Structural Measurements of *Sipunculus spp.* and the Physical and Chemical Characteristics of Its Habitat in Selected Coastal Areas in Negros Occidental

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"STRUCTURAL MEASUREMENTS OF *Sipunculan spp.* AND THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF ITS HABITAT IN SELECTED COASTAL AREAS IN NEGROS OCCIDENTAL"

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ABSTRACT

Sipunculans are less known marine worms and few studies have been conducted on it in the Philippines.

This study aims to describe the physico-chemical characteristics of *Sipunculan* habitat and their structural measurements in Barangays Sicaba, Cadiz City, Balaring, Silay City, and Capayawan, Cadiz Viejo, Negros Occidental.

Soil and water samples were collected from the subtidal zone. The soil was tested for sulfur, iron and available phosphorous, pH and organic matter while the pore water was tested for nitrite, phosphate, ammonia and pH and the surface water for Fecal Coliform and dissolved oxygen (DO).

Sicaba soil has 3.199411±0.217ppm of phosphorus, 22.62931±1.003ppm of iron, 190.7016±2.606ppm of sulfur, a pH of 7.75, and 2.47% of organic matter. Balaring soil has 3.142617±0.045ppm of phosphorus, 19.92555±1.912ppm of iron, 200.4758±2.475 of sulfur, a pH of 7.92, and 2.62% of organic matter. Capayawan soil has 3.331931±.381ppm of phosphorus, 26.20298±0.870ppm of iron, 224.5161±3.536ppm of sulfur, a pH of 8.08, and 1.28% of organic matter.

Sicaba water has 460MPN per 100mL of fecal coliform and a DO of 1.78. Balaring water has ≥2,400 MPN per 100mL of fecal coliform and a DO of 2.08. Capayawan has ≥2,400 MPN per 100mL of fecal coliformand a DO of 2.68.

Sicaba worms are about 28.5833±2.22g, 19.9542±1.30 cm long and has a circumference of about 3.7667±0.22cm. Balaring worms are about 146.25±8.02g, 23.583±1.72cm long and has a circumference of about 6.5625±0.21cm. Capayawan worms are about 174.25±10.88g, 46.68±2.72cm long and has a circumference of about 7.55±0.22cm.

The worms in Sicaba, Capayawan and Balaring live in a slightly basic environment with high levels of phosphorus, iron, nitrite, phosphate, ammonia and low levels of sulfur. Dissolved oxygen is low with high levels of fecal coliform and sufficient amount of organic matter.

The worms from Capayawan are relatively longer, heavier, and thicker than those in the other two sites.

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CHAPTER 1

INTRODUCTION TO THE STUDY

A. Background of the Study

The phylum *Sipuncula,* are bilaterally symmetrical, unsegmented, coelomate worms with cylindrical or sac-like body and a muscular introvert which is retractable. The phylum is divided into four families namely, *Sipunculidae, Golfingiidae, Asphonosiphonidae,* and *Phascolosomatidae.* (*Richmond)*

Sipunculids are found at all depths in all seas, feeding off bottom detritus. While some burrow into sand and mud, others live in crevices in rocks, or in empty shells. Still others bore into rock. Like any other aquatic worms, they eat algae, diatoms or miscellaneous plant and animal detritus. They swallow surrounding sand to digest bits of animal and plant tissue.

About 320 species have been formally described, all marine and mostly from shallow water. They are the best examined sipunculan species, used as model organism for anatomy, physiology, biochemistry, and ecology. They are also used as bait in some parts of the world.

Sipunculus nudus is one species of the phylum *Sipuncula* and these worms are found to be striving in the shores of Brgy. Balaring, Silay, Negros Occidental. They are locally known as *Sasing* and are used as an occasional delicacy by the people. In Vietnam, Sa sung or sea worm is fried and is consumed as drinking snack. It is also used as an ingredient that gives a special flavor to the Hanoian soup pho. *Sasing* is harvested in sub tidal or sub littoral zone, the area of the shore exposed during low tide, which according to the natives are 200 meters away from the shore line.

Aside from its wonderful taste, the said worm is rich in proteins and calcium, and is cleaner compared to the usual crustaceans that we eat (*Ang-Lopez 2007*). *Sasing* contains 24.44 mg of calcium per 100 g sample. Its calcium content is comparable to that in Spanish mackerel. It contains 6.95 mg iron per 100 g sample. The iron content in *Sasing* is comparable to the oyster, and is much lower than in mussel.

Other than Brgy. Balaring, the worms are said to be found also in Sicaba, Cadiz City, Negros Occidental and in Capayawan, Cadiz Viejo, Negros Occidental. These worms are also used as delicacies and as feeds for other marine organisms. The descriptions of the worms by natives of the places are quite similar but are not exactly the same.

The uncertainties on the species of the worms from the three mentioned places and the reason why certain species of worms are only found in some places and not in other places gave an open topic for discussion and a problem to be solved.

This study aimed to determine the physical and chemical factors that are present in the sand and water of Brgy. Balaring, Capayawan, and Sicaba through soil and pore water analysis. This study also aimed to know the exact *Sipunculan* species thriving in the three places and to determine the average diameter, length, weight and color of the worms.

B. Statement of the Problem

This study described the structural measurements of *Sipunculus spp.* and the physical and chemical characteristics of sand and water in its habitat in selected areas in Negros Occidental.

Objectives of the Study

This study aimed to identify the physical and chemical characteristics of the habitats of *Sipunculus spp.* and to describe the structural measurements of the different *Sipunculus spp.* from selected coastal areas in Negros Occidental.

This study specifically aimed to:

1.) Determine the sand's: (a) sulfur concentration, (b) total phosphorus, (c) available iron, (d) pH, (e) amount of organic matter, and (f) particle size;

2.) Determine the pore water's (a) ammonia concentration, (b) nitrite-nitrogen concentration, (c) phosphate concentration, and (d) pH

3.) Determine the surface water's: (a) amount of Fecal Coliform and (b) amount of dissolved oxygen.

4.) This study also aimed to describe the morphological structure of the worm from each of the places by measuring its (a) length, (b) diameter, and (c) weight.

D. Significance of the Study

Sipunculus spp. has been found to be existent in some coastal areas in Negros Occidental. The implementation of the soil and water analysis and the interpretation of the results might provide the basic information on their growth requirement or existence. The description of the species composition and morphological characteristics of *Sipunculus spp.* might give additional knowledge on the differences of different species living in different coastal areas.

Basically, this study might give more information on the marine worm species in addition to the little known existing studies on them and might also provide baseline information on their habitat requirements for their growth for future related studies.

E. Scope and Delimitation

This study focused on the analyzing of the physical characteristics of the sand and water from the different places limited only in Western Visayas which include Brgy. Balaring, Silay, Negros Occidental; Sicaba, Cadiz, Negros Occidental; Brgy. These places were chosen based on the presence of the sipunculan worm.

The chemical analyses for the sand are sulfur, total phosphorus, and available iron; while the analyses for the pore water are ammonia, nitrate-nitrite, and phosphorus. These analyses were chosen because these are the only available analyses for sand and pore water in SEAFDEC, where we submitted the samples.

The physical analyses for the soil include pH, organic matter, and particle size; while for the water includes pH, and fecal coliform. These physical analyses were performed in the Philippine Science High School Microbiology Laboratory.

F. Definition of Terms

Marine worms - common name for annelids

Sipuncula - phylum of an aquatic worms that usually lives near the coast

pH - a measure of acidity or alkalinity in which the pH of pure water is 7, with lower numbers indicating acidity and higher numbers indicating alkalinity. (Encarta 2007)

- in this study pH refers to the reading of the pH meter after the glass electrode of the meter is dipped into the water suspension of 1:1 ratio.

Sulfur concentration – amount of a nonmetallic yellow element that occurs alone in nature or combined in sulfide and sulfate minerals. (Microsoft ® Encarta, 2006)

- in this study sulfur concentration is computed based on the readings after the sample is placed into the spectrophotometer

Total Phosphorus - the amount of phosphorus, symbol P, reactive nonmetallic element that is important to living organisms and has many industrial uses. (Microsoft ® Encarta, 2006)

- in this study Total Phosphorous is computed based on the readings after the sample is placed into the spectrophotometer

BOD-Biochemical Oxygen Demand - measures the rate of consumption of oxygen by organisms in the water over a 5 day period. Increases in BOD can be due to animal and crop wastes and domestic sewage. *Biochemical Oxygen Demand* is a common, environmental procedure for determining the extent to which oxygen within a sample can support microbial life. (Magenis, 1994)

- in this study BOD is the reading of the DO meter after the probe is dipped into the body of water

Shoreline - edge where water meets shore: the land where a body of water, especially the ocean, meets the shore (Microsoft® Encarta® 2006)

- the point where high tide meets the shore.

Fecal Coliform Test - a test for water that will identify the presence of E. coli in water.

- in this study Fecal Coliform is estimated using a table after broth with sample has been incubated for 24 hours.

Iron - is the measure of a magnetic malleable, silver white metallic element, which has atomic number 26 and is a transition element. It occurs naturally and is the fourth most abundant element on the earth's crust. (Microsoft® Encarta® 2006)

- in this study iron concentration is computed based on the readings after the sample is placed into the spectrophotometer

Organic matter - is matter that come from a recently living organism that is capable of decay or is composed of organic compounds. Mainly composes the soil. It is made up of decaying plants and animals as well as living organisms. (Winkeypedia)

- in this study this is computed after sand was heated for 2 hours at 150C weighed heated again at 360 C.

Nitrite-nitrogen concentration – are esters of nitrous acids and contain the nitrosooxy function group. (Winkeypedia)

- in this study nitrite concentration is computed based on the readings after the sample is placed into the spectrophotometer

Ammonia concentration – is the measure of a colorless, pungent gas, NH_3, which is highly soluble in water. A saturated aqueous (water) solution of ammonia contains 45 percent ammonia by weight at 0° C (32° F) and 30 percent at ordinary room temperatures. On solution in water, ammonia becomes ammonium hydroxide, NH_4OH, which is strongly basic and similar in chemical behavior to the hydroxides of the alkali metals.

- in this study ammonia concentration is computed based on the readings after the sample is placed into the spectrophotometer

Phosphate concentration – is the measure of a chemical, formula H_3PO_4, common acid of phosphorus that is the source of industrially important compounds called phosphates. They are used in the production of detergents fertilizers and water softeners

- in this study ammonia concentration is computed based on the readings after the sample is placed into the spectrophotometer

CHAPTER 2

REVIEW OF RELATED LITERATURE

This chapter is composed of the following topics: a.) The phylum *Sipuncula,* b.)its four divisions, and some common species. This also includes c.) sand and pore water, as well as d.) soil and water analysis with the corresponding subtopics.

A. *Sipuncula*

The *Sipuncula* are bilaterally symmetrical, unsegmented, coelomate worms with a cylindrical or sac-like body, or trunk, and a muscular introvert which is completely retractable, unlike the echiuran proboscis. The mouth opens at the tip of the introvert, usually associated with tentacles. Sipunculids are found at all depths in all seas, feeding off bottom detritus. (*Richmond)*

The body is completely unsegmented, and the intestine forms a twisted loop, with the anus on the side of the body. Typical of sipunculans is a forward (anterior) body section, the introvert, which can be retracted into the body by the retractor muscles. At the tip of the introvert (retracted in the specimen shown at the top of this page) is the mouth, which is surrounded by a ring of tentacles. The body cavity, or ceolom, of sipunculans is large and filled with fluid, in which are found free-floating cells known as hemerythrocytes as well as free-floating clusters of cells known as ciliary urns. Sipunculans have no circulatory or respiratory systems; the coelomic fluid transports both nutrients and oxygen to all parts of the body. Nephridia filter the coelomic fluid.

Sipunculans are sometimes thought to be a group of annelids, and have been classified within them. However, this phylogenetic hypothesis is contradicted by comparing the anatomy of annelids, sipunculans, and other animals. For instance, sipunculans have no trace of important annelid characters such as segmentation and chaetae (bristles). The characters they do share with annelids are not restricted to annelids and sipunculans, but are much more general.

An alternative hypothesis places the sipunculans closer to the mollusks -- the snails, clams, squids, octopuses, and so on. This seems unusual at first glance; sipunculans do not look anything like most mollusks, at least outwardly. However, sipunculans and mollusks share several characters in early development; for example, after fertilization and division of eggs, both mollusks and sipunculans have a characteristic arrangement of cells in the embryo, known as the "molluscan cross."

A third hypothesis considers the characters shared by mollusks and sipunculans to be primitive characters for a larger group known as the Trochozoa, which also includes the annelids.

Sipunculans have an extremely sparse fossil record -- with one possible exception. A group of fossils known as hyoliths is found in rocks through much of the Paleozoic. Hyoliths are conical shells; each conical shell bears a hinged "lid", or operculum, covering the opening. While these shells somewhat resemble molluscan shells, a few fossil hyoliths have been found that show traces of the intestine -- and the hyolith intestine is looped and coiled, much like that of living sipunculans. A few living sipunculans secrete a calcified cuticular plate, the anal shield, so it is not impossible that past relatives of the sipunculans secreted more extensive shells. (American Zoologist 1995)

The phylum is divided into four families and about 320 species are described.

A.1. *Sipunculidae*

The family *Sipunculidae* is made up of mostly large species. Usually with integumentary canals or coelomic sacs, four introvert retractor muscles and two nephridia. Species of the genera *Sipunculus* and *Siphonosoma* are common inhabitants of the eulittoral or shallow seas.

Sipunculus indicus

This worm has a trunk to 50 cm, 10-15 mm diameter with a swollen posterior, pinkish-white in color. Introvert to 3 cm, lacking hooks but covered with triangular, scale-like papillae. From 39 to 43 longitudinal muscle bands; two pairs of short retractor muscles originate just behind the nephridiophores. Spindle muscles are strong and unattached posteriorly. The nephridia are thin, long and fastened to body wall with the opening behind the anus. It is found in coarse sand in lower eulittoral. (*Richmond)*

Sipunculus nudus

Its trunk is 30 cm and is pearly-gray in color. Its introvert is short, lacking hooks and covered with triangular, scale-like papillae. From 28-33 longitudinal muscle bands; ventral pair of retractor muscles originates from muscle bands 2-5, dorsal pair originate from bands 9-12. Spindle muscle arises before the anus and is unattached posteriorly; rectal caecum is small or elongated; some 15 intestinal whorls; two contractile vessels without villi. Its habitat is in coarse sand in lower eulittoral and the distribution is worldwide in temperate and tropical seas.

They are 81.57 % moisture, 14.29% crude protein, 0.14% crude fat, 1.63% ash. They contain 24.44 mg of calcium/100 g sample. The calcium content in *S. nudus* is comparable to that in Spanish mackerel. They contains 6.95mg iron/100 g sample. The iron content in them is comparable to the oyster, much lower than in mussel.

Siphonosoma cumanense cumanense

Its trunk is up to 25 cm and is 7-11 mm in diameter. Its body is opaque with smooth skin, variable in texture and color which is often pinkish. The introvert is 1/3 of body length and is bearing 20-25 long tentacles, no scale-like papillae or hooks. From 18-24 longitudinal muscle bands with short retractor muscles, with ventral pair originating from muscle bands 2-3 and dorsal pair form band eight. The nephridia open well before the anus. It has transverse layers of peritoneum. It lives on muddy sand in the upper eulittoral and its distribution is pantropical.

Siphonosoma australe

Its trunk is up to 25 cm with straw to dark brown in color which is sometimes mottled. The papillae on the introvert are larger than on the trunk with numerous hooks (55-60 rows). There are 15-20 externally visible longitudinal muscles. The ventral retractor muscles originate 1/3 down the trunk and the weaker dorsals arise near the anus. The nephridia are not attached with the opening just before the anus. Its habitat is on muddy sand in upper eulittoral.

A.2. *Golfingiidae*

The family *Golfingiidae* have a mouth surrounded basically by tentacles and may be finger-like, leaf-like or branched, sometimes with only a few lobes altogether. The ring of the tentacles is usually broken mid-dorsally by a sensory lobe. There are no coelomic or integumentary sacs. The musculature of the body wall is longitudinal and continuous. There is ni anterior shield. The species under this family are the following:

Golfingia hespera
G. semperi
Phascolion robertsoni
Themiste robertsoni

A.3. *Asphonosiphonidae*

These are small worms with a horny or calcareous cap at anterior end of trunk, sometimes also with a caudal shield. Tentacles surround the mouth either in a complete crown or incompletely, but not in a horseshoe-shaped ring dorsal to mouth. The longitudinal muscle of the body wall may be divided in bands or be continuous. The species under this family are the following:

Cloesiphon aspergillus
Aspidosiphon elegans elegans

A.4. *Phascolosomatidae*

The tentacles is in a horseshoe-shaped ring which does not surround the mouth but lies dorsal to mouth, enclosing the sensory lobe. The longitudinal muscles of the body wall forms bands in all except one genus. The skin with conical to hemispherical papillae is often very prominent at both ends of the trunk. There are no anal or caudal shields. The species under this family are the following:

Phascolosoma pacificum
P. evisceratum
P. granulatum
P. scolops

B. Sand

Sand is a loose, incoherent mass of mineral materials in a finely granular condition, usually consisting of quartz (silica), with a small proportion of mica, feldspar, magnetite, and other resistant minerals. It is the product of the chemical and mechanical disintegration of rocks under the influences of weathering and abrasion. When freshly formed the particles are usually angular and sharply pointed, becoming smaller and more rounded by attrition by the wind or by water.

Sand is an important constituent of most soils and is extremely abundant as a surface deposit along the courses of rivers, on the shores of lakes and the sea, and in arid regions. One specific form of sand is the major ingredient in glassmaking. Other types of sand are used in foundries to make casting molds and in ceramics, plasters, and cements.

Sand is used as a grinding and polishing abrasive in the form of sandpaper, which is a sheet of paper covered on one side with sand or a similar abrasive substance. Sandblasting is an important technique used for cleaning stone or for smoothing rough metal surfaces by blowing a stream of sand under air or steam pressure. (Microsoft Encarta, 2005)

C. Interstitial or Pore Water

Sediment interstitial water, or pore water, is defined as the water occupying the spaces between sediment particles. Interstitial water might occupy about 50% (or more) of the volume of a depositional sediment. The interstitial water is in contact with sediment surfaces for relatively long periods of time and therefore, might become contaminated due to partitioning of the contaminants from the surrounding sediments. In addition, interstitial waters might reflect ground water – surface water transition zones in upwelling or down welling areas. In these areas their chemistry might be more reflective of ground or surface waters at the site. Therefore, flow, residence time and other physicochemical factors (e.g., pH, temperature, redox potential, organic carbon, sulfides, carbonates, mineralogy) might have varying roles in determining whether interstitial waters are contaminated.

In many depositional types of sediment, interstitial waters are relatively static, and therefore contaminants in the interstitial water and in the solid phase are expected to be at thermodynamic equilibrium. This makes interstitial waters useful for assessing contaminant levels and associated toxicity. Interstitial water is often isolated to provide either a matrix for toxicity testing and/or to provide an indication of the concentration and/or partitioning of contaminants within the sediment matrix. (U.S. Environmental Protection Agency Washington, 2001)

D. Soil Analysis

Soil is very complex. It is made up of ground-up rock mineral particles of varying sizes, dead organic material, and living organisms. Various tests can be conducted to study these components. The colorimetric analysis for organic material uses chemical reactions involving, among other things, dichromate ion, which forms colored complexes. The amount of color can be read with a spectrophotometer (spectro = the spectrum; photo = light; meter = to measure) and related to the amount of organic material in the soil. The more organic material there is in the soil, the more dichromate it uses up and the less dichromate there is left to form the colored complex, thus the lower the absorbance reading.

The mechanical analysis can give an idea of what soil type the particular soil being tested is. In a water suspension, heavier particles of sand and silt settle out quickly while clay, with smaller, lighter particles, stays in suspension for quite some time. Thus these two fractions can be separated. Sand can be graded into various sizes: Diameter very coarse 2.00 to 1.00 mm coarse 1.00 to 0.50 mm (500 :m) medium-grained 0.50 to 0.25 mm (250 :m)fine 0.25 to 0.10 mm (100 :m) very fine 0.10 to 0.05 mm (50 :m)

D.1. Soil pH

The soil pH measures active soil acidity or alkalinity. A pH of 7.0 is neutral. Values lower than 7.0 are acid; values higher are alkaline. Usually the most desirable pH range for mineral soils is 6.0 to 7.0 and for organic soils 5.0 to 5.5. The soil pH is the value that should be maintained in the pH range most desirable for the crop to be grown or for the organisms to live. A pH between 6.0 to 7.0 is a Good pH for worms. pH Over 7.0 or under 6.0 can be dangerous for composting worms like Red Worms, European Night Crawlers and other composting worms. The higher the pH the more alkaline the bedding is. The lower the pH the more acidic. It is best to have bedding that is right in the middle. 6.5 is the perfect pH.(Worm man's 2009)

D.2. Phosphorus

Phosphorus, symbol P, reactive nonmetallic element that is important to living organisms and has many industrial uses. The atomic number of phosphorus is 15, and its atomic weight is 30.974. Phosphorus is in group 15 (or Va) of the periodic table.

Phosphorus is widely distributed in nature and ranks 11th in abundance among the elements in Earth's crust. It does not occur in the free state but is found mostly as a phosphate, as in phosphate rock and apatite. It is also found in the combined state in all fertile soil and in many natural waters. The element is important in plant and animal physiology and is a constituent of all animal bones, in the form of calcium phosphate. (Microsoft ® Encarta, 2007)

D.3. Sulfur

It is another situation with phosphorus compared to nitrogen because of the chemical behaviour of this element. In the relevant media phosphorus normally occurs in the fifth oxidation level, that means as phosphate or deriving compounds. The organically fixed part plays a rather subdominant role. In dependency from the pretreatment of the material to examine and the concentration to phosphate the respective compounds are easily, less easily to slightly soluble. To record the single fractions respective gradual methods including a drastical digestion have to be existent; the latter to determine the total phosphorus content. In contrary to nitrogen you can speak about a certain phosphorus circulation. But this is much less dynamical and finally includes much longer periods.

Furthermore this circulation is determined through completely different chemical and biochemical processes. In phosphorus sinks e.g. such as lakes and coast waters the proportion between the phosphorus content of the sediment and the dissolved phosphorus in the water is a very broad with high contents in the sediment. In an oligotrophic body of water the biomass production is normally restricted by phosphorus during the vegetation period. The system has adjusted to this “situation of lack”. So the system can get out of its balance by relative low total inputs and the alga growth can be considerably animated which results in the well known appearance of eutrophisation. (Schernewski., 1994).

E. Water Analysis

Water is an essential resource for living systems, industrial processes, agricultural production and domestic use. Analysis of a natural body of water tells how clean or polluted it is. If there is damage to wildlife, the measurements will help pinpoint the cause and the source. (Anonymous)

The principal factors that are taken into consideration when determining water quality are: dissolved oxygen content (DO), turbidity, acidity & alkalinity, trace elements and nutrients such as nitrogen, phosphorus, halogens (chloride and fluoride ions), alkali metals (sodium and potassium ions), calcium and magnesium ions, and the microorganisms.

Water analyses are done by several methods. The most common types of measurements are gravimetric or weighing, electrochemical using meters with electrodes, and optical. Instrumental methods are becoming increasingly popular and instrumentation is getting smarter and easier to use with the inclusion of microprocessors. In the simplest case, a sample may just be placed in an instrument and a result read directly on the display. More often, some physical separation technique or chemical procedure is needed before a measurement is made, in order to remove interferences and transform the analyte, the target of the analysis, into a form which can be detected by the instrument.

E.1. pH

pH measures the acidity or alkalinity of water. pH of rain water is about 5.5-6.0. Typically, natural water has pH 6.5-8.5. A pH<5 (acidic water) is most damaging to eggs and larvae of aquatic organisms. Most aquatic life (except for some bacteria and algae) cannot survive pH<4.Natural alkalinity is due to $CO_2(g)$, HCO_3^-, CO_3^{2-} and OH^-, carbonate rocks such as limestone and dolomite increase alkalinity. Alkalinity is increased by caustic substances from industry (KOH, NaOH), soil additives in agriculture such as lime $Ca(OH)_2$, super phosphate which is mixture of $Ca(H_2PO_4)_2$ and $CaSO_4$, and soaps and detergents. Natural acidity is due to $CO_2(g)$, HPO_4^{2-}, $H_2PO_4^-$, H_2S, Fe^{3+}, other acidic metal ions, proteins & organic acids. Increases in acidity can be due to acids used in industry, acid mine drainage, acid rain.

E.2. Dissolved Oxygen (DO)

The Dissolved Oxygen test measures the current oxygen levels in the water. The Do level varies with temperature. DO levels are highest in the afternoon due to photosynthesis and lowest just before dawn. DO is lowered by an increase in temperature (as from a discharge of hot water form a power station), increases in aerobic oxidation (due to increases in organic matter from sewage or due to inorganic fertilizers such as phosphates and nitrate with over stimulate algal growth). Water with DO<1ppm is dead.

Oxygen dissolved in water is derived from the air and from the oxygen given off in the process of photosynthesis by aquatic plants. Adequate dissolved oxygen is necessary for the life of fish and other aquatic organisms. To maintain a varied fish fauna in good condition the dissolved oxygen concentration should be at least 5.0 ppm, Dissolved oxygen is also responsible for many of the corrosive problems in industry. (Centralized analytical Laboratory, 1981)

E.3. Coliform Test

Escherichia coli, commonly known as *E. coli*, is a species of bacteria normally present in human intestines. A recently recognized strain, *E. coli* 0157:H7, produces high levels of toxins that can cause kidney damage, as well as septicemia, or blood poisoning. Symptoms can include diarrhea, chills, headaches, and high fever, and in some cases the infection can lead to death, even with medical intervention. Illness from *E. coli* may develop from consuming undercooked beef, unpasteurized milk, or from handling food without washing hands after changing diapers. (Microsoft ® Encarta, 2007)

E.4. Nitrite-Nitrogen

Nitrite nitrogen is a measure of the nitrogen available to the plant in nitrate form. In high rainfall areas, sandy soil types and areas with warm winters, this measurement may be of limited value except at planting or side dress time. In the areas with lower rainfall, the nitrate test may be very beneficial.

Nitrite, an intermediate compound in the nitrogen cycle, may occur in water as a result of biological decomposition of proteins. When correlated with the concentration of other nitrogen forms, trace amounts of nitrite may indicate organic pollution. (Centralized analytical Laboratory, 1981)

E.5. Phosphate

Phosphorus is an element which is essential for the growth of organisms and it can often be the nutrient responsible for eutrophication. (Centralized analytical Laboratory, 1981)

Phosphorus, symbol P, reactive nonmetallic element that is important to living organisms and has many industrial uses. The atomic number of phosphorus is 15, and its atomic weight is 30.974. Phosphorus is in group 15 of the periodic table.

Phosphorus is widely distributed in nature and ranks 11th in abundance among the elements in Earth's crust. It does not occur in the free state but is found mostly as a phosphate, as in phosphate rock and apatite. It is also found in the combined state in all fertile soil and in many natural waters. The element is important in plant and animal physiology and is a constituent of all animal bones, in the form of calcium phosphate. (Microsoft ® Encarta, 2007)

CHAPTER 3

Methodology

A. Collection of Samples

The samples for soil and interstitial water analysis were collected from four different places in Western Visayas, namely:

1. Sicaba, Cadiz City, Negros Occidental;
2. Brgy. Balaring, Silay, Negros Occidental
3. Capayawan, Cadiz Viejo, Negros Occidental;

For the soil analysis, the samples were collected from the subtidal or sublittoral zone on the exact site or place where the worm species will be collected and at about 1 foot below the ground in the area exposed during low tides.

The soil samples were collected by using a corer and were then transferred in a clean pail with cover. The corer was placed and dug on the sand by rotating it while applying a downward pushing force. After reaching the desired depth of sand, the cork of the corer was closed to have a vacuum inside so that the sand would not fall. When the corer was pulled, a board was then immediately placed at the bottom of it to avoid the contact of the sand sample with the surface water. The corer was carried away from the water to the shore. The cork was opened and the water inside it was poured out. About one inch of the exposed area of sand was removed using a hand spade and the remaining sand inside it was placed inside a clean pail. However, not all of the remaining sand inside the corer was used as sample but about one inch thick of sand must be left inside. The first one inch before getting the sand sample and the remaining one inch of sand later were not included as samples for these parts were in contact with water. The samples were then brought to SEAFDEC for analysis.

Upon reaching SEAFDEC, the pore water which was used for most of the water tests that were done was taken from the soil samples in the pail by way of centrifuge.

For the water analysis, the water samples were collected on the site where the worm species were collected. The water was collected from the surface of the sea. The surface water samples will be used for coliform testing and dissolved oxygen testing. For the Dissolved Oxygen test, twice-rinsed standard DO bottles were used to collect surface water samples. The bottles were opened under water and the water was allowed to overflow from the top of the bottle and the stopper was placed at once allowing no air bubbles to be entrapped in the bottle. The method used for collecting samples for DO was the same for collecting samples for Coliform testing except that an amber-colored bottle was used for it.

B. Worm Sampling

This phase includes the sampling of *Sipunculus spp.* and the measurement of the length, diameter, and the weight as well its color, and the identification of its species.

The worm sampling was done by fishermen who regularly harvest these worms. Ten samples of the worms from each site were collected by the fishermen. After thirty minutes to one hour of sampling, the worms were washed with water and were damped dry by using a hand towel. The length and the diameter of the worms in centimeters were measured using a centimeter ruler. With a weighing scale, the worms were weighed in grams and the data gathered was then recorded. The color of the worm was then observed.

For the identification of the worm species, we used Matthew D. Richmond's book entitled *A Guide to the Sea Shores of Eastern Africa and the Western Indian Ocean Islands*. We used the data we have gathered as basis for the identification. We further verify this with an expert in SEAFDEC.

C. Soil Analysis

Sipunculus nudus burrows in the sand and are found from just below the tidal zone down to 2,953 feet (900 meters) deep. They swallow surrounding sand to digest bits of animal and plant tissue. By this information, it was concluded that the sand components and characteristics may affect the growth of this worm species. The sand was collected with the use of a corer and was placed ina clean pail with a cover and was brought to SEAFDEC for analysis. The following tests and analysis were conducted.

C.1. CHEMICAL

C.1.a. Sulfur

Sulfate ions in the monocalcium phosphate extractant were precipitated by Barium ions and the produced turbidity was measured spectrophotometrically at 420 nm.

This procedure yielded a standard curve that is linear up to 50 mg S L^{-1}. The detection limit is approximately 0.05 mg S L^{-1} in the extract. The determination limit is approximately 0.05 mg S L^{-1}.

The extraction procedure was the weighing of 10 g air-dry <10-mesh soil into n extraction vessel, pipette into the vessel 25 ml Extraction reagent which was the Calcium Phosphate solution, and shake for 30 minutes. One-fourth – teaspoon powdered charcoal was added and was shaken for an additional 3 minutes. It was then filtered and transferred a 10 ml aliquot into another flask.

To a 10 ml aliquot, 1 ml Seed solution was added and immediately swirled. The flask was placed on a magnetic stirrer and ¼-teaspoon Barium Chloride Crystals was added. It was then stirred for about a minute. An aliquot was transferred to a spectrophotometric tube and the absorbance was read using calibrated UV-VIS at 420 nm.

For the calculation, the SO_4-S content of the soil material, expressed in mg SO_4-S kg^{-1} was determined by multiplying the SO_4-S content in the extractant by 2.5.

C.1.b Available Iron

12.5g of air dried soil sample was placed into a 125mL Erlenmeyer flask. 50mL of 1N ammonium acetate was added and the solution was shook for 30 minutes then filtered. 10mL of extract was pipetted into each of the 2 flasks. 2mL of hydroxylamine hydrochloride and 2mL of orthophenenthroline solution was added to the first flask. 2mL of hydroxylamine hydrochloride and 2mL of water was added.the absorbance was then read to 510nm.

The standard curve was made by pipetteing 0, 5, 15, 25 and 45mLof standard Fe (5ppm) into 100mL volumetric flasks. This solution are equivalent to

0.0, 0.25, 0.75, 1.25 and1.75 ppm Fe. 2mL of hydroxylamine hydrochloride and 2mL of orthophenanthroline solution was added and was shaken in between. 1N ammonium acetate was added. The volume was then dlluted with distilled water. Absorbance was read at 510nm.

ppm Fe in soil= ppm Fe in solution x(Dv/Av) x (Ev/wt Sample)

C.1.c Available Phosphorus

The standard curve was made by pipetteing 0, 1,3,4,5 and 6mLof standard P solution (5ppm) into 50mL volumetric flasks. A sufficient amount of extractant was added to make the volume 10mL. 30mL of water was then added the 5mL of 1N sulfuric acid. Then the flask was shaken gently and 5mL of ammonium molybate reagent was added, then 0.25mL of stannous chloride was added. Then the absorbance was read at 660nm within 10 minutes.

2.5 g of air-dried soil was placed into a 125mL Erlenmeyer flask. Then 0.5g of activated carbon 50mL of extraction reagent was added. It was shook using a shaker and was then filtered. 30mL o water 5mL of 1N sulfuric acid was the added. The Erlenmeyer flask was then shook to remove bubbles. 5mL of ammonium molybate was added and then the neck of the flask was washed down with water then 0.25 mL Stnnous chlorid3e was added and the reading was took using a spectrophotometer at 660nm.

The concentration in ppm is computed by:

ppm P in soil= ppm P in solution x(Dv/Av) x (Ev/wt Sample)

C.2. PHYSICAL

C.2.a. pH

In measuring the pH of the soil, the chemicals that were used was the color-coded buffer solutions of pH 4.0, 7.0, and 10.0 are purchased from commercial sources; the electrode Internal Filling Solution which was the Thermo Orion's 3M KCl and the electrode soaking solution which was 5 g of KCl in a liter of pH 7 buffer solution.

A two-point calibration of the pH meter used fresh buffer solutions of pH 4 and 7 was prepared. The soil samples was mixed with distilled water by ratio of 1 part soil by 1 parts water by volume (g is to mL). The solution was mixed for 5 minutes using a glass or plastic rod and was allowed the soil to settle. This was done 5 times. The pH meter was then dipped into the solution for pH reading.

C.2.b. Organic Matter

A balance was tared and the 50-mL beakers were weighed. A 5 cm^3 of air-dried, 2-mm sieved soil was scooped into a beaker. It was then dried for a minimum of two hours at 150°C ±5°C. It was maintained at 100°C until weighed. The weight of the beaker plus the warm soil sample was recorded to ±1 mg. It was then heated at 360°C for two hours after the temperature reaches 360°C ±5°C. It was then cooled to 105°C and maintained at 105°C until weighed. The beaker was weighed and warm ash in a draft-free environment to ±1 mg. The results were then calculated and reported % LOI as percent organic matter to the nearest tenth of a percent.

Calculations:

Dried Soil ($Soil_d$) = (Wt of Beaker + Wt of Soil at 150°C) - Wt of Beaker

Ashed Soil ($Soil_a$) = (Wt of Beaker + Wt of Soil at 360°C) - Wt of Beaker

Percent weight loss on ignition (%LOI):

$$LOI(\%) = \frac{Soil_d - Soil_a}{Soil_d} \times 100$$

D. Water Analysis

Water analysis was done to determine the properties that the certain samples of water from the three distinct places possess. There were two types of water samples that were taken. Ine is the surface water sample and the other is the pore water sample.

For pore water extraction the sample was collected by collecting sand from the site by using a corer and placing it in a clean pail. Once arriving at SEAFDEC the sand is placed into test tubes which are then placed in the centrifuge machine. After centrifugation the pore water was separated from the sand and the water becomes the pore water sample. This pore water sample was for the Nitrite-Nitrogen, Phosphate, Ammonia and pH tests.

For Fecal Coliform and Dissolved Oxygen Determination, surface water was used.The following are the analyses that were performed.

D.1. CHEMICAL

D.1.a. Dissolved Oxygen Determination

The portable DO meter was turned on and was warmed up. The probe was dipped into the body of water and was allowed to stabilize. Once stable the reading was recorded. The probe was then rinsed. The DO was taken 5 times and the mean will be recorded.

D.1.b. Nitrite-Nitrogen

Five milliliters of pore water sample was pipetted into a test tube then 0.1 ml sulfanic acid solution was added and mixed. Sulfanic acid solution was prepared by diluting 30 ml glacial acetic acid to 100 ml with distilled water and dissolving 1 g sulfanilic acid on it.

One ten milliliter (0.1 ml) of alpha-naphthylamine solution was added. It was mixed and was left to stand for 10 minutes. Alpha-naphthylamine solution was formed by dissolving 0.5 g naphthylamine in 30% acetic acid.

Absorbance was read at 510 nm against nitrite-free water. The concentration of nitrite in the sample was calculated using the standard curve.

For the preparation of Standard curve, nitrite calibration standards were prepared in the range 0.02 – 0.30 ppm N by diluting to 5 ml the following volumes of the standard nitrite solution: 0.02, 0.04, 0.06, 0.08, 0.10, 0.15,... 0.30 ml. Then 0.1 ml of sulfalinic acid solution and alpha-naphthylamine solution was added then mixed. Absorbance was measured at 510 nm against distilled water after 10 minutes. The absorbance against concentration was plotted. M (slope) and b (x-intercept) were solved using the least square method.

$$x = My + b$$

where x = concentration

y = absorbance

$$M = \frac{n\sum xy - \sum x \sum y}{n\sum y^2 - (\sum y)^2}$$

$$b = \frac{\sum y^2 \sum x - \sum y \sum xy}{n\sum y^2 - (\sum y)^2}$$

D.1.c. Phosphate Determination

The pore water extacted was used in the performing of this test.

For the preparation of the standard curve, phosphate calibration standards were prepared in the range 0.02 – 0.03 ppm P by diluting to 5 ml with distilled water the following volumes of standard phosphate solution: 0.02, 0.04, 0.06, 0.08, 0.10, ... 0.30 ml and 0.1 ml 95% ethyl alcohol was added and mixed. 0.1 ml of mixed reagent was added, and was mixed again, and was left to stand for 20 minutes. Absorbance was measured at 880 nm after 20 minutes using distilled water as blank. The absorbance was plotted against concentration and M and b values were solved using the least square method.

Five milliliters of sample was pipetted into a test tube then 0.1 ml 95% ethyl alcohol was added and mixed. One ten milliliter (0.1 ml) mixed reagents was added, mixed, and left to stand for 20 minutes. Absorbance was read at 880 nm against distilled water. The amount of phosphate in the sample was determined using the standard curve.

D.1.d. Ammonia Determination

A standard curve was made by preparing ammonia calibration standards int eh rang 0.02-0.04ppm N by diluting to 5mL with filtered sea water the following volumes of the standard ammonia solution: 0.02, 0.04, 0.06, 0.08, 0.10, 0.40mL.

0.2mL phenol solution was added then 0.2 mL of sodium nitroprusside then 0.5mL of oxidizing solution was added then it was covered with aluminum foil and was left to stand for 1 hour.

After 1 hour the absorbance was read at 640nm using filtered water as blank. The absorbance and concentration were then solved.

5mL of the sample was pipetted ino the test tube and 0.2 mL phenol solution, sodium nitropusside, and 0.5mL of oxidizing solution was added and it was left to stand for 1 hour then the absorbance was read.

D.2. PHYSICAL

D.2.a. Fecal Coliform Test

The sampe for this test was taken by filling an amber colored bottle by opening the bottle and closing the bottle under the surface of the water and the water was placed in a cooler filled with ice and was transported to PSHS-WV for analysis

Single strength agar and 1.5 strength agar is prepared. 10 mL of agar was placed in 6 test tubes. In the first 3 0.1 mL of sample was placed. In the other 3 1 mL of sample was placed. In the 3 other test tubes, 10 mL of 1.5 strength agar was placed and 10 mL of Sample was placed. A fermentation tube was placed in each test tubes and it was made sure that there was no air bubbles in the fermentation tube. The test tubes were placed in the incubator and left there for 1 day then inspected for bubbles and based on the bubbles the amount of fecal coli form can be computed.

D.2.b. pH

No sample preparation is required other than equilibration to 25°C in a water bath prior to measuring pH. A sample size of 80–120 mL was adequate. An 8-ounce (250-mL) wide-mouth jar was used as a sample container allowing the sample to be capped and providing adequate space for the electrode pair and any stirring apparatus.

After calibration of the meter for the desired pH range and temperature equilibration of the sample in a water bath, pH of the pore water sample was determined. Verify that the sample temperature is 25 ± 0.25°C. Electrodes were washed with reagent water, blot and immersed electrodes in the stirred sample were measured. 2 minutes was used up for electrodes to equilibrate and meter reading to stabilize. The pH value was read to 0.001 pH unit and reported to the nearest 0.01 pH unit. The meter was recalibrated between each sample measurement. If multiple measurements before recalibration are desired, a maximum of three measurements are recommended to maintain the precision stated in the method. The sample pH value was reported to the nearest 0.01 pH unit, and the temperature of measurement, 25°C.

CHAPTER IV

RESULTS AND DISCUSSION

A. Results

This study was conducted to describe the physical and chemical parameters of the environment in which *Sippunculan* species would thrive in.

The dimensions of the worms were taken and the mean was taken. The test for soil, pore water, and water were conducted in SEAFDEC bio-tech laboratory.

A.1 Sand test Results

Capayawan has he highest concentration of phosphorous and sulfur and the lowest amount of organic matter. Balaring has the highest concentration of iron (Table 1).

Table 1. Soil test results of phosphorous, available Iron, sulfur, soil pH and percent organic matter. The results for Phosphorous Iron and sulfur are in the unit ppm. The results of the phosphorus, iron, and sulfur test are the average of 2 readings±sd.

	Sicaba, Cadiz	Balaring, Silay	Capayawan
Phosphorous	3.199411±0.217	3.142617±0.045	3.331931±.381
Iron	22.62931±1.003	19.92555±1.912	26.20298±0.870
Sulfur	190.7016±2.606	200.4758±2.475	224.5161±3.536
Organic Matter	2.47%	2.62%	1.28%
Soil pH	7.75	7.92	8.08

A.2 Pore water test results

Sicaba has a higher nitrite and phosphate concentration. Capayawan has a greater ammonia concentration and Balaring has the highest pH (Table 2).

Table 2. Mean nitrite phosphate ammonia and pH readings of the pore water from Balarin, Sicaba and Capayawan. Nitrite, Phosphate and ammonia readings are in ppm. The nitrite phosphate ammonia readins are the average of 2 readings±sd

	Sicaba, Cadiz	Balaring, Silay	Capayawan
Nitrite	0.044219±0.011	0.567631±0.049	0.123496±0.028
Phosphate	0.286853±0.0024	0.271201±0.030	0.139442±0.0028
Ammonia	-0.0022±0.004	0.006743±0.011	0.010245±0.010
pH	7.95	8.14	8.04

A.3 Water Tests

Sicaba has a relatively smaller amount of fecal coli form compared to Balaring and Capayawan and Capayawan has a relatively higher D.O. readings compared to Sicaba and Balaring (Table 3).

Table 3. Mean amount of Fecal Coli Form and D.O. readings from the places in Negros Occidental. DO readings are averaged from 5 readings.

	Sicaba, Cadiz	Balaring Silay	Capayawan Cadiz
MPN per 100mL	460	≥2,400	≥2,400
DO(ppm)	1.78	2.08	2.68

A.4 Worm size weight and Diameter

Worms from Capayawan (Table 4) are relatively longer than those from the other sites. Also they relatively weigh more and they have a bigger circumference.

Table 4. The mean length, weight and circumference of the worms in the three sites in Negros Occidental.

Place	Weight(g)	Length(cm)	Circumference(cm)
Sicaba, Cadiz City	28.5833±2.22	19.9542±1.30	3.7667±0.22
Balaring, Silay	146.25±8.02	23.583±1.72	6.5625±0.21
Capayawan Cadiz Viejo	174.25±10.88	46.68±2.72	7.55±0.22

B. Discussion

According to the results, the pH of the soil and the pore water is slightly basic or alkaline in nature which is natural to all sea waters. pH is lowest in the most productive regions of the sea where upwellings occur. This means that the *Sipunculan* species, like any other marine organisms, live on a basic environment.

Dissolved oxygen analysis measures the amount of gaseous oxygen (O2) dissolved in an aqueous solution. As dissolved oxygen levels in water drop below 5.0 ppm, aquatic life is put under stress. The lower the concentration, the greater the stress. Oxygen levels that remain below 1-2 ppm for a few hours can result in large fish kills. (KY Watcher Watch) As for Brgy. Balaring, Capayawan and Sicaba the DO content of water is very low which explains why fish can be found kilometers away from the area where the worms are found. This means that *Sipunculan* worms can tolerate low levels of oxygen.

The results on fecal coliform for Balaring and Capayawan showed that it counted greater than or equal to 2400, the greatest reading for this test, and Sicaba with 460, the second to the greatest result possible, shows that there is a greater chance of pathogens present in the water. This maybe true because a lot of people are leaving very near the shoreline. A person swimming in such waters has a greater chance of getting sick from swallowing disease-causing organisms, or from pathogens entering the body through cuts in the skin, the nose, mouth, or the ears. (Bradford Woods) *Sipunculan* worms living in this kind of environment is very much possible since these worms may actually feed on these bacteria by swallowing other organism.

Organic matter (or organic material) is matter that has come from a once-living organism; is capable of decay, or the product of decay; or is composed of organic compounds. Organic matter increase the nutrient holding capacity of the soil and is food for soil organism from bacteria to the worms. (Graham & Allan, 2001) Sand usually has about 2-3% of organic matter.

Iron is an essential component of enzymes and is copiously available in soil. This makes iron an essential nutrient for plankton growth. (Floor Anthoni, 2006) Planktons are one of the food digested by *Sipunculan* worms. This is the reason why there is a high level of iron in the sand in the site.

Nitrite, ammonia, phosphate, phosphorus, and sulfur are some of the main nutrients for plant growth.

The normal amount of phosphorus in a marine environment is 0.008. (Floor Anthoni, 2006) The results (3.199411±0.217, 3.142617±0.045and 3.331931±.381) showed that the amount of total phosphorous in the soil is relatively high. The amount of phosphate in water is 0.286853±0.0024, 0.271201±0.030 and 0.139442±0.0028, which is relatively higher to the normal amount. This may have caused the water to be slightly basic since phosphate is basic.

The normal amount of sulfur in a marine environment is 904 ppm. The results showed that the amount of Sulfur in the site is 190.7016±2.606, 200.4758±2.475 and 224.5161±3.536, which is relatively lower compared to the normal amount.

The amount of nitrite-nitrogen and ammonia in the pore water is relatively low.

Though nitrite, ammonia, and sulfur have low values, the planktons, which are being eaten by the worms, depend on the abundant amount of phosphate in water, and Phosphorus and Iron in the soil.

These worms from Balaring and Capayawan, based on their color, length, weight and size either belong to the species *Sipunculus nudus* or *Sipunculus Indicus.*

For the soil analyses, Capayawan, Sicaba, and Balaring relatively have the same amount of phosphorus and pH. Capayawan has a relatively smaller amount of organic matter compared to the other two sites and relatively higher amount of sulfur and iron. For the pore water analyses the amount of nitrite is relatively lower in Sicaba compared to the other two sites. The amount of phosphate in Capayawan is relatively lower compared to the other two sites. For amount of ammonia and pH are relatively the same. The amount of fecal coliform in Sicaba is relatively smaller compared to the other two sites. The amount of dissolved oxygen in Sicaba is lower than Balaring which is lower than the DO in Capayawan.

The worms in Capayawan Cadiz Viejo are relatively longer, thicker and heavier than those in Balaring and Sicaba although Capayawan has the relatively lowest amount of organic matter. The bigger worms in Capayawan might be explained by the fact that the iron and sulfur content there is relatively higher compared to the other two sites. Also the DO is higher in Capayawan.

In general, the worms in Sicaba, Capayawan and Balaring live in a slightly basic environment with high levels of phosphorus, iron, nitrite, phosphate, ammonia and low levels of sulfur. They live in environments with low dissolved oxygen and high levels of fecal coliform. They also live in an area with sufficient amounts of organic matter.

The worms from Capayawan are longer, weigh more, and have a larger circumference than those in Balaring, which are also larger than those in Sicaba.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study aimed to describe the physical and chemical characteristics of the habitats of *Sipunculus spp.* and to describe the morphological structures of the different *Sipunculus spp.* from the three coastal areas in Negros Occidental.

This study specifically aimed to:

1.) Determine the sand's: (a) sulfur concentration, (b) total phosphorus, (c) available iron, (d) pH, and (e) amount of organic matter;

2.) Determine the pore water's (a) ammonia concentration, (b) nitrite-nitrogen concentration, (c) phosphate concentration, and (d) pH

3.) Determine the surface water's: (a) amount of Fecal Coliform and (b) amount of dissolved oxygen.

4.) This study also aimed to describe the morphological structure of the worm from each of the places by measuring its (a) length, (b) diameter, and (c) weight.

A. Summary of Findings

1.) Sicaba soil has 3.199411±0.217ppm of phosphorus, 22.62931±1.003ppm of iron, 190.7016±2.606ppm of sulfur, a pH of 7.75, and 2.47% of organic matter. Balaring soil has 3.142617±0.045ppm of phosphorus, 19.92555±1.912ppm of iron, 200.4758±2.475 of sulfur, a pH of 7.92, and 2.62% of organic matter. Capayawan soil has 3.331931±.381ppm of phosphorus, 26.20298±0.870ppm of iron, 224.5161±3.536ppm of sulfur, a pH of 8.08, and 1.28% of organic matter.

2.) Sicaba pore water has 0.044219±0.011ppm of nitrite, 0.286853±0.0024ppm of phosphate, -0.0022±0.004ppm of ammonia, and a pH of 7.95. Balaring pore water has 0.567631±0.049ppm of nitrite, 0.271201±0.030ppm of phosphate, 0.006743±0.011ppm of ammonia and a pH of 8.14. Capayawan pore water has 0.123496±0.028ppm of nitrite, 0.139442±0.0028ppm of phosphate, 0.010245±0.010 of ammonia and a pH of 8.04.

3.) Sicaba water has 460MPN per 100mL of fecal coliform and a DO of 1.78. Balaring water has ≥2,400 MPN per 100mL of fecal coliform and a DO of 2.08. Capayawan has ≥2,400 MPN per 100mL of fecal coliformand a DO of 2.68.

4.) Sicaba worms are about 28.5833±2.22g, 19.9542±1.30 cm long and has a circumference of about 3.7667±0.22cm. Balaring worms are about 146.25±8.02g, 23.583±1.72cm long and has a circumference of about 6.5625±0.21cm. Capayawan worms are about 174.25±10.88g, 46.68±2.72cm long and has a circumference of about 7.55±0.22cm.

B. Conclusion

The worms in Sicaba, Capayawan and Balaring live in a slightly basic environment with high levels of phosphorus, iron, nitrite, phosphate, ammonia and low levels of sulfur. They live in environments with low dissolved oxygen and high levels of fecal coliform. They also live in an area with sufficient amounts of organic matter.

The worms from Capayawan are longer, weigh more, and have a larger circumference than those in Balaring, which are also larger than those in Sicaba.

C. Recomendations

It is recommended that further studies be made on:

1. The physico-chemical characteristics of other places in Negros Occidental with *Sipunculus spp.*
2. The other methods of *Sipunculus spp.* preparation for food.
3. Effect of large coastal communities on *Sipunculus spp.* .
4. The effect of urea on *Sipunculus spp.*
5. What conditions will allow *Sipunculus spp.* to thrive.

APPENDIX A

Department of Science and Technology
PHILIPPINE SCIENCE HIGH SCHOOL WESTERN VISAYAS
Doña Lawa-an H. Lopez Campus
Bito-on, Jaro, Iloilo City

September 22, 2008

DR. EVALYN GRACE AYSON
Head, Research Division
SEAFDEC/AQD
Tigbauan, Iloilo

Dear Dr. Ayson:

Greetings!

We, Ma. Zarina Mae J. Yamog and Edward Victor De Juan, fourth year students of Philippine Science High School Western Visayas, will be conducting a research entitled, **"Species Composition and Morphological Characterization of *Sipunculus spp.* in Selected Coastal Areas in Iloilo and Negros Occidental"**, as a requirement for our research class.

Our study requires the following analysis:
Sulfur determination, total phosphorus, and available iron for soil;
Ammonia, nitrate-nitrite, phosphorus for pore water.

These tests require the use of a centrifuge and a spectrophotometer. Since our school does not have a spectrophotometer and we have a small centrifuge but we need to use a bigger one, we are humbly requesting for your permission for us to conduct our study in SEAFDEC/AQD within the dates specified in the schedule below.

Date	Time	Description of Work
September 27, 2008 (Saturday)	9:00 am – 5:00 pm	Sulfur, Total phosphorus and available iron content of soil
September 28, 2008 (Sunday)	8:00 am - 12:00 pm	Ammonia, nitrate-nitrite, phosphorus for pore water
October 4, 2008 (Saturday)	1:00 pm – 5:00 pm	Sulfur, Total phosphorus and available iron content of soil
October 5, 2008 (Sunday)	8:00 am – 5:00 pm	Ammonia, nitrate-nitrite, phosphorus for pore water

Our school requires us to do the analyses ourselves with adult supervision. Should you grant our request, may we also ask permission to do the analyses ourselves. We understand that your laboratory assistants don't have pay for overtime work during weekends and we are willing to offer an honorarium for their time.

Should you approve the above mentioned request, may we request from you a letter indicating such to be addressed to the Science Research Committee Philippine Science High School - Western Visayas Campus. You may fax it to telefax (033) 329 5644.

You may contact us through phone.
Call Philippine Science High School-Western Visayas Campus: 329 2011
The students' contact details:

Ma. Zarina Mae J. Yamog - 09212421537
Edward Victor De Juan - 09085241477

Hoping for your kind consideration.

Thank you very much!

Truly yours,

MA. ZARINA MAE J. YAMOG

EDWARD VICTOR DE JUAN

Noted by:

EDWARD ALBARACIN
Special Science Teacher 1
PSHS-Western Visayas

Dr. JOSETTE T. BIYO, Ph. D.
Director III

APPENDIX B

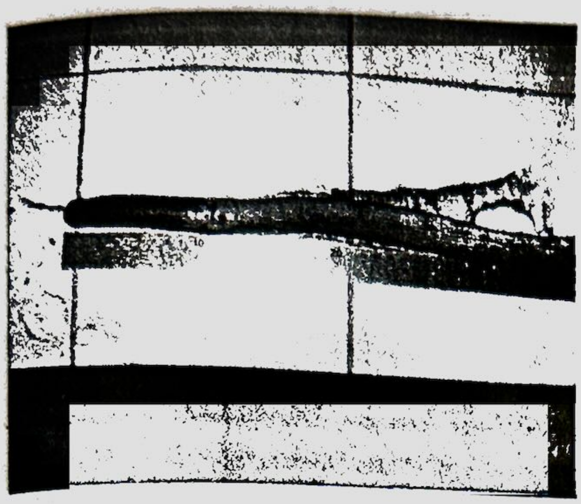


Figure 1 Worm from Balaring Silay

Figure 2 Balaring, Silay at low tide

Figure 3 Sicaba, Cadiz subtidal zone during low tide.

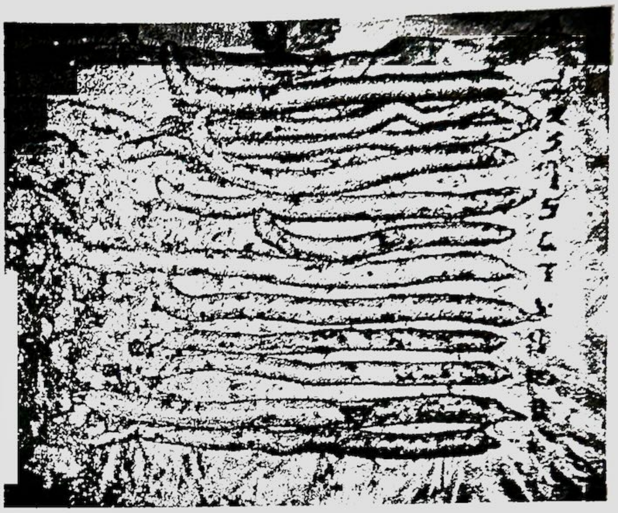


Figure 4 Worms from Sicaba Cadiz

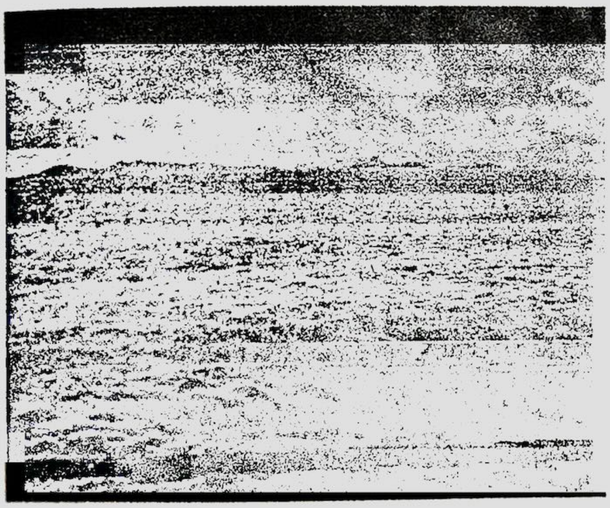


Figure 5 Capayawan Cadiz Viejo at low tide

Figure 6 Worms from Capayawan Cadiz Viejo

APPENDIX C

TABLE B. WORM DIMENSIONS

Worm #	Balaring Silay (Nov.15)		
	Weight	Length	Circumference
1	161	38	6
2	119	25	7
3	127	32	6
4	141	32	6.5
5	135	33	6
6	140	31	7
7	182	39	7.5
8	165	37	6.5
Sum	1170	267	52.5
Average	146.25	33.38	6.5625

Worm #	Capayawan Cadiz Viejo (Nov.12)		
	Weight	Length	Circumference
1	235	57	7.5
2	223	54	7
3	170	34	8.5
4	176	55.5	6.8
5	150	59.4	6.4
6	152	41.1	6.8
7	226	54	7.3
8	180	44.5	8
9	166	33.5	8.2
10	153	40.6	8.8
11	128	41	7.6
12	132	45.5	7.7
Sum	2091	560.1	90.6
Average	174.25	46.675	7.55

Worm #	Sicaba Cadiz City (Nov.11)		
	Weight	Length	Circumference
1	32	9.15	5
2	16	17	3
3	18	22.8	3.4
4	23	23	4.3
5	21	20.5	4.1
6	15	18.5	4
7	37	25	4.3
8	26	22.5	3.5
9	19	18	3.3
10	17	18.5	3.3
11	34	24.5	4.5
12	25	20	2.5
Sum	283	239.45	45.2
Average	23.58333	19.95417	3.766666667

APPENDIX D

TABLE C. Fecal Coli Form

Concentration	Sicaba Cadiz City			Balaring Silay City			Capayawan Cadiz Viejo		
0.1ml	x	x	/	/	/	/	/	/	/
1ml	/	/	/	/	/	/	/	/	/
10ml	/	/	/	/	/	/	/	/	/

APPENDIX E

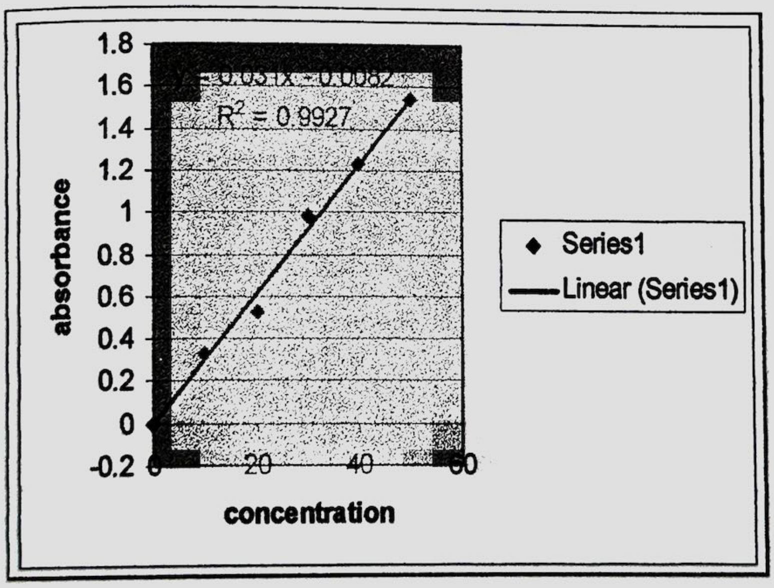
TABLE D. DO READINGS

Balaring Silay		Sicaba Cadiz City		Capayawan Cadiz Viejo	
D.O.	Temp	D.O.	Temp	D.O.	Temp
1.92	31.7	1.83	32.5	2.25	29.2
1.9	31.7	2.12	32.3	2.35	29.2
1.88	31.6	2.13	32.1	2.31	29.2
1.87	31.6	1.96	31.7	2.34	29.2
1.87	31.4	2.06	31.6	2.26	29.2

APPENDIX F

TABLE E SULFUR

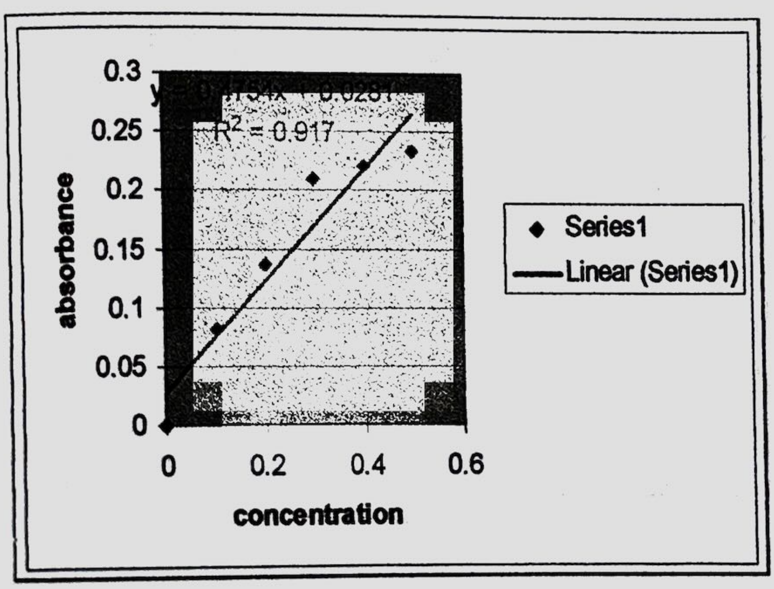
Cocentration	absorbance	net absorbance		
0	0.1144	0		
10	0.4454	0.331		
20	0.635	0.5206		
30	1.0916	0.9772		
40	1.3464	1.232		
50	1.651	1.5366		
		absorbance		concentration
balaring	trial a	1.2239	1.2239	39.74516
	trial b	1.2456	1.2456	40.44516
Sicaba	trial a	1.1145	1.1145	36.21613
	trial b	1.2338	1.2338	40.06452
Capayawan	trial a	1.3683	1.3683	44.40323
	trial b	1.3993	1.3993	45.40323



APPENDIX G

TABLE F. PHOSPHOROUS

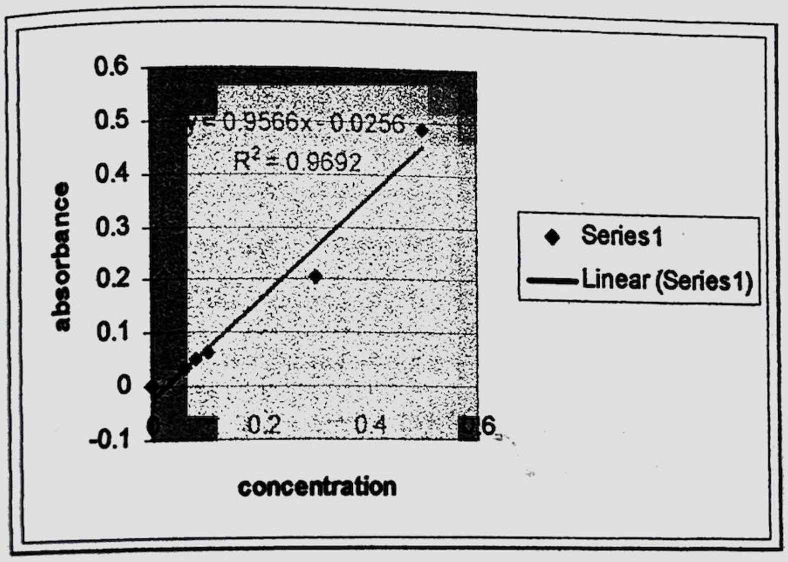
Cocentration	absorbance	net absorbance		
0	0.0566	0		
0.1	0.137	0.0804		
0.2	0.1929	0.1363		
0.3	0.267	0.2104		
0.4	0.2771	0.2205		
0.5	0.2905	0.2339		
0.6	0.3051	0.2485		
		absorbance		concentration
balaring	trial a	0.1591	0.1025	0.1565
	trial b	0.1561	0.0995	0.150189
Sicaba	trial a	0.1732	0.1166	0.186159
	trial b	0.1474	0.0908	0.131889
Capayawan	trial a	0.1602	0.1036	0.158814
	trial b	0.173	0.1164	0.185738



APPENDIX H

TABLE G. AMMONIA

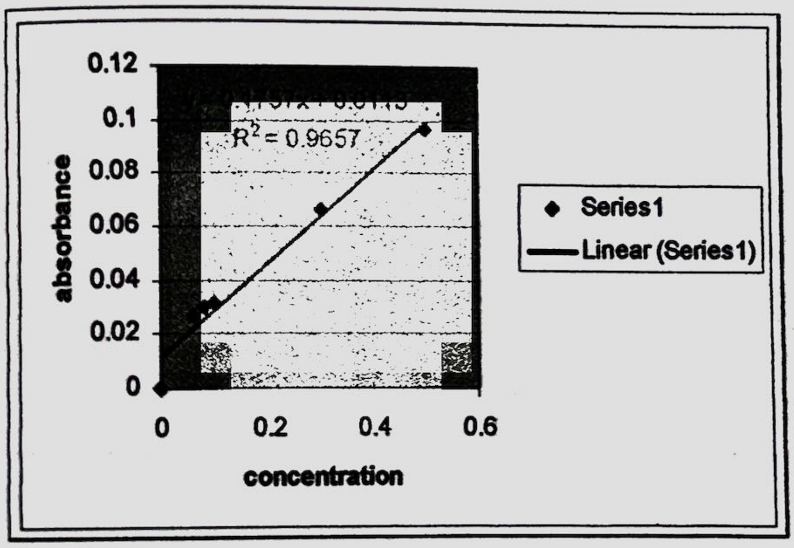
Cocentration	absorbance	net absorbance		
0	0.1121	0		
0.06	0.1454	0.0333		
0.08	0.165	0.0529		
0.1	0.1749	0.0628		
0.3	0.3163	0.2042		
0.5	0.6	0.4879		
		absorbance		concentration
balaring	trial a	0.1005	-0.0116	0.014635
	trial b	0.0854	-0.0267	-0.00115
Sicaba	trial a	0.0873	-0.0248	0.000836
	trial b	0.0815	-0.0306	-0.00523
Capayawan	trial a	0.1029	-0.0092	0.017144
	trial b	0.0897	-0.0224	0.003345



APPENDIX I

TABEL H.Phosphate

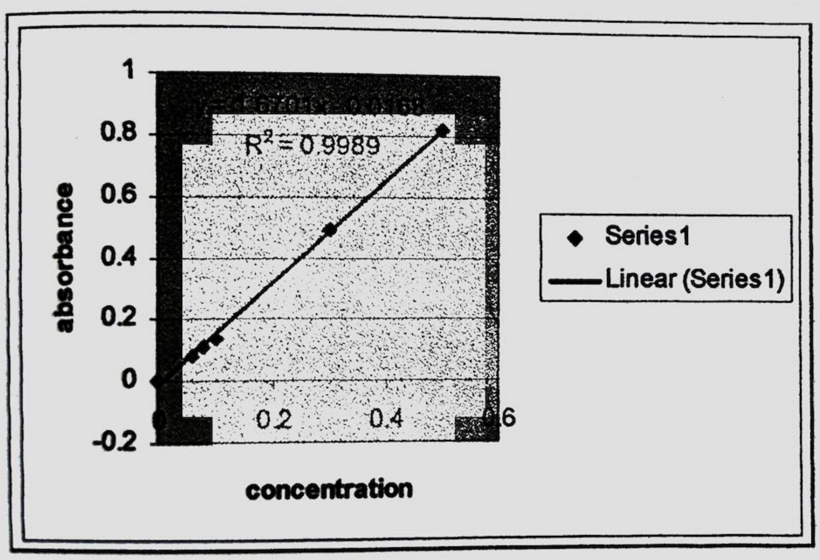
Cocentration	absorbance	net absorbance			
0	0.0698	0			
0.06	0.0969	0.0271			
0.08	0.0997	0.0299			
0.1	0.1014	0.0316			
0.3	0.1359	0.0661			
0.5	0.1659	0.0961			
		absorbance		concentration	
balaring	trial a	0.1345	0.0647	0.292544	
	trial b	0.127	0.0572	0.249858	
Sicaba	trial a	0.1338	0.064	0.28856	
	trial b	0.1332	0.0634	0.285145	
Capayawan	trial a	0.095	0.0252	0.067729	
	trial b	0.0957	0.0259	0.071713	



APPENDIX J

TABLE I.Nitrite

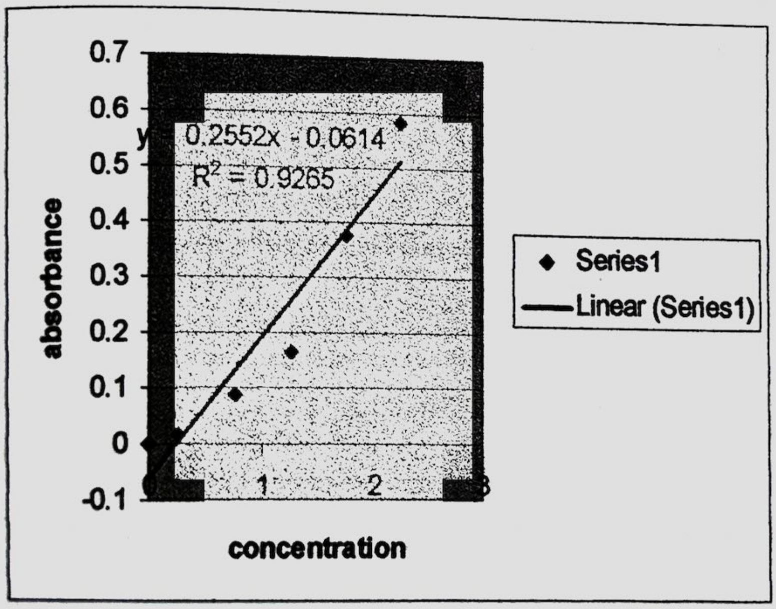
Cocentration	absorbance	net absorbance		
0	0.0964	0		
0.06	0.1788	0.0824		
0.08	0.2034	0.107		
0.1	0.2344	0.138		
0.3	0.5863	0.4899		
0.5	0.9155	0.8191		
		Absorbance		concentration
Sicaba	trial a	0.1615	0.0651	0.049039
	trial b	0.1454	0.049	0.039399
Balaring	trial a	1.0623	0.9659	0.588408
	trial b	0.9929	0.8965	0.546853
Capayawan	trial a	0.3053	0.2089	0.135142
	trial b	0.2664	0.17	0.11185



APPENDIX K

TABLE J. IRON

Cocentration	absorbance	net absorbance		
0	0.0674	0		
0.25	0.0818	0.0144		
0.75	0.1544	0.087		
1.25	0.2316	0.1642		
1.75	0.4446	0.3772		
2.25	0.6515	0.5841		
		absorbance		concentration
balaring	trial a	0.2144	0.2101	1.063871
	trial b	0.1799	0.1756	0.928683
				1.992555
Sicaba	trial a	0.1715	0.1672	0.895768
	trial b	0.2918	0.2875	1.367163
				2.262931
Capayawan	trial a	0.2092	0.2049	1.043495
	trial b	0.3453	0.341	1.576803



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