP) [3]. Methods have been developed to minimise this overestimation including a critical timing technique (‘the 6 second method’) in which the acid strength is adjusted prior to the formation of the complex [14] and complexing excess molybdate with a citrate–arsenate reagent [15]. Phosphorus containing organic compounds and condensed phosphates can also be determined using the molybdate reaction following chemical, photochemical, thermal or microwave digestion (see Section 4).

2. Natural waters

Phosphorus concentrations in natural waters fluctuate with changes in physico-chemical conditions and biological activity. In chalk-based catchments, for example, phosphorus is influenced by seasonal fluctuations in pH, dissolved carbon dioxide and total dissolved calcium concentrations [16]. Hydrological conditions also play an important role in aquatic phosphorus concentrations. The majority of phosphorus transport to catchments, from both diffuse and point sources occurs during short periods of increased discharge (e.g. storm events) [17,18], which demonstrates the importance of high temporal resolution monitoring during such events. Submersible or field-based instrumentation is desirable for monitoring dissolved phosphorus because it eliminates the need for sample collection and storage and, although such instrumentation is available [19,20], it is not used on a routine basis. Therefore, a comprehensive and effective sampling, sample treatment and analysis protocol must be adopted in order to minimise the physical, chemical and biological processes that can alter the physico-chemical forms of phosphorus during storage.

2.1. Sampling protocol

It is essential that the scientific objectives (e.g. determining bioavailable phosphorus, measuring seasonal phosphorus loads), safety issues and budgetary constraints are clearly identified prior to undertaking any sampling programme. Having established the scope of the exercise, an essential requirement of any sampling protocol is for the sample to be representative of the body of water from which it originates. It is therefore essential to adopt a well-organized protocol, which retains, as closely as possible, the original composition of the water body of interest. The protocol should be kept as simple as possible while minimizing the possibility of contamination or interferences. In rivers and streams, for example, samples should be collected from the water column at a series of depths and cross-sectional locations as individual grab samples or through the use of automated samplers for time series acquisition. Monitoring stations can be constructed to provide high quality supporting data (e.g. pH, dissolved oxygen, temperature, turbidity) in a judicious fashion via data acquisition/telemetry technology. It is also vital to avoid boundary areas, e.g. at the confluence of streams or rivers and below sewage treatment works, unless their impact on the system is being investigated. Point source phosphorus contributions from sewage treatment works, for example, can have a major affect on the overall water quality of freshwater systems [21]. Globally, phosphorus loading into receiving waters still occurs even though tertiary treatment measures (e.g. based on the reduction of phosphate by precipitation with iron chloride) are being implemented in some countries [22]. Other water bodies pose additional complications and these must be considered when designing a sampling protocol. In lakes and reservoirs, representative sampling is often difficult due to environmental heterogeneity, both spatial and temporal (e.g. seasonal thermal stratification). In order to study biogeochemical cycling in stratified water bodies appropriate depth profiling is required. For a complete study high spatial resolution sampling at the sediment–water interface is also essential but is not discussed further in this paper.

Location and frequency must also be considered when designing a sampling protocol. Site selection will ultimately depend on the problem to be addressed and safety and accessibility are of paramount importance. The frequency of sampling, from continuous to seasonal, will depend on the scientific objectives but will often be constrained by cost. For example, the highest phosphorus loadings in rivers and streams are generally correlated with intense, short-term discharges during autumn and winter months, while the lowest loadings occur in the summer months when discharge is low and biological activity is high [23,24]. In-water processes that affect phosphorus concentrations that must also be considered include plant, algal and bacterial turnover, anthropogenic inputs (e.g. sewage effluent), matrix considerations (e.g. water hardness) and resuspension of bottom sediments from increasing river discharge [21,25].

Prior to any sampling campaign it is essential to adopt an efficient cleaning protocol for all sampling equipment and storage bottles and continue this throughout the study. The walls of sample containers, for example, are excellent substrates for bacterial growth and therefore rigorous cleaning of all laboratory ware is necessary. For phosphate determination, it is recommended that containers be cleaned overnight with a nutrient free detergent, rinsed with ultrapure water, soaked in 10% HCl overnight, and then rinsed again with ultrapure water [26]. Containers should be rinsed at least twice with the water of interest prior to sample collection. In addition, sampling blanks should be taken to monitor and control the sampling process.

2.2. Sample preservation and storage

The overall effectiveness of any sample preservation and storage protocol depends on various factors including the nature of the sample matrix, cleaning procedures for sample containers, container material and size, temperature, chemical treatment (e.g. addition of chloroform) and physical treatment (e.g. filtration, irradiation of sample and pasteurization) [27–29].

Preliminary treatment often involves filtration which differentiates between the dissolved phase (operationally defined as that fraction which passes through a 0.45 or 0.2 μm filter) and suspended matter (that fraction collected on the filter) [30]. It is essential that filtration is carried out immediately after the sample is collected to prevent short-term changes in phosphorus speciation. Polycarbonate or cellulose acetate membrane filters are recommended for dissolved constituents in natural waters [31]. Filtration with a 0.2 μm filter is preferred as it removes the majority of bacteria and plankton that would otherwise alter dissolved phosphorus concentrations during storage [30]. It should be stressed however that some bacteria, as well as viruses, will pass through a 0.2 μm filter. As with sample containers, the filtration apparatus (including individual filters) must be cleaned prior to use with a similar acid wash/ultrapure water rinse procedure. The filtration procedure can be conducted under positive pressure or vacuum. However, excessive pressure gradients should be avoided as rupture of algal cells and the subsequent release of intracellular contents into the sample could occur. In samples of high turbidity it is important to minimise the sample loading to prevent clogging of filter pores.

Table 1 shows a summary of reported storage/preservation methods for phosphorus determination. Physical (i.e. refrigeration, freezing and deep-freezing) and chemical (i.e. addition of chloroform, mercuric chloride and acidification) preservation techniques have been used to help maintain the original phosphorus concentration during storage. It should be noted however that the use of chloroform is now discouraged in some countries because of toxicological risks. In addition, a variety of sample containers have been used including quartz, borosilicate glass, polyethylene, polypropylene, high-density polyethylene (HDPE) and polytetrafluoroethylene (PTFE).

For phosphorus determinations, however, it is difficult to select a generic treatment protocol due to the different effects of specific matrix characteristics (e.g., phosphorus concentration, hardness, salinity, dissolved organic matter and bacterial nutrient uptake) of the sampling location. In chalk catchments, for example, studies have shown that freezing samples is not the best treatment due to the possibility of phosphate being coprecipitated with calcite when thawing the samples [26,46]. Fig. 2a demonstrates this effect, showing an immediate (after 1 day) and continuing (up to 250 days) decrease in DRP concentration in samples analysed for phosphate after storage at −20 °C [26]. Storage at 4 °C is therefore recommended, together with the addition of chloroform to prevent biological growth. However, chloroform should not be used in samples with high organic matter content, as the release of cellular enzymes into the samples is possible [26]. Other studies have recommended immediate analysis after sampling [47] or analysis after a short storage period at 4 °C in the dark (maximum 48 h) [48–51].

In contrast to the extensive studies on phosphate stability during storage, the stability of dissolved organic phosphorus (DOP), as operationally defined, has not been widely studied. Fig. 2b–d show the stability of DOP (strictly this includes all acid hydrolysable phosphorus because acidic digestion conditions were used) from natural water samples (salinities 0, 14 and 32, respectively) over 32 days of storage. The DRP concentration on day 0 (1.17, 1.31 and 0.54 μM for salinities 0, 14 and 32, respectively) was subtracted from all results, which were based on sampling, autoclaving of sub-samples and storage of autoclaved and non-autoclaved sub-samples for subsequent analysis. They showed that there were no significant differences in DOP concentration if the samples were stored at −20 °C, autoclaved and analysed on the same day or if they were autoclaved immediately after collection and stored until analysed. The same trend (not shown) was also observed with phytic acid spiked (1.11, 1.50 and 0.45 μM for salinities 0, 14 and 32, respectively) standards and samples. These results suggest that storage at −20 °C is suitable for DOP determination but the final result is dependent on a reliable determination of the original DRP concentration. Freezing as a method for storage of unfiltered and filtered

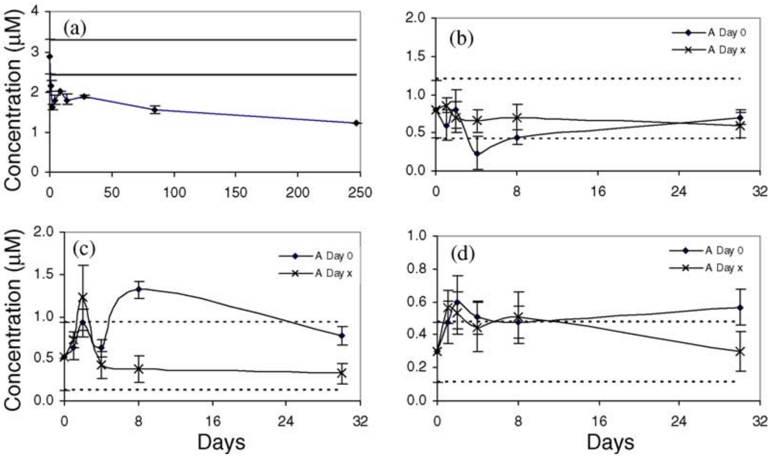


Fig. 2. Changes in the concentration of phosphorus species in natural water samples stored over time. (a) An immediate sharp decrease in DRP concentration in samples stored at −20 °C, followed by a gradual decrease over 250 days of storage. (b–d) The stability of DOP in natural water samples (salinities 0, 14 and 32, respectively) over 32 days of storage at −20 °C. A Day 0 are samples autoclaved on day 0 then stored until analysis, and a Day *x* are samples stored without treatment then autoclaved and analysed on day *x*. The dotted lines in each figure (solid lines in (a)) represent ±3 s of the measured DRP/DOP concentrations on day 0 (i.e. immediately after collection).

Table 1
Storage protocols for the determination of phosphorus species in environmental matrices (updated from [26] which was adapted from a table by Maher and Woo [75])

Phosphorus species	Matrix	Storage method	Maximum storage time	Comments	Ref.
FRP	Distilled, tap and lake water	Refrigerator (4 °C)	1 day	Polypropylene and polycarbonate containers suitable for storage. Glass containers sorbed phosphorus within 1–6 h	[32]
FRP	Standards added to rain water	Room temperature with $HgCl_2$ (0–50 $mg\,L^{-1}$)	3 days	$HgCl_2$ interfered with method when ascorbic acid was used as a reducing agent	[33]
FRP	River water	−10, 4, 20 °C with/without thymol (0.01%), KF (0.01%), TBT (0.001%), H_2SO_4 (0.05 M) or $CHCl_3$ (5 $mL\,L^{-1}$)	14 days	Samples showed no decrease in FRP if chloroform added and samples stored at 4 °C	[34]
FRP, TP	Open ocean water	Frozen (quick and slow), cooled (2 °C) with/without $HgCl_2$ (120 $mg\,L^{-1}$), phenol (4 $mg\,L^{-1}$) and acid (pH 5)	60 days	No significant change in TP concentration when samples frozen with/without acid	[35]
FRP	Coastal and estuarine waters	−10 °C, slow and quick freezing	365 days	Small change in FRP when samples were frozen. Quick freezing reduced losses	[36]
FRP, TP	Tap, lake and river waters	Room temperature, 4 °C, with the addition of $HgCl_2$ (40 $mg\,L^{-1}$), H_2SO_4 (0.05 M), and chloroform	16 days	Chloroform at 4 °C was suitable for only 8 days. No significant decreases in concentration (up to day 16) were shown in samples with $HgCl_2$ stored at 4°C	[37]
FRP	Sea water	Frozen at −40 °C initially, then stored at –20 °C	147–210 days	FRP concentration decreased in samples stored longer than 4 months	[38]
TP, TDP, FRP and TRP	Lake water	Refrigerator (4 °C)	180 days	No change in TP in samples for up to 6 months	[39]
FRP	Stream water	Frozen at −16 °C	4–8 years	No significant change in FRP concentration	[40]
FRP	Soil leachates	Room temperature (5–19 °C), refrigeration (4 °C) frozen (−20 °C) with/without $HgCl_2$ (40–400 $mg\,L^{-1}$) and H_2SO_4	1–2 days	Changes occurred within 2 days for all samples with smallest changes in samples stored at room temperature or 4 °C	[41]
FRP	Sea water	Pasteurization and stored at room temperature	18 months	FRP remained constant for 1 year. NH_4 losses after 3 days	[42]
FRP, TP	Stream water	Refrigerator (4 °C), H_2SO_4 (0.05 M), freezing with dry ice and subsequent analysis	8 days	Minimal change observed in highly concentrated (FRP > 1 $mg\,L^{-1}$) samples (1–3% loss after 8 days). 47% loss in FRP in lower concentrated samples	[43]
FRP	River water (chalk-based catchment), estuarine water (salinities of 0.5, 10 and 35)	Refrigerator (4 °C) with/without 0.1% (v/v) chloroform, −20 °C with/without 0.1% (v/v) chloroform, −80 °C without chloroform	247 days	For chalk-based samples, 4 °C with 0.1% (v/v) chloroform was the best treatment. Freezing is not recommended due to coprecipitation of inorganic phosphate with calcite	[26]
TP	River and canal water	Room temperature, refrigerator (4 °C) treatment to a pH of < 2 with H_2SO_4	28 days	No significant losses in TP concentration over the 28 day period for treated samples at 4 °C. No losses up to 7 days for room temperature (acidified) samples	[44]
FRP	Water extracts of poultry litter	Room temperature, freezing (−16 to −15 °C)	8 days	No significant losses in FRP concentration in samples stored at room temperature (up to 8 days). Freezing samples lowered concentration (up to 46%) for the 8 day period	[45]

samples for the determination of total and dissolved organic phosphorus has also been recommended by other workers [39,52–53].

3. Soils

Soil pre-treatment and storage can induce marked changes in the solubility of chemicals and therefore presents a critical control on subsequent analysis. This section focuses on phosphorus but it also has wider relevance for other elements. For example, water-extractable phosphorus is markedly influenced by even mild drying of soil. It has been known for some time that soil drying can render considerable concentrations of organic carbon soluble in water [54] and a similar effect was recently reported for phosphorus in a wide range of pasture soils from England and Wales [55]. In the latter study, 7 days air drying from approximate field moisture capacity at 30 °C increased concentrations of water-extractable organic phosphorus by up to 1900%. Organic phosphorus accounted for up to 100% of the solubilized phosphorus. This was at least partly derived from microbial cells, because a strong correlation existed between solubilized organic phosphorus and microbial phosphorus (Fig. 3). It has been reported that rapid rehydration can kill between 17 and 58% of soil microbes through osmotic shock and cell rupture [56] and the contribution of microbial lysis has been subsequently confirmed by direct bacterial cell counting in rewetted Australian pasture soils [57].

In addition to microbial lysis, the physical stresses induced by soil drying also disrupt organic matter coatings on clay and mineral surfaces [58], which may contribute to the solubilisation of both inorganic and organic phosphorus. Indeed, functional classification of water-extractable organic phosphorus from dry Australian pasture soils revealed similar proportions of microbially derived phosphate diesters and phytic acid from the non-biomass soil organic matter [59]. A similar mechanism probably occurs following freezing and thawing [60]. Such processes probably explain the increases in phosphorus extractable in bicarbonate following soil drying [61] because the high ionic strength of bicarbonate solution may reduce the degree of osmotic stress and associated lysis of viable cells compared to extraction with water [62]. The hypothesis that non-biomass organic phosphorus dominates in bicarbonate extracts is supported by the speciation of phosphorus in such extracts, which is dominated by phosphate monoesters and is, therefore, similar to the whole-soil organic phosphorus extracted in strong alkaline solution [63,64].

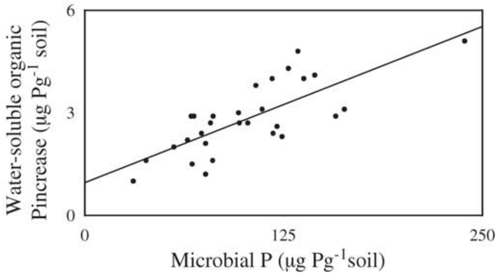


Fig. 3. The increase in water-soluble organic phosphorus after soil drying as a function of soil microbial phosphorus in a wide range of permanent pasture soils from England and Wales. Water-soluble phosphorus was determined by extracting soils at field moisture capacity with water in a 4:1 water:soil ratio for 1 h. Sub-samples were air-dried for 7 days at 30 °C and extracted in an identical manner. Adapted from [57].

The mechanisms by which soil drying could affect the solubility of non-biomass inorganic and organic phosphorus are poorly understood, but probably include both physical and chemical changes. Rapid rehydration of dry soils commonly causes aggregate breakdown [65], which increases the surface area for desorption by exposing surfaces and associated phosphorus protected within aggregates [66]. Such a process has been linked to increases in resin-extractable inorganic phosphorus following soil drying [67]. A more likely process is disruption of organic matter coatings on clay and mineral surfaces by the physical stresses induced during soil drying. This increases organic matter solubility and exposes formerly protected mineral surfaces, and has been attributed to increases in oxalate-extractable silica of up to 200% following drying of Swedish spodic B horizons [68]. Soil drying also increases the crystallinity of pure iron and aluminium oxides, which reduces the specific surface area and phosphorus sorption capacity of these minerals [69]. However, this is inconsistent with reports of increased sorption capacity of dried soils for phosphate and sulphate [70,71].

The effect of drying on phosphorus solubility does not appear to be consistent for all soils. In particular, phosphorus solubility in high organic matter soils may decrease following drying. For example, Schlichting and Leinweber [72] reported that phosphorus recovery from a German peat (pH 5.6) by a sequential fractionation procedure was markedly reduced by pre-treatment, including air-drying, freezing and lyophilization. The greatest reduction in phosphorus recovery followed lyophilization (phosphorus recovery was 75% when extracted fresh, compared with <50% from lyophilized samples) and even after storage at 4 °C for 3 weeks detectable changes were still observed.

The importance of specific artefacts that result from particular pre-treatments will vary depending on the study objectives. For example, assessment of plant-available phosphorus for fertilizer requirements is based on analysis of air-dried soils for practical reasons although field-fresh soils are needed to obtain meaningful data. This is impractical for most purposes, although refrigeration may be an acceptable alternative [58]. In this respect, there is a clear requirement for a detailed study of changes in phosphorus solubility during cold storage for several different soil types. Environmental soil phosphorus tests that involve water extraction routinely use air-dried soils and only measure inorganic phosphorus (e.g. [73,74]). The results of these tests will clearly vary depending on the moisture status of the soil prior to extraction and on the inclusion (or not) of organic phosphorus. If organic phosphorus is included in such tests, the standardization of soil moisture prior to extraction will be necessary. For details of extraction procedures for soil organic phosphorus see Turner et al. (this issue).

4. Digestion techniques

Digestion techniques for environmental samples are necessary for the determination of total phosphorus (TP) and total dissolved phosphorus (TDP). This is because many of the phosphorus species present contain P–O–P, C–O–P and C–P bonds that need to be broken down to release phosphorus as phosphate, which can then be determined using molybdenum blue chemistry [4]. The digestion technique must also be able to release phosphorus from biological material, e.g. algal cells and plant detritus and adsorbed/occluded P from sediments [75]. Traditional methods of digestion for natural water samples include fusion, dry ashing, perchloric acid, sulphuric acid–nitric acid and boiling on a hot plate, with more recent methods generally using autoclaving, UV photo-oxidation and microwave heating [75]. UV photo-oxidation can be used for organic phosphorus compounds in marine and freshwaters [47,76,77] but condensed polyphosphates present in the sample will not be broken down by UV photo-oxidation alone [2,3,78,79] and also need to be heated to 90–120 °C in the presence of acid [75]. To ensure that all polyphosphates present in the sample are decomposed, either boiling with HCl or potassium peroxydisulfate after UV irradiation is therefore recommended [80]. McKelvie et al. used an on-line UV photo-oxidation flow injection (FI) technique and found that results were comparable with a batch peroxydisulfate method [81].

Autoclaving methods are generally straightforward, give reproducible results and use sealed vessels that are less prone to contamination [75,82–84]. The following section is therefore a summary of different autoclaving techniques, combined with peroxydisulfate in either an acidic or alkaline media, for the determination of phosphorus in natural waters, soil solutions and sediments (see Table 2). Most methods described in Table 2 are based on spectrophotometric detection but ICP-MS and ICP-AES have, in recent years, been used to determine phosphorus in agricultural runoff waters and soils and results were comparable with spectrophotometric methods [128,129]. In addition, microwave digestion combined with ICP-MS detection has been used to determine phosphorus in marine environmental samples and plant leaves with good recoveries [130–132]. However microwave heating for batch sample digestion and in FI systems with spectrophotometric detection for on-line TDP and TP digestion [3] is less widely used than UV photo-oxidation or autoclaving.

4.1. Autoclaving

4.1.1. Alkaline peroxydisulfate

Menzel and Corwin first used autoclaving with peroxydisulfate in 1965 for the digestion of seawater samples [88]. Koroleff developed an alkaline peroxydisulfate alternative in 1969 [102], which was then slightly modified [101] and simplified by introducing a borate buffer [85]. This enabled the simultaneous determination of TP and total nitrogen (TN), as nitrogen bonds are only hydrolysed/oxidised in alkaline media [98]. Using a borate buffer, the pH is alkaline (ca. 9.7) at the start of the digestion process and becomes acidic (pH 4–5) as the sodium hydroxide decomposes [78,90,98]. Hosomi and Sudo also reported that pH change was important and in their method the pH decreased from 12.8 to 2.0–2.1 to ensure that even condensed polyphosphates were digested [92].

The alkaline method has also been used for particulate material but with relatively poor recoveries [133]. For example orchard leaves gave recoveries of 80–90% for TP and TN [91]. Higher recoveries can be obtained by decreasing the ratio of sample to peroxydisulfate [92]. Alkaline digestion of model phosphorus compounds has been found to be efficient for turbid water samples [125–127] although the concentration of suspended particulate material needs to be diluted to $<150\,\mathrm{mg\,L^{-1}}$ and difficulties can arise when this material is of soil origin rather than biological origin, e.g. algal cells and plant detritus. The alkaline method has therefore been used to determine TP in turbid lake waters and suspensions of particulate material [127].

Alkaline peroxydisulfate autoclaving, rather than acid peroxydisulfate, is recommended for the digestion of marine waters. This is because in the acid method, peroxydisulfate oxidises the chloride in seawater to free chlorine, thus reducing the oxidising power of the peroxydisulfate [104]. It is also recommended for the simultaneous determination of TP and TN.

4.1.2. Acid peroxydisulfate

An acid peroxydisulfate method developed by Gales et al. [134] has been adopted by the US Environmental Protection Agency [135]. Eisenreich et al. simplified the method [96] and various modifications of this approach are now used to digest different types of samples such as soil solutions, natural waters and river water [18,97,121]. The alkaline peroxydisulfate method for soil extracts is only appropriate if the total organic carbon concentration is $<100\,\mathrm{mg\,L^{-1}}$ and manganese is $<1\,\mathrm{mg\,L^{-1}}$. Above this manganese concentration, coloured solutions or precipitates are formed, which interfere with the digestion step [111]. This interference is avoided when using acid peroxydisulfate and solutions are colourless after digestion [84].

Pote et al. described standard methods for the determination of TP and TDP using sulphuric acid–nitric acid and peroxydisulfate digestions [136] and recommended the use of sulphuric acid–nitric acid digestion to achieve good recoveries for most samples. However this digestion method can be potentially dangerous if salts precipitate during digestion [89] and is less easy to control than the peroxydisulfate method [84,122]. Rowland and Haygarth compared a mild peroxydisulfate method to the more rigorous sulphuric acid–nitric

Table 2
Acidic and alkaline peroxydisulfate autoclave digestion methods

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Drainage waters	Digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 4 mL reagent added to 50 mL sample	30 min	115	Not reported	Not reported	Same method as [85]	[86]
Drainage waters	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	Not reported	Same method as [84]	[87]
Estuarine waters	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	1 h	120	Final pH 1.5–1.8	Orthophosphate, phenylphosphoric acid, phenylphosphorous acid	Same method as [88], but autoclaving time was increased from 30 min to 1 h. Quantitative recovery for model compounds at the 50 µg P level	[89]
Fresh and seawater	Acidic peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 4 mL reagent added to 50 mL sample. Alkaline peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 3 g H_3BO_3 in 100 mL 0.375 M NaOH. 5 mL reagent added to 50 mL sample	30 min	115	For alkaline method, initial pH ca. 9.7, final pH 4–5	Model compounds added to demineralised water and seawater:2-AEP (108, 77, 108, 88%), PTA (100, 70, 101, 95%), 5′-GMP-Na_2 (99, 93, 100, 94%), PC (98, 37, 99, 96%), FMN (99, 99, 100, 97%), G-6-P-Na (100, 95, 101, 92%), AMP (99, 94, 100, 93%), RP (100, 94, 103, 95%), PEP-3CHA (100, 100, 101, 101%), β-GLY (99, 100, 100, 96%)	Recoveries in parentheses are in the order: acidic demineralised water, acidic seawater, alkaline demineralised water, alkaline seawater. Acidic and alkaline peroxydisulfate methods [85] compared to continuous flow UV irradiation and high temperature combustion. Alkaline peroxydisulfate method recommended for marine waters	[90]
Fresh waters	Digestion reagent: 40 g $K_2S_2O_8$ and 9 g NaOH in 1 L distilled water. 5 mL reagent added to 10 mL sample	1 h	120	Initial pH 12.8, final pH 2.0–2.1	National Bureau of Standard Reference Material 1571 orchard leaves (98%), National Institute of Environmental Studies (NIES) Reference Material No. 1 pepper bush (96%), NIES Reference Material No. 2 pond sediment (100%), NIES Reference Material No. 3 chlorella (100%) all of concentration 50 mg L^{-1}. Model compounds:5′-ATP-Na_2 (99–100%), 5′-ADP-Na_2 (98%), TSPP (99–100%), SHMP (94–97%), STP (96–97%), G-6-P-K_2 (99–102%)	Analysed for TN and TP. Obtained higher recoveries for orchard leaves than [91]	[92]
Fresh waters	1 g $K_2S_2O_8$ and sufficient H_2SO_4 to make the sample 0.15 M acid	2 h	120	Not reported	Not reported		[93]
Lake waters	‘Strong’ acid: 25 mL 18 M H_2SO_4 and 1 mL 18 M HNO_3 in 1 L deionised water. 1 mL ‘strong’ acid and 2.5 mL aqueous 4% (w/v) $K_2S_2O_8$ added to 25 mL sample	30 min	Not reported, however in the UV digestion, sample maintained at 85 °C in the silica coil	Not reported	Dipotassium hydrogenphosphate (100%), STP (100%), AMP (100%)	Compared UV digestion to autoclaving. Recoveries for lake water samples were 100% for the peroxydisulfate digestion and 97% for the UV digestion	[94]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Lake, river and pond waters, raw sewage	Digestion reagent: 55 mL H_2SO_4 and 60 g $K_2S_2O_8$ in 1 L solution. 2.5 mL reagent added to 35 mL sample	1 h	Not reported	Not reported	G-1-P-K_2 (97.5%), G-6-P-K_2 (105%), DNA (sodium salt) (115%), AMP (95%), 5′-ADP-Na_2 (102.5%), SOP (100%), β-GLY (107.5%), TSPP (62.5%), STP (110%), SHMP (100%), disodium hydrogen orthophosphate (97.5%)	Autoclave method was compared to the hot-plate $H_2SO_4/K_2S_2O_8$ digestion. Autoclave method gave more precise values for model compounds than the hot plate procedure	[95]
Natural waters	Digestion reagent: 0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4. 1 mL reagent added to 20 mL sample	45 min	121 °C	Not reported	G-1-P (101.0%), G-6-P (103.1%), ATP (101.6%), NPP (101.9%), cAMP (101.8%), α-GLY (102.3%), myo-inositol 2-monophosphate (97.4%), PTA (85.6%), 2-AEP (99.2%), TSPP (99.5%), STP (97.7%), trisodium trimetaphosphate (98.8%), KHP (99.1%)	Method modified from [96]	[97]
Natural waters	Acidic peroxydisulfate digestion reagent: 5 g $K_2S_2O_8$ and 5 mL 4.5 M H_2SO_4 in 100 mL distilled deionised water. 0.8 mL digestion reagent added to 10 mL sample. Alkaline peroxydisulfate digestion reagent: 50 g $K_2S_2O_8$, 30 g H_3BO_3 and 350 mL NaOH in 1 L distilled deionised water. 1.3 mL digestion reagent added to 10 mL sample	30 min	120 °C	For alkaline method, initial pH ca. 9.7, final pH 4-5	NPP, α-GLY, G-6-P, tripolyphosphate, trimetaphosphate, ATP, 5′-GDP, 2-AEP. Recoveries shown in a figure, so precise values cannot be given. In general, recoveries ca. >58% for acidic method and ca. >26% for alkaline method	Compared acidic peroxydisulfate [85] and alkaline peroxydisulfate [98] autoclaving methods with magnesium nitrate high-temperature oxidation, magnesium peroxydisulfate high-temperature oxidation, and UV oxidation. Magnesium nitrate high-temperature oxidation was found to be the best method	[78]
Orchard leaves and aufwuchs	Digestion reagent: 13.4 g $K_2S_2O_8$ and 6 g NaOH in 1 L to give 200 mg peroxydisulfate per 15 mL aliquot. Other levels of peroxydisulfate also used (300, 400 and 500 mg)	1 h	100–110	Initial pH 12.00 for orchard leaf samples, final pH 2.5. Initial pH 12.8 for aufwuchs samples, final pH 3.7	National Bureau of Standards reference material 1571 (orchard leaf) (86.9–88.7% using 500 mg peroxydisulfate), and aufwuchs (93.6% using 300 mg peroxydisulfate, and 101.4% using 400 mg peroxydisulfate)	Analysed for TN and TP. Maximum recovery for orchard leaf when 500 mg peroxydisulfate was used, and 300 or 400 mg peroxydisulfate for aufwuchs	[91]
Pond water	Acidic peroxydisulfate digestion: 0.5 g $K_2S_2O_8$ and 1 mL H_2SO_4 solution (300 mL conc. H_2SO_4 in 1 L distilled water) added to 50 mL sample. Alkaline peroxydisulfate digestion: 5 mL 0.075 N NaOH and 0.1 mg $K_2S_2O_8$ added to 10 mL sample. After digestion, 1 mL borate buffer (61.8 g H_3BO_3 and 8 g NaOH in 1 L distilled water) added	30 min	110	Not reported	Water samples spiked with 0.2 mg L^{-1} KHP. Recoveries for acidic method were 88–113%, and for the alkaline method 85–112%	Acidic and alkaline peroxydisulfate methods same as [99]	[100]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
River water	Digestion reagent: 0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4. 1 mL added to 20 mL sample	45 min	121 °C	Not reported	Not reported	Method modified from [96]	[18]
River water	Digestion reagent: 20 g $K_2S_2O_8$ and 3 g NaOH in 1 L distilled deionised water. 5 mL reagent added to 5 mL sample	30 min	120 °C	Initial pH 12.57, final pH 2.0	KHP (99.6%), TSPP (97.2%), STP (99.2%), β-GLY (96.5%), SHMP (97.6%), G-1-P (99.5%), AMP (100.8%), ADP (98.9%), ATP (98.1%)	Results from this method were an improvement on the alkaline oxidation method for TN and TP of [101], which was in turn a modified method from [102]	[103]
Seawater	Two concentrations of $K_2S_2O_8$ added (4 and 40 mg mL^{-1}) to 10 mL sample acidified with sulphuric acid to pH 3	90 min	125	pH 3	Not reported	Three methods compared: autoclaving (acidic peroxydisulfate method based on [85]), UV irradiation and sequential use of both. The latter method gave the best recoveries	[104]
Seawater	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	30 min	120	Final pH 1.5–1.8	PFA (96.5%), 1-AEP (85.5%), 2-AEP (81.2%)	Compared their nitrate oxidation method with peroxydisulfate oxidation method from [88]	[105]
Seawater	Digestion reagent: 50g $K_2S_2O_8$, 30 g H_3BO_3, 350 mL 1 M NaOH in 1 L deionised water. 4 mL reagent added to 30 mL sample	30 min	110–115	Initial pH 9.7, final pH 5–6	KHP (0.25–7 μM)	Alkaline peroxydisulfate method for TP and TN based on [85]	[98]
Seawater	8 mL of 5% $K_2S_2O_8$ added to 50 mL seawater	30 min	120	Final pH 1.5–1.8	lecithin (101%), PC (98%), AMP (99%), zooplankton (100%)	Recoveries of model compounds relative to sulphuric acid-hydrogen peroxide digestion [106]	[88]
Sediments and soils	1 mL 5.5 M H_2SO_4, 0.4 g $K_2S_2O_8$ and 1 mL distilled deionised water added to 10–50 mg sample	1 h	130	Not reported	Not reported	Acid peroxydisulfate digestion compared to perchloric acid digestion	[107]
Sewage	Digestion reagent: 9 g NaOH and 40 g $K_2S_2O_8$ in 1 L distilled deionised water. 2 mL digestion reagent added to 10 mL sample	90 min	120	Not reported, however KCl/acetate buffer pH 4.5	Sodium dihydrogen phosphate (93% using 0.15 M KCl/acetate), STP (85% using 0.4 M KCl/acetate), TSPP (96% using 0.4 M KCl/acetate)	Anion exchange chromatography used to separate ortho- and poly-phosphates using either 0.15 or 0.4 M KCl/acetate as the eluting buffer. No polyphosphates detected in raw sewage samples	[108]
Soil extracts	Digestion reagent: 0.39 M $K_2S_2O_8$ and 0.6 M NaOH. 2 mL reagent added to 8 mL sample	1 h	120	Not reported	Not reported	Same method (La Chat method 30-115-001-1-B) as [109]	[110]
Soil extracts	Digestion reagent: 13.4 g $K_2S_2O_8$ dissolved in 1 L 0.3 M NaOH. 15 mL reagent added to 10 mL sample. Added 1.5 mL 0.3 M HCl and made up to 50 mL after autoclaving	30 min	110	pH 2	KHP, PTA dodeca sodium salt (99% for 0.1 mg L^{-1}, and 106% for 1.0 mg L^{-1})	Analysed for TN and TP. PTA dissolved in different extractants: water, 0.1 M $CaCl_2$, and 0.2 M H_2SO_4, and recoveries were comparable. Alkaline peroxydisulfate method appropriate for soil extracts when concentration of total organic carbon <100 mg L^{-1}	[111]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Soil leachate	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	Not reported	Same method as [84]	[112–116]
Soil leachate	8 mg $K_2S_2O_8$ and 50 μL 0.5 M H_2SO_4 added to 1 mL sample	1 h	120	Not reported	KHP (101%), PTA (76%), TSPP (95%), STP, 1-AEP (86%), G-6-P-Na (84%), 5′-ATP-Na_2 (69%)	Preconcentration and separation method for trace P compounds using a scaled down version of [84]	[117]
Soil solutions	Digestion reagent: 0.05 M H_2SO_4 and 16 g L^{-1} $K_2S_2O_8$. 1 mL reagent added to 1 mL sample	30 min	110	Not reported	Not reported		[118]
Soil solutions	Digestion reagent: 50 mg $K_2S_2O_8$ and 0.1 mL 5.5 M H_2SO_4 added to 1 mL sample. After digestion, solutions diluted to 10 mL with deionised water	1 h	120	Not reported	KHP, PTA (93.2–95.0% in concentration range 3.23–32.26 μM)	Acid peroxydisulfate digestion compared to sulphuric–perchloric acid, nitric acid, and nitric–perchloric acid digestion. Better recoveries were found for PTA using sulphuric-perchloric acid and acid peroxydisulfate digestion methods	[119]
Soil solutions	Digestion reagent: 13.4 g $K_2S_2O_8$ dissolved in 1 L 0.3 M NaOH. 15 mL reagent added to 10 mL sample. Added 1.5 mL 0.3 M HCl and made up to 50 mL after autoclaving	30 min	110	pH 2	Not reported	Same method as [111]	[120]
Soil solutions	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	45 min	121	Not reported	Not reported	Method modified from [96]	[121]
Soil solutions	0.15 g $K_2S_2O_8$ and 1 mL 0.5 M H_2SO_4 added to 20 mL sample	1 h	120	Not reported	PTA (89%), G-6-P-Na (89%), tetra-potassium pyrophosphate (102%), 5′-ATP-Na_2 (96%), AMP (96%), KHP	Acidic method compared to peroxide-Kjeldahl, and nitric acid-sulphuric acid digestions [122]. Acidic peroxydisulfate method found to be the best method	[84]
Surface runoff	0.5 g $K_2S_2O_8$ and 1 mL H_2SO_4 solution (300 mL conc. H_2SO_4 in 1 L distilled water) added to 50 mL sample	30 min	110	Not reported	Not reported	Same method as peroxydisulfate method in [99]	[123]
Surface runoff	$K_2S_2O_8$ and H_2SO_4	30 min	120	Not reported	Not reported		[124]
Turbid lake and river waters	Optimum digestion reagent: 0.27 M $K_2S_2O_8$ and 0.24 M NaOH. 2 mL reagent added to 10 mL sample	1 h	120	Final pH 2	NIES No 3 Chlorella (99–101% up to 100 μg P L^{-1}) and No 2 Pond sediment (98–104% up to 60 μg P L^{-1}, and 88% at 100 μg P L^{-1}). Model compounds added to distilled and lake water: KHP, G-6-P (113%), PTA (101%), α-GLY (108%), PEP (103%), 2-AEP (104%), PFA (106%), *o*-phosphonyl ethanolamine (109%), SHMP (114%), aluminium phosphate (23%)	Compared alkaline peroxydisulfate autoclaving method to microwave and hot-plate digestion and Kjeldahl digestion for TN and TP. Results showed that all methods used were suitable for turbid lake samples when suspended material is of biological origin	[125]

Table 2 (*Continued*)

Matrix	Digestion reactant	Digestion time	Digestion temperature (°C)	pH	Model compounds[a]	Comments	Ref.
Turbid lake and river waters	Optimum digestion reagent: 0.27 M $K_2S_2O_8$ and 0.24 M NaOH. 2 mL reagent added to 10 mL sample	1 h	120 °C	Final pH 2	NIES No 3 Chlorella (99–101% up to 100 μg P L^{-1}) and No 2 Pond sediment (98–104% up to 60 μg P L^{-1}, and 88% at 100 μg P L^{-1}). Model compounds added to distilled and lake water: KHP (93–99%), PTA (93–106%), 2-AEP (93–101%), α-GLY (94–102%), PFA (93–105%), *0*-phosphonylethanol (91–106%), PEP (93–117%)	Compared alkaline peroxydisulfate autoclave method to microwave digestion, and similar results were found	[126]
Turbid lake waters	Digestion reagent: 9 g NaOH, and 40 g $K_2S_2O_8$ in 1 L water. 2 mL reagent added to 10 mL sample	1 h	120 °C	Not reported	NIES No. 3 Chlorella (94–107% up to 100 μg P L^{-1}, and 90% at 250 μg P L^{-1}) and No 2 Pond sediment (92–109% up to 100 μg P L^{-1}, and 88% at 250 μg P L^{-1}). Model compounds added to lake water: KHP (99%), STP (96%), AMP (94%), β-GLY (103%)	Compared alkaline peroxydisulfate method to nitric acid–sulphuric acid digestion method [99]. Results showed no significant difference between the two methods	[127]
Water (overland flow)	Digestion reagent: 0.39 M $K_2S_2O_8$ and 0.6 M NaOH. 2 mL reagent added to 8 mL sample	1 h	120 °C	Not reported	Not reported		[109]

[a] With recoveries given in parentheses when reported.

acid method [122] for soil solutions and leachates. The latter method gave erratic recoveries and was more prone to contamination due to the open digestion vessels used [84]. Peroxydisulfate autoclaving is also safer than perchloric acid digestion [107,137]. The acid peroxydisulfate method generally gives good recoveries for model compounds and is simple and easy to use and is therefore recommended for TP and TDP determinations in natural waters and, particularly, soil solutions.

4.2. Model compounds

It is advisable to test the efficiency of any digestion method using a range of model phosphorus containing compounds that reflect different chemical bonds and stabilities and are representative of naturally occurring compounds (see Table 3). The majority of relevant compounds contain C–O–P and/or P–O–P bonds. Few compounds reported in the literature contain C–P bonds, which are very resistant to oxidation and hydrolysis [138].

Phosphonates are refractory organic phosphorus compounds and can be released into seawater from biological sources [78,90,139], and have been detected in soils [140] and soil leachate [117]. As phosphonates contain a strong C–P bond that is resistant to acid hydrolysis [139], they are useful compounds for recovery studies [78,90,97,125,126,139]. Condensed inorganic (e.g. sodium tripolyphosphate) and organic (e.g. adenosine-5′-triphosphate) phosphates and cocarboxylase [141] have also been shown to be resistant to UV irradiation alone [79]. With acid or alkaline peroxydisulfate autoclaving, however, these compounds have been successfully broken down [97,103,125,126].

Inositol phosphates are an important class of naturally occurring organic phosphorus compounds [142]. Phytic acid, for example, is one of the more resistant compounds to hydrolysis and is also one of the most refractory organic phosphorus compounds found in soils [75,119,141]. Other organic phosphorus compounds found in soil leachate and runoff are the sugar phosphorus compounds, e.g. D-glucose-1-phosphate and D-glucose-6-phosphate, which are labile [117]. Organic condensed phosphates, e.g. adenosine-5′-triphosphate and adenosine-5′-diphosphate are also important as they originate from all living systems, e.g. algae, bacteria, fungi, insects, plant and animal tissues [117].

It is therefore recommended that model compounds selected for digestion studies should include one with a P–O–P bond (e.g. sodium tripolyphosphate), a refractory C–O–P compound (e.g. phytic acid), a labile C–O–P compound (e.g. D-glucose-1-phosphate or D-glucose-6-phosphate), a refractory C–P compound (e.g. 2-aminoethylphosphonate), and a compound containing C–O–P and P–O–P bonds (e.g. adenosine-5′-triphosphate). Orthophosphate (e.g. as potassium dihydrogen orthophosphate) should also be used in all recovery studies as a method control [90]. One should also be aware that specific matrices may require additional model compounds. For example, acid soils and sediments may well contain phosphorus associated with iron or alu-

Table 3
Model compounds used in autoclave based digestion methods

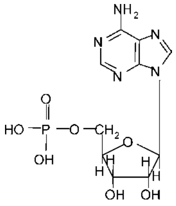
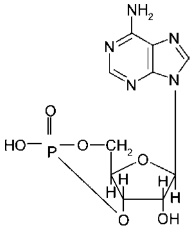
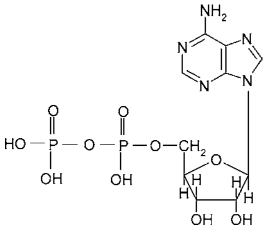
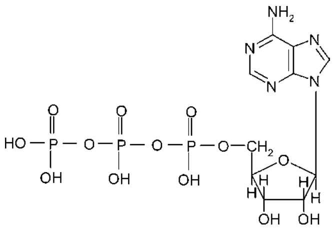
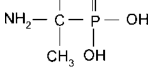
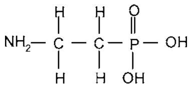
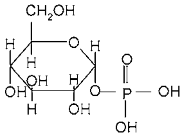
Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Adenosine-5′-monophosphate	Adenosine-5′-monophosphoric acid; 5-adenylic acid; adenosine phosphate; tert-adenylic acid; ergadenylic acid	AMP	$C_{10}H_{14}N_5O_7P$	
Adenosine-3′,5′-cyclic monophosphate	Adenosine-3′,5′-cyclophosphoric acid; cyclic AMP; 3′,5′-cyclic AMP	cAMP	$C_{10}H_{12}N_5O_6P$	
Adenosine-diphosphate		ADP	$C_{10}H_{15}N_5O_{10}P_2$	
Adenosine-5′-diphosphate (sodium salt)		5′-ADP-Na$_2$	$C_{10}H_{13}N_5O_{10}P_2Na_2$	Similar to ADP
Adenosine-5′-triphosphate		ATP	$C_{10}H_{16}N_5O_{13}P_3$	
Adenosine triphosphate disodium	Adenosine 5′-(tetrahydrogen triphosphate) disodium salt; adenosine 5′-triphosphate, disodium salt; adenosine 5′-triphosphate, disodium salt hydrate	5′-ATP-Na$_2$	$C_{10}H_{14}N_5O_{13}P_3Na_2$	Similar to ATP
1-Aminoethylphosphonate	1-Aminoethylphosphonic acid	1-AEP	$C_2H_8NO_3P$	
2-Aminoethylphosphonate	2-Aminoethylphosphonic acid	2-AEP	$C_2H_8NO_3P$	
Glucose-1-phosphate	Glucose-1-phosphoric acid	G-1-P	$C_6H_{13}O_9P$	

Table 3 (*Continued*)

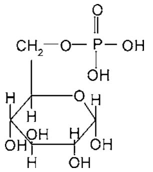
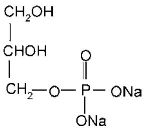
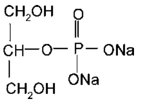
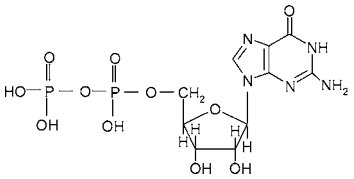
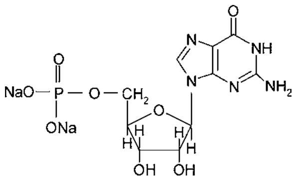
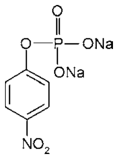
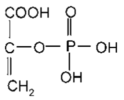
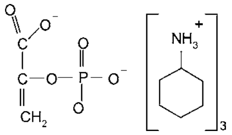
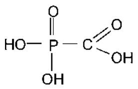
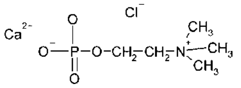
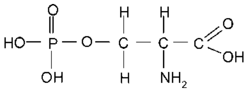
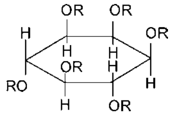
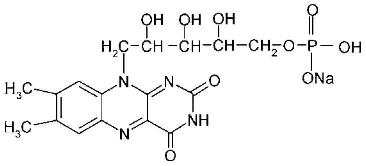
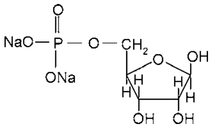
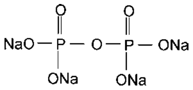
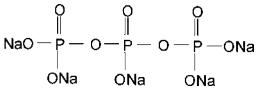
Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Glucose-1-phosphate dipotassium salt	Glucose-1-phosphoric acid (dipotassium salt)	G-1-P-K_2	$C_6H_{11}O_9PK_2$	Similar to G-1-P
Glucose-6-phosphate	Glucose-6-phosphoric acid	G-6-P	$C_6H_{13}O_9P$	
Glucose-6-phosphoric acid (dipotassium salt)	α-D-Glucose-6-phosphoric acid dipotassium salt	G-6-P-K_2	$C_6H_{11}O_9PK_2$	Similar to G-6-P
Glucose-6′-phosphate sodium salt		G-6-P-Na	$C_6H_{12}O_9PNa$	Similar to G-6-P
DL-α-Glycerophosphate disodium salt	rac-Glycerol 1-phosphate disodium salt; DL-α-glycerophosphate	α-GLY	$C_3H_7O_6PNa_2$	
β-Glycerophosphate disodium salt hydrate	Glycerol 2-phosphate disodium salt hydrate; sodium β-glycerophosphate	β-GLY	$C_3H_7O_6PNa_2$	
Guanosine 5′-diphosphate		5′-GDP	$C_{10}H_{15}N_5O_{11}P_2$	
Guanosine-5′-monophosphate disodium hydrate		5′-GMP-Na_2	$C_{10}H_{12}N_5O_8PNa_2$	
4-Nitrophenyl phosphate	*p*-Nitrophenyl phosphate	NPP	$C_6H_4NO_6PNa_2$	
Phospho(enol) pyruvate		PEP	$C_3H_5O_6P$	
phosphoenolpyruvic acid tri(cyclohexylamine) salt		PEP-3CHA	$C_3H_2O_6P\ (C_6H_{11}NH_3)_3$	
Phosphonoformate	Phosphonoformic acid	PFA	CH_3O_5P	

Table 3 (*Continued*)

Model compound	Synonyms	Abbreviation used in text	Chemical formula	Structural formula
Phosphoryl choline chloride calcium salt tetrahydrate	Phosphocholine chloride calcium salt tetrahydrate; calcium phosphorylcholine chloride	PC	$C_5H_{13}NO_4PCaCl{\cdot}4H_2O$	
Phosphoserine		SOP	$C_3H_8NO_6P$	
Phytic acid	Myo-inositol hexakis (dihydrogen phosphate); inositol hexaphosphoric acid	PTA	$C_6H_{18}O_{24}P_6$	 where R=PO_3H_2
Riboflavine-5′-monophosphate sodium salt	Riboflavin 5′-phosphate; FMN-Na	FMN	$C_{17}H_{20}N_4O_9PNa$	
Ribose-5-phosphate disodium salt dihydrate	D-Ribofuranose 5-phosphate	RP	$C_5H_9O_8PNa_2$	
Tetrasodium pyrophosphate	Sodium pyrophosphate; pyrophosphoric acid tetrasodium salt; diphosphoric acid, tetrasodium salt	TSPP	$Na_4O_7P_2$	
Sodium tripolyphosphate	Pentasodium tripolyphosphate dihydrate; sodium triphosphate; sodium polyphosphate; triphosphoric acid pentasodium anhydrous	STP	$Na_5P_3O_{10}$	
Sodium hexametaphosphate	Sodium metaphosphate; metaphosphoric acid, hexasodium salt; sodium polymetaphosphate	SHMP	$(NaPO_3)_n$	

minium phases, which are relatively resistant to oxidative dissolution [75].

4.3. Recovery studies using alkaline and acidic peroxydisulfate autoclaving

Typical phosphorus recoveries for a range of model compounds, digested using alkaline and acid peroxydisulfate autoclaving, are shown in Fig. 4. The alkaline peroxydisulfate digestion method can be used for the simultaneous determination of TP and TN [85]. This was chosen because the borate buffer ensures that the pH is initially alkaline, to break down nitrogen containing bonds, and becomes acidic during the digestion process to break down phosphorus containing bonds. An amount of 5 mL of digestion reagent (5 g potassium peroxydisulfate and 3 g boric acid dissolved in 100 mL 0.375 M sodium hydroxide) was added to 50 mL sample. The samples were then autoclaved for 30 min a 121 °C. Model compounds chosen were phytic acid, sodium tripolyphosphate and adenosine-5′-triphosphate, and were therefore representative of a refractory C–O–P compound, a P–O–P compound and a C–O–P and P–O–P bond containing compound, respectively. Recoveries were 89 ± 13% for phytic acid, 100 ± 13%

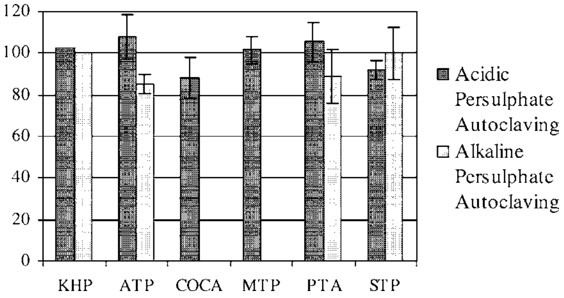


Fig. 4. Comparison of recoveries for a selection of model compounds using acidic and alkaline peroxydisulfate (40 g L^{-1}) autoclave digestions. KHP: potassium dihydrogen orthophosphate; ATP: adenosine-5′-triphosphate; COCA: cocarboxylase; MTP: methyltriphenylphosphonium bromide; PTA: phytic acid; STP: sodium tripolyphosphate. Error bars show ±3 standard deviations.

for sodium tripolyphosphate and 85 ± 4% for adenosine-5′-triphosphate.

The acid peroxydisulfate digestion method used was based on the method of Haygarth et al. [121]. One mL of 0.5 M sulphuric acid and 0.15 g potassium peroxydisulfate was added to 20 mL sample, and autoclaved for 45 min at 121 °C. The same compounds were used, plus two additional compounds that were not used in any of the autoclave methods listed in Table 2, but have been used in UV digestion studies, namely cocarboxylase containing C–O–P and P–O–P bonds [141] and methyltriphenylphosphonium bromide containing C–P bonds [143]. Recoveries were relatively low: adenosine-5′-triphosphate (74 ± 7%), cocarboxylase (68 ± 17%), methyltriphenylphosphonium bromide (93 ± 6%), phytic acid (60 ± 32%) and sodium tripolyphosphate (95 ± 4%). When the concentration of peroxydisulfate was increased from 8 to 40 g L^{-1} [81] however recoveries were greatly improved for adenosine-5′-triphosphate (108 ± 11%), cocarboxylase (88 ± 10%), methyltriphenylphosphonium bromide (102 ± 6%), phytic acid (105 ± 10%), and sodium tripolyphosphate (92 ± 5%). Peroxydisulfate concentration is the most important parameter, rather than digestion time or temperature, for improving recoveries, particularly for seawater samples [104].

4.4. Enzymatic degradation

Fig. 1 shows that DOP and total organic phosphorus (TOP) can be determined by difference following complete digestion, e.g. by autoclaving the sample (see Section 4.1). It is however desirable to be able to quantify specific organic compounds. To do this a more selective approach to digestion is required, such as the use of phosphate cleaving enzymes. This section therefore considers the use of acid and alkaline phosphatases and the particular sub-class of phytases.

Phosphatases belong to the class of enzymes called hydrolases [144] and their subclasses are alkaline phosphatase (EC.3.1.3.1) and acid phosphatase (EC.3.1.3.2). They hydrolyse phosphate monoesters to produce an alcohol and orthophosphate. Phosphatases play a key role in metabolic reactions such as the synthesis of organic phosphate compounds (transphosphorylation) and transport across cell membranes [145] and they have been isolated from a variety of sources. Alkaline phosphatase is the most studied phosphomonoesterase and has been isolated from, e.g. *Esherichia coli* [146,147]. Acid phosphatases show broad selectivity towards phosphomonoesters and have also been isolated from *E. coli* [146].

Strickland and Parsons established a classical method using phosphatase for the determination of phosphate [148] but this method was susceptible to product inhibition by reactive phosphate already present in the sample. McKelvie and coworkers immobilised *E. coli* onto CNBr-activated sepharose 4B beads in a FI system with an optimum pH of 8. The recovery of alkaline phosphatase hydrolysable phosphorus was low in natural waters but good in sediments [146]. They also applied alkaline phosphatase to soils [59]. Acid and alkaline phosphatase and phytase have been used in combination to investigate organic phosphorus speciation in soils [149].

Inositol hexaphosphate forms the bulk of extractable soil organic phosphorus [146,149,50]. Phytases (EC 3.1.3.8) are members of the family of histidine acid phosphatases [150,151] that are found in plants and micro-organisms, which catalyse the hydrolysis of phytate (myo-inositol hexakis-phosphate 1, 2, 3, 4, 5, 6) to less phosphorylated myo-inositol phosphates and free orthophosphates. Phytase from plant sources, e.g. wheat, first acts on the C_6 atom while that from microbial sources acts on the C_3 atom. McKelvie et al. [59,152] used a FI system with immobilised phytase for the determination of phytic acid in soils. Adenosine-5′-triphosphate was also hydrolysed but in low yields compared with phytic acid. Phytase has also been applied to the determination of phytic acid in the marine environment, but with low recoveries [152].

Enzymatic methods are important for assessing the potential biological availability of organic phosphorus but other methods are also needed for complete identification and this remains a challenging area of analysis.

5. Quality assurance and quality control

Phosphorus is a key determinand in most environmental monitoring and research programmes [153] and only accurate analytical data permits valid conclusions to be drawn about the phosphorus status of water bodies and soils. In addition to DRP it is also important to obtain accurate total phosphorus (TP) data because this parameter is used for load calculations, e.g. to determine discharges from sewage treatment works [18]. This has important implications regarding decisions on the installation (or not) of costly phosphorus removal technology. Programmes involving multi-national participation and international databanks [76] require adequate quality assurance/quality control (QA/QC) schemes to ensure the data integrity necessary for the comparison of data from various sources. Adherence to QA guidelines, participation in interlaboratory studies, use of reference materials (RMs) and certified reference materials (CRMs) are all means of achieving good data quality for phosphorus determinations [154,155].

5.1. *Certified reference materials*

A CRM is a reference material for which component values have been certified by a technically valid procedure and is accompanied by or traceable to a certificate or other documentation issued by a certifying body [156,157]. The use of CRMs is the most efficient way to measure and control accuracy [158] and can help produce reliable calibration and validation of measurement procedures [159]. CRMs can be either calibration CRMs, which are high purity substances or synthetically prepared mixtures, or matrix-matched CRMs, which can be natural samples or artificial samples simulating the composition of natural samples [158]. Few CRMs are commercially available for the determination of phosphorus species in environmental matrices (see Table 4), despite the need for such materials [155]. CRMs are not currently available for all environmental matrices routinely analysed for phosphorus species, such as estuarine waters, nor do they adequately span the range of phosphorus concentrations characteristic of environmental matrices. The National Research Council of Canada (NRCC) recognized the urgent need for CRMs for nutrients, including orthophosphate, for use in the marine sciences. MOOS-1, a natural seawater CRM available for the determination of nutrients in seawater, was developed in direct response to this need [159]. Analysis of MOOS-1 was carried out in 2002 by 25 expert laboratories participating in the 'NOAA/NRC 2nd intercomparison study for nutrients in seawater' [160]. Laboratories were predominantly selected on the basis of their previous satisfactory performance in a NOAA 2000 intercomparison study [159]. Flow and manual methods were used, all based on the spectrophotometric procedures of Strickland and Parsons [148]. Eighteen of the 25 laboratories achieved satisfactory *Z*-scores (see Section 5.2) for the determination of phosphate in seawater as shown in Fig. 5.

5.2. *Intercomparison exercises*

Inter-laboratory comparison studies are an essential feature of method development and validation [154] and play an important role in the certification of reference materials, such as described for MOOS-1 [159]. Performance in intercomparison studies undertaken by NOAA/NRCC in 2000 and 2002 [159,160] was used to assess the capabilities of international laboratories to quantify nutrients in MOOS-1, including orthophosphate. *Z*-scores [162] have been widely used for the statistical assessment of data in intercomparison exercises to give a comparative indication of performance with $|Z| < 2$ indicating satisfactory performance [160,163–166].

The main objectives of interlaboratory comparison studies are to determine inter-laboratory precision and accuracy and provide an impartial view of in-house quality control procedures. Participation can also identify best practise with respect to method, sample preparation, sample storage and training needs. The QUASIMEME project (Quality Assurance of Information for Marine Environmental Monitoring in Europe), now known as QUASIMEME Laboratory Performance Studies, was established to assist European Union labs in developing their QA/QC procedures to satisfy the data quality requirements of monitoring programmes in which they participated such as the International Marine Monitoring Programmes of the Oslo and Paris Commissions (OSPARCOM), the Helsinki Commission (HELCOM) and the MEDPOL programme [163,167]. Initially funded by the EU (1992–1996), the programme still continues by subscription of participating institutes. All institutes, worldwide, involved in chemical measurements in seawater are eligible to participate. The laboratory programmes for proficiency testing of most determinands are conducted twice per year and routinely include aqueous test materials containing orthophosphate and TP at concentrations similar to those found in estuarine, coastal and open water environments [168]. Regular testing is necessary to assure the quality of environmental data submitted since the performance of many laboratories does not remain constant [163,169]. The

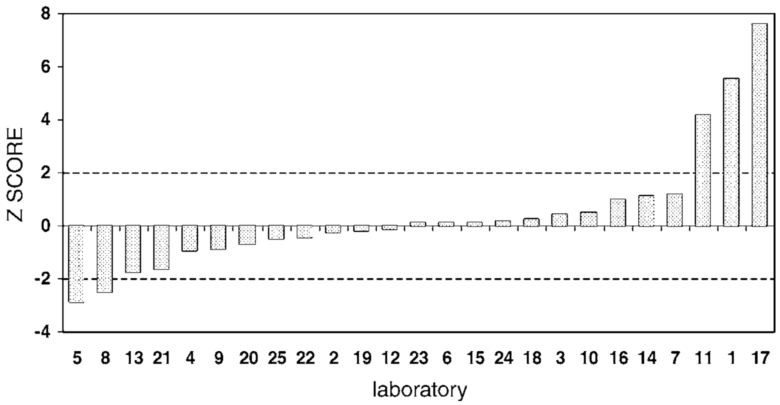


Fig. 5. Plot of *Z*-scores obtained by laboratories participating in the NOAA 2002 intercomparison study for the analysis of orthophosphate in MOOS-1. *Z*-scores calculated from the mean orthophosphate concentration, with the assigned value set at 1.6 ± 0.21 μM. $|Z| \leq 2$ represent the satisfactory *Z* score value for MOOS-1 [159,160].

Table 4
Commercially available CRMs for the determination of phosphorus species in environmental matrices

CRM	Matrix	Phosphorus species	Concentration	Comments	Supplier	Ref.
MOOS-1	Seawater	Orthophosphate	$1.56 \pm 0.07\ \mu\text{mol L}^{-1}$	Natural seawater sample, of Cape Breton Island, NS, Canada at a depth of 200 m	NRCC	[159,160]
QC RW1	Freshwater	Orthophosphate	$100\ \mu\text{g L}^{-1}$	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	[161]
QC RW2	Freshwater	Total phosphorus	$200\ \mu\text{g L}^{-1}$	Artificial sample, distributed as an ampoule to be 100 times with pure water	VKI	[161]
Australian natural water CRM	Natural water/freshwater	Orthophosphate	$27 \pm 0.8\ \mu\text{g L}^{-1}$	Natural water sample obtained from Christmas Creek in the Lamington National Park, Qld., Australia	Queensland Health Scientific Services	
		Total dissolved phosphorus	$37 \pm 1.2\ \mu\text{g L}^{-1}$			
BCR-616	Groundwater (high carbonate content)	Orthophosphate	$3.36 \pm 0.13\ \text{mg kg}^{-1}$	Artificial groundwater sample, prepared from ultrapure water, to which required salts were added; stabilized by autoclaving	BCR	www.irmm.jrc.be
SRM®-2702	Marine sediment	Total phosphorus	0.1552 ± 0.0.0066%	Material for SRM® was collected from Chesapeake Bay, USA, freeze-dried, seived at 70 µm (100% passing) and cone blended, then radiation sterilized and bottled	NIST	www.nist.gov
SRM®-1646a	Estuarine sediment	Total phosphorus	0.027 ± 0.0.001%	Material for SRM® was dredged from Chesapeake Bay, USA, freeze-dried, lightly deagglomerated and < 1 mm fraction ball milled and the < 75 µm blended and bottled	NIST	www.nist.gov
BCR-684	River sediment	NaOH-extractable P HCl-extractable Inorganic P Organic P Conc. HCL–extract P	$500 \pm 21\ \text{mg kg}^{-1}$ $536 \pm 28\ \text{mg kg}^{-1}$ $1113 \pm 24\ \text{mg kg}^{-1}$ $209 \pm 9\ \text{mg kg}^{-1}$ $1373 \pm 35\ \text{mg kg}^{-1}$	Material for the CRM was collected from the lower reaches of the River Po, Italy, then sieved and the <2 mm fraction was dried, lightly deagglomerated, crushed and hammer-milled and < 90 µm blended and bottled	BCR	www.irmm.jrc.be

assessment of the quality of data must be made at the time that the environmental samples are analysed. Such exercises provide vital information for improving the quality and performance of laboratories and a structure for developing robust analytical techniques. To this end, the QUASIMEME Laboratory Performance Study was designed to follow the IUPAC/ISO/AOAC international protocol for international testing [162,170]. All laboratories that submit data to the UK National Marine Monitoring Programme (NMMP) routinely participate in QUASIMEME as a means of external QA/QC of the data collected, including orthophosphate [171]. There have been several other national and international intercomparison exercises including the series of International Council for the Exploration of the Seas (ICES) exercises [164] and the Australian National Low level Nutrient (ANLLN) exercise.

5.3. Databases

Environmental monitoring and research programmes generate large amounts of information and can provide valuable databases of analytical information if appropriate QA/QC measures are used to preserve data quality. For example, databases have been generated from of the NMMP and the

'Winter Monitoring of the Western Irish Sea' programme [165] and both incorporated QA/QC schemes to ensure data integrity. Legislation such as the EU Water Framework Directive outlines an approach for managing water quality in the member states of the European Union which will require monitoring and environmental quality data (including P data) to be collected by member states and presented at the EU level. There is therefore the potential to add to the repository of data already held by the European Environmental Agency, and adherence to QA/QC practices such as intercomparison studies in conjunction with routine in-house use of RMs and CRMs is essential if such data are to be of practical use. Phosphorus data are also incorporated within larger assessment exercises dealing with broader issues such as water quality and eutrophication, e.g. the National Estuarine Eutrophication assessment in the United States [172].

6. Conclusions

Accurate determination of P species in environmental matrices is an important pre-requisite for understanding the biogeochemical cycling of the element. This in turn is essential for investigating the impact of phosphorus on ecosystem health. Key aspects of the analytical process for obtaining high quality phosphorus data are robust sampling and sample treatment protocols (see also Maher and Woo [75]). These cannot be universal due to the variability in behaviour of different matrices but nonetheless guidelines can be provided for aspects such as filtration, chemical treatment and storage conditions. For soils, wetting and drying have a considerable affect on phosphorus solubility.

In addition, for the determination of different phosphorus fractions and individual phosphorus containing compounds, particular attention needs to be given to the digestion process. Autoclaving (typically with peroxydisulfate in acid or alkaline media) is a widely used method that gives good recoveries but it is important to quantify this using a range of environmentally relevant model phosphorus containing compounds. Selective enzymatic degradation (typically using phosphatases) is a useful additional approach for the quantification of individual phosphorus containing compounds (or classes of compounds).

A critical aspect of the overall analytical process for any laboratory is participation in intercomparison exercises. This is particularly important for phosphorus determination due to the lability of the element in biologically active environmental matrices. To supplement such exercises the availability of more environmental certified reference materials is an important requirement. Finally, co-operation between analytical scientists and environmental scientists is fundamental to the generation of high quality, publicly available databases on the spatial and temporal variability of phosphorus species in aquatic and terrestrial ecosystems.

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