ABSTRACT

Research on catchment area have traditionally involved concentration and flux measurement to better understand weathering, transport and cycling of materials from land to sea. Potentially, modification of terrestrial environment can alter the carbon flow in a catchment. This research is to characterize dissolved inorganic carbon (DIC) in Sungai Kurau and Tasik Bukit Merah. A progressive depletion of ($\delta^{13}C$--DIC: -14.20 ± 0.47‰) towards downstream ($\delta^{13}C$--DIC: -24.44 ± 0.59‰) is observed. The trend indicates photosynthesis activity at the upper stream system where microbial respiration process is observed to occur at the Tasik Bukit Merah located at downstream area. The dynamic of carbon pathway is highly affected by allochthonous input and autochthonous process in the catchment system. Land use activities within the catchment can disturb the balance between biological and geological processes which control the carbon pool in Kurau catchment.

Keywords: Carbon-13; carbon cycle; photosynthesis; respiration; Tasik Bukit Merah

INTRODUCTION

Inland waters have been termed as regulator of climate change emphasizing the role they play in global biogeochemical cycle (Williamson et al. 2009). Inland waters can modulate the overall carbon (C) balance of entire landscape, not only acting both as source of C to the atmosphere through evasion of greenhouse gases, such as carbon dioxide ($CO_2$) and methane ($CH_4$) but also as sinks through C burial in sediment of lake and reservoir. In global contexts, carbon cycle has traditionally been presented as a number of reservoirs link by transfer pathways. Namely the atmosphere, terrestrial biomass, soil, the ocean and its associated biota and the lithosphere have been identified as primary carbon repositories (Mackenzie & Lerman 2006). The dynamic of the carbon cycle is primarily controlled by the watershed input and interim process as a result, the addition of the C or removed from the carbon pool (Lee 2014). The nature of the carbon cycle in tropical watersheds especially Malaysia remains poorly clarified. Few studies have addressed the contribution of Tropical River to the global carbon cycle and the resulting effects on worldwide climate. This is a subject of importance, given that tropical river provides more than 50% of the global water discharge, and therefore the largest percentage of the total fluvial carbon flux to the oceans (Meybeck 1993).
carbonate, the DIC will be dominated by HCO$_3^-$ at higher pH. CO$_2$ start dominate at pH above 9.

### STABLE ISOTOPE AS TOOL IN FINGERPRINT DETECTION

The isotopic composition of a carbon species can be used as an indicator of its origin and of subsequent processes leading to its modifications (Clark & Fritz 1997). This makes stable isotope analysis a valuable tool in environments where carbon is derived from various sources. All carbon within river is ultimately sourced from atmospheric CO$_2$, organic carbon, or carbonate mineral (Hope 1994; Meybeck 1993) each with a characteristic range of δ$^{13}$C values (Figure 1).

#### FIGURE 1. δ$^{13}$C values of carbon from various sources. Adapted from Dubois et al. (2010)

The carbon isotope composition of a compound can also under different modification (pathway process) subsequent to production. For example, soil CO$_2$ normally has an isotope composition which identical with the organic materials (Cerling et al. 1991). Biological processes can similarly influence the δ$^{13}$C values of various carbon species. Organism utilizing carbon such as photosynthesis process show a preference for $^{12}$C, due to its more rapid reaction rate owing to kinetic and thermodynamic considerations (Park & Epstein 1961). By product of biological activity also tend to be more δ$^{13}$C-depleted relative to the parent material. This is observed with plant uptake of CO$_2$ in which plant tissues can be δ$^{13}$C-depleted by more than 20‰, as well as with bacterial uptake of carbon substrates (Farquhar et al. 1989). In case of methane production, the latter process can fractionate carbon as -90‰ relative to precursor materials (¹²C) thus, enriching the δ$^{13}$C value (Whiticar et al. 1986).

### MATERIALS AND METHODS

Field work was carried out within the Malaysian Peninsular, in watershed located in the northern region of Perak state. The area of investigation is in the Batu Kurau district, which is located 20 km from Taiping at the northern region of Perak. Its landscape was built up millions of years of limestone formation and pristine river. The catchment size of Kurau is 151 km$^2$ and the Bukit Merah lake is 40 km$^2$ (Figure 2). It is one of the oldest reservoir in the country. The reservoir was built in 1902 with the capacity of 70 million m$^3$. The Bukit Merah Reservoir (BMR) was meant to serve the 24,000...
ha Kerian Irrigation Scheme mainly for rice cultivation. Approximately 10,000 farmers rely on rice cultivation industry. As well, the was very instrumental as fresh water resource to ~ 300,000 populations of Kerian and Larut Matang Districts since 1906. In addition, it also serves as the only unique native Malaysian Golden Arowana fish sanctuary in Malaysia.

**FIGURE 2. Map of Kurau Catchment**

**SAMPLING AND COLLECTION**

Sampling took place on May 2017. The sample was collected in 2 sub environments of Kurau catchment (BMR and Batu Kurau). Ten liter of water sample for δ¹³C-DIC were collected and pH were measured from each 11 points in the catchment (Figure 3). Precipitation of barium carbonate (BaCO₃) technique was conducted in the field or in-situ (Varlam et al. 2006).

**FIGURE 3. Map of sampling location. Adapted from Google Earth (2017)**
The primary technique used in this research project is the stable isotope technique. Stable isotope values are measured in units of per mil (‰), relative to the Vienna Pee Dee Belemnite (V-PDB) standard (IAEA 1993) based on the following equation:

\[
\delta^{13}C_{\text{‰}} = \left( \frac{R_{\text{Sample}} - R_{\text{std}}}{R_{\text{std}}} \right) \times 10^3
\]

where \( R_{\text{Sample}} \) is the \(^{13}\)C/\(^{12}\)C ratio in the sample of interest, and \( R_{\text{std}} \) is the \(^{13}\)C/\(^{12}\)C ratio in the V-PDB standard.

The Isotope Ratio Mass Spectrometer (IRMS) are used for isotopic analysis. It is a specialised mass spectrometer which produces precise and accurate measurements of variations in the natural isotopic abundance of light stable isotopes. The samples (Ba\(_2\)CO\(_3\)) were fed into the SERCON Water Equilibration System (WES) before analyses using IRMS (SERCON, 2007). The samples were flushed with Helium (He) gas with the custom setup group and 30 drops (± 300 µL) are injected into each vial. The sample is left for 60 min for a reaction. Depending upon the material the time of a reaction may have to be extended (Figure 4).

Sample-acid reaction:

\[
2H_3PO_4 + 3CaCO_3 \rightarrow Ca_3(PO_4)_2 + 3H_2O + 3CO_2
\]

The extracted CO\(_2\) gas was sampled by a specially designed sampling needle where the flow of He carrier gas pushed the gas mixture through the needle hole into the ionization chamber of the IRMS. The standard use in \(\delta^{13}\)-DIC analyses is NBS 18 (\(\delta^{13}\)C = -5.014), R022 (\(\delta^{13}\)C = -28.63) and IAEA-603 (\(\delta^{13}\)C = +2.46).

RESULTS AND DISCUSSION

Given that \(\delta^{13}\)C-DIC values of river and lake water of Kurau catchment range is 14.20 ± 0.47 until -24.44 ± 0.59‰. The pH values range from 4.93 ± 0.16 to 8.52 ± 0.11. The isotopic composition of DIC and pH in all sampling point are presented in Table 1. All data analyse using analysis of variance (ANOVA) on single factor and regression. Sample for \(\delta^{13}\)C-DIC mean is -19.53 with standard error of 0.90. The confidence level is set to 95% and overall standard deviation is 2.98. Each sample result differs with the values of ± 2.00‰. Sample for pH measurement mean is 7.00 with standard error of 0.35. The confidence level is set to 95% and overall standard deviation is 1.14. Each sample result differs with the values of ± 0.77.
TABLE 1. Isotopic properties of DIC in Kurau catchment

| Points | Location          | $\delta^{13}$C-DIC (‰) | pH     |
|--------|-------------------|-------------------------|--------|
| PT 1   | Upstream          | -16.82 ± 0.35           | 7.72 ± 0.60 |
| PT 2   | Bat Cave          | -22.80 ± 0.23           | 7.52 ± 0.51 |
| PT 3   | Confluent         | -19.93 ± 0.90           | 6.70 ± 0.09 |
| PT 4   | Sg Merah          | -22.39 ± 0.74           | 5.72 ± 0.18 |
| PT 5   | Railway           | -24.44 ± 0.59           | 4.93 ± 0.16 |
| PT 6   | Sg Kurau          | -19.78 ± 0.44           | 7.35 ± 0.05 |
| PT 7   | Dam Gate JPS      | -19.92 ± 0.26           | 7.99 ± 0.30 |
| PT 8   | Selinsing Dam Gate| -16.53 ± 0.54           | 8.22 ± 0.28 |
| PT 9   | Middle Lake       | -18.58 ± 0.29           | 8.52 ± 0.11 |
| PT 10  | Bkt Merah Resort  | -14.20 ± 0.47           | 6.34 ± 0.44 |
| PT 11  | Kmpg Selamat      | -19.44 ± 0.86           | 6.03 ± 0.27 |

*P ≤ 0.05
$R^2 = 0.50$

**BIOLICAL AND GEOLOGICAL SOURCE OF DIC**

In photosynthesis, CO$_2$ and H$_2$O are converted to organic matter by green plants, which use the solar light energy (photons) for this process (Berner & Berner 2012; Ishak 2014). This occurs during CO$_2$ diffusion into the leaf stomata and dissolution in the cell sap and during carboxylation (carbon fixation) by the leaf’s chloroplast where CO$_2$ is converted to carbohydrate (CH$_2$O) (Clark & Fritz 1997). Due to selection of different isotopologue of $^{12}$CO$_2$ (lighter) compared to $^{13}$CO$_2$ (heavier), this process causes the residual of $^{13}$CO$_2$ is enriched in the atmosphere (Wanninkhof 1985). The heavier $^{13}$CO$_2$ invaded into the stream and remain in the water due to differential partial pressure of CO$_2$ and under-saturated conditions in the water column (Karim et al. 2011). As a result, high concentration of $^{13}$CO$_2$ accumulated in the water causing the $\delta^{13}$DIC to be enriched. This happen because CO$_2$ have high solubility at low temperature (Weiss 1974).

**FIGURE 5. Isotopic signature of $\delta^{13}$C-DIC in Kurau Catchment**
However, based on pH 7.72 ± 0.60, geo-genic factor need to be considered such as dissolution of carbonate material from weathering factor which tend to enhance the δ¹³C (Stephens & Rose 2005). Hypothetically the source of DIC in PT 1 might come from the atmosphere that go through carbon fixation process and sink to the stream. Still, DIC derived from rock weathering do affect the isotopic values (Park & Epstein 1961). As the water move downward, δ¹³C-DIC show a depletion signature at PT 2 following respiration trend (Figure 5). Hypothetically, PT 2 will follow PT 1 signature since PT 2 is at the upstream river and quite close to each other. Depletion of δ¹³C-DIC at PT 2 is suspected due to CO₂ invasion in to the river because of less cover shade at the sampling point to promote photosynthesis process, causing more ¹²CO₂ dissolved in the water and additional factor such of low partial pressure, solubility of CO₂ and low water temperature.

Based on the pH value 7.52 ± 0.51, the river condition is slightly alkali due to presence of carbonate mineral, but it only have minor effect since invasion process is more dominant. PT 3 (confluence - Sg Kurau & Sg Ara) show slightly enrichment than PT 2, possibly preferential of ¹²CO₂ loss through evasion process and water mixing from Sungai Ara. PT 3 pH is at 6.70 ± 0.09 which is slightly acidic, might be due to addition of C3 organic matter to the stream since free CO₂ present pH below 7. Due to evasion process, the escape CO₂ effect the δ¹³C-DIC resulting more enriched value. Thus, the source of DIC at PT 3 might be the mineralization of DOC resulting free CO₂ in the system where based on field observation the water is turbid suggesting high transported of organic matter.

High depleted signature of δ¹³C - DIC was detected at PT 5 followed by PT 4 suggesting high microbial respiration may occur in the water column. Sungai Merah and surrounding terrestrial is suspected to be the source of organic carbon which cause active respiration process at the sampling points. This was observed, when microorganism uptake of carbon substrates it can be more -20‰. PT 5 show the highest respiration signature with δ¹³C - DIC -24.44 ± 0.59‰ compared to PT 4 with -22.39 ± 0.74‰. The cause of high respiration is suspected from palm oil plantation near to lake side which may have contributed to the addition of particulate organic carbon (POC) that washout into the lake. These POC are consumed by microorganism thus increasing the rate of respiration in the water (Stelzer et al. 2003).

In 2010, Department of Irrigation and Drainage (DID) Kerian has reported that there is a major land clearing for replanting by privately own property. Land clearing have the highest tendency for soil to leach down into the lake cause by surface run off with siltation (mixed with POC) thus degrading water quality (Andriesse & Schelhaas 1987). High respiration will promote more dissolved CO₂ (by product) in the water hence resulting a deplete signature of δ¹³C - DIC (Striegl et al. 2001). PT 6 and PT 7 show and intermediate range between respiration, evasion or aquatic photosynthesis process. The possible explanations are this might due to overlapping process between microbial respiration and evasion or aquatic photosynthesis process causing the value shift intermediately.

PT 9 show possible source from biogenic and geogenic where the isotopic signature falls under 3 possible source which is C3 organic matter, soil CO₂, aquatic DOC mineralization or CH₄ oxidation. However, the pH 8.52 ± 0.11, shown sign of carbonate mineral in the water column. As mention by Stephens and Rose (2005), addition of carbonate mineral will enrich the isotopic values. In general, since the isotopic value do not enrich, it is safe to assume the source of IC is from either C3 organic material, soil CO₂, aquatic DOC mineralization or CH₄ oxidation. PT 11 δ¹³C - DIC show a depleted signature suggesting respiration process and the pH is 6.03 ± 0.27 which is in the acidic condition. The possible source at PT 11 is from explanations aquatic DOC mineralization where this point is full of dead trees (swampy condition). The water is yellowish in color suggesting high in carboxic acid.

Unique fractionation of δ¹³C-DIC showed a possible of methanogenesis process at PT 10 and PT 8. High enrichment of δ¹³C-DIC are detected at PT 10 which is located near Bukit Merah Resort while PT 8 at it Selinsing Water Gate. It is hypothesized that the enrichment of δ¹³C-DIC is due to production of CH₄ through hydrogenotrophic methanogenesis (CO₂ reduction pathway) at PT 10 and the pH fall to 6.34 ± 0.44. Clark and Fritz (1997) explained, because of different metabolic pathway of methanogenic bacteria, it causes the carbon of CH₄ to fractionate more depleted and enrich for carbon of CO₂. Methanogenesis happen when inorganic oxidant such as nitrate, ferric iron or sulphate are depleted (Conrad 2005). There are 3 clues that may contribute to methanogenesis process based on our field observation and in-situ measurement (preliminary) in the lake which is: Present of micro-bubble when the sediment is tampered (suspect methane loss from the sediment through the atmosphere via ebullition); Low concentration of dissolved oxygen, 4-6 mg/L; and Low concentration of Nitrate, 1.2 mg/L. Hypothetically, the increase of CO₂ concentration and decrease oxygen are early sign of anoxic pathway in sediment respiration thus supporting CH₄ production (Holgerson & Raymond 2016). Talib et al. (2016) reported the concentration of total nitrogen (TN) have a decreasing trend from upstream to downstream (Figure 6).

Total nitrogen (TN) is the sum of NO₃, NO₂, organic nitrogen, and NH₄ (all expressed as N). TN have derived from various source such as soil runoff, fertilizer from cropland, septic discharge, runoff from animal manure, and industrial discharge. TN reduction (%) is inversely proportional to the distance from inlet (Sungai Kurau) in BMR. Table 2 shows a total of 61.84 t year⁻¹ of inorganic
nitrogen (NO₃, NO₂, and NH₄) are transported into the lake via Sungai Kurau. High source of inorganic nitrogen suggesting the present of anthropogenic activities at the upstream of the catchment. Anthropogenic activities caused high total suspended solid (TSS) input of sedimentation to BMR. Ismail and Najib (2011) reported 51,270 t year⁻¹ from Sungai Kurau and 2,831.9 t year⁻¹ Sungai Merah were transported into BMR. We can conclude that high TSS input bring more nutrient in BMR.

TABLE 2. Sediment and nutrient input of Bukit Merah Reservoir. Modified from Ismail and Najib (2011)

| Nutrient input      | NO₃  | NO₂  | NH₄  | PO₄  | TSS  |
|---------------------|------|------|------|------|------|
| Sungai Merah        | 4.17 | 0.15 | 2.16 | 5.05 | 2831.9|
| Sungai Kurau        | 39.81| 2.92 | 19.11| 68.32| 51270 |
| Total input (t year⁻¹) | 43.98| 3.07 | 21.27| 73.37| 54101.9|

*NO₃, nitrate; NO₂, nitrite; NH₄, ammonia; PO₄, phosphate; TSS, total suspended solid

Table 2 shows that high TSS carry more nutrient input into BMR especially Sungai Kurau. Inorganic nitrogen is total of TN, the data suggesting if the inorganic nitrogen follow the same trend of organic nitrogen in TN reduction over distance, this proof that low inorganic nitrogen (e.g. NO₃) may triggered the methanogenesis process at PT 10 causing the δ¹³C-DIC to be enriched. PT 8 also shown enrich value of δ¹³C-DIC but the pH is 8.22 ± 0.28 which is alkali. The possible explanation might be due to the presence of mineral such as calcium carbonate (CaCO₃) or magnesium carbonate (MgCO₃) near the dam outlet. DID (2010) also reported there are high sedimentation at PT 8 shallowing the outlet of the dam. This explanation might proof the source of IC is from carbonate mineral presence there.

CONCLUSION
This is the first hydrological study using stable isotope in Perak especially Kurau catchment. The results represent...
a snapshot of carbon cycle in Kurau catchment. The stable isotopic composition of DIC was studied as a potential tracer of IC generation and pathway process in Kurau catchment. These changes are attributed to combination of complex process in the carbon pool such as depleted biogenic source of δ13C (microbial respiration and decay), enriched geogenic source of δ13C (due to photosynthesis, evasion and methanogenesis). As well, the results indicate photosynthesis activity at the upper stream system where microbial respiration process is observed to occur at the Bukit Merah lake located at downstream area. The dynamic of carbon pathway is highly affected by allochthonous input and autochthonous process in the catchment system. Land use activities within the catchment can disturb the balance between biological and geological processes which control the carbon pool in Kurau catchment.

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