STABLE ISOTOPES OF CARBON, NITROGEN, AND SULPHUR AS POLLUTION SOURCE INDICATORS IN KUALA SELANGOR NATURE PARK

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ABSTRACT

Anthropogenic activities such as changes of land use for coastal development are the major factors affecting the sustainability of mangrove area. Mangrove forests in Kuala Selangor Nature Park (KSNP) is one of the areas that should be monitored for any potential source of pollution, and this can be performed by using stable isotopes as the important indicators. Vegetation samples from several palm and mangrove species were analysed for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ stable isotopes. The samples were collected from nine different locations comprising of KSNP area, water canal, residential area, sluice gate, aquaculture area, landslide area, agriculture area, jetty and river confluence area. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ value in the samples are found to be ranged from -33.05‰ to -13.33‰, +1.69‰ to +9.27‰, and +7.83‰ to +8.50‰, respectively. IsoSource modelling software was used and found that 50% is the highest source proportion identified in aquaculture location demonstrating that the area is the main pollution source in this study. The findings from this study could be used for further control measures by local authority in order to preserve the flora and fauna in KSNP.

Keywords: Carbon, nitrogen, sulphur, stable isotope, indicators, pollution source, Kuala Selangor Nature Park

INTRODUCTION

Stable isotope analysis (SIA) is a powerful technique for environmental assessment and monitoring that provides information about anthropogenic activities over time. Stable isotope compositions of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ change in predictable ways due to mixing and fractionation, giving insights into sources and cycling of these elements in biosphere (Fry, 2006). SIA has been widely used in mangrove ecosystem studies to better understand food web interactions (Abrantes et al., 2015), mangrove nutrient uptake (McKee et al., 2002), mangrove water use (Hayes et al., 2019), cycling of carbon (C) (Sasmito et al., 2020), nitrogen (N) (Fry and Cormier, 2011) and sulphur (S) (Raven et al., 2019).

The presence of pollutants in the food web depends on more than one factor and contamination of the surface water, sediment, or particulate matter, can affect the ecosystem structure and its trophic relationships (Bayen, 2012). Anthropogenic pollution of atmosphere causes diverse variations of carbon stable isotope ($\delta^{13}C$) values of plants. Plant vegetative parts during pollution frequently record a positive shift of $\delta^{13}C$, which is usually explained by photosynthesis at closed stomata (Savard, 2010). However, there are data suggested that the content of ^{13}C might be lower during pollution (Cada et al., 2016). Besides, man-made pollutants provide both enrichment (Pearson et al., 2000) and depletion (Kwak et al., 2009) of plants in ^{15}N. Therefore, nitrogen stable isotope ($\delta^{15}N$) values can be used as they can provide reliable information about the source (anthropogenic vs. natural) of nutrients within the coastal areas (Fry, 2006). The stable isotopes of sulphur ($\delta^{34}S$) are also important in the study of the effects of pollutant sulphur on the environment particularly to differentiate between organic and inorganic matter (Trust and Fry, 2002).

Human activities continue to affect coastal ecosystems throughout the world and the situation is very worrying. Excessive nutrient inputs (eutrophication) from intensive agricultural activity and growing populations within coastal regions usually result in rapid degradation of water quality and modifications of ecological features. Mangrove forests could play an important role in mitigating eutrophication in coastal settings as they have been described as nutrient limited ecosystems with a generally positive physiological response to nutrient addition (Reef et al., 2010). Mangrove plants, which can grow extensively and form stable stands within most protected coastal regions of the tropics and subtropics could be of particular importance for remediation.

Disturbances in mangrove areas not only affect recruitment, but can also change the cycling of C, N and S. Loss of mangrove trees and root structures can change organic matter inputs, sediment oxygenation and degradation of sediment organic matter. These changes alter overall sediment conditions, with consequences for benthic assemblages (Harada et al., 2019), sediment C and N stocks (Adame et al., 2018), and associated nutrient processes. Kuala Selangor Nature Park (KSNP) which is located in Northwestern Selangor, Malaysia also affected by anthropogenic pollution. It is the mangrove forest with many flora and fauna species. In this study, the aim is to determine C, N and sS stable isotope values in order to investigate the source of pollution from biota samples that have been collected from the study site.

MATERIALS AND METHODS

Area of study and sample collection

Kuala Selangor Nature Park (KSNP) (0320.0266,N,10114.4201,E) (Fig. 1), one of the attractive places in Northwestern Selangor, Peninsular of Malaysia is the habitat for 156 species of birds and frequently used as research for interested scientists and tourists (Rohaizah et al., 2020). In the present study, vegetation samples from several palm and mangrove species were collected from nine different locations comprising of KSNP area, water canal, residential area, sluice gate, aquaculture area, landslide area, agriculture area, jetty and river confluence area. These sampling sites are connected by Selangor river.
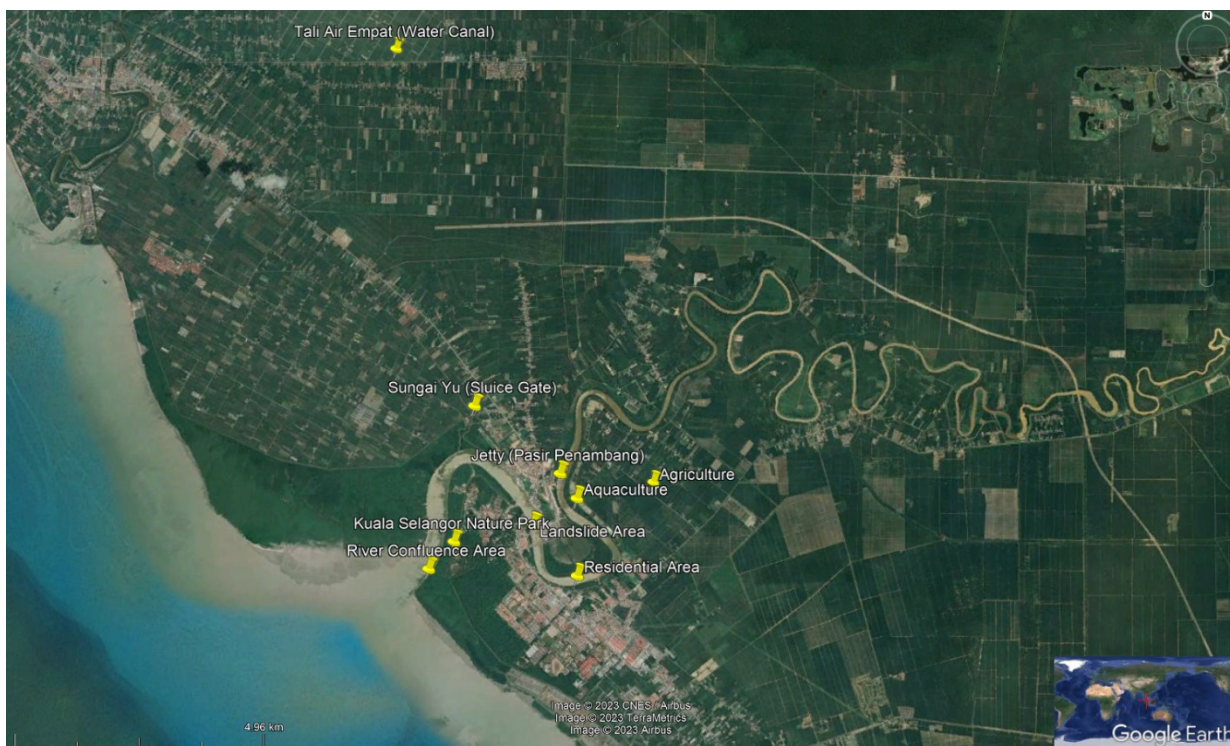
Figure 1. Location map of the KNSP sampling sites by Google Earth

Samples were collected during the dry season in February 2022. These plants were collected by hand, and carefully shaken to remove animal-related debris or soil particles. The plant samples also were clean using deionized water before kept in storage. They were then stored in clean containers and labelled accordingly to the sampling locations.

Sample Processing

All of the plant vegetation samples (leaves) were dried at 60 °C for 72 hours in oven (Memmert, Germany) and then ground to obtain homogeneity below 100 μm particles size. The samples were ground using ultra centrifugal mill ZM 200 (Retsch, Germany). Approximately 2 mg of samples were weighted and then inserted into tin capsules for bulk C, N and S isotopes analysis using Elemental Analyzer Isotope Ratio Mass Spectrometer (EA- IRMS) (Finlay, 2001).

Chemical Analysis

All the samples were analysed for $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ isotope ratios using EA-IRMS analyser. The analysis process is comprising of three stages namely combustion, reduction, and stable isotope ratio.

Combustion

Combustion which is the first process in the EA-IRMS will produce CO_2, H_2O, N_2, NO_x, SO_2 and SO_3 and volatile halogenated compounds. Therefore, the combustion tube is filled with tungsten trioxide (WO_3), which delivers additional oxygen to the reaction, prevents the formation of non-volatile sulphates and binds alkali and alkali earth elements.

Reduction

The carrier helium gas, with the combustion products, flows from the combustion tube to a reduction tube via a heated bridge. It is important that this bridge is heated to prevent H_2O condensing and reacting with the SO_3 and the SO_2. The reduction tube is filled with copper and facilitates the reduction of NO_x to N_2 and SO_3 to SO_2. Silver wool is also present in the reduction tube to react with any volatile halogen compounds that may have formed. From the reduction tube, the gas flows into an absorption tube which is filled with phosphorous pentoxide (Sicapent®) to remove water. After these reactions, the combusted sample has been quantitatively converted into the target gas species N_2, CO_2 and SO_2 used for detection of elemental concentrations and stable isotope ratios.

Stable Isotope Ratio

The gas stream (N_2, CO_2 and SO_2) passes into a gas chromatograph where components of interest are separated and then bled into a mass spectrometer where the isotope species are ionised then separated in a magnetic field. These isotopic species are detected separately and from their ratios, after then the level of ^{15}N, ^{13}C, ^{34}S are calculated.

Stable Isotope Analysis

Carbon, nitrogen and sulphur isotopes data are reported in a δ notation in per mil against the international reference material USGS 40, USGS 41a and IA-R025, respectively.

$$\delta \frac{13C}{12C} = \left(\frac{13C\ (sample)}{12C\ (standard)} - 1 \right) * 1000$$

The data quality of carbon, nitrogen and sulphur isotope analysis were rigorously monitored using a number of in-house standards, international reference materials and duplicated samples. In this case, we have developed sulphanilamide as our in-house standard.

IsoSource Mixing Model

IsoSource software was used to identify the contribution of different sources to the mixture. Users are required to insert the isotopic values of the sources and mixture, the source increment, and mass balance tolerance. Once completed, IsoSources generated output files that consist of all feasible source combinations and descriptive statistics of each source distribution for the analysis (Fig. 2). In this study, the isotopic values of water canal, residential area, sluice gate, aquaculture area, river, landslide area, agriculture area, jetty and river confluence area were categorised as potential sources that contributed to the pollution and KSNP was categorised as mixture. The source increment and the mass balance tolerance were set at 5% and 0.6% respectively in the IsoSource software. By default, the settings are 1% (increment) and 0.1 (mass balance tolerance) to determine the source contribution in pollution investigation study. However, users are able to modify the value for optimum data interpretation.
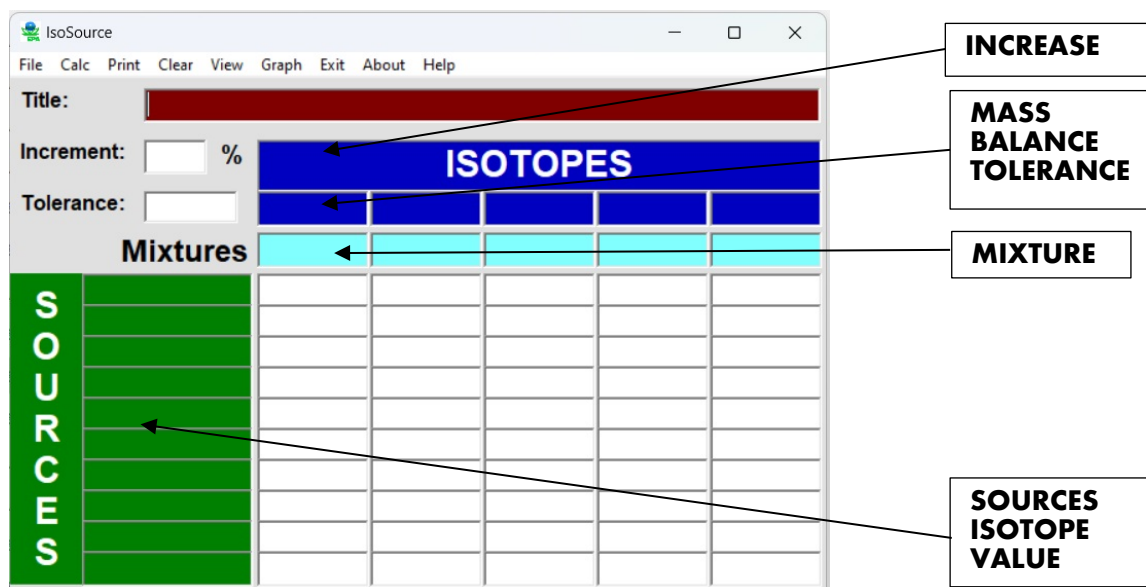
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Figure 2. The IsoSource mixing model

RESULTS AND DISCUSSION

Carbon, Nitrogen and Sulphur Isotopic Values

The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ value in the samples are ranged from -33.05‰ to -13.33‰, +1.69‰ to +9.27‰, and +7.83‰ to +8.50‰, respectively (Table 1).

Table 1. The carbon ($\delta^{13}C/^{12}C$), nitrogen ($\delta^{15}N/^{14}N$) and sulphur ($\delta^{34}S/^{32}S$) isotopic values of sources and mixture in the study

Sampling Locations	Stable Isotope Value (‰)		
	$\delta^{13}C/^{12}C$	$\delta^{15}N/^{14}N$	$\delta^{34}S/^{32}S$
Water canal	-13.33	8.79	8.27
Residential area	-25.63	6.60	7.83
Sluice gate	-30.94	7.40	8.45
Aquaculture area	-30.14	9.27	8.45
Landslide area	-29.42	7.63	8.22
Agriculture area	-33.05	2.23	8.18
Jetty	-28.33	8.98	8.46
River confluence area	-30.01	1.69	8.50
KSNP	-30.33	8.48	8.32

From the results, the samples collected from the residential area, sluice gate, aquaculture area, landslide area, agriculture area, jetty, river confluence area and KSNP are categorised as C3 plants since the $\delta^{13}C/^{12}C$ isotopic ratio are within –22 to −38 ‰ (Venkatesalu et al., 2008). The plant samples from water canal is categorised as C4 plants because the $\delta^{13}C/^{12}C$ isotopic ratio is within the range of –8 to −15 ‰ which is -13.33‰. Pollutants also are possible to cause the enrichment of ^{13}C isotope value. In certain cases, the ^{13}C signature of plant sample can be enriched by 2 ‰ in highly polluted area (Rikj and Ekblad, 2019).

Nitrogen stable isotope is very useful to be used as indicator to study nutrients derived from anthropogenic sources and it provide reliable information about the source of nutrients within coastal areas. From the results, aquaculture area shows the highest $\delta^{15}N/^{14}N$ value with 9.27 ‰. High $\delta^{15}N$ values arise due to the presence of excess nutrients in the environment allowing increased isotope fractionation via increased volatilisation of ammonia and increased microbial processing. Gritcan et al. (2016) reported that $\delta^{15}N$ values of around 10 ‰ are attributable to human and animal sewage, which is generally correlated with the presence of excess nutrients by eutrophication.

Sedimentary sulphate and sulphide minerals are the major sources of sulphur which available to plants. The results show that the $\delta^{34}S/^{32}S$ value of the samples are from +7.83‰ to +8.50‰. This result is in the range of – 50 to +10‰ indicates that the sulphur values are influenced by sedimentary sulphide. The sulphide in sediments is mainly derived from the products of sulphate-reducing bacteria, which are currently responsible for oxidising over half the organic matter flux reaching sediments. Pollution can cause strong depletion in $\delta^{34}S$ value in sulphide minerals that leads to the depletion of $\delta^{34}S$ value in plant (Tcherkez and Tea, 2013).

Source Contribution Rate Using IsoSource Software

The IsoSource software produced source proportion percentage of each location in the study that visualised possible pollution sources that contributed to KSNP area (Fig. 3).
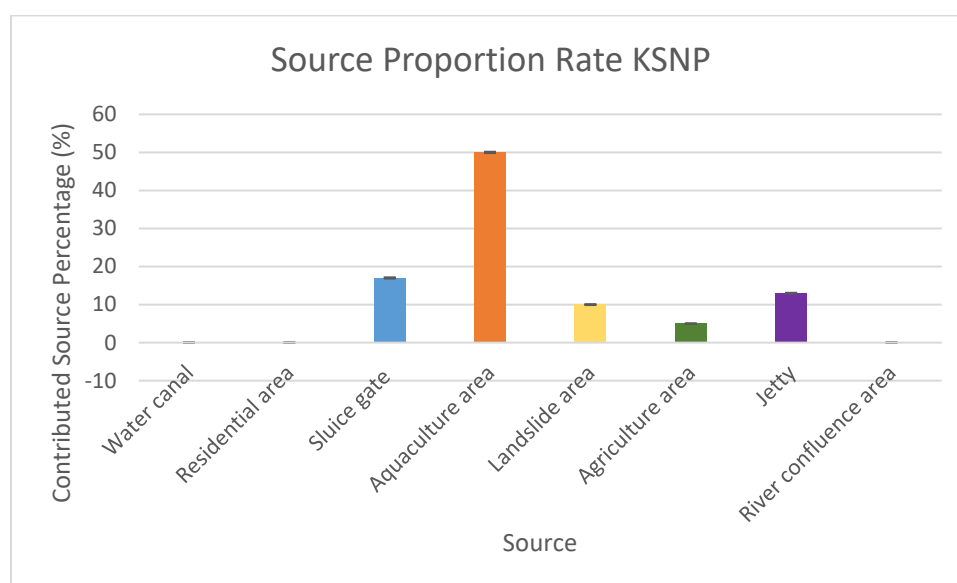

Figure 3. The contribution rate of the sources from different sampling locations in KSNP area

From the results, the highest proportion rate is from aquaculture area in the study with the value of 50%. The percent of frequency is shown in Fig. 4. The source is present between 0% and 65% and not present below 0% or more than 65%. The aquaculture area may be the main contributor to the pollution in KSNP is also supported by the high value of $\delta^{15}N$ at 9.27 ‰ which is due to the presence of excess nutrients that leads to increased volatilisation of ammonia and increased microbial processing. According to Singh et al. (2013), aquaculture and other anthropogenic activities such as illegal logging, land reclamation industrial and domestic use and pollutant runoff are threats to the mangroves.
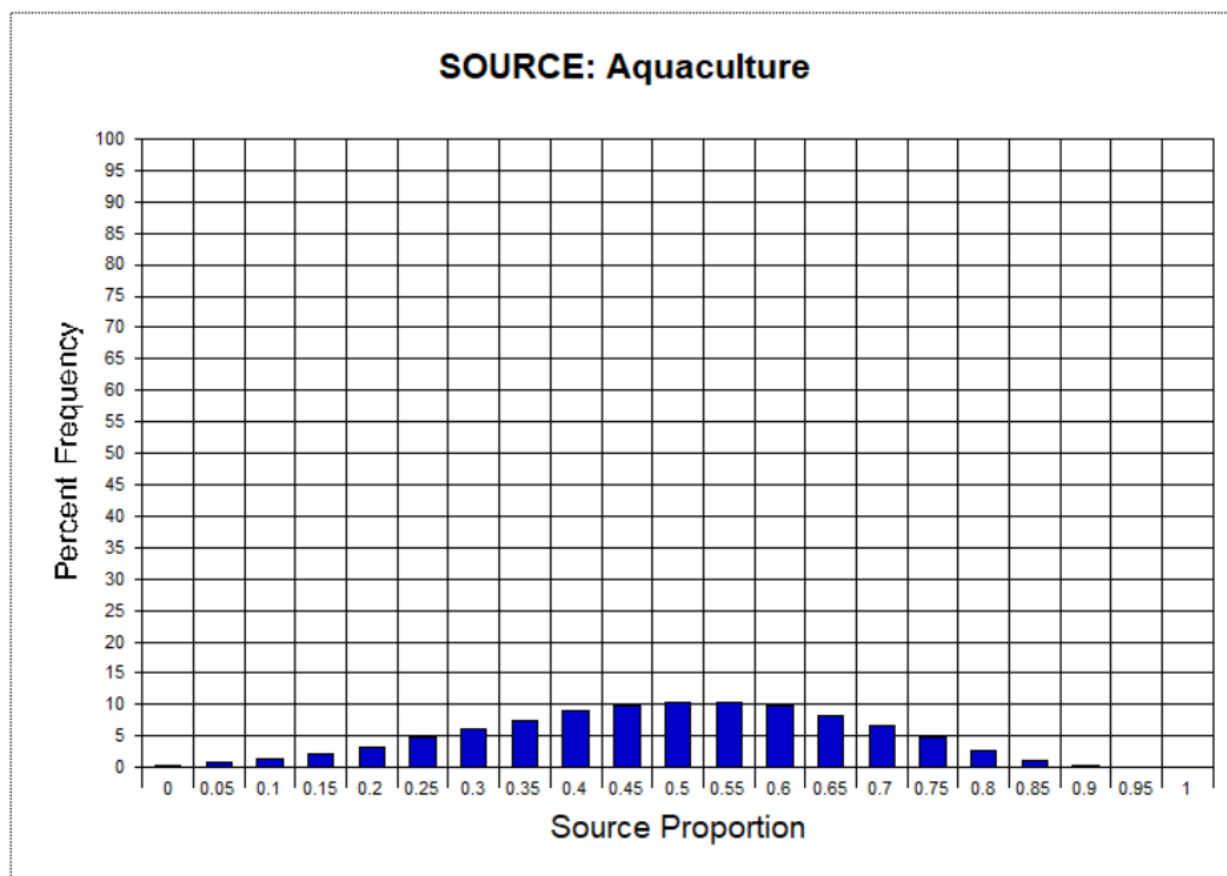
Figure 4. The most frequent proportion for aquaculture area is 50%

CONCLUSION

Mangroves can absorb N, C and S in coastal waters and its environment which are reflected in their tissue stable isotope values. Anthropogenically derived elements discharge into coastal waters, such as aquaculture runoff is possible to cause the major pollution problems in KSNP. The SIA is proven to be valuable method to determine source of pollution in an area. The pollution issues can potentially harm the flora and fauna species in the area and leads to the ecosystem imbalance. All the man-made activities should be controlled to ensure the sustainability of the ecosystem.

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