INTRODUCTION

1.0. Introduction

Despite an increased awareness by governments and general public of the need to protect all types of aquatic habitats, human impacts continue to impair the services that these ecosystems provide. There is a growing global concern over the increasing contamination of our aquatic environments from municipal, industrial, urban and agricultural sources (Munawar *et al*., 1989). Increased monitoring activities that focus on all major biological compartments are needed to quantify the present conditions of the Earth's aquatic resources and to evaluate the effectiveness of regulations designed to rehabilitate damaged ecosystems.

Increased awareness of the potential environmental hazards of nutrient loading in lakes and streams has generated a need for reliable laboratory techniques for water quality assessment (Cairn *et al*., 1979). As a result, existing procedures are being examined for their accuracy and relevance to current situations and new techniques are being developed so that changes in water quality can be rapidly detected. Bioassays, because of their relative simplicity, low cost and accuracy, have received a great deal of attention of late as potential diagnostic tools for water quality analysis. In addition to these advantages, bioassays provide a means, through direct observation of a living organism's reactions, of determining effects of pollutants on one or more aquatic life forms (Cairn *et al*., 1979).

Algae are an ecologically important group in most aquatic ecosystems but often ignored as indicators of aquatic ecosystem change (McCormick *et al.*, 1994). Because of their nutritional needs and their position at the base of aquatic foodwebs, algal indicators provide relatively unique information concerning ecosystem condition compared with commonly used animal indicators. It has long been recognised that algae are sensitive indicators of water quality and that any change in their population may provide as much information about water chemistry as the chemical determination themselves can (Rana, 1995). Bioassays involving algae have been used to determine the amount of algal growth a body of water can potentially support, as well as existence of toxicity of the water to algae (Cairn *et al.*, 1979). Such assays generally involve growth of a selected algal strain (or strains), in a water sample to be tested, under controlled laboratory conditions of light and temperature. Final biomass achieved over a specified time span, or growth rate of the test algae, is generally taken as an indication of the algal growth potential of the sample in question. Interest has been expressed in developing an algal assay which will serve as a standard procedure for water quality assessment (Cairn *et al.*, 1979).

Wastewater generated from agriculture and domestic sources contains high concentrations of organic matter, nitrogen and phosphorus, and causes eutrophication in receiving waters. Many algal species, especially *Chlorella*, are rather tolerant to organic pollution and will rapidly colonise any milieu rich in nitrogen, phosphorus and organic compounds. Microalgae have traditionally been used as a tertiary treatment process to remove inorganic nitrogen and phosphorus from wastewater after its organic matter (Biological Oxygen Demand and Chemical Oxygen Demand) has been reduced by conventional secondary treatments (Lavoie and de la Noue, 1985; Martin *et al.,* 1985; Oswald, 1988).

1.1. Environmental pollution

There is no doubt what causes chemical pollution: human eagerness to perform better and better with respect to production of food, energy and convenience products in order to ameliorate the way of living. This eagerness led to a tremendous growth in production of chemicals. For example, in the last two to three decades the production of pesticides in India alone increased more than 40 fold, whereas the production of dyes (30 fold), drugs (5-10 fold), petrochemicals (40 fold), fertilizers (30 fold) and metals (3 fold) increased considerably as well (Sampathkumar, 1977). It is evident that such increased production also leads to the release of toxic and hazardous wastes into the environment in liquid, solid or gaseous form. This cosmopolitan problem called environmental pollution is only recently recognised as a real problem and causes environmentalists headaches as to what approach is to be taken to conquer this phenomenon.

1.1.1. Water pollution

Comprising over 70% of the Earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. Without the seemingly invaluable compound comprised of hydrogen and oxygen, life on Earth would be non-existent: it is essential for everything on our planet to grow and prosper. Although we as humans recognise this fact, we disregard it by polluting our rivers, lakes, and oceans. Subsequently, we are slowly but surely harming our planet to the point where organisms are dying at a very alarming rate. In addition to innocent organisms dying off, our drinking water has become greatly affected, as is our ability to use water for recreational purposes. In order to combat water pollution, we must understand the problems and become part of the solution. According to the American College Dictionary, pollution is defined as: "to make foul or unclean; dirty." Water pollution occurs when a body of water is adversely affected due to the addition of large amounts of materials to the water. When it is unfit for its intended use, water is considered polluted. Two types of water pollutants exist; point source and nonpoint source. Point sources of pollution occur when harmful substances are emitted directly into a body of water. The Exxon Valdez oil spill best illustrates point source water pollution.

A nonpoint source delivers pollutants indirectly through environmental changes. An example of this type of water pollution is when fertilizer from a field is carried into a stream. Many causes of pollution including sewage and fertilizers containing nutrients such as nitrates and phosphates. In excess levels, nutrients over stimulate the growth of aquatic plants and algae. Excessive growth of these types of organisms consequently clogs our waterways, uses up dissolved oxygen as they decompose, and block light to deeper waters.

This, in turn, proves very harmful to aquatic organisms as it affects the respiration ability or fish and other invertebrates that reside in water.

1.1.2. The state of Malaysian freshwater environment

According to the Malaysian Environmental Quality Report (DOE, 2001), there are a total of 13 polluted rivers from 120 rivers monitored (increased from 12 in 1996), 47 slightly polluted (increased from 74 in 2000), and 60 clean rivers (increased from 34 in 2000) based on the Water Quality Index (WQI). Sources of pollution range from agro-based industries, manufacturing, livestock farming, domestic waste, earthworks and land clearing activities. A total of 93 rivers were polluted in terms of ammoniacal-nitrogen due to livestock farming and domestic waste, which constitute 80% of the polluted rivers. The increase in nitrogen concentration is considered to be amongst the main factor promoting eutrophication (James *et al.,* 1979).

1.2. Nitrogen

Nitrogen is a major constituent of the earth's atmosphere and occurs in many different forms such as elemental nitrogen, nitrate and ammonia. Natural reactions of atmospheric forms of nitrogen with rainwater result in the formation of nitrate and ammonium ions. While nitrate is a common nitrogenous compound due to natural processes of the nitrogen cycle, anthropogenic sources have greatly increased the nitrate concentration, particularly in groundwater. The largest anthropogenic sources are septic tanks, application of nitrogen rich fertilizers to turf grass, and agricultural processes. The increase in nitrate concentration in water resulting largely from the use of nitrogenous fertilizer is one of the most important problems in environmental protection (Jeanfils *et al.,* 1993). This contamination is now a worldwide problem, especially with respect to the quality of drinking water. Nitrate *per se* is not toxic, but it is the precursor of nitrite that can induce the methaemoglobinaemia in children (Terblanche, 1991).

1.2.1. Phosphorus

Phosphorus is a mineral nutrient which is essential for all forms of life. It plays a major role in energy transfer processes in the cells of living organisms. Phosphorus occurs naturally at low concentrations in surface waters and is an essential part of the food chain. Under these conditions it is derived from processes such as the weathering of rocks and the decomposition of leaf litter or other organic matter. Phosphorus concentrations vary considerably under natural conditions, depending on factors such as local geology, soil types and seasonal conditions. Concentrations can be expected to be much higher when wet weather conditions generate runoff and stream flows are high. It is important to consider stream flow rates and recent weather conditions when interpreting phosphorus results.

1.3. Eutrophication

Industrialised countries generate a great volume of urban and industrial wastewater. These effluents should not be dumped directly into rivers, lakes or the sea before treatment to reduce contaminants to environmentally safe levels (Martinez *et al.*, 1999). Special attention is required for inorganic substances which encourage vegetal growth, such as ammonium, nitrates and phosphates, contributing to the eutrophication of the bodies of water receiving the effluents. Eutrophication is generally accepted as the principal cause of the globally observed deterioration in marine coastal environments (Schramm, 1999).

In coastal areas, which are the centres of population and industrialisation and the primary recipients of nutrients from land, the typical symptoms of eutrophication can be observed. Among these, changes in marine plant communities and in particular the mass development or blooms of micro- or macroalgae are probably the most conspicuous effect of increased nutrient loads.

Eutrophication typically leads to increased growth in aquatic plants and algae (Moss, 1996). Algal blooms can significantly reduce the quality of waters used for domestic purposes. In addition, some algae produce toxins that can kill fish, mammals, and birds (Moss, 1996).

1.4. Tolerance of tropical microalgae to nitrogen enrichment

Nitrogen and phosphorus enrichment is one key factor that causes eutrophication. In view of this, the Algal Biotechnology Research Group from the University of Malaya has started a series of research to screen for microalgae that have potential use as biomonitors for nitrogen and phosphorus enrichment in freshwater ecosystems. For example, the tolerance of four tropical microalgae to nitrogen enrichment and their potential use for biomonitoring were investigated in an earlier study conducted by Foo *et al.* (2001). In the previous study, nitrogen levels ranging from 50 to 250 mM were tested in growth experiments lasting for 8 days.

The present study was an expansion from the previous one, using the same three chlorophytes (*Chlorella vulgaris* UMACC 001, *Scenedesmus quadricauda* UMACC 041 and *Ankistrodesmus convolutus* UMACC 101) grown at a lower range of nitrogen (0.03 to 18.75 mM) and phosphorus (0.02 to 12.5 mM) levels for a shorter growth period (96 hours). In the present study, the nitrogen sources used were nitrate ($NaNO_3$) a

xnd ammonium (NH_4Cl).

1.5. Objectives

The objectives of the present study are as follows:

1. To study the growth response of selected tropical microalgae to nitrogen and phosphorus enrichment in laboratory conditions.

2. To select suitable species for use in bioassay protocols for nitrogen and phosphorus enrichment in laboratory conditions.

The approaches used to achieve the above objectives are outlined in Figure 1.

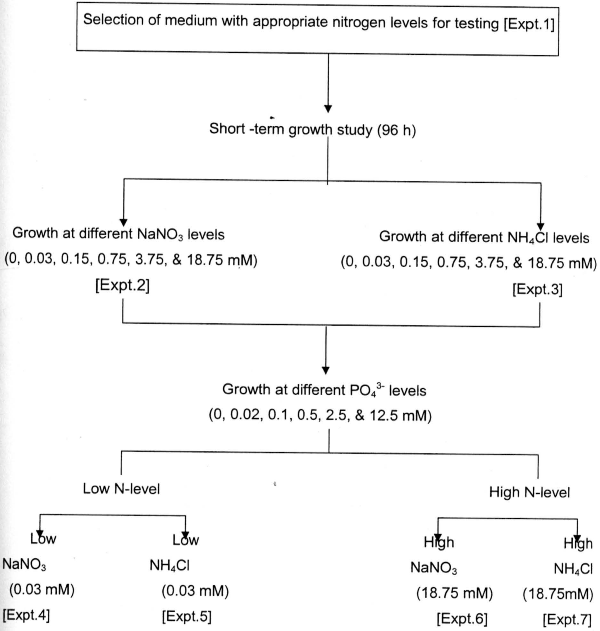

Figure 1: Outline of the study. All the experiments were conducted in laboratory conditions.