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Speciation of phosphorus fractionation in river sediments by explanatory data analysis

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ABSTRACT

This article investigates the controls on sediment phosphorus (P) speciation dynamics as a function of its fractionation into chemically defined operational pools along a river continuum. A total of 27 variables were analyzed in bed sediment samples collected for one year from six sampling points, along a 75 km river continuum (Louros River, NW Greece). Multivariate explanatory analysis of the complex experimental data matrix was performed in order to unravel the spatial pattern of P speciation. Non-parametric examinations were also applied in order to elucidate the temporal variations encountered in the speciation of P. The results suggest that inorganic P species control P bioavailability in space and time. Organic P was found to be very reactive among the various fractions thus changing its bioavailability but exhibited no temporal variation. The utility of the proposed approach in the differentiation of natural and anthropogenic P inputs and their classification to point and non-point sources is demonstrated presenting a significant improvement compared to mere fractionation analysis.

1. Introduction

Phosphorus (P) has long been recognized as a key factor responsible for eutrophication of fresh waters and for this reason knowledge of its concentration is an important parameter determining the eutrophic state of an aquatic ecosystem (Golterman and de Oude, 1991). However, knowledge of total P concentration is not always adequate to assess the risk associated with its presence in natural waters. That is because, although bioavailable P is mainly in the dissolved form, it occupies only a small fraction of total P, particulate P being a major fraction of total P. Therefore, the stability and chemical form of particulate P in association with the environmental conditions regulate its retainment and release from the sediment phase and determines the level of dissolved P in the water column (Pardo et al., 2003; House, 2003; Jarvie et al., 2005).

According to previous studies, in order to gain improved understanding on P distribution in a freshwater ecosystem special emphasis should be given to the determination of its chemical forms in the sediment phase (Fabre et al., 1996). This procedure, commonly referred as fractionation, strives to accomplish the classification of phosphorus according to physical (e.g. size, solubility), biological (e.g. bio-availability) or more frequently chemical (e.g. bonding, reactivity) properties that determine P involvement to the geological, chemical and biological processes. In order to differentiate the sediment P pool, chemical fractionation methods using various extracting agents have been presented (Cross and Schlesinger, 1995; Fabre et al., 1996; Ruban et al., 1999, 2001). The general principles behind most of the available procedures involve extraction of labile, reductant, metal bound, occluded and organic P.

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All these procedures, although accomplish a representative exemplification of P forms into chemically extractable pools, they do not account for the chemical species of P (speciation) in each of these fractions, in other words, they do not allow the differentiation of the distribution of P amongst defined chemical species. On the contrary, most of them either consider certain species to be present in a single fraction (e.g. organic P in NaOH extracts) or propose the separate differentiation of species (usually to inorganic and organic P) in the overall sediment sample irrespectively of its fractionation (De Groot and Golterman, 1993; Pardo et al., 2004). Therefore, the distribution of each P species that contribute to the measured total P concentration in each of these fractions is neglected. A possible reason is the difficulty in the differentiation between inorganic and organic P with the available sample treatment and extraction methods. Therefore, P speciation is defined each time on an operational basis as a function of the extractants and the fractionation protocol employed.

In view of the lack of published work on the speciation of P fractionation in river sediments, this study introduces an alternative method for the differentiation of the major P pools in the sediment phase, based on the investigation of P speciation in each operational fraction. Due to the large number of data, explanatory data analysis was conducted in order to accomplish a meaningful data reduction and interpretation of the multi-component chemical and physical interactions (Massart et al., 1998). A comparison with earlier reports on a part of these data enabled us to identify the advantages of using P speciation as an additional tool to conventional fractionation procedures.

2. Materials and methods

2.1. *Description of study area*

Samples for the purposes of this study were obtained from Louros River, NW Greece. This river is 75 km long with a catchment area of 780 km^2 of which 332 km^2 belong to the highlands and 448 km^2 to the lowlands. The river drains with a mean annual discharge of 19–28.6 m^3 s^{-1} from 1400 m over the sea level to Amvrakikos Gulf which is a RAMSAR protected ecosystem. The geographical coordinates of its estuaries are 20°46′ 42″E and 39°02′46″N. The underlying geology in the wider area is dominated by alluvial geological formations, calcitic and silicate tertiary deposits as well as hard limestones. The annual average catchment precipitation was 90.7 mm with minimum in August (no rainfall) and maximum in February (335.9 mm). The annual mean temperature during the study period was 17.9 °C and extreme values of 7.8 and 28 °C were registered in February and August, respectively.

Louros River receives treated domestic effluent and effluent from light industrial activities including meat processing, abattoirs, pig farms and a small quantity of olive mill wastewater mostly during the autumn and early winter months (October–January). Its water is the main source of irrigation for a cultivated land of 133.6 km^2 dominated by horticultural species, citrus trees/fruits, maize, cotton plants and olive trees. It is also used for aquaculture activities (mainly of trout, salmon and eel) while its tributaries cover the water supply demands of small settlements located around the river.

2.2. *Sampling procedure*

Six sampling points were selected based on the anticipated burden imposed by anthropogenic activities (Fig. 1): Point 1 is situated in the spring of the river and is considered to be unaffected by anthropogenic activities. Point 2 is situated after aquaculture units and point 3 near the village of Agios Georgios. All these sites are transitional between forested and agricultural areas. Point 4 lies near agricultural areas and just before areas with light industrial activities like meat processing industries and pig farms. Point 5 is situated after the meat processing units and at the estuary of the river in Amvrakikos Gulf and point 6 immediately after the estuary. The samples were collected in six sampling cruises during the period of 2002–2003 (11/02, 02/03, 05/03, 06/03, 09/03 and 11/03).

Surface sediment samples from a maximum depth of 5 cm were collected from the river cross section (water depth ≈1 m) in a total square area of 25 m upstream and downstream of the selected sampling point(s). The samples were stored in air-sealed plastic bags for transportation to the laboratory. All samples were air-dried and sieved through a 2-mm stainless steel-sieve and stored at 4 °C in thoroughly cleaned 250-ml glass jars covered with aluminum foil to avoid light exposure.

2.3. *Phosphorous fractionation*

Phosphorous separated into operationally defined pools using a sequence of increasingly strong reagents to successively remove more recalcitrant forms according to the modified method of Hedley et al. (1982) with the modifications proposed by Tiessen and Moir, (1993). Based on recent literature data, we decided to adopt two more modifications. The first was related to the water extractable P, where the use of resin was omitted since it is not necessary for the extraction of labile P, while water-extractable P is more closely related to bioavailable P compared to resin-extracted P (Sui et al., 1999). The second simplification compromised the removal of dithionite from the initially proposed bicarbonate–dithionite mixture for the extraction of bioavailable P in order to diminish co-extraction of inorganic P bound to the surface of $CaCO_3$, which may erroneously interpret part of $CaCO_3$-P as Fe–P (Jensen et al., 1998). Briefly, the fractionation procedure was performed as follows: 1 g of air-dried sediment was transferred to 50 ml flasks and sequentially extracted with (i) 30 ml deionized water (plant available and water-extractable P; TP-H_2O), (ii) 0.5 M $NaHCO_3$ (pH = 8.2) (weakly sorbed-bioavailable organic and inorganic P; TP-HCO_3), (iii) 0.1 M NaOH (strongly bound chemisorbed P-potentially bioavailable; TP-NaOH) and (iv) 1 M HCl (apatite or Ca-bound P-nonbioavailable; TP-HCl). Each extraction lasted for 16 h on an end-over-end mechanical shaker (180 cycles min^{-1}) at 25 °C. After each extraction, the supernatant was centrifuged for 1 h at 4500 rpm and then filtered through a Whatman No 42 (0.45 μm) filter paper.

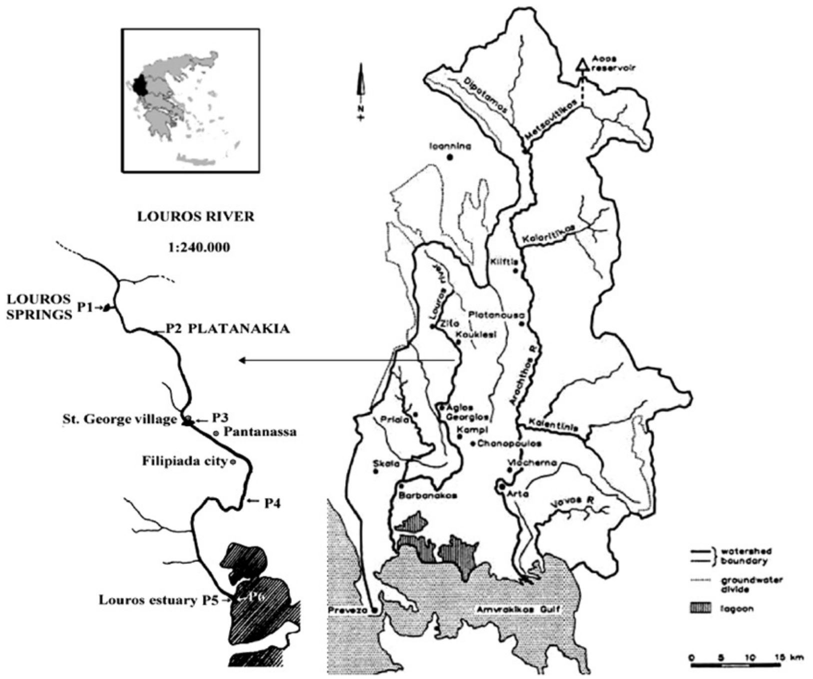

Fig. 1 – Map of Greece and magnified location map showing the catchment of the study area (Louros River) and the relevant bed sediment sampling sites.

Each filtrate was analyzed for orthophosphates, total inorganic P and total P according to the analytical methods described below. An aliquot (2 ml) of each fraction was transferred in a 10 ml vial so that all fractions were mixed. Total P was then determined to this sample according to the same analytical protocol. Samples not immediately analyzed were stored in dark at 4 °C for no more than 48 h (Jarvie et al., 2002b).

2.4. Analytical speciation of phosphorous

The determination of P species was performed as orthophosphate (PO) according to the method of Murphy and Riley (1962), considering recent guidelines for the elimination of interferences (Jarvie et al., 2002b). Measurements were performed with a Shimadzu UV-2100 spectrophotometer with matched quartz cells of 1 cm path length at 880 nm. Total inorganic P (IP) was determined according to the above procedure after acid digestion of the samples with sulfuric acid and heating at 90 °C for 2 h to ensure release of P from polyphosphates. The determination of Total P (TP) in each extract and in the mixed extract solution was based on a modified digestion method with excess of peroxydisulfate in strongly acidic conditions after 3 h of heating at $>90\,^{\circ}$C. Acid-hydrolyzable P (AP) was calculated from the difference between IP and PO.

2.5. Analytical methods

Sediment granulometric distribution was determined by dry sieving in stainless-steel sieves. Wet sieving was avoided since it could lead to the release of water extractable P which was one of the parameters studied. Three grain size fractions were separated: Gravel (>2 mm), sand (63 μm–2 mm) and silt/clay (<63 μm). Organic matter (OM) (as loss on ignition; LOI) and carbonate content determinations were based on weight losses after drying and combustion at 550 and 900 °C, respectively. Total concentrations of Ca, Mg, Fe and Al were determined after wet digestion with aqua regia for 2 h. Metal analysis was performed with a Shimadzu AA-6800 flame atomic absorption spectrometer.

2.6. Data treatment

Explanatory statistical tests were run in order to identify the different mechanisms that account for P behavior in the sediment phase. Detailed information regarding the statistical examinations can be found in previous reports (Vega et al., 1998; Alberto et al., 2001; Singh et al., 2004) so only a brief overview will be given herein.

Departures from normality were assessed with the aid of Kolmogorov–Smirnov tests for normal distribution. Since each value represented the level of each parameter at a certain point in time, these levels were considered to belong to different populations due to the fluctuation of the values with time. Therefore, to ensure normality, the $\log_{10}$ transformation of the values was decided (Zar, 1999).

Factor analysis (FA) and principal component analysis (PCA) were used to extract information from the most meaningful parameters (variables) which better described the overall data while afford data reduction with minimal loss of information. Redundant variables that do not contribute to the explanation of the observed variance were extracted with the aid of the anti-image matrix and the extraction communalitites. The anti-image matrix contains the negative partial correlations and they can give an indication of correlations which are not due to the common factors. In this line of thinking, factors that do not seem to fit with the structure of the other variables should be dropped. Extraction communalities are estimates of the variance in each variable accounted for by the factors (or components) in the factor solution. According to this method, variables with low extraction communalities should be considered for removal leaving those variables that hold a high value in the initial data matrix. Once the latent variables have been removed, principal components were extracted by the *R*- mode principal component method which mathematically transforms the original data with no assumptions about the form of the covariance matrix. For this analysis, the covariance matrix was diagonalized and the characteristic roots (eigenvalues) were obtained. The transformed variables or principal components (PCs) were obtained as weighted linear combinations of the original variables. To decide the number of PCs that aid to the interpretation of the underlying data structure the Kaiser criterion and the scree plot were used. The Kaiser criterion (Kaiser, 1960) dictates that only components with eigenvalues greater than unity should be retained which means that unless a factor extracts at least as much as the equivalent of one original variable, it should be dropped. The scree test (Cattell, 1966), which consists of plotting the eigenvalues against the number of the extracted components and finding the point where the smooth decrease of eigenvalues appears to level off to the right of the plot, eliminates components that contribute to factorial scree only.

Cluster analysis (CA) of the transformed data was applied to provide a better insight into the relationships among sediment components. The corresponding hierarchical dendrograms of the most representative variables carrying similar information about the objects were drawn using the complete linkage and Euclidean distances.

Discriminant analysis (DA) was used to classify and discriminate the effect of anthropogenic activities on sedimentary P dynamics. Three groups representing low, medium and high P load were selected and the discriminant function for each group was calculated as

$$f(G_i) = k_i + \sum_{j=1}^{n} w_{ij} p_{ij},$$

where i is the number of groups (G), k_i is the constant inherent to each group, n is the number of parameters used to classify a set of data into a given group, w_j is the weight coefficient, assigned by DA to a given selected parameter (p_j).

Multidimensional scaling (MDS) was deployed to compare and establish relationships between the sampling stations. For the latter analyses, data were restricted to those variables which provided statistically significant contribution to the total abundance. This criterion was verified through Pearson moment–product correlation and served to reduce the confounding effects of insignificant parameters. The procedure was applied to the $\log_{10}$ transformed data to downweight the influence of burdened stations and take into account unpolluted sampling sites as well. Average concentrations for each variable were used in the MDS ordination. The three-dimensional scatter plot was constructed from the first three ordination axes in MDS with each point representing the average sampling station structure for the overall sampling period. Finally, the Kruskal–Wallis ANOVA by ranks and the Median non-parametric tests were used to examine differences in the seasonal trend of P.

Statistical methods were performed with the aid of the automated modulus of SPSS 13.0 software package.

3. Results and discussion

3.1. *Development and evaluation of phosphorus speciation protocol*

An important source of variability when dealing with P speciation stems from the method used to differentiate between its basic components. Two approaches are most frequently applied to link the basic P species. The first, considers P speciation as a separate parameter in the sediment sample irrespectively of its fractionation while the second defines P speciation in relation to its fractionation.

The first approach assumes that TP is equal to the sum of inorganic and organic P content (TP = IP+OP) (Eq. (1)) or to the sum of soluble reactive P (defined as P determined by the molybdenum blue method) and the remaining non-reactive P (TP = NRP+PO) (Eq. (2)) (Owens and Walling, 2002; Selig and Schlungbaum, 2002). These two methods are practically the same the only difference being that in the first case all species are separately determined (PO, AP and OP) while in the second case only PO and TP are determined, the difference being NRP. When Eq. (1) is applied the importance of inorganic P to the TP content is accounted for and a clear differentiation between IP from OP is possible. The only limitation of this method lies with the analytical procedure that is used for the determination of inorganic P since it necessitates the acidic hydrolysis of the sample a procedure that may hydrolyze part of OP thus underestimate its value. Eq. (2) alleviates this drawback but does not account for AP which is co-estimated with OP. Therefore, NRP will always be higher than PO thus any data interpretation should be based on the examination of the relative percentage of NRP occupied in each fraction, which is very cumbersome and endangers significant loss of information.

The second approach is based on the assumption that either IP can be found in the HCl fraction ($TP = OP+TP_{HCl}$) (Eq. (3)) ignoring any IP species in other fractions or that almost all organic P is present in the NaOH fraction ($TP = OP_{NaOH}+IP$) (Eq. (4)) with trivial contribution from the other fractions (Ruban et al., 1999, 2001; Fang, 2000). Nevertheless, the simplification that one P species can almost quantitatively be recovered in a single fraction, is rather uncertain since both expressions define P linkage to the sediment (e.g. apatite P or humic bound P) and not to the species of P. Thus, the amount of TP extracted from each of these fractions cannot be ascribed only to one species.

With the above limitations in mind, and owing to the lack of a generally accepted solution to the issue of P speciation, all approaches, as expressed by the solution of their mathematical formulas, were applied to the available data set. The results from this study are tabulated in Table 1. As we can

Table 1 – Total phosphorus recovery with various speciation methods

Speciation method	Theoretical recovery (%)[a]	Experimental recovery (%)[b]	RSD (%)[c]	Eq no.
TP = IP+OP	100	93.1	4.6	(1)
TP = NRP+PO	100	100.2	4.1	(2)
TP = OP+TP_{HCl}	100	73.7	4.8	(3)
TP = IP+TP_{NaOH}	100	79.4	3.5	(4)
Proposed method	100	99.3	4.3	(1), (5)–(8)

[a] TP estimated as the sum of P in each fraction.
[b] TP determined in the mixed extract.
[c] RSD = Relative standard deviation. Average of triplicate measurements.

observe, Eqs. (3) and (4) failed to provide an efficient representation of P speciation yielding low recoveries. On the other hand, Eqs. (1) and (2) gave satisfactory recoveries although some loss of accuracy was observed. Since the same analytical procedure was employed for the determination of TP in all cases this loss could only be ascribed to the speciation method. According to the experimental values of Eq. (1), IP was always higher than OP in the NaOH extract (about 2–2.5 times) although the opposite behavior was anticipated (Selig and Schlungbaum, 2002). This implies that P bound to organic matter is hydrolyzed under the acidic conditions applied during IP determination thus overestimated the OP content. Eq. (2) alleviates this drawback since no acidic hydrolysis is required but it cannot be generalized to all fractions since they are expected to hold a significant amount of IP (especially the HCl fraction). In order to cope with this inconsistency Eq. (1) was decided for all fractions except for the NaOH extract were Eq. (2) was applied separately. In principle, TP is still defined as the sum of IP and OP (Eq. (1)) while the amount of a specific species (PO, IP and OP) is defined as the sum of this species in each fraction. However, according to Eq. (2) the IP-NaOH fraction is expressed by (or is equal to) PO-NaOH, therefore the latter is accounted in both the total PO and the IP content. Under this speciation method a total recovery of 99.3% was accomplished, which is very satisfactory and within the experimental error. The final form of the speciation approach is

$$TP = IP + OP, \tag{1}$$

where

$$IP = IP_{H_2O} + IP_{HCO_3} + IP_{HCl} + PO_{NaOH}, \tag{2}$$

$$OP = OP_{H_2O} + OP_{HCO_3} + OP_{HCl} + NRP_{NaOH}. \tag{3}$$

IP can further be divided to AP and PO according to the expressions:

$$AP = AP_{H_2O} + AP_{HCO_3} + AP_{HCl}, \tag{4}$$

$$PO = PO_{H_2O} + PO_{HCO_3} + PO_{HCl} + PO_{NaOH}. \tag{5}$$

3.2. *Analytical phosphorus speciation in sediments*

Once the data matrix has been re-constructed to meet the requirements of the proposed speciation method the distribution of TP between its inorganic and organic forms was assessed in more detail. Table 2 gathers the average data of sediment P speciation and fractionation. As will be discussed further below in more detail, the HCO_3 and HCl fractions are dominated by inorganic P while organic P is the predominant species in the H_2O and NaOH fractions.

3.2.1. *Phosphates*

Phosphates (PO) or orthophosphates is the lowest P species in the sediment. That is because in order for PO to reach the sediment they must be transformed to a particulate form, a procedure which necessitates they interaction with solids or the formation of water insoluble complexes (i.e. apatite). The distribution of PO in each fraction was decreasing according to the order: PO-HCl (46% of Total PO) > PO-HCO_3 (38% of Total PO) > PO-NaOH (13% of Total PO) > PO-H_2O (3% of Total PO). The relative distribution of PO to total P was following the same order: PO-HCl (16% of TP) > PO-HCO_3 (12% of TP) > PO-NaOH (4.4% of TP) > PO-H_2O (1% of TP).

The speciation profile of PO reveals that physical sorption plays a trivial role in the fixation of PO in the bed-sediment accounting for only 3% of the total PO found in the sediment. The lack of statistically significant correlation between TP-NaOH and PO ($r = 0.222$, $p < 0.05$) suggests that organic matter is not an important parameter in PO binding to the sediment. An increased amount of total PO extracted with HCO_3 ($r = 0.445$) suggests the formation of PO-metal oxides or hydroxides (FeOOH-PO). Last, the most important mechanism for PO sedimentation is apparently apatite formation which accounts for almost half of PO found in the sediment ($r = 0.559$). The tree diagram of Fig. 2 and the non-parametric correlation coefficients verify these interactions.

3.2.2. *Inorganic phosphorus*

The highest accumulation of IP was observed in the HCl fraction followed by HCO_3. Only a small percentage was measured in the H_2O and NaOH fractions. The distribution of PO in each fraction was decreasing according to the order: IP-HCl (50.4% of Total IP) > IP-HCO_3 (35% of Total IP) > IP-NaOH (7.1% of Total IP) > IP-H_2O (3.8% of Total IP). The relative distribution of IP to total P was following the order: IP-HCl (31% of TP) > IP-HCO_3 (21.8% of TP) > IP-NaOH (4.4% of TP) > IP-H_2O (2.3% of TP).

Table 2 – Phosphorus speciation and operational fractionation in Louros sediment: average concentrations (in $mg\,g^{-1}$) ± standard deviation ($n = 3$) and concentration ranges

Sampling point	Total P[a]	Inorganic P[b]	Organic P[b]	Phosphates	TP H_2O[c]	TP HCO_3[c]	TP NaOH[d]	TP HCl[c]
1	0.49 ± 0.04	0.28 ± 0.04	0.21 ± 0.05	0.14 ± 0.03	0.06 ± 0.04	0.16 ± 0.03	0.10 ± 0.05	0.17 ± 0.04
	(0.38–0.65)	(0.08–0.36)	(0.08–0.36)	(0.06–0.26)	(0.01–0.27)	(0.07–0.28)	(0.02–0.40)	(0.07–0.30)
2	0.62 ± 0.09	0.38 ± 0.06	0.24 ± 0.05	0.22 ± 0.07	0.05 ± 0.03	0.20 ± 0.03	0.06 ± 0.005	0.31 ± 0.04
	(0.34–0.98)	(0.20–0.55)	(0.14–0.47)	(0.03–0.43)	(0.01–0.21)	(0.11–0.33)	(0.05–0.08)	(0.17–0.46)
3	0.62 ± 0.08	0.39 ± 0.06	0.23 ± 0.03	0.23 ± 0.03	0.03 ± 0.01	0.22 ± 0.03	0.08 ± 0.02	0.29 ± 0.04
	(0.32–0.86)	(0.21–0.58)	(0.10–0.30)	(0.10–0.31)	(0.01–0.06)	(0.10–0.32)	(0.026–0.18)	(0.16–0.45)
4	0.47 ± 0.09	0.30 ± 0.08	0.17 ± 0.05	0.17 ± 0.05	0.05 ± 0.02	0.19 ± 0.03	0.06 ± 0.01	0.17 ± 0.07
	(0.27–0.78)	(0.19–0.70)	(0.06–0.41)	(0.03–0.42)	(0.03–0.15)	(0.09–0.34)	(0.03–0.09)	(0.03–0.47)
5	0.51 ± 0.05	0.31 ± 0.05	0.20 ± 0.02	0.18 ± 0.05	0.03 ± 0.01	0.17 ± 0.03	0.06 ± 0.01	0.25 ± 0.05
	(0.40–0.70)	(0.16–0.48)	(0.13–0.25)	(0.05–0.40)	(0.01–0.05)	(0.09–0.26)	(0.03–0.13)	(0.03–0.42)
6	0.44 ± 0.08	0.25 ± 0.04	0.19 ± 0.06	0.11 ± 0.02	0.03 ± 0.02	0.22 ± 0.02	0.03 ± 0.01	0.16 ± 0.01
	(0.24–0.72)	(0.18–0.39)	(0.02–0.38)	(0.01–0.18)	(0.01–0.13)	(0.18–0.28)	(0.01–0.06)	(0.026–0.42)

[a] Defined as the sum of inorganic and organic P or the sum of total measured P in all fractions.
[b] Defined as the sum of inorganic or organic P, respectively, in each fraction as specified in the text.
[c] The sum of inorganic and organic P in the respective fraction.
[d] The sum of phosphates and non-reactive P.

According to the Spearman correlation matrix, IP exhibited statistically significant positive correlation at the 95% confidence level with TP-HCO_3 ($r = 0.565$), TP-HCl ($r = 0.789$), organic matter ($r = 0.345$), $CaCO_3$ ($r = 0.567$) and Ca ($r = 0.611$). This implies that IP is associated with metal hydroxides and calcite precipitation. In addition, the grouping of IP with Mg (Fig. 2) and the significant correlation with TP- HCO_3 and organic matter, indicate that part of IP is affected by algal productivity (Gonsiorczyk et al., 1998).

3.2.3. *Organic phosphorus*

The distribution of OP in each fraction throughout the river was decreasing according to the order: OP-HCl (31.7% of Total OP) > OP-HCO_3 (30.3% of Total OP) > OP-NaOH (23.4% of Total OP) > OP-H_2O (14.5% of Total OP). The contribution of OP to the total P concentration was following the order: OP-HCl (12.1% of TP) > OP-HCO_3 (11.5% of TP) > OP-NaOH (9.1% of TP) > OP-H_2O (5.7% of TP).

These data show that although the relative proportions of OP in the H_2O and NaOH fractions is much higher than IP, most OP (in absolute values) is present in the HCO_3 and HCl fractions. The reason for this speciation profile probably lies with the sources of P or with the anerobic/anoxic P release mechanisms occurring in the bed sediment as a function of the redox conditions. However, non-parametric Spearman correlation analysis did not contribute in the effort to verify these mechanisms while cluster analysis although revealed a common grouping trend among the various OP species it did not contribute to the interpretation of its behavior.

3.3. *Pattern recognition between phosphorus speciation and fractionation*

The above discussion, although useful in recording the general mechanisms controlling for P mobility in the sediment, it does not allow us to extrapolate a more secure conclusion on the observed behavior. That is because many mechanisms may occur simultaneously and the univariate investigation of the correlated multivariate data, prone to both spatial and temporal variability, endangers significant loss of information. To overcome this uncertainty and obtain evidence on the prevailing mechanisms, pattern recognition techniques were employed.

Although many factors were experimentally determined, only those parameters exerting a significant effect on the observed behavior were of concern. The dendrogram of Fig. 2 shows that geochemical parameters form a separate group of inter-correlated variables which implies that they indirectly influence the observed variance of P pattern. However, CA although is capable of grouping variables that carry the same information together, it does not effectively handle the problem of multicolinearity (Thanasoulias et al., 2002) increasing the possibility of entering too many redundant variables into the DA model. Therefore, although CA is useful in identifying some interactions among the variables, it cannot be safely employed to remove the latent variables (Thanasoulias et al., 2003).

With reference to this problem, FA/PCA can be used to decide which variables are redundant irrespectively of their initial solution in the correlation matrix. This procedure

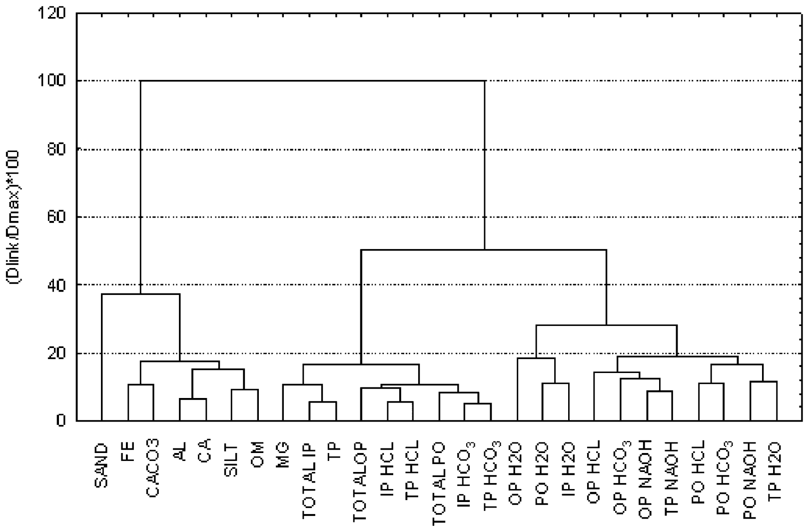

Fig. 2 – Hierarchical cluster dendrogram of P species and fractions in Louros River sediment.

further reduces the number of variables by extracting the latent of the correlated variables. However, since many variables were directly depended (species and fractions), direct application of FA/PCA with all variables did not provide a meaningful data reduction or interpretation of the observed variance. For this reason, the procedure for removing latent variables was based on the estimation of the extraction communalites and the negative partial correlations of the variables (anti-image matrix) (SPSS, 2005). Unless both criteria recommended rejection of a variable (data not shown for space saving reasons) no action was taken (Katsaounos et al., 2006). This analysis revealed that only 15 out of the 27 variables (55% reduction) were needed to explain the observed variance which is better than this observed when only P fractions were deployed.

Once the latent variables were identified and removed, PCA was applied to the remaining data to compare the compositional patterns between the analysed samples and the factors that influence each one. The criterion used to assess the efficiency of PCA was the Barlett's sphericity test on the correlation matrixes. Evidently, the calculated $\chi^2 = 560$ is statistically significant at $p = 0.05$ and 120 degrees of freedom indicating that the variables are not orthogonal but correlated, therefore allowing to reduce the dimensionality of the original data.

The scree plot for the given dataset (graph not shown) showed that only the first five components complied with the Kaiser criterion, whereas nine or ten components satisfied the scree test. Based on these data, the unrotated loadings of the experimental variables (15 out of initial 27) for the first five PC's were extracted (Table 3). The factor loadings are classified according Liu et al. (2003) as strong, moderate and weak corresponding to the absolute loading values of >0.75, 0.75–0.50 and 0.50–0.30, respectively. In our analysis, only moderate to strong loadings were considered disregarding weak loadings as uncertain.

As we can observe, all factors used in the PCA model were detected in the first five PCs with eigenvalues >1 explaining about 86.3% of the total variance. This verifies the validity of the applied methodology for the extraction of latent variables when inter-correlated variables exist in the same data matrix. The first PC accounting for 33.1% of the total variance showed high negative loadings for HCO_3 and HCl fractions originating, primarily from IP species possibly due to the high content of this species in these fractions. The second PC (19% of total variance) related the HCO_3 and NaOH fraction as a function of the OP content of the sediment. PC's 3 and 4 show that the relationship between H_2O and HCl fractions is based on the PO content while H_2O and HCO_3 on the OP content of the H_2O fraction. Finally, PC 5 reveals a reversed relationship between OP-NaOH and OP-HCl.

The positive loadings observed in PC2 between OP-NaOH and OP-HCO_3 suggests an interaction between those two fractions. The most apparent source of this interaction is the strong adsorption of phytate to FeOOH (De Groot and Golterman, 1993). Since the latter is extracted by alkaline bicarbonate it is possible that part of phytate–FeOOH is released to this fraction. However, this interaction alone cannot explain the common grouping of these fractions in the second PC, which accounts for 18.1% of the total variance. According to previous studies, this interaction stems from the sift of redox conditions in the upper sediment layer that lead to breakage and release of some organic P species, like humic-bound (Uhlmann et al., 1990), biogenic accumulated in microorganisms (mainly in the form of polyphosphate granules which are released as conditions sift from oxic to anoxic) (Waara et al., 1993), phospholipids (Baldwin, 1996) and phytate phosphatase (Golterman et al., 1998) towards the formation of P species extractable in mild alkaline conditions by ligand exchange (Fang, 2000). Since these conditions are offered by the HCO_3 solutions many researchers concluded that this fraction (redox sensitive bound P) can provide a qualitative indication of anerobic/anoxic P release (Gonsiorczyk et al., 2001; Selig and Schlungbaum, 2003).

An intriguing outcome of the PCA is that OP-HCl is negatively correlated to OP-NaOH (in PC 5) which is, in turn,

Table 3 – Unrotated loadings of the important experimental variables on the first five principle components (PCs)

	PC 1	PC 2	PC 3	PC 4	PC 5	Extraction communalities
TP H_2O	0.157	0.374	*0.596*	*0.606*	0.206	0.932
TP HCO_3	0.545	0.218	0.366	*−0.557*	0.018	0.964
TP NAOH	0.444	*0.653*	−0.058	0.016	−0.407	0.951
TP HCl	*0.729*	−0.145	*−0.601*	0.147	0.150	0.958
IP HCl	*0.726*	−0.279	−0.463	0.238	−0.146	0.911
TOTAL IP	*0.848*	−0.377	−0.029	−0.024	−0.107	0.899
PO H_2O	0.361	0.094	*0.586*	−0.203	0.333	0.831
PO HCO_3	0.514	−0.558	0.443	−0.270	−0.038	0.915
PO HCl	*0.752*	−0.406	0.057	0.337	−0.244	0.913
Total PO	*0.691*	−0.484	0.420	−0.045	−0.127	0.949
OP H_2O	0.151	0.259	0.369	*0.826*	0.062	0.934
OP HCO_3	0.387	*0.661*	0.153	−0.436	−0.051	0.916
OP NAOH	0.290	*0.667*	−0.221	−0.012	*−0.542*	0.958
OP HCl	0.410	0.062	−0.497	−0.089	*0.673*	0.950
Total OP	0.529	*0.696*	−0.125	0.052	0.328	0.891
Eigenvalues	5.303	3.045	2.260	1.871	1.328	—
% cumulative variance	33.10	51.20	66.30	78.00	86.30	—

Italic values represent moderate (>0.5) to strong loadings (>0.75).

positively related to OP-HCO_3 (PC 2) implying the formation of several recalcitrant organo-phoshorus species during HCO_3–-NaOH P exchange. These species (possible having C–P or C–O–P bonds) are chemisorbed and are not extracted by water or alkaline solutions. The presence of inert organic P compounds has already been observed in previous studies reporting the transformation of OP to more recalcitrant forms especially in areas receiving agricultural run-off (Lilienfein et al., 2000). The common group of OP-HCl with OP-HCO_3, OP-NaOH and TP-NaOH in the cluster dendrogram of Fig. 2 is also supportive to these interactions.

The remaining amount of OP, not involved in the above reactions, corresponds to organic P compounds or most probably organically bound P attached to the humic and fulvic acids which require strong alkaline media to solubilize, along with inorganic P compounds soluble in bases (Kozerski and Kleeberg, 1998).

In order to obtain more information on the observed behavior, the varimax rotated component matrix was also estimated. Rotation produces a new set of factors, each one involving primarily a subset of the original variables with as little overlap as possible, so that the original variables are divided into groups somewhat independent of each other (Massart et al., 1988) without affecting the goodness of fitting of the principal component solution. However, the rotated component matrix (not shown for brevity) provided no additional information on the inter-relations among the P species and fractions other than the obvious conclusion that each fraction forms a separate PC with a specific P species. This is not strange since the sum of the individual species make up the total P content of a fraction. This finding although does not provide any additional information with regards to the occurring mechanisms in the sediment phase, comes as a confirmation on the validity of the speciation method adopted herein.

3.4. *Importance of speciation to phosphorus concentration in the sediment and relation to its sources*

Based on the above treatment (identification of the important variables and their interactions) the classification of P concentration in three different groups was pursued. According to the Canada Province of Ontario, the concentration of 0.6 mg/g can be considered as a threshold for clean (<0.6 mg/g P), marginally polluted ($=0.6$ mg/g P) and polluted (>0.6 mg/g) sediment (Persaud et al., 1993). Three groups were therefore selected based on this concentration level for total P. The latent variables identified above were not considered in the DA modules in order to reduce uncertainty. With this approach the model could account for 90% of the total variance (including 15 parameters) in the original data (standard DA mode) which is very satisfactory. The results of this analysis are summarized in Table 4. Both the standard and the forward stepwise modes using 15 and 11 parameters, respectively, rendered the corresponding classification matrixes assigning 100% correct cases (Table 5). The 2D graph of the first two discriminant functions for the post hoc classification of the data with forward stepwise DA is depicted in Fig. 3. As we can observe, an excellent discrimination among the three P concentration levels was accomplished using only 11 out of the 27 variables imported in the initial data matrix. On the other hand, backward stepwise mode DA gave 88.8% correct assignations using only two parameters with a different match for each P concentration level. This is reasonable since the sum of IP and OP equal to TP upon which the differentiation has been based. However, the use of only

Table 4 – Classification functions and statistical parameters for disciminant analysis (DA) of *P* load in Louros river sediment

	Standard DA mode		Forward stepwise DA mode		Backward stepwise DA mode	
	Function 1	Function 2	Function 1	Function 2	Function 1	Function 2
Statistical parameters						
Chi-Square	88.7	22.1	89.7	23.1	53.4	5.4
Eigenvalue	12.0	1.3	9.8	1.3	3.4	0.2
Wilk's l	0.033	0.428	0.040	0.438	0.193	0.847
% Explained Variance	89.9	10.1	88.5	11.5	94.9	5.1
P values	0.000	0.077	0.000	0.011	0.000	0.020
Degrees of Freedom (df)	30	14	22	10	4	1
	Functions at group centroids[a]		Functions at group centroids[a]		Functions at group centroids[a]	
Group 1 <0.6 mg/g	2.91	0.53	−2.61	−0.53	1.63	0.15
Group 2 $=0.6$ mg/g	0.27	−2.47	−0.34	2.42	−0.32	−0.91
Group 3 >0.6 mg/g	−4.50	0.44	4.08	−0.41	−2.28	0.23

	Classification functions[b]			Classification functions[b]			Classification functions[b]		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Experimental parameters[c]									
TP H_2O $_{(F2)}$	−2.67	−16.92	−32.68	4.13	0.46	−3.52			
TP HCO_3 $_{(F1)}$	−203.84	−258.58	−156.28	−166.29	−213.61	−107.40			
TP NAOH $_{(F2)}$	−58.69	−72.46	−81.10						
TP HCl $_{(F1)}$	75.65	95.55	123.67						
IP HCl $_{(F1)}$	−135.51	−199.05	−184.53	−42.04	−81.90	−40.57			
Total IP $_{(F1)}$	146.33	303.78	292.18	46.13	168.59	126.71	−79.41	−66.41	−43.76
PO H_2O $_{(F1)}$	−24.60	−24.02	−27.52	−28.96	−33.04	−39.77			
PO HCO_3 $_{(F2)}$	−1.08	−8.57	−51.60	27.83	28.60	−3.48			
PO HCl $_{(F2)}$	13.03	14.24	−25.06	14.49	20.39	−11.23			
Total PO $_{(F1)}$	−2.16	−0.30	98.19	−48.79	−60.49	17.06			
OP H_2O $_{(F2)}$	−5.39	−0.75	6.19						
OP HCO_3 $_{(F1)}$	31.73	41.88	28.84	30.68	40.78	24.91			
OP NAOH $_{(F2)}$	8.69	10.55	3.83	−7.77	−9.82	−18.42			
OP HCl $_{(F1)}$	−34.39	−43.25	−49.81						
Total OP $_{(F2)}$	29.29	71.63	83.10	−20.24	9.18	17.14	−34.21	−23.52	−20.55
Constant	−131.96	−134.51	−119.80	−96.87	−90.11	−68.53	−42.03	−27.30	−14.59

[a] Unstandardized canonical discriminant functions evaluated at group means.

[b] Fisher's linear discriminant functions.

[c] F: Function. Parameter classification in the factor structure matrix (Function 1 and Function 2). Classifications according to the pooled within-groups correlations between the discriminating variables and the standardized canonical discriminant functions.

two parameters did not enable the differentiation between medium and high P concentrations assigning only half of the cases correctly.

Inspection of the parameters included in Table 5 shows that the distribution of P to inorganic and organic plays the most important role in the determination of P burden in the sediment (backward DA mode). Based on the classification in the factor structure matrix a second group of parameters (forward DA mode) that affects IP and OP can be detected. Inorganic P seems to be affected by the distribution of TP-HCO_3, IP-HCl, and PO while OP by the distribution of TP-H_2O, OP-NaOH and PO. Further, a less significant third group of four remaining variables is evident from the standard DA mode assignations.

Compared to our previous attempt, employing only P fractions, the present approach offers several advantages. Firstly it enables a very clear discrimination between P level a task which could only partially be achieved when only P fractions were included in the model especially for the higher concentration levels (Groups 2 and 3). Secondly, this discrimination is based only on P species and fractions thus leaving out the geochemical characteristics of the sediment thus reducing the number of analysis and the overall experimental effort.

Since IP is considerably dominant over OP for point and non-point sources, it can be inferred that the proposed approach enables the spatial profiling of P based on the point and non-point sources of sedimentary P. To validity of this conclusion was further assessed with the aid of multi-dimensional scaling to the important variables of the DA.

The three-dimensional scatter plot from the first three ordination axes (Fig. 4) shows that the proposed procedure

Table 5 – Classification matrix for discriminant analysis of phosphorus burden in Louros river sediment

P concentration threshold	% Correct	Groups assigned by DA		
		Group 1	Group 2	Group 3
Standard DA mode				
Group 1	100	18	0	0
Group 2	100	0	6	0
Group 3	100	0	0	12
Total	100	18	6	12
Forward stepwise DA mode				
Group 1	100	18	0	0
Group 2	100	0	6	0
Group 3	100	0	0	12
Total	100	18	6	12
Backward stepwise DA mode				
Group 1	100	18	0	0
Group 2	50	0	3	3
Group 3	91	0	1	11
Total	88.8	18	4	14

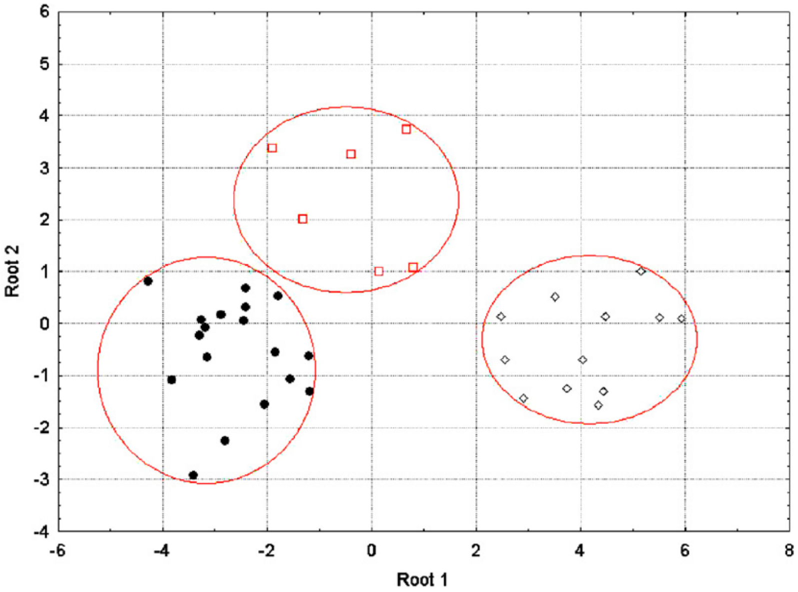

Fig. 3 – Canonical score graph of the first two discriminant functions for the post hoc classification of the data. Symbols: ● clean sediment ($<$0.6 mg/g), □ marginally polluted sediment ($=$0.6 mg/g) and ◇ P laden (polluted) sediment ($>$0.6 mg/g).

enables the differentiation of natural from non-natural inputs and their additional classification to point and non-point sources. According to the IP/OP ratio (Katsaounos et al., 2005), points 1 and 6 are influenced by natural inputs while points 2–5 by non-natural sources. As we can observe, points 2–5 form a separate group while points 1 and 6 are placed in the opposite sides of the graph. The latter trend is attributed to the different mechanisms controlling for P pattern but there are both of natural origin. In addition, points 2, 3 and 5 receive P from point sources (aquaculture and municipal effluent) while point 4 from non-point sources (agricultural run-off).

3.5. *Seasonal pattern of phosphorus speciation*

The temporal variation of P speciation as a function of its fractionation was examined with the aid of non-parametric analysis of variance by ranks (Kruskal–Wallis ANOVA) and the median test. More specifically, the Kruskal–Wallis ANOVA by ranks test was used to check whether the examined variables

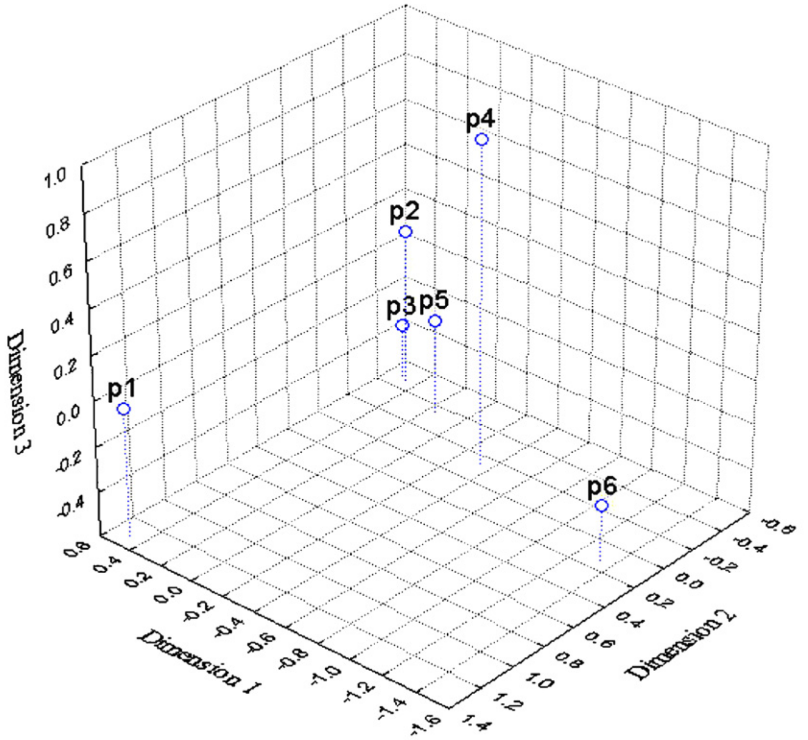

Fig. 4 – Three-dimensional scaling of sampling points as a function of sediment composition.

come from the same population (reflecting statistically significant differences among sampling trips). However, this test does not tell us how the samplings differed, only that they are different in some way. To characterize the kind of variation of each variable the median test was run in parallel. The combination of these methods has been shown to provide a more representative description of seasonal patterns in such complex data sets than DA while enable the indintification of temporal changes in a short-term basis. The results of this analysis tabulated in Table 6 reveal that IP controls the tempral patern of sediment P especially in the HCO_3 and HCl fractions which are dominated by IP. On the other hand, no OP species or fractions were found to exhibit seasonal variation throught the overall study period. This means that OP is accumulated in the bed sediment and although undergoes transformations from one form to antoher (as evidence by the interplay among the various fractions) it maintains a relatively uniform profile through the year. This is in agreement with the above discussion regarding the transformation of OP to recalcitrant forms that are accumulated into the bed sediment.

Samplings 1 and 6 (end of autumn) present the same trend with no significant fluctuation from the annual median value. In winter and summer months a decline in most P species and fractions is observed. During winter the increased flow reduces the deposition of particulate P or induce resuspension of fine sediment (House and Denison, 2002) that carries the highest P concentrations (Evans et al., 2004). In summer, increased P evasion is expected due to dissolution/desoprtion of particulate P into the soluble phase (Jarvie et al., 2002a) as a result of the reduction of dissolved phosphates in the water column to values below the Equilibrium Phosphate Concentration (EPC_0), a situation which known to induce mobilization of particulate P (House, 2003; Jarvie et al., 2005). Not surprisingly, the highest P accumulation was observed in the spring and at the beginning of the autumn due to the reduced flow that increases the sedimentation rate of the fine particles and to the intensification of the anthropogenic river-side activities, respectively.

4. Conclusions

The operational fractionation of P in river sediments was examined as means for indintiyfing the major spatial and tempral patterns associated with its presence in the sediments of Louros river. To accomplish a meaningfull data interpretation the speciation of P in each fraction was performed and a new speciation method was introduced that better described the distribution of P species among the fractions and in the overall sediment sample. Explanatory data analysis of the raw data matrix revealed that the appraoch introduced in this study offers significant advantages over mere fractionation analysis. In the first place, it enables a good interpretation of the observed behavior while offers the ability of discriminating among point/non-point and natural/non-natural P inputs simultaneously. Temporal changes in a short-term basis, were also determined and ascribed to specific species and fractions thus enabling the inditification of sources which contribute to the seasonal variation of P burden in the sediment.

Table 6 – Non-parametric identification of sediment component's variability in time

Sampling[a]	Flow	TP H_2O	TP HCO_3	TP HCl	IP HCO_3	IP HCl	OP H_2O	Org. matter	Sand	Silt/Clay	Al	PO	IP
S1 (Nov 2002)	+	=	−	=	=	+	+	−	−	−	−	+	=
S2 (Feb 2002)	+	−	−	−	−	−	−	−	+	+	+	−	−
S3 (May 2003)	−	−	+	+	+	+	−	+	+	+	+	+	+
S4 (June 2003)	−	=	−	−	−	−	=	+	−	+	−	−	−
S5 (Sept 2003)	−	+	+	=	+	=	+	+	−	+	−	−	+
S6 (Nov 2003)	+	=	=	−	=	+	+	−	+	−	+	+	=
Asymptotic Significance[b]	0.000	0.006	0.008	0.006	0.012	0.008	0.003	0.002	0.000	0.008	0.017	0.001	0.001

+: above annual median value.

−: below annual median value.

= : equal or about equal the annual median value.

[a] Classifications of values were performed with the Median test.

[b] Parameters with statistically significant effect are shown ($p<0.05$). Significance estimated from Kruskal–Wallis ANOVA by ranks test.

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