ZINC REMOVAL BY NATURAL MICROFLORA

IZWAN BIN ABDUL RAHMAN

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Engineering in Partial Fulfillment of the required for the
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Universiti Malaysia Pahang

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I declare that this thesis entitled “*Zinc Removal By Natural Microflora”* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ....
Name : IZWAN BIN ABDUL RAHMAN
Date : 28 APRIL 2009

Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Abdul Rahman Abdullah and Thahirom bt Md. Kehn
Loving brothers and sisters;
Hafizul, Shuhaida and Faizal

Supportive friends

For Their Love, Support and Best Wishes.

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ABSTRACT

Different kinds of heavy metals exist in the solution with different forms, so their treatment methods are also different. As for the treatment of heavy metal wastewater, many methods have been put forward at home and abroad, including chemical-treatment method, ion-exchange method, electrolytic method, adsorption method, reverse-osmosis method, evaporating concentration and biological treatment. This study used biological treatment to degrade zinc from wastewater. Zinc removal by natural microflora was studied by using sequencing batch reactor(SBR). 5 reactors were used in this studies; 1 10L reactor for accclimatization and 4 5L reactors for treatment. 1 month was used to acclimatize the microflora. Treatment reactors were run for 2 weeks. Every treatment reactor was set to run at specific loading rate which were ranging from 5 mg/L.d to 3 mg/L.d. This study was run to study the effects of different loading rate on the zinc waste treatment, to study the effects of zinc concentration to the growth of microflora and to study the degradation of zinc by microflora in soil.During the run, about 48-88% of zinc and about 30-48% of COD were removed. Mathematical calculation by Design Expert predicted that LR5 gives the maximum value of removal with 39.83% COD removal and 80.81% zinc removal at 486.74 mg/L suspended solid. This study shows that higher LR rate gives higher value of zinc and COD removal but gives lower value of SS.

ABSTRAK

Berlainan jenis logam berat wujud di dalam bentuk yang berbeza; jadi cara untuk merawatnya juga adalah berbeza. Bagi rawatan sisa buangan air logam berat pelbagai cara telah dicadangkan termasuklah cara rawatan kimia, cara rawatan penukaran ion, cara osmosis berbalik, meruapan kepekatan dan rawatan biologi. Kajian ini menggunakan cara rawatan biologi untuk menyinkirkan zink daripada sisa buangan air. Kajian tentang penyingkiran zink oleh mikroflora semulajadi telah dijalankan dengan menggunakan SBR. lima reaktor telah digunakan dalam kajian ini; satu reaktor 10L untuk menternak dan 4 5L reaktor untuk rawatan. satu bulan digunakan untuk menternak mikroflora tersebut. Reaktor rawatan dijalankan selama dua minggu. Setiap reaktor rawatan telah ditetapkan kadar beban yang khas iaitu dari 5 mg/L.h hingga 3 mg/L.h. Kajian ini dijalankan untuk mengkaji kesan kadar beban yang berbeza kepada rawatan zink, untuk mengkaji kesan kepekatan zink pada pertumbuhan mikroflora dan untuk mengkaji penyingkiran zink oleh mikroflora. Pada kadar beban 5, pengiraaan secara matematik oleh *Design Expert* meramalkan nilai maximum penyingkiran dengan 39.83% untuk COD dan 80.81% untuk zink pada nilai pepejal terampai 486.74mg/L. Kajian ini menunjukkan nilai kadar beban yang tinggi memberi nilai penyingkiran zink dan nilai penyingkiran COD yang lebih tinggi tetapi memberi nilai pepejal terampai yg lebih rendah.

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LIST OF ABBREVIATIONS

COD	-	Chemical Oxygen Demand
LR	-	Loading rate
SS	-	Suspended Solid

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CHAPTER 1

INTRODUCTION

1.1 Background

As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. (Lenntech, 1998)

Zinc (Zn) in the environment has both natural and anthropogenic sources. The fate and transport of Zn in soil, freshwater, and marine systems and the essential metabolic roles and toxicologic effects of Zn in biological systems have been reviewed by Nriagu and Davidson, 1998. The largest anthropogenic sources of Zn to the atmosphere are activities related to metal production; second-tier sources to the atmosphere include waste incineration, fossil fuel consumption, phosphate fertilizer, and cement production. Zn sources to air and water related to transportation activities include deicing salts, combustion exhaust, galvanized parts and railings, fuel and oil, brake linings, and rubber tires. Tire-wear particles have been recognized for several decades as a source of Zn to the environment (Landa et al., 2004).

1.2 Problem Statement

Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to zinc chloride can cause respiratory disorders. In the work place environment zinc contagion can lead to a flu-like condition known as metal fever. This condition will pass after two days and is caused by over sensitivity. Zinc can be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc the children may be exposed to it through blood or milk of their mothers (Lenntech, 1998)

Different kinds of heavy metals exist in the solution with different forms, so their treatment methods are also different. As for the treatment of heavy metal wastewater, many methods have been put forward at home and abroad, including chemical-treatment method, ion-exchange method, electrolytic method, adsorption method, reverse-osmosis method, electrodialytic method, evaporating concentration and biological treatment. However, limited by economic and technical reasons, the method mostly adopted at home and abroad for the treatment of heavy metal currently is the chemical method of neutral precipitation. But it has been proved by the application practice that the following problems are present in neutral precipitation method: (1) heavy metal wastewater generally appears acidic, which should not be drained out until neutral treatment with controlling its pH value over 10 by means of neutral precipitation method; (2) when amphoteric metals, such as zinc, lead, tin and aluminum, etc., coexist in the wastewater, along with the raise of waste-water pH value, the amphoteric metals will appear the tendency of redissolving(Xu, 2006)

This research is proposed to study the potential of mixed culture from soil in zinc removal.

1.3 Objective

The main objectives of this research are:

- To study the effects of different loading rate on the zinc waste treatment
- To study the effects of zinc concentration to the growth of microflora
- To study the degradation of zinc by microflora in soil.

1.4 Scope of Research

The scope of study in this experiment is to treat (degrade) zinc in wastewater. The wastewater that contains zinc was simulated with appropriate nutrients for microflora. The initial simulated wastewater was analyzed. Then, experiments were conducted separately in reactor with different loading rate. The efficiency of treatment for different zinc concentrations was evaluated in terms of water chemical oxygen demand (COD) and the changes in zinc concentration. The suspended solids parameter is used to measure the biomass quality inside the reactor.

CHAPTER 2

HEAVY METAL

2.1.1 Introduction

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain. Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. (Lenntech, 1998).

The production of metals results in the formation of emissions – unwanted solids, liquids and gases – directly (during mining and processing) and indirectly (associated with the consumption of raw materials and utilities, e.g. in the generation of electric power and manufacture of reagents and explosives). As mentioned earlier, in the overall supply chain of material needs, mineral resource extraction and processing are particularly critical stages for the potential release of gas, liquid and solid emissions. Ores from mining are often physically beneficiated then chemically transformed to extract metals and produce industrial materials. This requires significant amounts of energy. The impacts are exacerbated by the use of reagents, water and fuel. There are toxicity concerns with a number of metals (e.g. cadmium, lead, and mercury) and even metals that are biologically essential may also become toxic at high levels (e.g. zinc and copper). The issue of metal toxicity is considered in more detail later (Norgate et. al, 2006).

2.1.2 Characteristic of Zinc

Zinc is a lustrous bluish-white metal. It is found in Group 12 of the periodic table. It is brittle and crystalline at ordinary temperatures, but when heated to between 110°C and 150°C it becomes ductile and malleable; it can then be rolled into sheets. It is a fairly reactive metal. Although it is not abundant in nature, it is of great commercial importance. It is used principally for galvanizing iron, but is also important in the preparation of certain alloys, e.g., Babbitt metal , brass , German silver , and sometimes bronze (Columbia Encyclopedia, 2008)

Zinc is a small, hydrophilic, highly charged species, which cannot cross biological membranes by passive diffusion. Therefore specialized mechanism is required for both its uptake and release (da Silva and Williams, 1991)

It is a reasonable conductor of electricity. The density of zinc is 7.140 g/mL, which means the metal will sink in water and is relatively heavy. Most zinc production is based upon sulfide ores. These are roasted in industrial plants to form zinc oxide, ZnO. This may then be reduced with carbon to form zinc metal. Zinc burns in air at high red heat with evolution of white clouds of the zinc oxide. (Ophardt, 2003)

2.1.3 Zinc Sources

Zinc is a very common substance that occurs naturally. Many foodstuffs contain certain concentrations of zinc. Drinking water also contains certain amounts of zinc, which may be higher when it is stored in metal tanks. Industrial sources or toxic waste sites may cause the zinc amounts in drinking water to reach levels that can cause health problems. Zinc occurs naturally in air, water and soil, but zinc concentrations are rising unnaturally, due to addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal and waste combustion and steel processing. Some soils are heavily contaminated with zinc, and these are to be found in areas where zinc has to be mined or refined, or were sewage sludge from industrial areas has been used as fertilizer. Zinc is the 23rd most abundant element in the Earth's crust. The dominant ore is zinc blende, also known as sphalerite. Other important zinc ores are wurzite, smithsonite and hemimorphite. The main zinc mining areas are Canada, Russia, Australia, USA and Peru. World production exceeds 7 million tonnes a year and commercially exploitable reserves exceed 100 million tonnes. More than 30% of the world's need for zinc is met by recycling (Lenntech, 1998).

Zinc (Zn) in the environment has both natural and anthropogenic sources. The fate and transport of Zn in soil, freshwater, and marine systems and the essential metabolic roles and toxicologic effects of Zn in biological systems have been reviewed by Nriagu and Davidson, 1998. The largest anthropogenic sources of Zn to the atmosphere are activities related to metal production; second-tier sources to the atmosphere include waste incineration, fossil fuel consumption, phosphate fertilizer, and cement production (Figure 1). Zn sources to air and water related to transportation activities include deicing salts, combustion exhaust, galvanized parts and railings, fuel and oil, brake linings, and rubber tires. Tire-wear particles have been recognized for several decades as a source of Zn to the environment (Landa et al., 2004)

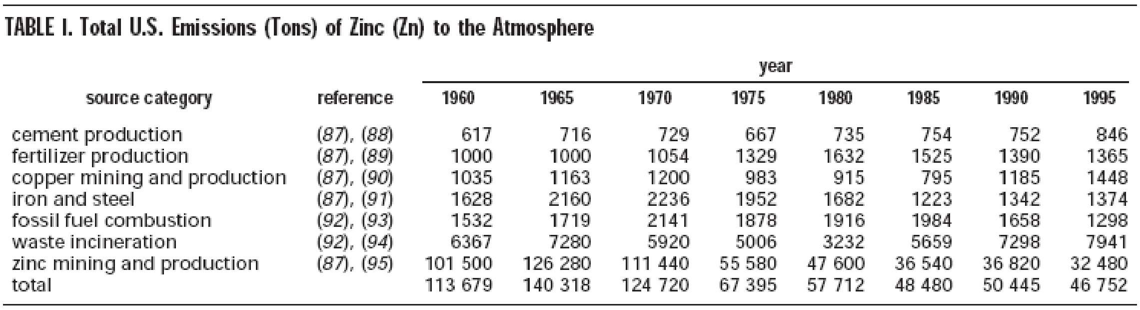


TABLE I. Total U.S. Emissions (Tons) of Zinc (Zn) to the Atmosphere

		year							
source category	reference	1960	1965	1970	1975	1980	1985	1990	1995
cement production	(*87*), (*88*)	617	716	729	667	735	754	752	846
fertilizer production	(*87*), (*89*)	1000	1000	1054	1329	1632	1525	1390	1365
copper mining and production	(*87*), (*90*)	1035	1163	1200	983	915	795	1185	1448
iron and steel	(*87*), (*91*)	1628	2160	2236	1952	1682	1223	1342	1374
fossil fuel combustion	(*92*), (*93*)	1532	1719	2141	1878	1916	1984	1658	1298
waste incineration	(*92*), (*94*)	6367	7280	5920	5006	3232	5659	7298	7941
zinc mining and production	(*87*), (*95*)	101 500	126 280	111 440	55 580	47 600	36 540	36 820	32 480
total		113 679	140 318	124 720	67 395	57 712	48 480	50 445	46 752

Figure 2.1.1: Total U.S. Emissions (Tons) of Zinc (Zn) to the Atmosphere

2.1.4 Disadvantages of Zinc

Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to zinc chloride can cause respiratory disorders. In the work place environment zinc contagion can lead to a flu-like condition known as metal fever. This condition will pass after two days and is caused by over sensitivity. Zinc can be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc the children may be exposed to it through blood or milk of their mothers (Lenntech, 1998)

Ingestion of zinc can affect the central nervous system, and birds with neurologic signs may have died as a result of colliding with walls or structures in the aviaries. The absence of histologic lesions usually associated with zinc toxicosis in affected birds may have been related to acute head trauma as the cause of death (Avian Medicine and Surgery, 2000)

Adverse effects of zinc deficiency are well documented and can lead to impaired immune and reproductive functions as well as impaired cognition (Yasui, 1987). Recently, however, attention has focused on the possibility of adverse effects of elevated concentrations of zinc, which can play a major role in cell damage following stroke (Koh et al., 1996) and may be a risk factor in Alzheimer's disease (Cuajungco et al., 2000). Zinc supplementation has increased in usage in the elderly, a population at risk for cognitive impairment, following the publication of a study (AREDS, 2002), which found that both antioxidants and zinc supplementation (with copper) can slow the loss of visual acuity in advanced stages of macular degeneration. Elevated zinc can also have adverse gastrointestinal effects in the elderly (Houston et al., 2001). Excessive zinc supplementation can result in toxic effects in adolescents and younger adults and impaired cognitive processes in infants (Hamadami et al., 2002). Penland et al. (2000) found cognitive impairment following zinc supplementation in women with low copper levels.

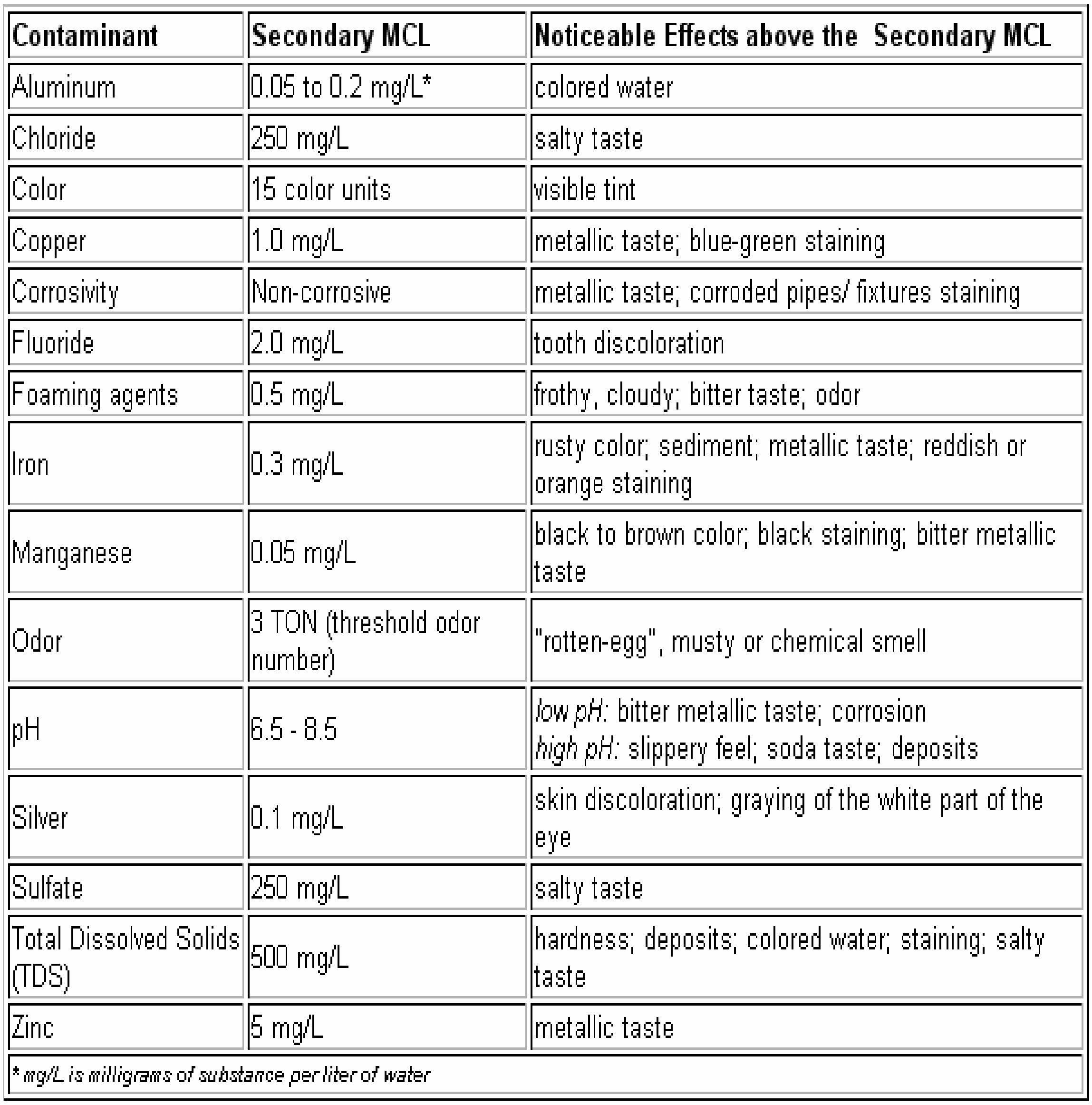
2.1.5 Zinc Level of Discharge

Environmental quality regulations are used to establish the maximum permissible concentrations of analytes that are allowed for a variety of medium (water, soil, sediments, fish, foodstuffs, etc.) before a site can be deemed to be polluted or contaminated. An obvious problem with such regulations are that they are recommendations and not always enforceable in law. Moreover, if water regulations are used as an example, an evaluation of the various international or national limits shows a wide degree of variability, and an inconsistency with regard to the list of analytes; e.g. for the sulphate limits (mg/l) of drinking water: EC (250), USEPA (250), WHO (400), Canadian (500) and Australia (400). In the case of heavy metals many limits do not exist or they cover wide levels which have only a limited use for the many types of environmental media under regulatory investigation (Ward, 2003)

Environmental legislation has been enacted in most countries and the standards have been made more stringent than ever including Malaysia. Malaysia’s DOE (Department of Environment) is empowered under the Environmental Quality Act 1974 (Amendment) 1985, to control and prevent pollution, as well as to protect and enhance the quality of the environment in Malaysia. Government has initiated programs to control scheduled waste management to safeguard the environment and the safety of the people.

In Figure 2, Standard A catchment’s are as referred to in this regulation shall be the areas upstream of surface or above subsurface water supply intakes, for the purpose of human consumption including drinking(Department of Environment, 2007) Standard B is applied to all areas beside areas stated for standard B. As for zinc, according to Figure 2, it is stated that the maximum concentration that can be released to the environment is at 2 mg/L for standard A and standard B.

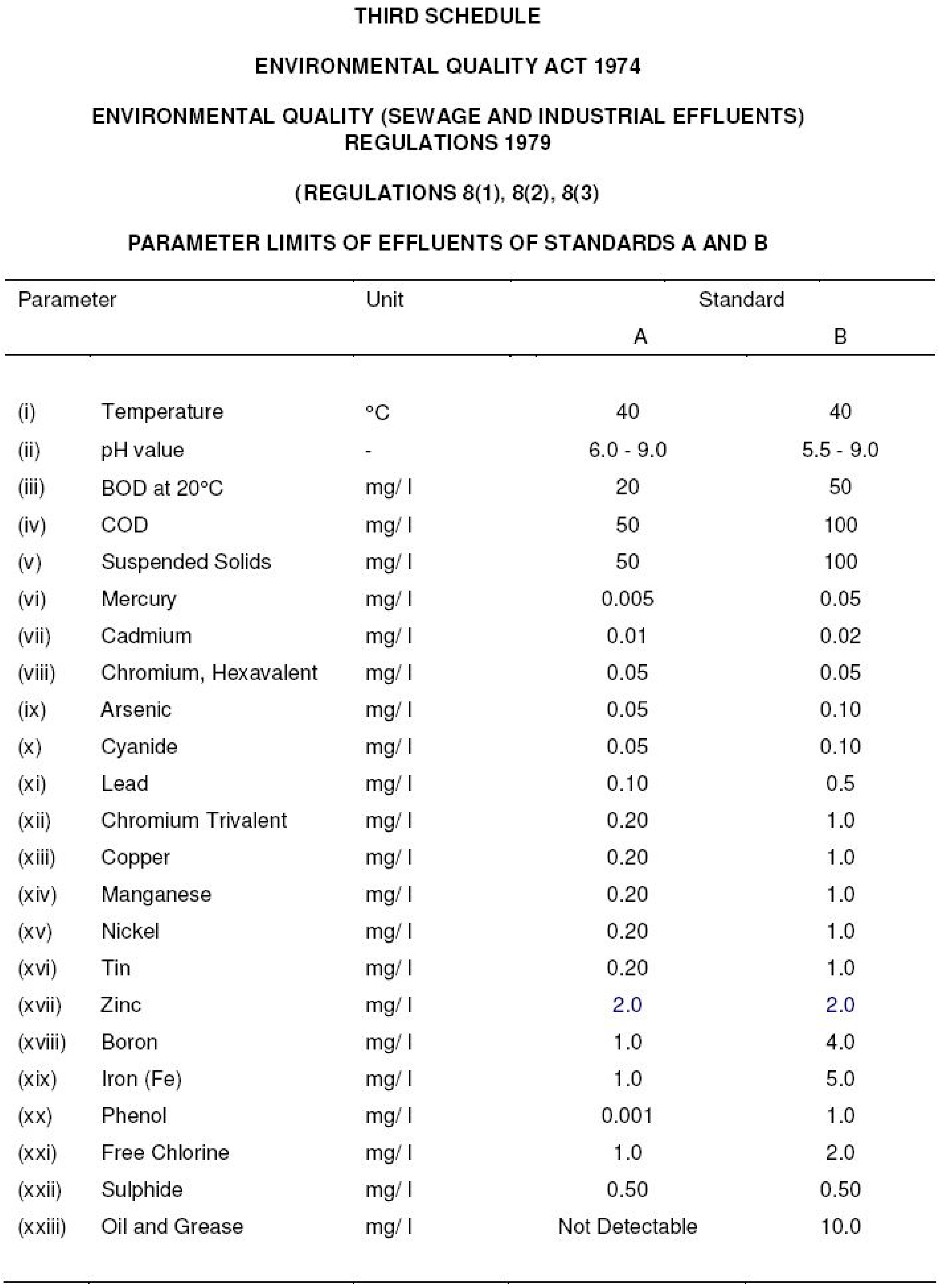
The U.S. Environmental Protection Agency (EPA) has established National Primary Drinking Water Regulations that set mandatory water quality standards for drinking water contaminants. These are enforceable standards called "maximum contaminant levels" or "MCLs", which are established to protect the public against consumption of drinking water contaminants that present a risk to human health. An MCL is the maximum allowable amount of a contaminant in drinking water which is delivered to the consumer.



Contaminant	Secondary MCL	Noticeable Effects above the Secondary MCL
Aluminum	0.05 to 0.2 mg/L*	colored water
Chloride	250 mg/L	salty taste
Color	15 color units	visible tint
Copper	1.0 mg/L	metallic taste; blue-green staining
Corrosivity	Non-corrosive	metallic taste; corroded pipes/ fixtures staining
Fluoride	2.0 mg/L	tooth discoloration
Foaming agents	0.5 mg/L	frothy, cloudy; bitter taste; odor
Iron	0.3 mg/L	rusty color; sediment; metallic taste; reddish or orange staining
Manganese	0.05 mg/L	black to brown color; black staining; bitter metallic taste
Odor	3 TON (threshold odor number)	"rotten-egg", musty or chemical smell
pH	6.5 - 8.5	*low pH:* bitter metallic taste; corrosion *high pH:* slippery feel; soda taste; deposits
Silver	0.1 mg/L	skin discoloration; graying of the white part of the eye
Sulfate	250 mg/L	salty taste
Total Dissolved Solids (TDS)	500 mg/L	hardness; deposits; colored water; staining; salty taste
Zinc	5 mg/L	metallic taste

** mg/L is milligrams of substance per liter of water*

Figure 2.1.2: Secondary Maximum Contaminant Levels



THIRD SCHEDULE

ENVIRONMENTAL QUALITY ACT 1974

ENVIRONMENTAL QUALITY (SEWAGE AND INDUSTRIAL EFFLUENTS)
REGULATIONS 1979

(REGULATIONS 8(1), 8(2), 8(3)

PARAMETER LIMITS OF EFFLUENTS OF STANDARDS A AND B

Parameter		Unit	Standard	
			A	B
(i)	Temperature	°C	40	40
(ii)	pH value	-	6.0 - 9.0	5.5 - 9.0
(iii)	BOD at 20°C	mg/ l	20	50
(iv)	COD	mg/ l	50	100
(v)	Suspended Solids	mg/ l	50	100
(vi)	Mercury	mg/ l	0.005	0.05
(vii)	Cadmium	mg/ l	0.01	0.02
(viii)	Chromium, Hexavalent	mg/ l	0.05	0.05
(ix)	Arsenic	mg/ l	0.05	0.10
(x)	Cyanide	mg/ l	0.05	0.10
(xi)	Lead	mg/ l	0.10	0.5
(xii)	Chromium Trivalent	mg/ l	0.20	1.0
(xiii)	Copper	mg/ l	0.20	1.0
(xiv)	Manganese	mg/ l	0.20	1.0
(xv)	Nickel	mg/ l	0.20	1.0
(xvi)	Tin	mg/ l	0.20	1.0
(xvii)	Zinc	mg/ l	2.0	2.0
(xviii)	Boron	mg/ l	1.0	4.0
(xix)	Iron (Fe)	mg/ l	1.0	5.0
(xx)	Phenol	mg/ l	0.001	1.0
(xxi)	Free Chlorine	mg/ l	1.0	2.0
(xxii)	Sulphide	mg/ l	0.50	0.50
(xxiii)	Oil and Grease	mg/ l	Not Detectable	10.0

Figure 2.1.3: Parameters Limits of Effluent of Standards A and B

2.2.1 Introduction

Microorganisms have been used in a number of biological treatment processes for metals remediation (Thompson and Watling, 1987; Macaskie and Dean, 1989; Smith et al., 1994). The microbe-based technologies can provide an alternative to conventional methods for metals removal (Gadd, 1992). Microorganisms can affect metallic contaminants directly or indirectly (pH changes, bioaccumulation, or biosorption) (Al-Shahwani et al., 1984; Beveridge and Doyle, 1992; Vesper et al., 1996). Interaction with microorganisms is an effective detoxification process for transition and heavy metals, utilizes both pure as well as mixed microbial cultures that can be dead or alive (Smith et al., 1994), works over a wide range of pH and temperature conditions, has low capital and operating costs, and minimizes waste (Churchill et al., 1995). This interaction includes both bioaccumulation and biosorption. Metals can be bioaccumulated by living organisms through complexation, coordination, ion exchange, chelation, and adsorption (Volesky, 1990).

Despite toxic stress, microorganisms have evolved several strategies to resist metal toxicity, removing or accumulating the metal through various physical-chemical mechanisms and transport systems of different specificity. Some of such strategies are biosorption (Volesky and May Phillips, 1995), alterations in membrane permeability to metals (Levine and Marzluf, 1989), the production of chelating agents (Butt and Ecker, 1987), energy-dependent efflux of metals (Nies and Silver, 1995; Tynecka and Malm, 1995), the biochemical transformation of metal ions (Yamamoto et al., 1992) and the precipitation of metals as insoluble salts (White and Gadd, 1996). In some cases, metal resistance is plasmid mediated (Mergeay et al., 1985; Silver and Walderhaug, 1992; Nies and Silver, 1995; Udo et al., 2000).

2.2.2 Microorganism from Soil

Whiting et al. (2001), employed natural soil microorganisms (bacteria and fungi) to increase the bioavailability of metals in contaminated soils. This has two advantages. First, microbial chelates are likely to occur predominantly in the vicinity