Environmental Pollution 182 (2013) 37–44

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

The influence of dissolved phosphorus molecular form on recalcitrance and bioavailability

Bo Li*, Michael T. Brett

Civil & Environmental Engineering, University of Washington, Seattle, WA 98105, USA

ARTICLE INFO

Article history:
Received 17 January 2013
Received in revised form
13 June 2013
Accepted 15 June 2013

Keywords:
Phosphorus
Bioavailability of phosphorus
Bioassay
Humic substances

ABSTRACT

Several studies have shown Soluble Reactive Phosphorus (SRP) analyses provide a poor index of dissolved phosphorus (P) bioavailability in natural systems. We tested 21 inorganic and organic P containing compounds with series of nutrient uptake and bioavailability bioassay experiments and chemical characterizations. Our results show that in 81% of cases, these compounds did not fit the classic assumption that SRP approximately equals Bioavailable P (BAP). Many organic compounds were classified as non-reactive, but had very rapid uptake kinetics and were nearly entirely bioavailable (*e.g.,* several nucleic acids, ATP, RNA, DNA and phosphatidylcholine). Several inorganic compounds also classified as non-reactive but had high bioavailability (*i.e.,* sodium tripolyphosphate and phosphorus pentoxide). Conversely, apatite was operationally classified as reactive, but had low bioavailability. Due to their tendency to alias as SRP, but recalcitrance and very low bioavailability, humic-(Al/Fe)-phosphorus complexes may play an especially important role in the dissolved phosphorus dynamics of natural systems.

1. Introduction

Phosphorus plays a critical role in the growth of algae and aquatic macrophytes, and is recognized as the most common limiting macronutrient in many freshwater systems (Redfield, 1934; Schindler et al., 2008). Elevated phosphorus (P) concentrations can lead to eutrophication resulting in excessive algal growth which can lead to a suite of negative responses including harmful algal blooms, hypoxia, and in severe cases fish kills (Smith et al., 1999; Lewis and Wurtsbaugh, 2008).

What compounds comprise dissolved P, and the relative bioavailability of this fraction for primary producers, is one of the most long-standing questions in freshwater and marine science (Hudson et al., 2000). Also, recent research has shown a high portion of the dissolved P in advanced P removal treatment plants is non-bioavailable, but it is unclear which P forms comprise the recalcitrant dissolved P (Reynolds and Davies, 2001; Li and Brett, 2012). It is often assumed that the soluble reactive P (SRP) fraction is nearly entirely bioavailable, and some authors have claimed that this is also the case for the total dissolved P fraction (Hatch and Reuter, 1999; Reynolds and Davies, 2001). Conversely, the classic results of Rigler (1968) and more recently Hudson et al. (2000) suggest measured phosphate may comprise only a minuscule portion of dissolved phosphorus. Because of its importance for aquatic ecosystems, a variety of methods have been developed to characterize the P forms that are most prevalent in natural systems (Murphy and Riley, 1962; Hedley et al., 1982; Cade-Menun et al., 2002). Although these fractionation techniques can identify certain broadly defined P pools, they cannot identify specific P containing compounds. This is important because different P species may vary greatly in their bioavailability for algae and bacterioplankton (Nausch and Nausch, 2006; Björkman and Karl, 2003). The large majority of studies use filtration and the acid-molybdate method to operationally define P pools according to whether they are "dissolved" (=soluble) and/or "reactive" (Murphy and Riley, 1962; Hedley et al., 1982; Cade-Menun et al., 2002). Many studies assume soluble reactive P (SRP) represents orthophosphate, and that this fraction can be readily utilized by aquatic primary producers (Reynolds and Davies, 2001). However, whether the SRP estimate given by the acid-molybdate method provides a reasonable estimate of orthophosphate concentrations has long been in dispute (Rigler, 1968; Hudson et al., 2000). Hudson et al. (2000) showed using Rigler ^{32}P bioassay experiments that SRP concentrations in 14 lakes exceeded actual phosphate concentrations by 2–3 orders of magnitude. These authors suggested the disparity between lake water SRP and phosphate concentrations could be artifacts due to

* Corresponding author.
E-mail address: libo@u.washington.edu (B. Li).

0269-7491/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2013.06.024

sample filtration releasing particulate P to the dissolved phase and the acidification step of the SRP analyses releasing bound phosphate. For example, the hydrolysis of non-orthophosphate in compounds such as phytic acid, riboflavin monophosphate and adenosine 5′ diphosphate, can contribute to the formation of heteropoly blue in SRP analyses (Boström et al., 1988; Kerouel and Aminot, 1996; Hudson et al., 2000).

Research has shown that various forms of P can be used as P sources and support primary production (Boström et al., 1988; Berman et al., 1991; Björkman and Karl, 1994, 2003) Dissolved non-reactive P, which is commonly assumed to represent dissolved organic P (DOP), represents a major phosphorus reservoir in soils and surface waters (Monbett et al., 2007). However, the DOP pool is generally poorly resolved because suitable measurement techniques are not available to deduce its composition. Studies examining the bioavailability of inorganic and organic phosphorus compounds to marine microorganisms identified several forms, such as adenosine-5′-triphosphate, adenosine monophosphate, and glucose-6-phosphate which can be readily converted to orthophosphate and thus made bioavailable (Hedley et al., 1982; Berman, 1988; Boström et al., 1988; Björkman and Karl, 1994, 2003). Research with freshwater phytoplankton showed some organic P forms can also support high algal growth rates (Boström et al., 1988; Berman et al., 1991; Cotner and Wetzel, 1992). When studying P-limited limnetic systems, Tarapchak and Moll (1990) showed several naturally occurring DOP compounds were hydrolyzed to orthophosphate and utilized by phytoplankton and bacteria. Although not well resolved, the fraction of DOP that is susceptible to enzymatic hydrolysis is considered to be an important component of the bioavailable P pool (Cooper et al., 2009; Monbett et al., 2009). However, to date, only a small number of defined P species have been analyzed using bioassays to characterize their uptake kinetics and bioavailability.

We examined the chemical and biological properties of a wide variety of pure phosphorus containing compounds, including inorganic, organic and humic substance associated P. The objective of these analyses was to determine how closely the bioavailability of pure phosphorus containing compounds corresponds to the conventional operational categorization used to classify the P present in surface water samples. We tested the conventional assumption that primary classification as SRP indicates that a phosphorus form is highly bioavailable. Bioassays were used to determine algal uptake rates and bioavailability, and these compounds were also assessed using the classic operational characterization scheme. Based on these results, a novel classification scheme for dissolved P was also suggested.

2. Methods

2.1. P containing compounds

A wide range of phosphorus containing compounds were used for this study based on their prevalence in nature and chemical classification (Table 1). Humic substances were obtained from International Humic Substances Society. These compounds were prepared as stock solutions and combined with a synthetic P-free nutrient medium as described by Miller et al. (1978) (SI Table 1). KCl instead of K_2HPO_4 was used as a potassium source to assure the compounds tested were the only P source for phytoplankton. Information for the P compounds used for these experiments and their final concentration in the test solutions are listed in Table 1. Fresh solutions were prepared prior to each chemical analyses and bioassay experiment.

2.2. Chemical analyses

The chemical analyses for each compound determined whether they were classified as reactive and/or dissolved according to the acid-molybdate spectrophotometric method described in Standard Methods 4500-P. This yielded the four classic operational categories, *i.e.,* total P (TP), soluble P (SP), total reactive P (TRP) and soluble reactive P (SRP). TP was determined after 45 min of autoclave-mediated digestion (120 °C, 100 kPa, with $K_2S_2O_8$ and H_2SO_4) of an unfiltered sample (APHA, 1967). TRP was determined using the same reaction on unfiltered samples without persulfate digestion. Samples for SP and SRP analyses (120 mL) were first filtered through a 0.45 μm polycarbonate membrane filter (Millipore®). SP was measured after persulfate digestion while SRP was determined without persulfate digestion. Soluble non-Reactive P (SnRP) was calculated as the difference between SP and SRP.

2.3. Algal bioassays

The freshwater alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was used for these experiments. As indicated by Standard Method 8111 (APHA, 1967), *P. subcapitata* was maintained in synthetic nutrient growth media prior to and during the bioassay experiments. Seven to ten days prior to the bioassays, algae cultures were centrifuged and resuspended into P-free medium to induce P-stress. Fifty mL of each test sample was placed into 125-mL Erlenmeyer flasks, which were acid-washed (0.1 M HCl) and autoclaved prior to each experiment. Standard media with known concentrations of KH_2PO_4 (0, 5, 10, 15, 20, 25, 30, 40 and 50 μg P L^{-1}) were incubated in triplicate to obtain a standard curve for the algal growth yield. Because the precision of this method is lower than for standard wet chemistry approaches, four replicates of each sample were incubated and the results averaged for the final calculations. Algal cell yield was linear in the 0–50 μg L^{-1} range ($r^2 \approx 0.99$).

Table 1
P species tested.

Category	Chemical name	Molecular formula	P content (μg P/L)	Producer, product number
Inorganic P	Aluminum phosphate (Al-P)	$AlPO_4$	71	ALDRICH, 341452
	Calcium phosphate (Ca-P)	$CaHPO_4$	58	MP Biomedicals, ICN 19380480
	Ferric pyrophosphate (Pyro-P)	$Fe_4(P_2O_7)_3$	59	MP Biomedicals, ICN211191
	Sodium tripolyphosphate (Tripoly-P)	$Na_5P_3O_{10}$	122	ALROS, AC39396
	Phosphorus pentoxide (P_4O_{10})	P_4O_{10}	103	ALROS, AC31582
	Apatite	$Ca_5(PO4)_3(OH,F,Cl)$	158	Alfa Aesar, 42535
	Ca-hydroxypatite	$Ca_5(PO4)_3(OH)$	219	Spectrum, C1264
Organophosphate	Adenosine 5′monophosphate (AMP)	$C_{10}H_{14}N_5O_7P$	105	Spectrum, AD113
	Guanosine diphosphate (GDP)	$C_{10}H_{15}N_5O_{11}P_2$	120	MP Biomedicals, ICN15121325
	Uridine diphosphate (UDP)	$C_9H_{14}N_2O_{12}P_2$	100	MP Biomedicals, ICN10120525
	Adenosine-5′-triphosphate (ATP)	$C_{10}H_{14}N_5O_{13}P_3Na_2{\bullet}3H_2O$	126	Affymetrix, NC9948088
	Deoxyribonucleic acid (DNA)		118	Spectrum, DE115
	Ribonucleic acid (RNA)		121	Spectrum, RI104
	Lecithin		67	Alfa Aesar, AA3648630
	Liposome	$C_{44}H_{88}NO_8P$	78	Sigma sterile pyrogen-free preliposome formulation 5
	Phytic Acid	$C_6H_{18}O_{24}P_6$	161	TCI, P0409
Humic substance	Elliott soil humic acid standard		261	IHSS, 1S102H
	Wackish peat humic acid reference		201	IHSS,1R107H
	Leonardite humic acid standard		663	IHSS, 1S104H
	Pahokee peat humic acid standard		503	IHSS, 1S103H
	Pahokee peat humic acid reference		520	IHSS, 1R103H