International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Landfill Leachate Toxicity Analysis with *Orechromis mossambicus* (Mozambique Tilapia): A Review

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Abstract

Landfill leachate contains more than 500 types of pollutants. The toxicity level of leachate discharge to the aquatic environment can be measured using living organism or bioassay as biological indicator. Bacteria, invertebrates, fish and plant are the common organism used. There are many types of fish being used in fish bioassay toxicity experiment such as *Anabas testudineus, Leporinus obtusidens, Orechromis mossambicus, Cyprinus carpio* and *Oryzias latipes.* Fish are considered as a secondary consumer and it is located at the end of the aquatic food chain. This paper provides an overview of fish bioassay experiment using *Orechromis mossambicus* or Mozambique Tilapia. *Orechromis mossambicus* is an omnivorous feeder in which it is able to clean up edible garbage in rivers and drainage or irrigation canals. In addition, it can grow and spawn fast as well as survive in low oxygen water which makes it suitable to be used in leachate toxicity experiment. The characteristic of this fish in toxicity experiment as a merit compared to other type of fish and baseline information of fish bioassay and the toxicity level of landfill leachate on *Orechromis mossambicus* were highlighted in this paper.

Keywords: landfill; leachate toxicity; tilapia; fish bioassay.

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1. Introduction

Toxicity assessment with bioassay method has been widely used to assess landfill leachate toxicity. It has been used to calculate the amount of substance an organism can be exposed to before adverse effects are observed [1]. Toxicity assessment using bioassay can represent either acute or chronic exposure [2]. Many organisms were used as biological indicator to assess the toxicity of landfill leachate such as bacteria, green algae, fish and others [1]. For example, several organisms such as crustaceans (*Artemia sp.*), invertebrate (*Daphnia magna* or flea), fish (*Geophagus brasiliensis* or pearl cichlid fish) and plant (*Allium cepa* or onion) were used to determine the toxicity of old landfill leachate in Brazil [3].

Landfilling is the main municipal solid waste disposal method used worldwide. Landfill leachate is produced when moisture from rain water, groundwater, surface water and snow (in temperate countries) enters the dumping site of landfill [4]. While moisture percolates through the waste, it carries along organic, inorganic, heavy metals, colloid, pathogen and other polluted matters [5]. Liquids which are not absorbed or evaporated will flow outward and downward, transporting contaminants from within the landfill. Leachate composition varies significantly among landfills depending on waste composition, waste age and landfilling technology [6]. It potentially contributes to environmental pollution which will affect groundwater and surface water [6]. The characteristic of leachate produced depends on the materials disposed in the landfill. Other factors that influence toxic contaminants in leachate are the decomposition of solid waste, precipitation rates, waste age, and landfill design and operation [7].

The toxicity level of leachate discharge to the aquatic environment can be measured using living organism as biological indicator. Fish bioassay is one of the common methods used and using *Orechromis mossambicus* or Mozambique Tilapia in the bioassay experiment is very rarely reported. *Orechromis mossambicus* or Mozambique Tilapia is a type of tilapia species that is also known generally as black tilapia. Due to its success as a type of fish commonly bred for consumption through aquaculture, its use has spread to tropical and subtropical countries around the world. This hardy fish can tolerate with high and low water temperatures, rears easily, has a broad, omnivorous diet, and has a firm texture and a mild taste which makes it very popular for consumption [8]. It is usually a medium-sized fish with approximate length of about 0.35 m for males and 0.25 m for females, laterally compressed, has long dorsal fins with 10-13 rays and spines [9]. Its scales are large along the snout and fore head and become smaller along the body [10]. Apart from being consumed as food, this fish also can be used in laboratory experiments because of its tolerance with any type of water condition and easy to handle.

This paper provides an overview of fish bioassay toxicity experiments previously reported in literature. The characteristic and morphology of Tilapia or *Orechromis mossambicus* as a merit in bioassay as compared to other types of fish was also highlighted. This review provides baseline information of fish bioassay and the toxicity level of landfill leachate with *Orechromis mossambicus*.

2. Methodology

Literatures published on toxicity assessment from 2001 to 2014 were reviewed. The literature searched was performed using Scopus and Google Scholar search engine with the following keywords: fish bioassay, tilapia and landfill leachate toxicity. The aimed was to gather information on fish bioassay using *Orechromis mossambicus*. In addition, the characteristic of *Orechromis mossambicus* and the toxicity assessment technique used were also highlighted in this paper as an advantage of using this fish in the toxicity experiment. There were 75 papers selected to be reviewed in this paper.

3. Landfill leachate

Landfill leachate mainly consists of large amounts of organic matter including dissolved organic matter, phenol, ammoniacal-nitrogen, phosphate, heavy metals, sulphide, hardness, acidity, alkalinity, salinity, solids, inorganic salts and other toxic compounds [11-16]. The complexity of these characteristics makes the leachate more difficult to manage. Improper waste disposal practice brings about various environmental issues namely environmental degradation and risks to human health to many developing nations in the world [17]. Without a proper lining system, open dumping landfill can leach out highly polluting liquid to the adjacent surface water bodies and also penetrates through the soil layers into the groundwater system [17]. It contains high level of organic pollutants in the form of high COD and TOC while dissolving various heavy metal elements [17-18]. Leachate is formed when moisture content in the landfill is high enough to produce liquid containing mixture of soluble organic compounds in the landfill. The leachate generation is highly dependent on soil types, waste composition, degree of compaction of the waste disposed, amount of landfill received, evapotranspiration, type of landfill and the age of landfill [17, 19]. In addition, leachate produced is also different from time to time. Leachate is produced in high amount during the biodegradation process. Usually the biodegradation of the leachate occurs at a higher rate during the first three to eight years of the landfill use.

Leachate is harmful and toxic to human and environment. Various metals classified as hazardous to the environment such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and cobalt (Co) also can affect human health at trace levels [2]. Leachate also contains toxic and carcinogenic compounds such as Pb, Ni, Cd and others [2]. Pb, which is potentially carcinogenic and toxic to human have an effect on the central nervous system which can cause muscle tremor, convulsions, paralysis, and coma [2, 20].

Other than that, organic compounds in the leachate also can cause mortality to hepatoxicity, immunotoxicity, carcinogenicity, and metabolic alterations on the aquatic organisms as well as humans that can lead to a decrease in the rates of reproduction and other activities in the body [3, 21-23]. Other types of toxic elements also detected in leachate sample such as halogenated aliphatic compound, phthalate esters, inorganic micro components, heavy metals and pesticides [21, 24]. Table 1 lists the common compounds found in landfill leachate.

4. Bioassay Experiment

Bioassay is a method used to measure the effect of certain substances on living organism. Chemical analysis only detects a small percentage of the toxic compounds present in an organism, but in bioassay toxicity tests, the toxicity effects on organisms can be detected even when the contaminants are not identified by the chemical analysis [1]. Bioassay has the ability to reflect the cumulative and synergistic effects of multiple compounds. There are many types of organism that has been used in bioassay experiment such as bacteria, invertebrates, fish and plant. Table 2 shows the various types of organisms that have been commonly used in bioassay experiments.

Table 1: Toxic elements found in landfill leachate

Halogenated aliphatic compound	**Inorganic microcomponents**
• Dichloromethane	• Calcium (Ca)
• *Cis*- 1, 2 – dichloroethylene	• Magnesium (Mg)
• 1,2 – dichloropropane	• Sodium (Na)
Phthalate esters	• Potassium (K)
• Diethyl phthalate	• Ammonium (NH_4)
• Di-isobuthyl phthalate	**Heavy metals**
• Di-n-buthyl phthalate	• Cadmium (Cd)
• Butylbenzyl phthalate	• Chromium (Cr)
• Di-(2-ethylhexyl)phthalate	• Lead (Pb)
• Dimethyl phthalate	• Nickel (Ni)
• Di-n-octyl phthalate	• Zinc (Zn)
• Diisononyl phthalate	• Mercury (Hg)
• Diisodecyl phthalate	• Copper (Cu)
Chlorinated phenols	**Metal organic compounds**
• Monochlorophenols	• Monobutyl tin
• Dichlorophenols	• Dibutyl tin
• Trichlorophenols	• Tributyl tin
• Tetrachlorophenols	• Monooktyl tin
• Pentachlorophenol	• Dioktyl tin
• 4-Nonylphenol	• Methyl mercury
• Nonylphenolmonoethoxylate	**Polycyclic Aromatic Hydrocarbons (PAHs)**
• 4-tert-Octylphenol	• Naphtalene
• Octylphenolmonoethoxylate	• 1-Methylnaphthalene
• 4-tert-Butylphenol	• 2-Methylnaphthalene
• 4-tert-Pentylphenol	• Acenaphthylene
• Bisphenol A	• Fluorene
Organochlorine	• Phenanthrene
• Polychlorinated biphenyls (PCBs)	• Anthracene
• Hexachlorobenzene (HCB)	• Pyrene
• Heptachlor epoxide (HCE)	• Fluoranthene
• Dichlorodiphenyltrichloroethane (DDTs)	• Benzanthracene

Pesticides • Lindane • 2, 4 – D • MCPA • MCPP (mecoprop) • 2,4,5 – T • 2,4 – DP (dichloroprop) • Bentazone • BAM (2,6- dichlorobenzamid)	• Chrysene

Source: [21, 24-27]

Table 2: Types of organisms used in bioassay experiment.

Species tested	Examples	Element tested	Reference
Bacteria	*Vibrio fischeri* (Luminescent bacteria)	Ammonia	[28]
	Photobacterium phosphoreum	Aromatic compounds	[1, 29]
Green algae	*Pseudokirchneriella subcapitata*	Ammonia, nonylphenyl, bisphenol A, or 4-*tert*-butylphenol	[30]
Fish	*Oncorhynchus mykiss* (rainbow trout)	Organophosphates	[31]
	Oreochromis niloticus	Heavy metals (Cr, Co, Cu, Ni, Zn, Pb, Cd)	[32]
Plant	*Lemna gibba and Lemna minor* (*Lemna Sp.*)	Ammonia	[1]
	Allium cepa	Biological oxygen demand (BOD), Iron (Fe) and manganese (Mn)	[3]
Invertebrates	*Daphnia magna*	Manufactured nanomaterials (ZnO, TiO_2, Al_2O_3, C_{60}, SWCNTs, and MWCNTs)	[33]
	Ceriodaphnia dubia	Ammonia, nitrite and nitrate	[23]

Each species of organism selected may have their strength and weaknesses as bioassay sample. For example, bacteria used for acute microbial test systems can be performed within a short period using small amount of sample volume amounting to several millilitres in quantity [28]. The bioassay using luminescent bacteria can be performed in 15 minutes compared to other organisms which required about 48 hours of testing. Moreover, the cost of toxicity assessment monitoring with bacteria is also reduced. Although the used of bacteria as bio indicator could determine organic compounds and ammonia, it reduced the sensitivity towards inorganic compounds [28]. Another limitation is bacteria such as *Vibrio fischeri* has low sensitivity in the screening of effluent toxicity, thus it is not suitable to determine the effect of effluent discharge [34].

In invertebrates, the toxicity testing took several days depending on the type of species used. For example, *Daphnia magna* takes about six to 10 days for acute toxicity test while *Ceriodaphnia dubia* takes about three to five days [1]. The most suitable invertebrate species used over the past 30 years to test the landfill leachate toxicity was *Daphnia magna* because of its sensitivity and it is easy to handle as they are immobilized species [1, 35].

5. Fish Bioassay Toxicity Experiment

Aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals or toxicants which exhibit changes in the aquatic environment [36]. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bio-indicator of environmental pollution [37-39]. Fishes are ideal indicator of contamination in the aquatic ecosystem because they filled in different trophic level, sizes and ages [40].

Moreover, fish is located at the end of the food chain in the aquatic ecosystem, thus it can reflect the water quality level status and act as an indicator of water pollution particularly heavy metals [41]. Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homeostasis of the fish can control, it results in death or cause damages in the fish opercula and may also cause physical damages to fish particularly on the skin, liver and gill surface [36]. Table 3 lists the review of fish bioassays. These are the examples of research paper recommending the using of fish for toxicity testing.

Table 3: Species of tested fish and type of pollutant tested in fish bioassay experiments.

Test species	Test compounds	Type of pollutants	Study location	Reference
Anabas testudineus	Heavy metals (Cd, Cu, Pb, Zn, Mn, K, Hg, Fe) and ammoniacal nitrogen	Sanitary landfill leachate	Malaysia	[17]
Leporinus obtusidens	Pb, Cr, Zn, Cl_2, BOD, COD, P, ammoniacal nitrogen, nitrogen and pH	Landfill leachate	Brazil	[42]
Pangasius sutchi	Heavy metals (Mn, Cu, Zn, Fe, Cr, Al)	Landfill leachate	Malaysia	[43]
Oreochromis niloticus	Heavy metals (Cr, Co, Cu,	Discharged water	Egypt	[32]

(Nile tilapia)	Ni, Zn, Pb, Cd)	containing agricultural, sewage, industrial and domestic wastes		
Oreochromis niloticus	Heavy metals (Cu, Cd, Zn, Pb, Ni)	River and lake	Malaysia	[44]
Orechromis mossambicus (Mozambique tilapia)	Metals, volatile fatty acids, monocyclic aromatic hydrocarbons, semi volatile organic carbon, organo phosphorus pesticides and alcohols	Landfill leachate	Malaysia	[18]
Oreochromis niloticus (Nile tilapia)	Zinc (Zn)	Lab based experiment	Egypt	[45]
Oryzias latipes (Japanese medaka)	Estrogenic activity, bisphenol A, 4-Nonylphenol, 4-tert-Octylphenol	Industrial waste landfill	Japan	[46]
Clarias Batrachus (Cat fish)	Cd and Hg	Industrial waste	India	[47]
Danio rerio (Zebra fish)	Physico-chemical characteristic	Landfill leachate	Brazil	[48]
Oreochromis niloticus (Nile Tilapia)	Zn, Cu and Pb	Lab based experiment	Malaysia	[49]
Cyprinus carpio L. (Common Carp)	Physico-chemical characteristic	Landfill leachate	Malaysia	[50]
Geophagus brasiliensis (Pearl chichild)	Biological oxygen demand (BOD), Iron (Fe) and manganese (Mn)	Landfill leachate	Brazil	[3]
Gambusia affinis	Pass, silicate, screen, methyl red, Cd, Cu, Ni, Zn	Textile dye wastewater	India	[51]
Fundulus heteroclitus	Benzo[*a*]pyrene (B*a*P)	Laboratory based experiment	Canada	[52]
Gambusia affinis	Methyl red, Cu	Textile wastewater	India	[53]
Sparus aurata and *Solea senegalensis*	As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, PCBs, PAHs	Sediment	Spain	[54]
Oryzias latipes	Physicochemical characteristics and heavy metals	Landfill leachate	Japan	[55]

Oryzias latipes (Japanese medaka)	Physicochemical characteristics	Landfill leachate	Japan	[56]
Channa punctatus	Mercuric chloride, heavy metal compound and malathion (organo phosphorus pesticide)	Laboratory based experiment	India	[57]
Melanotaenia nigrans	Cu	Laboratory based	Australia	[58]
Oryzias latipes	4-nonylphenols (NP) and 4-tert-octylphenol (OP)	Laboratory based	Japan	[59]

Most common species of fish used in bioassay experiments in tropical country like Malaysia is *Anabas testudineus, Pangasius sutchi*, *Cyprinus carpio*, *Orechromis niloticus* and *Orechromis mossambicus* [17-18, 43-44, 49-50]. Most of the studies test for physical characteristics of leachate and heavy metals contamination such as Pb, Cr, Zn, Fe and Mn, and other element such as phosphorus. In other country such as Brazil, species of *Leporine obtusidens*, *Danio rerio* and *Geophagus brasilienis* have been commonly used in bioassay experiments [3, 42, 48]. Studies from Egypt used *Orechromis niloticus* as their testing organism [32, 45]. The studies were performed to assess the heavy metals assessment in the fish body. In Japan, Japanese Medaka or *Oryzias latipes* is one of the common fish used in fish bioassay [46, 55-56, 59]. Goldfish is also being used to assess the toxicity. The elements tested are ammonium and sodium chloride coupled with the physico-chemical characteristic of the leachate. In India, *Gambusia affinis* was used to test the toxicity produced by textile wastewater [47, 51, 53, 57]. *Fundulus heteroclitus* was used in Canada to test the toxicity of Benzo[*a*]pyrene (B*a*P) in fish [52]. In Spain, *Sparus aurata* and *Solea senegalensis* were used to test for As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, PCBs, and PAHs toxicity in the sediment [54]. Last but not least, *Melanotaenia nigrans* was used in Australia to test copper toxicity [58].

Some of the reported studies showed variations in the result. This is due to differences in fish species, size, wet weight, length and environmental factor. Fish which is exposed to the increase of metal levels in an aquatic environment can absorb the bioavailable metals directly from the environment via the gills and skin or through the ingestion of contaminated water and food. All the pollutants may accumulate in the tissues and enter the human food chains and may cause a number of ill-health effects to human as the end user of the food chain [60-61].

6. Characteristic of Tilapia (*Oreochromis mossambicus)*

Fish is unique among the vertebrates, a consequence of having two routes of metal acquisition, from the diet and from the water. Fish can accumulate toxic substances from landfill leachate such as heavy metals from the water, into its body. Tilapia is a freshwater fish which belongs to Cichlidae family, the most widely distributed exotic fish in the world [62]. The family of Cichlidae is distributed across Africa, America, India and Ceylon and it includes over 100 species [32, 63]. *Oreochromis mossambicus* or Mozambique Tilapia generally is a medium-sized fish which has long dorsal fins, with about 10-13 rays and spines [9]. It has a large scale along the snout and fore head and small scales along the other parts of the body [10]. The coloration is a dull greenish yellow with weak banding pattern along the body [9]. The size and coloration vary depending on its captivity and diet. Some *Oreochromis mossambicus* look almost black in colour; females, non-breeding males and fry juveniles have a silvery colour on the scales [10]. The adult fish size varies according to its gender with females generally being smaller in size at approximately 0.25 m in length while males is larger being approximately 0.35 m in length. The male has an average weight of 2.5 pounds and the female ranges from 1.8-2 pounds as its maximum weight [9]. *Oreochromis mossambicus* can live in both brackish and salt water, can survive in a wide range of temperatures [9], and can live up to 11 years [10]. They are rarely found at high altitudes and are known to be tropical fish. The habitat range temperature for this species is between 17-35 ºC [64]. It is widely introduced, for example in south-east Asia including Taiwan, India and Japan and also in Trinidad and Tobago. *Oreochromis mossambicus* are omnivorous feeder. Juveniles tend to be carnivorous and eat fry and these fish occasionally cannibalize their own young [10]. Figure 1 shows the picture of *Oreochromis mossambicus.*

Figure 1: Mozambique Tilapia, *Oreochromis mossambicus* [65]

7. Toxicity test of landfill leachate with *Orechromis mossambicus* (Tilapia)

Several studies have been carried out to evaluate the impacts of landfill leachate using zebra fish [55] and Japanese Medaka fish [66]. Mozambique or black tilapia was used as the indicator to evaluate the landfill leachate toxicity in [18]. In Malaysia, the most common tilapia species found is Nile tilapia, red tilapia and black tilapia. All of these tilapias are consumed by Malaysian as food. In order to cater the fish demand supply, tilapias are breed in a large scale. The fish are reared in ponds, cages or pens and they grow well in fresh water and brackish waters. As with other types of tilapia fishes, *O. mossambicus* are omnivorous feeder in which it is able to clean up any edible garbage in rivers and in any drainage or irrigation canals. It is highly adaptable and is an easily cultured type of fish [62]. Due to its characteristic as omnivore’s feeder this fish has the potential to get contaminated from various types of pollutants and it can cause toxicity to the food chain.

Tilapia was chosen and highlighted in this paper because of high market demand for its edible nature and it is also vulnerable to leachate contamination [18]. In high scale tilapia seedling, the main advantage of tilapia is relatively low cost of production, mainly for fry and seed, and the quality of its flesh [36]. Furthermore, in the original extensive or semi-intensive culture systems, tilapias are more resistant to disease compared to other fish species [62]. On the negative side, the intensification of culture systems and resultant deterioration in the environment has caused an increase in parasitic and infectious disease problems to the fish. Although infectious diseases are caused by parasites, host and environmental factors may also contribute as a cause to the infections occurrence [62].

In addition, Mozambique tilapia can be one of the indicators for fish bioassay to determine the toxicity of leachate. The fish is a type of freshwater fish that can be found in the river and lake. For the supply purpose, many entrepreneurs used to seed the fish in the fish farm to cover the market demand. However, for people in the rural areas or those who likes fishing, they used to catch the fish in the lake or river to be consumed as a food. Study by [60] has determined heavy metals accumulation in *Tilapia sp.* fish in Tasik Mutiara, Puchong, Malaysia. The study showed that *Tilapia sp.* in the lake or river has the potential to be polluted with leachate and the safety of this fish for human consumption is dubious. Very limited research found dealing with *Orechromis mossambicus.* Several common researches are listed in Table 4.

Table 4: Toxicity assessment using *Orechromis mossambicus.*

Reference	Element tested	Sample tested
[18]	Metals, volatile fatty acids, monocyclic aromatic hydrocarbons, semi volatile organic carbon, organo phosphorus pesticides and alcohols	1) Leachate sample 2) Fish sample
[60]	Heavy metals (Al, As, Cd, Cu, Fe, Pb and Zn)	1) Water sample 2) Fish sample
[67]	As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Al, Ca, K, Mg and Na	1) Water sample 2) Fish sample 3) Sediment sample

8. Mechanism of pollutants absorption in fish

Over the past few decades, mechanism of pollutant absorption in fish has been studied. For example, a study by [37] has determined an accumulation rate of heavy metals in aquatic organisms. Heavy metals accumulated in the tissues of aquatic living can be a public health concern to both animals and humans [37, 68]. The permeability of the gills and body surface of the fish varies with temperature and other physical variables [69]. The absorption of metals by aquatic organism involves the transfer of metals to the circulatory system across the epithelial barrier of gills, digestive systems or integument [70]. This transfer across the epithelial cells consists of three ways. The first one is the uptake by the apical membrane, the interface with the external environment. Secondly, is the movement through the cell and interaction with intracellular ligands and lastly is the efflux across the basolateral membrane, the interface with the circulatory system. Organs that serve as the sites for uptake (e.g., gills, intestine and digestive gland) also tend to become concentrated with metals and therefore, exhibit relatively high potentials for bio-accumulation. The higher the metal concentration in the environment, the more it may be taken up and accumulated in the fish [71]. Water hardness (mainly related to calcium concentration) affects the uptake of metals across the gill epithelium [71]. The excessive uptake of nutritive metals such as Fe, Cu and Zn can be toxic to the fish [72].

Fish exposed to organo chlorine pollutants will be directly absorbed via gill membranes, and the gastrointestinal membrane via the food uptake [25]. All organo chlorine pollutants have the ability to bio concentrate and bio-accumulate. It was reported that the concentration of metals in fish tissues were not directly related to the corresponding concentrations in water [73], which means not all the metals taken up are accumulated in fish body because fish can regulate metal concentration in tissues to a certain degree. Accumulation of metals can be detected in different parts of the fish for example, the accumulation of Cu have been commonly detected in the liver of the fish while Hg have been detected in both liver and muscle tissues of the fish [74]. There are three possible routes for a substance to enter in a fish: through gills, food and skin [74]. Gill is the first targets of waterborne metals since it is exposed to the aquatic environment. Several studies also claimed that the major route of bio uptake for metals that are concentrated in fish is across the gill epithelium. The accumulated rate is depends on the balance between the uptake rate, metabolism of the chemical and excretion rate. The accumulation of metals in the fish body is via gills and the food consumption by the fish [75]. There are some metals that are essential for the fish. However, these metals can be a toxic to the fish when it is exceed the permissible level. Toxic effects of metals become worst when various metabolic activities inside the organism's body fail to detoxify. Table 5 shows the mechanism of pollutant accumulation in the fish.

Table 5: Mechanism of pollutants accumulation in the fish

Mechanisms	Organs involved	Reference
Via circulatory system	Gills, intestine and digestive gland	[70]
Via gills	Liver, kidney, gills and muscle	[71]
Via gills and intestinal epithelium	Gills and intestine	[72]
Via gill membranes and gastrointestinal membrane	Gills and intestine	[25]
Not stated	Liver and muscle	[73]
Via gill, food and skin	Gill	[74]
Via gills and food	Gills, liver, stomach, kidney	[75]

9. Advantages and disadvantages of fish bioassay

There are several advantages and disadvantages of fish, as biological indicator in assessing landfill leachate toxicity. In the aquatic food chain, fish is located at the end of the chain. This makes fish very suitable to act as the indicator in assessing the toxicity. Fish also can reflect the water quality level in certain places. Other than that, it also can detect the water toxicity even when the contaminants are not identified by chemical analysis. This is due to the natural behaviour of fish which it will response or behave in a certain way to the environmental changes. In many type of fish, tilapias were more resistant to disease than many other fish species although it is the original extensive or semi-intensive culture systems fish [1, 31]. However, in certain level, fish bioassay will have their limitation such as fish can damage easily if the homeostasis between the fish and toxic substances is uncontrolled. This situation may happen and also influenced by the type of fish used in the experiment. Then, like human, fish also have their diseases. The intensification of culture systems and resultant deterioration in the environment can caused an increase in parasitic and infectious disease problems to the fish [40]. Table 6 highlights the advantages and disadvantages of fish bioassay.

Table 6: Advantages and disadvantages of using fish bioassay

Advantages	Disadvantages
1) Reflect water quality level at certain places. 2) The original extensive or semi-intensive culture systems, tilapias were more resistant to disease than many other fish species. 3) Located at the end of food chain, fish can be an indicator to the water pollution in the river or lake. 4) Can detect toxicity even when the contaminants are not identified by chemical analysis. 5) Low cost of production especially when being reared in large scale for food supply	1) Fish can damage easily if the homeostasis between the fish and toxic substances is uncontrolled. 2) The intensification of culture systems and resultant deterioration in the environment can caused an increase in parasitic and infectious disease problems to the fish.

Source: [1, 31, 40]

10. Conclusion

In conclusion, *Oreochromis mossambicus* or Mozambique Tilapia is one of the suitable indicators for toxicity test. It can give more benefits compared to other organisms especially in water toxicity assessment. Characteristic and morphology of *Tilapia sp.* make them a suitable fish species for the leachate toxicity assessment. The pollutants contained in landfill leachate can be detected through this assessment and potential health problems to human can be addressed.

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