EFFECTS OF TRACE ZINC, COPPER AND CHROMIUM ON ORGANIC ADSORPTION CAPACITY AND ORGANIC REMOVAL IN ACTIVATED SLUDGE

By

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A Thesis for the Degree of Doctor of Philosophy

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To my parents

DECLARATION

I hereby declare that the thesis entitled "Effects of Trace Zinc, Copper and Chromium on Organic Adsorption Capacity and Organic Removal in Activated Sludge" is original and has not been submitted for other degrees or the like in this University or any other institutes. It does not contain any material, partly or wholly, published or written by others, except those references quoted in the text.

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Abstract

Heavy metals are commonly found in municipal sewage that contains industrial effluents. It is well documented that metal concentrations above toxic levels inhibit biological processes in municipal sewage treatment works and discharge of metals into surface waters can have severe impacts on the environment, ecosystems and public health.

However, the effects of trace metals at sub-toxic concentrations on biological sewage treatment processes and the mechanisms of their effects on COD removal and COD adsorption capacity (CAC) are less well documented. This study investigated and compared the effects of copper-, zinc-, lead- and chromium-laden wastewater at sub-lethal levels on organic removal efficiency in a simulated activated sludge culture, namely a sequencing batch reactor. The characteristics and mechanisms of adsorption of heavy metals at sub-lethal concentrations by activated sludge bioflocs and the effects of metals on COD removal efficiencies and CAC were also studied using batch activated sludge cultures and experiments.

Experimental results showed that adsorption capacity and rate of copper, zinc, lead and chromium on microbial flocs were much higher than that of

organic matters. Metals affected not only the adsorption rate of organic matter but also the CAC of the activated sludge. Metal-laden wastewater at sub-lethal levels affected the activated sludge process performance in organic reduction to different extents, depending on the operating hydraulic retention time (HRT). Metal ions acted as a strong competitor against organic matters for active sites on the bioflocs instead of acting as a toxic microbial inhibitor, thus hampering organic adsorption and affecting the COD removal efficiency under shorter HRTs. A mathematical model has been developed to predict values of CAC. Experimental results validated the proposed model.

Copper was found to have the highest effects on CAC and COD removal efficiencies, and therefore the mechanisms of adsorption and the effects of copper on the activated sludge microbes were investigated on a microscopic level. A Gram-negative bacterium, *Pseudomonas putida* 5-X was isolated from activated sludge. Cells with superficial layer removed, isolated cell envelopes, and the separated peptidoglycan layer were compared for copper adsorption properties with that of fresh cells. The treatment with 0.3 N HCl obviously enhanced the copper adsorption capacity, due to the degradation of the superficial layer-capsule. In intact cells, the partly metal-binding sites are inaccessible for heavy metal adsorption, but the isolated cell envelopes liberated much more metal-binding sites on cell surface and enhanced copper adsorption capacity. Although peptidoglycan layer of *P. putida* 5-x cell

envelope has some copper binding capacity, both the outer membrane and the inner membrane played an important role on the binding and adsorption of copper. Fresh cells, pretreated cells and whole cell envelopes of *P. putida* 5-x all could be described with *Freundlich* equation, but the adsorption model of separated peptidoglycan layer could be better described with *Langmuir* equation. The results indicated that the adsorption property of peptidoglycan layer with copper is different from that of intact cells or whole cell envelope.

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CHAPTER 1 : INTRODUCTION

1.1 WATER RESOURCE

Water resources include ocean and inland, surface and underground water bodies, and water vapour in the atmosphere. Out of the 1.36 x 10^{18} m^3 of water resources, more than 99 % of which are either saline water, locked in polar ice and rock formations, or geographically inaccessible. Furthermore, out of the 5 x 10^{13} m^3 of remaining water resources that are conveniently available for human consumption, a significant fraction of which are contaminated, do not meet potable water standards and cannot be readily used without elaborate treatment. (Ian and Liu, 1999)

Distribution of the water resource in the world is seriously uneven and unbalanced as some areas occupy much water resource but some do not. This renders some areas to have a high ratio of water volume per head, while other areas are less fortunate in this aspect. Take China for instance, the nation has 2711.5 x 10^9 m^3 of the water resource, which is ranked the sixth, in terms of the occupation of the water resource in the world. However, heavy metals, organic and other contamination from industrial, agricultural and municipal sources have render a substantial fraction of these water resources unavailable as potable water for human consumption. After taking this into consideration, China is only ranked the 121th in the list of water volume per head with a value of 2239 m^3/head. A recent study (Ian and Liu, 1999) warns that about thirty percent of the world population, including a significant fraction of the Chinese population, will run the risk of severe water shortage by the year 2025. Locally, in Hong Kong, the Water Supply Department (WSD, 1999) reported that pollutants, including heavy metals, have rendered the water resources from the southern China region less suitable for consumption and elaborate treatment is necessary. Therefore, recycle and reuse of wastewater and pollution control and quality of water resources become a major concern.

1.2 HEAVY METAL CONTAMINATION

Heavy metals, such as copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), silver (Ag), cadmium (Cd), and chromium (Cr), are commonly found in municipal sewage and industrial effluent. These common heavy metals are extensively used in the electroplating and metal-processing industries. Barium (Ba), lead (Pb), iron (Fe) and mercury (Hg), are the major waste constituents in the manufacturing of paints, paper, plastics, batteries, alloys, refractories, and scientific instruments (Lester *et al.*, 1979; Sawyer and McCarty, 1978).

The number of industries that discharge heavy metal laden wastes to domestic sewers has increased significantly during the past 30 years. In view of the toxic effects often caused by the presence of these wastewaters, even at very low concentrations, the general practice of combining pretreated or partially pretreated industrial and domestic wastes is being revaluated by a number of communities. The discharge of heavy metals into surface water can have severe effects on the environment, and metals may enter the food chain and eventually adversely affect human health. For nearly a century, industrial discharges of heavy metal-laden wastewater in relation to the environment and public health have been of concerned. Numerous investigations have then been carried out. Heavy metal residues in contaminated habitats may accumulate in micro-organisms, aquatic flora and fauna, which in turn, may enter into the human food chain, accumulate in the human body and result in serious health problems (Cooke *et al.*, 1990; Deniseger *et al.*, 1990).

Boudene (1978), Gatti *et al.* (1978), Lafontaine (1978), Lauwerys (1978), Norseth (1978), have showed the health effects of heavy metals on human. For instance, mercury causes headache and patresthesia of the tongue, lips, fingers and toes; chromium damages the liver and kidney.

1.3 EFFECTS OF HEAVY METALS ON ACTIVATED SLUDGE PROCESSES

Heavy metals are toxic to most microorganisms and often cause serious upsets in biological waste treatment systems. The interest of study on heavy metals in activated sludge microbes is their toxic effects. The toxicity of heavy metals in a given biological treatment system generally depends upon its species and concentration. Toxicity also depends upon the type of influent, its strength, and the extent of system acclimation. It has been reported that only soluble metal ions are able to cause toxicity (Sujarittanonta and Sherrard, 1981). The degree of toxicity has also been found to decrease with an increasing organic strength of wastewater (Bagby *et al.*, 1981). Other factors such as pH and sludge concentration have also been reported to affect the toxicity of metals, though to a lesser degree (Dilek and Yetis, 1992; Imai and Gloyna, 1990; Yetis and Gokcay, 1989). The successful implementation of effluent limitation guidelines for the metal-related industries leading to the lowering of metal concentrations in the effluent with no appreciable toxic effects on biological treatment processes (Patterson and Kodukula, 1984).

It is well documented that heavy metals at high concentrations are toxic to living organisms in aquatic environments. Numerous research studies have been conducted to investigate the toxic effects of heavy metals on biological treatment processes. It is widely recognized that metal-laden municipal sewage is toxic to biological processes in municipal sewage treatment plants. (Neufeld, 1976; Brown and Lester, 1979; Nelson *et al.*, 1981; Battistoni *et al.*, 1993; Codina *et al.*, 1994). It was also reported that microorganisms in activated sludge were inhibited by copper and zinc at concentrations of above 1 and 10 mg/L, respectively (Lamb and Tollerfson, 1973; Wong and Chang, 1991; Dilek and Gokcay, 1996; Dilek *et al.*, 1998). Sierp and Fransemeier (1933) investigated the effect of copper on an activated sludge system. They found that copper at a concentration of 1 mg/L had a detectable influence in increasing effluent turbidity and decreasing the extent of nitrification, but showed only a slight impact on the efficiency of BOD removal. Gellman (1998) also reported that 1 mg/L of copper affects the activated sludge process. He found that the copper toxicity decreased at a pH between 5 and 6.5. Suthirak and Sherrard (1981) and Zarnovsky *et al.* (1994) observed that activated sludge microorganisms and process efficiency were inhibited by cadmium, chromium and nickel at concentrations above 10 mg/L. The toxicity of heavy metal shock loads at around 20 mg/L on activated sludge was in a descending order of Hg, Ni, and Cd, and depended on sludge age and specific metal adsorption site on the microbial flocs (Battistoni *et al.*, 1993; Zarnovsky *et al.*, 1994).

In recent years, stringent effluent discharge standards have been implemented worldwide, requiring pretreatment of industrial effluents to substantially reduce the heavy metal contents. However, a small fraction of heavy metals, at trace concentrations, is inevitably discharged and ultimately reached the municipal treatment works. In general, heavy metals at trace concentrations meeting the discharge standards are believed to have no detrimental effect on microorganisms in activated sludge processes, and are taken up by microbial cells as essential micro-nutrients, involving such mechanisms as ion exchange, adsorption and complexation (Brown and Lester, 1979; Ghosh and Bupp, 1992; Lion *et al.*, 1988; Mullen *et al.*, 1989; Liehr *et al.*, 1994). On the other hand, Tan and Chua (1997) observed that heavy metals, such as copper and zinc, at concentrations of 1 mg/L and below could affect the COD removal efficiency of activated sludge process. Low concentrations of heavy metals could physically affect the adsorption and uptake of organic compounds in activated sludge. (Nelson *et al.*, 1981; Tan, 1993). However, published works on the effects of heavy metals, at sub-lethal concentrations, on the activated sludge microbes are relatively insufficient.

1.4 OBJECTIVES

In this study, the effects of copper-, zinc- and chromium-laden wastewater at sub-lethal levels on organic removal efficiency of activated sludge in a sequencing batch reactor (SBR) were investigated and compared. Chromium was selected to represent common toxic heavy metals, copper was selected for its high affinity towards adsorption sites on microbial cells, and zinc was selected to represent abundant and less toxic heavy metals in the environment. Sub-lethal concentrations for these three heavy metals were defined in accordance with the non-toxic drinking water standards from the World Health Organization (World Health Organization, 1980). The characteristics and mechanisms of adsorption of heavy metals at sub-lethal concentrations by activated sludge bioflocs and the effects metal ions on COD adsorption capacity (CAC) were examined. Batch experiments and examinations of activated sludge microbes were carried out for the study.

1.5 SCOPE OF THIS STUDY

The aforementioned objectives were achieved in three levels of studies. Firstly, the effects of three selected heavy metals, namely chromium (Cr), copper (Cu) and zinc (Zn) at sub-lethal concentrations, on CAC and COD removal efficiencies in a simulated activated sludge processes were investigated using a SBR system operated under different hydraulic retention times (HRTs). Chromium was selected to represent common toxic heavy metals, copper was selected for its high affinity towards adsorption sites on microbial cells, and zinc was selected to represent abundant and less toxic heavy metals in the environment. Sub-lethal concentrations for these three heavy metals were defined in accordance with the non-toxic drinking water standards from the World Health Organization (World Health Organization, 1980). The results are analyzed and reported in Chapter 4.

Secondly, the adsorption mechanisms of a selected metal, namely chromium, on activated sludge bioflocs and the adsorption isotherms were studied using shake flask cultures. Chromium was selected in this study because of the presence of Cr^{3+} and Cr^{6+} which have substantially different level of toxicity. The results are analyzed and reported in Chapter 5.

Thirdly, the characteristics of heavy metal uptake and adsorption of heavy metals on active sites on the cell surface structures were studied on a microscopic level. Copper was selected for this purpose because of its high affinity towards the active adsorption sites on the surface of the microbes. A common activated sludge microbial species, namely *Pseudomonas putida 5-x*, was isolated for this study. The results are analyzed and reported in Chapter 6.

CHAPTER 2 : LITERATURE REVIEW

This literature review is organised into four sections as follows:

1. The sources of heavy metals and their pollution problems in the water environment of Hong Kong;
2. The effects of heavy metals on activated sludge;
3. Relations between heavy metals and COD adsorption capacity; and
4. The development and operation of sequencing batch reactors.

Heavy metals have been defined in many ways (Nieboer and Richardson, 1980; Phipps, 1981). In general, chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), molybdenum (Mo), silver (Ag), mercury (Hg), cadmium (Cd), nickel (Ni) and metals with atomic mass larger than that of sodium are widely accepted as heavy metals. Besides, metals from groups IIA, IIB, IVB and VB of the periodic table, namely aluminum (Al), beryllium (Be), tin (Tn), thallium (Th), lead (Pb) and bismuth (Bi) as well as the metalloids arsenic (As), selenium (Se) and antimony (Sb) are often included toxic heavy metals. A more specific and most commonly used definition of heavy metals is metals with a density greater than 5 g/cm^3 (Passow *et al.*, 1961). Under this definition, 69 elements belong to the term of heavy metals.

16 out of the 69 elements are synthetic and these metals are actually of very diverse chemical properties. However, this definition of heavy metals was rather arbitrarily chosen from physical parameters (Martin and Coughtrey, 1982). This prompted Nieboer and Richardson (1980) to propose a scheme to avoid using the term 'heavy metals'. They suggested using three categories, namely, Class A, Class B and Boreline elements based on equilibrium constants that describe the formation of metal ion / ligand complexes. Class A elements which show preferences of ligands containing oxygen; Class B elements which show preferences of ligands with nitrogen or sulphur; Boreline elements of intermediate character between Classes A and B, which also reflect their Lewis acid properties (Phipps, 1981).

The more common 'heavy metal' as that defined by Passow *et al.* (1961) is adopted in the present study, and will be used throughout this thesis.

2.1 HEAVY METALS POLLUTION IN THE ENVIRONMENT OF HONG KONG

In Hong Kong, the major contribution of heavy metals is from the industrial discharge of effluent from metal finishing and electroplating industries (Environmental Protection Department, 1989). The chemical composition of electroplating effluent has been determined by Chiu *et al.* (1987) and the heavy metal contents and pH of wastewater discharged from sixteen local electroplating factories are summarised in Table 2.1. Electronics and printed circuit board industries are other local sources of heavy metal pollutants. The industries in Southern China that produce metal-laden wastewater are more diversified, and may also indirectly affect the Hong Kong environment. Barium-laden wastewater is discharged by paper and paints manufactories. Batteries and plastics industries produce cadmium wastewater. Chromium is commonly used in the manufacture of alloys, refractories, chemical catalysts and other chromic salts. Copper is applied as sulphate salt for control of algal growth in water supply reservoirs, while mercury is used in the production of amalgams, scientific instruments, batteries, arc lamps and gold and silver mining. Nickel, zinc, cobalt and silver are common metals for the electroplating processes. Zinc is also widely used for making galvanised pipes. Although lead is seldom used because of its toxicity, it is widely used in the past in the production of lead service pipes, lead based paints and

Table 2.1

Content of metals, cyanide and pH in electroplating effluent discharged from sixteen local electroplating factories (Chiu *et al.*, 1987)

Parameters	Range
Chromium	1-40 mg/L
Copper	1-30 mg/L
Nickel	3-365 mg/L
Zinc	4-250 mg/L
Aluminium	10-230 mg/L
Silver	2-3 mg/L
Cyanide	1-6 mg/L
pH	1.7-8.2

catalytic tetraethyl-lead gasoline for internal-combustion engines.

For the 2.5 million m^3 of water per day consumed in Hong Kong, 2.0 million m^3 become wastewater and are discharged each day. The heavy metals from the aforementioned sources, among other pollutants, will enter into the water environment either directly or via the sewerage works. Hopkins *et al.* (1964) stated that the environmental pollution problem associated with metals is one of redistribution by man's industrial and agricultural activities and the reason why the pollution of heavy metals is of particular interest is because heavy metals have strong attraction to biological tissues and, in general, the elimination process in biological systems is extremely slow (Oehme, 1978).

Different heavy metals have different paths to infiltrate or convert from one form or substance to another. For instance, methyl-mercury is produced from inorganic mercury as a consequence of microbial activity. Its compounds accumulated in fish tissues and transferred to brain tissues of human when consumed. Such compounds accumulated and stored in brain tissues have been linked to mental impairment and in some cases it has caused death. The well-known outbreak of methyl-mercury poisoning in Japan following the consumption of polluted fish and shellfish can be referred to Study Group on Minamata Disease (1968) and Tsuchiya (1969). More details on the mercury toxicology can be referred to Goldwater (1964) [history of mercury toxicology], Clarkson *et al.* (1972) [pharmacology and toxicology], Carty and Malone (1979) [chemistry of mercury in biological system], Swedish Expert Group (1971) [toxicology of methyl-mercury], and Friberg and Vostal (1972); Task Group on Metal Accumulation (1973); Task Group on Metal Toxicity (1976); WHO (1976; 1980); and Dickman *et al.* (1999) [toxicology and epidemiology].

For another example, as high as 3.4 mg/L of arsenic in well water in the Cordoba region of Argentina was measured. Such high level of arsenic has been recorded to cause 165 deaths. A high proportion of the deaths was due to cancer of the respiratory system and gastrointestinal tract (Pershagen, 1983). The toxicity of arsenic is dependent on its oxidation state, chemical form and solubility in the biological media (Subramanian, 1988). More details on the arsenic can be referred to a study conducted by Viraraghavan *et al.* (1999). Examples of poisoning from other heavy metals can be referred to Werrin (1963) for the antimony; WHO (1981) for the arsenic; Allen (1943) for the barium; Yamagata and Shigematsu (1970) for the cadmium and Glover (1970) for the selenium. Table 2.2 shows examples on the path of infiltration of some heavy metals and their impact on human health.

As the toxicity of heavy metals affects man, many water quality monitoring stations have been setup to monitor the contents of heavy metals in

Table 2.2

A summary of health impact induced by heavy metals

Metals	Path	Impact on the Human Health
As	*e.g.* soil → plants	*e.g.* increased risk of cancer of the lymphatic and hematopoietic system
Cd	*e.g.* soil → plants	*e.g.* kidney, liver, testis, nervous system, pancreas cardiovascular system damage
Cr	*e.g.* soil → plants	*e.g.* liver and kidney damage
Hg	*e.g.* soil → plants	*e.g.* headache, patresthesia, of the tongue, lips, fingers and toes
Ni	*e.g.* soil → plants	*e.g.* kidney damage
Pb	*e.g.* canned foodstuffs → human	*e.g.* lead-poisoning
Ti	*e.g.* tinned foodstuffs → human	*e.g.* vomiting and diarrhoea

inland water bodies in Hong Kong. There are 24 river water quality monitoring stations in Hong Kong, including Shing Mun River, Tai Po River, Tolo Habour, Channel Water Control Zone, Lam Tsuen River, Mui Wo River, Stanly Stream, Port Shelter, Junk Bay Water Control Zone, River Indus, River Beas, River Ganges, Yuen Long Creek, Kam Tin River, Tin Shui Wai Nullah, Fairview Park Nullah, Deep Bay Water Control Zon, Tuen Mun River, Tung Chung River, Pai Min Kok Stream, Sam Dip Tam Stream, Kau Wa Keng Stream and Kai Tak Nullah. These 24 monitoring stations cover main inland water bodies in Hong Kong providing a meaningful indication to water quality in Hong Kong. The locations of these 24 monitoring stations are shown in the Appendix I.

It has been shown by the Hong Kong Environment Protection Department (EPD) (1997; 1998; 1999) that the most serious heavy metal contents were at Kau Wa Keng Stream (Cd), Kai Tak Nullah (Cr), Yuen Long (Cu and Pb), and River Indus (Zn). A summary of the highest heavy metal contents measured between 1996 and 1998 is tabulated in Table 2.3.

These data in Table 2.3 shows that heavy metal disposal measured at two monitoring stations, i.e. Kai Tak Nullah and Yuen Long, was significantly reduced from high values in 1996 to low values in 1998. However, heavy metal disposal measured at other two monitoring stations, i.e. Kau Wa Keng

Table 2.3

A summary of heavy metals in water quality monitoring stations. (Data presented are in annual medians of monthly samples; figures in brackets are annual ranges) (Hong Kong EPD, 1997; 1998; 1999)

(a) Concentration of cadmium

Kau Wa Keng Stream – KW 3			
	1996	**1997**	**1998**
Cd **(μg/L)**	1.20 (0.10 – 2.40)	1.75 (0.70 – 3.70)	1.90 (1.20 – 8.20)

(b) Concentration of chromium

Kai Tak Nullah – KN4			
	1996	**1997**	**1998**
Cr **(μg/L)**	100 (1.0 – 610.0)	14.5 (1.0 – 100.0)	1.00 (1.0 – 11.0)

(c) Concentrations of copper and lead

Yuen Long – YL1			
	1996	**1997**	**1998**
Cu (µg/L)	156.5 (27.0 – 4,200.0)	40.5 (8.0 – 220.0)	24.5 (9.0 – 340.0)
Pb (µg/L)	13.0 (2.0 – 24.0)	4.0 (2.0 – 19.0)	2.0 (1.0 – 18.0)

(d) Concentration of zinc

River Indus – IN2			
	1996	**1997**	**1998**
Zn (µg/L)	345 (120.0 – 6,800.0)	380 (110.0 – 4,400.0)	855 (100.0 – 11,000.0)

Stream and River Indus, was significantly increased from low values in 1996 to high values in 1998. Standards for effluent discharge have been issued to guide and enforce the effluent discharge into drainage and sewerage systems, inland and coastal water (EPD, 1991). Different limits have been set for different water quality control zones. For instance, standards for effluents discharged into foul sewers leading to government sewage treatment plants are as shown in Table 2.4 (EPD, 1991), which shows that the allowable substrate concentrations of discharged effluent are decreased with the increase of the amount of discharged effluent. Compared with the substrate concentrations measured and specified, the accumulated substrate concentrations were significantly higher than the allowable concentrations specified.

Following the massive re-location of metal-related industries to the southern China region, concentrations of residual heavy metals in the watercourses in Hong Kong have been on declining trends.

In the case of marine water bodies, on the other hand, high concentrations of heavy metals in coastal waters (Chan *et al.*, 1974), rivers entering into estuaries (Wong *et al.*, 1980) and tissues of coastal marine organisms (Chan *et al.*, 1990; Chu *et al.*, 1990; Phillips, 1989) have been reported. Although concentrations of toxic metals in sea-water are usually below the concentrations which directly cause mortality, several investigations (Oakden *et al.*, 1984; Sullivan *et al.*, 1983; Lang *et al.*, 1980; Mirkes *et al.*, 1978) reported that sub-lethal concentrations may still affect the physiological and behavioural functioning of the organisms. Lo and Fung (1992) conducted a study on the pollution of heavy metals in the sediments at Hebe Haven of Sai Kung, Hong Kong. They found concentrations as high as 0.93, 120, 131, 42.3 and 50.9 mg/kg for cadmium, chromium, zinc, copper and lead, respectively. The corresponding enrichment factors were 13 (Cd), 7.0 (Cr), 2.9 (Zn), 5.2 (Cu) and 2.4 (Pb). Recently, Owen and Sandhu (2000) reported their measurements on heavy metal accumulation in Tolo Harbour, Hong Kong. They measured that chromium levels ranging between 14-30 mg/kg. Nickel ranged between 4-10 mg/kg in the Inner Tolo Harbour, but rose to 17 mg/kg at Tolo channel, probably due to definite migration patterns. Very low concentration of cadmium was reported. In addition to Tolo Harbour and Tolo channel, measurements near Tai Po and Centre Island (centre between Tai Po, Ma On Shan and Shatin) were also reported. Enrichment factors obtained for copper, zinc, lead and nickel were 4.2, 1.9, 1.3 and 3.3 respectively. A summary of the concentrations reported in Lo and Fung (1992) and Owen and Sandhu (2000) is shown in Table 2.5.

With respect to flux, the ranges of fluxs in μg/cm^2/yr reported by Lo and Fung (1992) were 0.006-0.18 (Cd), 2.5-22 (Cr), 6.6-20 (Zn), 3.0-6.4 (Cu) and

3.7-7.4 (Pb). The high chromium flux of 22 μg/cm^2/yr indicates the important contribution from the dyeing industry. Additionally, Lam *et al.* (1997) reported their study on the pollution of chromium, copper and nickel in coastal estuarine sediments by domestic and industrial effluents. In the overall metal content in all labile geo-chemical phases, the levels of chromium, copper and nickel in sediments of Victoria Harbour were significantly higher than those of Tolo Harbour. As Victoria Harbour was not a water quality control zone of Hong Kong, data were not complete and readily available. EPD (1997) showed that the sediments in Victoria Harbour and Tsuen Wan contained relatively high concentrations of copper (> 65 mg/kg) and mercury (> 75 mg/kg). Wong *et al.*, (1995) reported their measurements of heavy metals in Voctoria Harbour. The ranges of heavy metal concentrations measured were 98-259 mg/kg for zinc, 28900-34100 mg/kg for iron, 47-71 mg/kg for lead, 2.6-3.3 mg/kg for cadmium, 58-171 mg/kg for chromium, 45-922 mg/kg for copper and 24-64 mg/kg for nickel. EPD (1997; 1998 and 1999) showed the concentrations of chromium and copper at Tolo Harbour and such data are tabulated in Table 2.6. Table 2.6 shows that the concentrations of chromium and copper were significantly higher than those of allowable values specified by the EPD (1991).

In addition to the high concentrations of heavy metals in sediments indicating the pollution in Hong Kong waters, high concentrations of heavy metals were also measured from marine animals, *e.g.* fish. So *et al.* (1999) reported their measurements of heavy metals measured from rabbitfish as rabbitfish is one of the most common marine fish species that can easily be found either off shore or around the coastal region of Hong Kong (Chan, 1995). Besides, Shen *et al.* (1998) also investigated the concentrations of heavy metals, however, using tilapia collected from Shing Mun River. A summary of the results from So *et al.* (1999) work is shown in Table 2.7. The overall metal concentrations as shown in Table 2.7 were lower than the values specified by the local authority (Hong Kong Government, 1987). A scheme of water quality criteria proposed at an International Sediment Forum in 1982 suggested three categories for classifying contaminants. These three categories are:

- 'Target' class limiting the concentrations of copper and zinc to be 25 mg/kg and 180 mg/kg respectively;
- 'Standard' class limiting the concentrations of copper and zinc to be 70 mg/kg and 750 mg/kg respectively; and
- 'Limit' class limiting the concentrations of copper and zinc to be 400 mg/kg and 2500 mg/kg.

Table 2.4

Standards for effluents discharged into foul sewers leading to government sewage treatment plants reported (HK EPD, 1991)
(All units are mg/L)

Flow rate (m^3/day)	≤ 10	> 10 and ≤ 100	> 100 and ≤ 200	> 200 and ≤ 400	> 400 and ≤ 600	> 600 and ≤ 800	> 800 and ≤ 1000	> 1000 and ≤ 1500	> 1500 and ≤ 2000	> 2000 and ≤ 3000	> 3000 and ≤ 4000	> 4000 and ≤ 5000	> 5000 and ≤ 6000
Determinand													
pH	6 – 10	6 – 10	6 - 10	6 – 10	6 - 10	6 – 10	6 - 10	6 – 10	6 – 10	6 - 10	6 - 10	6 – 10	6 – 10
Temperature	43	43	43	43	43	43	43	43	43	43	43	43	43
Suspended solids	1200	1000	900	800	800	800	800	800	800	800	800	800	800
Settleable solids	100	100	100	100	100	100	100	100	100	100	100	100	100
BOD	1200	1000	900	800	800	800	800	800	800	800	800	800	800
COD	3000	2500	2200	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Oil & grease	100	100	50	50	50	40	30	20	20	20	20	20	20
Iron	30	25	25	25	15	12.5	10	7.5	5	3.5	2.5	2	1.5
Boron	8	7	6	5	4	3	2.4	1.6	1.2	0.8	0.6	0.5	0.4
Barium	8	7	6	5	4	3	2.4	1.6	1.2	0.8	0.6	0.5	0.4
Mercury	0.2	0.15	0.1	0.1	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Cadmium	0.2	0.15	0.1	0.1	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Copper	4	4	4	3	1.5	1.5	1	1	1	1	1	1	1
Nickel	4	3	3	2	1.5	1	1	0.8	0.7	0.7	0.6	0.6	0.6
Chromium	2	2	2	2	1	0.7	0.6	0.4	0.3	0.2	0.1	0.1	0.1
Zinc	5	5	4	3	1.5	1.5	1	0.8	0.7	0.7	0.6	0.6	0.6
Silver	4	3	3	2	1.5	1.5	1	0.8	0.7	0.7	0.6	0.6	0.6
Other toxic metals individually	2.5	2.2	2	1.5	1	0.7	0.6	0.4	0.3	0.2	0.15	0.12	0.1
Total toxic metals	10	10	8	7	3	2	2	1.6	1.4	1.2	1.2	1.2	1
Cyanide	2	2	2	1	0.7	0.5	0.4	0.27	0.2	0.13	0.1	0.08	0.06
Phenols	1	1	1	1	0.7	0.5	0.4	0.27	0.2	0.13	0.1	0.1	0.1
Sulphide	10	10	10	10	5	5	4	2	2	2	1	1	1
Sulphate	1000	1000	1000	1000	1000	1000	1000	900	800	600	600	600	600
Total nitrogen	200	200	200	200	200	200	200	100	100	100	100	100	100
Total phosporus	50	50	50	50	50	50	50	25	25	25	25	25	25
Surfactants (total)	200	150	50	40	30	25	25	25	25	25	25	25	25

Table 2.5

Summary of the concentrations (Lo and Fung, 1992; Owen and Sandhu 2000)

Metal	Concentration (mg/kg)				
	Tolo Harbour	Tolo channel	Near Tai Po	Near Centre Island	Hebe Haven
Ca	-	-	-	-	0.93
Cr	14 – 30	-	-	-	120
Cu	21	-	84	45	42.3
Ni	4 – 10	4 – 17	-	-	-
Pb	48	-	144	84	2.4
Zn	100	69	270	158	131

Table 2.6

Concentrations of chromium and copper in Tolo Harbour (HK EDP, 1997; 1998; 1999)

Minor stream in Tolo Harbour (Tai Po Kau Stream)			
	1996	**1997**	**1998**
Cr (µg/L)	1.0 (1.0 – 9.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)
Cu (µg/L)	1.0 (1.0 – 4.0)	1.0 (1.0 – 2.0)	1.5 (1.0 – 7.0)

Table 2.7

A summary of the concentrations of cadmium, copper, lead and zinc in tissues of rabbit fish collected in Tolo Harbour, Victoria Harbour and outside Hong Kong (So *et al.*, 1999)

	Cadmium concentration (mg/kg)			Copper concentration (mg/kg)			Lead concentration (mg/kg)			Zinc concentration (mg/kg)		
	Tolo Harbour	Victoria Harbour	Outside HK	Tolo Harbour	Victoria Harbour	Outside HK	Tolo Harbour	Victoria Harbour	Outside HK	Tolo Harbour	Victoria Harbour	Outside HK
Gills												
Mean	0.025	0.081	0.022	1.00	2.91	0.27	0.164	0.421	0.140	23.72	22.90	15.23
SD	± 0.019	± 0.300	± 0.014	± 1.12	± 4.34	± 0.06	± 0.169	± 0.253	± 0.077	± 10.56	± 10.27	± 4.93
Median	0.021	0.020	0.019	0.76	2.03	0.26	0.112	0.373	0.137	20.67	19.94	16.51
Range	0.004 - 0.101	0.001 – 2.221	0.008 - 0.053	0.04 - 7.08	0.08 - 25.1	0.17 - 0.34	0.004 - 1.021	0.126 - 1.442	0.043 - 0.292	9.47 - 65.90	9.69 - 57.10	3.42 - 19.46
Liver												
Mean	0.386	0.271	0.627	5.97	9.82	10.59	0.093	0.216	0.022	44.92	41.02	39.36
SD	± 0.203	± 0.201	± 0.580	± 3.73	± 10.69	± 15.93	± 0.099	± 0.420	± 0.013	± 13.31	± 28.96	± 16.91
Median	0.323	0.200	0.452	4.88	5.73	2.79	0.058	0.101	0.019	46.55	34.55	34.21
Range	0.035 - 1.010	0.014 – 0.816	0.152 - 1.775	1.85 - 21.14	1.35 - 53.03	1.65 – 46.93	0.003 - 0.543	0.005 - 2.909	0.007 - 0.040	22.33 - 75.57	6.71 - 234.95	11.79 - 66.32
Muscle												
Mean	0.006	0.010	0.004	0.21	0.42	0.15	0.009	0.105	0.007	4.63	6.79	4.63
SD	± 0.003	± 0.014	± 0.001	± 0.170	± 0.780	± 0.050	± 0.006	± 0.314	± 0.003	± 1.790	± 9.660	± 2.290
Median	0.005	0.005	0.004	0.18	0.21	0.16	0.015	0.031	0.005	4.20	4.34	3.99
Range	0.003 - 0.022	0.002 – 0.074	0.001 - 0.006	0.01 - 1.28	0.02 - 5.13	0.08 – 0.24	0.00 - 0.102	0.00 - 2.351	0.003 - 0.012	1.81 - 10.28	2.31 - 9.30	2.19 - 9.30

The concentrations of heavy metals measured in other parts of Hong Kong can be referred to in the publications by Ong Che (1999) [Mai Po] and Nicholson (1999) [Kat O, North Point, Yung Shue Wan and Central].

Chua *et al.* (1998) reported their experimental study on the characterisation of raw sludge, chemically modified sludge and anaerobically digested sludge in Hong Kong. Both physical and chemical analyses were carried out on the samples obtained from the field, including Chemically Assisted Primary Sedimentation (CAPS), raw sludge from Tai Po Sewage Treatment Plant (rTP), raw sludge from Yuen Long Sewage Treatment Plant (rYL), anaerobically digested sludge from Tai Po Sewage Treatment Plant (dTP), and anaerobically digested sludge from Yuen Long Sewage Treatment Plant (dYL). They reported the speciation of metals in various sewage treatment plants as shown in Tables 2.8 to 2.12.

Besides, they reported the total heavy metal contents in sludge in various sites as shown in Table 2.13.

Table 2.8

The speciation of metals (% extracted) in CAPS sludge (Chua *et al.*, 1998)

Phase	**Cu**	**Ni**	**Cd**	**Pb**	**Cr**	**Zn**
Adsorbed	1.99	9.31	8.76	11.2	2.30	21.92
Carbonate-bound	18.27	3.61	0.0	11.58	3.09	8.71
Organically-bound	13.02	18.14	18.99	26.86	15.3	27.8
Exchangeable	2.14	24.74	6.96	8.36	2.53	1.22
Sulphide-bound	34.7	14.41	11.3	11.12	5.06	20.20
Forms not extracted	29.88	29.79	53.99	20.36	71.18	20.14

Table 2.9

The speciation of metals (% extracted) in dTP sludge (Chua *et al.*, 1998)

Phase	**Cu**	**N**	**Cd**	**Pb**	**Cr**	**Zn**
Adsorbed	1.01	5.16	23.5	10.7	4.0	2.98
Carbonate-bound	5.77	4.23	0.0	9.53	2.26	21.89
Organically-bound	11.18	16.56	38.95	28.62	5.82	14.96
Exchangeable	6.76	16.37	14.95	24.04	8.97	2.7
Sulphide-bound	53.33	16.95	16.11	13.64	11.69	22.09
Forms not extracted	21.95	40.73	6.49	13.47	66.66	35.21

Table 2.10

The speciation of metals (% extracted) in dYL sludge (Chua *et al.*, 1998)

Phase	Cu	Ni	Cd	Pb	Cr	Zn
Adsorbed	3.84	3.97	16.02	13.8	3.14	3.34
Carbonate-bound	5.13	0.0	0.0	16.27	13.11	56.19
Organically-bound	7.83	12.44	11.92	27.83	36.88	18.38
Exchangeable	8.34	13.61	0.0	25.64	2.87	0.20
Sulphide-bound	42.78	24.69	16.44	14.05	33.07	11.72
Forms not extracted	32.08	45.29	50.6	2.41	10.93	10.17

Table 2.11

The speciation of metals (% extracted) in rTP sludge (Chua *et al.*, 1998)

Phase	Cu	Ni	Cd	Pb	Cr	Zn
Adsorbed	4.76	22.59	21.52	16.24	13.48	20.13
Carbonate-bound	6.14	15.85	16.70	0.0	28.16	18.78
Organically-bound	24.53	27.38	31.91	2.43	27.65	22.83
Exchangeable	12.99	22.39	14.51	29.44	13.82	3.00
Sulphide-bound	35.79	9.90	15.22	14.53	14.89	11.91
Forms not extracted	15.76	1.89	0.14	13.36	2.60	23.35

Table 2.12

The speciation of metals (% extracted) in rYL sludge (Chua *et al.*, 1998)

Phase	**Cu**	**Ni**	**Cd**	**Pb**	**Cr**	**Zn**
Adsorbed	3.04	13.80	14.78	17.78	11.05	1.04
Carbonate-bound	4.14	9.87	10.61	0.0	11.50	33.27
Organically-bound	8.95	14.58	20.96	20.94	16.56	25.37
Exchangeable	6.27	20.58	11.83	29.97	9.34	5.96
Sulphide-bound	30.56	14.23	12.86	23.03	33.17	27.86
Forms not extracted	47.04	26.94	28.96	8.28	18.36	6.55

Table 2.13

Total heavy metal contents in sludge in various sites.

(ppm in dry weight basis) (Chua *et al.*, 1998)

Values in parentheses are standard deviation of means of triplicates

Metal	**EPA range**	**CAPS**	**dTP**	**dYL**	**rTP**	**rYL**
Cu	84 – 17,000	2,069.98 (114.89)	107.37 (3.267)	100.78 (7.583)	63.88 (3.299)	92.63 (8.735)
Zn	101 – 49,000	545.94 (82.23)	454.4 (26.873)	5152.05 (195.70)	264.82 (35.96)	3,122.6 (103.43)
Ni	2 – 5,300	264.58 (9.463)	72.93 (6.396)	126.65 (21.507)	60.86 (7.074)	72.91 (4.986)
Cd	1 – 3,410	50.44 (3.483)	14.40 (0.548)	32.31 (2.898)	33.46 (3.458)	31.92 (1.803)
Cr	10 – 99,000	724.19 (35.08)	157.70 (32.873)	607.13 (15.335)	61.06 (21.60)	96.34 (9.954)
Pb	13 – 26,000	482.72 (47.31)	207.72 (3.777)	358.44 (11.809)	91.50 (88.58)	73.41 (13.593)

2.2 EFFECTS OF HEAVY METALS ON ACTIVATED SLUDGE

Besides the impact of heavy metal pollutants on human health and environmental ecosystems, the toxic effects on activated sludge processes in municipal sewage treatment plants have also received significant attention. Lester's work (1983) has already provided an extensive review on biological treatments. In this literature review section, topics to be reviewed are new and other than that given by Lester (1983). Toxic effects of heavy metals in wastewater treatment processes have been a subject of study for a long time. The toxicity of heavy metals in activated sludge depends mainly on two factors, namely, metal species and concentration. Other factors such as pH, sludge concentration, influent strength and type are also reported to affect the toxicity of metals, though to a lesser degree (Dilek and Yetis, 1992; Imai and Gloyna 1990; Yetis and Gokcay, 1989). The successful implementation of effluent limitations guidelines leading to the influent concentrations of heavy metals that are low enough to have no appreciable toxic effects on biological treatment processes (Patterson and Kodukula, 1984). Works have then been concentrated on the water-quality management and sludge-disposal management (Imai and Gloyna, 1993). With respect to the water-quality management, high efficiencies of metal removal from wastewater treatment processes are expected. In additional to the removal of heavy metals, adsorption characteristics and mechanisms of heavy metal uptake, microbiology, and oxygen uptake rate are also of interest as subjects of study. A summary of the effects of metal ions on activated sludge from previous investigations are summarised in Table 2.14. More recent and specific studies are shown in the following sub-sections.

2.2.1 Removal Efficiency of Heavy Metals

The removal efficiency of heavy metals in wastewater treatment processes has been extensively studied after discovering that the activated sludge could remove a large amount of metals from wastewater. Different removal efficiencies have been reported in a number of different and specifically designed investigations. In general, lower removal efficiencies were obtained from earlier investigations and higher removal efficiencies were obtained from later investigations. Brown *et al.* (1973) found that removal efficiencies of cadmium and chromium in the activated sludge process were poorer than those of copper, lead and zinc which are between 60 and 70%. Lower removal efficiencies of copper (30-33%), nickel (15-19%), iron (36-49%), manganese (32-33%) and mercury (10%) were measured and reported by Oliver and Cosgrove (1974). They also found that the removal of heavy

Table 2.14

A summary of effect of metal ions on activated sludge (Hanel, 1988)

Metal ion	Authors	Effects observed
Ag^+	Reimann (1969)	1 mg/L causes 2 % inhibition. 10 mg/L causes 57 % inhibition after two days (Ag as $AgNO_3$. in Sapromat)
Cu^{2+}	Barth *et al.* (1965)	Limiting concentration for continuous input 1.2 mg/L
	McDermott *et al.* (1963)	Limiting concentration for continuous input 1.0 mg/L Elevation of turbidity of treated effluent from 0.8 mg/L Limiting concentration for shock loads (4 hrs with t_{BB} = 6 hrs) 50 mg/L for $CuSO_4$. 10 mg/L for copper cyanide complex
	Reimann (1969)	10 mg/L causes 38 % inhibition. 50 mg/L causes 85 % inhibition of substrate respiration after five days (Cu^{2+} as $CuSO_4$. Sapromat)
Zn^{2+}	Neufeld (1976)	Above 40 mg/L deflocculation occurs
	Brown and Andrew (1972)	Under continuous input. 5-10 mg/L no adverse effect. 20 mg/L causes inhibition
	Barth *et al.* (1965)	Limiting concentration for continuous input 5-10 mg/L
	Offhaus (1968)	91.9 % inhibition of substrate respiration for 20 mg/L after 24-48 hrs. 81.6 % inhibition after 48-72 hrs. falling to 66.8 % after 72-96 hrs and 45.7 % after 96-120 hrs. Corresponding values for acclimatised sludge 46.6 %. 2.8 %. 16.0 %. 17.6 %. Measurements made in Sapromat with Zn as $ZnSO_4$
Ni^{2+}	McDermott *et al.* (1963)	Limiting concentration for continuous input 1-2.5 mg/L; with continuous input of 2.5-10 mg/L. BOD removal efficiency is 5 % lower
Hg^{2+}	Neufeld (1976)	Deflocculation occurs from 100 mg/L
Cr^{6+}	Bonomo (1974)	For continuous input at 10 mg/L. BOD_5 removal = 90 %. at 40 mg/L. BOD_5 removal = 83 %. at 60 mg/L. BOD_5 removal very low
	Moore *et al.* (1961)	Limiting concentration for continuous input: 10 mg/L at 50 mg/L; BOD_5 removal drops to about 3 % For shock loading: at 10 mg/L no effect: at 100 mg/L deflocculation
Cr^{3+}		Non-toxic: in tannery effluents at 40 mg/L Cr^{3+} and $B_{TS} \leq 0.5$ kg/kg d. BOD removal exceeds 90 % and Cr^{3+} removal 90 %
Cd^{2+}	Neufeld (1976)	Deflocculation occurs from 20 mg/L
	Weber and Sherrard (1980)	Limiting concentration for continuous input: 10 mg/L
Co^{2+}	Reimann (1969)	Total inhibition of respiratory activity at 2000 mg/L At 1 mg/L substrate respiration after 5 days reduced by 5 %, at 10 mg/L by 26 %, at 20 mg/L by 43 %, at 50 mg/L by 59 % (Co^{2+} as $CoCl_2$ in Sapromat)

metals by activated sludge occurred in two separate stages, namely, by primary settling of insoluble metals or metal sorption onto particulates and by sorption of dissolved metals or fine particulate metals onto the biological floc in the aeration tanks, with the subsequent settling out of the biological floc in the secondary clarifier. Stones (1977) reported that removal efficiencies of chromium, copper, lead, nickel and zinc did not exceed 45%. On adding cadmium, the removal efficiencies between 30 and 50% have been reported by Roberts *et al.* (1977). With decreasing the values of MLSS (mixed liquor suspended solid) from 1.40 down to 0.17 g/L, the removal efficiencies of cadmium were also decreased from 92 down to 25% (Port, 1978). Besides, Port (1978) reported that the removal efficiency depended on pH. For copper, less than 78% of removal efficiency was measured at pH less than 7 and the removal efficiency ranged between 78-98% was measured at the pH greater than 7. Moriyama *et al.* (1992) reported their study on the accumulation of zinc with different concentrations (0.5, 1.0, 2.5 and 5.0 mg/L) in activated sludge with different solid retention times (SRTs) (5, 10, 15 and 20 days). They found that the concentrations of zinc, cadmium, copper, chromium, lead, iron, manganese and nickel in activated sludge increased up to 10^5 times with and increase in the settled sewage heavy metals concentration. For all levels of zinc concentrations, the maximum concentration of zinc in the activated sludge was measured at the SRT of 15 days. Experimental results showed that the maximum concentration of zinc did not further increase by prolonging the SRT. The highest concentration ratio (heavy metal concentration in activated sludge / heavy metal concentration in settled sewage) for zinc, copper, chromium, lead and iron occurred at the SRT of 12 days. However, in these published literature, the changes in the organic degradation efficiency of the activated sludge, after adsorbing heavy metals, have not been studied nor reported. This leads to the objectives of this research project.

2.2.1.1 Heavy Metals Toxicity in Activated Sludge

Weber and Sherrard (1980) reported their experimental study on the effects of cadmium on the completely mixed activated sludge processes. Two concentrations of cadmium (5.15 and 9.98 mg/L) were selected for investigation on their toxic effects on activated sludge with different sludge ages (4.2, 5.6, 9.0 and 12.3 days for 5.15 mg/L; 5.1, 7.6, 10.6 and 13.1 days for 9.98 mg/L). From the study of 5.15 mg/L of cadmium, experimental results showed that the organic removal efficiency in the activated sludge process between influent and effluent averaged between 25 and 55 % and decreased with the sludge age. COD (chemical oxygen demand) removal efficiency was independent of sludge age among the sludge ages studied. Nitrification was observed at all sludge ages studied, however, it was not comparable with the control. From the study of 9.98 mg/L of cadmium, experimental results showed that effluent NO_3-N levels increased from 0.8 to 14.1 mg/L with the increase of sludge age. Sterritt and Lester (1981) reported their study on the influence of the sludge age on heavy metal removal in the activated sludge process. In their study, six sludge ages ranged between 3 and 18 days were selected. Ten heavy metals (cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, silver, and zinc) were selected and added continuously in the activated sludge system with different sludge ages to investigate their removal ability. They reported that cobalt, manganese and molybdenum removals were poor and were affected by changing the sludge with other sludge ages. The highest removal efficiencies for the other metals occurred at the 15 days of the sludge age, excepted for nickel which had the maximum removal efficiency occurred at the 18 days of the sludge age. Their results on removal in generally agreed with values reported by previous investigators, i.e. manganese and nickel (Oliver and Cosgrove, 1974), copper, lead and zinc (Stoveland *et al.,* 1979, Roberts *et al.*, 1977). They also reported that the removal of chromium generally followed the pattern of accumulation of MLSS as the sludge age increased. This implies that chromium was strongly associated with the biomass. A semi-quantitative comparison of affinity of the metals for the biomass between different metals was given as following, Cr > Cd > Ag > Pb > Zn > Cu > Ni, Co, Mn, Mo

Chang *et al.* (1986) reported that a high removal efficiency of dissolved organic matter, though exposed to constant input level of heavy metals, was maintained from acclimated sludge. Stephenson *et al.* (1987) measured that removals of cadmium and copper were always in excess of 75 %, however, removal of nickel was less than 40 %. The poor removal of nickel was also in agreement with previous observations (Lester, 1983). The toxic effect of different concentrations of nickel (II) (5.0, 10.0 and 25.0 mg/L) on activated sludge was studied by Yetis and Gokcay (1989). For the concentration of 5.0 mg/L of nickel, the removal efficiency was not adversely affected. The MLSS concentration measured was about twofold between the dilution rates of 0.18 and 0.4 h^{-1}. The critical dilution rate was observed at 0.58 h^{-1} from 0.32 h^{-1} at early stage. They pointed out an interesting observation that the MLSS concentration was increased with the increase of the dilution rate up to about 0.18 h^{-1}. For the concentration of 10.0 mg/L, the critical dilution rate was about 0.30 h^{-1}. For the concentration of 25.0 mg/L, the MLSS concentration was found to be a completely random manner. They showed that the removal efficiency of the activated sludge was not adversely affected by the presence of nickel (II) up to a concentration of 1.0 mg/L while the feed solution contained 1300 mg-COD/L. However, a concentration of 25.0 mg-Ni(II)/L reached toxic and lethal level and caused serious upsets in the system. Some stimulating effects occurred in the system when the feed solution contained 5.0 mg-Ni(II)/L.

Dilek *et al.* (1991) conducted a study on the effects of different concentrations of copper on organic removal efficiencies. They reported that removal efficiencies were not adversely affected when concentrations of copper ranged between 0.5 and 10 mg/L. A slight increase of the biomass yield can be detected up to a concentration of 10 mg/L. Dilek and Yetis (1992) extended the study by Dilek *et al.* (1991) on the removal efficiencies of various heavy metals with different concentrations, *i.e.*, copper (II) (1.0, 5.0, 10.0, 25.0 and 50.0 mg/L), nickel (II) (5.0, 10.0 and 25.0 mg/L) and chromium (VI) (1.0, 5.0, 10.0, 25.0 and 50.0 mg/L), using a laboratory-scale completely mixed activated sludge unit. Their results showed that removal efficiencies were not adversely affected by the concentration of copper (II), nickel (II) and chromium (VI) up to 10.0 mg/L, 10.0 mg/L and 50.0 mg/L, respectively. For the nickel (II), a concentration of 25 mg/L led to unpredictable toxicity that caused oscillations in the rate of growth of microorganisms. An interesting observation was noted for the presence of copper (II) at a concentration of 0.5 mg/L, which was the significant increase in the observed biomass yield. The MLSS concentration increased to 600 mg/L at around 0.13 h^{-1} from an initial of 500 mg/L. Therefore the presence of a concentration of 0.5 mg-Cu(II)/L stimulated the culture in terms of both growth rate and the biomass yield. According with the removal efficiencies from these works, the efficiency of metal removal, and hence metal toxicity, in activated sludge processes can be expressed as follows:

Pb > Cu > Zn > Cd, Cr > Ni

This sequence is similar to the sequence concluded by Lester *et al.* (1979). They reported that the order of decreasing toxicity was Cu > Cd > Pb > Cr. Apart from normal heavy metals, effects of trace heavy metals on activated sludge have also been studied. Battistoni *et al.* (1993) reported their laboratory study among four different conditions (5 - 20 days of sludge ages) on the uptake and toxic effects of heavy metals (mercury, cadmium and nickel) on activated sludge. In their study metal affinity sequence Hg > Cd > Ni was measured. They reported that mercury was preferentially adsorbed on the cell. Also they reported that cadmium was adsorbed on extracellular polymer limes and nickel was adsorbed on capsular polymers and cellular wall. Capsular polymers have been reported (Saunders and Dick, 1981) to remain attached to sludge floc and re-circulated, while slimes tend to exit from the plant with the colloidal and dissolved phase. It is therefore concluded that capsular polymers increased with sludge ages. This indicated that the toxicity of cadmium and nickel could be influenced by sludge ages, while no such effect was observed for mercury.

2.2.1.2 Effects of Heavy Metals on Organic Removal Efficiency in Activated Sludge

There has been contradictions in the published literature on the effects of heavy metals at trace levels, on the organic removal efficiency in activated sludge. One school of thought believes that heavy metals, at trace levels are essential for the growth of activated sludge microorganisms. The other school of thought believes that heavy metals, at trace levels, while not being toxic but still adversely affects the organic removal efficiency. However, the mechanisms that cause this adverse effect are seldom investigated.

Biochemical oxygen demand (BOD) removal has been reported to be significantly effected by even small concentrations (1-2 mg/L) of copper or chromium. Some investigations have been conducted to study the effect of heavy metals on BOD, e.g. Berthouex and Szewczyk (1984), Boston and Hure (1983) and Medine *et al.* (1981), Franke *et al.* (1981). McDermott *et al.* (1963) reported their study on the effects of copper on an activated sludge pilot plants. Their results showed that the suppression of BOD removal was less than 4 % when the copper concentration was 1 mg/L or below. Similar results as for BOD removal efficiency have been obtained for COD removal. Recently, Mittal and Ratra (2000) reported their extensive study on the toxic effects of metal ions (aluminum, copper, cobalt, mercury, nickel, lead and zinc) on BOD. Table 2.15 tabulates a summary of these results. They reported that microbial organisms (added as seed) were present along with the finite amount of different metal ion concentrations, however, the growth of microbial organisms as well as their population were affected by the presence of metal ions. As both aluminium and zinc have the largest oxidation potentials, they therefore were easily oxidised and consumed oxygen in addition to their requirement for the normal BOD process. This prompted to the overall increase of BOD in sharp contrast to the expected fall in their values. Conversely, oxidation of copper was observed only at a high concentration as its has a low oxidation potential. Cobalt, nickel and mercury apparently showed no increase of oxygen demand. The behaviour of lead was irregular. Oxidation potentials of the seven metals ions are reported as follows,

$$Al > Zn >> Co > Ni > Pb >> Cu >> Hg$$

Sujarittanonta and Sherrard (1981) reported their study on nickel toxicity in activated sludge using different concentrations of nickel (II) (1 and 5 mg/L) and COD (396, 391 and 787 mg/L), and sludge ages (5.2 to 16.9 days). For the study on 1 mg-Ni(II)/L and 396 mg-COD/L, the COD removal efficiencies were negligible at all sludge ages. The growth of microorganisms was depressed by nickel ions. This caused MLSS concentration lower than that obtained from the control aeration tank. For the study using 5 mg-

Ni(II)/L and 391 mg-COD/L, the COD removal efficiency was about 3 % less than that obtained from the control. In this case microorganism was increased with the increase of sludge ages. For the study using 1 mg-Ni(II)/L and 787 mg-COD/L, they observed that MLSS concentration was increased with the increase of the sludge age. Results of their study can be concluded as follows. Microorganisms in the activated sludge were inhibited by chromium and nickel at concentrations above 10 mg/L. The COD removal efficiency was not significantly affected with influent nickel concentrations as high as 5 mg/L and at a sludge age greater than 5 days. Apparently, the COD removal efficiency did spend on COD:Ni^{2+}. Besides, removal efficiency depended on microorganism concentration to nickel ratio, and operating sludge age.

On the other hand, the COD removal efficiencies ranged between 93.7 and 96.3 % were measured by Yamamoto and Win (1991). They found that no significant toxic affect on COD removal was observed when chromium content in the sludge was as low as 1 to 5 %. In their study, they found that the SRT of 20 days was the best among 10, 20 and 550 days adopted for removal of organic matter, nitrogen and chromium and the flux. The chromium removal efficiencies of 95.4 to 97.7 % were measured. Based on the investigation, they recommended that a volumetric organic loading of less than 8 kg-COD/m^3d should be kept when a SRT of 20 days is selected.

Table 2.15

A summary of BOD_5 exertion in presence of metal ions at 20 °C (Mittal and Ratra, 2000)

Sample no.	Type of sample	Change in BOD (inhibition / increase), % age						
		Nickel	Cobalt	Mercury	Aluminium	Zinc	Copper	Lead
3	(+) 0.1 mg/L	3.70(-)	9.80(-)	76.1(-)	1.30(+)	7.80(+)	0.60(-)	1.75(+)
4	(+) 0.25 mg/L	11.1(-)	13.0(-)	90.8(-)	7.90(+)	15.6(+)	3.00(-)	12.3(+)
5	(+) 0.50 mg/L	24.1(-)	18.5(-)	110(-)	11.3(+)	23.4(+)	3.50(-)	1.75(+)
6	(+) 0.75 mg/L	38.1(-)	22.5(-)	117(-)	17.1(+)	10.4(+)	11.9(-)	3.50(-)
7	(+) 1.0 mg/L	50.0(-)	30.7(-)	118(-)	Nil	6.30(-)	16.4(-)	8.77(-)
8	(+) 2.0 mg/L	-	37.5(-)	117(-)	11.8(-)	10.9(-)	20.9(-)	12.3(-)
9	(+) 5.0 mg/L	64.8(-)	41.6(-)	118(-)	52.6(-)	18.8(-)	31.3(-)	20.0(-)
10	(+) 10 mg/L	81.5(-)	45.6(-)	118(-)	61.8(-)		41.8(-)	29.8(-)
11	(+) 20 mg/L	90.7(-)	55.2(-)	118(-)	67.1(-)	28.7(-)	31.3(-)	14.0(-)
12	(+) 50 mg/L	109(-)	67.4(-)	118(-)	80.3(-)	37.5(-)	9.90(-)	7.00(-)
13	(+) 75 mg/L	-		117(-)	-		7.5(+)	1.75(-)
14	(+) 100 mg/L	114(-)	78.3(-)	118(-)	90.8(-)	46.9(-)	20.9(+)	-

Tan *et al.* (1994) used a sequencing batch reactor to study the effects of five different concentrations of copper (1, 3, 5, 10, and 30 mg/L) on the activated sludge processes. They reported that the concentration of 1 mg-Cu/L had little effect on the activated sludge. This agreed with previous literature, e.g. Dilek and Yetis (1992). Their experimental results showed that no significant adverse effect on the COD removal efficiencies was observed from adding different concentrations of copper (II) in the activated sludge system. They showed that the COD removal efficiency as high as 80 % was quite stable to be measured during the concentrations of copper at 3 and 5 mg/L. However, a faster rate of decline was induced at a concentration of 5 mg/L. A drastic decrease in the COD removal efficiency was measured with the concentrations of 10 and 30 mg-Cu/L. On the contrary, Tan and Chua (1997) observed that heavy metals, including copper (II), at sub-toxic concentrations as low as 1 mg/L also affected the COD removal in activated sludge.

As shown above, removal efficiencies as affected by a single heavy metal have been extensively studied. Removal efficiencies as affected by combined heavy metals have also been studied in numerous investigations. Bagby and Sherrard (1981) concluded that the COD removal efficiency at a given sludge age and influent COD concentration was decreased when the concentrations of cadmium and nickel were high. Lower sludge age also reduced the COD removal efficiency at a given influent concentration of heavy metals. In addition, they reported that the COD removal efficiency also decreased when the total influent metal concentration to total system suspended solids ratio increased. Beyenal *et al.* (1997) conducted a study on the removal efficiency of combined effects of copper and zinc on activated sludge processes. They reported that the effluent substrate concentration decreased with the increase of sludge age. This agrees with Bagby and Sherrard (1981). Their results also showed that the microorganism concentration increased with the increase of sludge age. Additionally, biokinetics of the reactions in the presence of heavy metals approached to that without heavy metals as the residence time got smaller and the effluent substrate concentration became larger. However, at large residence times and small effluent substrate concentrations, they found that the biokinetics of two sets of experiments diverted from each other significantly.

Chang *et al.* (1986) reported that the dissolved organic carbon (DOC) removal efficiencies of copper at concentrations of 1, 5 and 10 mg/L were slightly improved or remained at the same levels induced by cadmium. When the copper was increased to 50 mg/L, the DOC removal efficiency sharply decreased to 75 %.

2.2.1.3 Factors Affecting Removal Efficiency

Previous studies already showed that many factors affect the removal efficiency of metals in the activated sludge process. These factors can be divided into three groups, separately, operating parameters, physical or chemical factors and biological factors.

For operating parameters, a higher removal efficiency can be obtained by

(1) decreasing the sludge volume index (SVI) (Rudolfs and Zuber, 1953);

(2) increasing the age of sludge (Battistoni *et al.*, 1993; Rossin *et al.*, 1982; Sterritt and Lester 1981; Stoveland, 1978);

(3) increasing the suspended solids removal (Brown *et al.*, 1973);

(4) increasing the mixed liquor suspended solids concentration (Sterritt and Lester, 1981; Port, 1978); and

(5) increasing the settling time (Sujarittanonta and Sherrard, 1981; Bagby and Sherrard, 1981; Weber and Sherrard, 1980; Trahern *et al.*, 1980; Neufeld and Hermann, 1976, Brown *et al.*, 1973; Poon and Bhayani, 1971). Besides, the dissolved oxygen concentration is also one of the factors controlling the removal efficiency of metals (Barth *et al.*, 1965).

For physical and chemical factors, a higher removal efficiency can be obtained by

(1) decreasing the value of pH (Nelson *et al.,* 1981; Cheng *et al.,* 1975; Heukelekian and Gelman 1955; Sawyer *et al.,* 1955);

(2) increasing the metal ion concentration (Oliver and Cosgrove 1974);

(3) decreasing the metal solubility (Brown *et al.,* 1973);

(4) decreasing the metal valency (Stoveland, 1978); and

(5) increasing the size of particles (Chen *et al.,* 1974). In additionally, both concentration of complexing agents and temperature affect the removal efficiency.

For biological factors, concentration of extracellular polymers, metabolic intermediates and materials released as the result of lysis would affect the removal efficiency of heavy metals.

2.2.2 Mechanisms of heavy metal uptake

Published works on the characteristics of heavy metal uptake, especially at trace levels, by activated sludge microorganisms are limited, thus leading to the initiation of this research project. Nelson *et al.* (1981) proposed not only the surface complexation model, but also a speciation-distribution model, which is used to determine the metal species fraction, or the ratio of the metal species to total metal in the activated sludge system, including adsorption to solids. The speciation-distribution model can be expressed as Equation (2.1).

$$\alpha'(j)_n = \frac{\beta(j)_n[L(j)]^n}{1 + \sum_i \sum_n \beta(j)_n[L(i)]^n + \sum K_{A.M}\{S_m\}} \qquad (2.1)$$

where

$\alpha'(j)_n$ = species fraction for species $ML(j)_n$, including both soluble and surface complexes;

$\beta(j)_n$ = stability (overall formation) constant for metal-ligand complex;

$L(i)$, $L(j)$ = complexing ligand of type i and j respectively;

$K_{A.M}$ = conditional adsorption constant;

S_m = adsorbing surface of type m; and

n = ligand number.

They validated their proposed model using experimental data. Fristoe and Nelson (1983) adopted the speciation-distribution model to study cadmium in activated sludge using a full-scale wastewater treatment plant. They reported that:

(1) cadmium adsorption by activated sludge solids was quantifiable by determination of conditional adsorption constants valid at the pH and solution conditions specified;

(2) organic ligand complexation of cadmium in activated sludge was significant in the pH ranged of 5-8; and

(3) the speciation and distribution of cadmium in activated sludge could be quantified using an equilibrium constant approach.

Stephenson *et al.* (1987), based on the surface-metal complexation model, developed a new model to explain the mechanism of metal removal in activated sludge. Their results showed that removal efficiencies of cadmium and copper were higher than that of nickel. Removal mechanism for cadmium and copper was the interaction of particulate associated metal with the settleable biological solids. The low resolubilisation of copper by ethylenediaminetetraacetic acid and the calculated metal: ligand stoichiometry of less than unity. This indicates that copper had high affinity for mixed liquor. Stoichiometries greater than unity for cadmium and nickel indicate that removal of cadmium and nickel dissimilar to copper mechanistically.

Gourdon *et al.* (1990) also carried out an investigation on the mechanism of cadmium uptake in activated sludge. They reported that biosorption of cadmium below 30 mg/L was found to follow the Freundlich isotherm, which describes physical surface adsorption behavior. Biosorption increased strongly when the initial cadmium concentration was raised from 10 to 100 mg/L and increased with temperature between 5 °C and 40 °C. Besides, several observations were reported. Firstly, they found that more than 95 %

of total cadmium uptake was obtained within 5 minutes metal-sludge contact time. Secondly, biosorption strongly increased when the initial concentration of cadmium was raised from 10 to 100 mg/L. In the same concentration range of cadmium, the metabolic activity of the sludge was very significantly inhibited. Biosorption was found to increase with temperature between 5 °C and 40 °C and to decrease slightly (5 – 10 %) under pre-treatment of the sludge with metabolic inhibitors. These observations indicated that cadmium uptake was low and that adsorption to surface of the cells was the major mechanism of uptake.

Imai and Gloyna (1993) showed that the hypothetical sorption isotherm of chromium (III) composed of two straight lines with an initial slope of $K_{ad}/(1+K_c\,[FTOC]^2)$ and the second slope of K_{ad}, where K_c is the conditional stability constant for complexation and *FTOC* is the filtered total organic carbon. Their experimental results on the chromium adsorption isotherms showed linear relationship. This agreed with the hypothetical isotherm. Besides, this indicated that intracellular chromium (III) uptake can appropriately be described.

Chong and Volesky (1995) carried out an investigation on equilibrium batch sorption studies of combined heavy metals (copper + zinc, copper + cadmium, zinc + cadmium) using Langmuir-type model. Their results showed that the uptake of zinc decreased drastically when copper or cadmium was present. The uptake of cadmium was much more sensitive to the presence of copper than to that of zinc.

2.2.3 Microbiology in Metal-Laden Activated Sludge

Many different species of bacteria isolated from activated sludge have been shown (Sutherland, 1972; Wilkinson, 1958) and many of these bacterial genera and species have been targetted for investigation on their behavior in the presence of heavy metals. An extensive review on binding of soluble metal to bacterial polymers has been given by Lester (1983). The followings are a survey of literature that show recent investigations on the effects of shock load to ciliate species in activated sludge in the presence of heavy metals.

Madoni *et al.* (1994) studied the effects of eight ciliate species in activated sludge from heavy metals. These eight ciliate species were five of free-swimming forms (*Dexiostoma campyla, Drepanomonas revoluta, Paramecium caudatum, Uronema nigricans,* and *Spirostomum teres*) and three of crawling forms (*Aspidisca cicada, Euplotes affinis*, and *Euplotes patella*). Five heavy metals (lead, chromium, cadmium, copper and zinc) were selected. They reported that *Drepanomonas revoluta* and *Spirostomum teres* were the first and second sensitive to heavy metals among the eight ciliate species. The crawling ciliate Euplotes patella was the least sensitivity than other seven ciliate species. The difference of sensitivity between eight ciliate species was little when lead was added. A summary of their results is tabulated in Table 2.16.

Dilek and Gokcay (1996) reported their study on the microbiology of activated sludge treating wastewater containing nickel (II) and chromium (VI). A laboratory-scale once-through activated sludge unit treating simulated wastewater with different influent strengths (650 and 1300 mg-COD/L), nickel (II) (5 and 10 mg/L) and chromium (VI) (1 and 50 mg/L). A total of 6 combinations were examined. In each combination two types of bacteria were isolated from the activated sludge treating wastewater with different concentrations of heavy metals and influent strengths. Table 2.17 summarised their results.

Table 2.17 shows that Acinetobacter sp. was commonly presence at low organic loadings, i.e. 650 mg-COD/L with 5 or 10 mg-Ni(II)/L. Evidently Acinetobacter sp. was competed out when chromium is existence in the feed solution. It is also shown that very high biomass decay rates were dominated in where Flavobacterium lutescens, Proteus rettgeri, Arthrobacter simplex, and Pseudomonas mendocina. The sludge settleability for the nickel (II) alone at low influent strength (650 mg/L) was found to be poor, producing SVI values greater than 300; whereas SVI was below 100 in other cases.

As shown above, Yetis and Gokcay (1989) observed the critical dilution rate of three different concentrations of nickel (II) reacting with activated sludge. There is a similar case studied by Yetis and Gokcay (1989) and Dilek and Gokcay (1996), i.e. 5.0 mg-Ni(II)/L and 5.0 mg-Ni(II)/L + 1.0 mg-Cr(VI)/L. It is interesting to point out that the dilution rate reported in Yetis and Gokcay (1989) was 0.58 h^{-1} which is comparable with the value given in Dilek and Gokcay (1996), *i.e.* 0.61 h^{-1}.

Madoni *et al.* (1996) extended the study by Madoni *et al.* (1994) to investigate the shock load effects of heavy metals on ciliates of activated sludge. Fourteen species of ciliated protozoa and 2 species of testate amoebae were found in the tested protozoan community of the activated sludge (Madoni *et al.*, 1996). Eight out of 14 were sessile forms, 5 were crawling forms and the rest was free-swimming form. A summary of the sixteen protozoan species reported by Madoni *et al.* (1996) is tabulated in Table 2.18. In their study, they intended to investigate the shock load effect of cadmium (0 - 6 mg/L), copper (0 - 8 mg/L), chromium (VI) (0 - 400 mg/L), lead (0 -

8 mg/L) and zinc (0 -130 mg/L) on the ciliate community in the tested activated sludge (250 mL in a 500 mL glass flask).

In the presence of 5.23 mg-Cd/L, 8 of the 16 species were disappeared and the diminution of the cell density of the remaining species was observed. Consequently, cell mortality of the whole protozoan community was 50 %. A concentration of 6.12 mg-Cu/L caused 89 % of cell mortality in the whole community and 7 out of 16 species were disappeared. A concentration of 6.98 mg-Pb/L killed 65 % of the individual species, but caused the disappearance of only one species (*C. uncinata*). An 80 % mortality with the reduction of 9 species out of 16 was observed in the presence of 81 mg-Zn/L, and two species were still surviving in the presence of 145 mg-Zn/L. A 55 % mortality in the whole protozoan community with the reduction of 11 species out of 16 was observed in the presence of 150 mg-Cr(VI)/L. A concentration of 293 mg-Cr(VI)/L was necessary to cause a 90 % reduction in the number of organisms and to reduce richness to 8 species.

Table 2.16

A summary of results obtained (Madoni *et al.* 1994)

	24-hr LC 50 (95 % confidence limits) (µg/L)					
	Lead	**Chromium**	**Cadmium**	**Copper**	**Mercury**	**Zinc**
Aspidisca cicada	1,261 (1,136-1,475)	2,355 (2,029-4,285)	N/A	N/A	**	N/A
Dexiostoma campyla	1,099 (930-1,288)	3,293 (3,025-3,987)	N/A	N/A	**	N/A
Drepanomonas revoluta	875 (523-1,033)	45.6 (12.4-74.9)	194 (142-276)	1.75 (0.86-2.56)	5.37 (3.85-9.03)	2.54 (65.6-339)
Euplotes affins	2,323 (2,031-4,262)	2,725 (2,434-4,516)	N/A	N/A	**	N/A
Euplotes patella	2,177 (2,029-2,992)	9,472 (8,757-11,712)	N/A	N/A	**	N/A
Paramecium caudatum	2,260 (2,094-2,818)	2,567 (2,454-2,715)	N/A	N/A	**	N/A
Spirostomum teres	1,083 (906-1,210)	3,232 (3,124-3,371)	557 (479-658)	3.51 (2.20-6.71)	5.94 (4.47-7.74)	672 (538-928)
Uronema nigricans	1,616 (1,299-2,283)	2,177 (1,929-2,318)	N/A	N/A	4.30	N/A

(** means an overall range of 17.5 to 64 µg/L; N/A means not available.)

Table 2.17

A summary of the results (Dilek and Gokcay, 1996)

Ni (II) mg/L	Cr (VI) mg/L	D 1/Hour	COD mg/L	K_d	Microorganisms
5	-	1.16	650	0	Proteus rettgeri (facultative anaerobe) Acinetobacter sp. (obligate aerobe)
10	-	0.64	650	0	Acinetobacter sp. (obligate aerobe) Flavobacterium lutescens (facultative anaerobe)
-	50	-	1300	+++	Flavobacterium lutescens (facultative anaerobe) Proteus rettgeri (facultative anaerobe)
5	1	0.61	1300	+++	Flavobacterium lutescens (facultative anaerobe) Proteus rettgeri (facultative anaerobe)
5	50	0.46	1300	0	Bacillus cereus var. albolactis Klebsiella sp.
10	50	0.44	1300	+++	Arthrobacter simplex (obligate aerobe) Pseudomonas mendocina (obligate aerobe)

(D – dilution rate; K_d - decay constant)

Table 2.18

A summary of sixteen protozoan species (Madoni *et al.*, 1996)

Taxa	Cell number 25 μ/L			%
	Min.	Max.	Mean	
Free-swimming ciliates				
Drepanomonas revoluta	9	19	13	< 1
Crawling ciliates				
Aspidisca cicada	158	186	168	7.3
Aspidisca lynceus	1,422	1,672	1,507	67.8
Chilodonella uncinata	39	76	64	2.9
Euplotes sp.	28	53	38	1.7
Trochilia minuta	39	142	94	4.2
Attached ciliates				
Carchesium sp.	4	9	7	1
Epistylis sp.	1	8	4	< 1
Opercularia coarctata	45	53	50	2.3
Opercularia minima	131	233	179	8.1
Vorticella convallaria	43	72	60	2.7
Vorticella octava	27	57	39	1.8
Suctoria				
Podophrya sp.	1	1	1	<1
Tokophrya quadripartita	1		1	1
Testate amoebae	2	8	4	1
Total microfauna	1,950	2,590	2,229	100

A concentration of 26.2 mg-Cr(VI)/L caused only 2 out 16 species disappeared and lowered the protozoan density of the 8 %, whilst 68.8 mg-Cr(VI)/L produced the disappearance of 4 out of 16 species and raised the protozoan mortality to 42 %. These results agreed with Dilek and Yetis (1992). Their finding can be summarised as follows. Heavy metals change the structure of the activated-sludge microfauna by modifying both of density and species richness. Chromium (VI) and zinc were considerably less toxic than lead, copper, and cadmium. The order of toxicity of the five metals to the test microbial community was generally: Cd > Cu > Pb > Zn > Cr. This order is similar with the order specified by Lester *et al.* (1979)

Ciliated protozoan contributed over 9 % to the activated sludge biomass (Madoni *et al.*, 1994), and together with bacteria, played an important role in the purification process by removing the dissolved organic matter (floc-forming bacteria), and by clarifying the treated effluent (filter-feeding ciliates). These organisms were able to survive in the presence of heavy metal concentrations higher than those normally observed in the activated sludge, increase the likelihood that the purification process would be functioning when an additional amount of heavy metals entered the plant. Furthermore, the study provided information about the metal concentrations that affected the protozoan community in the activated sludge.

2.2.4 Oxygen Uptake Rate

The oxygen uptake rate (OUR) is one of the most important variables for estimating biological activity in activated sludge processes. The secondary influent waste to an aeration tank serves as food for the aerobic microorganisms. These microorganisms use oxygen to synthesize the food for new cell growth and energy and they respire CO_2. This process is similar to eat, grow, reproduce, breathe O_2, and respire CO_2 of human being. Aeration devices are constantly oxy-genating the tank solids. This biochemical oxidation of the sludge physically converts the activated sludge cells from a light, fluffy nature (when young) to a denser, more spherical cells possessing more rapid settling characteristics (when older). Mechanisms of activated sludge processes taking oxygen can be described as follows. The microorganisms are constantly using the dissolved oxygen (DO) in the mixed liquor. As aeration continues and the average sludge cells become older (higher age or MCRT), more and more oxygen is consumed. However, the rate of oxygen used can vary considerably upon sludge oxidation. If excess food is presence for the number of microorganisms in the system (a young or underoxidized sludge), the microorganisms will eat and reproduce extremely fast. Under this condition, microorganisms use a high rate of oxygen. This is the rapid log growth phase, and during this phase the sludge is not flocculant and has inherently poor settling characteristics. This condition is similar to sludge bulking, which is often disastrous to effluent quality. Excessive growth of filamentous organisms, many of which prefer low DO environments that can result from an extremely high OUR, may be observed with a phase contrast microscope. These microorganisms can link between floc particles and worsen sludge settleability.

As the food supply vanishes, the growth rate of the microorganisms also decreases. During this declining growth phase higher life forms are observed as the process age increases. Correspondingly, the OUR decreases although the overall amount of oxygen required increases. This properly oxidised sludge becomes more flocculant with desirable settling characteristics. This is the arena in which most activated sludge systems operate best, with the exception of high rate systems that operate in the rapid growth phase, or extended aeration systems that operate in the endogenous respiration phase. Relationships between the microorganisms, food, and OUR during BOD exertion and uptake rate within these various operational phases are shown in Figure 2.1.

If the food supply for the activated sludge process is too low, the microorganisms must compete for the existing food. Many of the microorganisms, then relying on internal cellular matter for food, become starved and die. In this phase of microbial growth, commonly called the endogenous phase, OURs are very low, though overall oxygen use still increases. Overoxidation causes dispersed flocs. The sludge cells take on a more spherical or grainly nature that increases the settling velocity. Because extended aeration systems normally stockpile higher solids (operating at a lower F/M (food / biomass) ratio and a higher MCRT) increased solids concentrations can slow settling because of hindered settling conditions, but the cells themselves are fast settling. This can be demonstrated by diluting a mixed liquor sample.

Generally, two approaches are used to estimate OURs. One is based on the use of sidestream respirographic sensors to estimate the respiration rate from a DO balance in a respiration chamber. The other is to identify the DO dynamics from DO probes placed in the place aeration tanks. Tyagi (1985) studied the effects of six different heavy metals (cadmium, nickel, zinc, chromium, lead and copper) and their mixture in the activated sludge process. They reported that three different types of inhibition were observed. A linear inhibition pattern was observed from cadmium. Inhibition of both lead and chromium showed parabolic (non-linear) modes. Zinc, nickel and mixture of metals indicated a hyperbolic (non-linear) inhibition pattern. The inhibition is defined as the ratio of the difference between OUR of the control system and OUR of the metal-laden system to OUR of the control system.

Their experimental results also showed that the specific growth rates decreased with the increase of the metal concentration.

Zarnovsky *et al.* (1994) reported their results of a study on the influence of cadmium on activated sludge activity indicating that maximum inhibition should be included in the assessment of metal toxicity. In their study, they observed that the maximum inhibition occurred at different time periods for acclimated and non-acclimated activated sludge. For the non-acclimated sludge, the maximum inhibition measured at the cadmium concentration of 3 mg/L was less than 40 % of those measured at higher cadmium doses. For the cadmium concentration of 10 mg/L, the maximum inhibition influence ranged between 5 and 9 hours for non-acclimated sludge and between 2 and 5 hours for acclimated sludge. Nevertheless, the maximum inhibition of activated sludge and the toxicity of cadmium decreased with the increase of the SRT. Besides, results showed that significant differences in the inhibition for both acclimated and non-acclimated sludge were at a shorter SRT.

Madoni *et al.* (1999) reported their study on the response of ammonium uptake rate and OUR from five heavy metals (cadmium (II), chromium (VI), copper (II), lead (II) and zinc (II)) in activated sludge. The toxic effect of each metal was measured after 1 and 24 hours exposure. Results of their study showed that higher values of ammonium uptakte than the values of

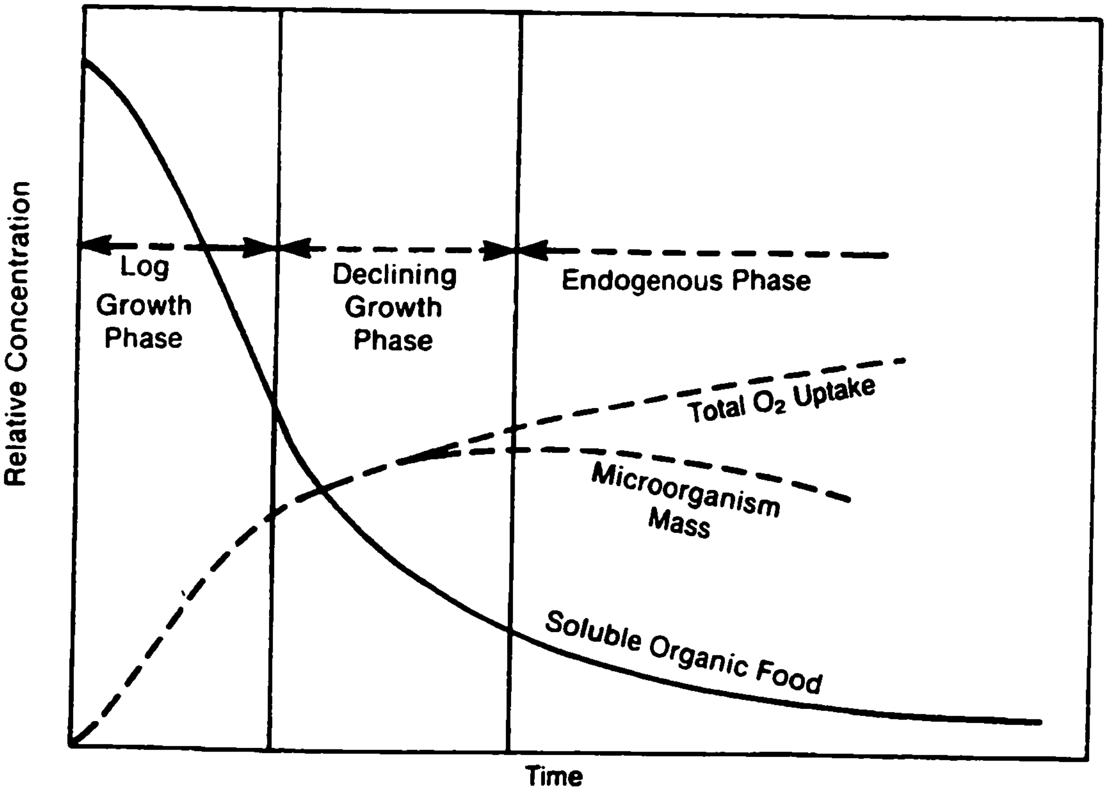


Figure 2.1

Relationships between the microorganisms, food, and OUR during BOD exertion and uptake rate (Metcalf & Eddy, 1991)

oxygen uptake rate were measured from the tests with cadmium (II), copper (II) and lead (II). This showed that a lower sensitivity of nitrifiers to those heavy metals in comparison with heterotrophs. Inhibitory effects of chromium (VI) and zinc (II) were quite similar to both ammonium and OURs.

2.3 ADSORPTION CAPACITY

Adsorption is one of the most important processes in water quality control. It may determine the fate and transport of pollutants in the aquatic environmental and it is a major unit process for effective removal of health related organic contaminants from drinking water and for advanced treatment of reclaimed wastewater for reuse. The adsorption process takes place in three steps: macrotransport, microtransport and sorption. Macrotransport involves the movement of the organic material through the water to the liquid-solid interface by advection and diffusion. Microtransport involves adsorption sites in the micropores. Adsorption occurs on the surface of the microorganisms and in the microprobes and mesopores (Metcalf and Eddy 1991). Sorption is the process by which a component moves from one phase to another across some boundary (Michael *et al.* 1994). It is the term used to describe the attachment of the organic material to the microorganisms. The driving forces that control adsorption include electrical attraction, a chemical affinity of the particular organic molecule for the adsorbent, van der Waal's forces and the hydrophobic nature of the organic. When the rate of sorption equals the rate of desorption, equilibrium has been achieved and the capacity of the microorganisms can be determined by calculating its adsorption isotherm. Equations that are often used to describe the experimental isotherm data were developed by Freundlich and Langmuir (Metcalf and Eddy, 1991). For the Langmuir isotherm, there are several assumptions including:

i) a fixed number of adsorption site being available,

ii) adsorption to be reversible,

iii) adsorption on the surface being mono-layer and adsorption only being gas molecules colliding with solid surface,

iv) all adsorption sites being equivalent, and

v) no interaction between adsorbed molecules. Figure 2.2 shows the mono-layer adsorption.

The Freundlich Equation is an empirical isotherm and describes as the multi-layer adsorption (see Figure 2.3).

A new term, namely, COD adsorption capacity (CAC) is recently introduced to represent and monitor the ability of activated sludge to adsorb organic matter (Tan and Chua, 1997). Additionally, CAC could be used for process decision on variation in air supply and feed pattern to the aeration units. Also CAC could be used to estimate the air the air supply to the aeration unit which is merely used for re-aerating the returned sludge. They reported that CAC of a healthy activated sludge increased with the flow along the aeration units. Tan *et al.* (1994) reported that biomass CAC was found to decrease with the increase of copper specific adsorption while the level of dosage was increased and the duration of operation was longer. The effect of heavy metals, chromium and zinc, on organic adsorption capacities has been studied by Chua (1998); Chua and Hua (1996) respectively. For the studies on zinc, the effect on CAC and the COD removal efficiency affected from different

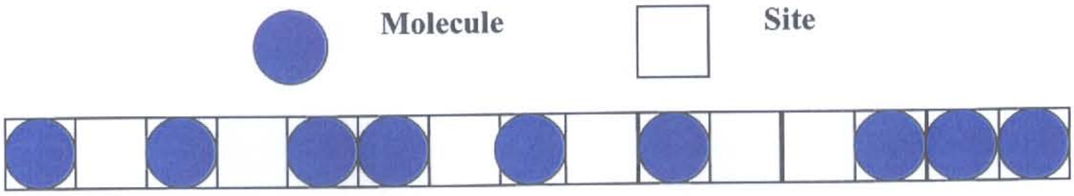


Figure 2.2

Mono-layer adsorption (Metcalf & Eddy, 1991)

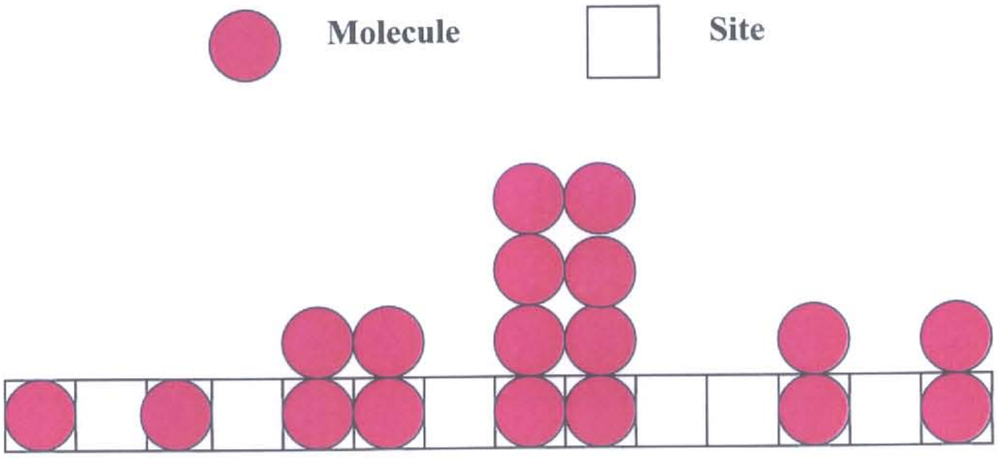


Figure 2.3

Multi-layer adsorption (Metcalf & Eddy, 1991)

hydraulic retention times (2.5 and 5 days) and conditions (with and without zinc) were studied.

Experimental results showed that the reactor stabilized within 15 days to have an average CAC of 39.7 mg/g with zinc from CAC of 53.8 mg/g without zinc. The COD removal efficiencies measured were 95.3 % and 91.9 % for the cases without zinc and with zinc, respectively. The difference of the COD removal efficiencies between the two cases was not significant. When the hydraulic retention time (HRT) was reduced to 2.5 days, residual COD level was increased leading to a drastic increase of CAC to a maximum of 103.8 mg/g. At the longer HRT, the sequencing batch reactor had sufficient period for biological assimilation so that organic adsorption was not a critical factor governing the performance of the sequencing batch reactor. Therefore the presence of zinc in the reactor affected the CAC, but had little effect on the COD removal.

For the studies on chromium, similar results as the studies on zinc were obtained. The reactor was stabilized within 12 days to have an average CAC of 43.6 mg/g with chromium and CAC of 49.6 mg/m without chromium. The corresponding COD removal efficiencies of the absence of zinc and the presence of zinc were 93.2 % and 93.7 % respectively. Residual COD level was dramatically increased to a maximum of 120.1 mg/g when the HRT was reduced to 2.5 days. The presence of chromium directly affected CAC, but had little effect on the COD removal as the reactor had sufficient period for biological assimilation at the longer HRT. Chua *et al.* (1999) reported the effects of four heavy metals (copper, zinc, chromium and lead) on CAC and the COD removal efficiencies. They reported that both CAC and the COD removal efficiency were decreased by metal-laden wastewater even at sub-lethal concentrations, particularly at shorter HRTs. Under longer HRTs, CAC was decreased by the metal-laden wastewater but no significant effect on the COD removal efficiency was observed. These results agreed with previous literature.

2.3.1 Adsorption isotherm

Adsorption isotherms are used to represent equilibrium distributions of metals between solution and bacterial solid phases. Nelson *et al.* (1981) proposed a surface complexation model to describe the adsorption of metal ions by a bacterial surface. An experiment was conducted to validate their proposed model. They found that pH was an important factor affecting metal adsorption. For cadmium and zinc, adsorption increased steadily from 15 to 20 % at pH of 4 to greater than 90 % at pH of 10. For copper, the maximum adsorption occurred in the range of pH at 7 and 8 and the adsorption decreased at higher and lower pH values. It is noted that there are some limitations in this model. A pertinent chemical reaction may be omitted if a complete chemical analysis of the wastewater was unknown, especially to the presence of soluble organic ligands which may strongly complex with heavy metals. Besides, adsorption constantly determined by this approach are conditional; that is, strictly valid only at the pH and chemical composition of the water in which they were determined and for bacterial solids cultured under particular conditions.

Imai (1988) based on Nelson *et al.* (1981) work to develop a model, namely, CRAS for the description of the chromium adsorption on activated sludge. Imai and Gloyna (1991) conducted a preliminary study on using CRAS for studying dynamic behaviour of chromium(III) in activated sludge. They validated the model for chromium(III) from experimental data and employed CRAS to study effects of chromium(III) on activated sludge (Imai and Gloyna, 1993). In their study two independent parameters, namely sludge ages and pH values, with three different values (5, 10, 15 days for sludge age and 6, 7 and 8 for pH values) were selected. They expressed the chromium(III) adsorption as a surface complex model using a conditional stability constant, K_{ad}. They found that chromium(III) adsorption significantly depended on both independent parameters. K_{ad} increased with the increase of pH from 6 to 8, especially from pH 7 to 8. Besides, K_{ad}

increased with also the increase of sludge age. These results agreed with the previous literature, e.g. Nelson *et al.* (1981). The removal efficiencies ranged from 54.6 % and 91.3 % were measured. Their measurements were comparable with values given in previous literature, e.g. 51 % ~ 98 % (Imai, 1988).

Imai and Gloyna (1996) further extended their study (Imai and Gloyna, 1991) on the effects of chromium(III) on activated sludge.

They found that

(1) Chromium(III) adsorption was in linear characteristics;

(2) the conditional stability constant, K_{ad}, increased with the increase of the sludge age;

(3) K_{ad} also increased with the increase of pH from 6 to 8, especially from 7 to 8; and

(4) K_{ad} increased with the increase of sludge age from 5 to 15 days.

The effect of pH on K_{ad} can be explained as that the chromium(III) adsorption played as hydrolyzed metals caution adsorption on a negatively charged surface. The bacteria were negatively charged at the neutral pH range. According to James and Healy (1972), a decrease in solution energy enhances the adsorption of hydrolyzable metal such as chromium(III) on a negatively charged surface as hydrolysis occurs. The effect of sludge age on K_{ad} shows obvious that chromium(III) adsorption on the bacteria depended on the organism's growth conditions. Chua and Hua (1997); Chua and Hua (1996); and Tan and Chua (1993) also reported that trace heavy metals could affect the adsorption and uptake of organic matters.

Oakley *et al.* (1981) simplified the adsorption isotherm with considering only the linear portion of the adsorption isotherm in their study. They showed that trace-metal (Cu and Cd) partitioning can be predicted from their laboratory study with the use of conditional equilibrium adsorption constants. However, the model has several limitations in the use of adsorption constants obtained from the adsorption isotherm. First, the amorphous hydrous oxides utilised are not thermodynamically stable phases but only metastable ones. Thus, true equilibrium is not attained. Second, the results with humic acid must be interpreted cautiously since the humic acid was removed by ultrafiltration rather than centrifugation. Humic acid in the natural environment probably adsorbs to some extend on other solids; thus the adsorption constants obtained may be higher than those obtained from a portion of the humic acid surface bounding to another solid.

Sheindorf *et al.* (1981) derived a Freundlich type multi-component adsorption isotherm and employed it successfully to describe adsorption data of various bi-component systems obtained from previous literature. Sheindorf *et al.* (1982) extended their study for organic pollutants adsorption from multi-component systems. Experiments were carried out to validate their proposed model. Good agreement of the experimental data was obtained for the bio-component system phenol-bromophenol.

Fristoe and Nelson (1983) reported both adsorption edges and isotherms of cadmium reacting with activated sludge from a study conducted on chemical modelling of heavy metals in activated sludge. For the adsorption edge, they found that cadmium adsorption increased by the increase of the activated sludge concentration or the decrease of the initial cadmium concentration. The curve of the cadmium adsorption edge was S-shape with an inflection point at about 50 % adsorption in the range of the pH between 5 and 6. The adsorption is less than 20 % at below pH of 4, greater than 80 % at above pH of 7, and between 90 % and 99 % at pH of 10. For the adsorption isotherm, straight lines were fitted using regression analysis, however, curves rather than straight lines were fitted for lower pH, i.e. pH = 3, 4, and 5. The slopes of the straight lines were conditional adsorption constant.

Adsorption isotherms are mainly expressed using Langmuir and Freundlich models. Some elements have been found to obey a particular model, e.g. cadmium using Freundlich isotherm and copper using Langmuir isotherm.

Adoption of the appropriate model requires to know what element is in the composition. However, some situations are that the composition is unknown so that either Langmuir isotherm or Freundlich isotherm is correct. Sheintuch and Rebhun (1988) carried out a study to investigate the adoption of adsorption isotherms for multi-solute systems with known and unknown composition. Three adsorption isotherms, namely the Ideal Adsorption Solution Theory, the Multi-component Freundlich Isotherm and the Empirical Freundlich Isotherm, have been assessed and compared for convenience and reliability of application. They stated that ways of using these expressions are depending on available information and experimental strategies. Besides, their study recommended to determine distributions of adsorption coefficients of representative background, using existing modern methods of fractionation, which will provide data for reliable estimation of adsorption equilibria.

It has been reported that cadmium uptake was better described by the Freundlich isotherm rather than the Langmuir isotherm (Chang *et al.*, 1995). Conversely, Chang *et al.* (1995) reported that copper uptake was better described by the Langmuir isotherm rather than the Freundlich isotherm. The Langmuir isotherm can be plotted as a rectangular hyperbola which is often described as "L-shape". Metal adsorption corresponds to an L-shape isotherm could be representative of 1:1 complex formation. However, "S-shape" isotherm has been observed in metal adsorption, e.g. Fristoe and Nelson (1983). These curves are sigmoid rather than hyperbolic and are variously attributed to a number of factors, including adsorption of the metal in the form of a complex from solution (Huang *et al.*, 1977), adsorption hydroxyl group rather than ionogenic carboxyl group (Steiner *et al.*, 1976), and competitive interaction with other adsorbed ions (Gould and Genetelli, 1978). Battistoni *et al.* (1993) concluded their study on uptake and toxic effects of heavy metals (cadmium, mercury and nickel) in activated sludge. Their results show that sludge ages did not affect the adsorption isotherm of mercury while sludge ages did affect the adsorption isotherm of both cadmium and nickel. For cadmium, affinity gradually decreased with sludge ages between 5, 10 and 15 days. In contrary, nickel uptake increased with the ages of sludge and showed remarkable difference between 10 and 15 days.

Sag and Kutsal (1996) carried out a study on the use of the competitive Langmuir model to chromium (VI) and iron (III) from binary metal mixtures by *R. arrhizus*. They found that the initial biosorption rates, equilibrium and ultimate metal removal decreased with increasing concentration of the other metal ion, the combined action of chromium (VI) and iron (III) ions on *R. arrhizus* was generally found to be antagonistic which is claimed to be the competition for adsorption sites on the cells and/or the screening effect by the second metal ion. For the metal uptake for both metal ions, the initial biosorption rates and equilibrium increased with increasing temperature, temperature increases influenced the uptake of chromium (VI) ions very sharply. Further, chromium (VI) ions dominated the competitive binding in the long-term uptake caused by chemisorption. The competitive Langmuir model agreed well with results found experimentally. The consistency of the competitive Langmuir model to the chromium (VI)-iron (III) system increased on increasing the competing metal-ion concentration.

Sag *et al.* (1998) extended Sag and Kutsal (1996) work onto a comparative study for the simultaneous biosorption of chromium (VI) and iron (III) on *C. vulgaris* and *R. arrhizus*. They concluded that combined effect of two metals on a biosorbent may be synergistic or antagonistic. As the initial biosorption rates and equilibrium metal removal decreased with increasing concentration of the other metal ion, the combined action of chromium (VI) and iron (III) on *C. vulgaris* and *R. arrhizus* was found to be antagonistic. This result agrees with their previous studies. The inhibitory effects of chromium (VI) and iron (III) ions on the adsorption capacity of *R. arrhizus* were higher than those of these metal ions on the adsorption capacity of *C. vulgaris*. The removal of chromium (VI) from the binary metal mixtures by *C. vulgaris* and *R. arrhizus* in the presence of iron (III) was always greater than the uptake of iron (III) under the same experimental conditions. The competitive Langmuir model was employed successfully to describe adsorption data of chromium (VI) and iron (III) ions on *R. arrhizus*.

Further, Sag *et al.* (2000) carried out an investigation of biosorption on lead, copper and zinc from bicomponent systems. Similar results as their previous study (Sag and Kutsal, 1996) that the initial biosorption rates and equilibrium metal removal decreased with increasing concentrations of the other metal ion, the combined action of lead (II), copper (II) and zinc (II) ions was found to be antagonistic have been found. They reported that there is only weak competition in the adsorptive capacity of lead (II) in the presence of copper (II) and zinc (II), whereas the uptake of copper (II) and zinc (II) is very much reduced by the addition lead (II), even at the optimum pH value for the biosorption of these metal ions. Such competitive adsorption data can be well characterised using the empirical Freundlich model.

2.4 SEQUENCING BATCH REACTORS (SBRs)

As the sequencing batch reactor (SBR) was used extensively in this work for the investigation of the effects of heavy metals on activated sludge, a section of literature review on SBR is justified.

Although conventional activated sludge processes are more commonly used in sewage treatment works in Hong Kong, SBR was however selected as the activated sludge simulator in this study because of its simplicity in configuration, operation and control, and its convenience in foam prevention and control.

The earliest activated sludge reactors were developed by Ardern and Lockett (1914) using the principle of SBR technology. However, systematic research on SBR technology did not begin until 1971 (Irvine and Davis). Other versions of batch processes were then developed in 1970s. For some reasons, the acronym SBR was used to cover a wide variety of batch processes. Although the SBR represents for various batch processes, the performance of different batch processes is different from each other.

The SBR belongs to the group of the so-called fill-and-draw reactors, including five phases

- Fill (static, mixed, or aerated);
- React (mixed or aerated);
- Settle;
- Decant / Draw, and
- De-sludge / Idle.

See the Figure 2.4

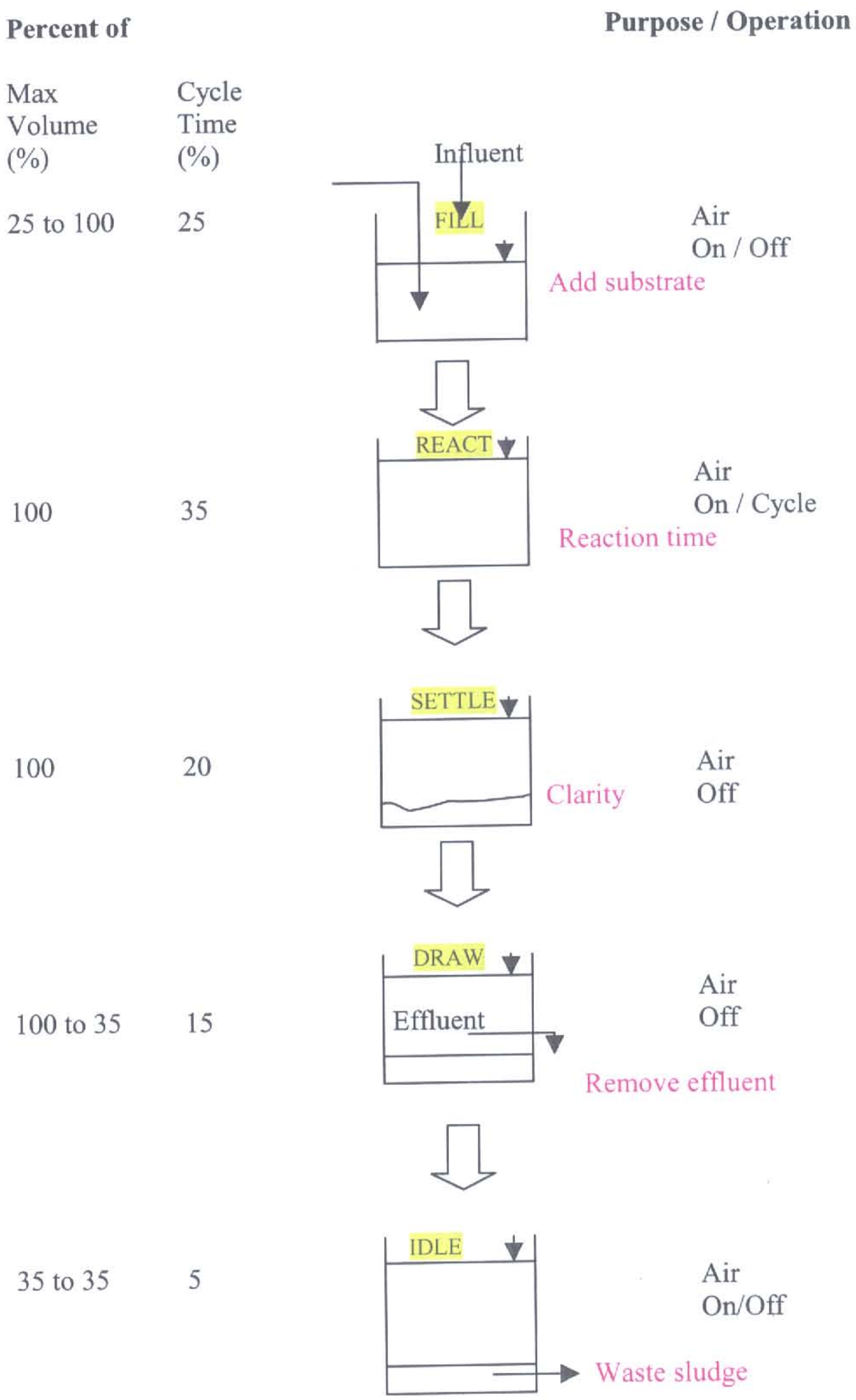


Fig 2.4
Each of the SBR steps (Metcalf & Eddy, 1991)

Table 2.19:

Typical operating sequence for a sequencing batch reactor

SBR operational procedures (Metcalf & Eddy, 1991)

Operational step	Description
Fill	The purpose of the fill operation is to add substrate (raw wastewater or primary effluent) to the reactor. The fill process typically allows the liquid level in the reactor to rise from 25 percent of capacity (at the end of idle) to 100 percent. If controlled by time, the fill process normally lasts approximately 25 percent of the full cycle time
React	The purpose of react is to complete the reactions that were initiate during fill. Typically, react takes up 35 percent of the total cycle time
Settle	The purpose of settle is to allow solids separation to occur, providing a clarified supernatant to be discharged as effluent. In a SBR, this process is normally much more efficient than in a continuous flow system because in the settle mode the reactor contents are completely quiescent.
Draw	The purpose of draw is to remove clarified treated water from the reactor. Many types of decant mechanisms are in current use, with the most popular being floating or adjustable weirs. The time dedicated to draw an range from 5 to 30 percent of the total cycle time, with 45 minutes being a typical draw period.
Idle	The purpose of idle in a multi-tank system is to provide time for one reactor to complete its fill cycle before switching to another unit.

The SBR is distinguished from other fill-and-draw systems by a number of idiomatic features. Basically, the SBR is filled and drawn within a period of time. After completion of the fill phase, variations in the influent of the treatment plant no longer any effect on the processes taking place in the reactor just filled (except to limit or extend the total time allowed for the processes to take place). After specifying the duration of the fill phase, time of the aerated and non-aerated react phase, and the time for the settle and draw phases can be selected according to specific process goals. The SBR cycle is continuously repeated beginning with the fill phase and ending with draw, or with an optional idle phase. By this the micro-organisms are periodically exposed to a defined and regulated variation of process conditions that can mitigate the impact of the forcing function previously defined. The amplitude and frequency of system's periodicity can be chosen according to specific long term goals (e.g. sludge settleability, size of the population of nitrifiers, denitrifiers, Bio-P-bacteria, etc.). Above implied that the SBR is a time-oriented technology rather than a space-oriented technology, it can be operated with great flexibility to accommodate variations in conditions and achieve the desired results (Ng, 1989).

2.4.1 Fill Phase

Fill provides for the addition of influent and may be static, mixed or aerated, depending on treatment objectives. Static fill results in minimum energy input due to the reduction in aeration time and high substrate concentration at the end of fill. Besides, static fill has a number of advantages, including the simultaneous removal of organic carbon, nitrogen and phosphorus, and effective prevention of filamentous bulking of activated sludge (Hsu, 1986; Herzbrun *et al.*, 1985). Mixed fill results in denitrification, if nitrates are present, a subsequent reduction of oxygen demand and energy in the beginning of aerobic reactions, a reduction of cycle time, and holds substrate concentration low, which may be important if biodegradable constituents exist which are toxic at high concentrations.

If no biological reactions occur during static fill (i.e. perfect separation of the influent substrate and the biomass), the substrate concentration in the SBR will reach a maximum at the end of static fill. That maximum concentration reached will equal the total substrate mass added during static fill plus any remaining at the end of idle divided by the total volume in the SBR at the end of static fill.

If mixed fill is selected, the substrate concentration, dissolved oxygen concentration, and nitrate concentration varying during the fill period. It is assumed that oxygen and nitrate are present in the SBR at the beginning of mixed fill and negligible concentrations in the influent, aerobic biological reaction would occur during the initial period of mixed fill, resulting in a reduction of dissolved oxygen and substrate. When oxygen is not available, nitrate will serve as the electron acceptor and anoxic biological reactions will degrade the substrate. Finally, fermentation or anaerobic biological reactions begin once oxygen and nitrate, the electron acceptors, are depleted.

Aerated fill is achieved by providing aeration during fill. The rate of substrate degradation is limited either by the biological reaction rate which is a function of biomass and substrate concentrations when dissolved oxygen concentration is above some minimum concentration, or a function of the rate at which oxygen is supplied from aeration. In the first case, the SBR reactor size is smaller, but the aeration system is larger and energy input greater. In the second case, a larger reactor is required, but the aeration system is smaller and energy input less. When biological reactions are limited by aeration rate, dissolved oxygen concentrations are nearly zero. This results in an additional benefit of greatest oxygen transfer efficiency.

Among the operating modes, the fill mode is one of the most important because, if properly controlled, a persistent waste may be made more degradable (Dennis and Irvine, 1979). Several studies have been carried out to investigate the effect of fill mode on the performance of the SBR treating various wastewaters. Kunz and Landis (1976) found that there were no variations in overall system performance for fill-and-draw reactors for sludge ages between 1-7 days. Irvine *et al.* (1977) developed a mathematical model to predict the values of total organic carbon (TOC), mixed liquor suspended solids (MLSS) and oxygen uptake rate. An experiment was carried out to validate their proposed mathematical model. Good agreement between measured and predicted values was obtained. An interesting observation was noted that during the longer fill time the TOC concentration decreases prior to the time that the reactor is full whereas the TOC concentration steadily rises during the fill period for the shorter fill time.

Dennis and Irvine (1979) showed that when the length of the fill time increases and the length of the react time decreases, the treatment in the reactor is similar to a continuously stirred tank reactor with varying liquid volume. When the length of the fill time decreases, the reactor is more likely a plug-flow reactor. Besides, they showed that there is a definite correlation between the settling velocity and the fill: react ratio – the greatest velocity occurred at a low fill time and a high react time while the lowest velocity occurred at a high fill time and a low react time. This is one of factors in the design of the SBR.

Yu *et al.* (1996) conducted a study on the effect of fill mode on the performance of SBRs treating various wastewaters, separately synthetic phenolic wastewater, synthetic wastewater containing polyvinyl alcohol (PVC) and effluent from a coke-plant wastewater treatment system. They showed under low-strength phenol conditions, the performance of the SBR with static fill was superior to the SBR with aerated fill in which filamentous bacteria developed. At high influent phenol concentrations, the SBR with static fill accumulated phenol during the fill period to concentrations inhibitory to microorganisms. Fill mode had no significant influence on the performance of SBRs treating PVC-containing wastewater. For the SBRs treating the effluent from a coke-plant-wastewater treatment system, fill mode had no significant impact on the performance of SBRs with respect to organic carbon removal and sludge settleability, but an operating mode which incorporated two anoxic periods, one ahead of the aeration and one after the aeration, was better than other operating modes in terms of nitrogen removal.

2.4.2 React Phase

Mixed react results in denitrification, if organics and nitrates are both present, reduction of oxygen demand, and anoxic or anaerobic conditions required for biological phosphorus removal. Aerated react results in completion of aerobic reactions, and bio-solids reduction, if aeration is extended.

2.4.3 Settle Phase

This is a secondary sedimentation of the traditional activated sludge process. After aerated and mixed react, separation is processed to isolate cells of activated sludge and clear water. The separation in the secondary sedimentation plant of the traditional activated sludge process is all directions, however, the separation in the SBR is static so the efficiency of the sedimentation is very high.

2.4.4 Decant / Draw Phase

This process is to dispose clear water after sludge sedimentation until the lowest level is reached. Most of the settled sludge at the bottom of the reactor is to be re-used at the next cycle of treatment. Excessive sludge is to be disposed. The water in the reactor can be used as recycle and diluting agent.

2.4.5 De-sludge / Idle Phase

The period after sedimentation and before a new cycle of sequencing batch reaction is called idle. According to the situation, mixing or aeration can be adopted. Under anaerobic, mixing not only saves energy but also maintains sludge activity. On purpose of the removal of phosphorus, disposal of residual sludge is generally carried out at the end of the settle and at the beginning of the idle. Morgenroth *et al.* (2000) carried out a study on the effect of long-term idle periods on the performance of SBRs. Their experiments showed that after 6 weeks of storage without aeration the sludge was still active with 55% of the initial volumetric ammonia oxidation rate. No adverse effects on sludge settleability were observed with an SVI below 140mL/g. Idle periods of 6 days had no effect on nitrogen removal. For longer idle periods, ammonia oxidation was not significantly effected but reactor operation had to be adjusted to prevent nitrite accumulation.

Besides, some studies have been carried out to investigate the effects of various phases to the performance of SBRs. Some investigations have been conducted to explore the performance of SBRs, e.g. Hoepker and Schroeder (1979) [effect of loading rate on effluent quality], Ketchum *et al.* (1987) [comparison of biological and chemical phosphorus removal], and Yamamtoo and Win (1991) [tannery wastewater treatment].

CONCLUDING REMARKS FOR LITERATURE REVIEW

- A more specific and most commonly used definition of heavy metals is an element with a density greater than 5 g/cm^3.

- A total of 24 water quality monitoring stations have been setup in Hong Kong. These 24 stations cover main watercourses in Hong Kong providing a meaningful indication to water quality, particularly on the levels of heavy metal contamination.

- Concentrations of some heavy metals measured from the 24 stations were very high. Besides, concentrations of some heavy metals measured from marine mammals in water in Hong Kong were very high too. These indicate that the water pollution from heavy metals in Hong Kong is serious.

- Previous studies showed that the presence of heavy metals at sub-lethal level also affected the performance of wastewater treatment, e.g. removal efficiencies. This prompts a need to investigate the effects and mechanisms of heavy metals at sub-lethal level on wastewater treatment.

- Some studies showed that the sludge age, suspended solid removal, sludge volume index, mixed liquor suspended solid concentration and settling time affect different extends of removal efficiencies of wastewater treatment.

- An indicator, namely CAC, was recently introduced the author of this thesis. Three applications of CAC have been introduced to monitor the ability of activated sludge to adsorb organic matter, to decide on variation in air supply and food pattern to the aeration units and to estimate the air supply.

- Adsorption isotherm is mainly modelled using Langmuir and Freundlich isotherms. Langmuir isotherm can be plotted as a rectangular hyperbola that is often described as "L-shape".

- Both different concentrations and toxicity of heavy metals affected the cilicate species in activated sludge.

- SBRs consist of five phases, separately fill, react, settle, draw, and idle phases.

- During the longer fill time the TOC concentration decreases prior to the time that the reactor is full whereas the TOC concentration steadily rises during the fill period for the shorter fill time.

- The settling velocity depends on the ratio of fill to react. The greatest velocity occurred at a low fill time and a high react time while the lowest velocity occurred at a high fill time and a low react time.

- Under low-strength phenol conditions, the performance of the SBR with static fill was superior, in terms of process stability and performance, to the SBR with aerated fill in which filamentous bacteria developed.

- At high influent phenol concentrations, the SBR with static fill accumulated phenol during the fill period to concentrations inhibitory to microorganisms.

- Fill mode had no significant influence on the performance of SBRs treating PVC-containing wastewater.

- For the SBRs which treats the effluent from a coke-plant-wastewater treatment system, fill mode had no significant impact on the performance of SBRs with respect to organic carbon removal and sludge settleability.

However, an operating mode which incorporated two anoxic periods, one ahead of the aeration and one after the aeration, was better than other operating modes in terms of nitrogen removal.

CHAPTER 3 : METHODOLOGY And MATERIALS

As stated in Chapter 1, the present study was designed mainly to investigate the effects of three selected heavy metals at sub-lethal concentrations on activated sludge processes. Chromium was selected to represent common toxic heavy metals, copper was selected for its high affinity towards adsorption sites on microbial cells, and zinc was selected to represent abundant and less toxic heavy metals in the environment. Sub-lethal concentrations for these three heavy metals were defined in accordance with the non-toxic drinking water standards from the World Health Organisation (World Health Organisation, 1980). The study was organized into three levels of experimental programmes. Firstly, the effects of heavy metals, including chromium (Cr), copper (Cu) and zinc (Zn) at sub-lethal concentrations, on CAC and COD removal efficiency in a simulated activated sludge process were investigated using two SBR systems operated under different hydraulic retention times (HRTs). Secondly, the mechanisms of metal adsorption on activated sludge bioflocs and the adsorption isotherms were studied using shake flask cultures. Thirdly, the characteristics of heavy metal uptake and adsorption of heavy metals on active sites on the cell surface structures, different superficial layers and different biochemical fractions in the layers were studied on a microscopic level. A common activated sludge microbial species, namely *Pseudomonas putida 5-X*, was selected and isolated for this study.

3.1 TEST PROGRAMS

3.1.1 Macro-Scale Study

3.1.1.1 Activated sludge process simulator

The term "Macro-Scale Study" was coined in this thesis to describe the process performance study of a laboratory-scale activated sludge simulator in the form of a sequencing batch reactor. The macro-scale study focused on the effect of heavy metals on the bulk of activated sludge microbes, hence, stability and well-being of the process. This is in contrast to the subsequent "Micro-Scale Study", which focused on the mechanisms of metal adsorption on the microscopic microbial cell surface binding sites.

Two identical 12-L sequencing batch reactors (SBR1 and SBR2) were used to simulate the operation of activated sludge processes. In each reactor, aeration and mixing were achieved by an air-diffuser system from the bottom of the reactor vessel (Fig. 1). In the seeding and start-up procedure of the reactors, returned activated sludge was collected from a local municipal sewage treatment plant, namely Taipo Sewage Treatment Works. The sludge was diluted ten fold, screened with a 2-mm sieve to remove coarse particles and introduced to seed the reactors. Mixed-liquor volatile suspended solids

(MLVSS) of the seeded reactors was maintained at around 3000 mg/L with a sludge age of approximately 10 days. A synthetic wastewater, prepared with glucose at 1000 mg/L was fed at 10 days HRT. The equivalent organic strength was 1000 mg/L COD (750 mg/L BOD_5). COD, instead of BOD, was used as the measurement for organic strength throughout this thesis because it allowed a large quantity of data to be obtained faster and more conveniently. These SBR systems were therefore started up at a food-to-microorganism (F/M) ratio of 0.03 mg BOD_5/mg MLVSS per day and a volumetric loading of 50 mg BOD_5/L per day. The SBRs were operated in a 24-h fill-and-draw cycle, which was similar to that described by Chambers (1993), Ng (1993) and Chua and Hua (1996). Each cycle consisted of five stages: FILL (5 min), REACT (6.5 h with aeration), SETTLE (1 h), DECANT (25 min) and IDLE (16 h) with 15-min aeration in every 2 h. The operating cycle was automated with timer-controlled feed, draw-off and aeration pumps. When the systems were acclimatized to the feed, as indicated by a stable COD removal efficiency, the HRT was reduced stepwise and a chromium-laden feed was introduced.

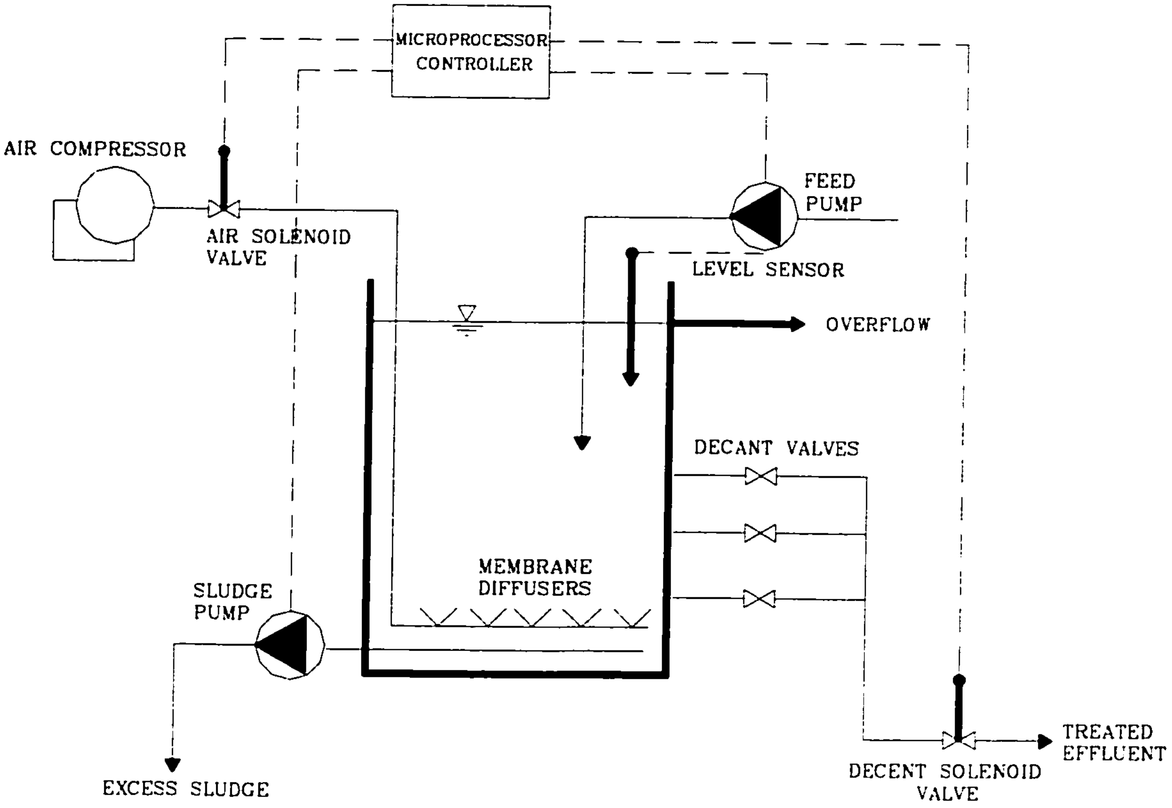


Fig 3.1

Schematics of Sequencing Batch reactor System – Activated Sludge Simulator

Experiments to investigate for the macro-scale study, it is aimed to investigate the effects of sub-lethal heavy metals on activated sludge processes with a larger scale with time-hours SBR systems.

In this macro-scale study, the SBRs were operated to investigate the relationship between HRT and CAC. The synthetic wastewater was fed initially to the system at 10 days of HRT. When the system was acclimatised to the feed of synthetic wastewater, indicated by a stable COD removal efficiency, the HRT was reduced stepwise to 5, 2.5, and then 2 days, allowing steady-state operation to be attained under each HRT. The metals-laden feed was prepared by dosing the synthetic wastewater with 0.05 mg/L of Cr^{3+}, 1.0 mg/L of Cu^{2+}, and 1.0 mg/L of Zn^{2+}. As different metals have different level of toxicity (Henry and Heinke, 1989), the concentration of metals selected in this study were made according to the maximum limit of the World Health Organization non-toxic drinking water standards (Table 3.1).

In addition to the fundamental experiments as described above, the SBRs were also operated in a series of experiments to study the reproducibility of the relationship between HRT and CAC. These experimental studies consisted of four stages including fourteen tests (namely 5 [HRTs] x 1 [without Cr^{3+}], 5 [HRTs] x 1 [with Cr^{3+}], 2 [HRTs] x 1 [without Cr^{6+}], and 2 [HRTs] x 1 [with Cr^{6+}]). The CAC and COD removal efficiency, as affected

Table 3.1

Maximum allowable heavy metal contents in drinking water (Henry and Heinke, 1989)

Metals	mg/L
Ba	1.0
Cd	0.01
Cr	0.05
Cu	1.0
Pb	0.05
Ag	0.05
Zn	5.0
Hg	0.002
Fe	0.3

by different HRTs with and without chromium in the SBRs, were recorded. In these tests with adding chromium, the chromium-laden feeds were prepared by dosing the synthetic wastewater with 0.05 mg/L of chromium (II) nitrate [$Cr(NO_3)_3 . 9H_2O$] and 0.05 mg/L of chromium trioxide [CrO_3] so that Cr^{3+} and Cr^{6+} laden feeds were produced. The overall layout of the laboratory set up of the SBR systems used in this study to simulate the activated sludge process is shown in Photo 3.1.

3.1.2 Mechanistic Study

In order to show that the effects of heavy metals, at trace level, on CAC and COD removal efficiency is not due to their toxicity, an experiment using Cr^{3+} and Cr^{6+} at 0.05 mg/L was designed. As Cr^{3+} and Cr^{6+} are of obvious difference in toxicity, if the extents of their effects on activated sludge are of the same order, then the effects must be due to physical mechanisms, instead of biological metabolic reasons.

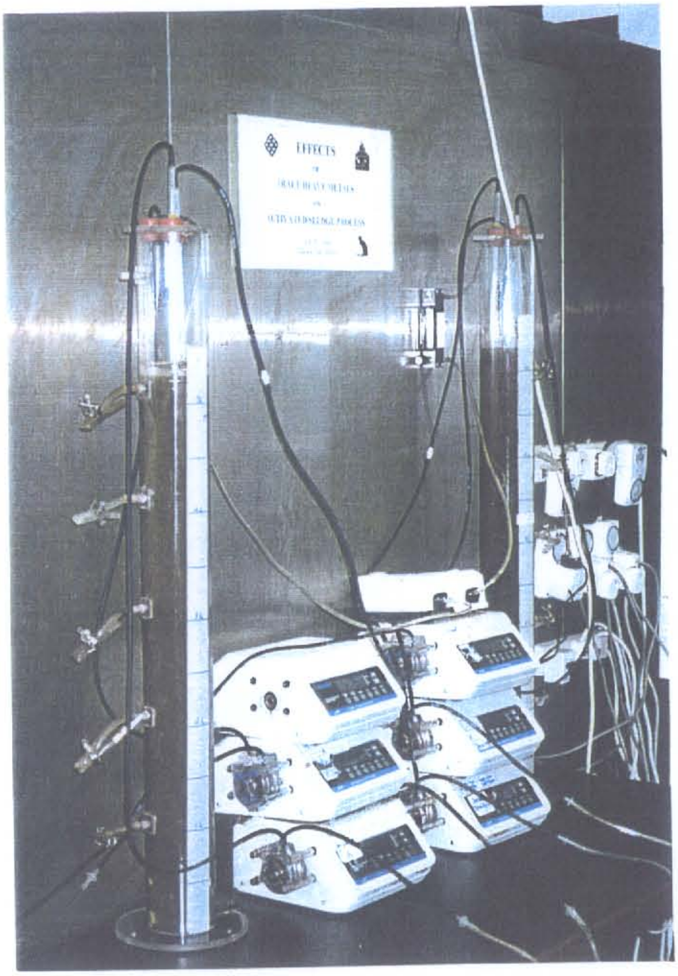


Photo 3.1

Operation of Sequencing Batch Reactor (SBR)

This experimental study consists of two stages including ten tests. These two stages were carried out to measure CAC and COD removal efficiency affected by six different concentrations of Cr^{3+} and Cr^{6+}. Similar to the previous experimental programmes, the chromium-laden feeds were prepared by dosing the synthetic wastewater with 0.05 mg/L of chromium (III) nitrate $[Cr(NO_3)_3 \cdot 9H_2O]$ and 0.05 mg/L of chromium trioxide $[CrO_3]$ so that Cr^{3+} and Cr^{6+} laden feeds were produced. Samples of activated sludge were first put into a cylinder and was fed with the chromium-laden feeds. 500mL of the resulting mixed liquors was then taken from the cylinder and placed in a 1-L beaker for settling for an hour. As biosorption is time and floc loading dependent, COD removed by instantaneous adsorption onto the cell wall has to be determined within a short period of time upon mixing the metal-laden sewage and the sludge. Therefore, the supernatant was collected and determined for its COD. The beaker was then placed on a magnetic stirrer and stirred at a rate of 300 rpm without aeration. Settled sewage taken from the cylinder was analysed for its COD. While the content in the beaker was stirred, 250 mL of the settled sewage was pumped into the beaker in exactly 1 minute by means of a peristaltic pump. At the end of one minute, the stirrer was stopped and the mixture left to stand for an hour. The supernatant was then collected and tested for its COD. CAC of the activated sludge taken before and after the aeration period from the cylinder was analysed for three consecutive days. The effluent quality on each day was analysed. Seven time intervals of CAC and COD removal efficiency were measured, separately, at 5, 10, 20, 30, 60, 120 and 240 seconds.

3.1.3 Microscopic Study

3.1.3.1 Microorganisms Culture

The bacterial strain used in this study was *Pseudomonas putida* 5-x, which was isolated from a local activated sludge plant. The inoculum used in the bacterial culture was prepared by inoculating a single mono-culture colony in 30mL of sulphate-limiting medium (SLM) in a 250-mL conical flask and incubated by shaking at 200 rpm for 24 hours at 30°C. A portion of 3 mL of the SLM containing 1% inoculum was then used for a culture in a 5-L automatic jar fermenter (Bioengineering Co.) at an agitation rate of 400rpm and an aeration rate of 1.5 L/min. The culture conditions were set at 30°C for 36~ 48 hours. The microbes were harvested by centrifugation at 4000 rpm for 10 min at 4°C. The cell pellets were washed twice using 100 mL of 10mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer.

Photo 3.2

Shaking of mixture by the AROS 160

3.1.3.2 Superficial Layer - Capsule

In order to remove the cell superficial layer, which may affect metal adsorption, 100 mg (dry weight) of *P. putida*5-x biomass was washed with 25 mL of 0.3N HCL by shaking at 200 rpm for 30 min at 25°C. The pretreated cells were harvested by centrifugation at 4000rpm for 20mins, and washed twice with 50 mL of 10mM MES buffer.

3.1.3.3 Preparation of Cell Envelopes

The pretreated cells were suspended in 10mM MES buffer, and then broken or ruptured by passing twice through a French Pressure Cell at 20,000 lb/in^2. The broken cell suspension was centrifuged at 3000rpm for 10 mins to remove whole cell. The supernatant was then treated with 5μg of deoxyribonuclease per mL and 10μg of ribonuclease per mL for 10min at 25°C, the envelope pellets were separated from supernatant by centrifugation at 15,000 rpm for 60min. The harvested cell envelopes were washed five times with 0.05 M, pH 7.2 HEPES (N-2-hydroxyethylpiperazine-N$'$-2ethanesulfonic acid) buffer, and then washed twice with distilled water.

3.1.3.4 Preparation of Peptidoglycan Layer from Cell Envelopes

The previously isolated cell envelopes were extracted with 4% SDS (Sodium dodecyl sulfate) solution for 30min at 25°C. The suspension was then centrifuged for 60min at 30,000rpm to separate the peptidoglycan layer. The pellets were then washed with 2% SDS, three times with 1M NaCL, and finally, five times with distilled water.

3.1.3.5 Determination of Copper Adsorption Capacity

50 mg (dry weight) of bacterial cell or 20 mg of cell envelope or peptidoglycan layer (dry weight) were added into a centrifuge bottle containing 100 mL of MES buffer with 50 mg/L or 100mg/L of Cu^{2+}. In the control bottle, no biosorbent was added. After equilibration by shaking at 200 rpm at 25 °C for 20 minutes, the biosorbents were removed by centrifugation at 8000rpm for 15 minutes at 25°C. The supernatant was transferred into a polyethylene bottle for the determination of Cu^{2+} concentration with a Perkin Elmer 3300 atomic absorption spectrophotometer. Adsorption capacity (AC) of Cu^{2+} expressed as mg Cu^{2+} removed per g cell dry weight and calculated by the following equation:

$$AC = \frac{(C_c - C_e)\,V}{W}$$

where C_c is the final Cu^{2+} concentration in the control (mg/L),

C_e, is the residual Cu^{2+} concentration after addition of the cells (mg/L),

W is the cell dry weight (g), and

V is volume of the Cu^{2+}-containing buffer solution (L).

3.1.3.6 Determination of Adsorption Isotherm for Copper Uptake

Biosorbents were added into the MES buffer solutions with initial Cu^{2+} concentrations of 10, 20, 30, 50, 80, 100 and 120mg/L, respectively. The Cu^{2+} adsorption capacities of different biosorbents were determined, and adsorption isotherm of different biosorbents were obtained by a graphical plot.

3.1.3.7 Transmission electron microscopy (TEM) analysis

Fresh cells and pretreated cell were harvested by centrifugation, dehydrated through an ethanol series and then embedded in SPURR. Thin sections of 60 nm were prepared using a Reichert Ultracuts (Leica, Wien, Austria) equipped with a diamond knife (Diatome 45°, Fort Washington, USA). Sections were then examined under transmission electron microscope. No fixative and stain was used in the preparation of the sections. For cell envelopes and peptidoglycan layer, carbon-Formvar coated nylon grids (400 mesh, Pelco International, Redding, CA, USA) were floated in aqueous suspensions for 30s, dried with blotting paper and then examined with a JEM-1200 EX-II TEM (JEOL Ltd., Tokyo, Japan) which was operated at 80 kV under standard conditions.

3.2 METHODS OF MEASUREMENT AND ANALYSIS

A number of essential water quality parameters have been measured in the various experimental programmes in order to monitor the performance of the sequencing batch reactors, the metal adsorption characteristics and microscopic mechanisms. These parameters include COD, MLVSS (mixed-liquor volatile suspended solids), and metal ion concentrations.

3.2.1 COD Removal Efficiency

COD is an indicator of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. There are several methods able to measure COD. COD in the sampled reactor liquor were measured in accordance with Standard methods (APHA, 1992).

COD removal efficiency was determined using Equation (2)

$$\text{COD removal efficiency} = (\text{COD}_{\text{inf}} - \text{COD}_{\text{eff}}) \times 100\% \qquad \text{Eq. (2)}$$

Where:

COD_{inf} = dissolved COD in the influent after the fill phase; and

COD_{eff} = dissolved COD in the effluent after the settle phase.

Dissolved COD was determined after filtering the sample through a 0.45-µm filter paper.

3.2.2 MLVSS

Mixed-Liquor Volatile Suspended Solids (MLVSS) was used in the tests because it provided an indication of the amount of microorganisms in the activated sludge. The alternative method, namely MLSS, was not used because it measures the total suspended solids, which may also included mineral salts and other inorganic matters. The MLVSS procedures were described as follows:

1. 10 mL sludge was collected.
2. A dried filter paper (ignited at 550 ± 50℃ for the determination of volatile solids) was weight A, mg).
3. The sample was filtered by pouring it into the three-piece funnel (Photo 3.3). Then the filter paper was evaporated on a steam bath overnight.
4. Filter paper was taken out, cooled and weighed (weight B, mg).
5. Filter paper was further ignited at 550 ± 50℃ for 15 minutes in a muffle furnace. It was then taken out and weighed (weight C, mg).

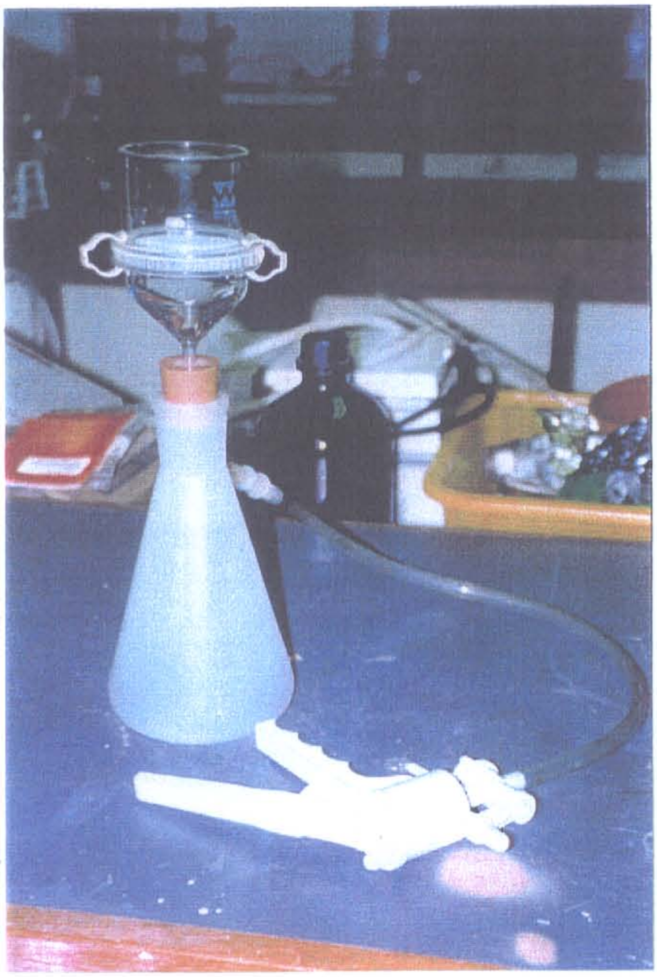


Photo 3.3

The three-piece funnel

Total suspended solids = (B – A) x 1000 x 1000 / 10 (mg/L)

Fixed suspended solids = (C – A) x 1000 x 1000 / 10 (mg/L)

6. The MLVSS was calculated by the following equations:

Volatile suspended solids (mg/L) =

Total suspended solids – Fixed suspended solids

3.2.3 Measurement of heavy metal concentrations

The concentrations of heavy metals, as previously described in Section 3.1.3.5, was measured by the atomic absorption spectrometry (Perkin Elmer 3300) and is shown in Photo 3.4. It resembles emission flame photometry in that a sample is aspirated into a flame and atomized. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a specific concentration range.

3.2.4 Determination of COD Adsorption Capacity (CAC)

The definition of CAC was adopted from that of Tan and Chua (1997), which defined COD Adsorption Capacity (CAC) was the parameter measured to

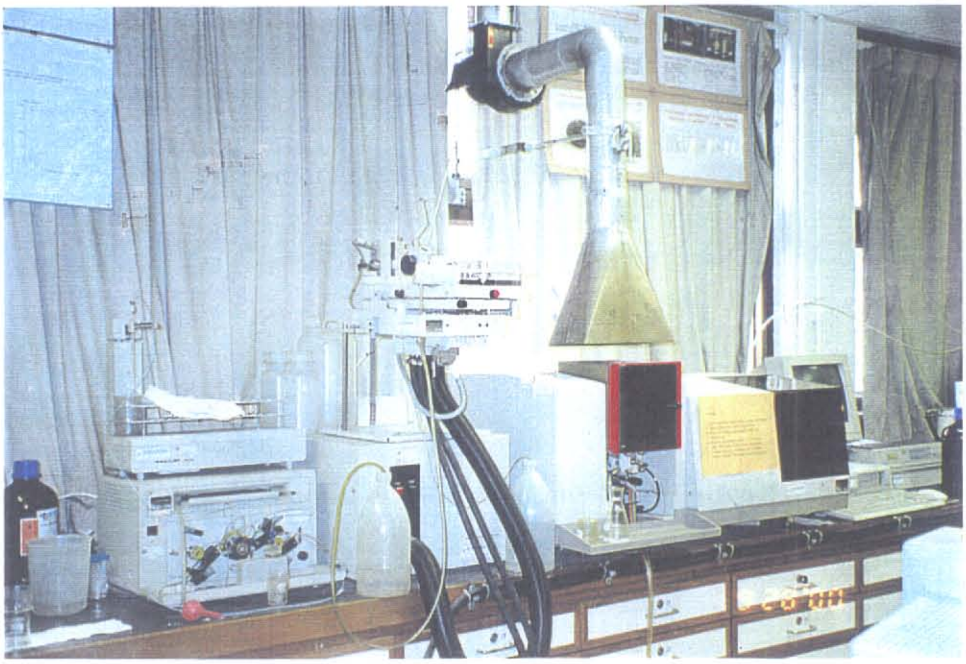


Photo 3.4

The atomic absorption spectrometry (AAS) monitor the ability of activated sludge to adsorb organic matters. The value of CAC was calculated from dividing the difference between theoretical dissolved COD after the fill phase and the actual dissolved COD measured at 10 minutes after the fill phase, by MLVSS or equivalently as Equation (1).

$$\mathrm{CAC} = \mathrm{A} / \mathrm{MLVSS} \qquad \text{Eq. (1)}$$

$$\mathrm{A} = [V_f / (V_f + V_r)]\, \mathrm{COD}_f + [V_r / (V_f + V_r)]\, \mathrm{COD}_r - \mathrm{COD}_{obs}$$

Where:

V_f = volume of feed in each operationg cycle (litres);

V_r = volume of the remaining reactor liquor after DECANT stage (litres);

COD_f = dissolved COD in feed (milligrams per litre);

COD_r = residual dissolved COD in remaining reactor liquor after DECANT (milligrams per litre); and

COD_{obs} = observed dissolved COD after initial 10 min during REACT stage (milligrams per litre).

MLVSS and COD in the sampled reactor liquor were measured in accordance with Standard methods (APHA, 1992). Dissolved COD was determined after filtering the sample through a 0.45- μ m filter paper.

CHAPTER 4 : MARCO-SCALE STUDY ON EFFECTS OF HEAVY METALS ON ACTIVATED SLUDGE PROCESSES

This Chapter presents the results that demonstrate the effects of three common environmental residual heavy metals, namely zinc, copper and chromium, on the COD removal efficiency and COD adsorption capacity (CAC) in SBR systems as activated sludge process simulators. In the first part of this experimental programme, the SBR systems were operated for an overall period of over 400 days under different conditions of HRTs and metal loadings. The process performance and stability under each set of operating conditions were studied and the effects under different heavy metals were compared. In the second part of the experimental programme, the effects of chromium were studied with greater details using Cr^{3+}- and Cr^{6+}-laden wastewater to demonstrate the effects of trace metals are mainly physical instead of biological toxic effects.

4.1 Performance of SBR in the Presence of Heavy Metals

4.1.1 Zinc-laden wastewater

The SBR systems were first operated for a period of 130 days with different HRTs and zinc loading conditions. Figure 4.1 shows the profile of SBR performance, in terms of CAC and COD removal efficiency, affected by zinc-laden wastewater. Table 4.1 tabulates the average MLVSS, CACs and COD removal efficiencies at various HRTs. After 30 days of start-up operation, the reactor was stable at 5 days of HRT. The average CAC was 53.8 mg/g and the corresponding COD removal efficiency was 95.3%. The reactor stabilised within 15 days after the zinc-laden wastewater was introduced. The average CAC and COD removal efficiency was 39.7 mg/g and 91.9%, respectively. The COD removal efficiency had not significantly changed while the CAC decreased by 26%. The residual COD increased when the HRT was reduced to 2.5 days, while led to a drastic increase of CAC. The CAC increased to a maximum level of 103.8 mg/g. When the system was operated at shorter HRTs, the average CAC and COD removal efficiency were 92.0 mg/g and 92.5%, respectively without zinc. However, with the presence of zinc, the average CAC and COD removal efficiency was reduced to 71.3 mg/g and 72.4%, respectively. It was equivalent to 23% and 22% drop in CAC and COD removal efficiency, respectively.

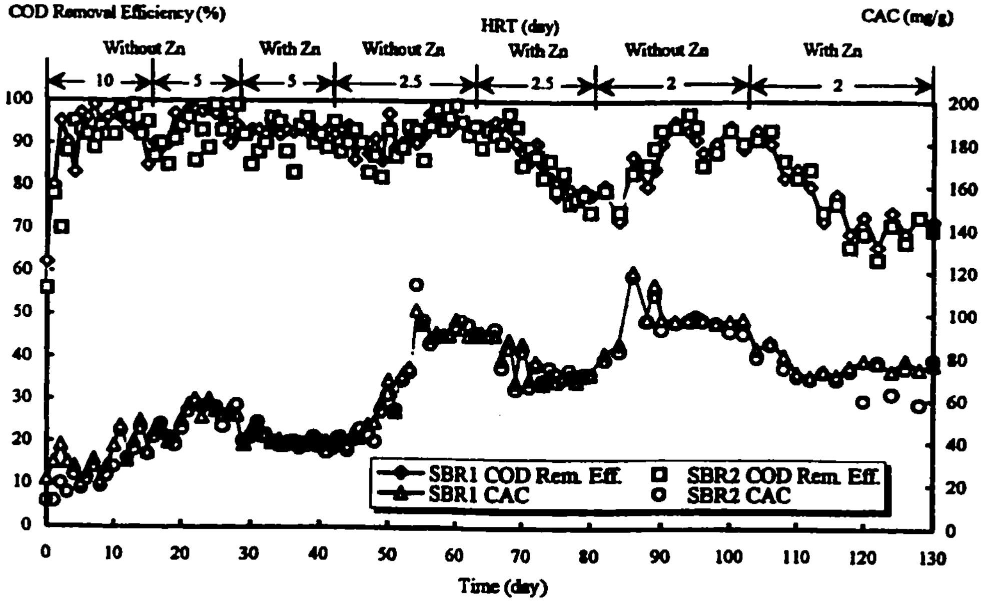


Figure 4.1

Performance of SBR affected by addition of zinc

4.1.2 Copper-laden wastewater

The SBR systems were similarly operated for a period of 130 days with different HRTs and copper loading conditions. Figure 4.2 shows the treatment performance of CAC and COD removal efficiency, affected by copper-laden wastewater. As that previously observed with zinc-laden wastewater, the performance of both the SBR systems was similar in response to the presence of copper ions. The average MLVSS, CACs and COD removal efficiencies of the two reactors under stable operation at various HRTs are summarised in Table 4.1. After 30 days of start-up operation to re-condition the systems from previous effects of the zinc-laden wastewater, these two reactors were stable at 5 days of HRT, the average CAC was 54.3 mg/g and the corresponding COD removal efficiency was 96.3%. In the presence of copper, these two reactors could be maintained stable within 15 days, the average CAC and COD removal efficiency was 39.3 mg/g and 93.7%, respectively. The COD removal efficiency had no significant change while the CAC decreased by 28%.

Table 4.1

The performances of SBR system affected by zinc, copper and chromium

HRT (day)	Zinc			Copper			Chromium		
	MLVSS (mg/L)	CAC (mg COD/g sludge)	COD removal rate (%)	MLVSS (mg/L)	CAC (mg COD/g sludge)	COD removal rate (%)	MLVSS (mg/L)	CAC (mg COD/g sludge)	COD removal rate (%)
5.0 without metal	1507	53.8	95.3	1492	54.3	96.3	1509	49.6	93.2
5.0 with metal	1482	39.7	91.9	1452	39.3	93.7	1520	43.6	91.8
2.5 without metal	1553	92.0	92.5	1569	93.2	91.6	1609	87.8	90.6
2.5 with metal	1598	71.3	72.4	1630	70.1	71.0	1503	74.6	77.9
2.0 without metal	1553	96.8	92.2	1530	97.7	90.2	1495	92.6	90.0
2.0 with metal	1528	75.6	70.8	1540	76.2	70.4	1500	77.8	73.8

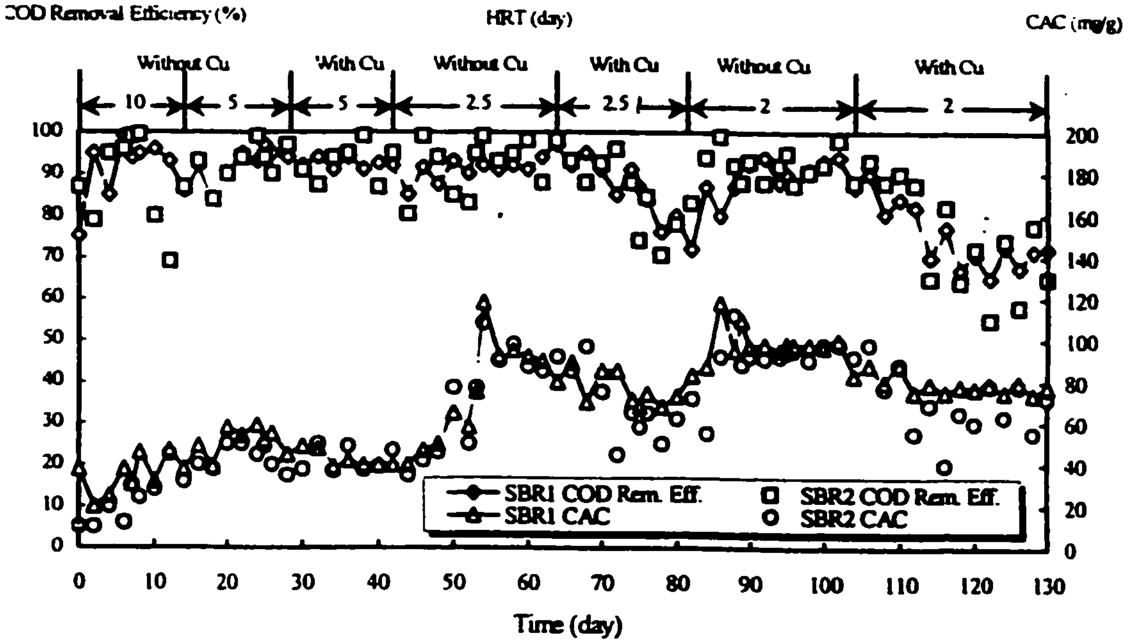


Figure 4.2

Performance of SBR affected by addition of copper

4.1.3 Chromium-laden wastewater

Similar observations were made during the 130 days of operation with chromium-laden wastewater. Figure 4.3 shows the performance of CAC and COD removal efficiency affected by chromium-laden wastewater. Table 4.1 summarises the average MLVSS, CACs and COD removal efficiencies of the two SBRs at various HRTs. After 20 days of start-up operation to re-condition the systems from previous effects of the copper-laden wastewater, these two reactors were stable at 5 days of HRT. The average CAC was 49.6 mg/g and the corresponding COD removal efficiency was 93.2%. In the presence of chromium, the average CAC and COD removal efficiency was 43.6 mg/g and 91.8%, respectively.

The COD removal efficiency had not significantly changed while the CAC decreased by 12%. When the HRT was reduced to 2.5 days, the residual COD increased as a result of a drastic increase of CAC to a maximum level of 120.1 mg/g , without chromium. Under stable operating condition at 2.5 days of HRT, the average CAC and COD removal efficiency was reduced to 74.6 mg/g and 77.9%, respectively, with chromium. These were equivalent to a 15% and 14% drop in CAC and COD removal efficiency, respectively. Similar results were obtained when the reactors were operated at 2 days of HRT. The observed average CAC and COD removal efficiency decreased 16% and 18%, respectively, in the presence of chromium.

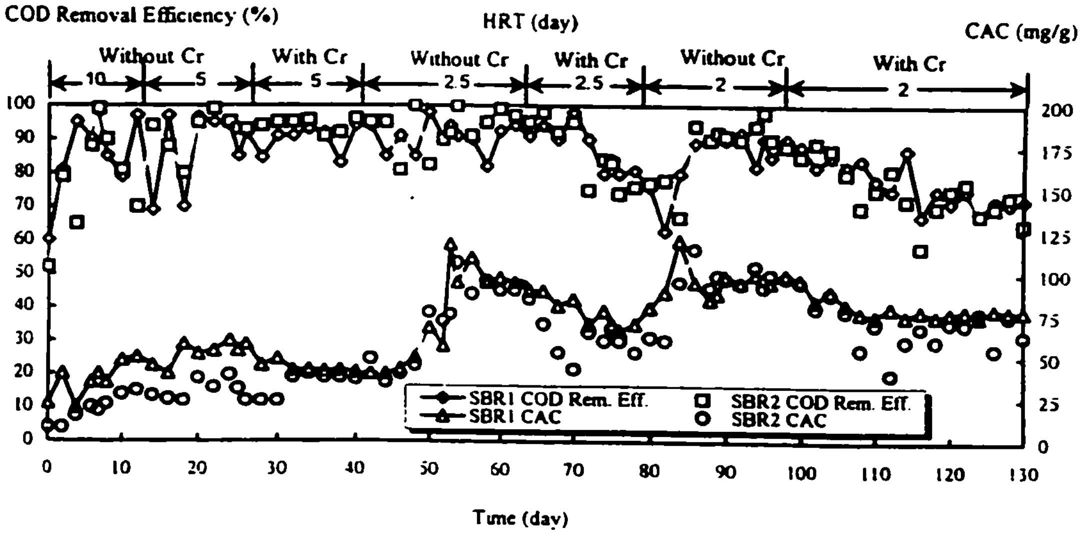


Figure 4.3

Performance of SBR affected by addition of chromium

4.1.4 Comparison of the effect of different heavy metals on activated sludge

As illustrated in Figures 4.1 to 4.3, the effects of heavy metals, at sub-lethal concentrations, on the COD removal efficiency was apparently similar, particularly at longer HRTs (e.g. 5 days). The different affinities, and hence different effects, of different heavy metals on the activated sludge reported by Lester *et al.* (1979) and Battistoni *et al.* were not observed. This was attributed to the fact that the concentrations of different heavy metals applied were in a similar order and relatively low concentrations, ranged from 1 to 5 mg/L. In addition, the retention of organic matters within the SBRs was sufficiently long for biological assimilation and therefore the effect on organic removal efficiencies was not obvious. The presence of heavy metals, at sub-lethal concentrations, would affect the CAC and had little effect on COD removal, particularly at this HRT of 5 days, while the overall effects on the CAC were also similar, decreased by 16 to 25%. The average CACs, under the effects of zinc, copper and chromium, were 62.2, 61.9 and 65.3mg/g, respectively, and the corresponding COD removal efficiencies were 78.3, 78.4 and 81.2%, respectively.

When comparing the effects of metal-laden wastewater on the CAC and COD removal efficiencies, for zinc, copper and chromium, at a shorter HRTs, namely 2.0 days, copper ions showed stronger effects on the CAC and COD removal efficiency than zinc ions. The effect of chromium on the CAC and COD removal efficiency was the lowest. These results were in agreement with the observations on relative affinities and relative toxic effects reported by Lester *et al.* (1979). Presence of copper and zinc affected the organic adsorption by activated sludge microbial ecosystem to similar extent, namely decreased by 20 to 25%, despite the fact that copper is generally known to be more strongly adsorbed on activated sludge bioflocs than zinc. This was in agreement with general observations from the batch adsorption experiments in the literature. Chua and Hua (1997) suggested that presence of copper in the incoming wastewater occupied specific active adsorption sites on activated sludge microbial cell surfaces and physically affected the configurations of neighbouring active sites on the activated sludge bioflocs, thus affecting the adsorption properties of organic compounds on the bioflocs. The detail adsorption mechanisms and the related effects will be investigated in Chapter 6.

Presence of chromium affected the CAC and COD removal efficiency to a lesser extent compared to the effects of copper and zinc. This was because chromium, although having a higher toxicity, was added at much lower concentration at sub-lethal level. These results illustrated that heavy metals present at sub-lethal concentrations affected the activated sludge through physical adsorption and competition against organic matters for active adsorption sites on the bioflocs, instead of biological toxicity. These will be shown more directly in the mechanistic studies in the subsequent Chapters.

Based on these fundamental results, it postulated that removal of COD by activated sludge is proceeded by a rapid adsorption of organic matters on certain active sites on the superficial layers of bioflocs, followed by a slower metabolic destruction of these organic compounds. Metal ions acted as a strong competitor against organic compounds for active sites on the bioflocs, and adsorbed metals altered the physical configuration of other active adsorption sites for organic matters, instead of acting as a toxic microbial inhibitor. Such competitive behaviour not only hampered the organic adsorption, but also affected the COD removal efficiencies in activated sludge processes, particular when the processes are operated under shorter HRTs.

4.2 Further Operations with Chromium-Laden Wastewater

In this extension of the study programme, the SBR systems were operated further with chromium-laden wastewater to pursue on the investigation of the effects on CAC and COD removal efficiency. Investigation was carried out in two aspects, namely (1) to observe the effects of trace chromium on CAC and process COD removal efficiency under very short operating HRTs (40 to 6 hours), and (2) to elucidate the effects of Cr^{3+} and Cr^{6+} on the activated sludge process.

4.2.1 Effects of chromium under shorter HRTs

Following the 20-day start-up operation and acclimatisation at 80 hrs (3.3 days) HRT, the hydraulic loading was increased step-wise and reactor systems reached a stable operation under a HRT of 40 hrs (approximately 1.7 days) after 30 days of operation. Figures 4.4 and 4.5 are constructed to show CAC and the COD removal efficiency measured and Table 4.2 summarises the operating conditions and measured parameters under stabilised operation at each of the chosen HRTs.

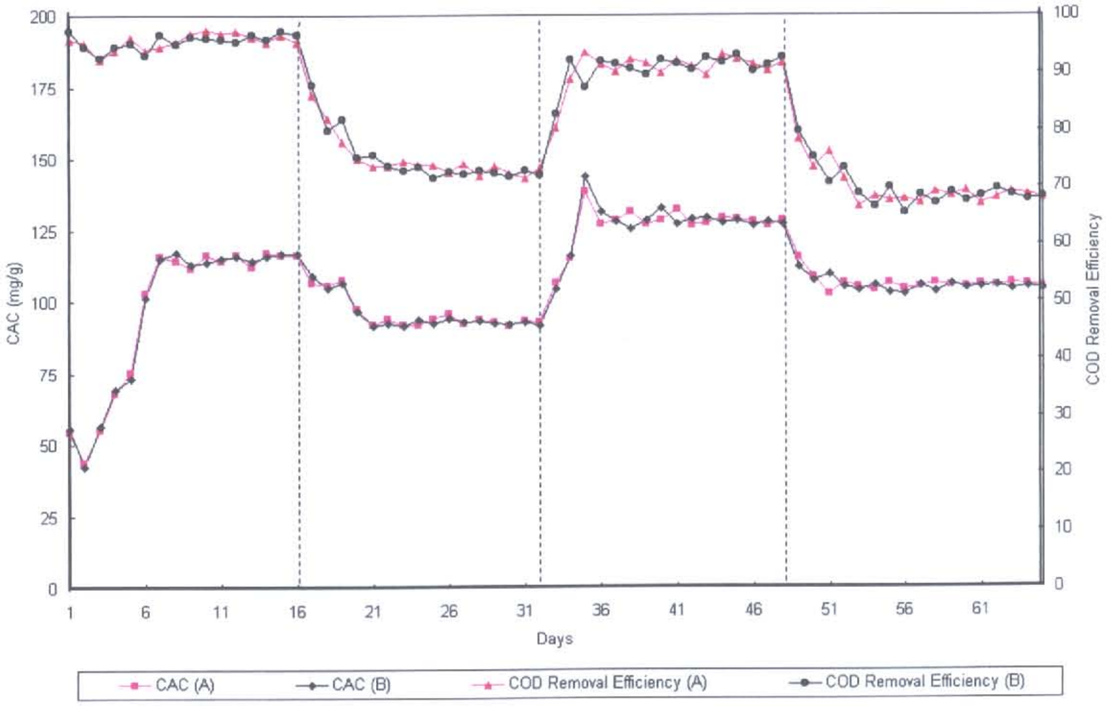


Figure 4.4

Performance of the activated sludge in the SBR with different HRTs

Figure 4.5

Performance of activated sludge in SBRs A and B under HRTs of 12 hrs and 6 hrs

Table 4.2

Average MVLSS, CAC and COD removal efficiencies of both studies.

HRT (hrs)	F/M ratio (mg BOD_5 / mg MLVSS per day)	Volumetric loading (mg BOD_5/L per day)	Presence of chromium (Cr^{3+})	MLVSS (mg/L)	Organic adsorption capacity (mg COD/L MLVSS)	COD removal efficiency (%)
40	0.21	300	No	1509	49.6	93.2
40	0.21	300	Yes	1520	43.6	91.8
20	0.39	600	No	1609	87.8	90.6
20	0.39	600	Yes	1503	74.6	77.9
16	0.51	750	No	1495	92.6	90.9
16	0.51	750	Yes	1500	77.8	73.8
12	0.5	1500	No	3000	115.0	96.2
12	0.5	1500	Yes	2980	93.0	73.3
6	1.2	3000	No	3016	128.4	91.3
6	1.2	3000	Yes	3008	105.5	68.2

Corresponding to the stabilised operation at the HRT of 40 hrs. the F/M ratio was 0.21 mg BOD_5/mg MVLSS per day. The average CAC was measured as 49.6 mg/g and the corresponding COD removal efficiency was measured as 93.2%. After the chromium-laden wastewater was distributed to the SBR, the reactor stabilised within 12 days with an average CAC of 43.6 mg/g and a COD removal efficiency of 91.8%. It had an equivalent drop of 12% on CAC while the COD removal efficiency was not significantly affected. A decrease of equivalent to 15, 16, 18 and 19% of CAC were measured for HRTs of 20, 16, 12 and 6 hrs. In addition, a corresponding decrease of 14, 19, 24 and 25% of COD removal efficiencies were measured. These results show that the presence of chromium ion affected both the COD removal efficiency and CAC to similar extents, especially when the systems were operated under relatively short HRTs. Besides, results also show that when the HRT was reduced from 40 to 6 hrs, the decreases in both CAC and the COD removal efficiency were increased while the decrease of CAC was less than the decrease of COD removal efficiency. The effect of HRT on the COD removal efficiency is therefore higher than that of CAC. As the flow rate in the SBRs was increased and the retention time was shortened, the reaction time taken for the activated sludge microbes to physically adsorb and biologically degrade the organic matters in the wastewater was reduced. Therefore, these results further substantiate the previous findings as described before, that the reaction of the SBRs becomes unstable when a short HRT is adopted, thus reducing the COD removal efficiency. As HRT was very short, the rapid physical adsorption of organic matters and hence retention of the organic matters in the reactor system became crucial for subsequent slow biological degradation of the organic matters. Therefore, presence of heavy metals competed against organic matters for active adsorption sites on the activated sludge microbes or bioflocs, thus hampering organic adsorption and degradation. These led to the observed phenomenon of more pronounced heavy metal effects on both the CAC and COD removal efficiency to similar extents under these relatively short HRTs.

When the HRT was reduced to 20 hrs, the F/M ratio was 0.39 mg BOD_5/mg MVLSS per day. In the absence of chromium, the residual COD level was increased leading to CAC having a maximum value of 120.1 mg/g. This further substantiated and agreed with the postulation given by Tan and Chua (1997) that biological degradation in activated sludge is preceded by surface adsorption on the bioflocs. Therefore, high residual organic concentration resulted in a stronger driving force for organic adsorption. Such observations were also observed from the results measured for the HRTs of 16, 12 and 6 hrs.

When the reactor was operated at the volumetric loading of 300 mg BOD_5/L per day and the relatively long HRT (40 hrs), retention of organic matters within the SBRs was sufficiently long for biological assimilation. As a result, organic adsorption was not a critical factor governing the SBR performance. Therefore, the presence of chromium affected CAC, but had little effect on the COD removal efficiency. At the volumetric loading of 600 and 750 mg BOD_5/L per day, the HRTs were 20 and 16 hrs, respectively. Compared to the normal SBR design ranges of 250-750 mg BOD_5/L per day and HRT of 0.5-2 days (Metcalf and Eddy, 1991), the SBRs were operating under relatively high loadings and short HRTs. Under such operation conditions, the presence of chromium caused a drop in CAC, and, as a result, affected the COD removal efficiency to a more significant and comparable extent. The variations in the COD removal efficiency were directly affected the variations in CAC under a short HRT.

These observations agree with the previous studies of a similar SBR systems fed with zinc- and copper-laden wastewater as described previously and also with that reported by others (Tan, 1993; Chua and Hua, 1996; 1997). Presence of copper or zinc affected the organic adsorption by activated sludge to similar extent, namely decrease of 20-25%, despite the fact that copper is generally known to be more strongly adsorbed on bioflocs than zinc. Chua and Hua (1997) stated that presence of copper caused changes in the surface properties on the activated sludge microbes or bioflocs for physical adsorption of organic compounds in the incoming wastewater, thus affecting the adsorption capacity, and hence the organic degradation efficiency, of organic compounds on the bioflocs. Presence of chromium affected CAC and the COD removal efficiency to a lesser extent compared to the effects of copper and zinc. This was because chromium, having a relatively high toxicity, was added in the synthetic wastewater at a much lower concentration compared to copper and zinc in the previous studies.

4.2.2 Effects of Cr^{3+} and Cr^{6+} on activated sludge

The toxicity of Cr^{3+} and Cr^{6+} are widely accepted to be significantly different, with the latter having significantly higher toxicity than the former. The SBR systems were further operated with Cr^{3+}- and Cr^{6+}-laden wastewaters in order to demonstrate and to substantiate the postulation that the effects of heavy metals, at trace concentrations, on activated sludge is mainly due to physical competitive adsorption, instead of biological toxicity. The observations and results measured from both SBR systems are shown in Figure 3 and summarised in Table 4.3.

When the two chromium solutions were distributed in the SBR A and SBR B respectively, decrements of both CAC and the COD removal efficiency were measured. For SBR A, the presence of Cr^{3+}, CAC was measured as 93.0 mg/g and the COD removal efficiency was measured as 73.3%. These were equal to a 19% drop in CAC and a 24% in the COD removal efficiency as compared to the results measured for the absence of chromium. For SBR B. the presence of Cr^{6+}, CAC was measured as 92.5 mg/g and the COD removal efficiency was measured as 72.8%. These were equal to a drop of 20% in CAC and a drop of 24% in the COD removal efficiency as compared to the results measured for the absence of chromium.

Similar pattern of results were observed on the CAC and COD removal efficiencies under the separate effects of Cr^{3+} and Cr^{6+} in SBR A and SBR B, respectively, despite the fact that Cr^{6+} has a significantly higher toxicity than Cr^{3+}. Under a sub-toxic level, the toxicity of chromium was no longer a significant factor that affected the activated sludge process performance. The effect of chromium on the activated sludge was only because of the competition between chromium and organic matters for the active sites on the bioflocs, agreeing with that observed and reported by others (Chua *et al.*, 1999). Chromium, in both Cr^{3+} and Cr^{6+} forms, acted as a strong competitor against the organic matters for the active adsorption sites on the activated sludge bioflocs, thus hampering CAC and affected the COD removal efficiency to similar extents under short HRTs.

When the HRT was reduced to 6 hrs, corresponding to a F/M ratio of 1.2 mg BOD_5/mg MLVSS per day, the CAC increased to a maximum of 132.2 mg/g and 132.4 mg/g for SBR A and SBR B, respectively. The increase of the

Table 4.3

Average MLVSS, CAC and COD removal efficiencies for SBRs A and B

HRT (hrs)	Reactor	F/M ratio (mg BOD_5/mg MLVSS per day)	Volumetric loading (mg BOD_5/L per day)	Presence of chromium	MVLSS (mg/L)	Organic adsorption capacity (mg COD/g MLVSS)	COD removal efficiency (%)
12	A	0.5	1500	---	3012	115.0	96.2
	B			---	3004	115.2	96.1
	A			Cr^{3+}	3000	93.0	73.3
	B			Cr^{6+}	2980	92.5	72.8
6	A	1.2	3000	---	3002	128.4	91.3
	B			---	2996	127.9	91.5
	A			Cr^{3+}	3016	105.5	68.2
	B			Cr^{6+}	3008	104.6	68.1

residual COD level caused the increase of CAC in the absence of chromium. As mentioned above, a higher residual organic concentration resulted in a stronger driving force from the bulk liquid phase towards the superficial layers of the activated sludge microbes, thus increasing the organic adsorption capacities.

During the operation, in the absence of chromium in SBR A, the average CAC and COD removal efficiency were 128.4 mg/g and 91.3%, respectively. The values measured in SBR A were 127.9 mg/g and 91.5%. However, in the presence of Cr^{3+}, the average CAC and COD removal efficiency measured from SBR A were 105.5 mg/g and 68.2%. These were equal to a drop of 18% in CAC and a drop of 25% in COD removal efficiency. In the presence of Cr^{6+} in SBR B, the average CAC and COD removal efficiency measured were 104.6 mg/g and 68.1% respectively. These were equal to a drop of 18% in CAC and a drop of 26% in COD removal efficiency. Similar results were also obtained on the effects of Cr^{3+} and Cr^{6+} on the activated sludge processes under the very short HRT of 6 hrs.

The rates of adsorption were derived at 0.5 to 6 minutes contact time in the activated sludge at room temperature and with copper and chromium initial concentrations at 2 mg/l and zinc at 4mg/l. It was observed that the adsorption capacity of copper, chromium and zinc reached the maximum level of 0.24, 0.18 and 0.15 mg/g-sludge, respectively, within 0.5 minutes and then remained stable in these levels although contact time had been extended to 6 minutes. These results provided evidence that the adsorption equilibrium of all metal ions in activated sludge was reached very quickly. Activated sludge completed for the metals within 0.5 minutes for all 3 metal ions during the aeration stage.

Metal ions adsorbed by activated sludge could be described by the Langmuir adsorption isotherm model or Freundlich adsorption isotherm model. The results of this study were fitted with the Freundlich adsorption isotherm model, which will be described in full in the next chapter. These isotherms were derived after 10-minute contact time at room temperature and copper and chromium concentration under 2 mg/l and zinc under 10 mg/l. From experimental results described previously, the order of adsorption capacity of the metals was found to be: Cu>Cr>Zn. This indicated that activated sludge adsorbed copper easily and has highest adsorption capacity for copper. The conditional adsorption constants (k and n) for the heavy metals used were calculated by experimental data and will be discussed later. The adsorption constant n indicates the effect of metal ion concentration on the adsorption capacity. It was observed that all constant n for the heavy metals used are slightly higher than published values. The higher n value indicated that the adsorption of heavy metals used in the experiment are more effectively adsorbed onto the active binding sites on the activated sludge microbes at low metal ion concentrations.

As metals adsorption on the surface of activated sludge microbes, it could have a competition with the organic matters in the wastewater and resulted in substantially reduced CAC. CAC of activated sludge was significantly affected by metal ions concentration of 0-2 mg/l of copper and chromium and 1-5 mg/l of zinc. It was found that CAC of activated sludge was decreased with an increase of metal concentration in the wastewater. For example, CAC with copper and chromium from 96.9 mg-COD/g-sludge to 76.4, 79.0, and 79.7 mg-COD/g-sludge, respectively when the concentrations of copper and chromium were increased from 0 to 2 mg/l, while CAC with zinc decreased to 77.3 mg-COD/g-sludge when its concentration was increased from 0 to 5 mg/l. This clearly indicated that with adsorption and accumulation of metals onto the surface of activated sludge microbes, metals competed with the organic substrates for the bioflocs binding sites and have effectively reduced the CAC of the activated sludge.

The CAC performances of activated sludge at wastewater without and with copper or zinc showed that the organic adsorption process of activated sludge was about 1 minute when wastewater without any metal and would delayed to 3 minutes when wastewater contained matals. It was also found that the rate of adsorption of organic matters was much slower than that of metal ions on activated sludge. Under steady state operation of the SBRs, CAC of activated sludge without metals kept stable around 68 mg-COD/g-sludge, while CAC of activated sludge with metals stabilized at 55 mg-COD/g-sludge. These results illustrated that although wastewater contained metal ions at the low sub-lethal concentrations in the SBR aeration tank, metal ions affected not only the adsorption speed of organic matters but also CAC of the activated sludge. When metals such as copper and zinc were present in the aeration tank, the CAC of the activated sludge was almost 20% lower than that without any metals.

It is important to note that metal ions affected activated sludge process in the organic matter removal efficiency and in the uptake of organic compounds, even if low concentrations of metals in the raw wastewater enter the simulated wastewater treatment process. Performance of the SBR in terms of COD removal efficiency and the variation in CAC were clear after the initial start-up operation. The reactor stabilized at 5d of HRT. The average CAC was 53.8 mg/g, and corresponding COD removal efficiency was 95.3. After the metal-laden wastewater was introduced, the reactor stabilized within 15d to an average CAC of 39.7mg/g and COD removal efficiency of 91.9%. The CAC decreased 26%, whereas the COD removal efficiency was not significantly changed. When the HRT was reduced to 2.5d, increased residual

COD level resulted in a drastic increase of CAC to a maximum of 103.8 mg/g. During the operations with different metals at the shorter HRT, the average CAC and COD removal efficiency under stable operation were around 92.0mg/g and 92.5%, respectively, in the absence of metal. The values were around 71.3mg/g and 72.4%, respectively, in the presence of metals. These were equivalent to a 23% drop in CAC and a 22% drop in COD removal efficiency.

The performance of the two SBR in terms of variations of CAC and COD removal efficiency demonstrated similarity between the performance profiles, which indicated consistency in the responses of these parameters under changing operating conditions. The average MLVSS, CACs and COD removal efficiencies of the two SBR systems under stable operation at each HRT gave similar trends. After the start-up operation at 10 days HRT and acclimatisation at 5 days HRT, the reactors reached the stable operations. The average CAC was around 54.3mg/g and corresponding COD removal efficiency was around 96.3%. After the metal-laden wastewaters were introduced, the reactors stabilized within 15 days to an average CAC of around 39.3 mg/g and COD removal efficiency of around 93.7. This was equivalent to a 28% drop in CAC, while the COD removal efficiency was not obviously changed. When the HRT was reduced to 2.5 days in the absence of metal, increased residual COD level resulted in a significant increase of CAC to a maximum of 118.6mg/g. During operation of the SBRs at the 2.5d HRT, the average CAC and COD removal efficiency under stable operation were 93.2mg/g and 91.6%, respectively, in the absence of metal, and 70.1mg/g and 71.0%, respectively, in the presence of metal. These represented a 25% drop in CAC and a 22% drop in COD removal efficiency. Similar results were obtained when the reactors were operated at 2.0 days HRT. Presence of different metals resulted in around 22% drop in CAC and around 22% drop in COD removal efficiency.

Based on these results and observations, it may be concluded that when the reactor was operated at the longer HRT of 5d, retention of organic matters within the SBR was sufficiently long for these slow bological assimilation, and organic adsorption was not a critical factor governing SBR performance. Therefore, presence of sub-toxic concentration metals such as zinc, copper and chromium, affected the CAC, but had little effect on COD removal. On the other hand, at the shorter HRT of 2.5d or shorter, presence of sub-toxic concentration heavy metals caused a drop in CAC, which in turn affected the COD, removal efficiency at a comparable extent. It is postulated that COD removal in activated sludge proceeded by a rapid adsorption the organic compounds on certain active sites of the bioflocs, followed by the metabolic destruction of the compounds. Zinc, copper and chromic ions possibly acted as a strong competitor against the organic compounds for active sites on the bioflocs, thus hampering organic adsorption and affected the COD removal efficiency under shorter HRTs. Comparing a drop in CAC and COD removal efficiency of zinc-laden wastewater and copper-laden wastewater at concentration 1 mg/l and HRT of 2.5days, it is clear that copper ions possesses a little stronger affect on the CAC and COD removal efficiency than that of zinc ions in activated sludge process. It was presumed that copper ions are a little easily adsorbed by bioflocs than zinc ions. This was in agreement with batch adsorption results, which will be described in the next chapter. On the other hand, at the volumetric loading of 200 and 250 mg BOD^5/l per day, the HRTs were 2.5 and 2 days, respectively. As the SBR system operation merely occupied 8h out 24-h fill-and-draw cycle. The effective volumetric loading were 600 and 750 mg BOD^5/l per day, respectively and the effective HRTs were 0.83 and 0.67 days, respectively. Compared to the normal SBR design ranges of 250-750 mg BOD^5/l per day and 0.5-2days HRT (Metcalf and Eddy, 1991), the SBRs were operating under relatively high loading and short HRTs. Under such operating conditions, the presence of sub-toxic metals, such as zinc, copper and chromic ions caused a drop in CAC, which in turn, affected the COD removal efficiency to a more significant and comparable extent. Presence of chromium affected the CAC and COD removal efficiency to a lesser extent compared to the effects of zinc and copper. This was because chromium, having a higher toxicity, was added in the synthetic wastewater at much lower concentration compared to zinc and copper. From the experimental results of SBR performances, it can be concluded that even if concentrations of metals were low, which were permitted in raw wastewater entering a wastewater treatment plant, they might also affect the activated sludge process in organic matter removal efficiency and uptake of organic compounds.

CONCLUDING REMARKS OF HEAVY METALS VS SBR PERFORMANCE STUDIES

- In this research project, two sets of experimental programmes were carried out to investigate the effects of HRT on the activated sludge process.

- In the first set of programme, it was found that both the CAC and the corresponding COD removal efficiency were decreased by adding metal-laden wastewater even at sub-lethal concentrations, particularly at shorter HRTs, namely 2.5 and 2 days.

- Under longer HRTs, namely 5 days, the presence of metal-laden wastewater caused a decreased of CAC but had no significant effect on the COD removal efficiency, because significantly long reaction time enabled slow and complete organic degradation rendering physical organic adsorption and metal competitive adsorption less important factors.

- Adsorption capacity and adsorption rate of copper, chromium, and zinc, at sub-lethal concentrations, on microbial flocs were much faster than that of organic matters. Therefore, heavy metals, at sub-lethal concentrations, affected not only the adsorption rate of organic matters but also the CAC and hence the COD removal efficiency of the activated sludge.

- In the second set of experimental programme, results show that the presence of chromium ion affected both the COD adsorption capacity and the COD removal efficiency to similar extents when the SBR systems were operated under relatively short HRTs, thus further substantiating the postulation on the mechanisms of organic adsorption and degradation, and the physical competitive effects of heavy metals.

- When comparing the effects of Cr^{3+} and Cr^{6+} on the activated sludge, it was shown that Cr^{6+} affected both CAC and the COD removal efficiency to a similar extent as Cr^{3+}.

- In general, the metal-laden wastewaters affected the performance of the SBR systems at different extends, depending on the operating HRT. Metal ions acted as a strong competitor against organic matters for active sites on the bioflocs, instead of acting as a toxic microbial inhibitor, thus hampering organic adsorption and affecting the COD removal efficiency. These effects were particularly obvious when the systems were operated under shorter HRTs.

CHAPTER 5 : MECHANISTIC STUDY ON HEAVY METAL ADSORPTION ON ACTIVATED SLUDGE

This Chapter presents the findings from an experimental programme designed to investigate the adsorption behavior of chromium and the effects of chromium on the activated sludge microbes and process. Different concentrations of Cr^{3+} and Cr^{6+} were added to the shake flask cultures of activated sludge. CAC and COD removal efficiency at several time intervals were measured and CAC data were used to fit into physical adsorption models. One set of CAC was selected and used to validate the mathematical model. A number of observations on the effects of chromium, under various conditions, on the activated sludge microbes have been noted.

5.1 EMPIRICAL EXPRESSIONS TO DESCRIBE THE EFFECTS OF Cr^{3+} AND Cr^{6+} ON CAC

In this specifically designed programme, a series of six sets of CAC and COD removal efficiency data, under the effects of different concentrations of both Cr^{3+} and Cr^{6+} were acquired. For the Cr^{3+}-laden shake-flask cultures, the results measured at the resulting chromium concentrations of 0.05, 0.1, 0.2 and 1 mg/L were used to formulate the mathematical model and the mathematical model was validated and the empirical coefficients were calibrated using the data acquired. Based on the data measured, the mathematical model for Cr^{3+} can be expressed as follows.

$$\mathrm{CAC} = \mathrm{A/MLVSS}$$

Eq. (5.1)

$$\mathrm{A} = [V_f/(V_f + V_r)]\mathrm{COD}_f + [V_f + V_r)]\mathrm{COD}_r - \mathrm{COD}_{obs}$$

Eq. (5.2)

where

V_f = volume of feed into the shake flask culture,

V_r = volume of the original culture,

COD_f = dissolved COD in the feed into the culture,

COD_r = residual dissolved COD in the original culture, and

COD_{obs} = observed dissolved COD after initial 10 minutes of shake flask culture.

$$CAC = K_l * 0.559^{0.699t} \qquad \text{Eq. (5.3)}$$

$$K_l = 102.26 - 17.06C$$

where

C is the concentration of chromium (III).

For the concentration at 2 mg/L, K_l equals to 101.41. Therefore, a set of estimated CAC can be obtained and is shown in Table 5.1. On comparing the predicted and measured CAC in Table 5.1, it is shown that the prediction of CAC is valid as the maximum difference between the measured and predicted CAC is less than 10%. The 10 % deviation is commonly acceptable for model fitting. Figure 5.1 shows the data measured at different concentrations of Cr^{3+}.

Table 5.1

A comparison of CAC affected by Cr^{3+} between measured and predicted

CAC affected by Cr^{3+} at the concentration of 2 mg/L			
Time	**Measured**	**Predicted**	**Difference (%)**
5 sec	40.55	38.09	6.07
10 sec	43.88	45.38	-3.42
20 sec	48.33	51.28	-6.10
30 sec	51.66	55.86	-.813
1 min	59.44	59.31	0.20
2 min	57.22	61.84	-8.07

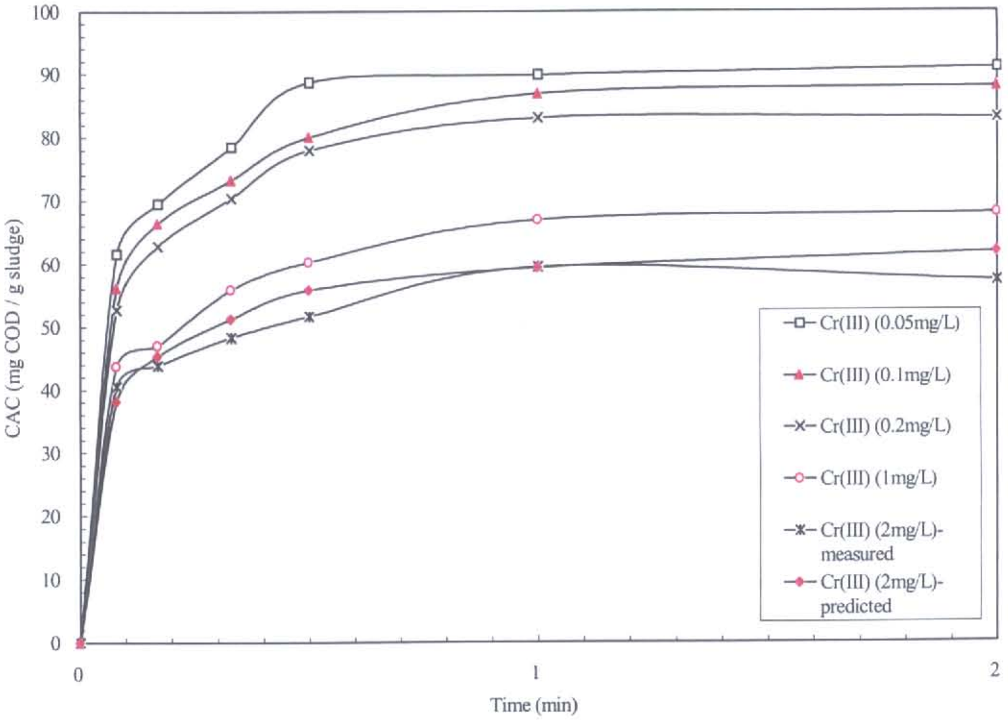


Figure 5.1

CAC affected by Cr^{3+} at different concentrations

For the Cr^{6+}-laden shake flask cultures, the results measured at the chromium concentrations of 0.05, 0.1, 0.5 and 1 mg/L were used to formulate the mathematical model. The adsorption model was similarly validated and the empirical coefficents was calibrated using the data acquired. The CAC expressions are as expressions 5.1 and 5.2. Based on the data measured, the mathematical model for Cr^{6+} can be expressed as follows.

$$CAC = K_2 * 0.6323^{0.6895'}$$

Eq.

(5.4)

$$K_2 = 92.167 - 4.24C$$

where

C is the concentration of chromium (VI).

For the concentration at 0.2 mg/L, K_2 equals to 91.319. Therefore, a set of estimated CAC can be obtained and is shown in Table 5.2. On comparing the predicted and measured CAC in Table 5.2, it is shown that the prediction of CAC is valid. Figure 5.2 shows the data measured at different concentrations of Cr^{6+}.

Table 5.2

A comparison of CAC affected by Cr^{6+} between measured and predicted

CAC affected by Cr^{6+} at the concentration of 0.2 mg/L			
Time	**Measured**	**Predicted**	**Difference (%)**
5 sec	56.50	57.74	-2.19
10 sec	65.34	66.57	-1.88
20 sec	70.39	73.43	-4.32
30 sec	77.97	78.58	-0.78
1 min	80.49	82.33	-2.29
2 min	83.02	85.02	-2.41

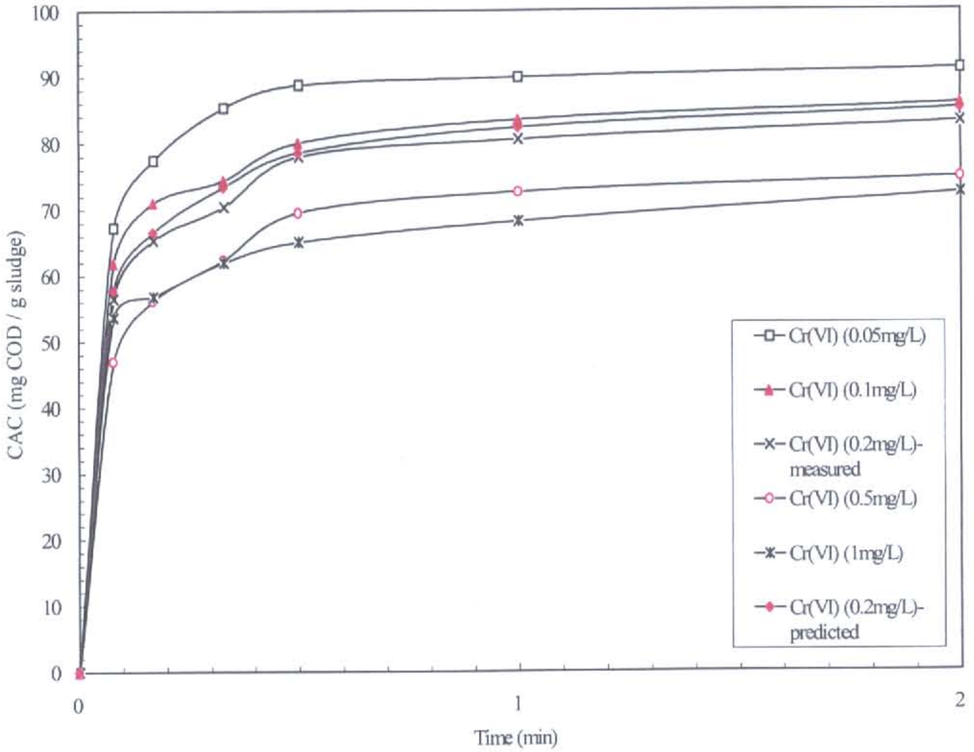


Figure 5.2

CAC affected by Cr^{6+} at different concentrations

Both the empirical mathematical expressions or adsorption models show that CAC decreases with the increase of the concentration of chromium.

Although it is know that the toxicity of Cr^{6+} is more than order higher than that of Cr^{3+}. But from these results on the time course of CAC graphs, they show that Cr^{6+} and Cr^{3+} at various concentration had the similar effects on CAC.

Therefore, the effects of metals on COD removal efficiency must be due to physical means and biological toxicity.

5.2 CHROMIUM ADSORPTION UNDER DIFFERENT CONDITIONS

Chromium adsorption behaviors, in terms of specific adsorption capacities, in the absence and presence of organic matters are separately shown in Figures 5.3 and 5.4. The corresponding adsorption rates are separately shown in Figures 5.5 and 5.6.

The general trends in the adsorption characteristics are similar for different chromium concentrations. In the absence of organic matters, the metal adsorption capacity (MAC) for chromium almost reached the maximum levels within 20 seconds. Sampling and analysis were carried out for another 4 minutes and the MAC remained at its stable levels. For the chromium concentration at 2 mg/L, the maximum MAC measured at 1.2 mg/g. When the chromium concentration was at 1 mg/L, the maximum MAC measured at 0.6 mg/g. Results show that higher MACs were achieved with the increase of the chromium concentration under the sub-toxic level, and the relationship is linear. Results also showed that the adsorption rate was increased by the increase of the concentration of chromium in the activated sludge cultures. This agrees with the results obtained by Imai and Gloyna (1990) in their study on metal adsorption by activated sludge microbes.

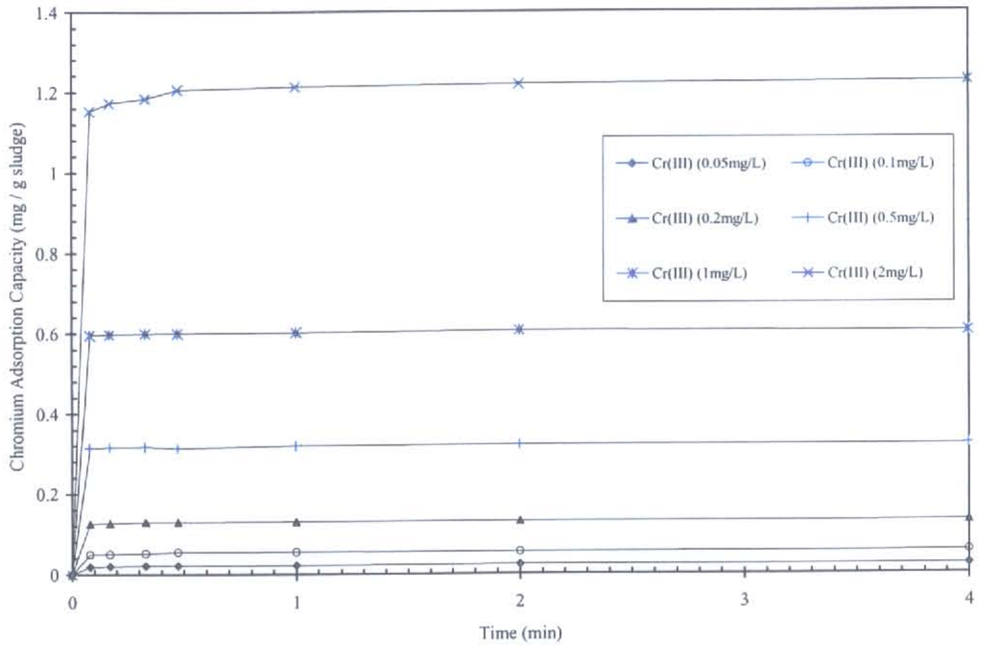


Figure 5.3
Chromium adsorption capacity without organic matters

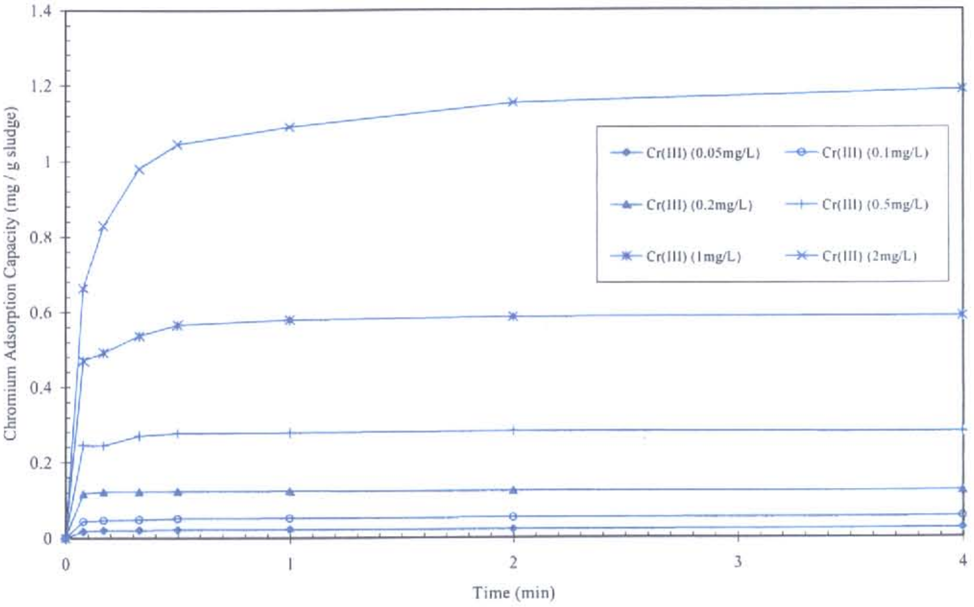


Figure 5.4
Chromium adsorption capacity with organic matters

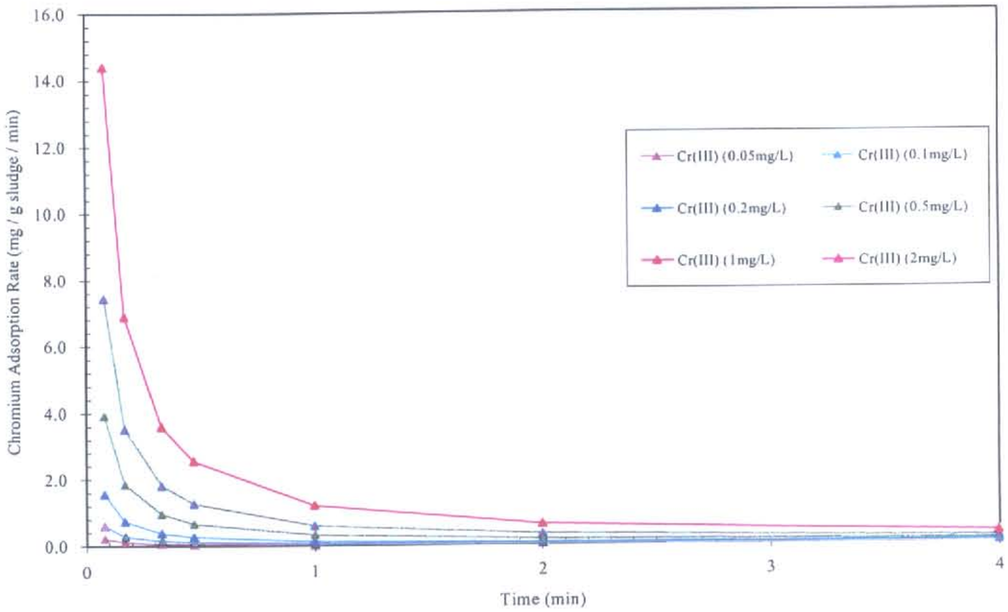


Figure 5.5

Chromium adsorption rate without organic matters

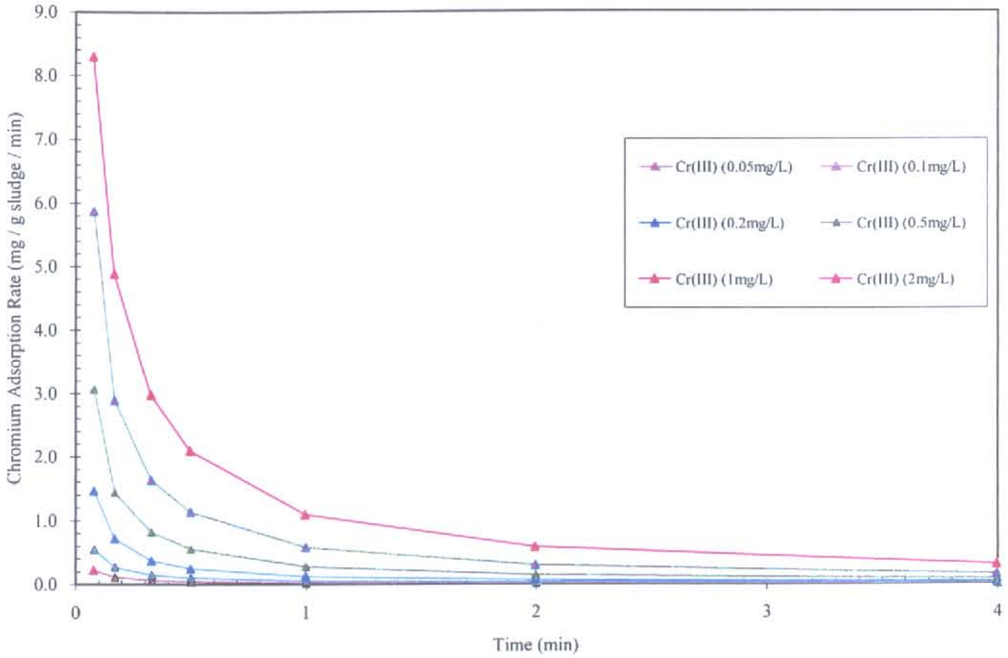


Figure 5.6
Chromium adsorption rate with organic matters

Figure 5.3 shows that there is a need of longer time for achieving the maximum MAC when the concentration of chromium was at 2 mg/L. Thirty seconds were required to reach a stabilised maximum MAC level for the culture with chromium concentration of 2 mg/L while 6 seconds were required for the culture with chromium concentration of 1 mg/L or below. This was because a higher concentration of chromium caused a stronger competition among the chromium ions for active adsorption sites on the activated sludge bioflocs. Therefore, a longer time was required for the chromium adsorption to reach a maximum level or attaining an equilibrium state as shown in the plateau in the adsorption profiles (Figures 5.3 and 5.4), with a chromium concentration of 2 mg/L.

Comparing the chromium adsorption behaviour shown in Figures 5.3 and 5.4. The presence of organic caused a delay in achieving maximum MAC or equilibrium state in the cultures with chromium concentrations of 2, 1 and 0.5 mg/L. These results substantiated the previous postulation that heavy metals acted as physical competitors against organic matters for active adsorption sites on the activated sludge superficial layers or surfaces of bioflocs. It is obvious from these shake-flask culture results that presence of one, metal or organic matters, adversely affected the adsorption capacity and rate of the other. However, there was no influence on the chromium adsorption when the concentrations of chromium were in the lower range, namely 0.2, 0.1 and 0.05 mg/L. The curves in Figures 5.5 and 5.6 show similar results. With the addition of organic matters, the chromium adsorption rate was decreased for the concentrations of chromium of 2, 1 and 0.5 mg/L. However, there was no change on the chromium adsorption rate when the concentrations of chromium were less than 0.2 mg/L. These observations agree with similar observations reported by Chua (1998). With the same amount of organic matters added, when the concentrations of chromium were higher (2, 1 and 0.5 mg/L), the competition between organic matters and chromium ions was stronger. As a result, the chances of neighbouring effects between the chromium ions and organic compounds, and physical obstruction and alteration of the configurations of the neighbouring active adsorption sites were increased. Therefore, the chromium ions had lesser chance for approaching the bioflocs. In general, presence of organic matters hindered both the rate and capacity of metal adsorption. On the other hand, with the same amount of organic matters, when the concentrations of chromium were lower (0.2, 0.1 and 0.05 mg/L), the competition between the chromium ions and organic compounds was relatively small or even negligible. Therefore, presence of organic matters caused no observable effect on the chromium adsorption when the concentration of chromium was less than 0.2 mg/L.

5.2.2 Adsorption of chromium in the forms of Cr^{3+} and Cr^{6+}

Chromium adsorption capacity in the activated sludge cultures (in the forms of Cr^{3+} and Cr^{6+} under different concentrations) in absence and presence of organic matters was shown in Figures 5.7 and 5.8, respectively. The corresponding profiles of chromium adsorption rates for absence and presence of organic matters are shown in Figures 5.9 and 5.10, respectively.

In absence of organic matters, the MAC of Cr^{3+} was higher than that of Cr^{6+} under the same chromium concentration. Figure 5.9 also shows that the chromium adsorption rate was higher for Cr^{3+}. These observations agree with the results obtained from Imai and Gloyna (1990). It is of interest to note that Cr^{3+} existed in a solution in a form of simple Cr^{3+} ion while Cr^{6+} existed in a form of compound ion (CrO_4^{2-}). As the activated sludge cells carried a negative charge with a value of pH that ranged between 5 and 9 (Busch and Stumm 1968), Cr^{3+} exhibited much greater affinity towards activated sludge microbes or bioflocs than Cr^{6+}. The adsorption mechanism for Cr^{3+} is a surface adsorption. However, for Cr^{6+}, the adsorption mechanism is a specific Cr^{6+} anion adsorption. The chromate (CrO_4^{2-}) was transported into the cell through the sulfate (SO_4^{2-}) transport system that involves a binding of a single polypeptide with a molecular weight of 32,000 (Imai and Gloyna 1990). As a result, the absorption capacity and the absorption rate of Cr^{3+} are higher than that of Cr^{6+}.

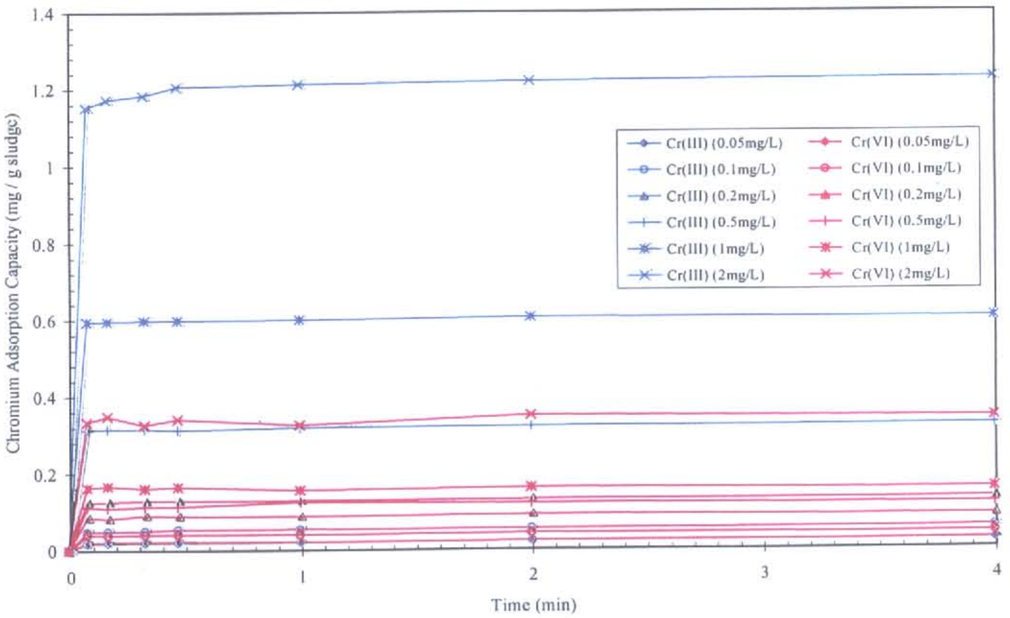


Figure 5.7
Chromium adsorption capacity for Cr^{3+} and Cr^{6+} without organic matters

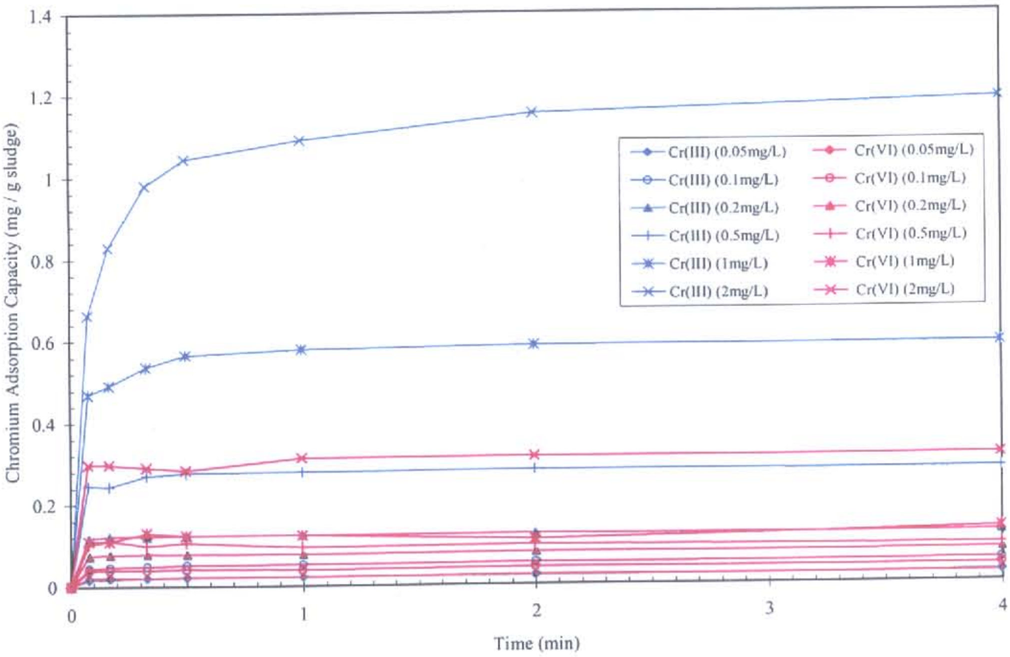


Figure 5.8
Chromium adsorption capacity for Cr^{3+} and Cr^{6+} with organic matters

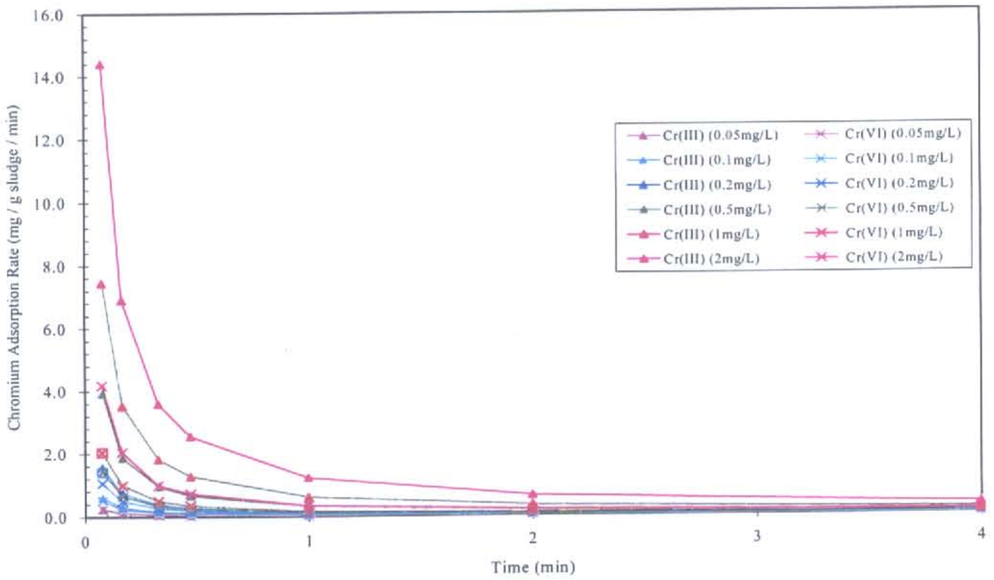


Figure 5.9
Chromium adsorption rate for Cr^{3+} and Cr^{6+} without organic matters

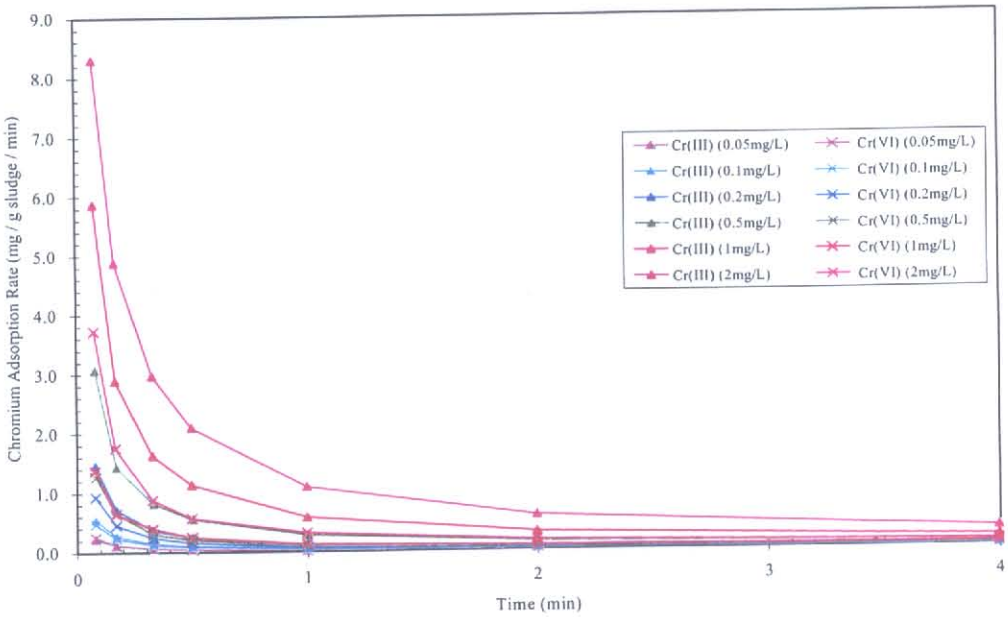


Figure 5.10

Chromium adsorption rate for Cr^{3+} and Cr^{6+} with organic matters

Comparing Figure 5.7 and Figure 5.8, it was found that the influence of organic matters on the chromium adsorption in the forms of Cr^{3+} and Cr^{6+} was similar. Competition existed between organic and chromium ions (Cr^{3+} and Cr^{6+}) for the active sites on the bioflocs. Therefore, for the culture sample with chromium concentration greater than 0.2 mg/L, organic matters caused a delay in the chromium adsorption no matter what type of the chromium ion existed. Similar results are shown in Figures 5.9 and 5.10. For both Cr^{3+} and Cr^{6+},the presence of organic caused a decrease in absorption rate when the chromium concentration was greater than 0.2 mg/L.

5.2.3 Adsorption isotherm

The adsorption behaviour of chromium ions (Cr^{3+} and Cr^{6+}) on activated sludge was tested with the Langmuir adsorption model (Chong and Volesky, 1995) and Freundlich adsorption model (O'Connor *et al.*, 1983). The data fitted better with the Freundlich adsorption isotherm model and the curves are shown in Figure 5.11. The slope and y-intercept of adsorption isotherm for Cr^{3+} and Cr^{6+} were different, namely 2.037 (slope for Cr^{3+}), 0.683 (y-intercept for Cr^{3+}), and 0.476 (slope for Cr^{6+}) and 0.856 (y-intercept for Cr^{6+}).

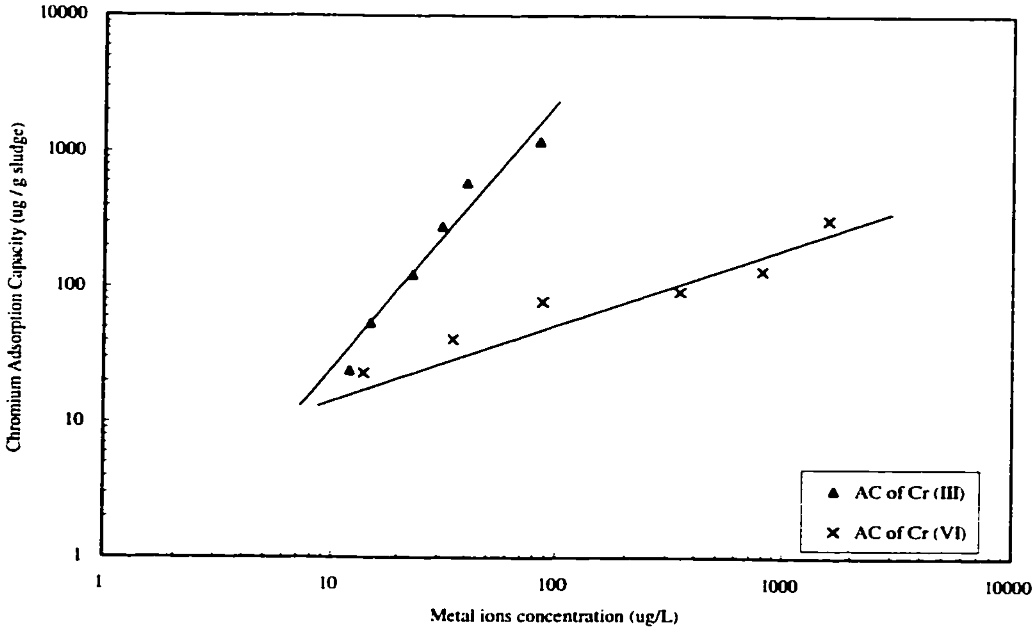


Figure 5.11

Adsorption isotherm for Cr^{3+} and Cr^{6+} under 25°C

5.2.4 Effect of chromium on CAC

Having interpreted the aforementioned results on the effects of presence of organic matters on metal adsorption rates and capacities for the understanding of the competitive behaviour between the metallic and organic species, it is now of greater interests and importance to investigate the spontaneous and instantaneous effects of presence of trace level of heavy metal, namely chromium, on the organic adsorption capacity (CAC). This is important in the understanding of the resulting adverse effects of metals on activated sludge process performance.

In absence of chromium, the CAC achieved a stabilised value of 110 mg/g at 1 minute after the commencement of shaking flask culture. However, with the presence of trace level chromium, there was a substantial drop (about 20 weight %) in the CAC value (Figure 5.12). The drop in CAC was increased with the increase of chromium concentration in the sample, in a linear manner. Similar observation was observed in the organic adsorption rate. The rate was decreased with the increase of resultant chromium concentration in the activated sludge culture sample. This agrees with the observation and postulation by Chua and Hua (1997) that the metallic and organic species are in a competitive relationship for physical adsorption sites and therefore the relationship between the extent of the metal effects on CAC and the metal concentration is linear. If, however, the effects of biological toxicity of metals set in, the relationship would have been non linear. This was because the extent of the metal effects on CAC would have been increased drastically when the toxic threshold is exceeded.

Additionally, it was found that the effects of Cr^{3+} and Cr^{6+} on the CAC and organic adsorption rate were similar (see Figure 5.13). This indicated that Cr^{3+} and Cr^{6+} ions affected the activated sludge in a similar manner and to a similar extent (see Figure 5.14), despite that these two forms of chromium are widely known to have very different level of toxicity. Under the selected sub-toxic levels, the toxicity of chromium and the different levels of toxicity of Cr^{3+} and Cr^{6+} no longer play an important role in the effects on CAC. The effect of chromium on activated sludge is most likely due to the physical competition between chromium and organic matters for the active sites on the bioflocs.

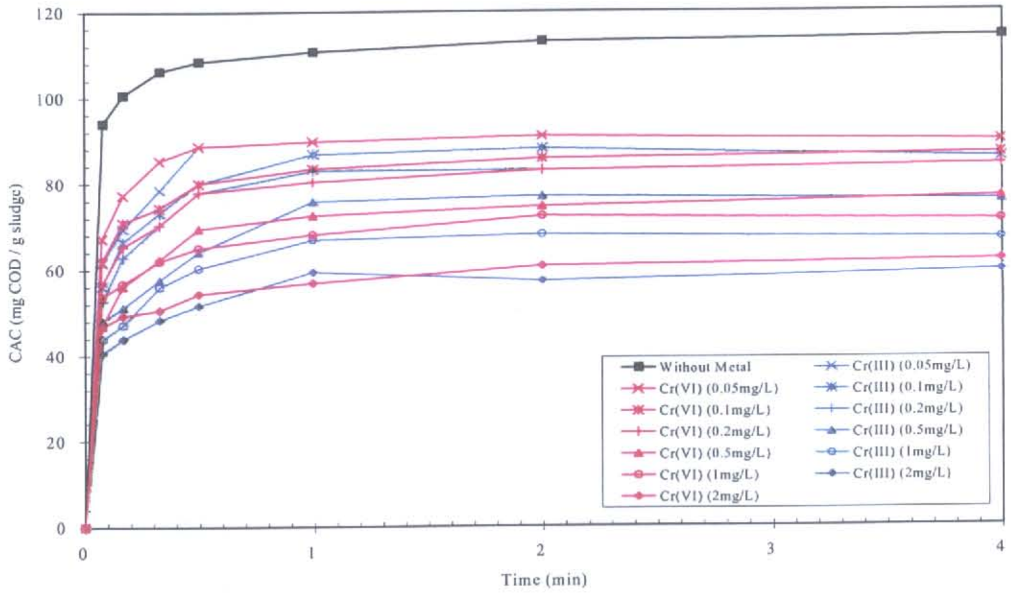


Figure 5.12

Performance of activated sludge in terms of CAC under different concentrations of chromium

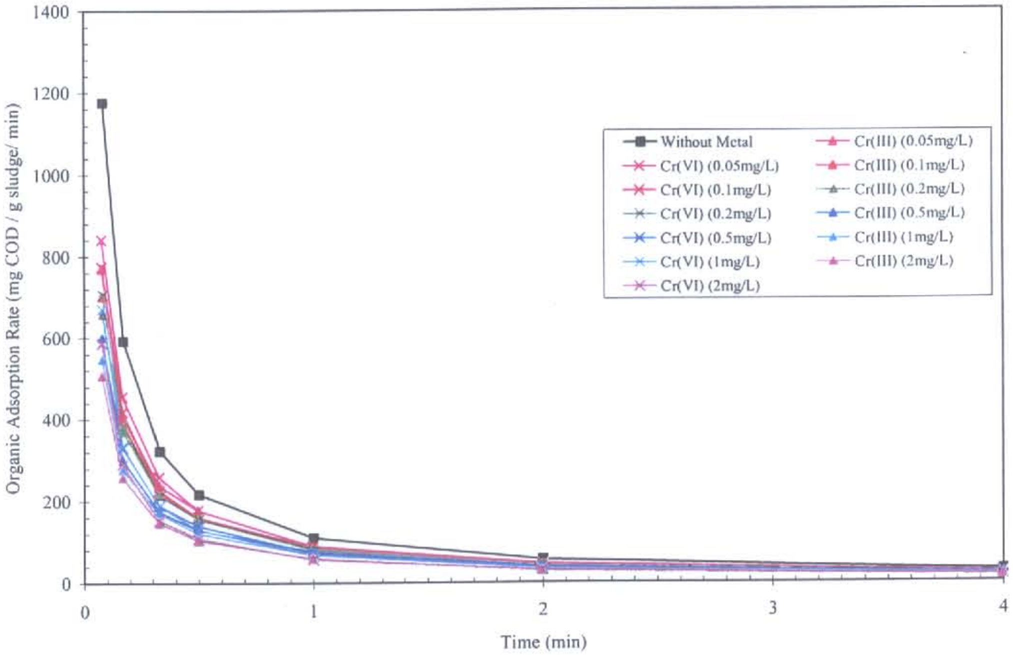


Figure 5.13

Performance of activated sludge in terms of organic adsorption rate under different concentrations of chromium

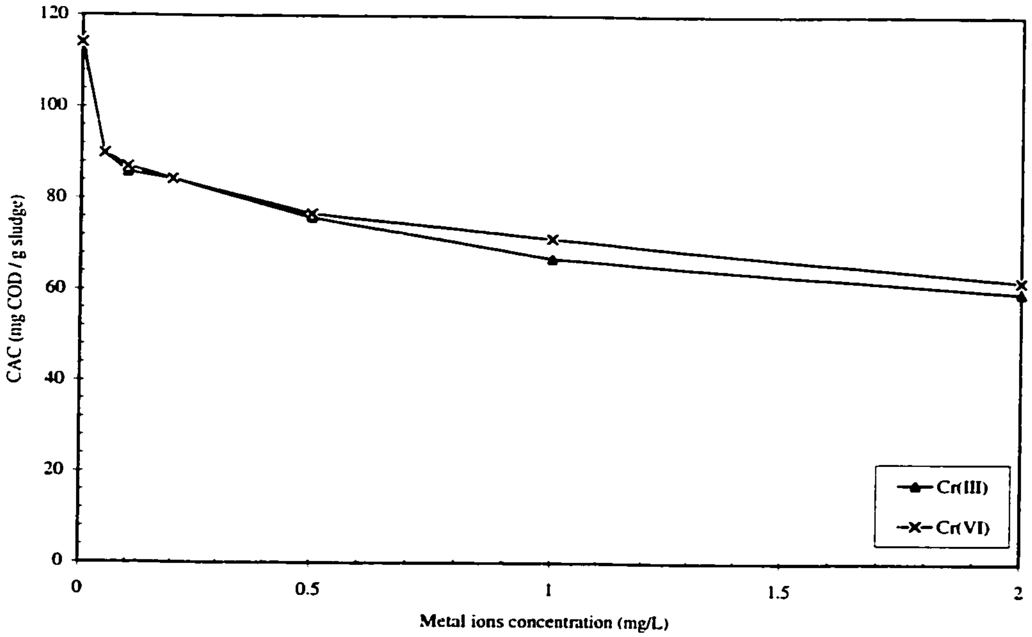


Figure 5.14

CAC under different concentrations of chromium

CONCLUDING REMARKS OF MECHANISTIC STUDIES

- In this research project, an empirical model was constructed, validated and calibrated for the prediction of CAC in activated sludge at different sub-lethal chromium concentrations.

- It was shown that the chromium adsorption rate decreased exponentially with contact time and with the concentration of chromium in the activated sludge.

- It was shown that the chromium adsorption capacity increased with the time up to a maximum level and with the concentration of chromium in the activated sludge.

- In absence of chromium, the CAC achieved a stabilised value of 110 mg/g 1 minute after the commencement of shaking flask culture. However, with the presence of trace level chromium, there was a 20 weight % drop in CAC. The drop in CAC increased with the increase of chromium concentration in a linear manner. Similar observation was observed in the organic adsorption rate. This substantiated that the metallic and organic species are in a competitive relationship for physical adsorption sites and therefore the relationship was linear. Biological toxicity of metals would have rendered the relationship non linear, because the extent of the metal effects on CAC would have increased drastically when the toxic threshold is exceeded.

- The adsorption behavior of chromium in activated sludge have been fitted with the Langmuir and Freundlich adsorption isotherm models. Nevertheless, the latter model showed better fit and was more consistent with the experimental results.

CHAPTER 6 : MICROSCOPIC-SCALE STUDY ON ADSORPTION OF HEAVY METAL ON ACTIVATED SLUDGE MICROBES

This Chapter presents the results of the microscopic-scale study on the effects of heavy metals on activated sludge microbes. Adsorption of metal on the different superficial layers and different biochemical fractions in the superficial layers of the microbial cells was investigated. A common activated sludge microbe, namely *Pseudomonas putida 5-x (P. putida 5-x)*, was cultured to study the adsorption of a common heavy metal, namely copper. The mechanism of Cu^{2+} adsorption by the biomass was studied by using the technique of transmission electron microscopy (TEM). TEM and X-ray analysis were carried to show the Cu^{2+} accumulation characteristics on the cell surface, in different fractions of the capsule, cell wall, and outer and inner plasma membranes. The characteristics of metal adsorption was modeled in the form of the Freundlich adsorption isotherm.

6.1 CHARACTERISTICS OF CELL SUPERFICIAL LAYERS AND THEIR ADSORPTION OF COPPER

The difference between the Cu^{2+} adsorption capacities of raw un-treated *P. putida* 5-x and that of cells pretreated with 0.3N of HCl showed that the pretreatment of cells could greatly increase the Cu^{2+} adsorption capacity in an order of more than 26 weight %, from 67.4mg/g to 85.3mg/g.

Initial Cu^{2+} concentration in water sample was changed while biomass in each sample was held constant. The relationships of Cu^{2+} concentration in equilibrium and the adsorption capacity of raw un-treated cells and pretreated cells were shown in Figure 6.1. From these results, the adsorption characteristics of pretreated cells and raw cells can be respectively modelled by two expressions as follows.

$$Log\, AC = 1.43 + 0.76\, Log\, C_e,$$

$$Log\, AC = 1.11 + 0.63\, Log\, C_e,$$

This illustrated that the Cu^{2+} adsorption and uptake by pretreated cells and fresh cells all obey the typical Freundlich adsorption isotherm as follows:

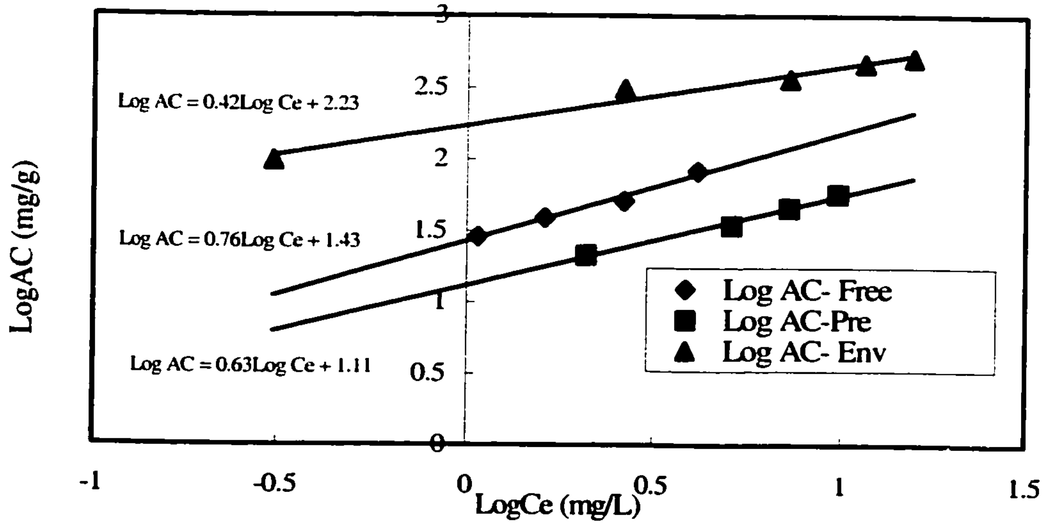


Figure 6.1

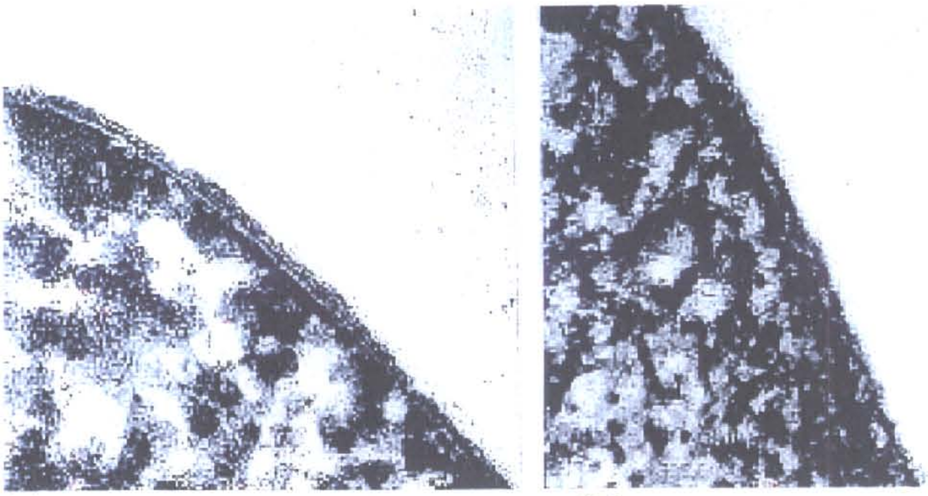
Cu^{2+} adsorption *isotherms* of fresh cell, pre-treated cell and cell envelope

$$\text{LogAC} = \text{Log } K_f + n \text{ Log } C_e.$$

These observations indicated that heavy metal uptake by activated sludge microbes is through mono-molecular layer surface adsorption on the cell outer surfaces, including different fractions of the capsule, cell wall and plasma membrane. The distribution of metal in these different fractions will be discussed later. The $K_{f\text{-}}$ values of pretreated cells and raw cells were 26.9 and 12.9, respectively, suggesting that the pretreated cells were better absorbents for Cu^{2+} than fresh cells.

A loosely attached outer capsular membrane on the outer rim of the raw cell wall of *P. putida* 5-x was examined by TEM (Figure 6.2 (a)). After treating with 0.3 N HCl, the outer capsular membrane appeared to be disintegrated or degraded and could not be seen (Figure 6.2 (b)). This indicated that the more effective adsorption of Cu^{2+} by pretreated cells was most likely related to the degradation and removal of the loose capsular membrane outside the cell wall, and the metal adsorption was mainly on active sites on the different fractions of inner firmly-attached cell wall and plasma membrane.

The outer loosely-attached capsule is a superficial and highly hydrated layer of Gram-negative cells. It has a water content of greater than 95 weight percent, and the solid structures are comprised of loosely arranged homopolymers or



(a)

(b)

Figure 6.2

TEM graphs of the cell surface

(a) Fresh cell

(b) Pretreated cell by 0.6 N HCl

heteropolymers of carbohydrates and proteins (Geesey and Jang, 1989). These polymers are physically very flexible and the configuration extends up to several hundred nanometres from the cell surface. This superficial structure is entirely bathed in water and can be easily removed by weaker eluent (Geesey and Jang, 1989). TEM analysis suggested that acidic pretreatment degraded and removed the outside capsule from cell surface and enhanced the Cu^{2+} adsorption and uptake of *P. putida* 5-x by a factor of 26 weight percent.

Theoretically, capsular carbohydrate and protein providing the electronegative groups, such as carboxyl and hydroxyl groups, may contribute to heavy metal ionic binding sites. But this contribution is limited due to the low density of electronegative groups in the outer loosely-attached capsule, which is more than 95 weight percent of water content. In general, two electronegative groups are needed to form an active site to bind a divalent Cu^{2+} through metal salt-bridging (Geesey and Jang, 1989). The presence of the metal salt-bridging in Cu^{2+} adsorbed capsule may induce a configuration change within the capsule, due to its highly hydrated structures and its physical flexibility. Therefore, the adsorbed metals may alter the physical and chemical properties, and the structural configurations of the cell surface, and hence, altering the other organic-binding sites on the cell wall. Furthermore, this may even hinder the normal transport of organic matters through the outer capsule into the inner cell wall and plasma membrane. Therefore, the active organic binding sites on the much denser inner layers may become inaccessible to the organic matters. In general, these results indicated that the adsorption and interaction of heavy metal, namely copper, with the binding sites in the outer capsule altered the capsular configuration, and the presence of metal salt-bridging limited the interaction of organic matters with binding sites on the inner layers, namely the cell wall and plasma membrane. These, in turn, led to the decreased organic adsorption capacity and organic removal efficiency of the activated sludge microbe, *P. putida* 5-x, in the presence of trace concentration of copper.

6.2 Cu^{2+} ADSORPTION BY ISOLATED CELL ENVELOPE OF *P. putida* 5-X

It has been well published in the literature that a number of separate studies on metal sorption by bacterial cells have shown that isolated cell walls from Gram-positive microbes have higher metal binding capacity than isolated envelopes of Gram-negative microbes. The metal binding capacity of the former is usually 10 times more than that of Gram-negative envelopes (Beveridge and Fyfe, 1985; Brierley, 1990). This observation is attributed to the difference between cell surface components and structure of Gram-positive and Gram-negative bacteria. However, due to the complexity of Gram-negative bacterial cell envelopes, difference may be seen on metal-binding capacity even among various Gram-negative cell envelopes.

In this study, 0.42g (dry weight) of separated cell envelope materials were obtained from 2g (dry weight) of *Pseudomonas putida* 5-x fresh cells (Fig.6.3). The Cu $^{2+}$ adsorption capacity of the cell envelopes reached 467mg/g in 100mg/L Cu^{2+} solution. This metal adsorption capacity for Cu^{2+} is 7 times more than that for fresh cells. It is interesting to note that in *P. putida* 5-x cells, the amount of cell envelope material is only about one fifth the overall weight of fresh cells (wt/wt). This means that on per weight basis, the total amounts of Cu^{2+} bound by 0.42g cell envelope material is much more

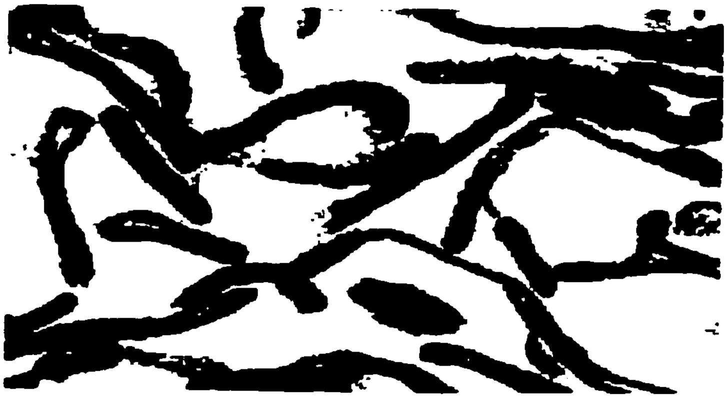


Figure 6.3

TEM graph of cell envelope of *P. putida* 5-x (x 50,000)

than by that of 2g fresh cells. Part of the metal-binding sites in intact cell surface are inaccessible for the binding of heavy metals, but after chemical and physical treatments, much more metal-binding sites in separated cell envelopes become accessible for effective metal binding. Similar to the fresh cells and pretreated cells that have been discussed previously, the Cu^{2+} adsorption isotherm of isolated cell envelope of *Pseudomonas putida* 5-x can also be described with *Freundlich* isotherm of $LogAC = Log K_f + n Log C_e$. The adsorption equation is as follows.

$$Log AC = 0.42 Log Ce + 2.23$$

The cell envelope materials have a K_{f} value of 169.7, which is 6 times and 13 times more than the acid-pretreated cells and fresh cells, respectively (Figure 6.1). These results show that the cell envelope of *Pseudomonas utida* 5-x is an excellent biosorbent, much better when compared to intact cells of *Pseudomonas putida* 5-x.

It has also been observed in this study that the Cu^{2+} adsorption capacity of the cell envelope of Gram-negative *P. putida* 5-x was in the same order as the Cu^{2+} adsorption capacity of typical Gram-positive bacteria reported by Beveridge and Fyfe (1985). This finding is in contrast with the generally accepted view that isolated cell walls from Gram-positive microbes have higher metal binding capacity, usually 10 time more, than isolated envelopes of Gram-negative microbes (Brierley, 1990; Beveridge and Fyfe, 1985). In general, the high metal adsorption capacity of Gram-positive bacterial cell wall is attributed to its thicker peptidoglycan layer which TA and TUA are emobedded. They all contain high dense electronegative groups, namely carboxyl groups, which could bind heavy metals (McLean *et al.*, 1990). However, the peptidoglycan layer of Gram-negative *Pseudomonas putida* 5-x is much thinner. It is interesting to observe the role of separated cell envelope such as peptidoglycan layer, spheroplast membrane, outer membrane, and plasma membrane on Cu^{2+} binding process, and to find out the reasons of such high Cu^{2+} adsorption capacity of cell envelope of the Gram-negative *Pseudomonas putida* 5-x.

6.3 Cu^{2+} ADSORPTION BY ISOLATED PEPTIDOGLYCAN LAYER FROM *P. putida* 5-X CELL ENVELOPES

Cell envelope consists of three structural components (Schiewer and Volesky, 2000), namely

(1) an outer membrane made of lipopolysaccharides, proteins and lipids,
(2) a plasma membrane made of lipids and proteins, and
(3) peptidoglycan layer.

The lipids, proteins and lipopolysaccharides in outer and plasma membrane can be easily dissolved with SDS solution, but the peptidoglycan layer is highly resistant to SDS extraction (Weidel and Peltzer, 1964). Therefore, the peptidoglycan layer, which is the main metal binding components in Gram-negative bacterial cell (Beveridge and Forsberg, 1982), was separated from cell envelope to test its Cu^{2+} adsorption properties.

Lipid analysis and Polyacrylamide Gel Electrophoresis analysis showed that separated peptidoglycan layer only containing trace amount of lipid and protein (Fig.6.4). The adsorption capacity of Cu^{2+} by peptidoglycan layer in 100mg/L Cu^{2+} solution was approximately 480mg/g (dry weight), which is not much more than whole cell envelope materials. This means that the

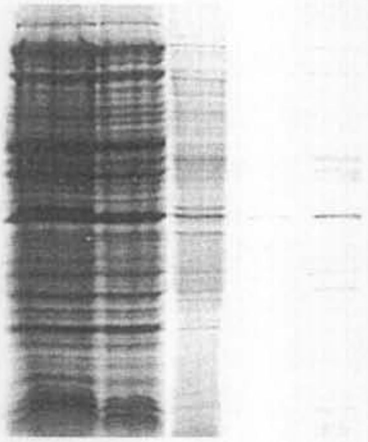


Figure 6.4

SDS-Page Results:

From left to right, lane one is fresh cell, lane two is broken cell, lane three is isolated cell envelope, lane four is separated peptidoglycan layer, lane five is Tritonx-100 undissolved cell envelope

peptidoglycan layer of Gram-negative cell envelope only plays a relatively minor role on Cu^{2+} binding. This also implies that the outer and inner membranes of *Pseudomonas putida* 5-x cell envelopes play a relatively important role on Cu^{2+} binding. This is possibly the main reason for such high Cu^{2+} adsorption capacity by the Gram-negative *Pseudomonas putida* 5-x cells. Therefore, the copper, or heavy metals in general, are mainly adsorbed on the outer lipopolysaccharide, protein and lipid membranes, and the inner lipid and protein plasma membrane of the cell envelope of active sludge microbes, and from which they affect the adsorption and uptake of organic matters.

One interesting observation in this study is that both the Cu^{2+} adsorption isotherms of the intact cells and whole cell envelope of *P. putida* 5-x can be described by the *Freundlich adsorption* equation. However, the adsorption model of separated peptidoglycan layer is better described by the *Langmuir* adsorption equation as follows.

$$AC = B * K\,Ce / 1 + K\,Ce$$

Figure 6.5 shows the specific adsorption capacity of the peptidoglycan layer at different equilibrium copper concentrations. These results, when fitted into the Langmuir adsorption isotherm, express the equation as follows.

$$AC = 2499\, Ce/1 + 4.91Ce$$

This indicates that the adsorption property of peptidoglycan layer for Cu^{2+} is different from that of intact cells and the other fraction of the cell envelope.

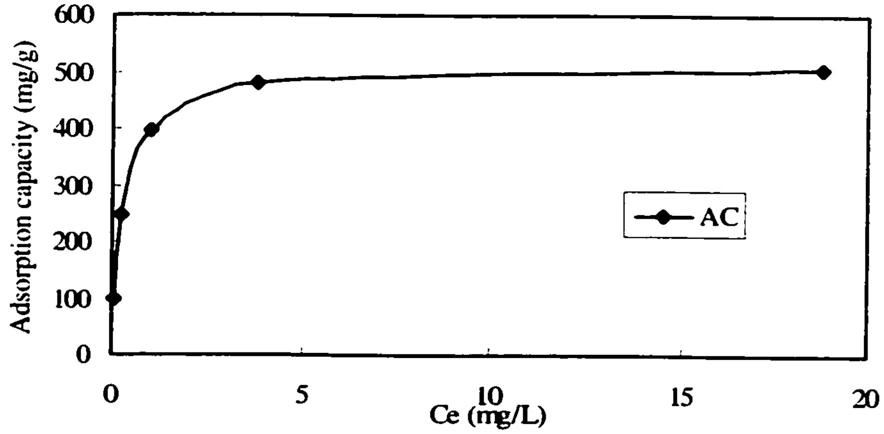


Figure 6.5

Cu^{2+} adsorption *isotherms* of separated peptidoglycan layer

CONCLUDING REMARKS OF MICROSCOPIC-SCALE STUDY

- It was shown that when pretreated with 0.3 N HCl, the Cu^{2+} adsorption capacity of *P. putida* 5-x cells was enhanced due to the degradation and removal of the superficial, high water-content capsule.

- The metal salt-bridging which was formed by bound Cu^{2+} induced a configuration change within the capsule and rendering the metal-binding sites on the cell wall are not accessible to organic matters.

- This, in turn, affected the organic adsorption capacity and organic removal efficiency of the activated sludge microbes.

- In intact cells, part of the metal-binding sites on the cell surface are not accessible for heavy metal adsorption.

- The isolated cell envelopes, however, after chemical and physical treatment, liberated more metal-binding sites on cell surface to enhance 7 times more Cu^{2+} adsorption capacity than intact cells. Peptidoglycan layer of *P. putida* 5-x cell envelope only plays a minor role in Cu^{2+} binding.

- The outer and inner membranes are the main fractions of the cell envelope that provide the active binding sites for Cu^{2+} binding and adsorption in the *P. putida* 5-x cells.

- Adsorption of copper by fresh cells, pretreated cells and the outer and inner membranes of the cell envelope of *P. putida* 5-x can all be described the *Freundlich* adsorption equation, but the adsorption model for the isolated peptidoglycan layer of the cell envelope is better described by the *Langmuir* adsorption equation.

- The above can be concluded be the adsorption property on the active binding sites of the peptidoglycan layer which is different from that of other fractions of the cells.

CHAPTER 7 : CONCLUDING REMARKS AND RECOMMENDATIONS

7.1 CONCLUDING REMARKS

In the macro-scale study on the effects of sub-lethal concentration of heavy metals on activated sludge process, namely SBR systems, two sets of experimental programmes were carried out to investigate the effects of HRT on the activated sludge process. In the first set of programme, it was found that both the CAC and the corresponding COD removal efficiency were decreased by adding metal-laden wastewater even at sub-lethal concentrations, particularly at shorter HRTs, namely 2.5 and 2 days. Under longer HRTs, namely 5 days, the presence of metal-laden wastewater caused a decreased of CAC but had no significant effect on the COD removal efficiency, because significantly long reaction time enabled slow and complete organic degradation rendering physical organic adsorption and metal competitive adsorption less important factors. Adsorption capacity and adsorption rate of copper, chromium, and zinc, at sub-lethal concentrations, on microbial flocs were much faster than that of organic matters. Therefore, heavy metals, at sub-lethal concentrations, affected not only the adsorption rate of organic matters but also the CAC. Hence, the COD removal efficiency of the activated sludge is also reduced. In the second set of experimental programme, results show that the presence of chromium ion affected both the COD adsorption capacity and the COD removal efficiency to similar extents when the SBR systems were operated under relatively short HRTs, thus further substantiating the postulation on the mechanisms of organic adsorption and degradation, and the physical competitive effects of heavy metals. When comparing the effects of Cr^{3+} and Cr^{6+} on the activated sludge, it was shown that Cr^{6+} affected both CAC and the COD removal efficiency to a similar extent as Cr^{3+}, despite the fact that it is more toxic.

In general, the metal-laden wastewaters affected the performance of the SBR systems at different extends, depending on the operating HRT. Metal ions acted as a strong competitor against organic matters for active sites on the bioflocs, instead of acting as a toxic microbial inhibitor, thus hampering organic adsorption and affecting the COD removal efficiency. These effects were particularly pronounced when the SBRs were operated under shorter HRTs.

In the mechanistic study on the adsorption of heavy metal on activated sludge microbes, an empirical model was constructed. It was subsequently validated and calibrated for the prediction of CAC in activated sludge at different sub-lethal chromium concentrations. It was shown that the chromium adsorption rate decreases exponentially with contact time and with the concentration of chromium in the activated sludge. The chromium adsorption capacity increases with the time up to a maximum level and with the concentration of chromium in the activated sludge. In the absence of chromium, the CAC achieved a stabilised value of 110 mg/g 1 minute after the commencement of shaking flask culture. However, with the presence of trace level chromium, there was a 20 weight % drop in CAC. The drop in CAC increased with the increase of chromium concentration in a linear manner. Similar observation was observed in the organic adsorption rate. This substantiated that the metallic and organic species are in a competitive relationship for physical adsorption sites and therefore the relationship was linear. Biological toxicity of metals would have rendered the relationship non linear, because the extent of the metal effects on CAC would have increased drastically when the toxic threshold is exceeded. The adsorption behavior of chromium in activated sludge have been fitted with the Langmuir and Freundlich adsorption isotherm models, and the latter showed better fit.

In the microscopic-scale study on the adsorption of heavy metal on the superficial layers on activated sludge microbes, it was found that pretreatment with 0.3 N HCl enhanced the Cu^{2+} adsorption capacity of *P.putida* 5-x cells due to the degradation and removal of the superficial, high water-content capsule. The metal salt-bridging formed by bound Cu^{2+} possibly induced a configuration change within the capsule and rendering the metal-binding sites on the cell wall are not accessible to organic matters. This, in turn, affected the organic adsorption capacity and organic removal efficiency of the activated sludge microbes. In intact cells, part of the metal-binding sites on the cell surface are not accessible for heavy metal adsorption. However, the isolated cell envelopes, after chemical and physical treatment, liberated more metal-binding sites on cell surface to enhance 7 times more Cu^{2+} adsorption capacity than intact cells. Peptidoglycan layer of *P. putida* 5-x cell envelope only plays a minor role in Cu^{2+} binding. The outer inner membranes are the main fractions of the cell envelope that provide the active binding sites for Cu^{2+} binding and adsorption in the *P. putida* 5-x cells.

Adsorption of copper by fresh cells, pretreated cells, outer and inner membranes of the cell envelope of *P. putida* 5-x all can all be described the *Freundlich* adsorption equation, but the adsorption model for the isolated peptidoglycan layer of the cell envelope is better described by the *Langmuir* adsorption equation. This implies that the adsorption property on the active binding sites of the peptidoglycan layer is different from that of other fractions of the cells.

In conclusion, results and observations from this work indicated that the adsorption of heavy metals, at sub-lethal concentrations, on activated sludge microbes are mainly physical mono-layer adsorption on active sites in specific biochemical fractions in specific superficial layers, which in turn hindered the adsorption, uptake and subsequent biodegradation of organic matters and led to significant adverse effects on activated sludge process performance.

7.2 LIMITATIONS AND RECOMMENDATIONS

Due to the time constraints in this research programme, a number of important aspects pertaining to the effects of metals on activated sludge process were not included in the study. These aspects are recommended as potential topics for future studies.

In this study, effects of single or individual heavy metal factor on activated sludge were investigated. However, in reality, the composition of wastewaters, including mixed municipal sewage and industrial effluents, often contain more than one type of metal. The combined effects of heavy metals on activated sludge may not be simply the algebraic sum of the individual effect of each metal. Therefore it is worth to extend the study and recommended for future works to investigate the combined effects of different combination of different heavy metals on the activated sludge. The usual combination of metals, such as zinc, nickel, silver, copper and chromium, that are common in electroplating effluents should be studied. The combined effects on activated sludge, and the competitive adsorption among different metals are of practical importance and will provide useful information for actual industrial applications.

In addition, the mechanism of effects of heavy metals on activated sludge process performance is not quantified nor explicitly expressed in a structured mathematical model. Future research should focus on the aspect of understanding the interactions between heavy metals, organic matters and activated sludge microbes in the form of a structured mathematical model, with metal concentrations, organic concentrations, HRT and other operating conditions as the controlled variables. This model, when validated and calibrated will be useful and can be applicable in process design, operation and optimisation.

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7 Chua, H., Lo, W., Wong, P.K., Bi, S.P. and S.N. Sin, **Studies on the Sorption and Desorption of Cu^{2+} from Wastewater by Magnetite-Immobilised Cells of *Pseudomonas putida 5-x.*,** *Science of the Total Environment* (2001, in press)

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9 Chua, H., **Sin, S.N.,** Tan, K.N., Lo, W. and Yu, P.H.F., (2000) **Novel Feast-Fast Technique for Filamentous Foam Control in Activated Sludge SBR Process – Introducing a New Foam Measurement Method,** *Water Science and Technology*, **42**(3-4), 95-102

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APPENDIX I

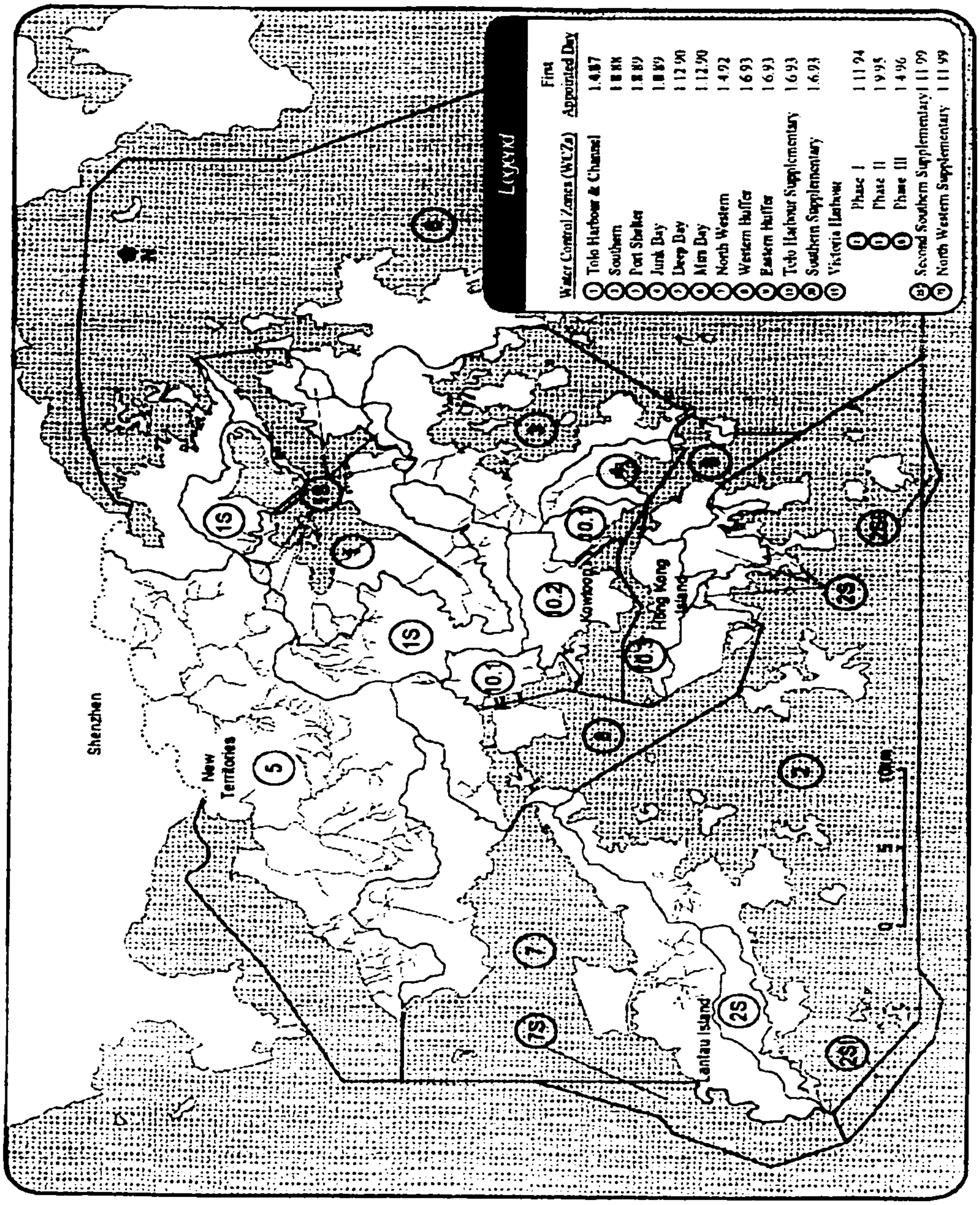

Figure A1
Water Control Zones in Hong Kong (after EPD 1998)

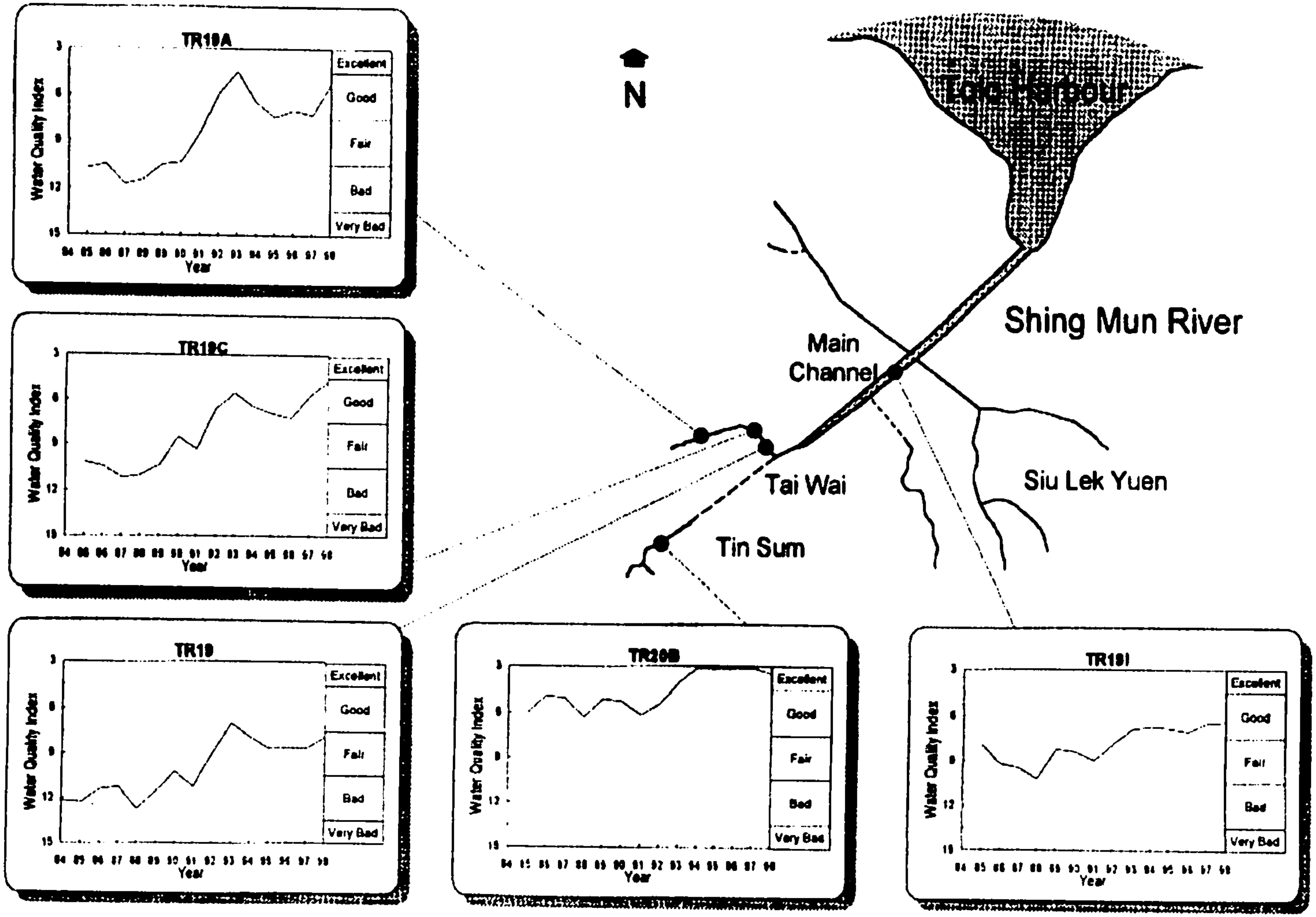

Figure A2
Water Quality Index of Shing Mun River (after EPD 1998)

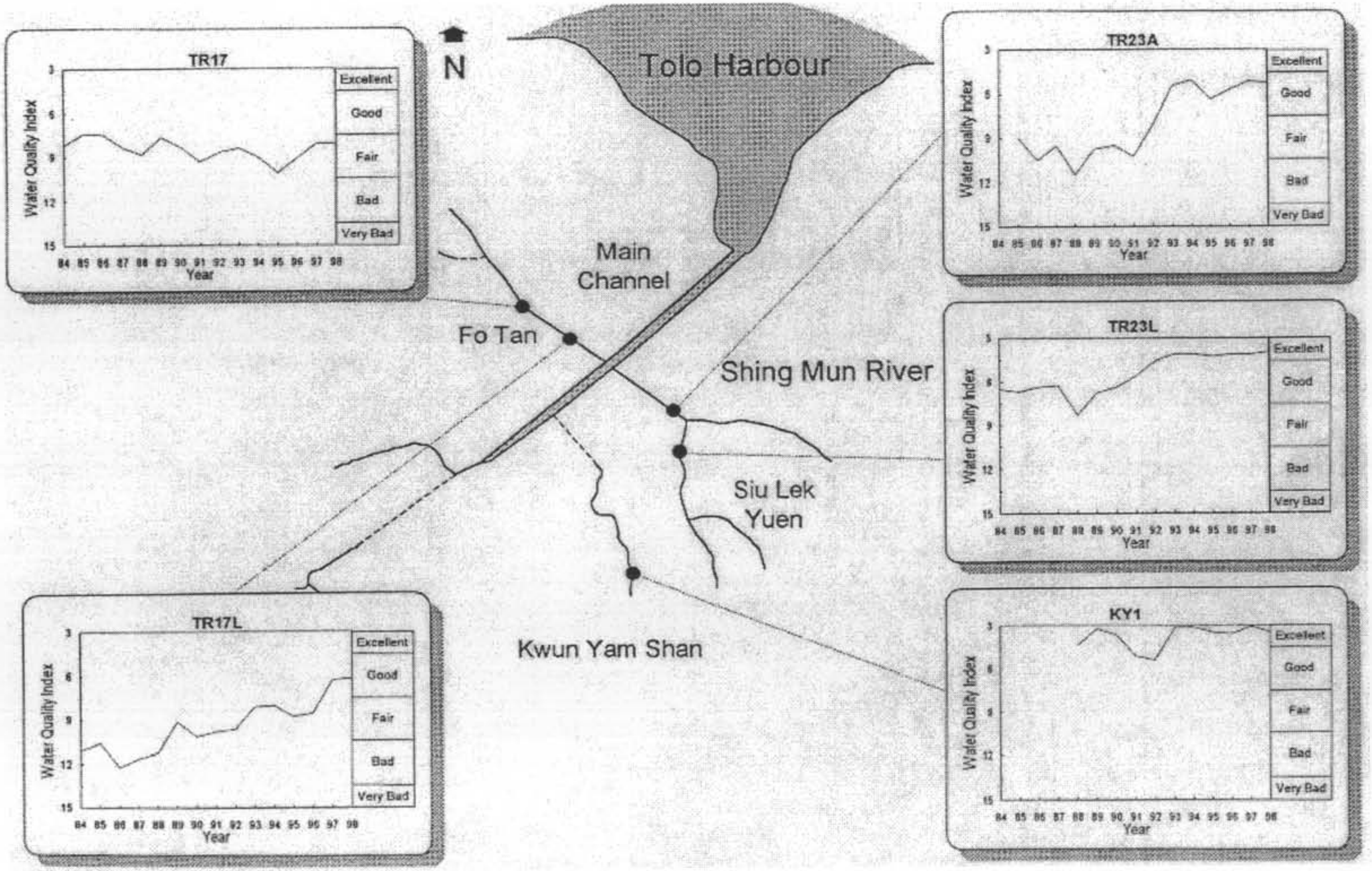

Figure A3
Water Quality Index of Shing Mun River (continued) (after EDP 1998)

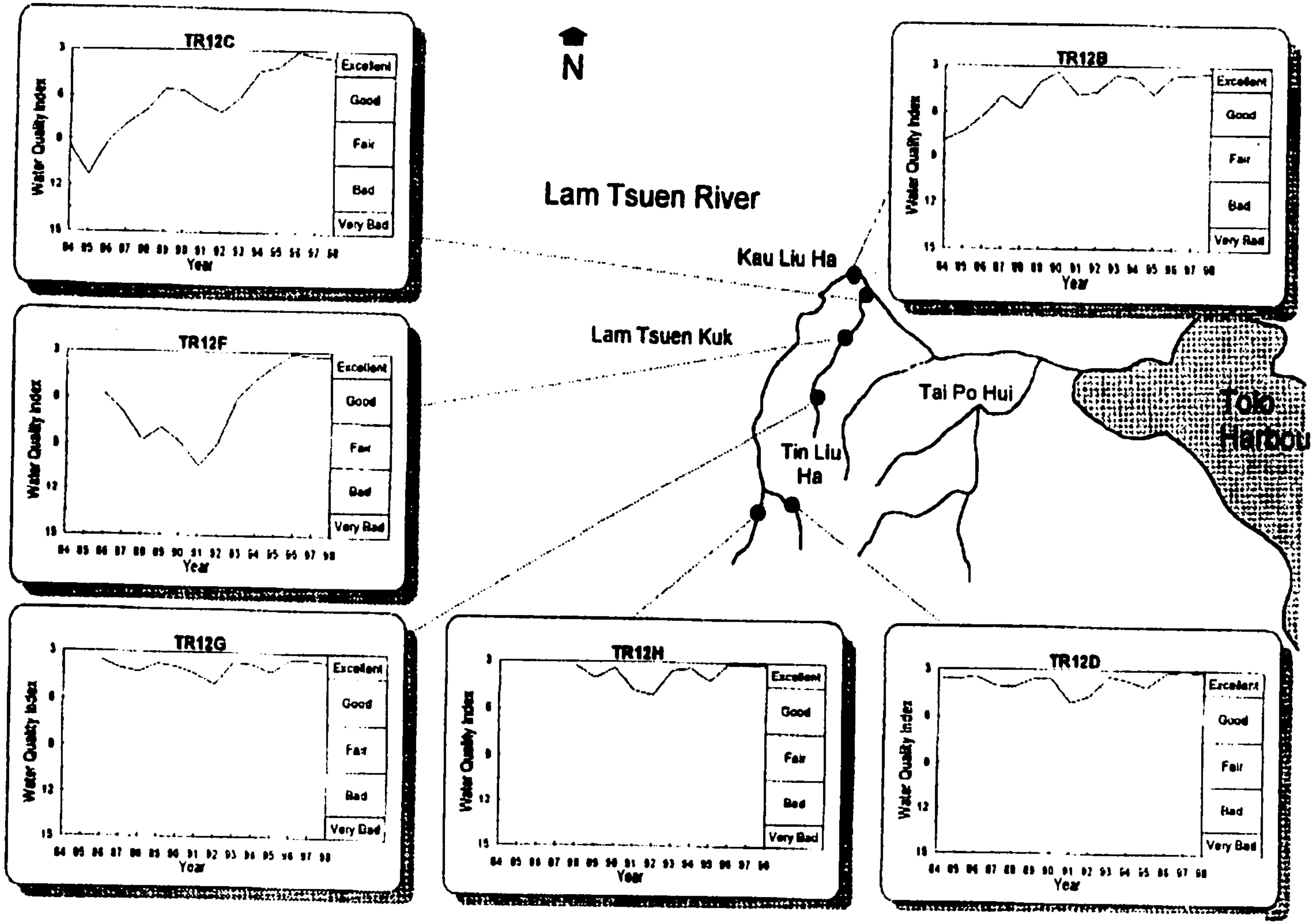

Figure A4
Water Quality Index of Lam Tsuen River (after EPD 1998)

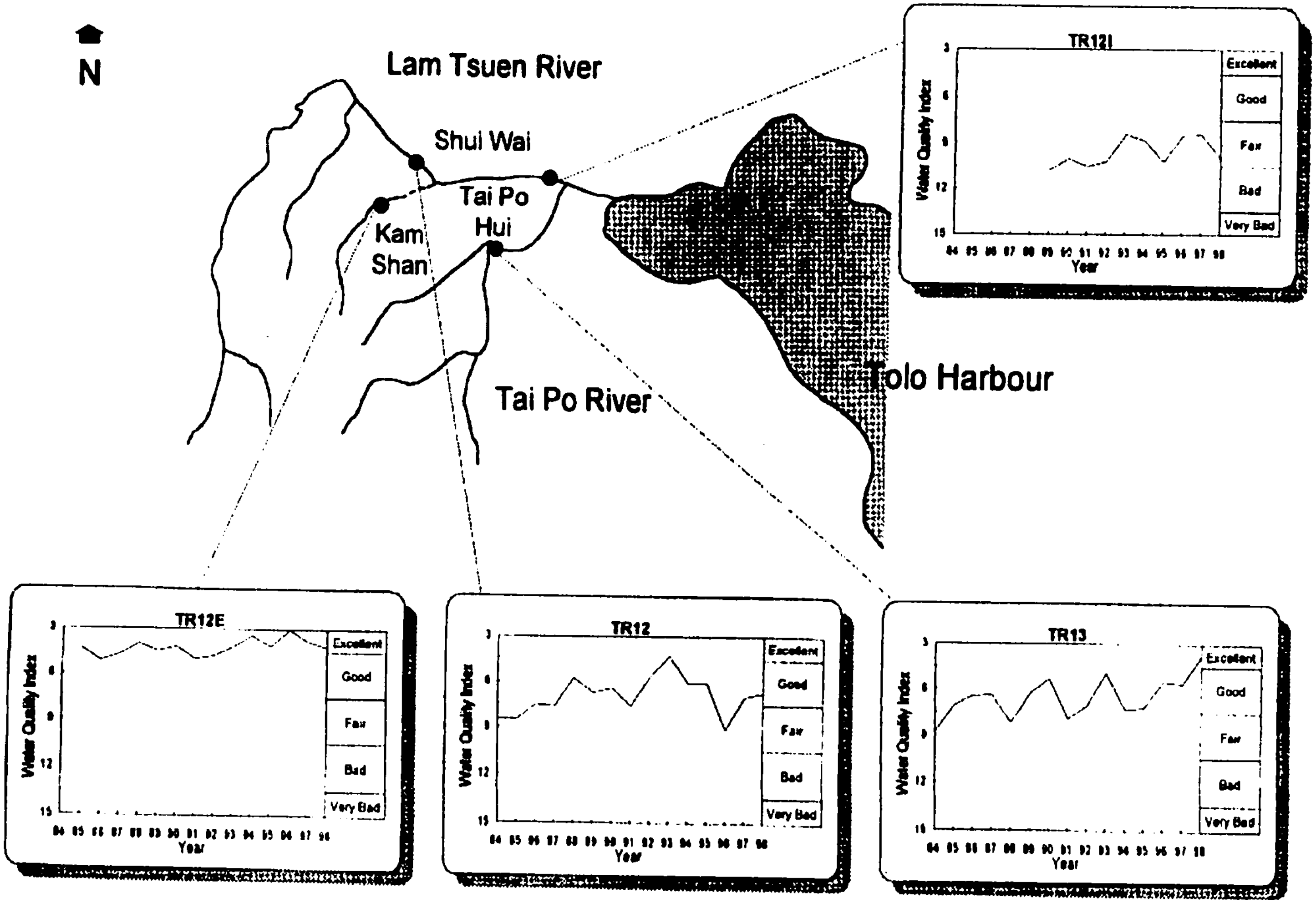

Figure A5
Water Quality Index of Lam Tsuen River (continued) and Tai Po River (after EPD 1998)

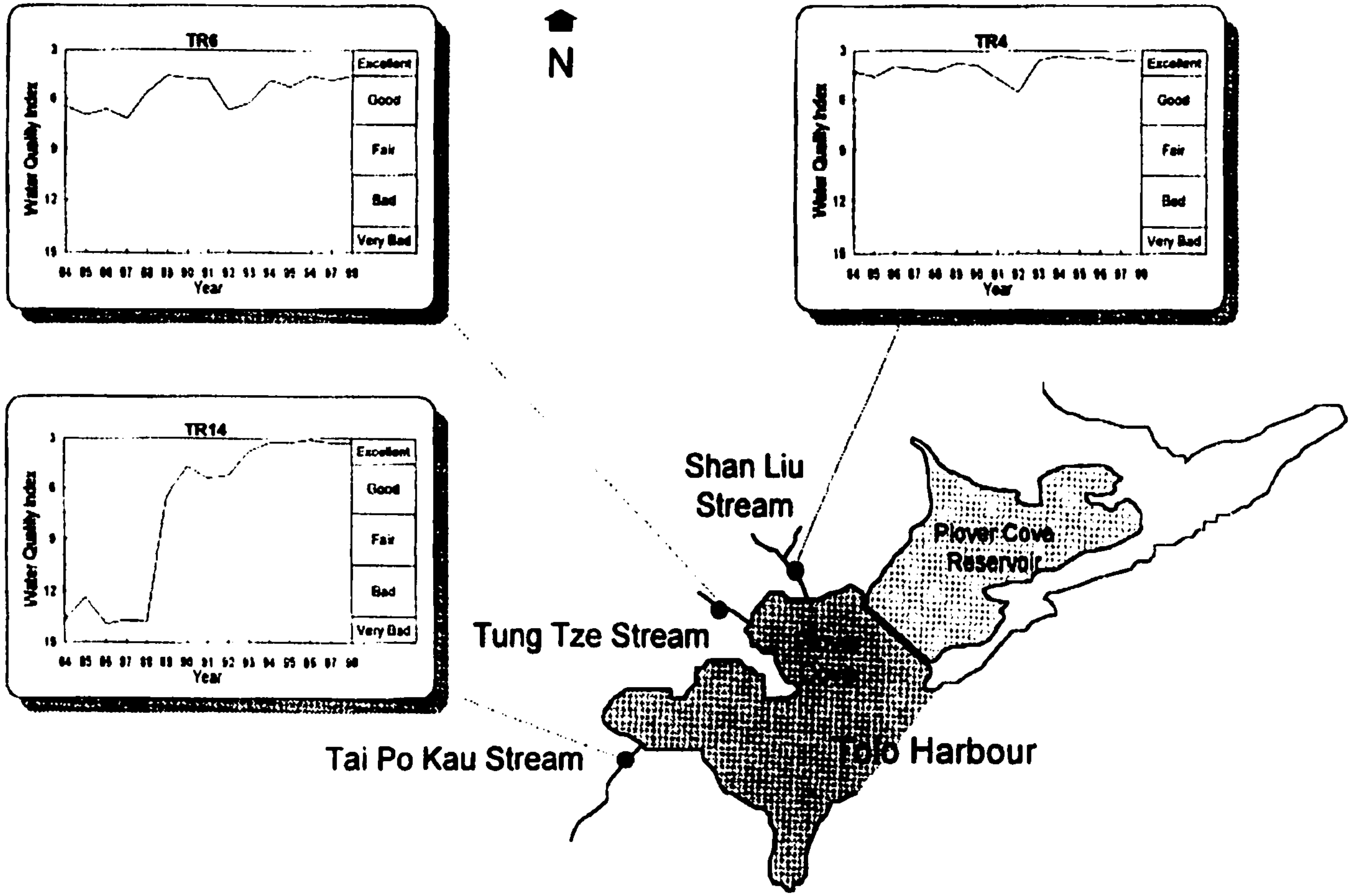

Figure A6
Water Quality Index of minor streams in the Tolo Harbour and Channel Water Control Zone (after EPD 1998)

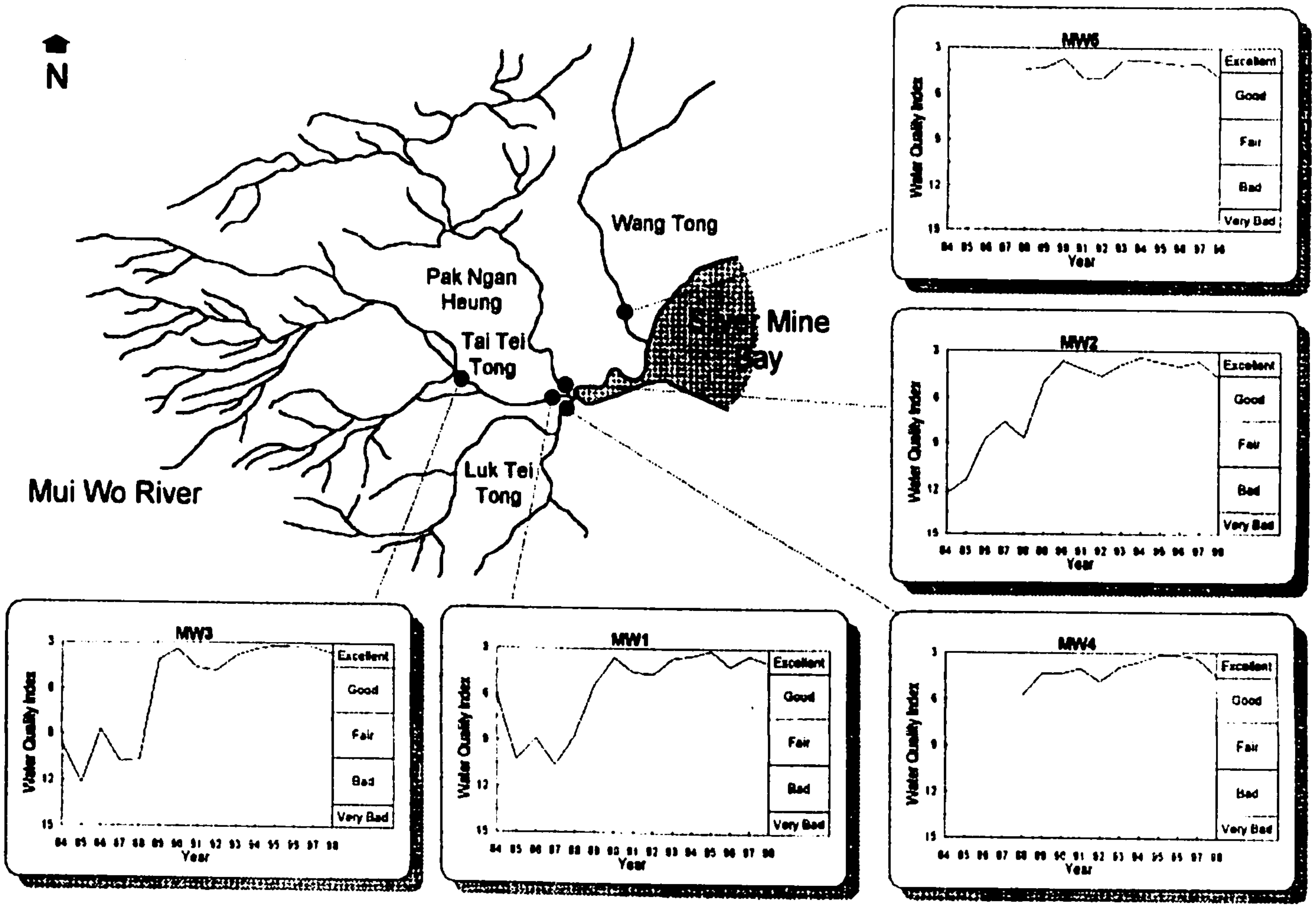

Figure A7
Water Quality Index of Mui Wo River (after EPD 1998)

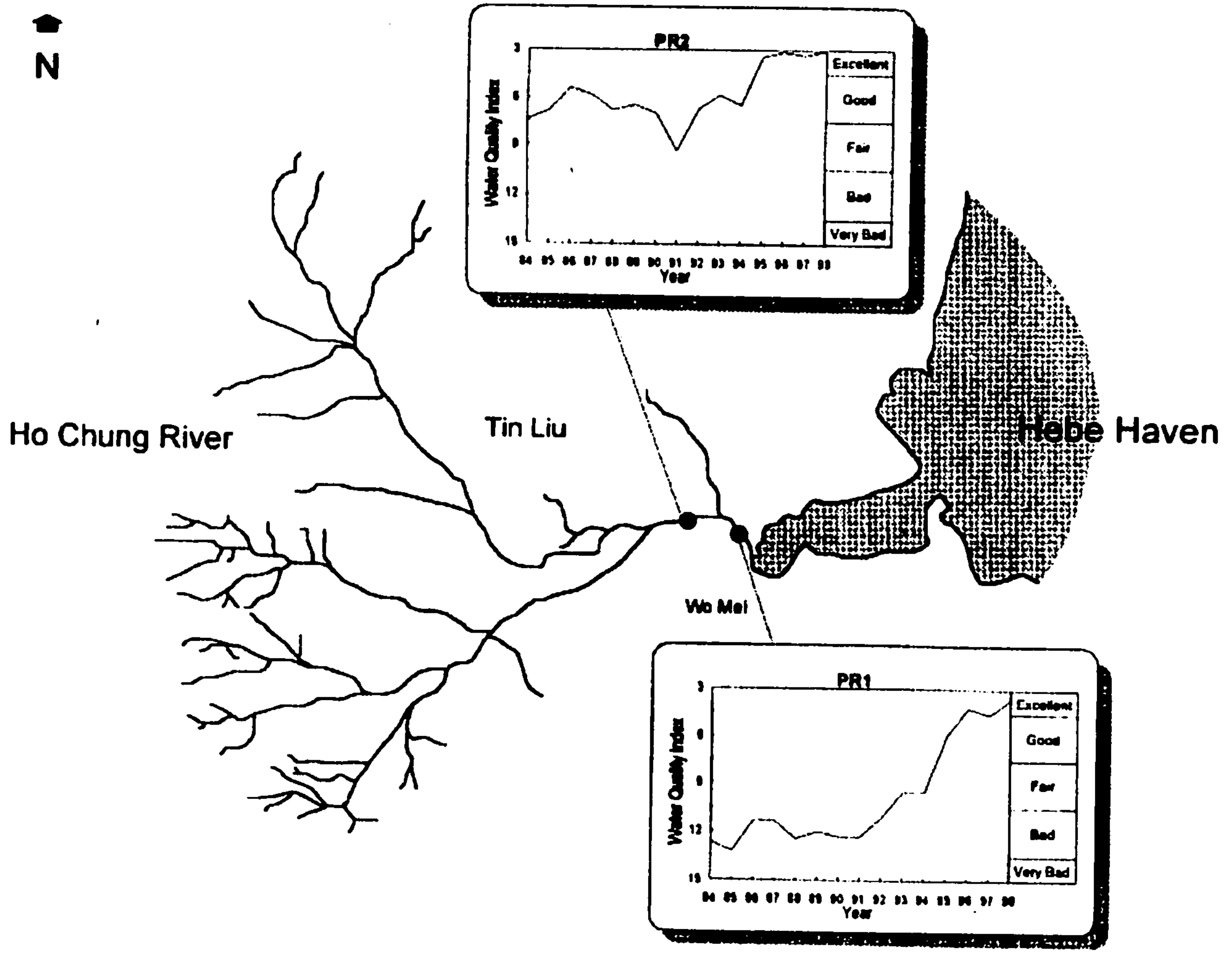

Figure A8
Water Quality Index of Ho Chung River (after EPD 1998)

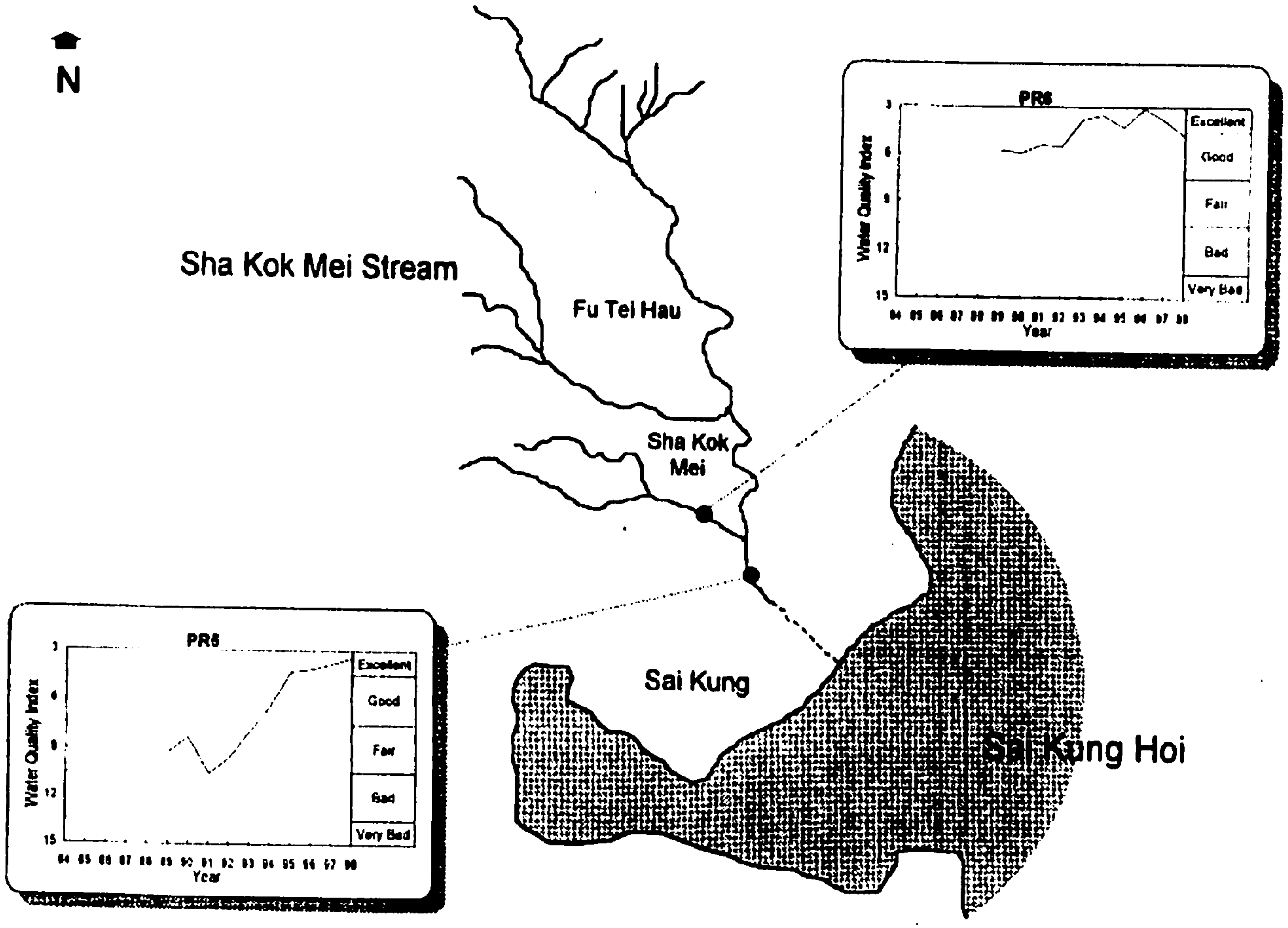

Figure A9
Water Quality Index of Sha Kok Mei Stream (after EPD 1998)

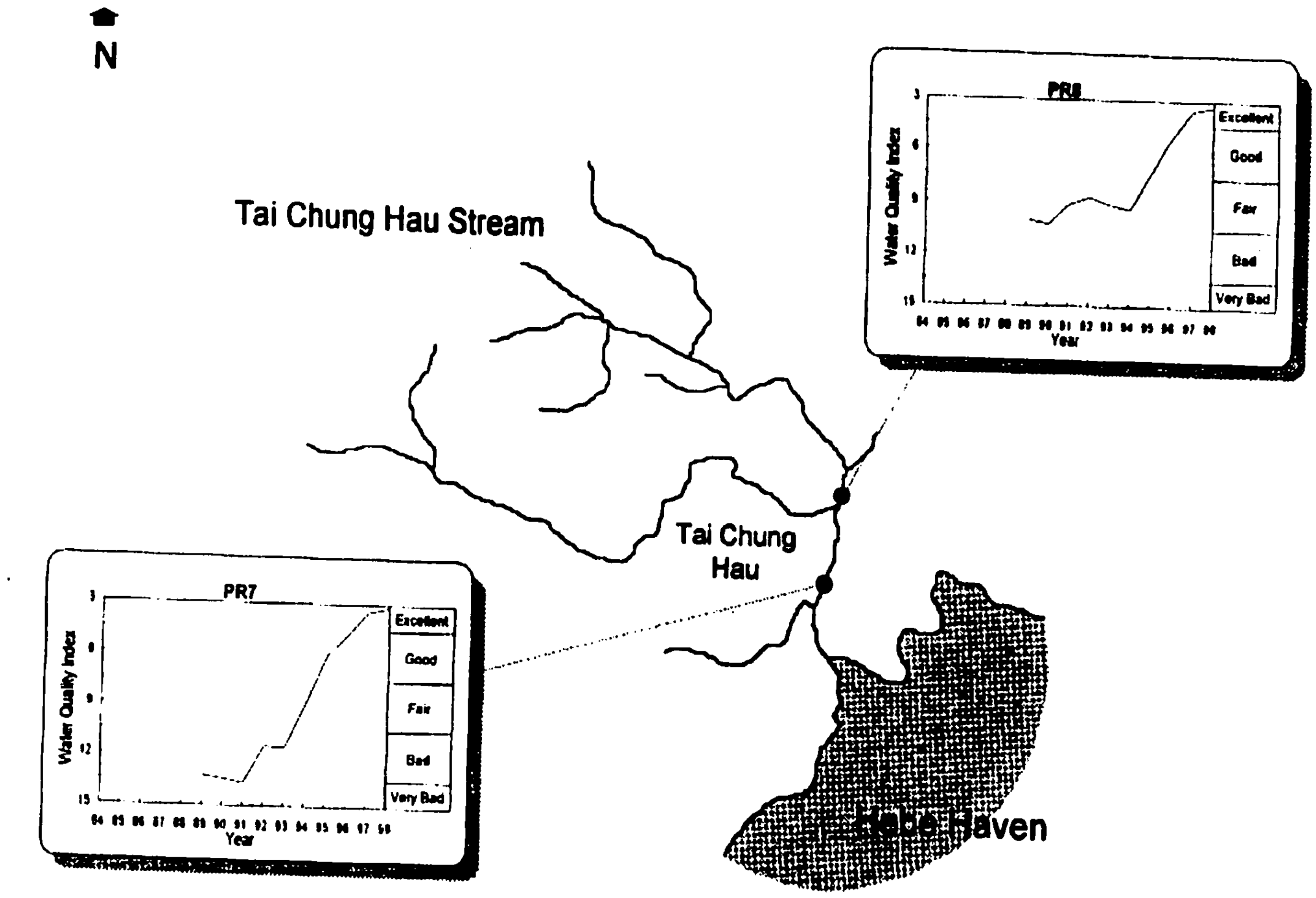

Figure A10
Water Quality Index of Tai Chung Hau Stream (after EPD 1998)

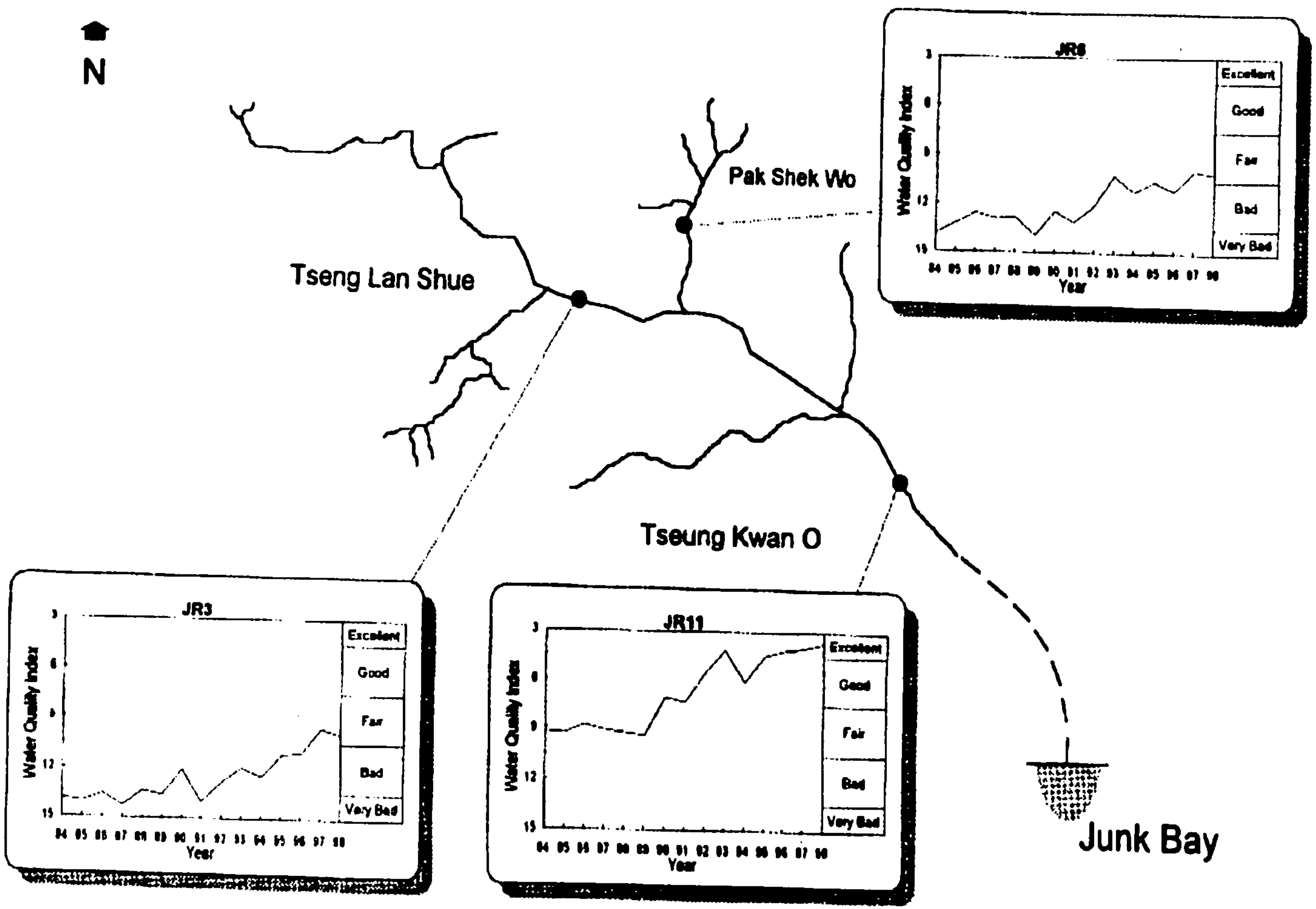

Figure A11
Water Quality Index of Tseng Lan Shue Stream (after EPD 1998)

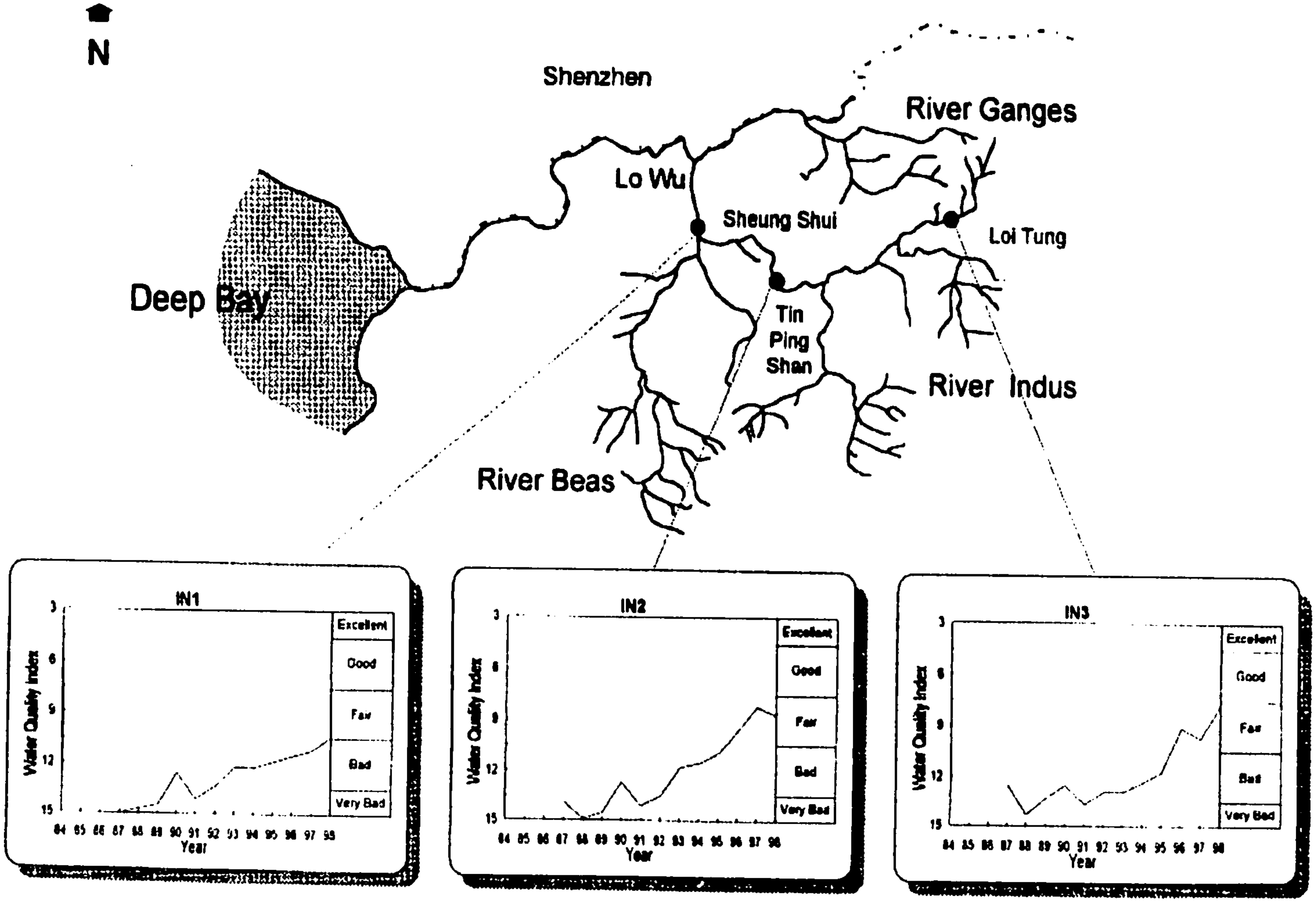

Figure A12
Water Quality Index of the River Indus (after EPD 1998)

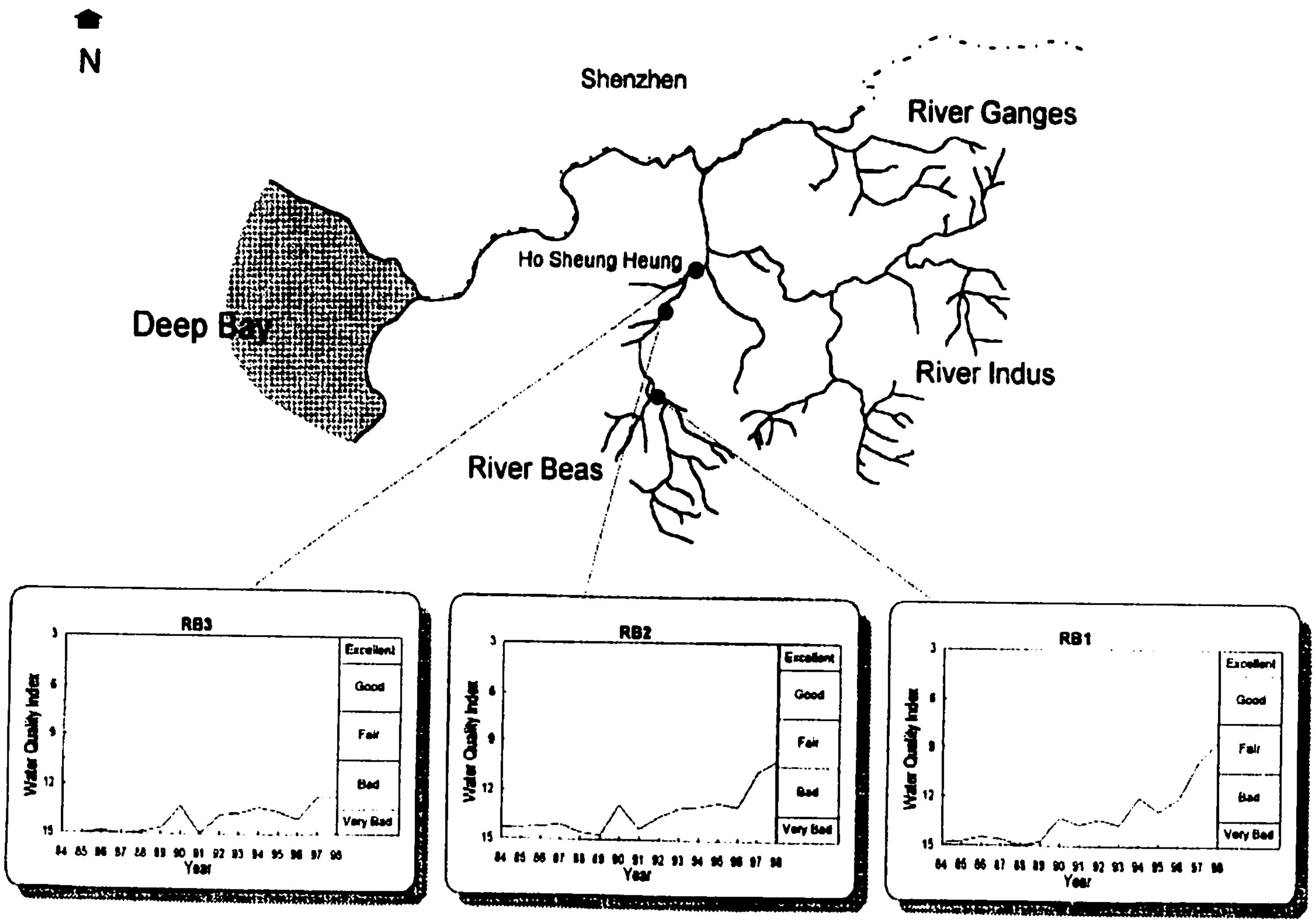

Figure A13
Water Quality Index of the River Beas (after EPD 1998)

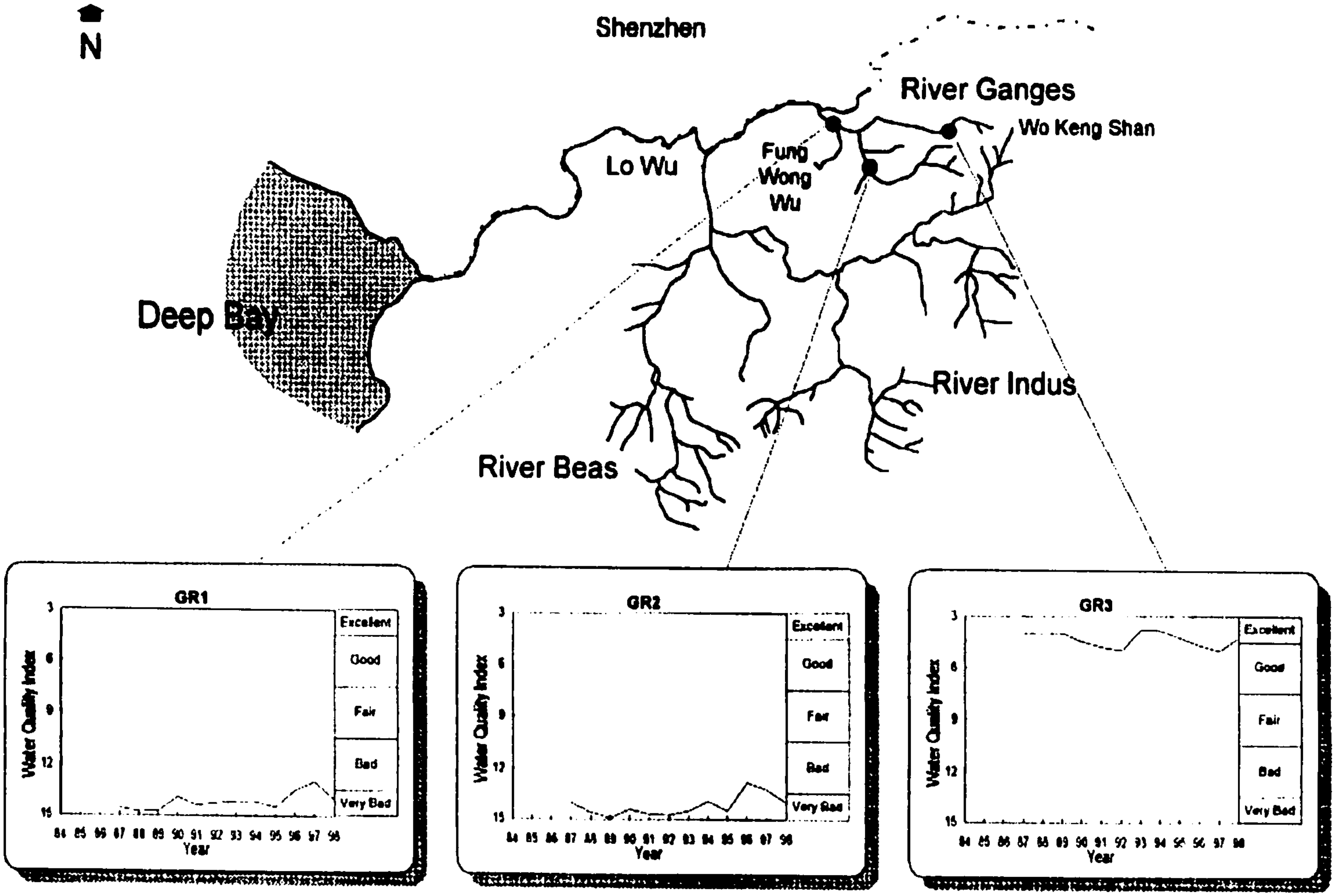

Figure A14
Water Quality Index of the River Ganges (after EPD 1998)

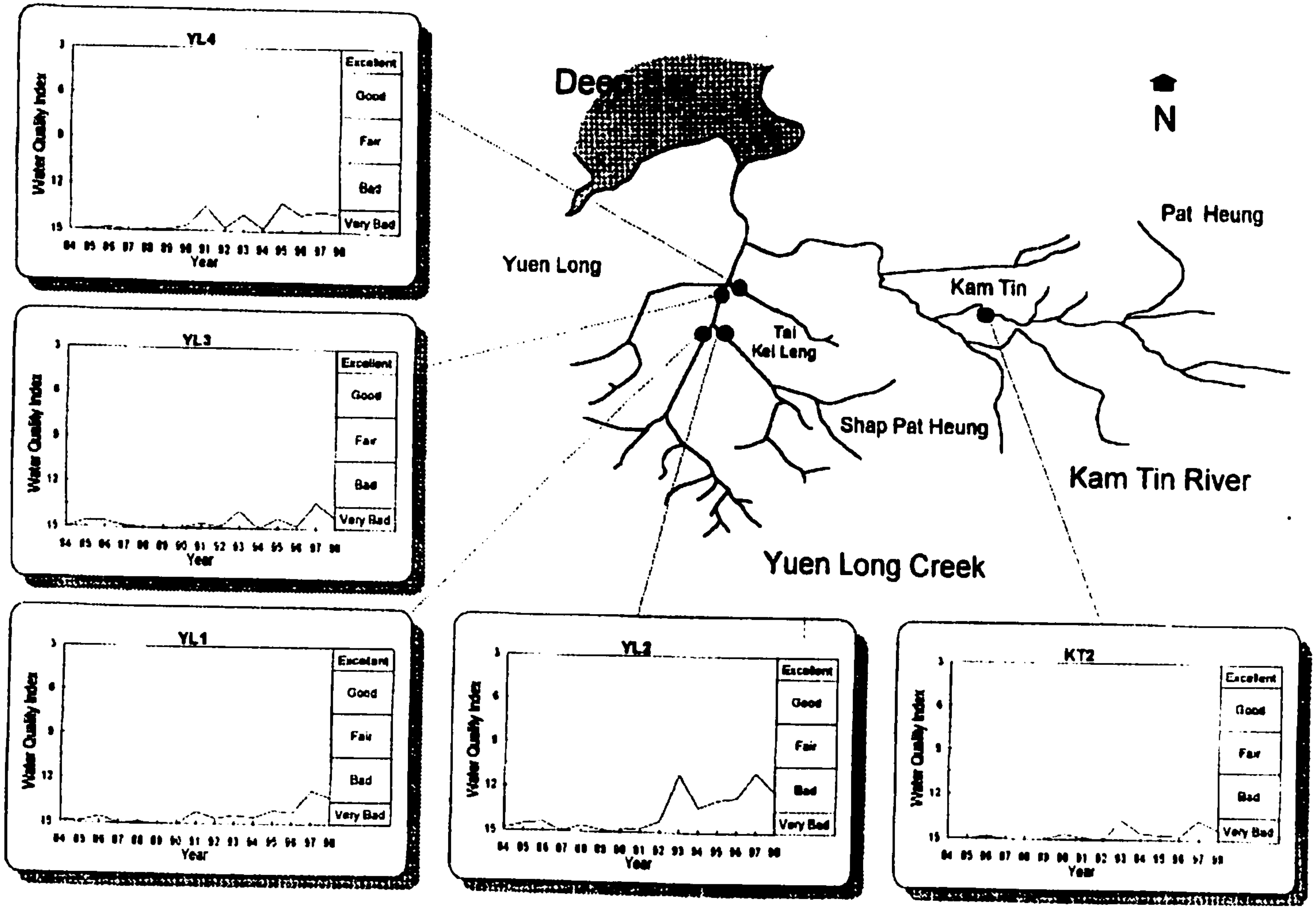

Figure A 15
Water Quality Index of Yuen Long Creek and Kam Tin River (after EPD 1998)

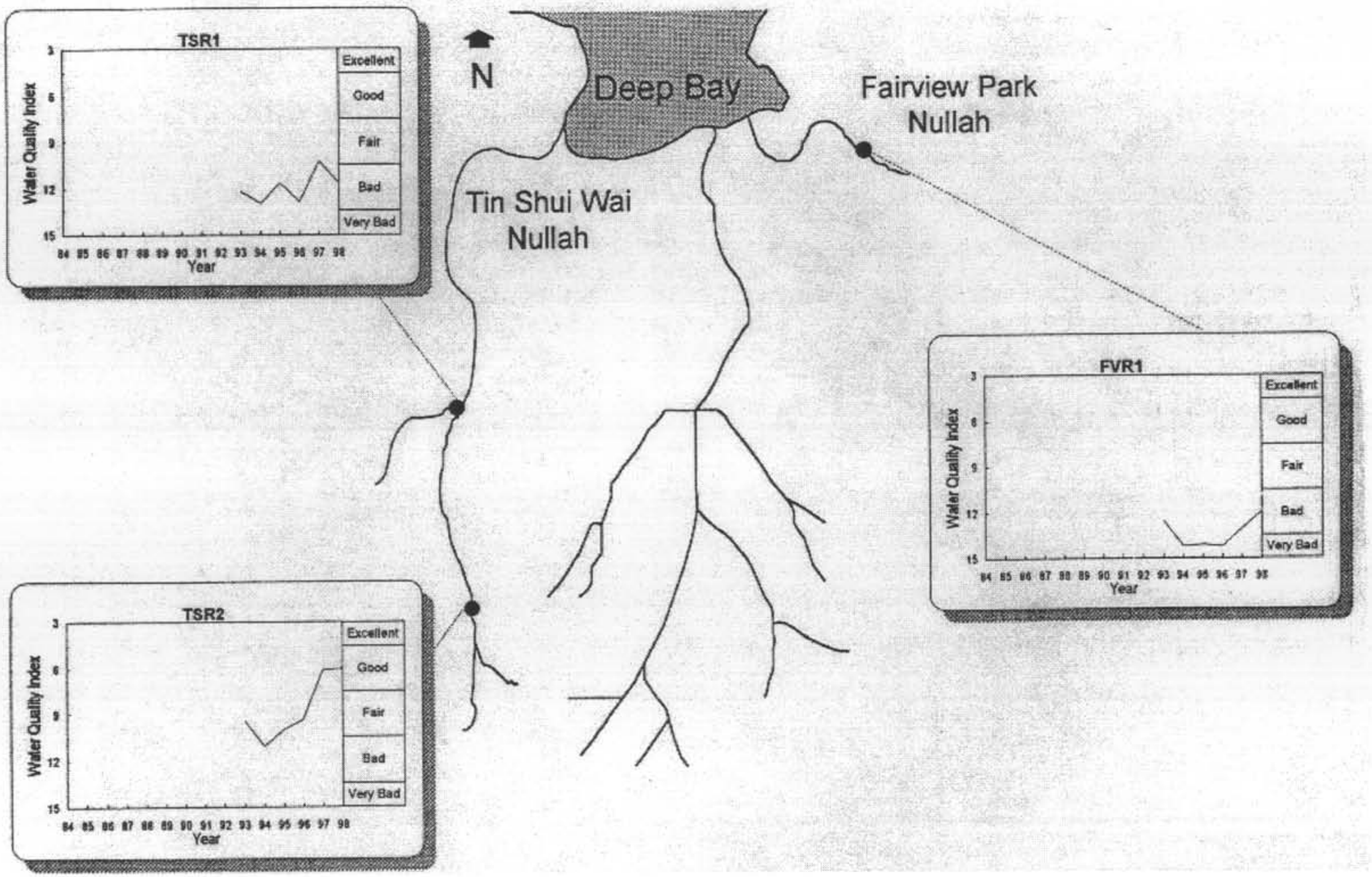

Figure A16
Water Quality Index of Tin Shui Wai Nullah and Fairview Park Nullah (after EPD 1998)

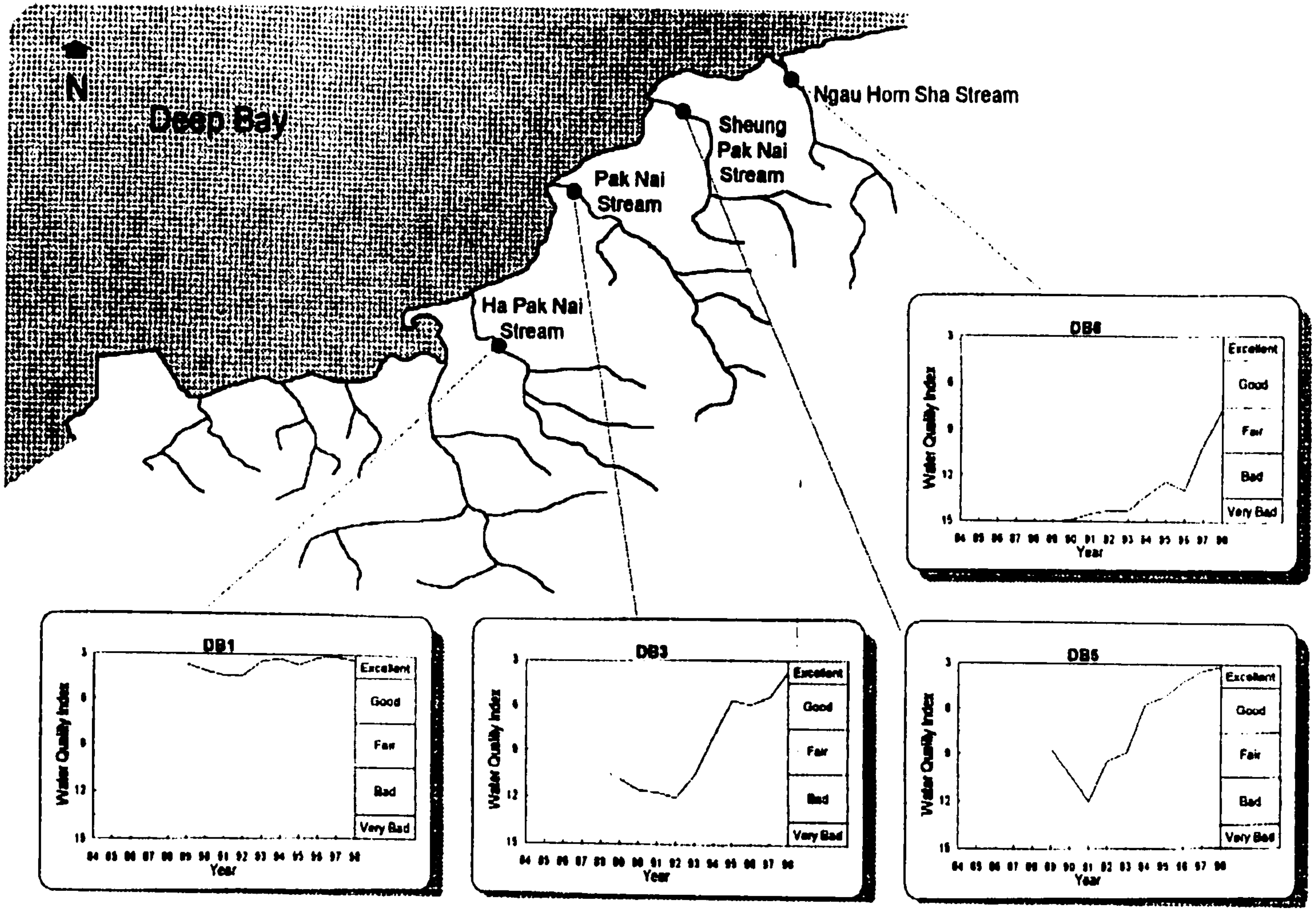

Figure A17
Water Quality Index of minor streams in the Deep Bay catchment (after EPD 1998)

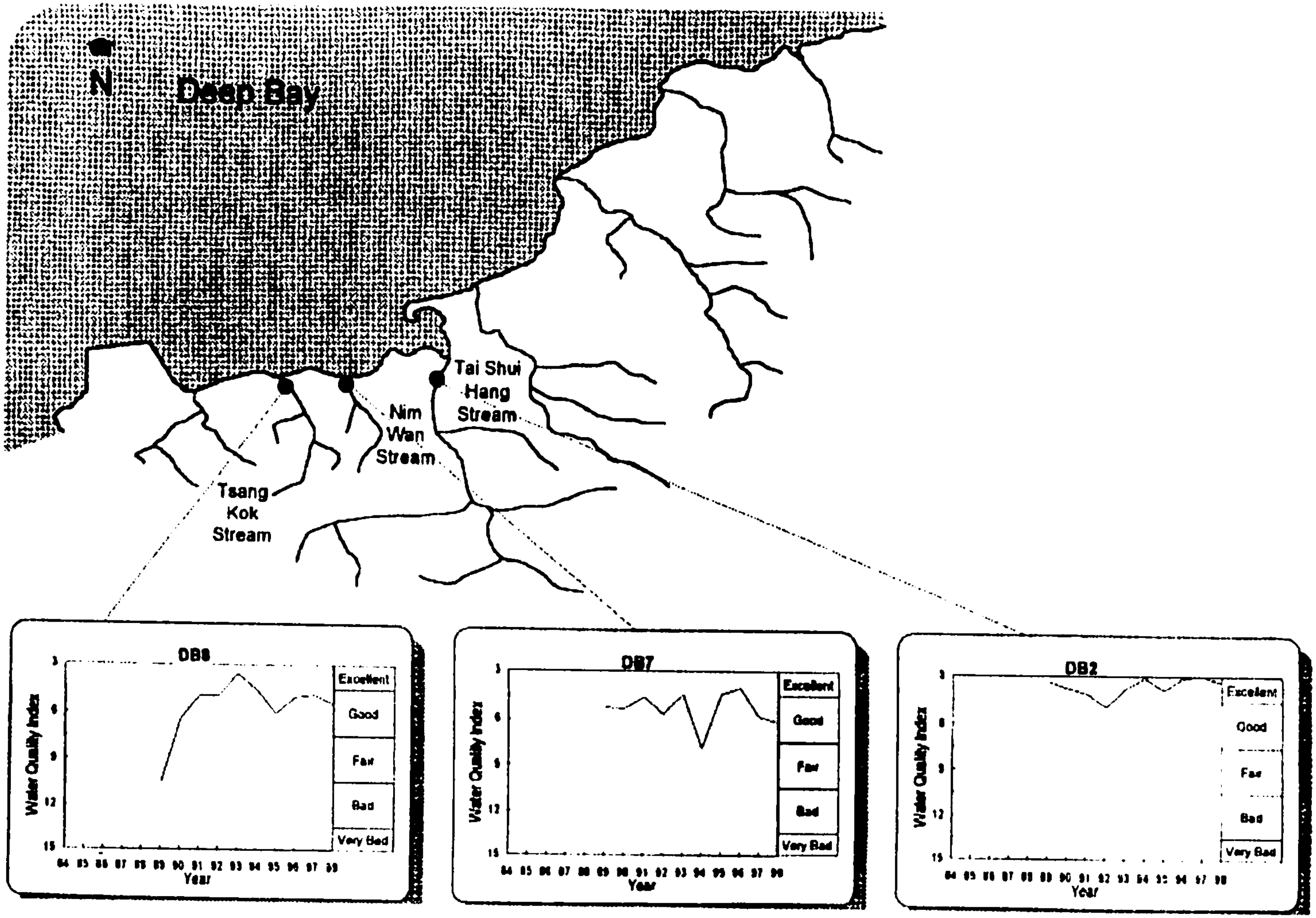

Figure A18
Water Quality Index of minor streams in the Deep Bay catchment (continued)(after EPD 1998)

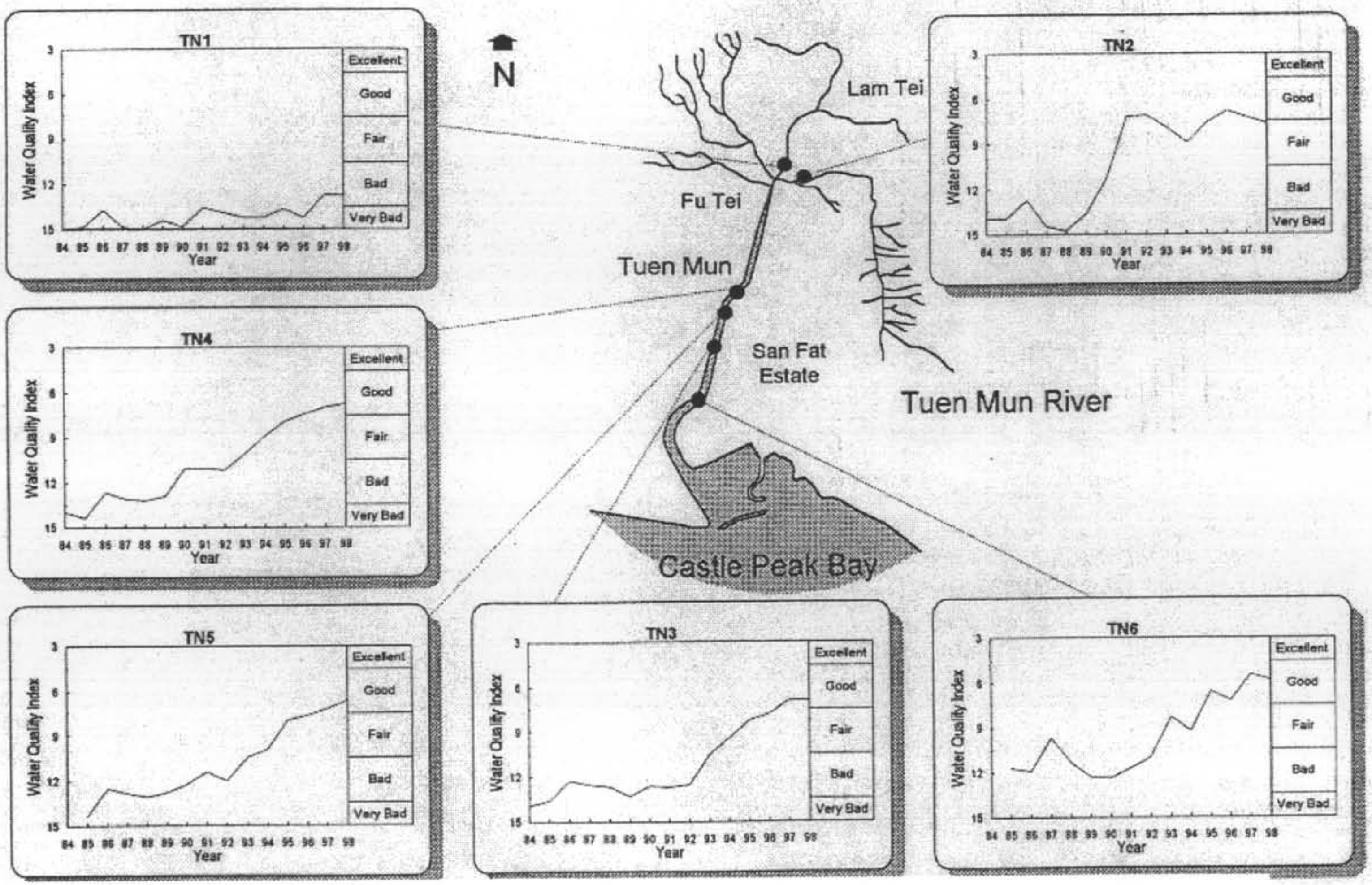

Figure A19
Water Quality Index of Tuen Mun River (after EPD 1998)

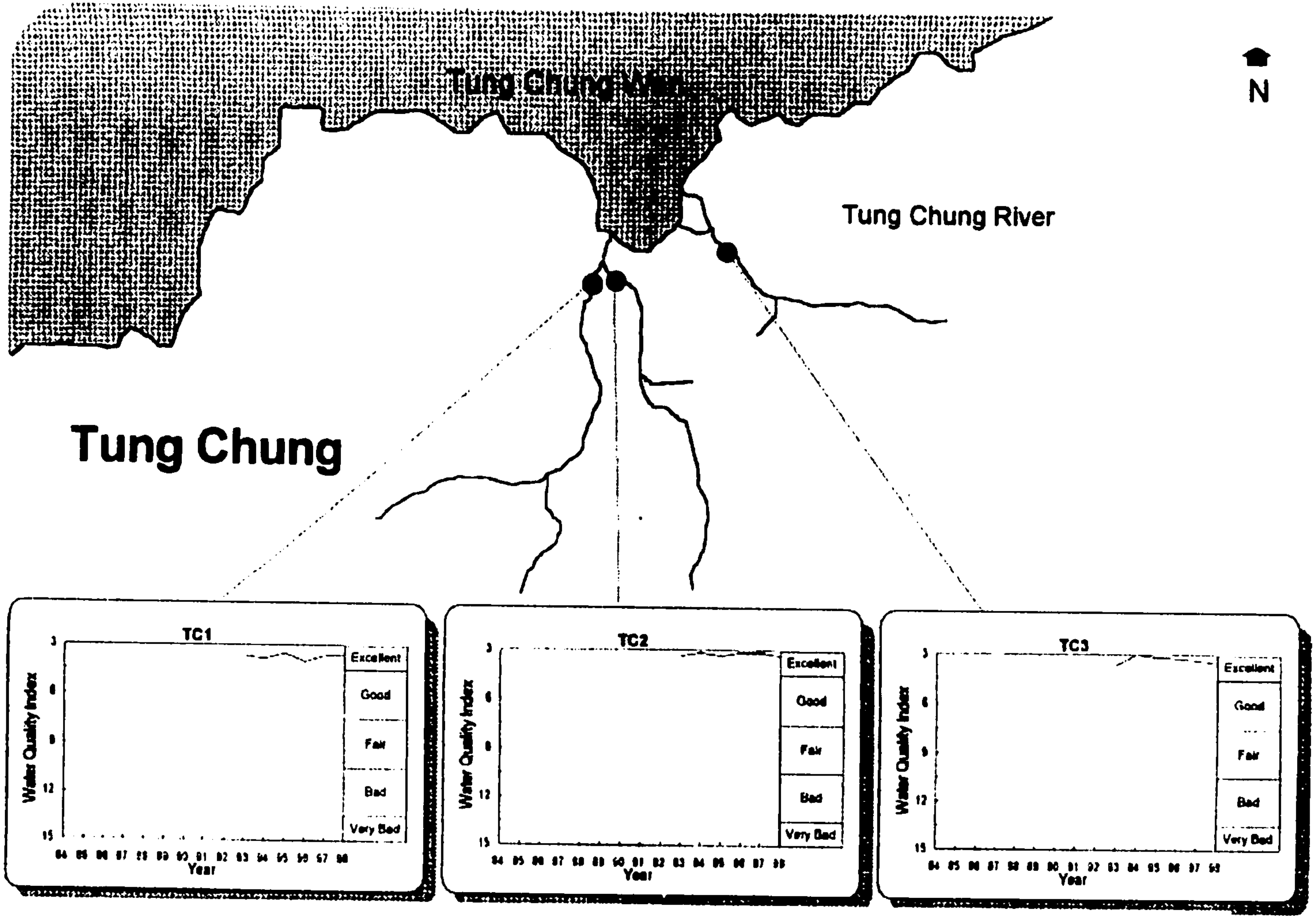

Figure A20
Water Quality Index of Tung Chung River (after EPD 1998)

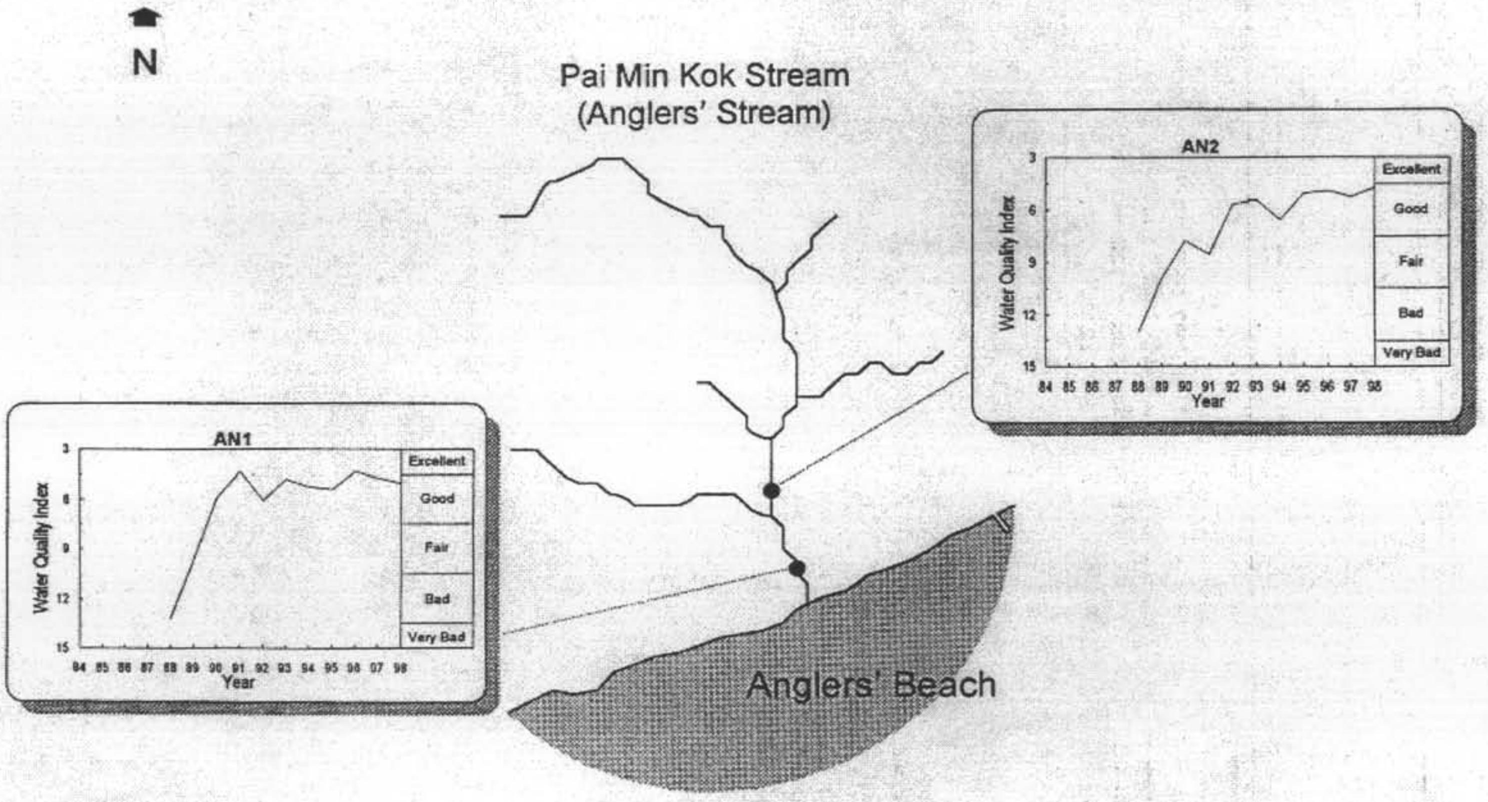

Figure A21
Water Quality Index of Pai Min Kok Stream (after EPD 1998)

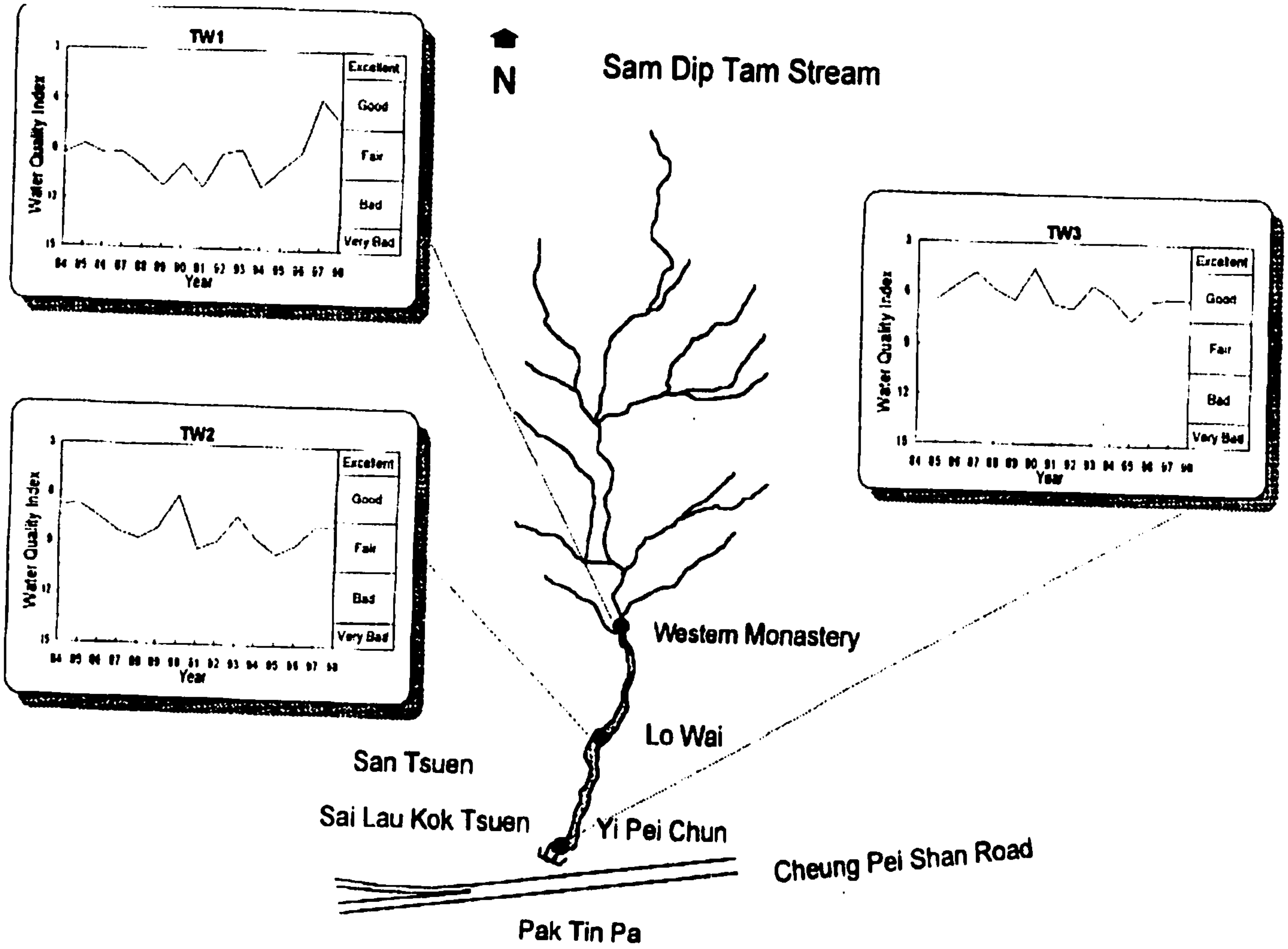

Figure A22
Water Quality Index of Sam Dip Tam Stream (after EDP 1998)

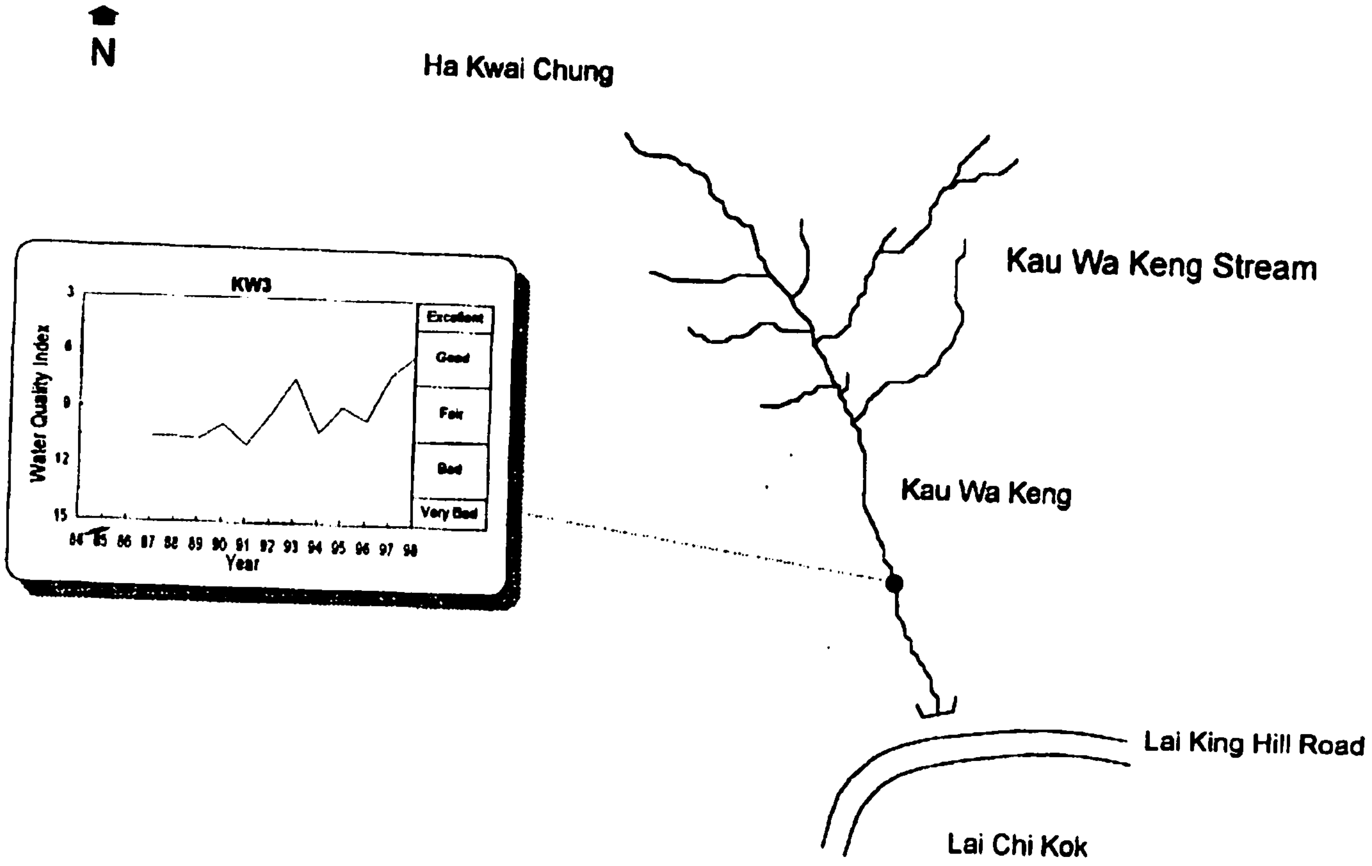

Figure A23
Water Quality Index of Kau Wa Keng Stream (after EPD 1998)

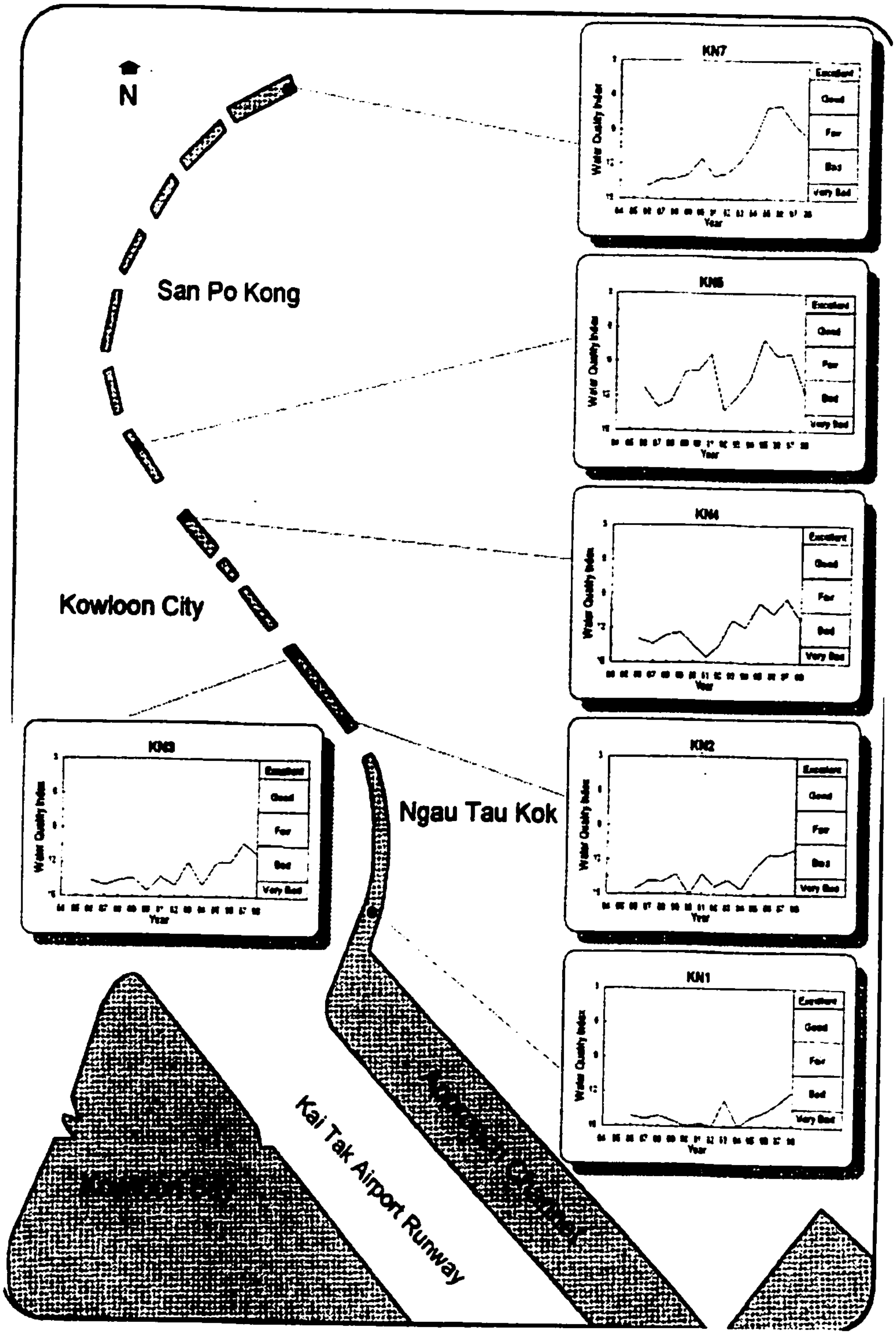

Figure A24
Water Quality Index of Kai Tak Nullah (after EPD 1998)

APPENDIX II : RAW DATA

SBR Performance Data under the Effect of Zinc

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
0	60.3	54.8	62.3	59.1	19.2	17.4	18.2	18.3	1.8	9.8
1	80.3	73.8	76.3	76.5	38.4	35.1	36.9	36.8	3.3	9.0
2	93.7	92.9	94.1	93.6	19.4	17.9	18.8	18.7	1.5	8.0
3	89.5	84.3	86.3	86.7	31.8	33.3	33.3	32.8	1.5	4.6
4										
5	96.1	96.3	95.7	96.0	38.6	35.3	35.1	36.3	3.5	9.6
6	84.7	80.3	82.3	82.4	33.2	35.1	34.3	34.2	1.9	5.5
7	78.5	71.8	79.3	76.5	35.1	33.1	35.2	34.5	2.1	6.1
8										
9	95.1	94.1	96.3	95.2	45.5	46.3	46.6	46.1	1.1	2.4
10	69.4	64.4	70.3	68.0	33.2	30.6	32.3	32.0	2.6	8.1
11	95.1	89.9	88.3	91.1	49.8	45.2	50.1	48.4	4.9	10.1
12										

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
13	91.1	86.3	88.9	88.8	42.5	43.9	44.1	43.5	1.6	3.7
14	91.8	83.3	87.7	87.6	46.4	42.3	43.3	44.0	4.1	9.3
15	86.4	79.8	83.2	83.1	39.3	41.1	39.3	39.9	1.8	4.5
16										
17	84.2	83.8	84.3	84.1	50.3	45.9	50.1	48.8	4.4	9.0
18	70.4	70.9	71.3	70.9	59.8	60.3	59.9	60.0	0.5	0.8
19	90.2	89.3	90.3	89.9	56.4	51.7	51.3	53.1	5.1	9.6
20										
21	94.8	92.1	93.3	93.4	52.5	53.3	53.2	53.0	0.8	1.5
22	94.3	93.1	93.9	93.8	54.6	49.9	53.1	52.5	4.7	8.9
23	95.9	93.3	96.1	95.1	60.3	54.9	54.3	56.5	6.0	10.6
24										
25	95.5	90.3	88.9	91.6	52.9	47.3	53.1	51.1	5.8	11.3
26	84.3	76.3	80.6	80.4	54.7	49.8	52.2	52.2	4.9	9.4
27	93.9	85.3	94.2	91.1	53.1	54.9	53.3	53.8	1.8	3.3
28										
29	92.1	93.3	92.6	92.7	44.3	40.1	44.4	42.9	4.3	10.0
30	83.5	76.8	83.3	81.2	46.6	47.3	46.4	46.8	0.9	1.9
31	91.3	83.3	87.6	87.4	44.8	40.9	40	41.9	4.8	11.4
32					40.4	41.1	40.1	40.5	1.0	2.5
33	91.8	83.8	87.3	87.6						
34	91.1	93.3	92.3	92.2	41.3	38.3	39.9	39.8	3.0	7.5

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
35	92.5	93.3	93.1	93.0	40.3	41.7	42.1	41.4	1.8	4.3
36					39.5	36.6	35.9	37.3	3.6	9.6
37	91.8	83.6	87.3	87.6						
38	81.8	76.3	79.9	79.3	39.6	36.9	40.1	38.9	3.2	8.2
39	92.3	92.9	92.1	92.4	40.3	42.1	41.3	41.2	1.8	4.4
40					39.3	36.2	39.8	38.4	3.6	9.4
41	92.3	93.3	92.6	92.7						
42	91.6	93.6	92.6	92.6	40.3	37.3	39.3	39.0	3.0	7.7
43	91.8	92.8	92.3	92.3	40.8	37.8	40.2	39.6	3.0	7.6
44					40.8	40.1	41.3	40.7	1.2	2.9
45	85.3	77.3	86.1	82.9						
46	90.3	81.3	86.1	85.9	43.8	39.9	41.1	41.6	3.9	9.4
47	87.3	89.3	88.3	88.3	48.8	49.3	48.8	49.0	0.5	1.0
48					50.8	45.7	50.1	48.9	5.1	10.4
49	85.3	79.3	82.8	82.5						
50	97.3	93.2	95.8	95.4	69.8	64.1	69.1	67.7	5.7	8.4
51	90.3	89.3	88.1	90.2	55.3	50.6	52.7	52.9	4.7	8.9
52					76.3	69.4	69.3	71.7	7.0	9.8
53	93.4	84.3	88.8	88.8						
54	91.1	82.5	86.6	86.7	118.6	109.3	112.8	113.6	9.3	8.2
55	93.1	86.9	94.3	91.4	94.3	95.7	94.3	94.8	1.4	1.5

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
56					110.3	111.6	111.1	111	1.3	1.2
57	93.9	94.4	94.6	94.3						
58	83.4	84.6	84.8	84.3	99.2	100.2	100.1	99.8	1.0	1.0
59	92.2	93.3	93.1	92.9	92.7	93.1	93.3	93.0	0.6	0.6
60					97.1	98.3	98.1	97.8	1.2	1.2
61	93.9	94.3	92.3	93.5						
62	92.5	93.8	94.3	93.5	91.9	83.2	87.3	87.5	8.7	9.9
63	93.3	93.6	94.1	93.7	91.6	83.6	92.2	89.1	8.6	9.6
64					92.9	94.3	93.3	93.5	1.4	1.5
65	92.3	92.1	93.3	92.6						
66	93.3	92.2	92.4	92.6	91.8	92.3	92.3	92.1	0.5	0.5
67	92.5	89.9	88.3	90.2	80.3	72.9	76.3	76.5	7.4	8.7
68					89.3	81.6	81.3	84.1	8.0	8.5
69	90.3	82.3	86.1	86.2						
70	98.3	89.3	96.5	94.7	85.3	86.3	85.1	85.6	1.2	1.4
71	90.3	93.2	92.3	91.9	70.3	63.9	70.1	68.1	6.4	9.4
72					79.3	72.9	76.7	76.3	6.4	8.4
73	83.3	86.3	86.1	85.2						
74	78.3	73.3	75.5	75.7	72.3	66.6	72.2	70.4	5.7	8.1
75	82.3	75.7	83.2	80.4						
76					72.3	66.9	68.8	69.3	5.4	7.8
77	78.3	72.1	74.9	75.1	72.3	66.8	66.3	68.5	6.0	8.7

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
78	79.3	72.4	79.3	77.0	69.2	70	68.3	69.2	1.7	2.4
79	80.3	72.8	81.3	78.1						
80					72.3	65.9	67.7	68.6	6.4	9.3
81	75.3	69.9	72.1	72.4	86.3	78.8	77.3	80.8	9.0	11.1
82	63.3	59.3	63.1	61.9	89.3	79.9	84.3	84.5	9.4	11.1
83	79.3	76.3	77.7	77.8						
84					120.3	110.7	121.5	117.5	10.8	9.2
85	83.3	86.3	86.1	85.2	95.3	90.3	93.3	93.0	5.0	5.4
86	88.3	89.3	88.8	88.8	96.3	97.3	97.7	97.1	1.4	1.4
87	89.3	86.9	88.1	88.1						
88					88.3	80.9	84.3	84.5	7.4	8.7
89	90.3	88.6	91.3	90.1	95.3	90.4	93.2	93.0	4.9	5.3
90	89.3	83.3	86.4	86.3						
91	92.3	86.8	92.1	90.4	98.3	99.1	99.3	98.9	0.8	0.8
92					96.3	89.4	93.8	93.2	6.9	7.4
93	90.2	83.6	87.2	87.0	99.3	90.6	99.1	96.3	8.7	9.0
94	81.3	73.6	81.2	78.7						
95	89.3	80.3	89.1	86.2	97.5	99.8	98.8	98.7	2.3	2.3
96					98.3	100.6	99.6	99.5	2.3	2.3
97	91.3	92.1	91.2	91.5	97.3	89.7	97.2	94.7	7.6	8.0
98	83.3	82.8	83.1	83.1						

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
99	90.3	89.3	89.9	89.9	98.3	90.6	93.3	94.1	7.7	8.2
100					100.3	91.5	95.5	95.8	8.8	9.2
101	89.3	82.9	89.1	87.1	97.3	98.8	97.8	98.0	1.5	1.5
102	80.3	72.9	75.5	76.2						
103	85.3	86.1	86.6	86.0	83.3	85.6	84.4	84.4	2.3	2.7
104					89.3	80.9	85.4	85.2	8.4	9.8
105	81.3	79.7	80.3	80.4	81.3	82.3	82.3	82.0	1.0	1.2
106	83.3	86.3	86.9	85.5						
107	80.3	80.9	80.3	80.5	83.8	78.8	84.1	82.2	0.3	0.4
108					78.8	80.3	79.3	79.5	1.5	1.8
109	82.3	82.2	80.9	81.8	76.8	69.9	72.1	72.9	6.9	9.5
110	75.3	76.1	75.6	75.7						
111	72.3	75.3	71.6	73.1	75.8	70.2	73.5	73.2	5.6	7.6
112					76.8	77.7	78.1	77.5	1.3	1.7
113	76.3	69.3	71.8	72.5	79.8	70.2	76.3	75.4	9.6	12.7
114	70.3	69.1	68.5	69.3	75.8	77.8	74.5	76.0	3.3	4.3
115	68.3	63.3	65.8	65.8						
116					78.3	72.3	74.8	75.1	6.0	8.0
117	66.3	68.3	67.7	67.4	76.3	77.1	76.6	76.7	0.8	1.0
118	70.3	64.8	70.8	68.6	76.3	72.9	74.5	74.6	3.4	4.5
119	72.3	70.3	71.1	71.2						
120					77.3	70.8	77.2	75.1	6.5	8.6

SBR Performance Data under the Effect of Zinc (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
121	70.3	63.8	65.8	66.6	75.3	70.9	73.2	73.1	4.4	6.1
122	72.3	73.3	72.7	72.8	78.3	79.2	78.8	78.8	0.9	1.1
123	70.3	72.2	71.3	71.3						
124					76.3	70.6	76.3	74.4	5.7	7.7
125	68.3	69.8	68.6	68.9	76.3	69.7	76.3	74.1	6.6	8.9
126	70.3	63.9	65.3	66.5	79.3	79.6	80.9	79.9	1.6	2.0
127	70.3	63.8	70.3	68.1						
128					76.3	77.2	76.6	76.7	0.9	1.2
129	69.5	71.3	70.3	70.4	77.3	70.8	73.8	74.0	6.5	8.8
130	70.5	71.6	70.3	70.8	76.3	71.7	72.3	73.4	4.6	6.3

SBR Performance Data under the Effect of Copper

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
0	52.6	52.8	56.6	54.0	9.2	9.4	8.9	9.2	0.5	5.4
1	80.6	78.8	76.3	78.6	8.4	8.5	7.9	8.3	0.6	7.2
2	78.7	77.9	74.1	76.9	9.4	8.9	8.7	9.0	0.7	7.8
3	65.5	68.4	66.3	66.7	21.8	23.3	23.3	22.8	0.5	2.2
4										
5	76.1	79.6	75.7	77.1	28.6	28.5	31.2	29.4	2.7	9.2
6	99.7	98.6	98.2	98.8	23.2	23.5	23.4	23.4	0.3	1.3
7	78.5	71.6	79.8	76.6	35.1	33.9	35.2	34.7	1.3	3.7
8	89.6	90.6	88.9	89.7	33.1	33.3	34.2	33.5	1.1	3.3
9	80.1	84.1	89.6	84.6	25.5	24.6	26.6	25.6	2.0	7.8
10	69.4	64.6	70.1	68.0	33.2	30.9	32.2	32.1	2.3	7.2
11	70.1	79.9	78.8	76.3	44.8	45.2	45.1	45.0	0.4	0.9
12										
13	91.1	88.6	88.9	89.5	42.5	43.2	44.1	43.3	1.6	3.7
14	91.8	93.6	87.9	91.1	39.4	40.3	40.3	40.0	0.9	2.2
15	93.4	89.8	93.2	92.1	34.3	34.1	35.9	34.8	1.8	5.2
16	86.6	87.4	85.9	86.6						
17	79.2	81.8	82.6	81.2	54.5	55.9	50.8	53.7	5.1	9.5
18	70.4	70.9	71.1	70.8	59.8	60.1	59.9	59.9	0.3	0.5
19	93.2	89.9	90.9	91.3	51.4	51.7	51.5	51.5	0.3	0.6
20	99.6	97.1	98.6	98.4	65.1	64.8	66.1	65.3	1.3	2.0

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
21	94.8	92.8	93.2	93.6	52.5	53.1	53.2	52.9	0.7	1.3
22	90.6	93.1	93.2	92.3	49.6	49.9	50.1	50.0	0.5	1.0
23	95.9	93.1	96.1	95.0	60.2	58.9	54.9	58.0	5.3	9.1
24	94.6	92.2	93.9	93.6	59.1	60.6	61.4	60.4	2.3	3.8
25	93.5	90.7	89.9	91.4	47.9	47.1	51.1	48.7	4.0	8.2
26	84.6	88.6	83.6	85.6	54.7	53.8	52.2	53.6	2.5	4.7
27	92.9	95.6	94.2	84.2	48.1	45.9	45.3	46.4	2.8	6.0
28										
29	91.1	93.1	92.2	92.1	39.3	40.8	40.4	40.2	1.5	3.7
30	94.5	96.8	93.6	95.0	51.4	47.9	46.9	48.7	4.5	9.2
31	91.6	93.6	89.6	91.6	44.8	42.9	40.9	42.9	3.9	9.1
32	95.6	94.1	91.6	93.8	35.4	40.1	40.1	38.5	4.7	12.2
33	91.8	93.8	89.3	91.6						
34	91.1	93.1	92.1	92.1	40.1	38.9	39.9	39.6	1.2	3.0
35	92.5	93.6	93.1	93.1	35.4	41.2	42.1	39.6	6.7	17.0
36	94.6	95.1	93.9	94.5	39.5	38.6	35.9	38.0	3.6	9.5
37	91.8	93.6	87.9	91.1	35.9	36.1	37.2	36.4	1.3	3.6
38	94.8	96.6	89.9	93.8	39.6	38.2	40.1	39.3	1.9	4.8
39	92.6	92.9	92.1	92.5	41.0	41.2	41.9	41.4	0.9	2.2
40	94.6	93.1	93.6	93.8	34.9	36.2	36.8	36.0	1.9	5.3
41	92.6	93.6	92.6	92.9						
42	94.6	93.6	92.6	93.6	44.0	37.9	39.1	40.3	6.1	15.0

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
43	80.8	82.8	82.6	82.1	40.5	39.7	41.2	40.5	1.5	3.7
44					35.4	34.2	34.1	34.6	1.3	3.7
45	85.6	87.3	86.1	86.3						
46	90.3	88.1	86.1	88.2	42.3	39.2	40.1	40.5	3.1	7.6
47	99.6	89.9	98.8	96.1	45.8	49.1	48.2	47.7	3.3	6.9
48					45.0	45.2	45.1	45.1	0.2	0.4
49	85.4	89.6	82.8	85.9						
50	82.2	83.2	85.8	83.7	74.6	76.4	69.1	73.4	7.3	9.9
51	89.7	89.6	88.1	89.1	55.2	55.6	57.2	56.0	2.0	3.6
52					72.1	69.4	69.8	70.4	2.7	3.8
53	99.4	94.6	98.8	97.6	75.1	76.2	74.9	75.4	1.3	1.7
54	91.1	98.2	86.6	92.0	105.1	103.2	110.2	106.2	7.0	6.6
55	93.1	96.9	94.6	94.9	95.3	95.7	94.9	95.3	0.8	0.8
56	91.6	93.6	92.1	92.4	89.1	91.6	91.1	90.6	2.5	2.7
57	93.9	94.4	94.1	94.1	97.1	98.2	96.9	97.4	1.3	1.3
58	83.4	84.8	84.8	84.3	99.2	102	101	100.7	2.8	2.8
59	99.2	99.3	93.9	97.5	92.7	93.1	93.1	93.0	0.4	0.4
60					92.7	91.8	91.2	91.9	1.5	1.6
61	93.9	94.1	92.6	93.5						
62	95.5	95.8	94.6	95.3	91.9	93.2	87.9	91.0	5.3	5.8
63	93.8	93.6	94.1	93.8	92.1	93.6	92.2	92.6	1.5	1.6
64					92.9	94.1	93.3	93.4	1.2	1.3

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
65	98.4	92.1	99.3	96.6	86.2	87.2	86.8	86.7	1.0	1.2
66	93.6	92.2	92.4	92.7	91.8	92.1	91.3	91.7	0.8	0.9
67	92.5	89.9	88.3	90.2	70.8	73.9	72.3	72.3	3.1	4.3
68	92.2	90.7	91.5	91.5	89.1	83.6	86.1	86.3	5.5	6.4
69	92.8	88.2	86.1	89.0	54.9	56.9	58.1	56.6	3.2	5.6
70	98.1	89.9	96.5	94.8	44.8	48.3	48.1	47.1	3.5	7.4
71	95.4	93.2	92.6	93.7	70.9	71.9	70.1	71.0	1.8	2.5
72					64.7	62.9	67.6	65.1	4.7	7.2
73	83.3	86.2	86.1	85.2	58.3	60.1	61.2	60.0	2.9	4.8
74	73.6	73.6	75.5	74.2	72.1	73.6	72.2	72.6	1.5	2.1
75	83.2	78.7	83.2	81.7	65.1	66.1	66.7	66.0	1.6	2.4
76					72.7	76.9	69.8	73.1	7.1	9.7
77	83.6	82.1	87.4	84.4	57.7	56.8	56.6	57.0	1.1	1.9
78	79.3	72.4	79.6	77.1	54.6	57.0	60.8	57.5	6.2	10.8
79	80.7	75.8	81.6	79.3						
80	73.6	78.1	71.9	74.5	62.7	65.1	62.7	63.5	2.4	3.8
81	77.2	69.9	72.1	73.1	86.1	87.8	87.7	87.2	1.7	1.9
82	61.5	59.9	63.1	61.5	60.8	67.9	68.4	65.7	7.6	11.6
83	67.8	76.7	67.7	70.7	94.1	90.1	89.7	91.3	4.4	4.8
84					121.0	117.1	125.2	121.1	8.1	6.7
85	83.2	84.4	86.1	84.6	105.9	109.3	103.2	106.1	6.1	5.7

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
86	93.6	89.3	88.8	90.6	91.6	93.2	90.7	91.8	2.5	2.7
87	91.3	88.9	88.1	89.4						
88					88.2	89.2	84.9	87.4	4.3	4.9
89	92.5	88.6	91.6	90.9	94.5	91.4	93.2	93.0	3.1	3.3
90	89.2	83.8	86.4	86.5						
91	92.8	86.8	92.1	90.6	93.8	89.1	89.9	90.9	4.7	5.2
92	90.3	91.2	94.8	92.1	96.1	98.4	98.2	97.6	2.3	2.3
93	95.2	93.4	87.2	91.9	94.9	90.6	90.2	91.9	4.7	5.1
94	81.3	83.6	81.8	82.2	103	99.9	100.7	101.2	3.1	3.1
95	91.8	80.4	89.1	87.1	97.5	98.2	98.1	97.9	0.7	0.7
96					93.8	96.2	96.1	95.4	2.4	2.5
97	91.7	92.1	91.2	91.7	97.2	87.9	92.2	92.4	9.3	10.1
98	98.3	98.2	93.1	96.5	98.2	96.1	96.2	96.8	2.1	2.2
99	89.8	89.6	89.9	89.8	97.8	96.2	93.9	96.0	3.9	4.1
100					101.0	96.5	98.5	98.7	4.5	4.6
101	84.9	82.9	89.1	85.6	96.7	98.2	98.2	97.7	1.5	1.5
102	80.3	76.9	75.5	77.6						
103	85.6	86.1	86.6	86.1	82.3	80.6	84.1	82.3	1.8	2.2
104	88.6	89.1	86.9	88.2	84.9	85.9	85.1	85.3	1.0	1.2
105	86.1	79.7	80.9	82.2	80.1	81.2	83.2	81.5	3.1	3.8
106	83.5	86.6	86.9	85.7	87	85.1	88.1	86.7	3.0	3.5
107	78.1	80.9	80.5	79.8	81.3	78.8	78.4	79.5	2.9	3.6

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
108					77.8	81.0	78.9	79.2	3.2	4.0
109	82.6	82.2	80.9	81.9	55.7	56.9	67.1	59.9	11.4	19.0
110	70.4	76.7	74.6	93.9						
111	74.7	75.9	71.6	74.1	40.7	47.2	47.3	45.1	6.6	14.6
112					46.2	47.7	48.1	47.3	1.9	4.0
113	81.6	79.7	78.8	80.0	64.7	67.2	67.6	66.5	2.9	4.4
114	70.4	69.1	68.5	69.3	60.7	67.8	67.4	65.3	7.1	10.9
115	68.7	63.9	65.8	66.1						
116	72.3	70.8	69.9	71.0	67.8	67.2	64.8	66.6	3.0	4.5
117	56.9	58.8	57.7	57.8	75.6	71.2	74.6	73.8	4.4	6.0
118	70.2	65.8	70.8	49.3	61.7	62.9	67.4	64.0	5.7	8.9
119	67.9	70.9	71.1	70.0	71.2	70.8	71.1	71.0	0.4	0.6
120					76.7	78.1	76.7	77.2	1.4	1.8
121	70.6	65.8	65.8	67.4	70.5	71.9	70.3	70.9	1.6	2.3
122	74.4	73.2	72.7	73.4	77.8	79.2	78.8	78.6	1.4	1.8
123	65.3	70.2	71.8	69.1	71.8	73.1	69.9	71.6	3.2	4.5
124					75.6	76.2	76.1	76.0	0.6	0.8
125	70.6	69.8	68.6	70.0	74.6	76.9	76.3	75.9	2.3	3.0
126	70.4	65.9	67.3	67.9	54.7	57.9	60.9	57.8	6.2	10.7
127	70.7	63.8	70.9	68.5						
128	73.2	69.9	70.1	71.0	75.6	76.7	75.6	76.0	1.1	1.4

SBR Performance Data under the Effect of Copper (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
129	64.5	71.1	70.3	68.6	62.7	60.8	57.8	60.4	4.9	8.1
130	70.5	71.9	71.3	71.2	70.6	70.1	71.2	70.6	0.6	0.8

SBR Performance Data under the Effect of Chromium

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
0	66.0	65.4	62.9	64.8	18.9	17.1	18.1	18.0	1.8	10.0
1	78.1	78.9	76.3	77.8	29.8	21.9	29.1	26.9	7.9	29.0
2	95.3	96.2	94.1	95.2	14.9	13.7	12.8	13.8	2.1	15.2
3	86.9	85.4	86.3	86.2	28.1	31.1	30.1	29.8	3.0	10.1
4	92.1	90.3	90.9	91.1	19.2	20.1	21.1	20.1	1.9	9.4
5	95.6	95.6	95.7	95.6	26.6	26.3	26.5	26.5	0.3	1.1
6	86.4	84.0	82.9	84.4	30.2	31.1	30.4	30.6	0.9	2.9
7	74.8	78.1	79.9	77.6	39.9	38.1	37.2	38.4	2.7	7.0
8	70.2	71.1	70.1	70.5	40.2	41.1	41.2	40.8	1.0	2.4
9	96.5	95.4	96.9	96.3	48.5	46.9	46.6	47.3	1.9	4.0
10	68.9	66.4	70.9	68.7	38.3	36.6	39.2	38.0	2.6	6.8
11	94.5	89.1	88.9	90.8	49.9	48.5	51.1	49.8	2.6	5.2
12	92.9	93.3	92.9	93.0	55.1	56.1	56.7	56.0	1.6	2.8
13	90.1	88.6	88.9	89.2	52.5	53.9	54.1	53.5	1.6	3.0
14	90.1	89.3	87.7	89.0	49.4	46.3	47.1	47.6	3.1	6.5
15	86.6	87.9	83.2	85.9	49.3	51.1	49.1	49.8	2.0	4.0
16	80.9	89.1	85.1	85.0	49.1	50.1	51.0	50.1	1.9	3.8
17	89.4	88.3	84.9	87.5	51.0	49.1	50.1	50.1	1.9	3.8
18	71.4	71.0	71.9	71.4	56.9	56.0	58.1	57.0	2.1	3.7
19	92.2	89.9	90.3	90.8	53.1	57.1	51.9	54.0	5.2	9.6
20	92.1	93.2	90.1	91.8	53.1	55.1	56.6	54.9	3.5	6.4

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
21	93.4	91.9	93.9	93.1	55.1	53.1	53.9	54.0	2.0	3.7
22	97.4	96.3	93.9	95.9	56.1	54.9	53.1	54.7	3.0	5.5
23	92.5	93.2	96.1	93.9	61.0	64.1	65.4	63.5	4.4	6.9
24	91.1	92.1	89.5	90.9	59.8	60.1	61.2	60.4	1.4	2.3
25	91.5	90.1	88.9	90.2	59.1	57.1	53.1	56.4	6.0	10.6
26	82.4	79.6	80.6	80.9	57.1	59.8	52.6	56.5	7.2	12.7
27	91.3	88.5	94.2	91.3	51.8	49.9	50.3	50.7	1.9	3.7
28	90.3	89.9	90.2	90.1	49.1	50.1	51.1	50.1	2.0	4.0
29	92.2	90.3	92.6	91.7	43.5	41.1	44.1	42.9	3.0	7.0
30	82.3	79.8	83.9	82.0	51.6	49.7	48.6	50.0	3.0	6.0
31	93.1	88.3	87.6	89.7	49.8	48.9	49.0	49.2	0.9	1.8
32	93.9	89.2	90.8	91.3	38.4	41.1	40.1	39.9	2.7	6.8
33	93.1	88.1	87.3	89.5	39.1	42.1	41.1	40.8	3.0	7.3
34	91.1	90.3	92.9	91.4	40.1	38.8	39.9	39.6	1.3	3.3
35	91.5	90.3	93.1	91.6	42.0	47.1	45.2	44.8	5.1	11.4
36	88.1	90.1	90.3	89.5	35.6	36.1	39.0	36.9	3.4	9.2
37	90.8	86.9	87.9	88.5	33.3	34.4	34.9	34.2	1.6	4.7
38	80.1	79.6	79.9	79.9	36.9	39.1	40.1	38.7	3.2	8.3
39	92.2	92.9	92.1	92.4	44.0	42.1	43.1	43.1	1.9	4.4
40	90.2	89.1	90.2	89.8	38.9	38.2	38.0	38.4	0.9	2.3
41	91.2	90.3	92.6	91.4	39.1	40.1	40.5	39.9	1.4	3.5
42	90.6	96.1	92.6	93.1	41.0	39.7	38.9	39.9	2.1	5.3

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
43	90.8	92.8	92.9	92.2	42.0	38.1	42.1	40.7	4.0	9.8
44	77.1	79.1	80.1	78.8	41.0	40.1	41.1	40.7	1.0	2.4
45	81.5	79.7	86.1	82.4	41.9	42.2	42.6	42.2	0.7	1.6
46	92.0	88.1	86.1	88.7	45.3	39.9	42.1	42.4	5.4	12.7
47	85.7	86.9	88.9	87.2	46.8	48.9	48.8	48.2	2.1	4.3
48	79.9	81.2	80.9	80.7	51.0	54.7	50.1	51.9	3.7	7.1
49	82.5	79.6	82.8	81.6	57.7	60.0	60.1	59.3	2.4	4.0
50	93.7	94.2	95.8	94.6	68.9	65.1	71.1	68.4	6.0	8.8
51	91.0	89.1	88.1	89.4	53.5	56.1	56.2	55.3	2.7	4.9
52	90.0	89.4	90.1	89.8	79.6	79.4	76.9	78.6	2.7	3.4
53	92.4	88.4	88.8	90.0	90.0	88.8	88.9	89.2	1.2	1.3
54	90.1	98.2	86.6	91.6	106.1	103.1	102.1	103.8	4.0	3.8
55	92.3	89.9	94.9	92.4	93.1	95.1	93.4	93.9	2.0	2.1
56	90.9	88.6	89.9	89.8	103.1	106.1	101	103.4	5.1	4.9
57	91.3	90.4	94.6	92.1	100.3	101.1	99.8	100.4	1.3	1.3
58	81.3	86.9	84.8	84.3	99.1	102.1	101.1	100.8	3.0	3.0
59	90.2	91.3	93.1	91.5	97.1	96.3	97.3	96.9	1.0	1.0
60	89	90.1	89.5	89.5	91.2	92.8	91.1	91.7	1.6	1.7
61	91.3	91.4	92.9	91.9	91.1	89.1	89.7	90.0	2.0	2.2
62	90.5	91.3	94.9	92.2	88.9	83.2	86.7	86.3	5.7	6.6
63	95.3	96.9	94.1	95.4	88.6	83.6	86.9	86.4	5.0	5.8
64	96.1	96.0	97.1	96.4	89.9	90.4	90.3	90.2	0.5	0.5

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
65	91.2	91.0	93.9	92.0	89.4	90.1	90.2	89.9	0.8	0.9
66	92.3	91.2	92.4	92.0	90.3	90.2	92.3	90.9	2.1	2.3
67	90.5	89.9	88.3	89.6	77.7	79.1	73.9	76.9	5.2	6.8
68	89.9	90.1	90.2	90.1	86.9	81.6	86.1	84.9	5.3	6.2
69	91.0	89.2	86.1	88.8	79.9	81.1	81.0	80.7	1.2	1.5
70	96.8	89.9	96.5	94.4	83.5	83.1	81.1	82.6	2.4	2.9
71	92.0	91.3	92.9	92.1	68.7	69.1	70.1	69.3	1.4	2.0
72	80.9	76.1	79.9	79.0	74.9	74.2	76.1	75.1	1.9	2.5
73	83.3	82.6	86.1	84.0	70.9	72.1	72.3	71.8	1.4	1.9
74	76.8	75.3	75.5	75.9	73.2	69.6	72.1	71.6	3.6	5.0
75	81.2	79.7	83.2	81.4	77.7	80.0	80.1	79.3	2.4	3.0
76	72.9	69.9	70.0	70.9	74.2	69.9	68.8	71.0	5.4	7.6
77	77.8	75.2	74.9	76.0	77.2	76.8	76.6	76.9	0.6	0.8
78	74.9	74.1	79.9	76.3	62.8	67.0	68.2	66.0	5.4	8.2
79	81.0	79.8	81.9	80.9	77.4	80.0	80.0	79.1	2.6	3.3
80	69.9	70.9	70.0	70.3	75.2	69.9	67.7	70.9	7.5	10.6
81	74.5	76.9	72.1	74.5	83.6	78.8	79.7	80.7	4.8	5.9
82	66.3	58.9	63.1	62.8	86.9	79.9	80.4	82.4	7.0	8.5
83	73.9	76.1	77.7	75.9	88.8	90.0	91.2	90.0	2.4	2.7
84	69.8	70.2	70.0	70.0	109.9	107.1	115.1	110.7	8.0	7.2
85	84.3	85.6	86.1	85.3	93.5	93.1	93.6	93.4	0.5	0.5

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
86	82.8	82.9	78.8	81.5	97.6	97.3	97.9	97.6	0.6	0.6
87	85.9	86.1	81.9	84.6	88.4	90.1	90.3	89.6	1.9	2.1
88	81.9	79.1	81.1	80.7	85.8	82.9	84.1	84.3	2.9	3.4
89	91.0	89.8	90.1	90.3	90.5	90.1	91.2	90.6	1.1	1.2
90	86.9	85.3	84.1	85.4	90.1	89.1	88.9	89.4	1.0	1.1
91	92.2	96.8	91.9	93.6	95.8	99.1	91.9	95.6	7.2	7.5
92	90.9	87.9	88.8	89.2	93.6	94.1	93.1	93.6	1.0	1.1
93	91.2	89.3	92.3	90.9	96.4	90.6	93.9	93.6	5.8	6.2
94	83.1	79.6	82.1	81.6	89.9	91.1	90.4	90.5	1.2	1.3
95	86.9	80.9	79.1	82.3	95.3	94.9	93.8	94.7	1.5	1.6
96	90.3	89.4	90.9	90.2	96.8	106	99.1	100.6	9.2	9.1
97	94.1	92.9	92.7	93.2	97.4	97.1	97.2	97.2	0.3	0.3
98	83.3	84.2	81.3	82.9	91.1	89.1	89.2	89.8	2.0	2.2
99	90.4	89.3	89.9	90.0	96.8	90.6	91.3	92.9	6.2	6.7
100	86.9	87.4	85.9	86.7	103.1	99.1	99.5	100.6	4.0	4.0
101	85.9	85.2	81.7	84.3	94.7	93.8	93.8	94.1	0.9	1.0
102	80.6	77.9	78.5	79.0	78.9	80.8	81.5	80.4	2.6	3.2
103	83.5	83.6	84.6	83.9	84.3	86.1	81.4	83.9	4.7	5.6
104	79.9	81.4	80.1	80.5	83.9	89.1	86.4	86.5	5.3	6.0
105	81.7	79.9	81.0	80.9	82.1	82.1	82.3	82.2	0.2	0.2
106	88.3	88.6	89.1	88.7	89.1	90.1	92.0	90.4	2.9	3.2
107	82.0	80.1	80.4	80.8	83.3	79.8	80.1	81.1	3.5	4.3

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
108	80.9	79.1	80.3	80.1	76.8	81.0	78.9	78.9	4.2	5.3
109	84.2	78.2	78.9	80.4	76.7	69.1	70.1	72.0	7.6	10.5
110	75.5	71.9	78.3	75.2	77.2	75.5	76.9	76.5	1.7	2.2
111	72.6	72.5	76.9	74.0	74.5	72.1	75.1	73.9	3.0	4.1
112	70.2	70.1	68.2	69.5	76.6	77.7	71.9	75.4	5.8	7.7
113	76.2	68.9	68.9	71.3	74.9	72.5	73.1	73.5	2.4	3.3
114	72.0	69.9	75.1	72.3	75.7	78.1	75.2	76.3	2.9	3.8
115	66.8	65.3	68.9	67.0	70.0	66.9	68.9	68.6	13.1	19.1
116	65.9	66.7	68.7	67.1	71.8	72.2	78.1	74.0	6.3	8.5
117	66.7	65.8	67.1	66.5	77.6	71.1	70.6	73.1	7.0	9.6
118	72.0	69.8	68.9	70.2	72.6	79.1	78.5	76.7	6.5	8.5
119	74.2	73.0	73.1	73.4	75.5	79.9	79.1	78.2	4.4	5.6
120	78.9	79.9	80.1	79.6	73.7	78.1	72.9	74.9	5.2	6.9
121	70.4	69.3	68.9	69.5	74.5	70.1	73.1	72.6	4.4	6.1
122	76.2	75.3	75.2	75.6	75.8	72.8	73.8	74.1	3.0	4.0
123	70.8	71.2	71.3	71.1	76.7	80.1	80.0	78.9	3.4	4.3
124	66.9	65.9	66.7	66.5	74.6	76.1	73.9	74.9	2.2	2.9
125	69.8	69.8	66.2	68.6	76.6	77.1	75.6	76.4	1.5	2.0
126	70.5	69.3	73.1	71.0	79.7	76.0	79.1	78.3	3.7	4.7
127	70.6	69.8	73.2	71.2	71.1	68.9	69.8	69.9	2.2	3.1
128	70.9	69.2	71.1	70.4	73.6	72.9	76.6	74.4	3.7	5.0

SBR Performance Data under the Effect of Chromium (Continues)

Day	SBR COD Removal Efficiency				SBR COD Adsorption Capacity					
	EFF1	EFF2	EFF3	EFFavg	CAC1	CAC2	CAC3	CACavg	Range	%Dev
129	65.9	67.1	67.0	66.7	74.7	78.1	78.3	77.0	3.6	4.7
130	74.5	76.0	77.3	75.9	76.8	77.1	76.3	76.7	0.8	1.0