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Reduced inorganic phosphorus in the natural environment: significance, speciation and determination

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Abstract

It is commonly assumed that phosphorus occurs almost exclusively in the environment as fully oxidized phosphate (primarily $H_2PO_4^-$ and HPO_4^{2-}, where the oxidation state of phosphorus is +V). Recent developments in the field of microbiology and research on the origin of life have suggested a possibly significant role for reduced, inorganic forms of phosphorus in bacterial metabolism and as evolutionary precursors of biological phosphate compounds. Reduced inorganic forms of phosphorus include phosphorus acid (H_3PO_3, P(+III)), hypophosphorus acid (H_3PO_2, P(+I)) and various forms of phosphides (P(−III)). Reduced phosphorus has been detected in anaerobic sediments, sewage treatment facilities and in industrial and agricultural processes.

Microbiological evidence suggests a significant role for reduced phosphorus species in metabolic processes and raises interesting questions regarding the biogeochemistry of this nutrient in the environment. However, the paucity of data on the presence and cycling of reduced phosphorus compounds in the environment requires attention in order to elucidate the role of these compounds in natural systems. This paper discusses the significance of reduced phosphorus in the natural environment, its speciation and methods of detection.

Keywords: Reduced phosphorus; Phosphite; Hypophosphite; Phosphine; Phosphate; Speciation; Detection methods

1. Introduction

Phosphorus (P) is an essential element for life on Earth and plays a vital role in cell physiology and biochemistry. It is also an important component of biomolecules including phospholipids, nucleic acids, proteins and polysaccharides. In the natural environment, P is frequently a limiting nutrient, often inhibiting primary productivity. Much research has been conducted to date to provide an understanding of how P is cycled through the environment. However, a true understanding of the dynamics of P in the natural environment requires expanded knowledge of its oxidation–reduction (redox) cycling. While other biologically relevant elements have well-defined redox mechanisms (e.g., denitrification for the conversion of nitrate to nitrogen gas), thus far, it has been assumed that P species do not undergo redox-reactions in the environment and exist solely in the phosphate, organic P esters (P–O–C bonds) and colloidal complex forms in aqueous systems [1–3].

The importance of P for microbial growth is well established, with phosphate and hydrolyzed phosphate esters being considered the most readily biologically available forms [2,4]. A synthesis of the cycling of phosphate in the environment is beyond the scope of this paper. Many recent review papers have discussed the phosphate cycles in great detail (see, for example [5–7]). Recent biochemical evidence, however, suggests that phosphite and hypophosphite are important alternative sources of P to organisms [8–10]. In these cases, various aerobic and anaerobic organisms were able to

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utilize the reduced P species as the sole source of P. A recent study found that phosphite oxidation coupled with sulfate reduction can be used as energy for metabolism in *Desulfotignum phosphitoxidans* [11,12], underscoring the importance of the reduced forms of P.

This paper presents an informative review of the importance of reduced P species, their speciation and enhanced measurement techniques based on the evidence currently in the literature. The first section describes the hypothesized importance of reduced P compounds to the onset of life on the primitive Earth. This is followed by a discussion of the various forms of reduced P compounds found in the environment today. Next, the speciation of reduced P compounds in various redox and pH environments is described. The last section details the analytical methods used to detect and quantify reduced P in the environment.

2. Phosphorus and the origin of life on Earth

The availability of P in the natural environment is essential to the formation of biologically relevant molecules, and subsequently, life on the primitive Earth. Its current abundance in the earth's crust is approximately 0.12%; however, almost all of the P on Earth is found in the form of minerals including apatites (chloro and fluoro), vivianite, wavellite, and phosphorites [13]. Apatite ($Ca_5(PO_4)_3$[F, OH or Cl]), the largest reservoir of phosphate on Earth [14–17], is relatively insoluble in water. Most of the P released into the current natural environment is present in particulate form [15,16,18–20] and is not biologically available, limiting primary production [19,21]. For these same reasons, it is postulated that, on the primitive Earth, phosphate would also have been limited, restricting the synthesis of biologically essential compounds [22].

Given the reducing environment on the primitive Earth, it has been proposed that a significant portion of the minute concentration of phosphate originating from minerals may have been initially converted to more reduced forms of P, which are more soluble than phosphate. For example, volcanic eruptions and associated lightning discharges may have produced hypophosphorus and phosphorus acids [22–25], while elemental P, phosphine and phosphorus acid were presumably produced during core formation [26]. De Graaf and Schwartz [24] showed that, under reducing conditions, 20% of the available phosphate in apatite is converted to phosphite, which would lead to a significant amount of bioavailable dissolved P in the environment.

Phosphite is stable in the absence of strong oxidizing agents [23,27] and its salts are relatively soluble [23]. Thus, it may have accumulated on the primitive Earth, where ultraviolet irradiation of solutions of phosphite in the presence of simple organic compounds (e.g., acetylene), leads to the synthesis of water-soluble polyphosphonic acids (e.g., methyl and ethyl phosphonic acids, vinyl phosphonic acid (VPA)) [23,28]. Phosphonic acids are also the only organic P compounds found in meteorites [29], suggesting an added (i.e., extraterrestrial) source of reduced P species on Earth. Further chemical reactions on the primitive Earth (e.g., VPA can undergo photolysis reactions generating oxygenated products) would be fundamentally important to the synthesis of biologically relevant molecules [28]. Thus, reduced P may have played a critical role in the onset of life on Earth. If this is the case, it is not surprising that researchers today are finding microorganisms that are capable of utilizing reduced sources of P in metabolism.

3. Reduced P in the environment

Phosphorus is found in nature in various forms including: (1) mineral forms; (2) organic forms such as phospholipids, nucleic acids, proteins, polysaccharides, nucleotide cofactors, and as phosphonates [30]; (3) dissolved inorganic forms such as pentavalent, trivalent or univalent dissolved species; (4) gaseous forms in the −III oxidation state; and (5) particulate or colloidal forms. In aquatic systems, for example, P occurs in both particulate and dissolved forms and can be operationally defined as total P (TP), filterable reactive P (FRP), total filterable P (TFP) and particulate P (PP). Total P is a measure of total dissolved plus particulate P and a useful predictor of mean chlorophyll concentrations in streams [31]. It is difficult to quantify particular P fractions due to differing water bodies and dynamic physico-chemical and biological instream processes. In chalk-based catchments, for example, seasonal fluctuations in pH, dissolved carbon dioxide and total dissolved calcium concentrations occur, possibly leading to inorganic P (and possibly part of the dissolved organic pool) being co-precipitated with calcium as calcite [32]. Discharge also plays an important role in determining P fractions in water bodies. During high discharge periods (e.g. storm events), for example, diffuse inputs of P have been shown to increase both as PP, total dissolved P (TDP) and/or FRP [33].

Inorganic P can exist in five different oxidation states (+V, +III, +I, 0, −III). Phosphate (i.e., P in the +V state) is a limiting nutrient distributed widely in the bio-, hydro-, and lithosphere. Thus, it is likely the case that organisms with the ability to utilize alternative sources of P, namely reduced P, may be at an ecological advantage under these limiting conditions.

Phosphite has been used as a fungicide and fertilizers as an indirect source of P to plants (it is presumed that oxidation of phosphite to phosphate by soil bacteria makes this nutrient available to higher plants) [34,35]. Phosphite is also used as a fumigant because it is toxic to insects at relatively low concentrations [36].

Phosphides or P compounds in the P (−III) state, are the most widely discussed forms of reduced P found in the literatures [34,37]. Phosphides are naturally found on Earth and in meteorites as schreibersite $(Fe,Ni)_3P$. Hydrogen phosphide (phosphine, PH_3) has been shown to exist in sewage sludge, sediments, soils, animal manure, marshes and

landfills. Glindemann et al. [38] detected phosphine in landfills, sewage works, compost processing and river sediments, and concluded that it is a ubiquitous trace gas emitted from the anaerobic biosphere. More recently, this same group of researchers was able to detect phosphine in remote air samples in the high troposphere [39]. Phosphine is formed naturally during the anaerobic decomposition of organic matter (i.e., splitting of proteins) containing P [13]. The production of phosphine by anaerobic bacteria in marshes however, has been disputed in the literature (see discussion in [40]). For example, Devai et al. [41] and Devai and Delaune [42] have reported significant phosphine emissions from sewage and waterlogged soils (on the order of 1 ng PH_3 m^{-2} h^{-1}). On the other hand, Burford and Bremner [40] and Brunner et al. [43] found no evidence for the production of phosphine and presented thermodynamic and metabolic arguments against its biogenic production. However, significant sorption of phosphine to solid matrices may inhibit its release from the matrix and its subsequent detection in off-gas samples [40,44].

The distribution of P species among environmental compartments (e.g., between water and sediments), significantly affects the bioavailability of these species to organisms. All sorption/desorption studies related to inorganic P species have been conducted exclusively using phosphate. These studies have found that P exists in many forms in sediments including absorbed onto organic matter (OM) and adsorbed on oxides and clays. Much of this association depends on particle type, size and OM concentration and overall charge (see Fig. 1) [1,2]. Among other factors, temperature [3], redox conditions [4,9,10,17], and mineralogy [45] affect the release and uptake of P in sediments. Specific geochemical processes include precipitation, dissolution, sorption, and desorption. The precipitation/dissolution reactions of phosphate have been studied extensively [46–52]. Sorption/desorption processes have also received considerable attention and are thought to control the uptake and release of phosphate from real soils [53]. A recent set of literature reviews provides a comprehensive discussion of the sorption and desorption of phosphate [54,55]. Sorption/desorption of reduced P species in natural aqueous-solid systems has not been investigated. Such studies would provide essential data on the cycling and bioavailability of P in the natural environment, as well as on the primitive Earth.

Phosphine may also be emitted during the storage of ferrous alloys and is produced as a result of hydrolysis reactions during manufacturing processes (i.e., production of gallium phosphide) [56]. Agricultural researchers have also been interested in the uptake and release of phosphine, a fumigant, in crops (see, for example [36,57]). Dumas, for example, found that after 220 days of aeration, even under high temperatures (i.e., 85 °C), phosphine was desorbing slowly from wheat [36], indicating that it is persistent in the environment.

Oxidation is believed to be the primary sink for phosphine gas. It may, for example, react with the hydroxyl radical in the atmosphere to ultimately produce water-soluble phosphoric acid [38], which may form cloud condensation nuclei leading to the wet deposition of P onto the Earth. This may be the only recognized redox biogeochemical cycling of P in the natural environment, although the contribution of phosphine to the overall P cycle is considered to be negligible and

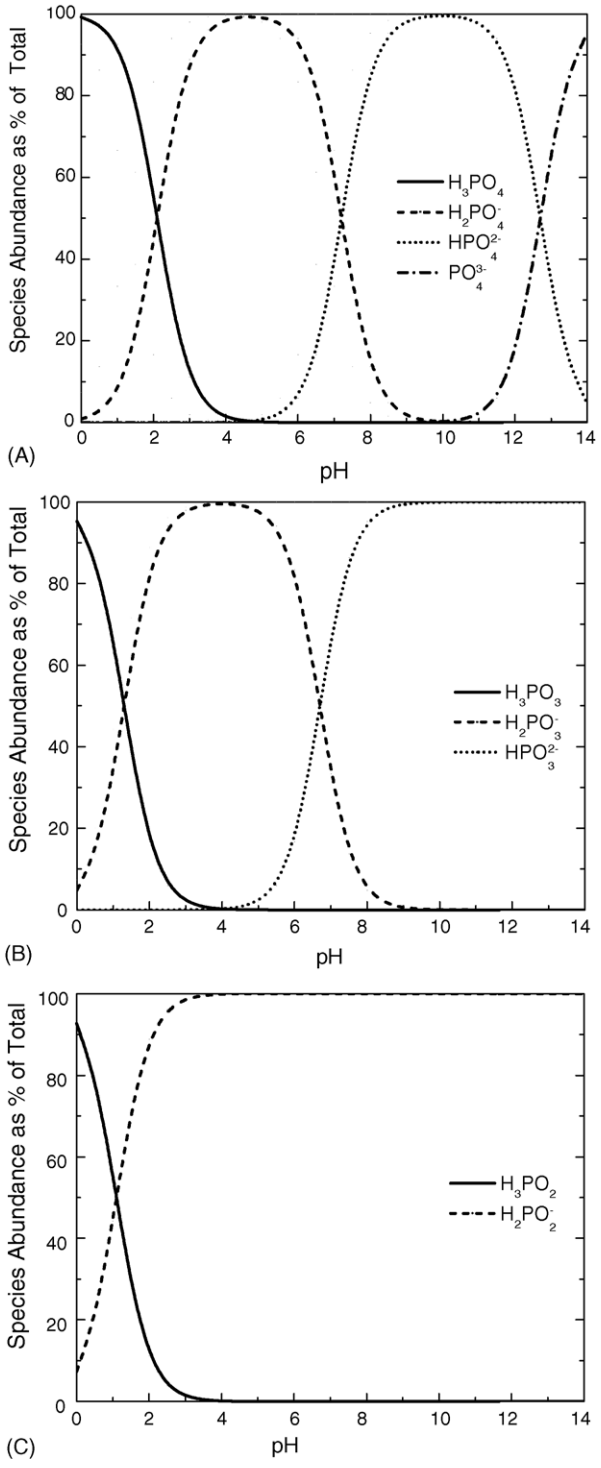

Fig. 1. Species abundance, as a percentage of the total concentration are shown for phosphate (A), phosphite (B), and hypophosphite (C) species. At circumneutral pH (typical of surface waters), the dominant species are $H_2PO_4^-$ and HPO_4^{2-} for phosphate, $H_2PO_3^-$ and HPO_3^{2-} for phosphite, and $H_2PO_2^-$ for hypophosphite.

is ignored [58]. Unfortunately, data on the occurrence, persistence, and fate of phosphine in natural waters, soils, and sediments are scarce. In these environments the presence of surfaces (i.e., particles) available for heterogeneous reactions may promote the oxidation of phosphine gas (either directly or by catalyzing other reactions) to other forms of P, including hypophosphite and phosphite, quickly eliminating phosphine from the environment.

Phosphonates are organic forms of phosphorus, characterized by a stable, covalent phosphorus–carbon bond (C–P) and are resistant to chemical hydrolysis and thermal decomposition [30,59,60]. These compounds are briefly mentioned here since they, like the reduced inorganic P species, have been demonstrated to serve as alternative sources of P to microorganisms [30]. Phosphonates are effective chelating agents and inhibitors of mineral precipitation and have many industrial applications (herbicides, detergent additives, medical applications to name a few). Of these compounds, 2-aminoethylphosphonic acid was the first to be identified in nature [61] and is found in the membranes of many animals, plants, and prokaryotes [62]. Bacteria have also been identified that can cleave the C–P bond and use the phosphonate as a P source [30,63–65]. However, most of the anthropogenic polyphosphonates are large molecules and are resistant to degradation by bacteria. The reader is directed to an excellent review of these organic P compounds by Nowack [59]. The remaining sections of this review will focus on the inorganic and gaseous forms of reduced P.

4. Speciation

Fig. 1 depicts the calculated equilibrium distributions of the various species of phosphate, hypophosphite, and phosphite as a function of pH. At the circumneutral pH of most natural waters and soils, the dominant P species according to equilibrium calculations are: $H_2PO_4^-$ and HPO_4^{2-} for phosphate, $H_2PO_3^-$ and HPO_3^{2-} for phosphite, and $H_2PO_2^-$ for hypophosphite. This speciation is based on the following pK_a values: for phosphate, pK_{a_1} = 2.1, pK_{a_2} = 7.2, and pK_{a_3} = 12.7; for phosphite, pK_{a_1} = 1.3 and pK_{a_2} = 6.7; and for hypophosphite pK_{a_1} = 1.1 [66]. The charge of each species will determine the environmentally relevant reactions (such as sorption/desorption) that may influence its mobility and distribution. Furthermore, the level of protonation of a given chemical species will influence its detection [27].

Given the redox conditions on the surface of the Earth, the predominant and stable form of P over all typical environmental pH values should be the pentavalent (+V) species (see Eh/pe-pH diagram in Fig. 2—equations used to generate Fig. 2 can be found in Table 1). Fig. 2 is a graphical representation of equilibria between chemical species as both a function of pH and redox potential (Eh or pe). Natural waters range between pH values of 4 and 10 and between Eh values of +1000 and −600 mV [67], suggesting that based on equilibrium considerations, reduced P should not be present in natural waters. However, despite the low Eh environments in which reduced P species are expected to exist, P in the −III oxidation state has been detected in the environment and has been shown to be relatively stable in the presence of oxygen and absence of other catalysts [37]. Furthermore, we have shown that standard solutions of P in the +III (phosphite) and +I (hypophosphite) oxidation states are stable for at least 15 days under high Eh, aerobic laboratory conditions [27]. If we, however, extend our definition of "natural waters" to include unusual and acidic aquatic environments such as those associated with acid mine drainage, the lower limit of Eh values in acidic environments (pH = 1 or lower, see, for example [68]) can be below −800 mV. In such environments, the fully protonated form of phosphite, H_3PO_3, is thermodynamically stable.

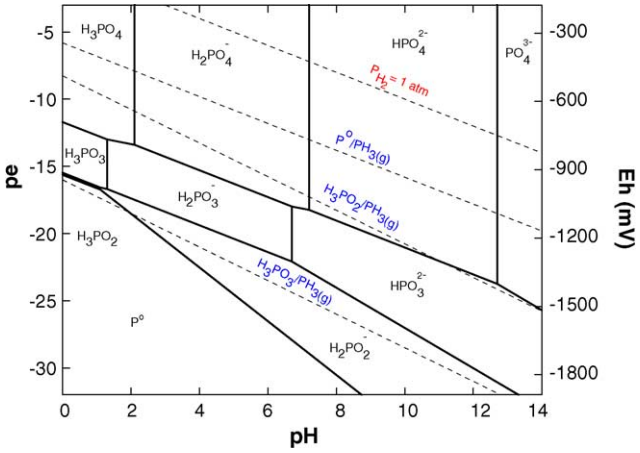

Fig. 2. Eh/pe-pH diagram for P species in water. For calculations involving equilibrium between reduced P species and phosphine (i.e., dashed lines including PH_3), an equilibrium concentration of 10^{-6} M for the reduced P compound was assumed. The dashed line with P_{H_2} = 1 atm is shown as a reference for a reducing environment on Earth.

Table 1
Half-reactions and thermodynamic constants involving phosphate, phosphite, and hypophosphite species

Reaction	E_h^0 (V)	pe^0	log K
$H_3PO_4 + 2e^- + 2H^+ \rightarrow H_3PO_3 + H_2O$	−0.69	−11.695	−23.390
$H_3PO_4 + 2e^- + 2H^+ \rightarrow H_2PO_3^- + H_2O$			−24.690
$H_2PO_4 + 2e^- + 2H^+ \rightarrow H_2PO_3^- + H_2O$			−22.590
$H_2PO_4^- + 2e^- + 2H^+ \rightarrow H_2PO_3^- + H_2O$			−29.290
$HPO_4^{2-} + 2e^- + 2H^+ \rightarrow H_2PO_3^- + H_2O$			−22.090
$PO_4^{3-} + 2e^- + 3H^+ \rightarrow H_2PO_3^{2-} + H_2O$			−9.390
$H_3PO_3 + 2e^- + 2H^+ \rightarrow H_3PO_2 + H_2O$	−0.913	−15.475	−30.95
$H_3PO_3 + 2e^- + 2H^+ \rightarrow H_2PO_2^- + H_2O$			−32.05
$H_2PO_3^- + 2e^- + 2H^+ \rightarrow H_2PO_2^- + H_2O$			−30.75
$HPO_3^{2-} + 2e^- + 3H^+ \rightarrow H_2PO_2^- + H_2O$			−24.05
$H_3PO_2 + e^- + H^+ \rightarrow P^0 + 2H_2O$	−0.922	−15.627	−15.627
$H_2PO_2^- + e^- + H^+ \rightarrow P^0 + 2H_2O$			−14.527
$P^0 + 3e^- + 3H^+ \rightarrow PH_{3(g)}$	−0.525	−8.898	−26.7
$H_3PO_2 + 4e^- + 4H^+ \rightarrow PH_{3(g)} + 2H_2O$			−42.322
$H_3PO_3 + 6e^- + 6H^+ \rightarrow PH_{3(g)} + 3H_2O$			−73.271

Eh values included in the table were taken from the literature [11], all other constants were calculated.

These observations suggest that the kinetics of chemical oxidation of these species may be relatively slow with respect to the equilibrium conditions depicted in Fig. 2. Thus, given potentially slow redox kinetics, we expect that reduced forms of P may exist in highly reducing microcosms in nature but that, thus far, have circumvented detection due to limitations in detection capabilities. Moreover, microorganisms (rather than chemical oxidants) are likely to be the catalysts or mediators of redox-reactions that rapidly cycle reduced P to phosphate in the environment. The strongest evidence for the existence of a detectable redox cycle for P is in the microbiology literature. The idea of the biological formation of reduced gaseous P compounds dates back more than 100 years [51], with Barrenscheen and Beckh-Widmansletter being one of the first groups to postulate the existence of anaerobic bacteria able to reduce organic P compounds to phosphine [52].

Adams and Conrad [69] were the first to report on the biological oxidation of phosphite. Since then, other researchers have also studied bacterial oxidation of phosphite and hypophosphite [65,70–72]. In most cases, the organisms were able to utilize either one or both of the reduced P species as the P source, oxidizing these compounds under both aerobic and anaerobic conditions. Evidence from *Pseudomonas stutzeri* WM88 also suggests that hypophosphite is oxidized to phosphate via a phosphite intermediate [73]. For example, the bacterial species *Agrobacterium tumafaciens*, *Bacillus caldolyticus*, *Escherichia coli*, species of *Pseudomonas* (including *aeruginosa*, *fluorescens*, and *stutzeri*), and *Serratia marcescens* and the yeast *Saccharomyces cerevisiae* have been described for their ability to oxidize phosphite [10,45,46,72] or hypophosphite [69,71–74]. These reports provide evidence for assimilatory metabolism of reduced P compounds. Therefore, phosphite and hypophosphite may be important sources of P to many other organisms and could be important in the cycling of P in general [11,12,30,63,65,70–73,75–84].

Research conducted by Metcalf and colleagues has furthered the field of reduced P biochemistry through the purification and characterization of an enzyme that catalyzes phosphite oxidation from *Pseudomonas stutzeri* WM88 named "phosphite dehydrogenase" or "PtxD" that is an NAD-dependent dehydrogenase [76]. In 2000, German researchers made the next key finding in the developing story of bacterial use of reduced P. A novel organism, *D. phosphitoxidans* was isolated that grew by the anaerobic oxidation of phosphite to phosphate coupled with the reduction of sulfate to hydrogen sulfide [11,12]. While previous reports had provided evidence for the assimilatory metabolism of reduced P compounds, this paper provided evidence for phosphite oxidation for energy metabolism. Furthermore, these researchers suggested that reduced P metabolism may have been an important activity on the early Earth and so reduced P metabolism could be an ancient evolutionary trait significant to the origin of life. Thus, the microbiological importance of reduced P compounds has been unequivocally established.

5. Reduced phosphorus determination

The first step in locating reduced P in the environment, which will help to elucidate the role of reduced P redox chemistry in natural systems, is to develop sensitive analytical methods of detection. Traditional methods of P determination are based on the reaction of P with acidified molybdate in aqueous solution to yield phosphomolybdate heteropoly-acid, which is then reduced using ascorbic acid or tin(II) chloride. The product is analyzed spectrophotometrically [85] to quantify the total amount of P in the sample. While the P determined here is defined as molybdate-reactive or FRP, other P-containing organic compounds and condensed phosphates can be determined following chemical, photochemical, thermal or microwave digestion [86]. Consequently, the molybdate method of P determination can be used to detect P in a variety of media including particles, natural waters, polluted waters, and aqueous extracts from plants, fertilizers, and other samples.

The traditional molybdate-active method of P determination is excellent for the detection of P in aqueous solutions, and when FRP was believed to be only phosphate, there was little need for new analytical techniques. However, the results of recent microbiological studies and measurements of environmental phosphine gas suggest that FRP may include not only phosphate, but a significant portion of reduced P oxyanions as well, which are indistinguishable from phosphate in the traditional molybdate-reactive method of detection. New methods of P determination must be established to distinguish various potential forms of FRP including the oxyanions phosphate, phosphite, and hypophosphite in aqueous media representative of natural waters, polluted waters, and biological extracts. It is less important that these methods isolate P in the form of phosphine, because phosphine is only slightly soluble in water. This section discusses modern analytical techniques with the potential of determining the amount of phosphite, hypophosphite, and phosphine.

There have been a number of studies describing the analysis of reduced P species in various environmental matrices, with Table 2 providing a selected compilation of methods and performance data from published work [27,81,87–94]. Established methods for the detection of P oxyanions are presented at the beginning of Table 2. These methods include ion chromatography, spectrophotometric determination in flow injection systems, and ^{31}P NMR. The ^{31}P NMR technique uses D_2O locking as a tracer for the oxidation of reduced phosphorus species [73]. It is not very sensitive, and is restricted to the laboratory; consequently, this work will not be discussed further in this paper.

Ion chromatography (IC) has been shown to be the method of choice by providing the selectivity, speed and affordability required for the detection of reduced P species. McDowell et al., for example, successfully used suppressed conductivity IC methods to detect hypophosphite and phosphite in a geothermal water matrix containing fluoride, chloride,

Table 2
Selected methods for the determination of reduced P species in environment samples

Reduced P species	Matrix	Method	Limit of detection[a]	Comments	Reference
$H_2PO_3^-$ and $H_2PO_2^-$	Synthetic geothermal water	Suppressed conductively ion chromatography	0.39, 0.83 μM	Addressed challenges associated with resolving $H_2PO_4^-$ from fluoride and $H_2PO_3^-$ from hydrogen carbonate contained in geothermal waters	[27]
	Synthetic waste waters	Single-column ion chromatography with indirect UV detection	1.25, 1.50 ppm	Optimized analytical conditions employed 4-amino-2-hydroxybenzoic acid (4 mM, pH range 5.5–6.5 at a flow rate of 2 mL min^{-1})	[87]
	Morpholinepropanesulfonic acid media	^{31}P NMR with D_2O locking	N/R	Appropriate for analysis in highly complex media used in microbiology studies	[73]
$H_2PO_3^-$	Liquid fertilizers	Spectrophotometric determination in a flow injection system	0.05% (w/v)	Online sample preparation consisting of the oxidation of $H_2PO_3^-$ by an acid permanganate solution heated to 50 °C	[89]
	Plant samples	Facile high performance ion chromatography	3–5 ppm	Samples analyzed within 24 h of preparation to avoid possible microbial growth	[91]
	Plant samples	Gas chromatography–mass spectrometry and ion chromatography	100 ng (IC), 100 pg (MS)	Samples extracted into formic acid-isopropanol, clarified by chloroform addition and partially purified by cation- an anion-exchange resins	[93]
PH_3	Sewage sludge cultures	Gas chromatography with a nitrogen–phosphorus detector (NPD)	N/R	Gas chromatographic analysis of phosphine in the headspace of cultures inoculated with sewage sludge	[81]
	Aluminium phosphide fumigated whole grain and soybeans	Suppressed conductively ion chromatography	0.010 μg g^{-1}	Method used converted PH_3 to $H_2PO_4^-$ and isolation by IC with eluent-suppressed conductivity detection	[88]
	Outgassing of semi-conductor devices, colonic gases	Packed column gas chromatography with alkali flame ionization detection	0.01 ng mL^{-1}	Method extended to the determination of phosphine in water	[90]
	Biogas, manure and sludge	Gas chromatography-thermionic specific detection	4 ng m^3	Sample preconcentration trap with glass beads employed	[92]
	PH_3–N_2 gas mixtures	Electrochemical oxidation on a SPE-based sensor	0–100 ppm range reported	Based on the oxidation of PH_3 on a solid polymer electrolyte (SPE)-Pt electrode. Potential for in situ investigations	[94]

[a] N/R: not reported.

bromide, nitrate, hydrogen carbonate and sulfate [27]. The listed common ions are present in both clean and polluted environmental waters, and consequently, methods established for the determination of reduced P in geothermal waters are readily extrapolated to other aqueous media. The McDowell et al. study reported a significant improvement in the limits of detection for hypophosphite (0.83 μM) and phosphite (0.39 μM), over existing ion chromatography methods [87,91,93,95–97]. If phosphite and hypophosphite are present in natural waters, estimated concentrations should be less than the estimated phosphate concentrations found in natural waters (0.3–30 μM [98]). Consequently, the submicromolar detection limits reported in McDowell et al. should be low enough to detect phosphite and hypophosphite in clean water, and can readily measure reduced P in more reducing environments such as sewage plants. Studies are currently underway in our laboratory to employ 2D analysis techniques with lower LODs and higher selectivity that will compliment the IC method of reduced P speciation.

Although ion chromatography is the best method that has been developed for quantitative analysis of separated reduced P oxyanions, there are some limitations. Suppressed ion chromatography is limited by its ability to resolve reduced P from other ions in solution including fluoride and hydrogen carbonate peaks [27]. One method of addressing this challenge is to use 2D analytical techniques that capitalize on the separation powers of ion chromatography, and use selective detectors such as mass spectrometers rather than the conductivity detection, which is less selective. For example, electrospray mass spectrometry is an excellent technique for the detection of highly electronegative compounds such as P oxyanions with sensitivity in the order of picograms. Alternatively, ICP–MS can be used in tandem with ion chromatography

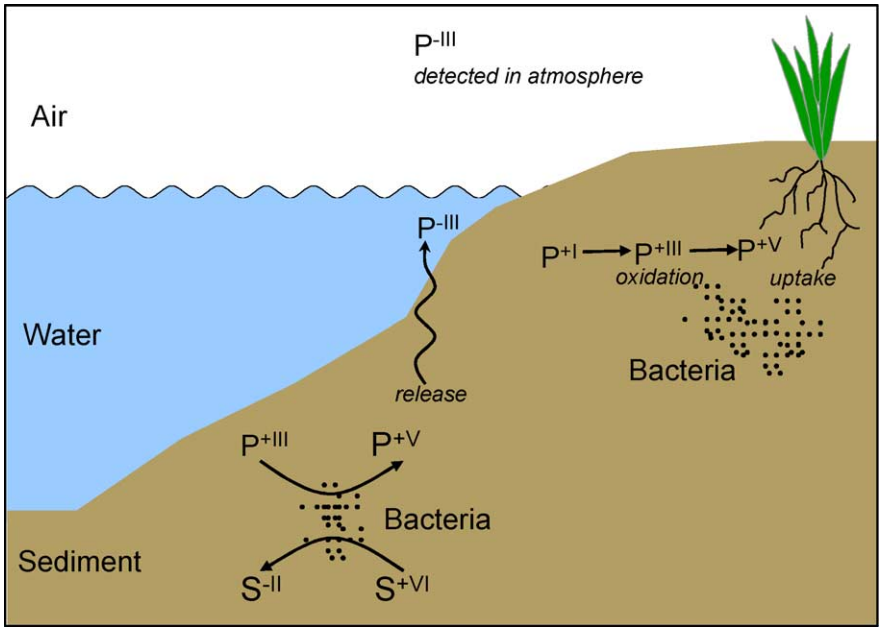

Fig. 3. Simplified diagram indicating the environments in which reduced P species have been found and the mechanisms by which these species are either taken up directly by organisms or are transformed to the most oxidized form of P, phosphate.

for elemental analysis of compounds eluding from the ion-exchange column.

For the analysis of reduced P oxyanions by chromatography, one must be careful to consider the amount of protonization of P oxyanion in an individual experiment based on the pH of the mobile phase [27]. As depicted in Fig. 1, this consideration is particularly important for phosphite, which may appear as either $H_2PO_3^-$ or HPO_3^{2-} at pH > 4. Misinterpretation of the data can result in miscalculation of the total concentration of each P oxyanion in the original sample.

Finally, analytical techniques must be developed that can perform in situ analysis of individual reduced P compounds. Commercial ion chromatographs are available for use in field research, however even these instruments require samples to be removed from the source prior to analysis. A preferred method of analysis is the monitoring of reduced P without removing the sample from its original environment.

Methods for the determination of reduced P as phosphine are more established than current methods for the detection of P oxyanions. Gas chromatography (GC) has been routinely used in the determination of phosphine. Chughtai et al. [90], for example, used packed column GC with alkali flame ionization detection for phosphine in water samples. More recent phosphine determination studies incorporated such techniques as cavity ring-down spectroscopy (CRDS) [99], GC-thermionic specific detection [92] and electrochemical oxidation on a SPE-based sensor [94]. The CRDS system used a near-infrared diode laser to probe absorption transitions with high sensitivity. The GC-thermionic specific detection system incorporated a preconcentration trap filled with glass beads and allowed the measurement of both free phosphine in biogas and matrix bound phosphine in manure and sludge. The SPE-based sensor offers direct sensing of phosphine gas, good linear relationships and long-term stability which may provide the basis for developing a practical phosphine gas sensor. Phosphine can also be detected with ion chromatography. Although PH_3 is not readily soluble in water, air can be treated with Br_2 to form phosphate, which is readily measured using ion chromatography [88].

Despite the promise of the techniques discussed above, none of these methods are currently appropriate for in situ analysis. It is possible that reduced P compounds are important in the cycling of P, but that their lifetimes are too short to measure significant concentrations hours or even days after removing the samples from their natural environments for laboratory analysis. It is also nearly impossible to design a standard storage protocol for collected natural water samples for reduced P determination due to the contrasting physicochemical and biological characteristics of different sample matrices. Biological uptake of reduced P species could also occur during storage as well as the possible adherence of species to sample storage containers. Hence, the need for sensitive and reliable in situ instrumentation is paramount.

Capillary electrophoresis is another chromatography technique that complements ion chromatography, and may be more appropriate than ion chromatography for on-site sample analysis. Typically capillary electrophoresis has better resolving power than ion chromatography, and smaller volumes of sample are required for analysis. A detailed review by Stover presented an overview on the analysis of oxyanions of P by capillary electrophoresis (CE) with an emphasis on electrolyte systems [100]. Capillary isotachophoresis (ITP) with conductivity detection has been applied to the separation of phosphite and hypophosphite using electrolytes with pH

3–6 [101,102]. Capillary electrophoresis is one of the latest additions to methods for the analysis of reduced P species and provides a powerful technique for separation and quantitation.

Flow injection (FI) techniques are potentially powerful tools for field-based and in situ analysis of reduced P. Flow injection has been applied to the determination of phosphite in fertilizers using an online sample preparation technique [89]. In this procedure, phosphite was oxidized to phosphate by an acidic permanganate solution with the phosphate generated determined spectrophotometrically by the molybdenum blue method. One possible method for the determination of reduced P using the molybdenum blue method is to determine the difference between total FRP measurements with and without sample oxidation prior to spectrophotometric analysis.

None of the established techniques can be used alone to establish unequivocally that reduced P is indeed an important component of P cycling in the environment, however a combination of these techniques can begin to answer this perplexing question. These previous works can be used as the foundation for the establishment of new 2D techniques that incorporate the best of existing techniques to create something even more powerful. In addition, the move towards field-based and in situ instrumentation is being paved by current advances in technology.

6. Conclusions

Much research has been conducted to provide an understanding of how P is cycled through the environment. However, most of the current literatures on the global biogeochemical cycles use “phosphate” and “phosphorus” interchangeably. Due to recent biochemical evidence, we believe that the inorganic reduced forms of P (e.g. phosphite, hypophosphite and phosphine) are important sources of P and could be important in the overall P cycle. In recent cases, for example, organisms were able to utilize the reduced P species as the sole source of P, oxidizing these compounds under both aerobic and anaerobic conditions. Phosphite and hypophosphite applied as fertilizers or pesticides/herbicides have been detected in anaerobic sediments, sewage treatment works and in industrial and agricultural processes. Moreover, phosphine has been shown to exist in sewage and marine sediments as well as in the atmosphere. Based on the above evidence, it is highly probable that reduced P compounds are important in the cycling of P. We propose here, a reduced P cycle (Fig. 3) based on the current state of knowledge and scientific evidence available. We believe that with better detection methods, the evidence for reduced P in the environment will increase and new contributions will be made to this initial diagram. However, due to such things as chemical kinetics, microbial activity, surface catalyzed reactions and possible storage effects, it is difficult to effectively measure the low concentrations present after removing the samples from their natural environments for laboratory analysis. Therefore, to fully understand the importance of reduced P, sensitive and reliable field-based or in situ methods of analysis are needed.

The scientific community has become too comfortable with phosphate as the only important form of P in the environment. We have accepted the paradigm of a redox-inert P and have overlooked potentially significant reduced P species. If reduced P is as significant as we have hypothesized in this review, then the evidence for these species in the environment will help expand the simplified reduced P cycle depicted in Fig. 3 and redefine the global biogeochemical cycle for P to include reduced P.

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