EFFECT OF DOMESTIC EFFLUENT, AGRICULTURAL AND INDUSTRIAL POLLUTION ON AQUATIC MACROINVERTEBRATES WITH EMPHASIS ON CHIRONOMIDAE (DIPTERA) AT COMMUNITY, INDIVIDUAL AND MOLECULAR LEVELS

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By

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LIST OF ABBREVIATION AND SYMBOLS

AbsFA	=	Absolute Fluctuating Asymmetry
ANOVA	=	Analysis of Variance
ASPT	=	Average Species Per Taxon
BOD	=	Biochemical Oxygen Demand
CCA	=	Canonical Correspondence Analysis
CFA	=	Composite Index of Asymmetry
cm	=	Centimeter
COD	=	Chemical Oxygen Demand
CTKR	=	Ceruk Tok Kun River
DA	=	Directional Asymmetry
df	=	Degree of Freedom
DI	=	Deformity Incidence
DMSO	=	Dimethyl Sulfoxide
DNA	=	Deoxyribonucleic Acid
EDTA	=	Ethyldiaminetetra acetic acid
ETOH	=	Ethanol
FA	=	Fluctuating Asymmetry
h	=	Hour
H_2O_2	=	Hydrogen Peroxide
JR	=	Juru River
KUR	=	Kilang Ubi River
KOH	=	Potassium Hydroxide
L	=	Left Side
LC	=	Lethal Concentration
LMA	=	Low Melting Agarose
mA	=	Milliampere
ME	=	Measurement Error
mg	=	Milligram
ml	=	Millilitre
mM	=	Millimolar
mm	=	Millimeter

min	=	Minute
MTSI	=	Modified Toxic Score Index
nd	=	Not Detected
NS	=	Not Significant
ppm	=	Part per Million
PCA	=	Principal Component Analysis
PR	=	Pasir River
PRR	=	Permatang Rawa River
R	=	Right Side
r	=	Correlation Coefficient
RDA	=	Redundancy Analysis
rpm	=	Revolution Per Minute
SE	=	Standard Error
SPSS	=	Statistical Package for Social Science
TBE	=	Tris Borate EDTA
TOM	=	Total Organic Matter
TSI	=	Toxic Score Index
TSS	=	Total Suspended Solids
TVE	=	Total Variance Explained
UV	=	Ultraviolet
V	=	Volt
WQI	=	Water Quality Index
µg	=	Microgram
µL	=	Microlitre
µm	=	Micrometer

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LIST OF PUBLICATIONS

International Journals

1. **Al-Shami, S.,** Che Salmah, M. R., Siti Azizah, M. N., Abu Hassan, A. & Ali A. (2010) Morphological deformities in *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in Juru River Basin, Penang, Malaysia. *Environmental Entomology*, **39 (1)**, 210-222.

2. **Al-Shami, S.,** Che Salmah, M. R., Siti Azizah, M. N. & Abu Hassan, A. (2010) Distribution of Chironomidae (Diptera) under the influence of several environmental parameters in polluted rivers of the Juru River Basin, Penang, Malaysia. *Journal of Environmental Sciences* **22 (10)**, xx-xx.

3. **Al-Shami, S.,** Che Salmah, M. R., Abu Hassan, A. & Siti Azizah, M. N. (2010) Fluctuating asymmetry of *Chironomus* spp. (Diptera: Chironomidae) larvae in association with water quality and metal pollution in Permatang Rawa River in the Juru River Basin, Penang, Malaysia. *Water, Air, and Soil Pollution* (**Online first,** DOI: 10.1007/s11270-010-0528-4).

4. **Al-Shami, S.,** Che Salmah, M. R., Abu Hassan, A. & Siti Azizah, M. N. (2010) Evaluation of mentum deformities of *Chironomus* spp. (Chironomidae: Diptera) larvae using modified toxic score index (MTSI) to assess the environmental stress in Juru River Basin, Penang, Malaysia. *Environmental Monitoring and Assessment* (**Online first,** DOI: 10.1007/s10661-010-1630-1).

5. **Al-Shami, S.,** Che Salmah, M. R., Abu Hassan, A. & Siti Azizah, M. N. Effect of Pollution on the diversity and abundance of benthic macroinvertebrates in Juru River Basin. *Ecotoxicology and Environmental Safety* (**Accepted**).

6. **Al-Shami, S.,** Che Salmah, M. R., Abu Hassan, A. & Siti Azizah, M. N. Detection of DNA damage of *Chironomus kiiensis* larvae exposed to Cd, Cu and Zn using the single cell assay. *Environmental Science and Pollution Research* (**In Review**).

7. **Al-Shami, S.,** Che Salmah, M. R., Abu Hassan, A. & Siti Azizah, M. N. Study on *Chironomus kiiensis* responses to sediments from rivers of north peninsular Malaysia in relation to pollution level: implication for ecotoxicological monitoring. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* (**In Review**).

Proceedings Including Oral and Poster Presentations

1. **Al-Shami S.,** Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2009). Distribution of Chironomidae (Diptera) under the influence of several environmental parameters in polluted Rivers of Juru River Basin, Penang, Malaysia. 17th International Symposium on Chironomidae. Nankai University, 6-10 July 2009, **Tianjin, China.**

2. **Al-Shami S.,** Che Salmah M. R., Siti Azizah M. N., Abu Hassan A. and Ali A. (2009). Incidence of deformities In *Chironomus* spp. larvae collected from Juru River Basin, Penang, Malaysia. 75th Annual Meeting of the American Mosquito Control Association. Hilton Riverside, 5-9 April 2009, **New Orleans, USA.**

3. **Al-Shami S.,** Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2008). Incidence of deformities In *Chironomus* spp. larvae collected from Juru River Basin. 6th IMT-GT UNINET Conference, Gurney Hotel, 28-30 August 2008, **Penang, Malaysia**.

4. **Al-Shami S.,** Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2008). Effect of pollution on the diversity and abundance of benthic macroinvertebrates In Juru River Basin. 4th USM- Life Sciences Postgraduate Conference, USM, 18-20th June 2008, **Penang, Malaysia.**

KESAN BAHAN BUANGAN DOMESTIK, PERTANIAN DAN PERINDUSTRIAN TERHADAP MAKROINVERTEBRATA AKUATIK DENGAN PENEKANAN KE ATAS CHIRONOMIDAE (DIPTERA) PADA TAHAP KOMUNITI, INDIVIDU DAN MOLEKUL

ABSTRAK

Kelimpahan dan kepelbagaian makroinvertebrata serta parameter air dikaji di lima sungai Lembangan Sungai Juru di utara semenanjung Malaysia; Sungai Ceruk Tok Kun (CTKR), Sungai Pasir (PR), Sungai Permatang Rawa (PRR), Sungai Kilang Ubi (KUR) dan Sungai Juru (JR). Sebanyak 23 genus makroinvertebrata dan sembilan spesies Chironomidae iaitu *Chironomus kiiensis*, *C. javanus*, *Polypedilum trigonus*, *Microchironomus* sp., *Dicrotendipes* sp., *Tanytarsus formosanus*, *Clinotanypus* sp., *Tanypus punctipennis* dan *Fittkauimyia* sp. telah dikenalpasti. Kesemua kelimpahan taxa chironomid kecuali *C. javanus* adalah berbeza secara signifikan di kalangan sungai-sungai tersebut (Ujian Kruskal Wallis, $P<0.05$). Mengikut pengkelasan Indeks Kualiti Air (WQI), CTKR, PR dan JR telah digolongkan dalam kelas III (sedikit tercemar), sementara PRR dan KUR termasuk dalam kelas IV (tercemar).

Model CCA mengasingkan makroinvertebrata kepada himpunan habitat sedikit tercemar (CTKR), habitat tercemar sederhana (JR) dan habitat tercemar (PRR, PR dan KUR). Sembilan belas parameter fizikal dan kimia yang diambil kira menjelaskan 62% dari jumlah keseluruhan varian. Biplot CCA menunjukkan bahawa chironomids *Dicrotendipes* sp. dan *Microchironimus* sp. cenderung kepada keadaan persekitaran yang mempunyai suhu air sederhana hingga tinggi, jumlah bahan organik (TOM), jumlah pepejal terampai (TSS), pH dan sulfat. Bersama dengan *C. kiiensis* dan *C. javanus,* kedua spesies ini agak toleran terhadap kepekatan Zn dan Cu. *Tanytarsus formosanus* sensitif terhadap perubahan kepekatan oksigen (DO)

terlarut. Walau bagaimanapun, *C. kiiensis*, *C. javanus*, *P. trigonus* dan *Tp. punctipennis* dipengaruhi secara negatif oleh kandungan oksigen di dalam air, menggambarkan ketahanan spesies tersebut terhadap kandungan oksigen yang rendah. Seterusnya, *C. kiiensis* dan *C. javanus* menunjukkan ketoleranan yang tinggi terhadap peningkatan kepekatan beberapa penunjuk pencemaran seperti TOM, TSS, permintaan oksigen biokimia (BOD), ammonia dan nitrat. Sebaliknya, spesies yang paling sensitif terhadap faktor-faktor tersebut adalah *T. formosanus*.

Kesan negatif parameter fizikal dan kimia sungai dan sedimen terhadap *Chironomus* spp. telah diselidik pada tahap individu dengan meneliti deformiti morfologi di bahagian kapsul kepala larva dari sungai-sungai yang tercemar iaitu PRR, PR dan KUR. Min deformiti tertinggi dicatat pada larva dari KUR (47.17%) diikuti oleh PRR (33.71%) dan PR (30.24%) Insiden deformiti yang dijelaskan oleh model RDA menunjukkan hubungan kuat dengan kandungan sedimen Mn dan Ni yang tinggi. Deformiti mentum dan epifaring pula berkorelasi tinggi dengan peningkatan TSS, jumlah aluminium, ammonium-N, penurunan pH dan oksigen terlarut. Insiden deformiti mentum yang telah digunakan untuk menghasilkan MTSI berdasarkan kepada TSI Lenat menunjukkan hubungan yang lebih kuat dengan jumlah insiden deformiti (DI). Model RDA seterusnya menyokong keberkesanan MTSI yang lebih tinggi dari TSI bagi menerangkan variasi kandungan logam berat di dalam sedimen sungai-sungai tersebut.

Ketidakstabilan tumbesaran *Chironomus* spp. berdasarkan kehadiran yang ditunjukkan dalam bentuk asimetri turun-naik (FA) dinilai pada larva yang dipungut dari PRR. Matrik FA dan indeksnya diukur pada tiga ciri yang dipilih; kelebaran mentum, panjang segmen antena pertama dan kedua. Ukuran bulanan kesemua ciri ini memastikan berlakunya FA. Model ordinasi RDA menunjukkan bahawa oksigen terlarut dan kualiti air sungai dalam nilai WQI berkorelasi secara negatif dengan semua indeks FA (FA, AbsFA dan CFA) kelebaran mentum larva, panjang segmen antena pertama dan kedua.

Genotoksisiti kepekatan sub-maut kadmium (0.1, 1, dan 10mg/l), kuprum (0.2, 2 dan 20 mg/l) dan zink (0.5, 5 dan 50 mg/l) ke atas larva *C. kiiensis* telah periksa selepas pendedahan selama 24 jam, menggunakan kaedah 'electrophoresis alkaline comet assay'. Kepekatan tertinggi Cd telah menunjukkan kerosakan DNA *C. kiiensis* yang lebih tinggi berbanding Cu dan Zn. Kesan bahan pencemar dalam sedimen telah diperiksa secara mendedahkan *C. kiiensis* kepada sedimen dari SR, PRR dan KUR selama 6, 12, 24 dan 48 jam. Tahap pencemaran yang diindeks sebagai jumlah kandungan parameter fiziko-kimia dan bahan pencemar di dalam air dan sedimen menunjukkan peningkatan dari SR ke PRR ke KUR. Kebanyakan larva *C. kiiensis* tidak dapat bertahan untuk jangka masa yang lama apabila terdedah kepada sedimen yang tercemar teruk (PRR hingga KUR). Analisis DNA menunjukkan kerosakan yang lebih besar dalam sel-sel yang diperolehi daripada larva yang diletakkan dalam sedimen tercemar terutama larva-larva dari KUR. Kesan keatas bahan genomik larva *C. kiiensis* meningkat seiring dengan pertambahan masa pendedahan.

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ABSTRACT

Abundance and diversity of macroinvertebrates as well as physico-chemical parameters of the water were investigated in five rivers of the Juru River Basin in northern Peninsula Malaysia; Ceruk Tok Kun River (CTKR), Pasir River (PR), Permatang Rawa River (PRR), Kilang Ubi River (KUR), and Juru River (JR). A total of 23 macroinvertebrate genera and nine chironomid species including *Chironomus kiiensis*, *C. javanus*, *Polypedilum trigonus*, *Microchironomus* sp., *Dicrotendipes* sp., *Tanytarsus formosanus*, *Clinotanypus* sp., *Tanypus punctipennis* and *Fittkauimyia* sp. were identified. Except for *C. javanus*, the abundance of chironomid species differed significantly among the rivers (Kruskal Wallis Test, $P<0.05$). Following the water quality index (WQI), CTKR, PR and JR were classified as Class III (slightly polluted rivers) while PRR and KUR fell in the Class IV (polluted) rivers.

The CCA model separated macroinvertebrates into assemblages of slightly polluted (CTKR), moderately polluted (JR) and polluted (PRR, PR and KUR) rivers. The 19 physico-chemical parameters considered in the model explained 62% of the total variance explained (TVE). The CCA biplot showed that the chironomids, *Dicrotendipes* sp. and *Microchironomus* sp., preferred environmental conditions with relatively moderate to high water temperature, Total Organic Matter (TOM), Total Suspended Solid (TSS), pH and sulphate. Together with *C. kiiensis* and *C. javanus,* these species were relatively tolerant to concentrations of Zn and Cu. *Tanytarsus formosanus* was sensitive to changes in dissolved oxygen concentration. However, *C. kiiensis*, *C. javanus*, *P. trigonus* and *Tp. punctipennis* were negatively influenced by

the oxygen content in the water implying their tolerance to low oxygen levels. In addition *C. kiiensis* and *C. javanus*, showed high tolerance to elevated concentrations of several pollution indicators such as TOM, TSS, BOD, ammonia-N and nitrate-N. In contrast, *T. formosanus* was very sensitive to these factors.

The negative effect of river physico-chemical parameters and pollutants in the waters and sediments on *Chironomus* spp. larvae was studied on the morphological deformities in parts of the head capsule (mentum, antenna, mandible and epipharyngis). The highest mean deformity was recorded in KUR (47.17%) followed by PRR (33.71%) and PR (30.34%). The total deformity incidence illustrated by the RDA model, was strongly correlated with high contents of sediment Mn and Ni. The mentum and epipharyngis deformity incidences were highly correlated with increase in TSS, total aluminum, and ammonium-N, and decreases in pH and DO. The mentum deformity incidences was utilized to develop a modified toxic score index (MTSI) based on Lenat's toxic score index (TSI) showed stronger relationship to total deformity incidence (DI).

Developmental instability in *Chironomus* spp. based on the presence of fluctuating asymmetry (FA) was assessed on larvae collected from PPR. The FA indices were measured for three selected traits; width of the mentum, the length of the first and the second antennal segments. Monthly measurements of these traits confirmed the occurence of FA. The ordination model RDA showed that dissolved oxygen and water quality in the river expressed as WQI negatively influenced all FA indices (FA, AbsFA and CFA) of the width of the larval mentum and the length of antennal segments one and two.

Genotoxicity of sub-lethal concentrations of cadmium (0.1, 1, and 10 mg/l), copper (0.2, 2, and 20 mg/l) and zinc (0.5, 5, and 50 mg/l) on *C. kiiensis* larvae was examined after 24 h exposure, using the alkaline comet assay electrophoresis. The highest concentration of Cd had caused higher DNA damage to *C. kiiensis* larvae compared to Cu and Zn. The effect of pollutants in sediments was inspected by exposing *C. kiiensis* larvae to sediments from SR, PRR and KUR for 6, 12, 24 and 48 h. Pollution level indexed by the amounts of physico-chemical parameters and pollutants in the water and sediment showed progressive increase from SR to PRR to KUR. Most of *C. kiiensis* larvae did not survive following long periods of exposure to highly polluted sediments (PRR to KUR). DNA analyses revealed greater damage in cells derived from larvae maintained in polluted sediments, particularly that of KUR. The effects on the genomic material of *C. kiiensis* larvae increased with increase in exposure time.

CHAPTER 1

INTRODUCTION

1.1 Background

Macroinvertebrates are defined as those invertebrates exceeding 0.5 mm body size, or large enough to be seen by the naked eye, and comprise mostly of insects as well as decapods, crustaceans, molluscs, leeches and oligochaetes (Wallace and Webster, 1996). Most of them live on or among streambed sediments, and hence are often referred to as macrobenthos, although the majority of stream insects have an amphibiotic life cycle and spend their adult stage on land (Dudgeon, 2008). Benthic macroinvertebrates have been widely applied as indicators of water quality in river management because they are affected not only by natural changes; including physical disturbance in the river but also by chemical and physical factors induced by human activities (Coimbra *et al*., 1996; Zamora-Munoz and Alba-Tercedor, 1996; Kay *et al*., 2001; Arimoro, 2009; Boonsoong *et al*., 2009; Canobbio *et al*., 2009).

Chemical analyses provide limited information on the effects of contaminants especially when they are present in water column at low concentration below the limits of analytical detection (Goodnight, 1973). However, utilization of the indicator organisms has several advantages over traditional chemical analyses for water quality assessment because these organisms live almost continuously in the water and respond to all environmental stressors, including synergistic combinations of pollutants (Morse *et al*., 2007).

As an important component of aquatic insect community, Chironomidae have been proven useful as biological indicators because of their response to physical and chemical changes in aquatic ecosystems (Dudley and Blair, 1992; Mousavi *et al*., 2003; Bhattacharyay *et al*., 2005; MacDonald and Taylor, 2006). Therefore, they are often included in most ecological and toxicological studies (Boothroyd, 1995; Janssens de Bisthoven and Gerhardt, 2003; Mousavi *et al*., 2003; Faria *et al*., 2008). Late instars of some chironomid larvae frequently develop alterations in their morphology when continuously exposed to stress or pollution conditions (Vermeulen, 1995). It is well documented that alterations in the morphology of chironomid larvae is indication to environmental pollution with heavy metals, radioactivity, organic compounds, and other xenobiotics (Bird, 1994; Vermeulen, 1995; Servia *et al*., 1998; Watanabe *et al*., 2000; Martinez *et al*., 2004; Servia *et al*., 2004a; Servia *et al*., 2004b). In this context, alterations in the morphology of chironomid larvae are extensively being used as bioindicator traits for assessing the aquatic pollution, specifically that relates to industrial wastes and agricultural runoff (e.g., deformities: Servia *et al*, 1998; Burger and Snodgrass, 2000; Meregalli *et al*., 2000; Pollet and Bendell-Young, 2000; Prygiel *et al*., 2000; MacDonald and Taylor, 2006; Veroli *et al*., 2010; fluctuating asymmetry (FA): Rettig *et al*., 1997; Bleeker *et al*., 1999; Allenbach *et al*., 1999; deformities and FA: Groenendijk *et al*. 1998).

Other effects of environmental pollutants at cellular and molecular levels such as DNA damage and induction of certain genes expression could also occur (Choi, 2004). The effects of pollutants are usually first manifested at the molecular and biochemical levels where the functioning of important biochemical pathways can be affected (McCarthy and Shugart, 1990). This disruption in function may, after a period of time, be expressed by developing morphological deformities or/and decrease in an organism's ability to grow, to reproduce or to survive (Choi, 2004).

Mitchelmore *et al*. (1998) reported that the exposures of aquatic species to genotoxic substances and processes can produce chemical or physical modifications to DNA, commonly measured as DNA adducts or DNA strand breaks. Detection of this DNA damage using the single cell gel (SCG, Singh *et al*., 1988) or comet assay has been widely applied to investigate the response of aquatic organisms including chironomids to organic and inorganic pollutants (Mitchelmore *et al*., 1998; Lee and Steinert, 2003; Lee and Choi, 2006; Lee and Choi, 2009; Park and Choi, 2009).

Dudgeon (2008) stated that many tropical countries lack resources for adequate sewage treatment, and considerable organic matter and other substances are released directly into streams. In Malaysia, the pollution of aquatic ecosystems has emerged as a critical ecological problem coinciding with rapid industrialization and urbanization. One of those polluted aquatic ecosystems is the Juru River which has been classified as "very polluted" based on the Water Quality Index (WQI) categorization by DOE (DOE, 1994). According to Lim and Kiu (1995), Lim and Seng (1997), Tan and Yap (2006) and Alkarkhi *et al*. (2008), the Juru Basin sediments are contaminated with heavy metals, such as Cd, Cu, Pb, and Zn. These contaminants are most likely a result of discharges from the light and heavy industries in the Perai Industrial Estate which established in the early 1970s (Mat and Maah, 1994).

Consequently, considerable efforts have been made in the past two decades to analyze chemical pollution in several Malaysian rivers (including the Juru River Basin) (e.g., Mat and Maah, 1994; Lim and Kiu, 1995; Lim and Seng, 1997). However, relatively much less attention has been paid to utilize aquatic organisms for purposes of environmental bioassessments (Morse *et al*., 2007). Presently, the DOE of Malaysia only uses water quality index for investigation of river pollution possibly due to the assumption that chemical investigation and WQI are more favourable approaches compared to biomonitoring using benthic organisms. Azrina *et al*. (2006) identified possible factors for the poor reliability on biological monitoring in Malaysia (1) measurement of water quality characteristics is a conventional technique which can give direct results on the pollution status of the water quality at the time of sampling, (2) lack of established taxonomical keys for the Malaysian macrobenthic invertebrates especially to the species level and (3) delays in the prevention and remediation of the polluted river ecosystem until a time when only the resistant bioindicators were found there. However, it is generally accepted that mere chemical analysis turn does not completely reveal the ‘‘health’’ of river ecosystems as far as the impacts of pollutants on the living organisms of the river ecosystem are concerned (Azrina *et al*., 2006; Morse *et al*., 2007; Arimoro, 2009; Boonsoong *et al*., 2009).

In the last few decades there has been an upsurge of interest in rapid assessment techniques for the biological monitoring of water quality in several developed countries (Chessman, 1995; Throne and Williams, 1997; Kay *et al*., 2001; Clements and Newman, 2002; Metzeling *et al*., 2003; Murphy and Davy-Bowker, 2005; Hicham and Lotfi, 2007; Rohasliney and Jackson, 2008; Canobbio *et al*., 2009; Song *et al*., 2009; Friberg *et al*., 2010). These methods emphasize a low cost approach, achieved by reduced sampling and more effective data analysis (Resh *et al*., 1995; Throne and Williams, 1997). The low cost of such approaches make them immediately attractive for use in developing countries (Resh *et al*., 1995; Che Salmah *et al*., 1999; Mustow, 2002; Azrina *et al*., 2006; Arimoro, 2009; Boonsoong *et al*., 2009), and other features enhance this suitability. In Malaysia, there have been very few studies conducted concerning effects of contaminants on aquatic invertebrates (Che Salmah *et al*., 1999; Azrina *et al*., 2006).

Furthermore, no such research has been emphasized on the application of chironomid larvae as bioindicators for water quality in Malaysia. This is in spite of the wide utility of larval head capsule abnormalities, such as deformation, phenodeviations, and asymmetries, to indicate the aquatic pollution-related stresses in several countries, such as Canada (Warwick, 1985; MacDonald and Taylor, 2006), Sweden (Wiederholm, 1984; Janssens de Bisthoven and Gerhardt, 2003), Spain (Servia *et al*., 1998; Servia *et al*., 2002 Italy (Veroli *et al*., 2010) and India (Bhattacharyay *et al*., 2005).

Chironomid larvae, specifically *Chironomus* spp. have been widely utilized in bioassessment of organic and inorganic pollutants at cellular and molecular levels (Choi, 2004; Ha and Choi, 2008; Lee and Choi, 2009; Park and Choi, 2009). Therefore, application of molecular biomarkers such as DNA damage has the potential to demonstrate the influence of different pollutants on the aquatic organisms. In Malaysia, there is a lack in application of all these empirical tools to demonstrate the influence of pollution on aquatic organisms including chironomid larvae.

1.2 Objectives

In view of limited information and practice of biological assessment of water quality in Malaysia, this research was undertaken in the Juru River Basin, one of the most polluted rivers in Malaysia. Investigation on the effect of domestic, agricultural and industrial effluents on chironomids at three different levels; community, individual and molecular, would produce a comprehensive set of ecotoxicological information. Evidently, that set of information (from cell to population) would provide better understanding on how the organisms are being affected and ways they respond to the pollution at various levels.

Thus, this study was conducted to achieve the following objectives:

1. To investigate the influence of industrial, agricultural and municipal stresses on the distribution and diversity of the macroinvertebrates in a reference ‘clean’ site (Ceruk Tok Kun River) and in polluted sites namely, Pasir River (PR), Permatang Rawa River (PRR), Kilang Ubi River (KUR), and Juru River (JR), all located in the Juru River system.

2. To investigate the effect of water physico-chemical parameters and organic matter and heavy metals of Cu, Zn, Mn and Ni on Chironomidae abundance and diversity in Juru River Basin.

3. To investigate the influence of anthropogenic and environmental stresses on morphological deformities in the *Chironomus* spp. larval head capsule (mentum, epipharyngis, mandibles and antennae) and develop a reliable toxic index using mentum deformities to assess the aquatic environment health.

4. To investigate the association between the developmental stability, expressed as fluctuating asymmetry (FA) in *Chironomus* spp. larvae, and water quality and heavy metal contents in the sediment of the Permatang Rawa River (PRR).

5. To detect DNA damage and genotoxic effects of heavy metals (zinc, copper and cadmium) and field-collected sediments from KUR and PRR rivers using the single cell gel electrophoresis (comet assay).

CHAPTER 2

LITERATURE REVIEW

2.1 Tropical Stream Ecology

Tropical streams differ from temperate streams in many aspects, such as evolutionary history, precipitation patterns, water temperature and diversity of riparian vegetation (Ometo *et al*., 2000; Gopal, 2005; Helson *et al*., 2006). The Asian natural inland aquatic ecosystems are diverse in their ecological patterns (Dudgeon, 2006; Gopal, 2005) and in particular riverine systems which are highly rich in their fauna (Dudgeon, 2000a; Dudgeon, 2000b). However, faunal biodiversity of Asian aquatic ecosystems is increasingly threatened by many factors all of which are related to human activities (Dudgeon, 1992; Dudgeon, 2000a; Dudgeon, 2000b; Gopal, 2005). The human influence is pervasive in all tropical rivers and, unfortunately, almost all large tropical rivers in Asia are polluted (Hynes, 1989, Dudgeon 1992).

Although tropical stream research has increased over the last two decades, there is still a huge gap in the knowledge of tropical streams compared with that of temperate streams (Jackson and Sweeney, 1995; Dudgeon, 2000a; Dudgeon, 2000b; Ometo *et al*., 2000; Mantel *et al*., 2004; Morse *et al*., 2007; Dudgeon, 2008). For instance, the taxonomy of tropical benthic macroinvertebrates including chironomids is poorly known compared to that of temperate regions (Cranston, 1995; Cranston, 2007). More information is required to better understanding of the dynamics of these streams.

2.2 Macroinvertebrates including Chironomidae in the Tropical Streams

2.2.1 Aquatic Insects and other Macroinvertebrates

The interest about the macroinvertebrate fauna of streams in the Asian region has increased in recent years (Dudgeon, 2006). The diversity and abundance of tropical macroinvertebrates have been investigated in many Asian countries such as Hong Kong (Mantel *et al*., 2004), Thailand (Boonsoong *et al*., 2009), Indonesia (Dudgeon, 2006) and Malaysia (Che Salmah *et al*., 1999; Siregar *et al*., 1999; Azrina *et al*., 2006).

The aquatic tropical habitats are characterized by a more even distribution of individuals among the macroinvertebrate species. Tropical aquatic communities not only contain many more species than temperate communities, but the most common species makes for a relatively small portion of the total community (Helson *et al*., 2006). In contrast, temperate communities are often dominated by a relatively few species which account for most of the individuals (Clements and Newman, 2002).

2.2.2 Chironomidae

Few studies have examined the changes in tropical river chironomid communities in response to eutrophication, pollution or the loss of riparian vegetation (Helson *et al*., 2006). Agricultural activities, through the use of fertilizers and organic manures, often lead to the nutrient enrichment of surface waters, as does the input of both treated and untreated domestic wastes (Harding *et al*., 1999).

In the temperate region, Pinder (1986) reported that family Chironomidae is the most widely distributed and frequently the most abundant group of insects in freshwater environment. However, there is a scarcity of studies on the ecological and biological aspects of tropical South-east Asian chironomids (Al-Shami, 2006).

Cranston (2007) investigated the chironomid fauna in selected water bodies in the southern part of Thailand from Ranong to Krabi. The chironomids in the rivers were found to be very diverse comprising of 29 species belonging to 15 genera in three subfamilies. Knowledge on Chironomidae in tropical areas of South East Asia particularly Malaysia and Singapore is not fully understood. Cranston (2004) documented that the first known Chironomidae species in South East Asia was the Javanese *Tanypus crux*. Karunakaran (1974) studied Chironomidae in Singapore and contributed further information about Chironomidae in this region. He reported many species from the subfamily Chironominae such as *Chironomus apucatus*, *C. crassiforceps, C. costatus, C. incertus*, *C. stupidus*, *C. tumidus*, *Polypedilum anticus*, *P. convexum*, *Tanytarsus* sp. Only one species and *Tanypus kraatzi* belonging to the subfamily Tanypodinae was reported. In Peninsula Malaysia, Bishop (1973) recorded three subfamilies, Chironominae, Tanypodinae, and Orthocladiinae, from a small river, Sungai Gombak in Selangor State. In addition, a single species of Diamesinae was recorded from a high elevation (above 3000 m) on Mountain Kinabalu in Sabah, Malaysia (Cranston, 2004).

2.3 Factors Influencing the Distribution and Diversity of Chironomidae and other Macroinvertebrates

Generally, all aquatic invertebrates respond quantitatively not only to the availability of trophic resources but to changes in their habitat and to physical and chemical variations of the water. The environmental factors, which control distribution of organisms in freshwater are current speed, the stability of water depth, light and temperature regimes and water quality (Hellawell, 1986). Each habitat is unique and presents its own opportunities for exploitation by suitably adapted organisms.

Physical heterogeneity, including the velocity of the current in a stream channel, is an important factor that may influence local biotic diversity, nutrient dynamics, algae and macrophyte distribution, retention and distribution of organic matter, predator-prey interactions, presence or absence of refugia during disturbance, and secondary production of invertebrates (Wallace and Webster, 1996).

2.3.1 Water Movement and Depth of Rivers

Water current speed is one of the important physical factors in running water that determines the distribution and diversity of aquatic macroinvertebrates including insects (Che Salmah *et al*., 1999; Wahizatul Afzan, 2004). Water movement creates high stratification in the water columns which in turn maintains high concentrations of DO. The continuous turbulent flow of stream waters is the dominant characteristic distinguishing them from lakes and ponds (Warren, 1971). Marsaulina *et al*., (1994) classified the water current speed into five classes; very fast (>0.1 m/sec), fast (0.05-0.1 m/sec), moderate (0.025-0.05 m/sec), slow (0.01-0.025 m/sec) and very slow (<0.01 m/sec). Living organisms (plants and animals) depend on water movement to deliver materials for their nutrition and respiration. Some stream animals obtain their food by straining it from moving water. Others depend on the current for renewal of the water bathing their respiratory surfaces, water that brings oxygen and carries away metabolites (Warren, 1971). However, water movement can be a destructive force in the lives of aquatic organism as it may tear them away from their substrate, move them to unsuitable locales, or fragment them. But in each aquatic environment, it prepares and maintains their place of life in ways for which they are adapted (Warren, 1971).

Water depth is also an important environmental variable, which can affect the spatial distribution and community composition of macroinvertebrates (Wahizatul Afzan, 2004). For instance, Ali *et al*. (2002) noted that the distribution ofsome species of chironomids displayed a strong, negatively significant correlation between distribution and water depth. Similarly, Shiozawa and Barnes (1977) reported that chironomid density decreased with increasing water depth in a lake ecosystem in their study on *Tanypus stellatus* and *Chironomus frommeri*. Other chironomid taxa such as *Glyptotendipes paripes* also showed significant inverse correlation with water depth (Ali, 1989).

2.3.2 Water Temperature

Many hydrobiologists consider the temperature regime to be the most important physical factor controlling the ecology of aquatic communities. The seasonal fluctuation of temperature controls the rhythm of the life histories of many animals (Hynes, 1970). The main significance of temperature on flowing waters is through its effect on metabolism and the capacity of water to hold dissolved oxygen (Dudgeon, 2008). Temperature is one of the major factors controlling rates of growth and development in aquatic insects. Adult body size of a number of insects depends largely on temperatures experienced during larval development (Hynes, 1970). In addition to a direct effect on metabolism, temperature is also likely to have indirect effect through its influence on food quality and quantity (Pinder, 1986).

Although Siregar *et al*. (1999) reported that the optimum range of water temperature for aquatic life is 27-30 ºC, water temperature in the South-east Asian tropics varies from as low as 24 ºC in the morning or during cloudy or rainy days and up to 40 ºC on hot sunny days (Ali and Ahmad, 1988). Ahmad *et al*. (2002) reported that the water temperature of the Linggi River in Negeri Sembilan, ranged from 22 to 29 ºC. However, higher range of water temperature (25.5 to 36 ºC) was reported in another tropical stream in Singapore (Johnson, 1967).

Different chironomid species exhibit different preferences for water temperature (Pinder, 1986). The stenothermic taxa (e.g., Diamesinae and Orthocladiinae) prefer cold water compared to warm eurytherms (several Chironominae and Tanypodinae) (Shiozawa and Barnes, 1977; Hirabayashi *et al.*, 2004).

2.3.3 Dissolved Oxygen (DO)

The hypolimnetic oxygen concentrations and trophic state are very important factors regulating aquatic insect distribution (Walker *et al*., 2003). Generally, the dissolved oxygen concentrations in tropical areas range from 0.8-8.8 mg/l and they play an important factor in determining the quality of water (Dudgeon, 2008). Usually, species richness increased with increasing amount of dissolved oxygen and clarity of water (Wahizatul Afzan, 2004). Siregar *et al*. (1999) concluded in their study of the distribution of aquatic insects and its correlation in the water quality in the Kerian river Basin, that the dissolved oxygen varied from a slightly low value of 4.40 mg/l to relatively a high value of 7.60 mg/l. This range is also suitable for most resident insects (Wallace and Webster, 1996).

Variation in dissolved oxygen preferences can be found among macroinvertebrate organisms including insects. Different preference to DO can be found even within the same taxonomical guild. For instance, odonate larvae *Pantala flavescens* was found to inhabit environment in the 4-6 mg/l of dissolved oxygen, whereas *Trithemis aurora* and *Libellago lineate* preferred living in running waters with higher concentration of dissolved oxygen (7-9 mg/l) (Che Salmah *et al*., 2002).

Some chironomid larvae have special adaptation to live in habitats with low dissolved oxygen. This is related to the possession of haemoglobin that enables them to survive at low oxygen concentration. Other chironomid larvae have developed unique behaviour to overcome oxygen deterioration. *Chironomus dorsalis* larvae have been reported to extend their dwelling tubes as much as 20 mm above the sediment surface in order to maintain an oxygen concentration of more than 7 mg/l at the tube opening (Pinder, 1986).

A number of variables affect the rate of re-aeration, such as velocity, temperature, depth, slope and channel irregularity (Mason, 1981). Moreover, the concentration of DO in lentic environments is also affected by pollution and industrial effluents (Coimbra *et al*., 1996; Dudgeon, 2008). This was corroborated by the study conducted by Ahmad *et al*. (2002) where DO in the highly polluted Linggi River in Negeri Sembilan, was found to range between 4.9 to 8.1 mg/l. In an earlier study, Johnson (1967) had observed a much lower range of 0.5 -2.23 mg/l. Mason (1981) reported that organic pollutant is the result when large quantities of organic compounds, which can act as substrates for microorganisms, are released into watercourses. During the decomposition process, DO in the receiving water may be utilized at a greater rate than it can be replenished, causing oxygen depletion and resulting severe consequences for the stream biota (Mason, 1981).

2.3.4 Biochemical Oxygen Demand (BOD)

According to Laws (1993), biochemical oxygen demand (BOD) is defined as the amount of oxygen consumed in a sample of water incubated in a stoppered bottle in the dark at 20 °C for 5 days. Since the sample is incubated in the dark, there is no possibility for photosynthesis to occur, thus the oxygen concentration would either remain constant or decline. A decrease in oxygen concentration occurs when there is respiratory activity of organisms and/or simple chemical oxidation of unstable substances such as ferrous iron or ammonia. The decline in oxygen concentration may be caused by both biological and chemical processes; hence it is referred to as biochemical oxygen demand (Laws, 1993). Excessive loads of dissolved and particulate organic matter result in increased biochemical oxygen demand and acute reductions in dissolved oxygen in the water column of streams (Dudgeon, 2008).

Based on their investigation on the highly polluted Linggi River, Ahmad *et al*. (2002) observed a high BOD ranging between 2.4 and 6.2 mg/l. Laws (1993) stated that the wastewater from industrial operations (such as pulp mills, sugar refineries and some food processing plant) is characterized by high concentrations of BOD. In such rivers, organisms that they are adapted to low levels of dissolved oxygen are abundant for example *Eristalis* spp., *Chironomus* spp., mosquito and oligochaeta (Goodnight, 1973; Mason, 1981). However, sensitive and moderately sensitive species such as caddisfly, mayfly and stoneflies nymphs would only be found in small numbers or gradually disappear (Laws, 1993).

2.3.5 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) is defined as the amount of oxygen consumed when the substances in the water are oxidized by a strong chemical oxidant (Laws, 1993). Song *et al*. (2009) found that most of the agricultural sites in France were characterized by high levels of COD that most likely have resulted from high loads of suspended organic and inorganic materials in the increased runoff from agricultural lands. The taxa richness and diversity of intolerant group of aquatic insects belonging to Ephemeroptera, Plecoptera and Trichoptera (EPT) were lower compared to forest sites. High concentrations of COD were reported in Malaysian rivers. For example, high COD range (12-52 mg/l) was reported in the polluted Linggi River (Ahmad *et al*. 2002). However, a much higher concentration range of COD (52.3-233 mg/l) was reported in the Langat River by Azrina *et al*. (2006).

2.3.6 pH

pH is one of the most widely investigated component in freshwaters. The acid rain and acid mine drainage are major factors contributing to pH changes in industrialized countries (Coimbra *et al*., 1996). Based on a study of 39 freshwater and tidal water habitats in the Southern parts of Peninsular Malaysia and Singapore, Johnson (1967) recorded characteristically soft, acidic, poorly buffered and calcium lacking water in these areas. He concluded that the pH value was complicated by the possibility of diurnal, seasonal and microtopographical variations. In many freshwaters, much of such variation results from the production and consumption of carbon dioxide by organisms affecting the bicarbonate buffer system. Distribution of macroinvertebrates is strongly affected by the variations in the pH values (Potts and Fryer, 1979) and decreased in pH (less than 5.5) is related to a general decrease in diversity of aquatic macroinvertebrates (Winner *et al*., 1980; Coimbra *et al*., 1996). However, high pH was also reported to affect the structure of the benthic communities (Coimbra *et al*., 1996). Collectors, shredders and predators from acidic streams are usually found in both low numbers and species richness (Hellawell, 1986). However, several large predators such as Odonata, Dysticidae, Corixidae and some Gerridae were less affected by acidic environment (Eriksson *et al.*, 1980; Bendell, 1988).

Many chironomid species are tolerant to variations of pH, within 6 to 9, but outside of this range, occurrence of fewer species is observed (Pinder, 1986). In the Kerian River, Siregar *et al*. (1999) found that some tolerant taxa from Chironomidae, Odonata and Lepidoptera survived in low pH environment. The ability to tolerate a wide range of pH seems to occur most frequently within the subfamily Chironominae and especially in the tribe Chironomini. For example, *C. plumosus* was typically the only species found in a series of lakes affected by acid mine-drainage when the pH was below 6 (Pinder, 1986).

In Malaysia as well as many other Asian countries, rice is the most important agricultural crop. As a common practice during rice cultivation processes, lime is applied to raise the pH of the soils (Ali and Ahmad, 1988; Al-Shami, 2006). When the effluents from rice fields enter the aquatic ecosystem, it contributes significantly to increase the pH above the normal range in those water bodies. Consequently, the fluctuation in the pH will affect the distribution and composition of aquatic macroinvertebrates (Heckman, 1979; Ali and Ahmad, 1988).

2.3.7 Total Suspended Solids (TSS)

Normal burden of suspended materials is derived from natural weathering and soil erosion by run-off. These enter surface waters by direct discharge, agricultural activities and forestry operations which may lead to increased loads of silt and soil particles entering streams and rivers (Hellawell, 1986). However, they are a threat to aquatic environment only when they are present over extended periods in ‘unusually’ large amounts, thereby changing the character of the habitat (Wahizatul Afzan, 2004). These particles may exert direct mechanical effects on organisms by increasing abrasion, clogging the respiratory surfaces of gills and interfering with feeding through inadvertent collection on feeding appendages or nets of filter-feeding invertebrates (Hellawell, 1986).

2.3.8 Total Organic Matter (TOM)

The distribution of aquatic insects especially Chironomidae depends on the availability of trophic resources (e.g. moss, filamentous algae, fine particulate organic matter, and leaves) (Berg, 1995). The abundance patterns of chironomids, and changes in species composition are known to be influenced by the quality and quantity of the food change in the particle regime, especially the organic matter coating the bottoms (Hirabayashi *et al*., 2004). Galdean *et al*. (2000) reported that the abundance of Chironomidae genera was related to the amount of detritus and the riparian vegetation in a tropical stream in Southeast Brazil. They observed that the Chironominae genera; *Chironomus*, *Fissimentum*, *Glyptotendipes*, *Goeldichironomus*, *Polypedilum*, *Stenochironomus* and *Tanytarsus* were prevalent and thrived on the fine and ultra-fine particles of organic matter.

2.3.9 Phosphate, Nitrate, Sulphate, Chloride and Ammonia Contents

Dudgeon (2008) reported that the amounts of phosphorus, nitrogen, and carbon in tropical streams and rivers are of special interest because of the regulatory role that these elements play in aquatic ecosystems. Elevated concentrations of phosphorus, nitrogen, and dissolved organic carbon were usually related to water pollution. Of greater fundamental interest is the concentration of these elements under natural conditions, and the factors that control their concentrations in nature. Relatively lower concentrations of particulate phosphorus were recorded in unpolluted water of the Amazon and Orinoco Basins. In basins of Asiatic rivers, particularly where land disturbance plays a major factor in liberating suspended solids to surface waters, the contents of the suspended solids together with all five elements were high (Downing *et al.*, 1999).

The concentration of nitrate is directly correlated with levels of dissolved oxygen in the water. Besides their study on several environmental, chemical, physical, and microbiological parameters in the Kelang River, Law and Mohammad Mohsin (1980) observed that nitrate concentration was high during the monsoon season as a result of fertilizer application or nitrate oxidation through the process of nitrification. Urban sources of nutrients, derived from domestic sewage and industrial wastes may contribute to the eutrophication of the river ecosystem (Mason, 1981). Detergents are an important source of phosphate in domestic sewage. Besides, agriculture effluents are another major contributor to nitrate loading in freshwaters (Mason, 1981; Ometo *et al.*, 2000). However, different crop cultivation causes significant differences in the concentrations of NO_3, NH_3 and PO_4 due to the varying needs for fertilizers (Ometo *et al.*, 2000).

The concentrations of chloride ions in the river streams are higher in urbanized than in rural areas. Ometo *et al*. (2000) reported that the human sewage is the main source of chloride in two tropical rivers in Brazil. Coimbra *et al*. (1996) investigated the water quality in Mediterranean River and showed that the concentrations of NO_3 and SO_4 in the downstream sites were almost 100 times higher than those in the reference sites. However, the concentration of PO_4 did not show