Environmental Toxicity and Evaluation

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Chapter 6
Environmental Toxicity and Evaluation

Lee Yook Heng, Lia Ooi, Izumi C. Mori, and Dedi Futra

Abstract The basic concept of environmental toxicity and its importance in the evaluation of ecosystem health will be introduced. Toxicity evaluation theory and practice will be briefly discussed. Traditional techniques such as bioassays for environmental toxicity evaluation will be introduced where the advantages and disadvantages will be presented. But the main focus of environmental toxicity evaluation will be on the use of more recent techniques for rapid environmental toxicity assessments such as toxicity biosensor and its basic concept, current applications, and future prospects.

Keywords Environmental toxicity · Toxicity measurement · Environmental security · Bioassay · Toxicity biosensor

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6.1 Understanding the Basic Concept of Toxicity

Toxicity refers to the capacity of a chemical (toxin) to affect an organism adversely. Environmental pollutants are usually appertained to the term xenobiotics, which are chemical compounds found in but not naturally produced within an organism or a biological system. Environmental xenobiotics mostly are wastes from anthropogenic activities (e.g., agriculture, mining, industrialization, urbanization, chemical spills, etc.), which can be grouped as pharmaceutical drugs, environmental pollutants, food additives, pesticides, carcinogens, antioxidants, hydrocarbons, and many more. The study of the toxicity effects of a chemical is known as toxicology. Classical toxicology, which was studied since centuries ago, is the study of the adverse effects of chemical, biological, and physical agents in biological systems which possess adverse effects on living organisms. The father of toxicology, Theophrastus Phillipus Auroleus Bombastus von Hohenheim (1493–1541), who was also known as Paracelsus, wrote "*sola dosis facit venenum*" (the dose makes the poison), which brought to the formation of the dose-response relationship.

Environmental toxicology (Entox) is an expeditiously expanding multidisciplinary field of science, concerned particularly in determination of the sources, fate, transformations, effects, and risks of toxicants on the environment, wildlife, and human health. Fig. 6.1 illustrates the major components of

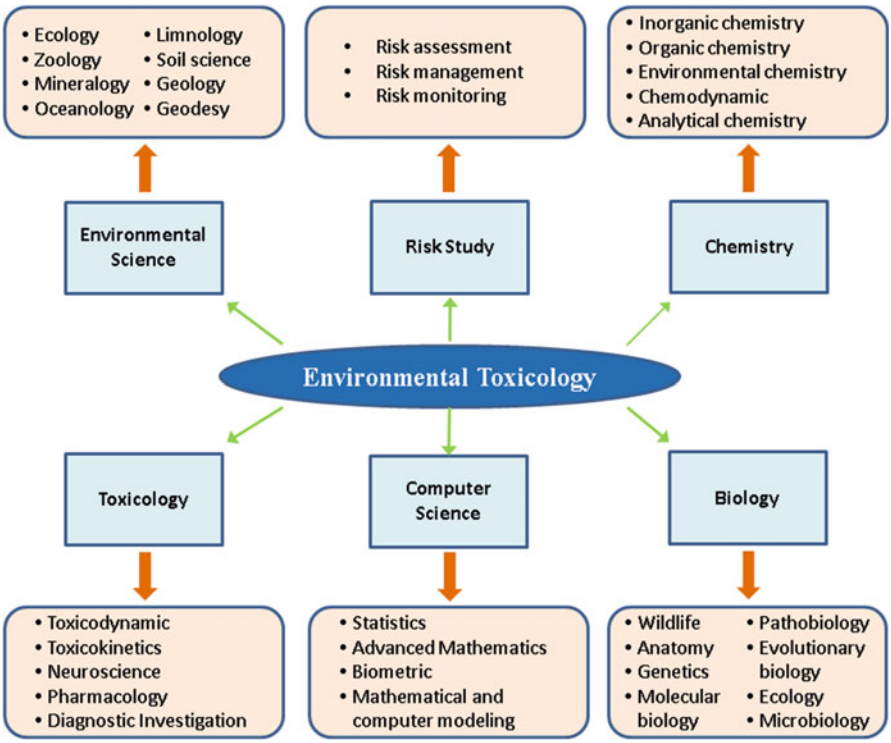

Fig. 6.1 Environmental toxicology and its components

environmental toxicology. Note that Entox is a multidiscipline; thus, its components consist of but not limited to those stated in the figure. The study of the toxic effects of environmental toxicants began in the 1960s, when Rachel Carson, the mother of environmental toxicology, who wrote the book *Silent Spring*, described the harmful environmental impacts of dichlorodiphenyltrichloroethane (DDT), which was widely used as insecticide. The book raised an unprecedented extend of public awareness among the US people regarding environmental toxicity and its impacts on the wildlife and human health. It eventually led to a nationwide ban on DDT usage and the formation of the US Environmental Protection Agency (US EPA) (Carson 2002; Paull 2013). In 1969, a French toxicologist René Truhaut (1909–1994) introduced the term "ecotoxicology" to describe the study of environmental toxicity which distinguishes it from classical toxicology (Truhaut 1977). Ecotoxicological studies provide invaluable information on potential hazards found in the environment via identification of important impacts of pollutants to living organisms.

Over time, urbanization and science development have brought industrialization and chemical invention to flourish. Hundreds of thousands of man-made chemicals have been created in the laboratory, and many more have been released into the environment every day. Researchers and the authorities are alarmed by the degradation of the environmental quality and observation of health impacts on organisms and human health in contaminated areas. Laws and legislations have been established, promulgated, and enforced for environmental protection. Numerous legislations have been made starting in the 1970s to reduce environmental toxicity. In other words, Entox is legislation driven. The National Environmental Policy Act of 1970 was the first piece of environmental legislation which brought to the foundation of US EPA. Clean Air Act of 1970, Federal Environmental Pesticide Control Act of 1972, Federal Water Pollution and Control Act of 1972, Safe Drinking Water Act of 1974, Toxic Substances Control Act of 1976, Resource Conservation and Recovery Act of 1976, Comprehensive Environmental Response, Compensation and Liabilities Act of 1980 (a.k.a. Superfund), and Clean Water Act of 1987 are several examples of acts created for environmental protection.

The degree of toxicity of a toxicant can vary depending on in which stage of the life cycle the exposed organism is and where it is found within the food web. Environmental toxicants enter a food chain via bioaccumulation and gradually increase in concentration when they move from one trophic level of the food web to another through biomagnification. These phenomena enable small amount of chemicals in the environment to be found in high dosage in the organisms sitting on top of a biomass pyramid. Environmental toxicant can enter the body of organism or human through several portals of entry, namely, inhalation, skin (or eye) contact, digestion, and injection. Three phases are involved when an environmental toxicant penetrates its way into an organism (Djuric 2015):

1. The exposure phase

   Comprises of all processes arising between the toxicants and the influence of environmental factors including degradation, biodegradation, chemical transformations, etc.

2. The toxicokinetic phase
   Comprises the absorption of toxicants into the organism followed by transportation, distribution, and accumulation of toxicants in the tissues and organs and the biotransformation of the toxicants
3. The toxicodynamic phase
   Comprises the interaction of (the bioavailable fractions of) toxicants with specific receptors in the organism, which eventually brought to the formation of a toxic effect.

Toxicity can be divided into three major categories based on the duration of time taken for the adverse effects to emerge, which are (i) acute toxicity, in which the harmful effects can be observed through a single or short-term exposure, where death is a major concern; (ii) subchronic toxicity, which results from repeated exposure for weeks or months and requires more than 1 year but less than the lifetime of the exposed organism for the effects to be observed; and (iii) chronic toxicity, which causes malignant effects to the exposed organism over an extended period of time. Bhopal disaster which happened in 1984 is a suitable example to study toxicity effects. The leakage of at least 27 tons of methylisocyanate (MIC) gas from a pesticide plant (Union Carbide India Limited) in Bhopal, India, exposed more than 500,000 people to the odorless, colorless, and deadly gas. It killed at least 3800 people immediately (acute effect) and 15,000–20,000 in the next three decades due to disaster-related deaths majorly caused by chronic inflammatory damage to the eyes and lungs (Knight and Riesenberger 2014; Monosson 2008).

The toxicity of a toxicant can also be classified as systematic toxicity (which affects the entire body or multiple organs of an organism) and target organ toxicity (which affects only specific tissues or organ in the organism) based on its target site in the organism. Target organ toxicity can be further categorized to dermal toxicity, ocular toxicity, hepatotoxicity, immunotoxicity, nephrotoxicity, neurotoxicity, reproductive toxicity, and so forth. Hydrogen cyanide is an example of systemic toxicant; it binds to cytochrome oxidase in the organism causing cellular hypoxia and rapid death (Monosson 2013). On the contrary, vinyl chloride monomer (VCM), a colorless gas which is majorly used in the production of polymer polyvinyl chloride (PVC), is an example of hepatotoxicant. Its hepatotoxicity has been long established since the 1930s. Studies in test animals and VCM worker found that even just a single short-term exposure to high dosage of VCM caused liver damage (Waite et al. 1930). Due to its high volatility, VCM has rarely been detected in surface waters. It was detected to have concentration of only 56 μg/liter even in highly polluted rivers in Osaka. However, in closed environment, VCM can be found in high concentrations in leachate (60,000 μg/liter), contaminated sand aquifer (56,000 μg/liter), groundwater (120,000 μg/liter), and well water (up to 200,000 μg/liter) in the vicinity of a PVC plant after 10 years of leakages (WHO 2004). This possesses environmental health risk to wildlife and human.

Dose, exposure, and response are the basic three factors that determine the adverse effects of a toxicant. "The dose makes the poison," and the *dose* of a toxicant is the amount of it that comes into contact with and is deposited within the body of a living organism. Threshold dose is the term used to describe the dose

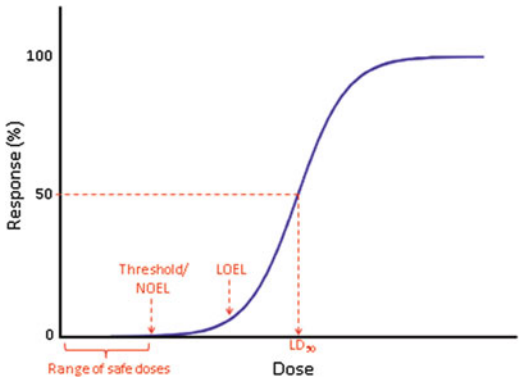

Fig. 6.2 Dose-response curve

level at which toxicity is first encountered. Any condition which provides opportunity for an external environmental agent to enter the body of an organism is referred to as *exposure*. Environmental exposure can be expressed as the amount of a xenobiotic in a unit of the media (e.g., mg/L for liquids, mg/g for solids, and mg/m^3 for air). *Response* is the biological effect of the chemical agent on the exposed organism.

A dose-response curve can be used to effectively display the dose-response relationship of an environmental toxicant to the response of the exposed organism. A dose-response relationship can be *graded* (for responses that are measured on a continuous scale in a single biological unit, e.g., heart rate) or *quantal* (for all-or-none responses in a population, e.g., mortality). Quantal dose-response curve is usually constructed in environmental toxicity study, where mortality rate of the exposed organisms is referred to as the response toward environmental toxicant exposure. The median lethal dose/concentration (LD_{50} or LC_{50}), the no-observed-adverse-effect level/concentration [NO(A)EL or NOAEC], and lowest-observed-adverse-effect level/concentration [LO(A)EL or LOAEC] of an environmental toxicant can be determined from a quantal dose-response curve (Fig. 6.2). Information from a dose-response curve is crucial to assist in assessing the risk of environmental toxicity.

From ecotoxicological aspects, *risk* refers to the chance of adverse effects to human health or to ecological systems resulting from exposure to an environmental stressor (USEPA 2015a). An environmental risk assessment (ERA) is a systematic process to predict and characterize the potential adverse effects to human health and the environment due to the exposure to a chemical substance. In 1983, the National Research Council (NRC) published NRC Red Book extensively describing about the four significant stages in risk assessment (RA): (i) hazard identification, (ii) dose-response assessment/hazard characterization, (iii) exposure assessment/consequence assessment, and (iv) risk characterization/risk estimation (NRC 1996). A comprehensive ERA should consist all of those listed key stages applying the conceptual model of the source-pathway-receptor. In this conceptual model, the pathway between the source of contamination (the hazard source) and the particular

ecosystem (the receptor) is investigated (RSC 2013). The pathway is the linkage between the receptor and the hazard; exposure risk exists when a pathway exists and vice versa. The presence and the concentration of a toxicant in an environmental medium, the probability of an ecological receptor to be exposed to the toxicant, and the inherent toxicity of the toxicant resulting from the exposure are three factors deciding the risk (USEPA 2015a).

ERA can be categorized into human health risk assessment (HHRA) and ecological risk assessment (EcoRA). In both RAs, information on the toxic properties of environmental stressors and dose-response relationships are crucial for the exposure risk to be accurately assessed. Although advanced analytical instruments are excellent in detecting even a very small amount of chemicals in environmental samples, only living organisms can demonstrate the toxicity effects of a toxicant (Cairns and Mount 1990). Toxicity information is usually obtained from animal studies, epidemiological investigation and clinical studies of exposed human populations, or accident case reports of exposed humans. A toxicity test encompasses a test organism (ranging from cellular material of microorganisms to higher-order organisms), a biological endpoint (ranging from physiological changes to death), an exposure period, and a dose or series of doses (EHS 2002). US EPA has developed extensive guidelines and reports on dose-response modeling and assessment. Two of the most popular guidelines on environmental toxicity studies are the whole effluent toxicity (WET) methods and aquatic toxicity identification evaluations (TIE).

WET investigates the toxicity of pollutants in a facility's effluent (wastewater) on aquatic organisms by measuring its effects from the aspect of survival, growth, and reproduction of the test organisms. It is practiced as part of the implementation of Clean Water Act which prohibits the discharge of toxic pollutants in toxic amounts into the environment. Several freshwater, estuarine, and marine organisms have been selected as test organisms for WET test, such as fathead minnow (*Pimephales promelas*), bannerfin shiner (*Cyprinella leedsi*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), water flea (*Ceriodaphnia dubia*, *Daphnia pulex*, *Daphnia magna*), sheepshead minnow (*Cyprinodon variegatus*), silversides (*Menidia beryllina*, *Menidia menidia*, *Menidia peninsulae*), and mysid shrimp (*Americamysis bahia*) (USEPA 2015b). An acute toxicity WET test predominantly includes exposure of test organisms to each of different effluent concentrations in a test duration ranging from 24 to 96 h (USEPA 2015c).

Similar to WET, TIE approach was developed for effluent evaluations, but its methods and techniques can be directly applied to different kinds of aqueous samples, for instance, sediment pore waters, ambient waters, waste leachates, and sediment elutriates (USEPA 1991). It consists of three phases which involved the characterization of the physical/chemical nature of the toxic constituents, the identification of toxicants, and the confirmation of the suspected toxicants. TIE assists in the characterization, identification, and confirmation of toxicant in aqueous samples. Water fleas (*Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*), fathead minnow (*Pimephales promelas*), scud (*Hyalella azteca*), and bluegill (*Lepomis macrochirus*) are some of the species of test organisms suggested to be used in TIE to identify acute and chronic effluent toxicity (USEPA 1991, 1993).

The emergence of sophisticated technologies in molecular and cellular biology has evolved the focus of toxicological studies from whole animal and population testing to cellular and molecular testing of the test organisms. In 2007, the Committee on Toxicity Testing and Assessment of Environmental Agents published a book titled *Toxicity Testing in the twenty-first century: A Vision and a Strategy*, which comprehensively illustrated the committee's vision for toxicity testing which will be conducted based on rapid *in vitro* assays. A toxicity testing which is pathway-based and consistent with the stages of RA stated in the 1983 Red Book has been developed (NRC 2007). Progressive toxicity testing tools such as high-throughput screenings, bioinformatics, functional genomics, computational systems biology, structure-activity relationships, molecular and genetic epidemiology, physiologically based pharmacokinetic models, stem cell biology, and so forth can be applied in assisting the modern risk assessment especially in environmental toxicity evaluation and in the effort to achieving *environmental security*, which is an environment protected from harm or adverse effects from natural or anthropogenic processes so that resources are sustained for future generations (DEFRA 2011; Krewski et al. 2011).

Improper handling of industrial wastes can result in irremediable and remorseful effects on wildlife and human health. The *Four Big Pollution Diseases of Japan* which occurred in 1912–1960s were all due to environmental toxicity as a result of inappropriate disposure of industrial wastes. These man-made diseases are Itai-itai disease, Minamata disease, Niigata Minamata disease, and Yokkaichi asthma. Itai-itai disease which first occurred in 1912 literally translates to "it hurts-it hurts disease." It was the first documented mass cadmium poisoning in the world (Rasnake 2009). Significant amounts of cadmium were released from the mining sites in the mountains into Jinzu River in Toyama Prefecture, Japan. Fish death and growth retardation in rice irrigated with the polluted water were observed. Local communities who were exposed to the polluted water and food experienced symptoms like debilitating pain, fragile bone, skeletal deformities, kidney failure, and eventually death. Mitsui Mining and Smelting Co., the mining company, was found guilty and was urged to pay health expense compensation to the victims and to clean up the polluted areas through soil restoration. The cadmium cleanup project began in 1979 which lasted for 33 years and finally marked its completion on 2012 after replacing 863 hectares of land in the Jinzu River basin (Kyoda 2012). Only 4 of its 196 victims were reported to be alive in 2012.

Minamata disease was another man-made disease reported in Minamata, Kumamoto Prefecture of Japan in 1956. It was identified as methylmercury poisoning when Chisso Corporation, a chemical factory, dumped the by-product of the chemical reactions into the waters of Minamata. Massive dead fish began to wash ashore in 1950, and cats in the area began to have convulsion and go mad (known as "the dancing cat disease" by the locals). The same symptoms appeared in human starting 1953 in people who ate the fish and seafood from the local waters. The victims suffered blindness, convulsions, incredible pain, and madness due to the neurotoxic effect of mercury poisoning (Hachiya 2006). In 1965, patients of Minamata disease were also reported in the Agano River basin in Niigata Prefecture (Niigata Minamata

disease) (MOE Japan 2002). It was until 1968 that Chisso plant was declared to be responsible for the outbreak of Minamata disease. Environmental toxicity by heavy metals affected numerous areas in Japan, which was then brought to the enaction of the Prevention of Soil Contamination in Agricultural Land Law of 1970 and Japanese Water Pollution Control Act of 1970.

6.2 Bioassay Methods of Ecotoxicity Assessment

Technological advance after the industrial evolution has made enormous changes to human lifestyle. Our daily life is getting abundant and we receive a benefit of the technologies. Lying behind the benefit, however, environmental problem arising from the technology has become a global concern.

Environmental pollution by a trace amount of hazardous chemical can be a problem that causes ecological casualties (Truhaut 1977). More than one hundred million of chemicals have already been registered to the Chemical Abstract Service database, the information division of the American Chemical Society. Millions of novel chemicals are being registered to the database annually (Binetti et al. 2008). Some of these chemicals that are potentially hazardous to the ecosystem are inferable to be discharged to the environment. Ecotoxicology (or Entox) aims at elucidating the fate of hazardous chemicals in environment and the toxic action of chemicals to the components of the ecosystem. Furthermore, it accumulates fundamental data of potential toxicants for the establishment of environmental regulations, which are eventually utilized for conservation and management of the biological resources of watersheds.

Assessment of toxicity of chemicals to a certain organism is an essential component of ecotoxicology. Bioassay (short for biological assay or biological assessment) is a practical means for assessing toxic effect of environmental samples and chemical products on a test organism. The purpose of bioassay is to determine toxicity parameters of water samples, chemical products, etc. Fishes, invertebrates, unicellular algae, and bacteria are often utilized as the test organisms in bioassays. Since evaluation of toxicity to a single organism is insufficient for comprehensive elucidation of ecotoxicity, the implementation of the batteries (a suite of bioassays) is recommended (Keddy et al. 1995). In practice, a combination of acute toxicity tests consisting of three organisms, alga, cladoceran (water flea) (e.g., genera *Daphnia* and *Ceriodaphnia*), and fish, is preferably used for a *battery test*. Each organism shares an ecological niche in the food chain, which is a structural aspect of the ecosystem. Since bioassays are carried out in a laboratory, the assay results are not necessarily appropriate to estimate the toxic effect at an actual pollution site. To understand the impact of a chemical on ecosystem, functional aspects of ecosystem in addition to structural aspects and specific property of the actual scene/site must be taken into account. Realistically, a method to comprehensively evaluate environmental toxicity having sights from both functional and structural aspects has not been established. Currently, ecotoxicity assessment relies on analyses of elementary

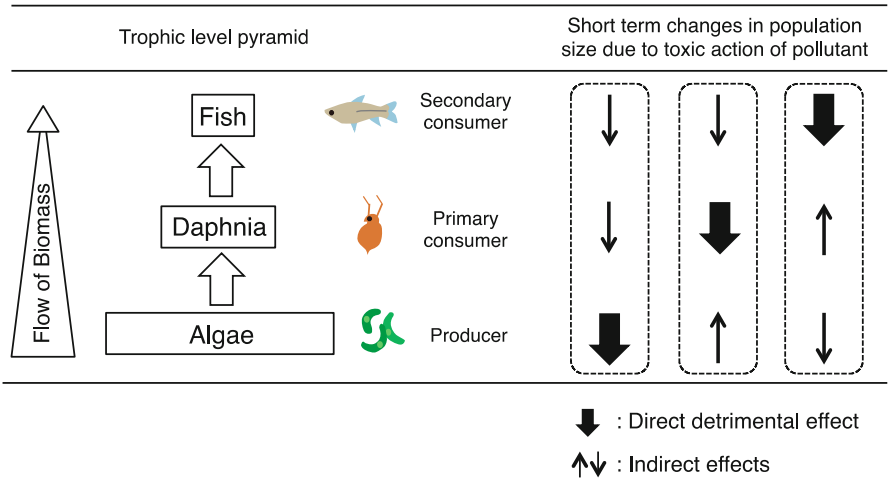

Fig. 6.3 Direct and indirect detrimental effects of pollutants on organisms through food chain. The short-term impact of pollutants on a population of a trophic level indirectly affects populations of other trophic levels through insufficiency of biomass flow or the decrease of feeding activity

process of toxic effects on each organism as a matter of fact. Biomonitoring that observes biota and ascertains whether the aquatic life is endangered is operated to complement bioassay. (USEPA 1978).

Since a species is linked to another species in the environment such as predator-prey relationship or competition for resources, therefore, an impact on a certain species may influence other species directly or indirectly in the ecosystem. The predator-prey relationship, in other words "food chain", is often taken into account for ecotoxicity evaluation (Fig. 6.3). Organisms in three different trophic levels, for example, producer (algae), primary consumer (cladoceran), and secondary consumer (fish), are preferably chosen as keystone species in freshwater aquatic ecosystem. Biomass is transferred to upper trophic level through feeding behavior. A detrimental impact on the population of an organism, which is immediately below trophic level, may cause a decreased biomass flow to the upper level. However, it should also be noticed that an ecological niche could be competed by several species. Hence, a chemical impact on a species might increase the population of other species through the reduced competitive pressure. On the other hand, a pollutant may exhibit detrimental effects on multiple species in the same level, which may cause a reduction of the population of the trophic level. Acute toxicity tests are practical but not flawless, as chronic and subchronic toxicity should also be taken into account.

Aquatic toxicity is examined under a static, semi-static, or flow-through condition. Static test is a test performed without replacing the test solution in the test vessel throughout the exposure period. Semi-static test is a test performed by replacing the test solution in the test vessel at a certain interval. Flow-through test is a test performed by constantly exchanging the test solution in the test vessel throughout

the exposure period. If the concentration of the test substance is unstable, a semi-static or flow-through test is recommended. Test chemicals added to the test solution can be lost by volatilization, photolysis, precipitation, or metabolism. The standard test method of the Japanese Ministry of the Environment recommends that the concentration of the test substance be preferably maintained at ≥80% of the initial concentration (METI 2013). Determining actual concentration of the toxicant in the test solution is a better indicator than nominal concentration.

Lethality is the most widely used indicator (endpoint) in toxicity tests. Lethality test possesses several very useful properties for a toxicity testing. Apparently, death is an important adverse effect on organisms. It is equally applicable to all test organisms, and any toxicant is applicable to the test. It can be measured rather easily without the use of specialized equipment. Terrestrial organisms are dosed by oral administration or hypodermal injection for toxicity tests. On the other hand, aquatic organisms are placed in a solution containing a toxicant for an aquatic toxicity testing. Therefore, the endpoint of aquatic toxicity test is LC_{50} instead of LD_{50}. An endpoint can be other parameters besides lethality. Toxic effect on cladocerans is examined on the basis of immobilization rate (the rate of organisms not to demonstrate swimming action). Halting swimming/moving does not necessarily mean it is dead. In this case, median effective concentration (EC_{50}) is used as the endpoint. Median inhibitory concentration (IC_{50}) is used as the endpoint, when inhibitory effect is examined. LC_{50}, IC_{50}, and EC_{50} are often determined by dose-response curve according to probit fitting or logit fitting (Casarett et al. 2001). These two plots give very similar value and are practically interchangeable for determination of LC_{50}, IC_{50}, and EC_{50}, while a marginal difference can be seen at higher or lower values, such as LC_{20} and LC_{80} (Fig. 6.4). Lowest-observable-effect-concentration (LOEC) and no-observable-effect-concentration (NOEC) are obtained by a statistical test such as Dunnett's procedure (Dunnett 1955).

A pollutant renders sublethal symptoms to aquatic organisms at lower concentrations. Histopathological abnormalities (e.g., dysplasia of filament cartilage of gills, glycogen depletion, or renal lesion of fish) and behavior alteration (e.g., swimming velocity of water flea, rostrum raising, and frantic swimming of fish)

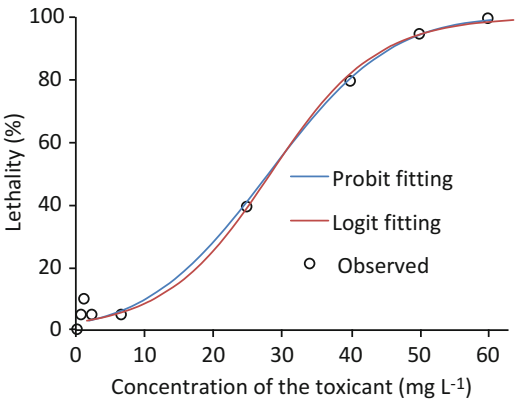

Fig. 6.4 Example of regression analysis of dose-response curve with probit model fitting and logit model fitting. Fitting curve was derived from the same data. The observed value was indicated with open circles (Data are modified from Mori et al. (2015))

are observable (Spies et al. 1996; Baillieul and Blust 1999). Reproductive toxicity is also an important endpoint of sublethal toxicity to aquatic organisms (e.g., Arcand-Hoy and Benson 1998; Fort et al. 2001).

The bioassay manual issued by USEPA (1978) describes a pithy, important, and yet pleasant notion for the selection of endpoints: "There are many effects other than death that can be and have been used. The possibilities are only limited by man's ingenuity, time, and money."

Test organisms of bioassays are favorable to be of significance to the local environment. Having a local/regional point of view, test organisms suited for Asian watershed have not been extensively developed. Bioassays with local organisms predict the impact on the ecosystems more relevantly, rather than foreign organisms. Criteria for suitable organism for bioassay are easiness to maintain in the laboratory, manipulable body size, reasonable sensitivity, constant and facile availability, and cost-effectiveness. Size does matter in such a way that small size of the animal makes the procedure easy under a limited laboratory space. Since the assays are carried out by an ordinary laboratory worker, the test organisms should not be too vulnerable to the laboratory condition. Test organisms with leveled quality must be available as the need arises with cheap price.

Meeting these criteria, a few papers have reported the utilization of local organisms suited for Southeast Asian aquatic environments in bioassays. Alkassasbeh et al. (2009) utilized common carp (*Cyprinus carpio*) to conduct a toxicity evaluation of landfill leachates. They used fries (immature fish) of which body length was approximately 3.8 cm. The fries were obtained from a local carp breeder. Common carp is easy to maintain and handle in a laboratory and is naturally distributed widespread in the world. Genetic evidence indicates evolutional differentiation of two subspecies of carp, *C. c. carpio* (European/Central Asia) and *C. c. haematopterus* (East/Southeast Asia) (Kohlmann et al. 2003). European/Central Asian carp and East/Southeast Asian carp were domesticated in very early days in history. How early was it? There is evidence that the Romans have already domesticated carp which became the ancestors of today's carp in the Danube River (Balon 1995). Amazingly, *C. c. haematopterus* have been cultivated as early as 4000 years ago during the Shang dynasty in China (Chia-nan 2015). The carp in North America were introduced from Europe in the late nineteenth century as foodstuff and became popular in game fishing (US National Park Service 2015). Today, people recognize carp in North America as an invasive species. The East/Southeast Asian subspecies of carp is a good candidate to assess freshwater toxicity to fish in Southeast Asian environments, although Suliasih et al. (2010) reported that common carp exhibit less sensitivity to landfill leachate than the other candidate fish, *Rasbora sumatrana*, as mentioned below.

Suliasih et al. (2010) and Budi et al. (2015) used a battery test consisting of the freshwater fish, *R. sumatrana*; the freshwater prawn, *Macrobrachium lanchesteri*; and tomato, *Solanum lycopersicum* for toxicity identification evaluation (TIE) of a landfill leachate. *R. sumatrana* is distributed in Malay Peninsula, Borneo, Sumatra, Thailand, and Cambodia (Shukor et al. 2008). It is easily procured at local aquarium shop as ornamental fish and as a feed for carnivorous fish; and it is easy to maintain

in the laboratory. The authors used adult *R. sumatrana* of which size is ~4.5 cm long and ~0.5 g in weight. In their study, in order to carry out TIE, the period of fish mortality testing was as short as 24 h (USEPA 1991), instead of 94-h test as suggested in OECD test guideline 203 (OECD 1992). These two studies used a riceland prawn, *M. lanchesteri*, instead of the popularly used cladocerans (e.g., USEPA 1991). *M. lanchesteri*, locally known as "*udang gantung*" (meaning hanging prawn), is sold at local aquariums as feed for carnivorous fish and sometime as foodstuffs and is distributed in Thailand, peninsular and state of Sabah in Malaysia, Laos, and Brunei (ZipcodeZoo 2015). The size of the prawn was ~2.5 cm long and ~0.1 g in weight, thus convenient to utilize. As it is procured readily and cheaply, it is a good candidate for the test organism in the area. Suliasih et al. (2010) exposed the prawn for 24 h to the dilution series of the leachate and determined the LC_{50}. Toxicity of a landfill leachate was successfully characterized by the phase I tests of TIE. They concluded that the leachate contained toxic compounds with various chemical characteristics. Tongbai et al. (2012) utilized *M. lanchesteri* to examine the toxicity of the organophosphate pesticide, chlorpyrifos. They tested the inhibitory effect of the pesticide on acetylcholinesterase (AChE) activity of the prawn extracts as well as the lethality test and examined neurotoxicity to riceland prawn. These domestic freshwater species are favourable candidates in regard to their local significance in Southeast Asian watershed.

OECD Guidelines for the Testing of Chemicals, Sect. 2, is a tool for assessing toxicity to biotic systems in the environment. It has test numbers with 3 digits starting from 200. Sects. 1 and 3 in the guidelines are on chemical properties, and Sect. 4 is for human health. Sect. 5 of OECD Guidelines is for other tests. Currently, 41 tests are available in the Sect. 2 (Table 6.1). These cover from aquatic to terrestrial organisms and broad kingdoms of organisms, including earthworms, insects, frogs, and avians; and not only on acute toxicity but also on longer exposure, soil respiration, and reproductive toxicity.

An approach of risk evaluation by chemical analysis becomes more challenging, since recent chemical pollution is characterized by wide variety of chemical species, relatively low amount, and broad spatial distribution. Each chemical in environmental samples is identified and quantified by chemical analysis. In order to identify and quantify hazardous metals, atomic absorption spectrometry (AAS) is sufficient. The number of organic compounds is so large, and thus separation of chemicals is usually necessary. Organic toxicants in samples are usually concentrated with solid-phase extraction (SPE) and fractionated utilizing a C_{18} reverse-phase cartridge and then analyzed with high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), or liquid chromatography-mass spectrometry (LC-MS). Other SPE cartridges such as styrene-divinylbenzene copolymer (Sep-Pak PS-2) or Oasis HLB are often used alternatives for C_{18}. It is not realistic to analyze all chemicals contained in the environmental samples. Toxicity data for many chemicals identified are usually not available. Remember that millions of chemicals are registered annually. Interactions of chemicals (additive, synergism, and antagonism) are not largely understood. Toxic effect of chemicals on organism

Table 6.1 List of OECD guidelines for the testing of chemicals (Sect. 2: Effects on Biotic Systems)

No.	Test name	No.	Test name
201	Freshwater Alga and Cyanobacteria, Growth Inhibition Test	221	*Lemna* sp. Growth Inhibition Test
202	*Daphnia* sp. Acute Immobilisation Test	222	Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)
203	Fish, Acute Toxicity Test	223	Avian Acute Oral Toxicity Test
204	Fish, Prolonged Toxicity Test: 14-Day Study	224	Determination of the Inhibition of the Activity of Anaerobic Bacteria
205	Avian Dietary Toxicity Test	225	Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment
206	Avian Reproduction Test	226	Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil
207	Earthworm, Acute Toxicity Tests	227	Terrestrial Plant Test: Vegetative Vigour Test
208	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	228	Determination of Developmental Toxicity of Dipteran Dung Flies (*Scathophagastercoraria* L. (Scathophagidae), *Musca autumnalis* De Geer (Muscidae))
209	Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation)	229	Fish Short Term Reproduction Assay
210	Fish, Early-life Stage Toxicity Test	230	21-day Fish Assay
211	*Daphnia magna* Reproduction Test	231	Amphibian Metamorphosis Assay
212	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	232	Collembolan Reproduction Test in Soil
213	Honeybees, Acute Oral Toxicity Test	233	Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment
214	Honeybees, Acute Contact Toxicity Test	234	Fish Sexual Development Test
215	Fish, Juvenile Growth Test	235	*Chironomus* sp., Acute Immobilisation Test
216	Soil Microorganisms: Nitrogen Transformation Test	236	Fish Embryo Acute Toxicity (FET) Test
217	Soil Microorganisms: Carbon Transformation Test	237	Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure
218	Sediment-Water *Chironomid* Toxicity Using Spiked Sediment	238	Sediment-Free *Myriophyllum spicatum* Toxicity Test
219	Sediment-Water *Chironomi*d Toxicity Using Spiked Water	239	Water-Sediment *Myriophyllum spicatum* Toxicity Test
220	*Enchytraeid* Reproduction Test	240	Medaka Extended One Generation Reproduction Test (MEOGRT)
		241	The Larval Amphibian Growth and Development Assay (LAGDA)

depends on the concentration of chemicals and species of organisms. Therefore, it is hardly predictable from chemical composition.

Bioassays possess several advantages over chemical analysis. They allow evaluating comprehensive toxicity of the samples, and characterization of the toxic action to test organisms. Chemical analysis data does not necessary predict the toxicity. Sometimes, bioassay is cost-effective as compared to chemical analysis. In addition, bioassays do not require special equipment in general. Nevertheless, bioassays also possess disadvantage over chemical analysis. The test result is not able to provide information of influential toxicity factors in the water sample. Some bioassays take long periods to obtain the results. Chemical analysis, bioassay, and biomonitoring are complementary to each other for risk evaluation of chemical in aquatic ecosystem.

It is required to handle a large number of water samples in order to conduct a watershed-wide risk evaluation. According to a case to watershed-wide examination of water by conventional method, Many samples through entire watershed were analyzed in Langat River (Selangor, Malaysia) (Juahir et al. 2011). Currently, it is difficult to say that the characteristics of bioassays are satisfactory to conduct a high-throughput toxicity test. A high-throughput procedure and cheaper running cost of bioassay are needed. As high-throughput bioassay requires a large number of organisms, ethics is also an issue in addition to the availability. Water sampling is also a practical difficulty. Comprehensive sampling of the watershed is not easy, and correcting some liters of water for each sampling site and processing in the laboratory are painstaking job. Due to these difficulties, watershed-wide risk evaluation utilizing bioassays have not been standardized yet. To make watershed-wide chemical risk evaluation realistic, several assignments should be cleared. To reduce the laborious effort to collect samples, minimization of sampling scale and accordingly optimization of bioassay for small volume are also requested. Bioassays are still time-consuming and costly. Maintenance of test organisms can be a burden. Alternative tests that take place in living organisms should be developed to solve these assignments. At the same time, they are ethically more apt than sacrifice organisms. To date, automation of the bioassay has not been applied. The automation may be a critical step for high-throughput toxicity evaluation. The development of toxicity-oriented biosensors would aid the development of high-throughput toxicity testing.

6.3 Biosensors for Environmental Toxicity Assessment

Biosensor is an analytical device which combines a biological recognition element material with a transducer to translate the biological signal to electric signal. This signal comes from a change in concentration of proton, release and uptake of gases, light emission, absorption, etc. brought about by the metabolism of the target compound by the biological recognition element (Lei et al. 2006; Malandain et al. 2005). Biosensors are the offspring of the combination between biotechnology and modern electronic. The biological element responds to the analyte, and the

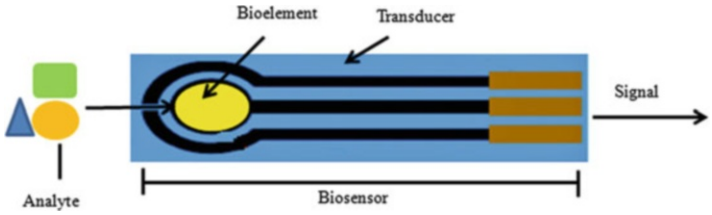

Fig. 6.5 Basic components of a biosensor with electrochemical transducer

transducer converts the biological signal to signals such as current, potential, and absorption that can be detectable by using optical and electrochemical method. Several transducers have always been explored in fabrication of a biosensor such as electrochemical (amperometric, potentiometric), thermal, piezoelectric, surface aquatic wave, and optical method (Lei et al. 2006; Su et al. 2011). Basic components of an electrochemical biosensor are shown in Fig. 6.5.

Toxicity biosensors are routinely used for the detection of environmental toxicants (Rogers 2006; Lei et al. 2006). Various biological matrices such as enzymes, antibodies, DNA, receptors, organelles, and microorganisms have been commonly used in the development of toxicity biosensor (Lei et al. 2006). Among these biological sensing elements, enzymes are the most popularly utilized recognition element, because of their unique specificity and sensitivity (Rogers 2006). However, the enzyme biosensor possesses several drawbacks, e.g., enzyme purification needed, high cost, and time-consuming. The in vitro operating environment could degrade enzyme activity (Rogers 2006; D'Souza 2001). In the meantime, the specific binding between antibody and antigen can be applied in immunosensor. The immunosensor can detect low concentration of analyte such as drugs, toxins, pesticides, herbicides, explosives, etc., while DNA biosensors have also been often developed for the detection of toxicants in environmental samples. The advantages of both antibody and DNA as the sensing elements are: good specification, high sensitivity, and good reproducibility (Rogers 2006; Lei et al. 2006). However, the antibody and DNA are low in stability, high cost, complicated (for DNA extraction) and time-consuming.

Another biological sensing element very commonly used to detect environmental toxicant is microorganism. A basic microbial biosensor is designed by immobilizing microorganism cell on a transducer surface to recognize toxicant in the environmental samples. The microorganisms such as algae, bacteria, and yeast are frequently used in the fabrication of toxicity biosensor, since they can be massively produced through cell culturing. Other advantages of the use of microorganism in the recognition sensing element are: the ability to detect a wide range of chemical substances, amenability to genetic modification, and broad operating pH and temperature range, making them ideal as biological sensing materials (Su et al. 2011). Compared to other cells from higher organism including plants, animals, and human beings, microorganism cells are easier to be mutated and have better viability and stability. The biosensor-based microbial cells greatly simplify the fabrication process and

enhance the performance of biosensors (Su et al. 2011; D'Souza 2001). Microbial cells consist of numerous enzymes and cofactors/coenzymes, endowing themselves with the ability to respond to a number of chemicals, which can be utilized as the signal for sensing purposes. However, microbial-cell biosensors are non-specific in detecting a specific type of toxicant, giving it a drawback.

The toxicity biosensor is usually fabricated via direct contact between microbial cell and the transducer. Therefore, immobilization of microorganism onto the transducer plays an important role in the development of the toxicity biosensor (D'Souza 2001). Many immobilization methods of microorganism involve adsorption, encapsulation, entrapment, covalent binding, and cross-linking technique (Lei et al. 2006; D'Souza 2001). Besides these methods, many novel immobilization techniques have been reported in the recent years in order to improve the performance and stability of the toxicity biosensor. Futra et al. (2014) have developed a microencapsulation technique, which is combined with cellulose nitrate membrane for the immobilization of *Aliivibrio fischeri* for the detection of heavy metals. Futra et al. (2015) proposed a natural polymeric film alginate modified cellulose membrane for the adsorption of GFP *Escherichia coli.* The GFP biosensor was applied to detect single and mixed toxicants in water samples. Ooi et al. (2015a) described an encapsulation strategy using *k*-carrageenan matrix for the immobilization of roGFP2 *Escherichia coli*. Yu et al. (2005) developed a sol-gel for the entrapment of *Moraxella* spp. cells. The resulting toxicity biosensor demonstrated improving cell activity and stability. Conducting polymer has also attracts great attention for its application in the immobilization of microorganism, due to its unique electrochemical properties (Ahuja et al. 2007).

The detection of toxicants in the environmental samples using toxicity biosensors has been successfully developed using various biological elements including DNA, antibody, enzyme, and microorganism. In this part, all the biological elements (DNA, antibody, enzyme, and microorganism) will be reviewed. Deoxyribonucleic acid (DNA) is anion polyelectrolyte, which is the carrier of genetic information and the foundation material of biological heredity. DNA consists of four bases type including adenine, thymine, cytosine, and guanine (Harteis and Schneider 2014). All of these bases will be coupled via hydrogen bonding to form double-strand DNA. The basic for genetic analysis of organism is based on the base pairs adenine/thymine and cytosine/guanine via hybridization reaction. The DNA sequences differ in living organisms and this criteria provides practical way to identify, evaluate, and diagnose various diseases (Harteis and Schneider 2014; Kerman et al. 2004).

Whole-cell biosensors are commonly utilized in evaluation of microbial habitat and provide measurements of the bioavailable fraction of toxicant compound. It is routinely used for the screening of toxicity in the environmental samples and also often used in early warning toxicity of aquatic system (Lei et al. 2006; Belkin 2006). It could produce measurable gene product encoded by reporter gene, which comes from natural microorganism or introduced by genetic manipulation. The reporter genes often used to evaluate environmental toxicity including *lacZ* gene from *Escherichia coli*, *lux* gene from *Vibrio fischeri*, and *gfp* genes from *Aequorea victoria* (Sorensen et al. 2006; Shin 2011). The biosensor based on microorganism

is usually sensitive to various toxicants and is very suitable for the evaluation of environmental toxicity, because enzyme and cofactor needed by microorganism for the detection of toxicant are available inside the cell of microorganism (Lei et al. 2006). The main advantages of microbials as sensing elements are its capability in detecting a wide range of chemicals and its amenability to genetic modification (Shin 2011). Microorganism-based toxicity biosensors have been developed to detect heavy metals, pesticides, and organic compounds. The summary of toxicity biosensor based on various microorganisms for the detection of toxicants is shown in the Table 6.2.

Optical toxicity biosensor based on bacterium has been reported by using marine bacterium *Aliivibrio fischeri* (Futra et al. 2014) and *Escherichia coli* transformed with green fluorescence protein (GFP) genes (Futra et al. 2015) for the detection of heavy metals in water samples. The optical biosensor was designed using microencapsulated alginate microsphere modified CNM (Futra et al. 2014) and alginate film functionalized CNM (Futra et al. 2015) transduced with spectrofluorimetric method. These biosensors were successfully utilized for the evaluation of trace metals with a low detection limits (at ppb to ppm level) and wide linear range. Ooi et al. (2015a, b) also developed a simple and easy optical biosensor based on GFP *Escherichia coli* using *k*-carrageenan as matrix immobilization which is used for the monitoring of sodium dodecyl sulfate in the drinking water and metaloids in water samples. This biosensor also obtained a low detection limit at ppm to ppb levels, with high reproducibility and stability. Eltzov et al. (2015) and Souiri et al. (2012) proposed other toxicity biosensors based on *Escherichia coli* bacteria that are constructed by using alginate bead (Eltzov et al. 2015) and ITO functionalized with PAH(PSS-PAH) (Souiri et al. 2012) for the detection of heavy metals in the water samples. These toxicity biosensors also demonstrated a low detection limit at pM level, with good reproducibility.

Electrochemical toxicity biosensor based on bacteria is also commonly used for the detection of heavy metal and benzene derivatives. The electrochemical biosensor was designed using alginate functionalized cellulose sulfate-PMCG (Schenkmayerova et al. 2015) and reported a low detection limit at uM level. An integrated electrochemical biosensor platform fabricated based on *Arthrospira platensis* bacteria immobilized on gold electrode via physical adsorption (Tekaya et al. 2013) evaluated toxicity based on the alkaline phosphatase activity (APA) from the cyanobacterium. This enzyme activity will be inhibited in the presence of heavy metals, conductivity was taken after addition of the substrate. The biosensor has obtained a low detection limit at fM level, with rapid response time.

Algae have been widely employed for the detection of heavy metals and pesticides in water via a simple optical biosensor based spectrofluorimetry. The optical-fluorescent biosensor developed using *Anabaena torulosa* was fabricated with pHEMA modified cellulose nitrate membrane and without pHEMA (Wong et al. 2013a, b) and designed using *Chlorella vulgaris* based on silica matrix (Nguyen-Ngoc et al. 2009). The optical biosensor based on algae have also achieved a wide linear range and a low detection limit at ppb level.

Table 6.2 The microbial biosensor recently used for the evaluation of various toxic materials

Material used for biosensor fabrication	Microorganism	Type of Biosensor	Toxicants	LOD	References
Alginate microspheres-CNM	*Escherichia coli* GFP	Fluorimetric	Cu(II)	0.04 ppb	Futra et al. (2015)
			Pb(II)	0.32 ppb	
			Pb(II)	0.46 ppb	
			Zn(II)	2.80 ppb	
			Ag(I)	720 ppb	
			Ni(II)	400 ppb	
			Co(II)	250 ppb	
Alginate film-CNM	*Aliivibrio fischeri*	Fluorimetric	Cu(II)	6.40 ppb	Futra et al. (2014)
			Cd(II)	1.56 ppb	
			Pb(II)	47 ppb	
			Zn(II)	320 ppb	
			Ag(I)	18 ppb	
			Ni(II)	2800 ppb	
			Co(II)	1700 ppb	
k-Carrageenan	*Escherichia coli* GFP	Fluorimetric	Sodium dodecyl sulfate	1.7 ppm	Ooi et al. (2015b)
Cellulose nitrate membrane	*Anabaena torulosa*	Fluorimetric	Cu(II)	1.19 ppb	Wong et al. (2013a)
			Pb(II)	0.03 ppb	
			Cd(II)	0.10 ppb	
			2,4-D	0.03 ppb	
			chlorpyrifos	0.03 ppb	

pHEMA-CNM	*Anabaena torulosa*	Fluorimetric	Cu(II)	1.41 ppb	Wong et al. (2013b)
			Cd(II)	0.25 ppb	
			Pb(II)	0.50 ppb	
			2,4-D	0.24 ppb	
			chlorpyrifos	0.12 ppb	
Physically adsorbed on GE	*Arthrospira platensis*	Conductometric	Hg(II)	0.01 fM	Tekaya et al. (2013)
Alginate bead	*Escherichia coli*	Fluorimetric	Cu(II)	1.00 pM	Eltzov et al. (2015)
			As(II)	0.01 pM	
			Zn(II)	0.01 pM	
Silica matrix	*Chlorella vulgaris*	Fluorimetric	Cd(II)	25 ppb	Nguyen-Ngoc et al. (2009)
			Paraquat	1.0 ppb	
Alginate- CS-PMCG	*Gluconobacter oxydans*	Amperometric	2-phenyl-ethanol	1.0 μM	Schenkmayerova et al. (2015)
ITO-PAH(PSS-PAH)- bacteria	*E. coli* strain PHL818	Impedimetric	Cd(II)	1.0 pM	Souiri et al. (2012)
			Hg(II)	1.0 pM	
Alginate-acrylic	*Lentinus sajor-caju*	Potentiometric	Permethrin	1 μM	Arip et al. (2013)
AFM-PVA gel	*Saccharomyces cerevisiae*	Potentiometric	BOD	1 ppm	Chiappini et al. (2010)

Note: *CNM* cellulose nitrate membrane, *pHEMA* poly(2-hydroxyethyl methacrylate), *GE* gold electrode, *CS* cellulose sulfate, *PMCG* poly(methylene-co-guanidine), *ITO* indium tin oxide, *PAH* poly-(allylamine hydrochloride), *PSS* poly-(sodium-4-styrenesulfonate), *AFM* acetate film membrane, *PVA* polyvinyl alcohol

Apart from algae, fungi have also been exploited for electrochemical biosensor constructed for the detection of pesticide and organic pollutant environmental samples (Arip et al. 2013; Chiappini et al. 2010). For instance, Arip et al. (2013) have proposed *Lentinus sajor-caju* based on encapsulated alginate grafted on acrylic microspheres and measured with potentiometry technique. The fungi biosensor demonstrated a wide linear response, with low detection limit at μM level; this contributed for the fungi to have high ability to degrade permethrin to yield the degradation product of 3-(2,2-dichlorovinyl)2–2-dimethylcyclopropanecarboxylic acid. Produced weak acid could be easily detected by the plasticizer-free H^+ selective membrane. Microbial biosensor for organic pollutant based on BOD and CO_2 production was also exploited using acetate film functionalized PVA gel and used for the immobilization of *Saccharomyces cerevisiae* (Chiappini et al. 2010). The proposed microbial biosensor was successfully utilized for the detection of BOD in a low detection limit at ppm level, with satisfactory result.

6.4 Future Prospect

Toxicity biosensor has employed various biological molecules as sensing element such as DNA, antibody, enzyme, and microorganism and is used for the detection of various environmental toxicants. It has also utilized various matrices for biomolecule immobilization to enhance and to improve analytical performance of toxicity biosensor. The toxicity biosensor can play an important role in biological early warning system for real-time toxicity detection and water quality assessment. Therefore, developing a simple and sensitive optical biosensor based on visual test and color change can be handy even for unskilled users making it easier for environmental toxicity testing. In the meantime, electrochemical biosensor is needed to create a simple and compatible electronic kit as assessment tool for environmental toxicity. From the literature, it is clear that toxicity biosensor has great potential and plays a helpful role in the detection of environmental toxicity in biological and aquatic system. Toxicity biosensor for the evaluation of environmental toxicity will continue to show advances with improvement in recognition elements such as antibody, DNA, enzyme, natural microorganism, and gene modification of microorganism. From the previous reports, the immobilization matrix used for the development of electrochemical toxicity biosensor always utilized semiconducting polymer and natural polymer. Therefore, conducting polymer and molecularly imprinted polymer can be good candidates as the immobilization matrix. Further research work is also still required to explore the potential of nanosensors and other novel approaches which could be used to assess environmental safety and also to improve environmental quality.

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