STUDY ON THE BIOLOGY AND HEAVY METAL TOXICITY ON CHIRONOMUS KIIENSIS TOKUNANGA AND CHIRONOMUS JAVANUS KIEFFER (DIPTERA: CHIRONOMIDAE)

by

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LIST OF SYMBOLS

SYMBOL	DESCRIPTION
º C	Degree Celsius
mg/L	Milligram per liter
mg/kg	Milligram per kilogram
cm	Centimeter
mm	Millimeter
ml	Milliliter
µm	Micrometer
%	Percent
mg	Milligram
g	Gram
dry solids/ml	Dry solids per milliliter
dry solids/L	Dry solids per liter
Cd	Cadmium
Cu	Copper
Ni	Nickel
Pb	Lead

LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
CL	Confidence limit
DO	Dissolved oxygen
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
DOE	Department of Environment
ASTM	American Society for Testing and Materials
USEPA	United State Environmental Protection Agency
INWQS	Interim National Water Quality Standards
WQI	Water Quality Index
pH	Power of hydrogen (Acid balance)
SPSS	Statistical Package for the Social Sciences
EC_{50}	Median effect concentration
LC_{50}	Median lethal concentration
LT_{50}	Median lethal time
EmT50	Median emergence time
96-h	96 hour
72-h	72 hour
48-h	48 hour
24-h	24 hour
12-h	12 hour
4-h	4 hour
2-h	2 hour
1-h	1 hour
10-d	10 days
L	Length
H	Height
W	Width
V	Volume
CHR	Corrected hydrometer reading
C.F	Correction formula
CI	Confidence interval
rpm	Revolution per minute

LIST OF APPENDICES

LIST OF PUBLICATIONS & SEMINARS

SEMINARS:

1) W. Ebau, Z. Din, and M.R. Che Salmah. Toxicity of Copper (Cu), Nickel (Ni), Cadmium (Cd) and Lead (Pb) on aquatic organism: a modified test using *Chironomus* sp. (2007). 12th Biological Sciences Graduate Congress (BSGC). Kuala Lumpur, Malaysia. (poster presentation)

2) Warrin Ebau, Z. B. Din, and Che Salmah M.R. Use of Tropical Chironomid Larvae (Diptera: Chironomidae) as indicator Organism for Aquatic and Sediment Toxicity Tests. International Conference on Environmental Research and Technology (ICERT 2008) Penang, Malaysia. [Accepted]. (oral presentation)

3) E. Warrin, Z.B. Din and M.R. Che Salmah. Toxicity of Copper (Cu), Nickel (Ni), Cadmium (Cd) and Lead (Pb) on aquatic organism: a modified test using *Chironomus kiiensis* Tokunanga and *Chironomus javanus* Kieffer. Seminar Tahunan NSF Kali Kelima, MOSTI (2008). (oral presentation)

4) E. Warrin, Z.B. Din and M.R. Che Salmah. Comparative Acute Toxicity of Waterborne Cadmium to Different Larval Stages of *Chironomus kiiensis* Tokunanga and *Chironomus javanus* Kieffer. Post Graduate PPSK/IPS Colloqium 2008. (oral presentation)

KAJIAN BIOLOGI DAN KETOKSIKAN LOGAM BERAT TERHADAP CHIRONOMUS KIIENSIS TOKUNANGA DAN CHIRONOMUS JAVANUS KIEFFER (DIPTERA: CHIRONOMIDAE)

ABSTRAK

Chironomus kiiensis Tokunanga dan *Chironomus javanus* Kieffer didapati sesuai untuk digunakan sebagai organisma ujian dalam kajian ketoksikan akuatik dan kajian ketoksikan sedimen. Kedua-dua spesis ini mempunyai kitaran hidup yang pendek (*C. kiiensis* = 20.86 hari and *C. javanus* = 15.14 hari) dan senang untuk dibiak di dalam makmal menggunakan kaedah dan makanan yang sama. Kedua-dua spesis ini didapati mempunyai empat peringkat instar dengan *C. javanus* mempunyai badan yang lebih panjang dan saiz yang lebih besar. Adalah sukar untuk membezakan kedua-dua spesis ini pada peringkat larval dengan menggunakan mata kasar kerana mereka kelihatan serupa. Ciri ketara yang membezakan *C. javanus* dengan *C. kiiensis* adalah bilangan gigi yang luar biasa (7 gigi) pada premandible. Walau bagaimanapun, kedua-dua spesis ini mudah dibezakan pada peringkat dewasa di mana *C. kiiensis* berwarna perang kekuningan manakala *C. javanus* berwarna hijau.

Kajian ini menunjukkan bahawa kesan ketoksikan logam-logam berat terhadap *C. kiiensis* dan *C. javanus* dipengaruhi oleh usia larva, jenis logam berat yang digunakan, tempoh ujian dan jenis ujian (sama ada organisma diberi makanan atau tidak). Instar peringkat awal *C. kiiensis* dan *C. javanus* secara umumnya lebih sensitif terhadap logam berat. Pemberian makanan semasa ujian didapati dapat melanjutkan kemandirian organisma yang diuji di mana ditunjukkan oleh nilai-nilai LC_{50} yang lebih tinggi. Dalam kajian ini, turutan kesan ketoksikan logam-logam

berat terhadap *C. kiiensis* dan *C. javanus* boleh disusun seperti berikut Cu > Cd > Pb > Ni. Walaupun kedua-dua spesies ini berkaitan rapat antara satu sama lain, mereka menunjukkan perbezaan yang signifikan dalam sensitiviti terhadap logam-logam berat dengan *C. javanus* merupakan spesies yang lebih sensitif ($p < 0.05$).

Tiga jenis sedimen, loam berpasir, loam liat dan loam lempung yang masing-masing dicampurkan dengan kuprum, kadmium, plumbum dan nikel diuji ke atas larva *C. kiiensis* dan *C. javanus* (yang berusia < 72 jam) selama 10 hari. Ujian dengan menggunakan *C. kiiensis* menunjukkan kesan toksik adalah dipengaruhi oleh jenis sedimen dan logam berat digunakan. Sedimen loam berpasir yang dicampurkan logam-logam berat mencatatkan nilai-nilai LC_{50} terendah diikuti dengan sedimen jenis loam liat dan loam lempung di mana turutan kesan ketoksikan dalam sedimen untuk logam-logam berat adalah seperti berikut Cd > Cu > Ni > Pb. Apabila ujian yang serupa dijalankan terhadap *C. javanus*, keputusan yang serupa dengan *C. kiiensis* diperolehi. Namun, turutan untuk ketoksikan logam-logam berat adalah tidak konsisten seperti dalam ujian yang melibatkan *C. kiiensis*. Secara keseluruhannya, kadmium merupakan logam yang paling toksik apabila *C. javanus* digunakan sebagai organisma ujian. Persamaan dalam turutan ketoksikan logam-logam berat untuk *C. kiiensis* dan *C. javanus* hanya dikesan dalam sedimen jenis loam berpasir.

Bagi kajian ini, beberapa perubahan terhadap keadaan ujian yang dicadangkan terdahulu (suhu, muatan beban ujian, sukatan ujian, usia organisma) dilakukan. Berdasarkan keputusan yang ditunjukkan oleh *C. kiiensis* dan *C. javanus* dalam makmal, spesis-spesis ini boleh digunakan sebagai organisma ujian bagi menggantikan spesis yang disyorkan: *C. tentans* and *C. riparius* yang tidak dapat dijumpai di Malaysia.

STUDY ON THE BIOLOGY AND HEAVY METAL TOXICITY ON <u>CHIRONOMUS KIIENSIS</u> TOKUNANGA AND <u>CHIRONOMUS JAVANUS</u> KIEFFER (DIPTERA: CHIRONOMIDAE)

ABSTRACT

Chironomus kiiensis Tokunanga and *Chironomus javanus* Kieffer were found to be suitable for use as test organism in both aquatic and sediment toxicity tests. Both species have a relatively short life cycle (*C. kiiensis* = 20.86 days and *C. javanus* = 15.14 days) and easy to culture under laboratory conditions using the same method and food. Both species were found to have four instar stages each with *C. javanus* having longer body and bigger in size. It is difficult to differentiate these two species at larval stages by naked eyes as they look similar. The apparent characteristic that differentiates *C. javanus* with *C. kiiensis* is the unusual teeth number (7 teeth) in the premandible. However, these two species were easy to differentiate at adult stage where *C. kiiensis* is yellowish brown in colour while *C. javanus* is greenish in colour.

In the present study, it was found that the toxic effects of the heavy metals towards *C. kiiensis* and *C. javanus* were influenced by age of the larvae, types of heavy metal used, exposure periods (24-h, 48-h, 72-h and 96-h) and test regime (feeding or non-feeding). The younger instar stages of *C. kiiensis* and *C. javanus* were generally more sensitive to the heavy metals. Feeding was able to increase the survival of the test organisms as shown by the higher LC_{50} values. In the present study, the toxic effects of the heavy metals on *C. kiiensis* and *C. javanus* can be

ranked as Cu > Cd > Pb > Ni. Even though the two species are closely related, they showed significant differences in sensitivity to the different heavy metals with *C. javanus* being the more sensitive species ($p < 0.05$).

Three types of sediment, sandy loam, clay loam and silt loam were separately spiked with copper, cadmium, lead and nickel and tested on larvae (< 72-h old) of *C. kiiensis* and *C. javanus* for 10 days. The test with *C. kiiensis* showed the toxic effects were influenced by the sediment type and heavy metal used. Metal-spiked sandy loam recorded the lowest LC_{50} values followed by metal-spiked clay loam and silt loam and toxicity ranking for the heavy metals were Cd > Cu > Ni > Pb. When similar tests were run on *C. javanus,* the toxicity ranking based on sediment type was similar with *C. kiiensis*. However, the toxicity ranking of the heavy metals was not consistent as with the test involving *C. kiiensis*. Overall, cadmium was the most toxic metal in all spiked-sediment for *C. javanus*. Similarity in metal toxicity ranking for *C. kiiensis* and *C. javanus* was only noticed in sandy loam sediment.

In this study, some changes to the earlier recommended test conditions (temperature, loading density, test volume, organism age) were made. Based on the performances of *C. kiiensis* and *C. javanus* in the laboratory, these species can be reared as test species to substitute the recommended species: *C. tentans* and *C. riparius* which are not native to Malaysia.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The aquatic ecosystem is highly complex and diverse. Each aquatic ecosystem is a dynamic product of complex interactions of living (biotic) and non-living (abiotic) components. In particular, one of its abiotic components: the sediment provides habitat, feeding, spawning, and rearing areas for many aquatic organisms such as the larval stages of aquatic insect Chironomidae.

Point source and non-point source pollutants which entered aquatic ecosystem not only harmful to the organisms that live in the water column but also to those that live in the sediment. These anthropogenic pollutants especially heavy metals will eventually deposit and accumulate in the sediment. Thus, it may be directly toxic to the aquatic organisms or bioaccumulate in the food chain.

Due to its ability to accumulate large amount of pollutants, sediment usually contains higher concentration of contaminants than the overlying water (Simkiss *et al.*, 2001). Therefore, aquatic and sediment toxicity tests are important in assessing the effect of contaminants on the aquatic organisms. The best candidates to conduct these tests are organism that closely associated with water and sediment.

In temperate countries, the larva of non-biting midge (Diptera: Chironomidae) commonly known as blood worms (chironomids) (i.e. *Chironomus tentans* and *Chironomus riparius*) are among the test organisms widely used in studies of both water-only toxicity and sediment toxicity. However those species that are recommended by standard methods such as by the United State Environmental Protection Agency (USEPA) (USEPA, 2000) and American Society for Testing and Materials (ASTM) (ASTM, 2005a, ASTM, 2005b) are not recorded to be native to Malaysia.

Toxicity study using larval chironomid as test organisms is still new in Malaysia. Published data is very limited whereby only a few reports concerning distributions of Chironomidae are available in Malaysia (Che Salmah and Abu Hassan, 2002, Che Salmah *et al.*, 1999). Taxonomic and biological studies on Malaysian Chironomidae are very limited (Yule and Yong, 2004). The search for potential local species suitable for toxicity test is extremely needed. Two species, commonly found in several habitats in Malaysia (Al-Shami *et al.*, 2006), *C. kiiensis* and *C. javanus* would make good candidates.

Database on median lethal concentration (LC_{50}) values of most of heavy metals are well established for recommended temperate chironomid species. These data bases are equally important for tropical chironomid species. Reish (1988) suggested that toxicity data from acute and chronic tests can be used to establish acceptable limits for the safe discharge of industrial effluent into the environment besides establishing local water quality criteria. It is frequently noted that the toxicity data of a pollutant varies between experiments as toxicity could be caused by chemical, physical, or biological factors or a combination thereof (Airas *et al.*, 2008, Munshi *et al.*, 2008, Bidwell and Gorrie, 2006, Brecken-Folse *et al.*, 1994, Postma *et al.*, 2002, Verriopoulos and Moraitou-Apostolopolou, 1989, Hamburger *et al.*, 1994, Leung and Furness, 1999).

In general, feeding of organisms should be avoided during test period however if needed it should be kept at minimum level. Literature available showed both of feed and non-feed were carried out for temperate chironomid species (De Haas *et al.*, 2004, Heugens *et al.*, 2006, Mangas-Ramírez *et al.*, 2001, Pascoe *et al.*, 1990).

A number of standard methods have been developed for assessing toxicity of contaminants associated with 'spiked' sediments using amphipods, midges, polychaetes, oligochaetes, mayflies, or cladocerans (USEPA, 2000, ASTM, 2005b). Several endpoints have been suggested in these methods to measure effects of contaminants in sediment including survival, growth, behaviour, or reproduction. Survival of test organisms after 10-d of exposures is the endpoint most commonly reported, however, the procedures using spiked sediment have been either inadequate, or not reported.

Apart from contaminants, many factors affecting the responses measured in toxicity tests could lead to flawed conclusions about sediment toxicity. For example, the physiochemical characteristic of sediments may affect the responses of organism especially those of burrowing types. Guidelines on selection of sediment for spiking procedure are not available as most of sediment toxicity tests are conducted with polluted sediment collected from the field (Berry *et al.*, 1996, Martínez-Tabche *et al.*, 1999, Milani *et al.*, 2003, Faria *et al.*, 2007).

The Department of Environment Malaysia in Year 2007 (Department of Environment, 2007a) reported that there are no strong evidence of heavy metal contamination in rivers or lakes in Malaysia. In order to conduct the sediment toxicity test, heavy metals were spiked in the natural sediment to fulfil this task. In order to study the effect of different sediment physiochemical on toxicity, three types of river sediments have been selected and spiked with heavy metals. Sediment toxicity test has been carried out for 10-d by using two selected chironomid species.

1.2 Significance of Study

Very little data is available on the toxicity of different chemicals on the indigenous Malaysian chironomids. This study is an attempt to investigate the sensitivity of Malaysian chironomids toward heavy metals and whether these species are suitable to be introduced as test organism in both aquatic and sediment toxicity tests. It is important to investigate the indigenous forms and develop testing protocols for the use of these organisms for sediment toxicological investigations in order to substitute the recommended species which are not native to Malaysia in the standard method.

Developing protocols using the indigenous Malaysian chironomid species is also of importance because many persistent chemicals tend to accumulate in sediments and evidence of impact on benthic communities exists in areas where water quality standards are not exceeded as mentioned earlier. Toxicity data from this study can be used to establish acceptable limits for the safe discharge of industrial effluent into the environment besides establishing local water quality criteria (Reish, 1988).

1.3 Objectives

The aims of this study can be stated as follows:

i. To study the biology of two potential test species in Malaysian chironomid, *C. kiiensis* and *C. javanus*

ii. To assess the toxicity of copper (Cu), nickel (Ni), cadmium (Cd) and lead (Pb) on four different life stages of *C. kiiensis* and *C. javanus*

iii. To investigate the effect of feeding on aquatic toxicity data of *C. kiiensis* and *C. javanus*

iv. To determine the 10-d sediment toxicity using three types of sediments for *C. kiiensis* and *C. javanus*

CHAPTER 2

LITERATURE REVIEW

2.1 Pollution of the Aquatic Environment

2.1.1 Water Pollution

Water pollution encompasses a wide range of human activities which add something to the water that affects the chemical composition, temperature, or microbial composition to such an extent that can cause harm to aquatic life (Lloyd, 1992). In many countries, water pollution is a very serious and visible form of environmental contamination.

As a result of very favourable government policies in the 1970s, there was an influx of investors into Malaysia resulting in expansion of various industrial sectors. Unfortunately, this tremendous transformation has its share of negative repercussions. Due to improper waste management and poor law enforcement, accelerated degradation of the freshwater systems was unavoidable. Each year, hazardous wastes such as heavy metals that are discharged from the industrial areas poses deadly threat to the aquatic ecosystem.

There are lots of evidences on the progressive deterioration of the water quality of various rivers due to the disposal of wastes into them especially in the urban and industrial areas. Sewage treatment plants (48.3 %), manufacturing industries (45.1 %), livestock industries (4.0 %) and agro-based industries (2.5 %)

are the principal contributors of pollutants in the Malaysian freshwater systems (Department of Environment, 2007b).

Department of Environment of Malaysia has classified a few rivers in this country as Class V and polluted based on the Interim National Water Quality Standard (INWQS) (Appendix 1) and Water Quality Index (WQI) (Appendix 2) (Department of Environment, 2007a). The rivers that are found heavily polluted are Menggaris River in Sabah, Jelutong River in Penang and Buluh River and Tukang Batu River in Johor. Most of the pollutants resulted from the anthropogenic source.

2.1.2 Sediment Pollution

Sediment has been defined as the particulate material lying below water, or for experimental purposes sediment could be formulated from particulate material (ASTM, 1994). According to Power and Chapman (1992), the sediments are divided into four main components which are the interstitial water (typically 50 % by volume of sediment), followed by inorganic phases (include rock fragment and minerals), organic matters and lastly is the anthropogenically derived materials including contaminants.

Sediments act as a reservoir for pollutants in the freshwater system and it is one of the possible media in aquatic monitoring. Apart from water, sediments are also responsible for nutrients and pollutant transportation in the aquatic environment. Contaminated sediments prolong the residence time of pollutants in the water column by accumulating organic and inorganic contaminants, and holding back their transport especially in lentic ecosystems.

Sediments are known to capture hydrophobic organic pollutants entering the water bodies (McCready *et al.*, 2006). It also serves as secondary, diffuse contamination to water column (Mighanetara *et al.*, 2009). From time to time, various contaminants from bottom sediments diffuse slowly into the lower water layer or the concentration of contaminants increased in pore-waters through dissolution or reduction when anoxic conditions occur in the bottom sediments (Bartram *et al.*, 1996). Therefore, sediments can provide a long- term record of toxic discharge (Chapman *et al.*, 1996).

The problem of polluted sediments is well known in both the freshwater and marine systems all over the world (Long and Chapman, 1985, Rand *et al.*, 2009). However, the complexity of the sediment-water column and sediment-biota interactions restricted the study on sediments toxicity (Dickson and John H. Rodgers, 1986, White, 1988).

Contaminated bottom sediments can have direct adverse impacts on bottom fauna. Polluted sediments can also be an enduring source of toxic substances to the environment and can affect not only organisms but also humans through the food chain or water or through direct contact (Coull and Chandler, 1992, Landrum and Robbins, 1990). These adverse impacts may be present even though the water quality of the overlying water meets the target criteria for water supply levels. There has been an increasing concern regarding the accumulation of toxic heavy metals in our environment that pose a threat to both public health and the natural ecosystem (Waiyaki, 1999, Hoke *et al.*, 1993, Gaston *et al.*, 1998).

A lot of resources in ecotoxicology have been assigned to the evaluation of sediment toxicity in view of the fact that sediments can act both as a sink and source of contaminants (Salomons *et al.*, 1987, Doe *et al.*, 2003). Sediment represents the primary repository for persistent, organic chemicals discharged into the aquatic environment as sediment created by the settlement of particles from the overlying water column and any chemicals that dissolved in the water column and absorbed by suspended particles will be trapped and deposited to the bottom of sediment (Adams, 1987).

The aquatic environment is vulnerable to contamination by high concentrations of anthropogically-introduced metals according to de Groot (1995) and the most abundant of these metals are copper, cadmium, nickel, lead and zinc. It has been well established that sediment has higher metal contaminants than the overlying water (Balasubramanian *et al.*, 1997, Burton, 1991, Tessier and Campbell, 1987). Thus the bottom dwelling organisms are exposed to higher risk of metal toxicity when compared to the water column organisms.

Sediment toxicity tests are being assessed in their ability to predict toxicity and metal bioavailability of individual contaminants in sediments to benthic organisms (Berry *et al.*, 1996, Burton, 1991, Dewitt *et al.*, 1996). At present, various whole sediment tests have been proposed but only a few standardized bioassays exist. According to Warwick (1990), for the European environment, the suitable test species are the benthic sludge worm *Tubifex tubifex* and the Dipteran *Chironomus riparius*, which both play major roles in many aquatic systems because they are widely distributed and abundant freshwater macroinvertebrates.

2.1.3 Heavy Metal Pollution (cadmium, copper, lead and nickel)

Level of heavy metals in the environment is increasing following their intensive domestic and industrial usage. Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water (Nies, 1999). To some extent, the word ‘heavy metal’ is imprecise, but includes most metals with an atomic number greater than 20, but excludes alkali metals, alkaline earths, lanthanides and actinides (Mason, 2002, Abel, 1996). Heavy metals are stable and persistent natural constituents of the earth's crust and cannot be degraded or destroyed (Netherlands Institute for the Law of the Sea, 1996).

Industrial and municipal wastewater and urban storm water commonly contain metals such as copper, cadmium, lead and nickel, all of which may be directly or indirectly released into the aquatic receiving systems. Although there are natural sources of heavy metals in the environment, their contribution is small and negligible compared to the input from human activities.

2.1.3.1 Cadmium

Cadmium has no known essential biochemical function and it is among the highly toxic heavy metals to living organisms (Alloway, 2001). The related incident to this metal resulted in the “Itai-itai” disease which happens in Japan due to the cadmium poisoning in irrigation water. Cadmium has many industrial uses such as in paints and pigments manufacture, batteries and as a stabilizer in plastics manufacturing (Fulkerson and Goeller, 1973). Another use is as anticorrosive agent for steel, iron, copper, brass, and other alloys.

Volcanic activities are the major natural source of both atmospheric and deep sea release of cadmium. This metal is commonly found in association with zinc and is distributed in the earth's crust with concentration of about 0.1 mg/kg (Waiyaki, 1999). However, the major input of cadmium to the aquatic ecosystem is from the non-ferrous metal mines.

The free ionic concentration of cadmium is biologicaly available to organisms and thus pose a threat especially to the benthic community. The presence of zinc has been shown to increase the toxic effect of cadmium on invertebrates (Wicklund, 1990). However, water hardness, salinity, chelating agents, and high organic content of water, tend to reduce the toxic effect of this metal. Carrol *et al.* (1979) verified that calcium reduces the acute toxicity of cadmium while increase in hardness of the water will reduce toxicity. Clubb *et al.* (1975) investigated the toxicity of cadmium to nine species of aquatic insects, but seven of the species tested were too insensitive to the effects of the metal for the LC_{50} to be determined.

The Interim Malaysia National Water Quality Standards (INWQS) for cadmium has been set at 0.01 mg/L (Department of Environment, 2007a). Its concentration that had been recorded in the Malaysian rivers are lower with values of 0.06 to 0.48 µg/g (Department of Environment, 2007b). In Malaysia, the mortality dose for cadmium poisoning is 10 mg (Food Act 1983, 2006).

2.1.3.2 Copper

Copper is the heavy metals that are observed to be in very high concentration in the aquatic environment due to a wide range of uses especially in the industrial.

There are natural sources of copper in the earth's crust and weathering of the rocks that contain copper causing the release of the metal into the environment. The natural abundance of copper in the earth's crust is approximately 60 mg/kg (Lide and Frederikse, 1993).

The potential sources of copper to the aquatic environment include smelting and refining industries: copper wire manufacturing, coal burning, iron and steel industries, and the manufacturing of printed circuit. Other anthropogenic sources are from agriculture (fertilizers, algaecides) and sewage (Sloof *et al.*, 1998).

Most of the copper that are released into the water system is in particulate form and usually will settle out, precipitate or absorb in the sediment or water column. Copper is toxic when present in high concentration, but because it is easily complexed by dissolved organic matter in solution, thus reducing the biologically available fraction. Giesy *et al.* (1990) found that the presence of organic matter decreased the accumulation of copper by the cladoceran, *Simocephalus serrulatus.*

Shaner and Knight (1985) found that alkalinity has a significant effect on the acute toxicity of copper-bearing sediments to *Daphnia magna*. The additions of humic acid further reduce the toxicity effect. Life stage has also been found to give impact on the toxicity of copper on organisms with the earlier instar being more sensitive. Gauss *et al.* (1985) reported that when the first and fourth instar midge of *C. tentans* were exposed in acute toxicity tests, the first instar appeared to be more sensitive with lower 96-EC_{50} values.

Based on the Malaysia Interim National Water Quality Standard (Department of Environment, 2007a) copper concentration in water supplies for drinking should not exceed the permitted level of 1.0 mg/L while the reported levels in Malaysian rivers are between 5.96 to 21.20 µg/L (Department of Environment, 2007b).

2.1.3.3 Lead

As with copper and cadmium, lead pollution is mainly due to anthropogenic input. Lead has been widely use in batteries, piping, paints, pigments and numerous other applications. The most destructive usage of non-recoverable lead is in the automotive industries as additives in the alkyl-lead fuel (Tsuchiya, 1979). In Malaysia, lead concentration in the environment has significantly decreased after the country switched to unleaded petrol.

Lead occurs naturally in the earth's crust in concentrations of about 13 mg/kg. The main entries of lead into the aquatic environment are through surface runoff and deposition of airborne lead. However, almost all of the lead is absorbed and tightly bound onto sediment and soil particle. Only a fraction is dissolved in the water column and pore-water, thus reducing its biological availability to organisms. The Malaysia Interim National Water Quality Standard (Department of Environment, 2007a) allowed only 0.05 mg/L of lead in the water supply for drinking purposes and the maximum level allowed in fish for consumption is 2.0µg/g (Food Act 1983, 2006).

In 1969, Warnick and Bell conducted a static bioassay on lead sulphate using the nymphs of stonefly (*Acroneuria lycorias*), mayfly (*Ephemerella subvaria*), and caddisfly (*Hydropsyche betteni*) as test organisms. It was found that LT_{50} of > 14 days for nymphs of stonefly was at 64 mg/L, LT_{50} of 7 days for nymphs of mayfly was at 16 mg/L and LT_{50} of 7 days for nymphs of caddisfly was at 32 mg/L. According to their findings, the nominal concentrations were unreliable after 96-h because of the decreasing metal concentrations in the test solution over the 2-week experimental period.

2.1.3.4 Nickel

Nickel is the 24th most abundant element in the earth crust. Its average concentration in the earth's crust is only about 0.008 % (Mason and Moore, 1982). Nickel is mainly use in the manufacture of stainless steel and other nickel alloys with high corrosion and temperature resistance. It has innumerable other uses, including catalysts, pigments, and in the production of nickel-cadmium batteries.

Nickel from the various industrial processes and other sources usually will end up in the aquatic system as waste water. It has been reported that nickel level in water-treatment plants was surplus the World Health Organization guidelines for safe drinking water level which is 0.02 mg/L (Sajidu *et al.*, 2007, World Health Organization (WHO), 1963). Normally, nickel that enters the aquatic ecosystem as soluble salts will be absorbed on clay particle or organic matter and are strongly bound together with the sediment. The Malaysia Interim National Water Quality Standard allowed only 0.05 mg/L of nickel in the water supply for drinking purposes (Department of Environment, 2007a).

Mathis and Cummings (1973) recorded nickel levels in sediments, water and biota in Illinois and reported concentration of < 0.01 mg/L dissolved in water and 3-124 mg/kg in sediments. Brkovic-Popovic and Popovic (1977) showed that water hardness will affect nickel toxicity. At a hardness of 0.1 mg calcium carbonate/litre, the 48-h LC_{50} was 0.082 mg nickel/litre. Increase in hardness to 34.2 mg calcium carbonate/litre and 261 mg calcium carbonate/litre increased the 48-h LC_{50} to 8.7 mg nickel/litre and 61.4 mg nickel/litre, respectively, suggesting a decrease in the toxic effect.

2.2 Factors That Affect Toxicity

There are numerous biotic and abiotic factors that affect the toxicity of pollutants. The biotic factors include the taxonomic group and organisms' size and age, while the abiotic factors consist of pH and alkalinity, dissolved organic carbon, water hardness, temperature, food availability and dissolved oxygen.

2.2.1 Biotic Factors

2.2.1.1 Taxonomic Group

Clearly, different taxonomic group will give different toxicity responses as the boundaries of maximum and minimum acceptable concentration for toxicants are different from one organism to another.

Toxicologists found that in aquatic bioassays, algae and aquatic macrophytes are usually less sensitive than aquatic animals. For aquatic animals, larval amphibians are generally more tolerant than fish, and fish are more tolerant to toxicants than arthropods (Mayer Jr and Ellersieck, 1988).

Generally, species within a given taxonomic family will show similarity in their responses to toxicants (Suter and Rosen, 1988). However, in the test organisms, species variations as well as variation in strains within the same species do occur.

Several studies have shown that even closely related species exhibit different toxicity responses. For example, Jeyasingham and Ling (2000) found that three species of chironomid from closely related family (Chironomidae) exhibited LC_{50}s to arsenic that differed at least by 3 fold in 96-h water exposures. *Chironomus zealandicus* was the most tolerant species followed by *Polypedilum pavidus* and *Chironomus* sp.*a*.

Toxicity tests need to be carried out with a wide range of species to avoid underestimation of toxicity values; some researchers suggested the use of a combination of different species, a multispecies test system.

2.2.1.2 Age and Size

Body size and life stage usually have a significant influence on toxic responses. In general, a larger body size and later instar stage will show lower sensitivity towards pollutants. Studies have shown that size of an animal do affect the levels of trace metals in organisms.

Earlier studies have shown that levels of metal in molluscs, including gastropods, are normally dependent on size (Boyden, 1974). According to Leung and Furness (1999) the concentrations of metallothionein, cadmium and zinc in periwinkles, *L. littorea* (as µg/g dry soft-body weight) decrease with increasing size.

Smaller individuals have higher concentration of metals than larger individual because smaller animals have larger surface area: volume ratio which can lead to faster chemical uptake per unit weight. A similar trend was found in several annelids, such as polychaetes where Bryan and Hummerstone (1973) found that smaller polychaetes such as *Heteromastus filiformis* tend to accumulate more metals per unit body weight than larger species, *Perinereis aibuhitensis*.

In some species an inverse relationship has been found between the weight of animal soft tissue and metal. Mouneyrac *et al.* (1998) analyzed cadmium, copper and zinc or metallothionein-like protein concentrations in the whole soft tissues of oysters, *Crassostrea gigas* and found a positive correlation with body size. Similarly, Martin (1974) analyzed various elements in whole-body samples of the crab *Cancer irroratus* and found that only Mn exhibited a significant positive correlation with body size.

2.2.2 Abiotic Factors

2.2.2.1 Dissolved Organic Carbon

Dissolved organic matter (DOM) are often quantified as dissolved organic carbon (DOC) playing an important role in affecting the transition of metal speciation. Organic carbon contents have been found inversely correlated with mean particle size distribution for sediment because organic carbon is often bound as a coating on sediment particles (Meador, 1991).

The effect of DOC on metal uptake and toxicity in aquatic organisms has been the subject of numerous studies (Daly *et al.*, 1990, Heijerick *et al.*, 2003, Meador, 1991, Penttinen *et al.*, 1998, Playle *et al.*, 1993). Most of these studies found a decrease in uptake or toxicity with increasing DOC concentration in both marine and freshwater systems.

DOC has been found to alter and reduce the bioavailability of metals to organisms by providing sorption sites. Metal such as copper was found to bind well with DOC and metals that are associated with organic matter are considered as non-bioavailable to aquatic organisms (Long *et al.*, 2004). McGeer *et al.* (2002) found as high as 6-fold increase in the 96-hour LC_{50} value of copper in the rainbow trout *Oncorhynchus mykiss* as a result of exposure to DOC. Clearly the copper complexing to DOC has significantly reduced the bioavailable metal.

2.2.2.2 Dissolved Oxygen Level

Dissolved oxygen (DO) is crucial for aquatic life and a minimum dissolved oxygen requirement (of 60 % saturation) is stated in the standard method for conducting toxicity test in the water column (ASTM, 2005a). Dissolved oxygen affects aquatic organisms by reducing their activities and metabolisms (Choi *et al.*, 2000, Hamburger *et al.*, 1994, Hamburger *et al.*, 1998, Holopainen and Penttinen, 1993, Penttinen and Holopainen, 1995) and retards their growth rate (Hanazato and Dodson, 1995).

Most aquatic animals (especially benthic organisms) are well adapted to low dissolved oxygen concentration. Organisms respond a decline in DO by decreasing their metabolic rate and thus the requirement for oxygen (Wu, 2002). Furthermore, the lower oxygen level has been shown to cause an additional stress to the aquatic organisms in the polluted environment (Airas *et al.*, 2008).

2.2.2.3 Food Availability

Food is vital for the survival and growth of organisms and a decline in feeding can retard growth and eventually causing death. Therefore, it is believed that food-limited animals are more susceptible to toxicant compared to well-fed animals. This is well documented in the research by Mangas-Ramírez *et al.* (2002) where they found that the LC_{50} of ammonia to *Daphnia pulex* was higher in the presence of algal food compared with when the food was absent.

In contrast, Pascoe *et al.* (1990) observed that the presence of food caused cadmium to be more toxic to *C. riparius* as a result of rapid binding of the metal to the food provided. In acute toxicity test, feeding should be avoided during the test period unless it is necessary (Hill *et al.*, 1993).

2.2.2.4 pH and Alkalinity

pH is an important parameter in the sense that it will affect the speciation and the solubility of many metals in water and thus their bioavailability to organisms (McDonald *et al.*, 1989). In general, acidification will increase the solubility of many metals and result in an increase of the free metal ion concentrations in the water. However, a decrease in pH will also cause increase competition between free metal ions and hydrogen ions for the same binding sites and will restrict the free metal ion activities (Odin *et al.*, 1995, Palawski *et al.*, 1989, Playle *et al.*, 1993). Apart from the chemical effects, in very acidic condition (< pH 5), the hydrogen ion itself may be detrimental to the aquatic organisms.

Several earlier studies have shown that toxicity of certain metals is positively correlated with increasing pH in several species of aquatic organisms. For example, Bervoets and Blust (2000) found that the uptake of metals in the midge *C. riparius* increased with increasing exposure pH with the exception of pH 9.0 and 10.0. The same trend was also detected in the study by Schubauer-Berigan *et al.* (1993) where they found an increase in toxicity with increasing pH for three aquatic invertebrate species.

In contrast, some other studies for other metals reported the reverse condition where the toxicity of metal increase with decreasing pH. Gerhardt (1994) studied the effect of iron and lead on the mayfly *Leptophlebia marginata* and found that both metals were more toxic at pH 4.5 than at pH 7. Similarly, Odin *et al.* (1995) investigated the toxicity of mercury on mayfly nymphs *Hexagenia rigida* and found the toxicity increase with decreasing pH in the water column.

2.2.2.5 Temperature

The effect of temperature on poikilothermic animals is crucial as they are unable to control their body temperature. Temperature is a critical factor since it has a combined effect on both chemical and physiological processes (André *et al.*, 2009, Heugens *et al.*, 2003, Postma *et al.*, 2002, Tsui and Chu, 2003). Ambient temperature variation can affect the metabolism (Hamburger *et al.*, 1994, Cherkasov *et al.*, 2006), toxicokinetics (Heinonen *et al.*, 2000, Honkanen *et al.*, 2001) and the physiological state (Heugens *et al.*, 2003) in both homeothermic and poikilothermic animals.

Temperature can affect the tolerance of toxicant in organisms in two general ways: Change in the ambient temperature can cause an increase in toxicity (Hodgson and Rose, 2004). For example, Lemus and Chong (1999) reported a higher copper toxicity in tropical juveniles fish, *Petenia kraussii* that was exposed to warmer temperatures. However, when white mullet *Mugil curema* are exposed to copper, the toxicity vary inversely with temperature.

Numerous studies been carried out to investigate the effect of temperature and metal uptake and toxicity in aquatic organisms (Heugens *et al.*, 2003, Leung *et al.*, 2000, Munshi *et al.*, 2008, Theegala *et al.*, 2007, Tsui and Wang, 2006, Witeska and Jezierska, 2003, Honkanen and Kukkonen, 2006, Heugens *et al.*, 2006). Generally, previous studies had found a positive correlation between toxicity and increasing temperature (Brecken-Folse *et al.*, 1994, Cairns Jr *et al.*, 1975).

2.2.2.6 Water Hardness

Hardness of water is mainly related to the amount of calcium and magnesium concentration present in the water. The calcium and magnesium that are present in the water compete the binding and transport sites at cell membrane surfaces of organisms with metal ion (Markich and Jeffree, 1994). As a result, increasing hardness causes a reduction in heavy metal toxicity.

Studies by toxicologists have shown that heavy metals are less toxic in very hard water as compared to hard water and soft water and are the most toxic in very soft water. This was agreed by Singh Rathore and Khangarot (2003) who determine the effects of water hardness and heavy metals concentrations on a freshwater *Tubifex tubifex* and found that heavy metals decreased with increasing water hardness with very soft water being the most toxic. The heavy metals were found an order of magnitude more toxic in soft than in hard water.

2.3 Aquatic Insects as Toxicological Tools

Benthic macroinvertebrates are often considered the best choice for test organisms in aquatic and sediment toxicity tests (Ankley, 1996, Day *et al.*, 1995, DeFoe and Ankley, 1998). Research and literature concerned with assessing toxicity with macroinvertebrate such as aquatic insects has expanded significantly in the last few years. For many years the aquatic insect community structures indices have been used as an effective and sensitive indicator of ecosystem pollution.

A review of the literature indicates substantial databases that exist on aquatic insect responses to nutrients, pesticide and other physiochemical perturbations (Faria *et al.*, 2007, Ristola *et al.*, 1996, Stuijfzand *et al.*, 1998). The indicator qualities of aquatic insects are widely recognized in detecting water quality and ecological changes due to anthropogenic activities. Previous studies using caddisfies (Trichoptera), stoneflies (Plecoptera) and mayflies (Ephemeroptera) proved that insect can be utilized as an effective indicator of ecosystem pollution (Che Salmah *et al.*, 2007, Che Salmah *et al.*, 1999).

Aquatic insects serve well as indicators of various environmental stresses such as fine inorganic sediment pollution (Fialkowski *et al.*, 2003, Taylor *et al.*, 1994) and are superior to measurement of chemical concentrations. Insects have an excellent potential as rapid, sensitive predictors of long-term toxicity because of their diversity and short, sensitive life cycle (Rosenberg and Resh, 1993).

In addition, the larvae and nymphs of some aquatic insect species have limited mobility and may remain in the same general area for many months thus they can provide important information on the environmental changes due to anthropogenic activities. Furthermore, these aquatic insect also have an intimate contact with the bottom sediments for extended periods of their life cycle and as such there is an increase in the likelihood for adverse effects occurring in the presence of polluted sediments (Minshall, 1988, Cummins, 1974, Neill, 1988).

There are several reports that have been published on the effects of metal contamination on benthic communities such as decreased in density (Diggins and Stewart, 1998, Winner *et al.*, 1980), reduction in number of sensitive taxa (LaPoint *et al.*, 1984), and changes in patterns of species distribution (Clements *et al.*, 1992). Their responses reflected both short and long term environmental condition within their niche.

2.4 Test Organisms with Reference to the Chironomidae

Selection of test organism in laboratory bioassays is important in order to get a good estimate of the probable damage cause from anthropogenic activities. Organisms that are proposed to be used in toxicity test must be of environmentally and economically important. Invertebrate that are frequently used in aquatic tests are daphnids, amphipods, mayflies, stoneflies and chironomids is also included in the list of standard method (ASTM, 2005a).

Chironomid larvae are commonly used as test organisms in assessing the toxicity in both natural (Giesy *et al.*, 1990, Hoke *et al.*, 1993, Pellinen and Soimasuo, 1993), spiked sediments (De Haas *et al.*, 2004, Brown *et al.*, 1996, Harrahy and Clements, 1997) and bioaccumulation of sediment-associated contaminants (Mäenpää and Kukkonen, 2006, Ankley *et al.*, 1994, Harkey *et al.*, 1994). Apart being test organisms in sediment toxicity test, chironomids have also been widely used in water-only tests (Stuijfzand *et al.*, 1998, De Bisthoven *et al.*, 2004, Stuijfzand *et al.*, 2000). Besides being used in laboratory experiments test organisms, chironomids are also used for in-situ bioassays (Soares *et al.*, 2005, Domingues *et al.*, 2008, Faria *et al.*, 2008, Faria *et al.*, 2006).

Environmental toxicology using chironomid is still new in Malaysia and there are not much effort been done to identify the species that can be used in toxicity test to substitute the recommended species in the standard method. Species that are recommended in American Society for Testing and (ASTM, 2005a) and United State Environmental Protection Agency (USEPA, 2000) are *C. tentans* (North America) and *C. riparius* (Europe) which are not native to Malaysia. Besides these two species, toxicity tests have also been conducted using *Chironomus maddeni* (Bidwell and Gorrie, 2006, Smith and Kokkinn, 2004), *Chironomus decorus* (Alaimo *et al.*, 1994, Hansen *et al.*, 1993, Maier and Knight, 1993, Malchow *et al.*, 1995) and *Chironomus plumosus* (Fargašová, 2003, Fargašová, 2001, Fargašová, 1998) as test organisms. Unfortunately, there is no record to show that these species are indigenous to Malaysia.

Chironomus javanus and *C. kiiensis* are both non-biting midges of the order Diptera and family of Chironomidae. Chironomidae is a large, cosmopolitan family, diverse forms but mostly are small and delicate flies with superficial resemblance to