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## Sediment Organic Phosphorus Fractionation and Spatial Variation in Langat River, Malaysia

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### ABSTRACT

Langat River in Malaysia is an important river with active development on its basin. Excess nutrients such as organic phosphorus introduced through these developments can change the trophic level of the river and by using simple and cost-effective methods, preliminary information on the status of organic phosphorus can aid in the understanding of the complex biogeochemical cycling of phosphorus in rivers. Water and sediment samples were collected from 10 points along the river. The proportion of organic phosphorus was quantified based on the fractions through sequential fractionation. The results indicate that the downstream section of the river has a high percentage of organic phosphorus (48.22%) compared to the upstream and midstream combined. The major fraction in all sites was the non-labile fraction comprising of 60–78% of the total organic phosphorus extracted. Humic acid derived from organic matter either autochthonous or allochthonous sorb organic phosphorus into the sediments and stays internal loading in the river. Moderately labile fulvic acid-bound organic phosphorus has lesser contribution in the sediments but may play an important role in increasing the dissolved phosphorus levels in the waters. Labile forms also contribute lesser in the sediments except when the river is closest to the sea due to higher sedimentation rate facilitated by tidal action. Organic phosphorus in the sediments of Langat River currently may not pose an immediate threat but presents a future risk to the ecosystem considering the river status in accumulating organic phosphorus.

### INTRODUCTION

In aquatic ecosystems, phosphorus (P) is an important but limiting nutrient. Excess P, especially in bioavailable forms, can lead to serious environmental problems like algal bloom and eutrophication, a consequence that does not exist in terrestrial ecosystems [1]. Rivers are complex systems and the important abiotic factors that control them are water and sediments. Particulate and dissolved P are found in the water, but the sediments can act as a sink or source of P depending on the present status [2]. Excess P introduced to rivers from runoffs, point-source human activities and the atmosphere are often removed from the water column by sediment sorption. However, this can be released again through changes in the physicochemical conditions and sediment resuspension [3]. Quantifying P fixed in sediments should then be a priority as the effect of the accumulated nutrient is of ecological significance.

Advanced techniques such as <sup>31</sup>P-NMR are costly to run and require specialist knowledge for interpretation. Due to limited access by some laboratories to sophisticated equipment like <sup>31</sup>P-Nuclear Magnetic Resonance (P-NMR) and X-ray Absorption Near End Structure spectroscopy (XANES) in identifying organic phosphorus species [4], fractionation methods are commonly used as an alternative. Although interpretations based on fractionation results are operationally defined and therefore not comparable between fractionation methods of choice, they still provide valuable information on the status of phosphorus pollution in aquatic environments [5-8].

Sequential fractionation is a method used commonly in agriculture to separate the forms of phosphorus available in the soil for crops. Using fractionation, the frequency of fertilizer applications can be estimated to ensure successful harvests. This is because P tends to get fixed in the soil due to the chemical associations with iron and aluminium oxides and can persist in

that form for years [9]. Applying this technique to organic phosphorus ( $P_o$ ) in river sediments can be informative by elucidating the composition of  $P_o$  present as it has been linked to P pollution status leading to eutrophication [10].

In the past, fractionation schemes that were used for sediments tended to pool organic phosphorus into one fraction and were often considered refractory or not bioavailable [4]. However, phosphorus dynamics in aquatic systems are complex and can interchange from one form to another through processes like mineralization and therefore require techniques that can elucidate the forms present in them [11]. Therefore, using a  $P_o$ -specific chemical fractionation method that is capable of differentiating between the various types of organic phosphorus present in Langat River can divulge more information on the complexity of organic phosphorus in aquatic systems while serving as a reference point for future temporal and spatial analysis of the river. To date, this is the first description of the organic phosphorus fractions in the sediments of Langat River. Thus, the objectives of this study were to identify the proportion of organic phosphorus fractions present in the sediment using sequential fractionation and lay the groundwork for future studies, recognizing possible sources that contribute to its distribution and discuss the potential environmental issues that may arise from excessive organic phosphorus in aquatic ecosystems.

## Materials and method

Langat River was selected as the study site due to its importance as well as the increased development that has been noted in recent years that has led to many pronounced land use changes at the basin [12]. 10 sampling sites were selected along Langat River ranging from upstream to downstream (**Fig. 1**). Sampling was conducted from Nov 2022 to Jun 2023. At each sampling station, water samples were collected using a horizontal water sampler while the sediments, approximately 5 – 10 cm depth, were either collected using a bucket in shallow waters (depth < 1 m) or an Ekman Grab sampler in deeper waters (depth  $\geq$  1 m). Both sediment and water samples were transported on ice before processing in the laboratory.



**Fig. 1.** Top map detailing the sampling sites used in this study along Langat River. Bottom map shows the geographic location of Langat River in the context of Peninsular Malaysia.

## Dissolved organic phosphate determination

Dissolved organic phosphorus concentration can only be determined after values for dissolved inorganic phosphate (orthophosphate) and total dissolved phosphate are first obtained. Therefore, the orthophosphate concentration was first determined using molybdenum blue assay [13]. Then the balance water samples were digested to determine the total phosphorus concentration dissolved in the water [14]. The orthophosphate values were then subtracted from the total dissolved phosphorus values and the remainder is considered dissolved organic phosphorus. Phosphate concentrations were measured using a DR900 Hach portable colorimeter unit and the detection limit was  $0.02 \text{ mg L}^{-1} (\text{PO}_4^{3-})$ . All values were then converted to represent phosphorus  $\text{P mg L}^{-1}$  instead of phosphate.

## Sediment organic phosphorus fractionation

Collected sediments were freeze-dried and crushed finely using pestle and mortar. One gram of the crushed sediments was then placed into 50-mL centrifuge tubes. Sequential extractions of the labile, moderately labile and non-labile forms of organic phosphorus were performed at room temperature. To extract the labile fraction, 50 mL of 0.5 M  $\text{NaHCO}_3$  (pH: 8.5) was added to the sediments and shaken for 16 h on a horizontal shaker. After shaking, the sediment solution was centrifuged at  $1900 \times g$  for 20 min and the liquid supernatant was filtered through Whatman 41 filter papers [15]. Orthophosphate was determined using molybdenum blue and the remainder was digested in an autoclave for 3 h with the addition of potassium peroxodisulphate,  $\text{K}_2\text{S}_2\text{O}_8$  and 0.5 M  $\text{H}_2\text{SO}_4$  to quantify the total phosphorus concentration [14].

The following solutions 1.0 M HCl (3 h), 0.5 M NaOH (16 h) were added sequentially and extracted following the same procedure as described above. Organic phosphorus extracted from 1.0 M HCl was considered moderately labile. Extracts recovered from shaking with 0.5 M NaOH were further processed to separate into fulvic and humic acids by acidifying the solutions to pH 0.2 using concentrated hydrochloric acid. The acidification results in the formation of two phases where the fulvic acid remains in the liquid phase while humic acid precipitates out. The concentration of humic acid is calculated by subtracting the fulvic acid concentration from the total phosphorus determined from the original 0.5 M NaOH extract.

Fulvic acid is considered moderately labile, while humic acid is considered non-labile. To further extract the remaining non-labile forms, the sediments were ashed at  $550^\circ\text{C}$  in a furnace for 1 h and then shaken for 24 h in 1.0 M  $\text{H}_2\text{SO}_4$ . The extracts collected here were digested as described above and the values obtained were taken directly as organic phosphorus. Organic phosphorus concentrations were determined as the result of the subtraction of total phosphorus and orthophosphate except at the final extraction step. Similarly, the phosphate concentration was measured using Hach DR900 portable colorimeter and the values were converted to represent  $\text{P mg kg}^{-1}$ .

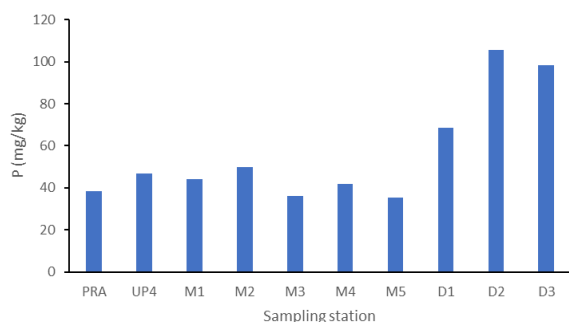
## Statistical Analysis

Pearson Correlation analyses were performed using SPSS version 29.0.

## RESULTS

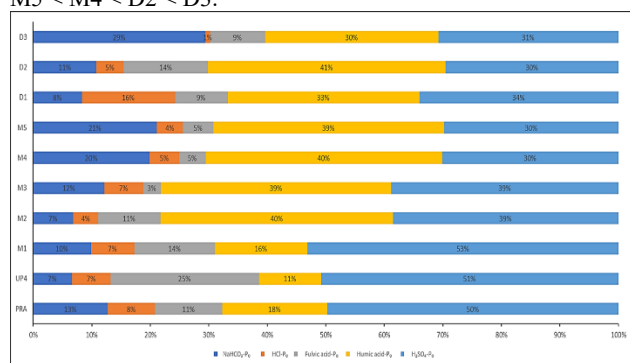
The total organic phosphorus shown in **Fig. 2** shows that site M5 (Kg. Teras Jernang, Kajang) has the least organic phosphorus load at  $35.20 \text{ mg kg}^{-1} \text{ P}$ . The highest was found at site D2 (Labohan Dagang, Banting) at  $105.7 \text{ mg kg}^{-1} \text{ P}$ . In general, the

order of increasing organic P load according to site is as follows; M5 < M3 < PRA < M4 < M1 < UP4 < M2 < D1 < D3 < D2. Significant organic P load is noticeable at the last three sites (D1= 68.39 mg kg<sup>-1</sup> P; D2= 105.7 mg kg<sup>-1</sup> P; D3= 98.19 mg kg<sup>-1</sup> P.



**Fig. 2.** Total organic phosphorus extracted from the sediments of Langat River. The total is the summation of organic phosphorus load from every fraction extracted.

The NaHCO<sub>3</sub> extracted fraction is the labile fraction of organic phosphorus. In Langat River, this fraction constitutes 7–29% of the organic P contribution at individual sites (**Fig. 3**). There is no clear observable pattern when compared across the sites studied but site D3 recorded the highest value at 28.86 mg kg<sup>-1</sup> P which is equivalent to 29% of the total P<sub>o</sub> recovered from the sediments of the site despite its large standard deviation of the mean. The site that recorded the lowest labile load was UP4 (3.10 mg kg<sup>-1</sup> P). The ascending order of labile organic phosphorus is as follows; UP4 < M2 < M1 < M3 < PRA < D1 < M5 < M4 < D2 < D3.



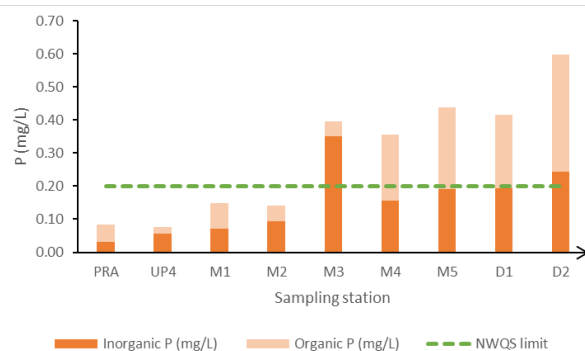
**Fig. 3:** Contributions in percentage of each organic phosphorus fractions extracted from the sediments of Langat River.

The moderate labile fraction is composed of two components with one being the extracts of HCl-P<sub>o</sub>. Overall, this fraction does not contribute much to the overall P<sub>o</sub> extracted from sediment and the percentage of contribution within each site ranges from 1–16%. The ascending order of load concentration for HCl-extracted P<sub>o</sub> are as follows; D3 < M5 < M4 = M2 < M3 < PRA = UP4 < M1 < D2 < D1 (**Fig. 4b**). Fulvic acid is categorized as moderate labile and is extracted alongside humic acid. On average, fulvic acid contributes significantly less than humic acid at each sampling site, with 3–25% of fulvic acid and 11–41% of humic acid at each site. Across all sites, D2 has the highest fulvic acid content compared to others. The amount of fulvic acid-associated P<sub>o</sub> extracted from the sediments of Langat River in ascending order is as follows; M3 < M5 < M4 < PRA < M2 < M1 < D1 < D3 < UP4 < D2 (**Fig. 4c**).

When divided into labile, moderately labile and non-labile fractions of organic phosphorus, the non-labile fractions

dominate all other types of extracted organic phosphorus found in the sediments. The values range from 60% to 78% (**Fig. 3**) of the total organic P extracted at the respective sampling site. However, as the non-labile fraction is composed of humic acid P and P that was extracted with a strong acid (H<sub>2</sub>SO<sub>4</sub>), there are differences in the contribution of both types to the non-labile fraction. Sites that are located at the upstream reaches have relatively lower humic acid contributions than the sites at the lower reaches. The P<sub>o</sub> load from humic acid in order of increasing concentration is as follows; UP4 < PRA < M1 < M5 < M3 < M4 < D1 < D3 < D2 (**Fig. 4d**). Meanwhile, the non-labile P<sub>o</sub> extracted with sulphuric acid in ascending order is as follows; M5 < M4 < M3 < PRA < M2 < D1 < M1 < UP4 < D3 < D2 (**Fig. 4e**).

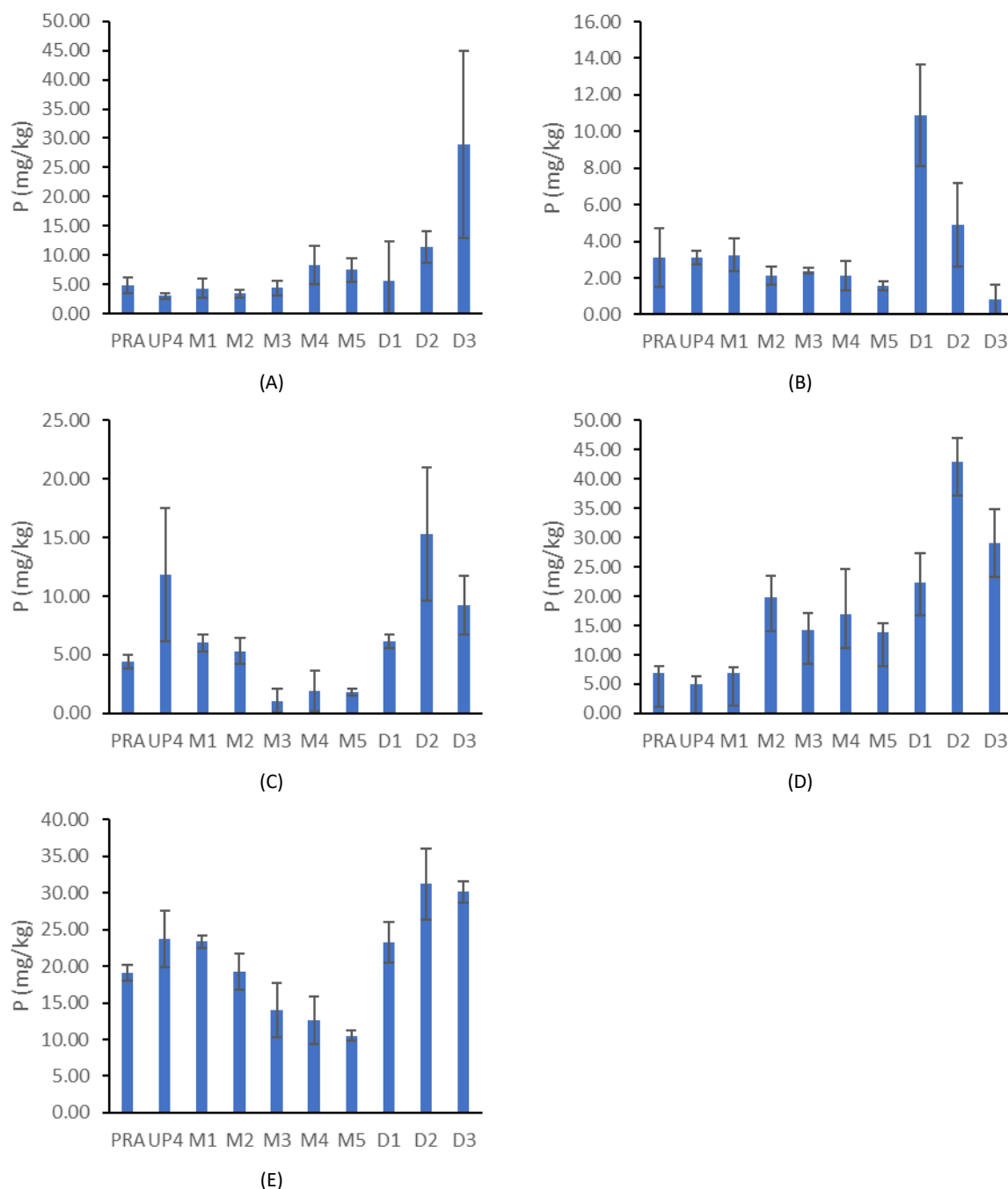
The total dissolved phosphorus found in Langat River in **Fig. 5** shows an increasing pattern towards the downstream with exception of sampling site D3 (Jugra, Banting). The dissolved organic phosphorus makes up the larger portion of total dissolved phosphorus in most of the sites exclusive of UP4, M2, M3 and D3. When compared with Malaysia's National Water Quality Standard guideline for acceptable phosphorus levels, Langat River only exceeds the standard at sampling stations M3 and D2. This is because the phosphorus that is measured is orthophosphate, which in this paper is defined as inorganic phosphate. However, when organic phosphate is taken into account, every sampling site beginning from M3 to D3 exceeds the 0.2 mg L<sup>-1</sup> P requirement.



**Fig. 5.** The total dissolved phosphorus in Langat River as accounted for by the dissolved organic and inorganic phosphorus. The dashed line indicates the 0.2 mg/L standard according to National Water Quality Standard Malaysia.

## DISCUSSION

Typically, total phosphorus (TP) has been used as a predictor of eutrophic events due to its linear relationship with chlorophyll *a* concentration, although this relationship is being challenged since it only exists within a range of P concentrations [16]. Recent research on organic phosphorus has shown that the bioavailable forms can be used as a reliable indicator of changing trophic conditions in freshwater ecosystems [10]. Additionally, as significant stores of P are found in the sediments, understanding the chemistry of P<sub>o</sub> here can help in better management of P input [17]. This study presents the first report of organic phosphorus fractions found in the sediments of Langat River. Based on the results gathered, the sediments of Langat River have substantial organic phosphorus.



**Fig. 4.** The sediment organic phosphorus load of every fraction (a)  $\text{NaHCO}_3$ -extract (labile) (b) HCl-extract (moderately labile) (c) Fulvic acid (moderately labile) (d) Humic acid (non-labile) (e)  $\text{H}_2\text{SO}_4$ -extract (non-labile). Error bars indicate standard deviation.

The total organic phosphorus load increases towards the lower reaches and this condition is facilitated by several factors including input from tributaries, sediment sorption capacity and land use changes [18, 8]. Comparing the downstream (D1, D2 & D3) with the rest of the river reach (seven other sites) revealed that the downstream section alone makes up 48.22% of the entire  $\text{P}_o$  load extracted from Langat River sediments (Fig. 2). Similarly, in a study conducted at the Kapuas River, Indonesia, [19] reported that organic matter, organic phosphorus and inorganic phosphorus all increased towards the downstream and corresponded with increased human activities. Human settlements and activities often concentrate at the middle and

lower reaches of major rivers that in turn act as drainage for the discharges that come from these activities [20]. Development in the Langat River Basin has been on the rise over the years and the downstream of Langat River is characterized by more industries, agriculture and residential areas that are known factors to contribute to the introduction of excess organic matter in the river. Conversely, the increase in human settlements places a higher demand on the river to supply clean water. According to [21], water from Langat River is not safe for human consumption without treatment due to chemical pollution and points that the current technology in use for treatment is not efficient. Although phosphorus in waters does not pose a direct health hazard to

humans, it is indeed a primary nutrient that controls biological productivity in aquatic ecosystems. As organic phosphorus mainly originates from biological processes like the decomposition of organic matter, areas with high biological productivity can end up with substantial organic phosphate which can exist in various forms in both the sediments and the overlying water [22].

In all the sampling sites, the non-labile fractions constitute the majority of the fractions extracted although the internal dynamics of this fraction change from the upstream sites to the lower stream sites especially with reference to the humic acid contribution of each site. The upper stream has lower humic acid content, but this increases further down the river. From site M2 onwards, the proportion of humic acid is either close to equal or more than the proportion of refractory  $P_o$  extracted from sulphuric acid. This is unlike the sites before M2 where the humic acid contributions range from 11–18%. Humic acids found naturally in rivers are organic matter derived from plant materials including algae in addition to bacteria [23]. On the other hand, increased humic acid can result from consequences of human activities, for example in Langat River, leaching of organic matter such as livestock manure from agricultural areas that settle at the lower reaches [24]. Simultaneously, sewage treatment plants have lower efficiency in removing humic substances and can comprise up to 28% of the effluent released [25].

In rivers, humic acids are heterogenous natural organic matter are found as a layer of coating on the sediments and due to its varied chemical properties, some can have higher P adsorption capacity than others [26]. Higher proportions of humic acids also indicate that the sediments act as sinks of P due to increased adsorption and therefore reduce the potential for internal loading in an aquatic environment [27]. The nature of humic acid explains why when its proportion increases towards downstream in Langat River, the organic phosphorus load extracted increased likewise. Additionally, it can be surmised that in Langat River currently, introduced  $P_o$  are being sequestered into sediments by humic acids and release from the sediment to the water is insignificant. However, consistent monitoring will shed more light on the temporal changes of sediment and water interaction with respect to P sorption and release.

Fulvic acids are also derived from organic matter and together with humic acid and humin are collectively called humic substances [28]. Fulvic acid differs from humic acid and humin on account of their lighter molecular weights and constant solubility in water regardless of changes in pH [29]. In Langat River, there is lesser fulvic acid percentage contribution than humic acid. Fulvic acid-bound organic P is also thought to have higher lability compared to organic P bound to humic acid and this makes it more bioavailable. According to [30], fulvic acid makes up 90% of dissolved organic matter that can be found in surface waters. Its lower contribution in sediments, similar to HCl-extracted  $P_o$ , suggests that the moderately labile fraction is an active contributor to the dissolved phosphorus in the waters of Langat River. Despite the lack of correlation between the moderately labile fractions and the dissolved phosphorus, there is a marked increase in total dissolved phosphorus beginning from sampling sites M3 onwards which corresponds to the significant decrease in fulvic acid contribution in sediments at sampling sites M3, M4 and M5. As part of the dissolved organic matter (DOM) in water, fulvic acid-bound  $P_o$  in Langat River may play an important role in the regulation of aquatic microorganism community structure and activity as it functions as a source of energy [31]. Equally, mineralization by microbial

action of fulvic acid-bound  $P_o$  can result in high concentrations of dissolved inorganic phosphorus which is an important factor leading to eutrophication [32].

Both the labile fraction and moderately labile fraction are the most active component of organic phosphorus found in the sediments [15]. The labile fraction is the highest at D3 and contributed 29% to the total organic P extracted from that site with a conspicuously large standard deviation of the mean. Environmental samples tend to have wide variations within them, and this is not only limited to studies on phosphorus. Other environmental studies like the metagenomics of bacteria also are affected where different community structures can exist when replicates are taken mere centimeters away due to environmental sporadicity [33].

In this study, the replicates were taken, whenever possible, from both riverbanks and the middle and then averaged to represent the site under study. The differences may arise when there are different land use activities at either banks or if tributaries form confluences at one of the banks. As these play an important factor in the transport of diffuse  $P_o$  sources, the collected data may represent these effects in the form of large variations. For example, at sampling site D3 (Jugra, Banting), Langat River flows in between Carey Island and the main landmass of Peninsular Malaysia. Carey Island is a holiday destination whereas the opposite bank is characterized by residential and agricultural land use. Both are interspersed with natural vegetation, however, these differences in land use can vary the  $P_o$  input into Langat River.

No significant relationship could be postulated between labile  $P_o$ , moderately labile  $P_o$  and the distribution of dissolved phosphorus (organic, inorganic and total) through Pearson's correlation. This lack of correlation may indicate that other physico-chemical or biological factors may influence the release and retention of labile organic phosphorus from the sediments into the water. Rivers are complex and its physico-chemical conditions change from upstream to downstream [34]. For sediment phosphorus to impact the waters, parameters such as the pH, minerals in sediment and redox conditions are important and these can change throughout the river [35]. In this study, both sites D2 and D3 have been observed to be affected by tides where the river flow can move in either direction depending on the tide. Particularly at D3 where the site is closer to backwater reach, deposition of sediment and particulates increases as the flow rate is moderated by the ocean and causing more labile  $P_o$  to be found there [36]. As labile  $P_o$  are susceptible to transformation, higher levels at the downstream coupled with increasing human activities in Langat Basin may negatively affect the river through eutrophication in the future if not closely monitored [37].

Langat River has significant stores of organic phosphorus in its sediments most notably at the lower reaches. The vast majority of organic phosphorus are non-labile and the sediments appear to sequester P inputs. However, mitigation efforts that are usually focused on removing orthophosphate should also consider the balancing act played by organic phosphorus. Non-labile organic phosphorus are deposits of P to be used by living organisms whenever orthophosphate is deficient [38]. Through bacteria mediated processes such as the release of extracellular phosphatase enzymes, non-labile forms can be converted to available forms thus sustain biological productivity even in reduced anthropogenic P input [39]. Thus, in Langat River, accumulated organic phosphorus can play an important role in ecological sustainability and risk.



## CONCLUSION

The organic phosphorus fractionation of the sediments in Langat River has been found to contain substantial organic phosphorus. The major type of organic phosphorus in the sediments is non-labile. Humic acid-associated organic phosphorus sorb introduced and natural P<sub>o</sub> making the sediments act as sinks and increase toward the downstream. Fulvic acid-associated organic phosphorus contributes less to the sediments but is more labile and may be present significantly in dissolved forms, playing a part in the internal loading of the river through microbial activity. Labile forms are higher at the downstream sites due to increased sedimentation rates and may be a cause for concern in the future. Through information gained from fractionation both environmental and anthropogenic factors were identified to contribute to the organic phosphorus load in Langat River sediments.

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## CONFLICT OF INTEREST

The corresponding author declares no conflict of interest on behalf of all the authors.

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