UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF MUTAGENIC ACTIVITY FROM RIVER WATER DUE TO INDUSTRIAL EFFLUENT: A REVIEW

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BY

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Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

ACKNOWLEDGEMENTS

Foremost, I would like to express my gratitude to my supervisor, Dr Rozaini Abdullah for guiding me throughout the completion of this thesis and also for imparting knowledge to help me understand the scope of this thesis. I would like to thank Dr Rozaini also for helping me change the direction of my study due to the Movement Control Order which severely impacted each and everyone of us during the pandemic, physically and mentally. Next, I would like to thank the staff of Environmental and Health Lab of Medical and Health Sciences Faculty in UPM, Miss Sofia and Miss Siti for assisting throughout the laboratory work although for a short period. They were very helpful in finding us equipments which we needed to proceed with the laboratory experiments. I would like to extend my gratitude to Dr Ho Yu Bin for being an excellent course coordinator by always keeping up with the latest news and information amidst the pandemic. Next I would like to thank my friends from UPM for always helping me and guiding me throughout the completion of thesis. Lastly, I would like to thank my parents for making me feel comfortable at home so that I could finish up my thesis amidst the pandemic. They were very understanding of all the needs I inquired of them.

In overall, thank you to everyone who inspired me directly or indirectly to complete this thesis.

Thank you.

ABSTRACT

ASSESSMENT OF MUTAGENIC ACTIVITY IN RIVER WATER DUE TO INDUSTRIAL EFFLUENT: A REVIEW

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Introduction: River water pollution is a deterioration of the surface water quality due to the influence of many types of pollutants in water body. One of the causes of river water pollution is the discharge of industrial effluents into the water bodies. These industrial effluents contain many contaminants such as heavy metals, acids and alkalis, dyes, aromatic amines and many more. The main health effect of interest from exposure to these river water contaminated with industrial effluents are changes in DNA or mutation, thus leading to cancer formation in living organism. **Objectives:** thus this study aimed to review the mutagenic activity present in river water due to discharge of industrial effluents by identifying the source and contaminant responsible in inducing mutagenic activity in river waters. In addition, this study aimed to assess mutagenic activity as one of the parameters in river water quality monitoring programme. **Methodology:** This review was done by incorporating keywords such as river water pollution, industrial effluent, mutagenicity and toxicity testing to search for research articles in the subscribed database such as Science Direct. **Results and Discussion:** It was found that the factors inducing mutagenic activity in river water includes the discharge of azo compounds or azo dyes, heavy metals and mixture of pollutants from varying sources. The assays such as Ames Test or *Allium cepa* test ere used in certain countries in their routine river water quality programme as screening process to assess the mutagenicity and lead to the detection of agents which causes DNA damage in organisms. **Conclusion:** In conclusion, agents causing mutation from industrial effluent has been identified from different sources. It is recommended to include the mutagenicity testing in annual river water quality programme in Malaysia to detect pollutants present in river water which causes DNA damage.

Keywords: River water pollution, Industrial effluent, Mutagenicity, Toxicity testing

ABSTRAK

PENILAIAN KEGIATAN MUTAGENIK DALAM AIR SUNGAI KESAN EFLUEN DARI INDUSTRI: KAJIAN TINJAUAN

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Pengenalan: Pencemaran air sungai adalah kemerosotan kualiti air permukaan yang disebabkan oleh pengaruh pelbagai jenis pencemaran dalam badan air. Salah satu penyebab pencemaran air sungai adalah pembuangan efluen industri ke dalam air sungai. Efluen industri ini mengandungi pelbagai bahan cemar seperti logam berat, asid dan alkali, pewarna, amina aromatik dan banyak lagi. Kesan kesihatan utama daripada pendedahan kepada air sungai yang tercemar dengan efluen industri adalah perubahan DNA atau mutasi, sehingga menyebabkan pembentukan barah pada organisma hidup. **Objektif:** Sehubungan dengan itu kajian ini bertujuan untuk mengkaji aktiviti mutagenik yang terdapat di dalam air sungai akibat pembuangan efluen industri dengan mengenal pasti sumber dan bahan pencemar yang bertanggungjawab dalam mendorong aktiviti mutagenik di perairan sungai. Di samping itu, kajian ini bertujuan untuk menilai aktiviti mutagenik sebagai salah satu parameter dalam program pemantauan kualiti air sungai **Metodologi:** Tinjauan ini dilakukan dengan memasukkan kata kunci seperti pencemaran air sungai, efluen industri, mutagenisiti dan ujian ketoksikan untuk mencari artikel penyelidikan dalam pangkalan data yang dilanggan seperti Science Direct **Hasil dan Perbincangan**: Didapati bahawa faktor-faktor yang mendorong aktiviti mutagenik dalam air sungai termasuk pembuangan sebatian azo atau pewarna azo, logam berat dan campuran bahan pencemar dari pelbagai sumber. Ujian seperti Ujian Ames atau Ujian *Allium cepa* digunakan di negara-negara tertentu dalam program kualiti air sungai rutin mereka sebagai proses pemeriksaan untuk menilai mutagenisiti dan membawa kepada pengesanan agen yang menyebabkan kerosakan DNA pada organisma. **Kesimpulan**: Kesimpulannya, agen penyebab mutasi dari efluen industri telah dikenal pasti dari pelbagai sumber. Adalah disarankan untuk menyertakan pengujian mutagenisiti dalam program kualiti air sungai tahunan di Malaysia untuk mengesan bahan pencemar yang terdapat di dalam air sungai yang menyebabkan kerosakan DNA.

Kata kunci: Pencemaran air sungai, efluen industri, Mutagenisiti, Ujian ketoksikan

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LIST OF ABBREVATIONS

DOE	Department of Environment Malaysia
DNA	Deoxyribonucleic acid
PAHs	Polycyclic Aromatic Hydrocarbons
IARC	International Agency of Research in Cancer
US EPA	United States Environmental Protection Agency
UV	Ultra-violet
CA	Chromosomal Aberrations
Pb	Lead
Ni	Nickel
Cu	Copper
Cr	Chromium
Mn	Manganese
Si	Silicon
Ti	Titanium
Cd	Cadmium
WQI	Water Quality Index

NWQS	National Water Quality Standards
DO	Dissolved Oxygen
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
NH3-N	Ammoniacal Nitrogen
SS	Suspended Solids
Zn	Zinc
Fe	Iron
Al	Aluminium
Sn	Tin

CHAPTER 1

INTRODUCTION

1.1 Background

Water pollution is a decline in water quality which usually resulted from human activities (Halder & Islam, 2015). It affects living organisms including plants, animals, humans and as well as the country's economy (Afroz & Rahman, 2017). According to a monitoring programme conducted by the Department of Environment Malaysia in 2017, it was revealed that 52% of river was found to be clean, 39% of the rivers was found to be slightly contaminated and 9% of them was found to be contaminated (Afroz & Rahman, 2017). The contaminations are mainly caused by anthropogenic activities such as agricultural activities, population growth, industrialization and urbanization (Singh & Gupta, 2017).

In general, the types of sources of water pollution can be classified into two types, which are point source and non-point source. Point sources are direct sources which can be identified easily however, non-point sources are mostly unidentifiable.

Effects due to water pollution can be identified immediately while some can take months or even years to be recognized (Singh & Gupta, 2017).

Industrial, agricultural and transportation activities are responsible in producing chemicals in large amounts which can be found widespread in air, water and soil mediums and lead to toxicity effects. For example, in the year 1915, study by Yamagiwa and Ishikawa reported the formation of tumours in ears of rabbits which was treated with tar (B. Liu et al., 2016). Various chemicals showed mutagenicity properties by causing mutations and cancers which was evident in animals with tumour growth after exposure to environmental mutagens (B. Liu et al., 2016). Economic development and technological advancements produce unwanted and undesirable industrial wastes and effluents (Mathur et al., 2005). These wastes which are improperly treated and disposed of into the environment, pose harmful impacts on human health, aquatic life and the environment as it is reported to be highly toxic in several studies (Bhat et al., 2019; Mathur et al., 2005; Sg et al., 2016). According to (Zeyad et al., 2019), the genotoxic assessment of industrial effluents on rivers shows existence of mixtures which is comprised of many types of toxic substances and chemicals which pose risk of hazard and carcinogenicity and also responsible for DNA damages in aquatic systems organisms. For an example, in the industrial area of Lucknow, the wastewater discharge from pesticide industry which was used for irrigation purposes exhibited strong mutagenicity in the soil which was irrigated with the wastewater (Zeyad et al., 2019).

One of the adverse effects of interest is the mutation effect due to exposure to these contaminated waters. The contaminants damage the germline which leads to problems in fertility and also mutations of generations to come in the future and might induce the cancer formation (Mortelmans & Zeiger, 2000). The toxic substances are often persistent in the environment. It affects human beings through food and water supplies. Usually, aquatic environment serves as the final receptor of many contaminants in relatively high amount. For instance, dies, petroleum-derived hydrocarbons, chromium and other substances constitute industrial discharges could result in the damage of DNA which can cause diseases and affect human offspring in exposed organisms (Bhat et al., 2019; J. Bianchi et al., 2011).

Some rivers in Asia are contaminated or polluted with frameshift-type mutagens and base substitution-type mutagens (Ohe et al., 2004). Few researchers have identified the contaminants which causes mutagenicity in river water such as heavy metals, heterocyclic amines, PAHs, pesticides and more. The chemicals which are released in to the water body are mostly carcinogens grouped as 1, 2A or 2B based on the International Agency of Research in Cancer (IARC) classification system. These chemicals are known to induce mutagenic activity (Ohe et al., 2004).

Chemical analyses are the basic method to assess industrial effluent for potential toxicity, however it has limitations such as it will not provide a complete evaluation of exposure to different types of organic toxins thus will not be able to reflect the joint toxic effects (Lv et al., 2015). Bioassays can be used to assess toxicity of complex mixtures without prior knowledge or information regarding the chemical composition of mixture (De Aragão Umbuzeiro et al., 2004). In order to screen for the mutation effect, Ames test, was started by Bruce Ames aimed to identify the chemical substances or agents that can induce or produce damage to genetics leading to gene mutations (Mortelmans & Zeiger, 2000). *Salmonella* strains which are histidine dependent, each carrying various mutations are used (Mortelmans & Zeiger, 2000). More specifically, strains TA97 and TA98 are used to identification of frameshift mutations while the strains TA100, TA102 and TA1535 are used for base-pair substitutions. Advantages of this test are it is simple to be performed, takes only two days or 48 hours to obtain result, low in cost and is easily transferrable to other laboratories (Zeiger, 2019). Apart from Ames Test, the use of plant bioassays are also considered to evaluate mutagenic risks of complex mixtures, identify presence of environmental mutagens and often adopted in monitoring related studies (Leme & Marin-Morales, 2009; Radi et al., 2010). One such example is the use of *Allium cepa* test to monitor the synergistic effects of mixture of contaminants or pollutants which includes hydrophilic and lipophilic chemicals and heavy metals (Radi et al., 2010). This is a fast and cost-effective procedure, easy to handle, provide reliable and good results and it can also be compared to other tests conducted in mammalian systems (Batista et al., 2016). This test also allows the assessment or evaluation of different genetic endpoints. The genetic classifications that can be evaluated by this test are mitotic index, chromosomal aberrations, nuclear abnormalities and micronucleus (Leme & Marin-Morales, 2009).

Therefore, this study is aimed towards assessing the mutagenic activity in river water which is contaminated with industrial effluent by identifying the source and contaminant causing mutagenic activity in surface water. Inclusion or the incorporation of mutagenicity assay as one of the parameters in river water quality monitoring programme will be assessed along with other water quality parameters.

1.2 Problem statement

Effluents or wastewaters are the by-products of rapid economic growth and industrialization, is detrimental to health of humans and the environment if disposed without adequate treatment (Bhat et al., 2019; Mathur et al., 2005; Sg et al., 2016). The rivers situated in urban area of developing countries are subjected to discharge of the effluents produced by industries (Suthar et al., 2009).

One of the most significant negative impact were related to pollution of environmental and caused by industrial activity was the Minamata disease in Japan. This case was due to methylmercury poisoning which was related with the consumption of contaminated seafoods such as fish and shellfish in a daily routine. The seafoods were contaminated with methylmercury, a by-product in manufacturing acetaldehyde. The chemical was found to be discharged into the sea (Hachiya, 2006).

In Malaysia, many reporting cases for pollution of river were reported due to industrial activities via discharge of effluents into rivers. The current case of pollution in Kim Kim River at Pasir Gudang Johor was due to dumping of chemical waste into the river next to the factory (News Straits Times, 2019). More than 6000 residents were impacted. In addition, the case of illegal and excessive discharge of industrial effluents from a battery factory into waterbodies in Kampung Jenjarom, Selangor was alarming due to the exposure to lead (News Straits Times, 2019). The Simin River in Negeri Sembilan was on the other hand, stirred panic among residents when it turned blue in colour after effluents contaminated with dye was discharged into the river from a nearby factory (News Straits Times, 2018).

Apart from the known effect of water pollution to human health such as infectious diseases like typhoid fever and cholera, poor blood circulation, skin lesions, vomiting and damage to the nervous system (Afroz & Rahman, 2017), several studies have proven that river water which is contaminated with industrial effluent do induce mutagenicity in humans.

1.3 Study justification

Surface water in general including seas, lakes and rivers tend to receive high amounts of effluents from agricultural, domestic activity and also industrial activity. The waters which are contaminated with unknown contaminants are used as drinking water by the public, used for agricultural purposes, recreational activities and also for religious practices globally (Ohe et al., 2004). The chemicals which are released into surface waters are carcinogens with the ranking of 1, 2A and 2B as according to the classification system of IARC. Most of the chemicals are known to have mutagenic activity (Ohe et al., 2004). Known or even unknown compounds of mutagenic becomes the components of the complex environmental mixtures and they exhibit harmful health impacts on humans (Ohe et al., 2004).

Mutation are inheritable changes or error in the genome or cell's genetic material (Clark et al., 2019; Strauss, 2010). This can result in adverse health effects and cause genetic disorders, cancer, development defects and cause aging (H. S. Kim & Lee, 2011; Verheyen, 2017). In another study, DNA damage are shown to alter or change the cell cycle and induces apoptosis and necrosis with proliferation of cell (Glatt, 2006). Mutations may occur through exposure to environmental chemical substances (H. S. Kim & Lee, 2011; Verheyen, 2017). It is also shown that around 70% of mutations which causes changes or alterations in amino acid in a protein are harmful (Verheyen, 2017). Mutations in germ cells causes genetically inherited diseases by the next generation and brought down by sexual reproduction (Clark et al., 2019; Verheyen, 2017). Mutations in somatic cells causes cancer and the mutant cell lines will remain with the organism where mutation occurred (Clark et al., 2019; Verheyen, 2017). Mutations in proto-oncogenes and tumor suppressor genes plays an important role in processes of cancer formation (Verheyen, 2017). Point mutations in proto-oncogenes induces transformation of normal cells into cancer cells while mutation in tumor suppressor gene or also referred as mutational inactivation or deletion have been shown to cause human tumors (Verheyen, 2017). This shows that carcinogenesis is formed through accumulation of genetic alterations (Verheyen, 2017). Mutational events such as proto-oncogenes and inactivation of tumor suppressor genes occurs as result of damage to DNA which is caused by exposure to chemicals (H. S. Kim & Lee, 2011). DNA damage is one of the many cell biological processes which forms the fundamentals of mechanism of carcinogenesis (H. S. Kim & Lee, 2011). The DNA damage or mutation in a carcinogenesis process only takes duration from minutes to days after exposure to chemicals (H. S. Kim & Lee, 2011).

Mutagens are known to cause changes to the deoxyribonucleic acid (DNA) which are not reversible and can be passed down through generations (Schrader, 2015). Reactive chemicals have presence of electrophilic groups which reacts with nucleophilic sites in DNA or proteins. Examples include, alpha, beta unsaturated aldehydes, hydrazines, nitrogen mustard and polyaromatic hydrocarbons (Verheyen, 2017). In another study, it is shown that the integrity of human genome is affected by exogenous chemical which induces covalent binding to the DNA, protein and lipids (H. S. Kim & Lee, 2011). Mutagens may cause mutation in somatic cells which eventually leads to cancer or the formation of malignant cells in human (Bendix, 1974; Phillips, 2018). Organisms inhabiting areas which are affected by discharge of effluents are able to suffer damage to the DNA and public utilizing the polluted or contaminated water are at risk of genotoxic effects and can develop cancer, cardiovascular diseases, teratology and aging (Batista et al., 2016; B. Liu et al., 2016).

This review is important to identify agents which could cause damage to the DNA and also for future public health diseases which are related to DNA damage, caused by irresponsible and illegal disposing of industrial effluents into water bodies. This would also serve as a reference for any remediation actions that should be taken on rivers contaminated with industrial effluents.

1.4 Conceptual framework

Figure 1.1 shows the conceptual framework of this review that is focusing on river pollution due to industrial activities which causes mutagenicity among humans.

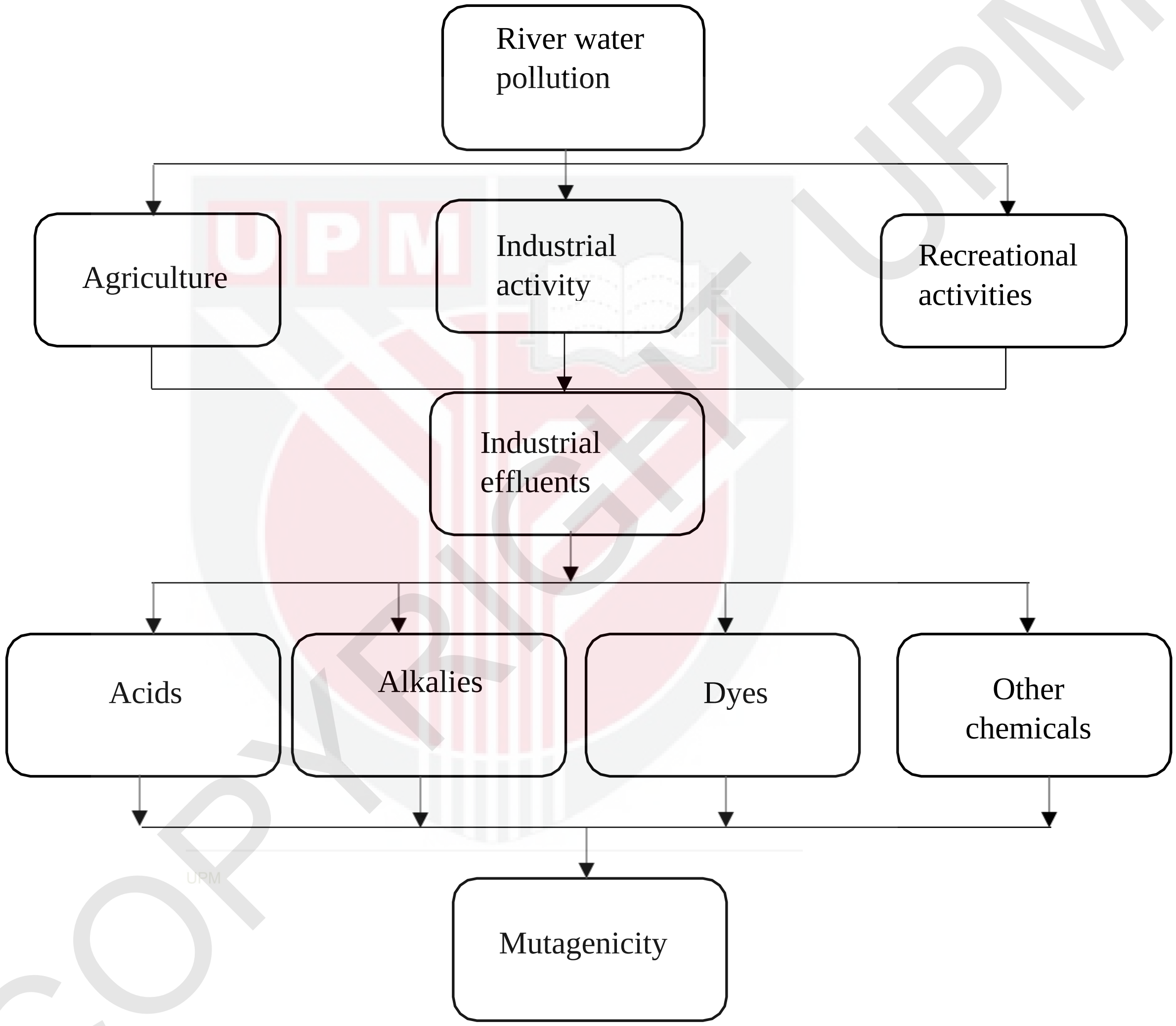

Figure 1.1: Conceptual Framework

1.5 Research objectives

General objective:

To review the mutagenic activity of river water contaminated with industrial effluent

Specific objectives :

1. To identify the source of mutagenic activity in river water samples contaminated with industrial effluent.
2. To assess mutagenic activity as a parameter in routine river water monitoring programme.
3. To assess the current situation in terms of river water pollution and river water monitoring system in Malaysia.

1.6 Hypothesis

There is positive mutagenic activity detected in river water sample contaminated with industrial effluent.

1.7 Definitions of terms

1. Mutagen

Mutagens are compounds of chemicals or radiation forms which causes changes which are not reversible and are heritable to the DNA (Schrader, 2015). They are known to be components of the complex environmental mixtures which can pose adverse health effects to human health (Ohe et al., 2004).

2. Mutagenicity

Mutagenicity is the production of genetic alterations which are transmissible(carcinogenicity). It is the ability of a chemical to induce damage genetically which can occur through few mechanisms which involves interactions with DNA namely adduct formations, base-substitutions, frame-shift deletions and intercalations. Chromosomal aberrations and changes in number of chromosomes are the examples of mechanisms involving both DNA and other cellular targets to induce genetic damages (Gadaleta et al., 2016). Mutagenicity can be tested using the bacterial reverse mutation test or often referred as Ames test which uses bacterial strains in need of amino-acid to detect gene mutations (point and frameshift mutations) (Gutzkow, 2015).

3. Water pollution

Water pollution is the deterioration in quality of water (Halder & Islam, 2015). Source of water pollution can be classified into two which is point source and non-point source. Pollutants originating from point source can be identified easily while non-point source pollutants originate from a combination of several sources (Afroz & Rahman, 2017; Singh & Gupta, 2017).

4. Industrial effluent

Industrial effluent is generated in the process of industrial production along with water loss of intermediate products, byproducts and pollutants (D. Li & Liu, 2019). The type and quantity of effluents or wastewaters generated continues to rise with the increased and rapid development of industry (D. Li & Liu, 2019).

CHAPTER 2

LITERATURE REVIEW

2.1 River Pollution

Malaysian rivers supplies around 97% of water in the country which includes as the source of drinking water, as a resource in industrial, domestic and activities related to recreation as well as waste disposal (Chin & Ng, 2015). Malaysia is blessed with a total of 329, 750 km^2 of land area with around 3000 river basins (Chin & Ng, 2015). With around 324 10^9 m^3 of rainwater annually, shortage of water supply is not a concern for the public. However, factors like increasing population, global climate change and river pollution problems affects water supply in the future. River pollution happens when it is affected badly by the influence of many types of materials or pollutants (Afroz & Rahman, 2017). Human activities in the form of industrial, agricultural and domestic purposes generates pollutants and causes the discharge of effluents into river (Poon et al., 2016). This degrades the river water quality. Disposal of heavy metals originating from commercial areas, markets, food centres and residuals of hydrocarbons from motor vehicles as well as silt loads from construction site gravely affecting urban rivers (Chin & Ng, 2015).

Types of source of river pollution can be classified into two kinds which are point and non-point sources. Point sources refers to clear and identifiable source, for an example effluent discharged from industries or oil spill from a tanker. Non-point sources on the other hand, are the ones which originates from a variety of sources and in a number of ways. For an example, runoff from agricultural fields and abandoned mines, urban wastes and activities which produces contaminants like logging or construction activities (Singh & Gupta, 2017).

Pollutants can be further categorized into two groups which are organic and inorganic. Pollutants which are organic includes insecticides, herbicides, organohalides, bacteria from sewage and livestock farming, food processing wastes, pathogens and volatile organic compounds. Pollutants which are inorganic includes heavy metals, silts from surface run-off, fertilizers such as nitrates and phosphates and chemical waste from industrial effluents (Singh & Gupta, 2017).

2.2 Industrial Wastewaters

Wastewater can be regarded as "used water" or "effluent". According to US EPA, wastewater can be defined as "water that has been used and contains dissolved or suspended waste materials" (Resource, n.d.). Water quality degradation is due to the combination of increase in discharge of untreated sewage, agricultural runoff and inadequately or improperly treated wastewater or effluents from industry. According to statistics, countries with high incomes treat around 70% of their industrial and municipal wastewater while countries with upper middle-income treat around 38% and 28% in countries with lower middle income. Only about 8% of wastewaters originating from industry and municipality are treated in low income countries (Resource, n.d.)

Wastewaters are made up of 99% of water and 1% of suspended, colloidal and dissolved solids. Municipal wastewaters contain broad range of contaminants like pathogenic microorganisms, nutrients, organic matters, heavy metals and emerging pollutants. Urban wastewaters or runoff contains contaminants like polycyclic aromatic hydrocarbons, rubber, heavy metals and suspended particulate matters. Agricultural runoff on the other hand, contains pathogenic microorganisms, nutrients from fertilizer, pesticides and insecticides. For wastewaters originating from industry, the contaminants largely are dependent on types of industry (Resource, n.d.). Industries which requires great amount of water for their manufacturing process are often located along the banks of rivers. These industrial effluents which contains acids, alkalis, dyes and other chemicals are often disposed into rivers (Singh & Gupta, 2017).

Wastewater affects various aspects such as environment, economy and health. The effects on environment includes, decreased biodiversity, degradation in aquatic ecosystem, foul odors, increase in greenhouse emissions and water temperatures and bioaccumulation of toxins. In economic sector, wastewater's impacts can be seen through reduced industrial and agricultural productivity, increase on financial burden due to healthcare and increase in costs of water treatment to name a few. Impacts on health sector can be seen through the increase in burden of disease due to degradation of water quality, decrease in bathing water quality and unsafe food (contaminated food products). A study have reported that effluent or wastewater discharges may cause DNA damage in organisms inhabiting contaminated areas (Batista et al., 2016; Zeyad et al., 2019).

2.3 Environmental Mutagens

Surface water including seas, lakes and rivers are often contaminated with large amounts of waste water or effluents released from various sources such as from industrial activities, agriculture and domestic source. These contaminated waters are used as drinking water, in agricultural activities, recreational related activities and also religious related activities (Ohe et al., 2004). Rivers situated in Europe, South America and Asia are said to be contaminated or polluted with direct and indirect- acting frameshift and base substitution type mutagens (Ohe et al., 2004). Increased partially treated or even untreated discharges or effluents from chemical industries, petrochemical industry, oil refineries are the source of the contamination. According to a study, mutagens are reported to be chemical compounds or forms of radiations like ultraviolet (UV) or X-rays. Mutagens may induce changes which are not reversible and heritable in deoxyribonucleic acid (DNA) (Schrader, 2015).

Mutagenic compounds become components of complex environmental mixtures which pose dangerous threats on human health. Environmental mutagens cause mutation in germ cells which results in increase of heritable abnormal genes in the population. Mutation in somatic cell results in formation of malignant cells in individuals (Bendix, 1974). Mutagens are activated metabolically to active forms by enzymes in mammalian cells. Original mutagens or known as promutagens are converted or changed to proximate mutagens before converted to ultimate mutagens which reacts readily with the DNA (Bendix, 1974). Exposure to mutagenic compounds are able to take place via intake of food and water and through sources in environment and occupations. Direct acting mutagens can interact or interfere with DNA without further modifications and indirect-acting mutagens become mutagenic as the unwanted by-products of transformation which is carried out by cellular detoxification pathways.

2.4 Mutagenicity

Mutagenic lesions become persistent when they are not detected by the DNA repair mechanisms, when any mistakes happen in the repair process or when the repair mechanisms are damaged extensively (Schrader, 2015). Due to subsequent cellular replications, the mutations become fixed on genome and gets inherited by all daughter cells. Through this, mutagenesis stretches over the lifetime of an organism. The effects of mutation largely depend on factors like target loci, size of mutation, period during cell cycle and compounding effects of the pre-existing mutations (Schrader, 2015).

When a mutation occurs in non-functional area of the DNA, there will be no impact and referred as silent mutation. When a mutation occurs in an actively transcribed region it will impact the gene expression and phenotype or lead to cell death, referred as lethal mutation (Schrader, 2015). Mutations interfere or disrupt in regulatory regions or gene coding sequences which results in altered gene expressions and protein function. In multistage development of cancer, mutations that occur in genes which promotes or inhibits growth (proto-oncogenes) and cellular replication (tumor suppressor genes) or code for the components of DNA repair pathways, plays an important role in it. The ageing process and associated degenerative diseases have been related with mutation accumulations over time which leads to less efficient cellular repair capabilities. Breast and colon cancers, cystic fibrosis and Huntington's disease are some of inheritable diseases which is due to mutation in germ cells thus leading to transmission of genetic diseases through generations.

Mutation can be divided to few classes such as extragenic, gene, chromosomal and genomic mutations (Schrader, 2015). Extragenic mutations occur outside of gene coding sequence which are mostly silent. Next gene mutations occur in coding sequence of gene. Point mutation includes base substitution causing transitions and transversions. Frameshift mutations occur when bases are lost or inserted which leads to production or synthesis of modified or inactive proteins and may be lethal to cell. When large gene sections are deleted, inverted or duplicated it is referred as block mutations. This leads to production of defective proteins. Chromosomal mutations involve deletion, translocation, duplication or amplification of whole genes. Genomic mutations includes duplication or loss of entire chromosomes (Schrader, 2015).

2.5 Mutagenicity Testing

Salmonella mutagenicity assay is often used to screen mutagenicity in environmental mixtures like river water. Strains which are often used includes the standard frameshift and base-pair strains, TA98 and TA100 respectively. The metabolically enhanced and highly sensitive TA98 and TA100 strains, YG1024, YG1041 and YG1042 which responds towards aromatic amines, nitro compounds are also used (Ohe et al., 2003). YG1041 (derivative of TA98) and YG1042 (derivative of TA100) are nitroreductase and *O*-acetyltransferase enhanced versions of the strains TA98 and TA100 respectively. The strain YG1024 is an *O*-acetyltransferase-enhanced version of strain TA98 (Ohe et al., 2003). The assay has helped in many key observations to identify presence and the type of mutagens. In a study conducted by White and Rasmussen (1998), the mutagenic activity in St. Lawrence River in Montreal was studied and compared with genotoxicity detected in different rivers in Asia and Europe (De Aragão Umbuzeiro et al., 2004). According to another study, Watanabe et al. (2002) reported that 87% of Japanese river samples analysed was found to be mutagenic (De Aragão Umbuzeiro et al., 2004). In Sao Paulo State, Brazil, Valent et al. (1993) reported that 14% of rivers were positive for mutagenicity especially for strain TA98.

Higher plants like *Allium cepa* (onion) are often utilized as a favourable model to evaluate the toxicity in industrial effluents and sludges and are frequently used in genotoxicity biomonitoring studies to monitor for possible synergistic effects of mixture of pollutants or contaminants which includes heavy metals, and hydrophilic and lipophilic chemicals (Bhat et al., 2019; Leme & Marin-Morales, 2009; Radi et al., 2010). This test is convenient as it detects different genetic endpoints which ranges from point mutations to chromosome aberrations (CA) in the cells of various organs and tissues such as the leaves, roots and pollens. The genetic endpoints includes phytotoxicity (root length and germination index), cytotoxicity (mitotic index), genotoxicity (chromosomal aberrations) and mutagenicity (micronuclei) (Bhat et al., 2019; Leme & Marin-Morales, 2009). This method is also cost and time effective, is sensitive in detecting different environmental pollutants, easy to analyse, have good reliability and reproducibility and has good correlation with other animal and microbial bioassays (Bhat et al., 2019; Leme & Marin-Morales, 2009; Radi et al., 2010). This test can be utilized in a routine monitoring of water pollution as it is a brief and cheap indicator of toxicity. It would provide information on the detection or presence of genotoxic and mutagenic contaminants in river waters through the demonstration of such contaminants to induce the chromosomal aberrations in *A.cepa* root cells (Radi et al., 2010). Among the endpoints assessed in this test are mitotic index, chromosome aberrations, nuclear abnormalities and micronucleus. The mitotic index is identified by total number of dividing cells in the cell cycle and have been used as a parameter to evaluate the cytotoxicity of some agents. Chromosome aberrations are identified by the changes in chromosomal structures or in total number of chromosomes. The changes can be spontaneous and also as a results of exposure to physical or chemical agents. DNA breaks, inhibition of DNA synthesis and the replication of altered DNA are factors which induces structural chromosomal alterations. Nuclear abnormalities are identified by morphological alterations in interphasic nuclei which is a result of action of the tested agents. Micronucleus is the most effective and simplest endpoint to evaluate mutagenic effects caused by chemicals (Leme & Marin-Morales, 2009). The classes of pollutants or contaminants which are sensitive to *A.cepa* test includes metals, pesticides, aromatic hydrocarbons, textile industry dyes, products used to disinfect drinking water, complex mixtures and other agents.

CHAPTER 3

METHODOLOGY

3.1 Search Criteria

This study was done by reviewing published research articles about mutagenicity observed in river waters due to industrial effluents. Many keywords were incorporated to garner many journals. The keywords used includes river pollution around the world and also in Malaysia which was reviewed to determine the cause and effect of river pollution globally. Next, "industrial effluent" was incorporated to gain further information on the types of effluents produced from various sources and the effect it brings to the environment in general. "Mutagenicity" was used to gain more information on what mutagenic activity is, on mutagens, and also the mechanism of mutation or mutagenicity. Next, "toxicity testing" was used to determine types of bioassays used to determine the mutagenic activity. The search engine used is subscribed by Universiti Putra Malaysia through EZaccess of Perpustakaan Sultan Abdul Samad. Science Direct, RSC e-books, PUBMED and NATURE JOURNAL.

3.2 Selection Criteria

Figure 3.1 shows the flowchart of the review process. Based on the keywords such as “river water pollution”, “industrial effluent”, “mutagenicity” and “toxicity testing”, around 196 articles were garnered. Then, from the 88 articles, the selections were narrowed down to the one related to mutagenic activity in river water due to industrial effluents which garnered around 61 articles. Finally, the 61 articles were selected to be used in this review paper based on selected criteria.

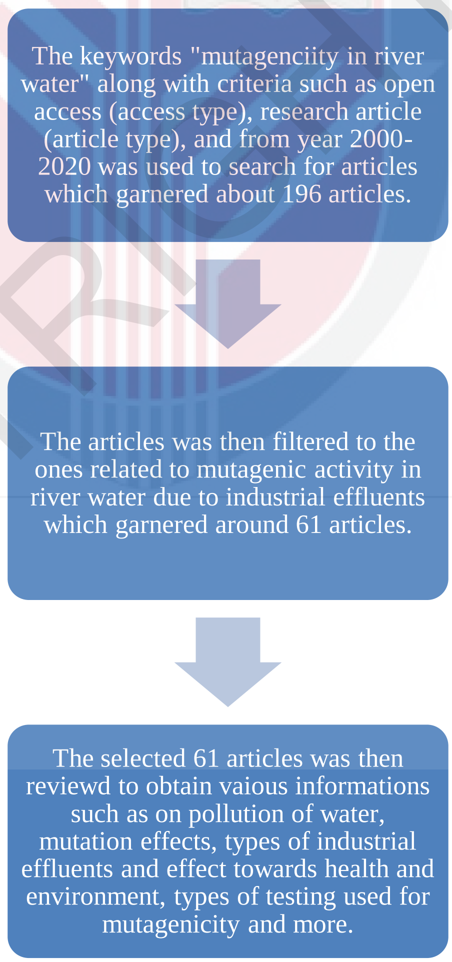

Figure 3.1: The flowchart of the review process.

CHAPTER 4

RESULTS

4.1 Source of mutagenic activity in river water samples contaminated with industrial effluents.

Table 4.1 shows the summary of studies which were conducted to detect presence of mutagenic activity of mutagens in river water which results from the dumping of industrial effluents or wastewaters into the water bodies. Studies on rivers which flows through metropolitan areas in North America, the Cristais River, Monjolinho River and Corrente River in Brazil were reviewed here to identify their source of mutagenic activity, to determine the contaminant or pollutant which induces mutagenicity in the river water.

Table 4.1 : Summary of studies conducted on river water contaminated with industrial effluents.

Place, country (year)	Sample characteristics	Mutagenicity test	Result	Type of industry	Chemical composition	Source
Rivers flowing through USA and Canada (2003)	21 sites on 6 major rivers were chosen to be sampled.	*Salmonella* mutagenicity assay or Ames Test using strains TA98, TA100, YG1024, YG1041 and YG1042 with and without metabolic activation (S9)	Rivers were contaminated with aromatic amines	Chemical manufacturing industry.	Aromatic amine	(Ohe et al., 2003)
Cristais River, Brazil (2004)	The samples includes industrial effluents from Industry A and Industry B, surface water, treated drinking water and sediments collected in Cristais River area.	Ames Test using strains TA98 and TA100 with and without metabolic activation (S9)	Industrial effluents of textile industry	Textile industry effluents	Azo dyes	(De Aragão Umbuzeiro et al., 2004)
Monjolinho River, Brazil (2011)	Five sites along Monjolinho River were chosen to be sampled	*Allium cepa* test.	Presence of heavy metals such as lead(Pb) and nickel(Ni)	• Mining activities, automobile emissions and burning of coal and leaded gasoline, paint, use of pesticides, batteries - Pb	Pb and Ni metal	(J. Bianchi et al., 2011)

				• Electroplating, zinc base casting and battery industries		
Corrente River (Brazil) (2016)	Four sites along Corrente River	*Allium cepa* and Micronuclei Test using fish	Mixture of pollutant from various sources.	Not specified	Mixture of pollutants from domestic sewage source, automotive mechanical wastes and family farming wastes.	(Batista et al., 2016)

4.1.1 Large rivers flowing through USA and Canada

According to (Ohe et al., 2003), several studies on rivers flowing through metropolitan areas that were conducted such as at Rhine River, Yodo River at Japan, Chao Phraya River at Thailand and at Ganges River in India showed significant levels of mutagenicity in surface water. This leads to the aim of this study which is to monitor a broad range of water samples from urban rivers located in North America. Hanging technique of blue rayon was used to collect samples from a total of 21 sites on 6 major rivers namely Providence River, Charles River, Potomac River, St. Lawrence River, Hudson River and East River (Ohe et al., 2003). The collection of samples was conducted during periods without precipitation to avoid the impacts of rain water. The blue rayon (5 grams) were hung in river for a duration of 24hours. They were then taken out, transferred in jars and placed on wet ice to the laboratory for further analysis. The mutagenic activity was assayed by employing standard plate-incorporation assay, which is the *Salmonella* mutagenicity test or Ames Test. The strains used includes TA98 (frameshift strain), TA100(base-pair strain) and the metabolically enhanced strains YG1042(TA98 derivative), YG1041(TA98 derivative) and YG1042(TA100 derivative) with and without metabolic activation (Ohe et al., 2003). The evaluation of mutagenicity employed the “modified two-fold rule” in which positive detection of mutagenicity needs a response of at least two-fold greater than the solvent control with combination of a clear concentration-response relationship.

A summary of the results are as follows. The strains YG1041 and YG1024 are more sensitive compared to the strain TA98 with S9 metabolic activation. 15 out of 21 samples were positive for mutagenicity in strain YG1041 with S9 mix. Strain YG1042 was more sensitive than strain TA100. 8 samples showed positive mutagenic activity in strain YG1042 with S9 metabolic activation. The results suggest that these rivers are contaminated with organic chemical which are able in producing mutations in the strains which are sensitive towards aromatic amine and nitroarene frameshift mutagens with three or more than three fused rings (Ohe et al., 2003).

4.1.2 Cristais River, Brazil

According to (De Aragão Umbuzeiro et al., 2004), Cristais River showed repeated mutagenicity in the routine river water quality-monitoring program in Sao Paulo state. This program employs the *Salmonella* mutagenicity assay as a parameter along with other parameters at sites where water is collected and treated for the purpose of drinking water. This study aims to identify the source of genotoxic contamination detected in Cristais River. The samples include effluents or wastewaters collected from two industries, raw river water, water which is treated and sample of sediment from the Cristais River. As a control site, Guarapiranga River were chosen as the raw river water did not exhibit any mutagenic activity for the mutagenicity testing under the same monitoring program. The liquid-liquid method was used to extract the effluents samples. The river and treated drinking water were extracted with the XAD4 at pH of neutral and acidic and also using blue rayon. The samples were tested with strains TA98, TA100, YG1041 and YG1042 for mutagenic testing (De Aragão Umbuzeiro et al., 2004).

Both raw and treated effluent did not exhibit mutagenic activity for strains TA98 and TA100 with and without S9 for Industry A. Industry B were mutagenic for raw and treated effluents for TA98 with and without S9 and TA 100 with S9. The surface water was mutagenic for TA 98 with and without S9. The drinking water was mutagenic for TA 98 with and without S9 and TA 100 without S9. The sediment was mutagenic for TA98 with S9.The mutagenicity detected in the YG-strains indicates that nitroaromatics or aromatic amines were causing the mutagenicity observed in the samples. Presence of mutagenic polycyclic compounds in water samples were indicated by the positive response for blue rayon extracts. The combination of different types of extraction procedures aids in the detection of the pollutions origins and also helps in the identification of the classes of chemical compounds which contributes to the mutagenic activity in the river. The textile dyeing facility which uses azo-type dyes was the sole identifiable source of mutagenic compounds (De Aragão Umbuzeiro et al., 2004).

4.1.3 Monjolinho River, Brazil

According to (J. Bianchi et al., 2011), the Monjolinho River supplies water to Sao Carlos city while receiving contributions from various streams which is impacted by domestic and industrial sewage. Among substances present in the sewage are oil, dies, metals, starch and pesticides. Sao Carlos on the other hand has economy which is based on industry and is the technological centre in Sao Paulo state. The Monjolinho River receives effluents or wastewaters from city of Sao Carlos which further deteriorates the water quality. The *Allium cepa* test was used in this study to assess the impacts of effluents from domestic activities and industry in Monjolinho River in different seasons of the year. Assay indicators such as cell death, chromosome aberrations and micronuclei in the meristematic cells of *A.cepa* was developed to monitor the water quality (J. Bianchi et al., 2011). Six sites of Monjolinho River were chosen to be sampled. The physical-chemical analysis of water was also determined such as pH, dissolved oxygen, electrical conductivity, and water temperature. Metals and inorganic nutrients in water samples were also evaluated together with rainfall measurement.

As for the result, all the sites showed mutagenicity through chromosomal aberrations except for site 1. The metal analysis revealed that increased values was observed at different sites for lead (Pb), nickel (Ni), copper (Cu), chromium (Cr) and manganese (Mn). The Pb metal can induce mutations and chromosomal aberrations in high plants and mammal cells, and this caused the significant presence of chromosomal aberrations in the analysis of *A.cepa* meristematic cells. Apart from Pb metal, Ni was also said to cause various types of chromosome damage like breaks, DNA gaps, sister chromatid exchange and other alterations. This study says that excessive amounts of Pb, Ni and Cu metals are the main reason for the changes or effects shown in *A.cepa* meristematic cells (J. Bianchi et al., 2011).

4.1.4 Corrente River, Brazil

According to (Batista et al., 2016) the Corrente River, is a major waterway in the district of Pedro II, Piaui, Brazil and often receives wastes from urban, automotive mechanical and family farming activities and is also the source of water supply for the public. The objective of this study was to assess the toxicity and mutagenicity of water samples of Corrente River. The water samples were collected at four sites along the river and during rainy and dry seasons. It is then assessed by analyses of microbiological, inorganic elements and physicochemical. The samples were then assessed for mutagenic activity with the *Allium cepa* test (toxicity, chromosomal abberations and micronucleus test) and fish (*Tilapia rendalli* and *Hoplias malabaricus*).

River water sample exhibited mutagenic effects for both the test, *A. cepa* and fish and toxicity effect in *A.cepa* test. The physicochemical parameter assessed showed increase in conductivity, hardness, chlorides, chemical oxygen demand and decrease in dissolved oxygen value at Site 3. Through the microbiological analysis, the total coliforms were seen at all sites except for Site 4. Faecal coliforms were detected at Site 1 and 2. Increase in concentration of Aluminium (Al), Silicon (Si) and Titanium (Ti) were observed at Site 2 while Chromium (Cr) and Nickel(Ni) were observed on Site 3. Site 1 shows high amount of Al, Si and Ti. Site 1 displayed the contribution of domestic sewage, Site 2 showed contribution of domestic sewage and automotive mechanical, Site 3 showed contribution of domestic sewage, automotive mechanical and family farming and lastly Site 4 has no anthropogenic contribution and was used as a control site. All the samples induced mutagenicity except for Site 4 which was observed through induction of chromosomal aberrations and micronuclei in *A.cepa* test. Apart from *A.cepa* test, mutagenicity of the various contributions at different sites was assessed using the peripheral blood of fish by Micronucleus Test. There was in increase in micronuclei in peripheral blood of both the fish species which was analysed. Site 3 has the highest average micronucleus frequency when compared to other sites. The observed mutagenic effects at all the sites except for Site 4, indicates that the observed effects are due to the complex mixture of pollutants or wastes comprising domestic sewage, automotive mechanical and family farming activities. The contaminants that can be found in domestic sewage are human excreta (pathogenic amicroorganisms), nutrients, organic matter and also emerging pollutants such as pharmaceuticals, drugs and endocrine disruptors. The family farming activities or agricultural wastewaters typically contains pathogenic microorganisms, nutrients from fertilizers, pesticides and insecticides. Since all the three sites show the same contribution of domestic sewage, it can be responsible for the observed mutagenic activity. the automotive mechanical contribution however, was not defined as in the type of activity, whether it is an industry or an anthropogenic activity done nearby the river. Assessing through the presence of heavy metals, Al, Si, Ti, Cr and Ni were found at various sites. The Al metal can be found at aluminium production industry, bauxite mining, can be found in paints and pigments, fertilizers and also textile industry (Goher et al., 2019). The Cr metal can be found in industries such as mining, electroplating, textile and tannery, can be found in paints and pigments and in fertilizers (Goher et al., 2019; Paul, 2017). Ni metal can be found in industries such as electroplating, zinc base casting and also in battery industries (Paul, 2017).

4.2 Mutagenicity assay as a parameter in river water quality programme.

Table 4.2 shows the countries which employed mutagenicity assay in river water to detect for mutagenicity activity.

Table 4.2: Countries which employed mutagenicity assay in river water to detect for mutagenic activity.

Country	Mutagenicity assay used	Mutagenic activity detected	Source
Brazil (2001)	Ames test	Yes	(Umbuzeiro et al., 2001)
China (2015)	Ames test	Yes	(Lv et al., 2015)
Cyprus (2012)	Ames test	No	(Katsonouri & Demetriades, 2012)
Argentina (2008)	Ames test and *Allium cepa* test	No	(Gana et al., 2008)

4.2.1 Significant river pollution cases

Minamata disease is a significant river pollution case as it adversely affected many residents (Hachiya, 2006). It was a methylmercury poisoning case which was related to the consumption of seafoods such as fish and shellfish in a large amount and on a daily basis. The seafoods was heavily contaminated with toxic chemical and this resulted in various clinical symptoms which was associated with level of exposure to the chemical. Hunter-Russel Syndrome, dysarthria, hearing impairment, ocular movement disturbance, tremors, disturbance of equilibrium are the symptoms displayed by severe cases among the residents. A predominant company armed with advanced technologies, Chisso was said to be the cause of the disease. The manufacturing of acetaldehyde which was synthesized through the hydrolysis of acetylene and using mercury as the catalyst generated methylmercury as a byproduct. After the discharge of methylmercury into the water bodies, it accumulated in the seafoods through absorption via gills or digestive tracts (Hachiya, 2006).

Next is the the Itai-Itai disease which was due to the exposure to cadmium (Cd). Cd was produced from human activities associated with industrialization. It was first recognized in 1960s in Japan and was characterized by osteomalacia and severe pain of bone and was associated with renal tubular dysfunction (Nishijo et al., 2017).

4.3 Data on river water quality of Malaysia.

The Department of Environment (DOE) conducted river water quality monitoring programme to determine the status of quality of river water and also to identify and determine any changes in the quality of river water. The monitoring programme is done by collection of water samples in designated regular intervals from designated stations for the in-situ and further laboratory analysis aimed to determine the physicochemical analysis and biological characteristics. Water Quality Index (WQI) is used as the indicator for level of pollution. The National Water Quality Standards for Malaysia (NWQS) is referred to indicate the suitability in terms of water uses. Parameters like Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (NH3- N), Suspended Solids (SS) and pH are incorporated in the WQI (Department of Environment (DOE), 2017).

Figure 4.1 illustrates the river water quality trend from the year 2008 to 2017. A total of 477 rivers were monitored and 219 (46%) of it were evaluated to be clean, 207 (43%) evaluated to be slightly polluted and 51 (11%) evaluated to be polluted. Based on Figure 4.1, it was shown that the number of polluted rivers increases from the year 2008 to year 2017 and the number of clean rivers on the other hand, is seen to be decreasing from the year 2015 to 2017 (Department of Environment (DOE), 2017).

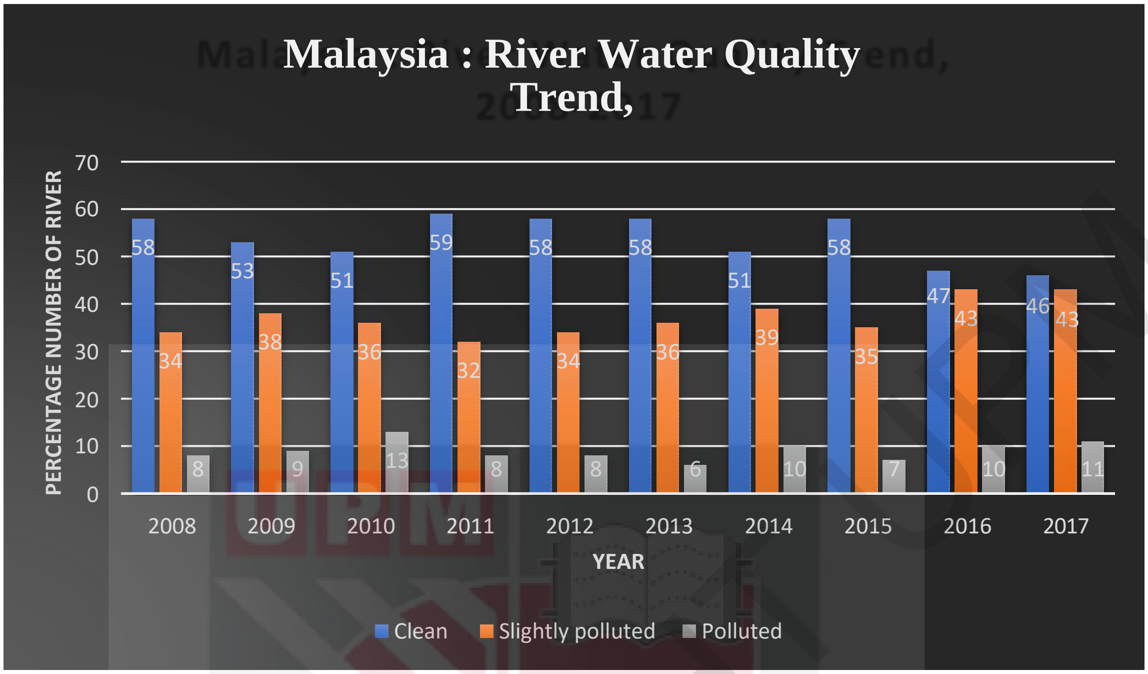

Figure 4.1 : The river water quality trend in Malaysia from year 2008 to 2017. (Department of Environment (DOE), 2017)

4.3.1 Significant river pollution cases in Malaysia

In Malaysia, one of the prominent river pollution case is the pollution in Kim Kim River due to chemical waste contamination. It is a case of illegal dumping or disposing of hazardous chemical wastes into the river which affected more than 2000 people and further prompted the closure of 111 schools. The case of toxic waste dumping into Kim Kim River are said to happen for several years. The residents have been reporting the incident to the authorities however no actions were initiated (Yap et al., 2019).

According to the Malay Mail (2019), the residents in Kampung Jenjarom, Kuala Langat, Selangor are said to be in danger of lead poisoning resulting from the exposure towards illegal disposing of heavy metal in rivers and streams nearby by a factory which has been operating at that place for the past five years. High level of contaminations were found among hair samples of residents and workers (Malay Mail, 2019).

4.3.2 Studies on presence of heavy metals, textile effluents and polycyclic hydrocarbons (PAHs) in Malaysian rivers.

According to a study in Malaysia (Shazili et al., 2006), it was found that sediments in Juru and Langat river are contaminated with Lead(Pb) and Zinc(Zn) while the Langat River was heavily contaminated with Cadmium(Cd). The concentration of Pb and Zn in Juru River in Penang are 117 µg g^{-1} dry wt. and 483 µg g^{-1} dry wt. respectively. The concentration of Zn and Cd in Langat River are 71-374 µg g^{-1} dry wt. and 3.0-37.9 µg g^{-1} dry wt. respectively. Source of metal pollution in environment is the manufacturing sector such as the metal finishing processes. This includes electroplating, etching and also preparation of metal components for different industries which can be identified as the source of wastes which have excessive amounts of Cd, Cu, Zn, Ni, Fe, Al, Mn, Cr and Sn (Shazili et al., 2006).

Another study which was conducted at *batik* factories to describe the effluents produced by the factory. *Batik* industries contribute a lot to Malaysia's economy however, the industry also generates effluents in large amounts with high pollutants concentration which requires treatment before discharging it into the environment. The Kelantan Department of Environment, revealed that the industry has the least compliance with the laws and regulations of the department. The various dyes and pigments used in the industry to impart colour to fabrics often produces coloured effluents. These effluents which contains dyes cause adverse effects to the aquatic biology, as they are a complex molecular structure and difficult to biodegrade. Physical parameters such as temperature, pH, dissolved oxygen, total dissolved solids and chemical oxygen demand were measured in the water samples collected from effluents before it is discharged. The results shows that the average level of temperature was 29.4 °C, the average pH was 8.45, average DO was 2.03 mg/L, average TDS was 5.15 mg/L and average COD was 1911.53 mg/L. The average value of COD was found to be higher than standard limit. The value of COD was found to be influenced by temperature, pH, TDS and DO of the effluent of batik. Effluents with high levels of COD when discharged, leads to depletion of dissolved oxygen, thus creating anaerobic condition. The study shows that the increase in demand for batik products increases the production of effluents thus creating it as one of the main source of pollution in Malaysia (Subki, 2011).

Another study conducted at Kim Kim River and Segget River shows that the concentration of PAH in Kim Kim River was classified as low to moderate while concentration of PAH in Segget River was classified as moderate to the level of pollution. The concentration of PAH in Kim Kim River ranged from 95.17 to 361.24 ng g^{-1} dry wt. while concentration in Segget River ranged from 330.09 to 552.76 ng g^{-1} dry wt. The Kim Kim River and Segget River which runs through the Johor state are often polluted with petroleum hydrocarbon contaminants which is the result of increase in usage of petroleum and its derivatives for energy due to industrialisation, urbanisation, agriculture and aquaculture (K. Kim & Rive, 2017).

CHAPTER 5

DISCUSSION

5.1 Source of mutagenic activity found in river water.

Based on this review, it was shown that the pollutants that might contribute to the mutagenicity in river water includes aromatic amines, discharge of textile industry effluent, heavy metals and a mixture of pollutants from various sources.

5.1.1 Aromatic amines

The incorporation of metabolically enhanced TA 98 and TA 100 derivatives, YG1024, YG1041 and YG1042 have proven to be more helpful in identifying the class of compounds producing the mutagenic activity in the river. These strains produce a more sensitive response to aromatic amines and nitro compounds (Ohe et al., 2003). Apart from the aid of these strains, the extraction method used in this study also helped in the determination of type of mutagens. The use of blue cotton by hanging technique adsorbs mutagens which have polycyclic planar structures (Ohe et al., 2003). The blue rayon which was initially blue cotton, is a copper phthalocyanine dye which is linked to cellulose allows the extraction of specifically planar carcinogens from variety of mediums like cooked meat, urine and faeces of humans and animals and also rivers which are exposed to carcinogens without the need of organic solvents. The dye adsorbs polycyclic compounds with at least three fused rings like heterocyclic amines, polycyclic aromatic hydrocarbons and aflatoxin (Yoxall et al., 2004). Aromatic amines are always regarded as an important class of environmental and industrial chemical and are reported to be carcinogens and mutagens (Benigni & Passerini, 2002) and are always recognized as a class of chemical which is of high concern to health of humans. Chemical industry manufacturing sectors like oil refining, production of synthetic polymers, adhesives and rubbers, pharmaceuticals, pesticides and explosives often apply the usage of aromatic amines. It can also be generated via combustion of organic materials, such as through emission of tobacco smokes (Gadaleta et al., 2016).

5.1.2 Discharge of Textile Industry Effluent

The Cristais River identified the textile dyeing industry as the contributor of the repeated mutagenicity found in the river. According to (Ning et al., 2014), the wastes which are produced or generated by textile industry causes detrimental effects to the environment. This industry releases particulate matter and dust, oxides of nitrogen and sulfur and also volatile organic compounds to the atmosphere causing air pollution (Lellis et al., 2019). The solid waste produced by this industry includes scraps of textile fabrics, yarns and discarded packaging. High loads of organic matters, micronutrients, heavy metal actions and pathogenic microorganisms constitute the textile sludge often produced in surplus volumes and in unwanted compositions (Lellis et al., 2019). The textile industry effluents however are the most damaging ones as they constitute about 80% of the total emissions generated in the industry. High level of biochemical oxygen demand (BOD), and Chemical Oxygen Demand (COD) can be detected in the effluents. BOD is the measure of organic pollution of water that can be biologically degraded while COD is the measure of total amount of oxygen that is required to oxidize organic material into carbon dioxide and water (D. Li & Liu, 2019). The textile industry effluents are difficult to treat as they contain large amount of pollutants like dyes, heavy metals, surfactants, solvents, detergents, recalcitrant compounds, dissolved solids, suspended solids, organic compounds, and have high pH (Mathur et al., 2005; Ning et al., 2014). This industries are said to produce effluents which are genotoxic and have been shown to be potent when compared to other industrial discharges (Mathur et al., 2005).

Several studies conducted have shown that effluents from textile and dye-related industries have mutagenic activity.

Importance should be given to the high amount of non-biodegradable organic compounds, the textile dyes. Dyes are soluble organic compounds especially those in classification of reactive, direct, basic and acids. Its ability to impart or give colour to a substrate is due to the presence of chromophoric groups in its molecular structures. The color in dyes used in textile industries causes aesthetic damages to water bodies and prevents penetration of light through water which eventually leads to reduction in rate of photosynthesis and dissolved oxygen levels this affecting the whole aquatic ecosystem (Lellis et al., 2019). Textile dyes are also toxic, mutagenic and carcinogenic agents, persist in environment as pollutants and provides biomagnification by crossing entire food chains (Lellis et al., 2019). Azo dyes are the group of dyes which are commonly used in the textile industry (Toxicology et al., 2017). Exposure to aromatic amine pose risks to human health especially associated with mutagenic properties or characteristics of aromatic amines (Toxicology et al., 2017). An important and common class of textile dye is azo dyes which releases aromatic amines through biotransformation (Toxicology et al., 2017). According to (Akhtar et al., 2016), exposure to textile effluents poses risks to human health such as mutations, genotoxicity and oxidative damage.

5.1.3 Heavy metals

The major contaminants or pollutants found in water are organic compounds which are volatile, biodegradable and recalcitrant, toxic metals, nutrients of plants, suspended solids, microbial pathogens and also parasites. Toxic metals are in need of attention as they accumulate through food chain and create environmental problems and as heavy metals cannot be degraded through natural processes, they tend to persist in soil and sediment and gradually released into the rivers (Goher et al., 2019; Paul, 2017). Presence of heavy metals in river can be due to natural processes and anthropogenic activities. The natural processes include atmospheric deposition, erosion, leaching, weathering of rocks and hydrodynamic processes (Goher et al., 2019; Paul, 2017). Various industries and domestic sewage contribute to the anthropogenic source of heavy metals in river water and it tends to increase in concentration with the increase of discharge from industries and domestic sewage. The metal industries, paints, pigments, varnishes, pulp and paper, tannery, distillery, rayon, cotton textiles, rubber, thermal power plant, steel plant, galvanization of iron products and mining industries including use of heavy metals in the agricultural practices are the examples of industries contributing heavy metals in river water (Goher et al., 2019; Paul, 2017).

Metals such as Co, Cu, Fe, Ni and Zn are essential in trace amounts for biochemical reactions and as nutrient in aquatic plants, animals and microorganisms (Goher et al., 2019). However, metals like Cd, Hg and Pb do not have any biological functions and are considered toxic. Arsenic, cadmium, chromium, lead, mercury and nickel are environmental pollutants that can cause adverse health effects especially cancer (Koedrith et al., 2013) as International Agency for Research on Cancer classified these metals as human carcinogens.

This study was conducted at different sites in Monjolinho River namely site 1, 2, 3, 4, 5, and 6. Site 1 is situated about 3 km away from the source of Monjolinho River, Site 2 is located at entrance of a Sao Carlos munipality's urban area and Site 3 is located in an urban area. Site 4 is located at the upper part of Monjolinho River, right after it drains the urban area, Site 5 is situated at middle part of river, in a rural area and site 6 is located in a rural area. Through metal analysis which was conducted with samples collected from different sites, high amounts of Pb, Ni, Cu, Cr and Mn were found at those sites. According to (Goher et al., 2019; Paul, 2017), source of Pb is said to be from mining activities, automobile emissions and burning of coal and leaded gasoline, paint, use of pesticides, batteries, municipal sewage, industrial wastes with high amount of Pb. Ni metal is released from natural processes such as volcanic eruptions and anthropogenic activities such as electroplating, zinc base casting and battery industries. Cu metal is generated from the electroplating industry, mining, smelting, refining, pigments and paints, alloys and solders, biocides, fertilizers and fuels and pesticides. Cr metal can be found in mining, electroplating, textile and tannery industries. Mn metal on the other hand can be detected in pigments and paints, alloys and solders, fuel and refineries, biocides, fertilizer, welding and ferromanganese production. High amount (exceeds limit) of Pb, Ni and Cu metal was detected at all sites, Cr was found at high amounts at site 4 and Mn was found to exceed the limits in site 6. Metals such as Pb, Ni and Cu which was found in excessive amounts are responsible for the effects or changes observed in *A.cepa* cells where chromosomal aberrations was detected at sites 2, 3, 4 and 5 while micronuclei was detected at site 2. The *A.cepa* meristematic cells which was exposed to samples with high amount of Pb and Ni levels, significant presence of chromosomal aberrations was detected. According to (J. Bianchi et al., 2011), Pb metal are known to induce mutation and chromosomal aberrations in mammal cells and high plants. Ni metal on the other hand, are known to cause various chromosome damages such as breaks, DNA gaps, sister chromatid exchange and other alterations or changes (J. Bianchi et al., 2011). Micronuclei which was observed at Site 2 also showed excessive amounts of Pb metal only. Thus, the mutagenicity detected at Site 2 can be related to the presence of Pb metal.

5.1.4 Mixture of pollutants from various sources

The Corrente River which is contaminated with various anthropogenic influence such as from urban, automotive mechanical and family farm waste. It is also the main water source for the population. All the Sites showed mutagenic effects in both *A.cepa* test and fish except for Site 4. Site 1 shows contribution of domestic sewage while Site 2 shows contribution of both domestic sewage and automotive mechanical. Site 3 shows contribution of domestic sewage, automotive mechanical and family farming while Site 4 has no anthropogenic contributions.

According to (Resource, n.d.), the typical contaminants or components in domestic wastewaters includes human excreta (pathogenic microorganisms), nutrients and organic matter. This wastewater might also contain emerging pollutants such as pharmaceuticals, drugs and endocrine disruptors. Mutagenic impurities are chemical reagents, starting materials, reaction intermediates, or by-products of reaction that can be introduced during pharmaceutical manufacturing processes. The mutagenic impurities exhibit potential to directly cause the DNA damage thus leading to mutations and causing cancer (X. Liu et al., 2018). Pharmaceutical products or compounds includes a diverse group of chemicals with their metabolites and transformation products (H. K. Khan et al., 2020). They persist in the environment through their use and disposal practices. The family farming activities or agricultural wastewater typically contains pathogenic microorganisms, nutrients from fertilizers, pesticides and insecticides used.

5.2 Mutagenicity as one of the parameters in river water quality monitoring programme.

The important river water quality parameter that is used worldwide are temperature, pH, dissolved solids, suspended solids, turbidity, dissolved oxygen, compounds of phosphorus and nitrogen, Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) (Gupta et al., 2017). The acceptable range of pH should be within 6.5 and 8.5 as according to standard such as World Health Organization(WHO). A pH of less than 6.5 causes synthesis of vitamins and minerals in human body to discontinue. A pH of more than 8.5, interferes the taste of water, making it saltier and furthermore a pH of more than 11 results in eye irritation and skin disorder. Dissolved Oxygen indicates the changes or alterations in biological parameters which can occur due to aerobic and anaerobic pathways. The

Biochemical Oxygen Demand (BOD) is used to determine the requirement of oxygen to stabilize the household and industrial wastes. By determining the BOD, effluents from domestic and industrial sources disposed or discharged into surface water can be assessed. The Total Dissolved Solids (TDS), is used to measure the amount of solid materials dissolved in surface water. Increased values can result in harmful effects to public health such as in the central nervous system, induces paralysis of the tongue, lips and face and causes irritability and dizziness. Nitrate- Nitrogen is contributed by agricultural activities which increases the nitrate concentration in surface water. High amounts of it can result in decrease in level of oxygen in water which in turn effects the aquatic life. Blue baby syndrome which occurs due to reaction between nitrite and iron in red blood cell which creates methaemoglobin which stops oxygen level. Turbidity in an increased amount will eventually cause pathogenic organisms to be harmful to humans and interfere in penetration of light and thus damage aquatic life and decreases surface water quality. Use of fertilizer in an increased amount is the source of phosphate and high levels of it causes damage of muscle, breathing problem and kidney failure. Increased levels in river causes eutrophication and depletion of dissolved oxygen concentrations (Gupta et al., 2017).

Different anthropogenic activities produce a complex mixture of substances which contains heavy metals, pesticides and contaminants present in domestic sewage. These are known to cause toxic effects such as genotoxic and mutagenic effects to organisms exposed to the mixture of pollutants and also affects the future generation since it is heritable (E. Bianchi et al., 2019; Leme & Marin-Morales, 2009). In order to identify the compounds which reacts with the DNA, genotoxic and mutagenicity assays are developed.

A study which was done at Sali River in Tucuman, Argentina with the aim of screening the surface waters of the river which is situated in industrial effluents or wastewaters discharge areas. This is to identify or detect the water quality problems by incorporating the short-term bioassays (Ames test). It is also to determine whether the water bodies have chemicals which can react with the DNA. Chemical analyses are also conducted routinely according to standard methods for dissolved oxygen, suspended solids and chemical oxygen demand. The results of this study showed that the surface waters did not exhibit any mutagenic activity in the Ames test, however micronucleus formation and the anaphase aberrations was observed in all the samples. This study shows that incorporation of mutagenicity assays to test surface waters and also effluents emanating from industries is beneficial in demonstrating that the pollutant mixtures contains many toxicants which are not identified and unregulated and which have adverse effects such as risk for carcinogenicity for human health. This study also suggests that additional assays should be implemented in all water quality monitoring programme to evaluate the presence of compounds which are genotoxic in environmental samples (Gana et al., 2008).

In another study at China, benefits of in vitro tests includes ability to evaluate biological effects of a mixture of pollutants, like acute and chronic toxic effects, mutagenicity and estrogenicity. The study which employs two in-vitro biological tests, the mutagenicity test and estrogenicity test showed varying mutagenic effects of the water bodies, lake water, river and treated drinking water. It was also mentioned that the use of these in-vitro tests were beneficial to comprehensively evaluate water quality (Lv et al., 2015).

A study which was conducted at Cyprus demonstrates the importance of implementing mutagenicity test on holistic monitoring of water quality. The holistic approach includes chemical, ecotoxicological and microbiological testings. The findings demonstrates that mutagenicity testing should be included in monitoring programmes to sustain and improve water quality in an area (Katsonouri & Demetriades, 2012).

One example of a water quality monitoring programme which incorporates the Ames Test as a parameter is the Environmental Agency of Sao Paulo State in Brazil. The Environmental Agency of Sao Paulo in Brazil have been utilizing the *Salmonella* mutagenicity assay since the year 1979 to assess the quality of surface water. To aid in the dissemination of information to the public, classifications such as low, moderate, high and extreme was proposed. The assay was then officially included in the Sao Paulo State Water Quality Monitoring Programme in 1998. An example of case where mutagenicity was detected repeatedly at several sites which prompted the need to implement remediation actions. The potential source was investigated in which a industrial discharge exhibiting high mutagenic level was identified as the source. Thus the industry was notified and enhancement and improvement of the quality of the treatment used for effluents were demanded. This was done to reduce or eliminate the mutagenicity found. The industry was also reminded to incorporate pollution prevention actions. The Water Treatment Plant was also warned about the crisis to prevent them from distributing the contaminated waters (Umbuzeiro et al., 2001).

The significant river pollution cases which occurred in Japan, the Minamata disease and Itai-Itai disease shows the importance to monitor the river water quality routinely by including the mutagenicity assays in the programme. This would ensure that preventive measures to minimize the adverse effect to health. Apart from that, source of the effluents discharged into the river can be identified and actions can be taken to minimize or treat the effluents properly before discharging into water bodies.

The advantages of using bioassay to test for mutagenicity such as Ames Test and *Allium cepa* test are as follows. The Ames Test is a reverse mutation assay which uses histidine dependent strains of *Salmonella typhimurium.* The strains each have different types of mutations in histidine operon and are modified to be more sensitive towards chemical mutagens. It is a required assay under the OECD guidelines for the genetic toxicology testing (Mortelmans, 2019). It was found that the test is simple to be performed, time and cost effective and can be transferred to other laboratories easily. The test is also effective in identifying carcinogenic chemicals which leads to prompt adoption and a requirement by regulatory authorities worldwide (Zeiger, 2019). The *Allium cepa* test are frequently used in monitoring studies as it is sensitive in identifying mutagens in various environmental mediums, can asses several genetic endpoints ranging from point mutations to chromosomal aberrations shown or observed in various cells of organs and tissues belonging to leaves, roots and pollen (Leme & Marin-Morales, 2009). This test is also shown to be short-term and cost-effective indicator of the toxicity in routine monitoring of water pollution. The substances present in surface water that could induce chromosomal aberrations in *Allium cepa* root cells are shown to be genotoxic or mutagenic substances (Radi et al., 2010).

Other types of mutagenicity testing includes Mammalian cell gene mutation test, Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, Mammalian chromosome aberration test, Mammalian micronucleus test, Knowledge or rule based Structure Activity Relationship (SAR) and the Quantitative or statistically based Structure-Activity Relationship (QSAR) (Verheyen, 2017).

5.3 Current situation in Malaysia.

Based on the trend of quality of river water in Malaysia, the number of rivers which are polluted is increasing from 2015 to 2017. According to DOE, the BOD, ammonial nitrogen and suspended solids remained dominant in terms of river pollution (Department of Environment (DOE), 2017). High value of BOD is an indicator of inadequate or improper treatment of sewage or effluents emanating from agricultural activities and manufacturing industries (Department of Environment (DOE), 2017). According to (Singh & Gupta, 2017), BOD is used to determine requirement of oxygen to stabilize the household and industrial wastes and effluents from industrial sources can be assessed.

Textile industry are said to employ more than 8000 various chemicals in many types of textile manufacturing processes and is a complicated industrial sector (Tounsadi et al., 2020). The textile industry effluents contains many toxic components and have a large quantity of chemicals such as acids, bases, salts, dispersants and many more (S. Khan et al., 2019). Effluents from textile industries are shown to exhibit mutagenic activity (Mathur et al., 2005).

PAHs have been identified to have mutagenic characteristics (Aziz et al., 2014; J. Li et al., 2010; Ning et al., 2014). The exposure towards PAHs through air, water and food items such as fish contaminated with PAH negatively affects human health (Aziz et al., 2014). The United States Environmental Protection Agency listed PAHs as priority pollutants (J. Li et al., 2010). These studies shows that Malaysian river waters needs to be assessed for the presence of chemical agents that can induce mutagenicity leading to DNA damage.

The river water pollution in Malaysia such as the Kim Kim River and Kampung Jenjarom River pollution indicates the importance of monitoring activities in continuous manner of river waters in Malaysia with the inclusion of mutagenicity assay in a routine monitoring programme. Apart from this, the presence of lead in the river in Kampung Jenjarom shows the need for mutagenicity testing in the water bodies as Lead is shown to induce mutagenicity (J. Bianchi et al., 2011).

CHAPTER 6

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

The sources of mutagenic activity in river water which was contaminated with industrial effluent were found to be aromatic amines, azo dyes, heavy metals such as Lead (Pb) and Nickel (Ni) and mixture of various source of pollutants such as from domestic wastes, automotive mechanical wastes and family farming activities wastes. The assays such as Ames test and *Allium cepa* test could be included in routine river water quality monitoring programmes.

6.2 Limitations

The limitations of this study includes the use of only a few keywords which limits the scope of study. The main keywords used in this review are “river water pollution”, “industrial effluent”, “mutagenicity” and “toxicity testing”. Apart from it, this review also focuses only on mutagenicity which is the DNA damage among human health than including more genetic effects brought upon by exposure towards river contaminated with industrial effluents.

6.3 Recommendations

The number of keywords should be increased to broaden the scope of search to a bigger scale. This would allow more topics to be encompassed and addressed in the review. A more comprehensive review on types of toxicity testing should be included to compare and assess each of the different toxicity testings adopted by different countries.

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