Seasonal Variability and Fractionation of P-Based Nutrients in Sungai Setiu Basin, Terengganu, Malaysia

(Variasi Bermusim dan Fraksinasi Nutrien Berasaskan P di Lembangan Sungai Setiu, Terengganu, Malaysia)

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ABSTRACT

A seasonal study of phosphorus (P)-based nutrients was carried out in Sungai Setiu basin which is located in the southern region of the South China Sea (Malaysia) from July 2010 until June 2011. Parameters measured were dissolved inorganic P(DIP), dissolved organic P(DOP) and total particulate P(TPP). In addition, DOP concentration was size fractionated into high molecular weight (HMW, 0.7 µm-100 kDa), middle MW (MMW, 100-30 kDa) and low MW (LMW, <30 kDa). The results show that the mean concentration of P-based nutrients ranged between 3.2-7.0 µg/L P for DIP, 5.6-12.1 µg/L P for DOP and 9.2-119.4 µg/L P for TPP. Higher mean concentrations of P were recorded at urban and agricultural areas. In addition, the distribution of P was affected by season as higher mean concentrations of P were observed during the north-east monsoon due to the surface runoff of nutrients during this heavy raining season. The fractionation study of DOP further revealed that HMW fraction was abundant in Sungai Setiu, making up approximately 60% of the total DOP. This high percentage of HMW DOP was recorded at stations in the vicinity of agriculture area and towns suggesting an important contribution from anthropogenic activities. The LMW DOP fraction was not significantly associated with chlorophyll-a, suggesting that the phytoplankton might not be the predominant contributor for LMW fraction in this river.

Keywords: Fractionation; monsoon and non-monsoon seasons; seasonal variability; P-based nutrients; tropical area

ABSTRAK

Kajian variasi nutrien berasaskan fosforus (P) telah dijalankan di lembangan Sungai Setiu yang terletak di kawasan selatan perairan Laut China Selatan (Malaysia) dari Julai 2010 hingga Jun 2011. Parameter yang diukur ialah tak organik terlarut P (DIP), organik terlarut P (DOP) dan jumlah partikulat P (TPP). Tambahan lagi, kepekatan DOP telah difraksikan mengikut saiz kepada berat molekul tinggi (HMW, 0.7 µm-100 kDa), MW sederhana (MMW, 100-30 kDa) dan MW rendah (LMW, <30 kDa). Keputusan menunjukkan kepekatan purata nutrien berasaskan P berjulat antara 3.2-7.0 µg/L P untuk DIP, 5.6-12.1 µg/L P untuk DOP dan 9.2-119.4 µg/L P untuk TPP. Kepekatan purata P adalah tinggi di kawasan perbandaran dan pertanian. Taburan P juga dipengaruhi oleh musim dengan kepekatan purata P adalah tinggi semasa monsun timur-laut disebabkan kehadiran nutrien larut lesap permukaan semasa musim hujan yang lebat. Kajian fraksinasi DOP seterusnya mendedahkan bahawa fraksi HMW adalah banyak di Sungai Setiu, merangkumi lebih kurang 60% jumlah DOP. Peratus HMW DOP yang tinggi direkodkan di stesen yang berhampiran dengan kawasan pertanian dan bandar yang mencadangkan sumbangan penting daripada aktiviti antropogenik. Fraksi LMW DOP tidak mempunyai hubungan yang bermakna dengan klorofil-a, bermaksud fitoplankton mungkin bukan penyumbang utama untuk fraksi LMW di sungai ini. Kami mencadangkan input organik nutrien ini seharusnya dipantau sebagai sebahagian daripada pengurusan sistem akuatik.

Kata kunci: Fraksinasi; kawasan tropika; variasi bermusim; musim monsun dan bukan monsun; nutrien berasaskan P

INTRODUCTION

Phosphorus (P) is a key constituent of all living matter and an essential nutrient in phytoplankton growth (Luu et al. 2012; Nausch & Nausch 2011; Suratman et al. 2010). P can come from a variety of natural sources associated with terrestrial weathering and plant growth and anthropogenic sources such as sewage, fertilizer runoff, soil erosion, animal waste, urban activities and industrial discharges (Ling et al. 2010; Shuhaimi-Othman et al. 2007; Tahir et al. 2008; Yang et al. 2009). A recent global model study, Nutrient Export from Watersheds (NEWS), derived estimates of total P fluxes using source estimates and transmission factors (Harrison et al. 2005). In the aquatic system, dissolved and particulate P compounds exist in inorganic and organic chemical forms. The modelling study of Harrison et al. (2005) fractionates the total P flux into dissolved inorganic (DIP) and organic (DOP) and particulate (PP) chemical components, a fractionation which is particularly valuable given the potentially different extents (or probably rates) of bioavailability of the different forms of P. However, the data base available for the calibration of such a model was rather limited, particularly for DOP

and remains so despite recent new data sets such as Yates and Johnes (2013). A feature of the NEWS model output is that the fluxes of DOP, dissolved organic nitrogen and carbon (DON and DOC, respectively) to the global ocean are particularly high in Southeast Asia, driven at least in part by the high river flows. However, the observational data base to provide evidence for high fluxes of dissolved organic nutrients in this region is very limited. Here we report the concentrations and forms of P in one north-east Peninsular Malaysian river system (Sungai Setiu) sampled along its length 12 times over a year. We also report results from sampling in other nearby river systems in order to evaluate if the results reported for the Setiu system sampled in detail are representative of rivers in this region.

In Malaysia, most of the water quality evaluations, especially for nutrient parameters, only measure the concentration of dissolved inorganic forms (Gasim et al. 2008; Khalik et al. 2013; Shuhaimi-Othman et al. 2007), due to the fact that the inorganic form is believed to be associated with eutrophication phenomenon (Jin et al. 2008; Luu et al. 2012; Xu et al. 2008). In contrast, not many studies have analysed the organic P because it is assumed to be composed mainly of refractory compounds resistant to biological degradation and unavailable as a source of nutrition for phytoplankton (Fagerberg et al. 2009; Rinker & Powell 2006; Suratman et al. 2010). As a consequence, the relative importance of aquatic DOP remains largely unknown. However, DOP concentration in aquatic system often comprises a significant fraction of the P pool and studies have also reported that the low weight fraction of DOP is released by phytoplankton and also can be utilised for their growth (Fagerberg et al. 2009; Harrison et al. 2005; Nausch & Nausch 2011; Yates & Johnes 2013).

DOP can be further divided into two classes i.e. highmolecular-weight (HWM) and low-molecular-weight (LWM), operationally defined by ultrafiltration membrane cutoff and DOP size fractionation has received increasing attention in studies of P cycling in aquatic system (Fagerberg et al. 2009; Nausch & Nausch 2011; Rinker & Powell 2006). It has been suggested that, HMW DOP could be of terrigenous origin and released during sediment resuspension (Cai & Guo 2009; Monbet et al. 2009; Suratman et al. 2008), while LMW DOP is of direct biological origin and potentially more bioavailable (Suzumura et al. 1998; Young & Ingall 2010).

This study is focussed on Sungai Setiu basin situated in Setiu district facing the South China Sea on the east coast of Peninsular Malaysia. The river is approximately 60 km long with a catchment area of about 960 km2 and originates in protected upland forests. The river is strongly influenced by the tropical monsoon, i.e. the wet north-east (NE) monsoon from October to March which can induce periods of continuous heavy rainfall for several days. Annual rainfall in this region is 3670 mm on average. For the sampling year in question the total rainfall was 3427 mm with an exceptional 1057 mm in March and 1816 mm falling in the normal monsoon period of October through January (MMD 2011).

The Setiu basin has undergone considerable development since early 2000. Human land use within this basin is of growing importance with agricultural activities important, especially in the lower reaches of the river. The Setiu district covered 130,436 hectares in total of area with an estimated population of 55,038 people with an annual population growth rate of 0.89%, corresponding to 5.42% of the total population of Terengganu state in the year of 2010 (SEPU 2010). The land used in Setiu district is dominated by forest reserve (43.0%) mainly in the headwaters of the river and agriculture (21.6%). In addition, the area covered by building was about 3.2% and only a small area was used by industrial activities (0.8%), while the rest of 31.4% was for other usage. The agriculture activities in Setiu consist of rice paddy, rubber, palm oil, tobacco, vegetable and fruit farming. In addition there is a shrimp aquaculture project which adjacent to station S1 at the downstream end of the catchment.

River flows are not routinely gauged on Malaysian rivers. However, there are two gauging records available for this region of NE Malaysia for the neighbouring Sungai Terengganu and Sungai Kelantan catchments which have broadly similar topography and land use. The annual average flows and areas of these two systems reported to be 206 m³s⁻¹ and 3340 km² and 527 m³s⁻¹ and 11900 km², respectively. We estimate the area of Setiu catchment to be 960 km² and scaling from that we estimate the annual flow of the Sungai Setiu system to be of the order of 59 m³s⁻¹.

In order to determine the spatial and seasonal variability of P-based nutrients and size fractions of organic P, a one year study has been carried out in Sungai Setiu basin. Sampling was done monthly to provide a general overview of the seasonality of composition in relation to the annual hydrological regime, although of course such a sampling scheme cannot capture the large scale and important short term variability in concentrations associated with short term changes in flow (Johnes 2007). The goal of the present study is to determine the relative importance of DOP and to consider the sources of P within this river system. By comparison to other Malaysian river systems in the same region, we also aim to determine if the results from the Setiu system are regionally representative.

MATERIALS AND METHODS

Samplings were conducted monthly from July 2010 until June 2011 at 10 sampling stations in Sungai Setiu basin (Figure 1). The sampling period covered the wet (NE) monsoon and dry south-west (SW) non-monsoon seasons to investigate the effect of the associated changes in flow on these P distribution. By conducting the spatial analysis of P at different stations in Sungai Setiu basin, the effect of land use activities on the P concentration in the water column can be investigated. Stations S7 and S8 in the upstream waters are in regions of protected forest with very little disturbance by human activity. Stations S2 and S3 are situated on another small tributaries of Sungai

Setiu where there is low density residential houses with no other major activity upstream of these stations. In the lower reaches of the river (stations S1, S4, S5 and S6), the agricultural activity such as paddy, rubber and oil palm plantation is more intense and there are small towns near station S6 (Permaisuri) and the river will receive waste water discharges directly.

Surface water samples (~0.5 m depth) were collected using a Van Dorn sampler and stored in high density polyethylene bottles and transported chilled to the laboratory. Prior to sampling, all high density polyethylene bottles were acid-washed, rinsed with deionized (DI) water and dried before used. Filtration was carried out through pre-combusted (450-500°C) 0.7 µm GF/F glass filters and the dissolved fraction was stored at -20°C until further analysis for the DIP and total dissolved P (TDP). Dissolved P was analyzed based on standard colorimetric method (Grasshoff et al. 1983) with manual absorbance measurements in a 5 cm path length cell. For the determination of TDP, alkaline digestion procedure was used to oxidise all forms of P to DIP which was then analysed as above (Grasshoff et al. 1983). The concentration of DOP was obtained from TDP concentration after subtracting the actual DIP concentration in the sample. We use the term DOP here as do Harrison et al. (2005), although we note that others use the term soluble unreactive P (SUP) to reflect the fact that the species liberated by the persulphate digestion may include polymeric inorganic P species that are unavailable to the routine DIP method. The unfiltered sample was digested similarly to TDP to determine the total P and total

particulate P (TPP) was then determined by subtracting TDP.

The organic size fractionation of P was carried out using the Millipore 8050 stirred cell ultrafiltration technique using 30 kDa and 100 kDa size cutoffs. Therefore, in this study, high molecular weight (HMW) DOP, middle MW DOP (MMW) and low molecular weight (LMW) DOP were operationally defined as the fraction with size 0.7 μ m-100 kDa, 100-30 kDa and <30 kDa, respectively. The aliquot obtained after the samples passed through the stirred cell were then oxidised by using UV digestion and subsequently analyzed by the standard colorimetric method (Grasshoff et al. 1983). The concentrations of DOP for each fraction were obtained after subtracting the respective DIP concentrations. In addition, the determination of chl-a concentration was performed spectrophotometrically based on Parsons et al. (1984) and is used here as a proxy of phytoplankton abundance (Rinker & Powell 2006).

Calibration of P was performed by using potassium dihydrogen phosphate (KH_2PO_4) standards in fresh DI water. The recovery tests based on standard addition of known concentration of inorganic P in DIP, DOP and TPP analyses yielded mean recoveries of 99.5%, 99.8% and 99.7%, respectively. About half the samples from each sampling trip were analysed in duplicate. The average precision from these duplicate samples for DIP, DOP and TPP determinations were within $\pm 2\%$. Detection limit for P-based nutrients in the current study, calculated as three times the standard deviation of blank (Christian 1994), was about 1 µg/L P.



FIGURE 1. Sampling stations in Sungai Setiu basin, Terengganu

RESULTS AND DISCUSSION

P-BASED NUTRIENTS DISTRIBUTION

The histograms for the P mean annual concentrations (DIP, DOP and TPP) present in the water column at each station are shown in Figure 2. The results showed that the mean concentrations of P-based nutrients ranged between 3.2-7.0 μg/L P for DIP, 5.6-12.1 μg/L P for DOP and 9.2-119.4 μg/L P for TPP. DOP and TPP are clearly important components of the P flux in this catchment as higher concentrations were recorded compared to DIP. Although, most of the P is transported in the particulate form, this probably will be trapped in the estuary and not reach the marine system. The DOP/DIP ratio is in the range of 1.5-2.9 and in general, there is an increase of this ratio in going from the upstream to downstream stations, especially in the middle reaches of this basin. This result suggested that the main fraction of the dissolved P is the organic P. Thus both DOP and TPP increase downstream in terms of absolute concentrations and in terms of their relative importance for P transport.

The results showed that higher DIP mean concentrations occurred in Sungai Setiu downstream of town and agricultural areas, reflecting the impact from the anthropogenic activities. Rothwell et al. (2010) found in their study that the dominant control on river water P is point source contributions from sewage effluent inputs, consistent with the NEWS model output (Seitzinger et al. 2005). Diffuse agricultural sources are also probably important, although the two sources are not easily separated in this sampling scheme since both urban and agriculture could be responsible for the increase in concentrations at the middle of this river basin. Pagliosa et al. (2005) in their study of rivers in Southern Brazil have reported a similar situation, in which urbanization resulted in the increase of urban effluents discharge and increases in the P level in the rivers. The high DIP concentrations at station S1 could be attributed to the introduction of shrimp aquaculture project near to this station either directly or indirectly, since a large area of Gelam forest had been cut down to create this facility (Sharma 2010). Similar phenomena of soil erosion associated with P transport have also been

reported from other studies (Duan et al. 2008; Puustinen et al. 2007; Yang et al. 2009).

In the largely undisturbed part of the catchment (stations S7 and S8), DOP represents about half of the dissolved P in the river water. The general pattern of DOP downstream is similar but not identical to that of DIP, indicating that there is clearly an anthropogenic source of DOP as well as DIP. Agricultural activity may contribute, since P-fertilizer or manure which may contain DOP is also commonly used in agriculture to improve crop production and soil properties (Guo et al. 2014; Turner & Rabalais 2003). Harrison et al. (2005) argue that diffuse agricultural sources are more important than point sources for DOP, but the similar downstream patterns here for DIP and DOP suggest that in this catchment at least the drivers of increasing concentrations downstream are similar for both components and to some extent also for TPP.

TPP is generally the dominant form of P. TPP concentrations increase markedly after station S7 but then remains rather constant downstream except for station S1. This increase after station S7 reflects a change in river bed from a rocky terrain around stations S8 and S7 to readily erodible banks at the other sites. During the present study, higher concentrations of total suspended solids (TSS) were observed in going from the upstream stations to downstream stations (data not shown). In addition, the ratio of TPP/TSS increased gradually from 0.012 in the upstream station (S8) to 0.049 in the downstream station (S1). These ratios suggested that an important source of particulate P was the bank erosion, but the amount of P in these eroding materials also increases downstream, probably reflecting increasing P abundance due to agricultural and human activity.

All three forms of P also exhibited seasonal variations with the highest and lowest concentrations of P-based nutrients observed during NE monsoon and SW nonmonsoon seasons, respectively as shown by average concentration at all sampling station for all stations in Figure 3. The general trend of increasing concentrations downstream is seen in all seasons. ANOVA analysis in the present study indicate that there were significant difference



FIGURE 2. Variation of mean concentrations of P-based nutrients in Sungai Setiu basin



FIGURE 3. Variation of mean P-based nutrients concentrations with monthly rainfall

between sampling periods (p<0.05) for these P-based nutrients, suggesting that there were marked variations of DIP, DOP and TPP concentrations particularly in response of rainfall during the wet monsoon season. Positive relationships between P-based nutrients and rainfall have been reported for many other rivers (Arreghini et al. 2005; Duan et al. 2008; Turner & Rabalais 2003; Yang et al. 2009). This could reflect mobilization of dissolved and particulate P from soils into the river (Arreghini et al. 2005; Duan et al. 2008). The unusual high rainfall in March produced a peak in DIP and DOP but not TPP. This might be due to the easily transported soils being mostly eroded during first flush of the monsoon rain in December, as observed in China (Yang et al. 2009).

P-based nutrients in this present study were compared with some selected studies that were reported in the literatures especially from the Malaysian area (Table 1). Particulate P was not analyzed in most of the selected previous studies except Sungai Kemaman (Malaysia), Changjiang River (China) and Wylye River (U.K.). It can be seen from the data in Table 1, P-based nutrients concentrations for Sungai Setiu were generally among the lowest in this compilation. Significantly higher concentration of P-based nutrients recorded at Sungai Serin was due to effluent from intensive animal farming (Ling et al. 2010). Nevertheless, as reported in the study of Sungai Marang and Sungai Kemaman basins, the possible sources of P compounds were similar to the present study as P-based fertilizer, organic manure and the soil erosion (Suratman et al. 2014; 2013). In the case of Changjiang River, the inputs of DIP were reported to be largely derived from point sources such as sewage from cities (Duan et al. 2008). The industrialization and urbanization surrounding Sungai Setiu basin was not as dramatic as that in the Changjiang River, therefore, there it is not surprising that lower DIP concentrations were recorded in Sungai Setiu. Concentrations of P compounds were also high in the Wylye River which was mainly attributed to the combined sources of agricultural runoff, sewage effluent, industrial discharge and wastes from livestock farming (Yates & Johnes 2013). In addition, the concentrations of DOP reported in this present study were at the low end of the range of the more extensive compilation of Harrison et al. (2005).

While the focus of this paper is on P and its fractionation, it is useful to compare the behaviour of P with that of N components using data obtained from the study by Hee (2012) on the same river system at the same time. The annual mean of nitrate concentrations at each station in this catchment are relatively low, increasing from 100 μ g/L N at station S8 to 162 μ g/L N at station S2 and with ammonia increasing downstream from a low mean concentration (10 μ g/L N) found at station S8 to about 29 μ g/L N at station S1. Higher mean concentration of ammonia was found at station S6 reaching 33 μ g/L N. This was probably due to the untreated waste discharges from the nearby town. DON mean concentrations increase downstream from about 70 μ g/L N at station S8 to 133 μ g/L N at station S1. Similar

TABLE 1. Comparison of P-based nutrients with those in selected study areas

Location	Phosphorus (µg/L P)			References
	DIP	DOP	TPP	
Sungai Setiu, Malaysia	3.2-7.0	5.6-12.1	9.2-119.4	Present study
Sungai Serin, Malaysia	40-4640	450-21100	-	Ling et al. (2010)
Sungai Marang, Malaysia	27-62	55-105	-	Suratman et al. (2013)
Sungai Kemaman, Malaysia	1.3-7.0	0.3-11.3	4.9-468.3	Suratman et al. (2014)
Changjiang River, China	6.2-31	5.6-15.2	19.22-201.5	Duan et al. (2008)
Wylye River, U.K.	70-5980	1-2030	1-1539	Yates and Johnes (2013)

to ammonia, higher mean concentration of DON was also observed at station S6 which recorded concentration of 184 µg/L N. As this paper emphasises dissolved organic nutrient, the correlation has been made between DON and DOP for all the individual data as shown in Figure 4. A significant positive correlation (R^2 =0.512, *n*=96) was observed between these two parameters and with an N/P ratio of about 15 on a mass basis. The correlation suggests that both DON and DOP are from similar sources and behave rather similarly as suggested by Harrison et al. (2005) in their global scale of dissolved organic nutrients.



FIGURE 4. Correlation between DON and DOP for all the individual data points

SIZE FRACTIONATION OF DOP

In order to investigate the sources of the organic fractions of P, three stations (i.e. stations S3, S6 and S8) were selected for size fractionation study of DOP. Three types of DOP fractions were determined, i.e. LMW, MMW and HMW. The mean percentage of LMW, MMW and HMW DOP were in the ranges of 25 to 33%, 6 to 18% and 58 to 61% of the total DOP, respectively, with HMW therefore the dominant component of the DOP (Figure 5). Based on the two-factor without replication ANOVA test, there were significant differences of LMW, MMW and HMW DOP percentages between sampling stations (p<0.05), which suggests the fractions of LMW, MMW and HMW DOP varied between stations.

Among the three stations, relatively high mean percentage of HMW DOP was observed at stations S3, S6 and S8 which were the areas of agriculture and town centre (stations S6) and low residential houses with no major



FIGURE 5. Variation of mean HMW, MMW and LMW DOP (%) at selected stations

activity (stations S3 and S8). Higher mean percentage of HMW DOP at station S6 suggests that some of the HMW DOP sources could be from anthropogenic inputs from adjacent agriculture area and town centre as suggested also by Monbet et al. (2009) in the Tamar River, U.K. However, since higher mean percentages of HMW were also recorded at undisturbed areas (stations S3 and S8) there must also be a non-anthropogenic sources of HMW DOP at these two stations.

In this present study, chl-a concentration was used to compare with the percentage of LMW DOP, as some studies have shown that this fraction has a positive correlation to chl-a through the direct release from phytoplankton (Nausch & Nausch 2011; Pakulski et al. 2000; Suratman et al. 2008). The fraction below 30 kDa which is defined here as LMW fraction was therefore plotted against respective



FIGURE 6. Relationship between LMW DOP with chl-a



FIGURE 7. Seasonal variation of DOP (%) for selected stations

chl-a concentrations. However, there was no significant relationship between LMW DOP and chl-a ($R^2=0.098$, n=30) (Figure 6). This suggests that phytoplankton did not play an important role in controlling the LMW DOP concentration in Sungai Setiu basin.

Figure 7 presents the seasonal variation for each fraction of total DOP. The results showed that there was no general trend of high or low percentages of DOP fractions with the rainfall as expected by Li et al. (2005). Li et al. (2005) have reported that the DOM fraction newly brought into the river source through the storm-water was mainly comprised of HMW humic molecules from such natural sources as soils and decayed vegetation of the forest-rich basin catchments. Thus, this will enhance the HMW fraction in the water column. In contrast, this was not observed in this study. In addition, the correlation tests between DOP fractions and the rainfall also show that there was a weak positive relation between all fractions (HMW, $R^2=0.192$; MMW, R^2 = 0.271; LMW, R^2 =0.170), which suggests that HMW, MMW and LMW DOP in Sungai Setiu basin were only weakly influenced by the rainfall. Although there is no correlation with the rainfall, it is interesting to see at station 8 there is almost no DOP in the MMW fraction except in December and March when there was very heavy rainfall. In addition, at the downstream area, there was an increased MMW fraction which suggests a signature of the anthropogenic impact at this area.

CONCLUSION

This study has shown that DOP represents an important component of riverine P transport in Sungai Setiu system. Lower concentration of DOP and DIP were recorded at the upstream stations and higher in going to the downstream stations, suggesting that anthropogenic activities contributed to the increase of dissolved inorganic and organic P concentrations in this basin. Concentrations of DIP and DOP increase with rainfall and hence presumably with flow, making the fluxes highly sensitive to water flow and climate. Most of the DOP was in the HMW fraction which constitutes about 60% on average. At the upstream station with least human disturbance there was very little DOP in MMW (mean 6 to 18%) and LMW (mean 25 to 33%). At stations S3 and S6 impacted by human activity the total DOP increases in all size fractions, but notably an increase in the MMW fraction. We suggest that DOP is therefore an important component of the P flux in Sungai Setiu. A comparison (Table 1) to other rivers in the region analysed by Ling et al. (2010) and Suratman et al. (2014, 2013) suggested this is the situation in other rivers in Malaysia and perhaps more generally at least in the SE Asian region, as suggested by the NEWS model system for the global dissolved P flux to the oceans (Seitzinger et al. 2005).

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