## Phosphonic Acid in Plant-Based Food and Feed Products Where does it come from?



Dr. Werner Nader October 2nd, 2024

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## **Eurofins Laboratories Worldwide**



## Eurofins Scientific Group



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1. Eurofins Dr. Specht Express

- Widely recognized pesticide competence center with over 50 years of experience;
- · Rapid and reliable screening
- Branches: fruits, vegetables, cereals, fruit juices & concentrates



- Broad portfolio of analytical services;
- Sampling, inspection and auditing services worldwide





### Pesticide residue legislations challenge international trade of food and feed

Werner Nader, Michelle Maier, Marco Miebach and Gabriel Linder

#### Abstract

The diversity of maximum residue levels (MRLs) for plant protection products among the countries worldwide challenges the international food trade. This article describes this diversity and its impacts on the rice supply chain based on the practical experience of an international inspection and analytical company and pesticide testing of 2,592 samples in 2019. Economic impacts of MRLs are illustrated with the example of Basmati rice imports into the EU, where Indian rice exporters lost estimated revenues of over 200 million USD from January 1st 2018 to August 31st 2019 due to a drop in the MRL for tricvclazole from 1 to 0.01 mg/kg. The article furthermore describes that certain substances in food are frequently interpreted as residues from agricultural practices and fall under the EU MRL regulation for pesticides. although they might be - and frequently are - of natural origin or are contaminants not related to agrochemical applications. Examples are high concentrations of the natural plant hormone indole-3acetic acid in cereal seeds, accumulation of bromide by Brazil nuts, phthalimide and mepiquat generated during food processing involving heat, chlorate from chlorinated water, and nicotine, diethyl-metatoluamide (DEET) and icaridin from the hands of workers during harvesting and further handling of the crop. Phosphonate can be introduced into the food by agricultural applications of the fungicide fosetyl or plant strengthening phosphonate salts. But it can be also of natural origin, as microorganisms produce the chemical in biogeochemical phosphorous cycles in various environments. Problems arise not only, when these chemicals exceed the legal MRLs. For organic food they are often interpreted as indicators of forbidden pesticide applications and in infant food they frequently exceed the stringent default MRL of 0.01 mg/kg of the EU. Regarding the occurrence of phthalimide and phosphonate results from the analysis of 3 210 tea and spice samples and 1 417 further food samples are presented, which were obtained in the period of 2017 to 2019.

#### 1. Introduction

Internationally, food safety legislations are diverse and the economic impacts of this diversity have been studied in detail by Bremmers et al. (2011) on the example of meat exports to the USA and the In 2005, the EU implemented harmonized MRLs European Union. Melo et al. (2014) describe the for plant protection products (Regulation (EC) No burden of regulations and standards on exporting 396/2005) followed by stringent enforcement and countries with Chilean fruit exports as an example reporting in the RASEF (Rapid Alert System for Food

García Martinez and Poole (2004) focused on the market barriers created by diverse fresh produce safety standards of the European retail chains on developing Mediterranean exporting countries.

#### www.researchgate.net/profile/Werner-Nader

84 | cereal technology 02/2020

Phosphonic Acid - Where does it come from?



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### Phosphonic acid in plant-based food and feed products - Where does it come from?

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#### ARTICLE INFO

#### ABSTRACT

Keywords: Phosphonate Phosphonic acid Fosetvl Organophosphonates Biogeochemical phosphorus cycle Organic agriculture Infant nutrition Phosphite

Salts of phosphonic acid (sodium or potassium phosphonate) or its ester fosetyl are highly effective systemic fungicides, which not only exhibit fungistatic activities, but also stimulate the natural defence systems of the plants against pathogens. Since EU legislation has approved potassium and sodium phosphonate as fungicides in 2013 and 2014, they are no longer permitted in organic agriculture. As a result, even minor contents of phosphonate in organic food samples are interpreted as an indication of a not permitted fungicide treatment. A maximum level of 0.01 mg/kg applies for food for infant nutrition. Here we present evidence from the scientific literature that phosphonate is also a natural compound, which occurs in significant amounts in the environment and is produced and consumed by microorganisms within the biogeochemical phosphorus cycle. Further potential sources for this chemical in the environment apart from agricultural fungicide applications are organophosphonates, as phosphonate occurs as a byproduct during their industrial production or during their microbial degradation in effluents from industry and households. An evaluation of Eurofins results from the analysis of phosphonate from 2017 to 2022 with methods achieving a limit of quantification (LOQ) of 0.01 mg/kg reveals that the compound was detected in 40.63% of all samples from organic and 43.42% from conventional agriculture, and in over 90% of organic samples of chickpeas, lentils, buckwheat, peanuts and wine. Furthermore, 97.3% of organic mushroom samples contain the compound and animal manure or compost used for cultivation is a potential source of the chemical. Consequently, other causes for phosphonate in food products than fungicide applications must be taken into account as well. Analysis of the ratio of the stable oxygen isotopes <sup>18</sup>O and <sup>16</sup>O was successfully applied to detect applications of mineral sulphur fertilizer in organic agriculture. Similarly, it

https://doi.org/10.1016/j.docodcont.2023.109701

# Phosphonic acid – a fungicide?



PAGE CONTENTS Status under Reg

Authorisation

Classification

**Toxicological information** 

Reference values		Source	Remark
ADI	1 mg/kg bw/day	Pt. A 19.02 RR may 2023	
ARfD		EFSA 2013	
AOEL	5 mg/kg bw/day	EFSA 2013	

Phosphonic Acid - Where does it come from?

#### Status under <u>Reg. (EC) No 1107/2009</u> Approved (repealing Directive 91/414/EEC Reg. (EU) 2020/2007 Maximum Residue Levels Dossier complete Reg\_(EU) 2018/155 Legislation Old legislation 08/953/EC Reg. (EU) No 832/2013 Toxicological information Date of approval 01/02/2014 31/01/2026 Expiration of approval RMS for Co-RMS for FR ongoing/upcoming FI ongoing/upcoming approval/renewal approval/renewal

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# Phosphonic acid – activator of the natural defence against pathogenic fungi

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Inicio / PROMOTOR DE AUTODEFENSAS / ACTION

## ACTION

Activador de Fitoalexinas

Fosfito de Potasio 840 g/L

Nutriente foliar líquido, con alta concentración de fosfito de potasio, 100% bioasimilable por la planta. Actúa sobre las plantas tratadas favoreciendo la producción de Fitoalexinas, sustancias que activan el sistema natural de defensa de las plantas contra patógenos que causan enfermedades como: tristeza, gomosis, mildiu, rancha, marchitez y otras podredumbres de la raíz y cuello de las plantas. Debe ser aplicado desde el inicio y durante el desarrollo del cultivo.

## **Maximum residue levels**

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## Food legislation in general

### Maximum residue levels under regulation (EC) 396/2005

Products to which MRLs apply	Phosphonic acid and its salts expressed as phosphonic acid(R) ① PLAN/2023/138 not yet applicable	Fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl) Reg. (EU) 2022/1324 applicable
Lemons	100	150
Limes	100	150
Mandarins	100	150
Others (2)	100	75
Tree nuts		
Almonds	1000	1500
Brazil nuts	400	500
Cashew nuts	400	500
Chestnuts	1000	1500
Coconuts	400	500
	1000	1500

## Organic and baby food

- Maximum level of 0.01 mg/kg for infant nutrition under (EU) No 2016/127 Not included in annex I of the implementing regulation (EU) No 2021/1165 for organic agriculture **Confusion within the EU over interpretation of residues in organic food and feed**
- Italy: 0.01 mg/kg maximum level for pesticides in general in organic by law, but 1 mg/kg for phosphonic acid under derogation in organic wines
- Netherlands: Control body SKAL requires investigation of farm inputs at PA findings of 0.01 mg/kg
- Germany: Orientation value of 0.05 mg/kg PA for annual and biennial and 0.1 mg/kg PA for perennial crop of the BNN eV, Association of Organic Processors, Wholesalers and Retailers, in general accepted

Eurofins Food Germany anonymized Data Base of all lab results

Data from 16,682,138 samples

Phosphonate results: > 120,000 samples Fosetyl results > 140,000 samples

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ByteOnion	Startseite
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Evaluation Results Parameter Evaluation ElimsExport Parameter in Test	Cl comparision   MRL Evaluation   Trendies   Exporte   Systemdata
Probenzahl: 16,682,138 Food Deutschland	Version: 1.041 3/7/2024

Phosphonic Acid - Where does it come from?



Percentage of positive phosphonate and fosetyl findings in samples from conventional and organic agriculture analysed by Eurofins since 2012 (with LOQ 0.01 mg/kg since 2017).

Cultivation	LOQ (mg/kg)	Sample number	≥ LOQ (%)						
Phosphonate									
Conventional	0,01	>10 000	43,42%						
	0.01-1	>80 000	33,92%						
Organic	0,01	>10 000	40,63%						
	0.01 - 1	>40 000	26,57%						
	Fosety	1							
Conventional	0.005-0,01	>40 000	1,08%						
	0.005-1	>100 000	1,49%						
Organic	0.005-0,01	>20 000	0,07%						
	0.005-1	>40 000	0,09%						

Source: Nader, Zahm & Jaschik, Food Control, 2023, https://doi.org/10.1016/j.foodcont.2023.109701

Phosphonic Acid - Where does it come from?

## Phosphonate findings in specific product types



			Conv	entional			Or	ganic		
		Sample	samples	Average	Maximum	Sample	samples	Average	Maximum	
		number	≥LOQ	conc.	conc.	number	≥LOQ	conc.	conc.	
	Product			m	g/kg			m	g/kg	
9	Almond	> 50	98,8%	11,366	55,1	< 50	100,0%	1,055	7,0	
· · · · ·	Mushrooms	> 100	71,0%	0,210	19,7	> 100	97,3%	0,045	0,1	
	Lentils	> 100	78,9%	0,655	18 7	> 750	97,2%	0,147	2,2	
	Wine	> 250	97,70%	4,257	50,8	> 50	96,7%	0,805	11,7	
	Chickpea	<b>Z</b> DU	97.3%	0.575	4.0	> 100	90.570	0.407	09	
<	Buckwheat	> 50	92,1%	0,111	0,3	> 50	90,5%	0,098	0,3	
	Peanut	- 250	96,0%	5,758	60,6	> 50	89,3%	4,430	42,4	
	Peas	> 100	53,6%	0,221	8,9	> 100	71,8%	0,114	0,9	
	Quinoa	> 50	83,9%	0,393	3,3	< 50	69,6%	0,049	0,6	
	Pineapple	> 250	90,5%	2,468	29,9	> 50	66,2%	0,257	1,3	Source: Nader, Zahm &
	Strawberry	> 500	74,5%	1,095	43,6	> 100	59,3%	0,050	0,6	https://doi.org/10.1016/i.foodc
	Apple	> 2500	68,6%	1,566	75,6	> 250	51,6%	0,040	4,3	ont.2023.109701
	Tomato	> 100	57,6%	0,996	26,4	< 50	50,0%	0,046	0,2	
	Rice	> 1000	41,6%	0,268	42,7	> 750	33,8%	0,011	0,4	
	Maize	> 100	51,8%	0,063	0,5	> 50	31,1%	0,016	0,5	
	Potato	> 100	69,7%	4,870	66,9	< 100	25,0%	0,080	0,8	
	Banana	> 5000	32,5%	0,051	10,8	> 2500	21,3%	0,018	4,5	
	Cucumber	> 100	72,9%	9,704	95,2	< 50	18,0%	0,119	1,3	
	Wheat	> 100	42,2%	0,037	1,8	> 50	17,3%	0,003	0,1	
	Sesame seeds	> 50	44,6%	0,082	0,7	> 50	16,1%	0,007	0,1	
Phosphonic Acid	Carrot	> 250	9,2%	0,042	4,5	> 100	1,8%	0,009	0,9	

## **Buckwheat from Gilgit area in Northern Pakistan**

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Phosphonic Acid - Where does it come from?

Phosphonic Acid - Where does it come from?



## Test results

XP103 Method	Pesticide-Screening Quechers LC-MS/MS DIN EN 15662:2018 mod., SPF-14.141, LC	C-MS/MS	
Subcontrac	ted to a Eurofins laboratory accredited for this test.		
Screen	ed pesticides	Not Detected	
XP207 Method Subcontrac	Pesticide-Screening Quechers GC-MS/MS DIN EN 15662:2018 mod., SPF-14.141, GC ted to a Eurofins laboratory accredited for this test.	C-MS/MS	
Screen	ed pesticides	Not Detected	
XP313 Method Subcontrac	Fosetyl/Phosphonic acid Internal Method SPF-14.188-4, SPF-14.18 ted to a Eurofins laboratory accredited for this test.	8, LC-MS/MS	
Fosety	1	< 0 .01	mg/kg
Phosp	honic acid	0.63	mg/kg
Fosety	l-Al (fosetyl+phosph. acid/salts as fosetyl) Regulatory Limit	0.84 2.0	mg/kg mg/kg *



1. Myth: Phosphonic acid is a relic of the reducing atmosphere on primitive earth and does not occur today naturally. If found in organic products, it must be a residue of fungicide applications.

**Fact is:** Phosphonic acid occurs ubiquitously and was detected in relatively high concentrations in wastewater, rivers, lakes and ponds, first reported in paddy fields in 1959. It is part of the biogeochemical phosphorus cycle and produced and consumed by microorganisms.

- 2. Myth: Phosphonic acid is a thermodynamically unstable chemical and oxidized once it gets into contact with oxygen.
- Fact is: It is kinetically stable with a half-life in oxygenated water of 1,000 up to 3,000 years.
- **3.** Myth: Phosphonic acid is rapidly assimilated and oxidized to phosphate by microbiota in soil and water and therefore does not occur in the aerobic environment with a high redox potential.
- **Fact is:** The persistence of phosphonate in soil is moderate to high with a period required for 90% dissipation, DT90, of 91 to 1,000 days. In the presence of phosphate genes for the assimilation of phosphonate are inactive in the bacteria.

#### PHOSPHATE REDUCTION IN THE PADDY FIELD 1.

#### PHOSPHATE REDUCTION IN THE PADDY FIELD I.

#### GORO TSUBOTA

Tochigi Agricultural Experiment Station, Utsunomiya RECEIVED MAY 19, 1959

The paper on the phosphate reduction in the among the known phosphorus compounds. paddy field has not yet been found. Although it seemed that its study was made by a Soviet investigator, K.I. RUDAKOV (2, 3), his research was not concerned with the paddy field and his original paper has not been available to the author.

During the study on some problems of phosphate fertilizer utilization, the author had come to presume, without referring the research of RUDAKOV, the possibility of occurrence of phosphate reduction in the soil under flooded condition.

The author presumed three levels of reduced compounds derived from phosphate, i.e., phosphate - phosphite - hypophosphite - phosphine,

Table 1. Redoxpotential in Each Level in the Course of Phosphate Reduction ⊿H Reaction En

Note: ( )\* N.A.LANGE: Handbook of Chemistry (1956) ( )\*\* G.CHARLOT: L'analyse Qualitative et les Re tions en Solution (1957)

H.PO.-H.PO.

H<sub>3</sub>PO<sub>3</sub>-H<sub>3</sub>PO<sub>3</sub>

H<sub>3</sub>PO<sub>2</sub>-PH<sub>3</sub>

-0.38

 $(-0.20)^{3}$ 

-0.67

(-0.50)\*\*

-0.34

Redoxpotentials in each level were calculated from the value of *AH* and shown in the the following table.

Considered from the values in the table, the possibility of microbiological reduction of phosphate in nature cannot be excluded. This phenomenon is considered to be of great significance in the study of relationship between soil and fertilizer in the flooded paddy field. If the phosphate reduction occurs in the paddy field, the transformation of phosphate into unavailable form for plants other than its absorption or fixation by soil will have to be studied. A. FUJIWARA (1) has informed the toxic effect of phosphite and hypophosphite upon the rice plant. And if phosphate is reduced to phosphine, the degree of dephosphorification and toxicity of reduced compounds of phosphate must be investigated from the standpoint of manure utilization in the soil.

The author and his collaborators have worked on this problem since 1957. In this paper, three items of the problem which the author studied under Prof. K. ARIMA at the Department of Agricultural Chemistry, University of Tokyo, will be reported. They are, 1) phenomenon of

#### Table 2. Mechanical Properties of the Soil Samples

-17.5

-30.9

-31.3

Soil	Sand 2~0.2mm	Fine sand 0.2~0.02mm	Silt 0.02~0.002mm	Clay <0.002mm	Soil classes	Apparent specific gravity
	70	70	%	70		
Utsunomiya	12.6	28.4	28.2	12.6	L	0.72
Sano	16.1	32.3	30.6	21.0	CL	1.06

#### Table 3. Chemical Properties of the Soil Samples

Soil	pН	с	N	$\begin{array}{c} \operatorname{Fe_2O_5}\\ \operatorname{Al_2O_3} & +\\ \operatorname{FeO} \end{array}$	Mn <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	C.E.C.	Absorption coeff. of P2O5	Effect of air- drying of the soil before flooding	Effect of tempera- ture elevation on the mineralisation of nitrogen
		%	70	70 1 70	mg/100g	%	me/100g		mgN	mgN
Utsunomiya	6.05	5.67	0.44	13.8 4.66	17.0	47.6	33.8	1992	18.1	22.9
Sano	6.42	1.95	0.22	14.1 8.42	76.4	54.0	20.8	504	8.4	10.1

THOSPHONIC AND - WHELE GOES IL COME HOM :



Air washing solution Sample (Water and HNOs

Absorbent (HNO<sub>3</sub>)

#### Fig. 1. The apparatus for aspiration. https://doi.org/10.1080/00380768.1959.10430888

dephosphorification in a paddy field model, 2) a electric lump in it. And evolved gas from the paddy reduction of phosphate in liquid culture medium. field model was suctioned with air flow at the rate and 3) soil microorganisms which participate of l/min. After a certain period, nitric acid solution in the phosphate reduction.

#### I. Dephosphorification in the paddy field model

The auther tried to detect phosphine, which is the end product of phosphate reduction, in the gas evolved from the flooded paddy field.

#### Materials and Methods

The soil samples used in this experiment were taken from the paddy field of volcanic ash soil rich in humus at IMAIZUMI, UTSUNOMIYA-city, and also from the field of alluvial soil at HORIGOME, SANO-city, TOCHIGI-pref.. General properties of the soil samples are shown in the Tables 2 and 3.

In brevity, the samples are described as UTSUNOMIYAsoil and SANO-soil.

I kg of the soil, 10 g of compost and 4.205 g of (NH4) HPO, were mixed well and put in the glass desiccator, the diameter of which is 23 cm. A funnel was inversely attached to the centre of its cover, so that evolved gas from the soil might be suctioned out easily. Absorption bottles of 200 ml volume were used and rubber stoppers through which glass tubes were inserted were fitted to them. The desiccators were kept at a constant temperature by a cover with

in the absorption bottles was evaporated to driness, the residue was dissolved in N H\_SO4, and phosphate was determined by the DENIGE's method, adjusting the acidity of the liquid to a constant.

#### **Results** and Discussion

1. Occurrence of dephosphorification. On November 7th 1957, the UTSUNOMIYA-soil and compost and (NH4). HPO4 were put in a desic cator with the sucking apparatus. Evolved ga from the flooded soil was suctioned with ai flow for sixteen days. The whole apparatu was covered and kept at 24 C.

The amount of phosphorus compound trappe in the first absorption bottle, which containe 200 ml of 5 N nitric acid, was shown in Tab

4.

Table 4. Extent of Dephosphorification in the Paddy Field Model (PaOs)/lkg of soil)

Soil comple	1st~3rd 4th~9th 10th~					
son sample	day	day	day			
Utsunomiya*	2.8	35.6	67.4			

Notes: One absorption bottle was used. This field was fertilized annually with organic fertilizers and manures.

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11

### Redox chemistry in the phosphorus biogeochemical cycle

### CrossMark Phosphonic acid in Florida waters

Depth, cm

0

15

25

River Front Park, 28° 4' 12.25" N, 82°

Sample

A-1

A-2

A 2 a

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PO43

95 (±1.5)

97 (+1.5)

08 (+1)

HPO2-

3 (±0.5)

1.05

0/+02)

H2PO2

2 (±1)

 $2(\pm 1)$ 

21+1)

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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved September 26, 2014 (received for review May 2, 2014)

Significance

the limiting nutrient, where it is broadly considered to reside as pentavalent P in phosphate minerals and organic esters. Exceptions to pentavalent P include phosphine-PH3-a trace atmospheric gas, and phosphite and hypophosphite, P anions that have been detected

recently in lightning strikes, eutrophic k termite hindguts. Reduced oxidation st phosphonates, characterized by C-P bo total organic dissolved phosphorus. been considered to be rare; however, reduced P compounds as sole P sources that between 10% and 20% of dissolve than+5 in water samples from central I samples bearing almost as much reduce tity of reduced P observed in the water here is broadly characteristic of similar scale, it accounts well for the concent phine and provides a rationale for the tion genes in nature. Phosphine is gene with thermodynamic equilibrium estal ation reaction of reduced P species. Co dissolved P inventory in Florida environ could hence be a critical part of the phot and in turn may impact global carbon (

SANS

phosphorus | redox chemistry | phosphona biogeochemistry

Life as we know it is dependent on in metabolism as energy-storing tors, in replication and transcription and DNA, and in cell structure as ph erals are the ultimate source of phosp ever, most phosphate minerals are dissolve at neutral pH and at room ten (P) is the limiting nutrient in many eco is especially slow compared with carb

Although inorganic phosphate and viewed as the prevalent compounds in C-P bonds, are ubiquitous, comprisin organic P in some natural samples (2) a stronger potential for electron shari based on the electronegativity different 2.2) compared with O (3.5) and P in potential for electron sharing, the fo phosphonates is thus less than +5; he a reduced oxidation state P (here after, environment. Phosphonates appear geochemical pathways, including a rol

acrobic methanogenesis in marine environments (3, 4). Ties between biogeochemical cycles, such as between P in phosphonates and C as methane, demonstrate that a thorough understanding of P geochemistry is necessary for understanding the biogeochemical cycling of other elements, such as carbon and nitrogen (5).

With the exception of the phosphonates, P is generally considered to be a redox-insensitive element as the reduction reaction is Freely available online through the PNAS open access option highly endergonic, and requires more free electrons than normally

15468-15473 | PNAS | October 28, 2014 | vol. 111 | no. 43 Phosphonic Acid - Where does it come from?

The element phosphorus (P) controls growth in many ecosystems as exist in the environment (6, 7). However, the reduced P compounds phosphite, hypophosphite, and phosphine are known to occur in nature (Fig. 1), and have origins that range from nonbiological (8, 9) to biological (10, 11). Phosphite and hypophosphite can be used by many microorganisms as sole P sources, suggesting there must

> Phosphorus is an important nutrient for living organisms. Phosphorus is generally considered to bear a 5+ oxidation state, but several lower redox states have been reported, including the toxic gas phosphine. We show here that the lower redox states of phosphorus are common in Florida water samples, and that based on the global concentration of phosphine, we might expect to see 5–15% of all dissolved phosphorus in a lower redox state.

Author contributions: M.A.P. designed research: M.A.P. and J.M.S. performed research: Z.A. ted new reagents/analytic took; MA.P. and J.M.S. analyzed data; and MA.P., LM.S. and ZA, wrote the paper. The authors declare no conflict of interast This article is a PNAS Direct Submission

<sup>1</sup>To whom correspondence should be addressed. Brail: mpasek@ud.edu

www.pnas.org	/cgi/doi/10	.1073/pnas.	1408134111
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B-1	0	56	5.73	0.3	BDL	BDL	100 (±5)
B-2	9	52	5.73	0.3	11 (±1)	10 (±2)	79 (±5)
B-3-g	19	-31	5.7	0.3	21 (±1)	17 (±1)	62 (±4)

Samples noted with "g" are from associated groundwater. Each sample was run in duplicate when possible, and errors (in parentheses) are reported as relative percent differences between runs, multiplied by the average percentage. BDL, below detection limit; n.d., not determined; TDP, total dissolved phosphorus.

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Table 1. Sample locations, characteristics, and reduced P contents given as percentages of TDP

" W

DH

8.1

8.1

TDP, mg/L

0.3

0.3

0.2

ORP, mV

213

242

nd

### Redox chemistry in the phosphorus biogeochemical cycle

### CrossMark Phosphonic acid in Florida waters

Depth, cm

Sample

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PO43

HPO2-

H-PO-

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Edited by David M, Karl, University of Hawaii, Honolulu, H, and approved September 26, 2014 (received for review May 2, 2014)

the limiting nutrient, where it is broadly considered to reside as pentavalent P in phosphate minerals and organic esters. Exceptions to pentavalent P include phosphine-PH3-a trace atmospheric gas, and phosphite and hypophosphite, P anions that have been detected recently in lightning strikes, eutrophic lakes, geothermal springs, and termite hindguts. Reduced oxidation state P compounds include the phosphonates, characterized by C-P bonds, which bear up to 25% of total organic dissolved phosphorus. Reduced P compounds have been considered to be rare; however, the microbial ability to use reduced P compounds as sole P sources is ubiquitous. Here we show that between 10% and 20% of dissolved P bears a redox state of less than+5 in water samples from central Florida, on average, with some samples bearing almost as much reduced P as phosphate. If the guantity of reduced P observed in the water samples from Florida studied here is broadly characteristic of similar environments on the global scale, it accounts well for the concentration of atmospheric phosphine and provides a rationale for the ubiquity of phosphite utilization genes in nature. Phosphine is generated at a guantity consistent with thermodynamic equilibrium established by the disproportionation reaction of reduced P species. Comprising 10-20% of the total dissolved P inventory in Florida environments, reduced P compounds could hence be a critical part of the phosphorus biogeochemical cycle, and in turn may impact global carbon cycling and methanogenesis.

phosphorus | redox chemistry | phosphonates | element cyding | biogeochemistry

ife as we know it is dependent on phosphate esters, which act in metabolism as energy-storing polyphosphates and cofactors, in replication and transcription as the backbone of RNA and DNA, and in cell structure as phospholipids. Phosphate minerals are the ultimate source of phosphate in the biosphere. However, most phosphate minerals are poorly soluble and slow to dissolve at neutral pH and at room temperature; hence phosphorus (P) is the limiting nutrient in many ecosystems. Phosphorus cycling is especially slow compared with carbon and nitrogen cycling (1).

Although inorganic phosphate and phosphate esters (P54) are viewed as the prevalent compounds in nature, phosphonates, with C-P bonds, are ubiquitous, comprising up to 25% of the dissolved organic P in some natural samples (2). The P in phosphonates has a stronger potential for electron sharing than the P in phosphates, based on the electronegativity difference between C and P (2.5-2.2) compared with O (3.5) and P in phosphates. With a greater potential for electron sharing, the formal oxidation state of P in phosphonates is thus less than +5; hence phosphonates represent a reduced oxidation state P (here after, reduced P) speciation in the environment. Phosphonates appear to be critical to some biogeochemical pathways, including a role for methylphosphonate in acrobic methanogenesis in marine environments (3, 4). Ties between biogeochemical cycles, such as between P in phosphonates and C as methane, demonstrate that a thorough understanding of P geochemistry is necessary for understanding the biogeochemical cycling of other elements, such as carbon and nitrogen (5).

With the exception of the phosphonates, P is generally considered to be a redox-insensitive element as the reduction reaction is Freely available online through the PNAS open access option highly endergonic, and requires more free electrons than normally

15468-15473 | PNAS | October 28, 2014 | vol. 111 | no. 43 Phosphonic Acid - Where does it come from?

The element phosphorus (P) controls growth in many ecosystems as exist in the environment (6, 7). However, the reduced P compounds phosphite, hypophosphite, and phosphine are known to occur in nature (Fig. 1), and have origins that range from nonbiological (8, 9) to biological (10, 11). Phosphite and hypophosphite can be used by many microorganisms as sole P sources, suggesting there must be an environmental source of these compounds (12).

In contrast to phosphite and hypophosphite, which are accessible as nutrients, phosphine is toxic to many organisms, although it is also a ubiquitous trace atmospheric gas that occurs at concentrations of about 1 ng/m3 on average (13). Variations in phosphine concentration are significant; the concentration of PH<sub>2</sub> in low-PH<sub>2</sub> environments is a factor of more than 10,000 less than those in high-PH<sub>3</sub> natural environments. The origin of phosphine may be linked to metal corrosion (14) in some environments, but in others, it is linked to microbial activity (15, 16). The link between phosphine and the other reduced P compounds, and to the organic phosphonates, is unclear, and is explored using thermodynamic models in Modeling of Relationship Between Phosphine, Phosphite, and Hypophosphite.

Between 10% and 67% of culturable bacteria are capable of using phosphite as a sole P source (12), including the critical primary producer Prochlorococcus (17). The ability of microbes to use reduced P as a sole P source suggests that a fraction of soluble P in the environment may be in a reduced oxidation state (18), possibly greater than 10% given the rate of occurrence of phosphite-using genes (12). To this end, we analyzed 32 Florida freshwater samples from six locations and at a variety of depths in the water column (Table 1: see Methods) for P speciation using high-performance liquid chromatography to separate P species coupled to detection with an inductively coupled plasma mass spect pometer (HPLC-ICP-MS). This method has been used previously to identify low-redox state P in Archean rocks, and the current work optimized the conditions for P speciation analysis in freshwater (19) (see Methods).

#### Florida Water Analysis

Phosphite and hypophosphite were detected in many Florida water samples (Table 1). In several samples, the phosphite and hypophosphite account for more than 25% of the total dissolved P (Fig. 2). The samples with lower oxidation-reduction potential, lower pH, and standing or stagnant water tended to bear the

#### Significance

Phosphorus is an important nutrient for living organisms. Phosphorus is generally considered to bear a 5+ oxidation state, but several lower redox states have been reported, including the toxic gas phosphine. We show here that the lower redox states of phosphorus are common in Florida water samples, and that based on the global concentration of phosphine, we might expect to see 5-15% of all dissolved phosphorus in a lower redox state.

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<b>River Front P</b>	Park, 28° 4' 12.25'	N, 828	" W				
A-1	0	213	8.1	0.3	2 (±1)	3 (±0.5)	95 (±1.5)
A-2	15	212	8.1	0.3	2 (±1)	1405	97 (±1.5)
A-3-g	35	n.d.	n.d.	0.2	2 (±1)	0 (±0.3)	98 (±1)
B-1	0	169	8.46	0.08	8 (±4)	3 (±2)	89 (±6)
B-2	20	168	8.46	0.3	2 (±1)	$1(\pm 1)$	97 (±2)
B-3-g	41	n.d.	n.d.	0.2	21 (±1)	5 (±0.5)	74 (±0.6)
Pithalachasco	otee River, 28° 14	26.15" N, 82° 4	0' 29.52" W				
A-1	0	8	6.49	0.2	7 (±1)	11 (±3)	82 (±8)
A-2-g	24	2	6.44	0.2	1 (±2)	10 (±3)	89 (±8)
B-1	0	6	6.49	0.2	6 (±1)	14 (±2)	80 (±8)
B-2	22	11	6.5	0.2	18 (±1)	7 (±2)	75 (±7)
B-3-g	44	13	6.51	0.2	3 (±2)	9 (±3)	88 (±8)
Hillsborough	River, 28° 1' 14.1	4" N, 82° 27' 13	.94" W				
A-1	0	64	8	0.09	3 (±2)	10 (±6)	87 (±17)
A-2	20	62	7.88	1.5	BDL	BDL	100 (±1)
A-3-g	39	62	7.84	0.04	6 (±5)	20 (±12)	74 (±20)
B-1	0	92	7.93	0.06	4 (±2)	15 (±9)	81 (±20)
B-2	14	90	7.85	0.04	BDL	24 (±14)	76 (±18)
B-3-g	28	88	7.51	0.01	BDL	BDL	100 (±20)
Pemberton (	Creek Retention P	ond, 28° 2' 4.74'	' N, 82° 15' 30.	71" W			
A-1	0	167	6.5	0.15	18 (±2)	33 (±3)	49 (±9)
A-2-g	15	-31	6.4	0.18	8 (±1)	33 (±3)	59 (±8)
B-1	0	195	6.5	0.16	10 (±2)	33 (±3)	57 (±8)
B-2-g	17	-12	6.1	0.22	23 (±1)	25 (±2)	52 (±6)
Muck Pond,	28° 13' 44.27" N,	82° 42' 83" W					
A-1	0	22	6.51	0.05	5 (±3)	11 (±6)	84 (±17)
A-2	18	41	6.49	0.06	4 (±3)	11 (±6)	85 (±17)
A-3-g	36	36	6.44	0.05	5 (±3)	9 (±6)	86 (±17)
B-1	0	11	6.76	0.05	5 (±3)	11 (±6)	84 (±17)
B-2	20	7	6.69	0.06	19 (±3)	9 (±5)	72 (±16)
B-3-g	41	5	6.65	0.05	12 (±3)	9 (±5)	79 (±17)
<b>River Front S</b>	wamp, 28° 4' 12.	81" N 022 021 51	73" W				
A-1	0	-138	5.73	0.3	3 (±1)	30 (±2)	67 (±5)
A-2-g	10	167	5.74	0.3	8 (±1)	16 (+2)	76 (±5)
B-1	0	56	5.73	0.3	BDL	BDL	100 (±5)
B-2	9	52	5.73	0.3	11 (±1)	10 (±2)	79 (±5)
B-3-a	19	-31	5.7	0.3	21 (+1)	17 (+1)	62 (+4)

TDP, ma/L

Table 1. Sample locations, characteristics, and reduced P contents given as percentages of TDP

DH

ORP, mV

Samples noted with "g" are from associated groundwater. Each sample was run in duplicate when possible, and errors (in parentheses) are reported as relative percent differences between runs, multiplied by the average percentage. BDL, below detection limit; n.d., not determined; TDP, total dissolved phosphorus.

Redox chemistry of the biogeochemical phosphorous cycle



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## New environmental model for thermodynamic ecology of biological **curofins** phosphine production

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### HIGHLIGHTS

### GRAPHICAL ABSTRACT

- Phosphine is a widely distributed trace gas.
- Phosphine is associated with anaerobic wetlands.
- · We analyse the thermodynamics of phosphate, phosphite and phosphine production.
- · A layered anaerobic ecosystem can produce phosphite and phosphine from phosphate.



## Quantities of phosphonate in selected products



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- Phosphonic acid is much more stable and ubiquitous in the environment than anticipated. We might expect to see 5–15% of all dissolved phosphorus in a lower redox state (Pasek et al., 2014, PNA).
- Natural sources for phosphonic acid in the environment:
  - ✓ Abiotic: Reactions of Schreibersite minerals with water, lightning strikes and geothermal fluids
  - Biotic: byproduct generated during the microbial degradation and synthesis of organophosphonates in reducing environments. Unwanted product of nitrogenase function? Rhizobia in legumes?

### Anthropogenic sources:

- ✓ Application of fosetyl and K- or Na-phosphonate
- Degredation of organophosphonates: glyphosate and ethephon, chelants discharged by industry and households into the waste water (< 35,000 tons/a in the EU alone)?</p>
- Byproduct of industrial chemical synthesis and reducing agent in some industrial metal electroplating processes
- ✓ Contaminant in mineral phosphorous fertilizers

## What can we do?



- 1. Differentiate between crop, where use of phosphonates is probable and where unlikely. Use the know-how of the practitioners in agriculture.
- 2. Check for alternative sources of phosphonate in the cultivation, e.g. from industrial or household water contaminations, use of glyphosate or ethephon on neighbouring fields, natural origin like in paddy fields.
- 3. Integrate phosphonate analysis of organic fertilizers, plant protection agents and particular irrigation water samples in the certification and control of farms and their inputs
- 4. Check for applications of mineral fertilizers, not permitted in organic cultivation
- 5. Investigate the production of phosphonic acid by microbial fermentation at industrial scale to get it approved for organic agriculture
- 6. More basic research is required to evaluate the phosphorus cycle.



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