

**TOXICITY AND INHIBITION EVALUATION OF
HAZARDOUS WASTES FROM METAL FINISHING
PROCESSES TO *VIBRIO FISCHERI*
İDRARIN GÜBRE OLARAK DEĞERLENDİRİMESİNDE
KLİNOPTİLOLİT ile İYON DEĞİŞİMİNİN YERİ
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TOXICITY AND INHIBITION EVALUATION OF HAZARDOUS WASTES FROM METAL FINISHING PROCESSES TO *VIBRIO FISCHERI*

SUMMARY

Industries generating hazardous wastes deal with very tough issues according to the properties of the wastes. One of them is toxicity which is the most important criterion of the hazardous waste. The assessment of toxicity of industrial chemicals, environmental pollutants, and other substances represents an important element in the protection of the health of the worker and member of communities because toxic pollutants can disturb the sustainability of natural ecosystem by variety of effects on species, populations, communities, and ecosystem processes, although such systems characterized by dynamic stability having some capacity to absorb pollutants. Metal finishing industry is the one of the most important industries dealing with the toxicity of the hazardous wastes. One of the most important environmental impacts of metal finishing industry's wastes is the leaching of pollutants to the surface and groundwater. In this research, process of a metal finishing industry was investigated and hazardous wastes of this industry were determined according to the US EPA's hazardous waste lists. Characterization of the wastewaters was done. Toxicological interaction between heavy metals and cyanide was investigated in concentrations determined in the characterization of electroplating bath sludge and its supernatant. Synergistic and antagonistic effects between the heavy metals and cyanide were quantified according to EC₅₀ values from BioToxTM test using *Vibrio Fischeri*. These synergism/antagonism and toxicological interaction were compared with the toxicity of sludge, the leachates of sludge extracted in respect of Toxicity Characteristic Leaching Procedure.

METAL SON İŞLEMLERİ ENDÜSTRİSİ PROSESLERİNDEN KAYNAKLANAN ZARARLI ATIKLARIN *VIBRIO FISCHERI*'YE OLAN TOKSİSİTESİNİN VE İNHİBİSYONUNUN DEĞERLENDİRİLMESİ

ÖZET

Zararlı atık üreticisi endüstriler için atığın özelliklerinden dolayı tanım, tespit, arıtma/depolama/uzaklaştırma prosedürleri ve yönetmeliklerdeki açıklar nedeniyle sorunlar vardır. Bu sorunlardan en önemlisi tehlike kriterlerinden birisi olan toksisitedir. Endüstriyel kimyasalların, çevre kirleticilerinin ve diğer maddelerin toksisitelerinin belirlenmesi işçi sağlığının ve topluluğun diğer elemanlarının korunmasında önemli bir adımı teşkil eder. Çünkü toksik kirleticiler türlerin, popülasyonların, toplulukların ve ekosistem proseslerinin üzerindeki çeşitli etkileri ile ekosistemin sürdürülebilirliğini tehdit ederler. Metal son işlemleri endüstrisi toksik özellikli tehlikeli atık üreten en önemli birincil üretim endüstrilerinden biridir. Metal son işlemleri endüstrisinin çevreye olan en önemli etkisi, kirleticilerin yüzeysel ve yeraltı sularına sızmasıdır. Bu proje kapsamında, bir metal son işlemleri endüstrisinin prosesleri incelendi ve bu endüstrinin zararlı atıkları ABD Çevre Koruma Örgütünün(EPA) zararlı atık listelerine göre tespit edildi. Bu endüstrinin proseslerinden kaynaklanan atıksuların karakterizasyonu yapıldı. Bu karakterizasyon sonucu ortaya çıkacak metallerin birbirleriyle ve siyanürle olan sinerjistik ve antogonistik etkileşimleri *Vibrio Fisheri* bakterilerini kullanan BioToxTM testi ile elde edilen EC₅₀ değerleri ile incelenmiştir. Bu etkileşimlerin arıtma tesisi oksidasyon ve kimyasal arıtım üniteleri sonrasında nasıl değiştiği arıtım kademelerinin çamuru ve üst suyunda yürütülen toksisite deneyleri ile ortaya koyulmuştur. Sinerjizm/antogonizm ve toksikolojik etkileşimler atıksuların, çamurdan Toxicity Characterization of Leaching Procedure (TCLP) metoduna bağlı kalınarak elde edilmiş ekstraktın toksisitesi ile karşılaştırılmış ve arıtım performansı değerlendirilmiştir.

1. INTRODUCTION

There is a growing concern about toxic wastewater discharges to aquatic environment in recent years in public especially in developed countries. These wastewaters should be considered as hazardous wastes due to the toxic characteristics. Although toxic wastewaters are treated according to the discharge standards of the water authority their toxic characteristics cannot be removed. Efficient tools in toxicity reduction programs include ecotoxicity tests, which are used to evaluate the level of effluent toxicity before and after treatment, and therefore the efficiency of operation. These tests are also helpful in determining the final effluent toxicity and propose improvement techniques enabling the establishment of goals in relation to treatment and thus reducing potential hazard of contaminants to aquatic ecosystems (Araujo et al. 2005).

There are lots of studies in literature containing individual toxic effects of toxicants, but receiving waters are generally exposed to toxicant mixtures, rather than single elements or compounds. This situation may substantially modify the effects of individual constituents. For example, the toxicity of heavy metal mixtures may differ from an additive (individual) response since synergistic or antagonistic interactions between two or more constituents in the mixture may make the overall toxicity more (or less) than that expected by simply summing the effects of each individual toxicant. Thus, even if the characteristics of individual chemicals are known, their behaviour in a mixture cannot necessarily be straightforwardly inferred (Mowat and Bundy 2002).

In this study toxic characteristics of wastewaters coming from metal finishing industry is investigated. The effect of change of the metal and the cyanide concentrations on toxicity of wastewater is also studied.

1.1. Aim and Scope

The aim and scope of the thesis are given in this section.

Detection of ecotoxicity of wastewaters from metal finishing process.

Monitoring ecotoxicity changes after some treatment steps.

Determination of the interaction of the metals and other compounds on ecotoxicity basis in a complex wastewater.

2. HAZARDOUS WASTE

2.1. Definition

The term hazardous waste gained acceptance starting about in 1970 and it became popular in mid-70s. Long before than the wastes that we know as hazardous were referred to by such terms as special industrial waste or chemical waste. The term hazardous waste by itself is ambiguous. A feature of any regulatory program is to provide a legal definition to determine what is and is not a hazardous waste. Developing a legal definition can take considerable effort with much disagreement (LaGrega et al. 2001). However, there is not any unique and universally accepted scientific definition of hazardous waste and it varies from one country to another (Blackman 1996; Chaaban 2001). One of the first established definitions which is still widely used is contained in US Resource Conservation and Recovery Act (RCRA) of 1976. RCRA considers wastes toxic and/or hazardous if they cause or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible illness; or pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed (Blackman 1996). United Nations Environment Program (UNEP) approved following definition for hazardous waste in December 1985 “Hazardous wastes mean wastes (solids, sludges, liquids and containerized gases) other than radioactive (and infectious) wastes which, by reason of their chemical activity or toxic, explosive, or other characteristics, cause danger or likely will cause danger to health or the environment, whether alone or when coming into contact with other waste (LaGrega et al. 2001). Hazardous wastes are defined by Talinli, 2000 as “Hazardous waste is a/any specialized and listed waste;

which has acute or chronic hazard potential described as “Flammable” ,”Toxic”, “Corrosive” and/or “Reactive” criteria,

which should be managed with all together with the social, political and economical aspects of the eco-system instead of conventional treatment and disposal techniques

because of its composition, constituents, physical form, fate and transport in the environment

which may be in forms of solid, liquid, slurry, sludge and pressurized gas

which may be a/hazardous substance that has been discarded or otherwise designated as a waste material, or one that may become hazardous by interaction with other substances”

2.2. Determination

Hazardous waste definition begins with an obvious point: in order for any material to be a hazardous waste, it must first be a waste. When the discarded material is defined as a waste, it should be classified if the waste is conventional waste such as wastewater, municipal solid waste, air emission, or not. The term “non-regular waste” or “solid waste” has been considered as intermediate waste which is obviously not conventional but probably hazardous and means any waste, whether in solid, sludge, slurry, liquid, or containerized gaseous physical form. The waste must be determined as hazardous or non-hazardous if the waste is identified as non-regular waste (Talinli et al. 2005). Hazardous waste determination and classification can be done by two methods: (1) lists and (2) tests.

2.2.1. Listing

The cornerstone of most regulatory programs is to itemize specific hazardous wastes into lists. Inclusion in such a list means that the waste is regulated as a hazardous waste. Some programs have prepared “exclusive” lists describing those wastes that are not hazardous, meaning anything not on the list is hazardous (LaGrega et al. 2001).

Hazardous waste listings describe wastes from very specific processes, wastes from very specific sectors of industry, or wastes in the form of very specific chemical formulations. The most convenient listing procedure is executed by USEPA and established lists serve as a base for other countries’ environmental administrations. Listing procedure and criteria of USEPA is given here.

Before developing a hazardous waste listing, EPA thoroughly studies a particular waste stream and the threat it can pose to human health and the environment. If the waste poses enough of a threat, EPA includes a precise description of that waste on one of the hazardous waste lists in the regulations. Thereafter, any waste fitting that narrative listing description is considered hazardous, regardless of its chemical composition or any other potential variable. For example, one of the current hazardous waste listings reads as: "API separator sludge from the petroleum refining industry." An API separator is a device commonly used by the petroleum refining industry to separate contaminants from refinery wastewater. After studying the petroleum refining industry and typical sludges from API separators, EPA determined these sludges were dangerous enough to warrant regulation as hazardous waste under all circumstances. The listing therefore designates all petroleum refinery API separator sludges as hazardous. Chemical composition or other factors about a specific sample of API separator sludge are not relevant to its status as a listed hazardous waste under the RCRA program.

Using listings to define hazardous wastes presents certain advantages and disadvantages. One advantage is that listings make the hazardous waste identification process easier for industrial waste handlers. Only knowledge of a waste's origin is needed to determine if it is listed; laboratory analysis is unnecessary to determine if it is RCRA-regulated. Analysis may be needed for other purposes, however.

The use of listings also presents certain disadvantages. For example, listing a waste as hazardous demands extensive study of that particular waste by EPA. EPA lacks the resources to investigate the countless types of chemical wastes produced in the United States, so the hazardous waste listings simply cannot address all dangerous wastes. The hazardous waste characteristics provide an important complement to listings by addressing most of the shortcomings of the listing methodology of hazardous waste identification.

Another disadvantage of the hazardous waste listings is their lack of flexibility. Listings designate a waste as hazardous if it falls within a particular category or class. The actual composition of the waste is not a consideration as long as the waste matches the appropriate listing description. For instance, some API separator sludges from petroleum refining might contain relatively few hazardous constituents and

pose a negligible risk to human health and the environment. Such sludges are still regulated as hazardous, however, because the listing for this waste stream does not consider variations in waste composition. Thus, the hazardous waste listings can unnecessarily regulate some wastes that do not pose a significant health threat. It is also possible for industries to substantially change their processes so that wastes would no longer meet a listing description in spite of the presence of hazardous constituents.

EPA has studied and listed as hazardous hundreds of specific industrial waste streams. These wastes are described or listed on four different lists. These four lists are:

The F list: The F list designates as hazardous particular waste streams from many common processes used in laboratories, automotive repair shops, retail outlets, and government facilities as well as several industrial and manufacturing wastes. F list wastes usually consist of chemicals that have been used for their intended purpose in an industrial-type process. That is why F list wastes are often referred to as "manufacturing process wastes." The F list wastes can be divided into seven groups, depending on the type of process or operation that created them. The seven categories of F-listed wastes are:

- Spent solvent wastes (F001 - F005);
- Wastes from electroplating and other metal finishing operations (F006 - F012, F019);
- Dioxin-bearing wastes (F020 - F023 and F026 - F028);
- Wastes from the production of certain chlorinated aliphatic hydrocarbons (F024, F025);
- Wastes from wood preserving (F032, F034, and F035);
- Petroleum refinery wastewater treatment sludges (F037 and F038);
- Multi-source leachate (F039).

The K list: The K list of hazardous wastes designates particular wastes from specific sectors of industry and manufacturing as hazardous. The K list wastes are therefore known as wastes from specific sources. K list wastes are manufacturing process wastes that contain chemicals that have been used for their intended purpose. To determine whether a waste qualifies as K-listed, two primary questions must be answered. First, is the facility that created the waste within 1 of the 17 different industrial or manufacturing categories on the K list? Second, does the waste match one of the specific K list waste descriptions? The 17 industries that can generate K list wastes are:

- Wood preservation;
- Inorganic pigment manufacturing;
- Organic chemicals manufacturing;
- Inorganic chemicals manufacturing;
- Pesticides manufacturing;
- Explosives manufacturing;
- Petroleum refining;
- Iron and steel production;
- Primary copper production;
- Primary lead production;
- Primary zinc production;
- Primary aluminium production;
- Ferroalloy production;
- Secondary lead processing;
- Veterinary pharmaceuticals manufacturing;

- Ink formulation;
- Coking (processing of coal to produce coke, a material used in iron and steel production).

In general, the K listings target much more specific waste streams than the F listings. For example, EPA recently added a number of listings to the organic chemicals manufacturing category of the K list. These new listings are for wastes from the production of carbamate chemicals. EPA estimates that only two dozen facilities nationwide produce waste streams covered by these new K listings. In contrast, F-listed spent solvent wastes are commonly generated at thousands of different sites and facilities. Industries that generate K-listed wastes, such as the wood preserving and petroleum refining industries, can also generate F-listed wastes. Typically, K listings describe more specific waste streams than F listings applicable to the same industry. For example, K051 and K048 designate as hazardous two very specific types of petroleum refinery wastewater treatment residues: wastewater treatment sludges created in API separators and wastewater treatment float created using dissolved air flotation (DAF) pollution control devices. The F037 and F038 listings complement these two K listings by designating as hazardous all other types of petroleum refinery wastewater treatment sludges and floats. These petroleum refinery listings illustrate that the K listings are typically more specific than the F listings, but they also illustrate that the two lists are in many ways very similar.

The P list and the U list: These two lists are similar in that both list as hazardous pure or commercial grade formulations of specific unused chemicals. The P and U listings are quite different from the F and K listings. For a waste to qualify as P- or U-listed, it must meet the following three criteria:

- the waste must contain one of the chemicals listed on the P or U list;
- the chemical in the waste must be unused;
- the chemical in the waste must be in the form of a "commercial chemical product," as EPA defines that term.

Typically, hazardous waste listings are narrative descriptions of specific waste streams and specific chemical composition are generally irrelevant to whether a listing applies to it. At first glance, the P and U listings seem inconsistent with these principles. Each P and U listing consists only of the chemical name of a compound known to be toxic or otherwise dangerous.

These four lists each designate from 30 to a few hundred waste streams as hazardous. Each waste on the lists is assigned a waste code consisting of the letter associated with the list followed by three numbers. For example, the wastes on the F list are assigned the waste codes F001, F002, and so on. Assigning the correct waste code to a waste has important implications for the management standards that apply to the waste. (Colorado Department of Public Health and Environment 1998).

Before listing any waste as hazardous, EPA developed a set of criteria to use as a guide when determining whether or not a waste should be listed. These listing criteria provide a consistent frame of reference when EPA considers listing a waste stream. There are four different criteria upon which EPA may base its determination to list a waste as hazardous. The four reasons why EPA may list a waste are:

The waste typically contains harmful chemicals, and other factors indicate that it could pose a threat to human health and the environment in the absence of special regulation. Such wastes are known as toxic listed wastes.

The waste contains such dangerous chemicals that it could pose a threat to human health and the environment even when properly managed. Such wastes are known as acutely hazardous wastes.

The waste typically exhibits one of the four characteristics of hazardous waste described in the hazardous waste identification regulations (ignitability, corrosivity, reactivity, toxicity).

EPA has cause to believe that, for some other reason, the waste typically fits within the statutory definition of hazardous waste developed by Congress.

EPA may list a waste as hazardous for any or all of the above reasons. Note that these four criteria do not directly correspond to the four different lists of hazardous waste. The majority of listed wastes fall into the toxic wastes category. To decide if a

waste should be a toxic listed waste, EPA first determines whether it typically contains harmful chemical constituents. If a waste contains chemical constituents which scientific studies show to have toxic, carcinogenic, mutagenic, or teratogenic effects on humans or other life forms, EPA then evaluates 11 other factors to determine if the waste stream is likely to pose a threat in the absence of special restrictions on its handling. These additional considerations include a risk assessment and study of past cases of damage caused by the waste. The results of the studies to determine to list a waste as hazardous are contained in the Background Documents published by EPA. These references may be consulted to help in making waste determinations.

Acutely hazardous wastes are the second most common type of listed waste. EPA designates a waste as acutely hazardous if it contains constituents that scientific studies show to be fatal to humans or animals in low doses. In a few cases, acutely hazardous wastes contain no such constituents, but are extremely dangerous for another reason. The criteria for designating a waste as acutely hazardous require only that EPA consider the typical chemical makeup of the waste stream. EPA is not required to study other factors, such as relative risk and evidence of harm, when listing a waste as acutely hazardous.

To indicate its reason for listing a waste, EPA assigns a hazard code to each waste listed on the F, K, P, and U lists. These hazard codes are listed below. The last four hazard codes apply to wastes that have been listed because they typically exhibit one of the four regulatory characteristics of hazardous waste. The hazard codes indicating the basis for listing a waste are:

- Toxic Waste (T)
- Acute Hazardous Waste (H)
- Ignitable Waste (I)
- Corrosive Waste (C)
- Reactive Waste (R)
- Toxicity Characteristic Waste (E)

The hazard codes assigned to listed wastes affect the regulations that apply to handling the waste. For instance, acute hazardous wastes accompanied by the hazard code (H) are subject to stricter management standards than most other wastes (Colorado Department of Public Health and Environment 1998).

2.2.1.1. Testing

Determination of hazardous waste by testing is conducted with detecting the characteristics of the waste is another method which needs proper analyses to define the waste as a hazardous waste. Although phytotoxicity, teratogenicity, bioaccumulation, mutagenicity are the characteristics of the hazardous waste because of the difficulties in testing protocols of these characteristics mentioned above, EPA decided to use 4 common characteristics to identify the hazardous waste: 1)ignitability 2)corrosivity 3) reactivity 4)toxicity (Talinli et al. 2005).

2.2.1.2. Ignitability

Ignitability is the characteristic used to define those wastes as hazardous that could cause a fire during transport, storage, or disposal. Examples of ignitable wastes include waste oils and used solvents.

A waste exhibits the characteristics of ignitability if a representative sample of the waste has any of the following properties (Liu and Lipták 1997):

It is a liquid, other than an aqueous solution containing less than 24% alcohol by volume, and has flash point less than 60°C (140°F), as determined by a Pensky-Martens Closed Cup Tester (using the test method specified in ASTM Standard D-93-79 or D-93-80) or by a Setaflash Closed Cup Tester (using the test method specified in ASTM Standard D-3278-78).

It is not a liquid and is capable, under standard temperature and pressure, of causing fire through friction, absorption of moisture, or spontaneous chemical changes and, when ignited, burns so vigorously and persistently that it creates a hazard.

2.2.1.3. Corrosivity

Corrosivity was chosen as an identifying characteristic of a hazardous waste because wastes with high or low pH can react dangerously with other wastes or cause toxic contaminants to migrate from certain wastes. Examples of corrosive wastes include acidic wastes and used pickle liquor from steel manufacture. Steel corrosion is a prime indicator of a hazardous waste since wastes capable of corroding steel can escape from drums and liberate other wastes.

A waste exhibits the characteristic of corrosivity if a representative sample of the waste has either of the following properties (Liu and Lipták 1997; Liu and Lipták 1999):

It is aqueous and has a pH less than or equal to 2 or greater than or equal to 11.5, as determined by a pH meter using an EPA test method. The EPA test method for pH is specified as Method 5.2 in “Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods.”

It is a liquid and corrodes steel (SAE 1020) at a rate greater than 6.35 mm (0.250 inch) per year at a test temperature of 55°C (130°F), as determined by the test method specified in NACE (National Association of Corrosion Engineers) Standard TM-01-69 and standardized in “Test Methods for the Evaluation of Solid Waste, Physical/Chemical Methods”.

2.2.1.4. Reactivity

Reactivity was chosen as an identifying characteristic of a hazardous waste because unstable wastes can pose an explosive problem at any stage of the waste management cycle. Examples of reactive wastes include water from TNT operations and used cyanide solvents.

A waste exhibits the characteristic of reactivity if a representative sample of the waste has any of the following properties (Liu and Lipták 1997):

It is normally unstable and readily undergoes violent change without detonating.

It reacts violently with water.

It forms potentially explosive mixtures with water.

When mixed with water, it generates toxic gases, vapours, or fumes in a quantity sufficient to present a danger to human health or the environment.

It is a cyanide- or sulphide-bearing waste which, when exposed to pH conditions between 2 and 11.5, can generate toxic gases, vapours, or fumes in a quantity sufficient to present a danger to human health or the environment.

It is capable of detonation or explosive reaction if subjected to a strong initiating source or if heated under confinement.

It is readily capable of detonation or explosive decomposition or reaction at standard temperature and pressure.

It is a forbidden explosive as defined in the 49 Code of Federal Regulations 173.51, or a Class A explosive as defined in the 49 Code of Federal Regulations 173.53, or a Class B explosive as defined in the 49 Code of Federal Regulations 173.88 DOT regulations.

2.2.1.5. Toxicity

To characterize the toxicity of hazardous wastes, a toxicity test should be applied. The Toxicity Characteristic Leaching Procedure test (TCLP), is designed to identify wastes likely to leach hazardous concentrations of particular toxic constituents into the groundwater as a result of improper management. During the TCLP, constituents are extracted from the waste to stimulate the leaching actions that occur in landfills. If the concentration of the toxic constituent exceeds the regulatory limit, the waste is classified as hazardous.

If the extract from a representative waste sample contains any of the contaminants listed in Table 2.1 (Liu and Lipták 1997) at a concentration equal to or greater than the respective value given, the waste exhibits the toxicity characteristic. A waste that exhibits the toxicity characteristic but is not a listed hazardous waste has the EPA hazardous waste number specified in Table 2.1. The TCLP test replaced the EP toxicity test in September 1990 and added 25 organic compounds to the eight metals and six pesticides that were subject to the EP toxicity test (Liu and Lipták 1997).

There are very significant gaps for TCLP. The main gap is seen in toxicity testing, because only 43 of the toxic chemicals are subject to the TCLP test. Thus, if a waste does not bear any of the 43 chemicals, the waste is not considered as hazardous, although it may be a hazardous waste (USEPA 1996). LC₅₀ or EC₅₀ bioassay analysis should be proceeded after leaching of solid wastes in order to determine the toxic characteristics of waste.

Table 2.1. Minimum Concentrations of Contaminants for RCRA Toxicity Characteristics

<i>EPA Hazardous Waste Number</i>	<i>Contaminant</i>	<i>Maximum Concentration (mg/L)</i>	<i>EPA Hazardous Waste Number</i>	<i>Contaminant</i>	<i>Maximum Concentration (mg/L)</i>
D004	Arsenic ^a	5.0	D036	Hexachloro-1,3-butadiene	0.5
D005	Barium ^a	100.0	D037	Hexachloroethane	3.0
D019	Benzene	0.5	D008	Lead ^a	5.0
D006	Cadmium ^a	1.0	D013	Lidane ^a	0.4
D022	Carbon tetrachloride	0.5	D009	Mercury ^a	0.2
D023	Chlordane	0.03	D014	Methoxychlor ^a	10.0
D024	Chlorobenzene	100.0	D040	Methyl ethyl ketone	200.0
D025	Chloroform	6.0	D041	Nitrobenzene	2.0
D007	Chromium	5.0	D042	Pentachlorophenol	100.0
D026	o-Cresol	200.0	D044	Pyridine	5.0
D027	m-Cresol	200.0	D010	Selenium	1.0
D028	p-Cresol	200.0	D011	Silver ^a	5.0
D016	2,4-D ^a	10.0	D047	Tetrachloroethylene	0.7
D030	1,4-Dichlorobenzene	7.5	D015	Toxaphene ^a	0.5
D031	1,2-Dichloroethane	0.5	D052	Trichloroethylene	0.5
D032	1,1-Dichloroethylene	0.7	D053	2,4,5-Trichlorophenol	400.0
D033	2,4-Dinitrotoluene	0.13	D054	2,4,6-Trichlorophenol	2.0
D012	Endrin ^a	0.02	D017	2,4,5-TP (Silvex) ^a	1.0
D034	Heptachlor (and its hydroxide)	0.008	D055	Vinyl chloride	0.2
D035	Hexachlorobenzene	0.13			

^aFormerly EP Toxicity Contaminants.

2.2.2. A rating system for determination of hazardous wastes

Talinli et al. (2005) proposed a new quantitative determination system in order to eliminate the subjectiveness of lists and characteristics tests. This system's conceptual framework is given in Figure 2.1. Mainly, two components exist in this approach: (1) hazard criteria of the hazardous waste in terms of ecological effects (Ee), (2) their combined potential risk (CPR).

To formulate the rating system, following assumptions are postulated;

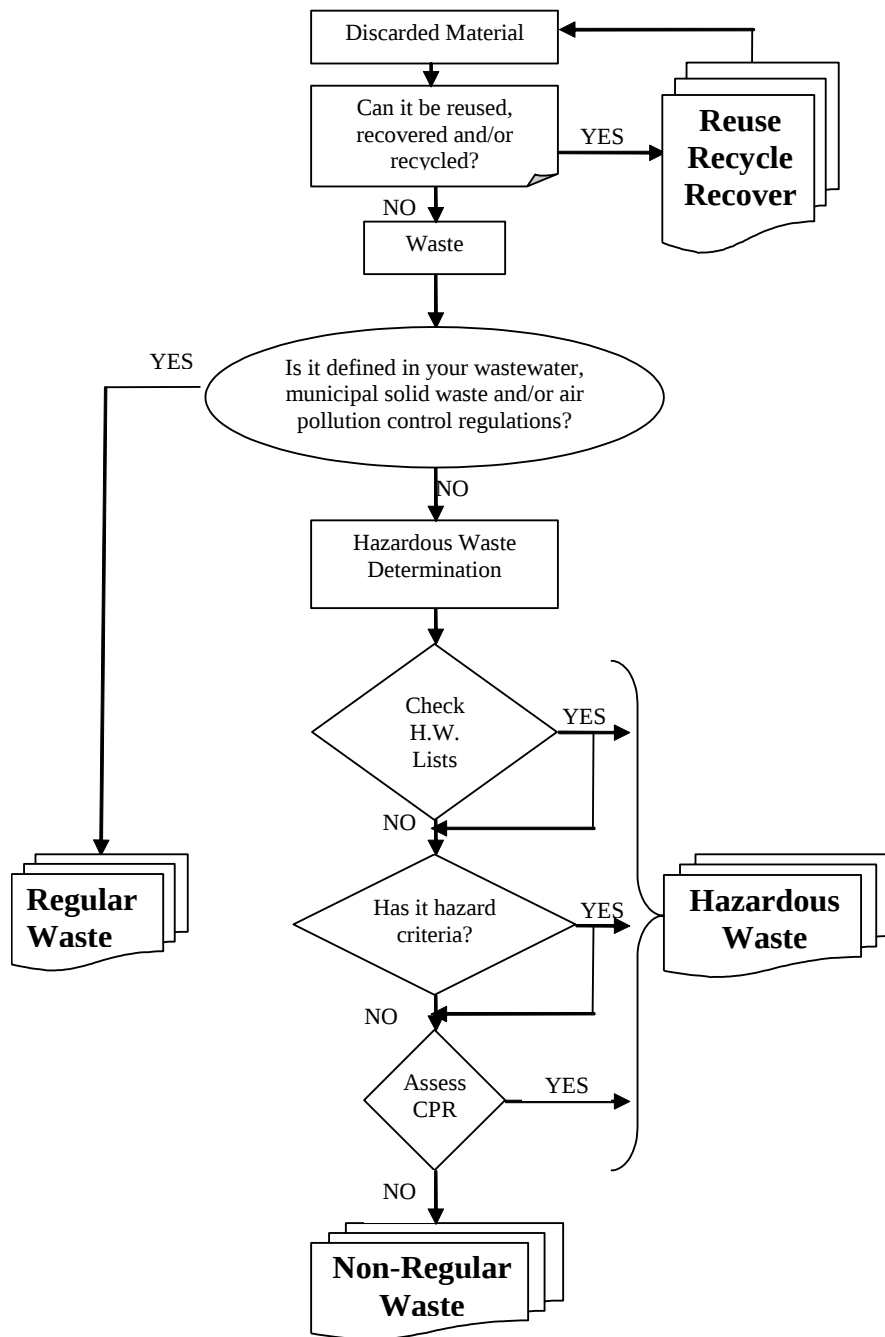


Figure 2.1. Conceptual Framework of Rating System

When the discarded material is defined as a waste, it should be classified whether the waste is conventional waste such as wastewater, municipal solid waste, air emission, or not. The term “non-regular waste” has been considered as intermediate waste which is obviously not conventional but probably hazardous. The waste must be determined as hazardous or non-hazardous if the waste is identified as non-regular waste.

Hospital and radioactive wastes are neglected in this inquiry, because they have their own control regulations and these wastes have already been identified as non-regular wastes.

Listing methodology of the hazardous waste and their lists published in different countries cannot be neglected. Thus a component consisting of established lists (L) also is used in rating system.

Ecological effects (Ee) includes primarily impacts of waste regarding its hazard characteristics such as toxicity, ignitability, corrosivity and reactivity. Physical form of the waste is another factor that affects the hazard characteristics.

Accumulative and synergistic effects and uncertain potential risks are included in combined potential risk (CPR) parameter. Components of this parameter are human health toxicity, carcinogenetic effects, infectious risks, and persistency associated with biodegradability, solubility, and bioaccumulation. Physical forms of the waste and exposure mode are also taken into account during the evaluation of these risks.

The critical components of this rating system are considered as cumulative functions of “Overall Rating Value” (ORV), because higher values of the specific components such as L, Ee, and CPR will lead to a higher value of ORV. In addition, the amount of the waste (Q) is a basic component in this rating system, so it should be a multiplier of the other components (Talinli et al. 2005).

3. TOXICOLOGY

3.1. General Information

Toxicology is fundamental science of poisons. A poison is generally considered to be any substance that can cause severe injury or death as a result of a physicochemical interaction with living tissue. As Paracelsus introduced in 14th century “All substances are poisons; there is none that is not a poison. The right dose differentiates a poison and a remedy”. However, all chemicals can be used safely if exposure of people or susceptible organisms is kept below defined limits; for instance if the chemicals are handled with appropriate precautions. If no tolerable limit can be defined, zero exposure methods must be used (Duffus and Worth 1996; Hodgson 2004).

Toxicity is a relative property reflecting a chemical’s potential to have a harmful effect on a living organism. It is a function of the concentration and composition/properties of the chemical to which the organism is exposed and the duration of exposure. Traditionally, toxicity data have been used in comparing chemical substances or the sensitivities of different species to the same substance. Information about the biological mechanism affected and the conditions under which the toxicant is harmful are also important for this comparison. Toxicity tests are therefore used to evaluate the adverse effects of a chemical on living organisms under standardized, reproducible conditions that permit comparison with other chemicals or species tested and comparison of similar data from different laboratories (Rand 1995).

Toxicity can be divided into the broad categories; direct and indirect. Direct toxicity results from the toxic agent acting more or less directly at sites of action and/or on organisms; indirect toxicity occurs as a result of the influence of changes in the chemical, physical and/or biological environment (e.g. changes in the quality and/or biological environment organisms or habitat changes and/or losses). Although most indirect toxicity on a population or community may be tracked back to direct toxicity

in a particular group and species, this is not always the case. Most experimental toxicology studies have been concerned with direct toxicity to individual species. The direct toxicity information gained is then used to estimate indirect effects or interpret site-specific situations (Rand 1995).

Some toxic effects are reversible and the others are irreversible. Effects may be reversible by normal repair mechanisms, such as by regeneration of damaged or lost tissue and recovery from narcosis. In many cases effects are reversible only if the organism can escape the toxic medium and find a toxicant-free environment. Serious damage or injury to an organism may be irreversible and may eventually result in death (Rand 1995).

Although basic toxicological principles are often summarized in the simple expressions “dose-response” or “concentration-response”, there are a number of vital assumptions (Rand 1995):

A cause-effect relationship exists. The effect or response in question is clearly direct or indirect results of the exposure of the organism(s) to the toxic agent(s) being examined.

A dose-response or concentration-response relationship exists.

The effect or response in question is in a result of the toxic agent(s) reaching and interacting with the site(s) of toxic action in or on the organism.

The amount of toxic agent reaching the site(s) of toxic action is some function of the exposure of the organism to the toxic agent or, in the case which a metabolite is the agent, its parent compound.

Above a real or statistically based effect threshold, the magnitude of the effect or response is proportional to the amount of toxicant reaching the site(s) of toxic action.

Effects can be quantified. Observed effects or responses of toxic action can be measured and quantified in a reproducible way that is relevant to the toxic processes under examination.

3.2. Causality

Causality is a critical toxicological as, for quantitative purposes, it must be reasonably certain that there is a causal relationship between the observed effect or response and the presence of the toxic agent. However, there may be some doubt about the identity of the chemical, which may have changed during the exposure, the actual exposure concentration in water, and the specificity of the response, because aquatic organisms may respond similarly to a variety of stresses. Thus, until proved, it is only a reasonable working presumption that the effect or response being observed is a result of exposure to the known concentration to the chemical (Rand 1995).

3.3. Dose-Response, Concentration-Response Relationships

Toxicity is a relative event that depends not only on the toxic properties of the chemical and the dose administered but also on individual and interspecific variation in the metabolic processing of the chemical. The first recognition of the relationship between the dose of a compound and the response elicited has been attributed to Paracelsus. It is noteworthy that his statement includes not only that all substances can be toxic at some dose but that “the right dose differentiates a poison from a remedy,” a concept that is the basis for pharmaceutical therapy (Hodgson 2004).

A typical dose-response curve is shown in Figure 3.1 (Hodgson 2004), in which the percentage of organisms or systems responding to a chemical is plotted against the dose. For many chemicals there will be a dose below which no effect or response is observed. This is known as the threshold dose. This concept is of significance because it implies that a no observed effect level (NOEL) can be determined (if lowest observed effect level is known) and that this value can be used to determine the safe intake for food additives and contaminants such as pesticides. Moreover, this curve is used for the determination of EC_{50} (the statistically derived exposure concentration of a chemical that can be expected to cause adverse response other than death in 50% of a given population of organisms under a defined set of experimental conditions), LC_{50} (the statistically derived exposure concentration of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions), and LD_{50} (the statistically

derived dose of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions) (Rand 1995; Duffus and Worth 1996; Walker 1996; Hodgson 2004).

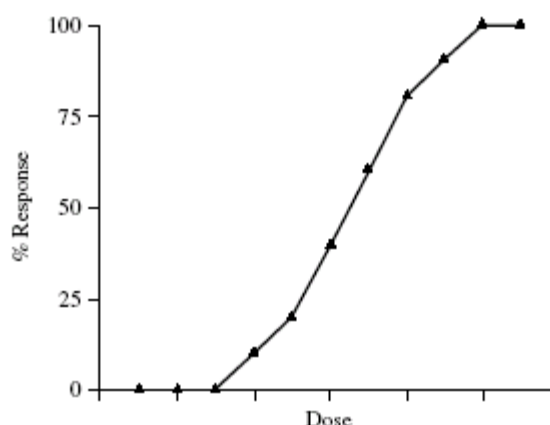


Figure 3.1. A Typical Dose Response Curve

3.4. Factors That Influence Toxicity

Any characteristic of the organism or the surrounding water that affects toxicity of a pollutant is considered to act as a modifying factor. Thus, species or the size of the organism reacting to the toxicant could be a biotic modifying factor. Physicochemical entities such as the pH or the temperature of the water could be abiotic modifying factors. The environmental or abiotic entities should properly be called masking factors defined by Fry as an identity which modifies the operation of a second identity on organism (Rand 1995).

3.4.1. Factors related to exposure

The concentration and time required to produce an adverse effect vary with the chemical, the species of organism, and the severity of effects. This contact between the organism and the chemical is called exposure. In the assessment of toxicity the most significant factors related to exposure are the type, duration, and frequency of exposure and the concentration of the chemical (Rand 1995).

Routes of exposure may increase or decrease the effect of the toxicant to organisms. Ingestion, inhalation and skin contact are the major exposure routes of the toxicants. Many substances are much more toxic by the inhalation route than oral intake or skin contact. This is due in part to the rapid uptake in lung that is directly transferred into blood stream. On the other hand, toxins may be detoxified by metabolic enzymes of the liver prior to reaching target organs. For instance, silica can cause a pulmonary disease known as “silicosis” after lung deposition of dust particles over an extended period of time, but is virtually harmless on ingestion (LaGrega et al. 2001).

Adverse or toxic effects can be produced in the laboratory or in the natural environment by acute or chronic exposure to chemicals or other potentially toxic agents. In acute exposure, organisms come in contact with the chemical delivered either in a single event or in multiple events that occur within a short period of time, generally hours to days. Acute exposure to chemicals that are rapidly absorbed generally produce immediate effects, but they may also produce delayed effects similar to those caused by chronic exposure. During chronic exposure, organisms are exposed to low concentrations of a chemical delivered either continuously or at some other periodic frequency over a long period of time (weeks, months, or years), measured in relation to the organism’s life cycle. Chronic exposure to chemicals may induce rapid, immediate effects similar to acute effects, in addition to effects that develop slowly (Rand 1995).

3.4.2. Factors related to the organisms

Species differ in susceptibility to chemicals. This difference may be due to differences in accessibility, with certain species effectively excluding a toxic medium for short period of time. In addition, rates and patterns of metabolism and excretion can substantially affect susceptibility. Differences in susceptibility to chemical agents among fish of different strains also result from genetic factors (Rand 1995).

Immature or young neonatal organisms often appear to be more susceptible to chemical agents than are adult organisms. Differences in rates of excretion of toxic chemicals may also be involved in age-dependent toxicity effects and the influence of difference in body size on toxicokinetics in general must be considered. However,

the reverse is also true. Embryos may be less sensitive than adults because, at particular stages, they may have protective or impermeable membranes (Rand 1995).

The toxicity of a particular chemical agent is traditionally evaluated on the basis of tests carried out with strong or healthy organisms. In order to extrapolate meaningful, relevant, and ecologically significant results from aquatic toxicity tests, not only appropriate tests but also appropriate organisms should be used. Several criteria should be considered in selecting organisms for toxicity testing (Rand 1995):

- Because sensitivities vary among species, a group of species representing a board range of sensitivities should be used whenever possible.
- Widely available and abundant species should be considered.
- Whenever possible, species should be studied that are indigenous to or representative of the ecosystem that may receive the impact.
- Species that are recreationally, commercially, or ecologically important should be included.
- Species should be amenable to routine maintenance in the laboratory and techniques should be available for culturing and rearing them in the laboratory so as to facilitate both acute and chronic toxicity tests.
- If there is adequate background information on a species, the data from test may be more easily interpreted.

3.4.3. Factors related to the chemical

The toxicity of a chemical agent can be influenced by its composition. Impurities or contaminants which are considerably more toxic than the chemical itself may be present. Impurities may vary from one batch of the chemical to another, so that the results obtained with a particular batch may not be reproducible. Therefore, toxicity tests are conducted with highly purified samples of a chemical agent (Hodgson 2004). However, recent researches indicated that toxicity of the mixture of pollutants may be equal to the sum of the fractional toxicities of the mixture of individual components or higher/lower than the sum due to synergistic/antagonistic interactions

(Utgikar et al. 2004). In order to evaluate adverse environmental impacts of discharges, toxicity differentiation due to interactions of chemicals in a mixture should be addressed.

Other factors that are directly related to the chemical are its physical and chemical properties such as solubility, vapour pressure, pH, and lipophilicity. These factors affect the persistence, transformation, bioavailability, and ultimate fate of chemical in water (Rand 1995).

3.4.3.1. Interactive Effects of Chemicals

The toxicity of a chemical may be increased or decreased by a simultaneous or consecutive exposure to another chemical. If the combined effect is equal to the sum of the effect of each substance given alone, the interaction is considered to be additive; e.g., the combinations of most organophosphorous pesticides on cholinesterase activity. If the combine effect is grater than the sum, the interaction is considered to be synergistic; e.g., carbon tetrachloride and ethanol on the liver and asbestos exposure and cigarette smoking on the lung. The term potentiation is used to describe the situation in which the toxicity of a substance is markedly increased by another substance that alone has no toxic effect (Lu et al. 2002). In some manner, potentiation is used in for synergism. In any case, if there is potentiation or synergism, the toxic effect should be greater than expected (Walker 1996). On the other hand, the exposure of an organism to a chemical may reduce the toxicity of another. Chemical antagonism denotes the situation wherein a reaction between two chemicals produces a less toxic product, such as chelation of heavy metals by dimercaprol. Functional antagonism exists when two chemicals produce opposite effects on the same physiologic parameters, such as the counteraction between CNS stimulants depressants (Lu et al. 2002). The general picture of interaction is illustrated in Figure 3.2 (Walker 1996), when two compounds are under consideration.

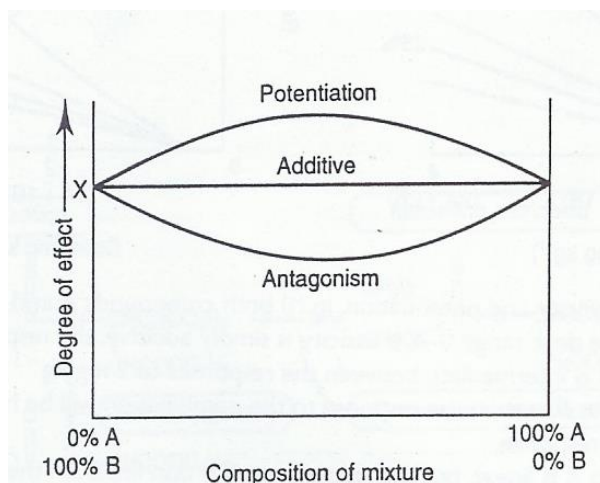


Figure 3.2. Interaction of Two Compounds

Relationship between dose and toxic effect for each of the individual components of a mixture should be taken into consideration. In particular, it is important to know whether there is a linear relationship between dose and toxic response (Figure 3.3) (Walker 1996). If this is the case, then increases in toxicity of combinations of chemicals which are substantially greater than additive should be considered as examples of potentiation. If, on the other hand, they are not linear, this conclusion does not necessarily follow (Figure 3.3). An enhancement of toxicity above that which is simply additive may merely reflect what happens when dose of an individual chemical (or chemicals) is increased, and may not therefore represents potentiation due to interaction between chemicals (Walker 1996).

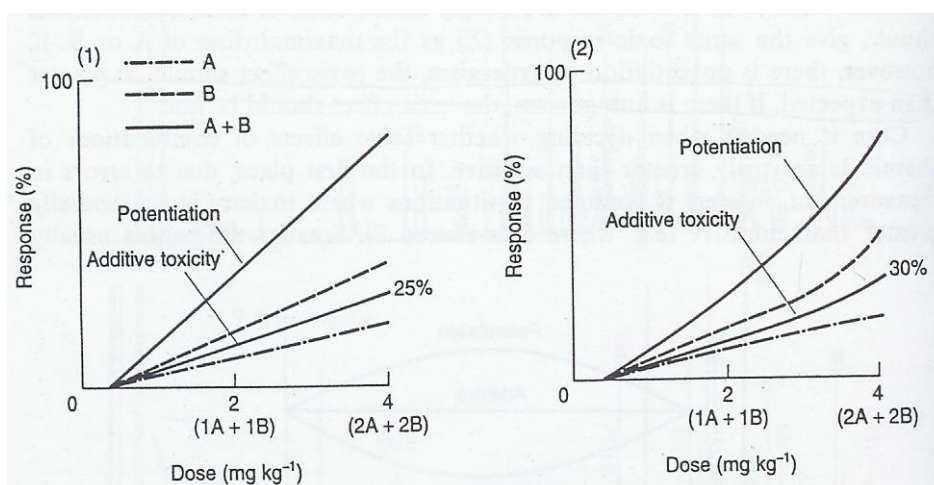


Figure 3.3. Additive Toxicity and Potentiation

3.5. Toxicity Test

Toxicology has been defined as the study of the effects of chemicals and other toxic agents on organisms with special emphasis on adverse or harmful effects. Toxicity tests are used to evaluate the concentrations of the chemical and the duration of exposure required to produce the criterion effect(s) (Rand 1995).

Although testing for toxicity, usually for the purposes of human health or environmental risk assessment, might be expected to be one of the more routine aspects of toxicology, it is actually one of the more controversial. Among the many areas of controversy are the use of animals for testing and the welfare of the animals, extrapolation of animal data to humans, extrapolation from high-dose to low-dose effects, and the increasing cost and complexity of testing protocols relative to the benefits expected. New tests are constantly being devised and are often added to testing requirements already in existence (Hodgson 2004).

An aquatic toxicity test is frequently called as a bioassay. A bioassay is performed to measure the degree of response produced by a specific level of chemical concentration. A biological assay (bioassay) is an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows its application to living matter (Rand 1995). Bioassays used in aquatic toxicology have taken a prominent position among analytical test for identifying and measuring environmental hazards. In particular, chronic toxicity tests have been developed for testing effluents, surface water, and sediment samples to estimate the safe or no effect sample concentration (Ostrander 1996).

Bioassays using luminescent bacteria are routinely used to assess the acute toxicity of environmental samples. Over the last 15 years, various applications using these organisms have been validated and recognized by several standards organizations. Luminescent bacteria possess several attributes that support their practical use for toxicity testing. Their small cell size provides a high surface-to-volume ratio which maximizes exposure potential. This structural characteristic plus (1) lack of membrane-aided compartmentalization; (2) location of most respiratory pathways (including enzymes required for bioluminescence) on or near the cell membrane; and (3) a metabolic rate 10 to 100 times mammalian cells, provide a dynamic metabolic

system which can be easily quantitated by measuring the rate of light output. The close association of the light production pathway with the bacteria's respiratory system provides a convenient and sensitive biological system for quantitating a metabolic inhibition due to the presence of toxic chemicals (Ostrander 1996) (Ren and Frymier 2003).

Most testing can be subdivided into *in vivo* tests for acute, sub-chronic, or chronic effects and *in vitro* tests for genotoxicity or cell transformation, although other tests are used and are described in this chapter. Any chemical that has been introduced into commerce or that is being developed for possible introduction into commerce is subject to toxicity testing to satisfy the regulations of one or more regulatory agencies. Furthermore compounds produced as waste products of industrial processes (e.g., combustion products) are also subject to testing (Hodgson 2004).

Toxicity test methods may be categorized according to length of exposure, test situation, criteria of effects to be evaluated, and organisms to be tested. The data generated in these tests may enable the researcher to determine the no observed effect concentration (NOEC) or no-effect concentration, which is the maximum concentration of the test material that produces no statistically significant harmful effect on test organisms compared to controls in a specific test. The lowest observed effect concentration (LOEC) or minimum threshold concentration (MTC) may also be obtained. This is lowest concentration that has a statistically significant deleterious effect on test organisms compared to controls in specific test. The effects evaluated are biological end points selected because they are based on process important to the survival, growth, behavior, and perpetuation of species. These end points differ depending on the type of toxicity test being conducted and species used. The statistical approach also changes with the type of toxicity test conducted (Rand 1995).

Acute Toxicity Tests: These are tests designed to evaluate the relative toxicity of a chemical to selected aquatic organisms upon short-term exposure to various concentrations of test chemical. Common effect criteria for fish are mortality; for invertebrates, immobility and loss of equilibrium; and for algae, growth. These tests may be conducted for a predetermined length of (time-dependent test) to estimate the 24- or 96-h LC50 or the 48- or 96-h EC50. An acute toxicity test may also have a

duration that is not predetermined, in which case it is referred to as a *time-independent* (TI) test. In a TI test, exposure of the test organisms continues until the toxic response manifested has ceased or economic or other practical considerations dictate that the test be terminated. For example the acute T1 test should be allowed to continue until acute toxicity (mortality or a defined sublethal effect) has ceased or nearly ceased and the toxicity curve indicates that a threshold or incipient effect concentration can be estimated (Rand 1995).

In the early development of acute toxicity tests, data were expressed as the *median tolerance limit* (TL_m or TL₅₀)- the test material concentration at which 50% of the test organisms survive for a specified exposure time (usually 24-96 h). This term has been replaced by median lethal concentration (LC₅₀) and median effective concentration (EC₅₀) (Rand 1995).

Chronic Toxicity Tests: The fact that a chemical does not have adverse effects on aquatic organisms in acute toxicity tests does not necessarily indicate that it is not toxic to these species. Chronic toxicity tests permit evaluation of the possible adverse effects of the chemical under conditions of long-term exposure at sublethal concentrations. In a full chronic toxicity test, the test organism is exposed for an entire reproductive life cycle (e.g., egg to egg) to at least five concentrations of the test material. Partial life cycle (or partial chronic) toxicity tests involve only several sensitive life stages; these include reproduction and growth during the first year but do not include exposure of very early juvenile stages. In full chronic toxicity tests exposure is generally initiated with an egg or zygote and continues through development and hatching of the embryo, growth and development of the young organism, attainment of sexual maturity, and reproduction to produce a second-generation organism. Tests may also begin with the exposed adult and continue through egg, fry, juvenile, and adult to fertilized eggs and criteria for effect include growth, reproduction, development of gametes, maturation, spawning, success, hatching success, survival of larvae or fry, growth and survival of different life stages, and behavior. The duration of a chronic toxicity test varies with the species tested; for instance, it is approximately 21 d for the water flea *Daphnia magna* and can be 275-300 d for the fathead minnow, *Pimephales promels* (Rand 1995).

From the data obtained in partial life cycle and complete life cycles test the *maximum acceptable toxicant concentration* (MATC) can be estimated. This is the estimated threshold concentration of a chemical within a range defined by highest concentration tested at which no significant deleterious effect was observed (NOEC) and the lowest concentration tested at which some significant deleterious effect was observed (LOEC). Because it is not possible to test an unlimited number of intermediate concentrations, an MATC is generally reported as being greater than the NOEC and less than the LOEC ($\text{NOEC} < \text{MATC} < \text{LOEC}$; e.g., $0.5 \text{ ppm} < \text{MATC} < 1.0 \text{ ppm}$). For regulatory purposes, the MATC is sometimes calculated as the geometric mean of the LOEC and NOEC, so it can be used as a point estimate (Rand 1995).

Toxicity assessment is the determination of the potential of any substance to act as a poison, the conditions under which this potential will be realized, and the characterization of its action. Risk assessment, however, is a quantitative assessment of the probability of deleterious effects under given exposure conditions. Both are involved in the regulation of toxic chemicals. Regulation is the control, by statute, of the manufacture, transportation, sale, or disposal of chemicals deemed to be toxic after testing procedures or according to criteria laid down in applicable laws.

Although for a variety of reasons extrapolation from experimental animals to humans presents problems, including differences in metabolic pathways, dermal penetration, mode of action, and others, experimental animals present numerous advantages in testing procedures. These advantages include the possibility of clearly defined genetic constitution and their amenity to controlled exposure, controlled duration of exposure, and the possibility of detailed examination of all tissues following necropsy (Hodgson 2004).

Although not all tests are required for all potentially toxic chemicals, any of the tests shown in Table 3.1 (Hodgson 2004) may be required by the regulations imposed under a particular law. The particular set of tests required depends on the predicted or actual use of the chemical, the predicted or actual route of exposure, and the chemical and physical properties of the chemical (Hodgson 2004).

Table 3.1. Summary of Toxicity Tests and Related End Points

I Chemical and physical properties
For the chemical in question, probable contaminants from synthesis as well as intermediates and waste products from the synthetic process
II Exposure and environmental fate
A. Degradation studies—hydrolysis, photodegradation, etc.
B. Degradation in soil, water, under various conditions
C. Mobility and dissipation in soil, water, and air
D. Accumulation in plants, aquatic animals, wild terrestrial animals, food plants, and animals, etc.
III In vivo tests
A. Acute
1. LD50 and LC50—oral, dermal or inhaled
2. Eye irritation
3. Dermal irritation
4. Dermal sensitization
B. Subchronic
1. 30- to 90-day feeding
2. 30- to 90-day dermal or inhalation exposure
C. Chronic/reproduction
1. Chronic feeding (including oncogenicity tests)
2. Teratogenicity
3. Reproduction (multi-generation)
D. Special tests
1. Neurotoxicity
2. Potentiation
3. Metabolism
4. Pharmacodynamics
5. Behavior
IV In vitro tests
A. Mutagenicity—prokaryote (Ames test)
B. Mutagenicity—eukaryote (<i>Drosophila</i> , mouse, etc.)
C. Chromosome aberration (<i>Drosophila</i> , sister chromatid exchange, etc.)
V Effects on wildlife
Selected species of wild mammals, birds, fish, and invertebrates: acute toxicity, accumulation, and reproduction in laboratory-simulated field conditions

3.6. Toxicity of Metals

A metal is defined by chemists as an element which has a characteristic lustrous appearance is a good conductor of electricity, and general enters chemical reactions as positive ions or cations (Walker 1996).

Although most metals occur in nature in rocks, ores, soil, water, and air, levels are usually low and widely dispersed. In terms of human exposure and toxicological significance, it is anthropogenic activities that are most important because they increase the levels of metals at the site of human activities.

Metals have been used throughout much of human history after industrial revolution to make utensils, machinery, and so on, and mining and smelting supplied metals for

these uses. These activities increased environmental levels of metals. More recently metals have found a number of uses in industry and medicine. These activities have increased exposure not only to metal-related occupational workers but also to consumers of the various products (Hodgson 2004).

Despite the wide range of metal toxicity and toxic properties, there are a number of toxicological features that are common to many metals. For a metal to exert its toxicity, it must cross the membrane and enter the cell. If the metal is in a lipid soluble form such as methylmercury, it readily penetrates the membrane; when bound to proteins such as cadmium-metallothionein, the metal is taken into the cell by endocytosis; other metals (e.g., lead) may be absorbed by passive diffusion. The toxic effects of metals usually involve interaction between the free metal and the cellular target. These targets tend to be specific biochemical processes and/or cellular and subcellular membranes (Hodgson 2004).

Metals are non-biodegradable. Unlike some organic pesticides, metals cannot be broken down into less harmful components. Detoxification by organisms consists of hiding active metal ions within a protein such as metallothionein, or depositing them in an insoluble form in intracellular granules for long-term storage or excretion in the faeces (Walker 1996).

3.6.1. Common toxic mechanisms and sites of action

Enzyme Inhibition/Activation: A major site of toxic action for metals is interaction with enzymes, resulting in either enzyme inhibition or activation. Two mechanisms are of particular importance: inhibition may occur as a result of interaction between the metal and sulfhydryl (SH) groups on the enzyme, or the metal may displace an essential metal cofactor of the enzyme. For example, lead may displace zinc in the zinc-dependent enzyme δ -aminolevulinic acid dehydratase (ALAD), thereby inhibiting the synthesis of heme, an important component of hemoglobin and heme-containing enzymes, such as cytochromes (Hodgson 2004).

Subcellular Organelles: Toxic metals may disrupt the structure and function of a number of organelles. For example, enzymes associated with the endoplasmic reticulum may be inhibited, metals may be accumulated in the lysosomes, respiratory

enzymes in the mitochondria may be inhibited, and metal inclusion bodies may be formed in the nucleus (Hodgson 2004).

Carcinogenicity: A number of metals have been shown to be carcinogenic in humans or animals. Arsenic, certain chromium compounds, and nickel are known human carcinogens; beryllium, cadmium, and cisplatin are probable human carcinogens. The carcinogenic action, in some cases, is thought to result from the interaction of the metallic ions with DNA (Hodgson 2004).

Kidney: Because the kidney is the main excretory organ of the body, it is a common target organ for metal toxicity. Cadmium and mercury, in particular, are potent nephrotoxics (Hodgson 2004).

Nervous System: The nervous system is also a common target of toxic metals; particularly, organic metal compounds. For example, methylmercury, because it is lipid soluble, readily crosses the blood-brain barrier and enters the nervous system. By contrast, inorganic mercury compounds, which are more water soluble, are less likely to enter the nervous system and are primarily nephrotoxics. Likewise organic lead compounds are mainly neurotoxics, whereas the first site of inorganic lead is enzyme inhibition (e.g., enzymes involved in heme synthesis) (Hodgson 2004).

Endocrine and Reproductive Effects: Because the male and female reproductive organs are under complex neuroendocrine and hormonal control, any toxicant that alters any of these processes can affect the reproductive system. In addition metals can act directly on the sex organs. Cadmium is known to produce testicular injury after acute exposure, and lead accumulation in the testes is associated with testicular degeneration, inhibition of spermatogenesis, and Leydig-cell atrophy (Hodgson 2004).

Respiratory System: Occupational exposure to metals in the form of metal dust makes the respiratory system a likely target. Acute exposure may cause irritations and inflammation of the respiratory tract, whereas chronic exposure may result in fibrosis (aluminum) or carcinogenesis (arsenic, chromium, nickel) (Hodgson 2004).

Metal-Binding Proteins: The toxicity of many metals such as cadmium, lead, and mercury depends on their transport and intracellular bioavailability. This availability is regulated to a degree by high-affinity binding to certain cytosolic proteins. Such ligands usually possess numerous SH binding sites that can outcompete other intracellular proteins and thus mediate intracellular metal bioavailability and toxicity. These intracellular “sinks” are capable of partially sequestering toxic metals away from sensitive organelles or proteins until their binding capacity is exceeded by the dose of the metal. Metallothionein (MT) is a low molecular weight metal-binding protein (approximately 7000 Da) that is particularly important in regulating the intracellular bioavailability of cadmium, copper, mercury, silver, and zinc. For example, in vivo exposure to cadmium results in the transport of cadmium in the blood by various high molecular weight proteins and uptake by the liver, followed by hepatic induction of MT. Subsequently cadmium can be found in the circulatory system bound to MT as the cadmium-metallothionein complex (CdMT) (Hodgson 2004).

4. INDUSTRIAL SURVEY

To provide general understanding of metal finishing industry, information pertaining to the industry size, product characterization, process survey, and waste survey presented in this chapter. Hazardous wastes from an organized industrial district for metal finishers have been studied in this study. Therefore, there are a number of different processes and their wastes, so a general industrial survey is given in this chapter.

4.1. General Information

The Standard Industrial Classification (SIC) code 34 is composed of establishments that fabricate ferrous and nonferrous metal products and those that perform electroplating, plating, polishing, anodizing, dying, and coating operations on metals.

Metal finishing operations are employed at some point during the manufacture of nearly all metal products. The most common of these include fabricated metal products, common machinery, electronic machinery, and household appliances.

Metal finishing companies are either captive shops or job shops. Captive shops perform finishing activities on the parts they manufacture, while job shops are a service industry that provides metal finishing for manufacturers. Captive shops are usually larger than job shops, and are prevalent in the automotive components industry (Thambiran 2002).

4.2. Product Characterization

The US Department of Commerce classification codes divide this industry by product and services. SIC code 34 is further divided as follows:

SIC 341 -Metal Cans and Shipping Containers

SIC 342 -Cutlery, Handtools, and General Hardware

SIC 343 -Heating Equipment, Except Electric and Warm Air, and Plumbing Fixtures

SIC 344 -Fabricated Structural Metal Products

SIC 345 -Screw Machine Products, and Bolts, Nuts, Screws, Rivets, and Washers

SIC 346 -Metal Forgings and Stampings

SIC 347 -Coating, Engraving, and Allied Services

SIC 348 -Ordnance and Accessories, Except Vehicles and Guided Missiles

SIC 349 -Miscellaneous Fabricated Metal Products.(USEPA 1995)

4.3. Process Survey

Metal Finishing comprises a broad range of processes and is performed on manufactured parts after they have been shaped, formed, forged, etc. The metal surfaces are prepared for finishing by cleaning, pickling, and dipping. A finish is any final operation applied to the metal object to alter its surface. This is done to increase corrosion or abrasion resistance, alter appearance, add hardness, or improve soldering. These characteristics can be significant for a product and could determine its quality and usefulness. Plating operations are typically carried out in batches. Processes used involve the cleaning, hardening or softening, smoothing, and conversion of the object's surface using chemicals. The metal objects are dipped into and removed from baths containing the plating solutions.

In general, the metal surface treatment and plating operations can be divided into three stages;

- surface preparation,
- surface treatment (finishing)
- post treatment.

4.3.1. Surface preparation

The surface of the metal may require preparation prior to applying a finish. Surface preparation, cleanliness, and proper chemical conditions are essential to ensuring that finishes perform properly. Without a properly cleaned surface, even the most expensive coatings will fail to adhere or prevent corrosion because of the roughness and porosity of the surface. Surface preparation techniques range from simple abrasive blasting to acid washes to complex, multi-stage chemical cleaning processes (USEPA 1995), (Thambiran 2002). A flowchart of a representative process used when preparing metal surfaces for finishing is given in Figure 4.1 (USEPA 1995).

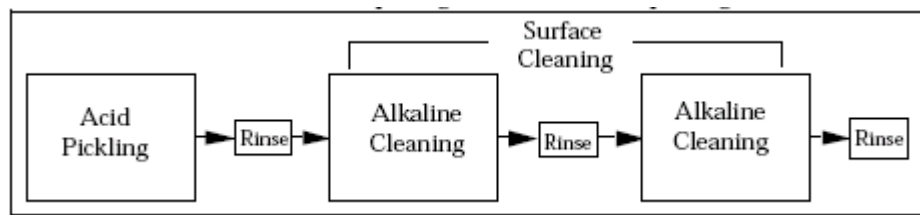


Figure 4.1. Process for Surface Preparation

Alkaline cleaning may also be utilized for the removal of organic soils. Most alkaline cleaning solutions are comprised of three major types of components: (1) builders, such as alkali hydroxides and carbonates, which make up the largest portion of the cleaner; (2) organic or inorganic additives, which promote better cleaning or act to affect the metal surface in some way; and (3) surfactants. Alkaline cleaning is often assisted by mechanical action, ultrasonics, or by electrical potential (e.g., electrolytic cleaning).

Acid cleaning, or pickling, can also be used to prepare the surface of metal products by chemically removing oxides and scale from the surface of the metal. For instance, most carbon steel is pickled with sulfuric or hydrochloric acid, while stainless steel is pickled with hydrochloric or hydrofluoric acids although hydrochloric acid may embrittle certain types of steel and is rarely used. The metal generally passes from the pickling bath through a series of rinses. Acid pickling is similar to acid cleaning, but is usually used to remove the scale from semi-finished mill products, whereas acid cleaning is usually used for near-final preparation of metal surfaces before electroplating, painting, and other finishing processes (USEPA 1995).

4.3.2. Surface treatment (finishing)

Surface finishing usually involves a combination of metal deposition operations and numerous finishing operations. Metal-ion-bearing solutions are commonly based on hexavalent chrome, trivalent chrome, copper, gold, silver, cadmium, zinc, and nickel. Many other metals and alloys are also used, although less frequently. The cleaners (e.g., acids) may appear in process wastewater; the solvents may be emitted into the air, released in wastewater, or disposed of in solid form; and other wastes, including paints, metal-bearing sludges, and still bottom wastes, may be generated in solid form. A diagram describing the general metal finishing process, including surface preparation, is provided in Figure 4.2 (USEPA 1995).

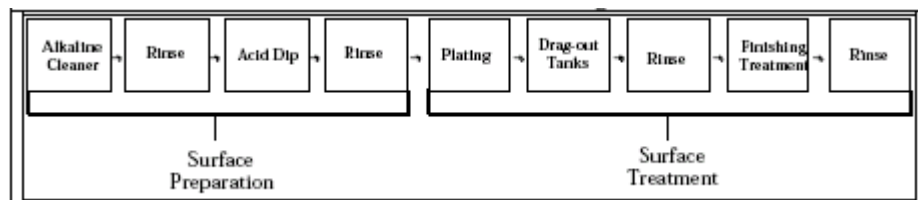


Figure 4.2. General Metal Finishing Process Including Surface Preparation

Basic metal finishing operations are;

Anodizing: Anodizing is an electrolytic process which converts the metal surface to an insoluble oxide coating. Anodized coatings provide corrosion protection, decorative surfaces, a base for painting and other coating processes, and special electrical and mechanical properties. Aluminium is the most frequently anodized material. Common aluminium anodizing processes include: chromic acid anodizing, sulphuric acid anodizing, and boric-sulphuric anodizing. The sulphuric acid process is the most common method. Following anodizing, parts are typically rinsed, and then proceed through a sealing operation that improves the corrosion resistance of the coating. Common sealants include chromic acid, nickel acetate, nickel-cobalt acetate, and hot water.

Chemical Conversion Coating: Chemical conversion coating includes chromating, phosphating, metal colouring, and passivating operations. Chromate conversion coatings are produced on various metals by chemical or electrochemical treatment. Solutions, usually containing hexavalent chromium and other compounds, react with

the metal surface to form a layer containing a complex mixture of compounds consisting of chromium, other constituents, and base metal. Phosphate coatings may be formed by the immersion of steel, iron, or zinc-plated steel in a dilute solution of phosphate salts, phosphoric acid, and other reagents to condition the surfaces for further processing. They are used to provide a good base for paints and other organic coatings, to condition the surfaces for cold forming operations by providing a base for drawing compounds and lubricants, and to impart corrosion resistance to the metal surface. Metal colouring involves chemically converting the metal surface into an oxide or similar metallic compound to produce a decorative finish such as a green or blue patina on copper or steel, respectively. Passivating is the process of forming a protective film on metals by immersion into an acid solution, usually nitric acid or nitric acid with sodium dichromate. Stainless steel products are often passivated to prevent corrosion and extend the life of the product.

Electroplating: Electroplating is the production of a surface coating of one metal upon another by electrodeposition. Electroplating activities involve applying predominantly inorganic coatings onto surfaces to provide corrosion resistance, hardness, wear resistance, anti-frictional characteristics, electrical or thermal conductivity, or decoration. The most commonly electroplated metals and alloys include: brass (copper-zinc), cadmium, chromium, copper, gold, nickel, silver, tin, and zinc. In electroplating, metal ions in either acid, alkaline, or neutral solutions are reduced on the work pieces being plated. The metal ions in the solution are usually replenished by the dissolution of metal from solid metal anodes fabricated of the same metal being plated, or by direct replenishment of the solution with metal salts or oxides. Cyanide, usually in the form of sodium or potassium cyanide, is usually used as a complexing agent for cadmium and precious metals electroplating, and to a lesser degree, for other solutions such as copper and zinc baths. The sequence of steps in an electroplating includes: cleaning, often using alkaline and acid solutions; stripping of old plating or paint; electroplating; and rinsing between and after each of these operations. Sealing and conversion coating may be employed on the metals after electroplating operations.

Electroless Plating: Electroless plating is the chemical deposition of a metal coating onto a plastic object, by immersion of the object in a plating solution. Copper and

nickel electroless plating is commonly used for printed circuit boards. The basic ingredients in an electroless plating solution are: a source of metal (usually a salt); a reducer; a complexing agent to hold the metal in solution; and various buffers and other chemicals designed to maintain bath stability and increase bath life. Immersion plating produces a thin metal deposit, commonly zinc or silver, by chemical displacement. Immersion plating baths are usually formulations of metal salts, alkalis, and complexing agents (e.g., lactic, glycolic, malic acid salts). Electroless plating and immersion plating commonly generate more waste than other plating techniques, but individual facilities vary significantly in efficiency (USEPA 1995).

4.3.3. Post treatment

Post treatment is the retouching part of whole process after surface treatment. Dying polishing, etching etc. are the most common post treatment types.

Painting involves the application of predominantly organic coatings to a work piece for protective and/or decorative purposes. It is applied in various forms, including dry powder, solvent-diluted formulations, and water-borne formulations. Various methods of application are used, the most common being spray painting and electrodeposition. Spray painting is a process by which paint is placed into a pressurized cup or pot and is atomized into a spray pattern when it is released from the vessel and forced through an orifice. Electrodeposition is the process of coating a workpiece by either making it anodic or cathodic in a bath that is generally an aqueous emulsion of the coating material. Polishing, hot dip coating and etching are processes that are also used to retouch the finished metal. Polishing is an abrading operation used to remove or smooth out surface defects (scratches, pits, or tool marks) that adversely affect the appearance or function of a part. Following polishing operations, area cleaning and washdown can produce metal-bearing wastewaters. Hot dip coating is the coating of a metallic workpiece with another metal to provide a protective film by immersion into a molten bath. Galvanizing (hot dip zinc) is a common form of hot dip coating. Etching produces specific designs or surface appearances on parts by controlled dissolution with chemical reagents or etchants. Etching solutions commonly comprise strong acids or bases with spent etchants containing high concentrations of spent metal. The solutions include ferric chloride,

nitric acid, ammonium persulfate, chromic acid, cupric chloride, and hydrochloric acid (USEPA 1995).

4.4. Waste Survey

General process diagram and waste sources are illustrated in **Figure 4.3** (USEPA 1995) for metal finishing industry.

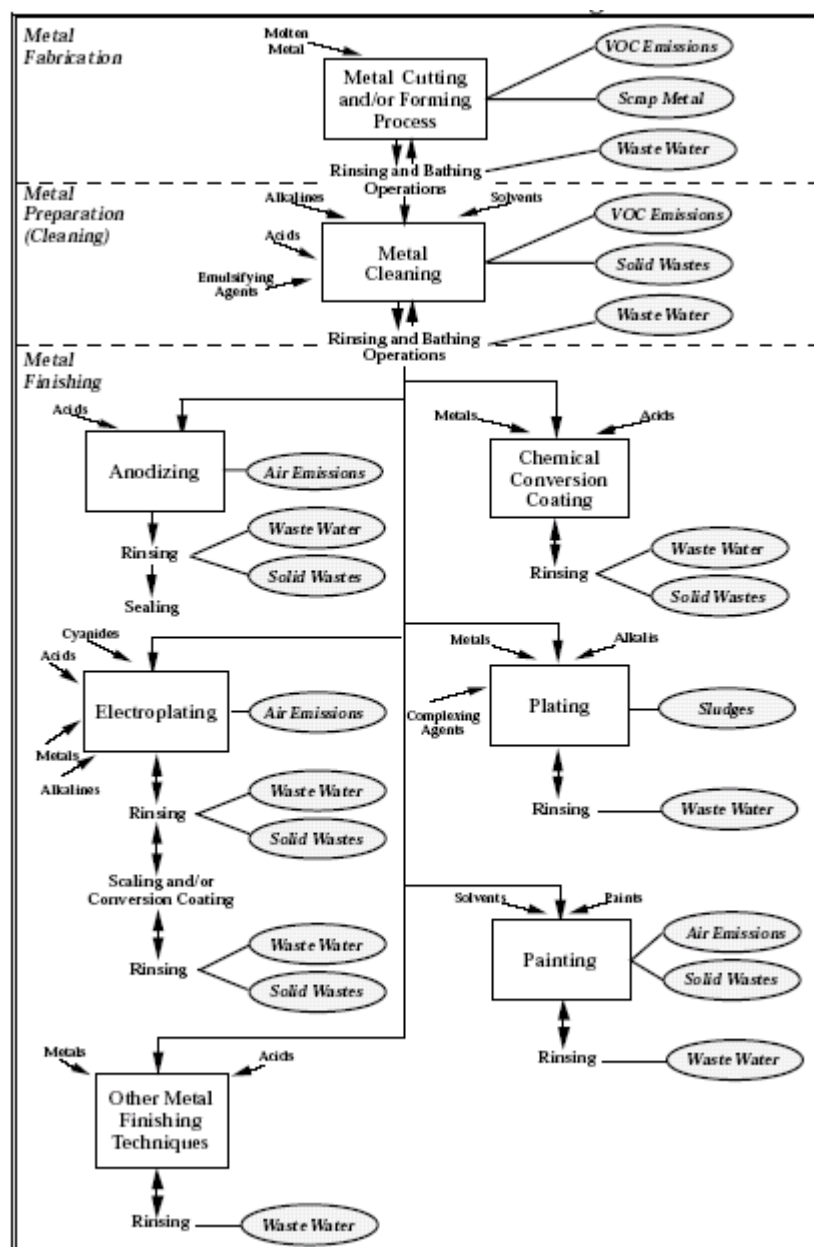


Figure 4.3. General Process and Waste Survey of Metal Finishing Industry

Surface preparation activities usually result in air emissions, wastewater, and solid wastes. The primary air emissions from cleaning are due to the evaporation of chemicals from solvent degreasing and emulsion cleaning processes. These emissions may result through volatilization of solvents during storage, fugitive losses during use, and direct ventilation of fumes. Wastewaters generated from cleaning are primarily rinse waters, which are usually combined with other metal finishing wastewaters (e.g., electroplating) and treated on-site by conventional hydroxide precipitation. Solid wastes (e.g., wastewater treatment sludges, still bottoms, cleaning tank residues, machining fluid residues, etc.) may also be generated by the cleaning operations. For example, solid wastes are generated when cleaning solutions become ineffective and are replaced.

Many metal finishing operations are typically performed in baths (tanks) and are then followed by rinsing cycles. Metal plating and related waste account for the largest volumes of metal (e.g., cadmium, chromium, copper, lead, and nickel) and cyanide-bearing wastes. Painting operations account for the generation of solvent-bearing wastes and the direct release of solvents (including benzene, methyl ethyl ketone, methyl isobutyl ketone, toluene, and xylene). Paint cleanup operations may contribute to the release of chlorinated solvents (including carbon tetrachloride, methylene chloride, 1,1,1-trichloroethane, and perchloroethylene).

Anodizing operations produce air emissions, contaminated wastewaters, and solid wastes. Mists and gas bubbles arising from heated fluids are a source of air emissions, which may contain metals or other substances present in the bath. When dyeing of anodized coatings occurs, wastewaters produced may contain nickel acetate, non-nickel sealers, or substitutes from the dye. Other potential pollutants include complexers and metals from dyes and sealers. Wastewaters generated from anodizing are usually combined with other metal finishing wastewaters and treated on-site by conventional hydroxide precipitation. Wastewaters containing chromium must be pre-treated to reduce hexavalent chromium to its trivalent state. The conventional treatment process generates a sludge that is usually sent off-site for metals reclamation and/or disposal. Solid wastes generated from anodizing include spent solutions and wastewater treatment sludges. Anodizing solutions may be contaminated with the base metal being processed due to the anodic nature of the

process. These solutions eventually reach an intolerable concentration of dissolved metal and require processing to remove the dissolved metal to a tolerable level or treatment/disposal.

Chemical conversion coating generally produces contaminated wastewaters and solid waste. Pollutants associated with these processes enter the waste stream through rinsing and batch dumping of process baths. The process baths usually contain metal salts, acids, bases, and dissolved basis materials. Conversion coating solutions may also be contaminated with the base metal being processed. These solutions will eventually reach an intolerable concentration of dissolved metal and require processing to remove the dissolved metal to a tolerable level.

Electroplating operations produce air emissions, contaminated wastewaters and solid wastes. Mists arising from electroplating fluids and process gases can be a source of air emissions, which may contain metals or other substances present in the bath. The industry has recently begun adding fume suppressants to electroplating baths to reduce air emissions of chromium, one of the most frequently electroplated metals. The fume suppressants lower the surface tension of the bath, which prevents hydrogen bubbles in the bath from bursting and producing a chromium-laden mist. The fume suppressants are highly effective when used in decorative plating, but less effective when used in hard-chromium plating. Contaminated wastewaters result from workpiece rinsing and process cleanup waters. Other wastes generated from electroplating include spent solutions which become contaminated during use, and therefore, diminish performance of the process. In addition to these wastes, spent process solutions and quench bathes may be discarded periodically when the concentrations of contaminants inhibit proper function of the solution or bath.

Electroless plating produces contaminated wastewater and solid wastes. The spent plating solution and rinse water are usually treated chemically to precipitate out the toxic metals and to destroy the cyanide. Electroless plating solutions can be difficult to treat; settling and simple chemical precipitation are not effective at removing the chelated metals used in the plating bath. The extent to which plating solution carry-over adds to the wastewater and enters the sludge depends on the type of article being plated and the specific plating method employed. However, most sludges may contain significant concentrations of toxic metals, and may also contain complex

cyanides in high concentrations if cyanides are not properly isolated during the treatment process.

Painting operations result in emissions, contaminated wastewaters, and the generation of liquid and solid wastes. Atmospheric emissions consist primarily of the organic solvents used as carriers for the paint. Emissions also result from paint storage, mixing, application, and drying. In addition, cleanup processes can result in the release of organic solvents used to clean equipment and painting areas. Wastewaters are often generated from painting processes due primarily to the discharge of water from water curtain booths. Sources of solid-and liquid-phase wastes include:

- Paint application emissions control devices (e.g., paint booth collection systems, ventilation filters, etc.)
- Equipment washing
- Disposal materials used to contain paint and overspray
- Excess paints discarded upon completion of a painting operation or after expiration of the paint shelf-life.

These solid and liquid wastes may contain metals from paint pigments and organic solvents, such as paint solvents and cleaning solvents. Still bottoms also contain solvent wastes. The cleaning solvents used on painting equipment and spray booths may also contribute organic solid waste to the wastes removed from the painting areas.

Wastewaters are often generated during other metal finishing processes. For example, following polishing operations, area cleaning and washdown can produce metal-bearing wastewaters. Hot dip coating techniques, such as galvanizing, use water for rinses following pre-cleaning and sometimes for quenching after coating. Hot dip coatings also generate solid waste, anoxide dross that is periodically skimmed off the heated tank. These operations generate metal-bearing wastewaters. Etching solutions are comprised of strong acids (e.g., ferric chloride, nitric acid,

ammonium persulfate) or bases. Resulting spent etchant solutions may contain metals and acids (USEPA 1995).

5. MATERIALS AND METHODS

5.1. Industrial

The primary business of the facilities involved in this study is the finishing of metal products. They are located in an organized industrial district (OID) for only metal finishers. The present OID contains about 190 facilities which have lots of different processes from electroplating to galvanizing. Therefore, the wastewater coming from these industries is very complex and containing lots of different hazardous chemicals and metals. The OID is established on 118,000 m² area and 92,000 m² of that is closed.

Wastewaters of these facilities are hazardous wastes due to their toxic and corrosive characteristics. Also, these wastes are listed in F type lists of USEPA as (Colorado Department of Public Health and Environment 1998):

F006: Wastewater treatment sludges from electroplating operations except from the following processes: (1) Sulfuric acid anodizing of aluminum; (2) tin plating on carbon steel; (3) zinc plating (segregated basis) on carbon steel; (4) aluminum or zinc-aluminum plating on carbon steel; (5) cleaning/stripping associated with tin, zinc and aluminum plating on carbon steel; and (6) chemical etching and milling of aluminum (T).

- F007: Spent cyanide plating bath solutions from electroplating operations (R, T).
- F008: Plating bath residues from the bottom of plating baths from electroplating operations where cyanides are used in the process (R, T).
- F009: Spent stripping and cleaning bath solutions from electroplating operations where cyanides are used in the process (R, T).
- F010: Quenching bath residues from oil baths from metal heat treating operations where cyanides are used in the process (R, T).

- F011: Spent cyanide solutions from salt bath pot cleaning from metal heat treating operations (R, T).
- F012: Quenching waste water treatment sludges from metal heat treating operations where cyanides are used in the process (T).

Moreover, wastewaters from metal finishing industry are reported as hazardous according to ORV of rating system (Talinli et al. 2005).

The wastewaters from the facilities come to a wastewater treatment plant (Figure 5.1). The objective of this plant is to remove the metals and the CN^- from the wastewater. To facilitate precipitation, firstly hexavalent Cr is reduced to trivalent Cr using NaHSO_3 which is then precipitated with Ca(OH)_2 in the precipitation tank. The CN^- in the wastewater is oxidized with HOCl which is then precipitated with Ca(OH)_2 in another precipitation tank

There were six sampling point along the treatment scheme. Liquid samples from influent, effluent, and different stages of the treatment plant, and sludge samples from precipitation tanks are taken. Metal and CN^- concentrations in all of the samples as well as in the extracts of sludges are analyzed. EC_{50} values of all of these samples are determined and the change in the toxicity according to the change in the metal and cyanide concentrations is investigated. The wastewater treatment plant scheme and the sampling points are illustrated in Figure 5.1.

Although all the experiments are conducted on the samples collected from the treatment plant, to monitor and investigate the treatment efficiency of the treatment plant is out of the scope of this study.

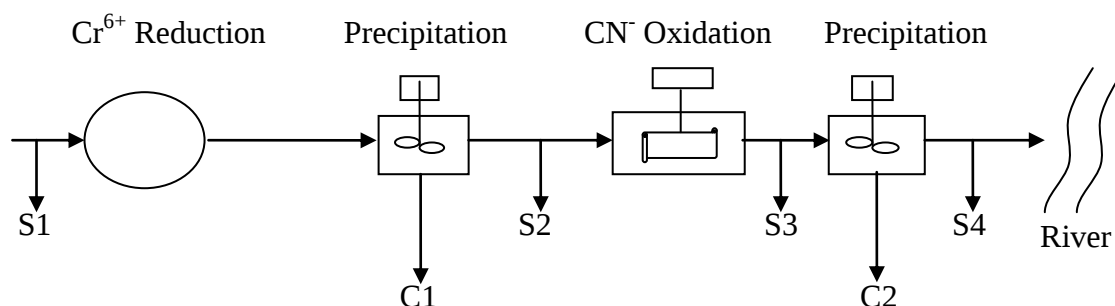


Figure 5.1. Wastewater Treatment Plant and Sampling Points

5.2. Experimental Work

During the study, toxicity tests, investigation of leaching behaviour, and characterization of wastes have been conducted.

5.2.1. Toxicity tests

Toxicity tests were conducted with BioToxTM toxicity bioassay test kit using *Vibrio Fischeri* luminescent bacteria. The inhibition of luminescence is determined by combining different dilutions of the test sample with luminescent bacteria. The decrease of light intensity is measured with Aboatox 1253 luminometer after a contact time (generally 5, 15, and 30 minutes). The inhibitory effect of dilutions is compared to a toxin free control to give the percentage inhibition. The value is plotted against the dilution factor and the resultant curve is used to calculate EC₅₀ of the sample.

Light production is the result of a chemical reaction involving the oxidation of a substrate, generally called luciferin, mediated by an enzyme called luciferase in the presence of an ionic cofactor; the intensity of produced light is proportional to the amount of reagents involved in the chemical reaction. A decrease in the intensity of the light produced therefore indicates alteration of one of the events leading to light production: either the chemical reaction (e.g., configurational inactivity of reagents), the expression of genes coding for the reagents, and/or any physiological control associated with the process (Deheyn et al. 2004). Basic oxidation and light production process is shown in Figure 5.2.

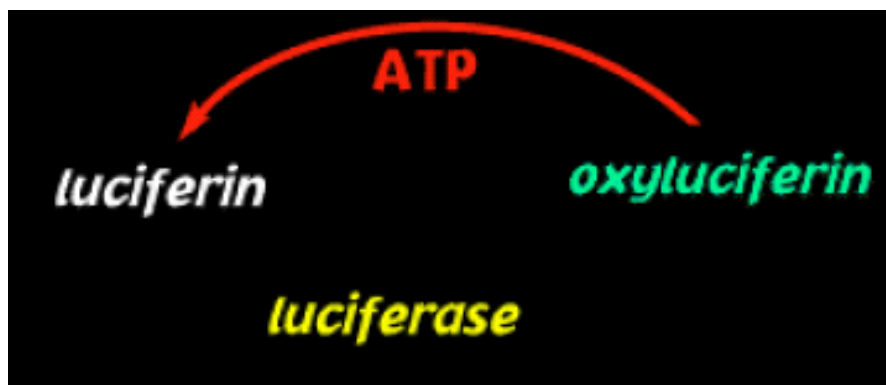


Figure 5.2. Basic Oxidation and Light Production Process

To optimally characterize and assess pollution, issues concerning both concentration and toxicity should be addressed. Microbial tests have been widely used in environmental toxicity screening due to similarity of complex biochemical functions in bacteria and higher organisms, ease of handling, short testing time, and reproducibility of results among laboratories. The use of bioassays to evaluate toxicity is strongly recommended in order to have a more direct and integrated assessment of environmental toxicity, since they depend upon factors such as pH, solubility, synergism/antagonism, and bioavailability (Mowat and Bundy 2001). *Vibrio Fischeri* is marine luminescent bacteria. The bioassay with *Vibrio Fischeri* requires only a short period of time to obtain reliable toxicity results which is one of its major advantages compared with other tests such as fish bioassays, which normally require several days. However, *Vibrio Fischeri* exhibits differing sensitivities for certain compounds depending on the duration of exposure. For example, the bacteria have shown less sensitivity to some water-soluble compounds, such as divalent metals, thereby requiring a 15 or 30-minute exposure time in order to observe any toxic response, whereas other compounds, such as organics, may affect the bacteria more rapidly, suggesting that a 5 or 10-minute exposure is optimal. Such toxicant-related time-dependent effects have also been reported for higher organisms and are difficult to avoid. The time-dependent effects on observed toxicity may be due to several processes that interact, including hydrolysis, photodecomposition, membrane diffusion, and chemical reaction with the substrate (Mowat and Bundy 2002). A 30-min exposure time was selected for use in this investigation.

Toxicity test were carried out in relevant with ISO 11348-3 standard test procedure. Pure cultures of bacteria in freeze dried form should be reconstituted with reconstitution solution in 15°C in a chiller. pH of the sample should be adjusted to 7 ± 0.2 if it is beyond the interval of 6-8.5. The salinity of the sample should also be adjusted to be equivalent to the 2% NaCl solution with Sample Diluent (20% NaCl). Several dilutions of the sample which would have been tested are prepared with dilution solution (2% NaCl) in order to make it react with *Vibrio Fischeri* and assess EC_{50} value. Light intensity of the cuvette containing only bacterial suspension is measured and then, immediately, sample is added to the cuvette. After exposure period light intensity is measured again (ISO 1999).

A blank sample with no toxicant (control) was used for all sets of experiments to correct for the time-dependent change in the light production of the bacteria themselves in order to isolate the toxic effects of the sample alone, as well as to account for small effects due to dilution arising from sample transfer, pipette error, and introduction of reagents (Mowat and Bundy 2002).

The BioToxTM Software performs automatically all calculations needed according to the equations below.

$$KF = \frac{IC_t}{IC_0} \quad (5.1)$$

$$INH\% = 100 - \frac{IT_t}{KF \times IT_0} \times 100 \quad (5.2)$$

KF = Correction Factor

IC_t = Luminescence Intensity of Control after Contact Time

IC_0 = Initial Luminescence Intensity of Cuvette Containing Bacterial Suspension just before Addition of Control Sample

IT_t = Luminescence Intensity of Sample after Contact Time

IT_0 = Initial Luminescence Intensity of Cuvette Containing Bacterial Suspension just before Addition of Test Sample

5.2.2. Leaching behaviour of wastes

Leaching behaviours of sludges and mobility of the metals in the sludges are determined regarding to Toxicity Characteristic Leaching Procedure (TCLP), Method 1311, in SW-846 with Zero Headspace Extractor (ZHE). The ZHE allows for liquid/solid separation within the device, and effectively precludes headspace. This type of vessel allows for initial liquid/solid separation, extraction, and final extract filtration without opening the vessel. The vessels shall have an internal volume of 500 ml, and be equipped to accommodate a 90 mm 0.6 µm pore sized filter.

5.2.3. Characterization of wastes

Metal analyses of wastewaters and leachates are conducted with flame atomic absorption spectrophotometry after digestion of them according to Standard Methods for Water and Wastewater Analysis.

To determine the metal concentrations in the sludges, they are digested according to USEPA's digestion procedure for soil and sediment samples for ICP, Flame and furnace atomic absorption is used. Then, metals of digestates are analyzed using flame atomic absorption spectrophotometry.

The COD analyses of samples are conducted according to ISO 6060 method.

CN⁻ analyses are carried out with ion selective electrode according to Standard Methods for Water and Wastewater Analysis.

6. RESULTS AND DISCUSSIONS

Results of all of the samples are given and discussed in this chapter. There are 4 liquid and 2 sludge samples. Their toxicity characteristics and the interaction of heavy metals, cyanide, and organic matter of the samples are discussed. The sampling points are given in Figure 5.1.

6.1. Sample S1

This sample is the influent of the treatment plant. The pH of the sample is 2.13. When it is adjusted to 7 ± 0.2 in order to conduct toxicity analysis, a precipitation is occurred. Therefore, it is allowed to precipitate and then both metal analysis and toxicity tests are conducted to both supernatant and mixed liquor of S1. Metal, COD, CN^- analysis and toxicity test results are given in Table 6.1.

Table 6.1. Metal and COD Analyses and Toxicity Test Results of Sample S1

(mg/l)	S1	S1 Supernatant
pH	2.13	6.88
Zn	261	130
Ni	104	56
Cu	57	14.9
Cr	64	3
Fe	30	0.91
Mn	4.39	4.04
Cd	0.63	0.19
CN^-	19.4	-
COD	565	470
EC_{50} (%)	4.47	5.83

Apparently, metal concentrations decrease with increasing pH due to the precipitation. This is increased EC_{50} value which means decreased toxicity of wastewater.

While about half of zinc and nickel is precipitated, most of copper, chromium and iron are precipitated. No considerable change on the concentrations of manganese and cadmium is observed although pH is increased. A slight decrease of COD is also observed. This may be occurred due to the adsorption of organic matter to the metal flocs.

Due to the precipitation of metals EC_{50} value of S1 is lower than EC_{50} value of supernatant of S1. EC_{50} interpolation curves are convenient to common S-shaped (sigmoid) toxicity curves for both S1 and supernatant of S1. R^2 value of linear regression of the test results for S1 is 0.92 and supernatant of S1 is 0.99. Two points of test results are beyond 95 % confidence limit for S1. If these points are removed, EC_{50} value slightly (0.3 %) changes. All points of the test results are in the 95 % confidence limit for supernatant of S1. Interpolation curves of S1 and supernatant of S1 are given in Figure 6.1 and Figure 6.2.

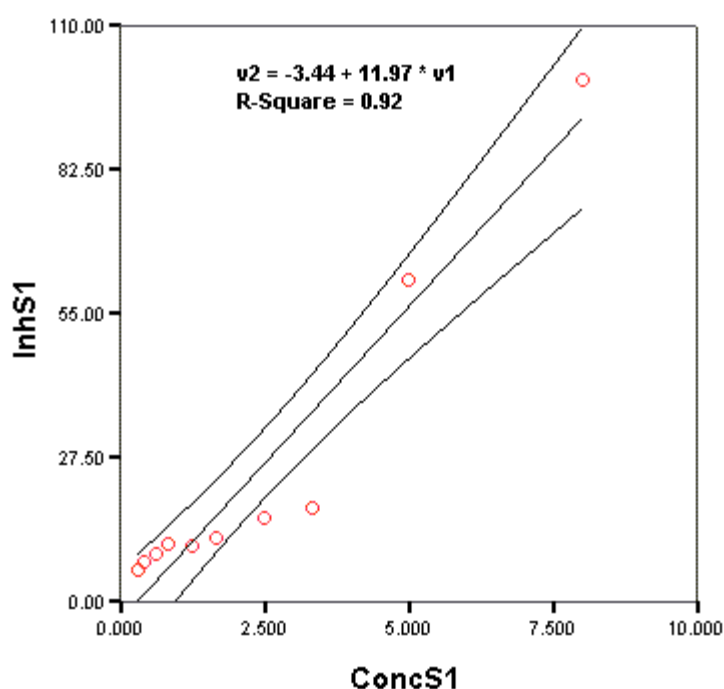


Figure 6.1. EC_{50} Interpolation Curve of S1

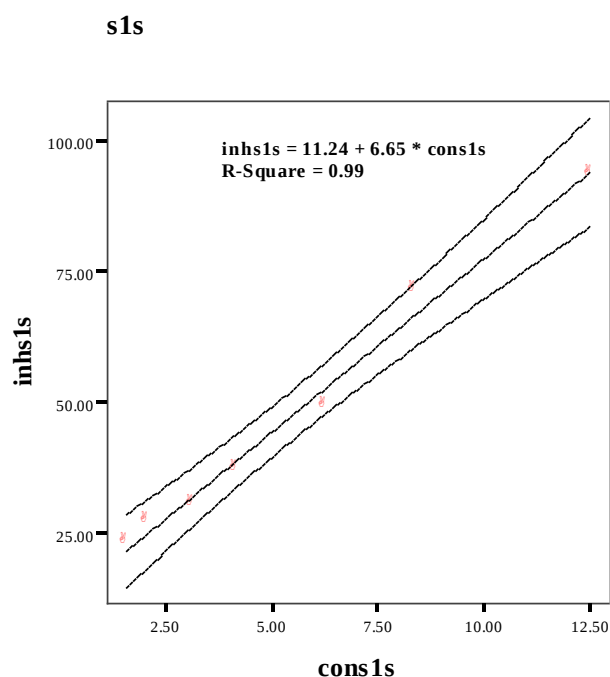


Figure 6.2. EC₅₀ Interpolation Curve of Supernatant of S1

6.2. Sample S2

This sample is collected from the effluent of first precipitation tank of the treatment plant. The pH of the sample is 3.45. When it is adjusted to 7 ± 0.2 in order to conduct toxicity analysis, a precipitation is occurred as well as S1. Therefore, it is allowed to precipitate and then both metal analysis and toxicity tests are conducted to both supernatant and mixed liquor of S2, too. Metal and COD analysis and toxicity test results of S2 and supernatant of S2 are given in Table 6.2.

Table 6.2. Metal and COD Analyses and Toxicity Test Results of Sample S2

mg/l	S2	S2 Supernatant
pH	3.45	7.01
Zn	231	173
Ni	152.8	124
Cu	65	23
Cr	76	19.7
Fe	57	12.4
Mn	3.46	3
Cd	0.49	0.3
COD	565	530
EC ₅₀ (%)	1.3	11.97

Apparently, metal concentrations decrease with increasing pH due to the precipitation as well as S1. This is increased EC₅₀ value which means decreased toxicity of wastewater.

Precipitation percentage of metals in S2 is less than Precipitation percentage of metals in S1. Slight decreases are observed on zinc and nickel concentrations. No considerable change on the concentrations of manganese and cadmium is observed although pH is increased. An important part of copper, chromium and iron is precipitated. A slight decrease of COD of S2 is also observed. This may be occurred due to the adsorption of organic matter to the metal flocs.

Due to the precipitation of metals EC₅₀ value of S2 is lower than EC₅₀ value of supernatant of S2. EC₅₀ interpolation curves are convenient to common S-shaped (sigmoid) toxicity curves for both S1 and supernatant of S1. R² value of linear regression of the test results for S1 is 0.99 and supernatant of S1 is 0.95. One point of test results is beyond 95 % confidence limit for supernatant of S2. If this point is removed, EC₅₀ value slightly (0.5 %) changes. All points of the test results are in the 95 % confidence limit for of S2. Interpolation curves of S2 and supernatant of S2 are given in Figure 6.3 and Figure 6.4.

S2

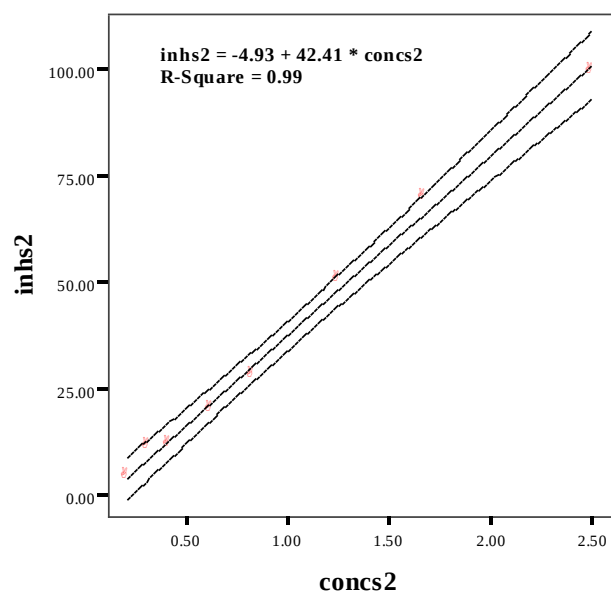


Figure 6.3. EC₅₀ Interpolation Curve of S2

s2s

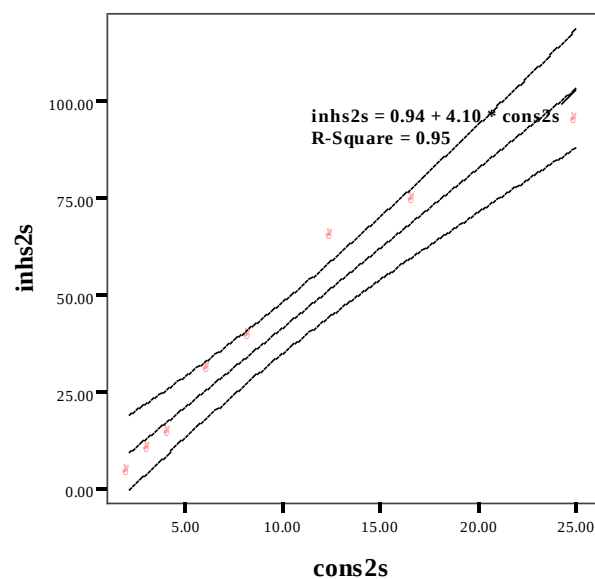


Figure 6.4. EC₅₀ Interpolation Curve of Supernatant of S2

6.3. Sample S3

This sample is collected from the effluent of the oxidation tank of the treatment plant. The pH of the sample is 6.81. Therefore, adjustment of pH is not required.

There are not any precipitated metals in the sample. Metal and COD analysis and toxicity test results of S3 are given in Table 6.3.

Table 6.3. Metal and COD Analyses and Toxicity Test Results of Sample S3

mg/l	S3
pH	6.81
Zn	204
Ni	150
Cu	46.2
Cr	19.1
Fe	14.1
Mn	5.6
Cd	0.7
CN ⁻	0.06
COD	530
EC ₅₀ (%)	1.77

EC₅₀ interpolation curves are convenient to common S-shaped (sigmoid) toxicity curve for S3. R² value of linear regression of the test results for S3 is 0.95 and all of the points of test results are in the 95 % confidence limit for S3. Interpolation curve of S3 is given in Figure 6.5.

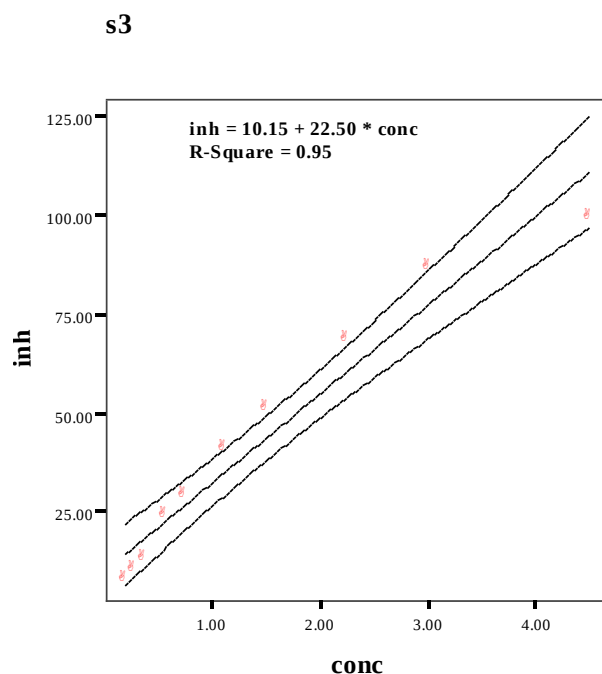


Figure 6.5. EC₅₀ Interpolation Curve of S3

6.4. Sample S4

This sample is collected from the effluent of the treatment plant. The pH of the sample is 5.88. After the adjustment of pH, any precipitation of metals in the sample is not observed. Metal and COD and toxicity test results are given in Table 6.4.

Table 6.4. Metal and COD Analyses and Toxicity Test Results of Sample S4

mg/l	S4
pH	5.88
Zn	0.1
Ni	0.4
Cu	1.3
Cr	0.3
Fe	<0.5
Mn	<0.3
Cd	<0.2
CN ⁻	0.06
COD	540
EC ₅₀ (%)	4.22

All the metal concentrations are proper to discharge the wastewater to a receiving water body, while COD concentration is not. Apparently, since there is no organic matter removal unit in the treatment plant, the COD concentration of effluent wastewater is more or less the same as the COD concentration of influent wastewater. According to İSKİ standards for discharge to sewer system, not only metal concentrations, but also COD concentration are proper to discharge of wastewater to sewer system.

EC₅₀ interpolation curves are convenient to common S-shaped (sigmoid) toxicity curve for S4. R² value of linear regression of the test results for S4 is 1.00 and all of the points of test results are in the 95 % confidence limit for S4. Interpolation curve of S4 is given in Figure 6.6.

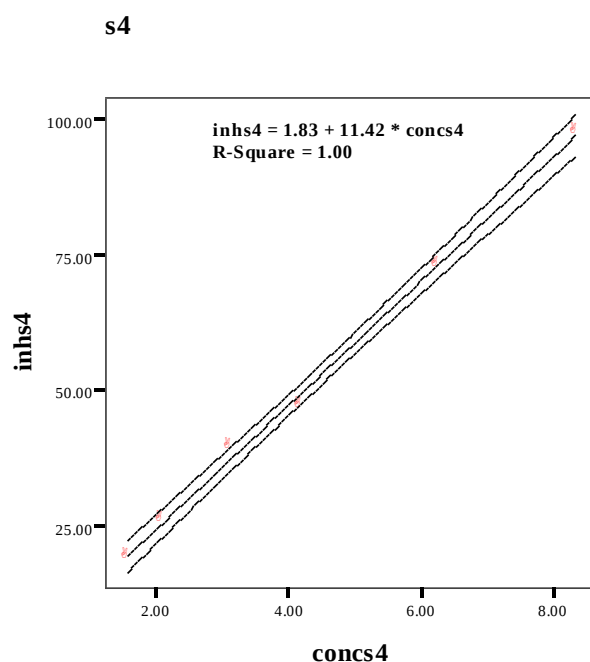


Figure 6.6. EC₅₀ Interpolation Curve of S3

6.5. Sample C1

This sample collected from first precipitation tank is in sludge form. The mobility of metals from sludge to extract is found using TCLP. Metal and COD and toxicity test results of C1 are given in Table 6.5.

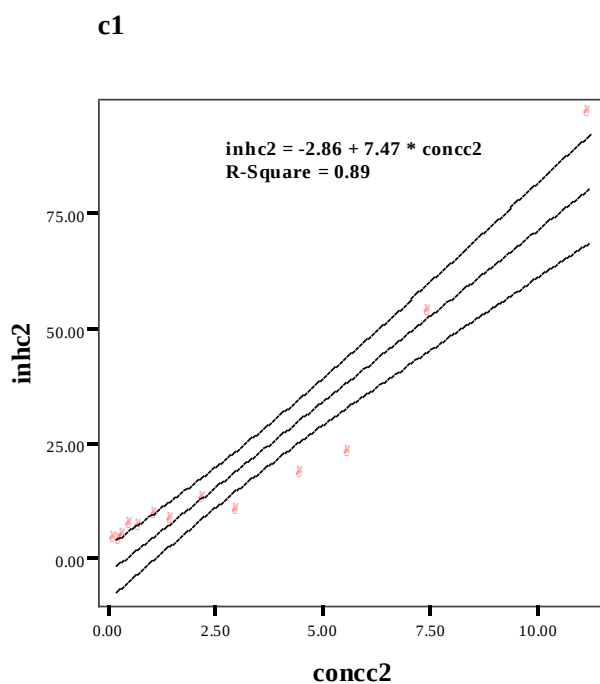
Table 6.5. Metal and COD Analyses and Toxicity Test Results of Sample C1

mg/l	C1
Zn	26.3
Ni	57.5
Cu	14.3
Cr	0.3
Fe	5.4
Mn	<0.3
Cd	<0.2
COD	200
EC ₅₀ (%)	7.1

The assessment of the mobilities is done using the influent metal concentrations to the treatment plant. It is apparent that the concentrations of metals in the sludges of treatment plant units cannot be more than influent concentrations.

The most mobile metals are nickel, zinc, and copper. The mobility of chromium, iron, manganese, and cadmium is not significant. Organic content's mobility is moderate.

EC₅₀ interpolation curves are convenient to common S-shaped (sigmoid) toxicity curve for C1. R² value of linear regression of the test results for S4 is 0.89. Four points of test results are beyond 95 % confidence limit for C1. If this point is removed, EC₅₀ value slightly (0.2 %) changes. Interpolation curve of C1 is given in Figure 6.7



6.6. Sample C2

This sample collected from second precipitation tank is in sludge form. The mobility of metals from sludge to extract is found using TCLP. Metal and COD analysis and toxicity test results of C2 are given in Table 6.6.

Table 6.6. Metal and COD Analyses and Toxicity Test Results of C2

mg/l	C2
Zn	2.2
Ni	2.6
Cu	33.3
Cr	3
Fe	29.2
Mn	<0.3
Cd	<0.2
COD	95
EC ₅₀ (%)	5.3

The assessment of the mobilities is done, same as C1, using the influent metal concentrations to the second precipitation tank. It is apparent that the concentrations of metals in the sludges of treatment plant units cannot be more than influent concentrations of the metals to the treatment unit.

The most mobile metals for C2 are copper and iron. The mobility of chromium, zinc, nickel, chromium, manganese, and cadmium is not significant. Organic content's mobility is also moderate for C2.

EC₅₀ interpolation curves are convenient to common S-shaped (sigmoid) toxicity curve for C1. R² value of linear regression of the test results for S4 is 0.88. All of the test results are in the 95 % confidence limit for C2. Interpolation curve of C2 is given in Figure 6.8.

c2

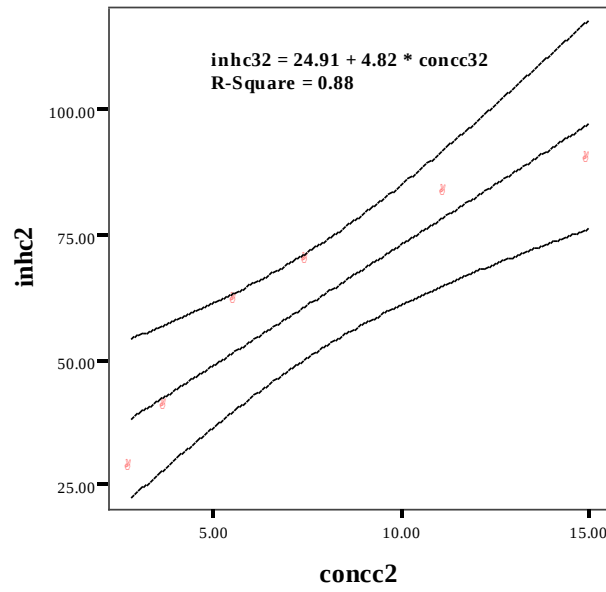


Figure 6.8. EC₅₀ Interpolation Curve of C2

7. CONCLUSIONS

7.1. Interaction of Metals and Cyanide

Wastewaters have very complex pollutant matrices. Each pollutant may affect each other by means of toxicity. Therefore, complexity increases with increasing number of elements. Interaction of metals and cyanide by means of toxicity in the wastewater samples is assessed in this section. Metal concentrations in the wastewater diluted up to its EC₅₀ value are compared with individual EC₅₀ values of the metals. Some significant differences identified during this comparison. The interaction of metals and cyanide for S1 and S1S is indicated in Table 7.1 and for S2 and S2S in Table 7.2.

Table 7.1. Interactions of Metals and Cyanide for S1 and S1S

(mg/l)	S1	conc*%EC ₅₀	S1S	conc*%EC ₅₀	Individual EC ₅₀ s (mg/l)
Zn	261	10.40	130	6.76	0.7 (Choi and Meier 2001)
Ni	104	4.16	56	2.89	17.7 (Choi and Meier 2001)
Cu	57	2.28	14.9	0.77	0.5-2 (Choi and Meier 2001)
Cr	64	2.56	3	0.16	16-58 (Choi and Meier 2001)
Fe	30	1.20	0.91	0.05	22 (McCloskey et al. 1996)
Mn	4.39	0.18	4.04	0.21	450 (Newman and McCloskey 1996)
Cd	0.63	0.03	0.19	0.01	4.7 (Gutierrez et al. 2002)
CN ⁻	19.4	0.78	-		5 (Liu et al. 2002)
EC ₅₀ (%)	4.47		5.83		-

For copper there is no difference in means of toxicity between being alone and being in a mixture. Copper has the same effect on *Vibrio Fischeri* in mixture with being individual. There is an additive response for copper when it is in this wastewater.

Toxic effect of zinc is lower when it is in a wastewater (complex mixture) then when it is alone. This means the zinc's participation to toxicity of the wastewater is relatively low. Accordingly, there may be an antagonistic interaction.

There are very big synergistic interaction between all metals and cyanide increasing toxicities of individuals in the mixture. The individual 30 minute EC₅₀ values of nickel, chromium, iron, manganese, cadmium, and cyanide is much higher then EC₅₀ values in the wastewater.

Table 7.2. Interactions of Metals and Cyanide for S1 and S1S

(mg/l)	S2	conc*%EC ₅₀	S2S	conc*%EC ₅₀	Individual EC ₅₀ s (mg/l)
Zn	231	2.54	173	18.51	0.7 (Choi and Meier 2001)
Ni	152.8	1.85	124	17	17.7 (Choi and Meier 2001)
Cu	65	0.72	23	2.51	0.5-2 (Choi and Meier 2001)
Cr	76	0.84	19.7	2.11	16-58 (Choi and Meier 2001)
Fe	57	0.63	12.4	1.33	22 (McCloskey et al. 1996)
Mn	3.46	0.04	3	0.33	450 (Newman and McCloskey 1996)
Cd	0.49	0.01	0.3	0.03	4.7 (Gutierrez et al. 2002)
EC ₅₀ (%)	1.3		11.97		-

When the same assessment method is used for S2 and S2S, we can easily achieve correlated results.

Synergistic effect for nickel, chromium, iron, manganese, and cadmium

Antagonistic interaction for zinc

Additive response for copper.

Antagonistic response to zinc in the wastewater is shown in Figure 7.1 comparing EC₅₀ values in the wastewater with the individual EC₅₀.

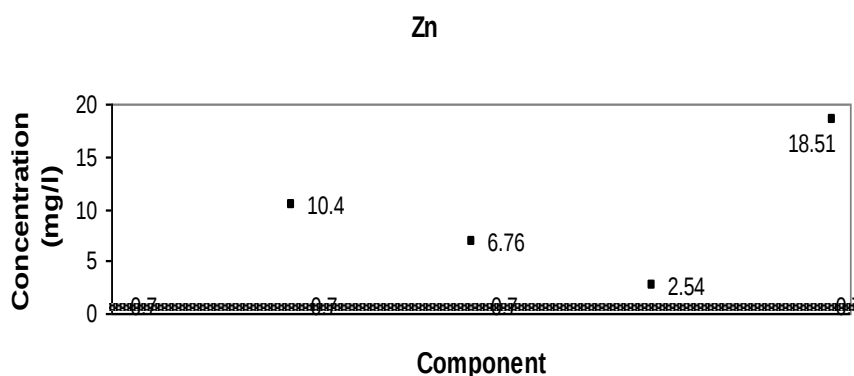


Figure 7.1. Response to Zinc

Individual EC_{50} of zinc is given in literature as 0.7 mg/l, but the EC_{50} s in the wastewater differentiate about 5 to 27 times more than individual EC_{50} value which indicates zinc's participation to the toxic characteristic of the wastewater is much lower than it would be. This concludes that there is antagonistic interaction decreasing zinc's toxicity between zinc and some other material in the wastewater.

Synergistic response to cadmium and chromium in the wastewater is shown in Figure 7.2 and Figure 7.3 respectively comparing EC_{50} values in the wastewater with the individual EC_{50} .

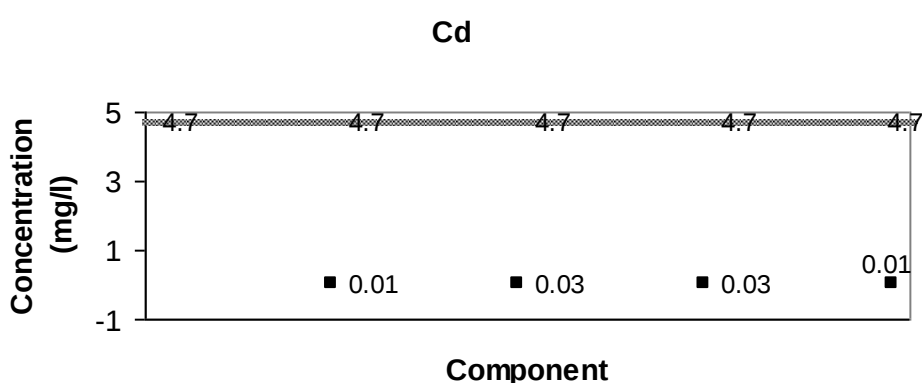


Figure 7.2. Response to Cadmium

Individual EC_{50} of cadmium is given in literature as 4.7 mg/l, but the EC_{50} s in the wastewater differentiate about 100 to 400 times less than individual EC_{50} value which indicates cadmium's participation to the toxic characteristic of the wastewater is much higher than it would be. This concludes that there is synergistic interaction increasing cadmium's toxicity between cadmium and some other material in the wastewater.

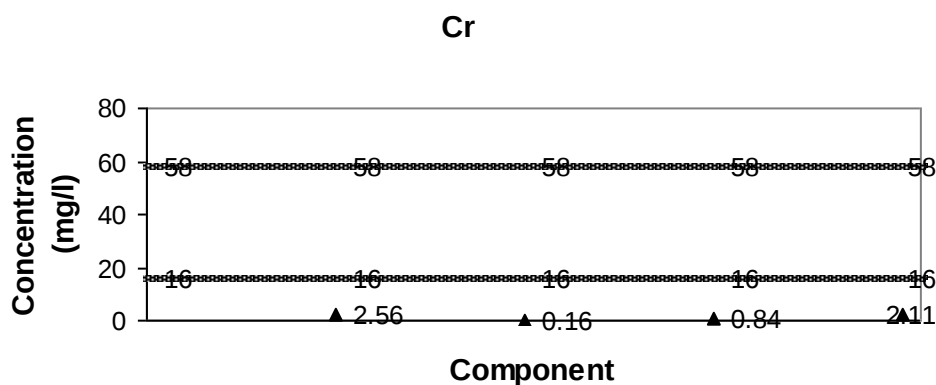


Figure 7.3. Response to Chromium

Individual EC_{50} of chromium is given in literature in the interval of 16 to 58 mg/l, but the EC_{50} s in the wastewater differentiate about 10 to 100 times less than individual EC_{50} values which indicates chromium's participation to the toxic characteristic of the wastewater is much higher than it would be. This concludes that there is synergistic interaction increasing chromium's toxicity between chromium and some other material in the wastewater.

All of these interactions show us that toxic materials' behaviour varies, when they are in a complex mixture. These variations cannot be estimated, interpolated, or assessed without any experimental data. All mixtures have their own toxic characteristic and toxic characteristics of all materials in a mixture depend mixture composition.

7.2. Evaluation of Entire Data

Metal, cyanide, COD concentrations and EC_{50} values of each sample are given in Table 7.3.

Table 7.3. Concentrations and EC₅₀ Values

(mg/l)	S1	S1 Supernatant	S2	S2 Supernatant	S3	S4	C1	C2
pH	2.13	6.88	3.45	7.01	6.81	5.88		
Zn	261	130	231	173	204	0.1	26.3	2.2
Ni	104	56	152	124	150	0.4	57.5	2.6
Cu	57	14.9	65	23	46.2	1.3	14.3	33.3
Cr	64	3	76	19.7	19.1	0.3	0.3	3
Fe	30	0.91	57	12.4	14.1	<0.5	5.4	29.2
Mn	4.39	4.04	3.46	3	5.6	<0.3	<0.3	<0.3
Cd	0.63	0.19	0.49	0.3	0.7	<0.2	<0.2	<0.2
CN ⁻	19.4	-	-	-	0.06	0.06	-	-
COD	565	470	565	530	530	540	200	95
EC ₅₀ (%)	4.47	5.83	1.3	11.97	1.77	4.22	7.1	5.3

EC₅₀ values are generally increasing with decreasing metal and cyanide concentrations which means toxicity is decreasing with decreasing concentrations. However, some samples do not suit this conclusion. For instance, although there is no significant change in metal concentrations between S1 and S2-S3 couple there is four times decrease in EC₅₀ values. It is apparent that the compounds of the wastewater are unknown other than metals, cyanide and COD and it is impossible to analyze them. Moreover, only COD concentration which is a collective parameter is known to show organic content, so organic content distribution is unknown and it is also impossible to analyze them. In the light of these constraints, there may be some conclusions conducted such as organic materials' structure may be changed into a more toxic form, some small changes in metal concentration may trigger the toxic response, and addition of treatment chemicals in order to reduction and precipitate of metals such as NaHSO₃ and Ca(OH)₂ may cause toxic effect to *Vibrio Fischeri*.

EC₅₀ value of S4 is about the same as EC₅₀ value of S1, although metal and cyanide concentrations of S4 are much lower than S1's. It is predicted that this is due to the action of HOCl, present in S4, as an oxidizing agent, which eliminates CN⁻ in the wastewater. Moreover, there are also metals in low concentrations. This amount of concentrations may also have adverse effects.

EC₅₀ changes throughout the treatment plant are shown in Figure 7.4.

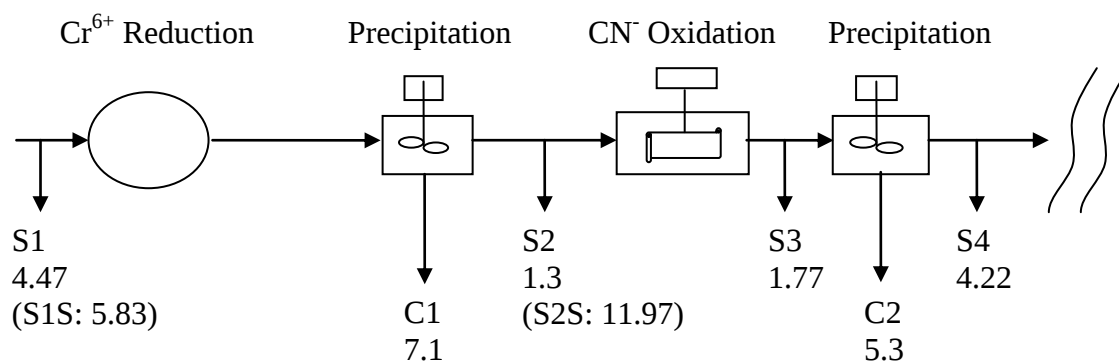


Figure 7.4. The Treatment Plant with EC_{50} Values of Each Sample

Although the scope of the study was not the monitoring the treatment efficiency it can be also concluded, with regard to monitoring the treatment plant; that, (1) there is no organic content removal unit in the plant. Therefore, all of the organic content goes without treatment. (2) Although treatment plant removes metals and cyanide from wastewater it has already toxic effects, so treatment plant cannot remove the toxicity of wastewater. (3) The treatment plant is not well designed and operated.

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