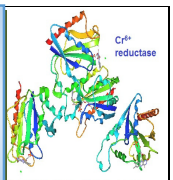
HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



Rapid Ecotoxicological Tests Using Bioassay Systems - A Review

Halmi, M.I.E.

Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Mohd. Izuan Effendi Halmi,
Department of Land Management,
Faculty of Agriculture,
Universiti Putra Malaysia,
43400 Serdang,
Selangor, Malaysia.
Email: m_izuaneffendi@upm.edu.my/zuanfendi88@gmail.com

HISTORY

Received: 16th March 2016
Received in revised form: 2nd of April 2016
Accepted: 5th of May 2016

KEYWORDS

Ecotoxicological Tests
pollution
metal
bioassay
bacteria

ABSTRACT

The rise in pollution cases globally is expected to increase in line with industrialization. Monitoring activities for pollutants have been hampered by the astronomical costs of instrumental-based approach. This has resulted in the intense research on low cost biomonitoring systems using enzymes, organisms including microorganisms. Only positive samples are sent for instrumental analysis; dramatically cutting the cost of instrumental analysis. This review attempts to outline and give due recognition to several selected bioassay systems that have been tested for their applicability using polluted water samples as a routine first line-of-defense. This includes small aquatic organisms-based assays, enzymes especially proteases and bacterial-based systems using respiratory dye or luminescence systems as a method for toxicant detection.

INTRODUCTION

A survey by the Malaysian Department of Environment in 2010 shows that water pollution has grown to a dangerous level. The presence of toxicants such as heavy metals, pesticides, organic and inorganic solvents generated from industrial and agricultural activities is becoming worse due to Malaysia striving to become an industrial country (DOE, 2010). Toxicants can cause potentially harmful effects to human beings, aquatic organisms and food webs because some of them cannot be degraded [1]. Many of them are carcinogenic or mutagenic. Hence, there is a need for a simple and fast procedure to screen for the presence of toxic substances from industrial effluents, polluted rivers and other polluted locations [2].

Toxic compounds can be detected by the use of instruments such as Atomic Absorption Spectrophotometer (AAS), Inductive Coupled Plasma (ICP), Flow Injection Mercury System (FIMS), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). These instruments are widely used for environmental monitoring but present many limitations and hurdles such as high cost, time consuming and logistics. Bioassays using bioluminescent bacteria show many advantages compared to other conventional methods and are suitable for preliminary screening of toxicants in the environment. The use of this screening tool has the advantage of indicating the real impact of all chemicals present in a given sample or ecosystems. The top recommended bioassay system by United States Environmental Protection Agency USEPA is bioassay using bioluminescent bacteria as this system is sensitive to many toxicants while being rapid and simple to operate (USEPA, 2004). Bioluminescence is an amazing phenomenon where living organisms have the special ability to emit light in nature. The metabolic process involved in bioluminescence requires several enzymes such as luciferase (EC number: 1.13.12.7) with luciferin as substrate [3]. This bioassay works on the principle that bioluminescence is reduced in the presence of certain toxicants. The inhibition of luminescence occurs when one or more enzymes involved in this reaction are inhibited after reacting with toxicants. The detection of toxicants using this bacterium is faster compared to other bioassays such as using *Daphnia magna*, rainbow trout and other microbial assays because the test result can be obtained in less than 30 minutes.

An example of a commercially produced bioassay using bioluminescent bacteria is the Microtox® system. This system uses the bacterium *Vibrio fischeri* which has an optimal assay temperature of 15°C [4]. Bioassay using *V. fischeri* has an intrinsic disadvantage due to the requirement of an exact assaying temperature of 15°C with a deviation of a few degrees

Celsius from the optimal dramatically affecting luminescence. The exact assaying temperature requires a refrigerated water bath making the assay not suitable for field applications and making the system more expensive and instrument dependent.

Global water pollution

Water quality issues are a major challenge facing humanity in the twenty first century. The rapid industrialization of developing countries, though contributing to economic development, has resulted in heavy losses to economic welfare in terms of effects on agricultures activities, human health and ecosystem at large through water pollution. Water pollution poses a serious challenge due to its impact on a large number of economic activities [5].

Water pollution is caused by a number of organic and inorganic compounds such as pesticides, heavy metals, sodium dedocyl sulfate (SDS) and phenol which are harmful to humans, plants, animals and aquatic environments [6]. The presence of these toxicants in the water basin is a result of industrial activities. They are toxic, carcinogenic, mutagenic and persistent because they have the ability to accumulate in the food chain, ultimately in fish. Toxicants can easily enter the human body by many pathways and can do harm to human health if consumed every day or at high concentrations [7].

The most infamous case related to water pollution is the mercury poisoning in Minamata, Japan in 1956. This tragedy was due to the long term effects of mercury-polluted wastes dumped into the Minamata bay [8]. Many residents developed symptoms of methyl mercury poisoning such as numbness in their limbs and lips, shaking in their arms and legs and eventually some suffer brain damage [9]. More recently, China is confronting some of the most serious environmental pollution issues. This country has undergone exceptionally rapid development over recent years leading to a dramatic rise in pollution and environmental damage. The harbor area of the coastal region in Xiamen [10] and Pearl River [11] have been found to be contaminated by a number of metals and organic pollutions as a result of industrialization that has been occurring in the delta of the river.

India also experiences the same problems faced by China. The problem has led to the deterioration of water quality in India. Rapid industrial growth has given rise to waste discharge into certain rivers which were responsible for the degradation of estuarine and coastal waters [12]. The discharges of partially treated and untreated wastes from the urban and rural area in India is a major source of pollution of coastal waters [13].

Water pollution in Malaysia

Environmental pollution is one of the major problems as Malaysia is shifting to a developed industrial country. Rapid industrialization has caused detrimental effects due to the increase in the variety of hazardous wastes generated from many industrial outlets located near polluted locations [14]. According to the Department of Environment, Malaysia, (DOE, 2002) the sources contributing to this problem include agriculture, agro-based industries, urbanization activities, solid waste disposal, industrial wastes, urbanization activities, deforestation and shipping activities.

In 2004, the major exports of Malaysia were electrical and electronic machinery, petroleum and natural gas, chemical products, palm oil and textiles [15]. As a result, many Malaysian rivers have polluted due to untreated wastes being channeled by irresponsible factories into the rivers. The river quality index for the rivers in Malaysia showed an increasing number of class 4 and 5 (increasing pollution) rivers while rivers for class 1 and class 2 (decreasing pollution from class 5 to class 1) are decreasing as a result of rapid industrial activities [16]. The lack of awareness of the Malaysian people on the importance of rivers contributes to severe water pollution problems.

A survey in 2010 by the Malaysian Department of Environment showed that out of 1,055 monitoring stations, 527 (50%) were found to be clean, 417 (40%) slightly polluted and 111 (10%) polluted. The number of clean rivers decreased from 306 rivers to 293, slightly polluted rivers decreased from 217 to 203 while the number of polluted rivers increased to 74 from 54. DOE has identified the sources contributing to river pollution in 2010, which comprise of manufacturing industries (44.57%), sewage treatment plants (49.27% inclusive of 790 Network Pump Stations), animal farms (70%) and agro-based industries (2.46%) [16]. The wastes products generated from these activities were found to be dumped into drain outlets from factories and these wastes flow into the rivers or sea. These disposal activities caused adverse effects to rivers in Malaysia with the Juru River being the most notorious. The Juru River located in Penang has been classified by Department of Environment (DOE) and World Health organization (WHO) as the dirtiest river in south East Asia. According to the report, the water is toxic and unsafe for drinking even after being boiled. This river index remains in class 5 over several years indicating that this river is much polluted and little is done to remediate the river [17]. The sediment collected from this river contains dangerous heavy metals such as mercury, copper, lead and zinc [6,18,19]. These contaminants originated from the Prai Industrial Estate that was first established in the early 1970s ([20]. Other polluted rivers in Malaysia include Sungai Dondang and Jejawi (Penang), Kempas, Tukang Batu, Rembah, Sungai Benut and Sungai Pasir Gudang (Johor), Sungai Kelang, Sungai Buluh and Sungai Sepang (Selangor), Sungai Batu (Perak), Sungai Miri (Sarawak) and Sungai Jimah (Negeri Sembilan). Elsewhere, water analyses done by the Department of Environment on Sungai Samit, Sungai Tuaran, Sungai Sedili Besar, Sungai Kempas in Sabah showed the presence of high levels of heavy metals such as cadmium, silver and zinc [16].

Organic pollution from the industrial sector also contributes towards pollution in the form of xenobiotic pollutants such as phenolic compounds, oil and grease [16]. Since Malaysia is an oil and gas producer , oil pollution could not be avoided especially since Malaysia partially owns the Straits of Malacca which is the busiest waterway in the world. Contamination occurs mostly because of human errors. For instance, two oil tankers collided with each other in the coastal areas of the Straits of Malacca spilling almost 150 tons of diesel making the case to be one of the largest hydrocarbon spills ever reported [21].

The Department of Environment has reported that 1,709 metric tonnes of phenol and phenolic wastes were generated in 2005. The National Guidelines for Raw Drinking Water Quality had stated that the maximum permissible limit for phenolic compounds in drinking water is 0.002 mg/L, and many groundwater wells in Malaysia have phenolic levels exceeding this limit, thus indicating a widespread pollution due to phenol and phenolic compounds [22]. The widespread and the high intensity of organic and non-organic pollutions in Malaysia make monitoring an important agenda.

Toxicity of toxicants

Toxicity is the ability of substances to cause harmful effects towards living organisms. A chemical is considered toxic when the amount required to give harmful effect is relatively small. Otherwise, the chemical can be considered as non toxic when a large amount is needed to give harmful effects [23].The toxicants can affect a single cell, a group of cells, an organ system, or the entire body. Toxicants can be found in many forms such as heavy metals, pesticides, organic and inorganic chemicals. Many of the toxic substances enter the body through the skin, eyes, throat and nose. The internal organs most commonly affected by toxicant are the liver, kidney, heart, nervous system and reproductive systems. The toxicity effects toward living organism depend on the amount ingested, inhaled or absorbed [24].

Toxicity can be studied by measuring the effect of toxicants on targets such as the organism, tissue and cell. Toxicity studies can be divided in acute, sub-chronic and chronic toxicities. Acute toxicity is referred to as the effects of toxicants in a short term period [25]. Acute toxicity studies are defined in several terms such as the median lethal dose; LD_{50} (dose of substances that kills 50% of the test animals), IC_{50} (concentration of the toxic compound which kills 50% of the test animal in a given time) and EC_{50} (concentration of toxicants or drug which induces a response halfway between baseline and maximum after specific exposure time) [26]. Sub-chronic toxicity is referred to the effect of toxicants to target organisms after more than one year but less than the lifespan of the organisms being studied. They will be observed for changes occurring such as food consumption and weight. Chronic toxicity is the ability of the toxicants to give certain effects towards living organisms over a long period of time and usually at levels far below that of acute toxicity.

Heavy metals

Heavy metals are elements that exhibit metallic properties with higher molecular weights. They include transition metals, metalloids, lanthanides and actinides. Heavy metals have a density five times greater than water. They exist naturally in the ecosystem in many forms. There are more than 20 types of heavy metals, but only four are classified as extremely dangerous to human life which are mercury, lead, cadmium and arsenic (As). These four heavy metals are toxic at very small concentrations [27].

The growth and metabolism of living organisms can be effected by the excessive amount of heavy metals because they have the ability to interfere with biochemical functions of enzymes. Heavy metals will react with enzymes and inhibit sulfhydryl (SH) enzyme systems [28]. A disruption of enzymatic activity can seriously affect the function of organs or tissues. Some of these metals such as copper, zinc and iron are essential to human life and play important roles in human metabolism. Other heavy metals can be considered as xenobiotics because they have no significant role in human physiology such as mercury and lead. Heavy metals can enter the human body through a variety of routes. They can be inhaled as tiny particulates or ingested through food or drink. These metals tend to be deposited in bones, liver and kidney for many years and they are capable of causing cancer, hypertension and renal toxicity [29]. Furthermore, when the dosage taken is high, people develop symptoms such as headaches and weakness. Heavy metals have also been identified as a factor affecting human reproduction systems [30]. Exposures to different heavy metals produce cellular impairments at structural and functional levels in male and female reproduction system. Heavy metals could interfere with the production of sperms in the testis, thus reducing male fertility [31]. Babies can suffer from the effect of heavy metals such as having low weight, heart defects, brain damage, growth retardation and others because heavy metals can be transferred from mother to baby through the placenta and breast milk [6,32].

Xenobiotics

Xenobiotics ("xeno" is Greek for foreign) are man-made chemicals that are present in the environment and they pollute the environment if they are present at high concentrations. The existence of these xenobiotics can cause many problems towards the environment as well as human beings [33]. Xenobiotics can be non-xenobiotics to certain organisms but it can be a xenobiotic to another organism. For example, *Acinetobacter* sp. strain AQ5NOL was able to tolerate and use phenol as a sole carbon sole at concentration more than 1500 mg/L but at this concentration, it can inhibit and effect the growth of other bacteria [34].

Xenobiotics can be divided into two categories which are biodegradable and non- degradable xenobiotics. Biodegradable xenobiotics mean that it can be degraded by the reaction of microorganism and other related reactions. Meanwhile, non-degradable xenobiotics cannot be degraded by biological reactions in the environment. Examples of xenobiotics include hydrocarbons, pesticides, synthetic polymers, synthetic solvents and alkyl benzyls [35]. Mammals have special metabolisms to prevent xenobiotics from giving harmful effects via xenobiotics transformation systems by a series of enzyme reactions before they are being eliminated through urine [36].

Pesticides

Pesticides are mixtures of substances being used for preventing and destroying pests. From 2006 to 2007, more than one billon kilograms of insecticides were sold to the agricultural sector world-wide [37]. Many of these pesticides eventually enter into the aquatic environments through rivers, the atmosphere, agricultural run-offs and industrial point sources. They are designed to control pests but they can also be very toxic to other organisms such as plants and animals including humans. Pesticide pollution can occur through the discharge of pesticides from manufacturing plants, accidental spills and natural processes such as dilution, surface run offs and leaching [37–41]. Sometimes the by-product of pesticides can be more toxic than its parent forms or be more persistent in the environment [42].

Pesticides can be divided into families such as organochlorines, organophosphates and carbamates. Examples of organochlorines include dichlorophenyltrichloroethane (DTT) and cyclodiene compounds. Organophosphates and carbamates largely replaced organochlorines throughout the years. Both of them react by inhibiting acetylcholinesterase. Examples of carbamates that are widely applied in agriculture include carbaryl (Sevin) and carbofuran (Furadan). Examples of organophosphates include parathion (Baladan M) and malathion (Celthion) [43–46].

Monitoring of toxicants in the environment

The rapid development of industrial and agriculture activities contributed to increasing environmental pollution in this world. Since toxicants are dangerous to the environment and living organism, the presence of these toxicants should be monitored. In order to detect them in the environment, scientists have developed numerous methods to monitor a variety of toxicants. Monitoring methods can be divided into two different types: conventional and bioindicator/bioassay methods [19,47].

Conventional detection of toxicants

Rapid and continuous detection of environment contaminant in waterways is important for protecting the natural environments and public health and for the management of treatment systems. Standard analytical methods using AAS, HPLC and GC have been traditionally applied for analyzing contaminants. These methods are very selective and sensitive because of the ability to detect the toxicants at very low concentrations. However, these methods suffer from many disadvantages because the sample water may contain thousands of different chemicals. So, chemical analysis will be very expensive if every single chemical needs to be analysed [48]. Moreover, most of the conventional methods require a long time for analysis, requires a skilled technician and not very user friendly. Therefore, fast and simple methods with high accuracy to detect toxicants in the environment may provide a better alternative [6].

Atomic Absorption Spectrometry and Induced Coupled Plasma

Atomic absorption spectrometry (AAS) and Inductive coupled plasma-optical emission spectrometry (ICP-OES) are the most widely used instruments to measure heavy metals. These instruments can detect heavy metals to parts per trillion. They require skilled technicians, are expensive to purchase and maintain and not amenable to field work analysis [32].

High performance liquid chromatography (HPLC)

High performance liquid chromatographic is a chromatographic technique used to separate compounds. HPLC is used in biochemistry to identify, quantify and purify individual components from a mixture. It uses different types of stationary phases and consists of a pump that moves the mobile phase and analyte through the column. The detector will detect and provide information of retention time for the analyte. HPLC is applied for detection of certain type of compounds such as pesticides, dyes, bioactive compounds and organic chemicals [49].

Gas Chromatography (GC)

Gas chromatography is an instrument that can separate and quantify volatile and hydrophobic compounds. In gas chromatography, the mobile phase is a carrier gas. The carrier gas is an inert gas such as helium or unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on inert solid support [50].

Bioindicators / bioassay of toxicants

Biological indicators or bioindicators can be defined as changes that occur at the organism, population or assemblage level when it has been exposed to toxicants [51]. Organisms in a polluted environment accumulate certain pollutants in its tissue that reflects the level of pollution when the organisms are subjected to chemical analysis. Bioindicators tell us about the effects of different toxicants in the ecosystems and about how long a problem has been present [52]. Organisms such as crustaceans, fish and microorganisms are monitored for changes in terms of biochemical, physiological and behavioral patterns that may indicate problems within their ecosystems.

A bioassay can be defined as the use of living organisms or their products such as enzymes or antibodies to detect toxicants. Bioassays have been widely used in ecotoxicology studies, especially in the detection of hazardous substances such as heavy metals, pesticides and others [53]. Bioassays are inexpensive, rapid and sensitive to a wide range of toxicants [54]. Examples of bioassays successful applied in ecotoxicology studies are those that use *D. magna,* rainbow trout, Microtox® based on *V. fishcheri* and Xenoassays™ based on the proteases [6,55–62].

D. magna

D. magna is a standard organism used for toxicity testing. They are among the most favourable test animals in aquatic toxicology [63]. Daphnia is easy to culture because it only needs water that contains bacteria. They have a very short reproductive life cycle, giving birth to a new young within the first week of life. The disadvantages of this method are that the required exposure time with toxicants is more than 24 hours compared to less than 30 minutes for microbial assays using bioluminescent bacteria and inhibitive enzyme assays [63–65,65].

Rainbow trout

Rainbow trout is a species of salmon native to tributaries of the Pacific Ocean in Asia and North America. They survive in water from 3°C in the winter until 21°C in the summer, but the optimum temperature range is from 10 to 16°C. Rainbow trout is used as a bioassay in many studies because it is sensitive to heavy metals [66]. However, this method requires a rearing system and facilities and the assay temperature is not suitable for tropical countries like Malaysia.

Inhibitive enzyme assay using proteases for heavy metals detection

Inhibitive enzyme assay using proteases have been developed using casein-coomassie-dye-binding as the color development reagent. Proteases such as papain [6], bromelain [32], trypsin [67] and many others [57,58,61,68] have been used to detect heavy metals in the environment. These proteases are temperature stable, pH and solvent tolerant and suitable to be applied in field works on polluted sites in tropical countries. The assays collectively are sensitive to mercury (Hg^{2+}), silver (Ag^{2+}), lead (Pb^{2}), zinc (Zn^{2+}), copper (Cu^{2+}) and cadmium (Cd^{2+}) at the sub parts per million level. The bioassay has been trademarked as Xenoassay™ [19,47]. Emerging new bioassays are cholinesterases. These enzymes including acetylcholinesterase and butyrylcholinesterase have been shown to be sensitive to heavy metals and have been utilized for monitoring of heavy metals in several selected water-polluted rivers and aquatic bodies [27,69–75].

Microbial bioindicators/bioassay

Microbial bioindicators/bioassays provide a simpler, rapid and less expensive method compared to the conventional method using ICP-OES, HPLC and others [76]. Bioassays using bacteria have been commercialized such as the MTT assay, the Polytox™ and the Microtox™ assays. These commercialized bioassays are designed to detect a broad spectrum of toxic inorganic and organic pollutants.

MTT assay

The assay is dependent upon the capacity of the bacterium *R. meliloti* in reducing the tetrazolium dye MTT (**Fig.** 1) and the inhibition of this process by many toxic chemicals [48].

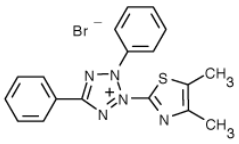


Figure 1. Structure of MTT.

The reduced MTT exhibits and increase in absorbance at 550 nm if the dye is reduced. The color of the respective MTT-formazan derivative is purple-blue. This reduction can be followed with a simple spectrophotometer. MTT has a chemical formula is $C_{18}H_{16}BrN_5S$, and a molecular weight of 414.32. The disadvantages of the assay are it is time consuming and takes almost 4 hours to be completed and since the mixture has to be incubated at 37°C, the assay cannot be used to detect volatile toxic compounds [48]. Studies have suggested that MTT is reduced by the cytochromes and especially NAD(P)H reductase [77–79]. The mechanism of inhibition is thought to involve the electron transport system residing in the cytoplasmic membrane of bacteria. As hydrophobic toxic chemicals can permeate and disrupts bacterial cellular membrane, this affects cytochrome activity.

The production of NADH is inhibited in the presence of xenobiotics and heavy metals causing a reduction in respiratory activity measured by the MTT assay. Two nontoxic cations; magnesium and calcium inhibited MTT reduction in the original Botsford's MTT assay. As these two ions are commonly found in the environment, the chelators EGTA (ethylene glycol-bis-(β-aminoethyl ether) N,N,N'N'-tetraacetic acid) and EDTA (ethylenediamine tetraacetic acid) had to be added to prevent this problem. The inclusion of these chelators decreases cell viability and reduce the toxicity of heavy metals ad these two chelators also bind to toxic metals. In order to exploit the advantages of the MTT assay for heavy metals without the inherent weakness of inhibition by non-toxic ions, newer bacterial-based MTT assays system have been recently developed that are not inhibited by these ions [80–82].

Polytox®

Polytox® is a blend of bacterial strains originally isolated from wastewater. The principle of this assay kit is based on the reduction of the respiratory activity of the dehydrated cultures in the presence of toxicants such as heavy metals. It is specifically designed to assess the effect of toxic chemicals on biological waste treatment. However, Polytox® is expensive and is difficult to maintain. It also needs a sophisticated computer program to analyse data and a highly trained technical personnel to run the tests [83].

Microtox®

The Microtox® bioassay system is originally developed by Beckman Instruments Inc. The bioassay's strongest attribute is its convenience as a primary screening test for a wide spectrum of toxicants. The Microtox® procedure can be used for testing either water (marine or fresh) or sediments [84].

Microtox® is based on bioluminescent bacteria *V. fischeri* which have an optimum temperature of 15°C for assay and maintenance [85]. It is the most recommended bioassay system by the United States Environmental Protection Agency (USEPA) and hence the most reported bioassay method for toxicants [85–89]. This assay is still relatively expensive due to the instrumentations needed to run the test and to maintain 15°C as temperatures fluctuations of more than 1°C has been reported to affect strongly on the IC_{50} of toxicants [48]. Hence, Microtox® is not suitable to be used in real time field works due to the precise temperature maintenance requirement. Recently, SDIX inc., the company that produces Microtox® has developed a field work-friendly system called Delta Tox® using a *V. fischeri* strain that allows ambient temperature testing of toxicants [90]. Delta Tox® is unpopular due its lower sensitivity in detecting heavy metals compared to Microtox® and is rarely reported in the literature. Thus, there is a need for the development of bioluminescent bacteria that has a broad range luminescence temperature stability as well as high sensitivity towards toxicants.

Bioluminescence inhibition assay

A bioluminescent inhibition assay is often chosen as the first screening method in a test battery experiment compared to other bioassays. This is based on speed and cost considerations. In addition, the assay protocol is usually simple and fast because the result can be obtained within 15 minutes [87]. *V. fischeri* is the most used luminescent strain due to its sensitivity towards a wide range of toxicants compared to other bacterial assays and have good correlation with the other toxicity bioassays such as enzyme inhibition, nitrification inhibition, algae, and so on. This bacterium is widely used for toxicity assessment of various environmental samples in the form of a commercial luminescence assay, Microtox® [91]. The assay temperature of this bacterium must be maintained strictly at 15°C and a few degree deviation from the optimal dramatically affect results. In order to maintain the temperature, the use of an expensive refrigeration unit is required which complicates its use on site. Due to this limitation, many more luminescent bacteria have been isolated and include *Vibrio fischeri* (DSMZ 7151/ NRRLB-11177) [92], *Photobacterium leiognathi* [93], *Vibrio harveyi* strain 525 [94], *Vibrio fischeri* strain 4172 [95], *Photobacterium phosphoreum* MT10204[96], *Vibrio harveyi* strain 525 [95], *Photobacterium* sp. Lub-1 [97], *Pseudomonas fluorescens* strain Shk1 [98], *Vibrio logei* [99], *Pseudomonas fluorescens* ATCC-13525 [100], *Photobacterium phosphoreum* strain 496 [101], *Photobacterium* sp. strain MIE [19] and*Vibrio* sp. isolate MZ [102].

Bioassay using bioluminescent bacteria works based on the reduction of luminescence in the presence of toxic compounds. The reduction of luminescence occurs when the luciferase enzyme and several related enzymes (e.g. electron transport chain) are inhibited which interferes with the metabolic processes of bioluminescent bacteria [103]. The inhibition of luminescence depends on the concentration of the toxicants that exist in the samples. If the polluted samples contain high concentrations of toxicants, the bioluminescent bacteria will lose all of their ability to produce luminescence because most of the enzymes involved in luminescence are inhibited. The effect of toxicants can be determined within 30 minutes or less depending on the type of toxicants because prokaryotic bioluminescent bacteria have short incubation times and have faster metabolic activity compared to eukaryotic cells.

According to [104], bioluminescent species can be found in most of the major marine phyla from bacteria to fish. For instance, comb jellies have the highest proportion of bioluminescent species. On the other hand, diatoms and arrow

worms have none or a small number of luminescent representatives. For example, microscopic plankton known as dynoflagellates increase in population when red tide occurs during the summer months in Southern California. These dynoflagellates glow in the dark when disturbed by currents or waves, resulting in a brilliant light show of turquoise glowing waves. This occurs as they break along the shoreline at night. During this red tide phenomenon, the dynoflagellates decrease the levels of oxygen and light in the water. Consequently, the organisms such as kelp, plankton, and fish die as the changing environment is poor and unhealthy for them to live in. This matter will cause the organic matter to decay and provide a suitable environment for bacteria, especially for luminescent bacteria to grow [104].

Bioluminescence and the mechanisms involved

Bioluminescent bacteria constitute a heterogeneous group of microorganisms, mainly representing the family Vibrionacea and they have the ability to emit light in the marine environment [105]. Luminous bacteria are present in the sea as free-living organisms, as saprophytes, and as symbionts in light organs of certain marine organisms. The biochemistry of the light reaction associated with luminous bacteria has been extensively studied, and many reviews on the topic have been published [3,106]). Bioluminescent bacteria are widely distributed in marine, freshwater and terrestrial environments. All of them are Gram negative bacteria [107]. The species usually used for bioluminescence inhibition assay includes *V. fischeri, P. phosphoreum, Vibrio harveyi and Pseudomonas fluorescens* [108].

Luciferase enzyme is responsible for the production of bioluminescence. A suite of genes dubbed “lux” genes code for the enzyme and other components of the luminescent system. Bacterial luciferase is a heterodimer composed of two different polypeptides, called alpha and beta with molecular masses of 40 kDa and 37 kDa, respectively. Both polypeptides are encoded by the luxA and lux B genes. [109]. Luciferase catalyzes the oxidation of a long-chain aliphatic aldehyde and reduced flavin mononucleotide ($FMNH_2$) with the reduction of molecular oxygen and the liberation of excess free energy in the form of a blue-green light at 490 nm:

$$FMNH_2 + RCHO + O_2 \text{ ----> } FMN + RCOOH + H_2O + \text{light (490 nm)}$$

In most luminescent bacteria, cell density can prompt luminescence. This condition is called quorum sensing and a key factor is the number of bacteria that are present. At low cell densities of less than 100 living bacteria per milliliter, luminescent bacteria do not produce luminescence, whereas luminescence is induced at high cell densities of above 1000 living bacteria per milliliter [110].

V. fischeri

V. fischeri is a gram negative rod shaped bacterium. This bacterium was isolated from the marine environment. It is free living or living in symbiosis with other marine organisms such as in the bobtail squid and some species of fish. This bacterium has the ability to produce bioluminescence and is motile by means of flagella [109].

***Photobacterium* sp.**

Photobacterium sp. is a gram negative bacterium living symbiotically with other marine organisms. This bacterium emits bluish-green light through bioluminescence reaction. *P. phosphoreum* is psychro tolerant which means it can tolerate low temperatures, typically from 4°C to 35°C [111–113].

Application of bioluminescent bacterial assay

Bioluminescence bacterial assay can be used for toxicity measurement for almost all kinds of samples such as organic and inorganic compounds. This assay has been applied as a screening tool for toxicity determination of effluents, water samples from a polluted river, dye wastewater and effluent from paper mill [114]. A study done by [115] showed that this type of assay has been successfully applied on sediments from a lagoon although an extraction method was required in order to apply this method. However, the system is limited for field works due to instrumental mobility limitations. Hence, there is a need for a bacterial strain that has broad optimal range for activity and at the same time highly sensitive for toxicants is needed.

CONCLUSION

Rapid ecotoxicological tests using bioassay systems is fast becoming the method of choice for large scale monitoring of toxicants in the environment. Microorganisms and their biological product such as enzyme have been successfully utilized to monitor toxicants. The simplicity, rapidity, near real time capability and low-skilled required to operate and use bioassay systems are clearly an advantage over the sole used of instrumental methods *per se*. More tests are needed to ensure the reproducibility of bioassay systems. The most excellent mode of use of the existing bioassay systems is an integration to current instrumental approach with bioassay systems becoming a first screening method or the first line of defense against toxicants in the environment. Only positive samples are sent for instrumental validation making the whole exercise a much cheaper method.

REFERENCES

1. Rieger PG, Meier HM, Gerle M, Vogt U, Groth T, Knackmuss HJ. Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence. J Biotechnol. 2002;94(1):101–123.
2. Isenberg DL, Bulich A. Environmental monitoring: use of luminescent bacteria. Chem Saf. 1994;211–226.
3. Cormier MJ, Totter JR. Bioluminescence. Enzymic aspects. Photophysiology. 1968;4:315–53.
4. Warne M, Boyd E, Meharg A, Osborn D, Killham K, Lindon J, et al. Quantitative structure-toxicity relationships for halobenzenes in two species of bioluminescent bacteria, Pseudomonas fluorescens and Vibrio fischeri, using an atom-centered semi-empirical molecular-orbital based model. SAR QSAR Environ Res. 1999;10(1):17–38.
5. Reddy VR, Behera B. Impact of water pollution on rural communities: An economic analysis. Ecol Econ. 2006;58(3):520–37.
6. Shukor Y, Baharom NA, Rahman FA, Abdullah MP, Shamaan NA, Syed MA. Development of a heavy metals enzymatic-based assay using papain. Anal Chim Acta. 2006;566(2):283–9.
7. Zheng L, Liu G, Chou CL. The distribution, occurrence and environmental effect of mercury in Chinese coals. Sci Total Environ. 2007;384(1–3):374–83.
8. Harada M. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology. 1978;18(2):285–8.
9. Takeuchi T, Morikawa N, Matsumoto H, Shiraishi Y. A pathological study of Minamata disease in Japan. Acta Neuropathol (Berl). 1962;2(1):40–57.
10. Klumpp DW, Humphrey C, Huasheng H, Tao F. Toxic contaminants and their biological effects in coastal waters of Xiamen, China.:: II. Biomarkers and embryo malformation rates as indicators of pollution stress in fish. Mar Pollut Bull. 2002;44(8):761–9.

11. Shi J, Ip C, Zhang G, Jiang G, Li X. Mercury profiles in sediments of the Pearl River Estuary and the surrounding coastal area of South China. Environ Pollut. 2010;158(5):1974–9.
12. Verlecar XN, Desai SR, Sarkar A, Dalal SG. Biological indicators in relation to coastal pollution along Karnataka coast, India. Water Res. 2006;40(17):3304–12.
13. Clark A, Turner T, Dorothy KP, Goutham J, Kalavati C, Rajanna B. Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. Ecotoxicol Environ Saf. 2003;56(3):390–7.
14. Abdullah AR. Environmental pollution in Malaysia: trends and prospects. TrAC Trends Anal Chem. 1995;14(5):191–8.
15. Ibrahim M. Persistent Organic Pollutants in Malaysia. Dev Environ Sci. 2007;7:629–55.
16. DOE. Malaysia Environmental Quality Report 2014. Department of Environment, Ministry of Natural Resources and Environment, Malaysia; 2015.
17. Al-Shami SA, Md Rawi CS, Ahmad AH, Abdul Hamid S, Mohd Nor SA. Influence of agricultural, industrial, and anthropogenic stresses on the distribution and diversity of macroinvertebrates in Juru River Basin, Penang, Malaysia. Ecotoxicol Environ Saf. 2011;74(5):1195–202.
18. Lim PE, Kiu MY. Determination and speciation of heavy metals in sediments of the Juru River, Penang, Malaysia. Environ Monit Assess. 1995;35(2):85–95.
19. Halmi MIE, Jirangon H, Johari WLW, Abdul Rachman AR, Shukor MY, Syed MA. Comparison of Microtox and Xenoassay light as a near real time river monitoring assay for heavy metals. Sci World J. 2014;2014.
20. Al-Shami SA, Rawi CS., HassanAhmad A, Nor SA. Distribution of Chironomidae (Insecta: Diptera) in polluted rivers of the Juru River Basin, Penang, Malaysia. J Environ Sci. 2010;22(11):1718–27.
21. Ku Ahamad KE, Halmi MIE, Shukor MY, Wasoh MH, Abdul Rachman AR, Sabullah MK, et al. Characterization of a diesel-degrading strain isolated from a local hydrocarbon-contaminated site. J Environ Bioremediation Toxicol. 2013;1(1):1–8.
22. AbdEl-Mongy MA, Shukor MS, Hussein S, Ling APK, Shamaan NA, Shukor MY. Isolation and characterization of a molybdenum-reducing, phenol- and catechol-degrading *Pseudomonas putid*a strain amr-12 in soils from Egypt. Sci Study Res Chem Chem Eng Biotechnol Food Ind. 2015;16(4):353–69.
23. Duffus JHWHG., Worth HG. Toxicology and the environment: An IUPAC teaching program for chemists. Pure Appl Chem. 2006;78(11):2043–50.
24. Silva AL., Barrocas PR., Jacob SC, Moreira JC. Dietary intake and health effects of selected toxic elements. Braz J Plant Physiol. 2005;17(1):79–93.
25. Burton Jr GA. Assessing the toxicity of freshwater sediments. Environ Toxicol Chem. 1991;10(12):1585–627.
26. Freshney I. Application of cell cultures to toxicology. Cell Biol Toxicol. 2001;17(4):213–30.
27. Aidil MS, Sabullah MK, Halmi MIE, Sulaiman R, Shukor MS, Shukor MY, et al. Assay for heavy metals using an inhibitive assay based on the acetylcholinesterase from *Pangasius hypophthalmus* (Sauvage, 1878). Fresenius Environ Bull. 2013;22(12):3572–6.
28. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. Int J Phys Sci. 2007;2(5):112–8.
29. Tripathi RM, Raghunath R, Krishnamoorthy TM. Dietary intake of heavy metals in Bombay city, India. Sci Total Environ. 1997;208(3):149–59.
30. Gerhard I, Monga B, Waldbrenner A, Runnebaum B. Heavy metals and fertility. J Toxicol Environ Health A. 1998;54:593–612.
31. Chowdhury AR. Recent Advances in Heavy Metals Induced Effect on Male Reproductive Function—A Retrospective. Al Ameen J Med Sci. 2009;2(2):37–42.
32. Shukor MY, Masdor N, Baharom NA, Jamal JA, Abdullah MP., Shamaan NA, et al. An inhibitive determination method for heavy metals using bromelain, a cysteine protease. Appl Biochem Biotechnol. 2008;144(3):283–91.
33. Parkinson A. Biotransformation of xenobiotics. McGraw-Hill New York; 2001.
34. Ahmad SA, Shamaan NA, Arif NM, Koon GB, Shukor MYA, Syed MA. Enhanced phenol degradation by immobilized *Acinetobacter* sp. strain AQ5NOL 1. World J Microbiol Biotechnol. 2012;28(1):347–52.
35. Mehrotra S, SANDHIR R, Chandra D. Degradation of Xenobiotics and Bioremediation. Environ Microbiol Biotechnol. 2004;59.
36. Varanasi U, Stein JE. Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ Health Perspect. 1991;90:93.
37. Abhilash PC, Singh N. Pesticide use and application: An Indian scenario. J Hazard Mater. 2009;165(1–3):1–12.
38. Begum G. Assessment of biochemical markers of carbofuran toxicity and recovery response in tissues of the freshwater teleost, *Clarias batrachus* (Linn). Bull Environ Contam Toxicol. 2008;81(5):480–4.
39. Begum G. Enzymes as biomarkers of cypermethrin toxicity: Response of *Clarias batrachus* tissues ATPase and glycogen phosphorylase as a function of exposure and recovery at sublethal level. Toxicol Mech Methods. 2009;19(1):29–39.
40. Tham LG, Perumal N, Syed MA, Shamaan NA, Shukor MY. Assessment of *Clarias batrachus* as a source of acetylcholinesterase (AChE) for the detection of insecticides. J Environ Biol. 2009;30(1):135–8.
41. Begum G. Organ-specific ATPase and phosphorylase enzyme activities in a food fish exposed to a carbamate insecticide and recovery response. Fish Physiol Biochem. 2011;37(1):61–9.
42. Palanisami S, Prabaharan D, Uma L. Fate of few pesticide-metabolizing enzymes in the marine cyanobacterium Phormidium valderianum BDU 20041 in perspective with chlorpyrifos exposure. Pestic Biochem Physiol. 2009;94(2–3):68–72.
43. Sabullah MK. Acetylcholinesterase from *Osteochilus hasselti* for the detection of insecticides and heavy metals. Universiti Putra Malaysia; 2011.
44. Sabullah K, Ahmad SA, Ishak I, Sulaiman MR, Shukor MY, Syed MA, et al. An inhibitive assay for insecticides using the acetylcholinesterase from *Osteochillus hasselti*. Bull Environ Sci Manag. 2013;1(1):1–4.
45. Hayat NM, Sabullah MK, Shukor MY, Syed MA, Dahalan FA, Khalil KA, et al. The effect of pesticides on cholinesterase activity by using fish as a biomarker. Nanobio Bionano [Internet]. 2014 [cited 2015 Jan 12];1(1). Available from: http://journal.hibiscuspublisher.com/index.php/NAB/article/view/50
46. Sabullah MK, Sulaiman MR, Shukor MYA, Syed MA, Shamaan NA, Khalid A, et al. The assessment of cholinesterase from the liver of *Puntius javanicus* as detection of metal ions. Sci World J. 2014;2014.
47. Shukor M, Masdor N, Halmi M, Kamaruddin K, Syed M. Near-real-time biomonitoring of heavy metals using the xenoassay® system. Proc Annu Int Conf Syiah Kuala Univ-Life Sci Eng Chapter. 2013;3(1).
48. Botsford JL. A simple assay for toxic chemicals using a bacterial indicator. World J Microbiol Biotechnol. 1998;14(3):369–76.
49. Raj DS, Prabha RJ, Leena R. Analysis of bacterial degradation of azo dye congo red using HPLC. J Ind Pollut Control. 2012;28(1):57–62.
50. Gaspare L, Machiwa JF, Mdachi SJM, Streck G, Brack W. Polycyclic aromatic hydrocarbon (PAH) contamination of surface sediments and oysters from the inter-tidal areas of Dar es Salaam, Tanzania. Environ Pollut. 2009;157(1):24–34.
51. Kohler A, Schneider S. Macrophytes as bioindicators. Arch Hydrobiol Suppl. 2003;147(1–2):17–31.
52. Markert B, Wappelhorst O, Weckert V, Herpin U, Siewers U, Friese K, et al. The use of bioindicators for monitoring the heavy-metal status of the environment. J Radioanal Nucl Chem. 1999;240(2):425–9.
53. Thomulka KW, McGee DJ, Lange JH. Use of the bioluminescent bacterium Photobacterium phosphoreum to detect potentially biohazardous materials in water. Bull Environ Contam Toxicol. 1993;51(4):538–44.
54. Bitton G, Koopman B, Agami O. MetPAD™: a bioassay for rapid assessment of heavy metal toxicity in wastewater. Water Environ Res. 1992;834–6.

55. Shukor MY, Masdor N, Baharom NA, Jamal JA, Abdullah MPA, Shamaan NA, et al. An inhibitive determination method for heavy metals using bromelain, a cysteine protease. Appl Biochem Biotechnol. 2008;144(3):283–91.
56. Shukor MY, Baharom NA, Masdor NA, Abdullah MPA, Shamaan NA, Jamal JA, et al. The development of an inhibitive determination method for zinc using a serine protease. J Environ Biol. 2009;30(1):17–22.
57. Baskaran G, Masdor NA, Syed MA, Shukor MY. An inhibitive enzyme assay to detect mercury and zinc using protease from *Coriandrum sativum*. Sci World J [Internet]. 2013;2013. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84886463804&partnerID=40&md5=56fe4ef11ba50ff5275953a2612962c1
58. Gunasekaran B, Sulaiman MH, Halmi MIE, Amir S, Roslan MAH, Jirangon H, et al. An inhibitive determination method for heavy metals using tomato crude proteases. Asian J Plant Biol. 2013;1(1):10–4.
59. Wahab SMA, Gunasekaran B, Shaharuddin NA, Johari WLW, Halmi MIE, Said NAM, et al. A novel method for the determination of mercury in herbal preparation using an inhibitive assay based on the protease papain. J Environ Microbiol Toxicol. 2013;1(1):1–4.
60. Gunasekaran B, Kasim MHM, Salvamani S, Shukor MY. Field trials on heavy metals using alpha-chymotryopsin enzyme assay. J Environ Microbiol Toxicol. 2014;2(1):25–34.
61. Sahlani MZ, Halmi MIE, Masdor NA, Gunasekaran B, Wasoh H, Syed MA, et al. A rapid inhibitive assay for the determination of heavy metals using α-chymotrypsin; a serine protease. Nanobio Bionano. 2014;1(2):41–6.
62. Shukor MY, Anuar N, Halmi MIE, Masdor NA. Near real-time inhibitive assay for heavy metals using achromopeptidase. Indian J Biotechnol. 2014;13(3):398–403.
63. Bodar CWM, Van Leeuwen CJ, Voogt PA, Zandee DI. Effect of cadmium on the reproduction strategy of Daphnia magna. Aquat Toxicol. 1988;12(4):301–9.
64. De Coen WM, Janssen CR. The use of biomarkers in Daphnia magna toxicity testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. J Aquat Ecosyst Stress Recovery Former J Aquat Ecosyst Health. 1997;6(1):43–55.
65. Diamantino TC, Guilhermino L, Almeida E, Soares AMVM. Toxicity of sodium molybdate and sodium dichromate to *Daphnia magna* Straus evaluated in acute, chronic, and acetylcholinesterase inhibition tests. Ecotoxicol Environ Saf. 2000;45(3):253–9.
66. Davies TD, Pickard J, Hall KJ. Acute molybdenum toxicity to rainbow trout and other fish. J Environ Eng Sci. 2005;4(6):481–5.
67. Shukor MY, Baharom NA, Masdor NA, Abdullah MPA, Shamaan NA, Jamal JA, et al. The development of an inhibitive determination method for zinc using a serine protease. J Environ Biol. 2009;30(1):17–22.
68. Kaira M, Halmi MIE, Shukor MY. Use of microorganisms, enzymes and plant proteases for heavy metals biomonitoring- a mini review. Asian J Plant Biol. 2013;1(1):20–4.
69. Sabullah MK, Ahmad SA, Sulaiman MR, Shukor MY, Syed MA, Shamaan NA. The development of an inhibitive assay for heavy metals using the acetylcholinesterase from *Periophtalmodon schlosseri*. J Environ Bioremediation Toxicol. 2013;1(1):20–4.
70. Sabullah MK, Sulaiman MR, Shukor MYA, Syed MA, Shamaan NA, Khalid A, et al. The assessment of cholinesterase from the liver of *Puntius javanicus* as detection of metal ions. Sci World J. 2014;2014.
71. Hayat NM, Shamaan NA, Shukor MY, Sabullah MK, Syed MA, Khalid A, et al. Cholinesterase-based biosensor using *Lates calcarifer* (Asian Seabass) brain for detection of heavy metals. J Chem Pharm Sci. 2015;8(2):376–81.
72. Sabullah MK, Ahmad SA, Shukor MY, Gansau AJ, Syed MA, Sulaiman MR, et al. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. Int Food Res J. 2015;22(2):435–54.
73. Sabullah MK, Sulaiman MR, Abd Shukor MY, Shamaan NA, Khalid A, Ahmad SA. In vitro and in vivo effects of *Puntius javanicus* cholinesterase by copper. Fresenius Environ Bull. 2015;24(12 B):4615–4621.
74. Sabullah MK, Sulaiman MR, Shukor MS, Yusof MT, Johari WLW, Shukor MY, et al. Heavy metals biomonitoring via inhibitive assay of acetylcholinesterase from *Periophthalmodon schlosseri*. Rendiconti Lincei. 2015;26(2):151–8.
75. Hayat NM, Shamaan NA, Sabullah MK, Shukor MY, Syed MA, Khalid A, et al. The use of *Lates calcarifer* as a biomarker for heavy metals detection. Rendiconti Lincei. 2016;Article in Press:1–10.
76. Attar H, Afshar S. Design of Sensible Biosensor for Rapid Detection of Biocides in Potable Water. Asian J Biotechnol. 2010;2(2):120–6.
77. Sowerby JM, Ottaway JH. The enzymic estimation of glutamate and glutamine. Biochem J. 1966;99(1):246–52.
78. Altman FP. Tetrazolium salts and formazans. Prog Histochem Cytochem. 1976;9(3):1–56.
79. Berridge MV, Tan AS. Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. Arch Biochem Biophys. 1993;303(2):474–82.
80. Ahmad F, Halmi MIE, Baskaran G, Johari WLW, Shukor MY, Syed MA. Inhibitive bacterial MTT assay for river monitoring of heavy metals. Bioremediation Sci Technol Res. 2013;1(1):1–7.
81. Halmi MIE, Ahmad F, Hashim AK, Shamaan NA, Syed MA, Shukor MY. Effect of bacterial growth period on the sensitivity of the MTT assay for silver. J Environ Biol. 2014;35(2):353–5.
82. Isa HWM, Johari WLW, Syahir A, Shukor MA, Azwady AN, Shaharuddin N, et al. Development of a bacterialbased tetrazolium dye (MTT) assay for monitoring of heavy metals. Int J Agric Biol. 2014;16:1123–1128.
83. Elnabarawy MT, Robideau RR, Beach SA. Comparison of three rapid toxicity test procedures: Microtox,® polytox,® and activated sludge respiration inhibition. Toxic Assess. 1988;3(4):361–70.
84. Johnson BT. Microtox® acute toxicity test. In: Small-Scale Freshwater Toxicity Investigations: Volume 1 - Toxicity Test Methods. 2005. p. 69–105.
85. Girotti S, Ferri EN, Fumo MG, Maiolini E. Monitoring of environmental pollutants by bioluminescent bacteria. Anal Chim Acta. 2008;608(1):2–29.
86. Ronco AE. Development of a bioassay reagent using *Photobacterium phosphoreum* as a test for the detection of aquatic toxicants. World J Microbiol Biotechnol. 1992;8(3):316–8.
87. Girotti S, Bolelli L, Roda A, Gentilomi G, Musiani M. Improved detection of toxic chemicals using bioluminescent bacteria. Anal Chim Acta. 2002;471(1):113–20.
88. Hsieh C-Y, Tsai M-H, Ryan DK, Pancorbo OC. Toxicity of the 13 priority pollutant metals to *Vibrio fisheri* in the Microtox® chronic toxicity test. Sci Total Environ. 2004;320(1):37–50.
89. Onorati F, Mecozzi M. Effects of two diluents in the Microtox® toxicity bioassay with marine sediments. Chemosphere. 2004;54(5):679–87.
90. Liang XU, Sheng Z. The Application of Deltatox~ Water Toxicity Detector in the Emergency Monitoring of Water Pollution. Pollut Control Technol. 2009;
91. Bulich AA. Use of luminescent bacteria for determining toxicity in aquatic environments. Aquat Toxicol. 1979;667:98–106.
92. Gellert G, Stommel A, Trujillano AB. Development of an optimal bacterial medium based on the growth inhibition assay with *Vibrio fischeri*. Chemosphere. 1999;39(3):467–76.
93. Ulitzur S, Lahav T, Ulitzur N. A novel and sensitive test for rapid determination of water toxicity. Environ Toxicol. 2002;17(3):291–6.
94. Mariscal A, Peinado MT, Carnero-Varo M, Fernández-Crehuet J. Influence of organic solvents on the sensitivity of a bioluminescence toxicity test with *Vibrio harveyi*. Chemosphere. 2003;50(3):349–54.
95. Peinado MT, Mariscal A, Carnero-Varo M, Fernández-Crehuet J. Correlation of two bioluminescence and one fluorogenic bioassay for the detection of toxic chemicals. Ecotoxicol Environ Saf. 2002;53(1):170–7.
96. Lee HJ, Villaume J, Cullen DC, Kim BC, Gu MB. Monitoring and classification of PAH toxicity using an immobilized bioluminescent bacteria. Biosens Bioelectron. 2003;18(5–6):571–7.

97. Hong Y, Chen Z, Zhang B, Zhai Q. Isolation of *Photobacterium* sp. LuB-1 and its application in rapid assays for chemical toxicants in water. Lett Appl Microbiol. 2010;51(3):308–12.
98. Ren S, Frymier PD. Toxicity of metals and organic chemicals evaluated with bioluminescence assays. Chemosphere. 2005;58(5):543–50.
99. Girotti S, Ferri EN, Fumo MG, Maiolini E. Monitoring of environmental pollutants by bioluminescent bacteria. Anal Chim Acta. 2008 Feb 4;608(1):2–29.
100. Dutka BJ, Kwan KK. Comparison of three microbial toxicity screening tests with the microtox test. Bull Environ Contam Toxicol. 1981 Jul;27–27(1):753–7.
101. Watanabe H, Hastings JW. Inhibition of bioluminescence in *Photobacterium phosphoreum* by sulfamethizole and its stimulation by thymine. Biochim Biophys Acta BBA - Bioenerg. 1990 Jun;1017(3):229–34.
102. Zahaba M, Halmi MIE, Ahmad SA, Shukor MY, Syed MA. Isolation and characterization of luminescent bacterium for sludge biodegradation. J Environ Biol. 2015;36(6):1255.
103. Choi SH, Gu MB. A portable toxicity biosensor using freeze-dried recombinant bioluminescent bacteria. Biosens Bioelectron. 2002;17(5):433–40.
104. Widder E a. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. Science. 2010 May 7;328(5979):704–8.
105. Hastings JW, Greenberg EP. Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria. J Bacteriol. 1999;181(9):2667.
106. Hastings JW. Bioluminescence. Annu Rev Biochem. 1968;37(1):597–630.
107. Hastings J, Potrikusv CJ, Gupta SC, Kurfürst M, Makemson JC. Biochemistry and physiology of bioluminescent bacteria. Adv Microb Physiol. 1985;26:235–91.
108. Thomulka KW, McGee DJ, Lange JH. Use of the bioluminescent bacterium Photobacterium phosphoreum to detect potentially biohazardous materials in water. Bull Environ Contam Toxicol. 1993;51(4):538–44.
109. Engebrecht J, Nealson K, Silverman M. Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri. Cell. 1983;32(3):773–81.
110. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol. 2005;21:319–46.
111. Kaiser KL., Palabrica VS. Photobacterium phosphoreum toxicity data index. Water Qual Res J Can. 1991;26(3):361–431.
112. Halmi MIE, Johari WLW, Amir S, Sulaiman R, Azlina A, Shukor MY, et al. Monitoring of heavy metals level in fish using *Photobacterium* sp. strain MIE. Bioremediation Sci Technol Res. 2013;1(1):19–22.
113. Abd Rachman AR, Halmi MIE, Shukor MY. Amplification of new isolated luciferase gene from marine *Photobacterium strain* MIE by using specific PCR. J Environ Microbiol Toxicol. 2014;2(1):35–7.
114. Parvez S, Venkataraman C, Mukherji S. A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals. Environ Int. 2006;32(2):265–8.
115. Salizzato M, Bertato V, Pavoni B, Ghirardini AV, Ghetti PF. Sensitivity limits and EC50 values of the Vibrio fischeri test for organic micropollutants in natural and spiked extracts from sediments. Environ Toxicol Chem. 1998;17(4):655–61.