TOXICITY ASSOCIATED WITH SEDIMENTS FROM MALAYSIAN ESTUARINE ENVIRONMENT

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BY

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ABSTRACT

With the rapid industrialization in the region marine pollution assessment in South East Asia is an ongoing and increasingly important environmental science. One of the areas of concern being sediment toxicology, for it has been identified to be a sink for most pollutants entering the aquatic environment. Owing to the scarcity of information on sediment toxicity bioassays, there is a need to identify species as test organisms for this region.

For the purposes of this study, an effects based approach known as Sediment Quality Triad was employed, where three components of environmental concern, namely sediment chemistry, sediment bioassays and benthic fauna alterations were investigated at a chosen location. The study area was a portion of coastline in Peninsula Malaysia with three estuaries, two of which, Sg. Juru and Sg. Perai, have been identified as receiving pollutants from the rivers feeding them. The third, Sg. Tambun, was included for comparison purposes. A reference location of minimal or insignificant contamination and reference sediment from a test organism collection site were also tested to obtain background or reference contamination values. Emphasis was placed on the bioassay portion of the triad to observe the applicability of local test animals to obtain reliable data from established toxicity tests and protocols.

The data for each study site comprising sediment chemistry, sediment bioassays and benthic macroinfauna composition were compiled and normalized to the

reference values to obtain ratio-to-reference figures and presented in a triangular format. the area of which depicts the degree of pollution induced degradation .

Three local species were tested with reference toxicants. the sea urchin (*Diadema setosa)*: oyster (*Crassostrea iredalei*) and mud crab (*Scylla serrata*). all had acceptable sensitivity and while the sea urchin was most sensitive to copper while the mud crab was most sensitive to cadmium. With regard to sediment bioassays, an amphipod. *Photis longydactylus*. seemed very promising for gross sediment testing. while a polychaete worm. *Perinereis nuntia*. has potential to being amenable for laboratory culture and subsequent use in chronic bioassays.

The sediment quality of the three sites revealed Sg. Juru to be most polluted and toxic. but the in situ effects on the benthic community needs to be investigated further. Sg. Perai was moderately toxic and is most likely being contaminated by something other than metals. Sg. Tambun was the least polluted. and least toxic. with a better assemblage of benthic fauna.

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List of Abbreviations and Symbols

EC :	Effective Concentration. An estimate of the toxicant concentration that would cause an observable adverse effect in a given percentage of test organisms.
LC 50:	Lethal Concentration 50. Concentration at which 50 % of test organisms are alive or dead.
SP:	Sungai Perai (Perai River)
SJ:	Sungai Juru (Juru River)
ST:	Sungai Tambun (Tambun River)
AVS:	Acid Volatile Sulfide
BDL:	Below Detection Limit
ASTM:	American Society for Testing and Materials
RTR:	Ratio to Reference

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 General Outline

Marine pollution monitoring and assessment has increased in dimension and importance with economic development and industrialization. Rivers, lakes and oceans have served as dump sites, since their capacity to receive, disperse and "hide" the unwanted material from sight was perceived as an acceptable practice. But there have been repeated, occasionally fatal, environmental disturbances such as the Minnamata Episode in the 1960's (Takeuchi, 1972), which have shown that these aquatic environments are not an all absorbing entity. The fate of these pollutants has been investigated and the finding suggests that most of the insoluble portion of the wastes in the aquatic systems have settled on the bottom closely associated with sediment particles. Therefore sediments are a major repository for persistent toxic substances discharged into the aquatic environment (Baker, 1980; Chapman, 1986) and assessment of sediment quality has become a subject of intensive research and monitoring programs.

Sediment is comprised of detritus, inorganic, or organic particles eventually settling on the bottom of an aquatic system. Natural and anthropogenic chemicals present in aquatic systems may enter the sediment by precipitation, and /or adsorption to particles in the sediment. The processes involved are dependent on the nature of the aquatic system and the sediment layer. Therefore, sediment is a matrix of materials and can be relatively heterogenous in terms of its physical, chemical and biological characteristics.

Problem sediments typically contain toxic levels of persistent contaminants, many of which have the potential of being lethal (e.g., heavy metals, chlorophenols) or have long term deleterious effects, including reproductive impairment/birth defects (e.g.polychlorinated biphenyls, polyaromatic hydrocarbons and dioxins). Reductions in, or changes to, sedimentary organisms, which are a major food source for other ecologically important and commercially important trophic level organisms such as crabs, shrimp and fish, have been a result of toxic sediments. Further, bottom-dwelling organisms such as crabs and bottomfish may develop cancerous lesions as a result of contact with problem sediments (Hargis, *et al*; 1984).

Chemical analyses of sediments from polluted sites have revealed unsafe contaminant levels, but most of them are strongly bound to the sediment matrix, *e.g* organic components such as fluoranthene to organic carbon while metals such as like cadmium have been shown to adhere to acid volatile sulfides (AVS) in sediment (Swartz *et al.* and Di Toro *et al.* in Lamberson *et al.* 1992). Considerable published data indicate that total metal concentrations on sediments are not good estimators of the "free" and bioavailable fraction of the total chemicals present.

This bioavailable fraction has the potential of being taken up in food chains or released to the water column when the solubility equilibrium shifts or the sediment is microbiologically degraded, resulting in the movement of these pollutants into the trophic food chains.

The bioavailability and toxicity of sediment-sorbed contaminants are linked via three potential sources: the sediments themselves, overlying water and interstitial (pore) water (Boese et al., 1990). The role that benthic systems, both biotic and abiotic, play in sequestering contaminants is well recognized (Baker, 1980). However, the key to sediment assessment is bioavailability; since sediments may contain relatively high concentrations of toxic compounds without leading to adverse effects on organisms living in the sediments. The fate of contaminants in a sediment-water system is highly dependent on their sorptive behavior which, in turn, affects bioavailability and toxicity. The only means of measuring bioavailability is by measuring or determining a biological response, for instance through bioassay testing.

Researchers have devised various approaches to investigate the threat of pollution from contaminated sediments, through the assessment of sediment quality. One of the first organized efforts of the scientific community to address emerging technical and regulatory issues on sediments was a workshop in 1984 on the "Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems" (Dickson *et al.*,1987). Since then considerable progress has been made in assessment methods. One of these approaches is the sediment quality triad, an effects based method which integrates environmental chemistry, biological observation and biological

experimentation to determine pollution induced degradation (Chapman et al., 1987; Long, 1989). Specifically, it involves three separate components, each of which can comprise one or more measurement end points. Sediment chemistry analyses which measure contamination, laboratory toxicity tests which measure effects under standardized conditions (bioassay) and assessments of resident community alteration (generally the benthic infauna) which measures field conditions (observation). (Chapman, personal communication).

Sediment chemistry deals with the conditions within specific sediments, the concentration of a chemical or suite of chemicals in these sediments and how these interact to yield a given chemical environment. Sediment conditions include grain size, organic content, pH, redox potential (Eh), chemical characterization and presence of other chemicals. A complete understanding of the chemical environment in sediment cannot be solely determined by chemical measurements.

The amount of organic carbon in sediments regulates the partitioning of non ionic organic compounds between particulate and interstitial water phases, and organic carbon content has been shown to have a substantial effect on the toxicity of these conpounds to benthic organisms. Swartz *et al.* (1990) found that the LC_{50} of total sediment fluoranthene increased with the concentration of sedimentary organic carbon, indicating that the contaminant was less bioavailable in more organically enriched sediments. The benthic fauna and the aquatic conditions above the sediment will also influence the chemical composition of these sediments.

Sediment bioassays are a direct measure of the potential toxic effects of the contaminated sediment on biota. The different end points of the various tests illustrate the wide range of damage that could ensue from contaminated sediments. While some tests are short term and measure the lethality of the pollutants, some are chronic tests, and illustrate the changes caused by pollutants to life cycle history parameters of the aquatic macro and meiofauna. Hence, they also deliver valuable information on problems related to recruitment and the reductions in aquatic life populations especially those of economical and ecological value.

The structure of natural marine communities is widely used for the detection and monitoring of anthropogenic impacts on the sea. The community level of biological organization is most commonly used for environmental impact studies, since it is, in practical terms, the most ecologically relevant (Warwick, 1993). Monitoring at lower levels of organization reflects the condition of the organism at the time of sampling, whereas the structure of an assemblage of organisms reflects the integrated conditions over a period of time. On the other extreme, monitoring at higher levels such as the ecosystem is simply not feasible.

In the community approach to pollution impacts, usually only one component of the community is examined. A wide variety of biotic components have been used including plankton (phytoplankton and zooplankton), fish (demersal and pelagic), soft bottom macrobenthos and meiobenthos, hard bottom epifauna (corals) and motile macro and meiofauna. One of the most widely used components, which is also the biota for this study, is the macrofauna. The reasons underlying the choice are

:-

a. They are closely associated with sediments

b. They are relatively non-motile and are, therefore, useful for studying local effects of pollutants (Bilyard,1987)

c. Their taxonomy is moderately simple and, as indicated in literature, their response to perturbation at taxonomic levels higher than species has been studied more extensively than any other component of the biota.

d. Quantitative sampling is relatively easy.

e. There is extensive research literature on the effects of pollution on macrobenthic communities which includes sensitive species such as echinoderms and arthropods.

As part of the sediment quality triad approach, the structure of the macrobenthic community has contributed significantly to the investigation on the Palos Verdes Shelf, California by Swartz *et al.*,(1986). Species richness, biomass, and density of the benthos was significantly reduced.

This study is significant because of its holistic approach in determining the pollution effects that have persisted for many years in certain Malaysian coastal estuarine areas, which are ecologically important for fishery recruitment and conservation in Malaysia. It will focus on the estuaries of the Juru and Perai Rivers, where there have been numerous short, as well as long term studies linking the decline in coastal fisheries with wastewater discharges from nearby factories, despite the apparent compliance of these factories to standards under the existing regulations. Most of these studies have examined contaminant levels in the water and in tissues of cockles and other shellfish. Some recent work by Din,(1995) on contaminants in sediment revealed the intertidal areas along the coast of Penang island to have a more

clayey texture and thus higher absolute metal concentrations. The study also included benthic fauna composition but there was no attempt to correlate metal concentrations with diversity or abundance of taxa .

The Juru estuary with the adjoining mudflats served by the Perai River is an area of considerable importance for pollution research. In the early 1960's Sg. Juru was a river with a wide variety of freshwater and brackishwater fishes and was an important fishing area for fisherman using traditional gear. However, with the opening up of the Juru Industrial Zone and the subsequent establishment of factories (textile, paint, insecticide, canning, metal plating etc.) the fishery declined and at present the estuary supports only an extensive natural and reseeded blood cockle (*Anadara granosa*) bed.

1.1.2 OBJECTIVES

The study used a sediment quality triad approach to assess the sediment quality of the Juru and Perai River estuary. Emphasis was placed on toxicity testing since a prime objective in undertaking this research was to select and determine the suitability of local tropical invertebrates as test organisms for established sediment toxicity testing schemes. This objective was approached using the larvae of the following invertebrates: the black spiny sea urchin, tropical oyster and mud crab. In addition, a local species of amphipod was selected for gross sediment testing and culture of a common polychaete was initiated.

A further objective was to make the necessary adjustments to the established testing schemes such using a shorter test duration which would be more suitable for the warmer tropics. While doing this an attempt was also made to culture indigenous juvenile polychaete worms. as has been done with *Neanthes arenacodenta* in North America. Laboratory cultured juvenile polychaeteous worms have served very well as test organisms for measuring toxicity of organic pollutants. Another objective was to determine confounding factors that affected toxicity testing in the tropics. *i.e.* effects of the silt/clay fraction. or total organic carbon on metal bioavailability.

On a broader perspective the present study was initiated as a field trial for the Sediment Quality Triad , where preliminary data was collected on the three separate components of the triad. each of which comprises one or more measurement end points: sediment chemistry analyses which measure contamination, laboratory toxicity tests. which measure effects under standardized conditions (experimentation), and assessments of resident community alteration (generally the benthic infauna). which measure field conditions (observation). The data were then interpreted and integrated to draw conclusions based on "weight of evidence" to assess the sediment quality of the chosen study area.

1.2 LITERATURE REVIEW

In this section investigations carried out by other researchers on the three components of sediment quality triad are reviewed. The individual components;

bulk sediment chemistry, sediment toxicity bioassays and benthic infaunal composition are reviewed separately.

1.2.1 Sediment Assessment - Sediment Quality Triad Approach

Sediment toxicity tests were first developed in the 1970's and were recommended in the U.S. Environmental Protection Agency/ U.S. Army Corps of Engineers Implementation Manual for dredged material evaluation (Lamberson,1992). Methodology proliferated in the 1980's, and sediment toxicity tests were recommended, along with chemical and community structure analysis, to provide information on the ecological impact of sediment contamination. This combination method or sediment quality triad approach is both descriptive and numeric. The sediment quality triad approach has been used in marine coastal waters on the west coast of North America such as Puget Sound, San Francisco Bay, and Vancouver Harbor (Long and Chapman, 1985) In the Puget Sound sediments, chemical analyses revealed high contaminant concentrations that were acutely toxic and had reduced benthic species diversity (Swartz *et al.*, 1982; Becker *et al.*, 1990). Thompson *et al.* (1989) recorded mortality and reductions in somatic growth rate and gonad production in sea urchins exposed to contaminated sediments from Southern California. However, the sediment quality triad approach was most useful in investigations by Chapman and Long (1990) while working with the San Francisco Bay sediments where synoptic measurements of chemical concentrations, sediment toxicity and benthic community structure analysis revealed a pattern of toxicity related to chemical contamination.

1.2.2 Bulk Sediment Chemistry

The toxicity of contaminated sediments may be modified by abiotic factors, in addition to the absolute concentration of specific chemicals. Chemical factors that may influence the apparent toxicity of a sediment-associated chemical include sorption to particulate or dissolved organic matter, the physical chemical form of the compound (including the ionic state), the presence of other ions (e.g. salinity, acid volatile sulfides, hardness) and concentrations of limiting compounds (e.g. dissolved oxygen, ammonia, hydrogen sulfide). Interactions among these factors and between geochemical and physical variables may future modify sediment toxicity (Lamberson *et. al.*, 1992). Metal and metalloid dynamics between sediments and interstitial and overlying waters are particularly complex. Considerable published data indicate that total metal concentrations on sediments are not good estimators of the " free" and bioavailable fraction of the total chemical present (Di Toro *et al.*, 1991; EPA, 1989; Karickhoff, 1981). Different sediments can differ by a factor of 10 or more in toxicity for the same metal concentration. To use toxicity estimates based on chemical measurements there needs to be a way to estimate the bioavailable fraction of the total present. A number of approaches to determine metal bioavailability associated with sediments have been reviewed or tried, including carbon normalization and sorption of metals in oxic freshwater sediments to particulate carbon and the oxides of iron and manganese (Jenne, 1987).

Recently the dominant role of the sediment sulfides in controlling metal bioavailability has been demonstrated (Di Toro *et. al.*, 1990; Ankley,1991). Sulfides are common in many freshwater and marine sediments and are the

predominant form of sulfur in anaerobic sediments. Sulfides have the ability to complex with metal ions to form water insoluble precipitates resulting in a lack of toxicity even when high metal concentrations are present in some sediments. It has been shown that the solid phase sediment sulfides that are soluble in cold acid, termed acid volatile sulfide (AVS), are a key factor for controlling the toxicity of cadmium, nickel and several other heavy metals (Di Toro *et. al.*,1990 and 1991).

Total organic carbon in sediments regulates the partitioning of non-ionic organic compounds between particulate and interstitial water phases, and work done by Adams *et. al.* (1985) and Landrum (1985) has shown organic carbon content to have a substantial effect on the toxicity of these compounds to benthic organisms. Swartz *et. al.*,(1990) found that the LC_{50} of total sediment fluoranthene increased with the concentration of sedimentary organic carbon, indicating that the contaminant was less bioavailable in more organically enriched sediments. De Witt *et al.*, (1992) found that the source of organic matter in sediment had relatively little effect on the toxicity of fluoranthene, which suggests that the bioavailability of non-ionic organic chemicals in sediment may be predicted from knowledge of the whole sediment concentration of the contaminant and the sediment organic content.

The bioavailability of metals in sediment can also be affected by the binding of the metal ions to the sediment constituents. Metal ions can be free in solution, complexed to dissolved or colloidal organic materials in sediment interstitial water, or bound to sediment particles. Kemp and Swartz (1988), demonstrated that the interstitial water concentration, rather than the total bulk concentration in the sediment, determined the toxicity of cadmium to the amphipod *Rhepoxynius abronius*.

In sediment, metal ions that are bound to sediment constituents may be unavailable to sediment dwelling organisms. Thus, sediments with relatively high concentrations of metals might have unexpectedly low toxicity. Metal ions also have an affinity to iron and manganese oxides and organic carbon in sediments, and in oxidized sediments, the presence of these substances might determine bioavailability. Swartz *et al.* (1986) showed that the toxicity of cadmium in a sandy sediment was inversely correlated with the concentration of organic carbon in the form of sewage sludge. Likewise the partitioning of metals to acid volatile sulfide (AVS) has recently been shown to be a major factor controlling the availability of toxic metals in reduced sediments.

To date, the factors that control the form in which metals exist in sediment systems have not been clearly delineated, suggesting that one master factor (e.g. organic carbon for nonionic organics) does not exist for all conditions, but that several are active.

1.2.3 Sediment Toxicity Tests

A wide variety of studies utilizing the biological assessment of sediment toxicity in marine sediments have been tabulated (Chapman and Long,1983; Chapman, 1986, 1988; Chapman *et al.*,1985). Responses have ranged from sublethal physiological effects, such as changes in respiration, to alterations in community structure and function. The duration of the tests ranges from a few minutes to more than a year, and the quantities of sediment tested ranges from a few grams to over a metric ton (Swartz, 1989). Only a few of these methods have been standardized *i.e.*,

published as ASTM Standard Guides. (1990) or Puget Sound Estuary Program. (1991) and are currently in common use as sediment toxicity tests.

Commonly used sediment toxicity tests may be classified as "acute" or "chronic" and test whole sediment. suspended sediment. sediment liquid phases (*e.g.*. pore water. interstitial water), or sediment extracts (*e.g.* elutriates, solvent extracts). Tests of whole or solid-phase sediment differ from tests with other sediment phases in that the whole. intact sediment is used to test the exposed organisms (ASTM. 1990). whereas suspended - sediment tests utilize a slurry of sediment and water to expose the organisms where sediment particles are held in suspension by stirring or agitation of the sediment/water mixture. Sediment elutriate tests on the other hand examine the toxicity of a liquid supernatant withdrawn after suspended-sediment particles settle.

Acute lethality tests have been developed for amphipods. cumaceans. copepods. shrimps. isopods. echinoderms. bivalves. polychaetes. and fish. With regard to this study. reviews on amphipods. bivalves. echinoderms and crustaceans are discussed.

Amphipods: *Sediment toxicity test using amphipods*

Amphipods are one of the most sensitive of benthic species and are among the first to disappear from benthic communities in sediments impacted by pollution (Swartz *et al.*, 1982). They were the most sensitive of several taxa tested in a multispecies whole sediment test. Amphipods appear to have an important functional role in benthic ecosystems and they are the principal benthic prey of many fishes. Photocephalids were the dominant burrowing invertebrate prey of seven out of eight

fish species (Manzanilla and Cross. 1982) and the Dungeness crab (*Cancer magister),* (Swartz *et al.* 1985). Oliver *et al.* (1982) identified phoxocephalids as important infaunal predators of small benthic invertebrates. Phoxocephalids thus occupy an unusual and functionally important trophic position as the prey of epibenthos and predator of the meiobenthos.

Most whole-sediment tests with amphipods are short- term static tests conducted for 10 days, (Swartz et al. 1985; ASTM. 1990) with the primary endpoints being mortality and the ability of test survivors to rebury in clean sediment at the end of the exposure period. The latter endpoint is a sublethal measure of the test organism's ability to survive under real-world conditions, because a benthic amphipod that is unable to bury will most likely be swept away from a suitable habitat by water currents or be consumed by predators.

Standard guidelines (ASTM. 1990) have been published for 10-day tests with *Ampelisca abdita, Eohaustorius estuaries, Grandidierella japonica,* and *Rhepoxynius abronius.* The test developed by Swartz *et al.* (1985) is a 10 day static experiment conducted with seawater of > 25 ppt salinity at 15° C under constant light. Test methods have also been developed for *Leptocheirus plumulosus* and are being developed for other species, such as *Eogammarus confervicolus,* generally following the ASTM guidelines. Reish and LeMay (1988) recommended using *G. japonica* or *Corophium insidiosum* in short-term exposures to test dredged material in southern California. Procedures for longer-term tests have been used for *G. japonica* (28 days) by Nipper *et al.*, (1989) while *G. lutosa* and *G. lignorum* have been used for 39 to 90 days (Connel and Airey. 1982). *A. abdita,* a species common in Malaysia, has been

used in a 56 day test duration (Scott and Redmond.1989). In longer term tests, amphipods are fed and endpoints include mortality, growth, and reproduction. Chronic test procedures are under development at several laboratories, but unfortunately are not yet standardized for any species of amphipods.

Chapman (1986) published the results of an inter-laboratory comparison of an amphipod sediment toxicity test carried out to determine variability of the test using the same protocol. The outcome was increased confidence in the robustness of the test from several standpoints, acceptable survival and behavior (emergence and reburial) of controls, determination of the rank order of toxicity, and agreement of mean values for survival and behavior.

The following genera of amphipods have been identified to inhabit the coastal seabed along the east coast of Penang Island, Malaysia: *Arrhis, Ampelisca, Erichtonius, Gammarus, Hyale, Liljeborgia, Melita, Monoculodes, Perioculodes, Photis, Stenothoe* and *Tritella* (Ong and Din, 1994). The density of the most common ones were 20 -30 in 0.1 m^2 bottom surface area as compared to *R. abronius* (800-2000 amphipods/m^2) in Yaquina Bay, Oregon (Swartz *et al.*, 1985).

From the above list, *Photis longydactylus* was chosen for this study . for it's abundance among the estuarine seaweed *Gracillaria sp.* and its ability to bury in soft sediment within 10 - 15 minutes

Bivalve mollusks : *Sediment Elutriate Test Using Oyster Embryos*

The bivalve larvae test procedure (APHA, 1985; ASTM, 1989) is a well established and reliable indicator of water quality. Two species recommended for

testing on the U.S west coast are the Pacific oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis*. The Pacific oyster embryo bioassay was initially developed by Woelke (1972), and at present is the principal water-quality bioassay used in the North Sea monitoring programs for the effects from dissolved hydrocarbons (Stagg, 1991). Modifications have been made to the method by many researchers, including Bourne *et al.* (1981) for use in particular regions and using different species of indigenous bivalves.

For the South East Asian countries two species that occur well distributed in most areas are *Crassostrea iredalei* (Faustino), and *C. belcheri* (Sowerby). There has been well documented hatchery production of eyed larvae for both species (Wong, 1989). Some work was carried out on reference toxicant testing (copper and cadmium) using *C. belcheri* at the Sains University in Penang, Malaysia in 1993 but the results were not published.

Echinoderm: *Sediment Elutriate Test Using Sea Urchin Embryo*

The echinoderm embryo test (Dinnel *et al.*, 1989; Puget Sound Estuary Program, 1991) is similar to the bivalve larvae test. Echinoderm species recommended include purple sea urchins (*Strongylocentrotus purpuratus*), green sea urchin (*S. droebachiensis*), sand dollars (*Dendraster excentricus)*, and the Atlantic urchin (*Arbacia punctata*). In the tropical and subtropical parts of the Indo Pacific a common species is *Diadema setosum* (Leske), and the organisms egg and embryo have been proposed and used by Kobayashi (1971) in marine pollution bioassays. The methodology was later improved to enhance the sensitivity, and to simplify it by

observations of the first cleavage and pluteus formation (Kobayashi. 1990). The same species has been selected for use in this study.

Crustacea: *Mud Crab Bioassay Using Zoea Larvae*

To date there have been relatively fewer toxicity tests using mud crab larvae as test organisms as compared to the other invertebrates. One species that has been well tested is the zoea I stage larvae of Dungeness crab, *Cancer magister* (Dana). Martin *et al.* (1981) exposed the larvae to toxic heavy metals for 96 hours. The metals used as toxicants include arsenic. cadmium. chromium, copper, lead, mercury, silver, nickel. selenium and zinc. Another species that has been used as an indicator of the effects of insecticides on estuarine biota is the commercially important blue swimming crab. *Callinectes sapidus* (Rathbun). (Bookhout and Costlow, 1975; Costlow. 1979). McKenney and Costlow (1981) used the same test organisms megalopae larvae to study the effects of salinity and mercury on survival and normal development of early life stages.

For the purposes of this investigation. larvae of a local species of mud crab *Scylla serrata* Forskall was used as the test organism. This mud crab constitutes a very important crab fishery throughout the entire Indo- Pacific region and they are caught mainly from brackishwater mangrove swamps (Jamari, 1994). Since early 1960's there has been successful larval and post larval culture of this species at the Fisheries Research Institute in Malaysia (Ong. 1966).

Culture of Polychaete Worms for Chronic Testing

Polychaetes have been utilized as marine bioassay animals frequently in the past because of their importance in the subtidal benthos where they usually constitute 50 per cent of the number of species and specimens of macroinvertebrates. Metallic ions enter the marine environment from a variety of natural and anthropogenic pathways. These ions generally become attached to sediment particles and are eventually deposited on the ocean floor. Here they can enter the food web through ingestion by detrital feeders such as polychaetes. Due to their preference for highly organically enriched sediments, polychaetes are often found living at high densities in coastal environments where they have the ability to accumulate xenobiotics from the external environment into their body tissues. They play a fundamental role as prey items for a wide range of commercially important fish species and, in mudflats and similar coastal environments, for wading birds.

Several polychaete species have proven to be valuable laboratory bioassay organisms for pore water (Akesson, 1980; ASTM, 1994a; Reish, 1984) and sediment toxicity (ASTM, 1994b; Dillon et al.,1993). One species, *Neanthes arenaceodentata* Moore has been widely used in marine toxicological research (Dillon and Moore,1993).

The life cycle and culture methods (Reish, 1980) are well documented and it is well suited for use in sediment toxicity tests for the following reasons :

a. It maintains intimate contact with the sediment throughout its life cycle.

b. It is a sediment ingester

c. It is well suited for monitoring reproductive end points because, unlike most nereid polychaetes, it has no planktonic trocophore larvae.

d. The whole life cycle can be completed in the laboratory, producing cultures of test organisms of known age and background.

Species of polychaetes that have been used successfully in acute as well as chronic testing include *Neanthes arenaceodentata, Nereis sp.* and *Capitella capitata. Neanthes* is not found in the tropics but the other two species are common. The nereid polychaete chosen to start laboratory culture for bioassay purposes in the present study is *Perinereis nuntia*, which is found living at high densities in coastal environments in Malaysia; there have been no previous culture attempts for this species.

1.2.4 Benthic Infaunal Community Structure

The marine macrobenthos responds in a consistent pattern to changes in the level of sediment organic enrichment (Pearson and Rosenberg, 1978). Benthic assemblages near major sources of organic material contain no macrofauna or only a few pollution-tolerant, opportunistic species that may be very abundant. In the mid 1980's , however there was a strong sense of disillusionment with the benthic community monitoring approach among both environmental managers and biologists (McIntyre, 1984). They were worried that this approach was labour intensive and expensive while the scientists felt it was not always possible to separate changes brought about by natural environmental variables and those caused by anthropogenic

activity. A series of valid and objective protocols for sampling and analyzing communities is now beginning to emerge (Clarke, 1993). Also, for many groups of benthic organisms such as macrobenthos and meiobenthos, effects of perturbations were detectable at high taxonomic levels (Gray et al.,1990; Warwick et al. 1990).

Warwick (1993) reviewed the various measures of community stress and classified them into four groups addressing the problem in four main ways:

1. Comparing community structure at that location with some theoretical expectation.
2. Comparing the community structure at that location with an empirical 'training data set derived from known community responses to impacts elsewhere.
3. Comparing attributes of the structure of the community that respond differently to the effects of pollution or disturbance, one acting as an internal control against the other.
4. Identifying properties of community structure that are extremely conservative in unperturbed communities (unlike diversity and species composition) and which are modified in a predictable way by perturbation.

Satsmadjis (1985) established empirical relationships between the numbers of individuals and species in unpolluted macrobenthic communities with two key environmental variables, sediment granulometry and water depth, described by an index. This index could be modified by the effects of pollution and is known as the coefficient of pollution. Besides this approach there are various other methods, namely the use of indicator organisms (Rosenberg, 1978); abundance-biomass comparison plots (Lambshead *et al.*, 1983); size distributions (Warwick, 1984) and phylum level meta-analysis (Warwick and Clark, 1993). Of the above the most recent

phylum level meta-analysis was the most practical and robust. Agard (1993) found that this method also worked well in a tropical environment (Trinidad. West Indies). Also. a very significant feature of this approach was the phyletic composition of the macrobenthic communities was not significantly influenced by sediment type or water depth and therefore disturbance effects could be detected by multivariate methods (Warwick and Clarke.1993).

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 SEDIMENT SAMPLING

Sediments that were to be tested for toxicity were examined for their physical properties and chemical constituents. Since the selection of sampling stations and methods of sample collection, storage, transport, and manipulation can potentially influence both the characterization of the chemical and physical properties and the toxicity or bioaccumulation tests, methods prescribed by environmental regulators were used.

2.1.1 Definition of the Study Area and Study Site

The study area was the portion of mainland coastal waters of the State of Penang which faces Penang Island (inset of Fig 2.1). This stretch of coastline is fed by three rivers that discharge industrial and shipyard runoff and is in the vicinity of a bridge that links Penang Island to the mainland. This study area has had pollution studies carried out in the 1970's and 1980's as it is a receiving site for contaminants.

The study area is an estuary fed by three rivers, two of which (Sg. Juru and Sg. Perai) receive discharges of contaminants and the third (Sg.Tambun) was an uncontaminated field control since there were no upstream discharges into this river. Figure 2.1 illustrates the location of the three study sites in the study area. The first site

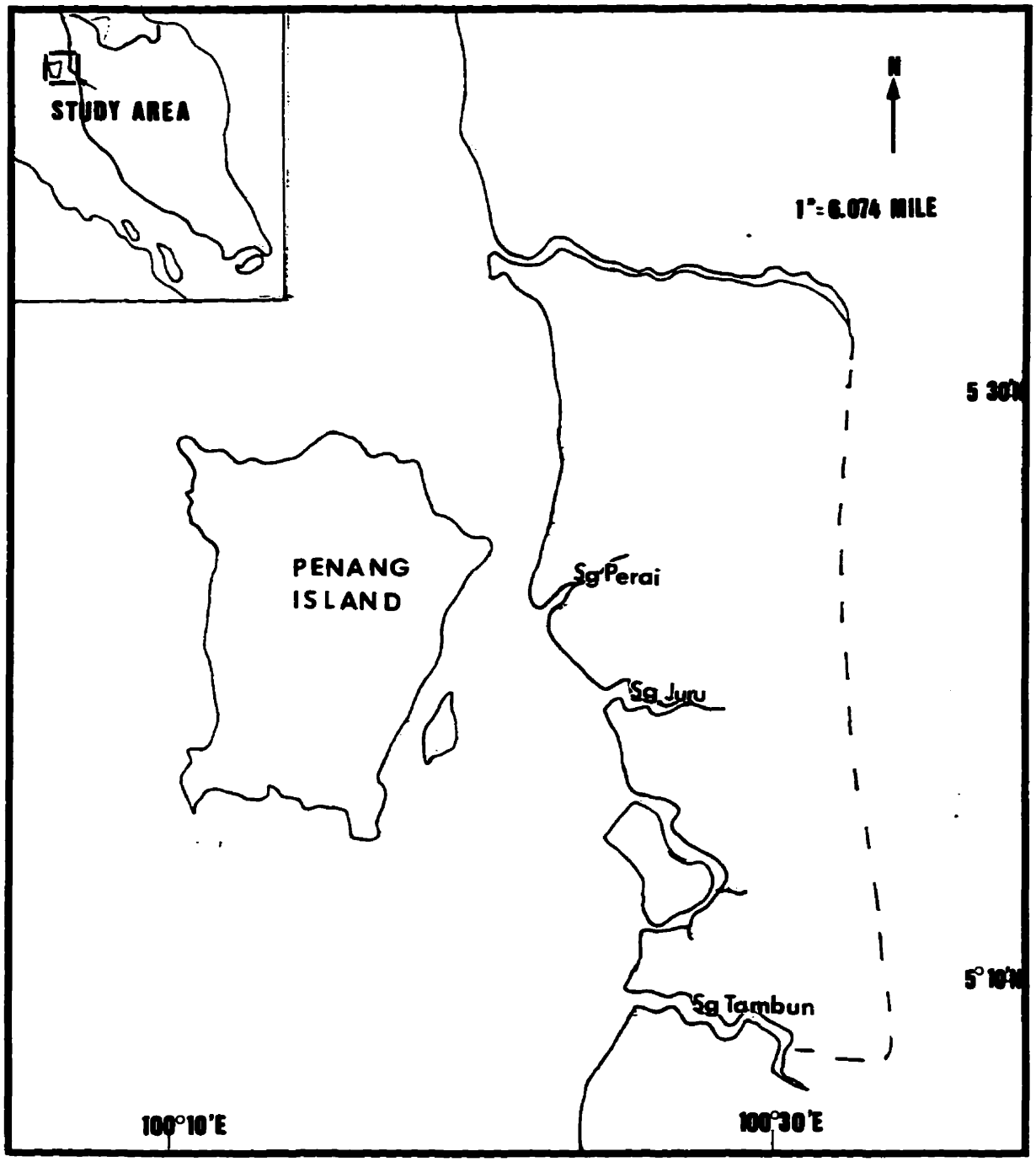


Figure 2.1: Map of the province of Penang showing the study sites.

Figure 2.1: Map of the province of Penang showing the study sites.

was the estuary of Sg. Perai (Perai River). The river receives discharge of organic waste from nearby sugar plantation and a ship repair facility at the mouth discharging metal and paint washings. A few kilometers upstream the river is used for aquaculture of primarily brackishwater fish species.

The second site was the estuary of Sg. Juru (Juru River) located about 5 kilometers down the coast from the first site. This river receives a greater amount and variety of contaminant discharges and most of the factories in a nearby industrial zone discharge into this river.

The third site was the estuary of Sg. Tambun (Tambun River) 5 kilometers along the coast from the second site. The river was relatively clean since there was little anthropogenic contribution of contaminants upstream . This site was chosen primarily to serve as a field control.

2.1.2 Determination of Deposition Zones

For monitoring and assessment studies, the location of fine-grained sediments is often a priority. These sediments are generally located in zones of deposition, have higher organic carbon content than other particle size fractions, and they are usually associated with higher levels of contaminants than other particle size fractions (Baudo *et. al.*, 1990; Suedel and Rogers, 1991; Power and Chapman, 1992).

A study by the Malaysian Nuclear Energy Unit in 1992 using radionucleotides determined the direction and deposition of suspended particles within a 24 hour period discharged from the Sg.Perai. The study revealed that there

was a net drift in a southerly direction owing to currents in the narrow stretch of water between the island and mainland and most of the fine particles were deposited in the stretch of mudflat between Sg. Juru and Sg. Tambun.

2.1.3 Field Measurements and Observations

The following information recommended by Mudroch and MacKnight, (1991) was recorded at the time of sediment sample collection.

a) Time and date of the collection of the sample

b) Ambient weather conditions

c) Type of sediment collection device and any modifications made during sampling

d) Details pertaining to unusual events which occurred during the operation of the sampler (e.g., possible sample contamination , equipment failure, unusual appearance of sediment integrity, etc)

e) Description of the sediment including texture and consistency, color, odor, and presence of biota.

Physical parameter measurements and organic carbon determinations were done within a day of sampling, while toxicity tests were run within two weeks of sampling, following the guidelines on sample storage time for bioassays. Heavy metal analyses were carried out much later but within two months from the sampling date which is within the six month time frame given in the ASTM,1992. The following metals could be determined using the equipment at the Fisheries Research Laboratory, Malaysia :- copper, cadmium, lead, zinc, aluminum and manganese. Samples were also sent to the Geological Survey Department Malaysia, laboratories for heavy metal

analysis as a means of cross checking the above data. In addition arsenic, mercury, iron and copper, cadmium, lead, zinc, aluminum, manganese were included in the analyses made at the Geological Survey Department laboratory. All chemical and physical analyses were run in triplicate.

2.1.4 Sampling

In line with the objectives of the project four sites were chosen for sampling (three for test sediments and one as a reference site). The test sediments were from the estuaries of three rivers two of which are known to discharge effluent water from industrial zones upstream and the third has had no pollution reports so far. The reference station of Tanjong Tokong is located also on an estuary in Penang Island, and has sediment similar in grain size and physical characteristics to the test sediments. The sediment from this station was used as a reference only for chemical characterization of the test sediments. Figure 2.1 indicates the sampling locations and the sampling design was rather simple since the objective was not to investigate sources of pollution or detect hot spots but merely to characterize the sediment. Therefore, a cluster of five subsamples for each station were collected and the grid location using GPS (Global Positioning System) was marked on the map. The GPS coordinates are given in Appendix I .

Sampling was done using an Ekmann grab and surface samples of 2 cm. thickness were composited in clean polyethylene bags, placed in plastic buckets, stored with ice packs in ice chests and brought back to the laboratory within two hours

of sampling time. Surface sediments were scooped with plastic spoons wrapped in acid washed polyethylene sheets.

The following measurements were recorded at the sampling sites: ambient temperature, water temperature, salinity, absence/presence of precipitation, tical flow and color of sediment surface (*i.e.* oxidized/reduced).

In the laboratory, samples collected were examined for large animals, stones and shells which were removed. The observations on color and odor of the sediment were recorded, then the sediment samples were mixed and homogenized thoroughly by stirring in rectangular plastic trays with plastic spoons lined with acid washed polyethylene sheets until a smooth texture and appearance was obvious. Duration of mixing was standardized to 30 minutes and maintained for all stations. Subsamples were taken for storage and stored at -20°C for metal analysis and -4°C for toxicity testing.

2.1.5 Sample Storage

Subsamples were taken for toxicity testing, chemistry determinations and physical characterization. Samples for toxicity tests were stored in polyethylene bottles at 4°C for a maximum of two weeks prior to testing. Samples for chemical determinations were stored either in glass bottles for organochlorine analyses or polyethylene bags for metal analyses. Both were stored at -20° C until analysis. Physical properties of the samples were determined immediately and work on percent moisture, grain size and total organic carbon (TOC) determinations were done on the day of collection or the next day, in which case the sediment was stored in dark

bottles at 4^0C. Recommendations proposed by Environment Canada (1993). as Guidance for the Collection and Preparation of Sediments for Physicochemical Characterization and Biological Assessment were followed during sampling. transportation and storage of sediment samples.

Besides the above samples . one grab collection made with a Ponar grab with an opening measuring 19 x 19 cm. was collected at each station for benthic faunal composition determination. The grab samples were not composited and immediately stained with rose bengal. In the laboratory, the samples were sieved through 0.5 mm mesh. followed by sorting and identification to the generic level.

2.2 SEDIMENT CHARACTERIZATION

2.2.1 General Description of Physical Appearance and Conditions

The texture (fine. intermediate, coarse),color (brown. gray, black). temperature and salinity of the sediment for each station was recorded. Temperature and salinity were measured with an SCT (salinity, conductivity and temperature) meter. Besides observations on the appearance and odor from hydrogen sulfide in the samples. presence of any large organisms or shellfish were also noted since the Juru estuary has extensive blood cockle beds. Subsamples were then taken for total organic carbon (TOC) and grain size determinations.

2.2.2 Total Organic Carbon (TOC)

Total organic carbon was determined by the estimation of organic carbon by the wet oxidation method (Holme and McIntyre, 1971). Digestion of 1g dry soil was done with concentrated chromic acid in a boiling water bath for 15-20 minutes. The cooled mixture was then titrated with 1N ferrous sulfate solution using diphenylamine as the end point indicator. Per cent carbon present was calculated using the following,

$\% C = \{(v_1 - v_2)/w\} \times 0.003 \times 100$

where v_1 represents the volume of normal potassium dichromate, v_2 the volume of ferrous sulfate and w the weight of sediment used.

2.2.3 Grain Size and Percentage Moisture

Sediments for particle size were air dried, accurately weighed to 100g and gently heated with 6 % hydrogen peroxide in a water bath to remove organic matter. It was then soaked overnight in 1litre of 0.01N sodium hydroxide, after which it was gently stirred with a mechanical stirrer for 30 minutes. The mixture was drained through a 45 μm sieve to remove the clay fraction as defined by Brown and McLachlan (1990), and dried at 100^0 C. Particles of different sizes were fractionated through a stack of test sieves placed in a shaker (Endecotts, Octagon 200) for 30 minutes. Sand retained within each sieve was weighed and expressed as a percentage of the dry weight of the total sample. Silt and clay fractions were determined by sedigraph measurements.

Percentage moisture was calculated from dry weights obtained by drying about 2 g sediment at 60 ^{0} C until constant weight.

2.2.4 Trace Metal Analysis

The following metals were determined in 1 g of dried sediment that had been digested according to the method prescribed by Lorring, (1991). The sediment of > 63μ was digested in teflon beakers with the addition of aqua regia and hydrofluoric acid in the ratio 1:3 and the mixture was heated on hot plates until complete digestion was observed.. Metal concentrations were determined using an atomic absorption spectrophotometer (Perkin - Elmer model 5000). To monitor digestion efficiency, analysis of a standard reference sediment, BCSS- 1 was done with each batch of sediment samples.

2.3 TOXICITY TESTING

2.3.1 Selection of test organisms

Preliminary observations were made on various invertebrates collected in the tidal zone. These were held under laboratory conditions, in aerated aquaria (sometimes with sediment). Invertebrates that were collected include two species of isopods, shrimps, sea urchin, three species of polychaete worms and amphipod.

Since they were not feeding on commercial feed, isopods became difficult to keep alive and active for more than a week and were discarded. In addition it was not possible to collect enough numbers of similar sizes. Shrimps have been reported to be cannibalistic if not fed properly and were also discarded. With polychaetes, only one was collected in large enough numbers and was thus chosen for

culture to generate juveniles for testing. Based on the above observations , background literature. their availability and abundance. the following test organisms were selected for this study and some of them are shown on Figure 2.2.

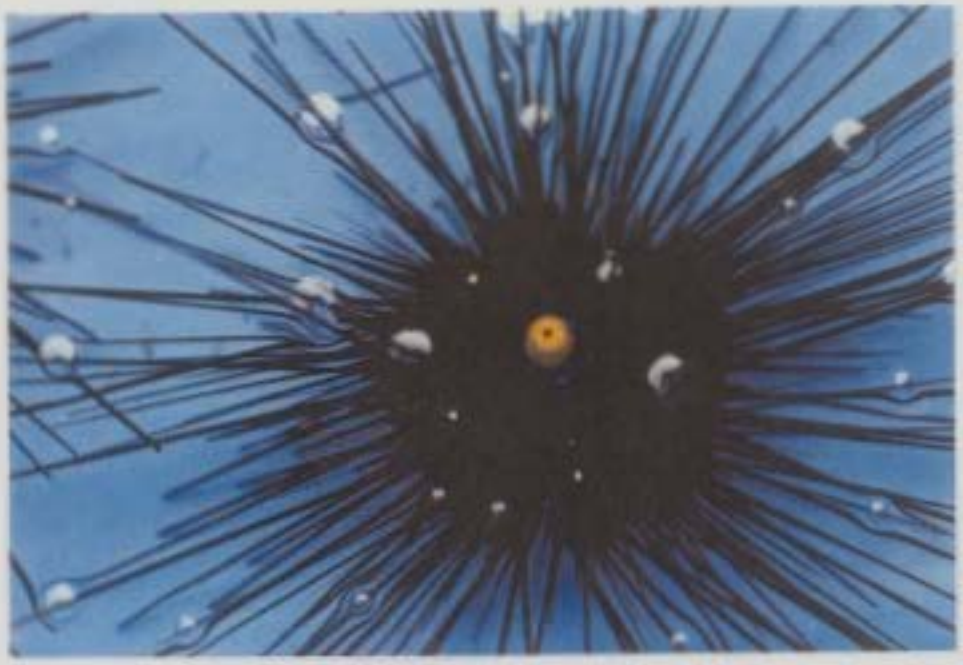
A. Black spiny sea urchin (*Diadema setosum* Leske).

B. Polychaete worm (*Perinereis nuntia* var. *brevicirris* Grube)

C. Mud crab (*Scylla serrata* Forskall) zoea larvae.

D. Amphipod (*Photis longidactylus*).

E. Tropical oyster (*Crassostrea iradelei* Faustino).



A: Black Spiny Sea Urchin (*Diadema setosum*)

B: Mature Polychaete Worm (*Perinereis nuntia)*

C: Mud Crab (*Scylla seratta* Forskall) Zoea Larvae

D: Amphipod (*Photis longidactylus*)

Figure 2.2: Invertebrates used in the reference toxicants and sediment bioassays

2.3.2 Sea Urchin (*Diadema setosum*) Bioassay

Test procedures summarized below were based on work done by Kobayashi. (1993 and 1994): Dinnel & Stober. (1987): and ASTM.(1980) recommendations with modifications where appropriate.

Collection and Holding Conditions of Adult Sea Urchins

Twenty adult sea urchins were collected for gamete collection for each trial from the Pulau Payar Marine Park reefs. They were collected just before or after spring tide to ensure successful spawning (Kobayashi. 1994) and kept in large fiberglass tanks with filtered sea water and were fed red algae (*Gracillaria* spp.). Salinity measurements of water in the holding tanks was monitored to ensure that it was within 27 - 30 ppt while temperature readings indicated little variation. simulating the conditions at the collection site

Spawning

Spawning was induced by mild electric stimulation administered from a 6 volt direct current source. It was recommended in the paper by Kobayashi (1993) that spawning be induced within 2- 3 days post collection. since the urchins don't respond well after a greater period of time.

Each experiment required sperm from 4 to 6 males and eggs from 2 to 3 females combined to give representative gametes and average viability for each spawning batch. Fertilization trials were run to determine sperm to egg ratio that resulted in 90 % fertilization in the controls . This was to ensure that there was no

excess of sperm that might overcompensate significantly for toxicant effects especially in the case of the sperm bioassay. Sperm counts were done by adding a few drops of 1 % glacial acetic acid to sperm solution dilutions and counting was done using a 10 µl in hemocytometer. Eggs were counted from 0.5 ml. aliquots of solution in Sedgewick - Rafter cells.

Sea urchins were used for two types of tests *i.e.* the sperm bioassay and the embryo bioassay. In the sperm bioassay, sperm were exposed to the toxicant test solution for one hour, at the end of which eggs were added to the test solution and the experiment stopped after 20 minutes when the fertilization membrane was formed. For embryo bioassays, fertilized eggs were introduced to test solutions. Experiments were conducted to determine different end points as indicators of toxic response and the following time durations, which correspond to different stages of embryonic development were used as stop times for end point measurements.

Time	Embryonic Development Stage
10 to 20 minutes	fertilization membrane
50 min. to 1 hour	first cleavage
5 hour	blastula formation complete
48 hour	pluteus formation complete

Testing With Reference Toxicant

Test solution concentrations were ascertained from literature and range finder tests and the concentrations for the two chosen metals were as follows:

Copper	0.002 ppm to 0.10 ppm
Cadmium	0.05 ppm to 5 0 ppm

There were a total of seven concentrations tested for each metal and three replicates for each concentration. Test solutions were made up from serial dilutions of 1 ppm copper and 10 ppm cadmium prepared from Merck stock solutions. The diluent used was sterile sea water at 30 ppt salinity. Metal concentration was determined at the end of the experiment to observe any losses during testing.

For the sperm bioassay. 10 µl. of sperm solution was added to the test solution and the appropriate volume of egg solution. usually 0.5 ml. to give 200 - 300 eggs. was added after one hour. The experiment was stopped at the end of 20 minutes with the addition of a few drops of 2 % formalin when the fertilization membrane had formed. In the case of embryo bioassay 200 - 300 fertilized eggs were exposed to the various test concentrations in 10 ml. test solutions and the experiments for the different end points were stopped at their respective time intervals. Test conditions are given in Appendix II

End point measurements were based either on the percentage of abnormal larvae or unfertilized eggs whichever was appropriate for each test concentration. and the Effects Concentration (EC 50) value was calculated from Effl.

program following procedures described by Stephen (1977). Prior to performing EC50 calculations, mean percent abnormality and mean percent unfertilized egg data were corrected for the control response using Abbot's formula:

$$\textbf{Adjusted Test Response} = \frac{\text{\% test response - \% control response}}{\text{100 - control response}}$$

Sediment Elutriate Testing with Sea Urchin Embryo Larvae

Sediment samples, partitioned for toxicity tests, were homogenized again by mixing in polyethylene troughs and large animals and stones were removed before 20 g aliquots of sediment were transferred into 500 mL test jars. 200 mL. of filtered sterile sea water of 30 ppt salinity was added to each jar, the jars were shaken on a mechanical shaker for 4 hours and 300 ml. sea water was added to make a final volume of 500 ml. The test jars were set aside for another 4 hours to allow the suspended sediment to settle. Each experiment had 3 replicate sediment samples from each study site and reference site (using sand from the marine park where adult urchins were collected). The controls were made up with sterile, filtered sea water and water quality test jars had reference sediment in them. About 3 to 4 ml. of fertilized egg solution, which gave about 3000 eggs per test jar, was added and the jars capped loosely and set aside in laminar flow cupboards for 48 hours. Mild aeration was maintained throughout the test duration and water quality, especially dissolved oxygen and pH, were monitored daily .

At the end of 48 hours, the free floating larvae could be discerned by the pink tint from the echinochrome. The clear suspension above the sediment interface (volume V) was decanted very gently and then a few drops of 1%

formaldehyde was added prior to counting. Counting was accomplished by mixing and removing 0.5 ml aliquots into counting wells and counting under low magnification. Five aliquots were counted for each test jar.

$$\text{Total number of abnormal larvae} = \frac{\text{sum of abnormal larvae x V}}{2.5}$$

$$\text{Total number of larvae alive} = \frac{\text{sum of abnormal and normal larvae x V}}{2.5}$$

All larvae that were collected from the suspension were considered live for % survival figures, since dead larvae were assumed to have sunk to the sediment layer.

2.3.3 Oyster (*Crassostrea iredalei*) Larvae Bioassay

Test procedures summarized below were based on work done in Puget Sound Estuary Program, (1991); APHA, (1985); and ASTM, (1989) recommendations.

Collection , Holding and Spawning of Adult Oysters

The oysters were collected from grow out facilities located in river estuaries just prior to their natural spawning season which is between late July and September. They were cleaned, especially the shells to remove barnacle growth and attached vegetation. Males and females (not distinguishable until spawning) were placed together in long troughs and submerged in sterile filtered sea water of 25 ppt. Temperatures in the troughs were raised 2 - 3 ^{0} C higher by addition of warm water which induces them to spawn. Adults showing gamete release were transferred to beakers of 1 litre capacity for isolation of sperm and eggs. After fertilization by

mixing. the eggs were washed by sieving through nytex mesh (0.5 micron) to remove yolk and other debris. Aliquots of 0.5 ml. solution were counted on Sedgewick Rafter cells to ascertain numbers.

Determination of Optimal Salinity Range

Owing to the fact that oysters were collected from areas that experienced large fluctuations in salinity (17 - 28 ppt), it was necessary to run a few experiments to ascertain the optimal salinity range for larval development.

Four salinity test solutions were prepared ranging from 15 ppt to 30 ppt. Test solutions were prepared by diluting sterile filtered sea water of 30 ppt using distilled water to reach salinities of 15.20 and 25 ppt. About 300 fertilized eggs were introduced into each 10 ml test solution and the experiment was stopped at 48 hours when the D veliger stage was reached. All tests were conducted in triplicate.

Testing With Reference Toxicant

As with sea urchins. about 200 to 300 fertilized oyster eggs were added to test solutions of similar concentration ranges as before. The volume of test solution was again 10 mL and the reference toxicants used were copper and cadmium. The test was static and the test conditions are listed in Appendix I. The end point was arrived at the end of 48 hour when the free swimming proddisonch larvae stage or D - shaped veliger stage was reached. The experiment was stopped by the addition of a few drops of iodine which kills as well as stains the shell for easy counting. End point observations were based on normal formation of the D hinge in the shell morphology.

Counting was done by removing a 0.5 ml aliquot of mixed suspension volume (V) and placing it in a counting well. Five aliquots were counted for each test and the total number was arrived at by counting the total number of larvae and multiplying by V/2.5

Sediment Elutriate Testing Using Oyster Larvae

A procedure similar to the one used for sea urchins was used for the oysters. 20g of homogenized sediment was shaken with 500 ml of sterile filtered sea water for 4 hours and allowed to settle . Then 1 -2 ml of fertilized egg solution (2000 - 3000 embryos) was added to the test jars which were capped loosely and left to stand in laminar flow cabinets for 48 hours to reach the D veliger larvae stage. Mild aeration was maintained throughout the test period but the flow rate was kept minimal so as not to disturb the test solution or sediment. Each experiment was run in triplicate with sea water controls, reference control (reference sediment was collected from an area near the oyster culture beds) and test jars for water quality measurements. At the end of 48 hours when the D-shaped veliger stage was reached the water suspension (volumn V) above the sediment layer was decanted off slowly without disturbing the sediment surface and a few drops of iodine was added to it to kill the larvae and aid counting. Five subsample aliquots of 0.5 ml were counted for each test jar and normal / abnormal larvae recorded .

$$\text{Total abnormal larvae} = \frac{\text{sum of abnormal larvae} \times V}{2.5}$$

$$\text{Total number of larvae alive} = \frac{\text{sum of all larvae} \times V}{2.5}$$

All larvae that were collected from the suspension were considered live and used to calculate the mean survival since dead larvae were assumed to have sunk to the sediment layer.

2.3.4 Mud Crab (*Scylla serrata* Forskall) Larvae Bioassay

Collection of Mud Crab Larvae

Culture of mud crab is an ongoing project at the Malaysian National Prawn Fry Center and adult berried females are brought in from the wild and kept in captivity until they release the larvae. These zoea larvae of 4 -6 days were transported in well aerated troughs to the lab where they were acclimatized for 2 to 3 days and fed *Artemia* nauplii. Only larvae showing active movements were used for the test.

Experiments were run using the reference toxicants copper and cadmium and 20 larvae were exposed for each test solution. Test solution volumn was again 10 ml and metal concentrations were prepared from Merck concentrates using filtered, sterile sea water of 30 ppt salinity as diluent. End points in these experiments was mortality at 24 and 48 hours and LC 50 values were calculated using a computer software program called Effl.

2.3.5 Bulk Sediment Testing Using Amphipods

The amphipod chosen belongs to the family Corophidae, species *Photis longidactylus*. Test procedures summarized below were based on the test protocol for amphipods recommended by Environment Canada (1992)

Amphipod collection

Sediment toxicity tests using mud crab larvae gave poor survival rates in the controls and was abandoned. In its place, amphipods collected from an estuary were used. The amphipods were actually inhabiting among the stalks of seaweed (*Gracillaria sp.*) but were observed to burrow in soft mud when placed in the test jars.

The amphipods were kept among red algae in glass aquarium tanks for 5 days prior to testing. They were tolerant to salinities up to 27 ppt. Reference toxicant tests could not be carried out since they were unable to survive without a substrate.

Four sets of experiments were done to determine a suitable test duration. The tests were static with no water change or feeding and they were stopped at 4, 6 , 8, and 10 day intervals with 3 replicates for each duration. Tests jars were set up with 3 cm thickness of sediment (20g) and filtered sea water was added slowly down the side of the jars so as not to disturb the sediment surface until the jars were 3/4 full. Test jars were left to stand overnight with mild aeration and 20 amphipods were seeded randomly into each test jar the next day. The jars were capped loosely to prevent entry of animals or foreign material into the test solution. Each experiment had sediment from the three sampling sites and one reference sediment collected from the location where the amphipods were taken. For each experiment three test jars from each station were removed at intervals of four, six eight and ten days and the number of surviving amphipods were removed by gentle sieving (0.5 mm mesh) in troughs of water of the same salinity and counted. Movement of legs when viewed under a dissecting microscope was an indication of viability.

2.4 BENTHIC FAUNAL COMPOSITION

2.4.1 Sampling and Identification

Sampling for the benthic infauna was carried out approximately two months after the sampling for bioassay testing. No major changes in weather conditions or storms, that might have affected the study site, were noted over this time period. Sampling location was identified with the GPS location. An Ekmann grab, with an opening of 19 x 19 cm was used to collect five samples from each study site. Samples were immediately fixed in 5% formalin and stained with rose bengal. Each sample was processed separately in the laboratory. The samples were sieved through 0.5 mm mesh, remaining material was sorted and recovered organisms were placed in containers in 70 % ethanol and later identified up to generic level. The number of species (g) and number of individuals (i) were recorded for each sample.

There are numerous ways to measure community stress, and for this study the question was 'are there pollution induced changes in the benthic fauna composition'. Species composition and diversity vary so much naturally from place to place that it was necessary to use an index that takes into account factors which induce natural variability such as the sediment texture and water depth. The index chosen for this purpose was the coefficient of pollution. It was also for this reason that the taxa were identified to the class level since it has been found that disturbance effects can be detectable at higher taxa, while there is enormous natural variability at the species level (Warwick and Clark, 1993).

2.4.2 Calculation of coefficient of pollution

The coefficient of pollution, p. is an index established from empirical relationships between the numbers of individuals and species in unpolluted macrobenthic communities (Satsmadjis.1985). The index p is calculated from a series of empirically derived integrated equations given below:

coefficient of pollution ,	$p = g'/[g(i/i_o)^{1/2}]$
theoretical number of species,	$g' = i/(0.0124i + 1.63)$
theoretical number of individuals.	$i_o = (-1.0187s'^2 + 2.63s' - 4.0)(2.20 - 0.0166h)$
calculated sand equivalent	$s' = s + t/(5 + 0.2s)$

in which s and t are the % sand and % silt in the sediment sample, g and i are the actual number of species and individuals from a sample area d m^2 respectively, and h is the depth of water at each station. This index was found to be modified by the effects of pollution and has come to be known as the coefficient of pollution. In this study for the purposes of the sediment quality triad presentation this index would represent the benthic fauna alteration.

2.5 Statistical Treatment of Bioassay Data

The statistical tests used were an ANOVA followed by William's test to compare test value means to control and establish if they were significant. Tukeys test or mean comparison was used to compare test values to each other. The tests were performed using the program Toxstat, release 3.2.

CHAPTER 3

RESULTS

3.1 Field Conditions at Study Site

The following site conditions recorded at the time of sampling are provided in Table 3.1.

Table 3.1: The depth, temperature, salinity, dissolved oxygen and pH of the three sites at the time of sampling

Site	Date	Water Depth	Water Temp	Salinity	Dissolved O_2	pH
Sg.Perai	16/06/94	2.5 metre	29° C	31 ppt	4.8 mg/L	7.5
Sg.Juru	16/06/94	3.0 metre	29° C	30 ppt	4.8 mg/L	7.3
Sg.Tambun	16/06/94	3.0 metre	28° C	30 ppt	5.4 mg/L	8.0

The data in Table 3.1 indicates all sites were similar for the parameters measured. The salinity values were high due to the sampling time coinciding with the incoming tide. Dissolved oxygen values indicate there is good mixing and circulation in the water.

3.2 Sediment Characterization

3.2.1 Physical Characteristics of Sediment

The physical features of the sediment from the three study sites are given in Table 3.2. A reference from Tanjong Tokong, a site with no pollution record and located in an undisturbed area was included for comparison.

Table 3.2 : Physical Features of Test Sediment from Sampling Sites Compared to a Reference from Tanjong Tokong .

Station	TOC %	Moisture	% Sand	% Silt	% Clay	Texture	pH
SP	0.8	47%	71.6	7.5	20.9	clayey-sand	7.6
SJ	1.2	47%	57.7	12.1	30.2	"	7.6
ST	2.2	45%	50.3	14.2	35.5	"	7.7
Reference	4.6	52%	7.5	28.3	64.2	sandy-clay	7.8

While having the same texture. the sediment composition varied between the sites. Sg. Tambun had the highest organic carbon load followed by Sg. Juru and Sg. Perai. Sg. Perai had a higher sand content than both Sg. Juru and Sg. Tambun which were similar with a higher percentage of fine sediment. The reference sample was of a different texture with more fines and a higher organic carbon content.

The sediment types show a general trend from sandy with little organic carbon at Sg. Perai to silty sand at Sg. Tambun with comparatively more organic carbon. Sg. Juru while being located in between the two had an intermediate composition.

3.2.2 Trace Metal Composition

The metals detected in the analyses of the sediment samples from the three sites plus the reference is given in Table 3.3. The values are presented in two forms. The first is the absolute concentrations of the metals in sediment. These values were then normalized by dividing the metal values with the sum of percent clay and silt for that particular site. All values are given in parts per million (ppm) dry weight.

Table 3.3 : Trace metal concentrations in bulk sediment and after being normalized for percent fines (silt and clay)

Absolute Values

Station	Cu	Pb	Zn	Mn	Cd	Cr	As	Hg	%Fe
Sg.Perai	8±2	13±5	70±11	174±3	<0.1	15±2	5	0.04±*	1.1
Sg.Juru	69±8	29±8	98±9	181±6	<0.1	24±4	5	0.14±•	2.1
Sg.Tambun	9±3	16±7	73±9	205±3	<0.1	22±7	5	0.06±•	1.6
Reference	11±2	30±4	84±6	446±5	<0.1	38±3	5	0.01±·	1.9

* 0.02; • 0.05; • 0.02 · 0.005

Normalized Values

Station	Cu	Pb	Zn	Mn	Cd	Cr	As	Hg	% Fe
Sg.Perai	0.28	0.46	2.46	6.13	BDL	0.53	0.18	.0014	0.04
Sg.Juru	1.60	0.69	2.32	4.28	BDL	0.57	0.12	.0013	0.05
Sg.Tambun	0.18	0.32	1.47	4.12	BDL	0.44	0.10	.0012	0.03
Reference	0.12	0.32	0.91	4.82	BDL	0.41	0.05	.0001	0.02

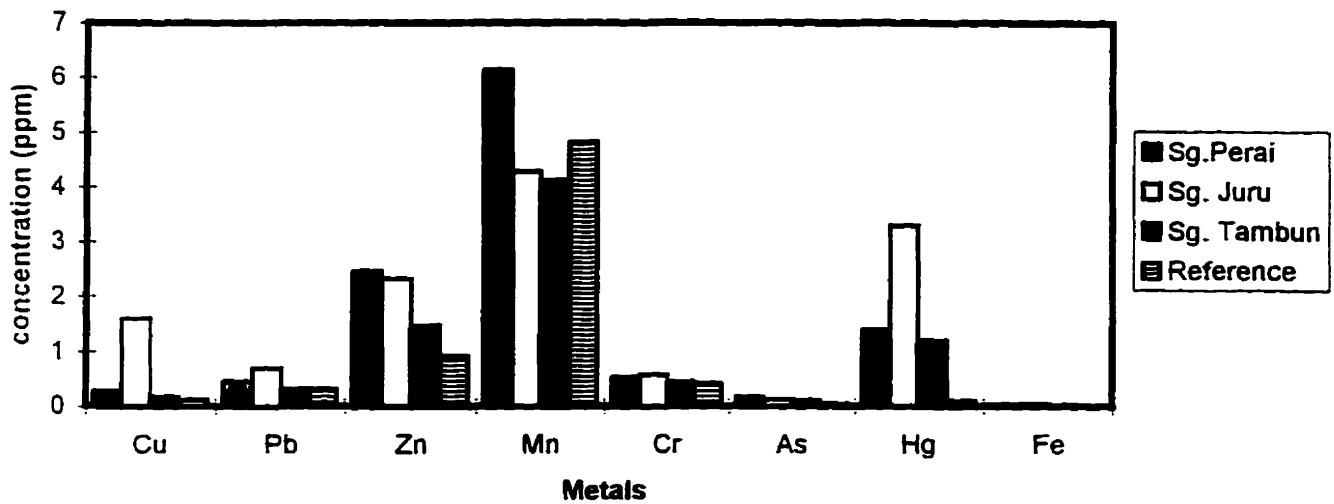
Major elements such as manganese and iron showed little difference in concentrations between the sites. Metals such as copper, arsenic and mercury were very much higher in the sediment from study sites than the reference site. Cadmium was below the part per million range for all sites and below the detection limits of the instrument used. Sg. Juru has the highest concentration (5x) of copper , lead, chromium, and mercury when compared to Sg. Tambun and Sg. Perai. Sg. Perai had relatively high values for zinc and manganese but these metals are seldom toxic at this concentration.

Figure 3.1 illustrates metal concentration levels for the three sites.

3.3 Toxicity Tests (Bioassays)

The endpoints used for the different test organisms were percentage of normal and abnormal larvae and the mean percentage survival. Results are presented as average of three or five values as indicated.

Figure 3.1:Trace metal concentrations in study area sediment normalized to percent fines



3.3.1 Sea Urchin Tests

The sea urchin was used primarily for two types of tests (a) reference toxicants and (b) sediment elutriate tests. The end point measurements at 48 hours were percentage of malformed larvae versus normal development. The following photographs (Figure 3.2) illustrate the differences observed in their development. A well developed fertilization membrane, a distinct and equally dividing intact cell embryo and the pluteus larvae with well developed skeleton in an A shaped formation with intact membranes were considered to show normal development. Absence of fertilization membrane, irregular cell division, exogastrulation and stunted or apollo shaped pluteus larvae were considered abnormal.

Reference Toxicants

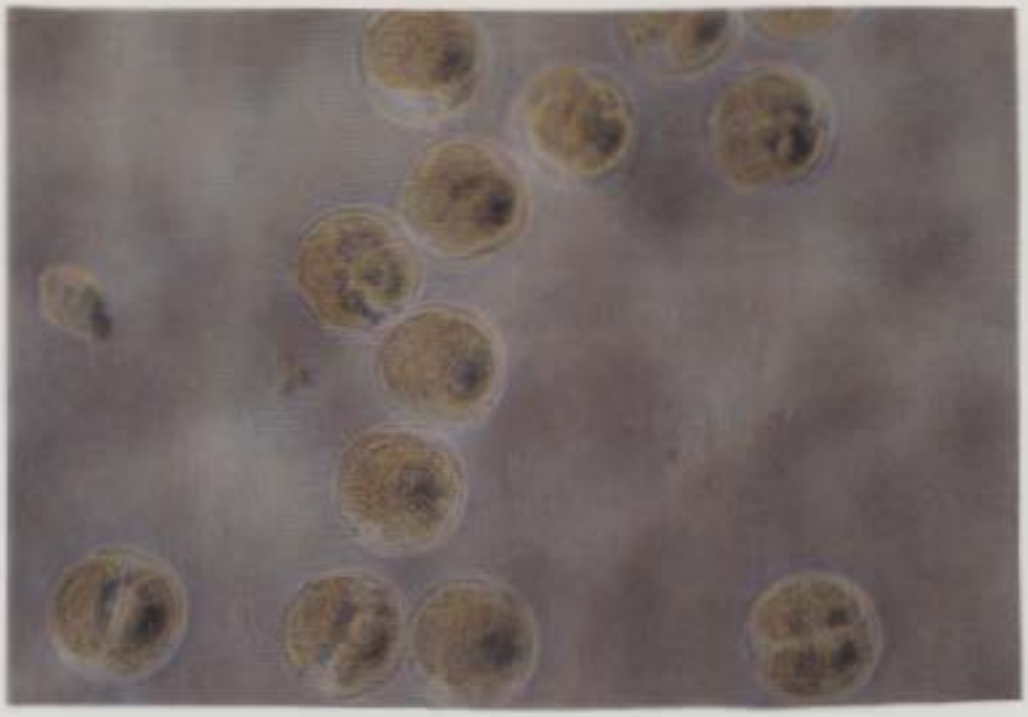
All reference toxicant trials were run using filtered sterile seawater with a temperature of 29-31^0 C, salinity of 27-28 ppt, pH of 7.2 -7.8 and dissolved oxygen of 3.3-4.5 mg/L. These conditions would impose no stress on the organisms for the duration of tests. The results of statistical tests showed that the data was normally distributed and had homogenous variances. Significant differences between test concentrations and controls were carried out using the William's test and are given in Appendix IV. The EC 50 values were calculated using the EFFL computer program (IBM/AT Version 1.0) following procedures described by Stephen (1977). The values are presented below in Table 3.4. The percentage abnormal and normal development of sea urchin larvae for all the test concentrations are given in Appendix III.

Table 3.4: EC 50 Values for Reference Toxicants to Sea Urchin Larvae

Test endpoint	Reference Toxicant (μg/ml)	
Sea urchin Sperm Bioassay	**copper**	**cadmium**
EC 50	**0.07**	**0.95**
Lower 95% CI	**0.06**	**0.80**
Upper 95% CI	**0.08**	**1.13**
1st Cleavage stage		
EC 50	**0.012**	**0.31**
Lower 95% CI	**0.008**	**0.24**
Upper 95% CI	**0.017**	**0.41**
Blastula stage		
EC 50	**0.069**	**1.03**
Lower 95% CI	**0.044**	**0.89**
Upper 95% CI	**0.109**	**1.18**
Pluteus larvae stage		
EC 50	**0.043**	**1.15**
Lower 95% CI	**0.031**	**1.04**
Upper 95% CI	**0.061**	**1.27**

Note: **The EC 50 values and their 95% confidence intervals were based upon nominal concentrations and were calculated according to Stephen (1977).**

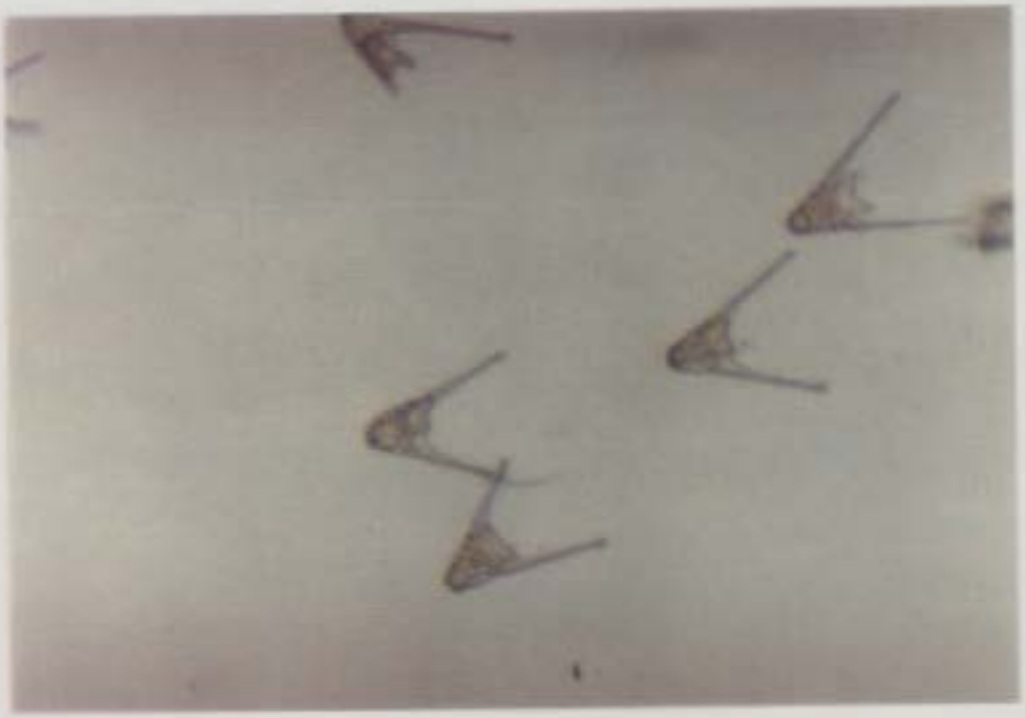
Results of analysis of variance indicated significant differences in endpoints occurred for all concentrations at p = 0.05. William's test showed that mean values for normal development between test concentrations and control were significant at p = 0.05 for cadmium concentrations above 0.5 ppm and copper concentrations above 0.02 ppm. The EC 50 values for copper are very much lower than those for cadmium. Comparing the mean percentage of normal development of the four stages of sea urchin development (Fig. 3.3 and 3.4) it is obvious that the first cleavage stage with EC 50 values of 0.012 ppm and 0.31ppm for copper and cadmium respectively, was the most sensitive for both reference toxicants, followed by the pluteus larvae stage. As a further confirmation of the sensitivity of these two stages, the statistical tests indicate a significant difference in mean abnormal larvae values between control and the lower concentrations of the test solutions for the two stages while there was no significant difference (p=0.05) in percent abnormal larvae between controls and the lower concentrations (0.05-0.20 ppm cadmium and 0.001-0.01 ppm copper) for the sperm fertilization and gastrula stages.



Sea urchin embryo showing irregular first cleavage

Sea urchin embryo showing exogastrulation

Retarded sea urchin larvae at 48 hour interval (mostly prepluteus)



Well developed sea urchin pluteus larvae at 48 hour time interval

Figure 3.2: Normal and abnormally developed sea urchin larvae

Figure.3.3 The percentage of normal sea urchin larvae at various stages of development while being exposed to a range of copper concentrations

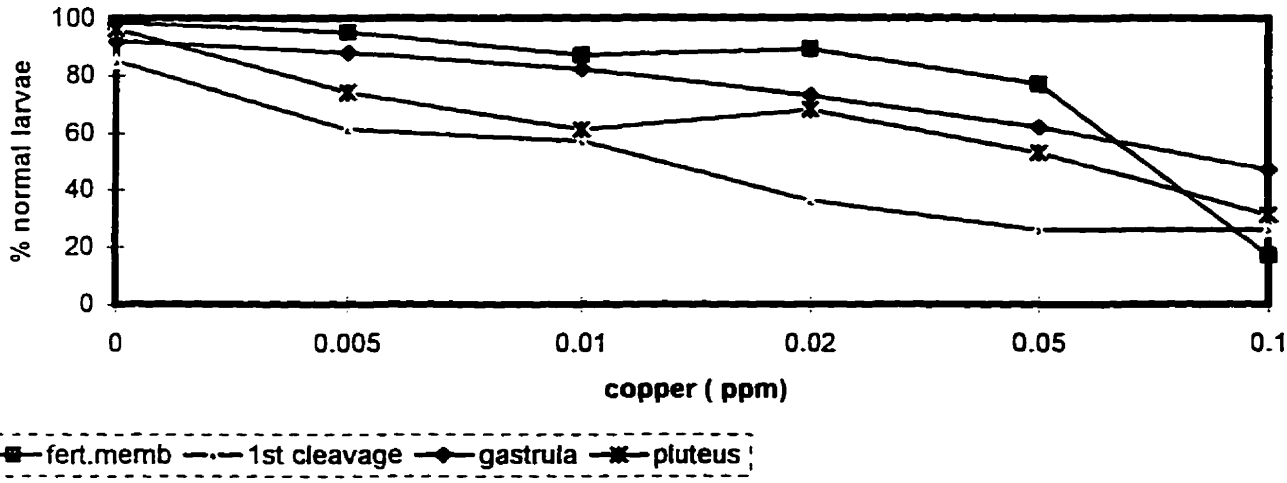
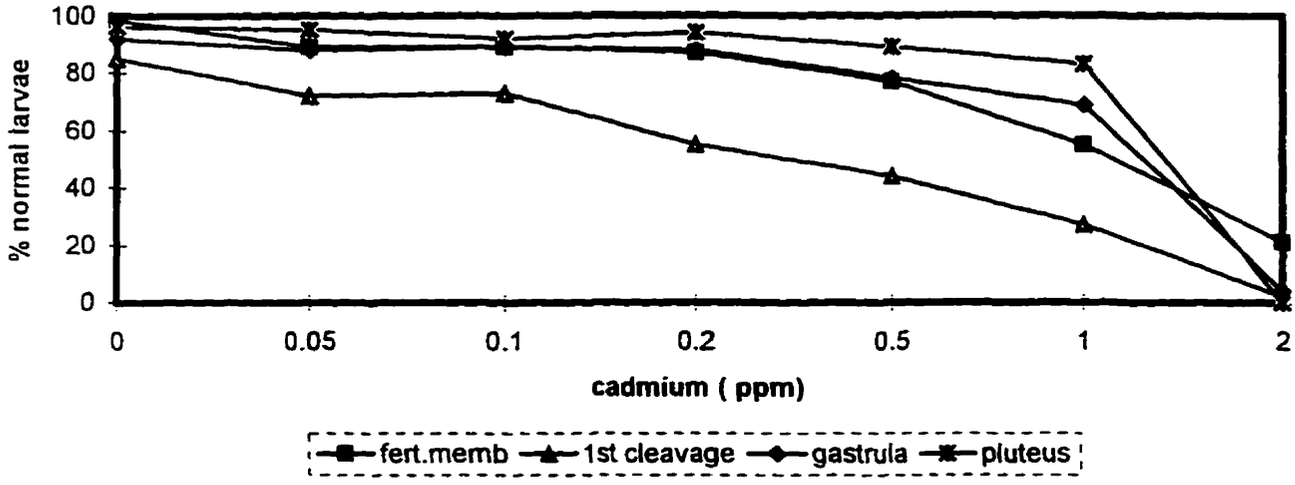


Figure3.4: The percentage of normal sea urchin larvae at various stages of development while being exposed to a range of cadmium concentrations



Sediment Elutriate Test

Table 3.5 lists the mean percentage of normal and abnormal pluteus larvae at the end of the 48 hour test for the three study sites, one reference site and one sea water control.

Table 3.5: Sea Urchin - Sediment elutriate bioassay for the three study sites and the reference site for three trials using 3000 fertilized eggs per test.

Station	Mean % survival	Sig.	% mean normal larvae	% mean abnormal larvae	Sig.
Sg. Perai	37.2 ± 1.5	*	85.9 ±	14.1±	*
Sg. Juru	0	*		-	*
Sg. Tambun	78.8 ± 2.2		86.5±	13.5±	*
Reference	41.1 ± 8.3	*	95.3±	4.7±	
Seawater Control	75.9 ± 5.7		92.9±	7.1±	

Table 3.6: The temperature, salinity, pH and dissolved oxygen levels in the test containers

Station	Temperature °C	Salinity(ppt)	pH	Dissolved O_2 (µg/ml)
Sg. Perai	28 - 29	29	7.6	4.0-5.4
Sg. Juru	28 -29	29	7.6	4.3-5.0
Sg Tambun	29.5- 30	29	7.7	4.0-5.0
Reference	28.5- 30	30	8.0	4.0-5.0

The mean survival values for Sg. Perai ranged from 300 to 450 and were significantly lower (p = 0.05) than the control. but not significantly different from the reference station (Table 3.5). The reference station had a lower survival than expected and this could have been due to the higher pH (Table 3.6) since the reference sediment had a large proportion of arogonite carbonate from the coral reefs on which the adult urchins live.

Sg Juru had no survivors at the end of 48 hours and therefore it was not possible to determine percent abnormal larvae. It was assumed that it had 100 % abnormal larvae. Sg.Tambun had survival values almost in the same range as the control and much higher than the reference.

Analysis of variance indicated significant differences (p=0.05) in percent abnormal sea urchin larvae between the study sites and the control and reference stations with the three study sites having higher values. The results indicate the Sg. Tambun sediment has the least effect and is not significantly different from the sea water control. Sg. Perai had a lower survival rate but the larval development was as normal as the reference site. In the case of Sg. Juru all died.

3.3.2 Oyster Larvae Tests

Oyster larvae were used in two types of bioassays *i.e.* the reference toxicants and sediment elutriate test. The end point observation to deduce normal and abnormal development was the formation of the D shaped shell for the veliger stage of the larvae. Malformed shells exhibited serrated edges or chipped appearance without a straight hinge on one side. The photographs in Figure 3.5, demonstrate the normal and abnormally developed shells.

Optimum Salinity Range

Mean values of normal and abnormal oyster larvae after exposure of the fertilized eggs to the different salinities for 48 hours are illustrated below (Table 3.7):

Table 3.7 - Oyster larval development in different salinity ranges in laboratory trials for 48 hours.

Salinity (ppt)	Normal Larvae(%)	Abnormal Larvae(%)
15	68	32
20	88	12
25	89	11
30	73	27

In the case of the 30 ppt test solution, most of the shells were hollow and appeared white. Based on the above results, the salinity range for tests using oyster larvae was maintained at 25 ppt for the bioassays.

Irregular development of straight hinged D-stage shell for oyster larvae

Well developed hinge at D-stage for oyster larvae

Figure 3.5: Normal and abnormal D shaped prodissonch oyster larvae

Reference Toxicant

Percentage of normal and abnormal oyster larvae for all the test concentrations are given in Appendix III. The data were normally distributed with a homogenous variance. The test concentration produced significantly different results from the control values (Williams test. Appendix IV). The calculated EC 50 values in ppm for copper and cadmium tested with oyster larvae are given in table 3.8.

Table 3.8: EC_{50} for reference toxicants on oyster larvae after 48 hours exposure

Test Endpoint D-hinge veliger larvae	copper	cadmium
EC 50	0.081	0.46
Lower 95% CI	0.060	0.41
Upper 95% CI	0.108	0.52

As with sea urchins copper appears to be more toxic than cadmium. the oyster being less sensitive, with an EC 50 value of 0.081 ppm as compared with 0.012 ppm for first cleavage of the sea urchin. It was more sensitive in the case of cadmium with a lower EC 50 value, 0.46 ppm when compared with three stages of sea urchin larval development which are fertilization membrane, gastrula and the pluteus larvae (Table 3.4).

Suspended Sediment Tests

Table 3.9 lists the percentage survival, and the mean percentage of normal / abnormal veliger oyster larvae in suspended sediment tests.

Table 3.9: Oyster Larvae - Laboratory suspended sediment bioassay conducted over 48 hours.

Station	Mean survival	Sig.	% Normal Larvae	% Abnormal Larvae	Sig.
Sg. Perai	709±11	*	81.8	18.2	
Sg. Juru	636±9	*	85.2	14.8	
Sg. Tambun	784±21	*	75.1	24.9	*
Reference	980±26		80.9	19.1	
Sea water control	1164±34		86.8	13.2	

Table 3.10: The temperature, salinity, pH and dissolved oxygen levels in the test containers.

Station	Temperature ^{0}C	Salinity (ppt)	pH	Dissolved O_2 (µg/ml)
Sg. Perai	29-30	27-28	7.5	4.2-4.5
Sg. Juru	29-30	26-27	7.6	4.0-5.0
Sg. Tambun	29-30	26-27	7.5	4.5-5.0
Reference	28-29	27-28	7.7	4.2-4.4

Mean survival values for all stations were significantly lower (p=0.05) than controls and reference station but there was no significant difference between the sites. As was the case in the bioassays with sea urchins, the order of toxicity with regard to mean survival was Sg. Juru > Sg. Perai > Sg. Tambun. A different trend was observed for percentage abnormal larvae with Sg Tambun > Sg. Perai > Sg. Juru but none of the values were significantly different (p=0.05), from control or reference

except for Sg Tambun which had 24.9 % abnormal larvae (Table 3.9). Water quality measurements were in the acceptable range (Table 3.10).

3.3.3 Mud Crab Bioassay

In the case of mud crab bioassays there were only reference toxicant tests since gross sediment trials with these larvae yielded poor results. The end point in this test was mortality and test animals were assumed dead if there was no movement in legs when gently prodded.

Reference Toxicant

Statistical tests for homogeneity of variance and normality of distribution as well as Williams test for significance of test results with controls are given in Appendix IV. The percentage mortality for each test concentration is given in Appendix III . The calculated LC 50 values are :-

Table 3.11: LC_{50} for reference toxicants on mud crab larvae after 48 hours exposure

Test Endpoint	copper (ppm)	cadmium (ppm)
LC 50	0.080	0.078
Lower 95% CI	0.070	0.066
Upper 95% CI	0.093	0.092

The LC 50 value of 0.078 ppm (Table 3.11) for cadmium is the lowest among three invertebrates (Figure 3.7) tested and is as toxic as copper (Figure 3.6) . The EC 50 values were reproducible within a narrow margin for repetitive test runs.

3.3.4 Bulk Sediment Testing Using Amphipods

Table 3.12 gives the mean percentage survival values for the amphipod tests at the different test durations.

Table 3.12 : Amphipod-Gross sediment bioassay for the three study sites and the reference site for three trials using 20 amphipods per test.

Station		% Survival			Sig.
	4 day	6 day	8 day	10 day	
Sg. Perai	50-60	30-50	10	10	
Sg. Juru	20-25	15	0	0	*
Sg. Tambun	50-65	15-20	15	10	*
Reference	50-75	40-60	40-50	40-50	
Sea water control	60	0	0	0	
Water quality of test containers	S% : 28-30	DO_2 : 4.9-5.4	Temp: 30° C	pH: 7.8-8.1	

Figure3.6: The percentage survival or percentage normal larvae of three invertebrate species whose fertilised eggs were exposed to a range of copper concentrations

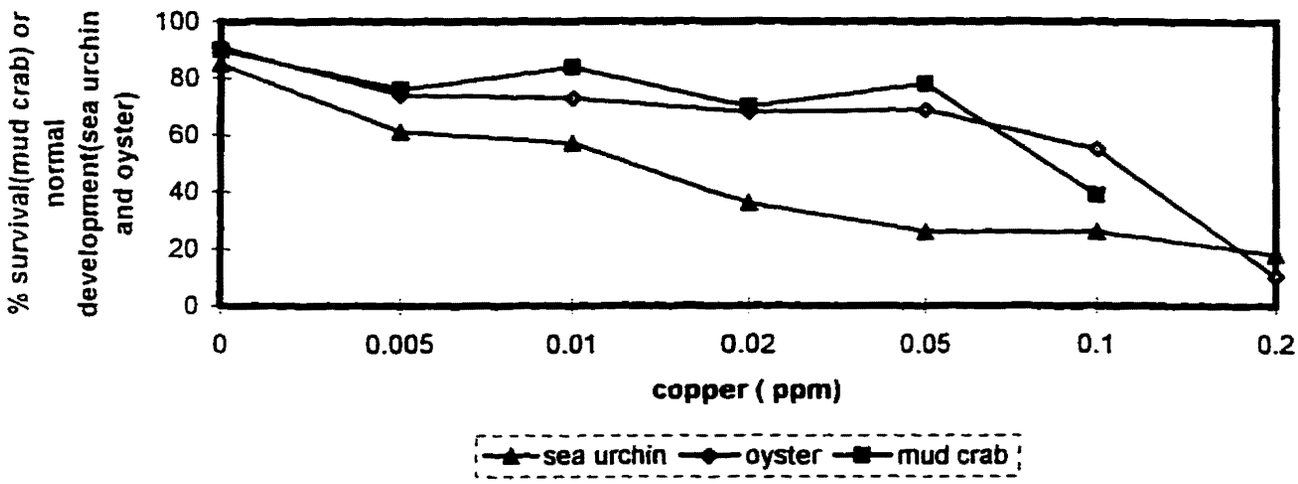
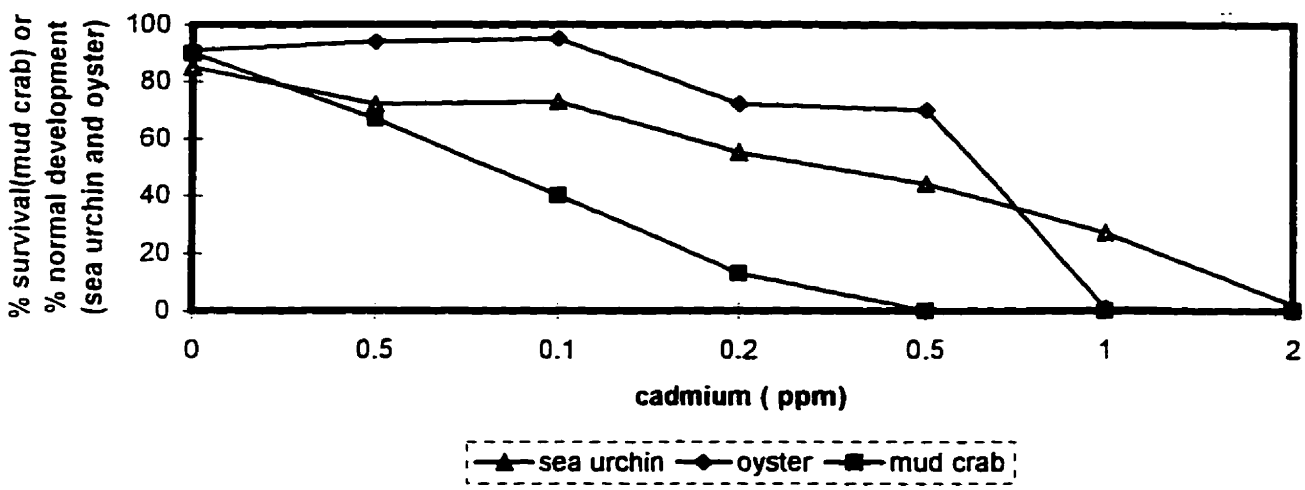


Figure 3.7: The percentage survival or percentage normal larvae of three invertebrate species whose fertilized eggs were exposed to a range of cadmium concentrations



The amphipods did not survive the in sea water control . demonstrating the need for a substrate to bury in. Also. considering the fact that the percentage survival for reference sediment was 40-60 on the sixth day . the test animals must have been under stress not caused by sediment toxicity. Even so, considering the data for the 4 and 6 day period, Sg. Juru was clearly not conducive to their survival. but Sg. Perai demonstrated lesser toxicity then Sg. Tambun. Since this was the only gross sediment testing where the test animal was intimately in contact with the sediment. the most likely cause of this observation can be attributed to grain size effects since Sg. Tambun had higher percent fine grained silt and clay (Table 3.2). The texture of the reference sediment *i.e* sediment from the location where the animals were collected. was more coarse.

Results of statistical tests demonstrate significant increases in amphipod mortality in the sediment from Sg. Juru and Sg. Tambun when compared to reference and Sg. Perai.

3.4 Benthic Fauna Composition

The abundance and diversity of the various benthic organisms in 0.04 m^2 sediment collected from the three stations (five substations for each site) are tabulated in Table 3.13.

ST- Sg. Tambun; SJ- Sg. Juru; SP- Sg. Perai

Table 3.13: Benthic Infauna Composition at Three Study Sites (15 sub stations)

Class/ Subclass	ST 1	ST 2	ST 3	ST 4	ST 5	SJ 1	SJ 2	SJ 3	SJ 4	SJ 5	SP 1	SP 2	SP 3	SP 4	SP 5
Oligochaeta	5					1							1		
Polychaeta	3	6	1	2	4	3	4	1	1		1	1		3	1
Bivalvia		9	4	2		6	2	5		2	1			11	
Gastropoda	1	2	1	2	1	4	2		1		1	6	5	8	
Crustacea		3	1	2		10	2				1	1	8		
Ophiuroidea	4	5	2	1											
Echiurida				1											1
Malacostraca															1
Arachnida		1													
Cirripedia		1				2	1								
Nematode		11		7	2	3	2		1				1		2
Hirudinea			2												
Sipunculida						3									
Turbellaria							1	1							2
Total No.sp,g (Diversity)	4	8	6	7	3	8	7	3	3	1	4	3	4	3	5
Total No. Individuals ,i (Abundance)	13	38	11	17	7	32	14	7	3	2	4	8	15	22	7
Coeff. of pollution, p	4.7	3.5	2.9	3.0	4.9	3.5	2.6	4.5	3.1	7.6	3.1	5.7	5.4	8.6	3.2

The benthic fauna composition was rather poor with very low abundance and diversity values. There was no dominant class for most subsamples but there was a slightly higher number of nematodes (21) for Sg Tambun while Sg.Perai had more (22) gastropods. most of which were dog whelks.

The diversity of taxa indicates Sg Juru to have three subsamples with less than 3 taxon while most of the taxa in the study site were from the first two substations SJ1 and SJ2.

Examination of the distribution of the different organisms among the three stations reveals one very important observation which is the presence of brittle stars (Class: Ophiuroidea) in Sg Tambun and not in the other two stations. Brittle stars are usually found in clean reference areas as dominant species (Swartz et al . 1986). Likewise the absence of amphipods in all three stations indicate they are all contaminated to some extent.

Coefficients of pollution 1.5 -2, 2-3, 3-4, and 4-8 are regarded as indicating slight, moderate, heavy and very heavy pollution, respectively (Satsmadjis, 1982). Therefore, since most of the p values were between 3.0 to 7.0, they indicate the sites to be heavy and very heavily polluted even using this taxonomic level of identification. Tukeys test for mean comparison of p values between the sites shows no significant difference.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1. Bulk Sediment Chemistry

Sediment from all three study sites were of a similar texture, that being clayey sand with Sg. Perai having more sand than clay. Their physical properties were rather similar and demonstrate no adverse fouling or anoxic condition. Cockles and some crabs were the only large invertebrates that were picked up in the sediment while sampling.

Organic carbon values were not significantly different between the sites and followed the % silt fraction ratios closely. Metal concentrations at Sg. Juru were the highest for copper, lead, chromium and mercury (Table 3.3). This is in agreement with the literature where the metals are mainly present in the clay/silt particles with grain size less than 0.063 mm (Goldberg, 1974; Forstner and Wittman, 1979. Araujo *et al.* 1988) as there were higher metal concentrations in sediments with higher clay fractions such as the reference site and Sg. Juru. Metal concentrations from this study had values higher by an order of two for zinc and manganese while copper concentrations were three times higher than those determined in another study (Din and Jamaliah). Concentrations of lead and chromium were lower in the present

study. Comparisons with metal concentrations done in other parts of the country (Table 4.1) show the values from the present study are in a similar range with the exception of copper.

Table 4.1: Range of metal concentration (μg/g dry weight) in the study sites compared to values from other studies conducted in Malaysia.

Metal	Present Study	Penang Island [1]	Straits of Malacca [2]
Arsenic	5.0	1.47-3.37	12.0 ± 6.12
Cadmium	< 0.1	0.036-0.067	0.112±0.06
Chromium	15-38	37.5-76.5	45.16±21.08
Copper	8-69	8.43-13.18	10.76±4.30
Manganese	174-446	71.9-108.0	386.4±160.2
Lead	13-30	14.2-34.7	30.17±8.65
Zinc	70-98	20.5-42.3	65.92±24.47

1- Din and Jamaliah, 1995

2- Din, 1992

Normalization of metal levels to percent fines as suggested by Chapman (personal communication) gave a metal distribution pattern with Sg. Juru having higher values for copper, lead, chromium, mercury and iron followed by Sg. Perai and Sg. Tambun respectively (Table 3.3; Figure 3.1). This could be attributed to the factory discharges from processes involving electroplating and paint production. The Sg. Perai site had higher values for zinc and arsenic which may have originated from the repair and maintenance operations in the shipyard located further up the estuary.

A more complete picture of the sediment contamination would have been achieved if analyses for hydrocarbons and pesticides were carried out. Also the availability of metals to the benthic population would be better understood if acid volatile sulfides had been determined for it has been demonstrated that they play a dominant role in controlling metal availability (Ankley, 1991). These are two factors to be considered in future investigations of a similar nature.

4.2 Bioassays

The bioassay tests were divided into two areas of concern , (a) the sensitivity of the test organisms to reference toxicants and (b) their utilization in sediment toxicity tests.

4.2.1 Sensitivity of Test Organisms to Reference Toxicants

Each of the three invertebrates used, had advantages over the others in certain areas thus making it difficult for choice ranking. They would suit specific tests depending on the objective of the tests. The most sensitive test for copper was the sea urchin bioassay with first cleavage as the end point (Figure 3.6) whereas for cadmium, the mud crab gave the lowest LC 50 value (Table 3.11; Figure 3.7).

Response curves for cadmium show a distinct increase in mortality or abnormality, as the case may be, in the EC/LC 50 value range which reflects the acute toxicity of this metal. In the case of sea urchin embryo with the different stages as end points, the first cleavage stage and the sperm bioassay were most suitable for toxicity testing owing to their sensitivity (Table 3.4, Fig 3.7). The two cell stage and the fertilization membrane are clearly observable so any abnormalities are easily detected.

The blastula and pluteus have more features. thus complicating the distinction between normal and abnormal larvae.

There have been different findings with regard to the most sensitive stage of the sea urchin embryo from various studies using different species and also between invertebrate species. This could be expected since they were all of different species. Dinnel and Stober (1987) reported that the relative sensitivities of the various bioassay organisms they tested to measure sewage toxicity were: sperm assays > oyster embryo abnormality > oyster embryo mortality > crab zoea mortality. In the case of sea urchins. Kobayashi (1994) found the sensitivity increased as the fertilized eggs developed into the pluteus larvae. The present report finds the following sequence for copper: first cleavage stage > pluteus larvae > sperm bioassay followed by oyster embryo and crab zoea which had almost similar sensitivity. In the case of cadmium it was the opposite, with crab zoea > oyster embryo > sea urchin first cleavage > sperm assay > pleuteus larvae. Pastorak *et al* (1994) conducted a variability study on EC 50 values for various test species and one reference toxicant (cadmium chloride) and reported oyster embryo to be more sensitive than sea urchin. The EC 50 values reported by them were 0.94 µg/ml for oyster embryo which is close to the present finding of 0.46 µg/ml but their value for sea urchin pluteus was 27.8 ug/ml while the present study gave a value of 1.15 µg/ml.

Sea urchin larval development was observed to be retarded rather than being abnormal by exposure to metal concentrations. This feature was especially noticeable during test runs using very ripe eggs characterized by a yellowish-orange color. In one trial run using such eggs. at the end of 48 hours the eggs had developed

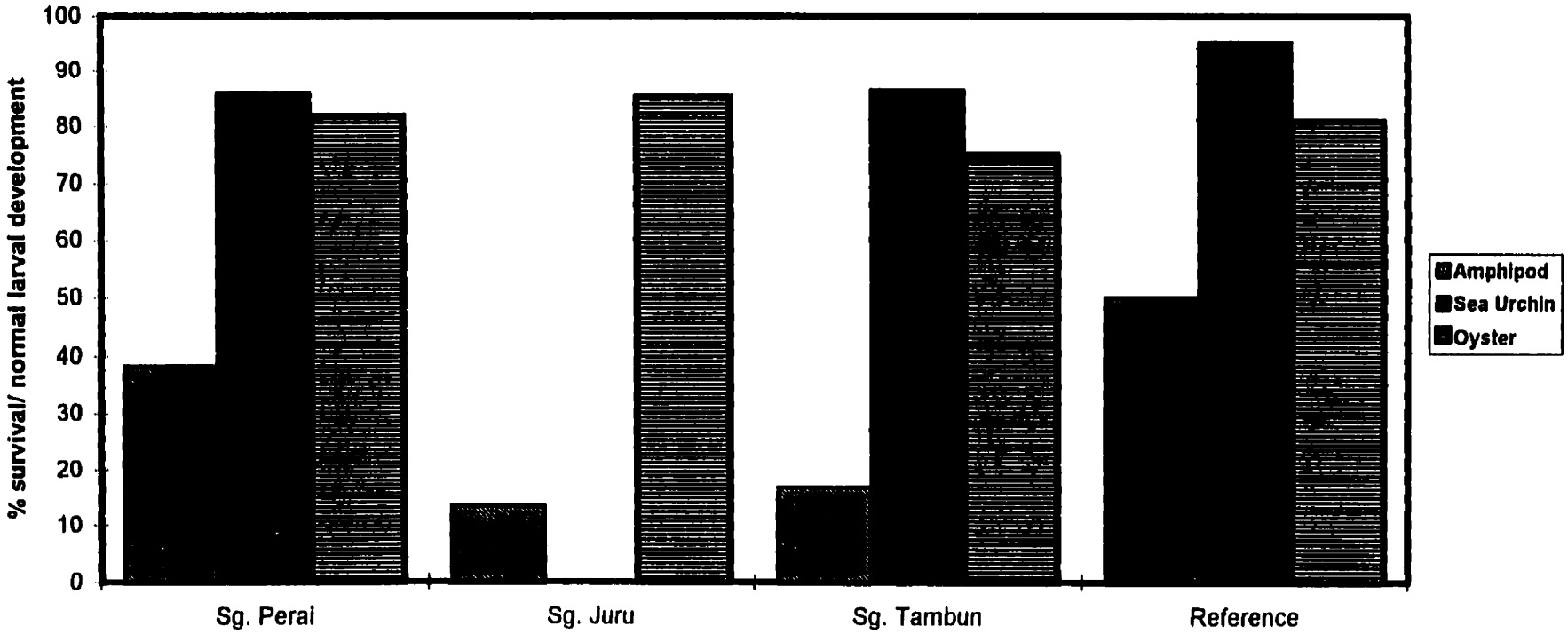
to pluteus in the controls and the lowest metal concentrations. but the development was retarded at various stages with the highest concentrations having all blastula and the intermediate ones having proportions of prepluteus and blastula.

These qualities of the test organisms are points to consider when selecting suitable test animals. The optimum salinity range determined for oyster larvae development was in agreement with work done using a closely related species the mangrove oyster *C. belcheri* (Devaki N. . personal communication)

4.2.2 Sediment Bioassay

On the whole there were three sediment bioassays with five end points. The sea urchin sediment elutriate test identified Sg . Juru to be most polluted followed by Sg. Perai and Sg. Tambun (Fig.4.1). In the mean survival end point for sea urchin test. Sg. Tambun had survival values similar to the controls demonstrating no lethal toxic effects while Sg. Juru and Sg. Perai both had values significantly lower than the control. Again, for the same test but with abnormal larval development as end points. the differences between the stations were insignificant but the survival as well as abnormal larval development values were significantly higher than the sea water controls and reference sediment. The order of toxicity for sea urchin sediment bioassay was Sg. Juru > Sg. Perai > Sg. Tambun. In addition to the feasibility of this species of sea urchin as a test organism it is possible to observe color differentiation between the test jars at the end of 48 hours since the echinochrome in the free floating pluteus larvae gives the suspension above the sediment layer a pink tint. This feature

Figure 4.1. :The percentage survival or normal development of three invertebrate larvae in sediment bioassay



could serve as a rapid test and a spectrophotometer reading might be used to give standard curves.

The reference sediment for the sea urchin test gave lower survival than expected since it was not maintained in an ideal condition nor did it simulate conditions at the collection site since the sediment had been kept for about a week in an aquarium with little water change and, therefore, it indicated a higher pH value resulting from the breakdown of arogonite calcium carbonate from the coral chips in the sediment.

Sediment bioassays using oysters produced survival values for the study sites that were all significantly lower than the control. However, except for Sg.Tambun, for the abnormal larvae end point the results were not significantly higher than the control.

The gross sediment bioassay using amphipods gave results indicating Sg. Juru to be most polluted followed by Sg. Tambun and then Sg. Perai. The test gave acceptable results for a test duration of six days since the survival in the reference control sediment was only 50% after six days. Before responses can be ascribed to contaminant effects, the tolerance of this new test species to natural variations in sediment characteristics has to be established as had been done for *Rhepoxynius abronius* (Swartz *et al*, 1985). Some of these features include particle size distribution, organic enrichment, and interstitial water salinity. Mean amphipod survival in fine uncontaminated field sediment can be 15% lower than survival in native sediment (DeWitt *et al*, 1988). In this study the possibility arises that the grain size effects from the higher percentage of silt and clay in Sg. Juru and Sg. Tambun

could have contributed to the increased amphipod mortality. It is possible to separate the effects of fine particles and chemical contaminants in test sediments by using regression based statistical models (DeWitt *et al.* 1989). The variable ambient conditions at the natural habitat also have to be determined to improve the choices made with reference to density of test organism. temperature and salinity.

4.3 Polychaete Worm (Perinereis nuntia) Culture

Historically. the nereid polychaetes have frequently been used for assessing the ecotoxicological impact of estuarine and marine contaminants, probably due to their abundance in areas potentially most vulnerable to pollution (Pesch and Hoffman. 1983; Reish. 1984). This particular species is one of the most abundant types inhabiting the shorelines of the Malaysian coast. Collection of the adults and holding them in laboratory tanks was rather simple and straightforward.

For the use of this organism in fertilization bioassays, there was no difficulty in collecting (via excise spawning) enough eggs and sperm for experimental purposes. Differentiation between fertilized and unfertilized egg was straightforward due to the rapid formation of the fertilization membrane and a jelly coat around the egg.

Culture of this species was more difficult in comparison to *Neanthes* owing to its swarming behavior prior to spawning which makes it difficult to contain the fertilized eggs and collect the juveniles. Also. identifying the gravid *P.nuntia* amongst the numerous polychaete species inhabiting the shallow coastal waters was not easy and there were some experiments which had to be abandoned when the

fertilized eggs did not develop any further because the adults were of different subspecies.

Further research is needed to investigate the diet, photoperiod manipulation for induced spawning and holding the adults in a water medium for any length of time as with *Platynereis dumerilii* (Hutchinson *et al*, 1995). When the species is amenable to laboratory culture it would be an ideal candidate for bioassay testing as has been done with *Neanthes arenacoedentata*.

4.4 Benthic Fauna Composition

The benthic fauna for all three sites are low in abundance and moderately poor in species diversity when compared the Straits of Penang Island (Ong and Din, 1995). This could be attributed to sediment texture and stress from salinity gradients and also, in part, to anthropogenic impacts. Mixed sediments generally do support a higher diversity than pure sand (Gray, 1974) and, in this case, all three sediment samples had more sand than silt or clay thus reducing the diversity and abundance. There was no indication of organic pollution which is usually typified by the dominance of some polychaetous species (Grassle and Grassle, 1974).

Sg. Tambun had on the average more species per sediment sample than Sg. Juru or Sg. Perai (Table 3.11). Sg. Juru had two subsamples with higher species composition and abundance, while the Sg. Perai subsamples all had poor species composition. This pattern would suggest that the higher silt and clay ratio and the lower toxicity values for Sg Tambun are both contributory to the observed species

composition of this site which includes the presence of brittle stars (Class Ophuiroidea, Table 3.11). Sg Perai on the other hand had higher toxicity values and was more sandy in texture thus exhibiting a poorer benthic faunal composition with the exception of dog whelks. Sg.Juru which had the highest in toxicity values also had more silt and clay compensating for it, thus it had some samples that were poor and some average in macrofauna abundance and diversity.

In comparison with benthic faunal data from another investigation (Ong and Din, 1995) on six sites in the neighbouring areas of Penang Island (Table 4.2), the study site shows a rather diminished community both in diversity and abundance. The sampling done for this earlier study at Sg Juru covered a wider area and had 12 sampling stations, but it still shows a minimal diversity (4 -11) but a wide range in abundance (7 - 341) depending on the exact location and sediment texture of the sample (Table 4.2). It gives a strong indication of the altered state of the benthos for the site.

Table 4.2 Benthic fauna composition in 0.1m ^{2} area of study site * compared to values obtained from other studies conducted in the province of Penang.

	SP*	SJ*	ST*	SP	JU	GE	PJ	BM	SJ
# Species	11.4	13.2	16.8	4-5	5-20	11-45	8-35	14-19	4-11
Abundance	33.6	34.8	51.6	7-220	14-117	80-727	36-202	93-170	7-341

Note: a) Values from present study were summed up for each site(5 stations) and multiplied by factor 3/5 to obtain data for

b) SJ for both investigations refer to Sungai Juru

4.5 Ratio to Reference Presentation of Sediment Quality Triad

Initial attempts to analyze and present Triad results in a meaningful way involved the use of bar graphs (Long and Chapman, 1985). Subsequently this was changed to summary indices or ratios for each of the three components, normalized to a reference value {called a ratio - to - reference (RTR) determination}, followed by presentation of the data in a triangular format (Chapman, 1990, 1992).

The results of the three components of the triad in this study, trace metal values normalized to percent fines, the three sediment bioassay results and the coefficient of pollution values for the three sites were normalized to their respective reference values (Table 4.3). In the case of the coefficient of pollution ratios a reference value of 1.0 depicting no pollution was used in the sediment quality triad.

The values for each study site were averaged and the presented in a triangular format with the area of the triad and the corresponding conclusion.

Table 4.3: Ratio to reference (RTR) values of three triad components for the study sites calculated from metal concentrations, sediment elutriate bioassay and coefficient of pollution values.

Metal Contamination: normalized values for study site ÷ values for reference site (from Table 3.3).

	Cu	Pb	Zn	Mn	Cr	As	Hg	Fe	RTR
SP	2.33	1.44	2.70	1.27	1.29	3.60	12.7	2.0	**3.41**
SJ	13.3	2.16	2.55	0.88	1.39	2.4	30	2.5	**6.89**
ST	1.50	1.00	1.62	0.85	1.07	2.0	10.9	1.5	**2.56**

Sediment Toxicity: % mortality and % abnormal larvae from bioassays ÷ by reference values (from Tables 3.5; 3.7 and 3.8).

	Sea urchin (Mortality)	Sea urchin (Abnormal)	Oyster (Mortality)	Oyster (Abnormal)	Amphipod	RTR
SP	2.6	3.0	14.55	0.95	1.8	**4.58**
SJ	4.15	-	18.2	0.77	2.0	**6.28**
ST	0.88	2.9	10.8	1.30	1.78	**3.53**

Coefficient of pollution values (Table 3.13) from study sites ÷ 1 (depicting no pollution)

Station	1	2	3	4	5	RTR
SP	3.1	5.7	5.4	8.6	3.2	**4.35**
SJ	3.5	2.6	4.5	3.1	7.6	**4.26**
ST	4.7	3.5	2.9	3.0	4.9	**3.81**

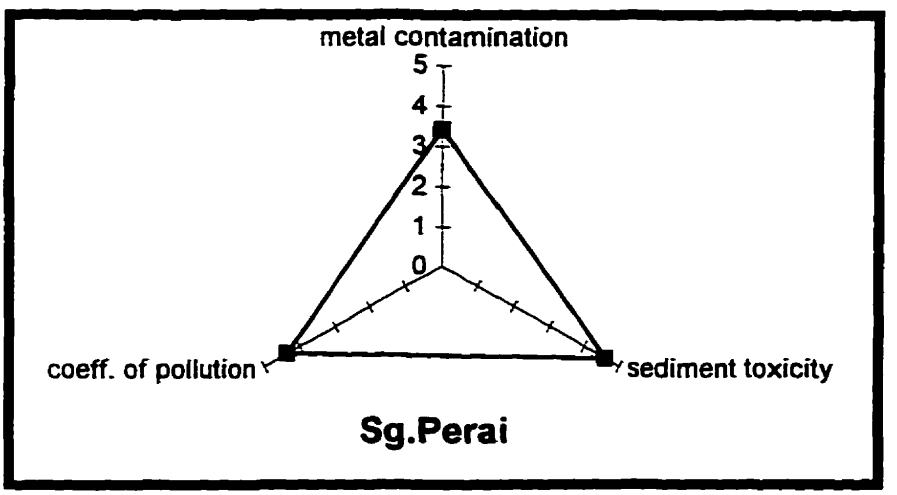
Sg. Tambun was chosen *a priori* for being less polluted since it does not receive industrial or agricultural waste upstream. The triad (Figure 4.3) with an area of 16.12 revealed this site to have the lowest RTR values (Table 4.3) for metal contamination (2.56), sediment toxicity (2.65) and coefficient of pollution (3.81), but the values were higher than the reference value of 1. It therefore indicates this site to be slightly contaminated by metals, and in terms of sediment toxicity, it was moderately toxic while the benthic faunal composition demonstrates slight pollution. Possibly the presence of contaminants other than metals contributed to the toxicity. This site being the last one in a southward direction is most likely receiving contam inants from the estuaries to the north as the net current flow along the coast is in a southerly direction.

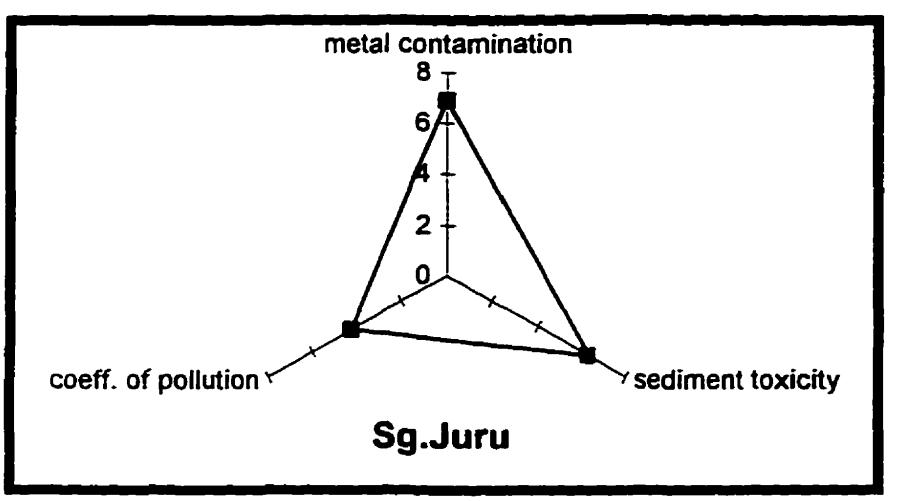
Sg. Perai with a triad area of 25.19 (Figure 4.3) was intermediate in it's RTR values (Table 4.3) with 3.41 for metal contamination; 4.58 for sediment toxicity and 4.35 for coefficient of pollution. It was only slightly higher than Sg. Tambun but here again the sediment toxicity was caused by more than metal contamination. There was also a corresponding paucity in benthicfauna, thus measurement of aromatic

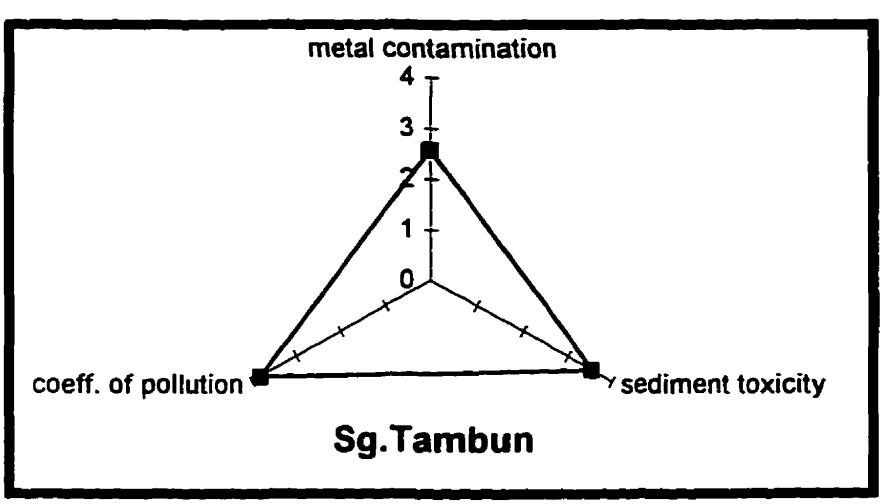
hydrocarbons or pesticides would have provided more information since this river also receives waste from a sugar cane plantation.

Sg. Juru had the highest RTR values (Table 4.3) with 6.89, for metal contamination, 6.28 for sediment toxicity and 4.35 for coefficient of pollution. The triad for Sg. Juru distinctly showed the toxicity of the sediment with one of the contributors being metals. The benthic fauna represented by the coefficient of pollution was not very different from the other two sites, therefore the contaminants are bioavailable but their *in situ* effects are not demonstrable. It suggests further investigation into the benthic faunal community alteration.

Figure 4.2:Sediment quality triad for study sites derived from RTR values







4.6 CONCLUSION

Assessment of the sediment quality based on chemical contamination would have led to the conclusion that the metal concentrations. with the exception of copper in Juru. were not elevated to a detrimental level when compared with metal levels in other parts of the country and the region. Thus, this site would not be viewed as seriously polluted if sediment chemistry data alone were to be taken into account. With regard to the bioassay data. two of the three sediment bioassays and four endpoints show Sg Juru to be the most toxic. followed by Sg Perai. In the case of sea urchin bioassay the Sg. Juru sediment elutriate had no surviving larvae which seems a gross overestimation of the natural situation. The abnormal development of oyster larvae showed the two sites to be equally toxic with the third clean site. Sg. Tambun. to be more toxic while the mean survival values indicate a toxicity ranking similar to the sea urchin and amphipod results. Some of this data could be discounted as using inappropriate test organisms or having end points that are not indicative of *in situ* effects.

This discrepancy becomes insignificant when the macroinfauna composition shows the third site to be relatively clean as indicated by the presence of echinoderms. It also revealed the benthic assemblage for all three sites to be rather poor which could have been inaccurately inferred to mean they were equally polluted.

Therefore, the holistic nature of the sediment quality triad approach has been demonstrated to provide a more accurate assessment of sediment quality.

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Appendix I

Coordinates of sampling stations using Global Positioning System

Study Site	Sampling Station	Latitude	Longitude
Sungai Perai	A	05° 23' 04"	100° 22' 11"
	B	05° 23' 05"	1000 22' 10"
	C	05° 23' 06"	1000 22' 13"
	D	05° 22' 56"	1000 22' 14"
	E	05° 22' 52"	1000 22' 12"
Sungai Juru	A	05° 20' 08"	100° 23' 31"
	B	05° 20' 06"	100° 23' 37"
	C	05° 20' 05"	100° 23' 39"
	D	05° 20' 04"	100° 23' 40"
	E	05° 20' 05"	100° 23' 46"
Sungai Tambun	A	05° 17' 02"	100° 24' 26"
	B	05° 17' 00"	100° 24' 21"
	C	05° 16' 56"	100° 24' 35"
	D	05° 16' 55"	100° 24' 46"
	E	05° 16' 55"	100° 24' 51"

Appendix II

Test Parameter	Test Condition
1. Test Type	Static
2. Salinity	28+/- 1 ppt
3. Temperature	29+/- 1 C
4. Light Quality	Ambient laboratory illumination
5. Light intensity	Moderate
6. Test chamber size	25 ml vol. flat bottom evaporating dish
7. Test solution volumne	10 ml
8. Renewal of test solution	none
9. No. of eggs/ chamber	300
10. Replicate test chamber	3
11. Feeding regime	None during test
12. Aeration	None during test
13. Dilution water	Filtered, UV- sterilized natural sea water
14. Test Duration	48 hrs
15. Effects Measured	Normal/ abnormal dev. of larvae
16. Test Acceptability	U.S.EPA: >70% mean control fertilization
	Env.Can: >50% mean control fertilization

Appendix III

Percentage of normal larvae developing from fertilized eggs of invertebrates exposed to copper and cadmium metal
D.setosa Leske

Nominal test concentration (ug.ml^{-1})	Percentage normal developement	
	Mean	SD
Fertilization membrane stage		
Dilution water control	98.5	2.0
Copper sulfate		
0.005	95.0	1.8
0.01	87.0	2.5
0.02	89.0	2.7
0.05	77.0	8.6
0.10	17.0	3.0
Cadmium chloride		
0.05	89.0	4.1
0.10	89.0	3.2
0.20	87.0	1.7
0.50	77.0	5.2
1.00	55.0	7.0
2.00	21.0	9.4
5.00	18.0	10.2
First cleavage stage		
dilution water control	85.0	5.0
Copper sulfate		
0.005	61.0	6.6
0.010	57.0	9.6
0.020	36.0	15.5
0.050	26.0	9.5
0.100	26.0	7.7
0.200	18.0	4.3
Cadmium chloride		
0.050	72.0	4.2
0.100	73.0	3.7
0.200	55.0	6.0

0.500	44.0	10.6
1.00	27.0	5.0
2.00	2.0	0.3
Gastrula stage		
dilution water control	92.0	5.9
Copper sulfate		
0.001	86.0	5.2
0.002	85.0	0.4
0.005	88.0	5.2
0.10	82.0	7.1
0.20	73.0	18.8
0.50	62.0	31.6
0.10	47.0	17.8
Cadmium chloride		
0.05	88.0	1.4
0.10	89.0	1.1
0.20	88.0	2.1
0.50	78.0	4.0
1.00	69.0	8.6
2.00	4.0	5.0
5.00	2.0	0.2
Pluteus larvae stage		
dilution water control	96.2	3.5
Copper sulfate		
0.001	76.0	2.8
0.002	84.0	1.3
0.005	74.0	3.4
0.010	61.0	5.0
0.020	68.0	4.0
0.050	53.0	4.8
0.100	31.0	3.9
0.200	32.0	1.0
Cadmium chloride		
0.050	95.0	1.6
0.100	92.0	2.5
0.200	94.0	1.3
0.500	89.0	1.6
1.00	83.0	5.4
2.00	0.0	

C.iredalei Faustino

D-hinged veliger stage

dilution water control	91.0	4.3
Copper sulfate		
0.005	74.0	3.6
0.010	73.0	1.5
0.020	68.0	5.6
0.050	69.0	1.8
0.100	55.0	2.2
0.200	10.0	2.0
Cadmium chloride		
0.050	94.0	3.8
0.100	95.0	4.6
0.200	72.0	8.6
0.500	70.0	8.3
1.000	1.0	1.4

S.serratta Forskall

Zoea larvae mortality

dilution water control	90.0	2.9
Copper sulfate		
0.005	76.0	10.4
0.010	84.0	6.0
0.020	70.0	13.6
0.050	78.0	6.2
0.100	39.0	23.0
Cadmium chloride		
0.050	67.0	11.2
0.100	40.0	8.6
0.200	13.0	12.0
0.500	0.0	

APPENDIX IV

Significance Tests for Sediment Bioassays

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.133	0.133		
2	Reference	0.187	0.187	-2.469	
3	Sg. Perai	0.183	0.183	-2.315	
4	Sg.Juru	0.143	0.143	-0.463	
5	Sg.Tambun	0.250	0.250	-5.401	

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	3			
2	Reference	3	0.053	40.0	-0.053
3	Sg. Perai	3	0.053	40.0	-0.050
4	Sg.Juru	3	0.053	40.0	-0.010
5	Sg.Tambun	3	0.053	40.0	-0.117

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0254	0.0064	9.143
Within (Error)	10	0.0073	0.0007	
Total	14	0.0327		

Critical F value = 3.48 (0.05,4,10)

Since F > Critical F REJECT Ho:All groups equal

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 1	4	3	2	5
1	Control	0.133	0.133	\				
4	Sg.Juru	0.143	0.143	.	\			
3	Sg. Perai	0.183	0.183	.	.	\		
2	Reference	0.187	0.187	.	.	.	\	
5	Sg.Tambun	0.250	0.250	*	*	.	.	\

* = significant difference (p=0.05) . = no significant difference
Tukey value (5,10) = 4.65 s = 0.031

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	0.133	0.133	0.133
2	Reference	3	0.187	0.187	0.171
3	Sg. Perai	3	0.183	0.183	0.171
4	Sg.Juru	3	0.143	0.143	0.171
5	Sg.Tambun	3	0.250	0.250	0.250

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.133				
Reference	0.171	1.712		1.81	k= 1, v=10
Sg. Perai	0.171	1.712		1.91	k= 2, v=10
Sg.Juru	0.171	1.712		1.94	k= 3, v=10
Sg.Tambun	0.250	5.288	*	1.96	k= 4, v=10

s = 0.027
Note: df used for table values are approximate when v > 20.

sea urchin normal development
File: B:\TOXSTAT\SEAURCNO. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	26268.653	6567.163	311.520
Within (Error)	15	316.212	21.081	
Total	19	26584.866		

Critical F value = 3.06 (0.05,4,15)
Since F > Critical F REJECT Ho:All groups equal

sea urchin normal development
File: B:\TOXSTAT\SEAURCNO. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	92.925	92.925		
2	reference	95.325	95.325	-0.739	
3	Sungai Perai	85.875	85.875	2.171	
4	Sungai Juru	0.000	0.000	28.622	*
5	Sungai Tambun	86.450	86.450	1.994	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

sea urchin normal development
File: B:\TOXSTAT\SEAURCNO. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	reference	4	7.662	8.2	-2.400
3	Sungai Perai	4	7.662	8.2	7.050
4	Sungai Juru	4	7.662	8.2	92.925
5	Sungai Tambun	4	7.662	8.2	6.475

sea urchin normal development
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ANOVA TABLE

Amphipod -Sediment Bioassay
File: B:\TOXSTAT\AMPHIPOD. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
reference	50.000				
Sungai Perai	38.333	1.905	*	1.86	k= 1, v= 8
Sungai Juru	15.000	5.715	*	1.96	k= 2, v= 8
Sungai Tambun	15.000	5.715	*	2.00	k= 3, v= 8

s = 7.500
Note: df used for table values are approximate when v > 20.

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	849816.400	212454.100	796.006
Within (Error)	5	1334.500	266.900	
Total	9	851150.900		

Critical F value = 5.19 (0.05,4,5)
Since F > Critical F REJECT Ho:All groups equal

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	780.000	780.000		
2	reference	418.500	418.500	22.128	*
3	Sungai Perai	365.000	365.000	25.402	*
4	Sungai Juru	0.000	0.000	47.744	*
5	Sungai Tambun	777.000	777.000	0.184	

Dunnett table value = 2.85 (1 Tailed Value, P=0.05, df=5,4)

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	3375.000	1125.000	16.071
Within (Error)	16	1120.000	70.000	
Total	19	4495.000		

Critical F value = 3.24 (0.05,3,16)
Since F > Critical F REJECT Ho:All groups equal

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Reference	52.000	52.000		
2	Sg Perai	62.000	62.000	-1.890	
3	Sg.Juru	82.000	82.000	-5.669	
4	Sg.Tambun	82.000	82.000	-5.669	

Dunnett table value = 2.23 (1 Tailed Value, P=0.05, df=16,3)

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Reference	5			
2	Sg Perai	5	0.616	1.2	-10.000
3	Sg.Juru	5	0.616	1.2	-30.000
4	Sg.Tambun	5	0.616	1.2	-30.000

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F

Between	3	3375.000	1125.000	16.071
Within (Error)	16	1120.000	70.000	
Total	19	4495.000		

Critical F value = 3.24 (.05,3,16)
Since F > Critical F REJECT Ho:All groups equal

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 1 2 3 4
1	Reference	52.000	52.000	\
2	Sg Perai	62.000	62.000	. \
3	Sg.Juru	82.000	82.000	* * \
4	Sg.Tambun	82.000	82.000	* * . \

* = significant difference (p=.05) . = no significant difference
Tukey value (4,16) = 4.05 s = 70.000

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Reference	5	52.000	52.000	52.000
2	Sg Perai	5	62.000	62.000	62.000
3	Sg.Juru	5	82.000	82.000	82.000
4	Sg.Tambun	5	82.000	82.000	82.000

Amphipod-Gross Sediment Test
File: Amp Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Reference	52.000				
Sg Perai	62.000	1.890	*	1.75	k= 1, v=16
Sg.Juru	82.000	5.669	*	1.83	k= 2, v=16
Sg.Tambun	82.000	5.669	*	1.86	k= 3, v=16

SOURCE	DF	SS	MS	F
Between	4	247.597	61.899	0.927
Within (Error)	10	667.820	66.782	
Total	14	915.417		

Critical F value = 3.48 (0.05,4,10)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Oyster Bioassay-sediment,normal larval development
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TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 5 2 3 4 1
5	Sungai Tambun	75.100	75.100	\
2	reference	80.967	80.967	. \
3	Sungai Perai	81.800	81.800	. . \
4	Sungai Juru	85.233	85.233	. . . \
1	control	86.833	86.833	. . . . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (5,10) = 4.65 s = 66.782

Oyster Bioassay-sediment,normal larval development
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	86.833	86.833	86.833
2	reference	3	80.967	80.967	82.667
3	Sungai Perai	3	81.800	81.800	82.667
4	Sungai Juru	3	85.233	85.233	82.667
5	Sungai Tambun	3	75.100	75.100	75.100

Oyster Bioassay-sediment,normal larval development
File: B:\TOXSTAT\OYSTERNO. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

control	86.833			
reference	82.667	0.624	1.81	k= 1, v=10
Sungai Perai	82.667	0.624	1.91	k= 2, v=10
Sungai Juru	82.667	0.624	1.94	k= 3, v=10
Sungai Tambun	75.100	1.758	1.96	k= 4, v=10

s = 8.172
Note: df used for table values are approximate when v > 20.

Amphipod -Sediment Bioassay
File: B:\TOXSTAT\AMPHIPOD. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	2772.917	924.306	16.432
Within (Error)	8	450.000	56.250	
Total	11	3222.917		

Critical F value = 4.07 (0.05,3,8)
Since F > Critical F REJECT Ho:All groups equal

Amphipod -Sediment Bioassay
File: B:\TOXSTAT\AMPHIPOD. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	reference	50.000	50.000		
2	Sungai Perai	38.333	38.333	1.905	
3	Sungai Juru	13.333	13.333	5.988	*
4	Sungai Tambun	16.667	16.667	5.443	*

Dunnett table value = 2.42 (1 Tailed Value, P=0.05, df=8,3)

Amphipod -Sediment Bioassay
File: B:\TOXSTAT\AMPHIPOD. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	reference	3			
2	Sungai Perai	3	14.819	29.6	11.667
3	Sungai Juru	3	14.819	29.6	36.667

2	Reference	50.000	50.000	\
3	Sg.Perai	54.000	54.000	. \
4	Sg.Juru	80.000	80.000	* * \
5	Sg.Tambun	80.000	80.000	* * . \
1	Control	100.000	100.000	* * * * \

* = significant difference (p=0.05) . = no significant difference
Tukey value (5,20) = 4.23 s = 96.000

Amphipod-Gross Sediment Test
File: A:\AMPHIPOD. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	5	100.000	100.000	100.000
2	Reference	5	50.000	50.000	66.000
3	Sg.Perai	5	54.000	54.000	66.000
4	Sg.Juru	5	80.000	80.000	66.000
5	Sg.Tambun	5	80.000	80.000	66.000

Amphipod-Gross Sediment Test
File: A:\AMPHIPOD. Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	100.000				
Reference	66.000	5.487	*	1.72	k= 1, v=20
Sg.Perai	66.000	5.487	*	1.81	k= 2, v=20
Sg.Juru	66.000	5.487	*	1.83	k= 3, v=20
Sg.Tambun	66.000	5.487	*	1.85	k= 4, v=20

s = 9.798
Note: df used for table values are approximate when v > 20.

Oyster Larvae-Suspended Sediment Bioassay
File: A:\OYSTER. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0254	0.0064	9.143
Within (Error)	10	0.0073	0.0007	

Oyster Bioassay-sediment,normal larval development
File: B:\TOXSTAT\OYSTERNO. Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	247.597	61.899	0.927
Within (Error)	10	667.820	66.782	
Total	14	915.417		

Critical F value = 3.48 (0.05,4,10)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Oyster Bioassay-sediment,normal larval development
File: B:\TOXSTAT\OYSTERNO. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	86.833	86.833		
2	reference	80.967	80.967	0.879	
3	Sungai Perai	81.800	81.800	0.754	
4	Sungai Juru	85.233	85.233	0.240	
5	Sungai Tambun	75.100	75.100	1.758	

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

Oyster Bioassay-sediment,normal larval development
File: B:\TOXSTAT\OYSTERNO. Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	reference	3	16.481	19.0	5.867
3	Sungai Perai	3	16.481	19.0	5.033
4	Sungai Juru	3	16.481	19.0	1.600
5	Sungai Tambun	3	16.481	19.0	11.733

Oyster Bioassay-sediment,normal larval development
File: B:\TOXSTAT\OYSTERNO. Transform: NO TRANSFORMATION

ANOVA TABLE

control	94.125				
reference	94.125	0.370		1.75	k= 1, v=15
Sungai Perai	85.875	2.172	*	1.84	k= 2, v=15
Sungai Juru	43.225	15.308	**	1.87	k= 3, v=15
Sungai Tambun	43.225	15.308	**	1.88	k= 4, v=15

s = 4.591

Note: df used for table values are approximate when v > 20.

Sea Urchin -Suspended Sediment Bioassay
File: A:seaurchin Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.323	0.569	0.328
2	Reference	0.213	0.462	0.267
3	Sg.Perai	3.370	1.836	1.060
4	Sg.Juru	0.000	0.000	0.000
5	Sg.Tambun	28.653	5.353	3.090

Sea Urchin -Suspended Sediment Bioassay
File: A:seaurchin Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	19653.477	4913.369	754.510
Within (Error)	10	65.120	6.512	
Total	14	19718.597		

Critical F value = 3.48 (0.05,4,10)
Since F > Critical F REJECT Ho:All groups equal

Sea Urchin -Suspended Sediment Bioassay
File: A:seaurchin Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	7.133	7.133		
2	Reference	4.733	4.733	1.152	
3	Sg.Perai	14.400	14.400	-3.488	
4	Sg.Juru	100.000	100.000	-44.571	
5	Sg.Tambun	13.667	13.667	-3.136	

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

Sea Urchin -Suspended Sediment Bioassay
File: A:seaurchin Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	2			
2	reference	2	46.561	6.0	361.500
3	Sungai Perai	2	46.561	6.0	415.000
4	Sungai Juru	2	46.561	6.0	780.000
5	Sungai Tambun	2	46.561	6.0	3.000

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	849816.400	212454.100	796.006
Within (Error)	5	1334.500	266.900	
Total	9	851150.900		

Critical F value = 5.19 (0.05,4,5)
Since F > Critical F REJECT Ho:All groups equal

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 4	0 3	0 2	0 5	0 1
4	Sungai Juru	0.000	0.000	\				
3	Sungai Perai	365.000	365.000	*	\			
2	reference	418.500	418.500	*	.	\		
5	Sungai Tambun	777.000	777.000	*	*	*	\	
1	control	780.000	780.000	*	*	*	.	\

* = significant difference (p=0.05) . = no significant difference
Tukey value (5,5) = 5.67 s = 266.900

Survival Mean for Sediment Bioassay- Sea urchin
File: B:sur Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

2	reference	984.667	984.667	2.075	
3	Sg. Perai	715.000	715.000	6.505	*
4	Sg. Juru	612.333	612.333	8.191	*
5	Sg. Tambun	772.333	772.333	5.563	*

Dunnett table value = 2.47 (1 Tailed Value, P=0.05, df=10,4)

Survival Mean for Sediment Bioassay Using Oyster
File: B:four Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	reference	3	150.369	13.5	126.333
3	Sg. Perai	3	150.369	13.5	396.000
4	Sg. Juru	3	150.369	13.5	498.667
5	Sg. Tambun	3	150.369	13.5	338.667

Survival Mean for Sediment Bioassay Using Oyster
File: B:four Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	499202.933	124800.733	22.449
Within (Error)	10	55592.000	5559.200	
Total	14	554794.933		

Critical F value = 3.48 (0.05,4,10)
Since F > Critical F REJECT Ho:All groups equal

Survival Mean for Sediment Bioassay Using Oyster
File: B:four Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 4 3 5 2 1
4	Sg. Juru	612.333	612.333	\
3	Sg. Perai	715.000	715.000	. \
5	Sg. Tambun	772.333	772.333	. . \
2	reference	984.667	984.667	* * * \
1	control	1111.000	1111.000	* * * . \

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* = significant difference (p=0.05) . = no significant difference
Tukey value (5,10) = 4.65 s = 5559.200

Survival Mean for Sediment Bioassay Using Oyster
File: B:four Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	1111.000	1111.000	1111.000
2	reference	3	984.667	984.667	984.667
3	Sg. Perai	3	715.000	715.000	715.000
4	Sg. Juru	3	612.333	612.333	692.333
5	Sg. Tambun	3	772.333	772.333	692.333

Survival Mean for Sediment Bioassay Using Oyster
File: B:four Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	1111.000				
reference	984.667	2.075	*	1.81	k= 1, v=10
Sg. Perai	715.000	6.505	*	1.91	k= 2, v=10
Sg. Juru	692.333	6.877	*	1.94	k= 3, v=10
Sg. Tambun	692.333	6.877	*	1.96	k= 4, v=10

s = 74.560
Note: df used for table values are approximate when v > 20.