

**Behavior and Distribution of Heavy metals in Soil
and Sludge and Recovery of Metals by
Phytoremediation and Biosurfactants Application**

2013

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Chapter 1 General Introduction

1.1 Background

Environmental pollution became a popular issue in the world with the rapid development of modern industry and agriculture. Then it began to draw major public attention how to resolve the problem of the environmental pollution in the recent years ^[1, 2]. It is well known that the major forms of environmental pollution include air pollution, water pollution, soil pollution and so on. Among them, it is regarded that soil pollution is most difficult to treat due to its features (potential and permanent natures) and complicated matrix ^[3, 4]. The most significant contaminants in soil are organic matters such as pesticide and fertilizer, and heavy metals such as lead (Pb) and mercury (Hg).

Heavy metal-polluted soil can be caused by many sources ^[5]. For example, Pb-pollution can be caused from lead paint, aviation fuel and gasoline, Cr- and Cd-pollution may be from rechargeable batteries, and Ni-pollution may be occurred in some electroplate sludge. Any pollution can produce a number of harmful effects on environment and/or human health directly or indirectly by all kinds of paths. On the other hand, sludge, as the byproduct of sewage treatment, is applied as fertilizer for land because of containing abundant nutrient element. However, there is also a large quantity of hazardous materials such as pathogenic microorganisms and heavy metals. So it is important to control that heavy metals do not get into water sources, and to ensure that there is no accumulation of heavy metals in the top soil ^[6].

Based on the above-mentioned, the monitoring and determination of harmful pollutants such as heavy metals (including REEs, Th and U) in soil and sludge

environment is very significant to protect the soil environment from the adverse effects of pollution. In addition to heavy metals, the monitoring of Aluminium (Al) is also significant in soil environment because Al is considered as a primary factor that reduces plant growth in acid soils. Furthermore, it is worthy to develop and find some effective methods to remove and recovery heavy metals from the polluted soil and sludge.

1.2 Rare Earth Elements, Thorium and Uranium

Rare earth elements (REEs) are a set of seventeen chemical elements in the periodic table, specifically they are the fifteen lanthanides, scandium, and yttrium. Because of the systematic variation of their behavior due to “lanthanide contraction”, the relative abundance of the REEs is widely used to study geochemical processes ^[7]. The lanthanide elements traditionally have been divided into two groups, the light rare earth elements (LREEs) are lanthanum through europium ($Z = 57$ through 63), and the heavy rare earth elements (HREEs) are gadolinium through lutetium ($Z = 64$ through 71). Although yttrium (Y) is the lightest REE, it is usually grouped to the HREEs because of chemically and physically similar properties ^[8, 9] (Table 1-1).

The rare earth elements are often found together, and are difficult to extract and separate each other. Commercial market demands for REEs have arisen due to widely application in industry and agriculture such as catalysis of engine, permanent magnet, phosphor of illumination and so on in recent years^[10, 11]. So the shortage of trace metals including REEs (and the problem of stable supply for these metals) has been concerned. Mining of REEs is increasing. At the same time, environment problem caused by REEs have received great attention. For the conservation of the scarce resources and protection of the environment, it is important to monitor the

concentration of the REEs in soil and to survey the recovery method of the REEs which may be released to the environment ^[12, 13].

Thorium (Th) and Uranium (U) are natural radionuclide and sources of nuclear power. Thorium is estimated to be about four times more abundant than uranium in the Earth's crust. These two elements were widely distributed in nature^[14], and they have produced serious environmental consequences as radioactive byproducts from toxic pollutants and hazardous waste materials, accompanying with mining, refining, and recycling of rare earth elements ^[15-18]. Therefore, investigating the concentrations of Th and U in soil environments is important from a radiation protection viewpoint. The proper management of the U tailings by phytoremediation has been performed recently ^[19], however, there only a few causes at present.

1.3 Heavy Metals

Heavy metals are a group of metals that occur naturally, and most of them can be found in the rocks and soil in the world. Natural weathering of rocks and soil can break them down and release heavy metals into environment. Heavy metals exist in nature at certain levels under normal circumstances ^[20-22] (Table 1-2). However, soils may become contaminated when the amount of heavy metals does beyond the limit. It may be due to the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition. Among heavy metals found at contaminated sites, most commonly are lead (Pb), chromium (Cr), nickel (Ni), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and arsenic (As).

For living things, some metals such as iron (Fe), chromium (Cr) and copper (Cu) are needed in small quantities to keep people and animals healthy, whereas some heavy metals such as lead (Pb) and mercury (Hg) are never desirable in any amount. At any case, any heavy metal can be toxic to living things at an elevated level.

Lead (Pb)

Lead is metal belonging to group IV and period 6 of the periodic table. It is naturally occurring and bluish gray metal usually found as a mineral combined with other elements, such as sulphur (i.e., PbS, PbSO₄), or oxygen (PbCO₃). Lead is not an essential element for life. It is well known to be toxic after uptake from food, air, or water as it can accumulate in individual organisms, and its effects have been more extensively reviewed than the effects of other trace metals. Lead can cause serious injury to the brain, nervous system, red blood cells, and kidneys^[23].

The most serious way of exposure of lead to soil is through direct ingestion (eating) of contaminated soil or dust. Studies have shown that lead does not readily accumulate in the fruiting parts of vegetable and fruit crops (e.g., corn, beans, squash, tomatoes, strawberries, and apples). Higher concentrations are more likely to be found in leafy vegetables (e.g., lettuce) and on the surface of root crops (e.g., carrots)^[24]. Generally, the risk of lead poisoning through the food chain increases as the lead level in soil rises above 300 ppm. Even at soil levels above 300 ppm, most of the risk is from lead contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant^[3]. Then it is necessary to remediate the contaminated soil by lead.

Chromium (Cr)

Chromium is the first element in transition metal of group VIB in the periodic table. It is one of the less common elements and does not occur naturally in elemental form, but only in compounds such as FeCr₂O₄. Major sources of Cr contamination include

releases from electroplating processes and the disposal of Cr containing wastes. Chromium can cause allergic dermatitis in humans ^[25]. In larger amounts and in different forms, chromium can be toxic and carcinogenic. The most prominent example of toxic chromium is hexavalent chromium (Cr (VI)). After chromium (VI) enters the body then reaches the blood stream, it damages the kidneys, the liver and blood cells through oxidation reactions. Chromium (III) is the other main form of chromium, and is essential for living organisms. Both can transform each other under certain conditions. Especially chromium migration is influenced by soil pH value. Chromium (III) mobility is decreased by adsorption to clays and oxide minerals below pH 5 and by low solubility above pH 5 due to the formation of $\text{Cr}(\text{OH})_3(\text{s})$. The leachability of Cr (VI) increases with increasing soil pH ^[26]. Chromium migration also depends on sorption characteristics of the soil, including clay content, iron oxide content, and the amount of organic matter. Generally, chromium can be strongly adsorbed iron and aluminum oxides on soil surfaces. It is worthy to note that Chromium can be transported by surface runoff to surface waters in its soluble or precipitated form. Then, soluble and un-adsorbed chromium complexes can leach from soil into groundwater.

Most of Cr released into natural waters is particle associated firstly, and is ultimately deposited into the sediment ^[27]. As chromium compounds were used in dyes, paints and the tanning of leather, these compounds are often found in soil and groundwater at abandoned industrial sites, so it is necessary and urge to cleanup and remediate the land.

Nickel (Ni)

Nickel belongs to the transition metals with atomic number 28. Native nickel is rarely found on Earth's surface because it is reactive enough with oxygen. Nickel

plays important roles in the biology of microorganisms and plants ^[28]. For example, urease (an enzyme that assists in the hydrolysis of urea) contains nickel. For animals, Ni is an essential foodstuff in small amounts, but it can be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer or dermatitis.

The major sources of nickel contamination in soil are metal plating industries, combustion of fossil fuels, and nickel mining and electroplating ^[29]. The larger part of Ni compounds that are released to the environment will adsorb to sediment or soil particles and become immobile.

Cadmium (Cd)

Cadmium is located at the end of the second row of transition elements. Cadmium occurs as a minor component in most zinc ores, and therefore is byproduct of zinc production. Cd is one of the most poisonous heavy metals (together with Hg and Pb) and is not known for any essential biological function. Cadmium contamination are from many common industrial such as fossil fuel combustion, battery production, phosphate fertilizers, cadmium pigments and coatings, iron and steel production, and electroplating ^[30-32].

The application of agricultural inputs such as fertilizers, pesticides, and biosolids (sewage sludge), the disposal of industrial wastes or the deposition of atmospheric contaminants increases the total concentration of Cd in soils, and the bioavailability of Cd determines whether Cd is uptaken by plant to a significant degree ^[33]. The main routes by which cadmium enters the body are inhalation of fine dust and fumes (tobacco smoking) or ingestion of highly soluble cadmium compounds (food intake) ^[34, 35]. The major threat to human health is chronic accumulation in the kidneys which lead to kidney dysfunction.

Copper (Cu)

Copper is a transition metal which belongs to period 4 and group IB of the periodic table. It can be found as either native copper or as part of minerals. It is an essential trace nutrient to all plant and animal life (not some microorganisms) at lower concentrations. Because of its role in facilitating iron uptake, copper deficiency can produce anemia-like symptoms. However, they are poisonous to higher organisms in sufficient amounts, which cause anaemia, liver and kidney damage, and stomach and intestinal irritation. Copper is mainly found in liver, muscle and bone in case of animal.

The interaction of Cu with environment is complex. Most Cu introduced into the environment is (or rapidly becomes) stable, and results in the form which does not pose a risk to the environment. In fact, Cu is not magnified in the body or bioaccumulated in the food chain. In the soil, Cu is strongly complexed to the organic matters, which indicates that only a small fraction of copper may be found in solution as ionic copper, Cu (II). The solubility of Cu is drastically increased at pH 5.5 ^[36], which is rather close to the ideal farmland pH of 6.0-6.5 ^[37].

In addition the above heavy metals mentioned, others metallic elements are toxic in excessive amounts to environment and human health. As a whole, the connection between soil contamination and metal uptake by plants is determined by many chemical and physical soil factors as well as the physiological properties of the crops. Soils contaminated with heavy metals may pose both direct (i.e. through negative effects of metals on crop growth and yield) and indirect (i.e. by entering the human food chain with a potentially negative impact on human health) threats.

1.4 Aluminium (Al)

Aluminium is the third most abundant element (after oxygen and silicon), and the most abundant metal, in the Earth's crust. It makes up about 8% by weight of the Earth's solid surface. Because of its strong affinity to oxygen, it is almost never found in the elemental state; instead it is found in oxides or silicates. Aluminium is the most widely used non-ferrous metal except iron. It is widely applied for the industries of transportation, packaging, construction, etc.

Despite its prevalence in the environment and wide application, aluminium salts are not known to be used by any form of life due to the possible risk to human health. Aluminium is evidence of some toxicity if it is consumed in excessive amounts although it is not as toxic as heavy metals^[38]. Studies have shown that consumption of acidic foods or liquids with aluminium significantly increases aluminium deposition in bone and the central nervous system, and causes osteopenia, neurotoxicity, even allergic^[39, 40]. Furthermore, aluminium exposure is considered as a risk factor for Alzheimer's disease^[41].

On the other hand, aluminium is considered as a primary factor that reduces plant growth in acid soils. Although it is generally harmless to plant growth in pH-neutral soils, the concentration of toxic Al^{3+} in acid soils increases and disturbs root growth and function^[42, 43]. Thus, it is necessary to learn the behavior and distribution of aluminium in soil, especially agricultural soil, for protecting the environment and remaining human health.

1.5 Biosurfactants

Biosurfactants are a structurally diverse group of surface-active substances synthesized by living cells. They are amphiphilic compounds which are the same with chemical surfactants, and contain hydrophobic and hydrophilic moieties that possess

the characteristic property of reducing surface and interfacial tension at the surface and interface respectively ^[44]. Due to their amphiphilic structure, biosurfactants increase the surface area of hydrophobic water-insoluble substances, and increase the water bioavailability of such substances.

Biosurfactant has been steadily increasing interest in recent years as an alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters) ^[45]. The reason for their popularity is primarily because of their diversity, environmentally friendly nature, higher biodegradability, possibility of large-scale production, selectivity, effectiveness under extreme conditions, and can be produced from industrial wastes and from by-products. ^[45, 46] The last feature makes cheap production of biosurfactants possible and allows utilizing waste substrates and reducing their polluting effect at the same time ^[47-50].

Biosurfactants are categorized by their chemical composition, molecular weight, physico-chemical properties, mode of action, and microbial origin. Based on the molecular weight, they are divided into low-molecular-mass biosurfactants including glycolipids, phospholipids and lipopeptides and into high-molecular-mass biosurfactants/bioemulsifiers containing amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers. Low-molecular-mass biosurfactants are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions ^[51, 52]. Examples of three kinds of common biosurfactants and their applications in environmental biotechnology are shown in Table 1-3 ^[53-60].

During the past few years, biosurfactants have gained importance in the fields of enhanced oil recovery, environmental bioremediation, food processing and pharmaceuticals. The examples of biosurfactant applications are listed in many review

papers ^[61-63]. In this paper, we discuss the potential roles and applications of biosurfactants mainly focusing on two aspects on removal of heavy metals. One is as elution agent to remove heavy metals in polluted sludge, and the other one is as enhancing agent used in phytoremediation of Pb-contaminated soil and sludge. With these specialized and cost-effective applications in environmental protection, biosurfactants can be useful molecules in future. Then, the two kinds of biosurfactants (saponin and sophorolipid) were collected in this work because of their following merits:

(1) Easy availability -- saponins are plant glycosides which are widely distributed in plants and sophorolipids can be produced by many avirulent yeasts ^[64, 65].

(2) Acceptable lower cost -- they can be produced with cheap raw materials which are available in large quantities (e.g. the market price of saponins (20% - 95%) from *Tribulus terrestris L.* is less than \$0.2/kg.) ^[66].

(3) Possibility of reuse -- e.g. the recovery and reuse has been reported by the precipitation method ^[67].

(4) Environmental compatibility -- they can be quickly biodegraded (generally in 2-3 weeks) ^[68]. Especially nonionic biosurfactants without charge have a better compatibility not to disturb the soil property than anionic biosurfactants with negatively-charge.

Furthermore, nonionic saponin and sophorolipid with the similar surface properties and the different structure were used for comparison. Some detail information about saponin and sophorolipid is introduced in Chapter 3.

1.6 Phytoremediation

Phytoremediation, also called green remediation, can be defined as an in situ

remediation strategy that uses plant to remove, contain or render environmental pollutants harmless. Phytoremediation technologies for remediation of heavy metal-contaminated soils include phytoextraction, phytovolatilization and phytostabilization. As it is a relatively new technology, phytoremediation is still mostly in its testing stages and as such has not been used in many places as a full-scale application. However, it has been tested successfully in many places around the world for different contaminants, and it can be used in conjunction with other remedial methods as a finishing step to the remedial process. However, until now there are very few relevant research on biosurfactants-enhancing phytoremediation of heavy metals in soil (with the method of soil culture) [69, 70].

1.7 The Purpose and Outline of the Thesis

The purpose of present study is at first to investigate the behavior and distribution of heavy metals in soil and sludge environment. The second is to establish the efficient recovery method of the metals from contaminated soil and sludge by phytoremediation and biosurfactants application (in batch and column experiment). Furthermore, in order to verify the effect of biosurfactants for phytoremediation of Pb-contaminated soil and sludge, the different dosage and kinds of biosurfactants (saponin and sophorolipid) were investigated using two kinds of plants (*Brassica juncea* and *Helianthus annuus*).

In this paper, there are 5 chapters.

In Chapter 1, the general introduction was stated.

In Chapter 2, the behavior and distribution of heavy metals including REEs, Th and U in agriculture soil collected from Niigata and Yamagata Prefectures, Japan and Qiqihar and Shangzhi cities in Heilongjiang Province, China, were studied.

Furthermore, some basic physical and chemical characteristics of the soil had been measured, such as pH, EC, CEC, moisture content and organic matter content. The results show that differences of the concentration and distribution characteristics of metallic elements are obvious between Chinese soil samples and Japanese soil samples, which may be largely attributable to the differences of the nature and formation history of soil, and of the surrounding environment as well as applied fertilizer.

In Chapter 3, the behavior and distribution of heavy metals in sludge from Niigata Prefecture, Japan and Qiqihar in Heilongjiang Province and Langfang in Hebei Province, China, had been investigated, and were compared with those in natural soil from the same sampling place. Moreover, the removal of heavy metals in polluted sludge by biosurfactants (saponin and sophorolipid) was carried out. The pH value and concentration of the biosurfactant solution in batch experiment and washing volume in column experiment were investigated to obtain optimum conditions.

In Chapter 4, the remediation of lead-contaminated soil and sludge using *Brassica juncea* and *Helianthus annuus* along with biosurfactants (saponin and sophorolipid) was performed in a plant environmental control system to learn the effect and mechanism of biosurfactant-enhanced phytoremediation. Effect of the different additional dosage and times of the two biosurfactants on biomass was surveyed. In addition, Pb concentration and Pb uptake amount in shoot and root, and the translocation of Pb from root to shoot were also investigated. Furthermore, plant growth regulators (Gibberellin A3 (GA3) and indole-3-acetic acid (IAA)) were applied to improve the biomass.

In Chapter 5, the conclusions of the thesis are presented.

Table and Figure

Table 1-1 REEs, atomic numbers, abundances and applications ^[8, 9]

Atomic Number	Element (Symbol)	Upper Crust Abundance /ppm	Applications
21	Scandium(Sc)	5.0	Light aluminium-scandium alloy for aerospace components, additive in Mercury-vapor lamps
39	Yttrium(Y)	22	Yttrium-aluminium garnet (YAG) laser, yttrium vanadate (YVO4) as host for europium in TV red phosphor, YBCO high-temperature superconductors, yttrium iron garnet (YIG) microwave filters, energy-efficient light bulbs
57	Lanthanum(La)	30	High refractive index glass, flint, hydrogen storage, battery-electrodes, camera lenses, fluid catalytic cracking catalyst for oil refineries
58	Cerium(Ce)	64	Chemical oxidizing agent, polishing powder, yellow colors in glass and ceramics, catalyst for self-cleaning ovens, fluid catalytic cracking catalyst for oil refineries, ferrocerium flints for lighters
59	Praseodymium(Pr)	7.1	Rare-earth magnets, lasers, core material for carbon arc lighting, colorant in glasses and enamels, additive in didymium glass used in welding goggles, ferrocerium firesteel (flint) products.
60	Neodymium(Nd)	26	Rare-earth magnets, lasers, violet colors in glass and ceramics, ceramic capacitors
61	Promethium(Pm)	-	Nuclear batteries
62	Samarium(Sm)	4.5	Rare-earth magnets, lasers, neutron capture, masers
63	Europium(Eu)	0.88	Red and blue phosphors, lasers, mercury-vapor lamps, NMR relaxation agent
64	Gadolinium(Gd)	3.8	Rare-earth magnets, high refractive index glass or garnets, lasers, X-ray tubes, computer memories, neutron capture, MRI contrast agent, NMR relaxation agent
65	Terbium(Tb)	0.64	Green phosphors, lasers, fluorescent lamps
66	Dysprosium(Dy)	3.5	Rare-earth magnets, lasers
67	Holmium(Ho)	0.80	Lasers
68	Erbium(Er)	2.3	Lasers, vanadium steel
69	Thulium(Tm)	0.33	Portable X-ray machines
70	Ytterbium(Yb)	2.2	Infrared lasers, chemical reducing agent
71	Lutetium(Lu)	0.32	PET Scan detectors, high refractive index glass

Table 1-2 Mean values and range of metallic elements in soil ^[20-22]

Element	Soil	Soil	Paddy Soil	Crust
Cr	70 (5~1500)	50 (3.4~810)	64 (16~337)	100
Co	8 (0.05~65)	10 (1.3~116)	9 (2.4~23.5)	~ 20
Ni	50 (2~750)	28 (2~60)	39 (9~412)	~35
Cu	30 (22~50)	34 (4.4~176)	32 (11~120)	55
Zn	90 (1~900)	86 (9.9~620)	99 (13~258)	40
As	6 (0.1~40)	11 (0.4~70)	9 (1.2~38.2)	2
Cd	0.35 (0.01~2)	0.44 (0.03~2.53)	0.45 (0.12~1.41)	0.15
Hg	0.06 (0.01~0.5)	0.28 (ND~5.36)	0.32 (ND~2.9)	0.08
Pb	35 (2~300)	29 (5~189)	29 (6~189)	15

Table 1-3 Three kinds of common biosurfactants and their applications in environmental aspects

Biosurfactant	Applications in Environmental Biotechnology	References
Sophorolipid	Recovery of hydrocarbons from dregs and muds; removal of heavy metals from sediments; enhancement of oil recovery	[53-55]
Rhamnolipid	Enhancement of the degradation and dispersion of different classes of hydrocarbons; emulsification of hydrocarbons and vegetable oils; removal of metals from soil	[53, 56-58]
Saponin	removal of metals and organic compounds from contaminated soil	[59,60]

Chapter 2 Behavior and Distribution of Heavy Metals Including REEs, Th and U in Agricultural Soil

2.1 Introduction

Soils are regarded as sinks for materials from the environment. In recent years, there has been growing concern over soil pollution, especially for soil pollution by heavy metals because most of heavy metals immobile in soil and accumulate in the topsoil. Then, heavy metals directly affect the quality of crops, vegetables and micro flora, or indirectly impact on environmental quality and human health via ground water, surface water, food chain and so on due to their toxic nature^[71-73]. Thus, the monitoring and determination of harmful pollutants such as heavy metals in natural environment is very significant^[74]. Hence, the distribution of heavy metals in soil has been widely studied in the world^[71-74].

In Japan, Yoshida et al.^[75] measured the concentrations of lanthanide elements, thorium (Th) and uranium (U) in surface soils, and discussed the relationship of soil type with soil utilization. Kato et al.^[76] determined the background levels and distribution of 19 elements in alluvial soils, and investigated the relationships among elemental composition, soil type and soil series. Yamasaki et al.^[77] and Takeda et al.^[78] have reported the background levels of 57 elements in 514 soil samples from Japan, and explained the variability of elemental composition from the viewpoint of different soil types and the influence of agricultural activities. Uchida et al.^[79-80] estimated soil-transfer factors of stable elements and naturally occurring radionuclides in upland field crops and rice collected in Japan. However, a few reports are available about chemical speciation and distribution of heavy metals including rare earth

elements (REEs), Th and U by sequential extraction procedure in case of agriculture soils from Japan.

Niigata and Yamagata Prefecture are two of the major grain-producing regions in Japan, and can be one of the important regions for investigating the behavior of metallic elements in agriculture soils. Therefore, the behavior of REEs, Th and U in agriculture soils of 2 sampling points (Shimofujiduka, Toyosato, Sakata City in Yamagata Prefecture, and Hebetsuka, Teradomari, Nagaoka City in Niigata Prefecture collected on April 2005, October 2005 and April 2006) was investigated in our laboratory^[81].

In this study, in addition to continuous determination of REEs, Th and U in agricultural field, the monitoring of such metallic elements as Al, Cr, Mn, Fe, Co, Ni, Zn, Cd and Bi is also carried out. Furthermore, other soil samples in Niigata and Yamagata Prefectures (Ueno, Sekikawa Village, Iwafune District in Niigata Prefecture, and Tateoka, Shinmachi, Murayama City in Yamagata Prefecture) are also taken and analyzed. At the same time, Heilongjiang Province is a main area of grain in China, so it is necessary to investigate the behavior of metallic elements in agriculture soil from the viewpoint of ensuring grain quality. Soil samples from Chaoxian village, Qiqihar City and suburban area of Shangzhi City in Heilongjiang Province were collected and analyzed.

The objective of this study is to investigate the behavior and distribution of heavy metals including REEs, Th and U in agricultural soil in Niigata and Yamagata Prefectures, Japan and in Heilongjiang Province, China, and is to investigate the factors for affecting the elemental distributions or concentrations of soils, and is also to use the outcome for environmental preservation. For these purposes, these elements in soils in paddy, upland field and no plow were partitioned into 6 fractions and

determined by a sequential extraction fractionation procedure as well as determination of total contents.

2.2 Experimental

2.2.1 Samples

The soil samples used in this thesis were collected from the agricultural field (paddy and upland field) in 5 sampling points: Toyasato, Sakata City (from 2005 to 2010) and Tateoka, Murayama City (from 2008 to 2010) in Yamagata Prefecture, and Ueno, Sekikawa Village, Iwafune District (from 2007 to 2010) in Niigata Prefecture, Japan and Chaoxian village, Qiqihar City (October, 2011) and Suburban area, Shangzhi City (October, 2011), China. The samples in Japan were taken twice a year, at spring (April) and autumn (October) season. The sampling points are shown in Fig. 2-1, and their geological characteristics are shown in Table 2-1. For reference, the surrounding soil in non-agricultural field in each area was also collected and analyzed. (Soils in Hebetsuka, Teradomari, Nagaoka City in Niigata Prefecture used in our previous paper^[81] were not collected and analyzed in this work because these fields have been lying fallow in recent years.)

The soil samples were taken from the topsoil (0-20 cm), and taken according to the method described in the manual of Science and Technology Agency^[82]. The method is the same as shown in our previous paper^[81] (i.e., soil samples in paddy field have been taken at 5 spots, then these were mixed up for analysis; and samples in upland field are mixture from those taken at 8 spots). The soils were air-dried at room temperature, ground, and passed through a 2 mm sieve to remove rocks, roots and other large particles^[75, 83].

2.2.2 Apparatus and Reagents

An ICP-MS instrument (Agilent HP4500) was used to determine the concentration of REEs, Th and U, and an ICP-AES instrument (SPS1500, Seiko Instruments Inc.) was employed to determine the concentration of other heavy metals. The operating conditions of the ICP-MS are the same as shown in our previous paper^[84] and that of ICP-AES are based on our other previous paper^[85].

Heavy metals including REEs, Th and U standard solutions used for making the calibration curve were prepared by diluting the standard solutions (XSTC-13 for heavy metals, Th and U, XSTC-1 for REEs; both $10\text{mg}\cdot\text{dm}^{-3}$ 5% HNO_3 solution) purchased from SPEX CertiPrep, Inc. (USA). All other chemical reagents, purchased from Kanto Chemical Co., Inc. (Japan) were of analytical grade. Water ($>18.2\text{ M}\Omega$) which was treated by an ultrapure water system (Advantec aquarius: RFU 424TA) was used throughout the work.

2.2.3 Measurement of pH, EC and Organic Carbon Content

Soil pH and EC (electric conductivity) were measured based on the method for soil testing by The Japanese Geotechnical Society^[86] (i.e., at a soil: water ratio of 1:2.5). In regard to pH, pH(KCl) as well as pH(H_2O) was measured by using $1\text{ mol}\cdot\text{dm}^{-3}$ KCl instead of distilled water. In this work, 75 cm^3 of ultrapure water (or $1\text{ mol}\cdot\text{dm}^{-3}$ KCl solution) was used for 30 g of soil sample. A pH-meter (Horiba F-21) and a conductivity meter (Horiba ES-12) were used to measure pH and EC, respectively. Organic carbon content of soil was determined by the Walkley-Black method^[87].

2.2.4 Determination of Metallic Elements Including REEs, Th and U in Soil Samples

All metals in soils were partitioned into six fractions with sequential extraction procedure mainly based on Sadamoto et al. [88] and Tessier et al. [89]. In this paper, these six fractions were denoted as F1, F2, F3, F4, F5 and F6, respectively. These fractions roughly correspond to the fractions based on Cao et al. [83], i.e., F(ws): water soluble, F(ec): exchangeable, F(cb): bound to carbonates, F(om): bound to organic matter, F(fm): bound to Fe-Mn oxides, and F(rd): residual described in our previous paper [81]. The outline of the sequential extraction procedure is shown in Table 2-2. For the initial step in this sequential extraction procedure, 7 g of dried soil sample in 100 cm³ polypropylene centrifuge tubes was used. Following extraction at each step, the mixture of soil sample and each extraction reagent was centrifuged (3000rpm×30min) using a centrifugal separator (Kubota Co. 5200). The supernatant solutions were separated, and the residues were washed with ultrapure water, and the extraction process at each step was repeated twice. Finally, the obtained solutions were evaporated to near dryness on a hot plate, redissolved by 1 mol·dm⁻³ HNO₃ and combined for analysis.

The residual fraction (F6) and total fraction were digested in a closed vessel (PTFE vessels) with the acid mixture of HNO₃ and HF by using “microwave digestion method” [79, 90, 91]. After digestion, samples were evaporated to near dryness on a hot plate. Then the residues were dissolved with HNO₃ and HF in a PTFE beaker and adding 30% H₂O₂ properly to yield the sample solution. The objective elements were finally redissolved by 1 mol·dm⁻³ HNO₃ for ICP-MS or ICP-AES measurement.

2.3 Results and Discussion

2.3.1 Soil pH, EC and Organic Carbon Content

Representative pH, EC and organic carbon content of soil samples at 5 sampling

places are presented in Table 2-3. From this table, the following matters are mainly found. (1) EC in the upland field is highest, and the value in the paddy field is higher than that in no plow field; although EC in the paddy field is highest or almost the same as the value in the upland field in case of Sekikawa. (2) The value of $\text{pH}(\text{H}_2\text{O}) - \text{pH}(\text{KCl})$ in the upland field is smallest, although the value is almost the same as the value in the paddy field in case of Sekikawa. Smaller value of $\text{pH}(\text{H}_2\text{O}) - \text{pH}(\text{KCl})$ is remarkable in Murayama, which is good agreement with the results that the content of organic carbon in the upland field in Murayama is especially large. (3) The content of organic carbon in the paddy field is generally higher than that in no plow field. Organic carbon content in soil samples from Shangzhi City is highest among 5 sampling places. (4) Most of the soil samples are weak acidic, but soil from Qiqihar City is slightly alkaline.

From these results, it is found at first that the concentration of total ions is largest in the upland field. The fertilizer containing phosphate, nitrate and potassium used in the soil of the upland field may be one of factors of higher concentration of total ions. However, in case of Sekikawa, EC is larger in the paddy field. It is known that some kinds of agricultural chemicals and fertilizer are used in the paddy field in Sekikawa, then the concentration of total ions is large in the paddy field as well as in the upland field.

Secondly, it is suggested that the soil fertility is highest in the upland field because it is recognized that the smaller the value of $\text{pH}(\text{H}_2\text{O}) - \text{pH}(\text{KCl})$ is, the higher the soil fertility is. Furthermore, $\text{pH}(\text{KCl})$ in the upland field is also larger than that in the paddy field or that in the non-agricultural field, indicating that the content of substituted H^+ and Al^{3+} in the upland field is large.

It is notable feature that in the soil of the upland field from Murayama, the content

of organic carbon is remarkably high, and the value of $\text{pH}(\text{H}_2\text{O}) - \text{pH}(\text{KCl})$ is remarkable small; which indicates that the fertility is particularly higher in the upland field from Murayama. Moreover, organic carbon content in all soil from Shangzhi is particularly high, which means the soil has very high fertility. If organic carbon content is regarded as one of the indexes of the fertility of soil, it can be found that the fertility of soil in Heilongjiang Province of China is richer than that of Japan.

Thirdly, it is generally known that there is much supply of organic matter due to the straw and root of rice, weeds, algae and so on in the soil of paddy field ^[92], and that the decomposition of organic matter by microorganism tend to be inhibited in the soil strongly affected by water ^[93]. Thus, these things might be the factors of higher content of organic carbon in the soil of the paddy field in this work.

In regard to the sampling places, it is noteworthy that the acidity of soil from Murayama is the largest (all soils: $4.5 < \text{pH}(\text{H}_2\text{O}) < 5.6$). It may be attributable to the fact that the soil in Murayama is derived from volcanic soil. In addition, $\text{pH}(\text{H}_2\text{O})$ of soil in the paddy field from Sekikawa is relatively small, which may be closely related to that the soil is yellow soil ^[94]. On the contrary, $\text{pH}(\text{H}_2\text{O})$ of soil collected from Qiqihar City is the highest among all soil samples due to weak alkaline characteristic of Aeolian sandy soil.

It is obvious that there are large differences of the values of pH, EC and organic carbon content of the soil among the different utilization and the different sampling points. It may be attributed to the difference of the soil parent materials, and physical and geography conditions.

2.3.2 Determination of Metallic Elements in Soil Samples

2.3.2.1 Determination of REEs, Th and U

Total concentrations of REEs in soil samples at 5 sampling places are shown in Table 2-4. In Table 2-4, the average value of each field (at each sampling place) during measurement period is presented. The relative standard deviation (RSD) of the triplicated analyses of each sample was less than 10 %. From this table, “Oddo-Harkins rule”^[95] (i.e., elements of even atomic number are more abundant than adjacent elements of odd atomic number) is generally found in all these samples.

Leedeey-normalized REEs concentrations (i.e., REEs patterns) for soils based on Table 2-4 are shown in Fig. 2-2. From this figure, the tendency of light-REE (LREE) enrichment was generally found in all samples, which is typical pattern of soil^[96] or shale^[97] (the representative of crust). Soil samples from Sakata, Murayama and Qiqihar, generally display nearly flat REE patterns, whereas Sekikawa and Shangzhi samples are characterized by concave and slight convex patterns. Moreover, the differences of REEs concentrations among sampling points are found, whereas no significant differences of REEs concentrations among soil utilizations can be detected. In other words, the concentrations of REEs are lowest in Shangzhi, those of light-REE (LREE) are highest in Qiqihar according to Sekikawa, and those of middle-REE (MREE) and those of heavy-REE (HREE) are highest in Murayama. It is worth noting that the slope is different between the soil samples in Japan and those in China, and it is sharper for the latter. It indicates that the geological differentiation of the soil in China may be bigger than that in Japan.

Among REEs, it is generally known that the extent of complexation with organic ligand is raised with the increase of atomic number^[98]. Namely, it is possible that the component of REEs complexed with organic ligand is high in the soil from Murayama. This is in good agreement with the fact that the content of organic carbon is remarkably high in the soil from Murayama. However, the concentrations of REEs

in soil from Shangzhi are lowest among 5 sampling points, although organic carbon content is very high. The differences of REEs concentrations found among sampling points may be largely attributable to the differences of the nature and formation history of soil, and of the surrounding environment as well as applied fertilizer.

In addition, to investigate the difference of REE concentrations (or REEs patterns) between in spring and in autumn (i.e., before and after cultivation throughout the year), leedey-normalized REEs concentrations (i.e., REEs patterns) for soils of Sakata in 2010 are shown in Fig. 2-3. REE patterns for soils of other years and other places (i.e., from Sekikawa and Murayama) are generally similar to those of these soils. No significant differences of REEs concentrations in soils can be observed before and after cultivation, although the concentrations may be raised in autumn.

Next, total concentrations of Th and U in soil samples at 5 sampling places are shown in Fig. 2-4. In Fig. 2-4, the average value of each field (at each sampling place) during measurement period is presented. The concentrations of Th and U in soils are not greatly varied regardless of sampling period (i.e., spring or autumn) in 3 sampling places in Japan (Table 2-5). From Fig. 2-4, the concentration of Th or U in Sekikawa is about 4 times higher than that in Sakata and Murayama and about 2 times higher than that in Qiqihar, which is even higher than the average value of Th and U in agriculture soil reported by Yoshida et al. ^[75]. Moreover, the concentration of U in Shangzhi is remarkably high. It may be mainly due to the difference of the parent materials of the soil.

No significant differences of Th concentrations in soils can be detected among soil utilizations. On the other hand, the concentration of U in agricultural field is remarkably larger than that in no plow field except for the upland field of Shangzhi, even reached to 1.8 times in the upland field of Sakata. Based on our analytical results

of U in some fertilizers shown in Fig. 2-5, it might be suggested that U from the fertilizers accumulate in soil to some extent. Additionally, the concentration ratio of U/Th in the agriculture soil is much higher than that in no plow soil in five sampling places. It is proposed that concentration ratios of U/Th in paddy field soils and upland field soils are much higher than those in Japanese crust and/or non-agricultural fields due to the effect of phosphate fertilizers [99]. It is also suggested that phosphate fertilizers contain 10-200 times more U than that in soils, but Th content in phosphate fertilizers is lower than that in soils [99, 100]. Then, high concentration ratios of U/Th in the agricultural soil in this work may be also due to the effect of phosphate fertilizers. Although the ratios are different among 5 sampling places, the mean value is about 0.29, which is slightly higher than the average value in Japanese soil [78]. On the other hand, the maximum value (0.60) is found in paddy field from Shangzhi, which indicates that the effect of phosphate fertilizers may be much large in China.

2.3.2.2 Determination of Aluminum and Some Heavy Metals

In this study, total concentrations of aluminum (Al) and some heavy metals (Cr, Mn, Fe, Co, Ni, Zn, Cd, Bi) in all soil samples are also preliminarily determined. The mean value during measurement period is shown in Table 2-6. The relative standard deviation (RSD) of the triplicated analyses of each sample was less than 10%.

From Table 2-6, the following results can be mainly obtained: (1) The concentrations of Al, Fe and Mn, whose content is considered to be mainly decided by the parent materials, are remarkably high. In particular, total contents of Al and Fe are between 6.5% and 11.7% of soil. Comparing among the sampling places, the concentrations of these elements are the highest in Murayama and the lowest Shangzhi. (2) The concentration of Zn in no plow field from Japan is higher than that

in the agricultural field. In other words, it means that the concentration of Zn decreased in the agriculture field. Comparing among 3 sampling points, the order of the concentrations is Sekikawa > Murayama > Sakata. On the other hand, the concentration of Zn is higher in agricultural field (paddy field and upland field) than that in no plow field from Qiqihar, and it is almost the same for soil samples from Shangzhi. These results show that the concentration of Zn maybe increase or remain balance in agricultural field due to the use of fertilizer or pesticide, atmosphere precipitation, irrigation and so on. (3) The concentrations of most heavy metals (except Zn and Mn) in soils are higher in paddy field in 5 sampling points. It means that irrigation is one of the dominant sources for introducing heavy metals into soil.

Comparing the concentrations between sampling periods, definite differences were not observed, although there are some elements whose concentrations increased after cultivation as shown in Table 2-7.

The following factors can be considered to alter the concentrations of metallic elements in soil:

- (i) supply from fertilizers and accumulation
- (ii) inflow of surrounding soil or water such as river water and precipitation
- (iii) drain away with water
- (iv) uptake by plant including rice plant

An increase of the concentration may be caused by the factor (i), whereas the opposite change (i.e., decrease of the concentration) may be caused by the factor (iii) or (iv). It cannot be presumed whether factor (ii) increases or decreases the concentration of metals depending on each case.

The concentrations of Zn in the agricultural chemicals and fertilizers used in the agricultural field are shown in Fig. 2-6. As known from Fig. 2-6, the significant

amount of Zn is contained in the fertilizers. Nevertheless, the concentration of Zn in the agricultural field is generally smaller than that of no plow field. In other words, it may be considered that the effect of (iv) is relatively large in the case of Zn. Moreover, the higher concentrations of most heavy metals in the soil from paddy field may be due to the fact that the paddy soil is generally argillaceous^[94], and clay mineral has relatively high capacity to hold metals.

In any case, the parent material of soil and regular agriculture activity as well as the above factors can affect the elemental compositions in soils as Han and Kingery^[101] pointed out.

2.3.3 Distribution of Metallic Elements in Soil Samples

2.3.3.1 Distribution of REEs, Th and U

The relative distributions of REEs among six fractions of soils in each field at 5 places are shown in Fig. 2-7 ((a) Sakata, (b) Murayama, (c) Sekikawa, (d) Qiqihar, (e) Shangzhi). In Fig. 2-7, the mean distribution proportion of each field (at each sampling place) during all measurement period is presented. To show the seasonal difference clearly, the distributions of REEs, Th, U among six fractions in soils from Sakata in 2010 are shown in Figs. 2-8 [Fig. 2-8-1 ((a) paddy (b) upland (c) no plow in April) and Fig. 2-8-2 ((a) paddy (b) upland (c) no plow in October)]. From these figures, the distribution characteristics among six fractions are not varied largely between spring and autumn (i.e., before and after cultivation throughout the year).

From Fig. 2-7 and Figs. 2-8, it can be seen that REEs mainly exist in the form of residual fraction (F6, i.e., silicate) for the soil samples in Japan. Furthermore, F5 occupies relatively large proportion fraction (8-34%), and F4 does certain proportion (6-10%). These results are also in fairly good agreement with those of Cao et al.^[83]

and our previous works ^[81] (i.e., April, November 2005 and April 2006). It is recognized that REEs usually exist as trivalent ions in soil environment and that organic complexes dominate at pH from 4 to 8 ^[102,103], then, F4 as well as F5 may be important fractions for investigating the behavior of REEs such as bioavailability and mobility of REEs. On the other hand, for the soil samples from China, the distribution characteristics of REEs in Qiqihar are similar with those in Japan except that F4 proportion is lower (1-6%). In case of the soil samples from Shangzhi, it shows distinctive difference with other soil samples. REEs mainly exist as residual fraction (F6) and Fe-Mn oxides fraction (F5). Especially F5 fraction occupies above 50% of REEs in no plow, and F6 fraction is almost equal with F4 fraction. In addition, F3 fraction in paddy and no plow also occupies a large portion of REEs (2-18%). According to Cao et al. ^[83], organic matter may play an important role in soil in determining the chemical distribution of REEs. It is accordance with the high organic matter content in soil samples from Shangzhi.

From these figures, it is also found that the proportion of F5 is generally larger in heavy-REE (HREE) than in LREE or in middle-REE (MREE). That is, HREE display higher affinity for free oxides than LREE or MREE. Among REE, it is known that HREE are easier to form hydroxide at lower pH than LREE or MREE ^[104]. Then, larger portion of HREE is considered to link with free oxides such as Fe-Mn oxides by coprecipitation.

The mean distributions of Th and U among six fractions of soils in each field at 5 points during all measurement period are shown in Fig. 2-9. From Figs. 2-8 and 2-9, it is found that F6 and F5 comprise almost all proportion (100%) for Th, and especially noting that F5 fraction occupies above 93% of total Th in upland field and no plow field from Shangzhi. It is considered that Th begins to precipitate as its hydroxides at

pH 3.5 and precipitate completely at about pH 6.0 ^[104], then free oxides such as Fe-Mn oxides may be important factors for controlling the mobility and bioavailability of Th.

On the other hand, it is noticeable that U in soils may be present as the fraction bound to organic matters (F4, 6-56 %) and inorganic matters such as carbonate (F3, 1-4 %) to some extent in addition to residual fraction (F6, 13-79 %) and free oxide compounds (F5, 7-47 %). However, each fraction of U has a wider range of percent ratio in soil samples from China than those from Japan; for example, F4 is 6-56% and 10-23%, respectively. Unlike F4, F5 and F6, F3 is considered to be easily-extractable fraction, and it is recognized that the uranyl ion may form complexes with carbonate ions at $\text{pH} \geq 5$. ^[105, 106] This can be important implications for the possibility of F3 (bound to inorganic matters) being significant fraction for investigating the effect of toxic metal on environmental impact.

Additionally regarding soil utilization, F4 and F3 in the paddy field account for higher proportion than those in the upland field or the no plow field, although the tendency is not found in the samples for Shangzhi, which may be related to the effect of surrounding water such as river water ^[107]. This result suggests that the effect of soil utilization as well as the chemical behavior of elements in soil (e.g., stability of compounds in soil environment) is one of important factors to affect the elemental distributions of soils.

However, further investigations to survey the factors influencing (or determining) the elemental distributions or concentrations of soils are needed. Thus, we are planning to take and analyze the surrounding water around each agricultural field.

2.3.3.2 Distribution of Aluminum and Some Heavy Metals

The relative distributions of Al and some heavy metals among six fractions of soils from Sakata in 2010 and from Qiqihar and Shangzhi in 2011 are shown in Figs. 2-10 [Fig. 2-10-1 ((a) paddy (b) upland (c) no plow in April) and Fig. 2-10-2 ((a) paddy (b) upland (c) no plow in October)] and Figs. 2-11 [Fig. 2-11-1 ((a) paddy (b) upland (c) no plow from Qiqihar in October) and Fig. 2-11-2 ((a) paddy (b) upland (c) no plow from Shangzhi in October)], respectively. The distribution characteristics of individual aluminum and some heavy metals fractions in soils from Japan at different sampling year were roughly similar to those shown in Figs. 2-10-1 and 2-10-2, and it is suggested that the distribution proportions among six fractions are not varied largely between spring and autumn (i.e., before and after cultivation).

From Figs. 2-10, it is at first found that these metals mainly exist in the form of residual fraction (F6, more than 50 % regardless of soil utilization or sampling season) as in the case of REEs, Th and U. From Figs. 2-11, it is observed that these metals mainly exist in F6 and F5 fractions (i.e., F5 and F6 are more than 60 % regardless of soil utilization or sampling season). It can be concluded that metallic elements in the soil from China is more easily affected by the variation of physical and geography conditions such as pH, and then easily released into the environment. In case of Al and Fe, F6 and F5 comprises more than 95 %. Thus, it is indicated that the risk for eluting heavy metals from soil to surrounding environment is rather small.

In regard to Mn, F2 and F3 occupies the relatively large proportion (5-25%) particularly in the soil of paddy field. In addition, F3 is also relatively large proportion fraction for Zn in soils from Sakata and Qiqihar, and for Co and Ni in soil from Qiqihar (particularly in the soil of upland field). It is generally recognized that the content of available (water-soluble) form in crops can be reflected to some extent by the metal content in F3 ^[100], then these results supply important information from the

viewpoint of environmental preservation. Furthermore, it is noticeable that F2 fraction of Zn is detected to some extent in case of the soil from no plow in contrast to being hardly detected in agriculture soil in Sakata. It may be suggested that the exchangeable fraction of Zn can be absorbed by plant including rice plant.

Other notable feature found in Figs. 2-10 and Figs. 2-11 is that Cr (and Bi) existed as F4 (bound to organic matter, 5-12% for Sakata; 14-40% for Qiqihar and 8-13% for Shangzhi) to some extent. Among organic matter, humic substances are considered as one of the main chemical species in the soil environment ^[108]. Previously, we have carried out laboratory model experiments on the interactions of Cr with humic substances ^[85], and confirmed that Cr(VI) (i.e., toxic, soluble species) is reduced to Cr(III) (i.e., less-toxic, less-soluble species) by humic substances. Then, in order to prevent the transfer of heavy metals into soils (or elution from soils) and/or to reduce absorption of elements by crops as much as possible, it is important to understand the role of humic substances in soils as well as determination of organic content.

Considering the above-mentioned, the distribution proportion of metallic elements among six fractions are varied largely depending on elements and soil utilization, although F6 (i.e., silicate) was relatively large proportion fraction for most elements. Then, it may be important to understand the characteristics of soil including elemental composition as well as the parent material and utilization of soil for considering the impact on the environment.

From this work, the behavior and distribution of metallic elements including REEs, Th and U in the agricultural field could be quantitatively clarified to some extent, although further continuous monitoring and investigation is needed. The data obtained and the method used in this work can be useful for environmental preservation.

2.4 Summary

The following matters have been mainly clarified from the behavior and distribution of metals in agriculture and non-agriculture soil in 5 sampling places:

(1) EC in the upland field is highest. The content of organic carbon in the paddy field is generally higher. Most of the soil samples belong to weak acidic soil, but soil from Qiqihar City is slightly alkaline. It is obvious that there are large differences of the values of pH, EC and organic carbon content of the soil among the different utilization and the different sampling points. It may be attributed to the difference of the soil parent materials, and physical and geography conditions.

(2) “Oddo-Harkins rule” of REEs and the tendency of LREE enrichment are generally found in all these samples. The concentration of Th is higher than U, and the concentration of U in agricultural field is remarkably larger than that in no plow field. No significant differences of REEs, Th and U concentrations are detected among soil utilizations, and/or before and after cultivation. The differences of REEs, Th and U concentrations may be largely attributable to the differences of the nature and formation history of soil, and of the surrounding environment as well as applied fertilizer.

(3) The concentrations of most heavy metals (except Zn) in soils are higher in paddy field. The concentrations of Al, Fe and Mn are remarkably high. Definite differences are not observed between sampling periods. The parent material of soil and regular agriculture activity as well as other factors can affect the elemental compositions in soils.

(4) The distribution characteristics of metallic elements among six fractions are not varied largely between spring and autumn. Most metallic elements mainly exist in the form of residual fraction in soil. The proportion of F5 is generally larger in HREE. F6

and F5 comprise almost all proportion (100 %) for Th. These results suggest that the distribution proportion of metallic elements are varied largely depending on elements and soil utilization

(5) Differences of the concentration and distribution characteristics of metallic elements are found between Chinese soil samples and Japanese soil samples.

Table and Figure

Table 2-1 Geological characteristics of soils used in this work

Location	Soil series group	Soil series	Parent material	Characteristics
a Toyosato, Sakata Town, Yamagata Prefecture	Gley soils	Alluvium	Unconsolidated sedimentary rock	Continuing reduction
b Tateoka, Murayama Town, Yamagata Prefecture	Gley soils	Volcanic soils	Unconsolidated sedimentary rock	Easy acidify and weathering High Organic matter content
c Ueno, Sekikawa Village, Niigata Prefecture	Yellow(paddy) Gley (field, no plow)	Aratano series Shibai series	Unconsolidated sedimentary rock	Weak binding energy of phosphate
d Chaoxian village , Qiqihar City, Heilongjiang Province	Aeolian sandy soil	--	Montmorillonite rock etc.	Low organic matter content High salt content Dry and lack of water in soil
e Suburban area, Shangzhi City, Heilongjiang Province	Black soil	--	Sedimentary rock, Basalt etc.	Big expansion coefficient High water-holding capacity High plasticity when wet state

Table 2-2 Sequential extraction procedure for fractional determination of metallic elements ^[83, 88]

Step	Fraction	Extraction reagents	Ratio of sludge:reagent	Extraction condition
1	F(ws), F1	Extrapure water	7:70	Shake 6 h, 30 °C
2	F(ec), F2	0.05 mol·dm ⁻³ Ca(NO ₃) ₂	7:70	Shake 24 h, 30 °C
3	F(cb), F3	2.5% CH ₃ COOH	7:70	Shake 24 h, 30 °C
4	F(om), F4	6% H ₂ O ₂	7:120	Water bath, 95 °C (Evaporate)
		2.5% CH ₃ COOH	7:70	Shake 24 h, 30 °C
5	F(fm), F5	0.1mol dm ⁻³ (COOH) ₂ + 0.175 mol dm ⁻³ (COONH ₄) ₂ + Ascorbic acid	7:210	Water bath, 6 h, 95 °C Occasional Shaking
6	F(rd), F6	HNO ₃ +HF		Microwave digestion

Note: F1, F(ws): water soluble; F2, F(ec): exchangeable; F3, F(cb): bound to carbonates; F4, F(om): bound to organic matter; F5, F(fm): bound to Fe-Mn oxides; and F6, F(rd): residual state

Table 2-3 Representative pH, EC and organic carbon content of soils at 5 sampling places

Sampling place	Period	Soil type	pH(H ₂ O)	pH(KCl)	EC / $\mu\text{S}\cdot\text{cm}^{-1}$	pH(H ₂ O)-pH(KCl)	Organic carbon content %
Sakata	Spring (2008)	Paddy	5.62	4.60	143.0	1.02	2.21
		Upland	6.31	5.40	628.0	0.91	0.882
		No plow	6.34	4.29	157.3	2.05	0.901
	Autumn (2008)	Paddy	5.40	4.40	266.0	1.00	2.34
		Upland	6.85	6.05	354.0	0.80	0.907
		No plow	6.46	4.44	53.1	2.02	0.834
Murayama	Spring (2008)	Paddy	5.30	4.00	7.62	1.30	3.63
		Upland	5.59	4.91	27.64	0.68	7.69
		No plow	4.96	3.77	8.62	1.19	2.93
	Autumn (2008)	Paddy	5.18	4.11	11.64	1.07	3.65
		Upland	5.54	4.88	24.99	0.66	7.58
		No plow	4.56	3.25	4.29	1.31	2.85
Sekikawa	Spring (2008)	Paddy	5.09	3.97	85.1	1.12	4.31
		Upland	6.51	5.3	78.4	1.21	2.67
		No plow	6.22	4.73	42.1	1.49	1.23
	Autumn (2008)	Paddy	4.92	3.95	104.7	0.97	4.31
		Upland	6.25	5.13	103.8	1.12	2.76
		No plow	6.00	4.56	43.6	1.44	1.21
Qiqihar	Autumn (2011)	Paddy	6.26	4.60	108.8	1.66	5.44
		Upland	7.96	7.13	242	0.83	6.05
		No plow	7.81	6.72	86.7	1.09	4.13
Shangzhi	Autumn (2011)	Paddy	5.15	3.72	96.2	1.43	7.19
		Upland	6.33	5.12	97.4	1.21	7.81
		No plow	5.53	4.09	65.9	1.44	7.57

Table 2-4 Mean concentrations of REEs ($\text{mg}\cdot\text{kg}^{-1}$) in soil samples in 5 sampling places

Sampling place	Soil type	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Sakata (n=11)	Paddy	13.5	30.0	3.26	13.1	2.91	0.914	2.97	0.456	2.76	0.553	1.68	0.231	1.58	0.229
	Upland	14.7	30.1	3.07	11.5	2.45	0.821	2.49	0.365	2.17	0.433	1.32	0.177	1.22	0.175
	No plow	12.9	26.5	2.89	11.5	2.44	0.863	2.51	0.386	2.31	0.463	1.42	0.195	1.33	0.192
Murayama (n=4)	Paddy	15.4	34.2	4.11	17.3	4.19	1.39	4.74	0.795	4.39	0.904	2.75	0.383	2.66	0.396
	Upland	17.0	36.7	4.45	18.1	4.05	1.39	4.60	0.723	3.83	0.772	2.40	0.332	2.32	0.351
	No plow	14.5	31.8	3.67	15.0	3.46	1.21	3.94	0.643	3.46	0.708	2.19	0.308	2.14	0.321
Sekikawa (n=4)	Paddy	19.7	50.9	4.49	16.7	3.31	0.640	3.06	0.475	2.63	0.537	1.66	0.248	1.75	0.275
	Upland	20.7	46.2	4.93	18.5	3.82	0.713	3.49	0.537	2.80	0.556	1.63	0.246	1.59	0.269
	No plow	21.5	47.1	5.16	19.4	4.06	0.718	3.68	0.566	3.02	0.578	1.74	0.241	1.71	0.248
Qiqihar (n=1)	Paddy	29.6	67.2	10.6	28.6	4.56	1.29	4.73	0.636	2.98	0.569	1.79	0.236	1.65	0.238
	Upland	30.3	87.0	9.63	25.5	3.80	1.16	3.99	0.517	2.39	0.456	1.44	0.191	1.33	0.191
	No plow	27.0	61.8	9.03	21.7	3.43	1.07	3.57	0.462	2.11	0.400	1.26	0.166	1.16	0.167
Shangzhi (n=1)	Paddy	7.22	24.4	1.70	6.81	1.62	0.963	2.04	0.279	1.41	0.292	0.923	0.146	0.996	0.159
	Upland	6.75	19.9	1.63	7.01	1.61	0.642	1.68	0.259	1.32	0.266	0.827	0.128	0.851	0.129
	No plow	6.48	20.8	1.57	6.41	1.13	0.458	1.18	0.258	0.983	0.290	0.748	0.097	0.679	0.102

* n is the number of soil samples from the same sapling point.

Table 2-5 Total mean concentrations (mg·kg⁻¹) of Th and U and U/Th at 5 sampling places

Sampling place	Period	Soil type	Th	U	U/Th
Sakata	spring	Paddy	4.14	1.37	0.33
		Upland	3.78	1.48	0.39
		No plow	3.82	0.82	0.21
	Autumn	Paddy	4.33	1.42	0.33
		Upland	4.14	1.48	0.36
		No plow	4.15	0.80	0.19
Murayama	spring	Paddy	3.73	1.75	0.47
		Upland	5.26	1.93	0.37
		No plow	3.12	1.29	0.41
	Autumn	Paddy	4.37	1.70	0.39
		Upland	4.31	1.69	0.39
		No plow	4.58	1.33	0.29
Sekikawa	spring	Paddy	19.7	4.42	0.22
		Upland	21.7	4.57	0.21
		No plow	23.5	4.11	0.17
	Autumn	Paddy	19.5	4.51	0.23
		Upland	22.1	4.46	0.20
		No plow	23.4	4.24	0.18
Qiqihar	Autumn	Paddy	13.4	3.40	0.25
		Upland	11.9	2.31	0.19
		No plow	11.2	2.08	0.19
Shangzhi	Autumn	Paddy	8.37	5.03	0.60
		Upland	9.11	3.88	0.43
		No plow	8.87	4.45	0.50

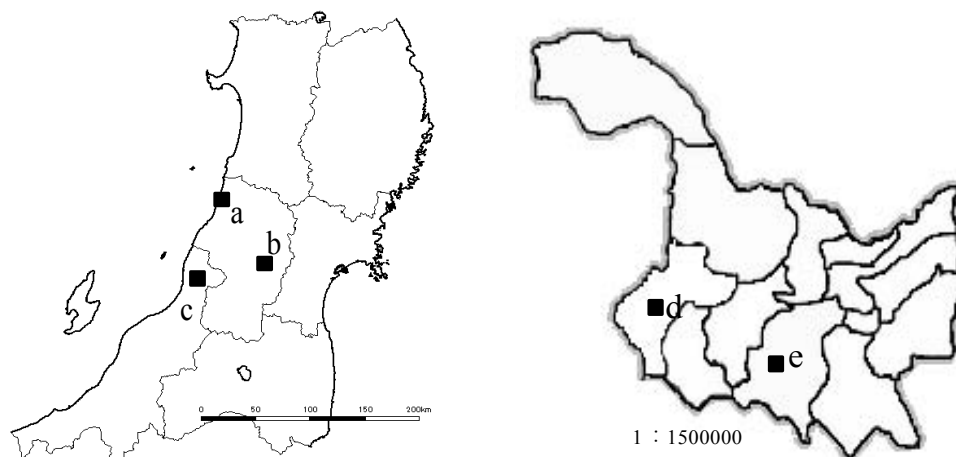
Table 2-6 Total mean concentrations (mg·kg⁻¹) of aluminum and some heavy metals

Sampling place	Soil type	Zn	Cd	Pb	Ni	Fe	Co	Mn	Cr	Al
Sakata (n=8)	Paddy	81.8	5.77	28.0	21.6	3.20×10 ⁴	11.2	642	44.9	6.51×10 ⁴
	Upland	84.3	4.65	26.0	13.2	2.14×10 ⁴	7.15	597	29.6	6.02×10 ⁴
	No plow	91.9	5.62	27.7	17.4	2.69×10 ⁴	8.36	694	50.8	6.39×10 ⁴
Murayama (n=6)	Paddy	105	8.00	24.7	12.9	4.49×10 ⁴	11.4	808	33.1	7.20×10 ⁴
	Upland	97.5	7.16	19.1	10.9	3.71×10 ⁴	10.5	936	29.7	7.62×10 ⁴
	No plow	162	6.39	15.8	12.4	3.00×10 ⁴	9.31	875	29.7	6.57×10 ⁴
Sekikawa (n=4)	Paddy	85.9	4.67	23.7	22.0	4.02×10 ⁴	7.16	325	60.8	6.30×10 ⁴
	Upland	176	4.34	27.1	11.3	2.78×10 ⁴	6.75	723	41.5	6.36×10 ⁴
	No plow	214	4.69	17.5	14.8	3.20×10 ⁴	7.14	618	36.6	6.90×10 ⁴
Qiqihar (n=1)	Paddy	64.8	2.69	14.4	15.1	2.65×10 ⁴	10.0	283	36.8	6.68×10 ⁴
	Upland	83.8	4.79	20.4	10.1	1.84×10 ⁴	6.40	338	45.1	6.58×10 ⁴
	No plow	44.7	3.78	11.6	11.0	1.58×10 ⁴	5.00	351	20.6	6.68×10 ⁴
Shangzhi (n=1)	Paddy	69.0	4.62	16.2	18.9	3.72×10 ⁴	17.1	634	58.7	2.99×10 ⁴
	Upland	74.0	2.98	10.8	14.1	2.75×10 ⁴	12.7	920	39.3	3.76×10 ⁴
	No plow	73.8	3.91	71.8	17.8	3.35×10 ⁴	12.8	665	49.0	5.86×10 ⁴

* n is the same meaning with that in Table 2-4.

Table 2-7 Total concentrations of aluminum and some heavy metals at 3 sampling places (mg·kg⁻¹) in 2010

Sampling place	Field	Period	Al	Cr	Mn	Fe	Co	Ni	Zn	Cd	Bi
Sakata	Paddy	April	6.35×10 ⁴	41.7	601	3.00×10 ⁴	16.5	19.7	78.4	8.70	40.8
		Oct.	7.33×10 ⁴	41.8	654	3.31×10 ⁴	10.2	20.7	90.8	9.28	43.4
	Upland	April	6.10×10 ⁴	27.9	634	2.15×10 ⁴	10.2	10.8	83.2	5.85	27.5
		Oct.	6.58×10 ⁴	28.4	601	2.16×10 ⁴	7.60	16.8	97.3	7.91	31.6
	No plow	April	6.68×10 ⁴	37.8	674	2.58×10 ⁴	11.3	13.4	103	7.05	38.6
		Oct.	6.83×10 ⁴	42.9	822	2.90×10 ⁴	12.4	18.5	114	7.89	45.4
Murayama	Paddy	April	7.38×10 ⁴	32.9	712	4.64×10 ⁴	15.1	15.3	122	11.5	31.2
		Oct.	7.10×10 ⁴	34.7	780	4.88×10 ⁴	10.8	17.0	123	11.4	36.6
	Upland	April	8.22×10 ⁴	26.6	910	3.62×10 ⁴	14.3	8.90	91.6	8.23	24.1
		Oct.	8.01×10 ⁴	25.3	978	2.58×10 ⁴	11.8	14.7	96.7	10.3	30.4
	No plow	April	6.74×10 ⁴	29.8	895	2.95×10 ⁴	13.1	11.5	153	7.50	28.4
		Oct.	6.92×10 ⁴	32.9	970	3.21×10 ⁴	9.97	15.1	162	7.97	35.5
Sekikawa	Paddy	April	6.25×10 ⁴	60.9	510	3.89×10 ⁴	7.32	21.1	86.4	4.70	52.3
		Oct.	6.38×10 ⁴	60.7	545	4.06×10 ⁴	7.09	22.6	92.4	4.82	55.1
	Upland	April	6.28×10 ⁴	42.3	724	2.81×10 ⁴	6.91	9.87	165	4.13	26.9
		Oct.	6.44×10 ⁴	42.1	721	2.84×10 ⁴	6.80	12.7	184	4.59	29.1
	No plow	April	6.88×10 ⁴	37.4	594	3.11×10 ⁴	7.32	13.4	203	4.67	23.2
		Oct.	6.93×10 ⁴	35.4	632	3.35×10 ⁴	7.21	16.5	221	4.89	25.7



(a) Toyosato, Sakata Town, Yamagata Prefecture, (b) Tateoka, Murayama Town, Yamagata Prefecture, (c) Ueno, Sekikawa Village, Niigata Prefecture, (d) Chaoxian village, Qiqihar City, Heilongjiang Province, (e) Suburban area, Shangzhi City, Heilongjiang Province

Fig. 2-1 Location of sampling points for soil

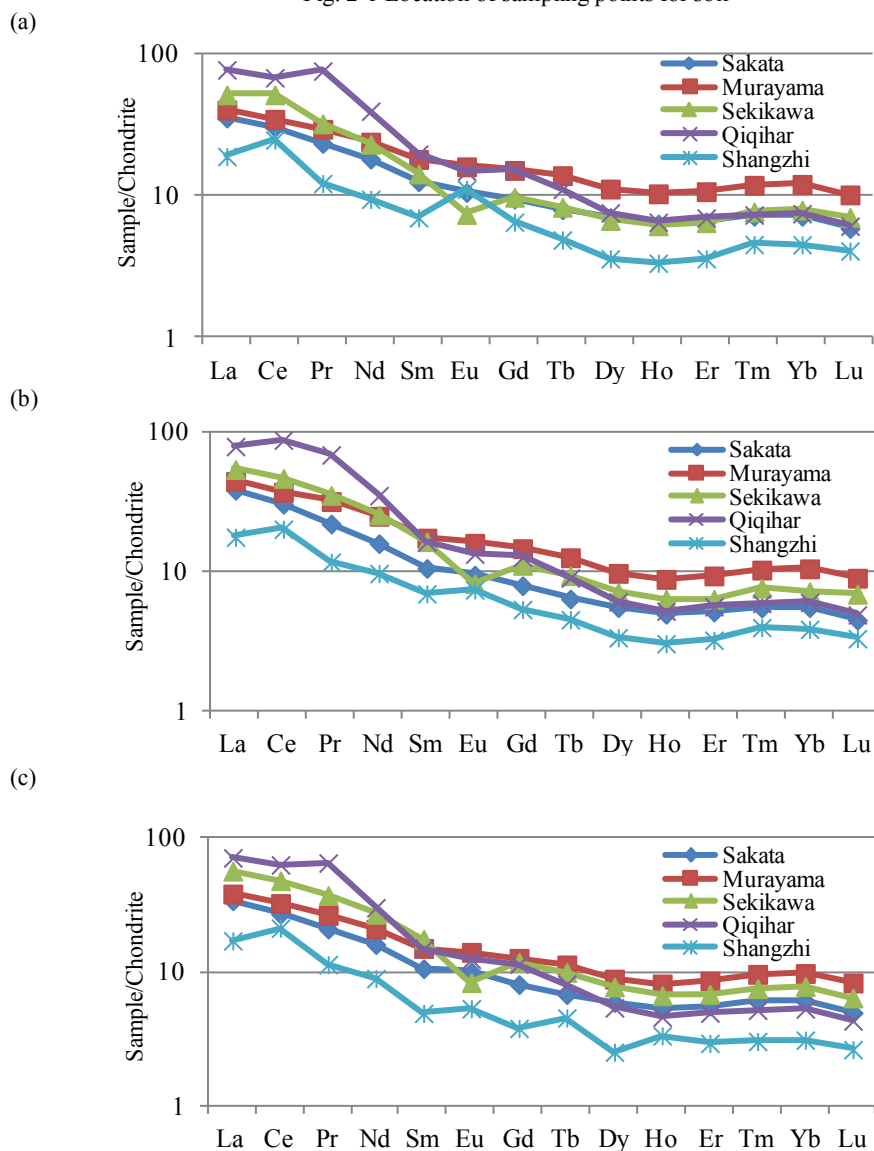


Fig. 2-2 Normalized mean REEs concentrations (REEs patterns) of soil samples at 5 sampling places during measurement period (a) paddy field (b) upland field (c) no plow

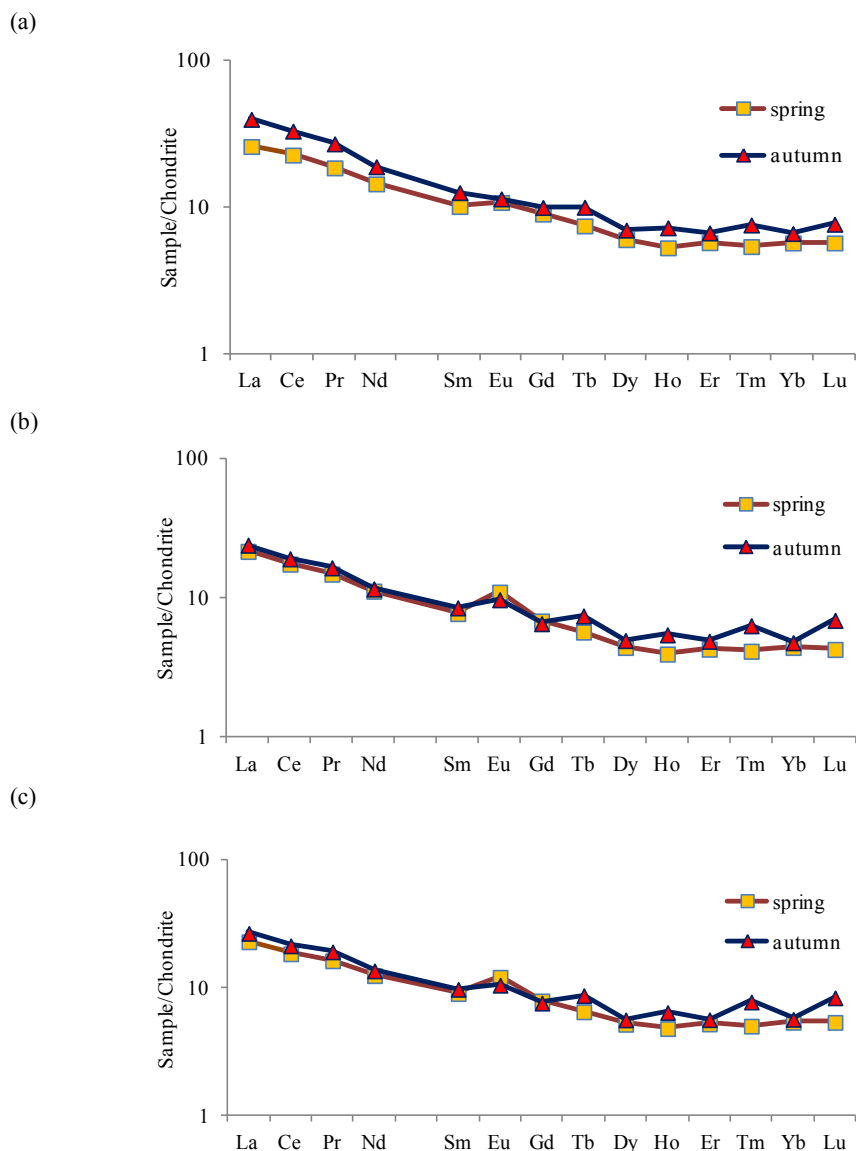


Fig. 2-3 Normalized REEs concentrations (REEs patterns) of soil samples from Sakata in 2010 (a) paddy field (b) upland field (c) no plow

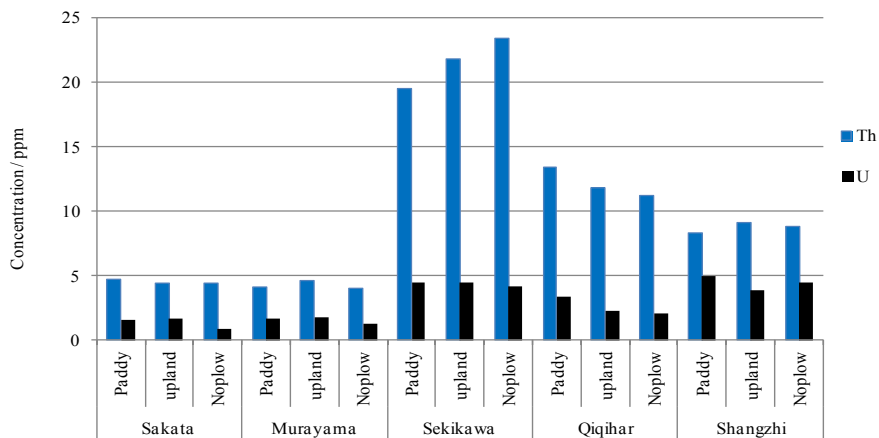


Fig. 2-4 Mean concentrations of Th and U in soil samples at 5 sampling places

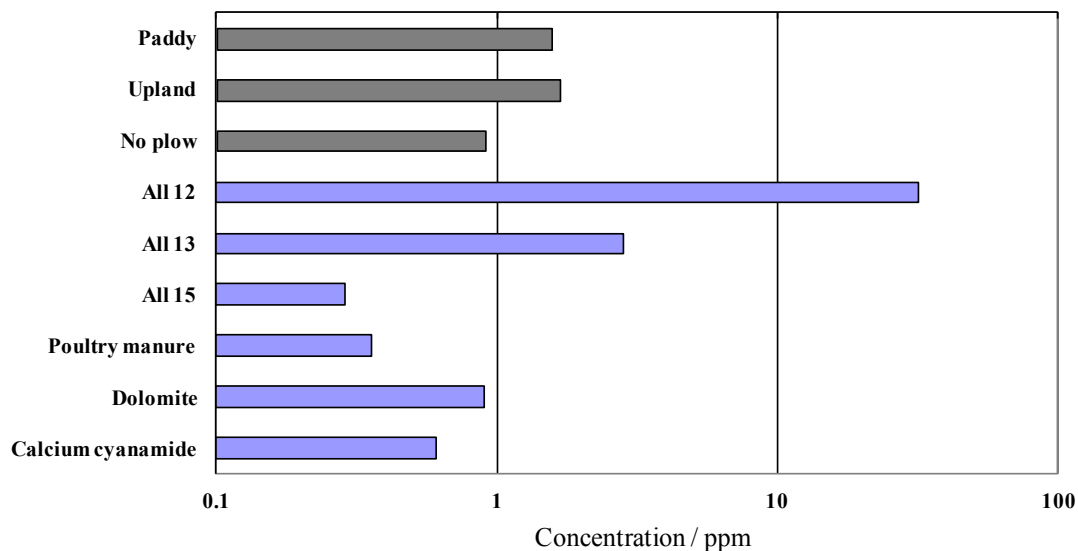


Fig.2-5 Concentrations of U in fertilizers used in the agricultural field along with those in soil samples from Sakata (“All 13” (containing N: 13 %, P₂O₅: 13 %, K₂O: 13 %), “All 15” (containing N: 15 %, K₂O: 15 %), “Poultry manure” (containing N: 4.0 %, P₂O₅: 5.0 %, K₂O: 2.5 %, and C/N=7.0), “Dolomite” (containing Alkali: 55.0 %, MgO: 15.0 %), “Calcium cyanamide” (containing N: 20.0 %, Alkali: 50.0 %), and agricultural chemical: “Diazinon” (containing: C₁₂H₂₁N₂O₃PS: 5.0 %, inert ore and binder etc: 95.0 %), Paddy, upland and no plow soil samples from Sakata.)

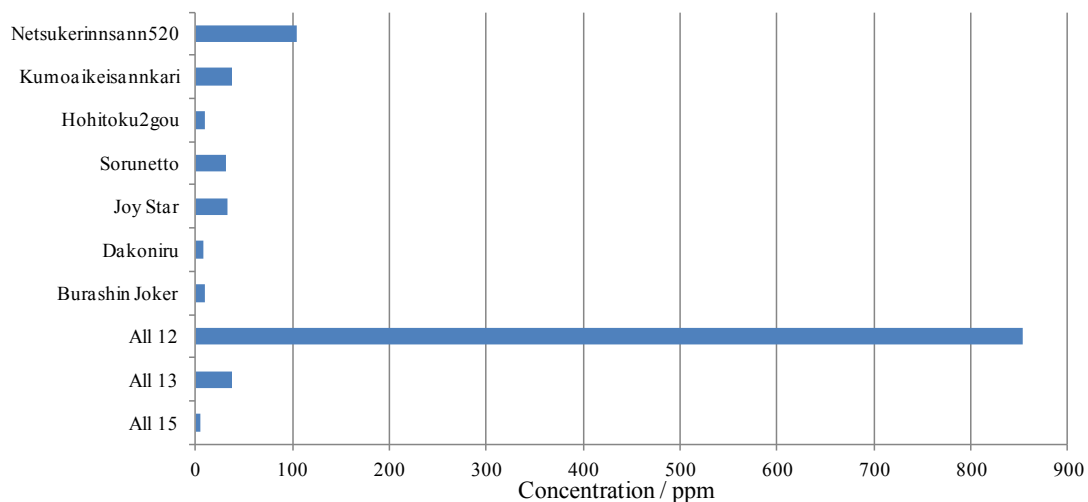


Fig. 2-6 Concentrations of Zn in fertilizers used in the agricultural field

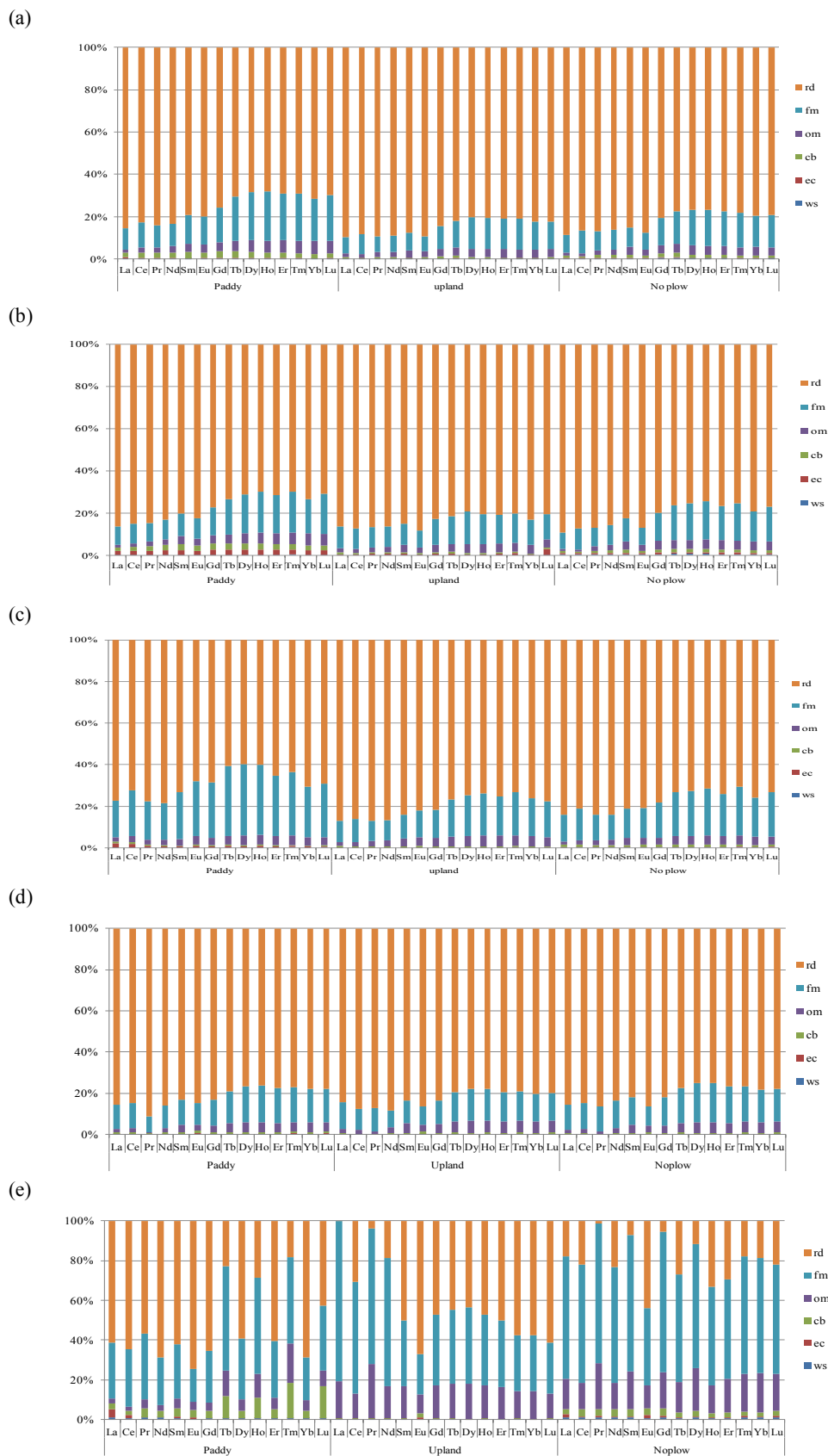


Fig. 2-7 Mean relative distributions of REEs among six fractions of soil samples at 5 sampling places during measurement period

(a) Sakata (b) Murayama (c) Sekikawa (d) Qiqihar (e) Shangzhi

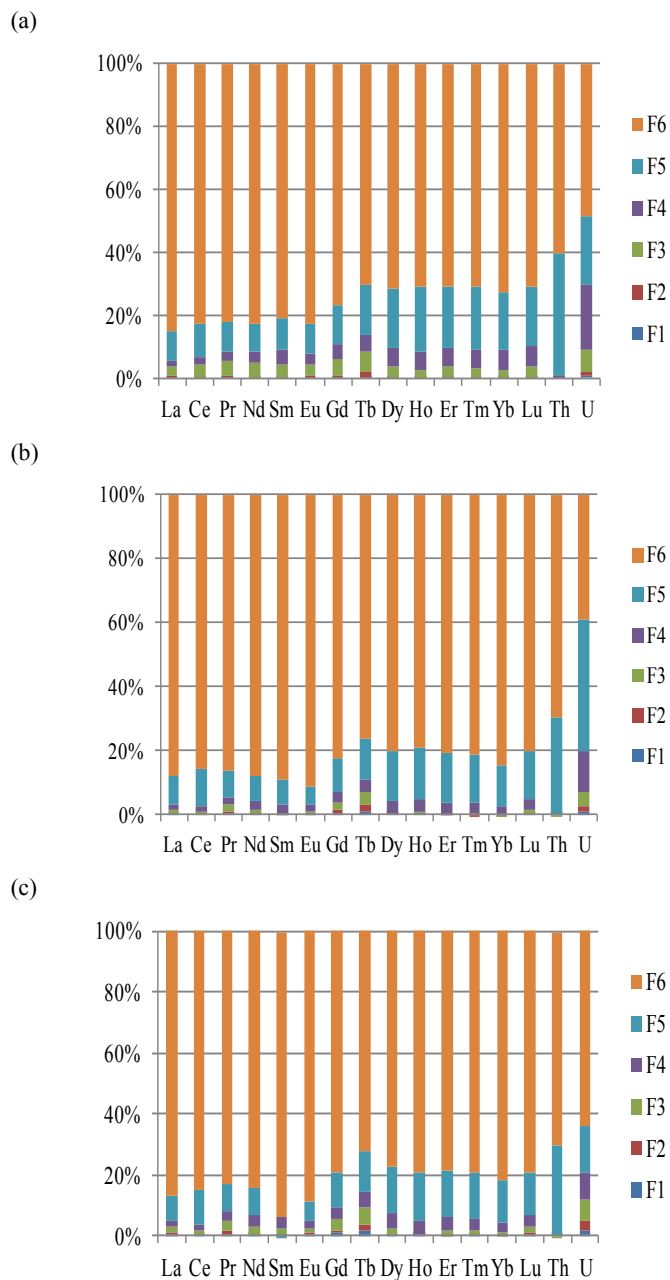


Fig. 2-8-1 The relative distribution of REE, Th and U among six fractions of soil from Sakata in April 2010
(a) paddy field (b) upland field (c) no plow

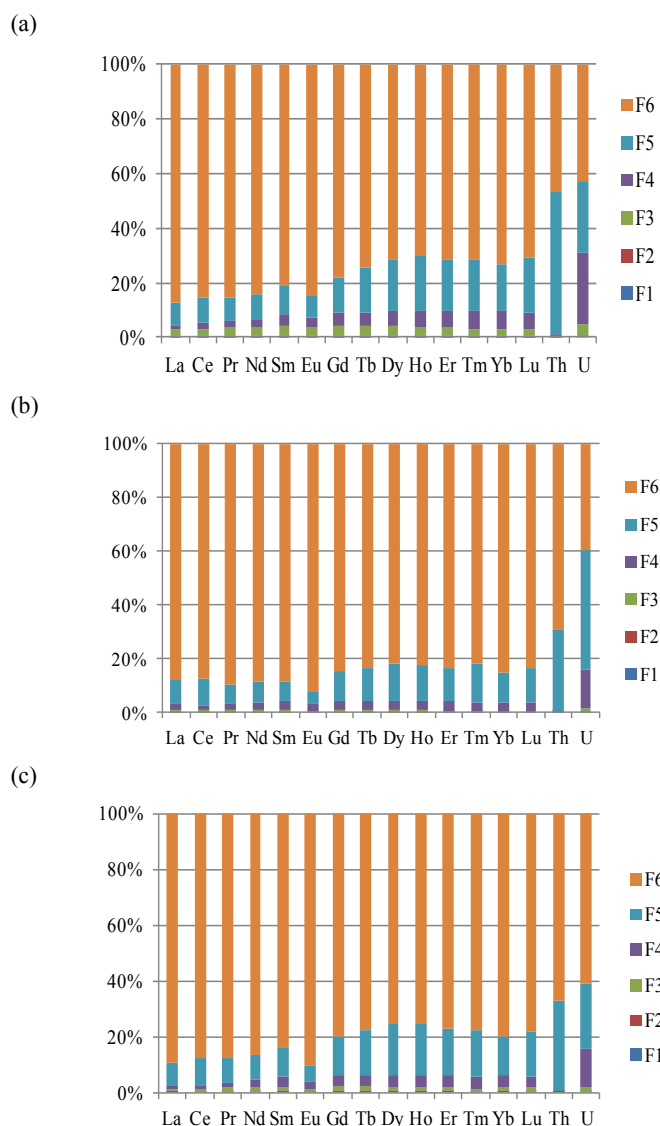


Fig. 2-8-2 The relative distribution of REE, Th and U among six fractions of soil from Sakata in October 2010
(a) paddy field (b) upland field (c) no plow

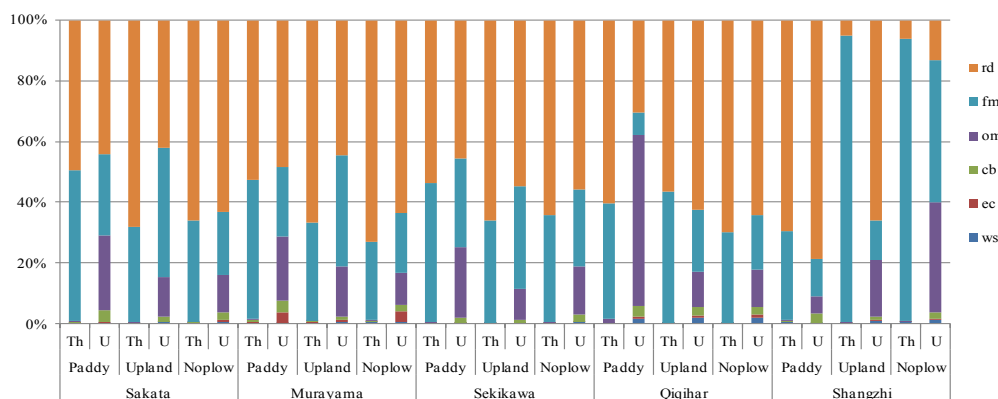


Fig. 2-9 Mean relative distributions of Th and U among six fractions of soil samples at 5 sampling places during measurement period

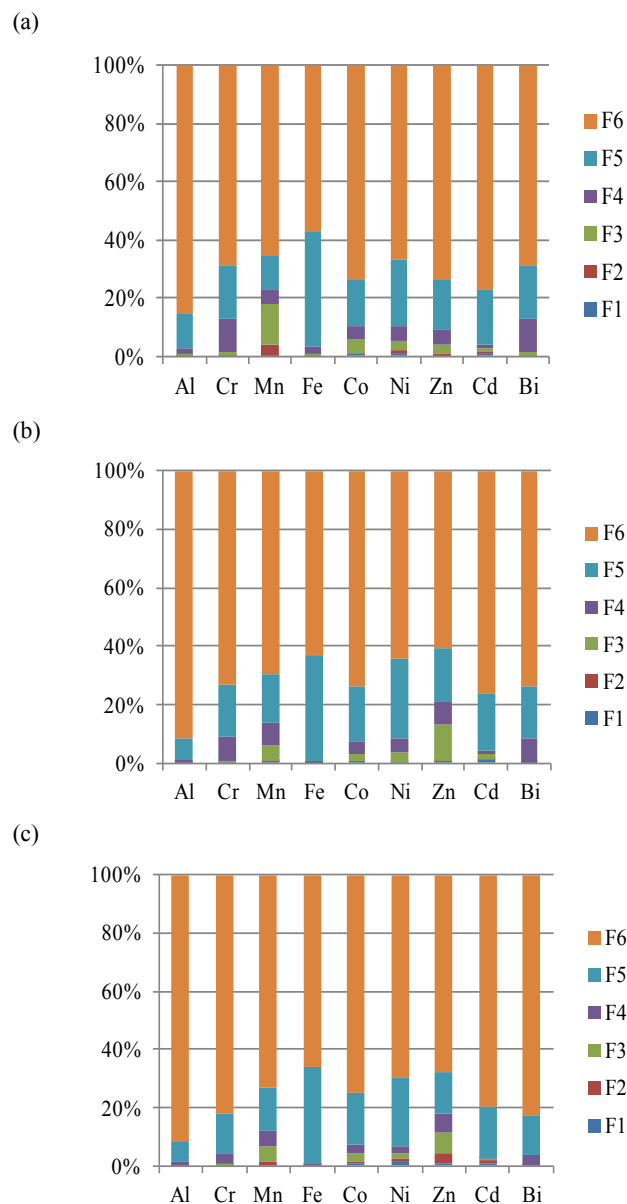


Fig. 2-10-1 The relative distribution of Al and some heavy metals among six fractions of soil from Sakata in April 2010 (a) paddy field (b) upland field (c) no plow

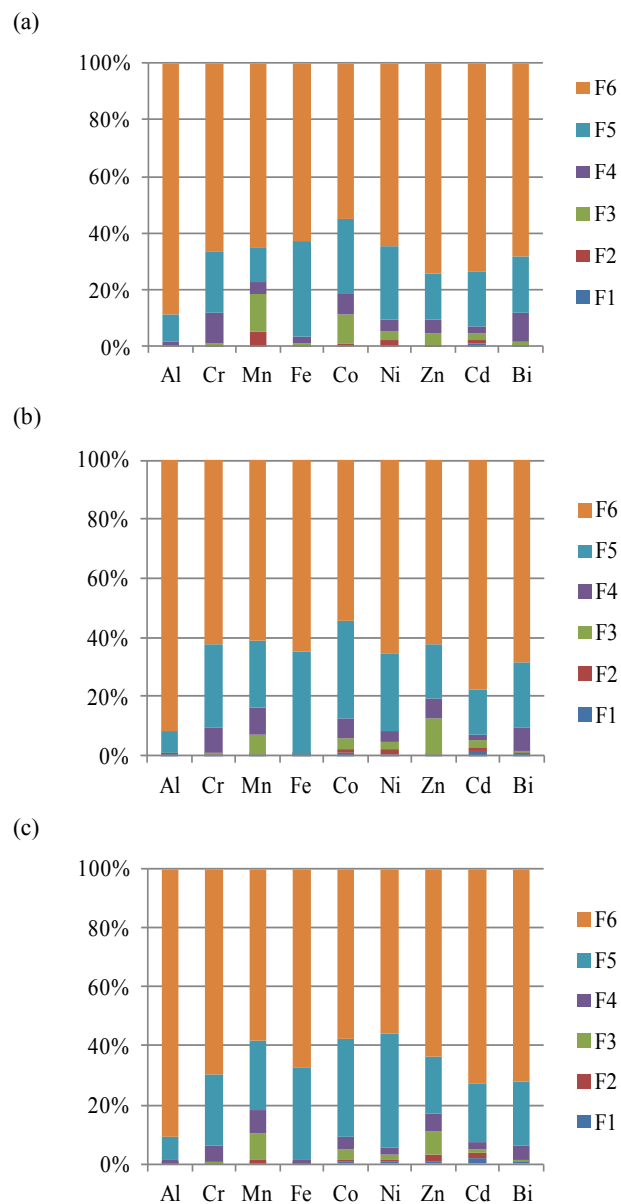


Fig. 2-10-2 The relative distribution of Al and some heavy metals among six fractions of soil from Sakata in October 2010 (a) paddy field (b) upland field (c) no plow

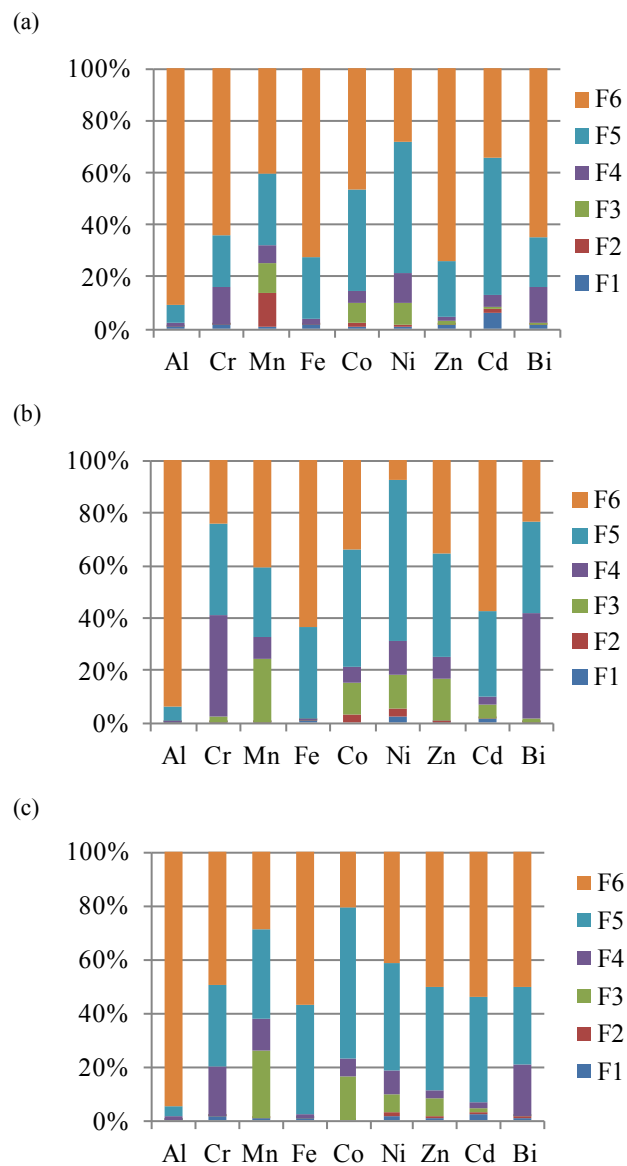


Fig. 2-11-1 The relative distribution of Al and some heavy metals among six fractions of soil from Qiqihar in October 2011 (a) paddy field (b) upland field (c) no plow

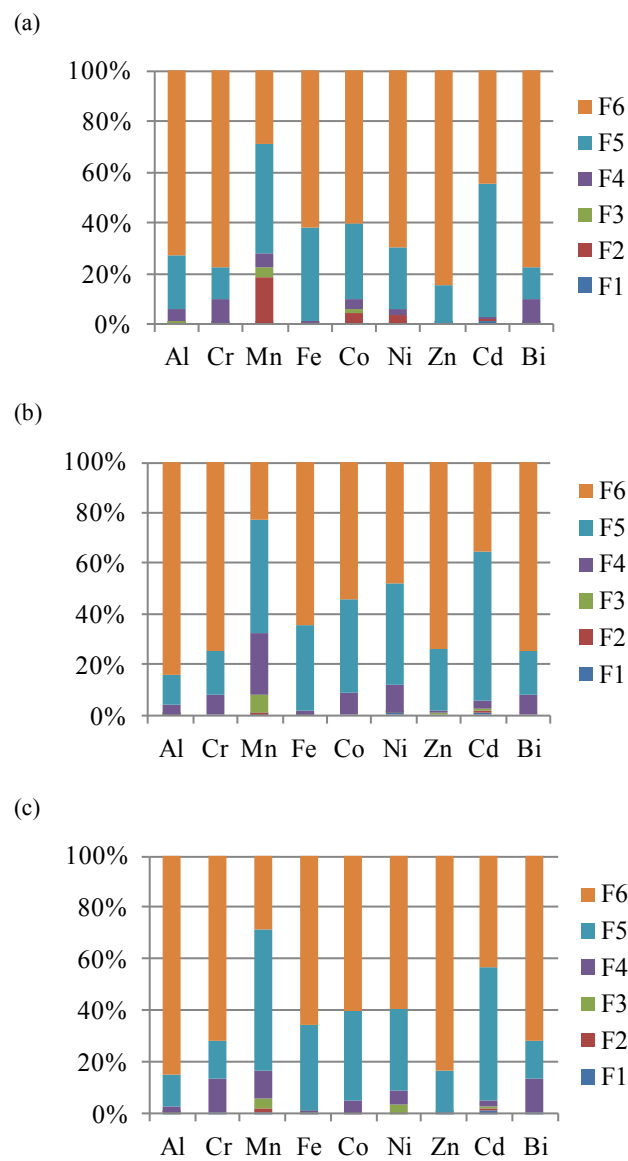


Fig. 2-11-2 The relative distribution of Al and some heavy metals among six fractions of soil from Shangzhi in October 2011 (a) paddy field (b) upland field (c) no plow

Chapter 3 Behavior and Distribution of Heavy Metals in Sludge and Recovery of Metals by Biosurfactants Application

3.1 Introduction

With the rapid development of industry, a large quantity of industrial sludge is settled down in wastewater treatment plants (WWTP) every year. The sludge must be treated and disposed in a safe and effective manner because it may be contaminated with toxic organic and inorganic compounds. Much of this sludge is treated using a variety of digestion techniques to reduce the amount of organic matter and the number of disease-causing microorganisms, then the nutrient-rich sludge is provided to use as agricultural soil for landscaping and garden planting or as natural fertilizer ^[109-111]. These techniques have reduced the amount of landfill and changed waste into resource ^[112,113]. However, the digested sludge cannot be directly used for practical use, because it may contain hazardous inorganic substances such as heavy metals and radioactive elements. For this reason, it is of significant importance to investigate the removal of these metals by eco-friendly method, and to study the behavior and distribution of heavy metals in sludge from an environmental protection and human health perspective. On the other hand, the demand for trace metals such as rare earth elements (REEs) in modern society has increased markedly in recent years. The shortage of trace metals including REEs and uranium (U) has been of concern and the investigation of new sources of these trace metals is important from a resources recovery point of view.

In recent years, the concentrations and distribution of heavy metals in sludge has been extensively studied ^[114-118]. Furthermore, the investigations of methods for the

removal of heavy metals from sludge have been widely carried out [6,119-121].

Total concentrations and fractions of heavy metals in sewage sludge from municipal and industrial wastewater treatment plants have been studied [114]. The results showed that the total concentrations of heavy metals in sludge varied greatly, and that there was no significant difference in total metal concentration between municipal and industrial wastewater treatment plants. Chen et al. [115] reported the bioavailability and eco-toxicity of heavy metals in municipal sludge by taking into consideration both the speciation of metals and the local environmental characteristics. From this work, it was found that only the sludge from Xia Wan sewage treatment plant showed elevated concentrations of heavy metals, and that the sludge from other plants showed low total concentrations of heavy metals except for a slightly higher concentration of Cd. The results of the sequential extraction procedure showed that Cu and Zn were principally distributed in the oxidize fraction, and that Pb was mainly in the residual fraction. Furthermore, the different types of sludge and the distribution of the heavy metals in sludge have been studied [116]. It was confirmed that the total concentrations of heavy metals did not exceed the limits set out by European legislation and that the stabilization method undergone by the sludge strongly influenced the distribution and the associated phases of heavy metals. The extractable forms of heavy metals in sludge from wastewater treatment plants have been determined to obtain suitable information about their bioavailability or toxicity [117]. In regard to current international legislation on the use of sludge for agricultural purposes, the concentrations of any metal did not exceed permitted levels. For most of the subject metallic elements, the increase of the concentrations was clearly found in two less-available fractions (oxidizable fraction and residual fraction) with the sludge treatment. In contrast, Ščančar et al. [118] determined the total and fractional

concentrations of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge samples from an urban wastewater treatment plant and showed that the sludge could not be used in agriculture due to the high total Ni concentration and its high mobility.

Currently, the removal of ultrasonic-assisted metals from sludge is applied widely. For example, Deng et al. ^[119] and Li et al. ^[120] investigated the removal or recovery of heavy metals from sludge using ultrasound-assisted acid. The results showed that ultrasonic treatment is a necessary and effective method for assisting the improvement of heavy metal removal. However, ultrasonic treatment has an effect on the physical and chemical properties of sludge to some extent, and is energy-consuming. In another study, Babel and Dacera ^[6] reviewed various methods for the removal of heavy metal from sewage sludge, including chemical extraction, bioleaching, electroreclamation, and supercritical fluid extraction (SFE). They compared the advantages and limitations of each, and gave a detailed analysis of their findings. A combination of two methods (i.e., bioleaching and electrokinetic remediation technology) for removing heavy metals from sludge has been also reported ^[121]. The combined technology can not only remove the heavy metals in the sludge but also make them be recycled, although it is energy-consuming to some extent.

In addition, soil washing with biosurfactants, as a kind of effective method for the removal of heavy metal in soil, has also attracted many researchers attention in recent years ^[68,122]. However, until now researches on removal of heavy metals in sludge with this method are few ^[123,124]. Therefore, two kinds of biosurfactants (saponin and sophorolipid) were collected, and the removal of heavy metals in polluted sludge was performed with soil washing in this work. The characteristics of saponin and sophorolipid are denoted in detail as following.

Saponin

Saponins are plant glycosides which are widely distributed in plants. They are amphipathic with the composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. Substantially pure saponin is a mixture of triterpene-glycosides extracted from the bark of the tree *Quillaja saponari* [125]. It is soluble in water and hot alcohol, but is insoluble in most organic solvents. Aqueous solutions will froth when shaken; the froth can be dispersed by alcohol or ether [126]. *Quillaja* saponin solutions are not autoclavable. Solutions have been found to be stable for about one month when stored at 2-8°C. Saponins are powerful emulsifiers having hemolytic ability, and therefore slightly toxic to some extent [127].

Saponin has been used for the removal of contaminants (organic compounds and heavy metals) in water and soil, although it was originally applied in medicine and pharmacology field.

Sophorolipid

Sophorolipids is a kind of extracellular biosurfactant produced by yeasts [64, 65]. They are comprised of one sophorose molecule (hydrophilic part) linked to one hydroxy fatty acid (lipophilic part) by one or two cross-links. They are mixtures with differences in acetylated degree of sophorose, hydroxy fatty acid length, and hydroxyl group position on the fatty acid.

Sophorolipids are classified as lactonic form and acidic form. Generally, the lactonic ones show better surface tension reducing propriety and biological activities, while the acidic ones display better foam formation ability and solubility and allow further modification at the free carboxylic acid end [128]. It has been shown that the lactonic form is necessary, or at least preferable, for many applications [129]. Currently sophorolipids have been applied in many specific fields such as the cosmetics, detergent, petroleum, pharmaceutical, environment industries, and therapeutics

according to the differences of surface and biological activity in different sophorolipid molecules^[130].

As mentioned above, most research has been mainly focused on toxic heavy metallic elements such as Cd, Pb, Cu and Cr as subject elements, and on the differences and characteristics of these elements according to different types of sludge or different treatment processes. However, few reports have been published about the behavior and distribution of REEs, Th and U. Moreover, there have been very few comparisons between concentrations of heavy metals in sludge and those in natural soil carried out. It is important to compare the concentration and distribution of metals in sludge with those in natural soil when considering the utilization of sludge as agricultural soil in the future. The purposes of this paper are (1) to investigate the behavior, distribution and characteristics of heavy metals including REEs, Th and U in sludge compared with those in natural soil; and (2) to study the removal/recovery process of heavy metals from polluted sludge with biosurfactant elution by batch and column experiments.

3.2 Experimental

3.2.1. Apparatus and Reagents

An inductively coupled plasma mass spectrum (ICP-MS) instrument (Thermo scientific X-Series) was used to determine the concentrations of REEs, Th and U, and an inductively coupled plasma atomic emission spectrophotometer (ICP-AES) instrument (SPS1500, Seiko Instruments Inc.) was employed to determine the concentrations of heavy metals (Zn, Cd, Pb, Cr, Ni and Cu). The operating conditions of the ICP-MS are the same as shown in our previous paper^[131] and that of ICP-AES are based on our other previous paper^[85].

Heavy metal standard solutions, including REEs, Th and U, were purchased from SPEX CertiPrep, Inc. (USA). Each working standard solution was prepared by diluting the original standard solution.

In this work two kinds of biosurfactants were used, saponin and sophorolipid. Saponin was purchased commercially from Sigma-Aldrich, Inc. (Germany). It is a nonionic biosurfactant, but includes the carboxyl group ($-\text{COOH}$) as shown in Fig.3-1 (a) based on the analysis of *quillaja bark* by Guo and Kenne ^[132]. Sophorolipid was supplied by State Key Laboratory for Microbial Technology (Shandong University, China). It is also a nonionic biosurfactant, and one possible structure of sophorolipid from *Wickerhamiella domercqiae* analyzed by Chen et al. is given in Fig. 3-1 (b) ^[133].

All other chemical reagents, purchased from Kanto Chemical Co., Inc. (Japan), were of analytical grade. Water ($>18.2 \text{ M}\Omega$), which was treated using an ultrapure water system (Advantec aquarius: RFU 424TA), was employed throughout the work.

3.2.2 Samples

There were four kinds of sludge used in the work. Among them, three kinds of sludge were directly collected from the plants. One sludge sample was collected in May 2010 from an industrial water treatment plant in Niigata, Japan. It was called the original sludge. The other two sludge samples were collected in September 2011 from a domestic water treatment plant in Qiqihar of Heilongjiang Province and from an electroplate factory in Langfang of Hebei Province, China. All sludge samples were air-dried and removed coarse sand and stone, then ground and sieved through 120 mesh (0.125mm).

The last sludge was artificially prepared in our lab, and called the polluted sludge. The polluted sludge sample was prepared by adding the solution containing three

kinds of metallic salts ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Ni^{2+} , 1000 ppm), $\text{Pb}(\text{NO}_3)_2$ (Pb^{2+} , 1360 ppm), and $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Cr^{3+} , 1000 ppm)) to the original sludge above-mentioned. The polluted sample was shaken for 3 days on a shaker at room temperature ($25.0 \pm 0.2^\circ\text{C}$), and subsequently centrifuged at 3000 rpm for 30 min using a centrifugal separator (Kubota Co. 5200). The supernatant was discarded and the polluted sludge was air-dried and sieved through 120 mesh (0.125 mm).

Basic characteristics of the sludge samples, such as pH, EC, moisture content and cation exchange capacity (CEC) were measured, based on the method for soil testing recommended by The Japanese Geotechnical Society [86]. For measuring organic matter content, 10g air-dried sludge samples were heated for 2 h at 105°C , then burned at 550°C in a furnace for 6 h. Organic matter content was estimated from the weight differences of the sludge before and after burning divided by the sludge weight before burning. Permeability is an important physical parameter to determine the feasibility of the soil flushing process. Therefore, the permeability of the sludge was also determined using the Unfirmed Water Head Test [134]. The specific surface area of each sample was measured using Micromeritics TriStar3000. The BET method and Langmuir method, as well as methods used in our previous work, were applied to determine the surface area [135,136].

For measuring total metal concentration, the sludge was digested with HNO_3 -HF by using the microwave digestion method as well as the case of digesting soil [81]. After this, the analysis of metallic elements was performed using ICP-AES or ICP-MS.

3.2.3 Distribution of Metallic Elements in Sludge

All heavy metals including REEs, Th and U in sludge samples were partitioned

into six fractions with sequential extraction procedures mainly based on Sadamoto et al.^[137] and Tessier et al.^[89]. In this paper, these six fractions: (1) water soluble, (2) exchangeable, (3) bound to carbonates, (4) bound to organic matter, (5) bound to Fe-Mn oxides and (6) residual were denoted as F1, F2, F3, F4, F5 and F6, respectively. The sequential extraction procedure is the same as shown in Table 2-2 (Chapter 2). For the initial step in this sequential extraction procedure, 7 g of dried sludge sample in 100 cm³ polypropylene centrifuge tube was used. Following extraction in each step, the mixture of sludge sample and each extraction reagent was centrifuged (3000rpm×30min) using a centrifugal separator (Kubota Co. 5200). This procedure is the same used in our previous work on soil^[138]. The concentrations of metallic elements in each fraction were determined with ICP-AES or ICP-MS.

3.2.4 Batch Test and Column Test

Effect of the concentration and pH value of biosurfactant solution on the removal of heavy metals in the polluted sludge and the electroplate sludge was investigated in batch experiments at room temperature (25°C). Each 1.0g of the polluted sludge or 0.5g of the electroplate sludge was weighed into a centrifuge tube, and 25 cm³ of biosurfactant solution, varying in initial concentrations from 1 to 50 g·dm⁻³, was added to each tube. The tubes were then shaken in a reciprocating shaker for 24 hours to attain equilibrium. The suspension was centrifuged (3000rpm×30min) using a centrifugal separator (Kubota Co. 5200). The supernatant solutions were separated, and dissolved with 1 mol·dm⁻³ HNO₃ after digestion for analysis (Digestion method was the same shown in 2.2.4 section.). Subsequently, the effect of pH value (varied from 2.5 to 6.5) was investigated with the same procedure as above.

Column tests were also conducted to remove heavy metals from polluted sludge

and the electroplate sludge at the optimum concentration and pH of the biosurfactant solution determined from the batch experiment. Permeability is a useful parameter for the flushing techniques like column experiments. Silica was mixed with the sludge (mass ratio of 4:1) to be compacted in column to improve the low permeability of sludge. 30g of each mixture was packed in a glass column (internal diameter of 1.5 cm and length of 30 cm), and two columns in total for each kind of sludge. Saponin solution and ultrapure water were prepared for mobilization and leaching of heavy metals from sludge at less than a rate of $0.2 \text{ cm}^3 \cdot \text{min}^{-1}$ using multichannel peristaltic pump. Each leachate was collected per one flush volume, then was digested and dissolved in $1 \text{ mol} \cdot \text{dm}^{-3} \text{ HNO}_3$ for analysis.

3.2.5 Recovery of Heavy Metals from Leachates

The precipitation method was applied by using $3 \text{ mol} \cdot \text{dm}^{-3}$ sodium hydroxide (NaOH) solution ^[67]. The leachate from the polluted sludge washed with saponin solution was used as a sample after determining the concentration of heavy metals. The pH value of the leachate was gradually increased because heavy metals were precipitated as hydroxide. The solution was allowed to stand for 24 hours before being centrifuged with a centrifuge, after which, the concentration of heavy metal was measured with ICP spectrometry.

3.2.6 Recovery of Saponin and Reuse

The leachates after washing with $25 \text{ g} \cdot \text{dm}^{-3}$ and $30 \text{ g} \cdot \text{dm}^{-3}$ saponin (pH 2.5) from the electroplate sludge were used as Sample1 and Sample 2 after determining the concentration of saponin by UV-Visible spectrophotometer (HITACHI, U-5100). Metals were separated from the leachates as precipitation to obtain saponin solution,

which is similar with the recovery method of heavy metals shown in 3.2.5 except that pH value of the leachate was adjusted to pH11. (The leachates before and after recovering were diluted by 100 folds for determining by UV-Vis.) The saponin solution recovered was adjusted to pH 2.5 again with $3\text{mol}\cdot\text{L}^{-1}$ HNO_3 , then reused to remove heavy metals from the electroplate sludge. The method was the same as the batch experiment as shown in 3.2.4.

3.3 Result and Discussion

3.3.1 Characteristics of Sludge

Some physical-chemical characteristics of sludge have been determined (Table 3-1). As shown in Table 3-1, pH value of the polluted sludge is lower than that of the original sludge, whereas EC is remarkably large. These results may be attributable to the fact that the polluted sludge was prepared by adding a solution containing three kinds of metallic elements (Pb, Ni and Cr). Furthermore, CEC of the sludge became small after the introduction of heavy metals. It is known that soil flushing proves effective only for permeable soil ($K > 1.0 \times 10^{-3} \text{ cm}\cdot\text{s}^{-1}$) or, to a lesser extent, slightly permeable soil ($1.0 \times 10^{-5} \text{ cm}\cdot\text{s}^{-1} < K < 1.0 \times 10^{-3} \text{ cm}\cdot\text{s}^{-1}$) [139]. The permeability of the sludge studied in this work ($K \approx 1.7 \times 10^{-5} \text{ cm}\cdot\text{s}^{-1}$) is much lower than the value of permeable soil, so quartz sand was added into the sludge to improve its permeability in column washing experiments. From the above-mentioned, it is perhaps obvious that the pH, EC and CEC values were changed by the introduction of metals.

Comparing with the original sludge each other, pH and EC values of the two sludge samples from China are higher. The pH (H_2O) and pH (KCl) values of the electroplate factor sludge are as high as 8.76 and 8.58. The EC values of the domestic water treatment plant sludge and the electroplate factor sludge are 27-fold and 89-fold

that of the original sludge, respectively. It indicates that there is a large amount of conductive matter in these sludge samples, which is also confirmed from the high CEC values. On the other hand, organic matter content in the electroplate sludge is similar with that in the original sludge, whereas that in the domestic sludge is larger, which is coincide with the characteristics of the domestic sludge.

3.3.2 Concentrations and Distribution of Heavy Metals in Sludge

The concentrations of heavy metals (Zn, Pb, Cd, Ni, Cr, Cu and Co) found in the sludge are listed in Table 3-2. The relative standard deviation (RSD) of the triplicated analyses of each sample was less than 5 %. From Table 3-2, the concentration of Zn was highest, and the total concentration of Pb, Ni, Cr and Cu did not exceed the limits in “The Criterion about the Waste Including Metals”^[140], but Cd is relatively high in the original sludge. For polluted sludge, the concentrations of uncontaminated elements (Zn, Cd and Cu) were almost unchanged; however, the concentrations of contaminated element increased remarkably. These results indicate that most of lead, chromium and nickel in solution have been introduced successfully into the sludge. The absorption rates of Pb, Ni and Cr in this study were 86.5%, 68.4%, 93.1%, respectively; which may be mainly attributed to competitive sorption onto the sludge. These results concur with those reported by Asha A, et al.^[141]. The metallic elements concentrations in the domestic sludge are similar with those in the original sludge, although the concentrations of Zn, Cr and Cu are a little high. On the other hand, they are extremely high in the electroplate sludge. The concentrations of Zn, Ni, Cr and Cu in the sludge are as high as $1.25 \times 10^4 \text{ mg} \cdot \text{kg}^{-1}$, $3.05 \times 10^4 \text{ mg} \cdot \text{kg}^{-1}$, $4.28 \times 10^4 \text{ mg} \cdot \text{kg}^{-1}$ and $2.53 \times 10^3 \text{ mg} \cdot \text{kg}^{-1}$, respectively. It suggests that the sludge has been severely polluted with heavy metals.

For reference, the concentrations of heavy metals in natural soil in Japan are also shown in Fig. 3-2(a) along with those of the original sludge. It was found that the concentrations of heavy metals in sludge are higher than those in natural soil A, B and C ^[138] (the natural soil A, B and C is no plow soil from Toyasato, Sakata town, Tateoka, Murayama town in Yamagata Prefecture, and Ueno, Sekikawa village in Niigata Prefecture, respectively). One possible reason for high concentrations in sludge is that the sludge was mainly precipitated from wastewater (containing many kinds of heavy metals), which is discharged from the industries such as paper manufacturing, petrochemical engineering, glass production, textiles and transportation. In particular, the concentrations of Cd and Ni in sludge are markedly higher (up to double) than those in natural soil. This suggests that heavy metals in sludge may tend to accumulate in agricultural soil if the sludge is used repeatedly.

The concentrations of heavy metals in natural soil from Qiqihar and Shangzhi as well as those of the domestic sludge in China are shown in Fig. 3-2(b). From Fig. 3-2(b), the similar characteristics in Fig.3-2(a) are also found, although the concentrations of Pb and Co are slightly lower than those in natural soil E (the natural soil is no plow soil in the respective sampling places). The concentrations of Zn, Cr and Cu are much larger in domestic sludge than those in natural soil D and E, which are caused by the wastewater from many kinds of paths such as small chemical factories, hospitals, municipal water for public, sanitary sewage and so on. However, the concentrations of all metallic elements in these samples do not exceed the limit of the national standards about pollutants in sludges from agricultural use, China ^[142].

The relative distribution of heavy metals is shown in Fig. 3-3-1 and Fig. 3-3-2. The results in Fig. 3-3-1, suggested that, in addition to the residual fraction, Pb, Cd, Ni and Cr mainly exist as Fe-Mn oxides fraction, Zn exists as carbonate fraction, and

Cu as organic fraction in original sludge. These results are in accordance with the distribution characteristics of heavy metals in soil ^[138, 143, 144].

From Fig. 3-3-2, the relative distribution characteristics of heavy metals in two sludge samples are obviously different from those in natural soil in China or those in original sludge. In domestic sludge, Pb almost completely exists as the residual fraction (F6) and Fe-Mn oxide fraction (F5), and Cu mainly exists as the residual fraction (F6) and organic fraction (F4). F5 and F4 are main proportion for other heavy metals. Furthermore, F3 and F1 for Ni, F3 for Zn and F1 for Co also exist as a certain proportion. On the other hand, Pb, Cd, Cr and Cu mainly exist as Fe-Mn oxides fraction (F5) in addition to the residual fraction (F6) in electroplate sludge. However, Zn, Ni and Co mainly exist as nearly equally proportion of F5, F4 and F3. It is noted that F3 proportion of metallic elements is high in the electroplate sludge, so it should be treated and disposed with caution because such elements in F3 are apt to be released into water environment and soil environment.

Comparing Fig. 3-3-1 (a) with Fig. 3-3-1 (b), the following results can be obtained. (1) The concentration of Ni in F2 (bound to exchangeable fraction) sharply increased; which suggests that Ni may be very harmful to the environment at the beginning period of pollution. (2) Heavy metals (Pb, Ni and Cr) were in relatively unstable fractions (from F1 to F5) at the early stage of pollution, and generally moved to the stable residual fraction (F6) with time and become difficult to remove from soil. Considering this, the remediation of polluted soil by heavy metals, should be carried out as soon as possible. (3) The dominant fraction (except residual fraction, F6) is different among elements; that is, the order of the relative distributions are “oxide fraction” > “carbonate fraction” > “organic fraction” for Pb, and “organic fraction” > “carbonate fraction” > “oxide fraction” for Cr. Ni does not show any dominant

chemical fraction although the general tendency is “organic fraction” > “oxide fraction” > “carbonate fraction” > “exchangeable fraction”. It indicates that Pb is easily adsorbed to Fe-Mn oxides, and that Cr is drawn to organic matter in sludge. (4) The dominant fraction of uncontaminated elements (i.e., Zn, Cd and Cu) hardly changed, although the relative distribution of F3 (bound to carbonate fraction) decreased in case of Zn.

Comparing Fig. 3-3-1 (a) and Fig. 3-3-1 (c), the proportion of residual fraction (F6) in natural soil C is relatively higher than that in original sludge. In contrast, it can be observed that the proportions of oxide fraction (F5) and carbonate fraction (F3) in original sludge are higher than those in natural soil C. Comparing Fig. 3-3-2 (a) and Fig. 3-3-2 (c, d), the proportion of organic fraction (F4) and F3 in the domestic sludge are larger than those in natural soil D and E. From these results, it is found that heavy metals in natural soil usually exist in a more stable state than those in sludge. It indicates that it may be hard for heavy metals in natural soil to permeate into groundwater or to be absorbed by crops. On the other hand, the high proportion of oxide fraction or organic fraction in sludge may be due to relative large contents of Fe and Mn (Table 3-3) or organic matter content in sludge.

In brief, the concentrations of heavy metals in sludge are larger than those in natural soils. The relative distribution of residual fraction in natural soil is higher than that in original sludge; while the ratio of oxide fraction or organic fraction in natural soils is lower than that in sludge.

3.3.3 Concentrations and Distribution of REEs, Th and U in Sludge

REEs, Th and U were also extracted from the sludge along with heavy metals, and determined with ICP-MS. The concentrations are shown in Table 3-4, the relative

standard deviation (RSD) of the triplicated analyses of each sample was less than 10 %. The relative distribution of REEs, Th and U is shown in Fig. 3-4 (Distribution characteristics of REEs, Th and U in natural soil A and B are similar to those in natural soil C, so the data for soil A and B are not shown in Fig. 3-4.). Judging from Table 3-4, the concentrations of REEs are variable depending on the kinds of sludge. That is, the concentrations in the original sludge are similar to those in natural soil C, and the concentrations in domestic sludge are between those in natural soil D and E, whereas the concentrations in electroplate sludge are much lower than those in natural D and E, while the concentrations of Th and U are smaller in sludge than those in natural soil C, D and E. On the other hand, the concentrations of metallic elements (except for HREE, i.e. heavy rare earth elements) in original sludge are higher than those in natural soil, which may be attributed to higher organic matter content in the original sludge.

Fig. 3-4 (a) and Fig. 3-4 (b) show the distribution characteristics of REEs, Th and U are generally similar to those of heavy metals. It is noted that, except for U which is higher, the proportion of carbonate fraction (F3) in sludge is lower than that in natural soil C. These results show that REEs, Th and U in natural soil exist in more stable states than those in sludge. In addition, high carbonate fraction of U in sludge is noticeable because the available content in crops is generally considered to be reflected, to some extent, by metal content in carbonate fraction ^[100]. Comparing Fig. 3-4(c) and Fig. 3-4(d), it can be found that the residual (F6) of REEs in domestic sludge is higher than that in natural soil D, whereas the proportions of F5 and F4 are lower than those in natural soil D. In addition, the exchangeable proportion (F2) of U is remarkably higher than that in natural soil D. For the electroplate sludge, the distribution characteristics of REEs, Th and U are generally similar to those of natural

soil D. However, F1 to F4 proportion of HREE and U can be obviously observed because of their very low concentration.

As mentioned above, the concentrations of REEs, Th and U in sludge are variable when compared with those in natural soils. Furthermore, heavy metallic elements including REEs, Th and U in sludge exist as more unstable fraction than those in natural soil.

3.3.4 Removal of Heavy Metals in Sludge

The removal of heavy metals in sludge (Pb, Ni and Cr in polluted sludge, and Zn, Ni, Cr, Cu, Mn, Al and Fe in electroplate sludge) was investigated with elution technology by using biosurfactant (nonionic biosurfactant sophorolipid and saponin). To quantify the factors influencing the removal efficiency of biosurfactant, the effects of the concentration and pH value of the biosurfactant solution in batch experiments and the washing volume of the biosurfactant solution in column experiments were tested in this work.

3.3.4.1 Batch Experiments

Effect of the concentrations of the biosurfactants solution on the removal efficiency of heavy metals are shown in Fig. 3-5-1 and Fig. 3-5-2, and the effect of pH value of the biosurfactants solution on the removal efficiency of heavy metals are shown in Fig. 3-6-1 and Fig. 3-6-2. For both biosurfactants, the concentration ranged from 1 to 50 $\text{g}\cdot\text{dm}^{-3}$, and pH ranged from 2.5 to 6.5. The removal efficiency of heavy metals by both biosurfactants generally ascended with increasing concentration and decreasing pH value, although the concentration (20.0 $\text{g}\cdot\text{dm}^{-3}$) and pH value (4.5) are optimal for most of heavy metals in electroplate sludge with sophorolipid solution.

However it was clear that saponin is more efficient than sophorolipid for two kinds of sludge. Although both biosurfactants are nonionic, the saponin used in this work contained the carboxyl group in saponin moiety ^[132]. For this reason, saponin reacts more easily with metallic elements, and to make metallic elements depart from the sludge surface into the soil solution. Because of this only the result using saponin are discussed in the following.

The removal efficiency is greatest when the concentration of saponin solution (i.e. $50 \text{ g}\cdot\text{dm}^{-3}$) is the highest (Fig. 3-5-1 (b) and Fig. 3-5-2 (b)). However, the polluted sludge is apt to produce colloidal precipitation due to the adsorption of biosurfactant molecules when the concentration is $50 \text{ g}\cdot\text{dm}^{-3}$. Because of this observation, an optimum saponin solution concentrations of $30 \text{ g}\cdot\text{dm}^{-3}$ for the polluted sludge and $50 \text{ g}\cdot\text{dm}^{-3}$ for the electroplate sludge were selected for the following column experiments.

From Fig. 3-6-1 (b) and Fig. 3-6-2 (b), it is shown that the removal efficiency is dependent on pH. When the pH value of saponin solution is higher than its pKa (4.6), the removal efficiency is low. It may be considered that sodium ions, which increased by adding NaOH to adjust pH of saponin solution, competes with heavy metals for saponin. In contrast, when the pH value was lower than its pKa, the removal efficiency abruptly increased. However, when the pH value was less than 3.0, the removal efficiency of Ni and Cr was reduced for the polluted sludge. This may be due to the amount of saponin adsorbed onto sludge which increased with decreasing pH because electrostatic attraction between saponin and sludge surface increases at low pH ^[67]. For this reason, a pH of 3.0 was applied in the following column experiments for the polluted sludge. On the other hand, the removal efficiency of metallic elements in the electroplate sludge was high even if the pH value is 2.5, which is not different

in the case of the polluted sludge. The electrostatic attraction between saponin and sludge surface does not play decisive role at low pH because of large quantity of metallic elements in the electroplate sludge.

3.3.4.2 Column Experiments

The concentrations of heavy metals removed from the two kinds of sludge with washing volume through column are illustrated in Fig. 3-7-1 and Fig. 3-7-2. In addition to biosurfactant solution, ultrapure water was used as eluent for the control. As seen in Fig. 3-7-1 (a), the removal of each metal showed a peak with the increasing of washing volume. In the case of Pb, Ni and Cr, 884, 460 and 552ppm respectively were removed overall from total loaded concentration. Fig. 3-7-1 (b) shows that, except some removal of Ni (58ppm) which is mainly existed as water soluble state, hardly any metals were removed with ultrapure water at the same pH value. The results indicate that saponin has high potential for the removal of heavy metals from polluted sludge compared to ultrapure water. After 12 washing volumes (one washing volume is about 6.2 cm^3), the total removal efficiency reached 73.2%, 64.2% and 56.1% for Pb, Ni and Cr, respectively.

In case of electroplate sludge shown in Fig. 3-7-2, the result (i.e., the effect of washing volume on removal of heavy metals) are much different from that shown in Fig. 3-7-1. The main points are as follows. (1) Any metals hardly outflowed with leachate at initial stage of washing (Fig. 3-7-2(a)), which may be attributable to the fact that heavy metals in electroplate sludge is exist mainly as more stable state throughout long-term contamination. Then, it is much difficult to remove heavy metals in electroplate sludge than those in the polluted sludge Therefore, more longer contact time is needed to make metals divorce from the sludge surface into leachate.

(2) The removal of most metals (i.e., Zn, Ni, Cr, Al and Fe) has been stepwise increased with the increasing of washing volume except for Cu and Mn. It may be attributable to the degree of difficulty of elution (that is, washed out the column) for metallic element among 6 different fractions (i.e., from water soluble (F1) to residual fraction (F6)). Because the concentration of Zn, Ni, Cr, Al and Fe is very high ($12.54 \text{ g}\cdot\text{kg}^{-1}$, $30.49 \text{ g}\cdot\text{kg}^{-1}$, $42.75 \text{ g}\cdot\text{kg}^{-1}$, $4.478 \text{ g}\cdot\text{kg}^{-1}$, $337.9 \text{ g}\cdot\text{kg}^{-1}$, respectively) in electroplate sludge, it remains far from the one washing volume in which the largest concentrations of these metals is reached and show a peak despite washing 30 washing volumes. On the contrary, the concentrations of Cu and Mn in electroplate sludge are low (2.528 and $1.355 \text{ g}\cdot\text{kg}^{-1}$, respectively), so the removal of them shows a peak through 30 washing volumes. (3) In case of Zn, Ni, Cr, Cu, Mn, Al and Fe ($1.127 \text{ g}\cdot\text{kg}^{-1}$, $10.23 \text{ g}\cdot\text{kg}^{-1}$, $10.34 \text{ g}\cdot\text{kg}^{-1}$, $1.632 \text{ g}\cdot\text{kg}^{-1}$, $0.7696 \text{ g}\cdot\text{kg}^{-1}$, $1.084 \text{ g}\cdot\text{kg}^{-1}$ and $44.29 \text{ g}\cdot\text{kg}^{-1}$, respectively), overall from total loaded concentration was removed, and almost the same removal efficiency was obtained as in the case of batch experiment (using $50 \text{ g}\cdot\text{dm}^{-3}$ pH 2.5 saponin solution as shown in Fig. 3-7-3). (4) The total removal efficiency of Zn, Ni, Cr, Cu, Mn, Al and Fe were 8.99%, 33.6%, 24.2%, 64.5%, 56.4%, 24.2% and 13.1%, respectively after 30 washing volumes. Although the removal efficiency is low, the removal amount of each metal is much large. Thus this method can be a promising method for the recovery of metals from the electroplate sludge. (5) Column washing method is more effective for the sludge which suffer small-scale (i.e., fewer kinds and low concentration of heavy metal) and short-term pollution than that for the sludge with large-scale (i.e., many kinds and high concentration of heavy metal) and long-term pollution. Then, in order to improving the removal efficiency and saving the time, other assisted technology such as ultrasonic can be considered to combine.

The results indicate that saponin is effective for removing the heavy metals from both the artificially polluted sludge and the actual electroplate sludge, and that saponin facilitates mobilization of metals selectively, and that the leaching behavior of biosurfactant is dependent on the characteristics of the metals and of the sludge. This may be due to the specificity of biosurfactant for each metal and the co-existence of metals in the sludge.

3.3.4.3 Confirmation of Fraction Removed by Saponin Solution

To confirm the fractions of heavy metals removed by column flushing with saponin solution, sequential extraction was conducted after the column washing. The concentrations of heavy metals in two kinds of sludge and the relative distribution of each heavy metal before and after the column washing are shown in Figs. 3-8-1 and Figs. 3-8-2. Fig. 3-8-1 (a) shows a remarkable decrease of total concentration was found for each heavy metal, and that the concentration in each fraction was also changed regardless of the kind of metal. The concentration of F1 for Pb and Cr slightly increased due to the residual saponin in the sludge which can further react with heavy metals in the extraction process. The concentrations of three elements in F3, F4 and F5 all decreased. Of the three fractions, however, F4 showed the smallest decrease. It may be that heavy metals in F3 and F5 could be more easily released than those in F4 under acidic conditions (pH 3). For the same reason, it is suggested that the removal efficiency of Cr (the proportion of this element in F4 was over 50% of total concentration) was the lowest among the three kinds of metals. From Fig. 3-8-1 (b), it is found that the proportion of the relative stable fraction of heavy metals became higher after column washing, and that the relative distribution characteristics of heavy metals were closer to those in natural soil.

The concentrations and distribution characteristics of heavy metals (Zn, Ni, Cr, Cu, Mn, Al and Fe) in electroplate sludge before and after column experiments compared with those in the polluted sludge are shown in Figs. 3-8-2. The concentrations of F2 for all metals and F3 for Zn, Al and Fe besides F1 increased due to the residual saponin. For example, the total distribution ratio of F1 and F2 for Ni and Mn increased from nearly zero to 5% and 12%, respectively. The concentrations of F5 and F6 for all elements decreased after the column experiment, and the concentrations of Ni, Cr, Cu and Mn in F3 also declined. It indicates that saponin can be effective to remove the metallic elements in residual fraction (F6) as well as those existed in carbonate fraction (F3) and oxide fraction (F5). In addition, it is noteworthy that F5 of Zn, Ni and Mn seems to react preferentially with saponin than F3 due to small F5 distribution ratio (as shown in Fig. 3-8-2(b)).

From the above-mentioned, saponin is more efficient than sophorolipid for the removal of heavy metals from sludge in this work. Saponin has selectivity for the mobilization of heavy metals, and mainly reacts with the F3 and F5 fractions of heavy metals. The removal efficiency of heavy metals for saponin are obviously different between the artificially polluted sludge and the actual electroplate sludge.

3.3.5 Recovery of Heavy Metals from Sludge

In order to recover heavy metals from the sludge leachates, the precipitation method by adding NaOH was firstly considered. Fig. 3-9 shows the recovery efficiency of heavy metals from the polluted sludge leachate at pH 9.2-12.9 using the precipitation method. At pH 10.9, the recovery efficiency of each heavy metal almost reached the maximum possible and was 89.7%, 91.1% and 99.1% for Pb, Ni and Cr, respectively. Due to the amphoteric nature of lead and chromium, their hydroxide

compounds (i.e., precipitate) are redissolved and this decreased the recovery efficiency (in addition to other issues such as the waste of alkaline solution) at excessively high pH (i.e. >11.5). Therefore, the optimal pH for recovery was considered to be about pH 10.9. That is to say, it is an effective method to use an alkaline solution for obtaining high recovery efficiency of heavy metals such as Pb, Ni and Cr.

The recovery of metallic elements in leachates from the electroplate sludge were also performed at pH 11.0 using the same precipitation method. No exact recovery efficiency of each element can be obtained from each leachate due to the competition of many elements. However, it is found that the higher the concentration of metallic elements is, the higher the recovery efficiency is. The maximum recovery efficiencies for Zn, Ni, Cr, Cu, Mn, Al and Fe were obtained 97%, 94%, 88%, 98%, 96%, 76% and 92%, respectively. Among them, the recovery efficiencies of Cr and Al were lower due to the amphoteric nature of their hydroxide compounds.

Although most Pb, Ni and Cr in the polluted sludge and a part of metallic elements in the electroplate sludge were removed with saponin by washing in the column, the residual concentrations are still higher than those in agricultural soil. Even still, this work has quantitatively shown that, to some extent, saponin could be an efficient sorbent for the removal of heavy metals from sludge. However, further investigations to survey the method for improving the removal efficiency of heavy metals, to elucidate the mechanism of the removal of heavy metals by surfactant, and to survey the selection of the optimum surfactant and the optimum conditions for the removal of heavy metals are needed in future research.

3.3.6 Recovery of Saponin from the Leachates and Reuse

According to Hong K. J., et al. [67], alkaline precipitation can also recover saponin from the leachates along with the recovery of heavy metals. The concentration of saponin in solution was determined by UV-Vis spectrophotometer ($\lambda_{\max} = 279.9\text{nm}$) and calculated by external standard method (Fig. 3-10). The ABS values, the concentrations and recovery efficiencies of saponin were listed in Table 3-5 before and after recovering, along with before and after reusing. From Table 3-5, the concentration of saponin in leachates decreased from initial concentration of $25\text{ g}\cdot\text{dm}^{-3}$ to $24.7\text{ g}\cdot\text{dm}^{-3}$ (or $30\text{ g}\cdot\text{dm}^{-3}$ and $28.1\text{ g}\cdot\text{dm}^{-3}$) due to the sludge adsorption. The 1st time recovery efficiencies of saponin were 86.6% for Sample1 and 93.6% for Sample 2. The 2nd time recovery efficiencies of saponin after reusing were 62.3% and 75.4%, respectively. It is obvious that the recovery efficiency becomes lower with increasing the recycling times. From these results, it is found that alkaline precipitation is effective method to separate saponin and heavy metals in the solution, and that losing a part of saponin may be attributed to the adsorption on hydroxide compounds surface.

The pH of the recovered saponin solution was adjusted to 2.5 with $3\text{mol}\cdot\text{dm}^{-3}$ HNO_3 . The solution was used once more to remove heavy metals in the electroplate sludge to investigate the reuse effect of saponin. The concentrations of several metals removed were compared with those from the batch experiment shown in Fig. 3-11. From Fig. 3-11, it is found that the recovered saponin remains the satisfactory ability of removing heavy metals from the sludge. It suggests that saponin should be predicted to withstand the repeated use, and hence it can be a promising material for saving the cost.

3.4 Summary

The behavior, distribution and characteristics of heavy metals including REEs, Th

and U in sludge from an industry water treatment plant and an electroplate plant were investigated and compared with those in natural soil from the same sampling places. Furthermore, the removal/recovery process of heavy metals in the polluted sludge and in electroplate sludge was studied with biosurfactant elution by batch and column experiments. Finally, the recovery and reuse of saponin was performed. Consequently, the following matters have been obtained.

(1) pH, EC (electric conductivity) and CEC (cation exchange capacity) of the sludge from China are higher than those of the sludge from Japan. It suggests that more conductive substances or ionic matter are included in the former sludge than in the latter sludge. It may be caused by the reason that more effective treatment of the wastewater is applied in Japan (than in China).

(2) The concentrations of heavy metals are greater than those in natural soils, although they are varied depending on the kinds of sludge. Furthermore, the concentrations of REEs, Th and U in sludge are more variable when compared with those in natural soils. The relative distribution of oxide fraction in original sludge is higher than that in natural soils in Japan. On the other hand, the relative distribution of the residual fraction in electroplate sludge is much lower than that in natural soil in China. Generally, heavy metallic elements in sludge have greater concentrations and exist as more unstable fraction than those in natural soil.

(3) Nonionic saponin is more efficient than sophorolipid for the removal of heavy metals from both the artificial polluted sludge and the actual electroplate sludge. Saponin has selectivity for the mobilization of heavy metals, and mainly reacts with F3 and F5 fractions of heavy metals. In other words, nonionic biosurfactants including the carboxyl group have high potential for the removal of heavy metals in sludge.

(4) The recovery efficiency of heavy metals (Pb, Ni and Cr) in leachates from the

polluted sludge reached about 90% - 100% by precipitation method with alkaline solution. However, the recovery efficiency (76% - 97%) was different for leachates from the electroplate sludge due to a large quantity of many metallic elements existed in leachates.

(5) Saponin can be recovered by alkaline precipitation. It can be predicted to withstand the repeated use, and hence it can be a promising material for saving the cost.

Table and Figure

Table 3-1 Physical-chemical characteristics of sludge

Parameters	Original sludge	Polluted sludge	Domestic sludge	Electroplate sludge
pH(H ₂ O)	5.78	4.47	6.39	8.76
pH(KCl)	5.08	4.42	6.21	8.58
EC, $\mu\text{S}\cdot\text{cm}^{-1}$	360	1.93×10^3	9.94×10^3	3.22×10^4
Moisture content, %	6.10	7.13	7.85	14.75
Permeability, $\text{cm}\cdot\text{s}^{-1}$	1.75×10^{-5}	1.72×10^{-5}	--	6.16×10^{-4}
Organic matter content, %	14.0	14.0	48.8	13.67
Cation exchange capacity (CEC), $\text{cmol}\cdot\text{kg}^{-1}$	33.6	30.5	1.07×10^2	1.76×10^2
BET specific surface area (SSA), $\text{m}^2\cdot\text{g}^{-1}$	36.0	29.4	--	--
Langmuir specific surface area (SSA), $\text{m}^2\cdot\text{g}^{-1}$	56.2	45.8	--	--

-- means the data was not determined or obtained.

Table 3-2 The concentrations of heavy metals in sludge (mg kg^{-1})

Samples	Zn	Pb	Cd	Ni	Cr	Cu	Co
Original sludge	232	33.5	11.2	32.7	53.0	50.7	13.8
Polluted sludge	233	1.21×10^3	11.5	716	984	51.2	11.2
Domestic sludge	691	46.2	10.0	26.9	121	108	6.42
Electroplate sludge	1.25×10^4	352	49.6	3.05×10^4	4.28×10^4	2.53×10^3	35.1

Table 3-3 The concentrations of Fe and Mn in original sludge and natural soil (mg kg^{-1})

Element	Or-sludge	Na-soil A	Na-soil B	Na-soil C	Do-sludge	El-sludge	Na-soil D	Na-soil E
Fe	4.73×10^4	2.69×10^4	3.71×10^4	3.20×10^4	4.30×10^4	3.38×10^5	1.58×10^4	3.35×10^4
Mn	769	694	936	618	509	1.36×10^3	351	665

Table 3-4 The concentrations of REEs, Th and U in original sludge and natural soil (mg kg⁻¹)

Samples	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Th	U
Or-sludge	22.3	48.6	5.54	28.6	5.13	1.28	6.40	0.764	3.75	0.728	2.18	0.297	1.90	0.283	7.90	2.33
Na-soil A	12.9	26.5	2.89	11.5	2.44	0.86	2.51	0.386	2.31	0.463	1.42	0.195	1.33	0.192	4.46	0.911
Na- soil B	14.5	31.8	3.67	15.0	3.46	1.21	3.94	0.644	3.46	0.708	2.19	0.308	2.14	0.321	4.09	1.31
Na- soil C	21.5	47.1	5.16	19.4	4.06	0.718	3.68	0.566	3.02	0.578	1.74	0.241	1.71	0.248	23.4	4.18
Do-sludge	20.7	44.1	6.95	14.7	2.64	1.04	3.02	0.37	1.65	0.32	1.01	0.13	0.90	0.13	5.39	1.86
Na-soil D	27.0	61.8	9.03	21.7	3.43	1.07	3.57	0.46	2.11	0.40	1.26	0.17	1.16	0.17	11.2	2.08
Na-soil E	6.48	20.8	1.57	6.41	1.13	0.46	1.18	0.26	0.98	0.29	0.75	0.10	0.68	0.10	8.87	4.45
El-sludge	2.79	2.51	0.527	1.96	0.0896	0.145	0.722	0.0248	0.0910	0.0157	0.0661	0.00707	0.0473	0.00724	0.158	0.136

Table 3-5 Recovery of saponin after removing heavy metals and reusing

Sample	ABS			Concentration of saponin (g dm ⁻³)			Recovery efficiency (%)	
	A0	A1	A2	C0	C1	C2	1st time	2nd time
1	0.429	0.371	0.268	24.7	21.4	15.4	86.6	62.3
2	0.489	0.457	0.369	28.1	26.3	21.2	93.6	75.4

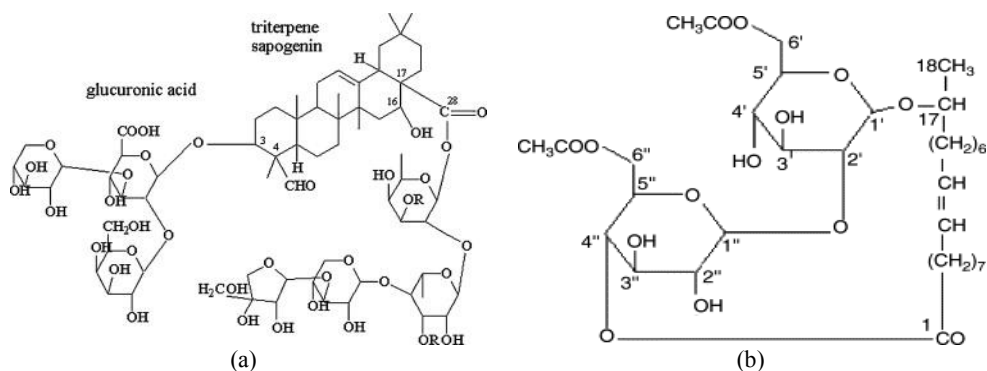


Fig. 3-1 One structure of saponin ^[131] and sophorolipid ^[85]

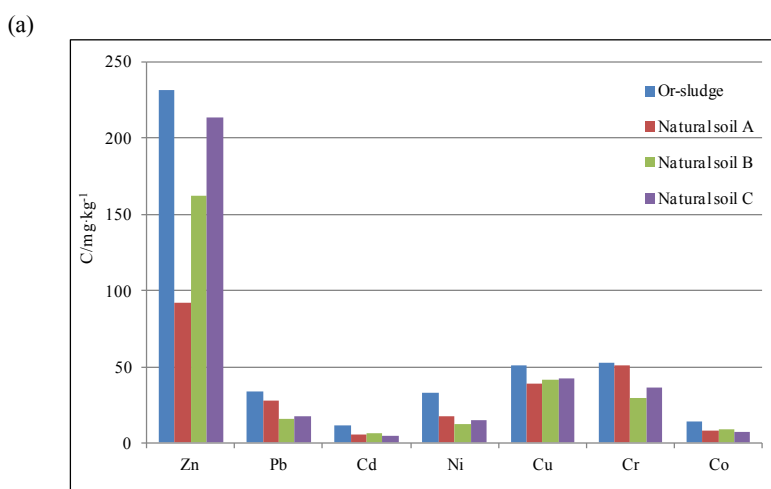


Figure 3-2 (a) Concentrations of several heavy metals in original sludge and natural soil ((A) Sakata and (B) Murayama in Yamagata prefecture in Japan, (C) Sekikawa in Niigata prefecture in Japan)

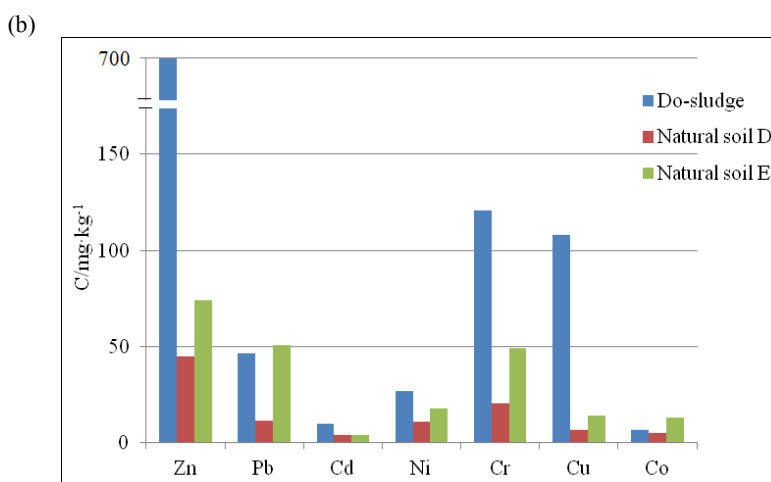


Figure 3-2 (b) Concentrations of several heavy metals in domestic sludge and natural soil ((D) Qiqihar and (E) Shangzhi in China)

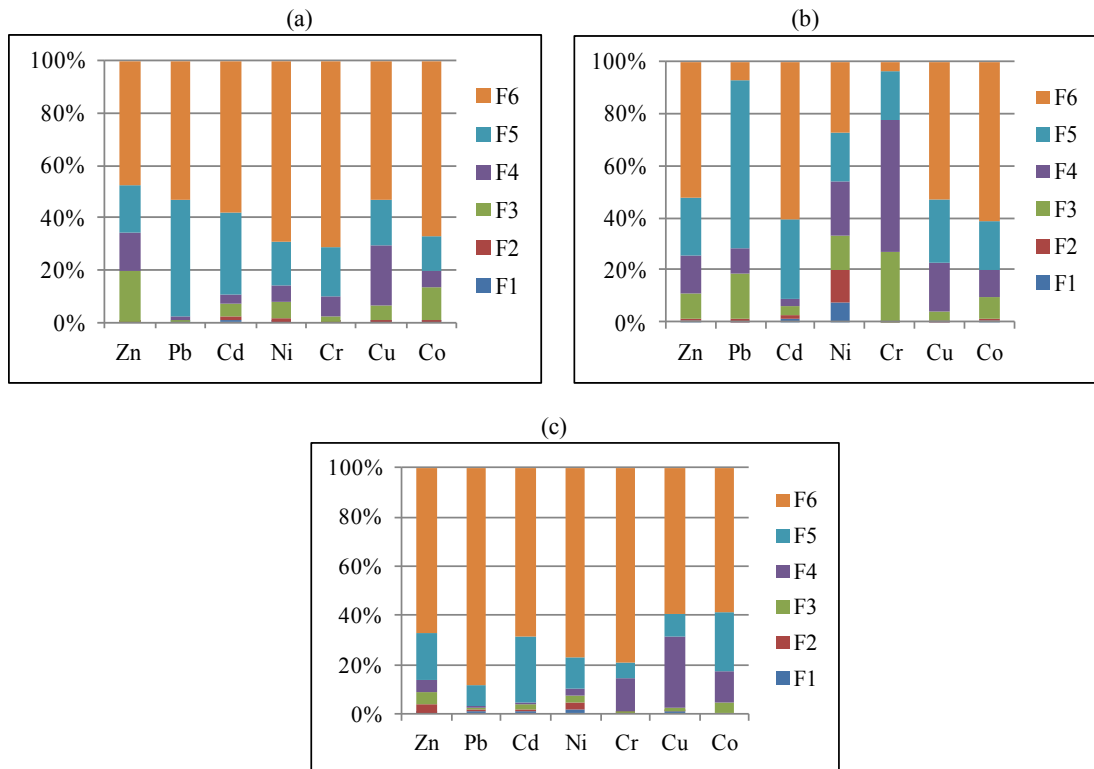


Figure 3-3-1 The relative distribution of heavy metals (a) Original sludge (b) Polluted sludge (c) Natural soil C

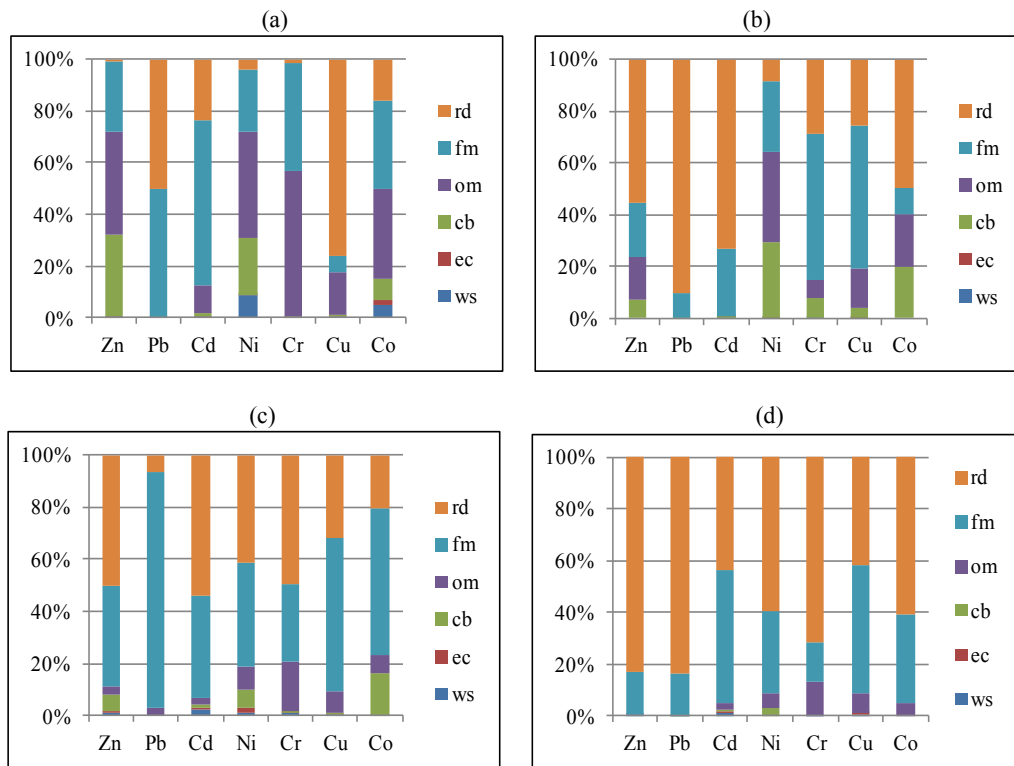


Figure 3-3-2 The relative distribution of heavy metals (a) Domestic sludge (b) Electroplate sludge (c) Natural soil D (d) Natural soil E

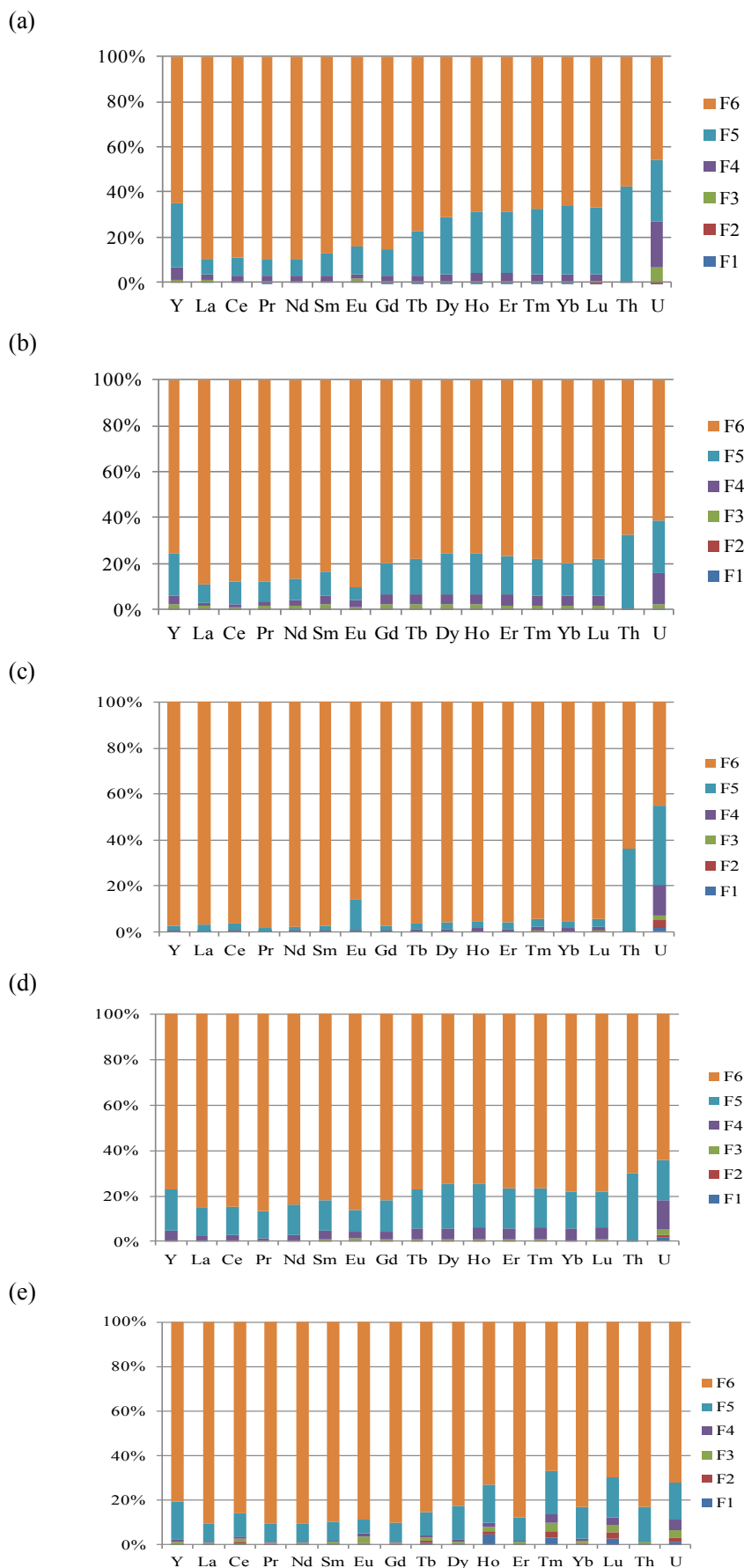


Figure 3-4 The relative distribution of REEs, Th and U in (a) Original sludge (b) Natural soil C (c) Domestic sludge (d) Natural soil D (e) Electroplate sludge

Chapter 3 Behavior and Distribution of Heavy Metals in Sludge and Recovery of Metals by Biosurfactans Application

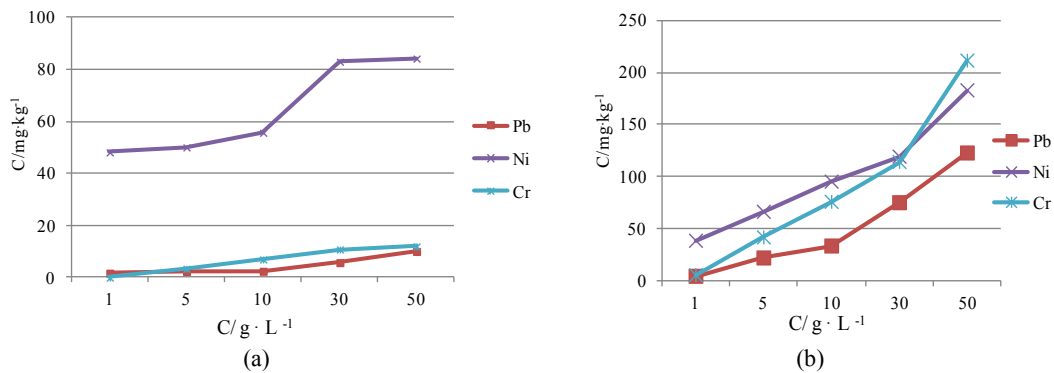


Figure 3-5-1 Effect of concentrations on removal of heavy metals in polluted sludge by batch experiments (a) sophorolipid (b) saponin

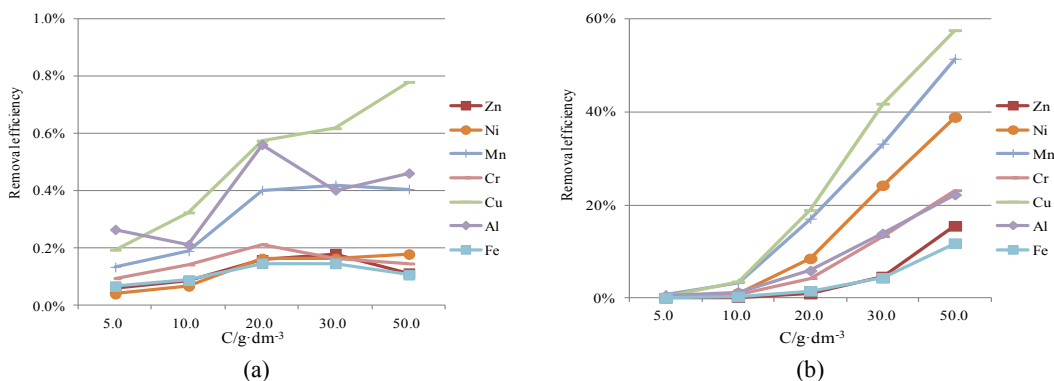


Figure 3-5-2 Effect of concentrations on removal of heavy metals in electroplate sludge by batch experiments (a) sophorolipid (b) saponin

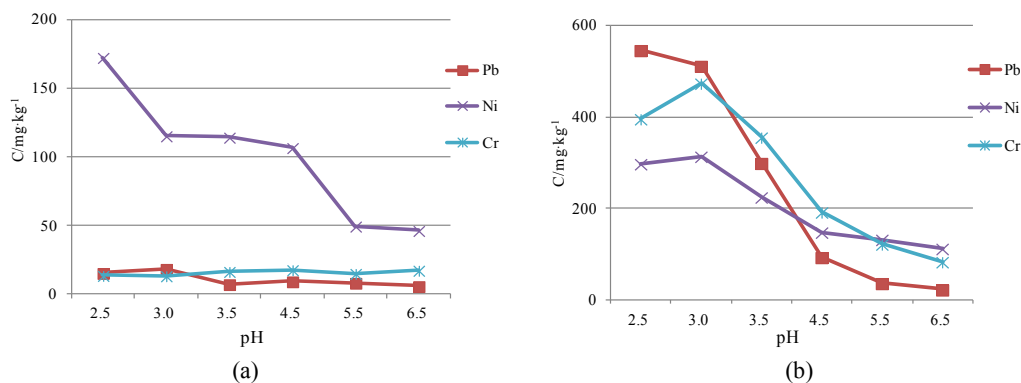


Figure 3-6-1 Effect of pH value on removal of heavy metals in polluted sludge by batch experiments (a) sophorolipid (b) saponin

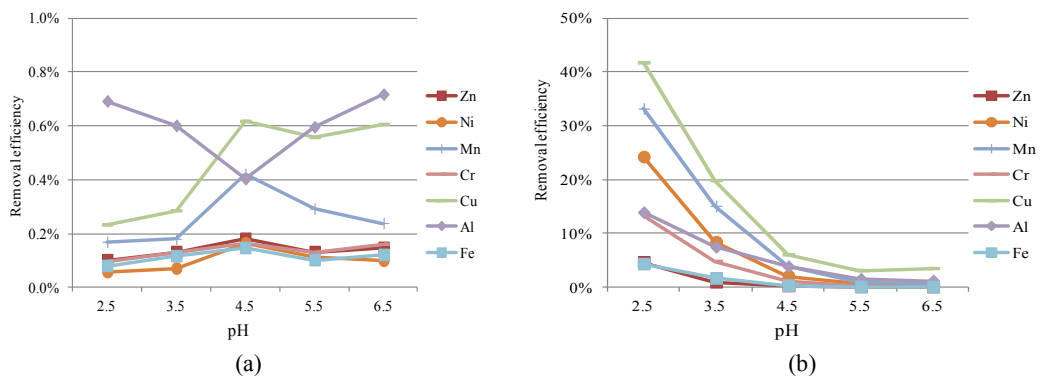


Figure 3-6-2 Effect of pH value on removal of heavy metals in electroplate sludge by batch experiments (a) sophorolipid (b) saponin

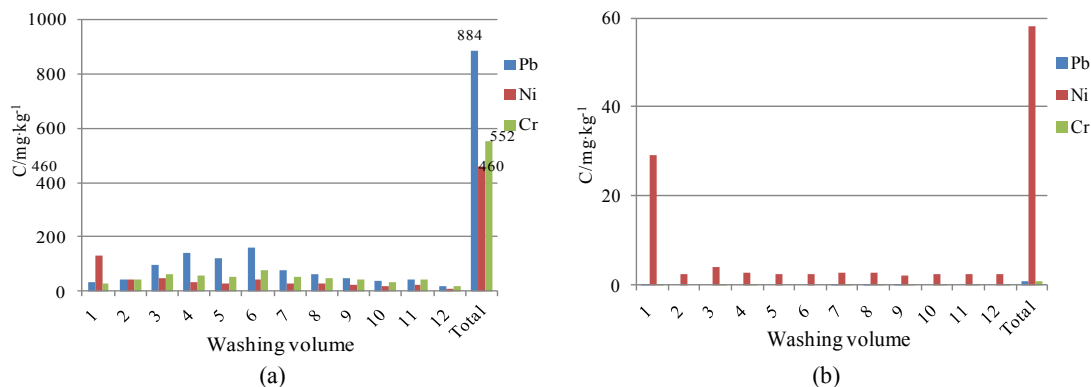


Figure 3-7-1 Effect of washing volume on removal of heavy metals in the polluted sludge by column experiments (a) saponin (b) ultrapure water (1w.v.= 6.2 cm³)

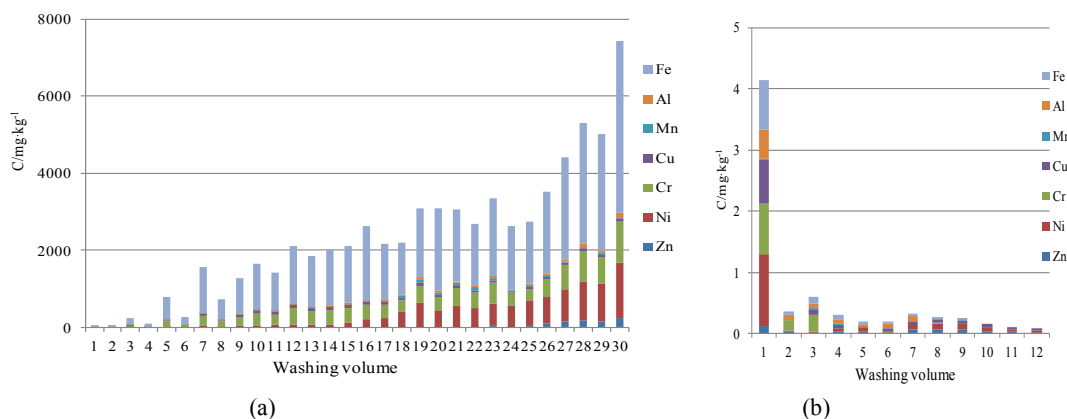


Figure 3-7-2 Effect of washing volume on removal of heavy metals in the electroplate sludge by column experiments (a) saponin (b) ultrapure water (1w.v.= 6.2 cm³)

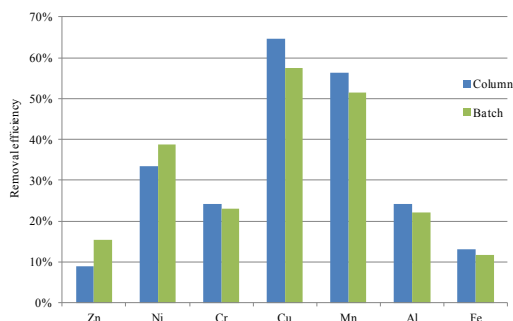


Figure 3-7-3 Removal efficiency of heavy metals in the electroplate sludge by batch experiment (one time, 0.5g sludge, pH 2.5, 5% saponin solution, 25ml) and column experiment (mass ratio of sludge and quartz sand=1:4, pH 2.5, 5% saponin solution, 30 volumes (1w.v.= 6.2 cm³))

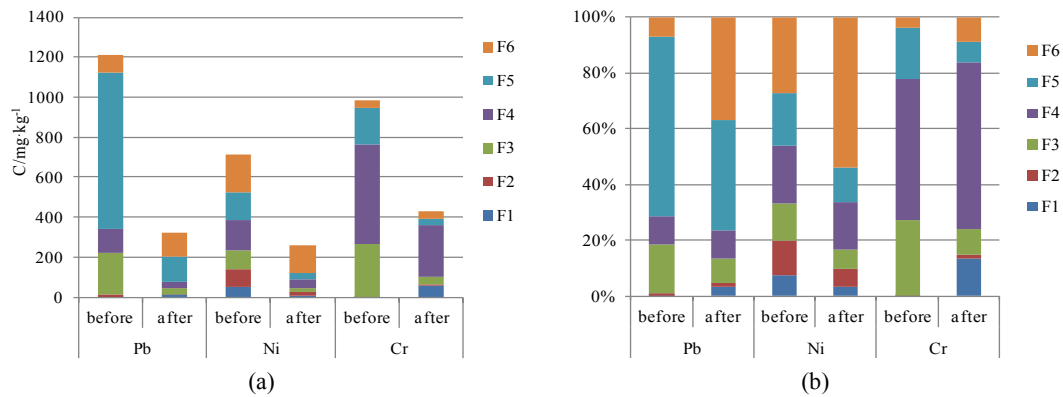


Figure 3-8-1 The concentrations (a) and the relative distribution (b) of heavy metals in polluted sludge before and after the column experiments

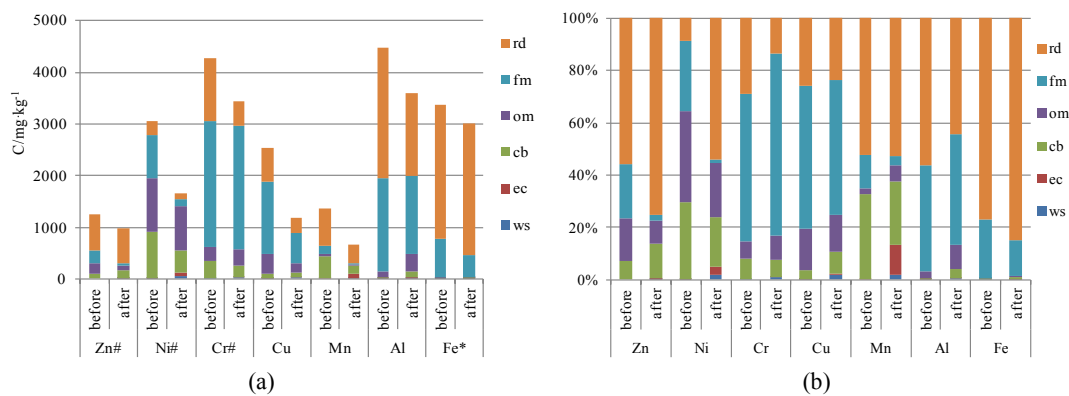


Figure 3-8-2 The concentrations (a) and the relative distribution of heavy metals in electroplate sludge (b) before and after the column experiments (# and * means 1/10 and 1/100 of the actual concentration)

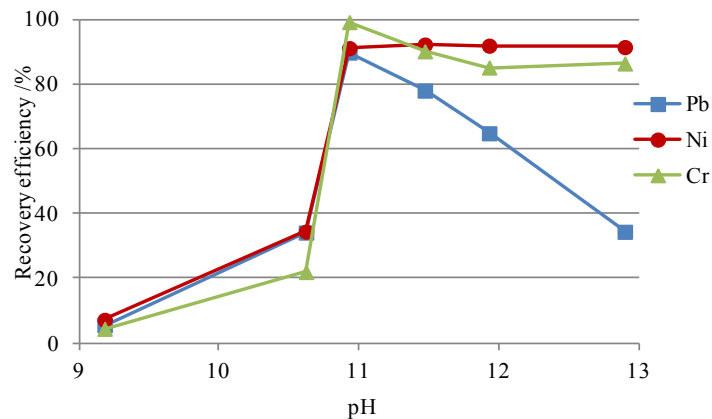


Figure 3-9 The recovery efficiency of heavy metals from the sludge leachate

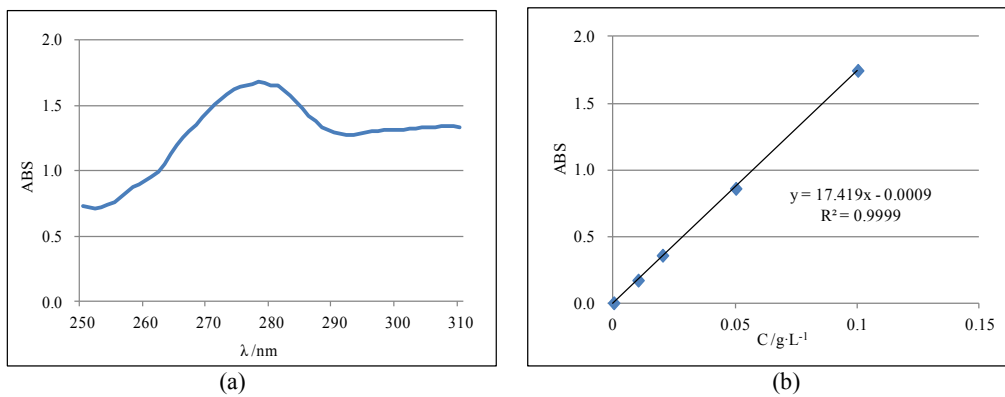


Fig. 3-10 The absorption curve (a) and calibration curve (b) of saponin

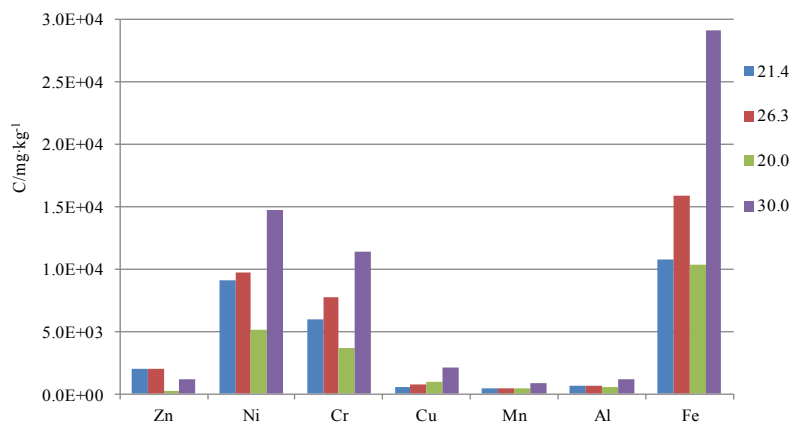


Fig. 3-11 Removal of heavy metals in the electroplate sludge with the recovered saponin
 -- 21.4 and 26.3 are the concentrations of saponin in solution recovered from Sample1 and Sample 2
 -- 20.0 and 30.0 are the concentrations of saponin in solution used in batch experiment

Chapter 4 Phytoremediation of Lead-contaminated Soil and Sludge Using Biosurfactants

4.1 Introduction

With the rapid development of industry and agriculture, the heavy metal-contaminated soil is becoming more and more serious in Japan. For example, the number of the polluted soil caused by lead (Pb) is increased from 1205 cases in 2005 to 1928 cases in 2007 based on the survey from Ministry of the Environment of Government of Japan ^[145]. In case of Niigata Prefecture, there are several reports about the polluted soil by heavy metals such as Pb, arsenic (As) and mercury (Hg) recently ^[146]. Then it is particularly important and urgent to remediate the polluted soil because metallic element do not degrade and persist almost indefinitely in the environment.

A number of remediation technologies have been developed for the remediation of heavy metal in contaminated soil. Generally, these remediation technologies are classified into three categories: physical treatments, chemical treatments, and biological treatment technologies; and each category is further divided into ex-situ and in-situ treatments. Ex-situ remediation is more thorough remediation techniques, but it costs more than in-situ remediation techniques during the excavation and transportation of the soil as well as through the remediation processes. In-situ remediation is less costly due to the lack of excavation and transportation costs, but these remediation techniques are less controllable and less effective ^[147]. Therefore, a combination of one or more of these approaches is often used for more cost-effective treatment of a contaminated soil.

Phytoremediation, which has the advantages of low-cost, environmental-friendly and minimal soil disturbance, has attracted more and more interest in recent years [148-152]. Phytoremediation makes use of the harvestable part of plants to remove pollutants. Any plant can uptake metals from soil theoretically, however, the overwhelming majority of plants generally remove only a small percentage of heavy metals from contaminated soil due to the low biomass and/or the weak translocation from root to shoot. Therefore, using plant with high biomass yields (such as *Brassica juncea*) along with adding chemically enhanced agent (such as surfactants) has been widely applied as a viable strategy for removing heavy metals from soils over a reasonable time frame [153-155].

Synthetic surfactants are more effective for metal-contaminated soil, so they have received increasing interest in recent years for enhancing the remediation of soil contamination [47,156-158]. However, their application are controversial because of their toxicity to soil or/and plant. Biosurfactants have similar properties with synthetic surfactants, and are nondestructive to the soil due to their producing from animal, plant or microbe [159]. Thus, they are considered to substitute synthetic surfactants. Currently, most of research on the application of soil remediation by biosurfactants is at a primary individual stage in the lab. Biosurfactants used mainly focuses on the four kinds of rhamnolipid, sophorolipid, surfactin and saponin, methods are used including soil washing, column flushing or hydroponics, contaminant refer to organic compounds and heavy metals. However, the relevant research of biosurfactants-enhancing phytoremediation of heavy metals in soil (with the method of soil culture) is very few. Raina M. Maier et al. [123] investigated the potential use of rhamnolipid to facilitate the uptake of copper-polluted soil by *Atriplex* and *Corn*. Interestingly, it is found that rhamnolipid were plant specific. Rhamnolipid enhanced

the uptake of copper in shoot in *Corn*, and decreased the shoot uptake of Cu in *Atriplex*. In addition, Sheng, X. et al ^[70] reported that biosurfactant-producing *Bacillus sp. J119 strain* was investigated for its capability to promote the plant growth and cadmium uptake of rape, maize, sudangrass and tomato in soil contaminated with different levels of Cd. Their study demonstrated that the tested strain could colonize the rhizosphere of all studied plants, but that its application enhanced the biomass and Cd uptake only in plant tissue of tomato. Using biosurfactant-enhancing phytoremediation of metal- contaminated soil offers the advantage of being nontoxic and easily biodegradable compared with some synthetic surfactants ^[160,161]. Moreover, biosurfactants have diversity of chemical forms, so they can be selected on the basis of their high specificity for different metals and their high metal removal efficiency.

The objective of this work is to confirm the effectiveness of biosurfactants (saponin and sophorolipid) for phytoremediation of Pb-contaminated soil and sludge with *Brassica juncea* and *Helianthus annuus*. The different additional dosage and time of the two biosurfactants were studied to obtain the optimum remediation effect. Furthermore, effect of plant growth regulators (GA3 and IAA) on the biomass of plant was also investigated. The concentration of Pb in *Brassica juncea* and *Helianthus annuus* was determined by ICP-AES.

4.2 Experimental

4.2.1 Apparatus and Reagents

A plant environmental control system (LPH-220N, Nippon Medical & Chemical Instruments Co. LTD, Japan) was used to cultivate plant. An inductively coupled plasma atomic emission spectrophotometer (ICP-AES) instrument (SPS1500, Seiko Instruments Inc., Japan) was employed to determine the concentrations of Pb. The

operating conditions of ICP-AES are based on our other previous paper ^[85]. Plant and soil samples were digested with a microwave digestion system (Speedwave4, ACTAC. Co. LTD, Japan).

Metallic salts, $\text{Pb}(\text{NO}_3)_2$, was purchased from Kanto Chemical Co., (Japan). Two kinds of biosurfactants used (saponin and sophorolipid) were the same with those mentioned in Chapter 3. Pb standard solutions used for making the calibration curve were prepared by diluting the standard solutions ($1000\text{mg}\cdot\text{dm}^{-3}$ in 6% HNO_3 solution) purchased from Merck Co. LTD (Japan). All chemical reagents used were of analytical grade. Water ($>18.2\text{ M}\Omega$), which was treated by an ultrapure water system (Advantec aquarius: RFU 424TA), was used throughout the work.

The nutrient solution was prepared by dissolving “Otsuka House No.1 and No.2” (Otsuka AfriTechno Co. LTD, Japan) in ultrapure water, then used as fertilizer during the period of the plant cultivation ^[162]. The preparation method was listed in the following:

(1) Stock nutrient solution, Weighed 300.0g Otsuka House No.1, dissolved it in ultrapure water, then transferred the solution to a 1000 mL volumetric flask, diluted with ultrapure water to volume, and mixed (Stock solution I). According to the above operation, 200.0g Otsuka House No.2 was dissolved then transferred to a 1000 mL volumetric flask, diluted the volume and mixed (Stock solution II).

(2) Working nutrient solution, Transferred 10 ml of Stock solution I and Stock solution II, respectively, to a 2000 mL volumetric flask, diluted with ultrapure water to volume, and mix. Adjusted pH value of the mixture solution during 5.8 - 6.0 with $3\text{ mol}\cdot\text{L}^{-1}$ KOH solution. The composition of the working nutrient solution is shown in Table 4-1.

4.2.2 Preparation of Pb-contaminated Soil and Sludge

The Pb-contaminated soil and sludge (the concentrations of Pb were $200\text{mg}\cdot\text{kg}^{-1}$ or $1000\text{mg}\cdot\text{kg}^{-1}$) used in this study was prepared by adding the solution containing $\text{Pb}(\text{NO}_3)_2$ to a certain amount soil, (i.e., Expanded Vermiculite) or the original sludge ($< 2\text{mm}$), then stirred well and air-dried. The soil was purchased commercially from Takamura Co. LTD (Japan), which has high moisture retention capability, air capacity and nutrient preserving capability. Physical and chemical characteristics of the Expanded Vermiculite are listed in Table 4-2. Their measure methods are the same as shown in our previous paper ^[163].

4.2.3 Uptake of Pb

4.2.3.1 Phytoremediation of Pb-contaminated Soil with *Brassica juncea*

The Pb-contaminated soil (the concentration of Pb were 0, 200 or $1000\text{mg}\cdot\text{kg}^{-1}$) was used to fill 500ml plastic pots (140 g soil per pot) and moistened with ultrapure water to reach approximately 80% water holding capacity. Each pot was planted with five seeds of *Brassica juncea*, and germinated in the plant environmental control system under the controlled conditions as shown in Table 4-3. The nutrient solution above-mentioned was used as basal fertilizers to supply nitrogen (N), phosphate (P) and potassium (K) for plants. Pots were watered every 2 days with the nutrient solution and ultrapure water according to water loss by weight to maintain 80% of water holding capacity. Following seedling emergence, the pots were thinned to one plant per pot. On the fourth week (4th week) or the seventh week (7th week), biosurfactants (saponin and sophorolipid) were added with the dosage of $0.1\text{g}\cdot\text{kg}^{-1}$ and $0.5\text{g}\cdot\text{kg}^{-1}$ soil, respectively. They were applied to the soil surface as solutions. Following the application of biosurfactants, watering was performed into soils every

day. On the other hand, the Pot treated without biosurfactants and/or Pb was kept as a control. All pot experiments were conducted according an orthogonal test design ($L_9(3^3)$) shown in Table 4-4. Each treatment was performed in duplicate with a random block design, thus, 30 pots were used.

Plant growth regulators (Gibberellin A3, (GA3) and indole-3-acetic acid (IAA)) were applied and sprayed on leaf surface to improve the biomass of *Brassica juncea* (Fig. 4-1). The spraying method of 10^{-6} mol·L⁻¹ GA3 or IAA are shown in Table 4-5. Furthermore, the effect of the additional dosage of two kinds of biosurfactants (0.05, 0.1, 0.5, 1.0 and 2.0 g·kg⁻¹) on the removal of Pb was investigated to confirm the optimum concentration of each biosurfactant for remediating Pb-contaminated soil.

4.2.3.2 Phytoremediation of Pb-contaminated Soil with *Helianthus annuus*

The phytoremediation of Pb-contaminated soil was also conducted by using *Helianthus annuus*. The experimental procedure was the same with that shown in 4.2.3.1 section.

Differences between two experiments were that only 1000 mg·kg⁻¹ Pb-contaminated soil was used, and that biosurfactants were applied to the soil surface on 4th week after seeding in this experiment. On the other hand, seedling of *Helianthus annuus* was performed before being transplanted to avoid the experimental error due to the differences of germination time and seed size. The seedling procedure was immersing seeds of *Helianthus annuus* in warm water for 24h, germinating on wet gauze for 3 days, planting the seeds in the soil mentioned in 4.2.2 section, and choosing and transplanting the same size plant with two leaves to the pots, and one plant per pot. At the same time, the effect of GA3 and IAA on the biomass of

Helianthus annuus was also investigated. The conditions of pot experiments are listed in Table 4-6.

4.2.3.3 Phytoremediation of Pb-contaminated Sludge with *Brassica juncea* and *Helianthus annuus*

1000mg·kg⁻¹ Pb-contaminated sludge 350g was filled into one pot, and 0.1g·kg⁻¹ biosurfactants were applied to the soil surface on 4th week after seeding. Other processes were the same with that shown in 4.2.3.1 section and 4.2.3.2 section. Experimental conditions of each pot were listed in Table 4-7.

4.2.4 Extraction and Determination of Pb in Plant Samples

Plants were harvested after 8 weeks growth by cutting stems 1 cm above the soil surface. The above-ground parts were considered as “shoot” and the parts below the ground were as “root”. Shoot and root samples were washed carefully with ultrapure water after tap water to remove any soil splash, and were oven-dried at 80°C for 24 h, and weighed then ground in a mortar. Dried plant samples (0.1g of each sample) were digested with 10 cm³ conc. HNO₃ (60-61%) and 3.0 cm³ conc. HF (46-48%) in a microwave digestion system for metal analysis. The digested samples were removed the acid completely on the hot plate, were diluted to 50 cm³ with 1 mol·L⁻¹ HNO₃ solution. The concentration of Pb in solution was determined by ICP-AES.

4.3 Results and Discussion

4.3.1 Phytoremediation of Pb-contaminated Soil with *Brassica juncea*

Effect of the dosage and the addition time of biosurfactants on phytoremediation of Pb-contaminated soil (with different Pb concentration) was investigated by *Brassica juncea*.

4.3.1.1 Biomass and Water Content

Water content of plants is an important parameter that reflects the growth of plants because all physiological activity of plants is enabled normally under only at certain water content. Water content in shoot (dry weight) is calculated by the formula (4-1).

$$\text{Water content \%} = ((\text{Fresh weight} - \text{Dry weight})/\text{Dry weight}) \times 100 \quad (4-1)$$

Effect of different factors on water content in shoot of *Brassica juncea* is shown in Fig. 4-2. From Fig. 4-2, the following matters were obtained. (1) Water content in shoot reduces with the increase of sophorolipid concentration, but it hardly changes with varying saponin concentration. (2) Water content obviously reduces at the level of 1000 mg·kg⁻¹ Pb regardless of the kind of biosurfactants (i.e., saponin and sophorolipid). They are reduced by 6.6% and 16.2% of that of the control, respectively. (3) Addition time of biosurfactant does not affect water content remarkably. (4) Water content in plant is lower with the addition of sophorolipid than that with the addition of saponin, which may be attributable to the difference of soil water holding capacity (or available water in soil).

The shoot and root biomass (dry weight) of *Brassica juncea* in all pots after 8 weeks growth are shown in Fig. 4-3. In the absence of biosurfactants, the biomass of the pots introducing Pb (Pot No.2 and No.3) exhibit clearly reduction compared with that of the control (Pot No.1). Furthermore, the reduction amount of the shoot and root are about 16% and 2% of the control, respectively. It indicates that excess Pb can inhibit the plant growth^[164], however, excess Pb in soil is generally nontoxic to plant. Pb is considered to be absorbed to soil mineral or to be precipitated as phosphate and carbonate, which leads to the low solubility and bioavailability of Pb^[165]. Thus, 1000 mg·kg⁻¹ Pb-contaminated soil was used in the following pot experiments to obtain an

obvious remediation effect under the normal growth conditions of *Brassica juncea*. From these experiments, it can be considered that only the addition of biosurfactants (saponin or sophorolipid) also led to a severe reduction of the biomass, especially in case of high concentration. The reducing biomass is larger when using saponin than that using sophorolipid at the same level. Moreover, it is found that the larger dosage addition of biosurfactant ($0.5 \text{ g}\cdot\text{kg}^{-1}$) by one time before seeding not only delay the germination time (later for 4 days than the other pots), but also inhibit the plant growth seriously as observed from the biomass in Pot No.8 and No.18. It is also noted that the shoot in Pot No.9 and No.19 began to turn yellow, withered and fell off after the addition of $0.5 \text{ g}\cdot\text{kg}^{-1}$ biosurfactants on 7th week (Fig. 4-4). Thus, it is inferred that the addition of biosurfactants on the 4th week is the optimum time for both saponin and sophorolipid. In addition, it may be considered that saponin have more strong inhibition for the growth of *Brassica juncea* than sophorolipid except the case of Pot No.9 and Pot No.19. When $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid was added, the difference of the biomass was not observed among those pots (Pot No.14, 15 and 16). These results suggest that the biomass is affected by the kind, the dosage and the addition time of biosurfactants as well as the concentration of Pb in soil.

4.3.1.2 Pb Concentration in Shoot and Root

The concentrations of Pb in shoot and root of *Brassica juncea* are shown in Fig. 4-5. The following matters were obtained: (1) Pb concentration in plant increased generally with the increase of Pb concentration in soil, and the concentration in root is much higher than that in shoot. It shows that Pb is easy to be accumulated in root of *Brassica juncea*, and that the translocation amount of Pb is small from root to shoot. (2) Pb concentration in shoot is obviously elevated when the biosurfactant dosage is

large, especially in case of pots with the addition of $0.5 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid, the concentration is above 5 times of that in non-addition pots. However, the addition of low concentration saponin inhibited Pb adsorption in shoot to some extent. On the other hand, Pb concentration in root was increased with the increase of the sophorolipid dosage. These results indicate that biosurfactants can improve the concentration of Pb in shoot and root of *Brassica juncea* and the translocation of Pb.

(3) For the addition time of biosurfactant, it is suggested that the earlier the biosurfactant is added, the higher the concentration of Pb in root is. (4) Under the same conditions, the concentrations of Pb in pots treated by sophorolipid are higher than those treated by saponin regardless of the part of plant (i.e., shoot and root). It can be concluded that sophorolipid is more effective for Pb adsorption and traslocation than saponin.

Ratio of metal concentration in shoot to the concentration in root of plant (i.e., translocation factors, TF) reflects directly the translocation ability of metal from root to shoot. Then, the TF of Pb is calculated and shown in Fig. 4-6. From Fig. 4-6, it is found that the TF ascended with the increase of biosurfactant concentration in soil regardless of the kind of biosurfactants (i.e., saponin and sophorolipid). The TF is highest with the addition of $0.5 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid, which shows that sophorolipid has strong translocation ability for Pb. These results indicate that Pb translocation is affected by the kind and dosage of biosurfactants, as the same with the results using EDTA ^[166].

4.3.1.3 Total Uptake Amount of Pb in Shoot and Root

Total uptake amount of Pb in shoot and root is calculated according to the formula (4-2),

$$T = C \times M \quad (4-2)$$

T, Total uptake amount of Pb in shoot (or root), μg

C, Pb concentration in shoot (or root), $\text{mg}\cdot\text{kg}^{-1}$

M, Biomass of shoot (or root), g

As shown in Fig. 4-7, total uptake amount of Pb elevated with the increase of Pb concentration in soil. It is the highest in pots without biosurfactant which indicates that the uptake amount mainly depends on the biomass. Total uptake amount reached the maximum when biosurfactant was added on the 4th week, and it was 254.8 μg and 266.5 μg for saponin and sophorolipid, respectively. In addition, for the additional dosage of biosurfactants, the total uptake amount was higher when 0.5 $\text{g}\cdot\text{kg}^{-1}$ saponin and 0.1 $\text{g}\cdot\text{kg}^{-1}$ sophorolipid were added, but it was lower than that in pots without the addition of biosurfactants. However, the interaction of each influence factor was not definitely elucidated in this study, then, further studies are needed (hereafter) to confirm the interaction for finding out the optimum additional dosage of the biosurfactants.

4.3.1.4 Effect of Plant Growth Regulators on the Biomass of *Brassica juncea*

From our other preliminary experiment (Data in the experiment are not shown here), it is confirmed that the addition of enhancing regulators, such as gibberellic acid (GA₃) and indole-3-acetic acid (IAA), improves the biomass in case of *Brassica juncea* as with the work of Hadi et al. ^[167] using maize (*Zea mays* L.). Then, the effect of the two kinds of plant growth regulators on the biomass of *Brassica juncea* under the same cultivating conditions was also investigated. *Brassica juncea* was planted in 1000 $\text{mg}\cdot\text{kg}^{-1}$ Pb-contaminated soil, and biosurfactants were added to the soil surface on 4th week after seeding. GA₃ and IAA (10^{-6} $\text{mol}\cdot\text{L}^{-1}$) were sprayed on the shoot

surface for 3 times by the following two methods. Method I: the plant growth regulators were sprayed for one time at the 15th day, 30th day and 45th day after seeding, Method II : the plant growth regulators were sprayed for one time at every 5 days before harvesting (Table 4-5). The effect of the addition of the plant growth regulators are shown in Fig. 4-8. In the pots added $0.5 \text{ g}\cdot\text{kg}^{-1}$ saponin, both GA3 and IAA improved the biomass, and GA3 was better than IAA. However, IAA led to the decrease of the biomass with Method II (Pot 24). The largest yield increased by 16.2% for shoot (Pot 21) and 13.3% for root (Pot 23), respectively. Method I was more effective than Method II. On the other hand, in the pots added $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid, the reduction of biomass was observed under the present conditions investigated. However, it is worth to note that the biomass in the pots only added $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid was much higher than the control, and that the increasing amount was 4.3% and 22.2% for shoot and root of the control, respectively. These results show that plant growth regulators cannot always increase the biomass of plants. Furthermore, it is found that the two kinds of biosurfactants improved the biomass of *Brassica juncea* comparing with the control when other cultivating conditions were identical. From the above-mentioned, the reduction of biomass observed in 4.3.1.1 section may be attributed to the difference and interaction of various influence factors.

4.3.1.5 Confirmation of the Optimum Dosage of Biosurfactants

To investigate the optimum dosage of biosurfactants, saponin and sophorolipid were added on the 4th week with the level of $0.05 \text{ g}\cdot\text{kg}^{-1}$, $0.1 \text{ g}\cdot\text{kg}^{-1}$, $0.5 \text{ g}\cdot\text{kg}^{-1}$, $1.0 \text{ g}\cdot\text{kg}^{-1}$ and $2.0 \text{ g}\cdot\text{kg}^{-1}$ to the pots along with $1000 \text{ mg}\cdot\text{kg}^{-1}$ Pb. From these results, it is shown that both saponin and sophorolipid in the concentrations studied ($0 - 2.0 \text{ g}\cdot\text{kg}^{-1}$) could obviously affect the growth of *Brassica juncea* (Fig. 4-9). The results also show

that the two kinds of biosurfactants can promote plant growth within a certain concentration range rather than having any phytotoxicity, which indicates their potential use in enhancing the remediation efficiency of Pb-contaminated soil. The maximum promotion was observed when adding $0.50 \text{ g}\cdot\text{kg}^{-1}$ saponin and $0.10 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid. The yield of *Brassica juncea* is 5.56 g per pot for $0.50 \text{ g}\cdot\text{kg}^{-1}$ saponin and 7.28 g per pot for $0.10 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid, which is much higher than the controls (4.59 g per pot). The result coincides with that reported by Lizhong Zhu, et al.^[168]. Moreover, it can be found that sophorolipid has a stronger acceleration for the growth of *Brassica juncea* than saponin. The reason why biosurfactants could promote the growth of plants is still unclear. One possible explanation is suggested: surfactants were observed to increase the permeability of the cell membrane, which led to a more efficient uptake of nutrients^[169].

The concentration of Pb in shoot obviously elevated when the concentrations of biosurfactants are less than $1.0 \text{ g}\cdot\text{kg}^{-1}$, which indicates that both saponin and sophorolipid can enhance the translocation of Pb from root to shoot. Pb concentration in shoot increased by 95.4% and 75.7% that of the control in pots with adding $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid and $0.1 \text{ g}\cdot\text{kg}^{-1}$ saponin, respectively. The reasons may be that biosurfactants can improve the activity of Pb in soil and increase the permeability of membrane of plant, then Pb is easy to be absorbed by plant^[168]. However, the concentration of Pb in shoot and root became decreased with the further increasing of the biosurfactants dosage, which is caused by excess biosurfactant influencing the normal physiological mechanism (such as photosynthesis and respiration, which was reflected by the sharply reduction of the water loss) of *Brassica juncea*^[170]. In addition, it is noteworthy that the concentration of Pb in shoot with adding sophorolipid is higher than that with adding saponin at the same dosage. On the

contrary, the result is opposite for that in root.

These results show that sophorolipid has much stronger translocation for Pb than saponin. The concentration of Pb in shoot and root were improved by the addition of biosurfactants. It can be concluded that adding $0.1 \text{ g}\cdot\text{kg}^{-1}$ biosurfactants is the optimum dosage from the uptake amount of Pb in shoot.

4.3.2 Phytoremediation of Pb-contaminated Soil with *Helianthus annuus*

The dosage of biosurfactant with adding $0.1 \text{ g}\cdot\text{kg}^{-1}$ and $0.5 \text{ g}\cdot\text{kg}^{-1}$ was performed to investigate the effect of saponin and sophorolipid on phytoremediation of Pb-contaminated soil with *Helianthus annuus*. Furthermore, the effect of GA3 and IAA on the biomass of *Helianthus annuus* was also investigated. Finally these results were compared with those of *Brassica juncea* used as remediation plant.

4.3.2.1 Biomass

The biomass in shoot and root of *Helianthus annuus* by using saponin and sophorolipid is shown in Fig. 4-10. From Fig. 4-10, it is found that (1) the biomass of *Helianthus annuus* increased with increasing of the biosurfactant dosage, and increased by 10% of the control biomass with adding $0.5 \text{ g}\cdot\text{kg}^{-1}$ saponin and by 17% with $0.5 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid. (2) GA3 further improved the biomass regardless of the kind and dosage of biosurfactants. On the other hand, IAA enhanced the biomass of *Helianthus annuus* in case of low dosage biosurfactant ($0.1 \text{ g}\cdot\text{kg}^{-1}$), however, it reduced the biomass when high dosage such as $0.5 \text{ g}\cdot\text{kg}^{-1}$ biosurfactant was used. The results are similar to those of *Brassica juncea* with GA3 and IAA, that is, plant growth regulators do not always improve the biomass. (3) the biomass of *Helianthus annuus* in pots is much smaller than that of *Brassica juncea*.

4.3.2.2 Pb Concentration in Shoot and Root

Pb concentration in shoot and root of *Helianthus annuus* with saponin and sophorolipid are shown in Fig. 4-11. The following matters were obtained: (1) Pb concentration in shoot and root of *Helianthus annuus* all elevated in pots with the addition of biosurfactant comparing with the control, the maximum Pb concentration was obtained with the dosage of $0.5 \text{ g}\cdot\text{kg}^{-1}$ saponin (increased by 5% of the control in shoot and by 43% in root) and $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid (increased by 22% of Pb concentration both in shoot and in root of the control). (2) The TF of Pb in *Helianthus annuus* is hardly change, even became small, with the addition of biosurfactant (Table 4-8). It suggested that two kinds of biosurfactant do not enhance the translocation of Pb from root to shoot, and the result is different from that in case of *Brassica juncea*. (3) Pb concentration in shoot and root of *Helianthus annuus* is much lower than those of *Brassica juncea*. Especially, it was about 1/10 of that in root of *Brassica juncea*. It indicates that *Helianthus annuus* is not necessarily a hyperaccumulator of Pb.

4.3.2.3 Total Uptake Amount of Pb in Shoot and Root

Total uptake amount of Pb in shoot and root of *Helianthus annuus* ((a) Saponin (b) Sophorolipid) is shown in Fig. 4-12. Total uptake amount of Pb increased with the increasing of the biosurfactant dosage regardless of the kind of biosurfactants (i.e., saponin and sophorolipid), and it depended on the biomass. The highest uptake appeared with adding $0.5 \text{ g}\cdot\text{kg}^{-1}$ saponin and sophorolipid. At this concentration, the uptake amount in shoot and in root was increased by 12.5% and 80.8% those in the control by adding saponin, and was 39.0% and 71.1% by adding sophorolipid. In addition, the increase in root is much higher than that in shoot, and the uptake amount

of Pb in *Helianthus annuus* is much lower than that in *Brassica juncea*. From these results, it was confirmed that *Brassica juncea* is the hyper-accumulating plant for Pb, but *Helianthus annuus* may be not.

4.3.3 Phytoremediation of Pb-contaminated Sludge with *Brassica juncea* and *Helianthus annuus*

The effect of biosurfactants (saponin and sophorolipid) on phytoremediation of artificially Pb-contaminated sludge was also investigated using *Brassica juncea* and *Helianthus annuus*. These results are shown in Fig. 4-13. (1) The characteristics of *Brassica juncea* biomass planted in polluted sludge were the same with those planted in polluted soil. That is, adding $0.1 \text{ g}\cdot\text{kg}^{-1}$ saponin reduced the biomass slightly, but adding $0.1 \text{ g}\cdot\text{kg}^{-1}$ sophorolipid improved the biomass by 20% of the control. On the other hand, the biomass of *Helianthus annuus* was almost unchanged with the addition of saponin and sophorolipid. (2) It was observed that the biomass of plants in polluted sludge was much smaller than that of plants in the polluted soil, which may be caused by the inherent nature of the sludge, such as low content of nutritive element, weak air permeability and high holding water capability. (3) The biomass of *Brassica juncea* in polluted sludge is almost two folds of that of *Helianthus annuus* under the same conditions, which is similar with the feature of biomass in plants cultivated in the polluted soil.

Biosurfactant enhanced the Pb concentration in root regardless of the plants used in this work (i.e., *Brassica juncea* and *Helianthus annuus*), and saponin was more effective than sophorolipid. Pb concentration in *Brassica juncea* was about 1/3 of that in *Helianthus annuus*, which was opposite with the results of Pb concentration of plants in polluted soil. The reasons may be that the root system of *Helianthus annuus*

is much stronger than that of *Brassica juncea*, so the former can absorb more Pb than the latter under rigorous growth environment. However, it is worth to note that the translocation of Pb from root to shoot was reduced according to the TF of Pb in plant.

Biosurfactants improved the uptake amount of Pb in plant, and saponin gave a better uptake than sophorolipid for root. However, sophorolipid has stronger translocation ability than saponin for shoot. The results suggest that the uptake amount of Pb mainly depends on the Pb concentration in plant. The uptake amount of Pb in *Brassica juncea* planted in the polluted sludge was about 1/10 of that in polluted soil, but the amount was similar level between in polluted sludge and in polluted soil in case of *Helianthus annuus*. This shows that *Helianthus annuus* is more suitable to remediate the Pb-contaminated sludge than *Brassica juncea*, and that the nature of soil plays an important role for the effect of phytoremediation.

The available Pb concentration in pots with no-addition of biosurfactant was 671.90 mg·kg⁻¹, 241.60 mg·kg⁻¹ and 336.41 mg·kg⁻¹ for Pot 38, Pot 51 and Pot 54, respectively (Fig. 4-14). The concentration increased obviously in all pots after adding biosurfactant, which was also observed with adding synthetic surfactants (EDTA) [171]. These results may be caused by the properties of biosurfactant, such as mulsification /de-emulsification, dispersion, foaming, wetting and coating. These properties make biosurfactant form complexes with metals at the soil interface in heavy-metal polluted soils, and make the metal remove from the soil surface leading to the increase of metal ions concentration and their bioavailability in the soil solution. The maximum available Pb concentration was increased to 170% of that in the control (Pot 55). It also clearly indicates the efficiency of biosurfactant for enhancing the phytoremediation effect of Pb-contaminated soil or sludge. From the above-mentioned, it is considered that the approach of applying biosurfactants to

metal-contaminated soil for increasing the phytoremediation efficiency is feasible.

4.4 Summary

The effect of biosurfactants (saponin and sophorolipid) on phytoremediation of Pb-contaminated soil and sludge by *Braasica juncea* or *Helianthus annuus* was investigated. Consequently, the following matters have been obtained:

(1) Biosurfactants (saponin and sophorolipid) can enhance the phytoremediation effect of Pb-contaminated soil or sludge by increasing the biomass and Pb concentration of plant.

(2) The biomass of *Brassica juncea* and *Helianthus annuus* is affected by the kind, the dosage and the addition time of biosurfactants as well as the Pb concentration in soil and the nature of soil.

(3) The increase of the biomass in shoot is larger than that in root due to the biosurfactant addition. On the other hand, the enhancement of Pb concentration in shoot is lower than that in root regardless of the kind of plants (i.e., *Brassica juncea* and *Helianthus annuus*).

(4) Plant growth regulators, GA3 and IAA, do not always improve the biomass.

(5) *Brassica juncea* is obviously more suited for the phytoremediation of Pb-contaminated soil than *Helianthus annuus*. On the contrary, *Helianthus annuus* is more effective for the Pb-contaminated sludge.

Table and Figure

Table 4-1 Nutrient solution composition ^[162]

Parameters	Concentration [mg kg ⁻¹]
Total nitrogen	260
NH ₄ ⁺ -N	23
NO ₃ ⁻ -N	233
P ₂ O ₅	120
K ₂ O	405
CaO	230
MgO	60
MnO	1.5
B ₂ O ₃	1.5
Fe	2.7
Cu	0.03
Zn	0.09
Mo	0.03
EC[dS·m ⁻¹]	2.6

Table 4-2 Physical and chemical characteristics of the Expanded Vermiculite

Parameters	Expanded Vermiculite
pH(H ₂ O)	7.20
pH(KCl)	5.17
EC [μS·cm ⁻¹]	23.8
Moisture content [%]	0.241
Organic matter content [%]	1.40
Cation exchange capacity (CEC) [cmol·kg ⁻¹]	11.5
Pb [mg kg ⁻¹]	Not detected
Zn [mg kg ⁻¹]	193

Table 4-3 Conditions of plant growth in the plant environmental control system

	Temperature [°C]	Relative humidity [%]	Photon flux density [lx]	Photoperiod [h]
Day	27	60	7500	12
Night	22	60	0	12

Table 4-4 Conditions in pot experiments of phytoremediation of Pb-contaminated soil with *Brassica juncea*

Pot [No.]	Pb [mg kg ⁻¹]	Saponin/Sophorolipid [g kg ⁻¹]	Adding time of biosurfactant [week]
1	0	0	-
2	200	0	7th
3	1000	0	4th
4,14	0	0.1	7th
5,15	200	0.1	4th
6,16	1000	0.1	-
7,17	0	0.5	4th
8,18	200	0.5	-
9,19	1000	0.5	7th

Table 4-5 Conditions in pot experiments of phytoremediation of Pb-contaminated soil with *Brassica juncea* with adding plant growth regulator

Pot	saponin [g kg ⁻¹]	sophorolipid [g kg ⁻¹]	GA3 [10 ⁻⁶ mol L ⁻¹]	IAA [10 ⁻⁶ mol L ⁻¹]
3	-	-	-	-
20	0.5	0	-	-
21	0.5	0	3 times*	-
22	0.5	0	-	3 times*
23	0.5	0	3 time [#]	-
24	0.5	0	-	3 times [#]
25	0	0.1	-	-
26	0	0.1	3 time*	-
27	0	0.1	-	3 time*
28	0	0.1	3 time [#]	-
29	0	0.1	-	3 time [#]

Note: * and [#] represents Method I and Method II, respectively.

Table 4-6 Conditions in pot experiments of phytoremediation of Pb-contaminated Soil with *Helianthus annuus*

Pot	saponin [g kg ⁻¹]	Sophorolipid [g kg ⁻¹]	GA3 [#] [10 ⁻⁶ mol L ⁻¹]	IAA [#] [10 ⁻⁶ mol L ⁻¹]
38	0	0	-	-
39	0.1	0	-	-
40	0.5	0	-	-
41	0.1	0	3 times	-
42	0.1	0	-	3 times
43	0.5	0	3 times	-
44	0.5	0	-	3 time
45	0	0.1	-	-
46	0	0.5	-	-
47	0	0.1	3 times	-
48	0	0.1	-	3 times
49	0	0.5	3 times	-
50	0	0.5	-	3 times

Note: [#] represents GA3 and IAA was sprayed by Method II.

Table 4-7 Conditions in pot experiments of phytoremediation of Pb-contaminated sludge with *Brassica juncea* and *Helianthus annuus*

Pot	Plant	Saponin [g kg ⁻¹]	Sophorolipid [g kg ⁻¹]
51	<i>Brassica Juncea</i>	0	0
52	<i>Brassica Juncea</i>	0.1	0
53	<i>Brassica Juncea</i>	0	0.1
54	<i>Helianthus Annuus</i>	0	0
55	<i>Helianthus Annuus</i>	0.1	0
56	<i>Helianthus Annuus</i>	0	0.1

Table 4-8 The TF of Pb in *Helianthus annuus*

Pot	Biosurfactant	Dosage[g kg ⁻¹]	TF
38	--	0	0.14
39	saponin	0.1	0.13
40		0.5	0.10
45	sophorolipid	0.1	0.12
46		0.5	0.14

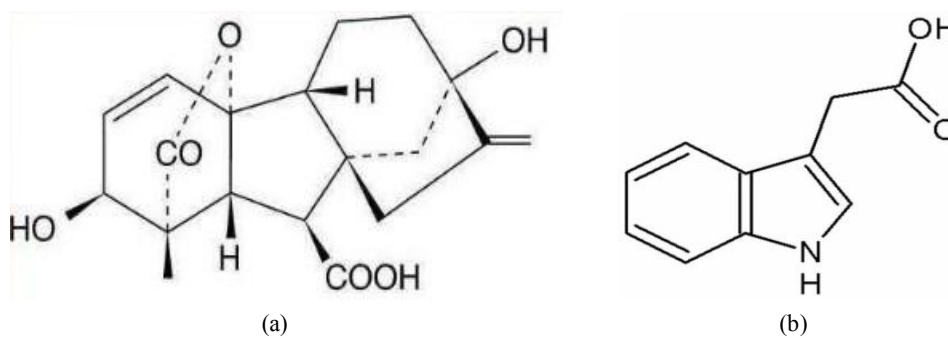


Fig. 4-1 The structure of (a) GA3 and (b) IAA

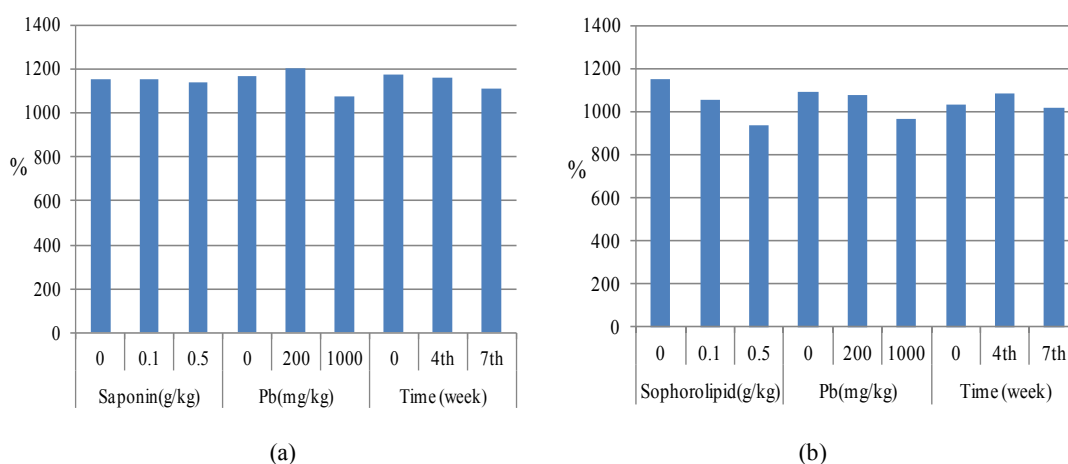


Fig. 4-2 Effect of different factors on water content of *Brassica Juncea* (a) Saponin (b) Sophorolipid

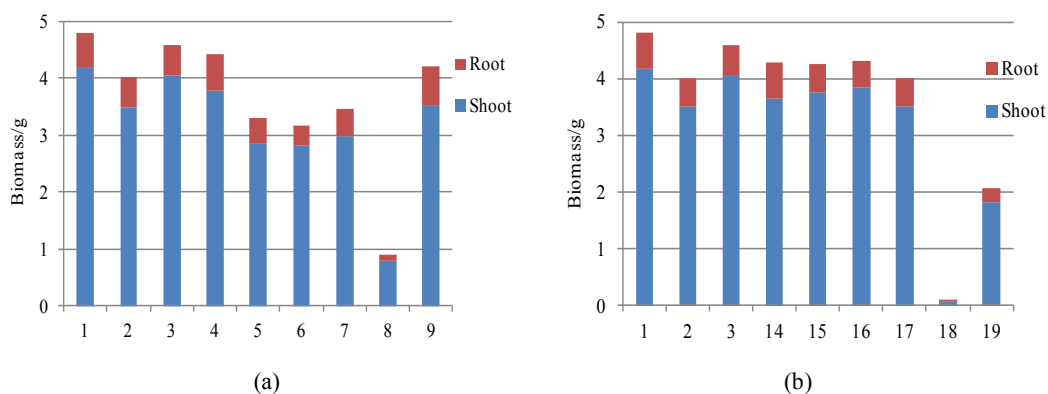


Fig. 4-3 The biomass of *Brassica Juncea* in shoot and root (a) Saponin (b) Sophorolipid



Fig. 4-4 The pictures of Pot No.9 and No.19 after the addition biosurfactants on the 7th week

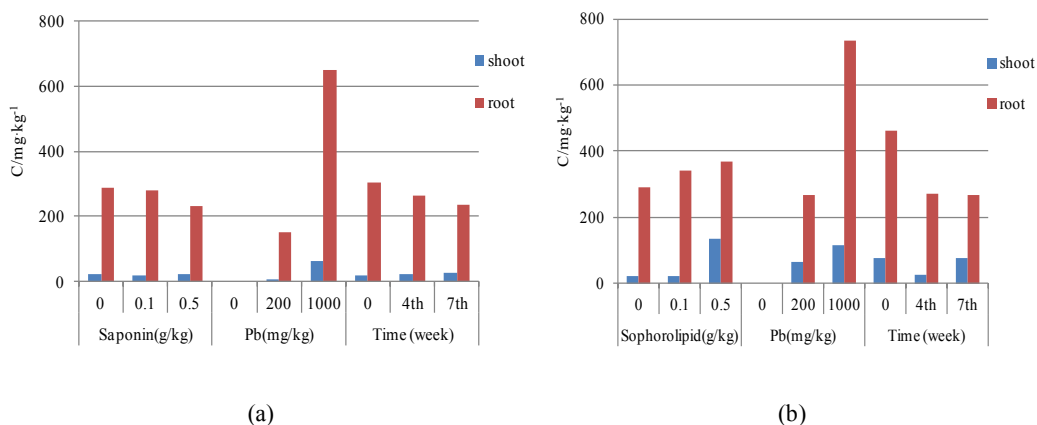


Fig. 4-5 Effect of different factors on Pb concentration in shoot and root of *Brassica juncea*
(a) Saponin (b) Sophorolipid

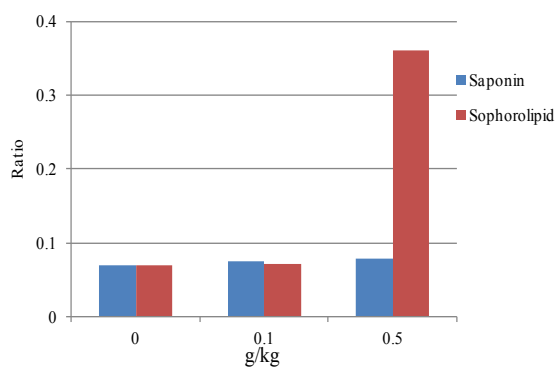


Fig. 4-6 Effect of the biosurfactants dosage on the TF of Pb in *Brassica juncea*

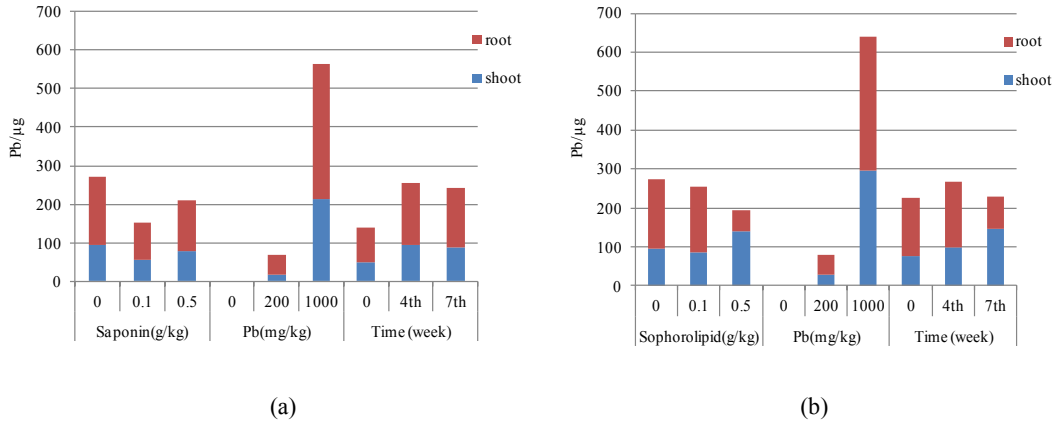


Fig. 4-7 Effect of different factors on uptake amount of Pb in *Brassica juncea* (a) Saponin (b) Sophorolipid

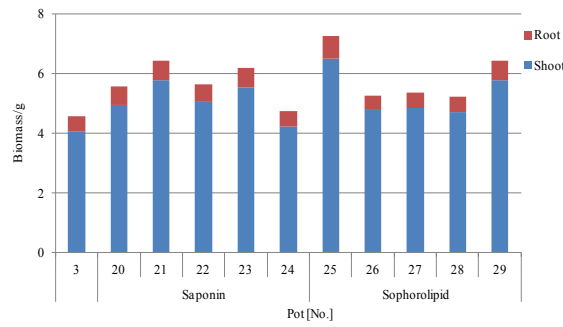


Fig. 4-8 Effect of plant growth regulator on biomass

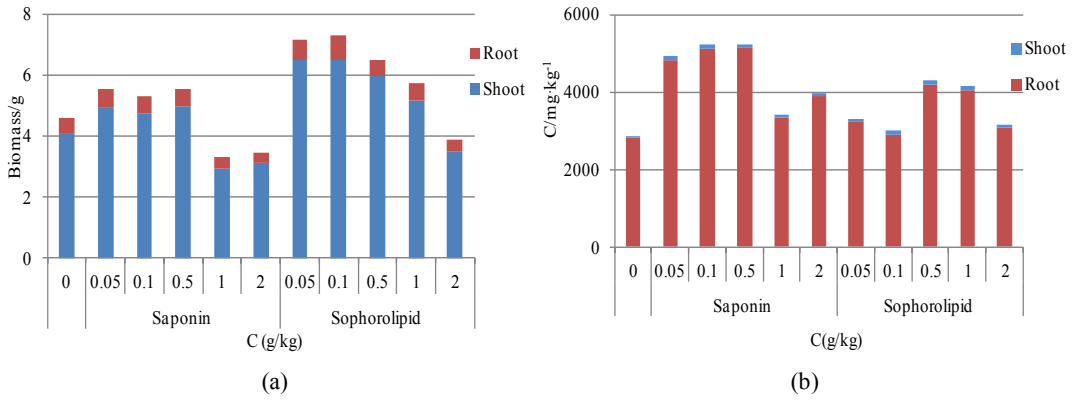


Fig. 4-9 Effect of the biosurfactant dosage on *Brassica juncea*
 (a) Biomass (b) Pb concentration (c) Uptake amount of Pb

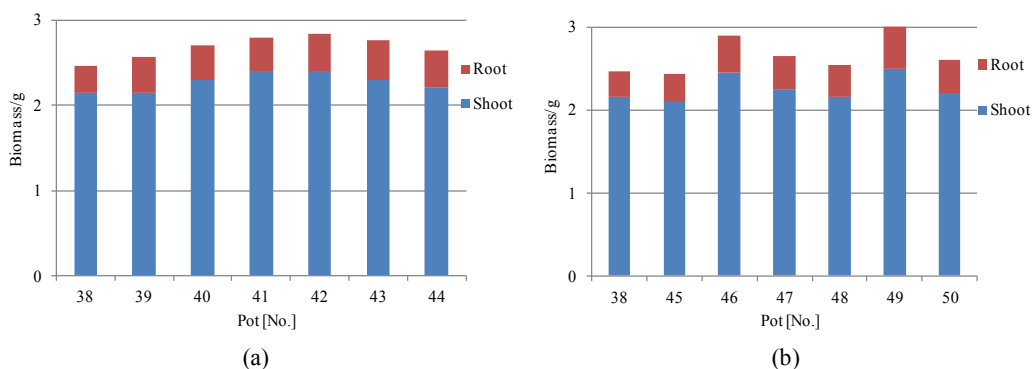


Fig. 4-10 The shoot and root biomass of *Helianthus annuus* (a) Saponin (b) Sophorolipid

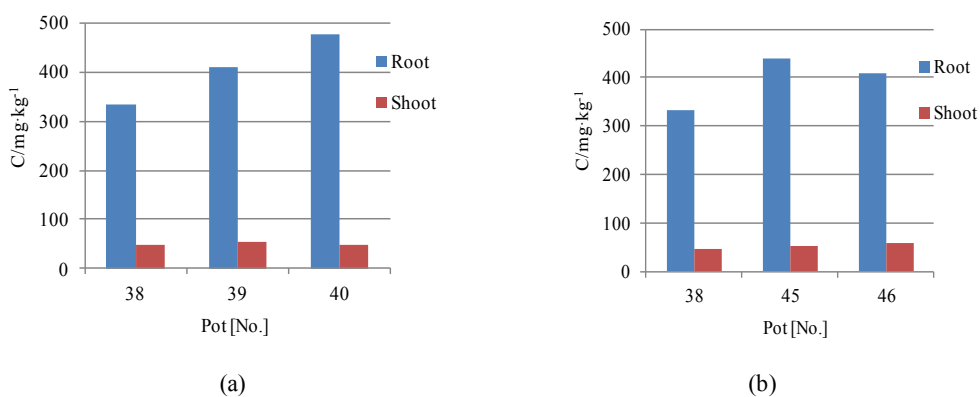


Fig. 4-11 Pb concentration in shoot and root of *Helianthus annuus* (a) Saponin (b) Sophorolipid

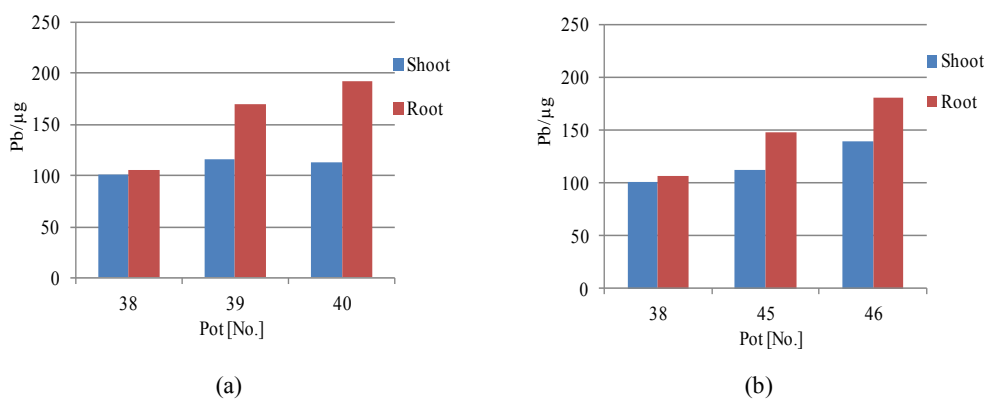


Fig. 4-12 Uptake amount of Pb in shoot and root of *Helianthus annuus* (a) Saponin (b) Sophorolipid

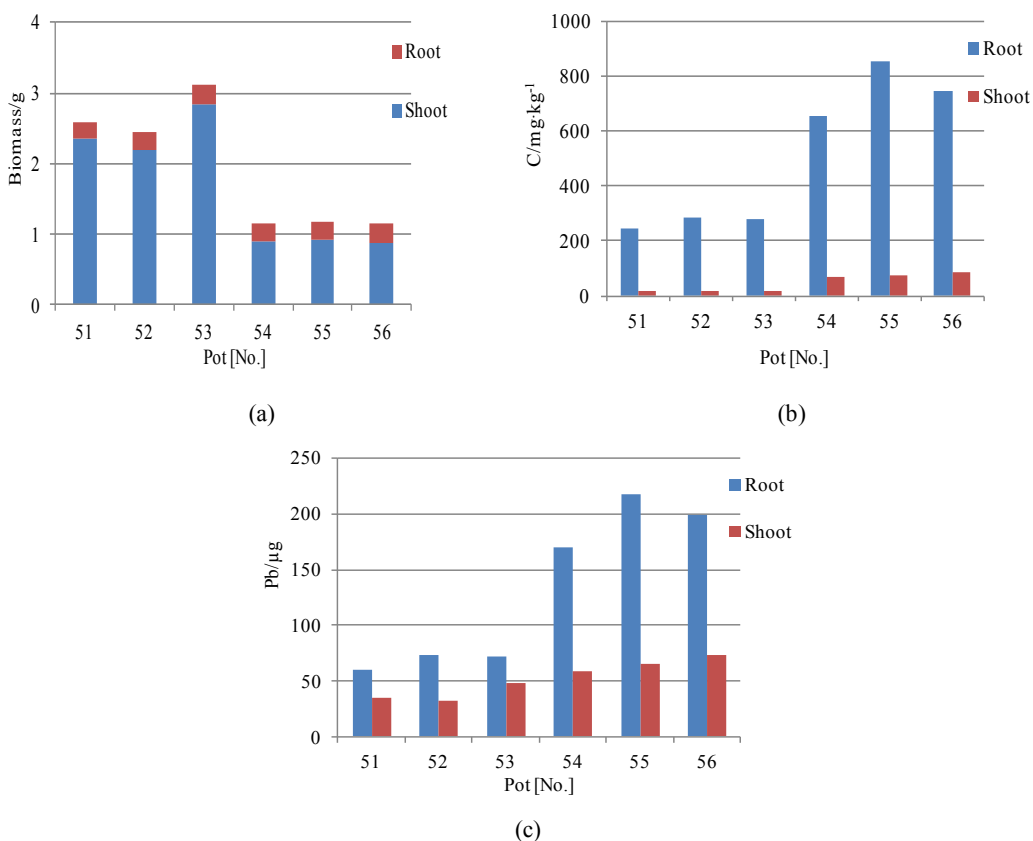


Fig. 4-13 Effect of the biosurfactant on phytoremediation of Pb-contaminated sludge
 (a) Biomass (b) Pb concentration (c) Uptake amount of Pb

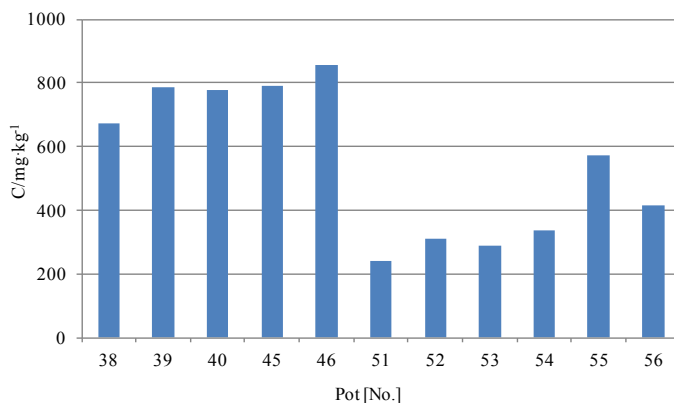


Fig. 4-14 Pb concentration in soil or sludge by 0.1 mol·L⁻¹ HCl extraction

Chapter 5 Conclusions

In the work of the thesis, the behavior and distribution of heavy metals including REEs, Th and U in soil and sludge environment have been investigated. Furthermore, the efficient recovery method of the metals from contaminated soil and sludge by phytoremediation and biosurfactants application (in batch and column experiment) has been established. In addition, The effect of biosurfactants in phytoremediation on Pb-contaminated soil and sludge was studied, the different dosage and kinds of biosurfactants (saponin and sophorolipid) were investigated using two kinds of plants (*Brassica juncea* and *Helianthus annuus*).

Consequently, the following matters have been mainly clarified.

(1) There are large differences of the values of pH, EC and organic carbon content of the soil among the different utilization and the different sampling points. It may be attributed to the difference of the soil parent materials, and physical and geography conditions. On the other hand, it is considered that more effective treatment of the wastewater is applied in Japan than in China from the characteristics of the sludge in two countries.

(2) No significant differences of REEs, Th and U concentrations are detected among soil utilizations, and/or before and after cultivation. The differences of REEs, Th and U concentrations may be largely attributable to the differences of the nature and formation history of soil, and of the surrounding environment as well as applied fertilizer. On the other hand, the concentrations of REEs, Th and U in sludge are more variable when compared with those in natural soils.

(3) The parent material of soil and regular agriculture activity as well as other

factors can affect the concentrations and compositions of heavy metals in soils. Definite differences of the elemental concentrations are not observed between sampling periods. On the other hand, the concentrations of heavy metals in sludge are greater and exist as more unstable fraction than those in natural soils.

(4) The distribution proportion of metallic elements in soils is varied largely depending on elements and soil utilization.

(5) Differences of the concentration and distribution characteristics of metallic elements are found between Chinese soil samples and Japanese soil samples.

(6) Nonionic saponin is more efficient than sophorolipid for the removal of heavy metals from the sludge.

(7) The high recovery efficiencies of heavy metals are obtained by alkaline precipitation. Saponin can also be recovered by alkaline precipitation. It can be predicted to withstand the repeated use, and hence it can be a promising material for saving the cost.

(8) Biosurfactants (saponin and sophorolipid) can enhance the phytoremediation effect of Pb-contaminated soil or sludge by increasing the biomass and Pb concentration of plant. The effect is affected by the kind, the dosage and the addition time of biosurfactants as well as the Pb concentration in soil and the nature of soil.

(9) Plant growth regulators, GA3 and IAA, do not always improve the biomass. *Brassica juncea* is obviously more suited for the phytoremediation of Pb-contaminated soil than *Helianthus annuus*. On the contrary, *Helianthus annuus* is more effective for the Pb-contaminated sludge.

The data obtained and the method used in this work can be useful for environmental preservation and recovery of resources.

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Acknowledgements

In the past three years and half, I have been studied in Kano Lab at the Graduate School of Science and Technology, Niigata University, Japan. Allow me to express my appreciations and thanks on my graduations!

Firstly, I would like to express my deepest gratitude to Associated Professor Naoki Kano for his sincerely guidance, valuable advice and encouragement, and I am very thanks to Professor Hiroshi Imaizumi for his help for me throughout my doctoral course. I would like to thank Professor Mineo Sato, Professor Tatsuya Kodama, Professor Kazuaki Yamagiwa and Associated Professor Naoki Harada for their advices and suggestions of this thesis.

Secondly, I also want to give my appreciations to peoples who give me many instruction, helping and encourage for my experiments. They are Kunihiko Fuzii, Manabu Ooizumi and Miyoko Masui in Office for Environment and Safety of Niigata University for the on the use of ICP-AES and ICP-MS, my co-members including Yoshikazu Nishimura, Yuichi Sato, Yoshimasa Higashidaira and my Chinese friends in our lab.

Finally, the best thanks to my parents and friends in China for their support of my studying abroad.