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A Dual Source of Phosphorus to Lake Sediments Indicated by Distribution, Content, and Speciation: Inle Lake (Southern Shan State, Myanmar)

Myat Mon Thin ¹, Elisa Sacchi ² , Massimo Setti ²  and Viviana Re ^{2,3,*} 

¹ Department of Physics, University of Mandalay, 73 Street, 05032 Mandalay, Myanmar; demomyatmonthin.edu@gmail.com

² Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 9, I-27100 Pavia, Italy; elisa.sacchi@unipv.it (E.S.); massimo.setti@unipv.it (M.S.)

³ Earth Sciences Department, University of Pisa, Via Santa Maria 53, 56126 Pisa, Italy

* Correspondence: viviana.re@unipi.it; Tel.: +39-050-2215812

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Abstract: In this study, grab and core sediments from Inle lake were collected and analysed for their water and organic matter (O.M.) contents. Total phosphorus (TP_{SMT}) and P fractions, namely inorganic-P (IP), organic-P (OP), P bound to Al, Fe and Mn oxy-hydroxides (Fe-P), and calcium-bound P (Ca-P) were determined by a sequential extraction procedure. TP_{SMT} varied considerably (152–1980 mg/kg), with minimum concentrations detected at sites away from the main inflow rivers and maximum concentrations in the floating gardens area. In core sediments, TP_{SMT} concentrations tended to decrease with depth, reaching values of <100 mg/kg. Concerning P forms, the overall abundance sequence in grab sediments was IP >> OP and Ca-P ≥ OP > Fe-P, whereas in core sediments it showed marked differences with depth and between sites. The relative abundance of the inorganic species (Ca-P, Fe-P) was controlled by the mineralogy of the sediments. While the TP_{SMT} distribution pointed to an increased anthropogenic input, the relative abundance of P species provided information on the P origin, incorporation processes, and evolution over time. This information, combined with chemical and mineralogical data, permitted to identify two different P sources: the agricultural input in the floating gardens area and the detrital input related to soil erosion.

Keywords: wetland; cultural eutrophication; sequential extractions; organic matter; endogenic Calcite; soil erosion

1. Introduction

Lake eutrophication is accelerated by nutrient pollution, one of the most widespread water quality problems worldwide [1]; it is estimated that it affects water quality in 28% of the lakes in Africa, 41% in South America, 48% in North America, 53% in Europe, and 54% in Asia [2]. The process of eutrophication can occur naturally over thousands of years [3]. Nevertheless, human activities have dramatically increased the level of eutrophication by discharging nutrients, such as phosphates and nitrates, into aquatic systems [4]. Phosphorus (P) is recognised not only as a primary nutrient for aquatic ecology but also as the most critical limiting nutrient for lake productivity [5]. Since its oversupply can lead to eutrophication of lakes [6,7], a reduction of the phosphorus content is usually essential to improve lake water quality and ecological state [3,8,9]. The main sources of phosphorus in lakes are external point and non-point sources, such as terrestrial runoff, rainfall, soil leaching, and industrial and municipal effluents. These are also indicated as the external load, to distinguish them from the internal load, corresponding to the release from sediments [3].

Sediments play an important role in the retention or release of P and have a major impact on the functioning of shallow lakes [10,11]. A number of different mechanisms might induce internal P release in shallow lakes, depending on a variety of physical, chemical, and biological factors [12,13]. In particular, sediments have been considered to play a significant role in the period following a reduction in external nutrient loading. They may serve as an important internal nutrient source until the pool of accumulated and releasable P has been buried deep and permanently in the sediment. Where the annual P retention is negative or considerably lower than predicted from empirical models [14], this internal load typically persists for about 10–15 years [15], although the duration is highly variable depending on the loading history and the capacity of the sediment to retain P [16].

Ecosystem studies on shallow lakes have identified two main alternative stable states: one is dominated by submerged macrophytes (clear-water state), and the other has a high algal biomass (turbid state) [17]. Authors have remarked that increased nutrient concentrations do not seem to have much effect on water transparency until they exceed a critical threshold, at which point the lake shifts abruptly from clear to turbid [18,19]. This critical nutrient level, which requires aquatic ecosystem modelling for its definition [20], depends on many factors such as lake size, depth, and climate and on whether any alternative stable states are present, characterised by different groups of primary producers [21]. Nevertheless, the identified shift between a clear and a turbid state is one of the most dramatic changes observed in shallow lakes subject to human-induced eutrophication. In this case, a positive feedback mechanism between phytoplankton production and P release acts to accelerate the transition into the alternative state [18,22,23].

To evaluate the extent to which P levels in lake sediments affect lake eutrophication, it is more efficient to consider the contents of different P fractions rather than total P content. Information about the different chemical P fractions in lake sediments is useful in understanding whether the sediment acts as an adsorber or a source of phosphorus. The association of phosphate with iron, aluminium, and calcium and the adsorptive properties of carbonates and clays are of special interest [24,25].

Wetlands are recognised as providers of ecological goods (e.g., water for irrigation and domestic use, fisheries, recreation) and services (e.g., carbon sequestration, flood control, groundwater recharge, nutrient and contaminant removal, biodiversity maintenance) but are threatened worldwide by rapid urbanisation, industrialisation, and agricultural intensification, resulting in a reduction of their areal extent and a decline in the functions they perform [26]. Inle lake (Southern Shan State, Myanmar) is a perfect example of an ecosystem that is suffering from environmental degradation due to the combined interaction of multiple stressors, such as changes in land use pattern, nutrient discharge from urban areas and agriculture fields, excess siltation from watershed erosion, and changes in microclimatic condition (e.g., temperature and rainfall patterns) [27,28]. In 2018, Inle lake was included in the list of wetlands of international importance as the fifth site of Myanmar (Ramsar convention; [29]), though it witnessed degradation in its water quality and lake status due to an increase in anthropogenic pressure and agricultural and industrial activities.

Concerning phosphorus as the main driver of eutrophication, previous studies mostly focused on the characterisation of Inle lake waters, providing relatively little information on the trophic status and on the environmental impact of the different anthropogenic activities. An assessment of surface lake water quality, conducted in 2004 [30], indicated that the concentration of $\text{PO}_4\text{-P}$ was relatively high (7.8 to 28.1 $\mu\text{g/L}$) and that phosphorus may derive from domestic and agricultural sources. This study concluded that the lake was eutrophic and suggested that more extensive studies be carried out due to the rapidly evolving socio-environmental conditions. Similar figures and conclusions were obtained by Lwin et al. [31], who found $\text{PO}_4\text{-P}$ concentrations of up to 69.3 $\mu\text{g/L}$ and identified some “hot spots” of contamination near the villages and in the floating gardens area. Mjelde et al. [32] conducted three extensive monitoring campaigns for water quality (at 14 lake stations, at the main tributaries and at the outflow) in different years and seasons, including phytoplankton and macrophyte assessments. The nutrient concentrations measured in the centre of the lake were generally low but increased close to the shores and in the inflow waters. Nevertheless, they indicated that most of the nutrients were

not available for phytoplankton growth, highlighting the role played by the large stands of aquatic macrophytes covering the lake bottom and suggesting that high amounts of phosphorus were likely to be stored in the sediments. Based on the nutrient concentrations, they concluded that Inle lake showed mesotrophic to semi-eutrophic conditions. Finally, in the frame of an integrated assessment of water resource quality on a catchment scale, Re et al. [33] analysed the P content of both surface and groundwater. Results indicated a low P content (median 15.6, average 17.1 µg/L) and a high N:P ratio, suggesting that P may be the limiting nutrient for this freshwater ecosystem. They attributed this low P concentration to endogenic Calcite precipitation, an effective process sequestering P in a mineral form and making it unavailable for the development of organic matter [34]. This internal mechanism of P removal, combined with the low water residence time, would prevent the accumulation of this nutrient in lake waters but would inevitably transfer it to sediments.

While there are only a few studies available that have addressed the P content in lake waters, there are even fewer that have fully characterised Inle lake sediments, with the exception of a small amount of mineralogical and isotopic data [35] and some pollen, charcoal, diatom, and ¹⁴C data from a very recent palynological study [36]. The aim of this study is therefore to fill this knowledge gap by analysing grab and core sediments to investigate the phosphorus content, speciation, distribution, and origin. The overall goal is to define the lake status, evaluate the role of sediments as a P source or sink, and highlight the danger represented by the changing environmental conditions and increasing anthropogenic impact, as a premise for the correct management of this valuable water body. Another paper [37] reports the mineralogical, morphological, and geochemical characterisation of the Inle lake sediments, allowing us to identify allogenic minerals and their associated chemical constituents, to describe the natural sedimentation patterns and to highlight the recent anthropogenic disturbances that may pose threats to the ecosystem and to the population.

2. Study Area

Inle Lake is located in Southern Shan State, Taungyi District, Nyaung Shwe Township (UTM 47Q 282178 N latitude, 282178 E longitude) at an elevation of about 884 m a.s.l. (Figure 1).

The basin has a humid subtropical climate with three seasons: March to June (summer), July to October (rainy), and November to February (winter). Average daily humidity ranges from 48% in March to 79% in the rainy season, and mean air temperature near the lake ranges from 22 °C to 31 °C [38]. Mean annual precipitation recorded from 2000 to 2013 is of 984 mm [39]. Rainy days are generally 80 per year, 70% of which occur during the southwest Asian monsoon season [36].

Inle lake is the second largest lake in Myanmar. Presently, the lake has an approximate length of 17.5 km and width of 5 km [28]. It is situated in a large, flat tectonic depression surrounded by mountain chains reaching 1200 m a.s.l. at their highest. The lake is fed by 30 streams, of which 17 come from the east and 12 from the west. The major inflow, from the north, is the Nanlit stream, which flows by the main town of Naung Shwe, located about 2 km N of the lake; the only outflow, to the south, is the Thanlwin River [40]. Other perennial inflows are the Kalaw and Indein streams, which are characterised by two remarkable birdfoot-like deltas ([41]; Figure 1), whereas the eastern lake side is steeper due to the proximity of the mountain range.

Inle lake has been classified as a shallow freshwater lake. Butkus et al. [27] report a depth that ranges from approximately 4 m in the hot dry season to 7 m in the rainy season. Nevertheless, in recent years, especially in 2010, the lake has experienced dramatic drops in the water level in summer [42]. A recent bathymetric survey indicated an average dry season open water depth of ~1 m in April 2015 and 2016 and an average rainy season depth of 2.2 and 2.5 m in October 2015 and 2016 and showed that the maximum lake depth (3.18 m) had halved since 1918 [28]. Su et al. [40] estimated a watershed area of 5612 km², a lake volume of 3.5×10^7 m³, an annual inflow water volume of 1.13×10^8 m³ year⁻¹, and a water residence of 0.32 years. More recently, Michalon et al. [28] quantified the entire lake flood zone (open water, floating gardens plus fringe wetland) as ~126 km² in the 2015 rainy season and ~94.4 km²

in the 2016 dry season, and, combining this information with bathymetric results, they estimated a lake volume of $122.6 \times 10^6 \text{ m}^3$ in October 2015 (highstand) and $50 \times 10^6 \text{ m}^3$ in May 2016 (lowstand).

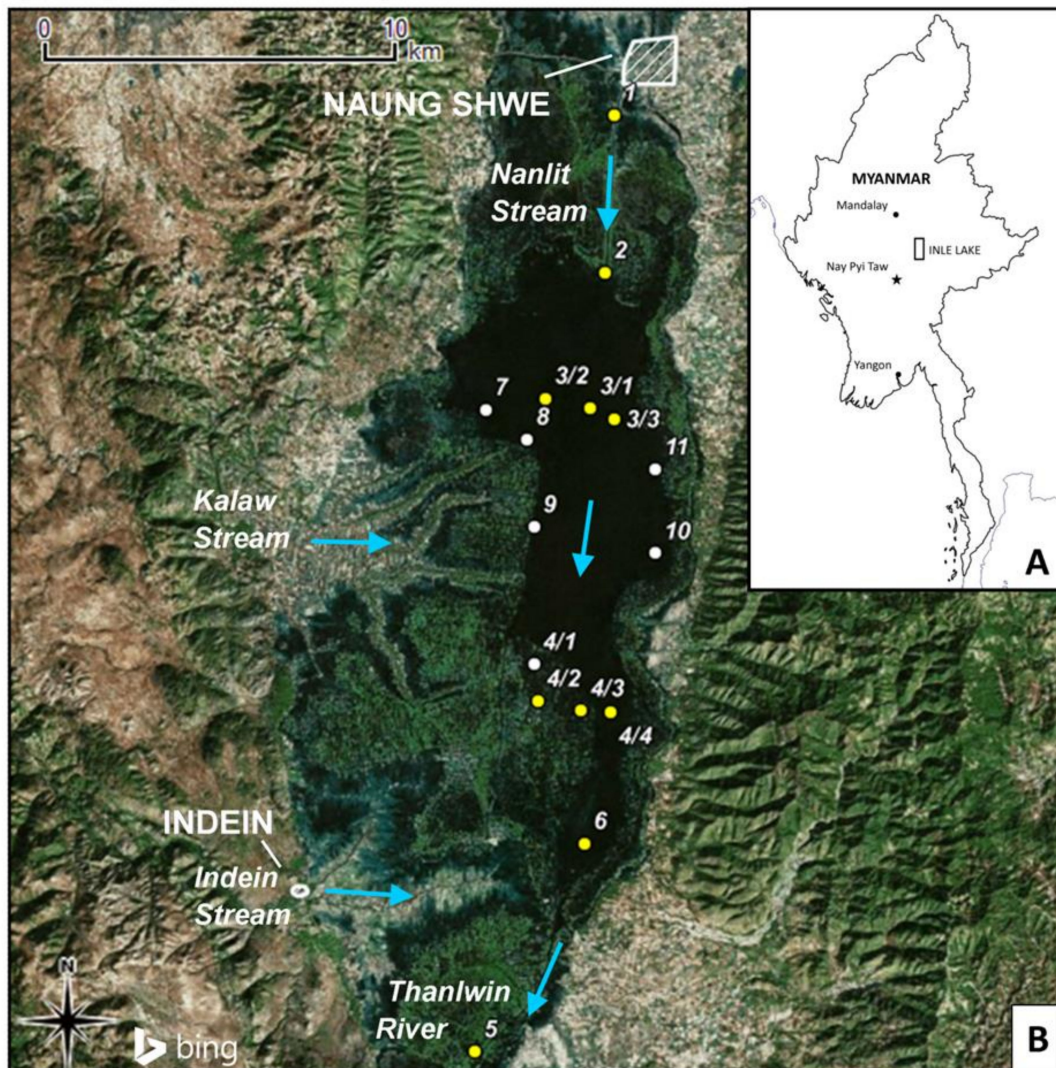


Figure 1. (A) Location of the study area in Myanmar; (B): location of the sediment samples (white dots = grab samples; yellow dots = grab and core samples). Blue arrows: surface water flow directions.

The land use pattern in the Inle Lake for 2003 reported about 38% (627 km^2) of the total area of 1647 km^2 under open water and only 24% (393 km^2) used for agriculture (floating gardens), the remaining 38% (627 km^2) being covered by floating vegetation, settlements and other forms of gardening and farming [43]. Concerning the catchment, a survey dated 2010 indicated that agricultural land occupies 42% of the area, forest with sparse canopies 16%, while other forms of land use and water surface areas account for 35%. Forest with dense canopies only remains on 7% of the catchment [43]. An analysis of land use and cover changes in the last 40 years indicates that the dominant landscape transformation processes were urbanisation (+203%), crop expansion (+34%) with a particular increase of floating gardens (+390%), land abandonment (+167%), deforestation (−49%), and wetland losses in marshlands (−83%) and water bodies (−16%) [44].

The basin is located in the Shan Plateau, which belongs to the Sibumasu block [45] and is mainly constituted by Permo-Triassic carbonates [46]. The catchment is located about 100 km E of the tectonically active Sagaing fault, an important continental transform fault between the India and Sunda plates [47]. In Myanmar, recent tectonic activity, and in particular the movement of the

eastern Himalayan syntaxis, condition the development of the hydrographic network [48]. Neotectonic studies [49,50] indicate that the lake catchment develops along a right-lateral strike-slip fault, parallel to the Sangaing fault. The Taunggyi normal fault borders the lake on the east side, causing the asymmetrical shape of the basin (Figure 1), and influencing the sediment transport and the development of the river deltas [49]. Moreover, due to the catchment topography, some areas are flooded during the monsoon period in response to the increase in the lake water level [28]. In the past, these floodable areas, mostly located to the N and W of the present-day open water surface (dark-green areas in Figure 1), were likely a swamp, and the straight course of the Nanlit stream, S of Naung Shwe, indicates that the northern sector is reclaimed land.

Anthropic activities on the lake sides and in its watershed are known to seriously threaten the Inle lake environment [27,30,33,40,41,44,51].

One of the main problems is the decrease in the open water surface, estimated to as much as 32.4% over a 65-year period (1935–2000) [41] and to about 23% in the period 1967–2014 [28]. This is partly due to an increase in sedimentation caused by deforestation in the watershed [40]. The resulting accelerated soil erosion mostly affects the western uplands characterised by unsustainable land use practices combined with a steep topography. The average soil loss rates are approximately quantified as 19.9, 10.1, and 26.2 t ha⁻¹ in the years 1989, 2000, and 2009, respectively [51]. Another reason for this shrinkage is the expansion of floating gardens, which are a typical agricultural system that makes the boundary between land and water remarkably unclear [28]. The system, locally known as *ye-chan*, involves the cultivation of hundreds of hectares of floating “islands” that produce enough tomatoes and other vegetables to drive the region’s economy [52]. Floating tomato gardens (a sort of hydroponic cultivation technique) used by the local residents are concentrated mostly in the western part of the lake. These gardens are created by using the dead parts of aquatic and marsh plants as a growing medium for planting fruits, flowers, and other vegetables. Once the fertility of these dead aquatic and marsh plants has been exhausted, they are transported to the perimeter of the lake, thus contributing to the apparent disappearance of the lake surface.

Another serious problem is the quality of the lake water. As in many other low-income countries, the population increase, with an exponential growth rate of approximately 2.3% per year [40], is not matched with adequate sanitation infrastructure. This anthropogenic pressure is expected to grow in the future because of the development of tourism in the area [53]. In addition, agrochemicals are widely used in floating gardens, especially for tomato cultivation [30], and the unregulated use of pesticides affects the transparency of the lake water, threatening the biological diversity of local flora and fauna [27,40]. More recently, Pradhan et al. [54] analysed the anthropogenic drivers of environmental change and comprehensively evaluated the state of the ecosystem. They confirmed a progressive decline in dissolved oxygen over the decade 2001–2011, related to high waste organic loading and anaerobic silt on the lake bottom, and reported that Inle lake was becoming shallower year by year because of sedimentation and deposition of dead biomass.

3. Materials and Methods

The field sampling was carried out in March 2014 (hot dry season). The sampling stations were selected on the basis of their position and environmental setting, to ensure full coverage of the lakebed area and proximity to potential sources of pollution (Figure 1, Table 1). Sixteen grab sediment samples were collected with a stainless-steel, Ponar type sampler, immediately placed in plastic containers, and tightly sealed to prevent oxidation. In addition, at selected locations, sediment cores, from 40 to 85 cm in length, were collected with a sampler prototype (handmade), which allows for careful recovery of the sediment–water interface [55] (Table 1). On the same day as collection, cores were cut into 5 cm long slices, wrapped with polythene foil to prevent oxidation, and put into a polyethylene container. All sampling tools were washed with lake water before the next sample was collected. The sediment samples were then transported to the laboratory at the Department of Earth and Environment Sciences,

University of Pavia, Italy. Due to their high organic matter content, grab samples were preserved frozen, while core sediment samples were stored in the dark in a refrigerator prior to analysis.

Table 1. Location, environmental setting, and potential pollution sources in proximity to the grab (G) and core (C) sediment samples reported in this study.

Site	Longitude (UTM)	Latitude (UTM)	Environmental Setting	Potential Nearby Pollution Sources	Samples Collected
1	47Q 0283427	2284690	Nanlit stream—inflow	Naung Shwe Township and motorboat circulation	G
2	47Q 0283122	2280515	Nanlit stream—lake entrance	Motorboat circulation	G, C
3/1	47Q 0282707	2276910	Inle lake—wide area—Centre	Detrital input from the Nanlit stream	G, C
3/2	47Q 0281508	2277202	Inle lake—wide area—West	None	G
3/3	47Q 0283305	2276640	Inle lake—wide area—East	None	G
4/1	47Q 0281104	2270131	Floating gardens area—Northern entrance	Floating gardens	G
4/2	47Q 0281190	2269152	Floating gardens area—Centre	Floating gardens and Kaylar village	G, C
4/3	47Q 0282356	2268878	Floating gardens area—Eastern entrance	Floating gardens	G, C
4/4	47Q 0283103	2268840	Inle lake—narrow area	Floating gardens	G
5	47Q 0279397	2259847	Lower Belu stream—outflow	Helon village	G
6	47Q 0282396	2265350	Inle lake—narrow area	None	G
7	47Q 0279908	2276921	Inle lake—Western bank	Floating gardens	G
8	47Q 0280995	2276119	Inle lake—Western bank	Floating gardens	G
9	47Q 0281155	2273786	Inle lake—Western bank	Floating gardens	G
10	47Q 0284373	2273034	Inle lake—Eastern bank	None	G
11	47Q 0284409	2275275	Inle lake—Eastern bank	Tourist resorts	G

In the laboratory, the sediment samples were dried at room temperature for 2–3 days. Large shells were eliminated by manual picking, and air-dried sediment was ground to a fine powder in an agate mortar. The full morphological, mineralogical, and geochemical characterisation of the sediment samples is reported elsewhere [37,56].

The water content was determined on 200 mg of air-dried sediment, heated at 105 °C for 2 h, cooled in a desiccator, and weighed again on an analytical balance. The organic matter (O.M.) content was subsequently determined by heating the oven-dried sediments at 450 °C for 3 h. This relatively low temperature of ignition can cause an underestimation of the total O.M., as the decomposition of the more thermally stable humified organic substances is only completed at 530–540 °C [57]. However, a lower temperature represents a compromise between a substantial, if not complete, destruction of O.M. and a limited mineral dewatering, which can be critical especially for clay-rich materials.

Total phosphorus content and P fractions were investigated by a sequential extraction procedure following the standards, measurements, and testing (SMT) protocol proposed by [58,59]. This procedure was selected for its simplicity and because results can be compared among sites, although some authors report that it may underestimate the NaOH-extractable P fraction due to partial resorption on carbonates [60]. The extracted solutions were analysed for their P content using the molybdenum blue method [61]. All reagents were analytical grade, and distilled water was used throughout to control the quality of analysis. All glassware was washed with hot diluted HCl and rinsed with distilled water before use. For the measurements, the spectrophotometer HACH DR 2800 was set at 880 nm, and cells with a light path of 20 mm were used [62].

In the SMT protocol, concentrated HCl-extractable, also indicated as total phosphorus (hereafter referred to as TP_{SMT}), was extracted from the sediment samples using 3.5 M HCl. The other P fractions considered in this study were the following:

1. Inorganic-P (hereafter referred to as IP), extracted using 1 M HCl;
2. Organic-P (hereafter referred to as OP), extracted on the same aliquot of IP, after calcination at 450 °C, using 1 M HCl;
3. NaOH-extractable P (also indicated as P bound to Al, Fe and Mn oxy-hydroxides, hereafter referred to as Fe-P), extracted using 1 M NaOH;
4. HCl-extractable P (also indicated as calcium-bound P, hereafter referred to as Ca-P), extracted on the same sample aliquot of the previous fraction, using 1 M HCl.

The concentration C , in mg/kg of P (dry weight) is derived from the equation:

$$C = (SV/(1000 \times m)) \times 10^3 \quad (1)$$

where S = P concentration in the extract in mg/L of P; V = volume of reagent used for extraction (20 mL), and m = mass of the test sample (200 mg dry weight).

Since it is assumed that

$$TP_{SMT} = IP + OP = (Fe-P + Ca-P) + OP \quad (2)$$

an internal control of the results was performed by comparing the sum of IP and OP with TP_{SMT} (mean error 15%), and the sum of Fe-P and Ca-P with IP (mean error 16%). Duplicates were analysed when the internal control indicated elevated errors (>30%) or when the results were close to the detection limit.

To further strengthen the interpretation of the P results obtained with the SMT protocol, they were compared with the results obtained by total digestion (TP_{TD}) and analysis with ICP-MS at ACTLABS Ltd. (Ancaster, ON, Canada) and with the results of the mineralogical composition of the sediments, determined by XRPD [63]. These data, available on a larger number of sites and sediment samples, are presented and discussed elsewhere [37,56]. To combine the P distribution patterns with the geochemical and mineralogical composition of the sediments, a Principal Components Analysis (PCA; e.g., [64]) was conducted using the SPSS software (version 16.0 for Windows®, SPSS Inc., Chicago, Ill., USA, [65]). The PCA was performed on 82 observations (i.e., all the grab and core sediment samples included in [63]) considering all the elements determined by TD and the mineral abundances (50 variables). Elemental concentrations below detection limits were substituted by a positive value of half the detection limit [66]. Factor loadings obtained from the PCA allowed us to identify the variables grouped in each factor. Furthermore, the factor scores of each sample were saved and further elaborated to classify the different sampling sites based on their affinity to the grouped variables.

4. Results

The water content of the collected lake sediments is generally low, ranging from 0% to 9%. However, there is a marked difference in O.M. contents at different sites, ranging from 5% to 60% (Table S1 in Supplementary Materials). The O.M. contents in sediment at sites 4/2 and 4/3 are higher than those at the other sites, which is likely to be due to the agricultural waste from the floating gardens (Figure 1).

In grab samples, TP_{SMT} varied considerably (Figure 2a), ranging from 152 to 1980 mg/kg, with the minimum concentrations detected at sites 3/2 and 11, located far from the main inflow rivers, and the maximum concentrations detected at sites 4/1, 4/3, and 4/2 in the floating gardens area. In core sediments, TP_{SMT} concentrations generally tend to decrease with depth (Figure 3), reaching values of <100 mg/kg at the bottom of the core at site 3/1. This trend is further confirmed by TP determined by total digestion data (TP_{TD}) available on a larger number of core samples [63]. The TP distribution

in the lake area and with depth in the sediment cores strongly suggests an increased input due to anthropogenic activities.

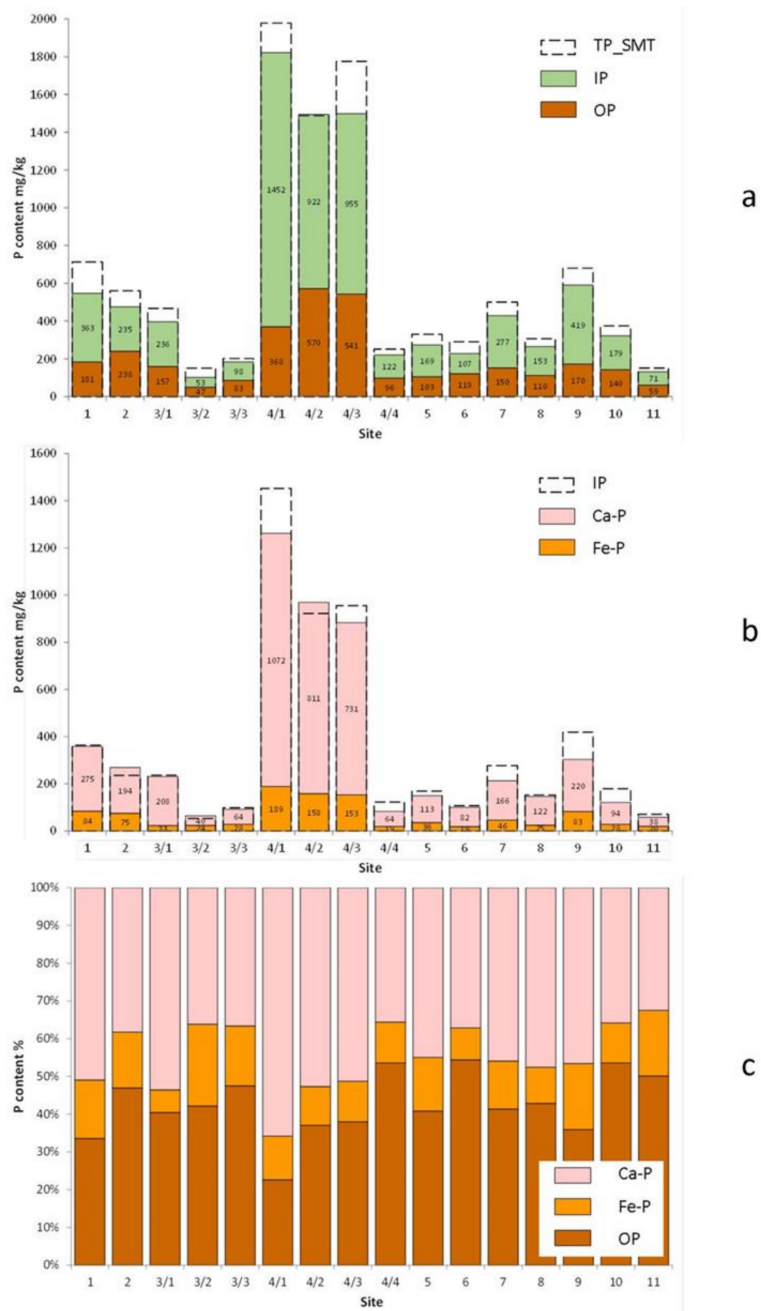


Figure 2. Total phosphorus (TP_{SMT}) and P forms determined from grab samples: (a) Inorganic-P (IP) and organic-P (OP), compared to TP_{SMT}; (b) calcium-bound P (Ca-P) and P bound to Al, Fe and Mn oxy-hydroxides (Fe-P) compared to IP; (c) percent contribution of OP, Fe-P and CaP.

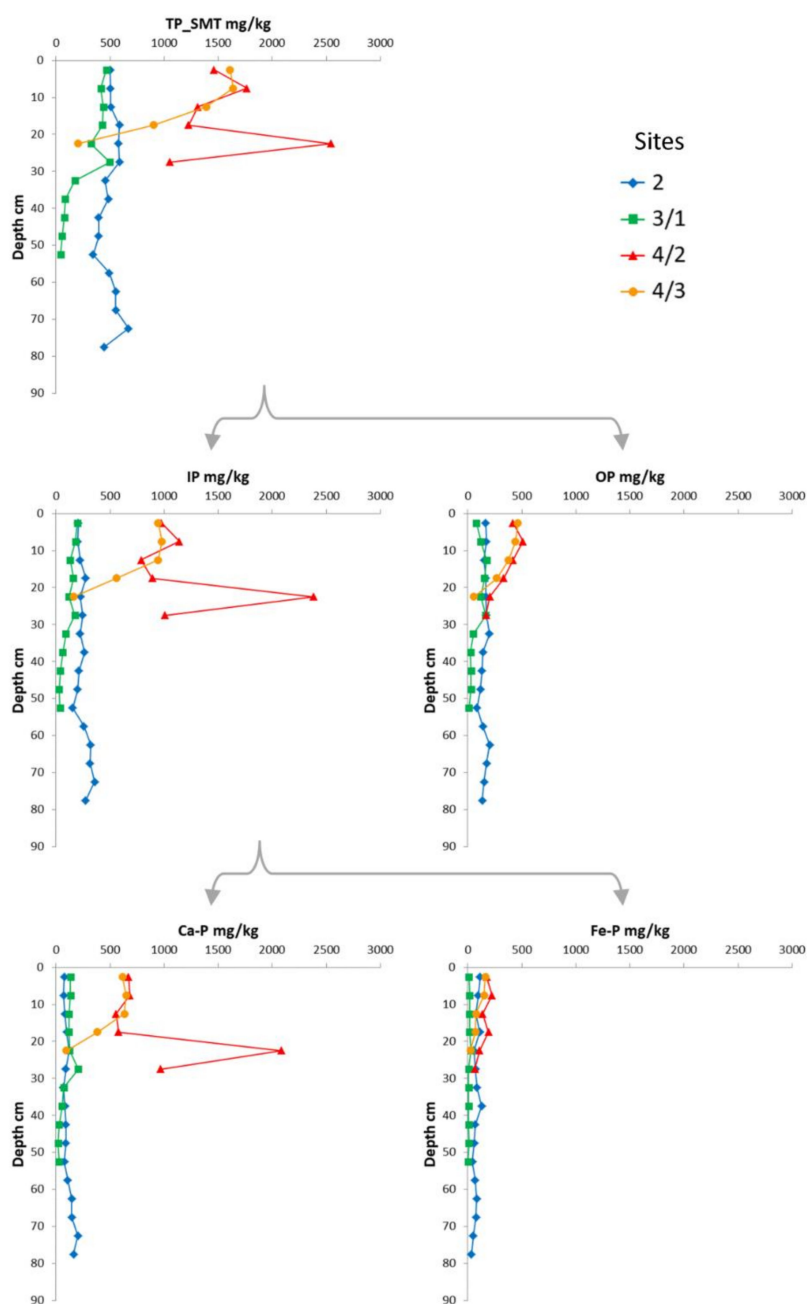


Figure 3. TP_{SMT} and P forms determined from core sediment samples and their variation with depth.

Concerning the P speciation, in grab sediments, OP and IP range from 47 to 570 mg/kg and from 53 to 1452 mg/kg, with an average concentration of 196 mg/kg and 363 mg/kg, respectively (Table S1). Within the Inorganic-P forms, Ca-P (range 38–1072 mg/kg) is dominant over Fe-P (range 19–189 mg/kg) (Figure 2b). The overall abundance sequence in grab sediments is generally IP >> OP and Ca-P ≥ OP > Fe-P. Therefore, assuming the sum of OP, Ca-P and Fe-P is equal to 100% P content of the sediment, these forms account for an average of 43%, 44%, and 13%, respectively (Figure 2c). In core sediments, all the P species tend to decrease with depth, as is observed for TP_{SMT} (Figure 3). Nevertheless, their relative abundance shows marked differences between sites. For example, the OP fraction accounts for more than 40% of TP_{SMT} at site 2, at the river inflow, and decreases to about 15% at the bottom of the core at site 4/2; Ca-P accounts for about 30% of TP_{SMT} at site 2 and reaches more than 80% at site 4/2; and Fe-P accounts for about 30% at site 2 and shows a mean value of about 10% at the other sites (Figure 4). These differences in absolute and relative concentrations for the P species

with the sample location and depth point to different P sources, incorporation, and evolution in time as preserved in the sediment record.

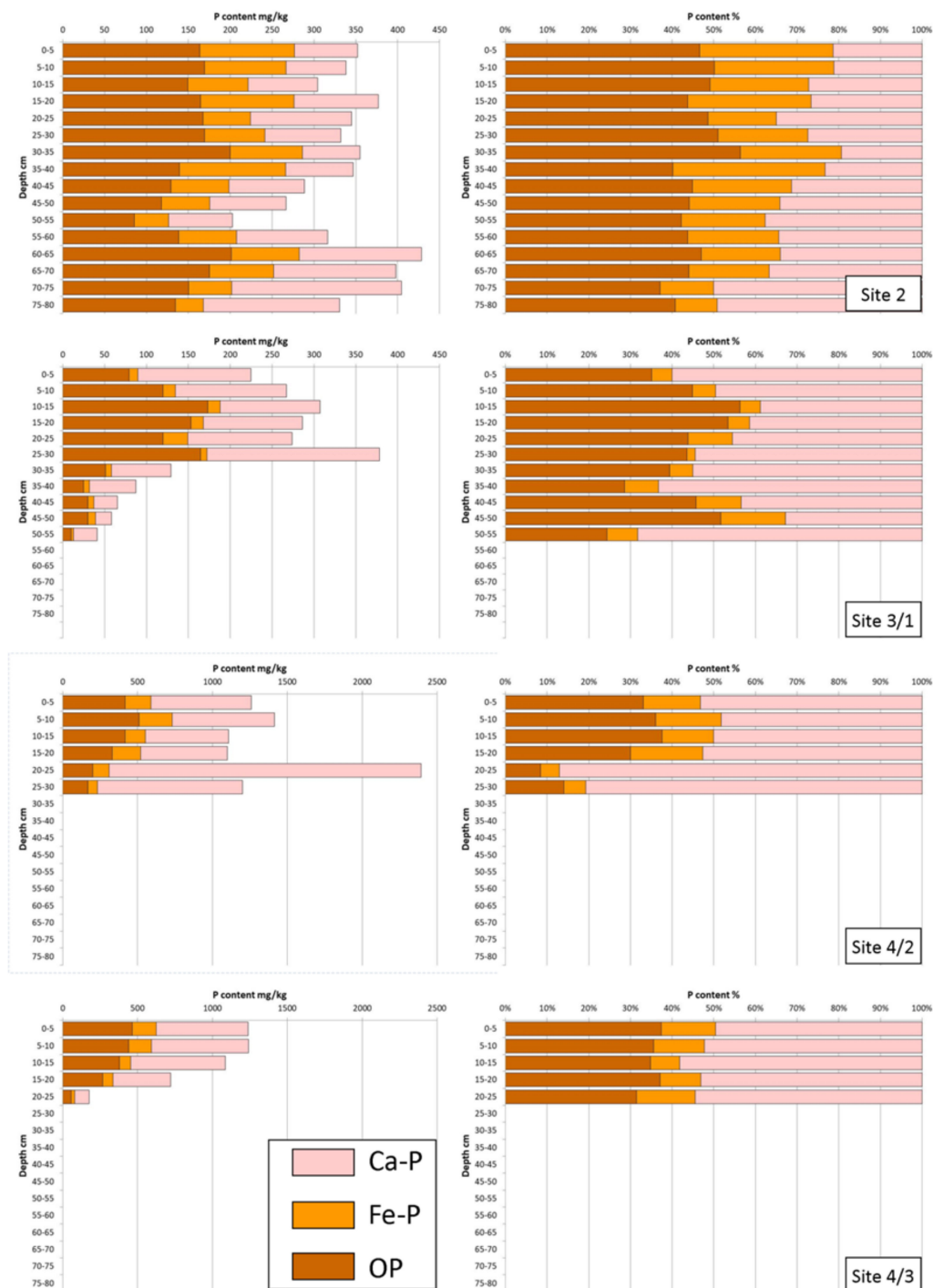


Figure 4. Concentration and contribution percentage of OP, Fe-P, and Ca-P in core sediment samples.

Concerning the PCA, the value of 1227×10^4 for the Bartlett chi-square statistic (1225 degrees of freedom and a minimum significance level of 0.000) applied for Bartlett’s sphericity test indicates the existence of a good correlation between the variables. Moreover, the measure of sampling adequacy (MSA) obtained by the Kayser–Meyer–Olkin (KMO) measure returns a rather high value (0.875), validating the PCA application. To reduce the overlap of the original variables over each factor, a

Varimax rotation was performed [67]. The PCA results are presented in Table S2 in Supplementary Materials, including the loadings, the eigenvalues, the amount of variance explained by each factor, and the cumulative variance.

5. Discussion

5.1. Identification of P Bearing Phases

The P forms determined using sequential extraction procedures are operationally defined and are related to specific reagents and procedures [60]. Nevertheless, the comparison with other parameters of the sediment (i.e., O.M. content, mineralogical composition, other chemical constituents) may allow for the derivation of the P related to a specific phase of sediment. This is of utmost importance since not all the P forms are likely to be released from the sediment and become bioavailable and are therefore likely to increase eutrophication.

Comparing TP determined with two different techniques (TP_{TD} and TP_{SMT}), a significant correlation between the two parameters is observed ($n = 50$; $r = 0.90$; $p \ll 0.01$). Nevertheless, TP_{TD} is always higher than TP_{SMT} , by about 30%, with a higher discrepancy for 6 grab sediments with high TP. If these data are eliminated, the correlation strongly improves ($n = 44$; $r = 0.99$; $p \ll 0.01$) and the discrepancy is reduced to about 7%. This correlation confirms that TP_{TD} and TP_{SMT} are both reliable and provide the same information and therefore can be used indifferently in the discussion.

A correlation matrix was calculated by taking into account the sediment data (water content, O.M. content, TP, P forms, Table S1) and the mineralogical composition (extracted from [63]). The results are presented in Table S3 in Supplementary Materials. It is interesting to note that the sediment data are all well correlated to each other. The most significant correlations ($p \ll 0.01$) are those between O.M. and water content, between TP_{SMT} and all the P forms, between OP and the water and O.M. contents, between IP and Ca-P, and between Fe-P and OP (Table S3). Furthermore, the mineralogical abundances are well correlated to each other, although this is partially expected since their content is expressed in % (these mineralogical correlations are discussed in detail in [37]). Little correlation is found between TP_{SMT} (and all P forms) and the mineralogical composition, with the exception of Hematite. This observation seems to confirm a possible organic origin for Hematite, rather than detrital, as discussed in [37].

More informative correlations are those observed between the relative abundance of the inorganic P forms (Fe-P or Ca-P) and the mineralogical data. Fe-P (%) is positively correlated ($p \ll 0.01$) with Kaolinite, Mica/Illite, and Quartz ($n = 54$, $r = 0.571$, $r = 0.556$, and $r = 0.427$, respectively) and negatively correlated ($p \ll 0.01$) with Calcite ($r = -0.516$) and Aragonite ($r = -0.387$). Accordingly, Ca-P (%) is positively correlated ($p \ll 0.01$) with Calcite ($r = 0.453$) and negatively correlated ($p \ll 0.01$) with Mica/Illite and Kaolinite ($r = -0.512$ and $r = -0.488$, respectively).

Kaolinite, Mica/Illite, and Quartz have been identified as allogenic phases, based on their morphology and occurrence in lake sediments. They are often present in coarse grains and aggregates, testifying to their origin from soil [37,56]. On the other hand, Calcite may have different origins, although morphological and hydrochemical data indicate that it is mostly related to endogenic precipitation [35,37].

The correlations observed between the sediment data and the absolute P content and forms (especially that between the O.M. and water content with OP) seem to confirm that the O.M. input may represent an important source of this nutrient to the system, coming both from soil erosion or due to agricultural activities. This finding is in agreement with [54], who suggested that the observed decline in dissolved oxygen in lake waters in recent years was related to the high waste organic loading and deposition of dead biomass, also contributing to the decrease in depth of the lake. In addition, our data suggest that, while the TP_{SMT} and the absolute P forms contents are determined by the location of the site in the lake area (i.e., to the proximity to the P source(s) to the lake), the relative abundance of the inorganic species is strongly controlled by the mineralogical composition of the particles, which in

turn is a function of the sediment depositional processes (i.e., whether detrital minerals or endogenic minerals dominate). As a consequence, we turned to the examination of the P distribution in the lake sediments.

5.2. Phosphorus Distribution in Lake Sediments

The spatial distribution map of TP_{SMT} was obtained by interpolating the results from grab sediments. Indeed, a comparison of these data with those of the top layers of the sediment cores (Table S1) shows a good agreement with the first 5–10 cm layers, and this is further confirmed by the comparison of the chemical and the mineralogical composition [37,63]. Such spatial interpolation, using the inverse squared distance method, provides the map shown in Figure 5.

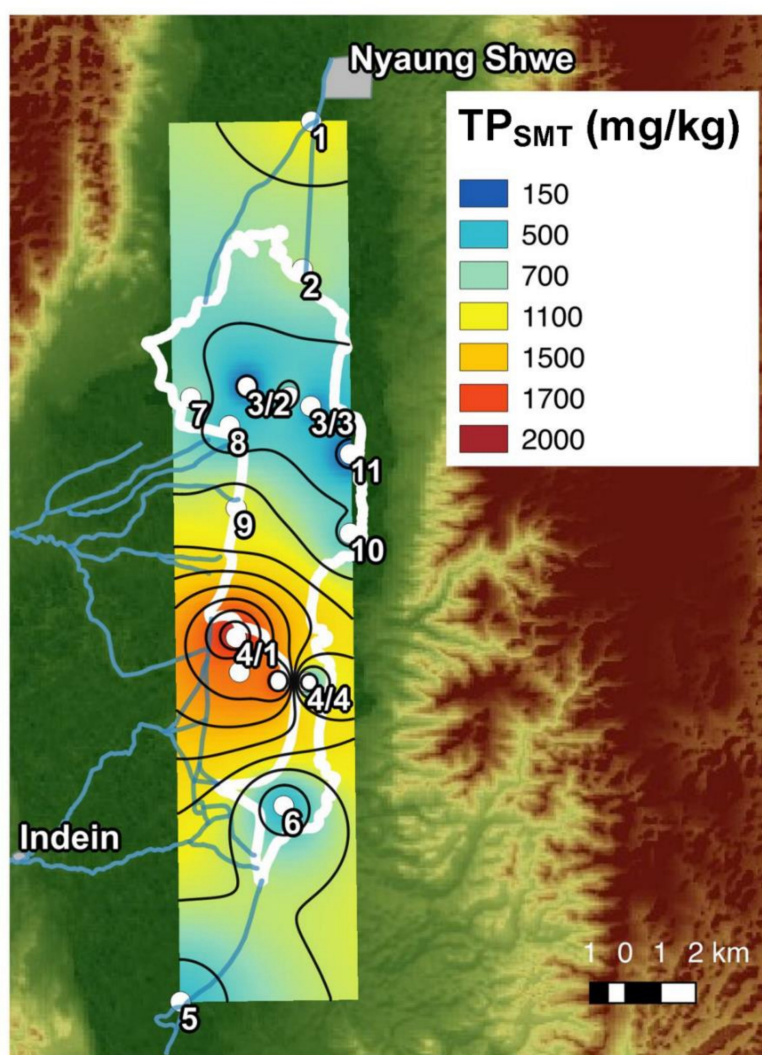


Figure 5. Spatial interpolation (inverse squared distance method) of TP_{SMT} data from grab sediments.

The highest contents of TP_{SMT} are found at sites 4/1, 4/3, and 4/2. These locations are within the floating gardens area: 4/1 and 4/3 are in the agricultural zone and 4/2 is located in the middle of the Kaylar village. At the first two locations, evidence suggests that the high P content is related to the use of synthetic fertilisers for cultivation. Indeed, the dominant P form here is IP and more specifically Ca-P, accounting for more than 50% (and up to 65%) of TP_{SMT} . In addition, Thin et al. [56] was able to detect Apatite grains in the sediments of these sites. Furthermore, site 4/4, located in proximity of 4/3 but towards the centre of the lake, where the floating gardens are absent, shows a much lower TP_{SMT} concentration. A similar agricultural origin for TP_{SMT} can be envisaged for site 9, also located

in proximity to cultivated areas, but with much lower concentrations. On the other hand, site 4/2 may also receive a contribution from civil sources (such as domestic and sewage effluents).

High TP_{SMT} concentrations are also detected at sites 1 and 2. These represent the sediment transported by the Nanlit stream and the lake sediment at the stream inflow into the lake, respectively. This allows the attribution of this TP_{SMT} to the detrital input contributed by the watershed, through soil erosion and transport by surface waters. It should be noted that at site 2, the second highest concentrations of OP and Fe-P were detected, following the highest concentrations at site 4. During soil erosion, orthophosphates can be leached out of the agricultural soils and adsorbed directly on the stream sediments or on suspended particles that later sediment in the stream. This adsorption is partly dependent on the availability of oxy-hydroxides of Al and Fe in the suspended particles [68] (Singh et al., 2005). Macroscopic and microscopic observations of the sediment particles reported by [56] and [37] highlight the presence of coarse grains and aggregates (often reddish brown), which are typical of tropical red soils, and a larger proportion of detrital minerals in these samples [63]. The influence of the detrital P input is likely to extend towards the centre of the lake, reaching site 3/1, which displays a distinctively higher TP_{SMT} than sites 3/2 and 3/3. Indeed, the Nanlit stream course, while crossing the northern part of the Inle lake basin, appears to have been rectified (Figure 1) and is periodically dredged to facilitate motorboat circulation [28]. The large input of sediments carried by the stream can also be observed in satellite images, where an important fan extends from the Nanlit entrance to the lake to more than 2 km south [35]. It is interesting to note that the TP_{SMT} distribution map for the lakebed area (Figure 5) compares nicely with the map of total P in surface waters reported by [33]. A significant ($p < 0.01$) correlation exists between the two parameters ($n = 13$, $r = 0.76$), despite the fact that sediments were sampled in summer 2014 and waters in winter 2015, and the same contamination hotspots are identified, corresponding to the Nanlit stream (site 1), its entrance to the lake (site 2), and the floating gardens area (sites 4/1, 4/2, and 4/3). Therefore, the sediments closely reflect the P content of surface waters and can be used to identify the areas of higher anthropogenic impact. Additionally, the correlation suggests that the sediments are presently acting as a sink for the P input to the lake.

Additional support to the identification of the sources is provided by the P distribution with depth in the sediment cores (Figure 3), which, as already described, is characterised by high concentrations in the top layers and low concentrations in the bottom layers. This is particularly observed in the cores collected at sites 4/3 and 3/1, where the lower bottom values could be representative of the natural P background before the anthropogenic disturbances. This observation is further confirmed by the analyses of TP_{TD} [63], also decreasing with depth and reaching the low values that characterise some uncontaminated sites located away from P sources (e.g., sites 3/2, 10, and 6). At site 2, the TP_{SMT} distribution does not significantly change with depth, which is likely to be because the sediment affected by the allogenic inputs by the inflow stream is very thick, and background values are not reached at depth (see also the discussion in [37]). This distribution of TP and P forms with depth confirms the anthropogenic disturbance of the P cycle at Inle lake.

5.3. Characterisation of the Anthropogenic Phosphorus Inputs

The discussion above suggests that the lowest TP_{SMT} values recorded in the bottom layers of core 3/1 and core 4/2 may represent the natural background concentrations and relative proportion of P before the advent of anthropogenic disturbances. If this is correct, an indication of anthropogenic P content and forms delivered to the lake can be obtained by subtracting these background values from the other sediment layers.

Due to the differences in the environmental setting of the lake area, we considered the bottom layers of core 3/1 as representative of the background value of TP_{SMT} and of the P forms for core 3/1 and core 2. Therefore, we subtracted these values from those of each layer to obtain the results shown in Figure 6. In core 2, the anthropogenic input is dominantly in the form of OP, accounting for more than 50% of the P forms, followed by Fe-P (between 25% and 10% from top to bottom) and Ca-P (between 15% and 45% from top to bottom). On the other hand, in the top layer of core 3/1, the dominant form is

still that of OP (around 45%), but the relative proportion of Fe-P is much lower (generally less than 10%) in favour of Ca-P, which accounts for about 50% of the anthropogenic P forms. The same calculation can be performed for cores 4/2 and 4/3, using the value displayed by the bottom layer of core 4/3 as a background value. In this case, the Ca-P form is the dominant one of the anthropogenic P forms (generally more than 50% but reaching up to 90%), followed by OP (around 35%) and Fe-P (10–15%).

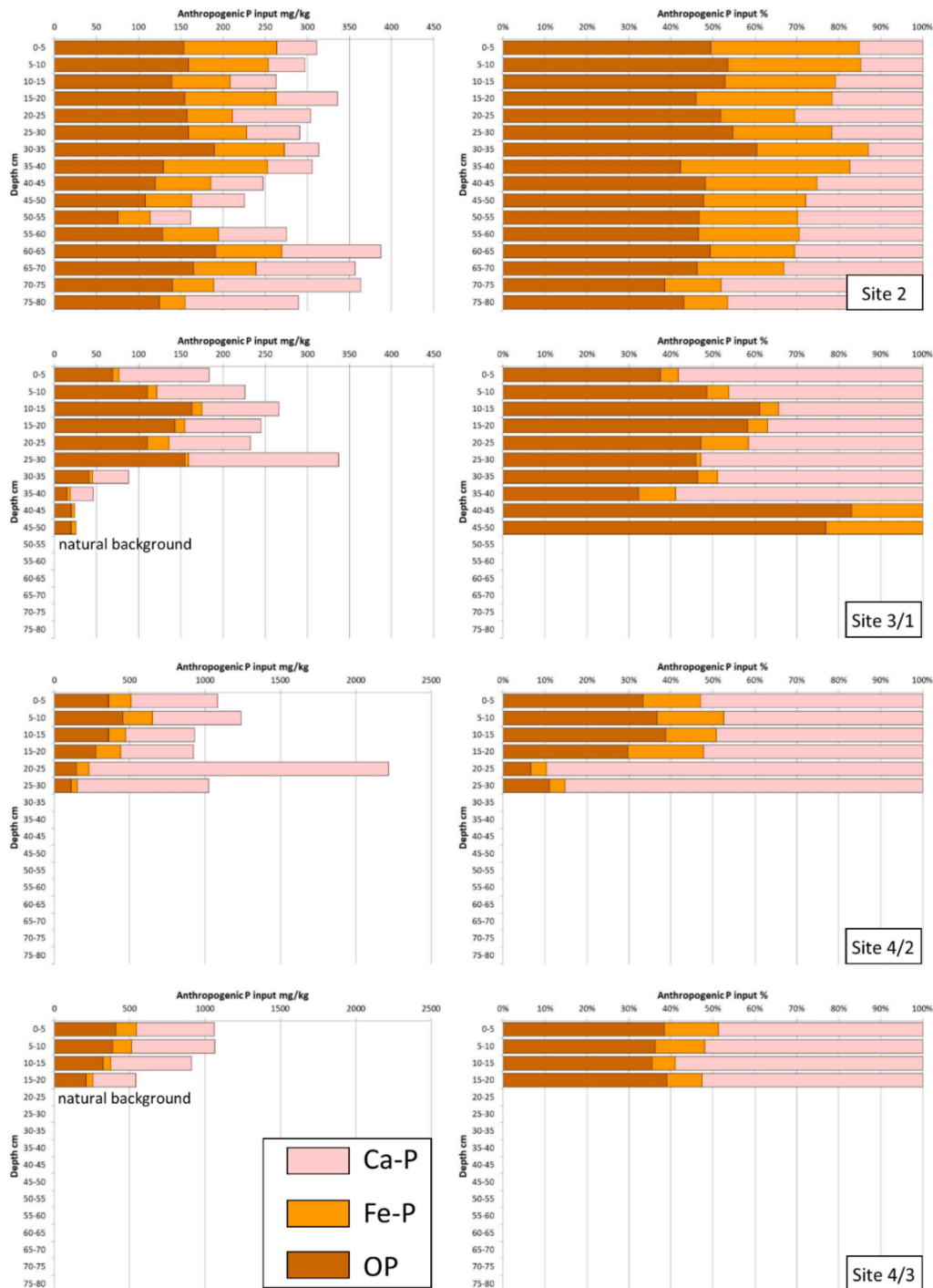


Figure 6. TP_{SMT} and P forms of the anthropogenic input in core samples, calculated by assuming that the bottom layer of core 3/1 represents the natural background concentration for cores 2 and 3/1, and core 4/3 bottom layer represents the natural background concentration for cores 4/2 and 4/3.

This information is crucial to understand the ability of the sediments to store or release P. Indeed, it is generally acknowledged that Ca-P, related to the presence of Apatite and Apatite-like minerals (including carbonates), is a relatively stable and non-bioavailable phosphorus form. Calcite is naturally present in the sediments of Inle lake, as it derives from the calcareous rock that is present in the watershed [46] and is precipitated from lake waters both as endogenic and as authigenic Calcite [33,35,37]. As a consequence, the high Ca-P contents detected in the floating gardens area should have little repercussions on the trophic state of the waters. Furthermore, due to the alkaline character of the Inle lake waters, it is likely that part of the P carried by the rivers or released by the sediments during O.M. degradation may be immobilised in endogenic or authigenic Calcite. This could be the case of the top layers of the core collected at site 3/1, where Ca-P is the most abundant P form.

On the other hand, OP can easily be released by the sediments during O.M. degradation. In this regard, the high percentage of the anthropogenic OP form, detected both at the Nanlit river inflow and in the floating gardens area, should be considered alarming. In the former case, the sources of OP are likely to be related to soil erosion and civil input from the city of Nyang Shwe; in the latter case, OP is derived from the dead parts of the cultivations. This conclusion is in agreement with the findings of [54], indicating a strong input of O.M. in the lake area and suggesting that its degradation may be at the origin of the decrease in DO registered in lake waters. The potential establishment of anoxic conditions is extremely worrying, as these could affect the Fe-P contents. Indeed, the reduction of Fe oxy-hydroxides in the sediment is generally acknowledged as the main mechanism by which P can be released from the sediments and can constitute the internal P load in eutrophic lakes [24,69]. In the case of Inle lake, it seems that oxygenated conditions at present are maintained in the water column throughout the year, as a combined effect of the low depth and fast circulation [33]. Nevertheless, the input of O.M. to the lake should be carefully monitored, and action should be taken to prevent shifting to anoxic conditions that could trigger a positive feedback mechanism of P release from the sediments [23].

5.4. Characterisation of the Dual Phosphorus Sources

As previously discussed, the spatial variation in P content with different sites can be ascribed to a set of differences in the natural setting and in anthropogenic impacts, such as agricultural practices (application of chemical fertilisers and pesticides) and soil erosion in the lake catchment areas. If so, not only P but also other elements should reflect these differences. The elaboration of the chemical and mineralogical data with the PCA may provide additional support to the interpretation.

The PCA analysis provided six principal factors with eigenvalues greater than 1, which explain a high percentage (around 90%) of the total variance in all samples (Table S2) and allow for the grouping of the variables in a geochemically reasonable and interpretable manner.

- Factor 1 (F1), accounting for 60% of the total variance, is composed of the high positive loading for silicates (Kaolinite, Mica/Illite, and Quartz) and numerous elements (Ba, Be, Li, Na, K, Rb, Cs, Al, Fe, Mn, Co, Cr, Cu, Ni, Pb, V, Zn, Tl, Bi, Sn, As, Ag, Sc, Ti, Y, Zr, La, Ce, Hf, and Th). This factor reflects the binding of these elements to allogenic minerals. It is also characterised by the high negative correlation with Calcite content. The strongly bipolar character of the sediment mineralogy reflects the antithesis between detrital input and endogenic Calcite precipitation (see also the discussion in [37]). High factor scores for F1 are observed at or near inflow water (sites 1 and 2), indicating a detrital origin of these elements and minerals contributed to the lake by the erosion of the rocks and soil in the watershed.
- Factor 2 (F2) resulting from the analysis explains 10% of the total variance and is mostly composed of the positive relation of P, U, Cd, Mo, and Sb. This factor can be considered as the “P pollution factor” due to the association of this element to other trace elements that are commonly found in phosphate fertilisers [70–72]. High scores of F2 are recorded at sites 4/1, 4/2, and 4/3, in the floating gardens area, further supporting this interpretation.

- Factor 3 (F3), accounting for 8% of the total variance, is associated with the positive loadings for W, Nb, and Ta and the negative loading for Dolomite. These elements are classified as High Field Strength Elements (HFSE) and have similar geochemical behaviour. This factor is not clearly attributable to a natural or an anthropogenic process. Dolomite displays a low communality value (0.300), indicating that the factors do not adequately explain its variance. The attribution to this factor is uncertain, partly due to the relatively low number of samples showing the presence of this mineral [63].
- Factor 4 (F4), accounting for 6% of the total variance, is characterised by the negative loading for Ca, Mg, and Sr. This factor can be assumed as the carbonate pole as indicated by the excellent correlation of F4 with Mg and Sr, while the lower correlation with Ca could be due to the fact that concentrations of these elements above 50% could not be adequately assessed with the analytical method used.
- Factor 5 (F5), accounting for 4% of the total variance, is associated with the negative loading for Aragonite and Hematite and may be related to authigenic minerals of a biogenic origin [37].
- Factor 6 (F6), accounting for 2% of the total variance, is composed of Au and S. This factor cannot provide significant information as the two elements are only present in very small amounts which are close to the detecting limit.

Overall, F1 + F2 explain more than 70% of the cumulative variance of the dataset. Concerning the dual origin of P, a clear distinction can be observed between sites by plotting TP_{TD} with respect to F1 scores (Figure 7). Two different trends of increasing P concentrations are observed. The first one (displayed by samples from sites 1 and 2), which also corresponds to an increase in F1 values, highlights the presence of a detrital input enriched in both major and trace elements in areas close to the inflow of major rivers and can be attributed to soil erosion. The second trend (displayed by samples from sites 4/1, 4/2, and 4/3), which is not associated to an increase in F1 values, is related to the impact of human induced pollution, mainly associated with agricultural activities in the floating gardens area and with civil sources of P. In this interpretation, site 4/1, displaying high P content but also a relatively high F1 score, is impacted by both the detrital inputs from the stream situated on the western side of the lake and by agricultural activities. It is worth noting that this conclusion, fully in line with that obtained with TP_{SMT} and the P forms contents, is derived from the elaboration of an independent dataset [63], further supporting the interpretation.

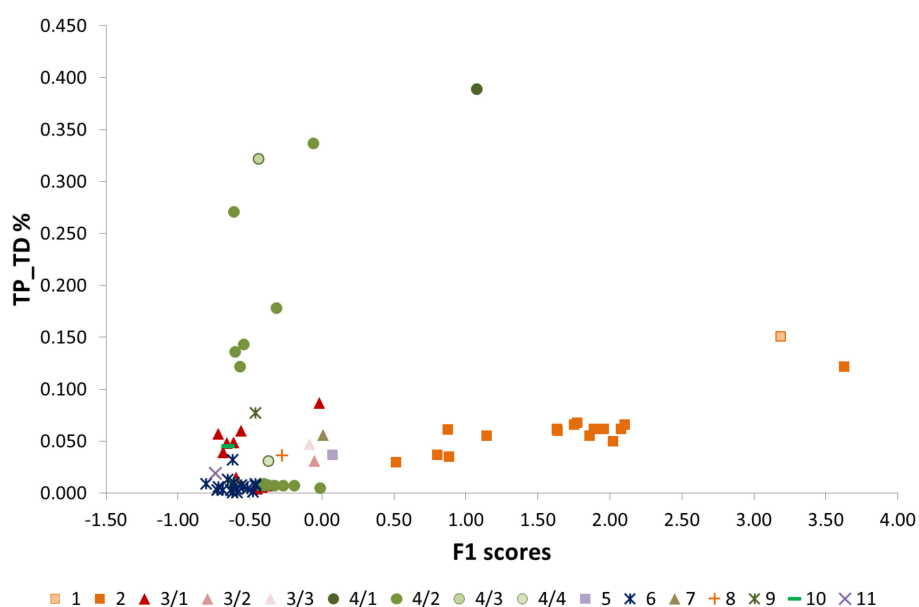


Figure 7. TP_{TD} versus F1 scores for all the sediment samples of this study, distinguished by site.

6. Conclusions

In this study, Inle lake sediments (both grab and core samples) were analysed for their water and O.M. contents, for their total phosphorus concentration, and P fractions, the latter determined by a sequential extraction procedure (SMT protocol).

The TP_{SMT} distribution in the lakebed area and with depth in the sediment cores (i.e., over time) pointed to an increased input due to anthropogenic activities. The main P contamination hotspots corresponded to the floating gardens area, the Nanlit stream, and its entrance to the lake. Concerning P forms, the overall abundance sequence in grab sediments was generally IP>>OP and Ca-P≥OP>Fe-P. In core sediments, the relative abundance of P species showed marked differences with depth and between sites, suggesting that information on different P sources, incorporation processes, and evolution over time was preserved in the sediment record. In addition, the relative abundance of the inorganic species (Ca-P, Fe-P) was strongly controlled by the mineralogical composition of the particles, which in turn was a function of the relative abundance of detrital and endogenic minerals.

Results allowed us to identify two distinct P sources. The first in terms of concentration is the anthropogenic pollution, mainly associated with agricultural activities in the floating gardens area. Nevertheless, the dominant anthropogenic P form identified here was Ca-P, a relatively stable and non-bioavailable form. In addition, the correlation between TP_{SMT} and the P content in surface waters suggests that the sediments are presently acting as a sink for the P input to the lake. Indeed, due to the alkaline character of Inle lake waters, part of the P carried by the rivers or released by the sediments during the O.M. degradation is trapped in endogenic or authigenic Calcite. The second source is the detrital input, enriched in both major (Al, Fe, Mn) and trace elements, mostly affecting the areas close to the inflow of major rivers and related to soil erosion. In this case, the dominant anthropogenic P form was OP, which is more labile and can easily be released during O.M. degradation. Moreover, this process could lead to the establishment of anoxic conditions in the future, which may promote the reduction of Fe oxy-hydroxides and trigger a positive feedback mechanism, further increasing the internal P load. At present, oxygenated conditions are maintained in the water column throughout the year, as a combined effect of the low depth and fast circulation [33]. Nevertheless, the changing climatic conditions and the anthropogenic disturbances are affecting sedimentation and are progressively reducing the open water surface and the depth of this shallow lake [37]. Therefore, action is needed to limit the O.M. input to the lake, to prevent it from shifting to anoxic conditions that would accelerate the transition towards a eutrophic state.

In conclusion, the characterisation of P content and forms in the sediments favoured a better understanding of the sources, the dynamics, and the potential threats posed by this key nutrient. This study represents a replicable approach for assessing the potential role of sediments as a P source for tropical alkaline lakes subject to increasing anthropogenic pressure and climate change. Moreover, the results of our study are fundamental in implementing an effective management strategy for a sustainable economic development of the area that preserves the ecological functions of Inle Lake, in line with the objective set by the Ramsar Convention for wetlands of international relevance.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/12/7/1993/s1>. Table S1: Water, Organic Matter, TP, and relative forms extracted from grab and core sediments from Inle lake. Table S2: Results of the Factor Analysis performed on the chemical and mineralogical data from grab and core sediments from Inle lake. Table S3: Correlation matrix of the sediment data (water content, O.M. content, TP, P forms, Table S1) and the mineralogical data extracted from Thin et al. [63]. Cc = Calcite; Qz = Quartz; M/I = Mica/Illite; Kaol = Kaolinite; Ar = Aragonite; Hem = Hematite; Dol = Dolomite. In red, correlations with $p < 0.01$; in blue, correlations with $p < 0.01$ and $r \geq 0.8$.

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