

■ Humic substances interactions with sedimentary phosphorus

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Sediments receive a large amount of organic matter in eutrophic environment. Phosphorus is often responsible for this high primary production. This is why phosphorus become trapped into the sediment, organic matter bounded or not. The aim of this study is focused on the role of humic substances in the biogeochemistry of sediment phosphate. Phosphate was extracted in sediments from two Mediterranean lagoons. A fractionation scheme allows the separation of different inorganic fractions followed by the extraction and by the separation of humic and fulvic acids. 21 – 34% of total phosphorus was co-extracted with humic substances. Indeed, phosphorus might either be complexed with these compounds or be an integral part of their molecule. Humic substances therefore play an important role in the phosphate storage.

ing primary production in aquatic environments. A weak phosphorus release from sediment into the water can enhanced seriously eutrophication. Since the concentration of humic substances in sediment is larger than the concentration of phosphate, even low binding affinities may change phosphate availability [2]. In freshwater, orthophosphate may be complexed with humic substances in the presence of iron [3]. Hirata [4] observed that complexes of metal ions and dissolved organic matter may be concentrated by adsorption onto mineral particles such as clays. Organic-bound phosphorus accounted for 6 – 19% of Total-P in coastal sediments [4].

In eutrophic environment, sediments receive a large amount of organic matter. The aim of this study is to focus attention on the role of humic substances in the biogeochemistry of phosphorus in sediment. Several fractionation schemes are used to characterise sediment phosphorus. They separate different fractions of inorganic phosphate and enable quantification of the organic P pool. Inorganic phosphate consists of iron bound- and calcium bound- phosphorus. However, interactions of organic matter with this “inorganic” phosphate, are rarely considered. Furthermore, the nature and reactivity of the organic pool are unknown. In this work, sediment phosphorus is extracted following a fractionation scheme that allows a certain characterisation of organic phosphorus. Chemical relations between phosphate and humic substances are discussed.

Abbreviations used

Total-P	total phosphorus
Inorganic-P	inorganic phosphorus
Organic-P	organic phosphorus
Fe(OOH)-P	iron bound phosphorus
CaCO ₃ -P	calcium bound phosphorus
Organic-P _{ac}	acid soluble organic phosphorus
Organic-P _{alk}	alkaline soluble organic phosphorus
Fulvic-P	organic phosphorus co-extracted with fulvic acids
Humic-P	organic phosphorus co-extracted with humic acids
Residual-P	residual phosphorus.

Introduction

Sediments are derived from various detritus such as suspended matter, plankton, micro-organisms and faecal pellets. Humic substances are formed during the decomposition of this organic matter. These substances may represent a major fraction of the organic carbon reservoir in recent sediment, between 40% and 70% [1]. Though the phosphate concentration of sediments amounts only to a few percent of their dry weight, this element plays an important role in control-

Material and methods

Sediments were collected in two Mediterranean French coastal lagoons (Thau and Méjean) characterised by their high organic matter concentration. In the Thau lagoon, a large amount of organic matter comes from oyster farming; in the Mejean lagoon, from macroalgae and phytoplankton. Table I gives some characteristics of the sediments.

Sedimentary phosphorus was fractionated following the extraction sheme (Fig. 1) described by De Groot & Golterman [5]. Chelating agents allow a specific extraction of the Inorganic-P with less destruction of the Organic-P [6], unlike most classical extraction schemes. Fe(OOH)-P is extracted with buffered CaEDTA/dithionite and CaCO₃-P subsequently with Na₂EDTA. In the next step, acid hydrolysable Organic-P (Organic-P_{ac}) is extracted with H₂SO₄ [7] and then the remaining Organic-P (Organic-P_{alk})

Table I. Mean concentration on organic carbon, total phosphorus, Kjeldhal nitrogen, calcium and total iron of Thau and Méjean sediments. Mean, standard deviation (sd) and number of stations sampled (n).

	Thau			Méjean		
	mean	sd	(n)	mean	sd	(n)
Organic-C (mg g ⁻¹)	34	16	(8)	55	8	(19)
Total-P (mg g ⁻¹)	0.7	0.3	(8)	0.8	0.2	(19)
N Kjeldhal (mg g ⁻¹)	4	2	(7)	4	1	(19)
Ca (mg g ⁻¹)	133	40	(30)	125	23	(19)
Total-Fe (mg g ⁻¹)	14	8	(30)	16	4	(19)

with 2 M NaOH, 90 °C for 30 minutes. The previous extraction of Inorganic-P prevent both the re-adsorption of Organic-P on iron hydroxydes and the precipitation with Ca. Next, humic acids are precipitated from the NaOH extract by acidification at pH = 1 then collected on a filter (Whatman, GF/F). Humic-P is defined as the part of Organic-P_{alk} that precipitates at pH = 1 with the humic acids (brown precipitate). Fulvic-P is defined as the part of Organic-P_{alk} that remains in the yellow solution after the acid precipitation. Humic-P, Fulvic-P and Residual-P are measured after 1 h K₂S₂O₈ mineralisation in an acid medium. Soluble orthophosphate is determined by colorimetry according to AFNOR standard T 90-023. Phytate (C₆O₂₄H₁₈P₆) is measured in the extract containing Fulvic-P using enzymatic hydrolysis with phytase [5].

Results and discussion

Inorganic phosphate was the major fraction in these sediments (Fig. 2). About 25% of P was extracted with CaEDTA/dithionite and is thus supposed to be iron bound phosphate. CaCO₃-P was the most important inorganic fraction related with the high concentration of Ca²⁺ in lagoon waters, which controls with the pH the solubility of orthophosphate [8].

Organic-P represented a substantial proportion of the Total-P (38% of Total-P in Thau and 28% in Méjean). This Organic-P pool was subdivided in three fractions: Fulvic-P attaining 60% of Organic-P whereas Humic-P and Organic-P_{ac} represented the remaining 40%. The extract called Fulvic-P was further analysed to determine the nature of phosphate. Orthophosphate hydrolysed by NaOH during the extraction accounted for 30% of Fulvic-P. Another 30% of Fulvic-P was identified as phytates using an enzymatic hydrolysis with phytase. The nature of the remaining 40% of Fulvic-P was unidentified but this fraction appeared to be less easily hydrolysable.

The yellow colour of the extract obtained with CaEDTA/dithionite showed the presence of humic substances. Francko & Heath [3] identified a functional class of complex phosphorus compounds that may release orthophosphate on UV exposure. This phosphorus compound had a high molecular weight and was eluted with yellow humic acids. The authors suggested that phosphate adsorbed onto ferric iron humic complexes may be released by

PHOSPHORUS FRACTIONATION

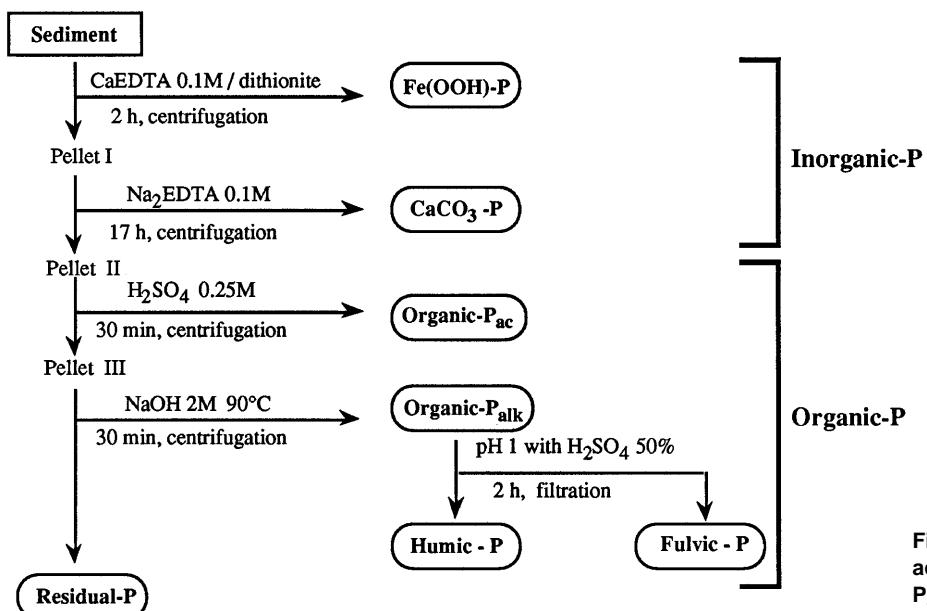


Figure 1. Fractionation scheme. Organic-P_{ac}: acid soluble organic phosphorus, Organic-P_{alk}: alkaline soluble organic phosphorus.

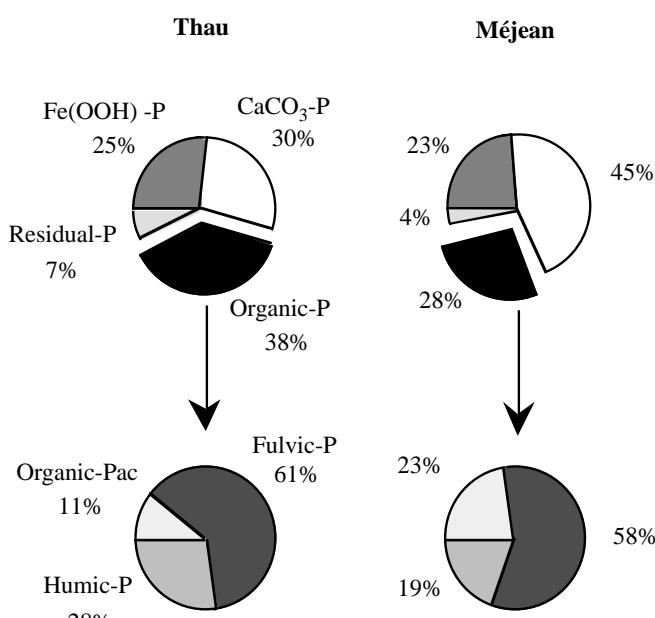


Figure 2. Phosphorus fractionation in Thau and Méjean sediments, in % of Total-P at the top and in % of Organic-P at the bottom. Organic-P_{ac}: acid soluble organic phosphorus, Organic-P_{alk}: alkaline soluble organic phosphorus.

photoreduction of iron. In sediments such complexes could be extracted with Fe(OOH)-P fraction. The extractability of this fraction may be affected by the presence of humic substances but information on this process is not available yet. Biochemical components such as nucleic acids, lipids and sugars may be included in the acid soluble Organic-P fraction [7]. Golterman et al. [9] proposed another extractant for acid soluble Organic-P: 0.5M trichloroacetic acid (TCA). Cold TCA (0 °C 30 min) extracts sugar bound phosphates whereas hot TCA (95 °C 30 min) extracts nucleic acid phosphate and polyphosphates [10]. All these compounds should be extracted with 0.5M H₂SO₄, as preliminary evidence of the nature of acid soluble Organic-P.

The phosphorus precipitated with humic acids (Humic-P) may be either part of their molecule or adsorbed on humic surface. Stevens & Stewart [11] separated by precipitation an acid-soluble and an alkali-soluble concentrated solutions of organic phosphorus. Alkali-soluble organic phosphate was co-chromatographed with humic acid. The authors concluded that phosphate may be either an integral part of humic acid or an organic phosphate/metal/organic matter complex. However, considering the extraction scheme used in this work, this phosphate should have already been partly extracted from sediments in the first step (Fe(OOH)-P).

Orthophosphate found in the extract containing Fulvic-P may be formed by alkaline destruction of the complexes

between fulvic and humic acids with orthophosphate. Phytate (inositol hexaphosphate) has been shown to be present in huge quantities (30–150 µg g⁻¹ of dry sediment) in lake sediments [5]. It is bound to iron hydroxides [5]. It could be partially bound to organic matter [12]. Several bacterial strains contain phytase enzyme which could produce or mineralise phytate. Phosphate release from phytate is proposed to explain the release of soluble orthophosphate from sediments when they turn anoxic [13]. The non identified 40% of Fulvic-P may be organic-bound phosphorus of high molecular weight (fulvic acids), that resist hydrolysis.

As phytate may be complexed to fulvic acid [12], humic-bound phosphorus accounted for about 80% of organic phosphorus (25% of Total-P). This value corresponds to 75% found by Nissenbaum [1].

Conclusion

In sediments, the nature of chemical bindings between phosphate and humic substances has rarely been studied. However the bioavailability of Humic-P and Fulvic-P is certainly controlled by these interactions.

Future research on the sediment phosphate mobility should take into account the influence of these substances. Here, two questions must be addressed: what is the nature of the different extracts obtained with this fractionation scheme and what is their mobility.

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