CHAPTER 1

INTRODUCTION

The growing development of agricultural, industrial, and urban activities has given rise to a number of environmental problems. A wide range of human activities contributes to the heavy metal pollution of aquatic environments. The major activities include mining and ore processing, coal and fuel combustion, industrial processing and agricultural processing (fertilizers, pesticides, herbicides, etc.). In the last decades, these activities have led to a dramatic increase in the amount of effluents discharged into natural environments. Among the types of chemical substances of environmental interest, heavy metals play a special role due to their toxicity and bioaccumulation properties [1, 2].

The retention of heavy metals by soil depends on factors such as the nature of mineral and organic constituents, the nature of heavy metal, the composition of the soil solution, and its pH. The processes of heavy metal retention by solid phases of soil and sediment are controlled by different mechanisms: adsorption to surface-active minerals and humic constituents (ionic exchange, specific adsorption); diffusion in the primary and secondary mineral structures; and precipitation [3].

In recent years, the fluxes of trace metals from terrestrial and atmospheric sources to aquatic environments have increased continuously. Heavy metal contaminants entered to aquatic environments can have dramatic effects on the bioavaiability and the toxicity of biological processes. The determination of heavy metal concentrations in environments is therefore very important with regard to their toxicity and biogeochemical cycles in environments [4, 5]. At present, the necessity to advance our knowledge of the mobility of chemicals in soils and the physicological activities of inorganic elements makes necessary not only the determination of total amount of trace elements but also the exact determination of their concentrations for different chemical forms. Speciation analysis normally refers to the determination of metal species and metalloids including organometallic compounds. As a matter of fact, speciation is very significant when applied to trace elements because analytical difficulties are related to the choice of relevant techniques for measurement of individual species [6].

1.1 Definition of Heavy Metals

The term "heavy metal" is entrenched in the literature of environmental pollution. A heavy metal is a metallic element with a density greater than 5 g/cm^3. After a new classification of heavy metals by Neibore and Richardson in 1980 [7], Martin and Coughtrey introduced a list of 27 elements, which commonly encountered as environmental pollutants under the heading "heavy metals". Heavy metal is rarely used while some terms such as trace metals, trace inorganic, microelements, and micronutrients have been used as synonym one [8]. Another name that has been used for a term of elements is "toxic metal". This name is even less appropriate because all trace elements are toxic to living organisms when they present in excess. For instance, cobalt, copper, manganese, and zinc are toxic at high concentration but they are an essential for normal healthy growth for plants, animals, and human [9, 10]. The World Health Organization (WHO) has classified cadmium and lead as members of the group of very toxic metals. This is because of their detrimental effects even at low levels. It should be noted, however, that all trace elements are predominantly toxic when their levels excess the limits of safe exposure [1, 5].

1.2 Background Information of Heavy Metals

1.2.1 Lead

Lead (Pb), an element of atomic mass 207.2 and atomic number 82, is the commonest of toxic heavy metal elements. In most inorganic compounds lead is in the +2 oxidation state. The salts of Pb (II), lead oxides and lead sulfide, are not readily soluble in water, with exception of lead acetate, lead chlorate, and, to some extent, lead chloride. Inorganic Pb (IV) compounds are unstable and strong oxidizing agents [11]. The lead concentration in soils is commonly found in the range of 2-200 mg/kg and average 16 mg/kg. However, lead compounds are generally insoluble and so poorly transferred from one medium to another [12].

Acute lead poisoning in humans causes severe distortion in kidney, reproductive system, liver, brain, and control nervous system. Mild lead poisoning causes anemia. Lead poisoning from the environmental exposure is thought to have caused metal retardation in many children. The victims may have headache and sore muscles and may feel generally fatigued and irritable [11]. The lead contamination in soil and water is a major problem for both human and animals. The lead level in normal surface soil is typically less than 40 mg/kg, but with increased values according to different sources, for example, metal mining (170-13,000 mg/kg), motorway soil (460-3,600 mg/kg), urban gardens (270-450 mg/kg), and sewage sludge farmland (80-300 mg/kg) [1]. The accepted average values of metal contents in environments and some human tissues are reported as shown in Table 1.1.

The WHO has listed the safe daily intake limit of lead concentration at 300 μg per person. This limit assumes that level of 30 μg/100 g of blood or less is safe [13]. On the other hand, the United State Environmental Protection Agency (U.S. EPA) has showed that the adverse affects of lead absorption by children occur at level of 10 μg/100 g of blood. The United Kingdom standard for lead in food has limited at 1.0 mg/kg, with the exception for baby food, which is 0.2 mg/kg. The EPA and the WHO have limited maximum admissible concentration of lead at 0.05 mg/L for drinking water [1, 14].

1.2.2 Cadmium

Cadmium (Cd), an element of atomic mass 112.4 and atomic number 48, is not known to be biologically essential or beneficial to human body. Cadmium at very low concentrations can be toxic to aquatic organisms. It is a rather rare element in the earth's crust and present in the concentration ranging of 0.1-0.5 μg/g (average 0.15 μg/g). Cadmium, like other heavy metals, may exist in a number of forms in water and other environments. These include free cadmium ion, inorganic and organic complexes, and cadmium associated with colloidal and particulate matters [14].

The effects of acute cadmium poisoning are very serious for human such as high blood pressure, the kidney damage, the destruction of testicular tissue, and the destruction of red blood cells. The toxic actions of cadmium can involve the replacement of zinc in certain enzymes that prevents the proper functioning of enzymes.

Table 1.1 Metal levels in environments and human tissues [1, 11].

Location	**Concentration (mg/kg, mg/L)**					
	Pb	**Cd**	**Zn**	**Cu**	**Mn**	**Cr**
Earth's crust[a]	15	0.2	125	75	975	150
Sediments[a]	20	0.5	80	57	760	130
Drinking water[b]	0.05	0.005	5.0	1.0	0.1	0.05
Agricultural soil[c]	50-300	1-3	150-300	40-140	-	-
Sewage sludge[c]	750-1200	20-40	2500-4000	1000-1750	-	-
River water[d]	3	0.02	20	5	7	1
Seawater[d]	0.03	0.1	10	2	0.2	0.05
Surface soils[d]	1.5-80	0.1-2.5	15-125	6-60	7-2000	5-1100
Plant[d]	0.3-10	0.02-0.5	12-60	1-12	20-240	0.02-0.2
Plant foodstuffs[d]	0.05-4	0.008-0.3	1.2-45	0.08-9	1.3.90	0.01-14
Wine[e]	0.3	0.01	5.0	5.0	-	-
Human tissues[f]:						
Brain	-	-	820	375	20	0.8
Kidney	-	-	4900	270	91	2.2
Liver	-	-	3800	680	130	1.5
Lung	-	-	1400	130	24	20
Muscle	-	-	4800	85	6	2.3
Skin	-	-	1000	120	22	41

[a] Average abundances of trace elements in solid phases (mg/kg) [11].

[b] Standards and guidelines for metals in environments recommended by WHO (mg/L) [11].

[c] Maximum admissible concentrations of toxic metals in sewage sludge recommended by CEC (mg/kg) [11].

[d] Typical natural trace element concentrations (mg/L, mg/kg) [1].

[e] Maximum admissible levels of metals in wine recommended by West-German Wine Ordinance (mg/L) [11].

[f] Concentrations of essential elements in human tissue (mg/kg) [11].

A disease specifically associated with cadmium poisoning has been recognized in Japan known as “itai-itai” (uoch-uoch). The EPA has classified cadmium as a probable human carcinogen. In the USA, the safety level of cadmium for drinking water has been set at 0.01 mg/L while the WHO and the U.S. EPA have limited the safety level at 0.005 mg/L [11].

1.2.3 Zinc

Zinc (Zn), an element of atomic mass 65.4 and atomic number 30, plays an important role as an essential trace element in all living systems. Normally, zinc levels in plants are in the range of 25-150 mg/kg with concentrations above 400 mg/kg being toxic [5]. Zinc is generally found in all human tissues and body fluids. It is essential for healthy growth and reproduction for human. Zinc deficiency symptoms have rarely been described for adults. The deficiencies of zinc can cause serious damage to the skin and the enzyme system of infants and young children. The deficiency of zinc in human leads to dwarfism, reduced rates of blood clotting and wound healing, skin abnormalities, and other problems [11].

The levels of zinc for human health have been limited at 15 mg/day for men and 12 mg/day for women. If large doses of zinc (10-15 times) are taken by mouth even for a short time, stomach cramps, nausea, and vomiting may occur. However, the EPA has determined that zinc is not classifiable as to its human carcinogenicity. The European Union (EU) has limited zinc values in soils and sewage sludge for use in agriculture at 150-300 mg/kg and 2,500-4,000 mg/kg, respectively. For drinking water, the maximum acceptable concentration has been set at 5.0 mg/L [11].

1.2.4 Copper

Copper (Cu), an element of atomic mass 63.5 and atomic number 29, is an essential trace element for life. It is a component of several metalloenzymes and other proteins. The copper-containing protein haemocyanin is also used to transport oxygen in arthropods, having a similar role to iron-containing haemoglobin [15]. Average concentration of copper in the earth's crust is about 50 mg/kg. It also occurs naturally in plants and animals. Copper is not toxic to animals, but toxic to plants and algae at moderate levels.

Copper is necessary for good health. However, very large single or daily intakes of copper can harm for human health. Long-term exposure to copper dust can irritate for nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea. Intentionally high intakes of copper can cause liver and kidney damage and even death. Very young children are sensitive to copper, and long-term exposure to high levels of copper in food or water may cause liver damage and death. Copper is not known to cause cancer. The U.S. Public Health Service (U.S.PHS) has limited the copper concentration at 1.0 mg/L for both natural and drinking waters. The recommended daily intake of copper in food is the range of 0.5-0.7 mg for children in the first year of life and the range of 2.0-3.0 mg or up to 5.0 mg for adults. The WHO has limited maximum admissible concentration of copper at 1.0 mg/L for drinking water. In the food products, the maximum admissible concentrations of copper have been limited at 24.2, 5.6, 127, and 19.8 mg/kg for fruits, mushrooms, tea, and coffee, respectively [11].

1.2.5 Manganese

Manganese (Mn), an element of atomic mass 54.9 and atomic number 25, is normally vital micronutrient for both plants and animals. In human, manganese is involved in the digestion and absorption of food through peptidase activity and in the synthesis of cholesterol and fatty acids. Elemental manganese does not occur naturally as a metal. Frequently, it is found in various salts and minerals in association with iron compounds. Introduction of manganese into environments by human activities is not serious problem because it is only slightly to moderately toxic to aquatic organisms in excessive amounts. The deficiency of manganese causes growth disturbances in plants and a yellowing of the needles of conifers.

The diseases of manganese poisoning are occurred widely for workers who have mainly occupied in manganese ore mills, smelting works, battery factories, and manganese mines. Manganese miners or steel workers exposed to high levels of manganese dust in air may have mental and emotional disturbances, and their body movements may become slow and clumsy. This combination of symptoms is a disease called manganism. Workers usually do not develop symptoms of manganism unless they have been exposed for many months or years. Manganism occurs because too much manganese injures a part of the brain that helps control body movements. Some of the symptoms of manganism can be reduced by medical treatment, but the brain injury is permanent. The WHO has limited maximum admissible concentration of manganese at 0.1 mg/L for drinking water [5].

1.2.6 Chromium

Chromium (Cr), an element of atomic mass 51.9 and atomic number 25, is an element found in many minerals. Chromium exists in all oxidation states form II to VI, but only the trivalent and hexavalent compounds and the metallic chromium are practically important [11]. Chromium as the hexavalent chromate ion is more toxic than the trivalent species. Cr (VI) compounds can reduce plant growth and cause skin inflammation in fish, mammal, and human. After a longer latent time, they produce lung cancer. Chromium (III) is considered essential trace element for the proper functioning of living organisms. It has reported to be responsible for the control of glucose and lipid metabolisms in mammal. Most agencies in as USA, Japan, Germany, and the WHO have limited the safety level of chromium at 0.05 mg/L for drinking water [11].

1.3 Sources of Heavy Metals in Environments

The primary source of heavy metals in environments is from naturally occurring geochemical materials. Natural processes, such as weathering of rocks and volcanic activity continually add heavy metals to the aquatic environment. In addition, this occurrence may be enhanced by anthropogenic activities such as rapid urbanization, industrialization, and mining operations [8]. The trace element contamination in surface soils and sediments increasingly occurs according to growing industrial and agricultural activities. In many cases, soils act as natural buffers controlling the transportation of trace element contaminants to the aquatic environment. Trace metal inputs arise from direct discharges of various kinds of contaminated wastes, disturbance of drainage basins, atmospheric precipitation, and agricultural activities [1, 5].

Most lead used by industry comes from mined ores or from recycled scrap metal batteries. It is used in the production of ammunition, in some kinds of metal products (cheat lead, solder, some brass and bronze product, and pipes), and in ceramic glazes. Some chemical containing lead such as tetraethyl lead (TEL) and tetramethyl lead (TML) were once used as gasoline additives to increase octane rating. Sources of lead in surface water or sediment include deposites of lead-containing dust from the atmosphere, wastewater from industries that handle lead (primary iron and steel industries and lead product), urban runoff, and mining piles.

Cadmium has the following uses: to galvanize metal parts, as a pigment in paints and plastics, in rechargeable nickel-cadmium (ni-cad) batteries, and as a catalyst and preservative in the plastics industry. Some alloys, solders, and pigments in ceramic glazes, oil paints, and other art supplies may contain cadmium. Cadmium contamination in soil, water, and sediment can occur through municipal wastewater, electroplating, metal processing, plastic and dye manufacturing, and the application of phosphate fertilizer. Cadmium enters plants and animals from soil and water thus entering the food supply.

Manganese comprises approximately 0.085-0.95% of the earth's crust at an average concentration of 950 mg/kg. Principal ores of manganese include pyrolusite (MnO_2), manganite ($Mn_2O_3.H_2O$), hausmannite (Mn_3O_4) and rhodocrosite ($MnCO_3$). Manganese in rocks and minerals is naturally released into the environment due to weathering processes. Rocks containing high levels of manganese compounds are mined and used to produce manganese metal. This manganese metal is mixed with iron to make various types of steel. Some manganese compounds are used in the production of batteries, as an ingredient in some ceramics, pesticides, and fertilizers.

Zinc is part of nature. Most rocks and many minerals contain zinc in varying amounts and zinc exists naturally in air, water and soil. The average natural level of zinc in the earth's crust is 70 mg/kg, ranging of 10 and 300 mg/kg. At some locations, zinc has been concentrated to much higher levels by natural geological and geochemical processes. The most commonly found zinc mineral is sphalerite (ZnS). Zinc metal is produced both from ores and from recycled zinc products. A major part of zinc present in surface waters is ultimately deposited in river sediments, estuaries and coastal areas where it binds to inorganic and organic matter, which reduces its bioavailability.

Copper and its compounds are common in the environment. Most copper compounds found in air, water, sediment, soil, and rock are strongly attacked to dust and dirt or imbedded in minerals that they cannot easily affect human health. Copper compounds are most commonly used in agriculture to treat plant diseases, like mildew, or for water treatment and as preservatives for wood, leather, and fabrics. High concentrations of copper may be found in soil because dust from these industries settles out of air, or waste from mining and other copper industries are disposed of on soil.

All compounds of chromium are colored; the most important are sodium and potassium chromates, dichromates, and potassium and ammonium chrome alums. Dichromates are used as oxidizing agents in quantitative analysis, also in tanning leather. Other compounds are of industrial values; lead chromate is chrome yellow, a valued pigment. Chromium compounds are used in the textile industry as mordants, and other industries for anodizing aluminum. Applications of chromium compounds are found in the following industries; printing (photomechanical reproduction processes), oil (as anti-corrosives), and fireworks (additive to the inflammable mixture) [11].

The overall role of trace elements in living organisms is directly related to the interactions within environments such as geological, biological, or marine systems. For instance, trace element composition in soils may significantly influence the elemental composition of the vegetation, which in turn influences that of animal or human tissues or fluids via the food chain. Figure 1.1 shows a general schematic of the movement of trace elements within the environment [1]. The cycling of trace metals involves a common set of biogeochemical processes as illustrated schematically in Figure 1.2. Processes that remove metals to solid phases are particularly important in migrating of the effects of contaminant metals introduced into aquatic ecosystems [16].

1.4 Physico-Chemical Forms of Heavy Metals in Sediments

Sediment consists of a complex mixture of soil phases that may include silica, clays, oxides of iron-manganese, solid organic matter, carbonates, sulfides, and minerals [17]. Heavy metals may be associated with sediments in the following ways:

i) loosely adsorbed, either as metal ions or as hydroxy-and carbonate species;

ii) precipitated or co-precipitated, particularly with iron and manganese oxides;

iii) strongly associated with solid organic matter; and

iv) incorporated in the crystalline structures.

Sediments do not always act as sinks for heavy metals. They may also release metals back to water depending on the presence of different geochemical phases [8].

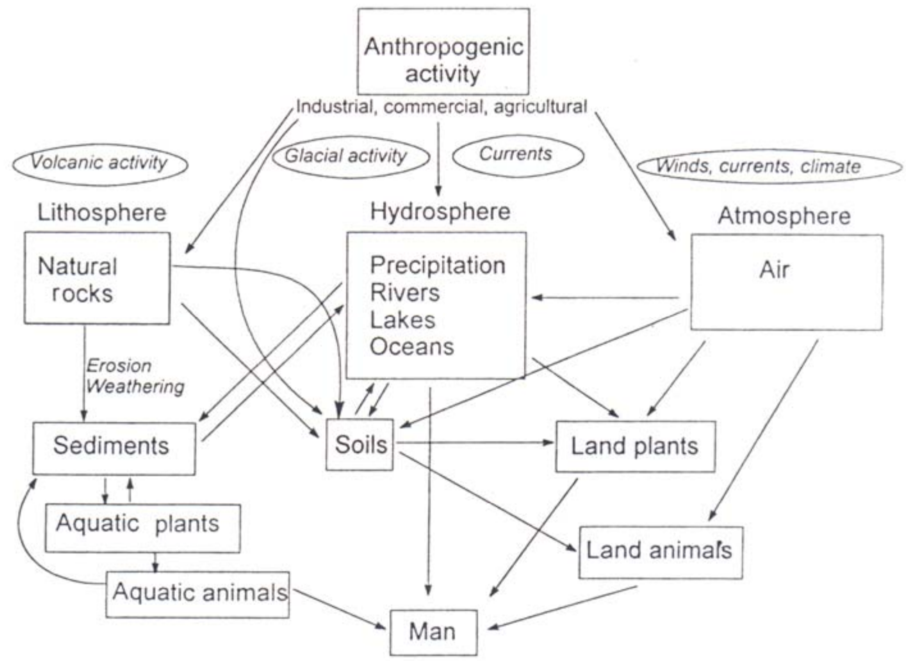

Figure 1.1 The movement of trace elements within the environment [1].

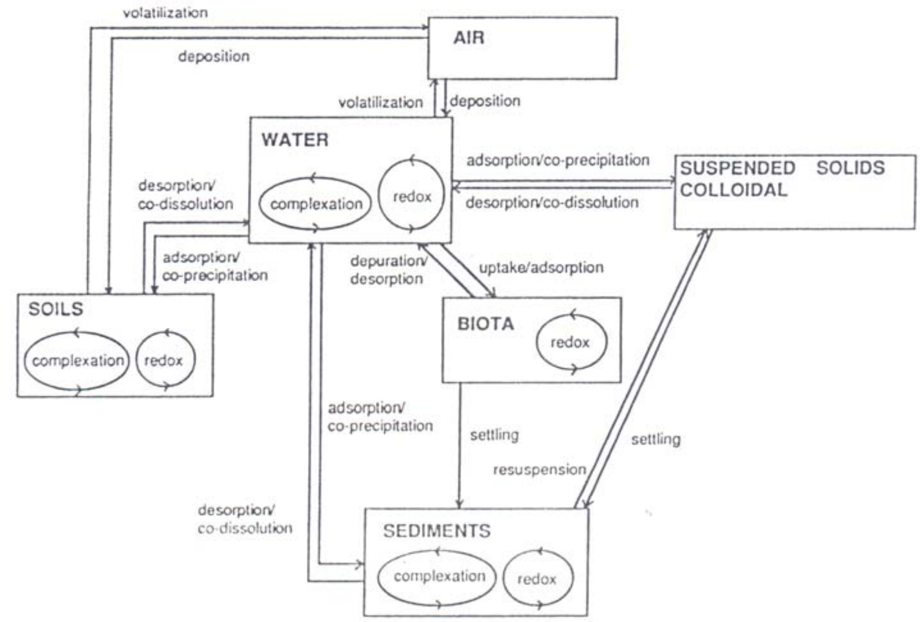

Figure 1.2 Schematic diagram of processes controlling the biogeochemical cycling of heavy metals in aquatic environments [16].

1.5 Speciation of Heavy Metals for Soil and Sediment Studies

Within the terrestrial ecosystem, soils play a major role in element cycling and accumulate heavy metals in concentration orders of magnitude higher than in water and air. Heavy metals take part in biogeochemical cycles and are not permanently fixed in soils; therefore, assessment of metal distribution in soils is a key issue in many environmental studies. The knowledge of metal binding with different soil phases and components is of major interest to assess the connections with other biotic and abiotic elements of the environment. It is widely recognized that to assess the environment impact of soil pollution, the determination of metal speciation will give more information about the potential for release of contaminants and further derived processes of migration and toxicity. Therefore, in geoenvironmental studies of risk assessment, chemical partitioning among various geochemical phases is more useful than measurements of total heavy metals contents [18]. In practice, environmental studies involving soil and sediment analysis are based on the use of leaching or extraction procedures (e.g. single or sequential extraction) [19].

Most studies dealing with particular metals in natural water systems (i.e., metals associated with suspended matter or bottom sediments) concern total metal concentration. Relatively few attempts have been made to evaluate the speciation of particular metals, i.e., the partitioning among the various forms in which they might exist. Use of total concentration as a criterion to assess the potential effects of sediment contamination implies that all forms of a given metal have an equal impact on the environment; such an assumption is clearly untenable [20].

Conceptually, the solid material can be partitioned into specific fractions that can be extracted selectivity by using appropriate chemical reagents. Several experimental procedures, varying in manipulative complexity, have recently been proposed for determining the speciation of particular trace metals. These procedures can be grouped into (i) methods designed to effect the separation between residual and non-residual metals only and (ii) more elaborate methods making use of sequential extractions. The former methods normally involve a single extraction and offer a better contrast between anomalous and background samples than does the determination of total metal concentration [20]. Single extraction methods provide some information e.g. metal availability for plant uptake and potential mobilization of metals under different extraction conditions. The use of a series of extracting solution with gradual increase in extraction strength was thereafter designed. This method is called sequential extraction. Both types of extraction procedures are applied using not only different extraction schemes but also different laboratory conditions.

1.5.1 Single Extraction Method

Single extraction procedures have been designed to dissolve phase whole element content is correlated with the availability of element to plants. This approach is well established for major elements and nutrients and it is commonly applied in studies of fertility and quality of crops, for predicting the uptake of essential elements, for diagnosis of deficiency or excess of one element in soil, in studies of the physico-chemical behaviour of elements in soil, and for survey purposes. Single extraction methods have been sometimes modified by changing types of extracting solutions such as NH_4OAc, $MgCl_2$, HNO_3, CH_3COOH, etc. Single extraction provides information of environmental effect, related to mobility and phytoxicity of heavy metals. Several environmental agencies undertake the single extraction method in evaluation of toxicity of solid waste. A large number of test procedures have been designed particularly for soil studies. These partly used organic chelators such as EDTA and DPTA, either as single extracting solution or in sequential extraction procedure [21].

1.5.2 Sequential Extraction Method

Sequential extraction method provides detailed information about the origin, speciation, mobility, and bioavailability of metals with different environmental conditions. Sequential extraction is based on subsequent elution by a series of chemical solutions, each of which digests a particular bonding form, thus releasing the percentage of elements fixed in the bonding form. Sequential extraction method can be used qualitatively to monitor metal potential bioavailability in response to changing environmental conditions [22]. The ability of various extracting solutions to release metal ions depends on their association with particular soil fractions. Extracting solutions like electrolytes, weak acids, and chelating agents release metals from coordination sites, while strong acids and other redox agents are capable of releasing additional quantities of metal as a result of the decomposition of the solid matrix.

In fact, the sequential extraction methods provide more information than single extraction and have several advantages [6].

i) extractive procedures applied are comparable to those occurring in nature. In natural environments, soils and sediments are subject to similar leaching procedures by natural and anthropogenic electrolyte solutions.

ii) the total sum of all fractions should be more-or-less 100%, so the results are self-checking.

iii) it is an essential tool in establishing element partitioning with natural samples.

iv) chemical extraction sequences can be used for the estimation of the potential remobilization of metals under changing environmental conditions.

Within a scheme of sequential extraction scheme, extracting solutions more commonly used are generally applied according to the following order: unbuffered salts, weak acids, reducing agents, oxidizing agents, and strong acids. Therefore, sediment phases might be called following extracting solution properties such as exchangeable, acid-soluble, reducible, and oxidizable. One of the most widely applied procedures was proposed more than 20 years ago by Tessier *et al.* It partitions elements into five operationally defined geochemical fractions including exchangeable, bound to carbonate, bound to Fe-Mn oxide, bound to organic matter, and residual [23]. The types of extracting solutions and extraction conditions proposed for sequential extraction method are summarized in Table 1.2, 1.3, 1.4, 1.5, and 1.6 for exchangeable, bound to carbonate (acid-soluble), bound to Fe-Mn oxide (reducible), bound to organic matter (oxidizable), and residual fractions, respectively. Modified Tessier's sequential extraction methods have been widely used to study the distribution of metals among various geochemical forms as shown in Table 1.7.

Table 1.2 Some extracting solutions and conditions commonly used for exchangeable fraction (F-I).

Reagent	Conc. (M)	pH	Temp. (°C)	Time (h)	Ref.
$MgCl_2$	1.0	7	RT	1	[3, 18, 20, 24-29]
$MgCl_2$	0.4	4.5	RT	1	[30]
$MgCl_2$	0.5	7	RT	20 min	[31]
NH_4NO_3	0.1	-	RT	2	[19]
$CaCl_2$	0.01	-	RT	2	[32]
HOAc	0.11	2	RT	16	[33-40]
HOAc	0.11	2	50	16	[41]
NH_4OAc	1.0	7	RT	2	[42]
NH_4OAc	1.0	7	RT	5	[43]
NH_4OAc	0.01	7	RT	2	[44]
NaAOc	1.0	8.2	RT	1	[45]

Table 1.3 Some extracting solutions and conditions commonly used for bound to carbonate fraction (F-II).

Reagent	Conc. (M)	pH	Temp. (°C)	Time (h)	Ref.
NaOAc/HOAc	1.0	5	RT	6	[3, 18, 20, 24-29, 31, 42]
NaOAc/HOAc	1.0	5	RT	5	[27, 45]
NaOAc/HOAc	0.5	5	RT	2	[30]
EDTA	0.005	-	RT	2	[19]
EDTA	0.01	-	RT	4	[32]
HOAc	0.1	3.5	RT	1	[43]

Table 1.4 Some extracting solutions and conditions commonly used for bound to Fe-Mn oxide fraction (F-III).

Reagent	Conc. (M)	Medium	pH	Temp. (° C)	Time (h)	Ref.
$NH_2OH \bullet HCl$	0.04	25% v/v HOAc	2	96	6	[3, 18, 20, 24, 28, 29, 31]
$NH_2OH \bullet HCl$	0.1	25% v/v HOAc	3	70	6	[30]
$NH_2OH \bullet HCl$	0.4	25% v/v HOAc	2	96	6	[25, 43]
$NH_2OH \bullet HCl$	0.04	25% v/v HOAc	2	80	6	[42]
$NH_2OH \bullet HCl$	0.1	HNO_3 2 M	2	RT	16	[26, 33, 34, 36, 38-40]
$NH_2OH \bullet HCl$	0.5	HNO_3 2 M	1.5	RT	16	[35]
$NH_2OH \bullet HCl$	0.1	HNO_3 2 M	1.5	50	16	[42]
$NH_2OH \bullet HCl$	0.05	HNO_3 2 M	2	RT	30 min.	[45]
$NH_4C_2O_4$	0.2	$H_2C_2O_4$ 0.2 M	3.3	20	4	[46]
$K_4P_2O_7$	0.1	deionized water	-	20	24	[28]

Table 1.5 Some extracting solutions and conditions commonly used for bound to organic matter fraction (F-IV).

Reagent	Extractable step	Ref.
30 % $H_2O_2^{*}$/0.02 M HNO_3/3.2 M NH_4OAc^{**}	1) 3 mL HNO_3+5 mL H_2O_2, 85 °C for 2 h 2) 5 mL H_2O_2, 85 °C for 3 h 3) 5 mL NH_4OAc, RT for 30 min.	[3, 18, 20, 26, 28, 29, 31, 42, 43, 45]
8.8 M $H_2O_2^{*}$/0.02 M HNO_3/3.2 M NH_4OAc^{**}	1) 10 mL H_2O_2, 85 °C for 1 h 2) 10 mL H_2O_2, 85 °C for 1 h 3) 50 mL NH_4OAc, RT for 16 h	[33-37]
9.8 M $H_2O_2^{*}$/0.02 M HNO_3/3.2 M NH_4OAc^{**}	1) 8 mL H_2O_2, 85 °C for 1 h 2) 8 mL H_2O_2, 85 °C for 1 h 3) 40 mL NH_4OAc 1 M, RT for 16 h	[41]
30 % $H_2O_2^{*}$/0.02 M HNO_3/3.2 M NH_4OAc^{**}	1) 3 mL HNO_3+5 mL H_2O_2, 90 °C for 2 h 2) 5 mL H_2O_2, 90 °C for 3 h 3) 5 mL NH_4OAc 3.2 M, RT for 30 min.	[44]
0.05 M Na_2-EDTA, pH 7	6 h for room temp.	[46]
0.1 M $Na_4P_2O_7$, pH 9.7	12 h for room temp.	[46]

*adjusting to pH 2 with HNO_3.

**prepared in 20% v/v HNO_3.

Table 1.6 Some extracting solutions and conditions commonly used for residual fraction (F-V) and total metal contents.

Reagent	Volume ratio (mL)	Procedure step	Ref.
HF/HClO$_4$	10/2 10/1 0/1	1) 10 mL HF+2 mL HClO$_4$, heated to near dryness 2) 10 mL HF+1 mL HClO$_4$, heated to near dryness 3) 1 mL HClO$_4$ to white fumes	[3, 20, 24, 38]
HF/HNO$_3$/ HClO$_4$	10/15/1	Microwave digestion at 400 W, 2 h	[34]
HF/HNO$_3$/ HClO$_4$	10/3/1	Microwave digestion at 750 W, 6 h	[25, 43]
HF/HNO$_3$/ HClO$_4$	15/4/2	Heat at 90 °C to near dryness	[31]
HCl/ HNO$_3$	7.5/2.5	Reflux for 2 h	[35]
HF/HNO$_3$	10/2	1) PTFE bomb at 180 °C, 6 h 2) 10 mL HNO$_3$ at 180 °C to dryness	[22]
HF/HNO$_3$	2/2	1) heated at 100 C, 12 h 2) 1.5 mL H$_2$O$_2$, at 70 C, 2 h	[36]
HNO$_3$ (7 M)	20	Heat at 80 °C, 6 h	[42]
HF/HNO$_3$	7/7	Microwave digestion	[18]
HF/H$_2$SO$_4$	10/1	Heated to near dryness at 80 °C	[29]
HCl/ HNO$_3$	3/1	Microwave digestion at 850 W, 50 s (four times)	[40]
HNO$_3$/HF/HClO$_4$/ HCl	4/1/1/1	Heat for 3 h in water-bath	[45]

Table 1.7 Extracting solutions used for sequential extraction schemes modified from Tessier’s method for the determination of heavy metals in environmental samples.

Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	References
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HClO_4$	Tessier *et al.* (1979) [20]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	-	Diks *et al.* (1983) [cited in 22]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HClO_4HCl$	Lee *et al.* (1984) [cited in 22]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HNO_3/HCl$	Gibson *et al.* (1986) [cited in 22]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HClO_4$	Rauret *et al.* (1989) [47]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	HNO_3	Oughton *et al.* (1992) [42]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HNO_3$	$K_4P_2O_7$	-	Howard *et al.* (1995) [27]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	HF/HNO_3	Raksasataya *et al.* (1996) [48]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HClO_4$	Perez *et al.* (1996) [49]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HNO_3/HClO_4$	Jones *et al.* (1997) [28]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_4C_2O_4$	$H_2O_2/\ NH_4OAc$	$HF/HNO_3/H_2O_2$	Mester *et al.* (1998) [50]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_4C_2O_4$	$H_2O_2/\ NH_4OAc$	HNO_3	Usero *et al.* (1998) [51]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	$H_2O_2/\ NH_4OAc$	$HF/HNO_3/HCl$	Gomez *et al.* (1999) [25]

Table 1.7 Extracting solutions used for sequential extraction schemes modified from Tessier's method for the determination of heavy metals in environmental samples (continued).

Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	References
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	-	Perez *et al.* (1999) [52]
NH_4OAc pH 7	NH_4OAc, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	-	Gomez *et al.* (2000) [43]
NH_4OAc pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HNO_3$	H_2O_2/ NH_4OAc	HNO_3	Ostrega *et al.* (2001) [44]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$HF/HClO_4$	Morera *et al.* (2001) [3]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$HF/HNO_3/HClO_4$	Li *et al.* (2001) [31]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$HF/HNO_3/HClO_4$	Perez *et al.* (2001) [53]
$MgCl_2$ pH 4.5	NH_4OAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$LiBO_3/HF$	Kurosaki *et al.* (2002) [30]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$HF/HNO_3/HClO_4$	Navas *et al.* (2003) [18]
$MgCl_2$ pH 7	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	HF/H_2SO_4	Poledniok *et al.* (2003) [29]
$MgCl_2$ pH 7 or NaAOc pH 8.2	NaOAc/HOAC, pH 5	$NH_2OH \bullet HCl/HOAc$	H_2O_2/ NH_4OAc	$HNO_3/HF/HClO_4/$ HCl	H. Akcay *et al.* (2003) [45]

1.6 Selection of Sequential Extraction Schemes

Since 1980, several schemes of sequential extraction methods have been widely proposed for soils or sediments or solid wastes as reported on several publications [20, 25, 37-41, 54]. The schematics of sequential extraction procedures may be performed three to eight steps [22]. The choice of the type of procedure depends on the aim of the study. To obtain information about the available fraction of metals, a single step procedure is usually applied. If more information is required, sequential extraction procedures are an essential tool for understanding the origin, mobilization, transport of trace metals [55]. The sequential extraction method proposed by Tessier *et al.* [20] has often been thoroughly studied by several researchers [3, 18, 24-27, 29, 30, 33, 43, 45, 47, 48]. This method has been usually employed as origin or modified extraction conditions for both conventional and microwave assisted extractions. The selection of extracting solutions for each fraction is described as below steps.

F-I: Exchangeable Fraction

Metals extracted from the exchangeable fraction would include weakly-sorbed metal species, particularly those retained on the soil surface by relatively weak electrostatic interactions and those that can be released by ion-exchange processes. Reagents used for this purpose are electrolytes in aqueous solutions such as salts of strong acids and bases or salts of weak acids and weak bases at pH 7 [23]. The most popular reagent is magnesium chloride (1 M $MgCl_2$) which combines the rather strong Mg^{2+} ion-exchange capacity with the weak complexing ability of Cl^-. This reagent does not attack organic matter, silicates, and metal sulfides.

Extraction with acetate salts (particularly ammonium acetate) has also been used frequently in soil and sediment studies. Divalent cations should, in general, be more effective than monovalent cations in removing exchangeable ions, but NH_4^+ promotes replacement of ions in the interlayer exchange sites of vermiculite. Other reagents showing similar properties have seldom been used such as nitrate salts ($Mg(NO_3)_2$, NH_4NO_3, KNO_3, etc.) and calcium salts ($CaCl_2$, NH_4Cl, $Ca(NO_3)_2$, etc.) [23].

F-II: Bound to Carbonate Fraction

Several works have shown that significant trace metal concentrations could be associated with sediment carbonates. In this partitioning fraction, released metals should be susceptible to pH changes [20]. The chemical properties of extracting reagents for this fraction should dissolve carbonate-bound metals. A buffered acetic acid/sodium acetate solution at pH 5 is generally used for several works. The pH of the extracting solution is usually kept acidic to prevent the precipitation of metal hydroxides. Metal fraction recovered in these conditions may be thought to have been present as co-precipitated with carbonate minerals but also as specifically sorbed to some sites of the surface of clays, organic matter, and Fe/Mn oxyhydroxides.

Ethylenediamminetetraacetic acid (EDTA) is sometimes used in particular cases, for example, the study of sludges or soils polluted by lead as a result of battery recycling. Because of its complexing ability, this reagent is less specific than the acetic acid/acetate solution and is more able to extract metal ions that were bound to organic matter. Its use is therefore generally preceded by the oxidation of organic matter [23].

F-III: Bound to Fe-Mn Oxide Fraction

Iron and manganese oxides exist as nodules, concretions, cement between particles, simply as a coating on particles; these oxides are excellent scavengers for trace metals and are thermodynamically unstable under anoxic conditions [20]. A mixture of reducing agent and an acid is normally employed to extract trace metals associated with oxides of Fe and Mn in soils and sediments. These forms of oxide, or so-called reducible phase, could range from amorphous to crystalline. Simultaneous extraction of Fe-Mn oxides can be achieved through the use of a 0.02 or 0.04 hydroxylamine hydrochloride solution ($NH_2OH{\bullet}HCl$) prepared in 25% v/v of acetic acid, at high temperature (96-100 °C). Its ability to dissolve the different metallic oxides depends on pH, concentration, extraction time, and temperature. Generally, the pH of the extracting solution is adjusted to be lower than 3 in order to enhance the reducing power of the reducing agents. Tessier *et al.* [20] have reported that hydroxylamine solution did not reduce the organic carbon content in sediment samples, but selectively attacked metal oxides. Thus, this type of extracting solution should selectively attack metal oxides and dissolve any metals from weakly to strongly bound.

Oxalate buffered solution (0.2 M oxalic acid/0.2 M oxalate solution) is generally used for characterization of iron phases such as iron, aluminium, and silicon oxides. In particularly, extraction procedure is employed at ambient temperature, in the dark, following the previous leaching of Mn oxides because this reagent becomes specific for amorphous ion phases. Another reducing agent that could dissolve even well crystallized Fe oxides in the pH range 7-8 is sodium dithionite solution ($Na_2S_2O_4$). In order to avoid FeS precipitation, it is necessary to add a strong ligand and, buffer solution to stabilize the pH and the oxidation potential during the whole extraction. Most studies employed a sodium citrate/sodium dithionite solution adjusted to pH 7.3 by $NaHCO_3$ at high temperature (80 °C). The extracting time is 15 min and the treatment is repeated once or twice, according to the content of extractable iron [23].

F-IV: Bound to Organic Matter Fraction

Trace elements may be incorporated in many forms of organic matter including organisms, organic coating on inorganic particles, and biotic detritus. In sediments and soils, the organic content comprises mainly complex polymeric materials known as humic substances and, to lesser degree, other products such as carbohydrates, proteins, peptides, amino acids, fats, waxes, and resins [23]. Under oxidizing conditions, organic matter can be degraded, leading to release of sorbed trace metals.

Within a scheme of sequential extraction, oxidizing agent is often used to dissolve the organic substances or any oxidizable matter such as oxides and sulfides of some metals. Hydrogen peroxide is generally used as extracting solution in this process. It is adjusted to pH 2 with dilute nitric acid solution in order to prevent metals scavenging by the formation of metal hydroxides at higher pH values. The oxidation process is promoted by heating for several hours. An important re-adsorption of released metals can occur during the extraction, and this step is followed by an extraction with a soft complexing agent such as NH_4OAc prepared in nitric acid solution.

The use of sodium hypochlorite (NaClO) as an oxidizing reagent in alkaline conditions leads to a better destruction of organic matter, minimizing the attack of amorphous constituents and clay minerals. Nevertheless, it can oxidize some Mn oxides to MnO_4^- ions. Extraction procedure is generally performed at pH 8.5 with a 0.7 M NaClO, at high temperature. As the hypochlorite ion is unstable in aqueous solutions and decomposes quite rapidly at high temperatures, it is advisable to use short extraction time (15 or 30 min.) This procedure is generally employed as the second step in fractionation schemes, after exchangeable step [23].

F-V: Residual Fraction

Once the first four fractions have been removed, the residual solid should contain mainly primary and secondary minerals, which may hold trace metals within their crystal structures. These metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature [20]. Digestion methods are commonly used involving different mixtures of strong acid solutions (HNO_3-HCl, HNO_3-H_2SO_4, HNO_3-$HClO_4$, etc.), occasionally in combination with HF in order to dissolve the silica matrix [56].

Aqua regia, HCl:HNO_3 (3+1 v/v), digestion procedure is considered adequate for analyzing total-recoverable heavy metals in soils of certain regions and is used to estimate the maximum element availability to plants. Residual elements that are not released by aqua regia digestion are mostly bound to silicate minerals and are considered unimportant for estimating the mobility and behavior of the elements [57]. Digestion methods, however, have several drawbacks. If open systems are used, there is risk of atmospheric contamination and loss of elements.

1.7 Selectivity and Re-adsorption Problems

Although sequential extraction procedures have extensively been employed in geochemical research, there are some questions about the accuracy of the extraction methods because the analytical results are affected by selectivity and re-adsorption problems [27]. Generally, it is difficult to associate a given extracting reagent with a particular physico-chemical phase. Instead, the specificity of an extracting solution is operationally defined according to what it extracts; e.g. acetate buffer extracts the "acetate buffer-extractable" phase. Although it is often believed that this particular phase includes carbonate minerals, these minerals may not be uniquely attacked by this reagent. This is the non-selectivity problem [26].

The second problem is trace element re-adsorption among phase during extraction, meaning that metals released by one extracting solution could associate with other undissolved solid components or freshly exposed surfaces within the time-scale of the extraction step [25]. The re-adsorption problem may lead to serious misinterpretation of extraction data because the metal concentration determined in the extract may not be representative of the metal levels in the sediment fractions.

Three experimental approaches have been used to assess the re-adsorption problem [25]:

(i) uses model sediments containing one, two or more synthetic phases;

(ii) uses natural sediments spiked with pure synthetic phases which provide fresh unoccupied sites for retention of any metals released during previous extraction steps; and

(iii) uses standard addition of metals dissolved in the extracting solutions over natural sediments.

1.8 Accuracy Investigation

1.8.1 Accuracy of Sequential Extraction Method

Although sequential extraction methods have long been widely used, very few works reported detailed tests on their accuracy and reproducibility [36]. The accuracies of sequential extraction method proposed by Tessier *et al.* [20] and some modified methods have been evaluated by comparing total metal concentrations from hot-acid digestion with the sum of metal from all fractions [37, 38, 41]. The percentage recovery (%) of the sequential extraction methods is also calculated as follow [37]:

$$\text{Recovery} = \frac{[S]}{[T]} x100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1.1)$$

Where, [S] is the sum of metal concentrations from all steps.

[T] is total concentrations found by hot acid digestion.

1.8.2 Accuracy of Hot-Acid Digestion

To check the accuracy of hot-acid digestion for obtaining total metal concentration, standard addition is employed. Heavy metal standard solution with known concentration is spiked to sample that is then analyzed to give a percentage recovery of this metal. The percentage recovery is also calculated as follow [36].

$$\%\ \text{Recovery} = \left(\frac{C_M}{C_S + C_A}\right) x\,100 \quad \ldots\ldots\ldots\ldots\ldots (1.2)$$

Where, C_M is metal concentration found for spiked sample.

C_A is metal concentration found for non-spiked sample.

C_S is concentration of spiked standard solution.

1.9 Aims of the Work

The purpose of this work was to optimize the sequential extraction method for determination of some heavy metals (zinc, copper, lead, cadmium, chromium, and manganese) in river sediments. The sequential extraction method proposed by Tessier *et al.* was particularly modified with the aim of finding extraction efficiencies similar to or better than that of Tessier's method. The proposed method was used to partition samples into five operationally fractions, namely exchangeable, bound to carbonate, bound to Fe-Mn oxide, bound to organic matter, and residual. The ratios of extracting solution volume per sediment weight (V/m), extraction times (T_E), and repetitive extractions for each extraction step were specially studied. The optimized sequential extraction method was then employed to determine heavy metals for each fraction in order to evaluate heavy metal distributions in sediment samples from the Kwai Noi River at Kanchanaburi Province.

1.10 The Relevancy of the Research Work to Thailand

Due to the ecotoxicity and mobility of heavy metals in the environment depending on their chemical forms rather than total contents, sequential extraction procedure was therefore widely developed in order to study metal speciation in different environmental samples. The aim of this research work is to determine heavy metal concentrations (Mn, Zn, Cu, Pb, Cd, and Cr) in different sediment phases and to evaluate the bioavailability and distribution of these metals in sediment samples collected from the Kwai Noi River at Kanchanaburi Province. The results obtained from this work could be used qualitatively to monitor metal potential bioavailability in response to changing environmental conditions.