공학석사 학위논문

유류 오염토양의 생물학적 정화를 위한 Biosolids의 전처리

Pretreatment of Biosolids for Bioremediation of Oil Contaminated Soils

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한국해양대학교 대학원

토목환경공학과

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ABSTRACT

A study required for the use of biosolids amendment for the remediation of oil contaminated soil was performed. The amendment was prepared by the cultivation of oil degrading bacteria using biosolids, and its effectiveness in remedying oil contaminated soil was evaluated. A study to remove the hazardous materials, such as heavy metals and pathogens, contained in biosloids was also performed to relief the riskiness when the amendment were applied to soil. For the studies on the preparation of the biosolids amendment, and its effectiveness for the remediation, functional microbial consortium was cultivated from biosolids mixed with total petroleum hydrocarbon (TPH) and sawdust in the first set of experiment. Further, the mixture was maintained by a humidifier over a period of 4 weeks to reduce the water loss. After the well growth of functional microbial consortium, the biosolids mixtures were applied to artificial oil contaminated soil and natural oil contaminated soil to test the TPH degradation. The application of biosolids mixture was more effective in artificial oil contaminated soil than the natural oil contaminated soil. The TPH concentration was rapidly reduced over 80% within a period of 7 days after biosolids mixtures application. However, in the case of natural oil contaminated soil, less than 2000 mg/kg TPH was remained after 65 days of biosolids mixtures application. For the study to remove heavy metals and pathogens contained in biosloids, phosphate amendments and ultrasonic treatment were used to immobilize the heavy metals and remove the pathogens from the biosolids. Potassium dihydrogenphosphate was used as a source of phosphate for metals immobilization. Before and after phosphate amendments, metal concentration in biosolids was analyzed by EPA 6010, EPA 3051 and selective sequential extraction methods for comparison of the results. The results showed that 50% of the metals immobilized by phosphate amendments. In addition, extractable level of metals was different in different methods. Ultrasonic treatment was used to increase the metal phosphate reaction as well was pathogens removal from biosolid. Two approaches were used in this experiment, at first phosphate amendments followed by ultrasonic treatment and at second ultrasonic treatment followed by phosphate amendments. Biosolids were treated ultrasonically in both experiments for 1hr by ultrasonic pipette washer machine. The results showed that biosolid treated with ultrasonic followed by phosphate amendments have higher metal immobilizing efficiency than the other method. Finally, pathogens could be removed from biosolids using ultrasonic treatment.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
ACS	Artificially oil-contaminated soil
CFU	Colony forming units
DS	Dried solid
EPA	Environmental protection agency
HM	Heavy metal
MPN	Most probable number
NCS	Naturally oil-contaminated soil
SSE	Selective sequential extraction
SSET	Selective sequential extraction total
TPH	Total petroleum hydrocarbon
TS	Total solid
US	Ultrasonic
VRB	Violet red bile
WWTP	Wastewater treatment plant

Chapter 1

INTRODUCTION

1.1. Background

In Korea, the biosolids produced from wastewater treatment plant has been dumped into several special sea areas apart from the coastline. However, the ocean dumping for the disposal of biosolids could not be used any more if London convention comes into effect in the near future. Therefore, the biosolids problem concerning its treatment and disposal is a major issue for environmental engineers, now. Traditional methods for the biosolids disposal including landfill, incineration and pyrolysis could be also considered, but these methods also have lots of adverse effects on environment due to the spread of some hazardous pollutants contained in the biosolids (Ting et al., 1999). On the other hand, soil contaminated with oil is also another serious concern to be solved for the environmental engineer. Usually, bioremediation is one of the cost-effective methods for the cleaning of the soil contaminated with oil (Wang and Bartha, 1994). However, lots of bacteria which can degrade pollutants originated from oil and some nutritive substances are required for the bioremediation of soil contaminated with oil. Then, the biosolids from a biological treatment of wastewater is an aggregate of myriad bacteria containing lots of nutritive substances needed in microbial growth, as well as organic matter. The organic matter and the nutritive substances could be easily utilized for the growth of other bacteria during fermentation under substrate limited condition. This indicates that the biosolids has a great potential converting into the materials (inoculums and nutritive materials) for the bioremediation.

Petroleum products are some of the most widely used chemicals in society today. With the massive quantity of fuel required to power automobiles and heat homes, and the number of times each gallon of petroleum is stored, transported, or transferred, accidents and leakages are unavoidable. Petroleum contamination results from leaking aboveground and underground storage tanks, spillage during transport of petroleum products, abandoned manufactured gasoline sites, other unplanned releases, and current industrial processes. As petroleum contains hazardous chemicals such as benzene, toluene, ethylbenzene, xylenes, and naphthalene, this contamination can be hazardous to the health of plants, animals, and humans (Zhou and Crawford, 1995; Liebeg

and Cutright, 1999; Ting et al., 1999; Vasudevan and Rajaram, 2001). One of the best approaches for restoring contaminated soil is to make use of microorganism able to degrade those toxic compounds in a bioremediation process. Bioremediation is an attractive approach of cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminant (Frankenberger, 1992). In this study, bioremediation of oil-contaminated soil was tried by using the biosolids amendment which was prepared by the cultivation of oil degrading bacteria after removal of hazardous compounds containing in biosolids.

Heavy metals containing in biosolids may cause severe problem when applying to soil. Heavy metals solubility is related to its mobility and bioavailability; immobilization techniques may reduce the solubility of heavy metal contaminants. Chemical immobilization is a remediation technique that decreases the concentration of contaminant by sorption or precipitation. Several studies have used number of remediation techniques for metal contaminated soil. The objective of this study was to find out a proper immobilization methods for heavy metals such as Cu, Cd, Cr, Pb and Ni using difference concentrations of phosphate amendments. The immobilization of heavy metals in biosolids by phosphate is extremely important before disposal to land application. Although the immobilization of heavy metals using phosphate amendments in soils has been very successful in many studies nevertheless, the effectiveness of using phosphate to immobilize heavy metals in biosolids as well as comparison of different analytical methods was not available in the literate.

Treated biosolids are often disposed by delivering it to a landfill, to land farms for spreading the biosolids over available land, by incineration, by disposal at sea or by use of treated biosolids for agricultural purposes such as a soil additive. Regardless of the disposal method employed on the biosolids derived from wastewater, any safe biosolids disposal methods requires the elimination, or at least the sufficient inactivation, of the harmful pathogens, bacteria or other microorganisms present in biosolids. Thus, some type of disinfecting method should be employed before the disposal or reuse of the biosolids. In this study, ultrasonic treatment was applied to remove pathogens from biosolids.

1.2. Objectives of the study

The overall objectives of this research were:

1. Cultivation of a functional microbial consortium for degrading oil contaminated soil.
2. Investigation of the effectiveness of biosolids on the remediation of oil contaminated soil after cultivation the functional group of bacteria.
3. Immobilization of heavy metals contained in biosolids using phosphate amendments.
4. Examination of the effect of ultrasonic radiation on the killing of pathogens and increasing of the metal-phosphate immobilization rate.

Chapter 2

LITERATURE REVIEW

2.1. Overview of biosolids

Biosolids, historically known as sewage sludge, are the solid organic matter produced from private or community wastewater treatment processes that can be beneficially used, especially as a soil amendment.

2.1.1. Biosolids sources and characteristics

A typical process for producing biosolids is given in figure 2.1. The water and organic content in waste activated sludge are reduced and stabilized by thickener, anaerobic sludge digestion, chemical conditioning and belt filter press processes. The final biosolids contains 80-90% water, heavy metals, pathogens as well as inorganic or organic matter.

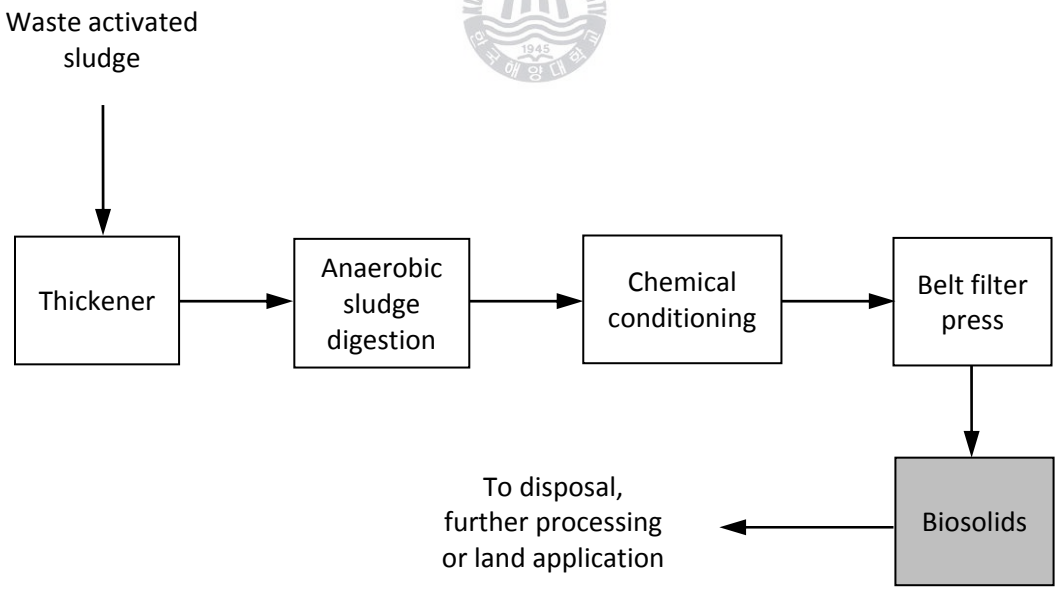


Figure 2.1. Typical process for producing biosolids in wastewater treatment plant.

To treat and dispose the biosolids produced from wastewater treatment plants in the most effective manner, it is important to know the characteristics of the biosolids. The characteristics depend on the origin of the biosolids, aerobically digested biosolids are brown to dark brown in color and have a flocculent appearance. The odor of aerobically digested sludge is not offensive; it is often characterized as musty. Well-digested aerobic sludge can be easily dewatered on drying beds. Anaerobically digested biosolids are dark brown to black in color and contain an exceptionally large quantity of gas. When thoroughly digested, they are not offensive, the odor being relatively faint and like that of hot tar, burnt rubber, or sealing wax. Primary sludge, when anaerobically digested, produces about twice as much methane gas as does waste activated sludge.

The typical chemical composition and metal content in digested biosolids are given in table 2.1 and 2.2.

Table 2.1. Typical chemical composition of digested biosolids.

Item	**Unit**	**Digested biosolids**	
		Range	**Typical**
Nitrogen	% of TS	1.6 – 3.0	3.0
Phosphorus	% of TS	1.5 - 4.0	2.5
Cellulose	% of TS	8 - 15	10
Protein	% of TS	15 - 20	18
Silica	% of TS	10 - 20	-
pH	-	6.5 – 7.5	7.0
Alkalinity	mg/L as $CaCO_3$	2,500 – 3,500	3,000
Organic acids	mg/L as HAc	100 - 600	200
Energy content	kJ TS/kg	9,000 – 14,000	12,000

Source: U.S. EPA 1979.

Table 2.2. Typical metal content of digested biosolids.

Metal	Dry biosolids (mg/kg)	
	Range	Median
Arsenic	1.1 – 230	10
Cadmium	1 – 3410	10
Chromium	10 – 99,000	500
Cobalt	11.3 – 2490	30
Copper	84 – 17,000	800
Iron	1,000 – 154,000	17,000
Lead	13 – 26,000	500
Manganese	32 – 26,000	260
Mercury	0.6 – 56	6
Molybdenum	0.1 – 214	4
Nickel	2 – 5,300	80
Selenium	1.7 – 17.2	5
Tin	2.6 – 329	14
Zinc	101 – 49,000	1,700

Source: U.S. EPA 1984.

2.1.2. Biosolids disposal methods

Land application

Land application involves the spreading of biosolids on the soil surface or incorporating or injecting biosolids into the soil. Land application has been practiced for decades and continues to be the most common method for using biosolids. Biosolids serve as soil enrichment and can supplement or replace commercial fertilizers. Nutrients (e.g., nitrogen and phosphorus), micronutrients including essential trace metals (e.g., copper, zinc, molybdenum, boron, calcium, iron, magnesium, and manganese), and organic matter in the biosolids are

beneficial for crop production, gardening, forestry, turf growth, landscaping, or other vegetation.

Biosolids generally have lower nutrient contents than commercial fertilizers. Biosolids typically contain 3.2 percent nitrogen, 2.3 percent phosphorus, and 0.3 percent potassium, while commercial fertilizers might contain 5 to 10 percent nitrogen, 10 percent phosphorus, and 5 to 10 percent potassium (Metcalf & Eddy, 1991). Nevertheless, the use of biosolids conditions the soil and reduces or eliminates the need for commercial fertilizers, thereby reducing the impacts of high levels of excess nutrients entering the environment. Furthermore, although biosolids contain metals, so do fertilizers, although data on metals in fertilizers are not comprehensive. States are only now starting to look at regulating metals levels in fertilizers, whereas metals in biosolids have been regulated for years.

Biosolids treatment before land application can involve digestion, composting, alkaline treatment, heat treatment, or other methods. Biosolids are treated to different levels, depending on the end use. In many cases, land application of biosolids is less expensive than disposal methods. Biosolids composting adds cost, but the resulting compost has a wide variety of uses, and a composting program has the potential to reduce municipal funding normally spent on purchasing soil amendments and/or provide high-quality compost to many other users. Furthermore, composting offers ease of storage and ease of application because of its semidry product, less odors, and more flexibility in land application due to its high quality.

Some of the uses for biosolids and biosolids composts include their application to various types of land including agricultural lands, forests, mine reclamation sites and other drastically disturbed lands, parks, and golf courses. Composted and treated biosolids are used frequently by landscapers and nurseries and by homeowners for lawns and home gardens. Agricultural land application of biosolids has worked well for many communities. Application of biosolids to forest lands, which currently involves a relatively small percentage of biosolids, can help shorten pulp wood and lumber production cycles by accelerating tree growth. At reclamation sites, biosolids help revegetate barren land and control soil erosion; relatively large amounts of biosolids are used to achieve these goals at reclamation sites. A growing market is the use of biosolids in manufactured soils, which can be used for erosion control, roadway construction, and parks. Composted and heat dried or pelletized biosolids used on public lands, lawns, and home gardens are often sold or given away in bags or bulk quantities; these forms are usually of excellent quality (with very low levels of metals and pathogens below detection levels), are easy to store and handle, and are usually in high demand.

Incineration

Incineration of biosolids involves firing biosolids at high temperatures in a combustor or combustion device. The volatile organic materials in the biosolids are burned in the presence of oxygen. Incineration reduces biosolids to a residue primarily consisting of ash, which is approximately 20 percent of the original volume. The incineration process destroys virtually all of the volatile solids and pathogens and degrades most toxic organic chemicals, although compounds such as dioxin may be formed, and products of incomplete combustion must be controlled. Metals are not degraded and are concentrated in the ash and in the particulate matter that is contained in the exhaust gases generated by the process. Air pollution control devices, such as high-pressure scrubbers, are required to protect air quality.

Surface disposal and landfilling

Surface disposal is defined as biosolids placed on an area of land where only biosolids are placed for final disposal. It does not include biosolids that are placed on land for either storage (generally less than 2 years) or treatment (e.g., lagoon treatment for pathogen reduction). It involves landfilling of biosolids in monofills (biosolids-only landfills), disposal in permanent piles or lagoons used for disposal (rather than treatment or temporary storage), and dedicated surface disposal practices. The difference between surface disposal and land application primarily involves the application rate. If biosolids are spread on land at greater than the agronomic rate, then the ability of the cover crop to retain nitrogen might be exceeded, and the excess nitrogen could migrate through the soil and contaminate ground water.

2.2. Heavy metals in biosolids

Application of sewage sludge to agricultural soil is a common practice because of low costs and recycling of nutrients achieved. However, this practice can pose a threat to environment and the major concern arises from the fact that sewage sludge, especially those from the heavily urbanized and industrialized areas, contains a relatively high concentration of heavy metals. Thus application of sewage sludge to agricultural soil may result in elevated concentrations of toxic metals, which may then threaten ground water quality and lead to food chain contamination. Pollutant limits of heavy metals for land application are listed on table 2.3 base on environmental protection agency of United States.

Table 2.3. Pollutant limits of heavy metals for land application.

Metal	Ceiling concentration (mg/kg)	Cumulative pollutant loading rates (kg/ha)	Annual pollutant loading rates (kg/ha/yr)
Arsenic	75	41	2.0
Cadmium	85	39	1.9
Copper	4,300	1,500	75
Lead	840	300	15
Mercury	57	17	0.85
Molybdenum	75	NL	NL
Nickel	420	420	21
Selenium	100	100	5.0
Zinc	7,500	2,800	140

NL: no limit. Source: U.S. EPA 1993 and 1994.

2.3. Pathogens in biosolids

2.3. 1. What are pathogens?

A pathogen is an organism or substance capable of causing disease. Pathogens infect humans through several different pathways including ingestion, inhalation, and dermal contact. The infective dose, or the number of a pathogenic organism to which a human must be exposed to become infected, varies depending on the organism and on the health status of the exposed individual.

2.3.2. Pathogens in biosolids

The four major types of human pathogenic organisms (bacteria, viruses, protozoa, and helminths) all may be present in biosolids. The actual species and quantity of pathogens present in the biosolids from a particular municipality depend on the health status of the local community and may vary substantially at different times. The level of pathogens present in biosolids also depends on the reductions achieved by the wastewater and sewage sludge treatment processes.

Table 2.4. Principal pathogens of concern in biosolid

ORGANISM	DISEASE/ SYMPTOMS
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning), typhoid fever
Shigella sp.	Bacillary dysentery
Yersinia sp.	Acute gastroenteritis (including diarrhea, abdominal pain)
Vibrio cholera	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli	Gastroenteritis
Enteric viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Rotaviruses	Acute gastroenteritis with severe diarrhea
Polioviruses	Poliomyelitis
Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.
Echoviruses	Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.
Reovirus	Respiratory infections, gastroenteritis
Astroviruses	Epidemic gastroenteritis
Caliciviruses	Epidemic gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Balantidium	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth worms	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Ascaris suum	May produce symptoms such as coughing, chest pain, and fever
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginafa	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Taenia solium	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

Source: Kowal (1985) and EPA (1989)

2.3.3. Survivability of pathogens

Wastewater generally contains significantly high concentrations of pathogens which may enter the wastewater system from industries, hospitals, and infected individuals. The wastewater treatment process tends to remove pathogens from the treated wastewater, thereby concentrating the pathogens in the sewage sludge. Like any other living organisms, pathogens thrive only under certain conditions. Outside of these set conditions, survivability decreases. Each pathogen species has different tolerance to different conditions; pathogen reduction requirements are therefore based on the need to reduce all pathogenic populations. Some of the factors which influence the survival of pathogens include pH, temperature, competition from other microorganisms, sunlight, contact with host organisms, proper nutrients, and moisture level.

Table 2.5. Survival times of pathogens in soil and on plant surfaces

Pathogen	Soil		Plants	
	Absolute maximum	Common maximum	Absolute maximum	Common maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	1 year	3 months	2 months	1 month
Protozoan cystsd	10 days	2 days	5 days	2 days
Helminth ova	7 years	2 years	5 months	1 month

Source: Kowal, 1985.

2.3.4. Reducing the number of pathogens

Pathogen reduction can be achieved by treating sewage sludge prior to use or disposal and through environmental attenuation. Many sewage sludge treatment processes are available that use a variety of approaches to reduce pathogens and alter the sewage sludge so that it becomes a less effective medium for microbial growth and vector attraction. Processes vary significantly in their effectiveness. For example, some processes (e.g. lime stabilization) may effectively reduce bacteria and viruses but have little or no effect on helminth eggs. The effectiveness of a particular process can also vary depending on the conditions under which it is operated. For example, the length of time and the temperature to which sewage sludge is heated is critical to the effectiveness of heat-based treatment processes.

Table 2.6. General approaches to controlling pathogens in biosolid

Approach	**Effectiveness**	**Process examples**
Application of high temperatures (temperatures may be generated by chemical, biological, or physical processes).	Depends on time and temperature. Sufficient temperatures maintained for sufficiently long time periods can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Helminth ova are the most resistant to high temperatures.	Composting Heat drying and heat treatment Pasteurization Aerobic digestion Anaerobic digestion
Application of radiation	Depends on dose. Sufficient doses can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Viruses are most resistant to radiation.	Gamma and high-energy electron beam radiation.
Application of chemical disinfectants	Substantially reduces bacteria and viruses and vector attraction. Probably reduces protozoan cysts. Does not effectively reduce helminth ova unless combined with heat.	Lime stabilization
Reduction of the sewage sludge's volatile organic content (the microbial food source).	Reduces bacteria. Reduces vector attraction.	Aerobic digestion Anaerobic digestion Composting
Removal of moisture from the sludge	Reduces viruses and bacteria. Reduces vector attraction as long as the sewage sludge remains dry. Probably effective in destroying protozoan cysts. Does not effectively reduce helminth ova unless combined with other processes such as high temperature.	Air or heat drying

2.4. Oil-contaminated soil and treatment technologies.

Oil pollution accidents are nowadays become a common phenomenon and have caused ecological and social catastrophes. Spill and leaks of petroleum products including gasoline, diesel fuel, and lubricating and heating oil often results in the contamination of soil and water.

2.4.1. The composition of the petroleum-degrading microbial population

The heterogeneous or heterotrophic microorganisms found in soils include naturally occurring populations that posses the ability to degrade petroleum products. This population imparts a large hydrocarbon assimilatory capacity to most soils.

Table 2.7 lists the genera of hydrocarbon-degrading bacteria and fungi isolated from soil. In decreasing order, Pseudomonas, Arthrobacter, Alcaligenes, Corynebacterium, Flavobacterium, Achromobacter, Micrococcus, Nocardia, and Mycobacterium appear to be the most consistently isolated hydrocarbon-degrading bacteria from soil. In decreasing order, Trichoderma, Penicillium, Aspergillus, and Mortierella were the hydrocarbon-degrading fungi to be most often isolated from soil. It is clear that bacteria and fungi are the principal agents of petroleum biodegradation in soil, but the relative contribution of each not clear.

Spore-forming bacteria generally have a negligible role in biodegradation. Although Bacillus strains have been isolated from contaminated soils, this may be due to their persistence in soil and subsequent spore germination during enrichment and isolation procedures. Also, a number of actinomycetes have been shown to have hydrocarbon-degrading abilities; however, these organisms do not seem to compete successfully in contaminated soils. The role of algae and protozoa is poorly documented in the literature and does not appear to be significant.

A unique group of hydrocarbon-degrading bacteria not included in table 2.7 is the methanotrophs, which posses a highly specialized C1 metabolism. The methanotrophs are strict anaerobes and typically metabolize petroleum products at rates one or two orders of magnitude lower than aerobic bacteria. Methanotrophs are ubiquitous in soil and become greatly enriched near natural or anthropogenic seeps of methane-containing natural gas.

Early researchers noted that the number of aerobic bacteria in an agricultural soil increased on application of a crude oil. Although the bacterial numbers increased, species diversity of aerobic microbes decreased with little effects on the anaerobic microbes. More recent investigations confirm these findings. Microbial numbers and activity are generally enhanced in contaminated soils.

Stimulation of microbial activity is positively correlated to increasing amounts of hydrocarbons in soil. A study reported that soil receiving an application of 39.2% of crude oil possessed the highest number of microorganisms relative to soil receiving less amounts of oil. Pinholt et al. showed that eight months after contamination, oil degrading bacteria in soil increased tenfold to almost 50% of the total bacterial count. In this case, no pronounced decrease in fungal species diversity occurred, although Scolecobasidium and Mortierella were selectively enriched, as were to a lesser degree, Humicola and Verticillium. Also, Jensen reported that oil treated soils possessed lower bacterial species richness than untreated soils. Populations of Arthrobacter and coryneforms such as Corynebacterium, Brevabacterium, Mytobacterium, and Nocardia showed strong positive responses to oil contamination.

Table 2.7. Genera of hydrocarbon-degrading bacteria and fungi isolated from soil.

Bacteria	**Fungi**
Achromobacte	*Acremomium*
Acinetobacter	*Aspergillus*
Alcaligenes	*Aureobasidium*
Arthrobacter	*Beauveria*
Bacillus	*Botrytis*
Brevibacterium	*Candida*
Chromobacterium	*Chrysosporium*
Corynebacterium	*Cladosporium*
Cytophaga	*Cochliobolus*
Erwinia	*Cynlidrocarpon*
Flavobacterium	*Dabaryomyces*
Micrococcus	*Fusarium*
Mycobacterium	*Geotrichum*
Nocardia	*Gliocladium*
Proteus	*Graphium*
Pseudomonas	*Humicola*
Sarcina	*Monilia*
Serratina	*Mortierella*
Spirillum	*Paecilomyces*
Streptomyces	*Penicillium*
Vibrio	*Phoma*
Xanthomonas	*Rhodotorula*
	Saccharomyces
	Saccharomyces
	Scolecobasidium
	Sporobolomyces
	Sprotrichum
	Spicaria
	Tolypocladium
	Torulopsis
	Trichoderma
	Verticillium

Source: Bossert and Barth

2.4.2. Chemical structure and biodegradability of petroleum

The chemical structures of the constituents present in the soils proposed for treatment by bioremediation are important for determining the rate at which biodegradation will occur. Although nearly all constituents in petroleum products are biodegradable, the more complex the molecular structure of the constituent, the more difficult and less rapid is biological treatment. Most low-molecular weight (nine carbon atoms or less) aliphatic and monoaromatic constituents are more easily biodegraded than higher-molecular-weight aliphatic or polyaromatic organic constituents.

Table 2.8. Chemical structure and biodegradability of petroleum product.

Biodegradability	**Example Constituent**	**Products In Which Constituents Is Typically Found**
More degradable	n-butane, l-pentane, n-octane	Gasoline
	Benzene, toluene, ethylbenzene, xylenes	
	Methyl butane, dimethylpentenes, methyloctanes	
	Propylbenzenes	Diesel fuel
	Decanes	
	Nonane	
	Naphthalenes	
	Dodecanes	Kerosene
	Fluoranthenes	
	Tridecanes	Heating fuels
	Pyrenes	
	Tetradecanes	Lubricating oils
Less degradable	Acenaphthenes	

Source: EPA 1994

2.4.3. Vapor pressure

Vapor pressure is important in evaluating the extent to which constituents will be volatilized rather than biodegraded. The vapor pressure of a constituent is a measure of its tendency to evaporate. More precisely, it is the pressure that a vapor exerts when in equilibrium with its pure liquid or solid form. Constituents with higher vapor pressures are generally volatilized rather than undergoing biodegradation. Constituents with vapor pressures higher than 0.5 mm Hg will likely be volatilized by the induced air stream before they biodegrade. Constituents with vapor pressures lower than 0.5 mm Hg will not volatilize to a significant degree and can instead undergo in situ biodegradation by bacteria.

As previously discussed, petroleum products contain many different chemical constituents. Each constituent will be volatilized (rather than biodegraded) to different degrees, depending on its vapor pressure. If concentrations of volatile constituents are significant, treatment of extracted vapors may be needed. Table 2.9 lists vapor pressures of select petroleum constituents.

Table 2.9. Vapor pressures of common petroleum constituents

Constituent	**Vapor Pressure (mm Hg at 20^0C)**
Methyl t-butyl ether	245
Benzene	76
Toluene	22
Ethylene dibromide	11
Ethyl benzene	7
Xylenes	6
Naphthalene	0.5
Tetraethyl lead	0.2

Source: EPA 1994

2.4.4. Product composition and boiling point

Boiling point is another measure of constituent volatility. Because of their complex constituent compositions, petroleum products are often classified by their boiling point ranges (rather than vapor pressures). In general, nearly all petroleum-derived organic compounds are capable of biological degradation, although constituents of higher molecular weights and higher boiling points require longer periods of time to be degraded. Products with boiling points of less than about 250^0C to 300^0C will volatilize to some extent and can be removed by a combination of volatilization and biodegradation. The boiling point ranges for common petroleum products are shown in table 2.10.

Table 2.10. Petroleum product boiling ranges

Product	**Boiling range (^{0}C)**
Gasoline	40 to 205
Kerosene	175 to 325
Diesel fuel	200 to 338
Heating oil	> 275
Lubricating oils	>290

Source: EPA 1994

2.4.5. Treatment technologies for oil-contaminated soil

Soil treatment technologies are often developed and evaluated in order to conform with regulatory demands, which may require or suggest that residual total petroleum hydrocarbon concentration in soil be reduced below 1000 mg/kg or, in some areas, below 100 mg/kg. Table 2.11 compares various features and the applicability of a variety of remediation technologies.

Table 2.11. Technology applicability

Technology	Applicability	Soil Type and Saturated Zone Characteristics	Variations	Cost	Permits
LPH recovery LPH withdrawal	All lighter-than-water petrochemicals except for the most viscous fuel and lube oils	Works better with more permeable soils	Total fluid extraction, passive bailers, dual pump recovery wells, thermally assisted LPH recovery, mop and disk skimmers	Variable	Groundwater discharge, product storage, and possibly, groundwater withdrawal
Vadose zone Soil vapor extraction	LPH less than about 0.5ft, contaminants with Vp>1 mmHg (BTEX, gasoline, MTBE, PCE, TCE, TCA, mineral spirits, MeOH, aceton, MEK, etc.)	Permeable soils, ROI>10 ft, depth-to-water greater than 3 ft	Thermally assisted venting, horizontal venting, surface sealing, passive vent points, closed loop venting, concurrent groundwater pumping for VOCs in capillary fringe	Low	Air discharge permit may be required
In situ percolation (bioremediation)	Any aerobically biodegradable chemical in the vadose zone	Works better in permeable soils; depth-to-water greater than 3ft	Oxygen and nutrients need to be supplied to the subsurface	Low to moderate	Air discharge permit may be required when soil venting used to provide oxygen
Excavation	All soils and contaminants	All soil types	Dewatering may be used to exposed soils in capillary fringe	High	On-site treatment of excavated soil may require permitting
Saturated zone Sparging	Contaminants in saturated zone with K_H>0.1 and Vp>1 mmHg; contaminants: BTEX, MTBE, PCE, TCE, TCA, mineral spirits,	Hydraulic conductivity>10^{-5} cm/s (silty sand or better); at least 5 ft of saturated thickness	Hot air, steam, and cyclic sparging, concurrent groundwater pumping	Low	Air discharge permit; water discharge if concurrent groundwater dumping
In situ bioremediation	Any biodegradable chemical in the saturated zone; inhibited by pH extremes, heavy metals, and toxic chemicals	Nutrients are transported better in more-permeable soil	Oxygen supplied by sparging or peroxide addition; nutrient addition with groundwater recovery and reinjection	Moderate to high	Water discharge for nutrient injection, air discharge if performed with sparging/venting
Excavation	All soils and contaminants	All soil types	Dewatering needed, groundwater containment may be used (slurry walls, sheet piles)	Very high	Permits for dewatering operations

Source: Ram, N.M, Bass, D.H., Falotico, R., and Leahy, M. *J. Soil Contam*. 2(2):167-189. Lewis Publishers, Boca Racon, FL, 1993.

Technology	Applicability	Soil Type and Saturated Zone Characteristics	Variations	Cost	Permits
Ground water recovery and treatment Groundwater recovery	Uses: (1) LPH recovery, (2) provides hydraulic control of contaminant plume, (3) pump and treatment technologies	Transmissivity, depth-to-water and saturated-zone thickness determine optimal strategy	Recovery wells, well points, interceptor trenches	Variable	Well installation, groundwater withdrawal and groundwater discharge
Liquid-phase carbon	Removal of compounds with low solubility/high adsorptivity	Sea groundwater recovery	High pressure (75 to 150 psi) and low pressure (12 to 15psi)	Low to high depending on contaminant loading	Water discharge permit
Air stripping	Compound with $K_H > 0.1$; contaminants with K_H between 0.01 and 0.1 may require an air-water ratio > 100	Sea groundwater recovery	Packed towers, low profile, heated and closed-loop air stripping; off-gas treatment may be required	Low if no off-gas treatment required	Air and water discharge permit
Advanced oxidation	Most effective on sulfide cyanide, double-bonded organics (PCE, TCE), BTEX, phenols chlorophenols, PCBs, PAHs, some pesticides	Sea groundwater recovery	Hydroxy/radicals produced by combinations of UV, ozone, and peroxide	Moderate to high	Water discharge permit
Bioreactors	Any biodegradable compound	Sea groundwater recovery	Fixed-film and suspended growth reactors	Moderate to high	Water discharge permit
Off-gas treatment Vapor-phase carbon	Adsorptive capacity generally increases with increasing molecular weight	NA	Pretreatment dehumidification; on-site regeneration	Moderate	Air discharge permit
Catalytic oxidation	Conventional units can treat all compounds containing carbon, hydrogen, and oxygen; concentrations should not exceed about 20% of the LEL	NA	Some units can treat chlorinated compounds. Exhaust gas scrubbing may be required	Moderate to high	Air discharge permit
Thermal oxidation	Compounds containing carbon, hydrogen, and oxygen; usually not amenable to halogen-containing compounds	NA	Exhaust gas scrubbing may be required	Moderate to high	Air discharge permit

Abbreviations: NA, not applicable; LEL, lower explosion limit; ROI, radius-of-influent; LPH, liquid-phase hydrocarbon; MTBE, methyl *tert*-butyl ether; PCE, perchloroethylene; TCE, trichloroethylene; TCA, trichloroethane; MEOH, methanol; MEK, methyl ethyl ketone; BETX, benzene, toluene, ethylbenzene, and xylenes; PCBs, polychlorinated buphenyls; PAHs, polyaromatic hydrocarbons.

Chapter 3

METHODOLOGY

3.1. Remediation of oil – contaminated soil using biosolids

The objective of this experiment is to cultivate a functional microbial consortium using biosolids and to evaluate its effectiveness for remediation of soil contaminated with diesel oil.

3.1.1. Sample collection and storage

For this study, dewatered biosolid sample was collected from Yeongdo municipal waste water treatment plant, Korea. Further, thickened biological sludge samples were collected from 3 different treatment plants as followed, Ulsan-Mipo petrochemical industrial complex (Sludge 1); Yeocheon petrochemical industrial complex (Sludge 2), and night soil treatment plant (Sludge 3). All the samples were collected and transferred to the laboratory within 2 hr and stored immediately at 4^{o}C. All the thickened biological sludge samples were centrifuged at 5000 rpm for 10 min to remove the superfluous water content. Commercially available saw dust was also been collected for this present study. Artificially and naturally oil contaminated soils were used to study the degradation of oil contamination. 10 kg of uncontaminated soil from Korea maritime university campus was collected; sieve (5 mm) to remove the organic debris and high density particles and added commercially available (TPH - total petroleum hydrocarbon) diesel oil to make the concentration of 5000 mg TPH/kg for artificial contaminated soil (ACS). Soil from Ulsan petrochemical complex industrial park was collected and used for natural contaminated soil (NCS). The concentration of petroleum hydrocarbon in this soil was ranged from 10,000 to 13,000 mg TPH/kg.

3.1.2. Experimental apparatus

Figure 3.1 shows the schematic diagram for the fermentation system cultivating functional microbial consortium degrading oil. The fermentation system was consisted of an air blower, a fermentation vessel (5 L), a humidifier, and an alkaline trap. The humidifier was filled with distilled water to maintain the water content of the air from the air blower. The alkaline trap was filled with 5% NaOH solution. A perforated plate was placed on the bottom of the fermentation vessel to supply humidified air uniformly into the vessel. The off

gas from the vessel was passed through the alkaline trap to collect carbon dioxide evolved from microbial respiration during the fermentation.

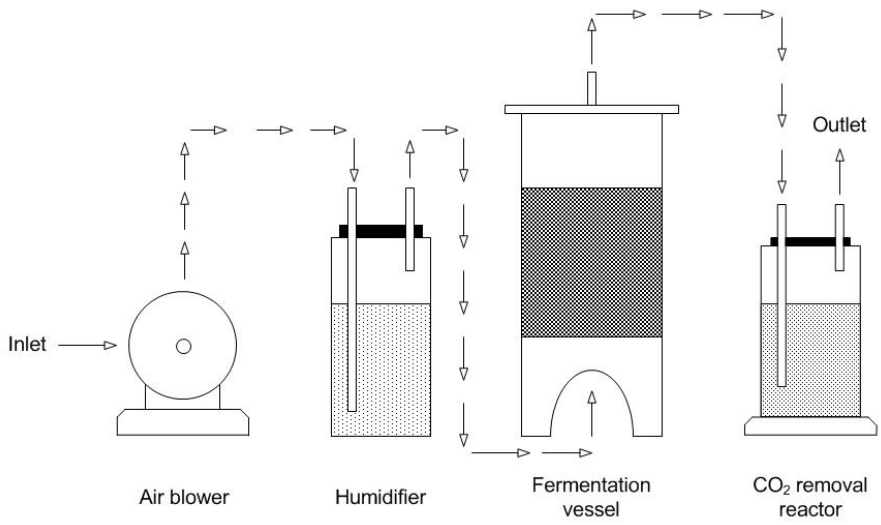


Figure 3.1. Schematic diagram of the fermentation system for cultivation of microbial consortium.

3.1.3. Experimental setup

Table 3.1 shows the initial condition of reactors 1, 2, 3 and 4 for fermentation. A fixed quantity of biosolid (1 kg), sludge (0.2 kg), sawdust (0.1 kg) and 12 g of TPH were added in each reactor except reactor 4, where same experimental setup maintained without sludge. While, R1, R2 and R3 were received sludge 1, sludge 2 and sludge 3, respectively. Here 12 g of TPH was added in each reactor to obtain oil degrading microorganisms and sawdust was added to reduce the water content. This TPH was making the concentration approximately 10,000 ppm in all reactors. All the reactors were mixed well and allow to fermentation process with continuous humidified air supply over the period of 4 weeks. Changes in diesel oil content, carbon dioxide production, water content and organic content were monitored every week upto 4 weeks study period. 50 g and 100 g of fermented products were removed from all the reactors after 4 weeks. Each mixed immediately with 1 kg of artificial oil contaminated soil (ACS) and natural oil contaminated soil (NCS) (Table 3.2). For comparison, 1 kg of ACS and NCS were maintained separately without addition of fermented products and considered as a control.

Table 3.1. Initial conditions of reactors for the remediation of oil-contaminated soil using biosolids.

	Re.1	**Re.2**	**Re.3**	**Re.4**
Biosolid	1 kg	1 kg	1 kg	1.2 kg
Sludge	0.2 kg [a]	0.2 kg [b]	0.2 kg [c]	NA
Sawdust	0.1 kg	0.1 kg	0.1 kg	0.1 kg
TPH[d]	12 g	12 g	12 g	12 g

[a] Sludge 1; [b] Sludge 2; [c] Sludge 3; [d] Total Petroleum Hydrocarbon; NA: Not added

Table 3.2. Amount of the final fermentation products mixed into soil contaminated with oil for its effectiveness test

Reactor	**Sample ID**	**Amount of Fermentation product (g)**	**Soil (ACS or NCS) (kg)**
	Control	NA	1.0
R1	1A	50	1.0
	1B	100	1.0
R2	2A	50	1.0
	2B	100	1.0
R3	3A	50	1.0
	3B	100	1.0
R4	4A	50	1.0
	4B	100	1.0

NA: Not Added

3.1.4. Analytical procedure

To analyze total petroleum hydrocarbon (TPH), 1 gram of the dried sample was mixed with 20mL extraction solution (S316, HORIBA) in 50 ml conical tube. Further, samples were extracted by vibration (Vortex-2 Genie) and centrifugation (HA-1000) for 15 min and 20 min, respectively. The extracted samples were injected into an oil analyzer (OCMA-300 HORIBA) to obtain TPH concentration.

The carbon dioxide trapped in the alkaline solution was determined by the change of pH in the alkaline solution. The analysis of organic matter is based on standard methods.

3.2. Immobilization of heavy metals in biosolid using phosphate amendments

The objective of this study is to reduce concentration of heavy metals in biosolids using potassium dihydrogenphosphate.

3.2.1. Experimental design

Bulk samples of fresh biosolids were obtained from Yeongdo Municipal wastewater treatment plant, Busan in sealable plastic container to maintain their original moisture content. Samples were transferred to the laboratory within 1 h of being collected and were stored at $4^{o}C$. Analytical grade of potassium dihydrogenphosphate (KH_2PO_4) form the source of phosphate was used as a chemical immobilization amendment for the present study.

5 concentrations of phosphate were used in this experiment (T1 to T5). 200 g of biosolid were added 6 numbers of 250 ml glass beakers. Each beaker received 0, 1.45, 2.9, 5.8, 10.15 and 14.5 g of KH_2PO_4. After the addition of KH_2PO_4 samples were mixed homogenously. 200 g of biosolid alone served as a control (C). The initial 1.45 g of KH_2PO_4 addition was selected by using the molar ratio between phosphate and all metals content in biosolid and further KH_2PO_4 additions are corresponding to the ratio of 1, 2, 4, 7 and 10. The detail calculation method of phosphate amount is given in table 3.3 and equation below.

Table 3.3. Briefly calculation amount of phosphate added to biosolid

Metals	**Metals concentration (mg/kg DS)**	**Atomic weight (g/mol)**	**Metals concentration (mmol/kg DS)**	**Metals-phosphate reaction**	**Require phosphate (mmol /kg DS)**
	C	*M*	$m_1 = C/M$		m_2
Cu	372	63.5	5.86	$3Cu^{2+} + 2PO_4^{3-} = Cu_3(PO_4)_2$	3.91
Zn	248	65	3.82	$3Zn^{2+} + 2PO_4^{3-} = Zn_3(PO_4)_2$	2.54
Cd	8.8	112	0.08	$3Cd^{2+} + 2PO_4^{3-} = Cd_3(PO_4)_2$	0.0.5
Cr	27	52	0.52	$3Cr^{2+} + 2PO_4^{3-} = Cr_3(PO_4)_2$	0.35
Pb	82	208	0.39	$3Pb^{2+} + 2PO_4^{3-} = Pb_3(PO_4)_2$	0.26
Ni	49	59	0.83	$3Ni^{2+} + 2PO_4^{3-} = Ni_3(PO_4)_2$	0.55
Fe	21,428	56	382.64	$3Fe^{2+} + 2PO_4^{3-} = Fe_3(PO_4)_2$	255.1
Mn	985	55	17.91	$3Mn^{2+} + 2PO_4^{3-} = Mn_3(PO_4)_2$	11.94
				Total	*274.7*

Molecular weight of KH_2PO_4: 136 g/mol = 136 mg/mmol

Total amount of phosphate: 274.7 mmol/kg DS equal to $274.7\frac{\text{mmol}}{\text{kg DS}} \times 136\frac{\text{mg}}{\text{mmol}} = 37{,}358\frac{\text{mg}}{\text{kg DS}} \approx 37.4\frac{\text{g}}{\text{kg DS}}$

Water content of biosolid: 86%

Total amount of phosphate base on wet weight = 37.4 * (1-0.86) = 7.26 g/kg

Amount of phosphate added to 200 g of biosolid: $7.26\frac{\text{g}}{\text{kg}} \times 0.2\text{kg} = 1.452\ \text{g} \approx 1.45\ \text{g}$

Beakers were covered with aluminum foil to prevent moisture loss as well as maintain field moisture content and kept in the laboratory at room temperature. After 1, 5 and 10 day incubation period, 10 g of biosolids samples were removed from the control and treatments for the analysis of heavy metals were determined by selective sequential extraction (SSE), EPA 6010 and EPA 3051 method. The detail analytical procedure of these methods is given in section 3.4.

Table 3.4. Amount of biosolid and phosphate amendments in each reactor.

	Control (C)	**T1**	**T2**	**T3**	**T4**	**T5**
Biosolid	200 g	200 g	200 g	200 g	200 g	200 g
KH_2PO_4	-	1.45 g	2.9 g	5.8 g	10.15 g	14.5 g

3.2.2. Biosolid characteristics

Table 3.5. Characteristics of biosolid obtained from Yeongdo wastewater treatment plant.

Component	**Unit**	**Concentration**
Water content	%	83%
pH	-	7.7
Cd	mg/kg DS	8.8
Cr	mg/kg DS	27
Cu	mg/kg DS	372
Mn	mg/kg DS	985
Fe	mg/kg DS	21,428
Ni	mg/kg DS	49
Pb	mg/kg DS	82
Zn	mg/kg DS	248

3.3. Reducing biological toxicity of biosolids using ultrasonic

The objective of this study is to remove pathogens and motivate the reaction between metal and phosphate in biosolids using ultrasonic.

3.3.1. Materials

The biosolid used in this experiment was collected from Nambu municipal wastewater treatment plant located in Busan, Korea. Characteristics of this biosolid are given in the table 3.6. The ultrasonic cleaner used for this experiment was Roronex Pr-140 with 35 kHz operational wavelength and 450W power. All chemicals including KH_2PO_4 and Selenite Broth were purchased from Kanto Chemical Co., Inc and Merck, respectively. For pathogen enumeration, 3M Petrifilm and Costar 3594 96-well count plate were also prepared.

Table 3.6. Characteristics of biosolid collected from Nambu WWTP.

Parameters	**Unit**	**Concentration**
Water content	%	83.6
Escherichia coli	CFU/g DS	5,200
Total coliform	CFU/g DS	30,250
Salmonella sp.	MPN/g DS	10,327
Cu	mg/kg DS	688
Zn	mg/kg DS	290
Cd	mg/kg DS	9
Cr	mg/kg DS	70
Pb	mg/kg DS	220
Ni	mg/kg DS	58

3.3.2. Experimental setup

Two kinds of experiments were conducted in this study. Removal of heavy metals and pathogens in phosphate amended biosolids using ultrasonic treatments was studied in experiment 1. Where, 2 g of KH_2PO_4 was mixed thoroughly with 200 g of biosolid in 500 mL glass beaker. This KH_2PO_4 amended biosolid kept inside the ultrasonic cleaner, which was previously filled with 1 L of tap water and it was make upto 10 cm height. This sample was exposed ultrasonically for 1 hr. Samples were removed using stainless steel spatula at different time periods started from 5, 10, 15, 30 and 60 min during the ultrasonic treatment without the instrument turned off. Care was taken to remove the samples. After 1 h ultrasonic treatment sample was kept it in laboratory at room temperature and removed the sample on 3rd day and 6th day. Control was maintained with phosphate and without ultrasonic exposure and samples removed for analysis simultaneously with ultrasonic exposed sample. The *Salmonella, Escherichia coli* and total coliform was enumerated during ultrasonic treatment at initial, 15 min, 30 min, and 60 min.

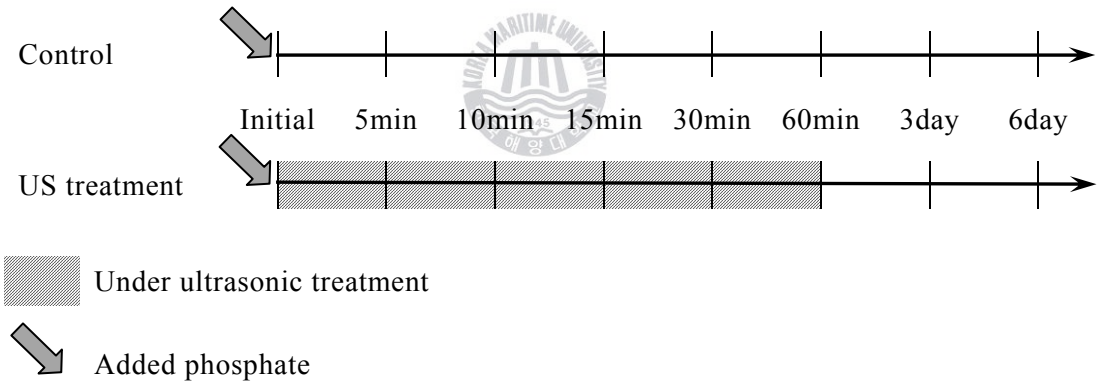


Figure 3.2. Experimental setup diagram for experiment 1.

For experiment 2, 200 g of biosolid in a 500 g glass beaker was treated ultrasonically for 1 h. During this time but after 1 h nearly 10 g of biosolids sample was removed from the beaker. After ultrasonic treatment beaker were removed from the ultrasonic and treated with 2 g of KH_2PO_4 and kept it at room temperature. Sample was removed at 1, 2 and 3rd day for the analysis of heavy metals content. In the case of control, 200 g of biosolids in a 500 g

glass beaker with the addition of phosphate and without ultrasonic exposure. Both the samples were removed at 1, 2 and 3rd day for the analysis of heavy metals content.

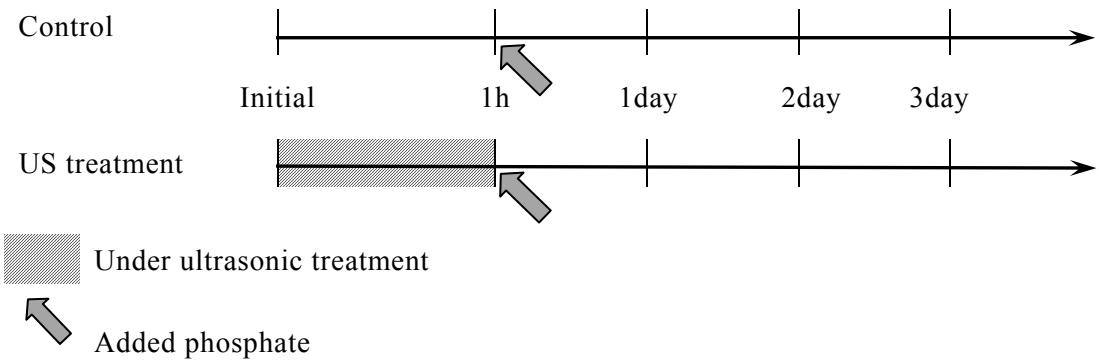


Figure 3.3. Experimental setup diagram for experiment 2.

3.3.3. Biosolids analysis

Heavy metals concentration in biosolids was analyzed according to EPA 6010 method. Pathogens enumeration method is given in the section 3.4 in this chapter.

3.4. Analytical methods

3.4.1. EPA 6010 method

For EPA 6010 method, a representative 1 g of dried biosolid was transferred into a conical flask, followed by the addition of 10 ml 1:1 HNO_3. After the addition, the sample was heated and reflux for 30 min without boiling. This step was repeated until 5 ml solution was evaporated. Sample was heated with covered beaker in a hot plate after the addition of 2 ml water and required quantity but not more than 10 ml of 30% hydrogen peroxide. After boiling, sample was added with 5 ml concentrated HCl and 10 ml water and turned to hot place for 15 min of additional refluxing without boiling. Finally the sample was diluted to 100 ml with distilled water and filtered

through Whatman paper No. 0.45-μm. The concentration of heavy metals in final solution was analysed using AAS (Mireg, 2000 model).

3.4.2. EPA 3051 method

The total metal contents of biosolids were determined by microwave-assisted acid digestion, according to EPA Method 3051 (US EPA, 1994b). A representative 1 g of dried sample was transferred into a teflon microwave digestion vessel, followed by the addition of 12 mL concentrated nitric acid. The sample vessels were put into a microwave digestion unit (Model Q45 Eviro Prep) and heated for 10 min. After digestion, the supernatant solutions were collected and passed through Whatman No. 0.45-μm filter. The filtered digestive solutions were then diluted to volume and analyzed by the AAS (Mireg, 2000 model).

3.4.3. Selective sequential extraction method

The selective sequential extraction (SSE) procedure used in this study is based on the methods of Chao (1972), Tessier et al., (1979), and Muller et al., (1986). Taking 2g dried biosolid sample, we used the following detailed sequential extraction procedure.

Fraction 1 – exchangeable

The samples were extracted at room temperature for 1h with 16mL of magnesium chloride solution (1M $MgCl_2$, pH 7.0) with continuous agitation.

Fraction 2 – bound to carbonates

The residue from fraction 1 was leached at room temperature with 16mL of 1M NaOAc adjusted to pH 5.0 with acetic acid. Continuous agitation was maintained and the time necessary for complete extraction was determined to be 3h.

Fraction 3 – bound to iron and manganese oxides

The residue from fraction 2 was extracted with 40mL of 0.04M $NH_2OH.HCl$ in 25% (v/v) HOAc. This fraction experiment was performed at 96 ± 3^0C with intermittent agitation for 6h.

Fraction 4 – bound to organic matter

To the residue from fraction 3, 20mL of 7M NaOCl (adjusted to pH 8.5 with HCl) was added, and the mixture was heated to 90±2^{0}C for 2h with occasional agitation. After centrifuge separation, a second 20mL aliquot NaOCl (adjusted to pH 8.5 with HCl) was then added and the sample was heated again to 90±2^{0}C for 2h with intermittent agitation.

Fraction 5 - residual

The residue from fraction 4 was digested with 12mL concentrated HNO_3 in a microwave digester (Model Q45 Eviro Prep) following the procedure recommended by the EPA 3051 for residual fraction.

After the prescribed time interval for each extraction, samples were centrifuged (4000rpm, 10min, HA-1000-3, Hanil Science Industrial Co..Ltd) and the supernatant filtered through a 0.45μm filter. The remaining solid sample was washed twice with distilled water before continuing with the next extraction step.

3.4.4. *Escherichia coli* and total coliform

Escherichia coli and total coliform was enumerated using commercially available Pertrifilm plates (3M Microbiology Products, USA) Petrifilm E. coli/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most E. coli (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and E. coli. About 95% of E. coli produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter).

Representative of 1-2 g biosolid (base on wet weight) was thoroughly mixed with 100 mL of pure water, and then 1 mL of water was transfer on Pertrifilm plates. After that, the plates were incubated at 35 ± 2^0C for 24 hr. At the end of incubation time, the number of *Escherichia coli* was indicated by blue colonies with gas on plates. Total coliform equal to sum of the number of *Escherichia coli* and red colonies showed on plates.

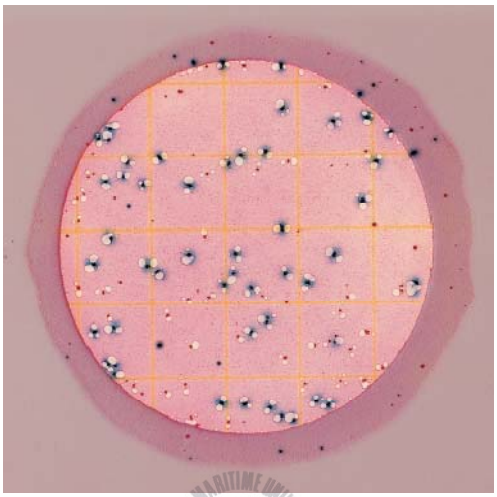


Figure 3.4. *Escherichia coli* and total coliform growth on Pertrifilm plates.

3.4.5. *Salmonella sp.*

The density of *Salmonella* was enumerated base on 5-tube MPN method, the Selenite Broth was considered as medium for *Salmonella* growth. In detail, 2.3 g of Selenite Broth was diluted to 100 mL of pure water, and then 180 µL of this solution was loaded onto every well of 96-well sterile plate using 8-channel pipette. For biosolids dilution, 1-2 g of biosolids (base on wet weight) was uniformly mixed with 100 mL of pure water. Therefore, 20 µL of this solution was transfer on first column of plates. The serial dilutions were made by transferring 20 µL from column i into 180 µL of medium in column i+1.

For analyses *Salmonella*, one sample was replicate 3 times by dividing the 96-well plates into three areas, A, B and C. Figure 3.5 displays three different areas and serial dilutions on the plate.

After transferring biosolids solution into proper well, the plates were cover by plastic film and incubated at 37^0C for 48 hr. The number of Salmonella and then was counted by light pink color appearing on plates.

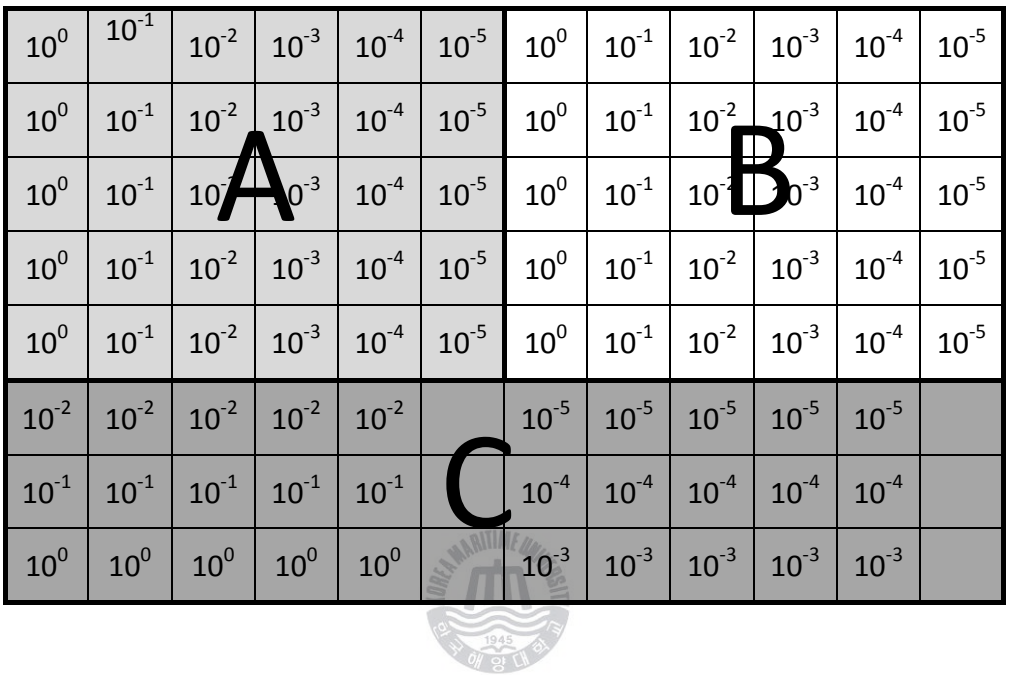


Figure 3.5. The dilution method using 96-well plates.

Chapter 4

RESULTS AND DISCUSSIONS

4.1. Remediation of oil – contaminated soil using biosolids

Table 4.1 shows the degradation of TPH in different reactors. Perusal of the results showed that in all reactors TPH concentration was decreased with increasing duration of time. In all reactors TPH concentration was rapidly decreased within a week time where 81.1 to 87.8% of TPH degraded. Further, the difference between the increasing durations in all reactors showed the percentage of degradation was decreased with increasing duration of time. The degradation percentage difference between the initial and 1st week was <20% whereas, in the case of 3rd and 4th week difference was <1 in all reactors. The degradation rate of TPH was somewhat higher in biosolids mixed with sludge and petrochemical industry complexes (R1 and R2) when compared to municipal wastewater treatment plant sludge mixture (R3) and the reactor without sludge (R4). At the end of the experiment, nearly 99% of TPH was reduced in all reactors which indicate that all functional microbial consortiums degraded the TPH and glowing well. In final quantity of TPH in R1, R2, R3 and R4 were 46.2, 50, 66.8 and 152.2 mg TPH/kg, respectively.

Table 4.1. Degradation of TPH (mg/kg) in different reactors

Time (weeks)	**Re.1** (mg/kg)	**Re.2** (mg/kg)	**Re.3** (mg/kg)	**Re.4** (mg/kg)
Initial	10,000	10,000	10,000	10,000
1	1,216	1,536	1,901	1,888.2
2	319.8	420.6	501.9	784
3	95.2	79.2	102	217.6
4	46.2	50	66.8	152.2

Fig.4.1 shows the CO_2 evaluation in all reactors according to time. There was no significant difference of CO_2 production between the reactors in all durations. Carbon dioxide production was drastically decreased within a week however, increased with increasing duration of time after 1st week up to the study period of 4 weeks. This increase may be decay and stabilization of organic matter by bacterial consortium in the biosolids. Perusal of the results indicates that the selectively cultivated bacterial consortium in all reactors degraded diesel oil as well as stabilization of biosolids is possible by the fermentation with addition of TPH as a carbon source.

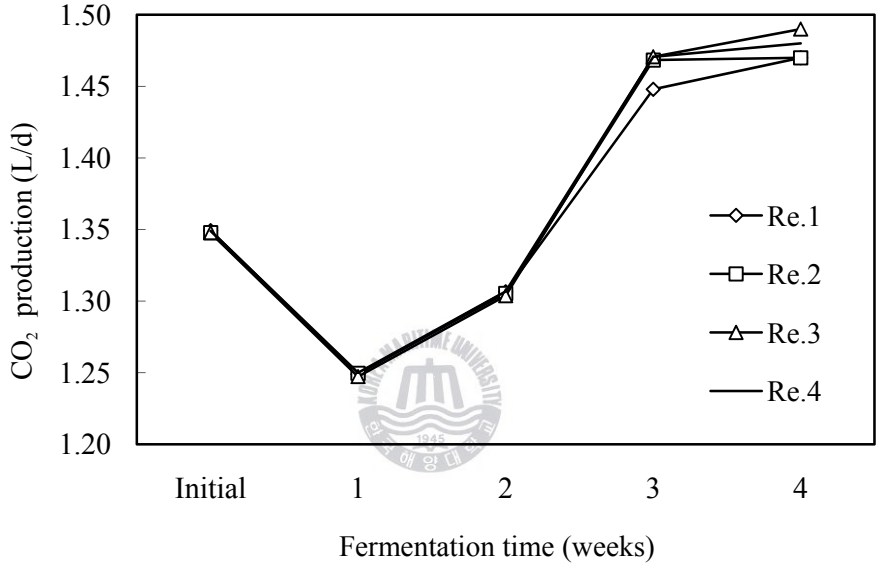


Figure 4.1. Amount of carbon dioxide evolved from the biosolids according to the fermentation time

Changes of TPH by the fermentation products in artificially oil-contaminated soil (ACS) during the effectiveness test are given in figure 4.2. The TPH concentration was decreased significantly within one week duration when compared to initial concentration, where added with 50 and 100 g of fermentation products obtained from all reactors. When compared to control in different amount of fermentation products from all reactors showed a significant difference. The volume difference was observed between 50 g and 100 g of fermentation products where, reduction of TPH concentration was slightly higher in 100 g (B) than 50 g (A). This indicates that the volume of fermentation product is a important factor for the activity of microbial consortium degrading diesel oil. After 4 weeks all fermentation products added with ACS were reached 55 ppm of TPH. Figure 4.3 shows the degradation of

TPH by fermentation products in naturally oil contaminated soil (NCS). In the case of NCS, TPH concentration was decreased with increasing duration of time. When compared to control both A and B fermentation products showed a significant difference.

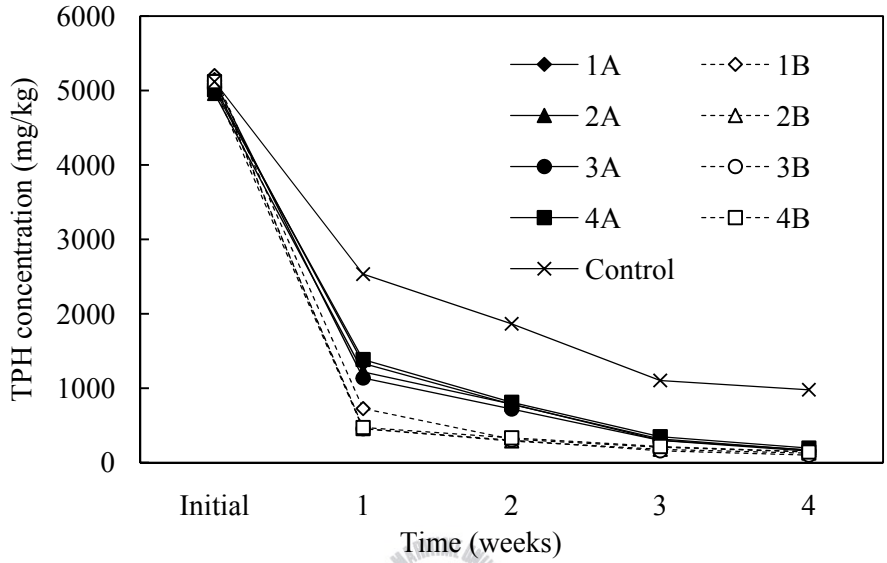


Figure 4.2. Degradation of TPH in ACS during the effectiveness test by different fermentation products.

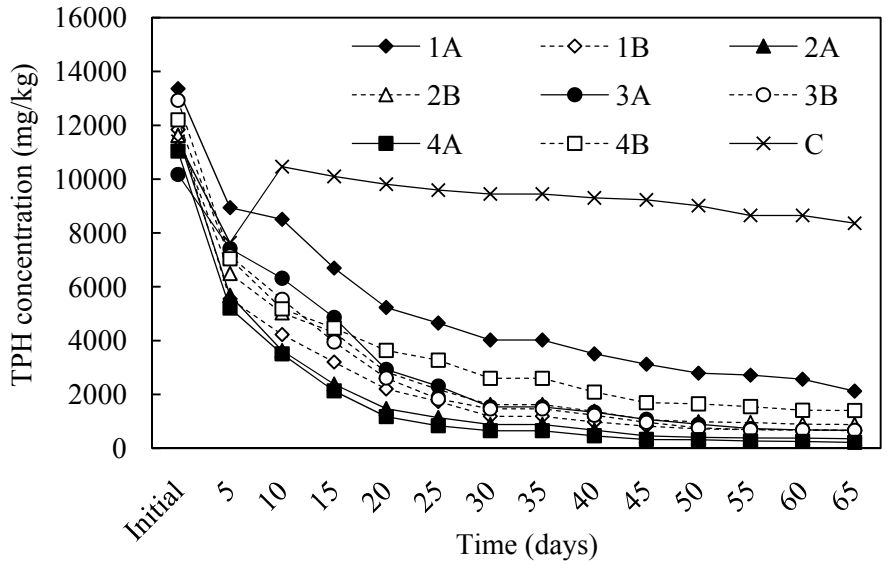


Figure 4.3. Degradation of TPH in NCS during the effectiveness test by different fermentation products.

Figure 4.4 shows the organic content in the soil mixed with fermentation products. The organic contents contained in the soil were slightly decreased according to the operation time, and the rates of the decrease were not affected by the amount of the fermentation product with the soil.

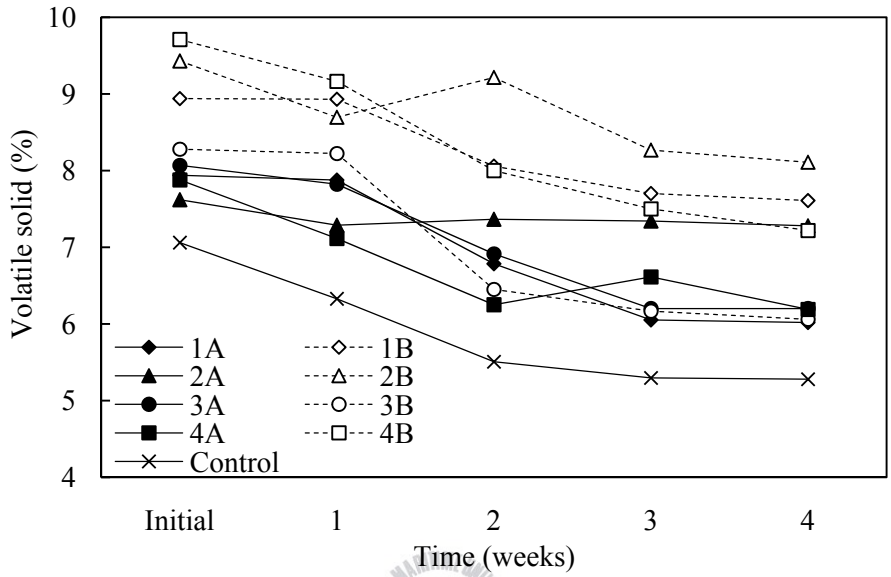


Figure 4.4. Percentage of volatile solids in ACS during effectiveness test by different reactors fermentation products.

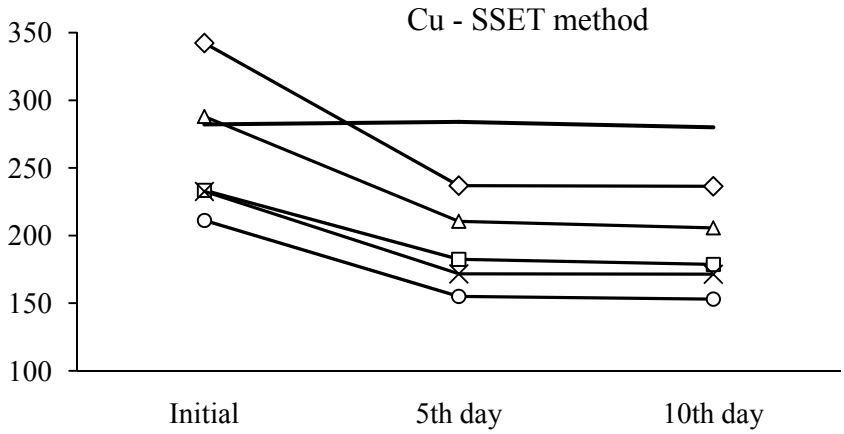
4.2. Immobilization of heavy metals in biosolid using phosphate amendments

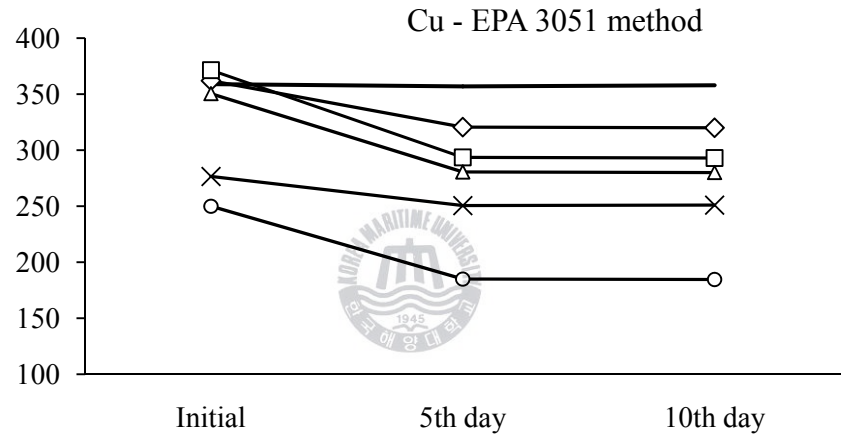
4.2.1. Effect of phosphate amendments on heavy metals immobilization.

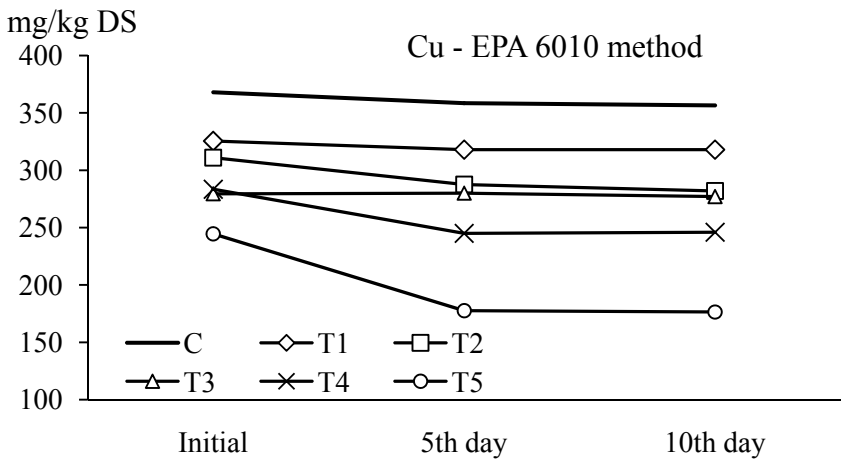
Figure 4.5 detail shows the heavy metals removal at initial, 5th day and 10th day in three different analysis methods. At initial, after 15 minutes mixing biosolid with phosphate, a significant amount of heavy metals was removed. The maximum capacity was observed in case of Pb at 46% removal in both SSE and EPA 6010 method. This result strongly demonstrated that metals can readily combine with phosphate for a short time (M. Sarioglu et al., 2005; Sona Saxena et al., 2005). After 5 days treatment, the higher immobilization capacity of heavy metals was also verified. In detail, the removal percentage ranged from 11% (Cu, T1, EPA-6010 method) up to 56.7% (Pb, T5, EPA-6010

method) with the exception of Cr and Ni in EPA 3051 method. For the next analysis at 10^{th} day, there was no difference in concentration of heavy metals compare to data at 5^{th} day. It was concluded that, the immobilization equilibrium was observed at 5^{th} day or before that time, in future work the study to identify exact equilibrium in between 5 days will be conducted.

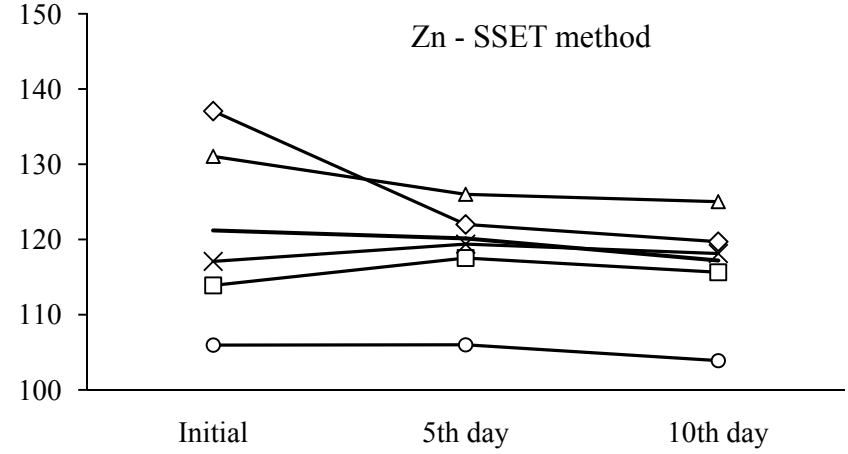
The relation between amount of phosphate added to biosolid and heavy metals removal capacity is clearly described in figure 4.6. The percent removal is calculated base on changes between heavy metals concentration in control sample (average value of initial, 5^{th} and 10^{th} day) and treatment sample at final day. For individual heavy metals, the equilibrium concentration of phosphate was detected in many cases, 2.9 g KH_2PO_4 (Cu and Cd in SSE method, Ni in EPA 6010 method), 1.45 g KH_2PO_4 (Cr and Pb in SSE method) and 5.8 g KH_2PO_4 (Pb in EPA 6010 method). The initial concentration of heavy metals in biosolid was followed the order Cu > Zn > Pb > Ni > Cr > Cd, however the removal capacity showed Cu ≈ Pb > Ni > Cr > Cd > Zn. This clue strongly proved that Zn is a heavy metal which is most difficult to immobilize using phosphate amendments. For total heavy metals, about 30% of metals was removed at the highest phosphate amount added (14.5 g KH_2PO_4). In addition, there was no observation of equilibrium phosphate concentration; those heavy metals have ability to combine with higher amount of phosphate in order to reach equilibrium.

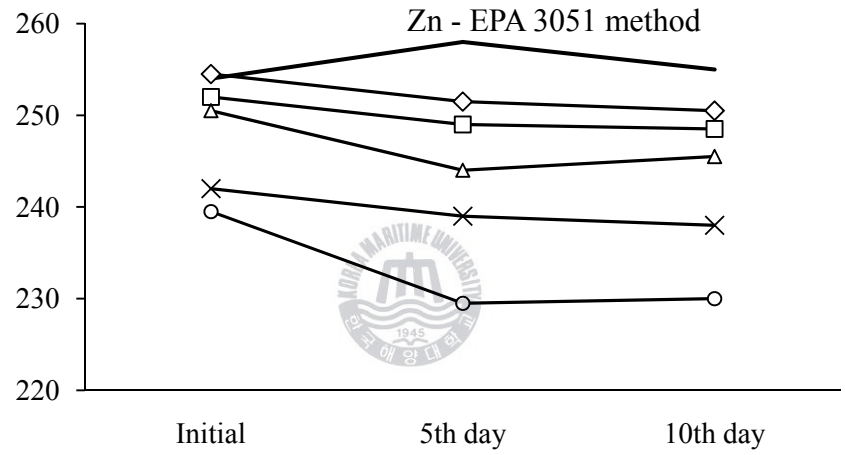

Cu - SSET method
350
300
250
200
150
100
Initial
5th day
10th day

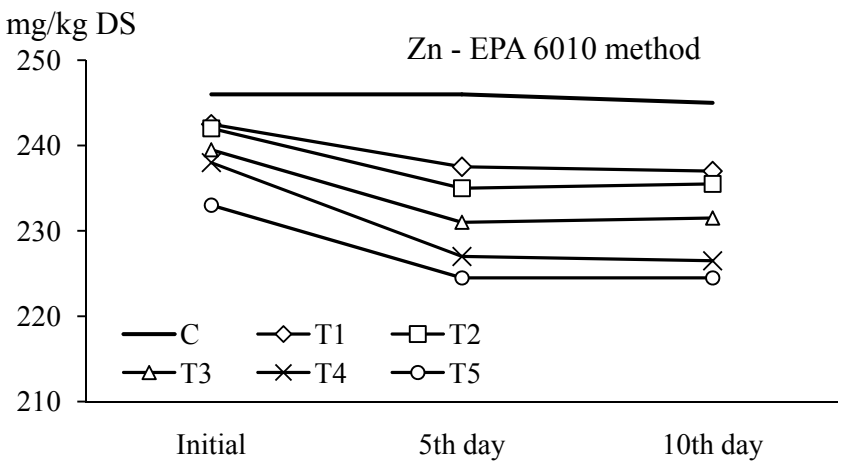

Cu - EPA 3051 method
400
350
300
250
200
150
100
Initial
5th day
10th day


mg/kg DS
Cu - EPA 6010 method
400
350
300
250
200
150
100
C
T1
T2
T3
T4
T5
Initial
5th day
10th day

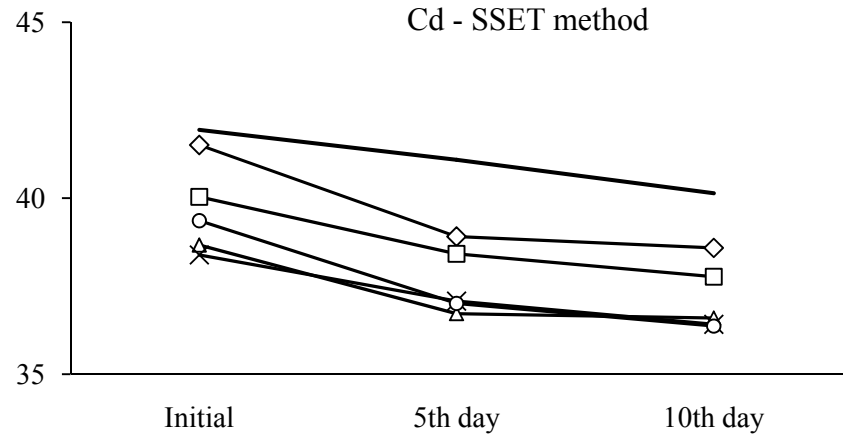
(a)

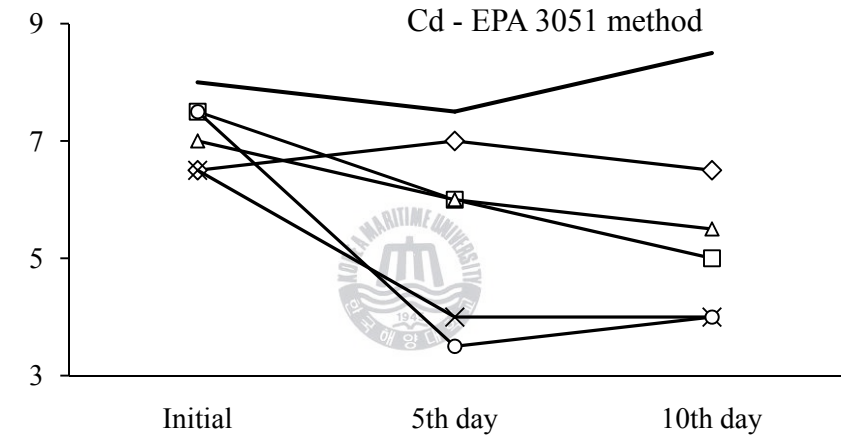

Zn - SSET method
150
140
130
120
110
100
Initial
5th day
10th day

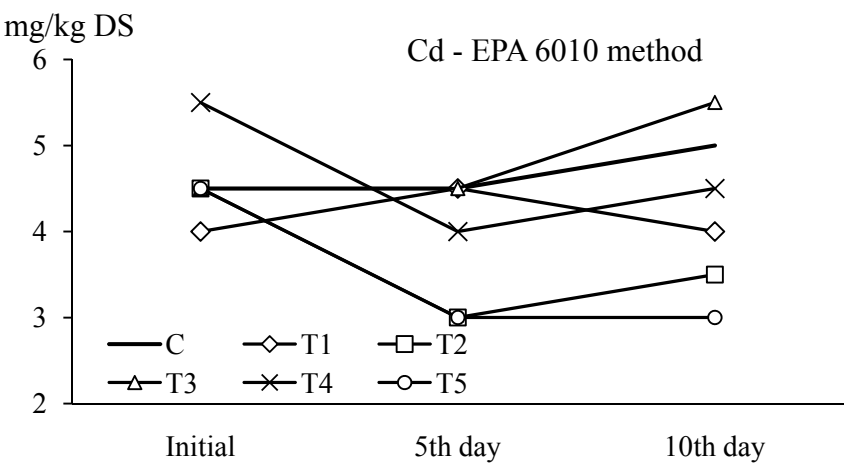

Zn - EPA 3051 method
260
250
240
230
220
Initial
5th day
10th day


mg/kg DS
Zn - EPA 6010 method
250
240
230
220
210
C
T1
T2
T3
T4
T5
Initial
5th day
10th day

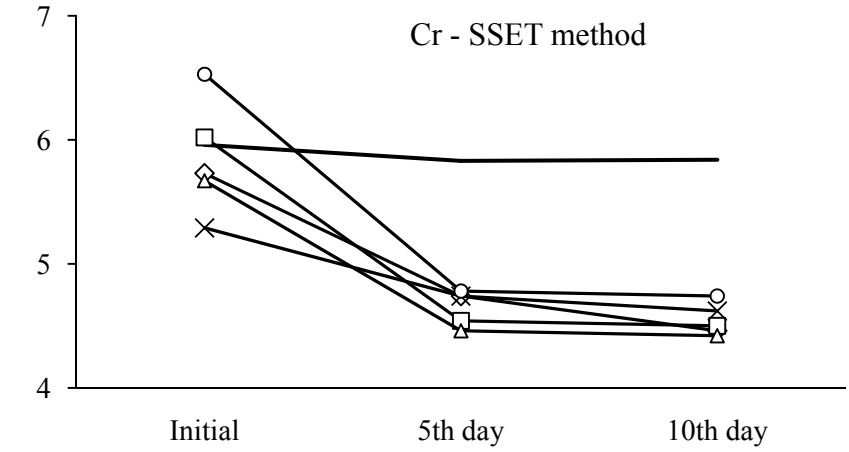
(b)

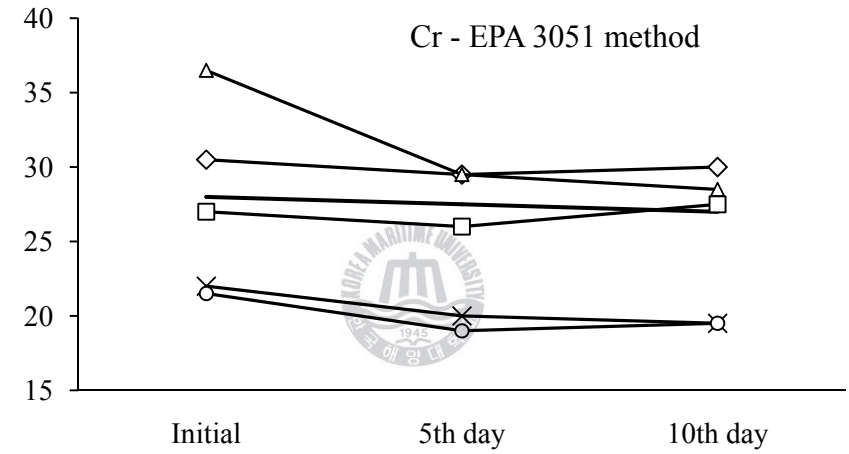

Cd - SSET method
45
40
35
Initial
5th day
10th day

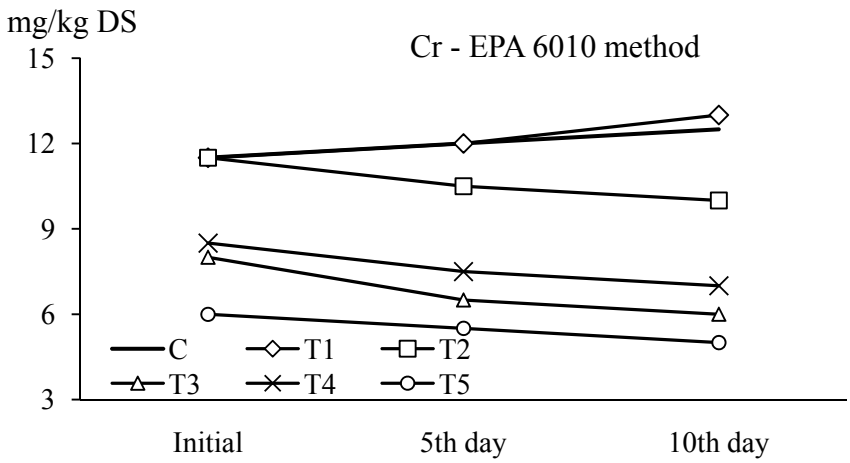

Cd - EPA 3051 method
9
7
5
3
Initial
5th day
10th day


mg/kg DS
Cd - EPA 6010 method
6
5
4
3
2
C
T1
T2
T3
T4
T5
Initial
5th day
10th day

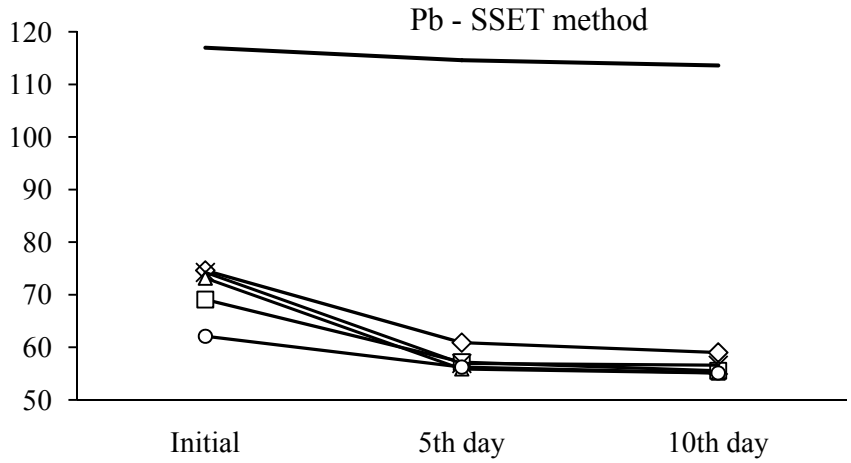
(c)

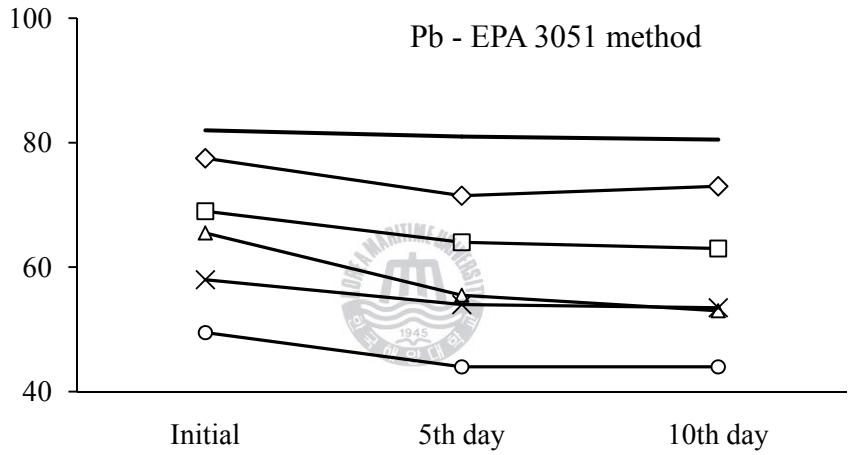

Cr - SSET method
7
6
5
4
Initial
5th day
10th day

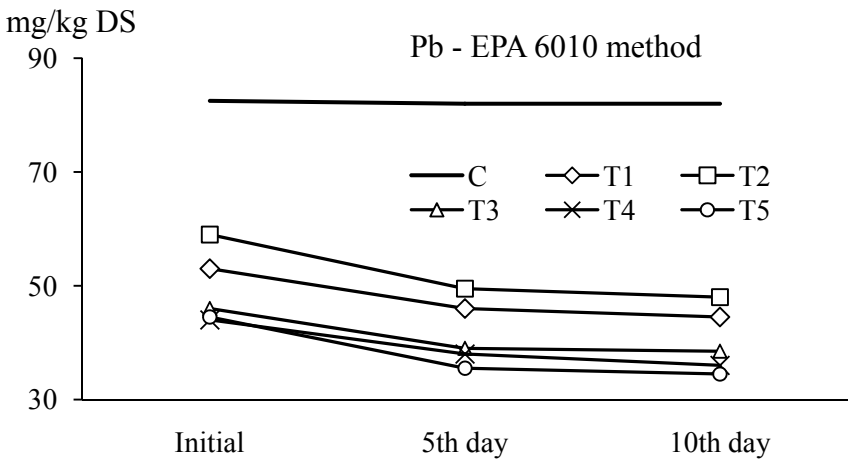

Cr - EPA 3051 method
40
35
30
25
20
15
Initial
5th day
10th day


mg/kg DS
Cr - EPA 6010 method
15
12
9
6
3
C
T1
T2
T3
T4
T5
Initial
5th day
10th day

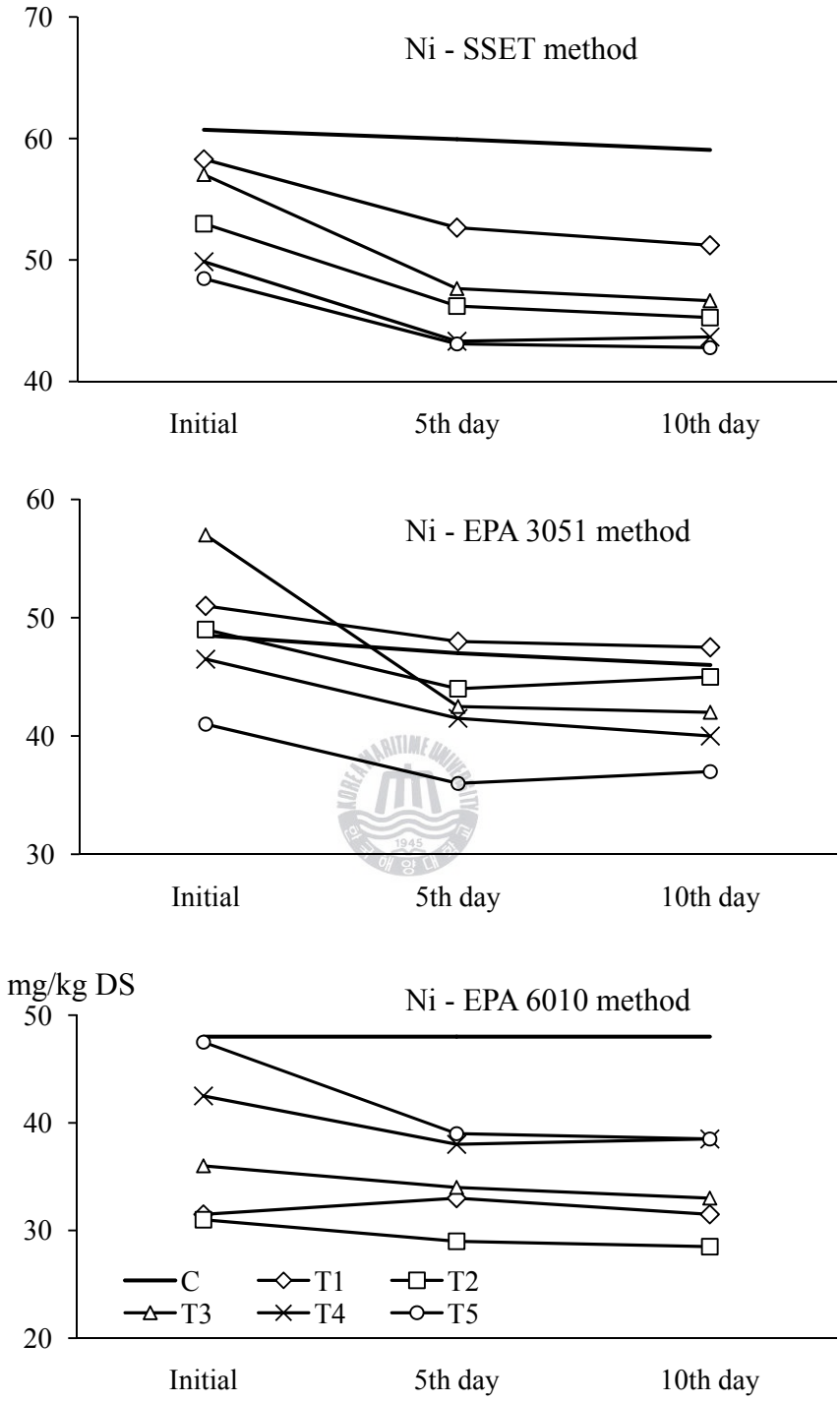
(d)


Pb - SSET method
120
110
100
90
80
70
60
50
Initial
5th day
10th day


Pb - EPA 3051 method
100
80
60
40
Initial
5th day
10th day

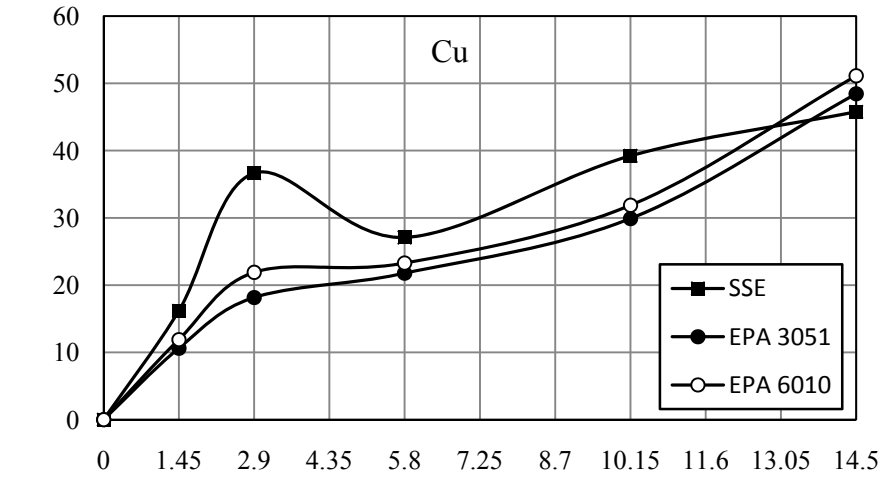

mg/kg DS
Pb - EPA 6010 method
90
70
50
30
C
T1
T2
T3
T4
T5
Initial
5th day
10th day

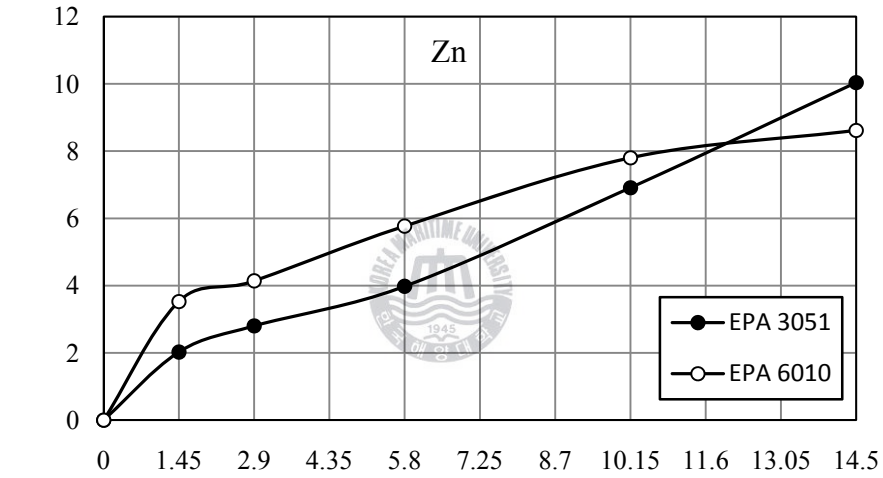
(e)

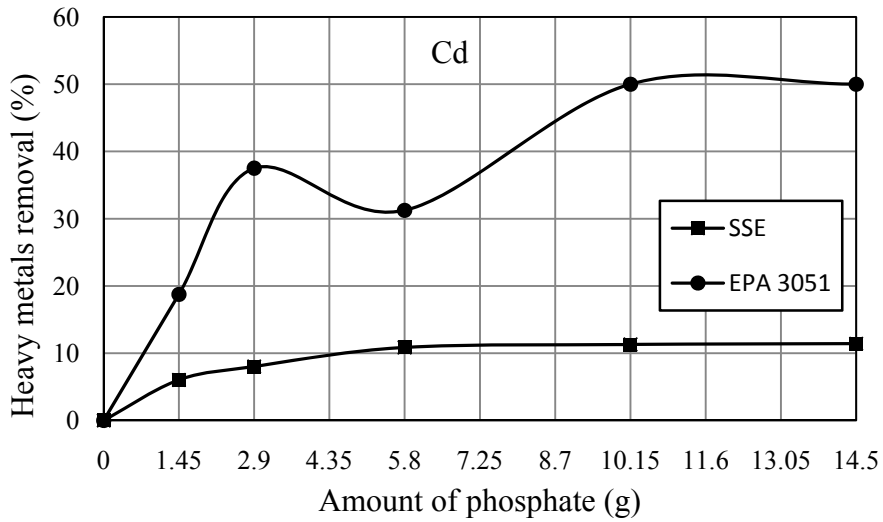


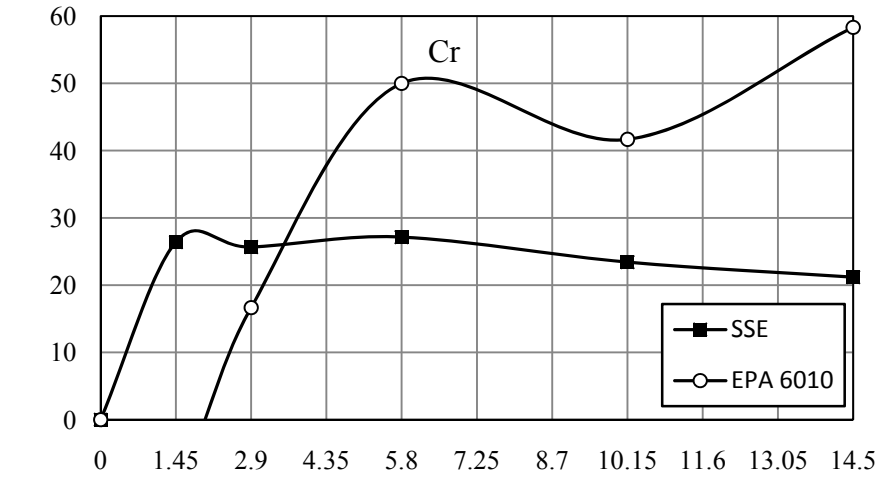
(f)

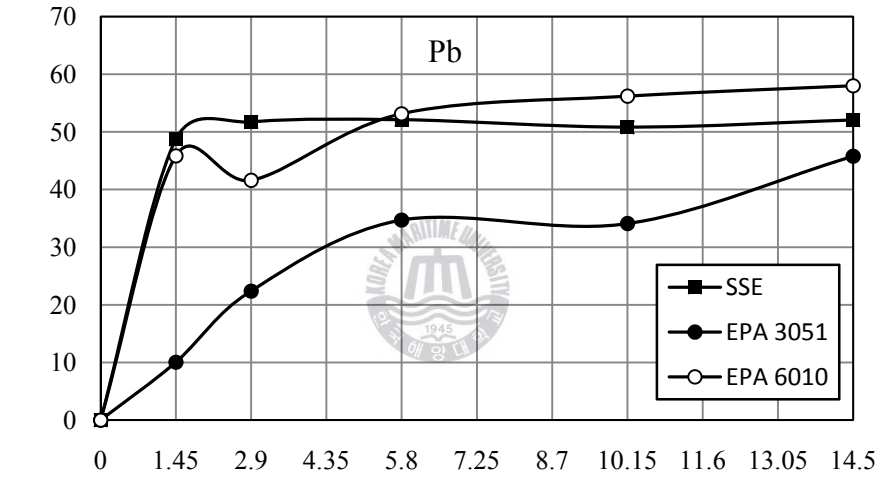
Figure 4.5. Heavy metals analyzed by EPA 3051, EPA 6010 and SSET method in biosolid amended with difference concentrations phosphate.

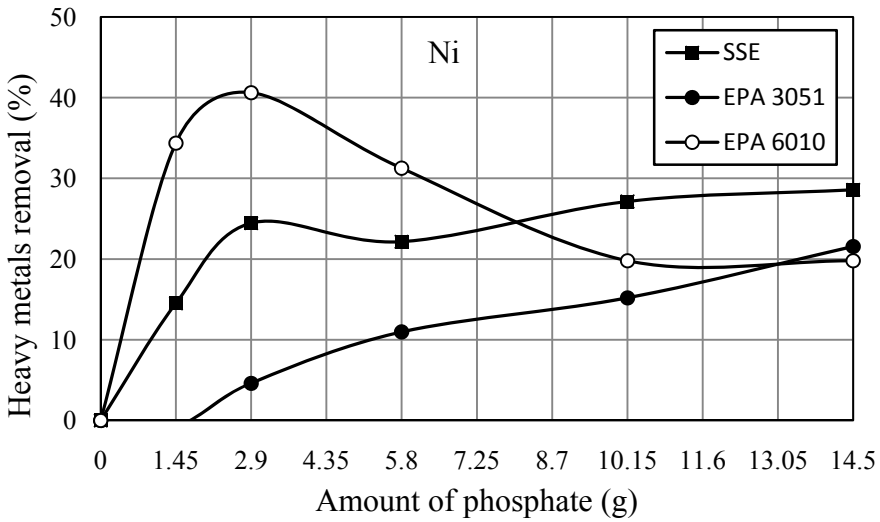

Cu
60
50
40
30
20
10
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
SSE
EPA 3051
EPA 6010


Zn
12
10
8
6
4
2
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
EPA 3051
EPA 6010


Cd
60
50
40
30
20
10
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
SSE
EPA 3051
Heavy metals removal (%)
Amount of phosphate (g)


Cr
60
50
40
30
20
10
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
SSE
EPA 6010


Pb
70
60
50
40
30
20
10
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
SSE
EPA 3051
EPA 6010


Ni
50
40
30
20
10
0
0 1.45 2.9 4.35 5.8 7.25 8.7 10.15 11.6 13.05 14.5
SSE
EPA 3051
EPA 6010
Heavy metals removal (%)
Amount of phosphate (g)

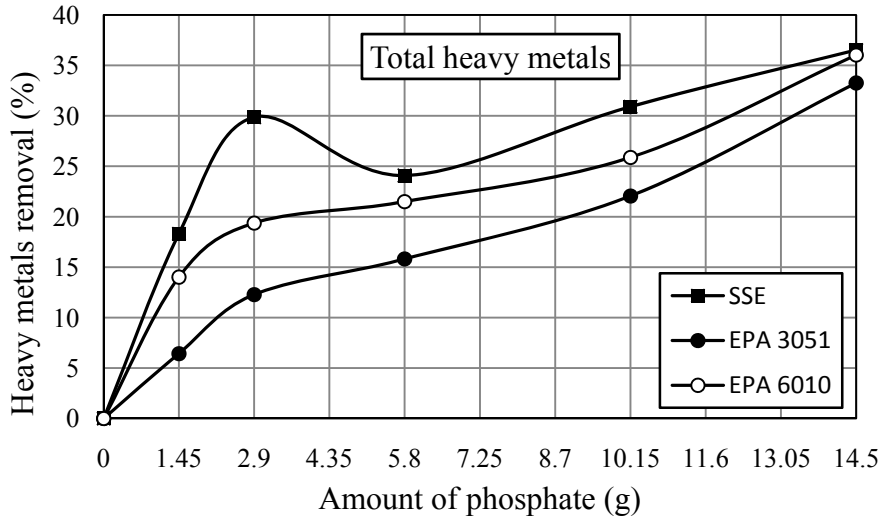


Figure 4.6. Effect of amount of phosphate on heavy metals removal capacity in biosolid according to EPA 6010, EPA 3051 and SSE methods.

4.2.2. Comparison of different analytical methods – Method limitation.

Difference between EPA 3051, EPA 6010 and SSE methods of total heavy metal extracted is presented in figure 4.7. This figure is plotted base on average of 3 data of heavy metals concentration in control sample collecting from initial, 5th day and 10th day data.

There was not much different between two EPA methods in case of Cu, Zn, Pb and Ni, however EPA 3051 method showed 1.7 and 2.3 times higher than EPA 6010 method in Cd and Cr, respectively. This difference may cause by interferences from EPA 6010 analytical procedure in term of analyzing low concentration of heavy metals. By next experiment, the comparison between two EPA methods will be approached by assessing higher concentration of Cd, Cr and lower concentration of other metals (Cu, Zn, Pb, and Ni).

In comparison between SSE and EPA methods, the differences could be divided into two groups, i) the extractable value of SSE was higher than EPA method in Cd, Pb and Ni; ii) the extractable value of SSE was lower than EPA method in Cu, Zn and Cr. Various authors reported the limitation of sequential

extraction method. For sample pre-treatment, the distribution of metals varies according to the drying method and the treatment time (H. Farrah et al., 1993). Bordas and Bourg (1998) studied the influence of freeze drying, air drying and oven drying (1050C) on superficial river sediment. None of the drying methods completely preserved the distribution of Cu, Pb, Zn and Cd in the various geochemical fractions, particularly when the metal content was low. The main modification observed was transfer from the exchangeable and the carbonated fractions to the reducible fraction. Air drying induced more modifications than freeze drying, especially in the exchangeable fraction. It also accelerated the crystallization of amorphous Fe oxides and the oxidation of Fe, Mn and S. However, the authors (Bordas and Bourg., 1998) concluded that these two drying methods caused less damage than oven drying in sample pre-treatment. A number of authors have established that the dissolution of iron oxides was incomplete during the reductive step of Tessier's scheme, leading to an overestimation of the residual fraction (C.Kheboian et al., 1987, P.P. Coetzee., 1993, M.J. La Force et al., 2000). Similarly, Martin et al (1996) considered that the recommended contact time is sometimes not sufficient for adsorbed species and that increased metal extraction during the following step is caused by kinetic effects.

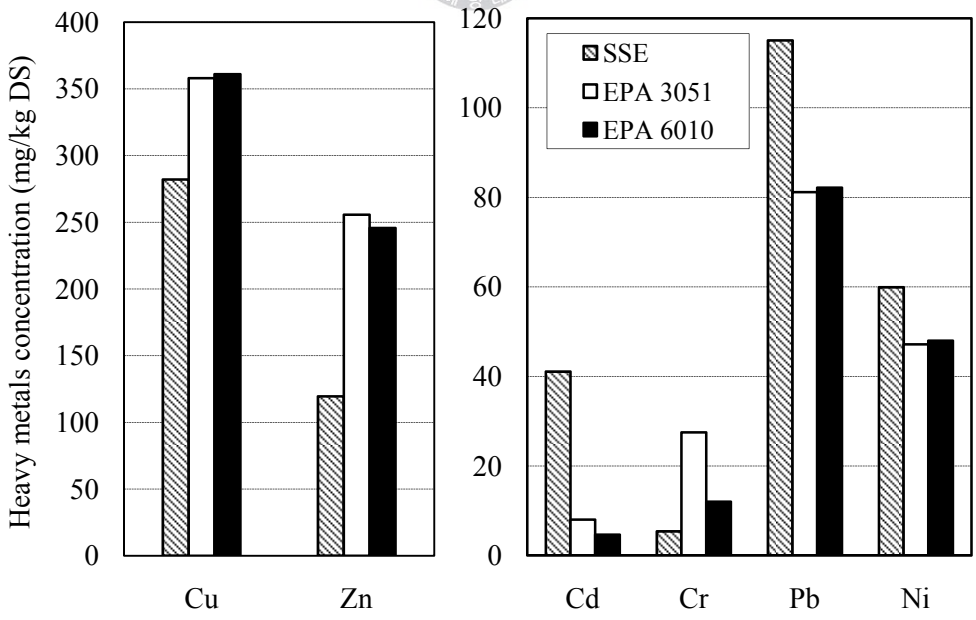


Figure 4.7. The difference of heavy metals concentration in control sample analyzed by EPA 6010, EPA 3051 and SSE methods.

4.2.3. Fractionation studies

Different fractionation of heavy metals recorded by using selective sequential extraction methods (SSE) for a period of 10 days in different phosphate amended biosolid is presented in figure 4.8. From selective sequential extraction results clearly showed that the extractability of metals depends on fractions. In all phosphate amendments, significant amount of heavy metals associated with F1 (for Pb and Ni), F3 (for Cd), F4 (for Cu, Zn and Ni) and F5 (for Cu, Zn, Pb and Ni) fractions. Contradictory, Pb in F2 and F3 fractions and Cr in F4 and F5 fractions were not extracted (below detection limit) in all phosphate amendments, where the quantity of extracted metals were similar to that of control.

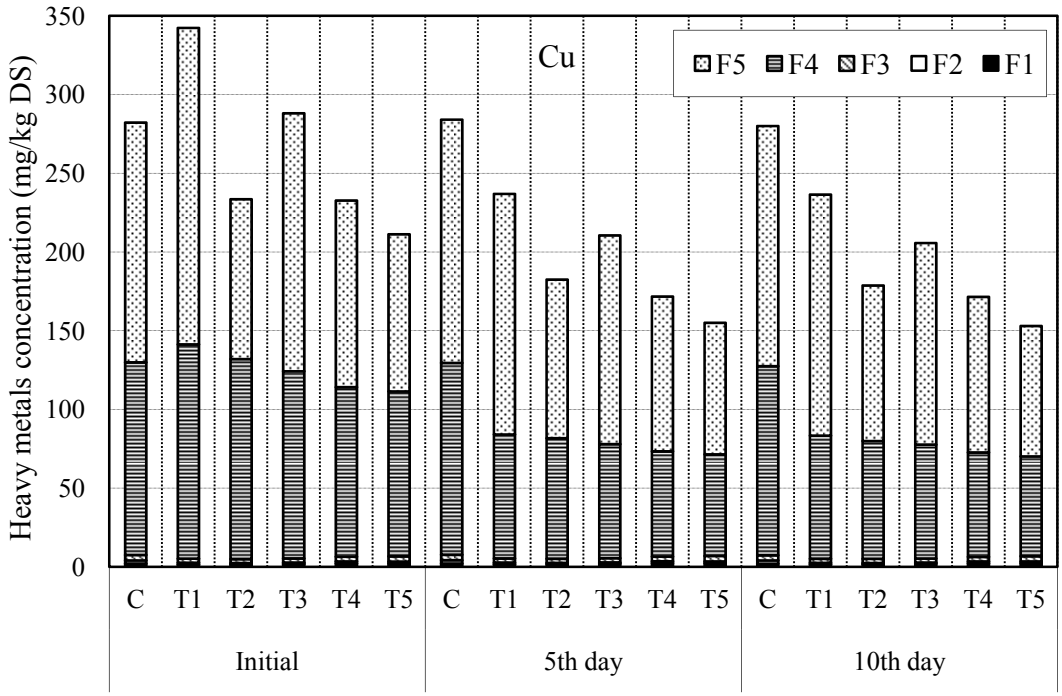
Copper in all phosphate treatments were primarily associated with F4 and F5 fractions. Low addition of phosphate (T1) to the biosolid significantly increased (22%) Cu level in the F4 and F5 fractions and significantly reduced higher phosphate treatments (nearly 50%) when compared to the control. Additionally, the fractions distributions of Cu in all treatments of biosolids during the 10^{th} day are similar to those at 5^{th} day. Compared to all methods, approximately 25% of the distributed level of total Cu was significantly reduced at higher phosphate treatments (T4 and T5). For the other three phosphate amendments, T1, T2 and T3, there were no statistically significant decreases or increase between the controls. Significant reduction was observed in the F5 fraction (mg/kg) of Zn in biosolid with higher amendment of phosphate (T5). However, the lower treatments of phosphate in F5 fraction (T1 to T4) not been made any specific decreasing of increasing trend. Fe-Mn oxide fraction (F3) of Cd in the biosolid with different application of phosphate was not problematic at all incubated times.

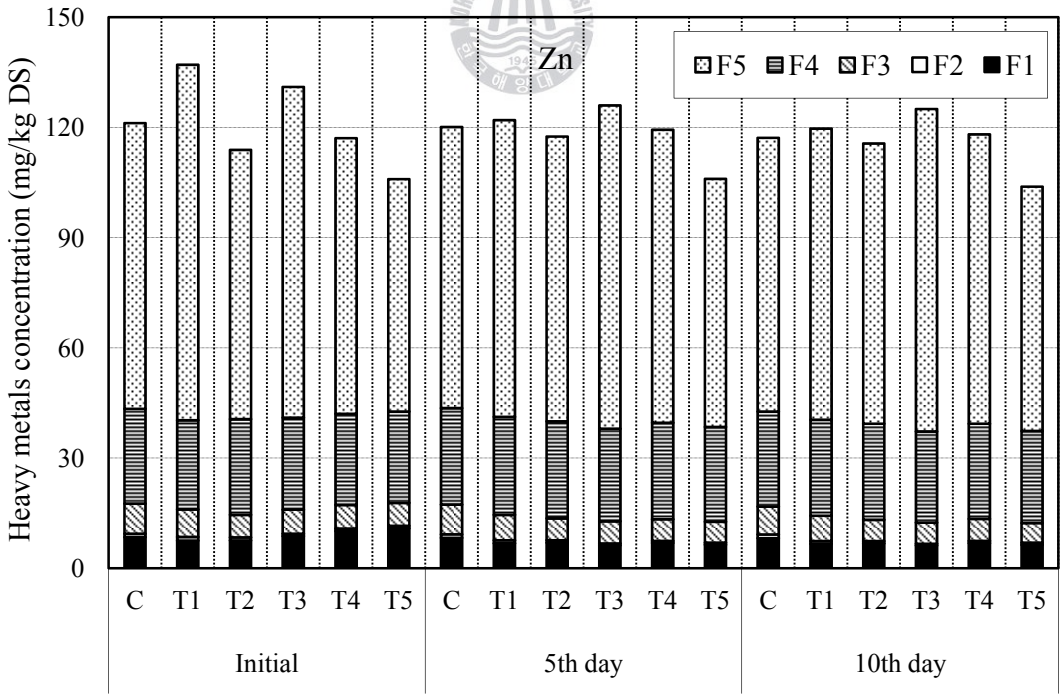
Various fractions of sequential extraction extracted from 67.5 to 100% of the metals, however, the extraction were differed in different fractions. For example, Cd in fraction F3 extracted 28.3 mg/Kg of Cd, which accounted 67.5% to the total Cd (41. Mg/Kg) obtained from all fractions. Both F4 and F5 fractions jointly extracted 97.3% of Cu and 87.4% of Zn when compared to total fraction. However, in the case of Ni, totally 82.8% of metal extracted by F1, F4

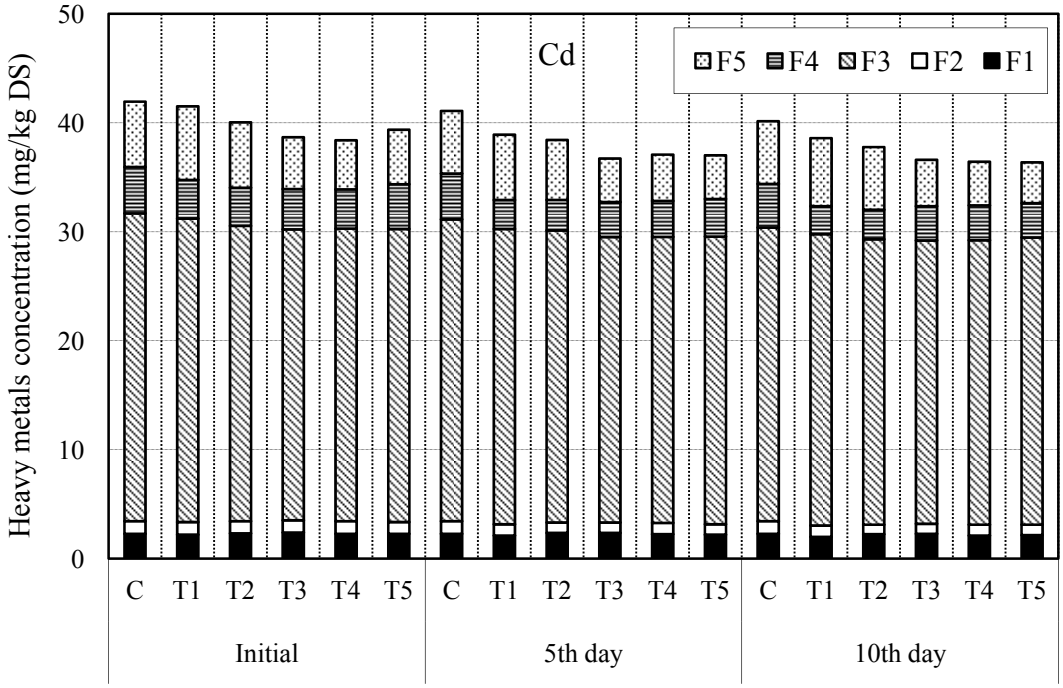
and F5 fractions and Cd showed 67.5% of metal extracted by F3 fraction. Although below detectable level of Cr in fractions F4 and F5, 100% of the Cr was extracted by other fractions (F1, F2 and F3). In the case of Pb, 90.6% was extracted by F1 and F5 fractions, even as F2 and F3 fractions values blow detectable level.

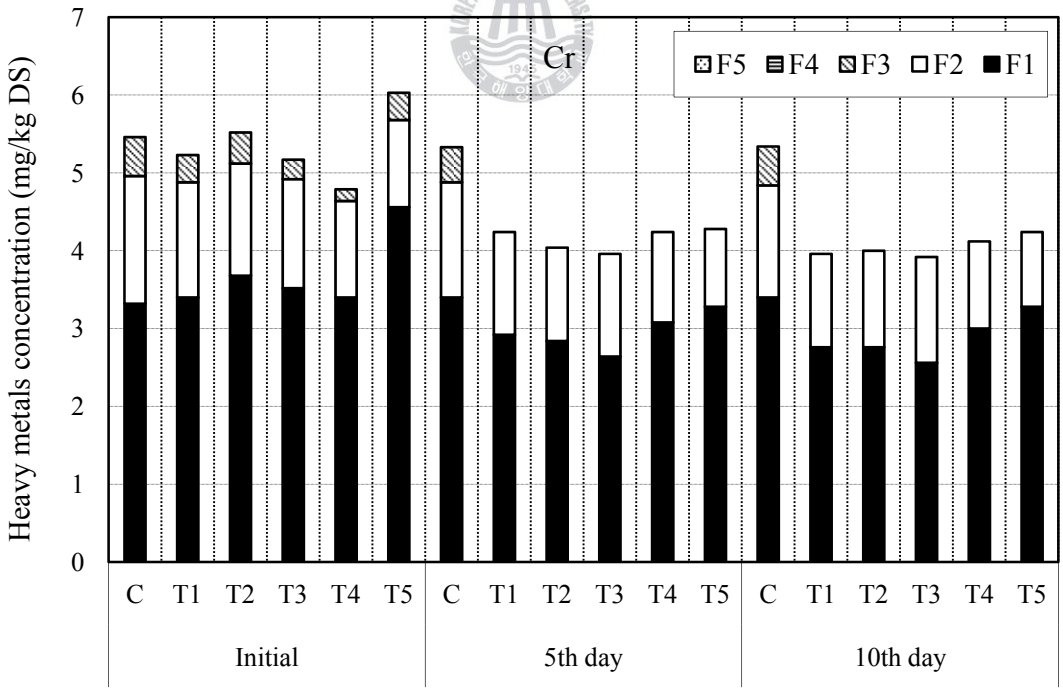
Fractions of F4 and F5 in all experiments at all incubated times in sequential extractions of Cr were recorded below detection level as like control. While, 5 and 10 day recorded value of Cr in F3 fractions at all P amendments showed the values were below detection limit, where control extracted 0.45 and 0.5 mg/kg at 5^{th} and 10^{th} days, respectively.

Results of the sequential extraction showed the extractable level of Pb mainly associated with F1 and F5 fractions in all phosphate amendments at all durations however, the level below detectable at carbonate (F2) and Fe-Mn oxide (F3) fractions. Based on the phosphate amount there was no difference of Pb extractions in biosolid between the phosphates concentrations however, compared to control all phosphate concentrations reduced nearly 50% of Pb at all durations. Distributed level of Ni mainly associated with F1, F4 and F5 fractions in all P amended biosolids at all durations. The results of the residual fraction (F5) of sequential extraction showed nearly 60% of metal reduced at higher treatment concentration of P in all durations.


Cu
F5 F4 F3 F2 F1
Heavy metals concentration (mg/kg DS)
350
300
250
200
150
100
50
0
C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5
Initial
5th day
10th day


Zn
F5 F4 F3 F2 F1
Heavy metals concentration (mg/kg DS)
150
120
90
60
30
0
C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5
Initial
5th day
10th day


Cd
F5 F4 F3 F2 F1
Heavy metals concentration (mg/kg DS)
50
40
30
20
10
0
C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5
Initial
5th day
10th day


Cr
F5 F4 F3 F2 F1
Heavy metals concentration (mg/kg DS)
7
6
5
4
3
2
1
0
C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5 C T1 T2 T3 T4 T5
Initial
5th day
10th day

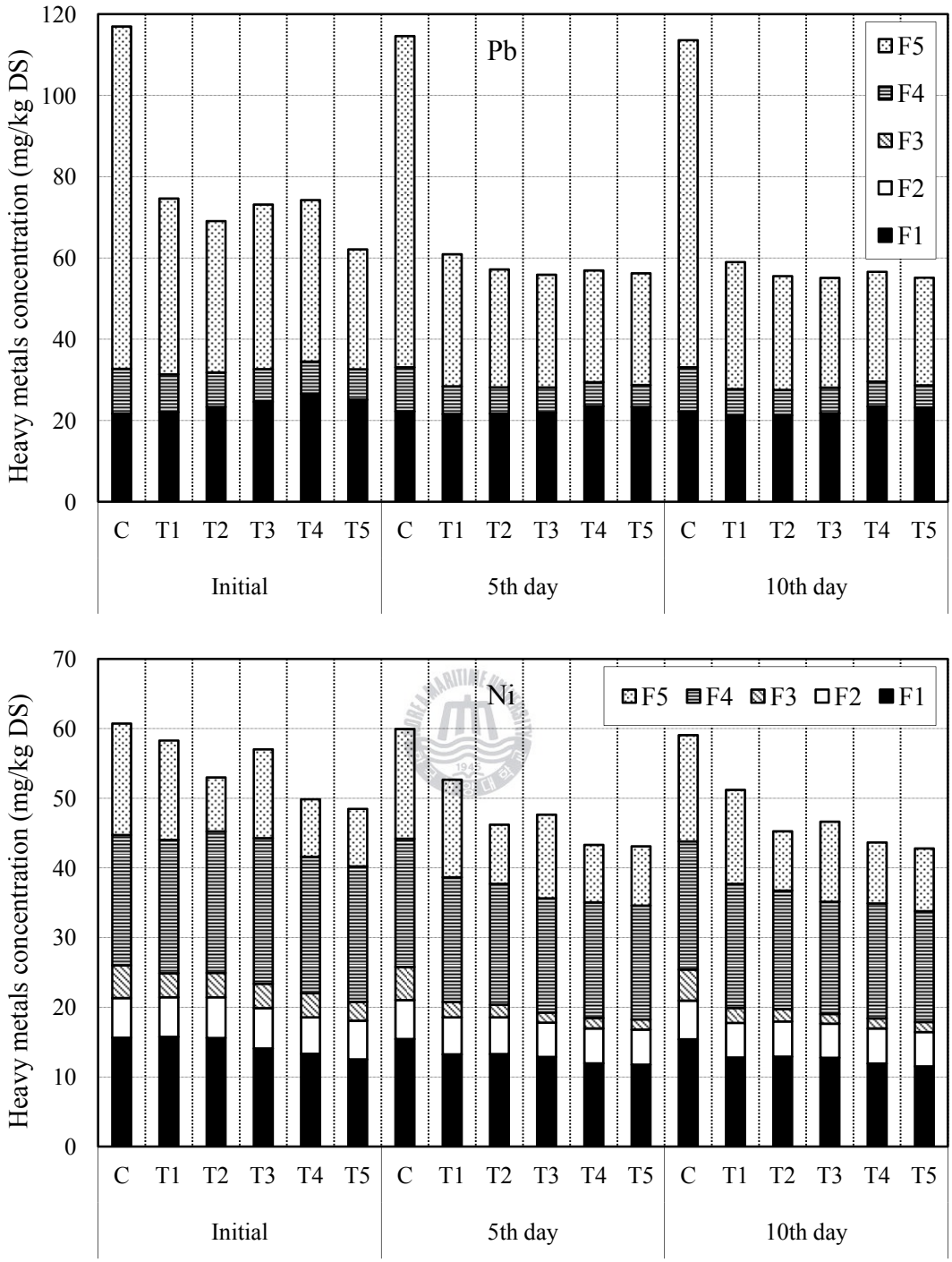


Figure 4.8. Fractionation of heavy metals in biosolid amended with difference concentrations phosphate over a period of 10 days. Metals analyzed by selective sequential extraction method.

4.3. Reducing toxicity of biosolids using ultrasonic

4.3.1. Effect of ultrasonic treatment on heavy metals immobilization

In experiment 1, after 5 minutes adding phosphate and treatment using ultrasonic, both of control and US treatment sample reduced heavy metals concentration from 3.57% (Cr, control sample) up to 50% (Pb, control sample). For US treatment sample, the percentage removal was 6.20, 9.52, 11.11, 32.69, 34.28, and 41.28% in Zn, Cu, Cd, Ni, Cr and Pb at 5 minutes, respectively. However, heavy metals tended to release the metal-phosphate combination after 15 minutes treatment due to electromagnetic energy (Figure 4.7). Various authors reported that ultrasonic is an effective methods for extraction of Pb (K.Ashley, 1995), Cr (B.R.James et al, 1995), and Cu, Zn, Cd, Ni (S.L.Harper et al, 1983) in environmental sample. The heavy metals release capacity at 30 and 60 minutes is detail described in following equation.

$$Re_i = \frac{(C_i - C_{15})}{C_{15}} \times 100$$

Where:

Re_i: heavy metals release capacity at time i, i= 15 or 30 minute (%)

C_i: heavy metals concentration in ultrasonic treatment sample at time i (mg/kg DS).

C_{15}: heavy metals concentration in ultrasonic treatment sample at 15 minute (mg/kg DS)

Table 4.2. Heavy metals release after 15 minute treatment using ultrasonic.

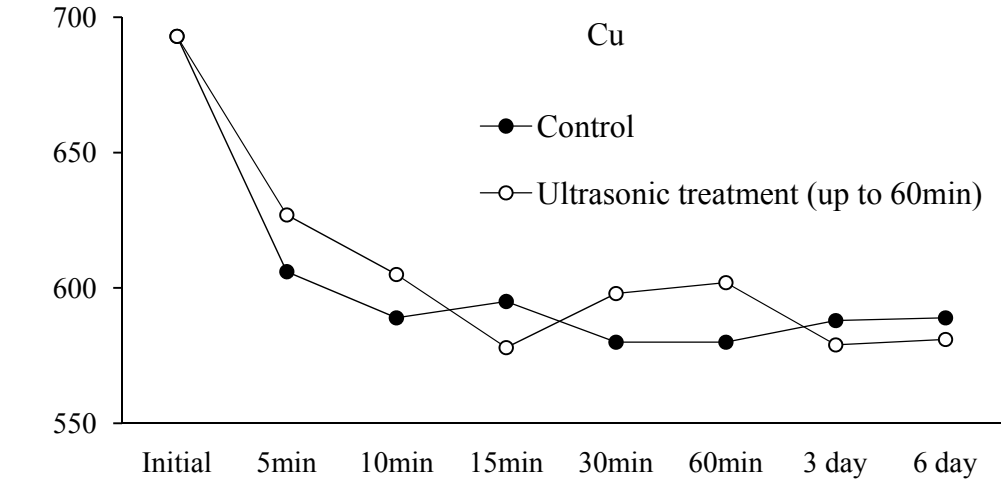
Heavy metals	Re_{30} (%)	Re_{60} (%)
Cu	3.46	4.15
Zn	0.37	0.37
Cd	0.00	0.00
Cr	6.09	10.97
Pb	4.76	11.90
Ni	10.81	24.32

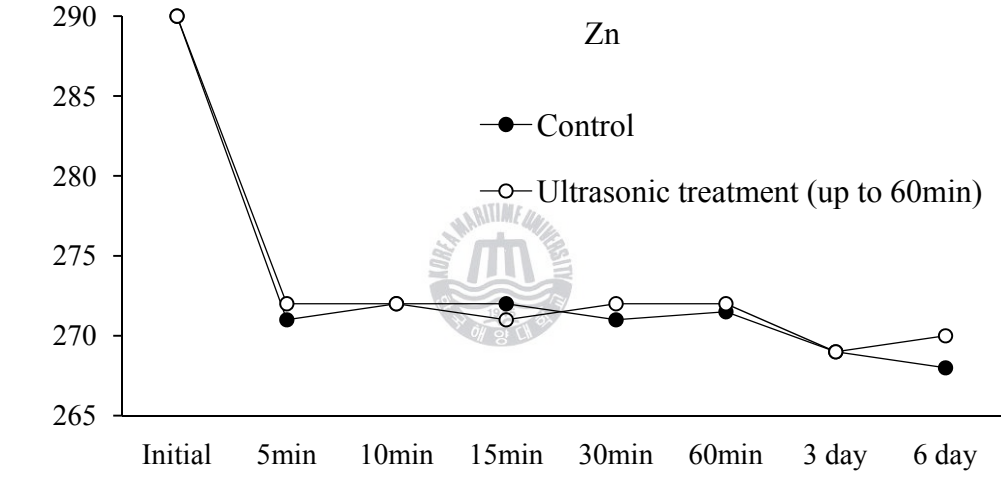
There was no observation of release of Cd from metal-phosphate compound after 15 minutes ultrasonic treatment. The maximum release capacity was observed in case of Ni with 10.81 and 24.32% after 30 and 60 minutes, respectively. After 3 and 6 days, the heavy metals capacity reached 50% in case of Pb-ultrasonic treatment sample. In descending, the observation of metals removal was Pb, Cr, Ni, Cu, Cd, Zn. The detail percentage removal of each metal at 3 and 6 day is given as the table 4.5.

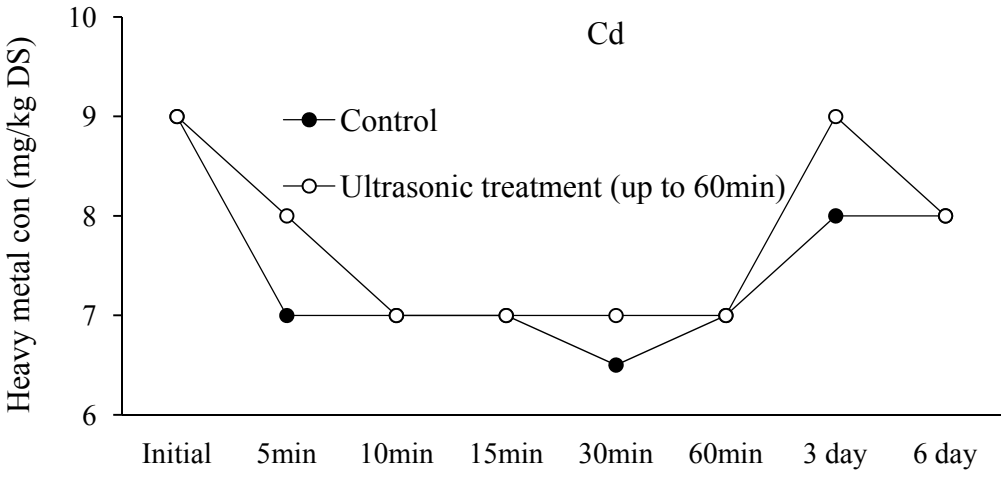
Table 4.3. Heavy metals removal at 3 and 6 day in experiment 1.

Heavy metals	**Heavy metals removal at 3 day (%)**		**Heavy metals removal at 6 day (%)**	
	Control	US treatment	Control	US treatment
Cu	15.15	16.45	15.00	16.16
Zn	7.24	7.24	7.58	6.89
Cd	11.11	0.00	11.11	11.11
Cr	41.43	30.00	48.57	40.00
Pb	47.70	50	44.03	41.74
Ni	30.77	13.46	32.69	23.07

The table above shows that, with the exception for Cu and Cd, the control sample had higher effective in heavy metals removal at 6 day compare to ultrasonic treatment sample. The most different removal percentage was observed in case of Ni, 32.69% removed in control sample, meanwhile only 23.07% removed in ultrasonic treatment. These results strongly prove that the treatment process which adding phosphate followed by ultrasonic treatment had a negative effect in remove heavy metals.


Cu
700
650
600
550
Control
Ultrasonic treatment (up to 60min)
Initial
5min
10min
15min
30min
60min
3 day
6 day


Zn
290
285
280
275
270
265
Control
Ultrasonic treatment (up to 60min)
Initial
5min
10min
15min
30min
60min
3 day
6 day


Cd
Heavy metal con (mg/kg DS)
10
9
8
7
6
Control
Ultrasonic treatment (up to 60min)
Initial
5min
10min
15min
30min
60min
3 day
6 day

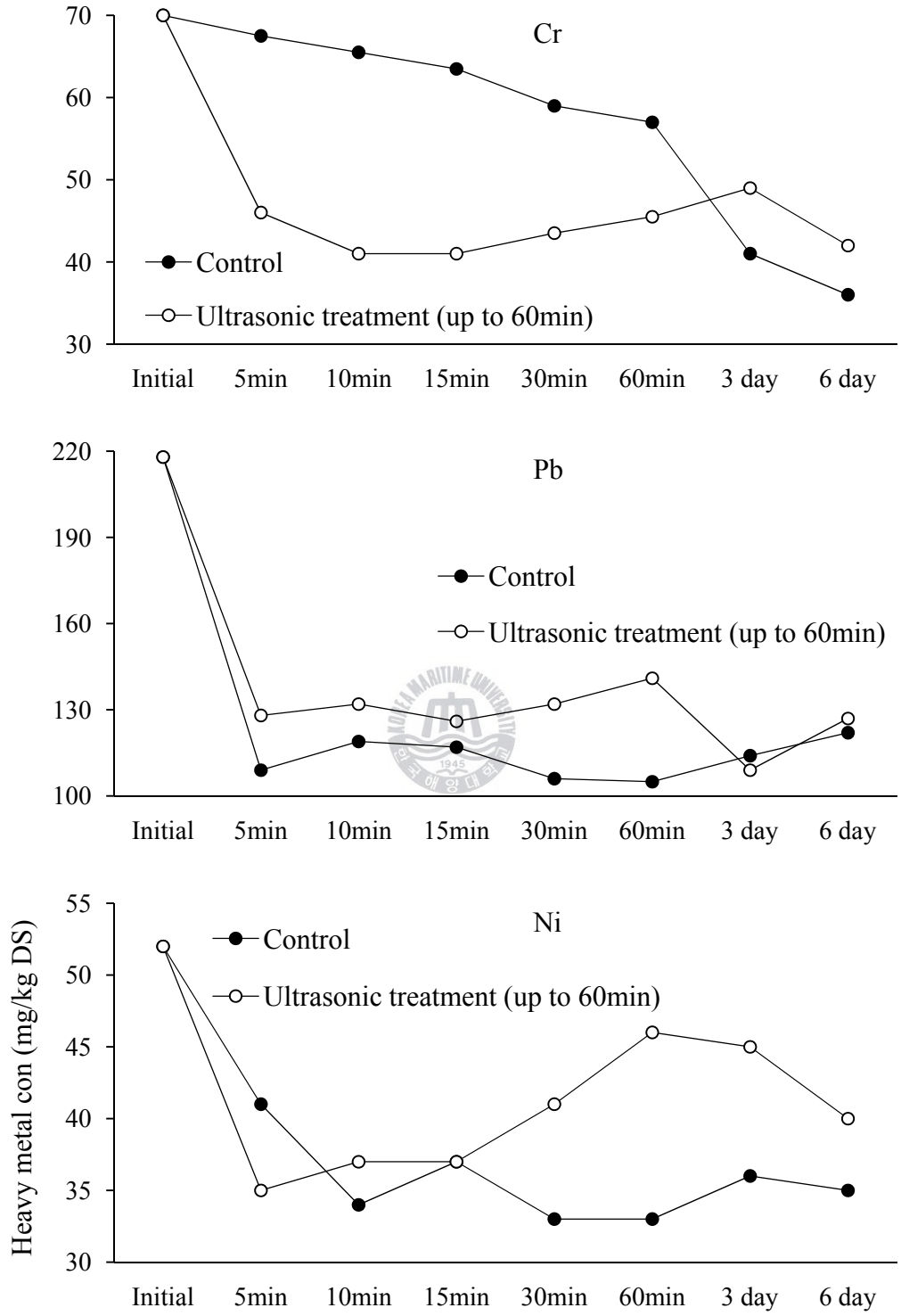


Figure 4.9. Effects of ultrasonic treatment and phosphate added on heavy metal concentration in experiment 1.

In experiment 2, there was a little variation of heavy metals concentration at initial and after 1hr treatment with ultrasonic cleaner (Figure 4.8). In detail, the increasing of metals concentration was observed in Cu, Pb, Zn at 0.89, 0.91, 3.58%; and Cr, Ni, Cd at 3.07, 3.44, 12.5% for decreasing of metals concentration at initial and after 1hr treatment, respectively. With the exceptional of Cr, heavy metals removal capacity in ultrasonic treatment sample was higher than those in control samples at 3 day. The detail percentage removal in control and ultrasonic treatment sample is listed in table 4.6.

Table 4.4. Heavy metals removal in control and ultrasonic treatment at 3 day in experiment 2.

Heavy metals	**Control (%)**	**Ultrasonic treatment (%)**
Cu	14.02	16.62
Zn	3.23	7.53
Cd	0.00	12.50
Cr	43.07	38.46
Pb	50.00	54.09
Ni	39.65	41.38

To compare the different effectiveness between two treatment approaches on heavy metals immobilization, figure 4.10 was plotted. Based on the data of heavy metals removal capacity at 3 day, the graph clearly describes the difference of experiment 1 and 2. In detail, there was a similarity in case of Cu (16.45% and 16.62%) and Zn (7.24% and 7.53%). In contrast, the significantly higher effective of experiment 2 method was detected in Cd (0 and 12.5%) and Ni (13.46% and 41.38%). Furthermore, the data Cr and Pb also demonstrates the negative effect of method used for experiment 2.

In conclusion, the best approach for heavy metals immobilization in biosolids is pretreated with ultrasonic and then adding phosphate.

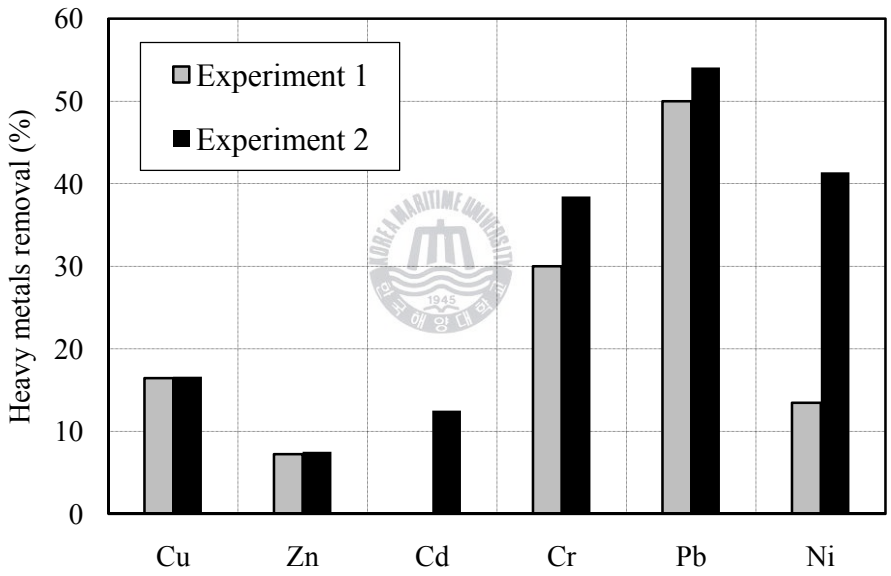
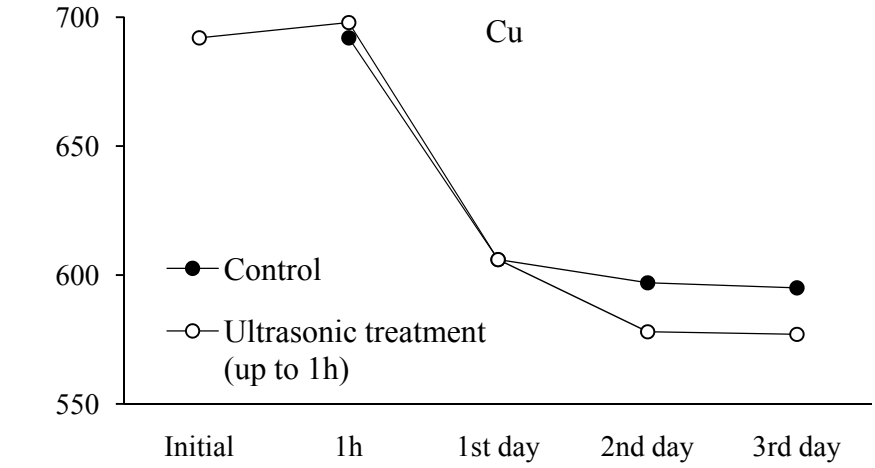
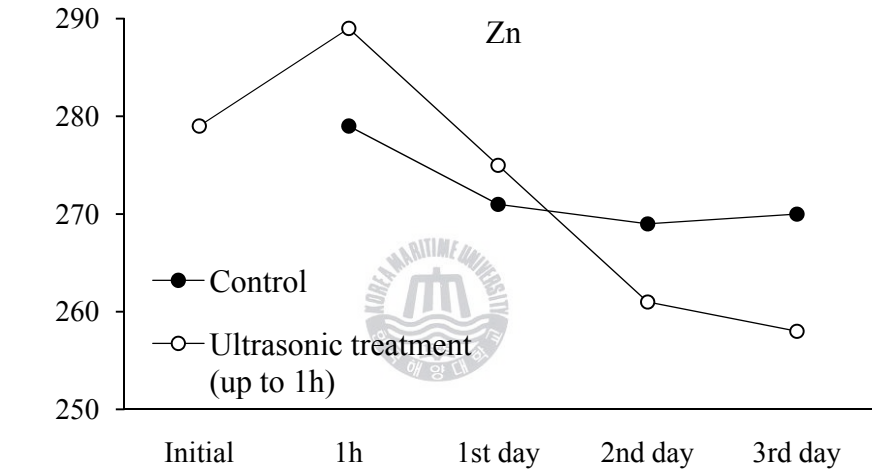
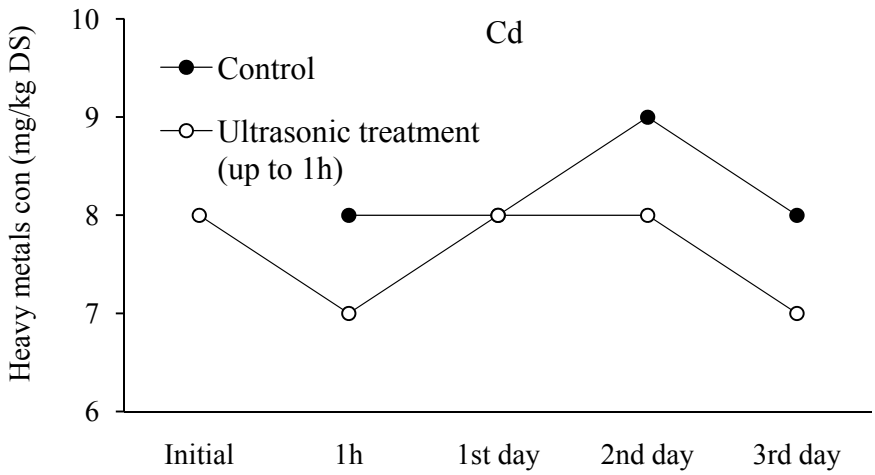


Figure 4.10. Comparison the heavy metals removal capacity after 3 days in ultrasonic treatment sample between experiment 1 and experiment 2.


Cu
700
650
600
550
Control
Ultrasonic treatment
(up to 1h)
Initial
1h
1st day
2nd day
3rd day


Zn
290
280
270
260
250
Control
Ultrasonic treatment
(up to 1h)
Initial
1h
1st day
2nd day
3rd day


Cd
Heavy metals con (mg/kg DS)
10
9
8
7
6
Control
Ultrasonic treatment
(up to 1h)
Initial
1h
1st day
2nd day
3rd day

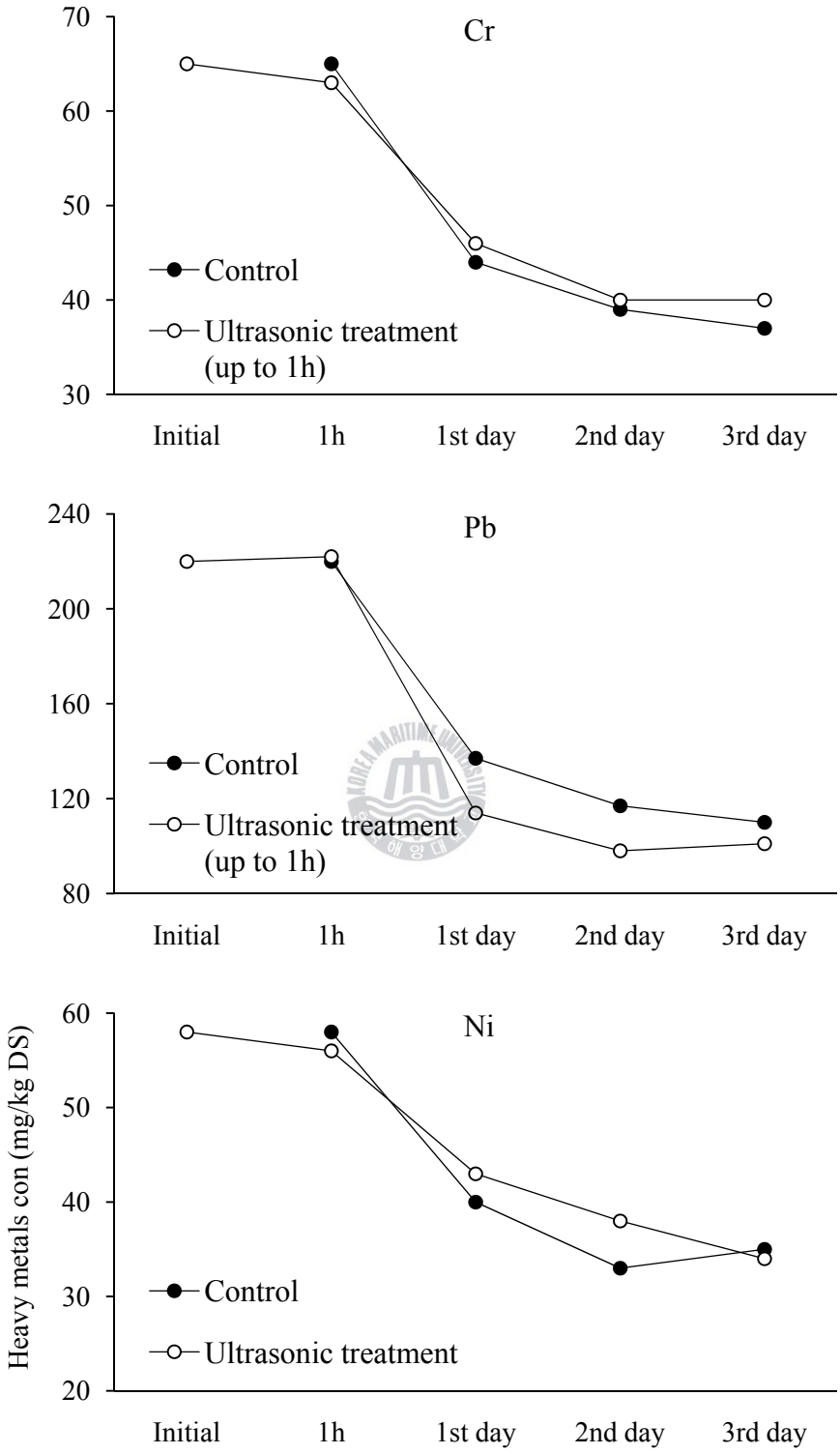


Figure 4.11. Effects of ultrasonic treatment and phosphate added on heavy metal concentration in experiment 1.

4.3.2. Effect of ultrasonic treatment on pathogens survival

The ultrasonic treatment method significantly effects on pathogens removal in biosolids. After 15 minutes treatment using electromagnetic wave energy, 50% of *Salmonella* was removal, and over 88% removal after 60 minutes (Figure 4.). The environmental regulation of biosolids of US.EPA-503 indicates class A standard for biosolids that are sold or given away in a bag or other container for application to land must meet one of these following requirements:

i) Either the density of fecal coliforms in the sewage sludge less than 1,000 MPN per gram total solids (dry weight basis).
ii) Or the density of *Salmonella sp.* Bacteria in the sewage less than 3 MPN per 4 grams of total solids (dry weight basis).

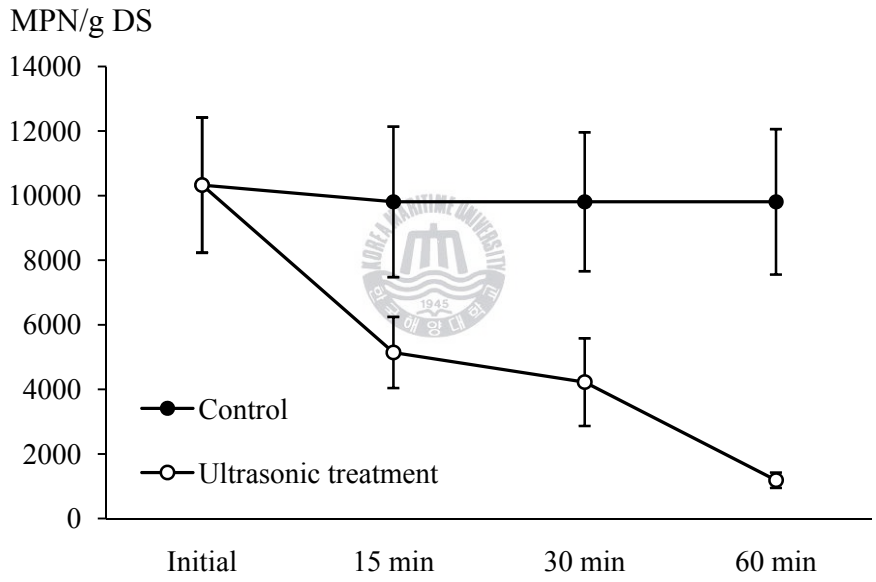


Figure 4.12. Reducing the number of *Salmonella* in experiment 1.

Although the number of *Salmonella* after 1hr treatment using ultrasonic was higher than standard, the density of fecal coliforms reached the standard. In experiment 1, the number of *Escherichia coli* was reduced 57.6 and 83.7% after 15 and 60 minutes treatment, respectively. The density of total coliform was also decreased over 76% at the end of ultrasonic treatment process. Adding amount of KH_2PO_4 may cause the effect of reducing number of *Escherichia coli* and total coliform in control sample.

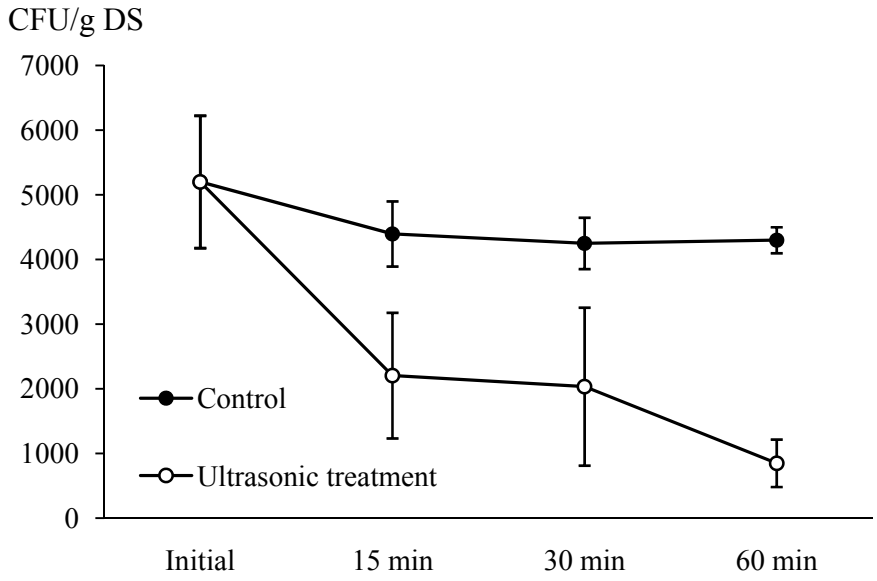


Figure 4.13. Reducing the number of *E. coli* in experiment 1.

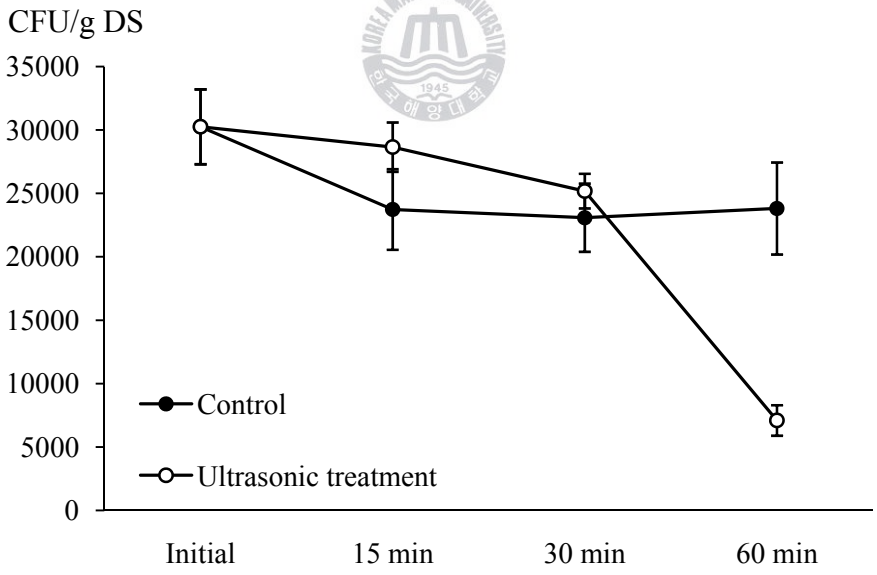


Figure 4.14. Reducing the number of total coliform in experiment 1.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

From the study required for the remediation of oil-contaminated soil using biosolids, it can be concluded that biosolids could be a good medium for the cultivation of the functional microbial consortium degrading oil, as well as inoculums, and the product obtained from the fermentation of the biosolids was effective for the remediation of soil contaminated with oil. The biological sludge obtained from wastewater treatment plant located in the petrochemical industrial park was slightly more effective for the production of microbial consortium degrading diesel oil, compared to the biosolids from municipal wastewater or night soil treatment plant. The degradation rate of diesel oil contained in the soil was significantly affected by the amount of the fermentation product mixed with the soil, but the oil contained in the soil could be degraded to about 55 mg TPH/kg within 4 weeks with 50 g of the fermentation product for 1 kg of the soil. The results of the heavy metals immobilization using phosphate amendments study concluded that Cu is the most predominant element in the biosolid. Phosphate amendments have the capacity to immobilize all the metals up to 50%. The increasing duration of time did not show any other immobilizing efficiency of P. In addition, the extractable level of metals different in different methods. Finally, ultrasonic treatment could be removed pathogens and enhance heavy metals immobilization.

5.2. Recommendations

Based on the extensive experimental data obtained, several recommendations for future studies can be outlined:

Remediation of oil-contaminated soil using biosolids

1. Study on the optimum conditions (pH, temperature, humidity, nutrients, diesel oil concentration) for cultivation of oil-degrading bacteria in biosolids is recommended.

2. This study had not counted the number of oil-degrading bacteria in soil and biosolids. In order to understand thoroughly the effects of environmental conditions on the growth rate of oil-degrading bacteria, the proper enumeration method would perhaps be useful.
3. Designing the pre-treatment and cultivation system for remediation contaminated soil using biosolid is the objective of next study.

Heavy metals immobilization using phosphate amendments

1. The present study focused only on potassium dihydrogenphosphate for heavy metals immobilization. The consideration on other types of phosphate or different materials such as apatite or fly ash should be investigated.
2. Further detailed work is needed to increase the immobilization or decrease the extractable level of metals with short term duration.

Reducing toxicity of biosolid using ultrasonic treatment

1. Comparison the effectiveness between ultrasonic and microwave treatment methods, consideration about energy consumption.

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