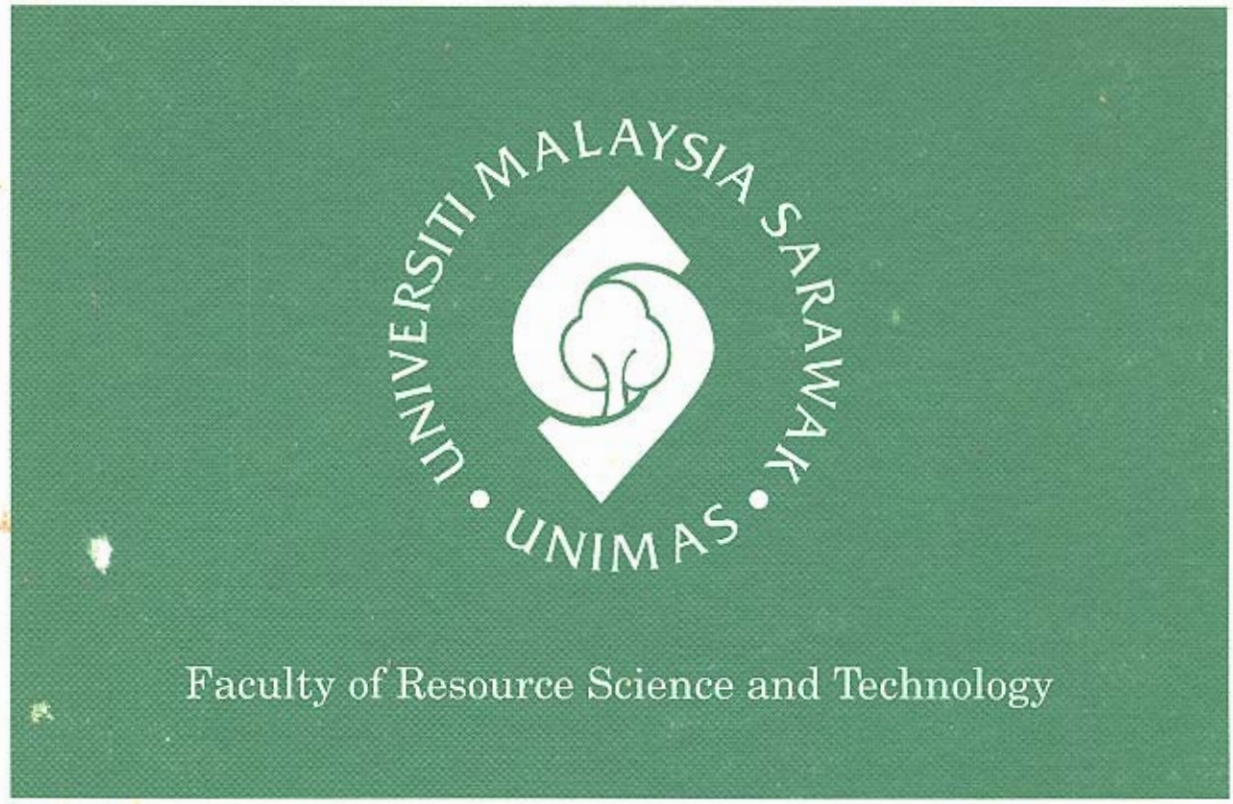

DISTRIBUTION OF INTERTIDAL NEMATODES ALONG THE COASTAL AREA OF THE WESTERN PART OF SARAWAK

Sarinah Binti Bujang

Bachelor of Science With Honours
(Animal Resource Science and Management)
2004

Faculty of Resource Science and Technology

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This project is submitted in partial fulfillment of
the requirements for the degree of Bachelor of Science with Honours
(Animal Resource Science and Management)

FACULTY OF RESOURCE SCIENCE AND TECHNOLOGY
UNIVERSITY MALAYSIA SARAWAK

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TABLE OF CONTENTS

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Sarinah binti Bujang

Animal Resource Science and Management
Faculty of Resource Science and Technology
University Malaysia Sarawak.

ABSTRACT

A study on the diversity of marine nematodes was conducted at the intertidal area of western part of Sarawak, Malaysia. The main objective of this study was to find genera composition of nematodes along the coastal area of western part of Sarawak. The approach was to perform a sampling of marine nematodes, measure physico-chemical and biological parameters of the water and sediment. Results obtained show that there are 27 genera of nematodes found in the samples. The most abundant genera in the study area were *Viscosia*, *Halalaimus*, *Daptonema*, *Monhystera* and *Anticoma*. The density of nematodes was approximately 91- 285 individual / 10cm^2. The basic data recorded in this study could be used in future pollution monitoring programme of the benthic environment at the intertidal area of western part of Sarawak.

Key words: Nematodes, physico-chemical parameters, community structure

ABSTRAK

Kajian kepelbagaian Nematoda marin telah dijalankan di kawasan perairan pantai barat Sarawak, Malaysia. Objektif utama kajian ini adalah untuk mendapatkan komposisi genus Nematoda di sepanjang kawasan perairan barat Sarawak. Pendekatan yang diambil ialah dengan melakukan persampelan Nematoda marin, mengukur parameter fiziko-kimia dan biologi terhadap air dan sedimen. Hasil kajian menunjukkan bahawa terdapat 27 genera Nematoda telah dijumpai di kawasan kajian. Genera yang paling dominan ialah Viscosia, Halalaimus, Daptonema, Monhystera and Anticoma. Nilai kepadatan Nematoda adalah sebanyak 91- 285 individual / 10cm^2. Kajian ini menjadi asas kepada kajian pemantauan pencemaran sekitaran bentik di perairan barat Sarawak pada masa akan datang.

Kata kunci: Nematoda, parameter fiziko-kimia, struktur komuniti

INTRODUCTION

Meiofauna is the preferred term to refer to the organisms that live interstitially (Nybakken, 1993). The Phylum Nematoda is one of the meiofauna that consists of small multicellular vermiform organisms which can be found in almost every conceivable environment (Platt & Warwick, 1988). Nematoda is the organisms that pass through a 500μm sieve but are retained on a 45 μm sieve. In marine sediments, nematodes are the most abundant animals (Higgins & Thiel, 1988).

Nematodes constitute a numerous and widely distributed group of fauna (Platonova & Gal'tsova, 1985). By virtue of their wide range of adaptations, marine nematodes have exploited all seashore and seabed habitats (Platt & Warwick, 1988). They are probably the most abundant multicellular animals alive today; nematode concentration of one million individuals per square meter are typically encountered in shallow-water sediments in both fresh water-and salt water, and concentration exceeding four million per square meter have been reported from some marine habitats (Pechenik, 2000).

Nematodes are distinguished from other organisms in that no clear-cut demarcation occurs between the head and the body (Platonova & Gal'tsova, 1985). Nematodes are nonsegmented, wormlike invertebrates lacking jointed appendages but possessing a body cavity and a complete alimentary tract (Thorp & Covich, 1991). Typically they are vermiform, bilaterally symmetrical pseudocoelomates, with four main longitudinal

epidermal cords, a triradiate pharynx, a circumenteric nerve ring, and no circulatory or respiratory organs (Bird & Bird, 1991).

Phylum Nematoda has two classes, containing about 185 families of pseudocoelomates and acoelomate worms (Pechenik, 2000). The main diagnostic characters are the presence of caudal glands (secreting a sticky fluid), bristles and conspicuous amphids (cephalic multifunction sense organs) in the majority of Adenophorea, being either absent or inconspicuous (amphids) in the Secernentea (Higgins & Thiel, 1988). A recent cladistic analysis using molecular data suggests that many of the morphological similarities among different nematode groups evolved through convergence, and that one of the two nematode classes, the Secernentea, may have evolved from within the other class, the Adenophorea (Pechenik, 2000).

The distribution and population dynamics of nematodes are influenced by the granulometric composition of sediment, size of capillary passages occupied by some species of nematodes, presence of sufficient quantity of interstitial water, effects of temperature, salinity, oxygen and others (Platonova & Gal'tsova, 1985). Temperature may have an indirect influence on the population dynamics of nematodes, for examples it may stimulate the development of growth of one or the other nutritional substance (Platonova & Gal'tsova, 1985). Many nematodes do not need a rich oxygen supply and may be regarded as facultative anaerobes (Higgins & Thiel, 1988).

Species richness and diversity vary among habitats, being greatest in sandy beaches with over 100 species being typical (Platt & Warwick, 1988). In muddy sites and in algal communities, the number of species is more typically in the range 30-70 (Platonova & Gal'tsova, 1985). In general, although seaweeds and low salinity mud have a relative low number of species, they may be dominated by only one or a few species whereas sand and high salinity mud may have more species, but none of them totally dominate the assemblage. According to Platt & Warwick (1983), the potential food items for nematodes include general organic detritus, decomposing organisms, bacteria, diatoms and other living organisms. Free-living nematodes may also have nascent commercial value as potential food sources in the aquaculture of some edible animals, such as penaeid shrimp and certain fish, and may soon be exploited as sensitive monitors of environmental contamination (Pechenik, 2000).

Nematodes play an important role as a bioindicator for polluted area (Moore & Bett, 1979). According to Tietjen (1977), there are relations between population density, composition and species diversity with physico-chemical factors within the study area. The information with the physico-chemical parameters is very important for indicating the area. Information on the marine nematode community in Sarawak is therefore very crucial before a marine pollution control programme is planned.

The objectives of this study were to investigate genera composition and distribution of nematodes in sediment. Apart from that, it was also aimed at studying the influence of physico-chemical parameters of the water on the nematode distribution and also to map

the distribution of nematodes along the coastal area of the western part of Sarawak. It is hoped that this study could serve as a basics for marine environment control programme in the future.

METHODOLOGY

Study Area

The study was conducted at an intertidal area of low tide level of the western part of Sarawak, from Tanjung Datu to Bako consisting of ten stations. The stations involved in this study are as shown in Table 1. The majority of the stations are sandy areas except Tanjung Melaban, which is a muddy area. The plants that dominate all of the areas are *Rhizophora, Pinus* and *Avicennie* sp.

Table 1: Location of the station

Number of Station	Name of Station	Coordinates (GPS Reading)
S1	Teluk Upas	109°39.205'E, 2°02.247'N
S2	Teluk Serabang	109°39.627'E, 1°59.149'N
S3	Siruk	109°44.050'E, 1°50.049'N
S4	Teluk Belungi	109°50.934'E, 1°46.527'N
S5	Sampadi	109°58.312'E, 1°42.289'N
S6	Rambongan	110°07.207'E, 1°42.037'N
S7	Santin	110°13.953'E, 1°42.480'N
S8	Bako	110°26.551'E, 1°43.429'N
S9	Moyan	110°36.053'E, 1°36.480'N
S10	Tanjung Melaban	110°47.178'E, 1°34.733'N

Field Sampling

Field sampling was conducted at ten stations (Figure1). All of the stations involve the intertidal area. Seven samples were taken at every station where three of the samples were for nematode analysis, two samples for chlorophyll *a* analysis, one sample for particle size analysis and one sample for total organic matter analysis. The physico-chemical parameters of the water were measured by using pH meter (model Jenway 3071), refractrometer (model Atago S-28) and dissolved oxygen meter (model Cyberscan DO 300 Series). The station was determined by using Global Positioning System (model Garmin GPS 12CX).

Perspex tube was used for sediment sampling at each station. The top 5cm of the sediment samples from the surface were taken. Samples taken for nematode analysis were preserved in 5% formalin and a drop of Rose Bengal.

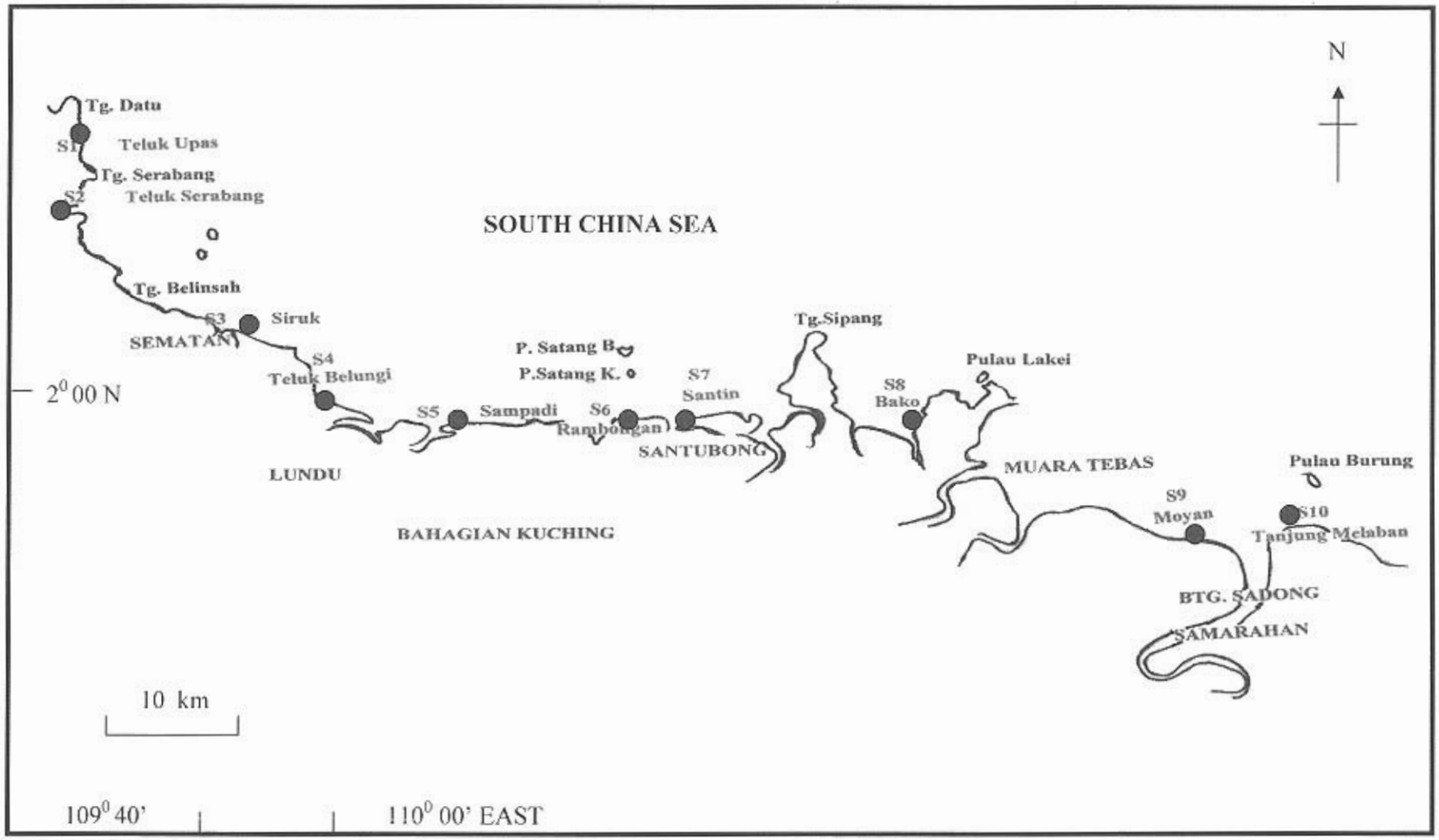

Figure 1: Ten stations in the study area along the western part of Sarawak.

Laboratory Process – Sediment Particle Size Analysis

For sediment particle size analysis, method used in determination of sand, silt and clay are the combination of dry and wet sieving (also known as pipette method), which is based on the settling rates of particle in distilled water column (Kilmer and Alexander, 1984). This method is applied with the following assumptions (adapted from Soil Survey Laboratory Handbook, 1992; Buchanan 1984). The principle of sedimentation analysis is simply that large particles will fall faster and farther then small particles through a column of distilled water in a given time.

Each of the samples was dried overnight in an oven at 60°C and 10.0g of the dried sample was weighed on an electronic balance (model Ohaus CT 200-S), and then were placed into a plastic flask in which 100ml of distilled water was added. The flask mouth was covered with a stopper and placed onto a mechanical rotator and left overnight to break up and separate any clumps and to separate, which may aggregate during drying process.

In order to remove sand fraction, the suspension was poured into a 50μm sieve. Funnel and 1 litre graduated cylinder were placed below the sieve to collect particles that can pass through sieve. The sediment retained on the 50μm sieve was washed to the edge of the sieve and slowly placed into a pre-weighed and labeled petri dish and dried.

The clay and silt fractions were determined by using pipette analysis. The solution was then diluted with distilled water to a volume of 1 litre and placed into a water bath at

26°C for 12-16 hours (overnight) to standardize the temperature of the solution. The solution was stirred and 25ml aliquots were then removed with a pipette based on the fixed time according to Stokes Law (Table 2). All the aliquots were placed into a pre-weighed and labeled petri dish then dried in oven at 105°C and weighed. Formula used in the calculating of fractions are as follows;

Percentage of clay = 100 X (RW_2) / TW

Where RW_2 = Sample dry weight (<2μm)

DV = Pipette volume

CF = 1000ml / DV

TW = sample total weight

Percentage of silt = (100 X (RW_{20} X CF) /TW)–Clay %

Where RW_{20} = Net weight / TW X 100

Percentage of Sand = Net weight / TW X 100

Coarse Silt % = 100 – (clay + fine silt+ sand) %

Table 2: Fixed time for pipette method according to Stokes Law.

Samples	Stirred	20μm	5μm	2μm
S1	0 min	4min 1sec	1h 4min 27sec	4h 30min
S2	2 min	7min 1sec	1h 7min 27sec	4h 33min
S3	8 min	13min 1sec	1h 13min 27sec	4h 39min
S4	14 min	19min 1sec	1h 19min 27sec	4h 45min
S5	20 min	25min 1sec	1h 25min 27sec	4h 51min
S6	26 min	31min 1sec	1h 31min 27sec	4h 57min
S7	32 min	37min 1sec	1h 37min 27sec	5h 3min
S8	38 min	43min 1sec	1h 43min 27sec	5h 9min
S9	44 min	49min 1sec	1h 49min 27sec	5h 15min
S10	50 min	55min 1sec	1h 55min 27sec	5h 21min

Note: h – hour, min – minute, sec – second.

Laboratory process – Total Organic Matter

Percentage of organic matter was determined by using method and outline according to Greiser & Faubel (1988). The analytical method involves drying the samples at low temperature, then combusting the organic content at high temperature. The temperatures for combustion should be about 450 to 500°C to avoid volatilizing bicarbonates (Greiser & Faubel, 1988). The weight change on combustion measured the organic content.

Sediment samples were removed from plastic bag and placed in a petri dish with the label and dried overnight in an oven at 60°C. Sediment samples then were grinded with pestle and mortar to break up clumps. The 0.5g sediment samples were put into a pre-weighed

crucible and burnt in the furnace (Model Felisa) ignited at 550°C for 8 hours. After the sample was sufficiently cool, they were removed from the furnace and placed in a desiccator (to prevent reabsorption of water). The sediment sample was reweighed after the crucible cooled down. The weight loss is the amount of organic matter.

Laboratory Process - Chlorophyll *a* Analysis

Chlorophyll *a* analysis from the sediment was based on Wasmund (1984). The sediment sample (1cm sediment on surface) with a known weight and water content was put into a mortar and homogenized by grinding and after that 5-10ml of 90% acetone was added. The sample was than homogenized again. Homogenized processes were done in a polystyrene box containing ice to maintain a cool condition in order to minimize evaporation of acetone solution. Suspensions were then transferred into a centrifuge tube and centrifuged at 4000 rpm for 30 minutes by using a centrifuge machine (model Kubota 8800).

Due to more complete pigment extractions, sediment samples were stored overnight in the dark prior to centrifugations. Spectrophotometer (model Secomam PRIM Light & Advanced) measured the optical density of the acetone extract before and after acidifications. Supernatants were decants into a cuvet and extinctions were measured at wavelengths given in equation below using uv-spectrophotometer (model Secomam PRIM Light & Advanced). For pheophytin determination, HCl were added to 1.5ml of extract volume and absorbance at 665nm was determined before and after acidification.

Turbidity blank was measured at 750nm. All values from 665, 664, 647 and 630 were subtracted from the blank value before calculating the pigment content.

Chlorophyll *a* was calculated using the following formula

$$\text{Chlorophyll } a \text{ (mg/m}^3\text{)} = \frac{26.7\,(E_0 - E_a) \times V}{Vs \times L}$$

Eo = absorbance before acidification at 665nm

Ea = absorbance after acidification at 665nm

V = Volume of water content of the samples plus acetone added

Vs = Volume of sediment sample

L = Path length (cm) of the spectrophotometer cell.

Laboratory Process - Nematode Extraction

Nematodes were extracted from the sediment taken by using sieving combination technique as recommended by Shabdin (1998). As for the sieving method, the preserved samples were washed through sieves of 500 μm and 45 μm by using tap water. Nematodes that were retained on 45 μm sieve concentrated by washing it to the edge of sieve and then washed into a grid petri dish. These specimens were then placed under a stereomicroscope for sorting, identification and enumeration (McIntyre & Warwick, 1984; Lardicci *et al.*, 1999). In slide preparation for nematodes, glycerol was used. The nematodes structure has been draw by using compound microscope (model Zeiss

Axioskop 50). Then the drawing was identified by using the keys in the literature of Platt & Warwick (1998).

Data Analysis

The species diversity (H') and the species evenness (J') of nematodes were measured using Shannon-Wiener Index (Krebs, 1989). The species richness was also calculated. The formulas used are as follows:

$$H' = - \Sigma (Pi) (\log 2 Pi)$$

$$J' = H' / \log_2 S$$

$$SR = (S-1) / \text{Ln } N$$

Where; S is the total of species

N is the total of individual

*P*i is the proportion of total sample belonging to *i*th species.

The correlation between physico-chemical parameters and nematode was carried out by using Statistical Packages for Social Science (SPSS).

RESULTS

Physico-chemical Parameter

The salinity of all stations involved in this study was found to be almost similar with one another. The highest salinity was at Station 1 (Teluk Upas) with a value 36 psu while the lowest salinity was at Station 10 (Tanjung Melaban) with a value 28 psu (Table 3). The difference of the maximum and minimum value of salinity is 8. There was no obvious difference in salinity among the stations.

The maximum value for temperature was at Station 6 (Rambongan) and the minimum value for temperature was at Station 7 (Santin). The maximum value was 33.1 °C and the minimum value was 27.4 °C (Table 3). The difference of the maximum and minimum value of temperature is 5.7.

The difference in the dissolved oxygen value among the stations was not obvious. The maximum value for dissolved oxygen was at Station 7 (Santin) with the value 8.65 mg/l and the lowest dissolved oxygen was at Station 9 (Moyan) with the value 6.63 mg/l (Table 3). The difference of the maximum and minimum value of dissolved oxygen is 2.02.

The difference between the maximum and minimum value of pH is 0.12 where the maximum pH value was 7.18 at Station 1(Teluk Upas) and the minimum pH was 7.06 at

Station 2 (Teluk Serabang) (Table 3). There was no obvious difference in pH value among the station because all of the values are within 7.06 -7.18.

Table 3: Physico-chemical parameters

Station	Salinity (psu)	Temperature (°C)	Dissolved Oxygen(mg/l)	pH
S1	36	28.10	8.62	7.18
S2	32	29.50	6.91	7.06
S3	30	31.20	7.96	7.17
S4	34	28.50	8.25	7.15
S5	32	30.80	8.33	7.16
S6	33	33.10	7.57	7.15
S7	32	27.40	8.65	7.13
S8	30	32.70	7.29	7.15
S9	31	30.40	6.63	7.12
S10	28	29.10	8.25	7.07

Notes: psu - practical salinity unit

Sediment Particle Size

The minimum percentage of sand (>50μm) was at Station 10 (4.5%) and the maximum was at Station 3 (99.7%) (Table 4). This was due to the condition of Station 10 that was a muddy area compared to other stations that were sandy area. The percentage of clay (2μm) with the minimum value was both at Station 3 and Station 6 (0%) while the maximum percentage was at Station 10 (28.0%). For the percentages of silt (5μm), the minimum percentage was at Station 3 (0.3%) and the maximum percentage was at Station

10 (67.5%). All stations were sandy except Station 10 that was muddy due to the percentage of sand which was more than 88% (Figure 2).

Table 4: Sediment Particle Size

Station	Sand (%)	Clay (%)	Silt (%)
S1	97.9	0.7	1.4
S2	98.3	0.9	0.8
S3	99.7	0	0.3
S4	97.3	0.6	2.1
S5	97.0	1.2	1.8
S6	97.5	0	2.5
S7	96.6	0.4	3.0
S8	97.2	0.8	2.0
S9	88.2	10	1.8
S10	4.5	28	67.5

Notes: sand= (>50µm); clay= (2µm), silt= (5µm)

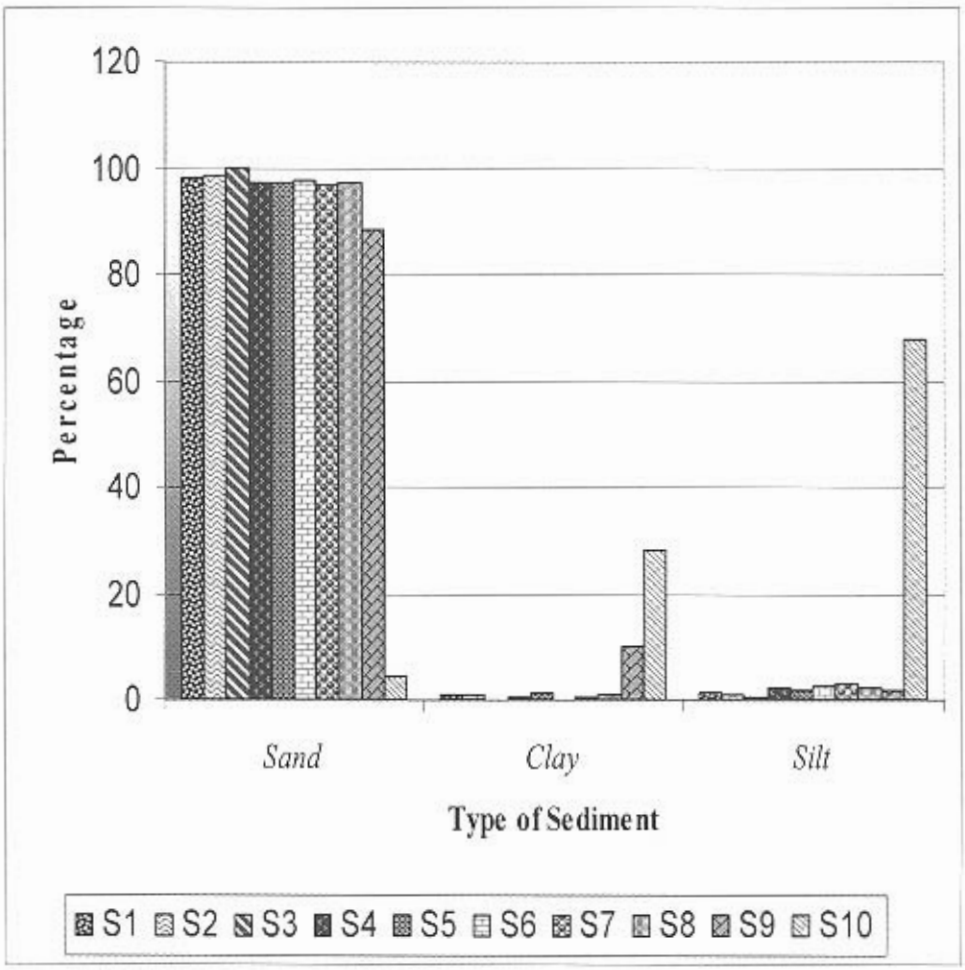

Figure 2: The percentage of sediment structure for every station.

Total Organic Matter

The maximum amount of organic matter was found at Station 10 (0.0876 g/g sediment) and the minimum amount of organic matter was at Station 1 (0.0101 g/g sediment) (Table 5). The difference of maximum and minimum value of Total Organic Matter is 0.0775. There was no obvious difference in organic matter among the stations.

Chlorophyll *a*

As shown in Table 5, the maximum value of chlorophyll *a* was at Station 4 (0.1907 mg/m^3) and the minimum value was at Station 7 (0.0153 mg/m^3). The difference of maximum and minimum value of chlorophyll *a* was 0.1754. The value of chlorophyll *a* among the station was inconsistent.

Table 5: Total Organic Matter and Chlorophyll *a*

Station	Total Organic Matter (g/g sediment)	Chlorophyll *a* (mg/m^3)
S1	0.0101	0.0305
S2	0.0588	0.1831
S3	0.0706	0.1373
S4	0.0123	0.1907
S5	0.0117	0.0229
S6	0.0150	0.0839
S7	0.0151	0.0153
S8	0.0258	0.1068
S9	0.0166	0.1030
S10	0.0876	0.0610

Nematode Species Composition

A total of 27 genera of nematodes have been found in the study area (Appendix 1). There are *Prochaetosoma, Metoncholaimus, Viscosia, Rhynchonema, Anoplostoma, Halalaimus, Monhystera, Calligyrus, Daptonema, Greeffiella, Anticoma, Spirophorella, Ptycholaimellus, Marylynnia, Aponema, Paracanthonchus, Chromadorita, Tricoma, Theristus, Xyzzors, Comesoma, Thallassironus, Haliplectus, Sphaerolaimus, Sabatiera, Stylotheristus* and Alpha (unidentified genus).

Figure 3 shows the percentage of nematodes at Station 1. The dominant genera found at Station 1 are *Monhystera* and *Viscosia*, each consisting of 15% of the total number. This was followed by *Halalaimus* (14%), *Metoncholaimus* (14%), *Prochaetosoma* (13%), *Rhynconema* (12%), *Anoplostoma* (10%) and *Calligyrus* (7%).

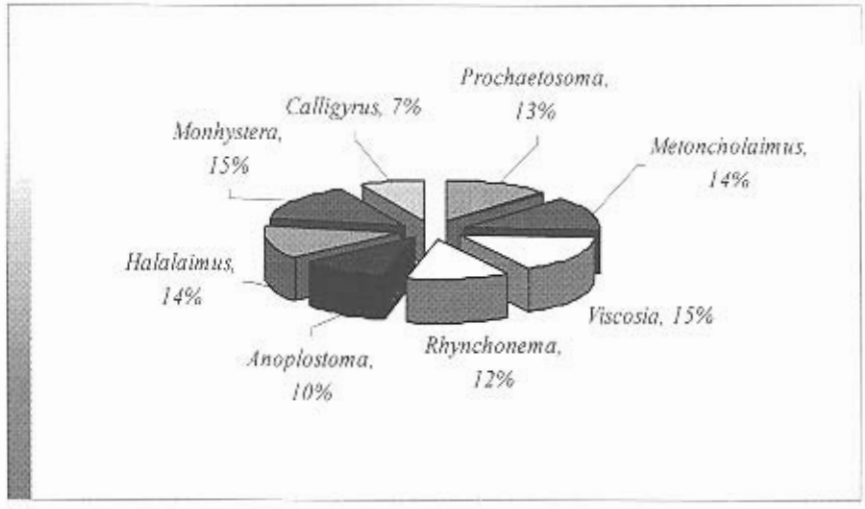

Figure 3: The percentage of genera at Station 1.