Removal of Plasticizer DEHP from Environmental Samples by Spent Compost of Mushroom *Pleurotus pulmonarius*

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Abstract

Plasticizers are additives used in the manufacture of plastics, and high residual plasticizer levels are encountered in the environment. Bis(2-ethylhexyl) phthalate (DEHP), being the most common plasticizer, is a suspected human carcinogen and an endocrine disruptor. Therefore, DEHP-contaminated soil, sediment and water samples were collected, and the bioremediation capacities of the spent compost of mushroom *Pleurotus pulmonarius* (SMC) were determined.

An industrial soil collected from a recycling factory was contaminated by DEHP and spilled diesel. The soils were divided into two batches for off-site *ex situ* bioremediation by SMC. This industrial soil was contaminated with 5.4–6.9 g/kg total petroleum hydrocarbons (TPH), 14.5–19.0 g/kg oil and grease and 95–99 mg/kg DEHP. The removal by 3% SMC amendment applied twice accounted for 56–64%, 31–33% and 51–54% disappearance of the TPH, oil and grease and DEHP contaminants, respectively. Beside chemical analysis, six bacteria and six fungi were inoculated into the sterilized soil samples for ecotoxicity tests. The original soil samples containing residual oil and DEHP contents were found to be more toxic than the SMC-treated soil. Thus SMC simultaneously degrades organic pollutants and

reduces toxicity in less than a month.

Besides, DEHP-contaminated sediment was collected from Kai Tak Approach Channel, Kowloon. This sediment contained 44.4-128.0 mg/kg DEHP and heavy metals 21.3-23.4 mg/kg Cd, 24.9-43.5 mg/kg Ni, 128.5-198.5 mg/kg Pb, 144.6-329.2 mg/kg Zn and 164.5-230.0 mg/kg Cu, and bore an unpleasant sewer smell. SMC and SMCE as strong oxidizing agents could decrease the S content of sediment significantly as well as calcium nitrate, and consequently lowered the evolution of nuisance gas hydrogen sulfide. With the treatment of the optimized combination of 2.25% SMCE and 0.25% nitrate, the malodor could be removed completely and the contents of H_2S and NH_3 in the air were decreased significantly. For the degradation of organic pollutants, the target pollutant DEHP showed a sharp decrease in the first week in the time effect experiment after the treatment with the combination of 2.25% SMCE and 0.25% nitrate, while the decrease of DEHP slowed down in the second week. The results may be attributed to the immediate degradation of DEHP by enzymes in SMCE. When raw sediment and nitrate-treated sediment were bioremediated with SMCE, larger fluctuation in DEHP removal was observed with the nitrate-treated sediment. It supports that nitrate and SMCE would act in optimum at a certain combination. The effect on mobilization of sediment heavy metals by

SMC or SMCE was also examined. Although some laboratory results suggested reduction of copper and lead, other results using different environmental samples of the sediment did not reproduce the results. Further investigation is needed.

An underground water sample contaminated with dibutyl phthalate (DBP, 127.5±20.7 μl/l) and DEHP (67.0±7.7 μl/l) was tested. One percent SMC could remove 94.2±3.6% and 100% for DBP and DEHP, respectively, within 1 h at room temperature. DBP and DEHP were degraded completely by SMCE except 0.2% SMCE for DBP after 24-h treatment. SMC had higher removal efficiencies than SMCE, because SMC had an integrated system of biosorption and biodegradation to remove DBP and DEHP. The sorption kinetics of DBP and DEHP by SMC could be described by the Freundlich monolayer model. Even after eight cycles of the sorption-desorption of DEHP, SMC maintained the 100% removal efficiency. Thus SMC is a good biosorbent for DEHP.

Using artificially spiked garden soil with DEHP and ultra-low sulphur diesel, the removal efficiencies of SMC were 41.7±9.8% and 36.1±8.4% for TPH and DEHP, respectively. SMCE which contained the water-soluble nutrients, SMC enzymes and micro-organisms had about half removal efficiency of SMC on the

same pollutant. The enriched SMC microorganisms also showed biodegradation of DEHP and diesel, so did the filtrate containing enzymes and nutrients of SMC. Thus SMC acted in multiple ways in bioremediation of DEHP: biostimulation and bioaugmentation. Besides, the immobilized lignolytic enzymes of the mushroom *P. pulmonarius* played a major role in biodegradation.

This study reveals the potential in applying SMC of *P. pulmonarius* in bioremediation of DEHP from the soil, sediment and water environments. More investigation and field studies would be appropriate for developing spent *P. pulmonarius* compost in environmental cleanup.

摘要

增塑剂是添加到高分子聚合物中增加塑性的添加剂，它的广泛应用加剧环境增塑剂的残余污染。邻苯二甲酸二乙基己酯（DEHP）是化工行业最常用的一种增塑剂，它可以导致人体致癌，激素系统紊乱，损坏生殖系统等。DEHP 几乎可以在任何环境中发现，因此，在本研究中我们收集到 DEHP 污染的土壤，海洋沉积物，及水的样品来研究凤尾菇生物修复的能力。

我们从玩具回收厂采集到重型机械泄漏的 DEHP 污染土壤，这些被污染的土壤同时也存在大量的机械柴油。污染的土壤被分为两批进行凤尾菇生物修复处理。这些土壤的污染程度是：总石油烃污染 5.4–6.9 g/kg，油脂 14.5–19.0 g/kg，DEHP 95–99 mg/kg。凤尾菇的种菇废料（SMC）在添加两次后去除 56–64%总石油烃，31–33% 油脂和 51–54% DEHP。除了进行化学分析外，六个细菌和六个真菌被接种进消过毒的土壤样品中进行毒性测试，发现被处理后的土壤明显有更高的微生物数量，表明土壤对微生物的毒性降低。因此，一个月内两次应用种菇废料可以明显降解有机污染物，并且能够降低土壤的毒性。

DEHP 污染的海洋沉积物采集于香港启德明渠进口道，其中 DEHP 有 44.4－128.0 mg/kg，重金属含量分别为镉 21.3-23.4 mg/kg，镍 24.9-43.5 mg/kg，铅

128.5-198.5 mg/kg，锌 144.6-329.2 mg/kg 和铜 164.5-230.0 mg/kg，同时这里的海洋沉积物样品散发出恶臭味。SMC 及 SMCE 都可以像硝酸钙一样显著降低海洋沉积物中的硫含量，硫含量的降低有利于硫化氢气体的去除，达到消除臭味的目的。2.25% SMCE + 0.25%硝酸钙的混合液能完全除掉臭味，并且显著的降低了从海洋沉积物中释放出来的硫化氢和氨气的含量。在处理有机污染物方面，2.25% SMCE + 0.25%硝酸钙的混合液使 DEHP 的含量在的一个星期很快减少，而在第二个星期 DEHP 的降解速度开始变慢，这些可能是由于 SMCE 中木质素降解酶在第一个星期的快速作用。在另外一个实验中，我们发现 SMCE 应用于硝酸钙处理过的海洋沉积物表现出很大的波动，因此 SMCE 菌与硝酸钙在某一个比例才能达到最优的处理效果。而在这些实验中，我们观察到 SMC 或者 SMCE 对铜(Cu) 和铅（Pb）等重金属有一定的去除能力，但是表现不一，因此 SMC 对重金属的作用还需要进一步的研究和观察。

本研究中 SMC 处理的污染水体含有 127.5±20.7 μl/l DBP 和 67.0±7.7 μl/l DEHP。在室温下，1%SMC 在一个小时内可以去除 94.2±3.6% DBP 和 100% DEHP。除了 0.2%SMCE 外，其它量的 SMCE 在 24 个小时后均能完全降解 DBP 和 DEHP。相比 SMCE，SMC 有更高的去除效率，这是因为 SMC 是一个生物吸附和生物降解的整合系统。SMC 对 DBP 和 DEHP 的吸附动力学可以用 Freundlich 模型来解释。循环利用 SMC 重复吸附 DEHP 的实验也证明了 SMC

在使用八次后依然能够完全去除水体中的 DEHP。因此对于 DEHP，SMC 是个很好的吸附剂。

在人为添加柴油和 DEHP 的土壤中，SMC 对总石油烃和 DEHP 的去除效率分别是 41.7±9.8% 和 36.1±8.4%，SMCE 有 SMC 去除污染物效率的一半。没有生物活性的 SMC 仍然能表现出去除污染物的效率，而 SMC 里的微生物表现出了生物降解 DEHP 的能力。因此 SMC 是通过生物添加和生物刺激两个途径进行生物修复污染物的。另外凤尾菇 SMC 中的木质素降解酶也是很重要的降解污染物的因素。

这些实验证明了凤尾菇的种菇废料从土壤，海洋沉积物及水体里去除 DEHP 的生物修复能力。因此应该进行更多理论实验及野外应用实验，从而对凤尾菇的种菇废料在环境污染治理中的发展提供更合理有效的方法。

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Abbreviations

AAS	Atomic absorption spectrophotometer
ABTS	2,2'-Azinobis-3-ethylbenthiazoline-6-sulfonate
AIA	Automated Wet Chemistry Analyzer
ANOVA	Analysis of variance
AR	Analytic reagent
ATSD	Agency for Toxic Substances and Disease Registry
BE	Biological efficiency
1st Cap	Tissue cap of the organ fruiting body from the first flush
2no Cap	Tissue cap of the organ fruiting body from the second flush
CUHK	The Chinese University of Hong Kong
Day 15 compost	Vegetative growth with the compost partially colonized
Day 28 compost	Vegetative growth with the compost fully colonized
DBP	4,4'-Dichlorobenzophenone
DEHP	Bis(2-ethylhexyl) phthalate
DMAB	3-(Dimethylamino)-benzoic acid
DNA	Deoxyribonucleic acid
DRE	Destruction and removal efficiency
EC	Enzyme Commission

EDTA	Ethylene diamine tetraacetic acid
1^{st} F	First flush of fruiting
2^{na} F	Second flush of fruiting
FW	Fresh weight
GC/MS	Gas chromatography-mass spectrometry
GC/MSD	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
hr	Hour
ICP	Inductively coupled plasma-atomic emission spectrometry
ISV	In Situ Vitrification
IU	International enzymatic unit
K_{oc}	Organic carbon partition coefficient
K_{ow}	Octanol-water partitioning coefficient
LD_{50}	Median lethal dose
Lac	Laccase
LiP	Lignin peroxidase
MBTH	3-Methyl-2-benzothiazolinone hydrazone
min	Minute
MnP	Manganese peroxidase

MS	Mass spectrometer
PAHs	Polycyclic aromatic hydrocarbon
PCBs	Polychlorinated biphenyls
PCP	Pentachlorophenol
POPs	Persistent organic pollutants
RC	Removal capacity
rpm	Revolutions per minute
SMC	Spent mushroom compost
1st SMC	First spent mushroom compost
2nd SMC	Second spent mushroom compost
1st Stem	Tissue stem of the organ fruiting body from the first flush
2nd Stem	Tissue stem of the organ fruiting body from the second flush
TOC	Total organic carbon
US EPA	United States Environmental Protection Agency
V	Voltage
VOC	Volatile organic compounds
wt.	Weight
w/v	Weight by volume

Chapter 1 Introduction

1.1 The definition and types of plasticizers

Plasticizers are additives which soften the materials (usually a plastic or a concrete mix) they are added to (http://en.wikipedia.org). Consumption of plasticizers accounts for about one third of the global plastic additives market. Plasticizers are often made of esters of polycarboxylic acids with linear or branched aliphatic alcohols of moderate chain length. Plasticizers work by embedding themselves between the chains of polymers, spacing them apart, and thus significantly lower the glass transition temperature for the plastic resulting in softer texture (Rahman and Brazel, 2004). The most common plasticizers are phthalates (or called phthalate esters) giving hard plastics like PVC the desired flexibility and durability.

A phthalate has the general structure of an esterified benzenedicarboxylic acid with two alkyl chains, which are used in situations where good resistance to water and oils is required. Some common phthalate plasticizers are bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), bis(n-butyl)phthalate (DnBP, DBP),

butyl benzyl phthalate (BBzP), and diisodecyl phthalate (DIDP) (http://en.wikipedia.org).

As the plastic industry continues to grow, so does the plasticizer industry. In the early 1990s, the annual US production of plasticizers averaged 2 billion pounds, of which 1.25 billion pounds were phthalates (Rahman and Brazel, 2004). By 1999, the global demand for plasticizers increased to 10.1 billion pounds, worth about US $7 billion (Rahman and Brazel, 2004). The current overall growth rate for production of plasticizers, as estimated in early 2000s, is about 2.8% per annum (Murphy et al., 2001; Rahman and Brazel, 2004). The estimated amount of DEHP manufactured in 1998 was approximately 260,529 tonnes in Japan (Tanaka, 2003).

1.2 Bis(2-ethylhexyl)phthalate (DEHP)

1.2.1 The physical-chemical properties of DEHP

Phthalate esters (Figure 1.1) comprise a group of compounds that are widely used as industrial chemicals. Among them, bis(2-ethylhexyl)phthalate (DEHP) is the most commonly used and persistent one (Wams, 1987). Phthalates account for 92%

of plasticizers produced worldwide while DEHP represents 51% of the phthalates. Therefore, DEHP will be introduced as the main plasticizer. DEHP is a colorless oily liquid at ambient conditions, its structure and physical properties are illustrated in Figure 1.2 and Table 1.1, respectively. The low vapour pressure (listed in Table 1.1) of DEHP indicates poor volatilization (Marttinen et al., 2003). Figure 1.2 shows that the stability of its molecular structure originates from the steric hindrance of the alcohol side chain, which renders it resistant to biodegradation (Staples et al., 1997a, b; Yuan et al., 2002).

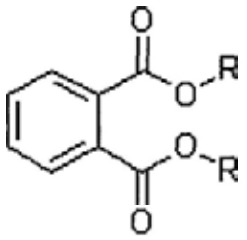


Figure 1.1 A common structure of phthalate esters where R is the aliphatic C-chain.

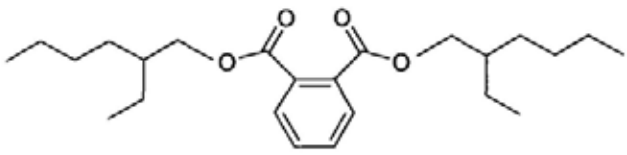


Figure 1.2 The chemical structure of Bis(2-ethylhexyl)phthalate.

Table 1.1 Physical properties of bis(2-ethylhexyl)phthalate (Müller et al., 2003).

Molecular weight	390.54 g/mol
Vapor pressure	1.32 mm Hg at 2000°C
Melting point	-50°C
Boiling point	230°C at 500 mm Hg
Color/Form	Colorless, oily liquid
Odor	Slight odor
Specific gravity	0.9861 at 20°C
Octanol/water partition coefficient	Log K_{ow} = 4.89
Aqueous solubility	0.003 mg/l

1.2.2 Sources and environmental concentrations of DEHP

The plasticizer can enter the environment through various routes (Jeng, 1986; Wams, 1987; Staples et al., 1997; Fatoki and Noma, 2002; Marttinen et al., 2003 a&b; Psillakis et al., 2004). During the production of DEHP, about 1% of it is lost into wastewater (Wams, 1987). During its distribution to the plastic producing industry, about 0.05% of it is lost into sewage system (Wams, 1987). During the production of plasticized PVC, about 0.8% is lost into wastewater (Wams, 1987). As DEHP is not chemically but only physically bound to the plastic structure, after disposal of plasticized PVC products to landfills, it may be leached by water percolating through landfills.

Plasticizers persist in the environment, e.g. DEHP may be found in almost all samples taken of the environment. DEHP is a rather stable compound in the natural environment, and the degradation and distribution parameters are shown in Table 1.2. Half life means the time required for a pollutant to lose one-half of its original concentration, and the pollutant is persistent if its half life is more than 120 days in soil (REACH, 2008). Bioconcentration factor is defined as the ratio of a test chemical's concentration in the tissues of an organism, to the chemical's concentration in the surrounding medium (e.g., usually water), when the chemical uptake is supposedly from this medium only. Bioaccumulation factor is defined as the ratio of a test chemical's concentration in the tissues of an organism, to the chemical's concentration in the surrounding medium (e.g., usually water), when all potential uptake mechanisms (e.g., from the organism's prey as food) are included. From this table, the half-lives in different phases indicate the persistence of DEHP, and bioconcentration factor reflects the distribution in our food sources (US Department of Health and Human Services, 2002; Müller et al., 2003; Kambia et al., 2003).

Table 1.2 Characterization of biodegradability of DEHP in environment (Müller et al., 2003).

		DEHP
Characterization of biodegradability		Readily
Half-life (d)	Air	1
	Surface water	50
	Soil	300
	Sediment, aerobic	300
	Sediment, anaerobic	Infinite
	Sediment, total	3,000
Partitioning coefficient	Organic C-water (K_{oc})	165,000
	Plant-water	1,940
Bioaccumulation (l/kg)	Aquatic biota	2,700
Bioconcentration Factor	Fish	840
	earthworms	1
Bioaccumulation Factor	Meat	0.0002
	milk	0.00006

1.2.3 Persistence of DEHP

DEHP is a rather stable compound in the natural environment. From table 1.2, DEHP has a long half-life. The low vapour pressure (listed in Table 1.2) of DEHP indicates poor volatilization (Marttinen et al., 2003). The metabolite of DEHP by abiotic transformation, oxidation, photolysis and chemical hydrolysis can be regarded as negligible (Wams, 1987; Staples et al., 1997a,b; Marttinen et al., 2003). Biodegradation is considered to be the most significant aquatic fate process for

DEHP (Staples et al., 1997a). Yet, the stability of its molecular structure (i.e. 2-ethylhexanol) renders it resistant to biological degradation (Staples et al., 1997a; Yuan et al., 2002). The tendency of DEHP to adsorb on solid particles because of its hydrophobicity may reduce the bioavailability of DEHP, and result in the lower degrading effectiveness of microorganisms, thus retarding the degradation process (Marttinen et al., 2003; Chang et al., 2004). Therefore DEHP persists in the environment because of resistance to biodegradation and sorption to soil organic materials, reducing bioavailability. Because of the recalcitrance, DEHP-contaminated sites show chronic pollution problems.

1.2.4 Routes of exposure

Exposure of the general human population to DEHP is about 30 μg/kg of body weight per day (Kambia et al., 2003). European Union Council (2001) prescribes that the limited intake of DEHP is 48 mg/kg/day without causing adverse effect on human health, while the criterion of USEPA does not exceed 20 mg/kg/day (Wang, 2005). The most likely route was through consumption of residues in water and food e.g. fish, seafood, meat, milk, cheese, eggs or food coming in contact with containers containing DEHP, and to a lesser extent by inhalation and dermal contact

(Steiner et al., 1998; Doull et al., 1999; US Department of Health and Human Services, 2002; Kambia et al., 2003; Lovekamp-Swan and Davis, 2003). The study carried out by the Berlin Environment Bureau indicates that the content of DEHP was hundreds mg/kg in dust gathered from 550 families. If dust contains 775 mg DEHP/kg, then the daily intake by a child with 13 kg body weight is 6 mg/kg (Wang, 2005).

Another high-risk population is patients who undergo medical procedures (US Department of Health and Human Services, 2002). For instance, hemophiliacs and dialysis patients receive dialysis treatments or blood transfusions from sources that have contacted DEHP-containing tubing or containers (US Department of Health and Human Services, 2002). Estimates of exposure levels indicate that the former may be exposed to 1 to 2 mg DEHP/day and the latter to an average dose of 40 to 75 mg/day (US Department of Health and Human Services, 2002). Patient undergoing long-term treatment can be particularly at risk of potential toxicity of DEHP due to regular exposure.

1.2.5 Toxicities of DEHP

The widespread occurrence and persistence of DEHP in the environment raises concern about its toxic effects on living organisms.

1.2.5.1 Acute toxicity

The reported lethal oral dose (LD_{50}) of DEHP ranged from 26,000 to 4,000 mg/kg in various animals, showing that the acute toxicity is low (Wams, 1987). For acute toxicity, it has been reported that the 96h-LC_{50} for the freshwater *Daphnia magna* was 11.1 mg/l. The 96h-LC_{50} for the midge, scud and bluegill exceeded the concentrations tested at 18, 32 and 770 mg/l respectively (Agency for Toxic Substances and Disease Registry, 1993). Call et al. (2001) demonstrated that DEHP has no significant survival reductions on the freshwater invertebrate midge (*Chironomus tentans*), amphipod (*Hyalella azteca*) and oligochaete (*Lumbriculus variegatus*). The acute toxicity test performed with green alga (*Selenastrum capricornutum*), epibenthic invertebrate waterflea (*Daphnia magna*) and mysid shrimp (*Mysidopsis bahia*), benthic invertebrate midge (*Paratanytarsus parthenogenetica*) as well as fish bluegill sunfish (*Lepomis macrochirus*) at concentration approaching its aqueous solubility by Adams *et al.* (1995) showed no acute toxicity on these living organisms. In brief, the lack of acute lethality

observed was owing to its very low water solubility and consequent inability to accumulate a critical body burden (Adams et al., 1995; Call et al., 2001).

1.2.5.2 Chronic toxicity

1.2.5.2.1 Adverse effects on reproduction system

Bis(2-ethylhexyl)phthalate (DEHP) is a well-known reproductive toxicant. It causes apoptosis and loss of spermatogenic cells, resulting in testicular atrophy. Its mechanism of inducing testicular atrophy has been associated with depletion of zinc in the testis. The loss of testicular zinc is followed by a decrease in the activities of enzymes containing zinc (Park et al., 2002).

DEHP also has adverse effects on female reproductive system. Lovekamp-Swan and Davis's study (2003) demonstrated that the ovary was a target site for DEHP which exerted primary functional alternation through suppression of estradiol production in the ovary, prolonged estrous cycles, and ending with anovulations in female rats (Lovekamp-Swan and Davis, 2003).

1.2.5.2.2 Carcinogenicity

DEHP was first listed in the third annual report on carcinogens by United States Department of Health and Human Services in 1983 (US Department of Health and Human Services, 2002). The carcinogenic potential of DEHP in rodents was established *in vivo* and was considered as the result of non-direct DNA-reactive mechanisms. DEHP induced morphological transformation of Syrian hamster embryo cells and inhibited gap junctional intercellular communication (GJIC), two mechanisms related to *in vivo* carcinogenicity. Hepatocarcinogenesis in response to DEHP is preceded by peroxisome proliferation, hyperplasia and hepatocyte DNA synthesis. The suppression of apoptosis has also been suggested to contribute to the hepatocarcinogenicity of DEHP. Mechanisms of apoptotic inhibition by DEHP were not clearly elucidated (Maire et al., 2005).

1.2.5.2.3 Endocrine disruption

DEHP is an endocrine disrupting chemical that instigates disturbance in the endocrine system (Petrović et al., 2001; Psillakis et al., 2004). DEHP inhibits binding to the estrogen receptor and is antiandrogenic (Moore et al., 2001). Moore et al.

(2001) suggested DEHP causes abnormal sexual development of male reproductive system in rats by acting as an antiandrogen primarily.

1.2.5.2.4 Regulations

DEHP has been classified as a probable human carcinogen (Group B2) by USEPA since 1987. USEPA has also listed it as a water priority pollutant (Marttinen et al., 2003). The priority pollutants are a set of chemical pollutants under EPA regulation, and 126 chemicals are in this current list. Pretreatment regulations have specified an effluent limitation of 5.0 mg/l on the combined bis(2-ethylhexyl)phthalate (DEHP), di-*n*-octyl phthalate (DNOP) and di-*n*-butyl phthalate (DNBP) ester concentration before discharge to a publicly owned treatment works. The USEPA also regulates DEHP under the Clean Water Act (CWA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), Superfund Amendments and Reauthorization Act (SARA), and Toxic Substances Control Act (TSCA). World Health Organization (WHO) recommended the limit of DEHP concentration for household water is 8 μg/l (Marttinen et al., 2003) and European Commission proposed the maximum acceptable value for sludge to be used in

agriculture is 100 μg/g dry weight (European Commission, 2000; Marttinen et al., 2003).

1.2.6 Treatment of DEHP

Active treatment methods of DEHP, such as adsorption, sonolysis, photocatalytic oxidation have been reported. The remediation methods can be classified into three categories: physical method, photochemical method and biological method. However, these methods are neither cost-effective nor rapid. Hence, there is a strong need to look for an alternative treatment process for such type of pollutants.

1.2.6.1 Biodegradation

Biodegradation is a widely accepted biological treatment method for environmental contaminants. It is the process by which organic substances are broken down by the enzymes produced by living organisms. The degradation of DEHP by numerous microorganisms, such as *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Moraxella* species, has been reported (Azarova et al., 2003). The di-ester is

hydrolyzed into the mono-ester by esterases (Wang et al., 1997), and then the mono-ester is converted into phthalic acid, which is the rate limiting step for biodegradation (Wang et al., 1997). Phthalic acid is further degraded to CO_2 and H_2O via pyruvate and succinate (Wang et al., 1997). However, once a plasticizer enters the natural environment, incomplete microbial degradation, releasing persistent and toxic metabolites such as adipic acid and 2-ethylhexanol happens (Figure 1.3). These two compounds are toxic to various species including mammals (Table 1.3) (Nalli et al, 2006).

Microbial degradability is due to its capability to utilize the pollutant as sole sources of carbon and energy, but its degradation pathway depends on the species and enzyme involved (Chang et al., 2004). This removal procedure encounters with certain disadvantages such as long time requirement to render DEHP harmless and complete degradation is hardly obtained (Murai et al., 1998). Both dead end metabolites exhibit acute toxicities, while DEHP does not exhibit acute toxicity (Table 1.3).

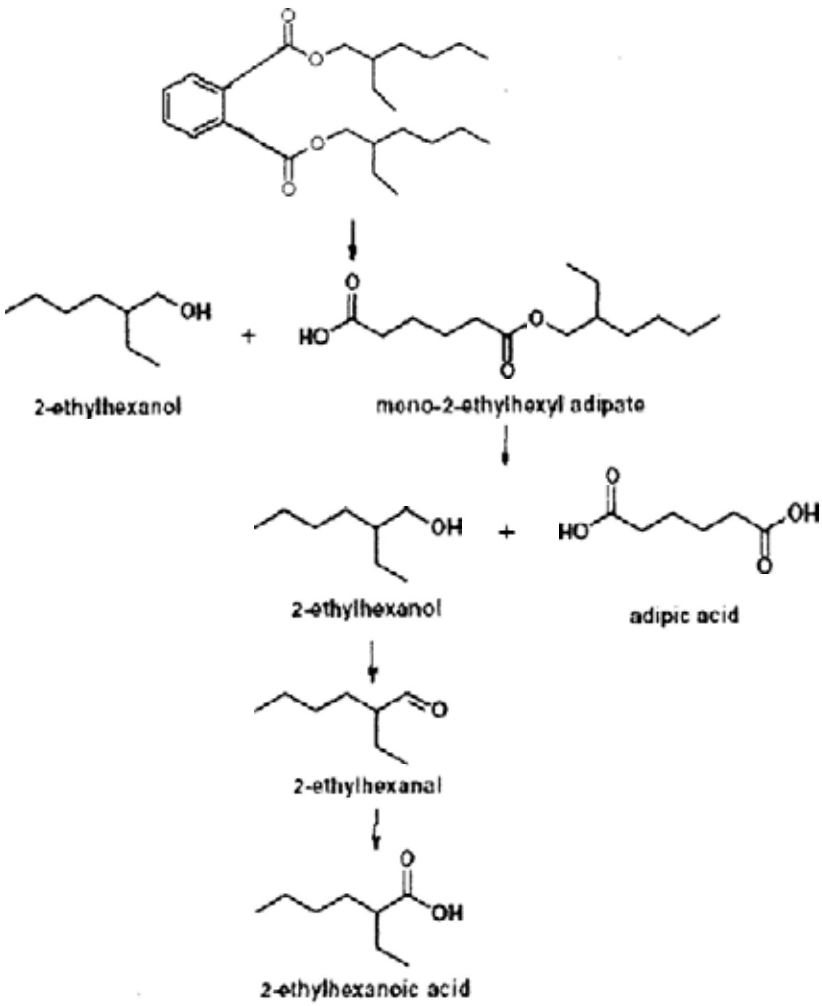


Figure 1.3 The metabolism of DEHP (Nalli et al., 2006).

Table 1.3 A comparison on toxicities of DEHP and its metabolites (MSDS; Horn et al., 2004).

Compounds	Microtox® EC50 (mg/l)	ORAL-RAT LD50 (mg/kg)
2-ethylhexanoic acid	43	3000
2-ethylhexanol	7.8	2046
DEHP	54	20000

1.2.6.2 Sewage treatment process

Activated sludge systems are frequently applied to remove DEHP from industrial wastewater. DEHP is removed by three different mechanisms: adsorption to primary and secondary sludge, biodegradation by the activated sludge process and anaerobic digestion (Marttinen et al., 2003). The residual DEHP concentration is found high in the sludge as DEHP has low water solubility and high octanol-water partition coefficient (Marttinen et al., 2003).

The reduction of DEHP concentration at several sewage treatment processes has been 60-100%, and the removal efficiencies vary (Marttinen et al., 2003). Moreover, treated sludge contains DEHP and necessitates proper disposal in further steps to avoid secondary pollution.

1.2.6.3 Adsorption

Adsorption is a physical process that occurs when contaminants from a gas or liquid solute accumulates on the surface of a solid or a liquid (adsorbent), forming a film of molecules or atoms (the adsorbate). Commercial adsorbents include activated

carbons, alumina, silica, bauxite, bentonite molecular sieves and ion exchange resins. Adsorption by activated carbon has also been used. For DEHP, the removal efficiencies of the systems based on adsorption to activated carbons were high, ranging from 90 to 100% (Wams, 1987). However, the material is expensive and it is not economical (Chan et al., 2004). Therefore, an alternative method is desired in order to treat this compound.

1.2.6.4 Advanced oxidation processes (AOPs)

The interest in developing advanced oxidation processes (AOPs) in chemical water treatment has grown in the recent years (Guzzella et al., 2003). These processes generate highly reactive hydroxyl radicals to oxidize various compounds in the water. These radicals are characterized by having a high oxidation potential (2.8 V) that can, in some cases, completely mineralize contaminants by converting them into CO_2 and H_2O. Several AOPs processes can generate the hydroxyl radicals including: UV- H_2O_2, Fenton's reagent, peroxone (ozone-H_2O_2), and titanium dioxide (TiO_2)-assisted photo-catalytic processes.

The UV-H_2O_2 oxidation system involves the single-step dissociation of H_2O_2 to

form two radicals. Hydroxyl radicals can oxidize organics by abstraction of protons producing organic radicals, which are highly reactive and can be further oxidized. Tawabini and Al-Suwaiyan (2004) reported that more than 98% of dimethyl phthalate (DMP) was removed after 45 min when the UV-irradiated solution was dosed with 136 ppm of H_2O_2.

1) $H_2O_2 \rightarrow 2OH^{\cdot}$

2) $H_2O_2 + H_2O \rightarrow HOO^{-} + H_3O^{+}$

3) $OH^{\cdot} + H_2O_2 \rightarrow HO^{\cdot}{}_2 + H_2O$

4) $OH^{\cdot} + HOO^{-} \rightarrow HO^{\cdot}{}_2 + OH^{-}$

5) $2\ HO^{\cdot}{}_2 \rightarrow H_2O_2 + O_2$

6) $2OH^{\cdot} \rightarrow H_2O_2$

7) $OH^{\cdot} + HO^{\cdot}{}_2 \rightarrow O_2 + H_2O$

$OH^{\cdot} + RH \rightarrow H_2O + R^{\cdot} \rightarrow$further oxidation

Fenton's reagent is a mixture of H_2O_2 and ferrous iron, which generate hydroxyl radicals according to the following reaction:

$Fe^{2+} + H_2O_2 \rightarrow F^{3+} + OH^{-} + OH^{0}$ (1)

The catalytic effect of Fe^{2+} can be enhanced by irradiating the solution with ultraviolet (UV) light.

$$Fe^{3+}+h_v \rightarrow Fe^{2+}+OH^0 \quad (2)$$

The results studied by AL-Tawabini (2003) demonstrated that photo-Fenton process was more effective and faster than Fenton's reagent in removing DnBP and that photolysis by UV irradiation was the dominant mechanism in degrading the compound. The results also show that enhancing the removal via UV irradiation was achieved by increasing either the temperature or the H_2O_2 concentration.

1.3 Spent mushroom compost

Oyster mushrooms including *Pleurotus pulmonarius* are the second most popular edible mushroom in the world. Therefore, spent *Pleurotus pulmonarius* compost is easily available (Chiu et al., 2000). Many roles for SMC have been considered: use of SMC in crop production, landscaping and horticulture, as a substrate component, the possibility of landfilling or incineration, as well as the possibility of using SMC as an energy feedstock (Williams et al., 2001; Watabe et al.,

2004).

1.3.1 Physico-chemical properties of SMC and its function

Usually, the main components of mushroom compost are wheat straw, sawdust, wheat bran, which is used as sources of carbon and energy by different metabolically interdependent microorganisms during composting. After composting through biological and chemical reactions, mushroom compost contains lots of organic matters. Soil organic matters (SOM) play an important role in nutrient availability and stability of soil aggregate (Entry et al., 1996; Hussain et al., 1999). Also, water-holding capacity increased with the increase in soil organic matters and carbonate levels. This might be due to the change of soil texture and increase of the percentage of silt in the soil (Hevia et al., 2003). Besides, organic matters in soil could also reduce the evaporation of water and increase the water use efficiency of spring wheat (Dong and Zhan, 2000). Therefore, supplementation of organic matters to farmlands is very important to agriculture.

Spent compost of mushroom *P. pulmonarius* has a relatively low bulk density (0.26 g/cm^3) and high porosity (86%) (Gong, 2004). These make SMC be used as a

soil conditioner (Chang, 1987). Amendment with SMC not only can decrease soil bulk density, clod and surface crust formation, but also can reduce diurnal temperature fluctuations, and increases soil pH, infiltration rate and soil water content (Ntougias et al., 2004). So it can have important benefits in developing soil structure and texture and improving aeration and drainage (Chen and Zeng, 2005).

Also spent compost of *P. pulmonarius* contains significant amounts of essential plant macro- and micro-nutrients and organic matter. SMC contains lots of N, P, K, which are essential for plant growth (Table 1.4) (Law et al., 2003; Chiu et al., 2005) and SMC is biodegradable. So the application of SMC can lead to increased levels of plant-available soil nutrition, and enhances plant biomass production (Ntougias et al., 2004). Moreover, its application into agricultural soils has promoted suppressiveness against various plant pests and pathogens (Ntougias et al., 2004). Therefore, spent mushroom compost could be used as a fertilizer and soil conditioner for the planting of trees and shrubs, especially, in vegetation establishment phases of land reclamation.

Table 1.4 Physico-chemical properties of the spent *P. pulmonarius* compost (Law et al., 2003).

pH	6.69±0.02
Ash content (%)	8.96±0.30
C (%)	25.61±0.08
H (%)	4.20±1.33
N (%)	2.42±0.02
S (%)	0.49±0.15
Chitin (%)	25.17±1.37
Minerals	
Manganese (mg/g)	0.46±0.04
Potassium (mg/g)	6.91±0.02
Calcium (mg/g)	14.75±7.01
Iron (mg/g)	0.34±0.08
Zinc (mg/g)	0.22±0.13
Sodium (mg/g)	1.30±0.11
Water-soluble anions	
Fluoride (mg/l)	4.43±1.25
Chloride (mg/l)	67.44±3.19
Nitrite (mg/l)	0.04±0.00
Bromide (mg/l)	2.17±1.13
Nitrate (mg/l)	0.20±0.09
Phosphate (mg/l)	30.44±1.30
Sulphate (mg/l)	20.55±0.62

1.3.2 Biological properties of SMC and its functions in organic pollutants remediation

Mushrooms secrete many extracellular enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase, which are immobilized in SMC and can oxidize organopollutants in soil (Chiu et al., 2000; Law et al., 2003; Ball and Jacksohn, 1995). The two major enzymes, laccase and manganese peroxidase (MnP), secreted by *P. pulmonarius,* show substrate non-specificity by Fenton reactions so that they are able to mineralize a wide range of highly recalcitrant organopollutants (Cajthaml et al., 2002).

Laccase uses oxygen molecules to oxidize phenolic compounds to very reactive, free radicals. The presence of primary mediating substrates extends the substrate range of laccases to non-phenolic aromatics by forming potent radicals which co-oxidize non-phenolic lignin compounds. As illustrated in Figure 1.4, laccase uses oxygen as an electron acceptor to remove hydrogen radicals from phenolic hydroxyl groups. Consequently, free radicals formed can undergo rearrangements which lead to subsequent non-enzymatic reactions include alkyl-aryl cleavage, oxidation of benzyl alcohols, cleavage of side chains and aromatic rings, etc. (Palmieri et al.,

2000; Shleev et al., 2003). Although laccase possesses a relatively low redox potential, the other two lignin-degrading enzymes which do not favour the oxidation of non-phenolic compounds, extend their substrate ranges by appropriate redox mediators.

As shown in Figure 3, the substrate spectrum can further be expanded with the involvement of a mediator such as 2,2'-azino-bis-(3-ethylebenzo-thiazole-6-sulfonic acid (ABTS) and 1-hydroxybenzotriazole (HBT) which are low molecular weight compounds and can be easily oxidized by laccases to produce very unstable and reactive cationic radicals. These radicals can then oxidize more complex substrates, e.g. non-phenolic compounds which originally cannot be oxidized by laccases, before returning to their original state (Mester and Tien, 2000; Torres et al., 2003).

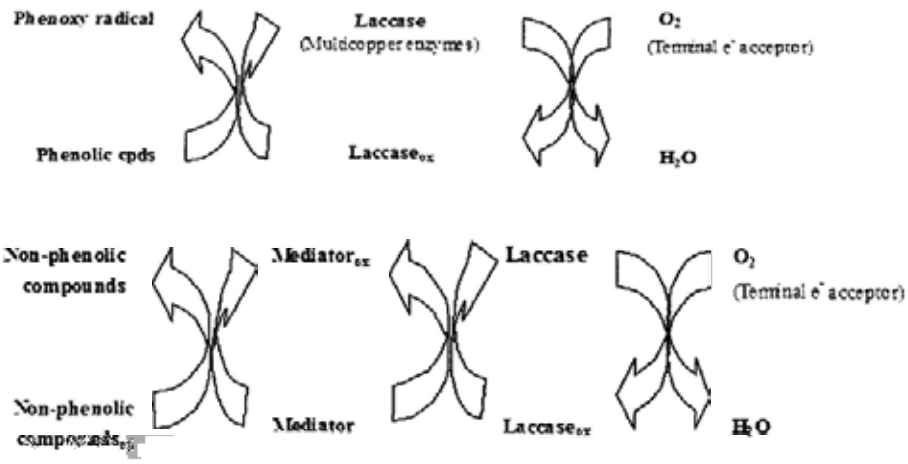


Figure 1.4 The catalytic cycle of laccase (Law et al., 2003).

Manganese peroxidases are iron-containing glycoproteins. The catalytic cycle of MnP (Figure 1.5) is initiated by binding of hydrogen peroxide or an organic peroxide to the native ferric enzyme and oxidized to a two electron oxidized intermediate, MnP Compound I and a water molecule. Subsequent reduction of MnP Compound I to MnP Compound II and finally MnP Compound II to the native MnP and release the second water molecule is accomplished by the oxidation of Mn (II) to Mn (III) (Sundaramoorthy, 1997; Mester, 2000). Mn (III) generated is unstable. Therefore it chelates with organic acids such as oxalate, a metabolic product of lignin-degrading fungi. This stable complex is capable of oxidizing various phenolic substrates and it can act as a stable diffusing oxidizer to oxidize substrates at a distance away from the active site of MnP (Sundaramoorthy et al, 1997; Banci et al., 1999; Martinez, 2002).

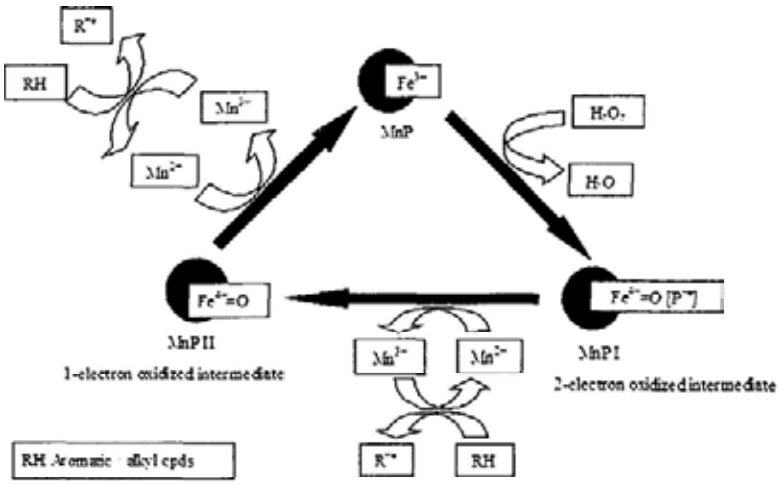


Figure 1.5 The catalytic cycle of manganese peroxidase (Law et al., 2003).

Some surveys have indicated that 200 mg/kg PAH-contaminated soil samples treated with SMC of *Pleurotus pulmonarius* had significantly been reduced in 2 days under continuous shaking at 80℃ (Lau et al., 2003). 5% of SMC of *Pleurotus pulmonarius* could remove 89.0% of 100 mg PCP/l within 2 days at room temperature (Law et al., 2003). Eggen indicated that the soil treated with SMC of *Pleurotus ostreatus*, incubated for 7 weeks, resulted in the removal of 86% of total 16 PAHs (Eggen, 1999). Recently SMC was discovered to degrade DDE, a deadend metabolite of banned pesticide DDT in soil (Gong et al., 2006). The capacity of white rot fungi for biodegradation of recalcitrant organic pollutant has generated a considerable research interest in this field. Therefore, Chiu et al. (1998) have compared the ability of various mushrooms (*Armillaria gallica, A. mellea, Ganoderma lucidum, Lentinula edodes, Phanerochaete chrysosporium, Pleurotus pulmonarius,* a *Polyporus* sp., *Coprinus cinereus* and *Volvariella volvacea*) to remove PCP from liquid with SMC of *Pleurotus pulmonarius*. The results showed that *A. mellea* strain had the highest degradative capacity (13 mg PCP g^{-1} mycelium). However, its role as a plant pathogen and slow growth rate would greatly limit its practical application. In contrast, P. *pulmonarius* grew fast and has a complete degradation pathway for PCP. In comparison, *Pleurotus* SMS harbouring both

bacteria and fungi reached a higher degradative capacity (19 mg PCP g^{-1}) in only 3 d. Therefore, use of *Pleurotus* SMC for bioremediation of organopollutant-contaminated sites seems promising.

1.4 Aim of study

To examine the potential capacity of spent compost of *Pleurotus pulmonarius* in bioremediation of contaminated environmental samples with DEHP. Four objectives were set to:

(1) Evaluate the pollutant removal capacity of SMC on contaminated soil with DEHP and diesel collected from Lau Fou Shan;

(2) Evaluate the pollutant removal capacity of SMC/SMCE on contaminated sediment with DEHP and heavy metals from Kai Tak Approach Channel;

(3) Evaluate the pollutant removal capacity of SMC/SMCE on contaminated water with DEHP and DBP, which provided by China Light and Power Company.

(4) Study the mechanisms of SMC on the removal of pollutant by artificial contaminated soil.

Chapter 2 Using SMC to treat environmental soil samples with DEHP and diesel

2.1 Introduction

2.1.1 Contaminated soil

Soil contamination takes place due to human activities and by natural processes (Gadepalle et al., 2008). Agricultural soil is one of the most frequently contaminated soils around the world due to pesticide application and sewage irrigation which introduces many contaminants such as organics (petroleum hydrocarbons, hydroxybenzene, etc.), metals (Cr, Pb, etc.) (Jacks et al., 2000; Liu et al., 2005). The remediation of agricultural soils which have the direct risk for humans is therefore imperative.

In this study, contaminated soils were collected from an industrial area with mixed factories practicing waste recycling, manufacturing of portable toilets and manufacturing of heavy instruments in Lau Fau Shan, the New Territories of Hong Kong. It is not uncommon that mixed and persistent organic pollutants can be found in contaminated soil. Beside 95.6±7.6 mg/kg DEHP in Lau Fau Shan soil exceeding the intervention value (60 mg/kg for total phthalates), 5337±111 mg/kg total petroleum hydrocarbon (TPH) exceeding the Dutch C level (5000 mg/kg) also was found in Lau

Fau Shan soil for this experiment. There were totally 80 m^3 contaminated soils dug in China Light & Power Company Limited (CLP), Tuen Mun, New Territories, Hong Kong. The soil was contaminated with 1153±244 mg TPH/kg, exceeding the Dutch B level.

2.1.1.1 DEHP in contaminated soil

Bis(2-ethylhexyl) phthalate (DEHP), the most common plasticizer, is a suspected human carcinogen, could damage liver and kidney, and might damage the development of reproductive organs (Moore et al., 2001; US Department of Health and Human Services, 2002; Lovekamp-Swan & Davis, 2003; Maire et al., 2005; Shailaja et al., 2008). The persistent nature made sewage sludge containing 55.1-163.3 mg DEHP/kg (Alatriste-Mondragon et al., 2003). However, environmental degradation rates of phthalates are slow and therefore do not play an important role in the removal under typical environmental conditions by hydrolysis, photodegradation and biodegradation (Staples et al., 1997; Asaoka et al., 2000). It is reported that more than 41% of the DEHP in sludge amended soil disappeared through mineralization after one year (Maden et al., 1999). DEHP has been found to be the most persistent and stable pollutant among phthalates (Shanker et al., 1985) with 75–90% remaining in soil after six months of incubation at room temperature (Kirchmann et al., 1991). DEHP has a strong tendency to adsorb to soil and sediments, as the solubility in water is very low. Therefore, the widespread DEHP arouses concern.

2.1.1.2 Oil in contaminated soil

Oil as a widely used product is one major chemical contaminant in soil (Yateem et al., 1997; Sarkar et al., 2004; Molina-Brarahona et al., 2004; Nalli et al., 2006). 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 (Sarkar et al., 2004; Kermanshahi Pour et al., 2005). Residual oil as represented by total petroleum hydrocarbons (TPH) could reach 20,000 mg/kg in an old industrial site near Barcelona, Spain (Sabaté et al., 2003). Spilled oil also introduces hazardous chemicals such as solvents benzene, toluene, ethylbenzene and xylenes, and polyaromatic polycyclic hydrocarbons (PAHs) (Table 2.1) (Liebeg and Cutright, 1999; Vasudevan and Rajaram, 2001; Sarkar et al., 2005). The Songhua River was contaminated by toxic benzene due to an explosion of a petrochemical plant on Nov. 13, 2005. The slick of cancer-causing benzene moving along China's Songhua River could pose a long-term risk to human health, contaminate the food chain and damage the region's fragile ecosystem. Because the residual level of benzene was at multiples of the national safety levels, the government halted the city's water supply for four days starting from Nov. 24 in face of the health hazard after consumption. The China government suffers a lot of economic loss on this event including the health damage to the environment and compensation paid to USSR and reputation damage owing to the slow and ineffective action strategy in handling environmental disasters.

Table 2.1 The pollutants in gasoline and the remediation criteria in soil (USEPA, 2002).

Pollutant	LD50 (oral-rat)	Dutch "B" standard (soil)
Benzene	930 mg/kg	0.5 mg/kg
Toluene	636 mg/kg	3 mg/kg
Ethyl benzene	3500 mg/kg	5 mg/kg
Xylene	4300 mg/kg	1 mg/kg
Naphthalene	533 mg/kg	5 mg/kg

2.1.2 Bioremediation of contaminated soil

Natural degradation of these persistent organic pollutants is slow as the indigenous microorganisms may not primarily target the persistent pollutants as a food source (Sarkar et al., 2004), and therefore remediation is needed (Staples et al., 1997; Asaoka et al., 2000). Bioremediation based on the microbial and/or plant metabolic activities has certain advantages over landfill disposal and incineration; For example, the conversion of toxic wastes to non-toxic end products, reduces health and ecological effects. Bioremediation, also, performs the treatment *in situ* without disturbing native ecosystems and is usually of low cost (Sarkar et al., 2004). Bioremediation, in terms of landfarming, biopiling using indigenous micro-organisms, biostimulation by stimulating the microbial growth and metabolism, bioaugmentation by enriching the microbial flora has been applied to treat oil-contaminated sites and DEHP sites (Al-Awadhi et al., 1996; Liebeg and Cutright, 1996; Cho et al., 1997; Vasudevan and Rajaram, 2001; Molina-Barahona et al., 2004; Shailaja et al., 2008).

There are two main treatment formats in bioremediation, *ex situ* and *in situ*. For *ex situ* treatment, there are landfarming, biopile and composting. Actually, landfarming is quite similar to biopile. Both of them are above-ground engineered systems that use oxygen to stimulate the growth and reproduction of aerobic bacteria to degrade the petroleum constituents adsorbed to soil. However, landfarms are aerated by tilling or plowing, but biopiles are aerated by forcing air to move by injection or extraction through slotted or perforated piping placed under the pile (Biowise et al., 2000). Composting refers to the addition of compost's primary ingredients to contaminated soil, where the compost matures in the presence of the contaminated soil (Semple et al., 2001). While for *in situ* applications, it includes bioslurry, bioventing and natural attenuation (Jorgensen et al., 2000; USEPA, 2004). One of the major obstacles in bioremediation of soils contaminated with synthetic organic compounds is the failure of laboratory remediation schemes to simulate the impact of field soil conditions on both the contaminant and the microorganism (Ward and Singh, 2004a, b). So the technology of bioremediation still needs further study and improvement.

2.1.3 The aim of this study

In this study, SMC was applied into environmental soil sample contaminated with diesel and DEHP. The pollutant removal capacity of SMC was evaluated with keeping moisture in natural situation, and tilling effect also was observed.

2.2 Material and Methods

2.2.1 Production of spent mushroom compost

Pleurotus pulmonarius strain PL-27 was cultured for SMC preparation. The cultivation compost of oyster mushroom *Pleurotus pulmonarius* was consisted of: sawdust (agent: Tai Wo Trading Company, China), wheat bran (agent: Wing Hing Loon, Tai Po, China), lime and sugar (food grade, Guangxi Metals and Minerals Import and Export Corporation, China) mixed in a ratio of 85:13:1:1 (w/w/w/w). After mixing, the constituents were then watered with 60% tap water (modified from Ching, 1997). The well-mixed cultivation compost was undergone fermentation for two weeks and then packed into autoclaved bags. After sterilization at 121°C for 1.5 hours, the substrate was inoculated with the oyster mushroom *P. pulmonarius*. The compost was then incubated in the dark at 28°C in mushroom cultivation complex, Department of Biology, The Chinese University of Hong Kong. When the mycelium had fully colonized the substrate (about 1 month), the bags were torn open to let the culture fruit. After mushroom harvest, the residue was called spent mushroom compost (SMC).

2.2.2 Characterization of spent mushroom compost

Mushroom yield was recorded for each flush. Ten bags of compost were taken at different stages of the mushroom cultivation: the compost fully colonized with white mycelia (day 28), compost at the first flush of fruiting (1^{st} F), first spent mushroom

compost (1st SMC) which is the compost after the harvest of the first flush of fruiting bodies, compost at the second flush of fruiting (2nd F) and second spent mushroom compost (2nd SMC) which is the compost residue after the harvest of the second flush of fruiting bodies. Fruiting bodies from both flushes were also collected and divided into tissues cap and stem for later analysis. After collection, compost material and the fruiting bodies were freeze dried. Protein and lignolytic enzymes in compost and organ fruiting bodies were extracted with distilled water in 1: 60 ratio (w/v) for three hours at 200 rpm and 4°C. The crude enzyme extract was filtered using Whatman No.1 filter paper and collected. The protein contents and the lignolytic enzymes activities were quantified with appropriate protein and enzyme assays mentioned below.

2.2.2.1 Protein assay

The protein content was measured by Folin-Phenol method with a micro-Lowry protein assay kit (Sigma P-5656). Seventy five µl protein standards/samples were mixed thoroughly with 750 µl Lowry Reagent solution. The solution was allowed to stand at room temperature for 20 minutes. Then 375 µl Folin Reagent Working Solution was added to the tubes and mixed and the colour of solution was allowed to develop for 30 minutes. The absorbances of standards (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml bovine serum albumin standard solution) and samples against blank were measured at a wavelength of 750 nm. The protein concentration of the samples were calculated from the protein standard curve according to their absorbance.

2.2.2.2 Laccase assay

The laccase activity was measured by ABTS assay modified from Lang et al. (1997) and Gálvez et al. (2000). The reaction mixture contained 1 mM 2,2'-azinobis-3-ethylbenthiazoline-6-sulfonate (ABTS), 100 mM succinic-lactic acid buffer (pH 4.5) and 100 µl sample, in a total volume of 1 ml. The reaction (formation of cation radical) rate was monitored at 420 nm (E_{max} = 36,000 M^{-1} cm^{-1}) at 25°C for a total of 3 minutes. A spectrophotometer (Milton Roy SP3000) fitted with time scan function was used. The reaction was initiated at the moment of sample addition and the increase in absorbance was monitored every 15s in the following 3 min in order to determine the slope of the rate of color change, which indicated the rate of catalytic reaction. An international enzymatic unit (IU) was defined as 1.0 µmol product formed per minute under the assay conditions were used to express the enzyme activities.

2.2.2.3 Manganese peroxidase assay

Manganese peroxidase was measured by MBTH/DMAB assay modified from Castillo et al. (1993) and Lang et al. (1997). The reaction mixture contained 0.07 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH), 0.99 mM 3-(dimethylamino)-benzoic acid (DMAB), 0.3 mM $MnSO_4$, 0.05 mM H_2O_2, 100 mM succinic-lactic acid buffer (pH 4.5) and 100 µl sample, in a total volume of 1 ml. The reaction (purple indamine dye product formation) was initiated by addition of H_2O_2, measured at 37°C at a wavelength of 590 nm (E_{max} = 53,000 M^{-1} cm^{-1}). A spectrophotometer fitted with time scan function

was used. Time zero was registered at the moment of addition of H_2O_2 (initiation of the reaction) and the increased absorbance was recorded every 15 seconds in the following 2 minutes.

2.2.2.4 Calculation of activities and specific activities of laccase and manganese peroxidase

Amount of product formed = Change of absorbance/ (Path length * Extinction coefficient)

Enzyme activity = Amount of product formed/Reaction time

Specific enzyme activity (enzyme purity) = Enzyme activity/Protein amount

where,

Path length of 1 ml cuvette = 1.

Extinction coefficient for laccase product = 36,000 and manganese peroxidase product = 53,000.

2.2.3 The application of SMC in field soil contaminated with petroleum and DEHP

2.2.3.1 Soil collection

Two batches each of one m^3 of mixed contaminated soils were collected from an industrial area with mixed factories practicing waste recycling, manufacturing of portable toilets and producing heavy instruments in Lau Fau Shan, New Territories,

for recycling resulted in heavy pollution with spilled oil and residue Bis(2-ethylhexyl) phthalate (DEHP), the most common plasticizer. A total of approximately 2 tons of soil was transported to CUHK.

2.2.3.2 Off-site *ex-situ* bioremediation of mixed contaminated soil

The first batch was contaminated with 6945±270 mg TPH/kg, 19007±706 mg oil and grease/kg and 99.2±17.1 mg DEHP/kg. The second batch was contaminated with 5367±84 mg TPH/kg, 14493±1455 mg oil and grease/kg and 95.4±5.8 mg DEHP/kg. Both TPH and DEHP contents exceeded the Dutch intervention values (5000 mg TPH/kg (Dutch C level) and 60 mg/kg for total phthalates).

One m^3 of the contaminated soils was divided into 2 groups of three piles for control or treatment. Five percents SMC were manually mixed into the contaminated soil in the treatment group. Tilling was also done to the control group for comparison. The initial soil moisture content was kept at 30% by spraying with tap water. The piles of soil were then watered with 25-l water weekly. Six samples of each 100 g were collected randomly from a pile and mixed together to form a composite sample of the pile at weekly interval for chemical soil analyses. During the treatment period, the effect of tilling to increase aeration was also monitored while SMC was added for the second time to speed up the biodegradation.

2.2.3.3 On site measurement of the physical characteristics of the air and soil

2.2.3.3.1 Air temperature and moisture

Air temperature and moisture were recorded using a thermohygrometer (Traceable Hygrometer S1930L).

2.2.3.3.2 Light intensity

Light intensity was recorded using light intensity meter (Li-Cor L1-250).

2.2.3.3.3 UV intensity

UV intensity was recorded using a UV meter (Spectroline DIX Series).

2.2.3.3.4 Soil temperature

Soil temperature was recorded using a thermometer.

2.2.3.4 Ecotoxicity of treated soil

2.2.3.4.1 Isolation and identification of bacterial and fungal isolates

2.2.3.4.1.1 Isolation

Aseptic techniques were performed for isolation of microorganisms in this part. One gram of soil sample (soil samples collected from contaminated area, Tsing Yi site

and Lau Fau Shan site) was weighed in an autoclaved test tube with 10.0 ml of sterilized 0.85% sodium chloride solution. Then this mixture was mixed with vortex and serial dilution (10^{-2} to 10^{-7}) was performed. One hundred microliter of each dilution of microbial solutions was transferred as an inoculum into Nutrient Agar plate (Difco) for bacterial growth. Another 100 µl of each dilution of microbial solutions was transferred as an inoculum into Potato Dextrose Agar PDA plate (Difco) for fungal growth. The solution was spread evenly on the plates with a glass spreader. For bacterial growth, the plates were incubated at 30°C for 2 days. Different bacteria were isolated according to their different shapes and colours. PDA plates were incubated at 28°C for 3 days. Similarly different fungi were selected. Microbes isolated were cultured again for purification in broth medium. The bacterium was first grown in Luria Broth (LB) medium at 30°C for 24 hours at 200 rpm. The cultures of bacteria were centrifuged and were washed with 0.85% NaCl for three times to remove residual nutrient, and freeze-dried for DNA extraction. The fungi were first grown in Potato Dextrose broth (Difco) at 28°C for 5 days at 200 rpm. Then the biomass was collected by filtration aseptically and washed by autoclaved deionised water for three times to remove residual nutrient, and freeze-dried for DNA extraction.

Table 2.2 Composition of NA plate (Difco).

Chemicals	Amount (g/l)
Peptone	5
Beef Extract	3
Sodium chloride	8
Agar	12

Table 2.3 Composition of PDA plate (Difco).

Chemicals	Amount (g/l)
Potato Starch	4
Dextrose	20
Agar	15

Table 2.4 Composition of Luria Broth (LB).

Chemicals	Amount (g/l)
Tryptone (Difco)	10
Yeast extract (LabM)	5
Sodium chloride (BDH)	5

Table 2.5 Composition of Potato Dextrose Broth (Difco).

Chemicals	Amount (g/l)
Potato starch	4
Dextrose	20

2.2.3.4.1.2 DNA extraction

The genomic DNA of a microorganism was extracted by NucleoSpin Plant DNA extract kit (740570250, Macherey & Nagel, Düren, Germany). Freeze-dried DNA powder was transferred to a 1.5 ml eppendorf and 400µl buffer C1 were then added to the tube. The mixture was vortexed thoroughly and incubated for 30 min at 60°C. The mixture was centrifuged for 5 min at 10000 rpm and then 300 µl of the clear lysate were transferred to a new tube with 300 µl buffer C4 and 200 µl ethanol. The mixture was mixed by inverting the tube 2-4 times, and the content was transferred to a NucleoSpin

Plant column with a 2 ml centrifuge tube. The sample was centrifuged for 1 min at 10000 rpm and flowthrough was discarded. 400 µl buffer CW were added onto the NucleoSpin Plant column and centrifuged for 1 min at 10000 rpm. Flowthrough was discarded. Another 200 µl buffer C5 were pipetted onto the NucleoSpin Plant column. The sample was centrifuged for 2 min at full speed in order to remove buffer C5 completely. The NucleoSpin Plant column was placed in a new 1.5 ml centrifuge tube. 100 µl elution buffer CE (preheated to 70°C) was added onto the membrane and the column was incubated for 5 min. The sample was centrifuged for 1 min at full speed to collect the DNA. DNA extraction was stored in -20°C for further use.

2.2.3.4.1.3 Polymerase chain reaction (PCR)

PCR were performed with a total volume of 20 µl of reaction mixture which was consisted of: 2.5 µl 10X Reaction Buffer, 2.5 µl $MgCl_2$, 1.6 µl 2.5 mM dNTPs, 0.5 µl 10 M primers, 0.1 µl 1.5 U Taq DNA polymerase (Thermoprime Plus) (cat no. AB-0301, ABGene), 12.3 µl ultra pure water and 0.5 µl DNA sample. PCR amplifications were performed in a PTC-100 Programmable Thermal Controller (MJ Research Inc., USA). For fungal 18s rDNA amplification, primers ITS1 and ITS2 were used and PCR program consisted of: 94/95 °C, 56 °C for 1 min and 72 °C for 1 min for 36 cycles with the last extension time lengthened to 10 min. The independent amplification reactions were done for each fungal DNA sample. For bacterial 16S rDNA amplification, specific PCR program consisted of 95°C for 1 min; 58°C for 1 min and 70°C for 1 min for 39 cycles with the last extension time lengthened to 10 min.

2.2.3.4.1.4 Agarose gel electrophoresis of DNA

After PCR amplification, PCR product was resolved by 2% agarose gel electrophoresis with Tris-acetate acid-EDTA (TAE) as buffer. The PCR reaction mixture sample was mixed with an appropriate amount of 6X agarose gel loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanole) to give a final concentration of 1X solution. The marker used was GeneRuler^{TM} 100 bp DNA Ladder Plus, ready-to-use (MBI Fermentas). One microliter DNA ladder was mixed with 1 µl loading buffer together with 4 µl water. Then the sample was loaded into the slots of the gel which was placed into the electrophoresis tank and covered by 1X TAE buffer. A voltage of 170 V was applied when the bromophenol blue has migrated for an appropriate distance through the gel. Afterwards, DNA was visualized using ethidium bromide staining. Gel image was captured using a gel documentation system (BIORAD Gel Doc 1000 with the Quantity One 1-D analysis software (version 4.6) (Bio-Rad).

2.2.3.4.1.5 Putative identities of the amplified DNAs

1). Purification of PCR products (Gene clean)

PCR products of expected size were purified by using GENECLEAN® II KIT (BIO 101, Inc.). Three volumes of sodium iodide stock solution were added to PCR product. One to five µl GLASSMILK® suspension was added and mixed well. After 5

minutes incubation at room temperature, the solution was centrifuged at 14,000 x g for 1 min. Supernatant was discarded and the pellet was resuspended in 20µl New Wash Solution (GLASSMILK®/DNA Complex). The pellet was recovered by centrifugation as done before. After the pellet had been washed for three times by vortex and centrifugation, it was allowed to dry under vacuum. Ten µl preheated (70°C) ultra pure water was used to resuspend the dried pellet and the mixture was allowed to stand for 5 minutes to elute PCR product. Supernatant (purified product) was collected after 2 min centrifugation. The purified PCR product was then checked for its quantity by gel electrophoresis and then undergone cycle sequencing in order to increase product amount. The DNA was labeled with different fluorescent dyes using DNA sequencing kit.

2). Sequencing

One µl forward primer of the target gene, 4 µl dRhodamine Terminator and 2 µl purified DNA template together with ultrapure water were mixed in order to make up to a final volume of 10 µl. The thermal profile for cycle sequencing was: 25 cycles of DNA denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and 60°C for 4 min (Table 2.6). Cycle sequencing products were transferred to PCR tube with 26 µl EtOH mixture (500 µl of 95% ethanol with 20 µl sodium acetate (pH 5.2)). The mixture was incubated in ice for 15 minutes for precipitation. Supernatant was discarded after centrifugation at 14,000 x g for 25 min. 180 µl 70% ethanol was transferred into PCR tube and vortexed well. Supernatant was discarded after centrifugation for 20 min. To the pellet, 12 µl Hi-

Dye Formamide was added and then the solution was vacuum dried for 5 min. The mixture was mixed by vortex and spinning down. DNA samples were denatured at 95°C for 2 minutes using thermal cycler PTC-100TM. Samples were placed in ice bath immediately for 5 minutes to prevent fusion of DNA strands. After spinning down, samples were ready for analysis by the MegaBACE 1000 DNA sequencer (Amersham pharmacia Biotech, Buckinghamshire, UK).

Table 2.6 The thermal profile of cycle sequencing.

Step	Procedures
1	96°C for 10 sec 50°C for 5 sec Repeat 25 cycles 60°C for 4 min
2	Hold at 4°C
3	Spin down the contents of the tube

3). Identification of sequence

Putative identification was made by homology search with Genbank using BLAST function (http://www.ncbi.nlm.nhi.gov/blast/Blast.cgi).

2.2.3.4.2 Indigenous bacterial ecotoxicity test

Six species of bacteria isolated from contaminated soil were used, including a *Methylobacterium* sp., a *Pseudomonas* sp. and a *Bacillus* sp. from the Tsing Yi soil samples (isolated by Miss Carmen Ho, 2004), and a *Pseudomonas* sp., a *Flavobacterium*

sp. and a *Bacillus* sp. from Lau Fou Shan soil samples. The bacterium was first grown in Luria Broth (LB) medium at 30°C for 24 h at 200 rpm. The cultures were centrifuged and were washed with sterilized 0.85% NaCl for three times to remove residual nutrient. One ml of each bacterial suspension was then inoculated into 2 g of autoclaved soil and incubated at 30°C for 3 days. The bacterial count on LB agar plate was done before and after the incubation by spread plate method. The difference between the changes in the number of bacterial colonies of control and treatment were used as an indicator of soil toxicity. The colonies were counted on dilution plates with 20-300 colonies. Bacterial colony forming units were calculated per g of soil.

2.2.3.4.3 Indigenous fungal ecotoxicity test

Six species of fungi isolated from contaminated soil samples were used, including a *Trichoderma asperellum*, *Trichoderma harzianum* and *Fusarium solani* (isolated by Miss Carmen Ho, 2004) from the Tsing Yi soil samples, and a Basidiomycete sp., and two *Penicillium glabrum* strains from Lau Fou Shan soil samples. The fungi were first grown in PDA broth (Difco) at 28°C for 5 days and were aerated by shaking at 200 rpm. Then the biomass was collected by filtration aseptically. Then the biomass was washed by autoclaved deionised water for three times to remove residual nutrient. The biomass was put into a sterilized blender with double volume of autoclaved ultra pure water and the biomass was blended into slurry form as an inoculum. One ml of inoculum was then inoculated into 2 g of autoclaved soil respectively. After incubation at 28°C for one week, the sample was extracted with 10

ml with acetone/hexane mixture (1:3; v/v) (Analytical grade, Lab-Scan) shaking at 200 rpm for 2 hrs. After the first extraction, it was repeated with another 10 ml acetone/hexane mixture for another 2 hours. The solvent was transferred in a 50 ml round bottom flask and concentrated at 60°C by a rotary evaporator with a vacuum pump (Rotavapor R-114, Büchl, Switzerland). One ml of 99% HPLC grade hexane was used to redissolve the sample after evaporation. The sample was filtered by a 0.45 μm filter (Acrodisc syringe filters 4CR PTFE) into gas chromatography vial for gas chromatography-mass spectrometry (GC-MSD) measurement (Shimadzu GCMS-QP5050A). The ergosterol content of 1 ml inoculum and the changes in the ergosterol content of control and treatment soil were used as a measure of soil ecotoxicity. The GC operating conditions and temperature profiles were as follows: temperature was raised to 80°C for 1.5 minutes and heated from 80°C to 290°C at 10°C/min and then maintained at 290°C for 5 minutes. The ergosterol content was measured before and after the incubation.

2.2.4 Soil characterization

2.2.4.1 Soil pH, electrical conductivity, and salinity

Ten gram of air-dried soil sample were extracted with 25 ml ultra-pure water (pH = 5.5) in 50 ml conical flask. The mixture was shaken at 200 rpm for 30 minutes. Then the liquids were filtered through Whatman no.1 filter paper (Allen, 1989; Landon, 1991). The prepared samples for pH, electrical conductivity and salinity were measured by pH

meter, conductivity and salinity meter, respectively. A pH electrode connected to Orion 410A+ pH Meter measured the pH value of the liquid sample. The electrical conductivity was measured by a Jenway Conductivity & pH Meter (4330, Jenway). One hundred microliter of each liquid sample was added into a Hand Held Salinity Refractometer with Automatic Temperature Compensation (A366ATC, Vista) with a pipetteman. Then the salinity of the sample was recorded from the reading in the meter. Between samples, ultrapure water was used to rinse the refractometer.

2.2.4.2 Moisture

About 10 g of soil samples were added into a pre-weighed Petri dish and then placed in a 105°C oven until constant weight (Allen, 1989). After cooling in desiccators, the dried soils were weighed and the water contents of the samples were calculated by mass difference.

2.2.4.3 Total organic carbon contents

A thin layer of oven-dried soil was placed into a preheated (900°C in oven) ceramic boat. The weight of soil was recorded. The carbon content of the sample was measured by a Total Organic Carbon Analyzer (Shimazu, 5000A). The carbon content was measured on a weight percent basis.

2.2.4.4 Soil texture

Soil texture is of major importance in determining nutrient storage in the rooting profile. The following table (Table 6) shows the particle diameters of the three main particle size groups, sand, silt and clay. The Bouyoucos hydrometer method (Allen, 1989) is adopted in textural analysis, which measures the decrease in density of the suspension as particles settle. Soil texture is determined with a hydrometer by determining the percentages of silt, clay and sand in soil samples, because the different particle sizes lead to different precipitate speeds. The larger particle size of sand results in the first precipitate, and then is the sedimentation of silt, and finally is the sedimentation of clay.

Fifty grams of air-dried 2 mm sieved soil sample were weighed and added into a container of a high-speed stirrer. Twenty five ml 5% Calgon solution (50 g sodium hexametaphosphate/l (Sigma P8510, pH = 9)) and 400 ml tap water were added. After stirring for 15 minutes, samples were transferred to a 1 l cylinder and diluted to 1 l with tap water. Then, a paddle was used to stir for 1 minute. A Bouyoucos soil hydrometer was used to commence timing for readings. Readings were taken at 4 min 48 seconds (for silt and clay content) and 5 hours (for clay content). For every degree above 19.5°C to each reading, 0.3 units were added. The soil moisture was determined at the time of weighing. The sand, silt and clay contents were expressed as percentages and the soil textural class was determined following the classification of International Society of Soil Science. Table 2.7 shows the particle size distribution in ISSS.

Table 2.7 Particle size distributions of sand, silt and clay in International Scale (International Society of Soil Science).

Fraction	Particle diameter (mm)
Sand	2.0-0.02
Silt	0.02-0.002
Clay	<0.002

2.2.4.5 Total nitrogen and total phosphorus contents

One g of air-dried soil was weighed into a digestion tube. Five ml 69% nitric acid, 1 ml 37% HCl and 0.5 ml 98% sulphuric acid were added (modified from Allen, 1989). Samples were heated in a heating digestion block (VELP DK 42/26) with the program of 1 hour at 160°C and 2 hours at 380°C. The samples were cooled down and diluted to about 10 ml with ultrapure water. Then the liquids were filtered through Whatman no. 1 filter paper and diluted to 50 ml with volumetric flask. Three sample blanks were also carried out in the same way. The total nitrogen and phosphorus contents were determined by San^{++} Automated Wet Chemistry Analyzer (AIA) (Skalar).

2.2.4.6 Available ammonium and nitrate contents

Available ammonium and nitrate were measured by San^{++} Automated Wet Chemistry Analyzer (AIA) (Skalar) after extraction with 2M potassium chloride (KCl) (BDH) (Allen, 1989; Dahnke and Johnson, 1990). Ten g of air-dried 2 mm sieved soil and 50 ml 2 M KCl solution were put in a 200 ml conical flask. Then the mixture was

shaken at 180 rpm for 15 minutes. Three blanks were run with 2 M KCl only. The suspension was allowed to settle and was filtered through Whatman no. 1 filter paper before measurement.

2.2.4.7 Available phosphate content

Available phosphate was determined by AIA after extraction with Troug's reagent ($(NH_4)SO_4$ buffer, pH = 3.0) (Troug, 1930). One g of air-dried 2 mm sieved soil and 50 ml Troug's reagent were put in a 200 ml conical flask. The mixture was shaken at 180 rpm for 30 minutes. Three blanks were run without soil. The suspension was allowed to settle and filtered through Whatman no. 1 filter paper before measurement.

2.2.4.8 Soil bacterial and fungal population

Aseptic techniques were performed for the procedures in this part. The method is the same as 2.2.3.4.1.1 section. The result was presented as the colony forming unit (cfu) per gram of dried soil.

2.2.4.9 Extraction of DEHP and organic pollutants

2.2.4.9.1 Extraction procedures

The DEHP residues were extracted from the tested samples using the national standard methods described by Zheng and Obbard (2003). Five gram soil were

extracted with 10 ml dichloromethane (DCM) (Analytical grade, BDH) shaking at 200 rpm for 2 h. After the first extraction, it was repeated with another 10 ml solvent for another 2 hours. The extract was transferred in a 50 ml round bottom flask and then concentrated at 60°C by a rotary evaporator with a vacuum pump. One ml of acetone (HPLC grade, Labscan) was used to redissolve the residue after evaporation. The extract was filtered by a 0.45 μm filter (Acrodisc syringe filters 4CR PTFE) and kept at -20°C for gas chromatography-mass spectrometry (GC-MSD) measurement.

2.2.4.9.2 GC-MS conditions

Separation of sample components was performed on a 0.25 mm (i.d.) × 30 m HP-1 methyl siloxane capillary column coated with a 0.25 μm film (Hewlett Packard HP19091Z-413). The conditions and temperature profiles for PAHs analysis are listed in Tables 2.8 and 2.9. DEHP was resolved at 27.06 min by GC-MSD.

Table 2.8 Temperature profile for GC-MSD for sample analysis.

Oven situation	Rate (°C/min)	Temperature (°C)	Hold time (min)	Run time (min)
Initial	/	60	1.5	1.5
Ramp	8	300	10.0	41.5
Post run	/	60	0.5	42.0

Table 2.9 Conditions of GC-MSD for DEHP analysis.

Parameter	Condition
Carrier gas	High purity helium
Column flow rate	2 ml/min
Column pressure	122.2 kPa
Oven temperature	60°C
Oven equilibrium time	1.5 min
Injection temperature	250°C
Interface temperature	250°C
Final temperature	300°C
Ramp temperature	8°C/min
Hold time	10 min
Split mode	Splitless
Solvent cut	4 min

2.2.4.10 Oil and grease content

Oil and grease content was determined by a gravimetric method modified from Juteau *et al.* (2003). One gram oven-dried soil sample was suspended in 10 ml ultrapure water, acidified with 2-3 drops of 37% hydrochloric acid (Merck) and extracted five times with 10 ml ethyl acetate (Analytical grade, Labscan). Extracts were dehydrated with anhydrous sodium sulfate (Fischer Scientific) and filtered using syringes with PTFE 0.45 μm-micro filter into a preweighed 100 ml round-bottomed flask. After evaporation with a rotary evaporator kept at 30°C, the flask was put into a deep freezer for 2 hours and freeze dried with a freeze dryer (Labconco, Missouri, USA) under

vacuum for 3 days, and then weighted. The oil and grease content of the sample was calculated based on the weight difference of the flask.

2.2.4.11 Total petroleum hydrocarbons (TPH)

Total petroleum hydrocarbons content was analyzed by a gravimetric method (modified from USEPA 1664 Reference Method). Five gram oven-dried soil sample were suspended in 10 ml ultrapure water, acidified with 2-3 drops of 37% hydrochloric acid (Merck) and extracted three times with 10 ml n-hexane (Analytical grade, Labscan). Extract was dehydrated with anhydrous sodium sulfate (Fischer Scientific) and filtered using syringes with PTFE 0.45 μm-micro filter. After five gram of silica gel 60 (Merck) were mixed with the extract, the extract was filtered using Whatman No.1 filter paper to remove silica gel and soil into a preweighed 50 ml round-bottomed flask, and the solvent was evaporated with a rotary evaporator kept at 40°C. The flask was freeze-dried by freeze-dryer for 3 days. The TPH content of the sample was calculated based on the weight difference of the flask.

2.2.4.12 Total heavy metal analysis

All glassware and plastic wares were acid-treated in an acid bath made with 10% hydrochloric acid (HCl) (Merck, Germany) for 24 h. All of them were rinsed with deionized water and transferred to a water bath for at least 5 hours and then oven-dried. The heavy metals were extracted from the samples by microwave digestion system

(Milestone ETHOS Microwave Digester) to solubilize metal ions into solution (modified from USEPA 3051 reference method). For every sample, 0.2 g oven-dried sample was weighed and put into a TFM vessel and 18 ml 69% HNO_3 (BDH) was added. The TFM vessel was sealed and heated in the microwave digester. Temperature program was first increased to 170°C in 15 min and then increased to 180°C in 10 min. After cooling in fume hood, 10 ml of ultrapure water were added. Then, the solution was filtered through a Whatman No.1 filter paper and diluted with ultrapure water in 25 ml volumetric flask. The digested samples were stored in PE bottles at 4°C until measured. The heavy metals contents were analyzed by inductively coupled plasma (ICP) spectrophotometer (ATOMSCAN16 ICP-AES, Thermo Jarrell Ash) or Atomic Absorption Spectrophotometer (AAS) (Polarized Zeeman Z-8100 AAS, Hitachi).

2.2.4.13 Extraction efficiency

Extraction efficiency (EE) (%) = $(A_e/A_i) \times 100$ %

EE is the percentage of target DEHP or heavy metal extracted from the soil; A_e is the amount of chemical (mg) adsorbed on soil and A_i is the amount of chemical (mg) added into the soil.

In order to check the extraction efficiencies of DEHP and heavy metals, 3 replicates of blank sample and 3 replicates of soil sample with known concentrations of

chemicals spiked were prepared. The extraction efficiency can be calculated by the equation:

([sample added with chemical] - [blank sample])/ Conc. of chemical added x 100%.

The extraction efficiencies of DEHP and heavy metals are listed in the Table 2.10 .

Table 2.10 Extraction efficiencies of DEHP and heavy metals in wheat. Data are presented in mean $\pm$ SD of 5 replicates.

Pollutant	Extraction Efficiency (%)
DEHP	94.3±2.8
Pb	96.5±4.8
Cu	97.3±3.1
Ni	94.9±3.5
Zn	100.8±6.3

2.2.5 Statistical analysis

Data were presented in mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine any significant difference among the datasets of the treatments and the control. If only two sets of data were compared, student t test was used instead of ANOVA. The statistical analysis was carried out with the Statistical Analysis System version SPSS 11.5. If there was significant difference among the groups ($p<0.05$), ranking of the groups was performed with Tukey test ($p=0.05$) and letters a, b, c, etc. represent the ranking from the highest to the lowest.

2.3 Results

2.3.1 Characterization of spent mushroom compost

2.3.1.1 Mushroom yield

Two flushes of fruiting bodies were collected and weighted. The mushroom yield in the first flush (462±134 mg/g compost) was more than that in the second flush (191±100 mg/g compost). The biological efficiency (BE), calculated as the percentage yield of fresh mushroom over the dry weight of the substrate, was 23.7±4.5 %.

2.3.1.2 Protein content

As shown in Figure 2.1 (a) and (b), the protein contents of the fruiting bodies from both flushes were higher than those in compost, and the protein contents in the caps were more than those in stems. In addition, protein was accumulated in the compost along cultivation.

(a) (b)

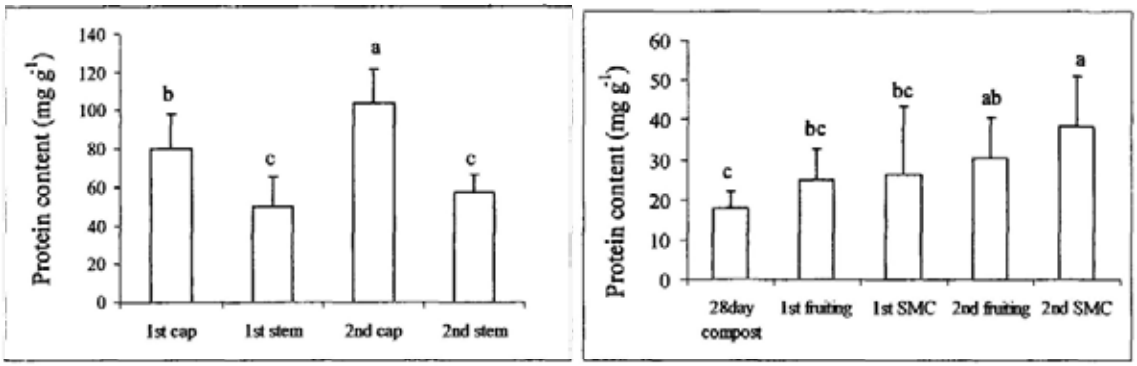
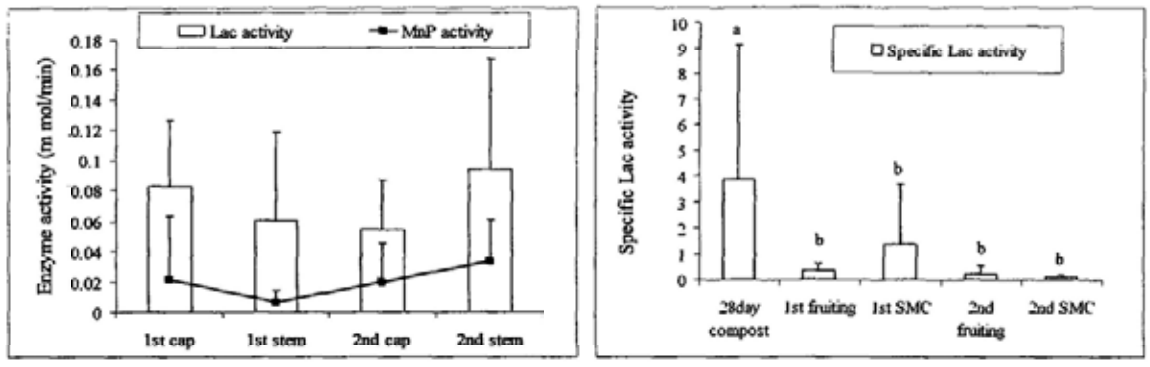


Figure 2.1 The change in protein contents of *Pleurotus pulmonarius* along artificial cultivation by solid-state-fermentation. (a) Mushroom composts at different stages of solid-state-fermentation, and (b) fruiting bodies harvested from the two flushes. Data are presented in mean ± SD of 10 replicates. Means with the same letter are statistically similar (one way ANOVA with Tukey test, $p < 0.05$).

2.3.1.3 Specific lignolytic enzymes activities

Specific laccase and manganese peroxidase (MnP) activities could be detected in caps and stems from both flushes, but the enzymes showed relatively lower activities than those in compost (Figure 2.2 (a)). Specific laccase and manganese peroxidase activities did not show the significant difference in composts at different stages of solid-state-fermentation, but in general they were maximal during 28 day cultivation and declined sharply at fruiting stage and rose in the intercrop period between two flushes (Figure 2.2 (b) & (c)). Although the two enzymes exhibited similar patterns of regulation, specific laccase was around 2 to 10-fold more active than specific MnP in the corresponding developmental stages.

(a) (b)



(c)

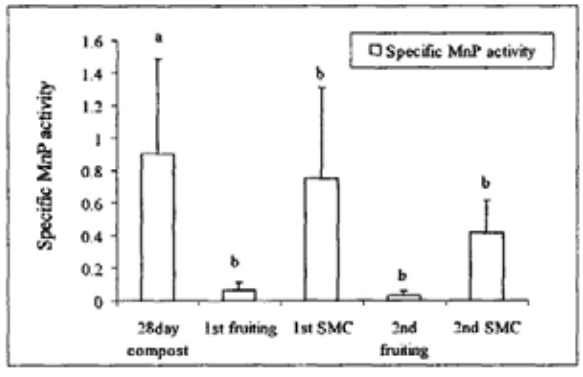


Figure 2.2 The change in lignolytic enzyme activities of *Pleurotus pulmonarius* along solid-stage-fermentation. (a) Fruiting bodies harvested from the two flushes, (b) and (c) Mushroom composts at different stages of solid-state-fermentation. Data are presented in mean $\pm$ SD of 10 replicates. Means with the same letter are statistically similar (one way ANOVA with Tukey test, $p < 0.05$).

2.3.2 Soil characteristics

The physical-chemical properties of SMC with undetectable TPH and DEHP contents and the contaminated soils are listed in Table 2.11. Both TPH and DEHP contents in soil were higher than safety guidelines Dutch C level (5 g/kg soil) for TPH

and 60 mg/kg soil for DEHP. There are 430±46, 136±38 and 477±24 mg/kg for lead, copper and zinc in initial soil. These metal contents exceeded the Chinese standards of soil quality (GB 15618-1995).

2.3.3 Off-site *ex-situ* bioremediation of batch 1 mixed contaminated soil

2.3.3.1 Soil and air temperature, light intensity and UV conditions

The soil and air temperatures on site are plotted against time in Figure 2.3. The soil temperature and air temperature fluctuated during monitoring but followed similar patterns between them. Due to the coming of winter, air and pile temperatures decreased. The range of soil temperature was from 25°C to 32°C. There was the great fall at day 70 for the UV 365 nm, which was due to the wet and cloudy weather, and also at that day there was lower air temperature (Figure 2.4). Moreover, higher temperature and good climate result in the peaks for UV 365 and 254 nm at day 65.

Table 2.11 The physical-chemical properties of SMC and batch 1 mixed contaminated soil. Data are presented in mean ± SD of 5 replicates.

Parameters		SMC	Contaminated soil
Total organic carbon (%)		21.7±1.7	4.9±0.3
$NO_{X\ water\text{-}soluble}$ (mg/kg)		231±21	14±0
$NH_{3water\text{-}soluble}$ (mg/kg)		276±51	6±1
$N_{Kjeldahl}$ (mg/kg)		2227±381	670±102
$P_{water\text{-}soluble}$ (mg/kg)		205±26	47±14
P_{total} (mg/kg)		769±162	249±24
K^+ (mg/kg)		2826±396	619±28
pH		7.3±0.2	7.9±0.1
Electrical conductivity (μS/cm)		2397±321	214±14
Salinity (‰)		32.0 ± 1.0	1.1±0.2
Moisture (%)		59.1±1.0	15.5±1.1
Stone (%)		—	37.2±5.0
Soil texture	Sand (%)	—	69.8±0.4
	Clay (%)	—	8.0±0.6
	Silt (%)	—	22.1±0.3
	Texture class	—	Sandy loam
Bacteria ($\times 10^5$ CFU/g)		1132±263	53±9
Mold ($\times 10^3$ CFU/g)		563±85	24±4
Total petroleum hydrocarbon (TPH) (mg/kg)		—	6945±270
Oil & grease (mg/kg)		—	19007±706
DEHP (mg/kg)		—	99.2±17.1

" — " not detected

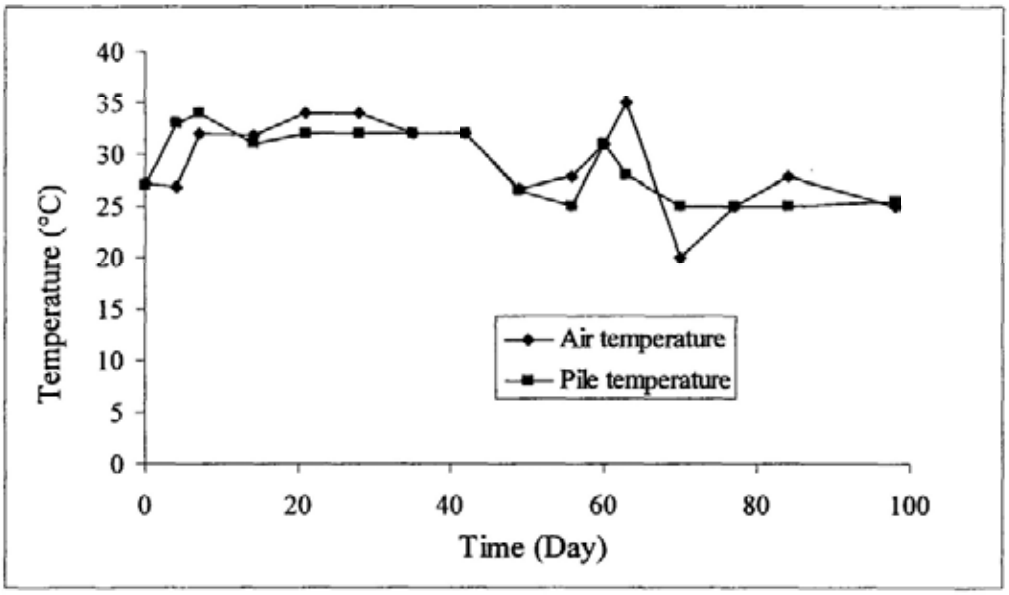


Figure 2.3 The soil and air temperatures measured at 14-15 o'clock during monitoring in batch 1 mixed contaminated soil.

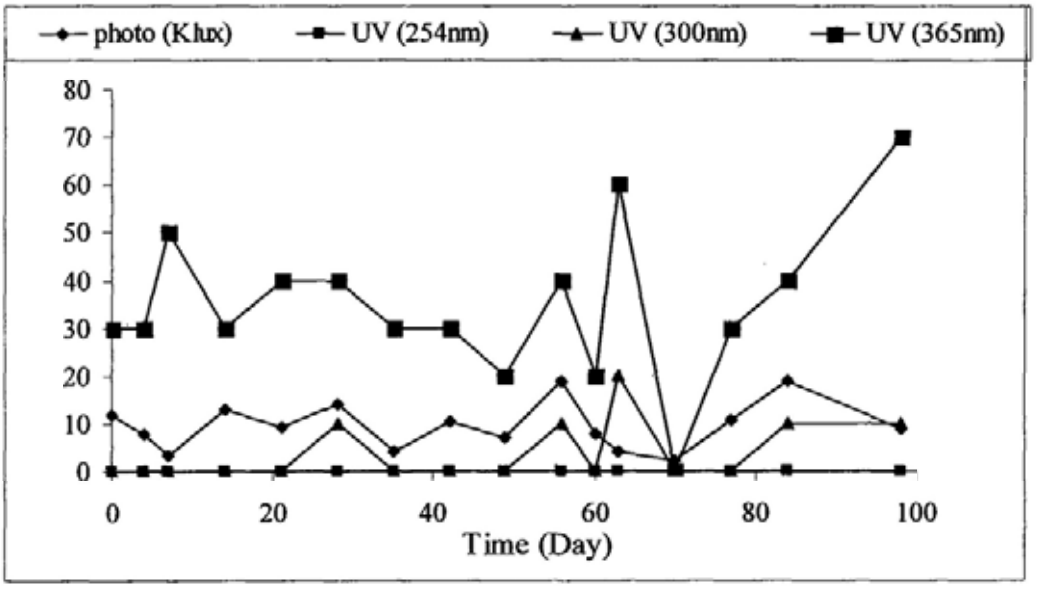


Figure 2.4 The site physical properties measured at 14-15 o'clock during monitoring in batch 1 mixed contaminated soil.

2.3.3.2 The changes of pollutants and microbial population in soil

In batch 1 mixed contaminated soil, tilling was practiced on day 35 and 77. SMC was added at day 0 and 56 to the treatment group. Figure 2.5 shows the decrease in pollutant TPH and oil and grease contents in the treatment process in comparison to the spontaneous decrease in the control. It took 100 days to complete the removal of the TPH to reach Dutch B level (1000 mg/kg). The content of TPH in control soils had a slight drop in this study. The disappearance of TPH might be due to the presence of indigenous degrading microorganisms (Figure 2.6). In treatment soils, the content of TPH had a rapidly drop after the first addition of SMC, and the second addition of SMC gave a similar effect on the decrease of TPH content. However, the first tilling has no significant effect on degradation of pollutant, while the second tilling had a slight effect on the removal of TPH. Compared these two tilling stages, higher moisture (Figure 2.7) was observed in soils during the second tilling stage, which stimulated the growth of mold population with lower air temperature (Figure 2.6 (b)). Therefore, mold population may contribute to the degradation of pollutants. In addition, bacterial population increased by *Pleurotus* compost began to drop since the bacterial population after inoculation might have exceeded the carrying capacity of soil, but the bacterial population in treatment soils was still higher than that in control soils after 3 months of cultivation (Figure 2.6 (a)). As shown in Figure 2.3, the content of oil and grease showed a similar decreasing tendency. For another persistence pollutant DEHP, the degradation of this compound was successful as well (Figure 2.8). After first the addition of SMC, the content of DEHP had dropped below the intervention value 60

mg/kg. No effect of tilling was found in the degradation of DEHP as in the case with TPH.

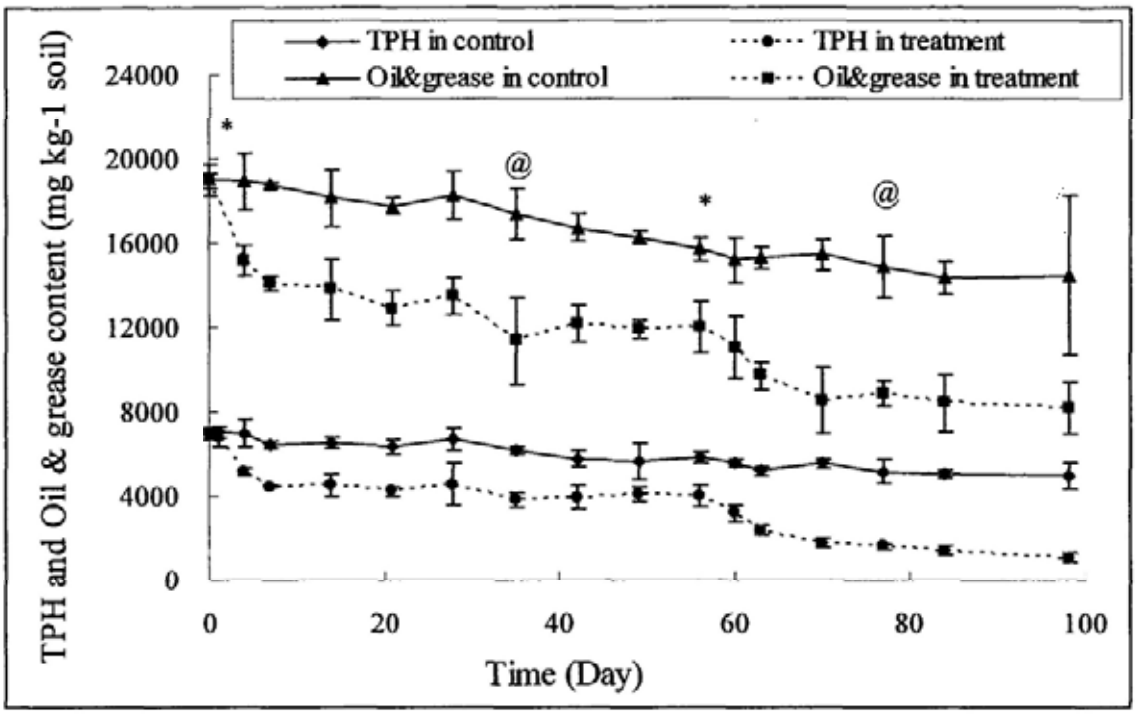
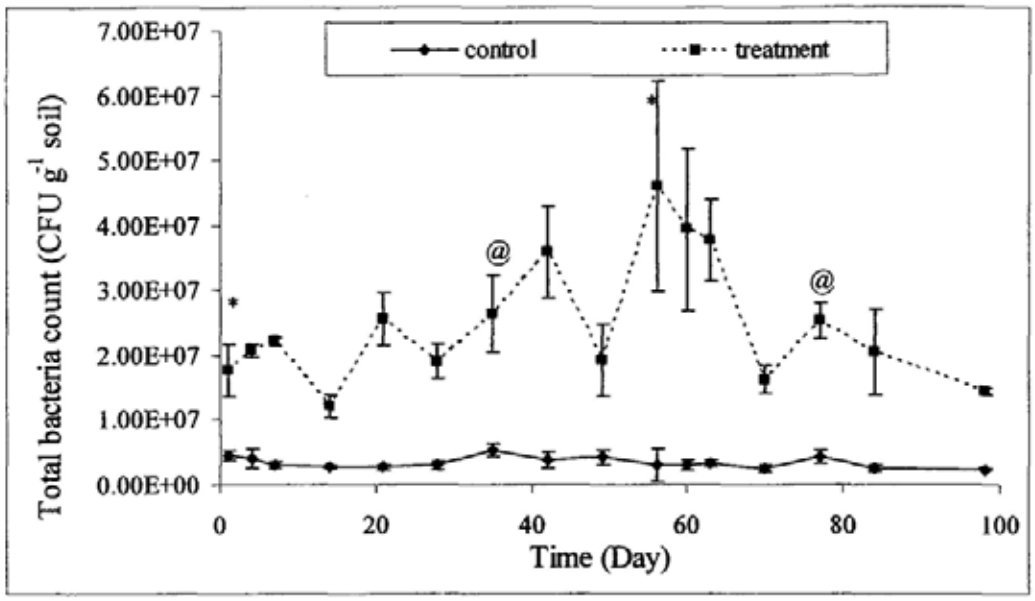


Figure 2.5 The change of TPH and oil and grease contents of the batch 1 soil under treatment in contrast to control. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment or tilling in control; @, tilling.

a)



(b)

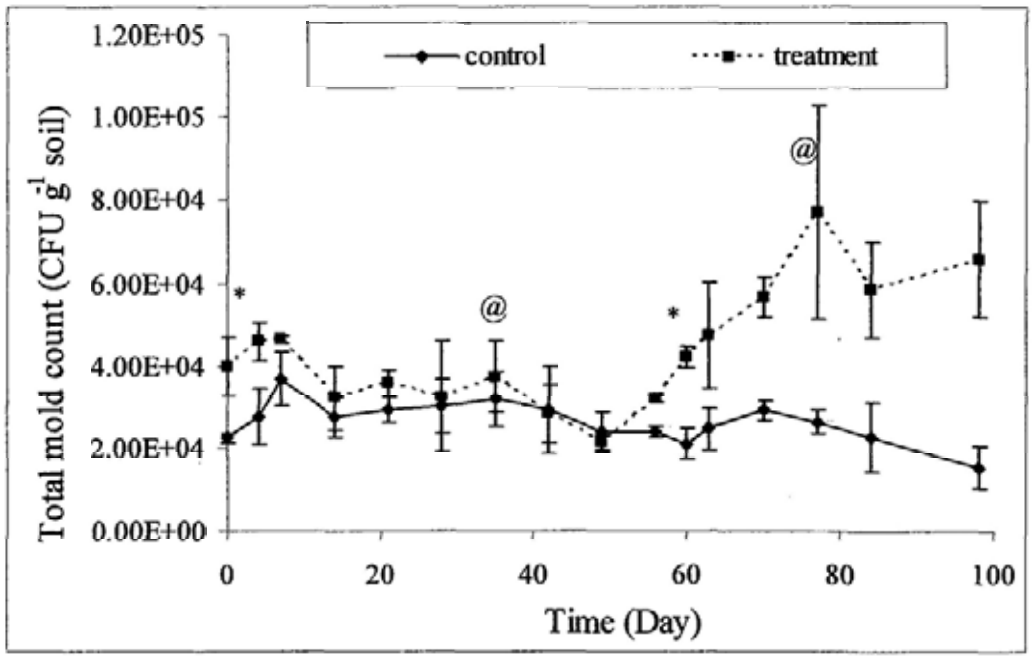


Fig. 2.6 The change of (a) total bacteria population and (b) total mold population in control and treatment of the batch 1 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment or tilling in control; @, tilling.

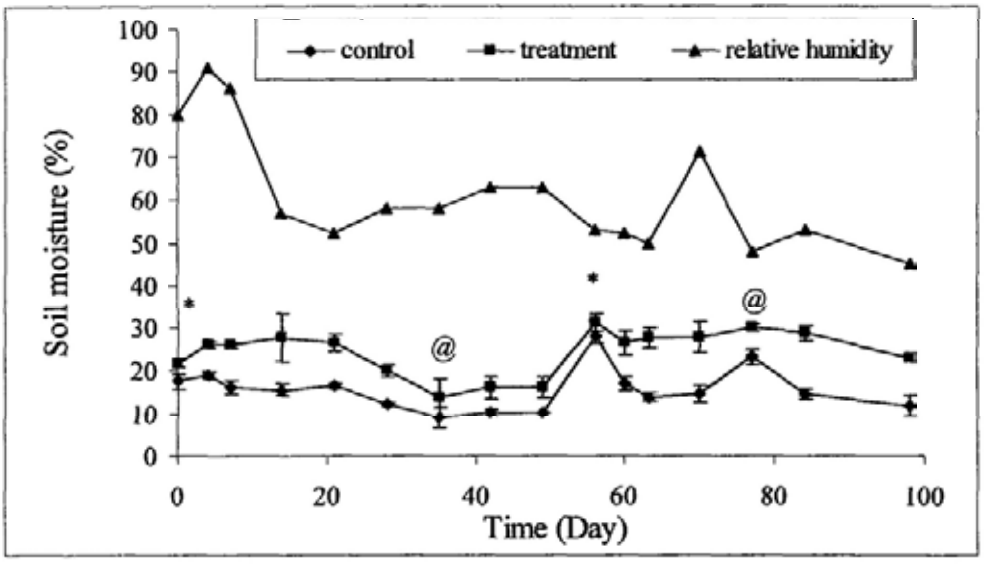


Figure 2.7 The change of soil moisture under treatment in contrast to control in Batch 1 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment or tilling in control; @, tilling.

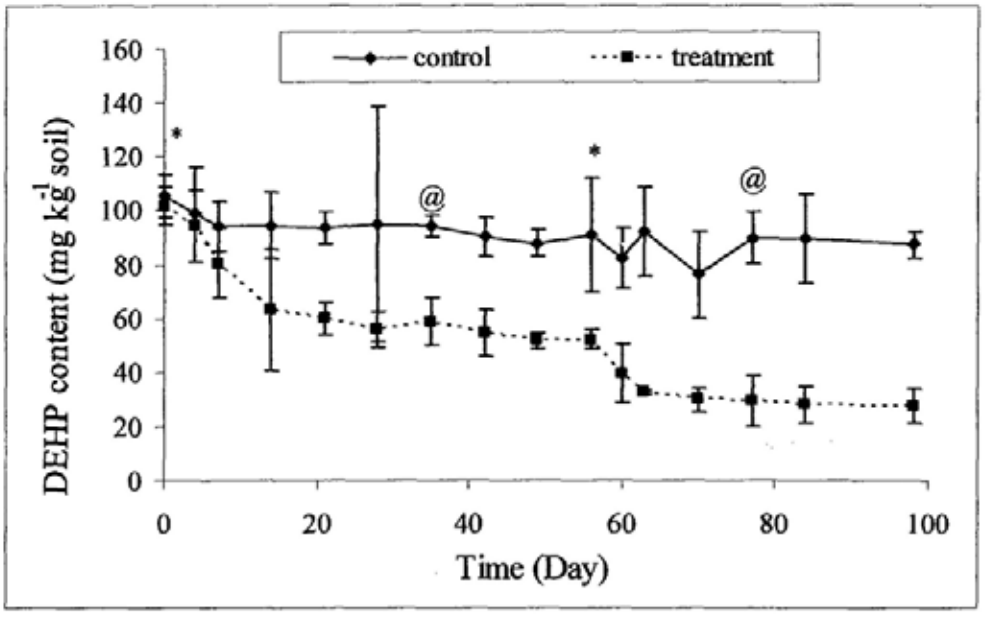


Figure 2.8 The change of DEHP contents of the soil under treatment in contrast to control in Batch 1 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment or tilling in control; @, tilling.

2.3.3.3 The changes of nutrients and physical properties in soil

Soil nutrients were significantly higher in the treated soil than those in control soils, such as TOC, nitrogen and phosphate contents, which may be attributed to the slow release of nutrients from SMC (Table 2.12). There was also an increase in electrical conductivity. The initial soil pH was slightly alkaline (pH 7.92±0.05). When SMC was added into soil, pH value could be neutralized and dropped to 7.02±0.08 and 7.04±0.04 at the first and second addition respectively. Therefore, after treatment, pH value of treatment piles had dropped from 7.90±0.06 to 7.74±0.06, showing a neutralizing trend compared with initial soil (Figure 2.9). In addition, due to the higher water holding capacity of SMC, the soil during and after treatment could maintain higher moisture. Higher moisture and nutrients could stimulate the growth of microbes, which may contribute fundamentally to biodegradation of petroleum and DEHP. Furthermore, the application of SMC decreased stone percentage and increased clay percentage when compared with control and initial soil samples. This implies that the addition of SMC could change soil texture and improve soil structure. As a consequence, improvement of soil properties could provide better conditions for bioremediation.

Table 2.12 Modification of the physico-chemical properties of the batch 1 mixed contaminated soil treated by SMC. Data are presented in mean ± SD of 3 replicates. (Treatment time: 100 days)

Parameters		Before treatment		After treatment	
		control	treatment	control	treatment
Total organic carbon (%)		4.7±0.2	4.9±0.4	$4.4±0.2^{A}$	$5.4±0.2^{B}$
$NO_{X\ water\text{-}soluble}$ (mg/kg)		$13.5±0.0^{a}$	$13.7±0.2^{a}$	$14.1±0.1^{bA}$	$14.4±0.1^{bB}$
$NH_{3water\text{-}soluble}$ (mg/kg)		5±0	$6±1^{a}$	$5±0^{A}$	$7±0b^{B}$
$N_{Kjeldahl}$ (mg/kg)		622±108	718±85	$655±186^{A}$	$1002±99^{B}$
$P_{water\text{-}soluble}$ (mg/kg)		63±2a	$52±20^{a}$	93±8b	117±4b
P_{total} (mg/kg)		245±14	254±35	$228±27^{A}$	$341±49^{B}$
K^{+} (mg/kg)		621±42	$616±13^{a}$	652±27	$742±60^{b}$
pH		7.95±0.02	$7.90±0.06^{a}$	7.83±0.07	$7.74±0.06^{b}$
Electrical conductivity (μS/cm)		202±8	222±10	$206±17^{A}$	$293±59^{B}$
Salinity (‰)		1.2±0.3	1.2±0.3	$1.3±0.3^{A}$	$2.3±0.3^{B}$
Soil moisture (%)		$17.6±1.9^{a}$	$15.6±0.8^{a}$	$12.0±2.4b^{A}$	$23.0±1.0^{bB}$
Stone (%)		35.3±0.3	38.5±6.5	35.0±4.3	35.1±4.2
Soil texture	Sand (%)	69.8±0.4	$70.5±0.5^{a}$	$70.1±0.6^{A}$	$66.9±0.8^{bB}$
	Clay (%)	8.0±0.6	$7.7±0.2^{a}$	$8.1±0.5^{A}$	$9.9±0.4^{bB}$
	Silt (%)	22.1±0.3	21.8±0.4	21.9±1.0	23.3±0.6
	Texture class	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Bacteria ($\times10^{5}$ CFU/g)		$46±7^{a}$	$57±9^{a}$	$42±8b^{A}$	$284±72^{bB}$
Mold ($\times10^{3}$ CFU/g)		23±1	$144±5^{a}$	$15±0^{A}$	$65±14^{bB}$

Data indicated with different small letter superscripts show significant difference at ($P < 0.05$) within the same control or treatment groups at initial and final stages. Data indicated with different capital letter superscripts show significant difference at ($P < 0.05$) between the control and treatment groups at initial or final stages.

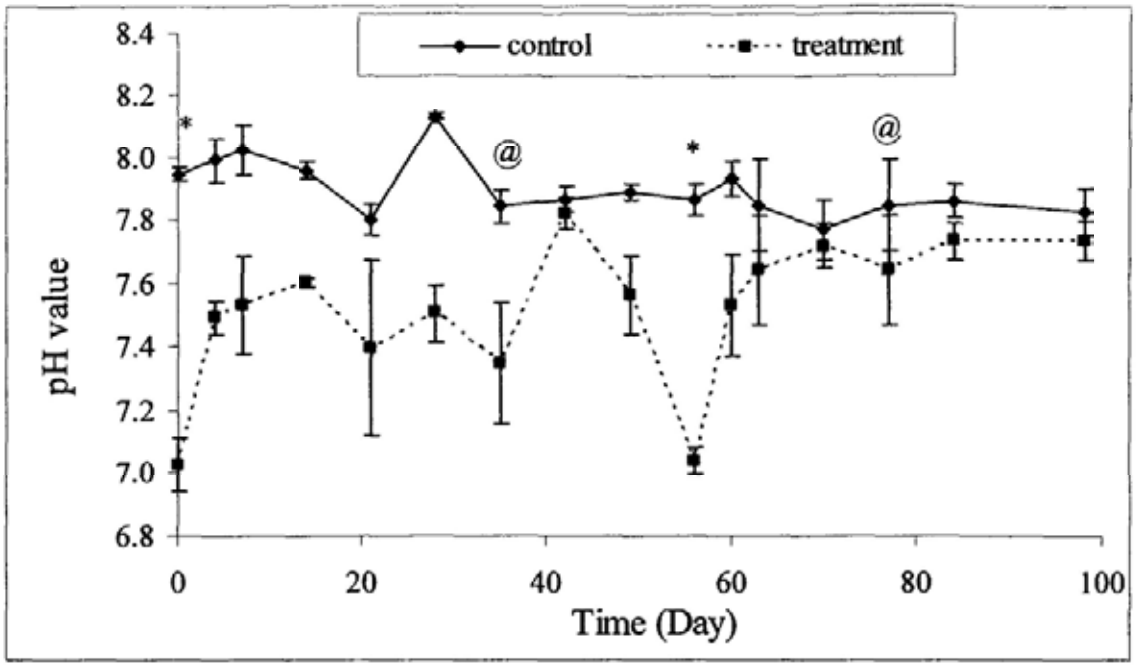


Figure 2.9 The change of pH values of the batch 1 soil under treatment in contrast to control. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment or tilling in control; @, tilling.

2.3.3.4 Yield and quality of the harvested mushrooms and wheat

In addition, during this experiment, mushrooms were harvested and their DEHP and heavy metal contents were measured. The results were 0.36±0.40 and 22±7 mg/kg for DEHP and copper, respectively, while other heavy metals in mushroom could not be detected. In addition, there were no significant differences in soil heavy metals contents before and after treatment. This result indicates that the application of SMC has no effect on the removal of heavy metals in soil.

After treatment, wheat seeds were sown in treated soil, and the wheat growth and harvested seeds were compared with the wheat grown in agricultural soil. The results show that the average weight of 10 heads in treated soil did not differ from those grew in agriculture soil. Also, the 100 seeds weight did not show significant difference between wheat in both types of soil (Figure 2.10). The growth of wheat in terms of the height did not differ between treated soil and agriculture soil (Figure 2.11). The results in figure 2.12 show the effect of soil contaminated with heavy metals on the wheat seeds. The wheat in treated soil contained more heavy metals than those in normal agricultural soil. This reflectes the accumulation of heavy metals in wheat from soil (Figure 2.12).

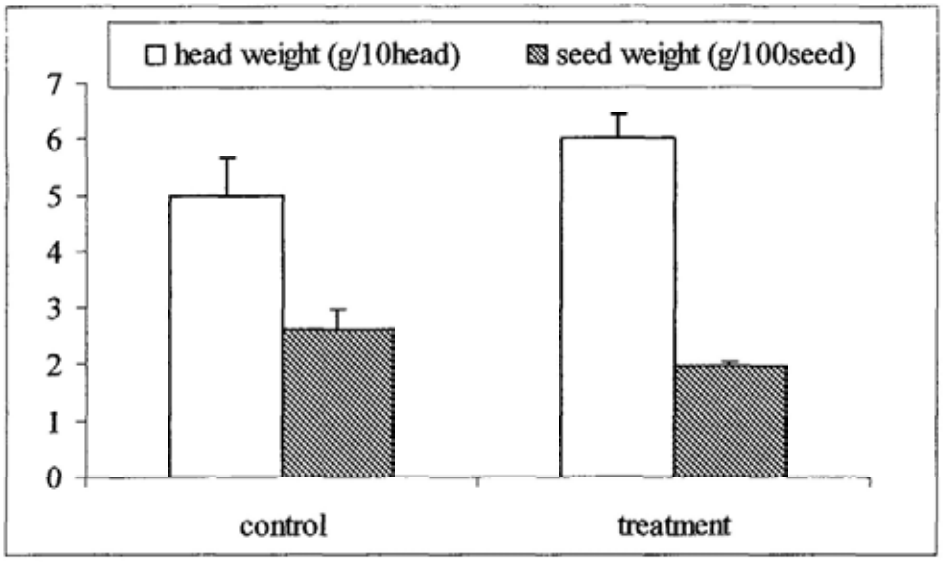


Figure 2.10 Wheat quality grew in treated soil in contrast with normal agricultural soil. Data are presented in mean $\pm$ SD of 3 replicates. Data do not show significant difference at 5% levels after Student T test.

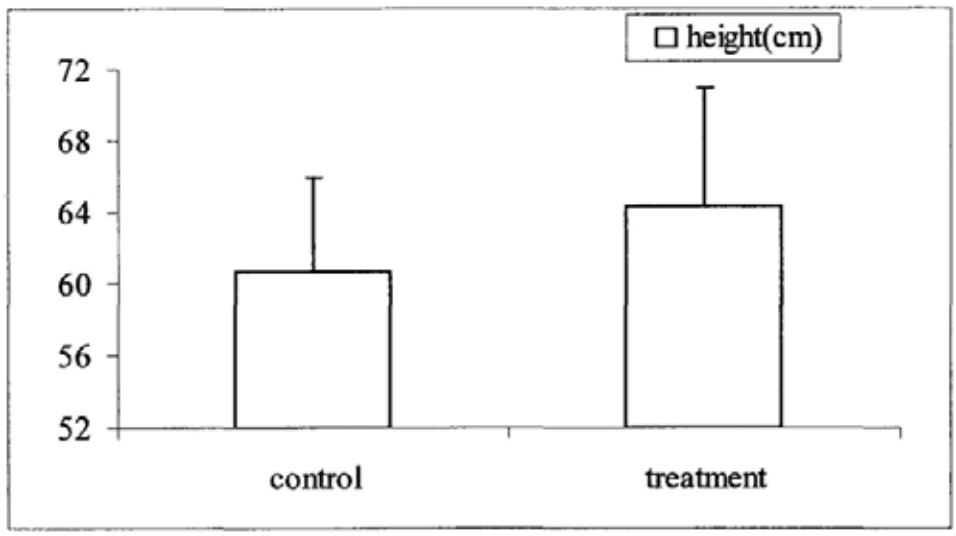


Figure 2.11 Wheat height grew in treated soil in contrast with normal agricultural soil. Data are presented in mean ± SD of 3 replicates. Data do not show significant difference at 5% levels after Student T test.

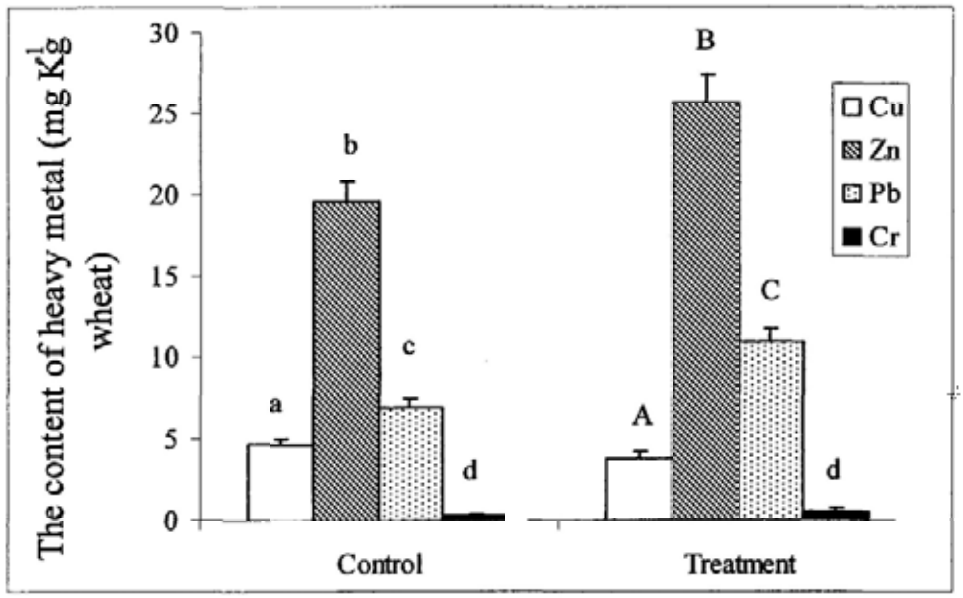


Figure 2.12 The heavy metal contents in wheat grown in normal soil and treated soil. Data are presented in mean ± SD of 3 replicates. Data indicated with different small or capital letters show significant difference at 5% levels after Student T test.

2.3.4 Off-site *ex-situ* bioremediation of batch 2 mixed contaminated soil

2.3.4.1 Soil and air temperature, light intensity and UV conditions

The soil and air temperatures on site were plotted against time in Figure 2.13. The soil temperature and air temperature had similar patterns between them. However, the temperature was lower than that in batch 1 soil under treatment due to the seasonal change.

2.3.4.2 The change of pollutants and microbial population in soil

Batch 2 mixed contaminated soil was used to evaluate the capacity of SMC on bioremediation without tilling between two applications of SMC. At the second week after the first addition of SMC, the same amount of SMC was applied into soil again. The contents of TPH, oil and grease, and DEHP were not different between initial and final samples in control piles in such short period of incubation time, 28 days (Figure 2.15 & 2.16). However, both TPH and DEHP showed rapid drop in treatment piles with a linear rate. TPH content had dropped below Dutch C level 5000 mg/kg after first application of SMC, and second application of SMC promoted the degradation of TPH again (Figure 2.15). This shows the stronger removal capacity of SMC. After treatment, TPH content had decreased to the Dutch B level 1000 mg/kg. The degradation of DEHP also could be observed from Figures 2.16 and 2.17. DEHP content was reduced by 36%

after the first application of SMC and dropped to 43.9 $\pm$ 0.9 mg/kg after the second application of SMC.

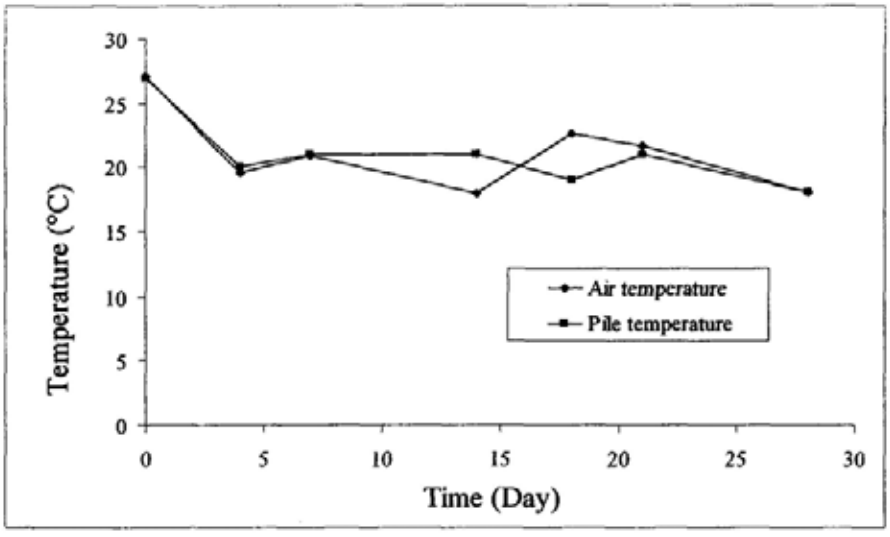


Figure 2.13 The soil and air temperatures measured at 14-15 O'clock during monitoring in Batch 2 mixed contaminated soil.

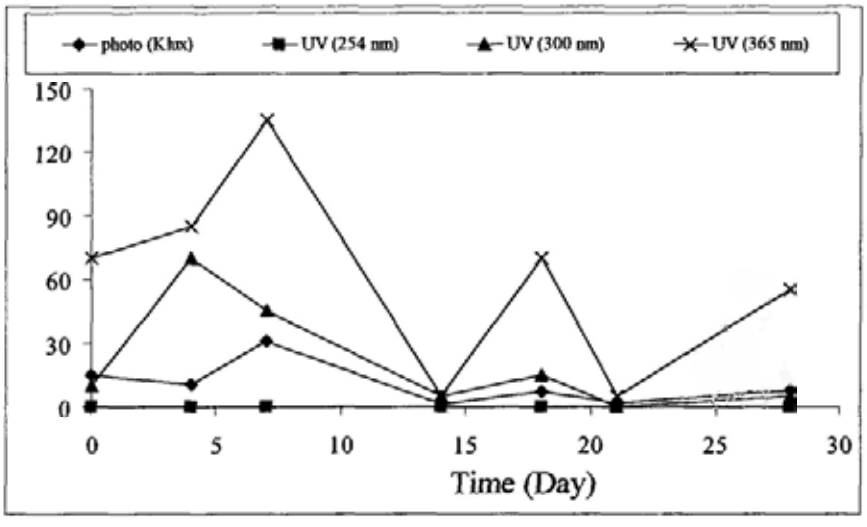


Figure 2.14 The site physical properties measured at 14-15 O'clock during monitoring in Batch 2 mixed contaminated soil.

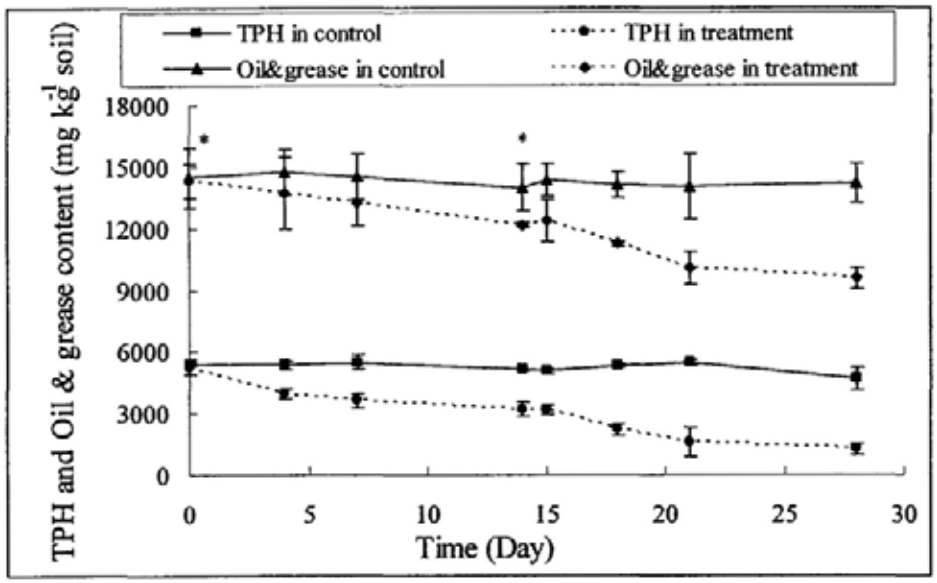


Figure 2.15 The changes of TPH and oil and grease contents of the batch 2 soil under treatment in contrast to control. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment and tilling in control.

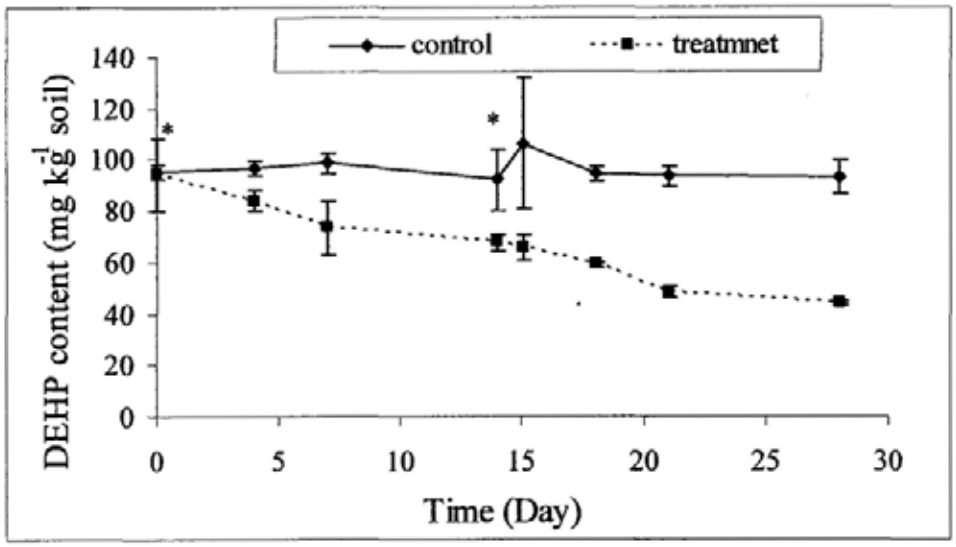


Figure 2.16 The change of DEHP content of the batch 2 soil under treatment in contrast to control. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment and tilling in control.

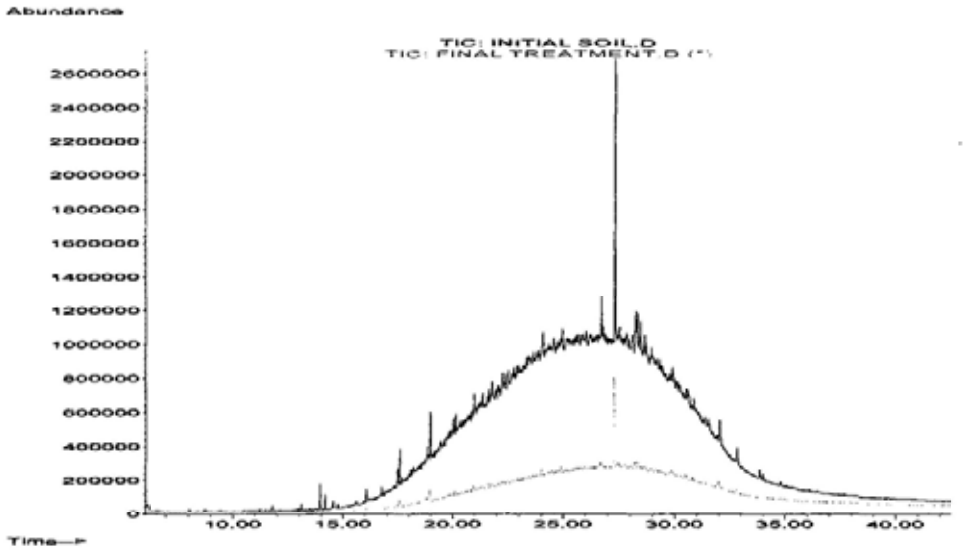
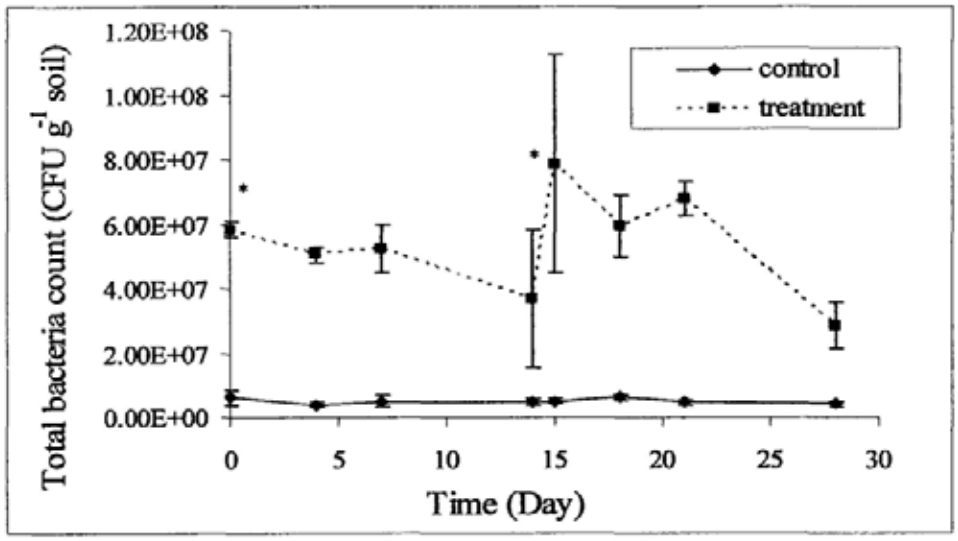


Figure 2.17 The GC profiles of control (higher curve) and treatment (lower curve) at day 28 by GC-MS for Batch 2 soil.

For microbial population (Figure 2.18), every addition of SMC could increase the population of microbes. With time bacterial population dropped rapidly, but at the end of treatment the bacterial population was still higher than the population of bacteria in control soils. Mold population increased due to the good conditions during treatment, such as higher moisture, more nutrients and so on (Figure 2.19). These characteristics and results were similar to the Batch 1 experiment.

For soil properties, although the nutrient contents in batch 2 soil were not significantly improved in contrast to the results shown in batch 1 soil (Table 2.13), total Kjeldahl nitrogen and total potassium content in treatment soil still showed significant improvement. In addition, pH was dropped by SMC treatment as in batch 1 soil experiment (Figure 2.20). All of above improvements were helpful to stimulate the activities of microbes and further to benefit the degradation of pollutants.

(a)



(b)

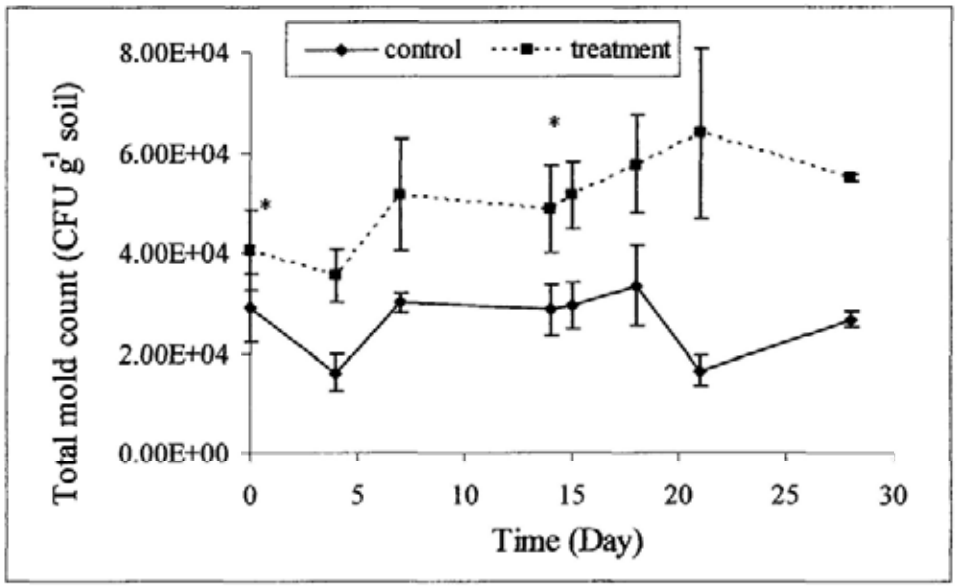


Figure 2.18 The change of (a) total bacteria population and (b) total mold population in control and treatment of the batch 2 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment and tilling in control.

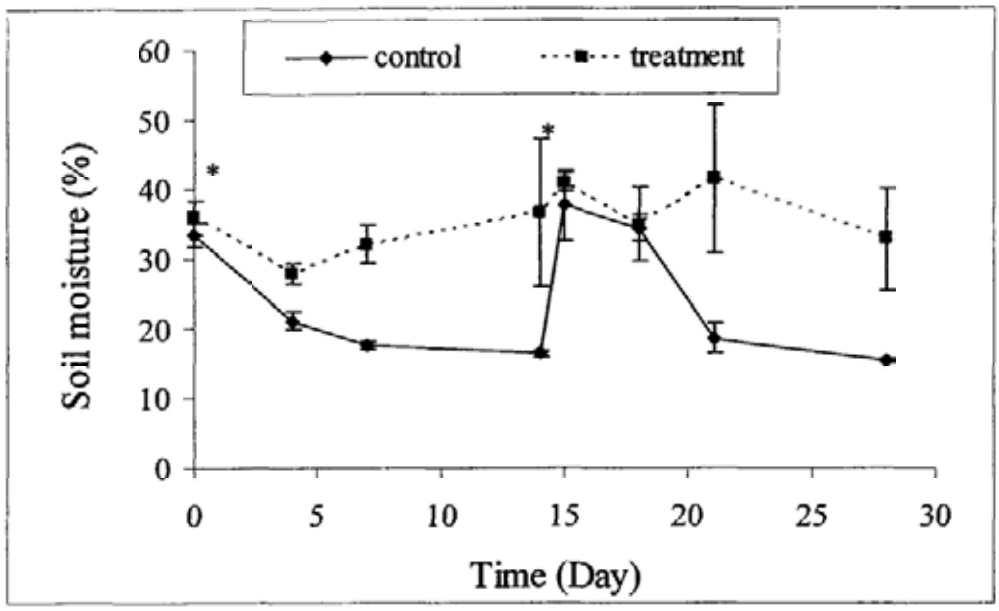


Figure 2.19 The change of soil moisture in control and treatment in Batch 2 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment and tilling in control.

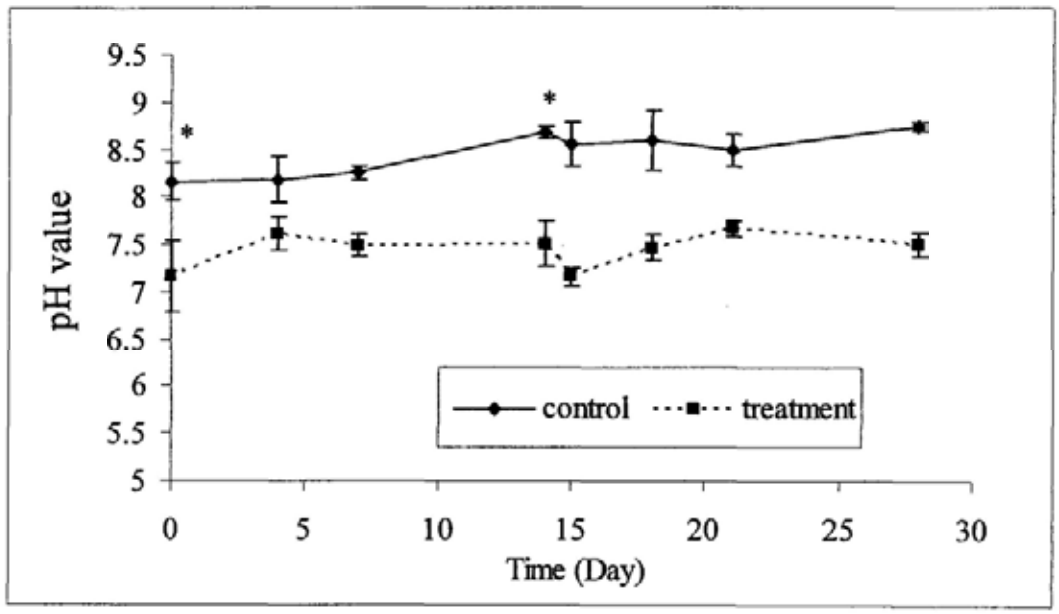


Figure 2.20 The change of pH value of the soil under treatment in contrast to control in Batch 2 soil. Data are presented in mean ± SD of 3 replicates. *, SMC application in treatment and tilling in control.

Table 2.13 Modification of the physico-chemical properties of the batch 2 mixed contaminated soil treated by SMC. Data are presented in mean ± SD of 3 replicates. (Treatment time: 28 days)

Parameters		Before treatment		After treatment	
		control	treatment	control	treatment
Total organic carbon (%)		4.3±0.1	4.3±0.1	4.4±0.1	5.0±0.8
$NO_{X\ water\text{-}soluble}$ (mg/kg)		13.6±0.0^{a}	13.7±0.1^{a}	14.1±0.1^{bA}	14.3±0.1^{bB}
$NH_{3water\text{-}soluble}$ (mg/kg^{1})		5±1	7±3	4±0^{A}	7±1^{B}
$N_{Kjeldahl}$ (mg/kg)		593±100	588±165^{a}	651±231^{A}	1062±243^{bB}
$P_{water\text{-}soluble}$ (mg/kg)		64±2	65±0	65±1	63±2
P_{total} (mg/kg)		242±114	254±69	186±58^{A}	324±63^{B}
K^{+} (mg/kg)		609±47	622±42	609±19^{A}	698±49^{B}
pH		8.16±0.20^{a}	8.06±0.12^{a}	8.75±0.04^{bA}	7.51±0.13^{bB}
Electrical conductivity (μS/cm)		192±16	180±18^{a}	187±4^{A}	249±302^{bB}
Salinity (‰)		1.2±0.3	1.2±0.3	1.2±1.0	2.3±1.0
Soil moisture (%)		25.1±0.9^{a}	26.4±1.3	13.3±0.2^{bA}	24.5±4.0^{B}
Soil stone (%)		38.3±1.2	32.2±4.4^{a}	36.2±3.0	45.2±5.8^{b}
Soil texture	Sand (%)	66.9±0.5	71.4±0.3^{a}	69.1±3.1	66.3±1.1^{b}
	Clay (%)	7.7±0.3	7.4±0.0^{a}	8.5±0.8^{A}	10.4±0.7^{bB}
	Silt (%)	22.0±0.6	21.2±0.3	22.4±2.3	23.3±0.7
	Texture class	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Bacteria (×10^{5} CFU/g)		63±23	52±14^{a}	42±8^{A}	284±72^{bB}
Mold (×10^{3} CFU/g)		29±7	24±3^{a}	15±0^{A}	55±1b^{B}

Data indicated with different small letter superscripts show significant difference at ($P < 0.05$) within the same control or treatment groups at initial and final stages. Data indicated with different capital letter superscripts show significant difference at ($P < 0.05$) between the control and treatment groups at initial or final stages.

2.3.4.3 Yield and quality of the harvested mushrooms and the greening of treatment soil by *Wedelia chinensis*

The DEHP content of mushrooms harvested from the treatment soil was 0.65±0.19 mg/kg, which was more than the content of DEHP in mushrooms from the first batch soil. The copper content was 12.8±10.7 mg/kg in mushrooms and other heavy metals were not detected similarly.

Moreover, after successful treatment, greening was done in the treatment soil. Revegetation area was covered by transplanting *Wedelia chinensis*. Although *Wedelia chinensis* needed a longer adaptive period, it had an obviously faster growth rate and had a good effect on the greening of soil (Figure 2.21).

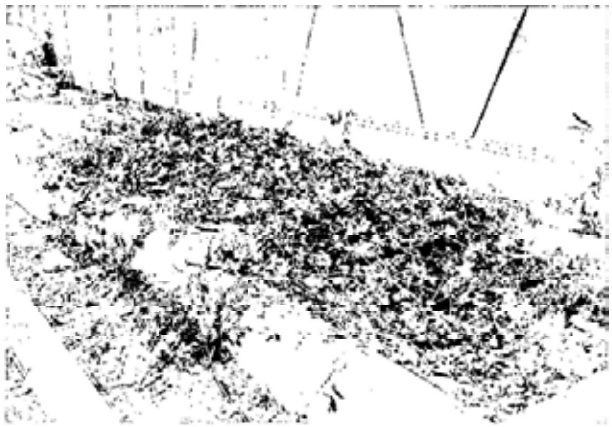


Figure 2.21 The green cover by *Wedelia chinensis*.

2.3.5 Soil ecotoxicity

Results of soil ecotoxicity on bacterial growth of these two experiments were shown in Tables 2.14 (a) and 2.15 (a). It was interesting that these bacteria could tolerate the contaminated soils and even used organic pollutants as carbon source for growth. In general, the relative bacterial growth was higher in treatment soils than in control soil in these two experiments. This indicates that these bacteria could grow better after treatment. The same bacterial species isolated from different soils showed different relative growth. The growth of *Pseudomonas aeruginosa* isolated from Lau Fau Shan was enhanced after treatment, but no significant difference between control and treatment soils in both experiments. However, the same species (*Pseudomonas aeruginosa*) isolated from Tsing Yi showed significantly higher growth after treatment, which was owing to the stronger viability of this bacteria. *Flavobacterium* sp. grew better in soil after treatment in both experiments, but only in batch 1 soil showed a significant increase. For other bacteria, *Bacillus cereus* and *Methylobacterium* sp. were sensitive to the toxicity of soil.

Results of soil ecotoxicity on fungal growth of these two experiments were shown in Tables 2.14 (b) and 2.15 (b). *Fusarium solani* and *Trichoderma harziauum* could grow better after treatment in both experiments, indicating that pollutants in soil induced toxicity on these two fungal species, and the growth of these two fungi were inhibited by the toxicity of original soil. However, *Trichoderma asperellum* tolerated the contaminated soil, because this fungal species did not show higher population growth after treatment. The relative growth of one *Penicillium glabrum* isolate from Lau Fau

Shan soil was not significantly higher than that in control, due to the large standard deviation.

2.4 Discussions

2.4.1 Enzymes in spent mushroom compost

The laccase and manganese peroxidase activities were measured in caps, stems and composts. The results in Section 2.3.1 demonstrated higher enzyme activities in the first spent mushroom compost than those in the second spent mushroom compost, while the mushroom yield in the first flush was twice to triple of that in the second flush. Spent mushroom compost is a by-product from commercial mushroom growers. As with spent mushroom compost, pre-fruitbody compost can be obtained from commercial mushroom growers. The laccase activity found in the mushroom compost was nearly two to ten folds more than manganese peroxidase. It has been reported that laccase is the dominant extracellular enzyme in liquid cultures for *Trametes versicolor* and *Pleurotus ostreatus* which are related species of *Pleurotus pulmonarius* (Evans and Hedger, 2001). These extracellular enzymes could degrade organic pollutants (Laine and Jorgensen, 1997; Eggen, 1999; Lau et al., 2003). The optimum temperatures of immobilized laccase and manganese peroxidase were 45°C and 75°C respectively (Lau et al., 2003). Thus, these enzymes could work under high temperatures.

Table 2.14 Reduction in toxicities of the batch 1 mixed contaminated soils treated by SMC tested by (a) bacteria and (b) fungal.

(a)

	Bacterial species	**Relative growth (%)**	
		Control	Treatment
Isolates from Lau Fau Shan	*Bacillus cereus*	196±158[a]	1453±282[b]
	Flavobacterium sp.	132±49[a]	824±366[b]
	Pseudomonas aeruginosa	142±118	269±150
Isolates from Tsing Yi	*Bacillus cereus*	112±40[a]	451±252[b]
	Methylobacterium sp.	249±173[a]	1536±426[b]
	Pseudomonas aeruginosa	144±21[a]	1016±449[b]

(b)

	Fungi species	**Relative growth (%)**	
		Control	Treatment
Isolates from Lau Fau Shan	Basidiomycete sp.	103±12[a]	139±3[b]
	Penicillium glabrum	121±29	151±34
	Penicillium glabrum	111±15[a]	123±14[b]
Isolates from Tsing Yi	*Fusarium solani*	96±7[a]	129±11[b]
	Trichoderma asperellum	96±8	125±16
	Trichoderma harziauum	89±8[a]	132±16[b]

Data are presented in mean ± SD of 3 replicates. Data indicated with different small letter superscripts show significant difference at ($P < 0.05$) within the control and treatment group for the initial or final set of data.

Table 2.15 Reduction in toxicities of the batch 2 mixed contaminated soils treated by SMC tested by (a) bacteria and (b) fungal.

(a)

	Bacterial species	Relative growth (%)	
		Control	Treatment
Isolates from Lau Fau Shan	*Bacillus cereus*	89±19	189±94
	Flavobacterium sp.	95±20	145±48
	Pseudomonas aeruginosa	141±49	399±177
Isolates from Tsing Yi	*Bacillus cereus*	133±45[a]	373±95[b]
	Methylobacterium sp.	154±85[a]	471±127[b]
	Pseudomonas aeruginosa	108±7[a]	296±46[b]

(b)

	Fungi species	Relative growth (%)	
		Control	Treatment
Isolates from Lau Fau Shan	Basidiomycete sp.	99±25[a]	201±56[b]
	Penicillium glabrum	117±22	193±40
	Penicillium glabrum	103±20[a]	154±5[b]
Isolates from Tsing Yi	*Fusarium solani*	86±24[a]	141±10[b]
	Trichoderma asperellum	123±18	153±14
	Trichoderma harziauum	71±23[a]	148±29[b]

Data are presented in mean ± SD of 3 replicates. Data indicated with different small letter superscripts show significant difference at ($P < 0.05$) within the control and treatment group for the initial or final set of data.

2.4.2 Soil temperature and air temperature

Although the soil temperatures fluctuated during treatment, the maximum soil temperature was 32°C (Figure 3.1) in batch 1 soil experiment which was still within the optimal temperature range (30 to 40°C) for biodegradation of petroleum (Bossert and Bartha, 1984). However after one-month treatment the temperature decreased in batch 1 soil experiment due to the change of season, which did not benefit for the growth of pollutant-degrading bacteria. In batch 2 soil experiment, during the whole period of treatment, the soil temperature was below 30 °C, which also did not benefit the degradation of pollutants by bacteria, but the lower temperature is favorable for the growth of mold population.

2.4.3 The removal of petroleum hydrocarbons and DEHP

In the controls of batch 1 and batch 2 soil experiments, the TPH reduction was about 11% after one month due to volatilization, abiotic loss of diesel fuel and the degradation by native indigenous microbes, which was similar to the report of Margesin and Schinner (1997). Some strains, such as *Bacillus subtilis* and *Pseudomonas aeruginosa*, had been reported to be isolated from contaminated soil and had the capacity of degradation of petroleum hydrocarbons (Das and Mukherjee, 2005; Mukherjee and Das, 2005; Das and Mukherjee, 2007). DEHP was more persistent than petroleum hydrocarbons, and only less than 5% were removed after one month in

control pile, which completely meet the half-life of DEHP in soil (300 days) (Müller et al., 2003).

However, in both batch 1 and batch 2 soil experiments, addition of SMC resulted in the fast removal of petroleum hydrocarbons and DEHP. The main reason was attributed to enzyme activities in SMC. Previous reports demonstrate the degradation capacities of enzymes in white rot fungi (Eggen, 1999; Cajthaml et al., 2002). Collins et al. (1996) demonstrated *in vitro* oxidation of anthracene and benzo(a)pyrene catalyzed by purified laccase enzymes from *Trametes versicolor*, although the role of laccase remains unclear. After one week of SMC application, the removal rates of DEHP and petroleum began to slow down. This may be due to the inactivity of enzymes in soil. However, the contents of DEHP and petroleum still showed slow decline. This may be due to the microorganisms capable of degradation.

In batch 1, tilling led to spontaneous degradation of 17% DEHP, 30% TPH and 24% oil and grease in 98 d in control pile. However, in treatment pile, beside the application of SMC, tilling at days 35 and 77 did not enhance the removal except day 77 tilling for TPH. Yet tilling raised transiently the soil communities to different extents. Therefore, tilling is found not a steady practice to increase the soil micro-organisms for speeding up biodegradation.

2.4.4 Microbial population and removal of pollutant in soil

In control pile, there were about 50 x 10^5 cfu bacteria and 2.5 x 10^4 cfu mold/g soil. It has been reported that if the indigenous microorganisms capable of degrading target contaminant is less than 10^5 cfu/g soil, bioremediation will not occur at a significant rate (Forsyth et al., 1995). After the addition of SMC to contaminated soil, the microbial population was immediately stimulated, resulting in a 4 to 10 fold increase of bacterial population and 2 fold increase of mold population. This indicates that the addition of SMC introduced microbes into soils. However, the bacterial population began to decrease, which was due to the carrying capacity of soil and tolerance of microbes to contaminated soil (Molina-Barahona et al., 2004). Moreover, the decline of temperature did not benefit the growth of bacteria. After 3-month treatment in batch 1 and 1-month treatment in batch 2, the bacterial populations were still higher than those in control. Mold population maintained a higher level during the second addition of SMC period in batch 1 soil experiment and the whole experiment period in batch 2 soil. This may be due to the higher moisture and lower temperature. In addition, increase in microbial population had the relationship with the increase of nutrients in soil, as well as the reduction of pollutants (Sabaté et al., 2004; Sarkar et al., 2005).

2.4.5 The nutrients status and physical condition of soil

SMC contained significant amounts of essential nutrients (N, P and K) and organic matter. N and P were understood to be the primary limiting nutrients in microbial degradation of petroleum hydrocarbons (Ting et al., 1999; Sarkar et al., 2005). After treatment, total Kjeldahl N, available N and total P were increased significantly in

treated soil. This indicates the application of SMC could improve the contents of nutrients in soil. The increase of nutrients could stimulate the growth of pollutant-degradation microbe. Moreover, SMC has a relatively low bulk density, and the addition of SMC as a bulking agent affected the contaminant degradation efficiency (Elektorowicz, 1994). The addition of SMC into soil could increase oxygen diffusion and mineral nutrients availability, as well as carbon source and mechanical support surface for bacterial adsorption (Piehler et al., 1999; Molina-Barahona et al., 2004). Thus, SMC greatly improves the physicochemical soil characteristics to speed up microbial adaptation and selection (Jørgensen et al., 2000; Molina-Barahona et al., 2004). In addition, the optimum pH range for hydrocarbon degradation is reported to be from 6.5 to 8.5 (Sarkar et al., 2005). In this study, although pH value of the original soil was less than 8.5, the application of SMC could buffer the pH value near to 7.0, which should be better for the growth of microorganisms.

2.4.6 The reduction of soil ecotoxicity

Environmental hazard assessment is usually performed by chemical analysis. However, chemical data alone are not sufficient to evaluate the biological effects (Petanen and Romantschuk, 2003; Plaza et al., 2005). Toxicity assessment at the end of bioremediation can provide valuable and complementary information to the chemical analysis (Ahtiainen et al., 2002). Several biological toxicity tests were used to evaluate the toxicity of treated soil. Usually, soil quality tests using microorganisms are promising tools for risk-based corrective action (Plaza et al., 2005). Indigenous

microbes were used to evaluate the ecotoxicity of soil as they are more indicative and representative than model organisms (Reynoldson et al., 1994; LaPoint and Waller, 2000; Preston and Shackelford, 2002). Microorganisms have several advantages for use in toxicity testing, like simple, rapid, sensitive and inexpensive (Bitton and Dutka, 1986; Bernand et al., 1996). In this study, these species showed resistance to the toxicity of contaminated soil. They maintained normal populations in control pile, and they showed a slight increase of relative growth rate due to the long time for natural degradation of pollutants in soil. Most of these species could grow better in treated soil, though they showed different relative growth. Higher relative growth indicates the reduction of toxicity to microorganisms. It has been reported that fungal treatment of creosote-contaminated soil has been shown to a reduction in toxicity (Baud-Grasset et al., 1993; Baud-Grasset et al., 1994), and several reports on the toxicity of biodegraded oil by bioremediation treatments also showed that bioremediation treatments strongly decreased toxicity (Wang and Bartha, 1990; Claxton et al., 1991; Cho et al., 1997). Some studies reported that the degradation of DEHP by numerous microorganisms, such as *Bacillus*, *Psuedomonas*, *Micrococcus*, and *Moraxella* species, could release persistent and toxic metabolites such as adipic acid and 2-ethylhexanol (Murai et al., 1998; Azarova et al., 2003; Chang et al., 2004; Nalli et al, 2006). However, in our study, through the chemical analysis, these two toxic metabolites, adipic acid and 2-ethylhexanol, were not found, and in general, the whole toxicity of soil was reduced.

2.4.7 The revegetation of soil with wheat and *Wedelia chinensis* after bioremediation

After treatment, wheat and *Wedelia chinensis* were transplanted into treatment soil. The normal growth of wheat and well cover of *Wedelia chinensis* indicate that the treated soil did not adversely affect the growth of plants. However, heavy metals in treated soil resulted in the higher content of heavy metal copper in wheat.

Chapter 3 Using SMC to treat sediment samples contaminated with DEHP and heavy metals

3.1 Introduction

3.1.1 Contaminated sediments

Sediments in our rivers, lakes and oceans or channels serve as sinks of pollutants carried by the incoming water such as heavy metals and organic pollutants (Hong et al., 1995; Zheng & Richardson, 1999; Wong et al., 2000; Leivouri, 2003; Tolosa et al., 2004; Wong et al., 2005; CEDD, 2006).

The ex-Hong Kong International Airport, popularly known as Kai Tak Airport, has been the international airport of Hong Kong until July 6, 1998. Kai Tak was located in the north of Kowloon Bay in Kowloon, Hong Kong. According to the baseline sediment quality report (2005), sediments at Kai Tak Approach Channel (KTAC) were highly contaminated with heavy metals (Table 3.1). Moreover, 99.0±21.7 mg DEHP/kg sediment was measured exceeding the sediment standard level (60 mg/kg). Odor and noise are the most common environmental complaints received from the community during the operation of the Kai Tak Airport. Malodor is usually generated from anaerobic digestion of organic matter leading to high contents of hydrogen sulfide, volatile organosulphurous compounds, methane, ammonia and other compounds (Arup Scott Wilson Joint Venture, 2002). Some are toxic, corrosive gases. The potential

environmental impacts include the followings: unpleasant odor would cause potential health impact to surrounding sensitive receivers and affect the potential uses of surrounding land.

Table 3.1 The concentrations of heavy metals and high molecular weight PAHs in sediment at KTAC (Arup Scott Wilson Joint Venture, 2002).

Area	Heavy Metals(mg/kg dry weights)*								
	Cr	Cd	Cu	Hg	Ni	Pb	Ag	Zn	As
A1	120	2.1	330	0.49	45	110	20	710	4.4
A2	310	3.3	1,200	1.4	98	140	18	820	5.3
A3	390	3.7	1,600	1.6	120	150	16	900	5.1
A4	68	1.5	230	0.43	29	100	14	760	3.7
Average	222	2.7	840	1.98	73	125	17	798	4.6
LCEL ^	80	1.5	65	0.5	40	75	1	200	12
UCEL ^	160	4	110	1	40	110	2	270	42

☐ LCEL<value<UCEL

☐ Value>UCEL

*Result represents samples collected from 10-30 cm section.

Area	HMW PAHS* (ug/kg dry wt.)	LCEL^ (ug/kg dry wt.)	UCEL^ (ug/kg dry wt.)
A1	<170	1,700	9,600
A2	<170		
A3	<170		
A4	<170		
Average	<170	N/A	N/A

LCEL<value<UCEL

Value>UCEL

*Result represents samples collected from 10-30 cm section.

^ UCEL(Upper Chemical Exceedance Level) and LCEL(Lower Chemical Exceedance Level) as stated in Works Bureau Technical Circular No.3/2000 – Management of Dredged/Excavated Sediment.

3.1.1.1 DEHP in sediment

Phthalate esters (PAEs) are the major additives used as plasticizers in the manufacture of plastics to provide the desired flexibility and durability (Peakall, 1975; Rahman et al., 2004). The United States Environmental Protection Agency (USEPA) has classified the most common PAEs as priority pollutants, and PAEs are endocrine disrupting compounds. It has been reported that PAE concentrations in surface marine waters and river sediments ranged from 0.1 to 300 μg/l (Mayer et al., 1972; Giam et al., 1978; Gledhill et al., 1980; Yuan et al. 2002; Chang et al., 2005), and from 0.1 ng/g to 100 μg/g (Thuren, 1986; Tan, 1995; Yuan et al. 200; Chang et al., 2005) 2, respectively. Yuan et al. (2002) reported that 0.5–23.9 μg/g of DEHP was detected in sediments taken from various rivers in Taiwan, suggesting that contamination of river sediment by this compound is common. It is generally agreed that microbial activity is the major mechanism responsible for the degradation of phthalate esters in soils and sediments and is more effective under aerobic than anaerobic conditions (Yuan et al., 2002). DEHP, one of the most common PAEs, has lower degradation rate under anaerobic conditions compared to other PAEs (Moore et al., 2001; Yuan et al. 2002; Chang et al., 2005; Shailaja et al., 2008). Moreover, under anaerobic conditions, hydrogen sulfide and ammonia are generated from the organic decomposition process within the sediments, causing odor nuisance to the general public.

3.1.1.2 Heavy metals in sediment

Sediments are normally mixtures of several components including different mineral species as well as organic debris (Habes, 2006). Sediments represent one of the ultimate sinks for heavy metals discharged into the environment (Hollert, 2003). Heavy metals accumulate in the sediments through complex physical and chemical adsorption mechanisms depending on the nature of the sediment matrix and the properties of the adsorbed compounds (Leivouri, 1998). Hong Kong Government reported that 49-130 mg/kg lead were found in the sediment of Tolo Harbour, and 27-210 mg/kg copper were detected in the sediment of Victoria Harbour in 2005 (www.epd.gov.hk). These contaminants have a negative impact on the integrity and sustainability of the marine ecosystems. Waterbird eggs surveyed in the interior area in southern China, including Hong Kong, bore 0.6–3 μg/g Hg (dry wt), reaching/exceeding the level (0.5 μg/g) of concern (USDI, 1998; Lam, 2005).

3.1.2 Treatment of contaminated sediment

Sediment treatment options include *ex-situ* treatment, e.g. removal of sediments, and *in-situ* treatment of the sediments, e.g. capping of sediments, solidification (Murphya, 1999; Catherine, 2001). However, for *ex-situ* treatment, there are many limitations, such as not directly applicable to the current situation according to the 'Protect Harbour Ordinance' for Victoria Harbour into where Kai Tak falls, environmental impacts during dredging, and large quantity of contaminated sediment for disposal and so on (www.cedd.gov.hk). *In-situ* treatment also has many shortcomings: difficult for repeated use, difficult for maintenance dredging or destruction to seabed

and so on (www.frtr.gov/matrix2/section1/toc.html). *In-situ* treatment using calcium nitrate was proposed for sediment treatment for removal of the odor nuisance in Shing Mun Nullah. In the study by Chan et al. (2004), the effectiveness was assessed. However, the test results did not show reduction in the organic content and heavy metals in sediment but hydrogen sulfide was reduced after treatment (Chan et al., 2004). There was, however, no long term monitoring of treatment effectiveness, which remains uncertain. In Hong Kong there are standards for dredged sediment for dumping. However, the sediments are not categorized. Therefore, the China standard is used as a reference in this study. Table 3.2 shows the marine sediment quality standard of China (Table 3.2; GB 18668-2002).

3.1.3 Aim of this study

To investigate the treatability of sediment contaminated with DEHP, heavy metals and nasty odor by spent compost of oyster mushroom, *Pleurotus pulmonarius*. In addition, SMC water extract (SMCE) and nitrate also were tested and compared with SMC.

Table 3.2 Marine sediment quality standard in China (GB 18668-2002).

Item	Indicator for Classes		
	First	Second	Third
Wastes and other	No industrial and municipal wastes, no macroscopic plant detritus and animal carcass at bottom of sea		No discernible industrial and municipal wastes, no discernible macroscopic plant detritus and animal carcass at bottom of sea
Color, odor and structure	Nil color and odor; natural		
Coliform (/g wet weight) ≤	200		
Fecal coliform (/g wet weight) ≤	40		
Pathogen	No pathogen with shellfish culture as food for human being		
Hg ($\times 10^{-6}$ g/g)	0.20	0.50	1.00
Cd ($\times 10^{-6}$ g/g)	0.50	1.50	5.00
Pb ($\times 10^{-6}$ g/g)	60.0	130.0	250.0
Zn ($\times 10^{-6}$ g/g)	150.0	350.0	600.0
Cu ($\times 10^{-6}$ g/g)	35.0	100.0	200.0
Cr ($\times 10^{-6}$ g/g)	80.0	150.0	270.0
As ($\times 10^{-6}$ g/g)	20.0	65.0	93.0
Organic carbon ($\times 10^{-2}$ g/g)	2.0	3.0	4.0
Sulfide ($\times 10^{-6}$ g/g)	300.0	500.0	600.0 (0.06%)
Total petroleum hydrocarbons ($\times 10^{-6}$g/g)	500.0	1000.0	1500.0
Hexachlorocyclohexane ($\times 10^{-6}$g/g)	0.50	1.00	1.50
Dichlorodiphenyltrichloroethane ($\times 10^{-6}$g/g)	0.02	0.05	0.10
Polychlorinated biphenyls ($\times 10^{-6}$g/g)	0.02	0.20	0.60

3.2 Material and methods

3.2.1 Sediment collection

Sediments were collected from Kai Tak Approach Channel (KTAC). The Kai Tak International Airport was closed on 6th July 1998, but was still not redeveloped in 2006. Beside the spilled petroleum in the runway, sediments at KTAC were contaminated by heavy metals, organic pollutants and petroleum hydrocarbons (CEDD, 2006). Bis(2-ethylhexyl)phthalate (DEHP), the most common plasticizer, was the main and persistent organic pollutant found in sediment of KTAC. The nuisance gases particularly hydrogen sulfide were generated by the anaerobic decomposition of organic matter in sediments (www.pland.gov.hk). These pollution problems adversely affected the urban redevelopment of East Kowloon in Hong Kong.

3.2.2 A comparison on pollutant removal capacities of SMC/SMCE, nitrate and H_2O_2

SMC was the residue after harvest of the edible mushroom while SMCE was the water extract of SMC prepared by distilled water.

A microcosm was set up with at 1:1 height ratio of the seawater and sediment collected from Kai Tak Approach Channel in an enclosed container (12 cm×12 cm×15 cm) to create an anaerobic environment as in the field. One percent calcium nitrate (weight: wet weight of sediment, g/g) dissolved in distilled water ($Ca(NO_3)_2$:water=20

g:100 ml), 1% hydrogen peroxide, or SMCE was applied using injection, while solid SMC was added and mixed with sediment. The dose of nitrate used was according to the recommendation of Chan et al. (2004) (calcium nitrate: sediment S content = 8: 1; mass ratio). Different amounts of SMC, 0.5%, 1%, 1.5%, 2% (weight: wet weight of sediment, g/g), were tested to treat the sediment DEHP. SMCE was initially added to the sediment with different concentrations in terms of the equivalent amounts of SMC. After preparation of SMCE, different amounts of SMCE with the same volume to nitrate treatment could be got through dilution with distilled water, and then were injected into sediment. In the control in parallel, an equivalent amount of distilled water was also injected to the sediment to simulate the effect of volume increase, and the disturbance of injection to the reaction mixture.

This microcosm system was carried out in the laboratory at room temperature. After 7 days, the physical parameters were measured for water samples, and the collected sediment samples were oven-dried or freeze-dried for further study.

3.2.3 Dose response experiment with SMC/SMCE+Nitrate on the removal of pollutants from sediment

The mixtures of SMC/SMCE+nitrate were used to remove pollutants and odor from contaminated sediment. The same design as section 3.2.2 was applied in this experiment. The maximal amount of SMC/SMCE applied in this experiment was 3% (g/g sediment), while nitrate was 1%. Different ratios (Table 3.3) were designed with five replicates.

The liquid treatments (SMCE+nitrate) were injected into sediments with a fixed volume. SMC was manually mixed with the sediment and let settle in the containers. The containers were incubated at 25±2°C for 7 days. Sediment and water samples were taken for the further chemical and physical analyses.

Table 3.3 The ratios of SMC/SMCE and nitrate designed in dose response experiment.

	Control	Treatment				
SMC/SMCE	0	3%	2.25%	1.50%	0.75%	0
Calcium nitrate	0	0	0.25%	0.50%	0.75%	1%

3.2.4 Time effect experiment with the optimal amount of SMCE+Nitrate

In this experiment, larger Sigma containers (Plant cell culture box, catalog number: P-5557, Sigma, Louisiana Plastic industries) were used with 1:1 height ratio of the seawater and sediment. Considering the natural marine condition, this simulation experiment employed an open system. Every week the water lost by evaporation was restored twice with distilled water to maintain the initial total volume of the reaction mixture. In treatments, the combination of 2.25% SMCE+0.25% nitrate and 3% SMCE were added into contaminated sediment to monitor the time effect of treatment, respectively. Control setup was injected the same volume of distilled water. Control and treatments with three replicates were monitored for one month. Every week sediment and water samples were collected randomly for further analyses.

3.2.5 A comparison of the effects of different amounts of SMCE on raw sediment and sediment treated with nitrate

Raw sediment and sediment treated with nitrate were collected from the field and the removal capacity of SMCE was evaluated on these two different sediments. Five ratios 0%, 1%, 2%, 3% and 5% each with 5 replicates were tested. This experiment was carried out in environmental columns (15 cm×15 cm×50 cm) with 1:1 height ratio of the seawater and sediment, and run for 4 weeks in this laboratory. Every week sediment and water samples were collected randomly for physical and chemical analyses.

3.2.6 Sediment characterization

3.2.6.1 Moisture

About 10 g of soil samples were added into a pre-weighed funnel with a filter paper. Excess water was let run out about 5min. Then the whole setups were weighted and placed in a 105°C oven until constant weight (Allen, 1989). After cooling in a desiccator, the dried sediments were weighed and the water contents of the samples were calculated by mass difference.

3.2.6.2 Sediment bacterial and fungal population

Aseptic techniques were performed for the procedures in this part. The method is mentioned in Section 2.2.3.4. The result was presented as the colony forming unit (cfu) per gram of dried sediment.

3.2.6.3 C, H, N and S contents in sediment

The C, H, N and S contents in sediment were determined with 2.000 mg of freeze-ground sample by mortar and pestle in liquid nitrogen (AD-4 autobalance, Perkin Elmer) and placed into the CHNS/O Analyser (PE 2400, Perkin Elmer).

3.2.6.4 Total nitrogen, total phosphorus, available ammonium and nitrate, available phosphates

The nutrients in sediment were determined by AIA after digestion or extraction. The method is the same to the soil measurement description in Section 2.2.4.

3.2.6.5 Extraction of DEHP and organic pollutants

The method of DEHP extraction in sediment is the same as the soil sample. Five gram sediment was extracted with 10 ml dichloromethane (DCM) (Analytical grade, BDH) shaking at 200 rpm for 2 h. This setup was repeated with another 10 ml solvent for another 2 h. The solvent was concentrated at 60°C by a rotary evaporator with a vacuum pump. One ml of acetone (HPLC grade, Labscan) was used to redissolve the sample after evaporation. The acetone was filtered by a 0.45 μm membrane (Acrodisc

syringe filters 4CR PTFE) for gas chromatography-mass spectrometry (GC-MSD) measurement.

GC-MS conditions and temperature profiles for DEHP analysis were the same with Tables 2.8 and 2.9.

3.2.6.6 Total heavy metal analysis

Total heavy metal analysis in sediment was performed according to the description in Section 2.2.4.

3.2.6.7 Gas, dissolved oxygen and turbidity measurements

The odor gases H_2S and NH_3 from sediment were measured by a gas detector (serial no. SE106-006299, BW technology, Canada) when collecting samples. Turbidity and dissolved oxygen were measured by turbidimeter (EC-TN1001R, Infra-Red Turbidimeter, Eutech Instrument, Singapore) and dissolved oxygen meter (YSI 5100, Ohio, USA) before taking samples, respectively.

3.3 Result

3.3.1 The characteristics of KTAC sediment and SMC

Table 3.4 shows the physico-chemical properties of KTAC sediment, KTAC water, SMC and SMCE used in the present study. The KTAC sediment was C-rich reaching 7.9% but was still lower than those of SMC and SMCE. The KTAC sediment also contained $N_{Kjeldahl}$, small amount of water-soluble NO_x, total P, water-soluble P and K. Yet, the KTAC sediment was highly contaminated with heavy metals and DEHP. The KTAC sediment contained 0.8±0.2% S content which was the source of the nuisance odor in the KTAC site. This batch of sediment had metals Cu and Pb exceeding the severe effect levels (110 and 250 mg/kg for Cu and Pb), and metals Cr, Ni, Zn exceeding the lowest effect levels (26, 16 and 120 mg/kg for Cr, Ni and Zn) (USEPA, 2007). Both SMC and SMCE contain abundant nutrients such as total carbon, nitrogen, phosphate and potassium. They have higher electrical conductivity and salinity, and have a neutral pH value. Heavy metals and organopollutant contents of SMC do not exceed the standard levels. Although SMC contained Fe, Zn and Cu whose levels did not exceed any environmental guidelines, the nutrient content of SMCE was comparatively lower than SMC and did not contain the heavy metal ions (Table 3.4).

Table 3.4 The physico-chemical properties of sediment and seawater samples collected from Kai Tak Approach Channel and SMC and SMCE used in this study. Data are presented in mean ± SD of 5 replicates.

Parameters	SMC	SMCE	Seawater	Sediment
Total organic carbon (%/mg/l)	20.4±2.2	27.3±2.0	1.7±0.8	7.9±0.9
$N_{Kjeldahl}$ (mg/kg / mg/l)	1632±218	—*	—	41.3±5.9
NO_X $_{water\text{-}soluble}$ (mg/kg / mg/l)	198±26	31.9±5.7	6.2±0.6	2.4±0.3
NH_4^+ $_{water\text{-}soluble}$ (mg/kg / mg/l)	267±27	51.1±1.0	70.9±6.6	12.3±1.5
P_{total} (mg/kg / mg/l)	612±32	—	—	7.6±1.3
$P_{water\text{-}soluble}$ (mg/kg / mg/l)	66.6±33.4	8.5±2.0	9.0±4.1	2.8±0.4
K^+ (mg/kg / mg/l)	267±87	30±9	517±12	3525±1449
Cd (mg/kg / mg/l)	nd**	nd	0.31±0.01	22.5±16.3
Ni (mg/kg / mg/l)	nd	nd	0.18±0.11	34.8±5.4
Pb (mg/kg / mg/l)	nd	nd	nd	154.5±36.9
Zn (mg/kg / mg/l)	8.1±1.4	nd	nd	207±81
Cu (mg/kg / mg/l)	4.8±1.7	nd	nd	202±25
Cr (mg/kg / mg/l)	nd	nd	nd	102±8
Fe (mg/kg / mg/l)	0.3±0.2	nd	0.4±0.0	2345±41
Electrical conductivity (μS/cm)	2241±29	1698±302	32877±8753	82937±15522
Salinity (‰)	32±1.0	32±1.0	25±6	50±4
pH	7.2±0.4	7.3±0.4	7.1±0.2	7.8±0.1
DEHP (mg/kg / mg/l)	nd	nd	nd	99.0±21.7

mg/l for SMCE and KTAC water; mg/kg for SMC and KTAC sediment.

*—, not determined; **nd, not-detectable

3.3.2 A comparison on pollutant removal capacity of SMC/SMCE, nitrate and H_2O_2

3.3.2.1 Removal of DEHP

In Figure 3.1, the highest curve of the overlapping gas chromatograms of the sediment samples after treatment is the profile for control. The green line shows the sediment treated by 1% SMC, and the lowest profile was for the sediment treated by 2% SMCE. The GC chromatograms indicated the decrease of organopollutants after treatment by SMC and SMCE. In addition, removal of DEHP was assessed by gas chromatographic analysis of the organic extract, and the retention time of DEHP in GC profile was about 27.06 min. The results, which represent the fate of DEHP already present in the sediment, are shown in Figure 3.2. There was no significant difference among the datasets.

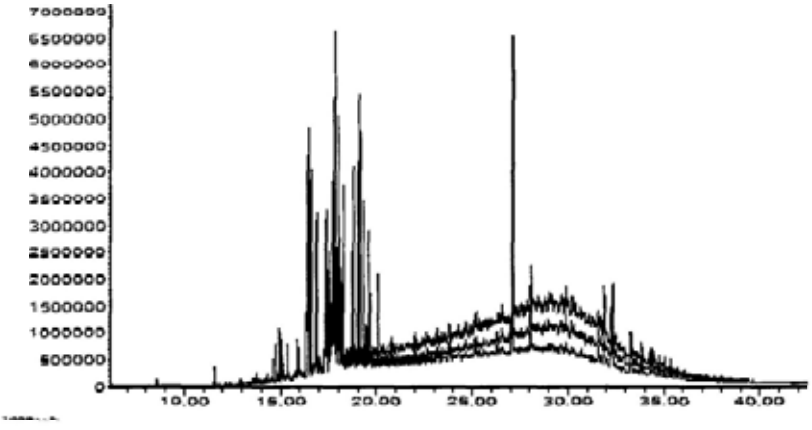


Figure 3.1 GC profiles of sediments after treatment. Black line is the profile for control while green line shows the profile for treatment by 1% SMC, and blue profile is for the sediment treated by 2% SMCE. The retention time of DEHP is 27.06 min.

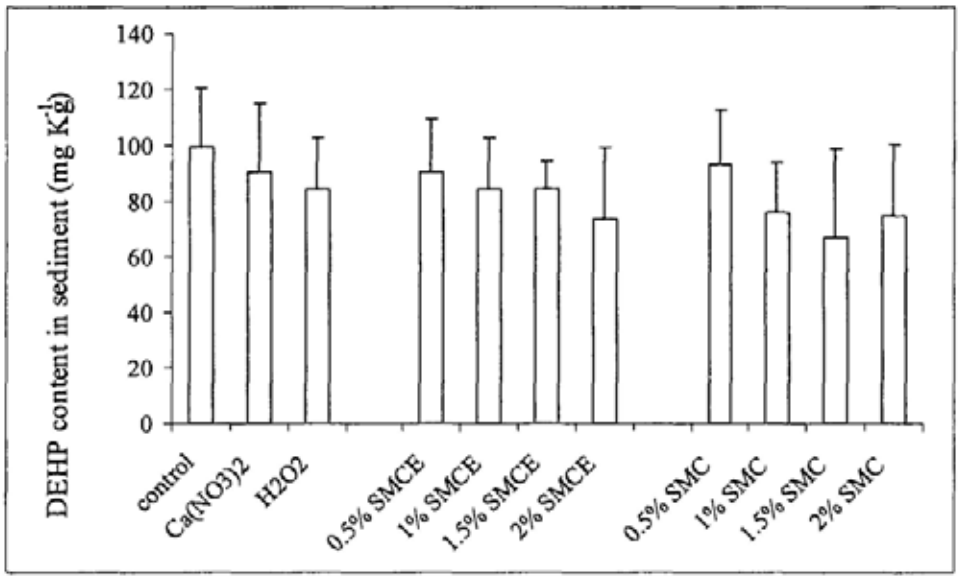


Figure 3.2 DEHP content in sediment after 7 days different treatments. Data are presented in mean ± SD of 5 replicates. There is no significant difference between different treatments at 5% levels after one-way ANOVA.

3.3.2.2 Removal of heavy metals

Figures 3.3 and 3.4 show the concentrations of heavy metals in sediment after different treatments. Neither the injection of hydrogen peroxide nor calcium nitrate affected the metal profiles (Figures 3.3 and 3.4). In comparison, results showed the slight effectiveness of SMC and SMCE in mobilizing copper bond to sediment after 7 days. One percent SMC gave a removal of 43% copper, and there was a significant difference between control and 1% SMC treatment. However, SMC and SMCE had no effects on other heavy metals, which had a close relationship with the characteristics of SMC and the forms and species of heavy metals binding to sediment.

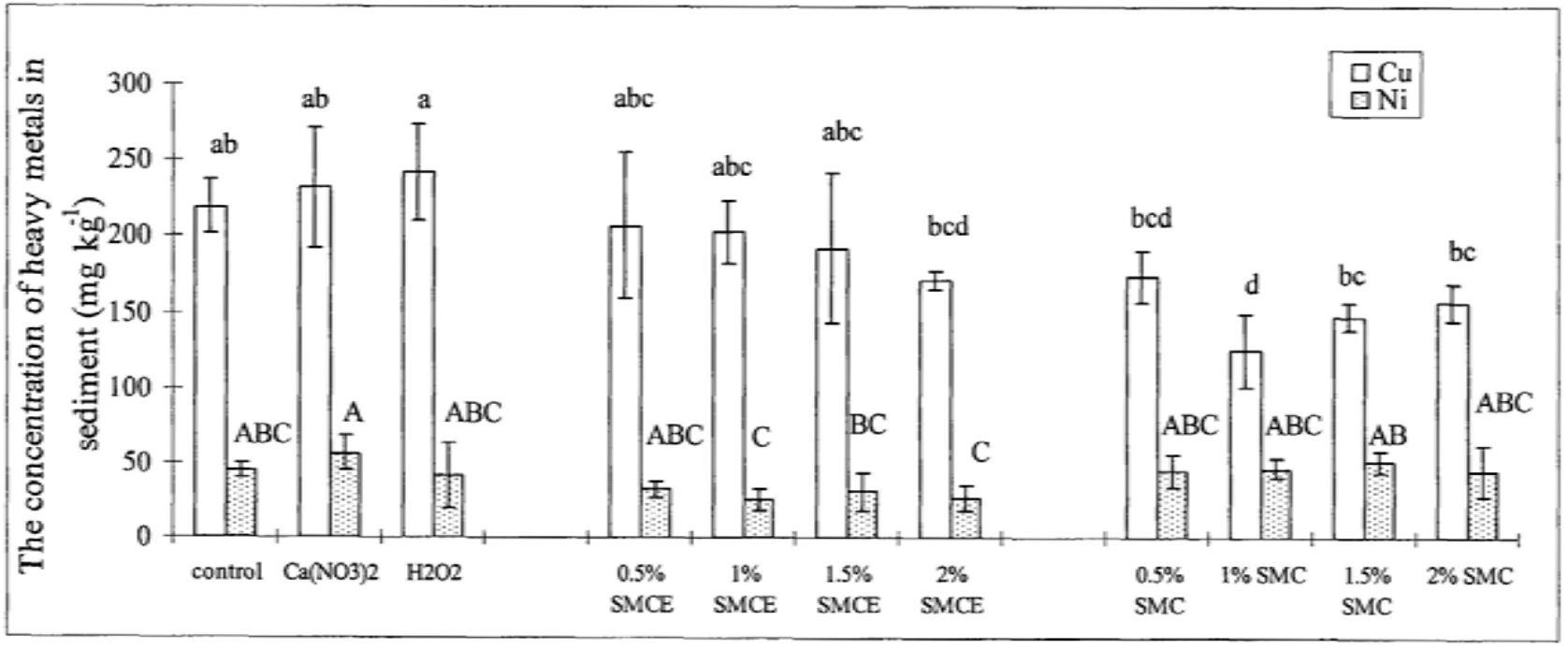


Figure 3.3 Copper and nickel contents in sediment after 7-day treatment.

Data are presented in mean ± SD of 5 replicates. Means indicated with different small and capitals letter superscripts show significant difference at $P < 0.05$ within different treatments on copper and nickel contents (One way ANOVA with Tukey test, $p < 0.05$).

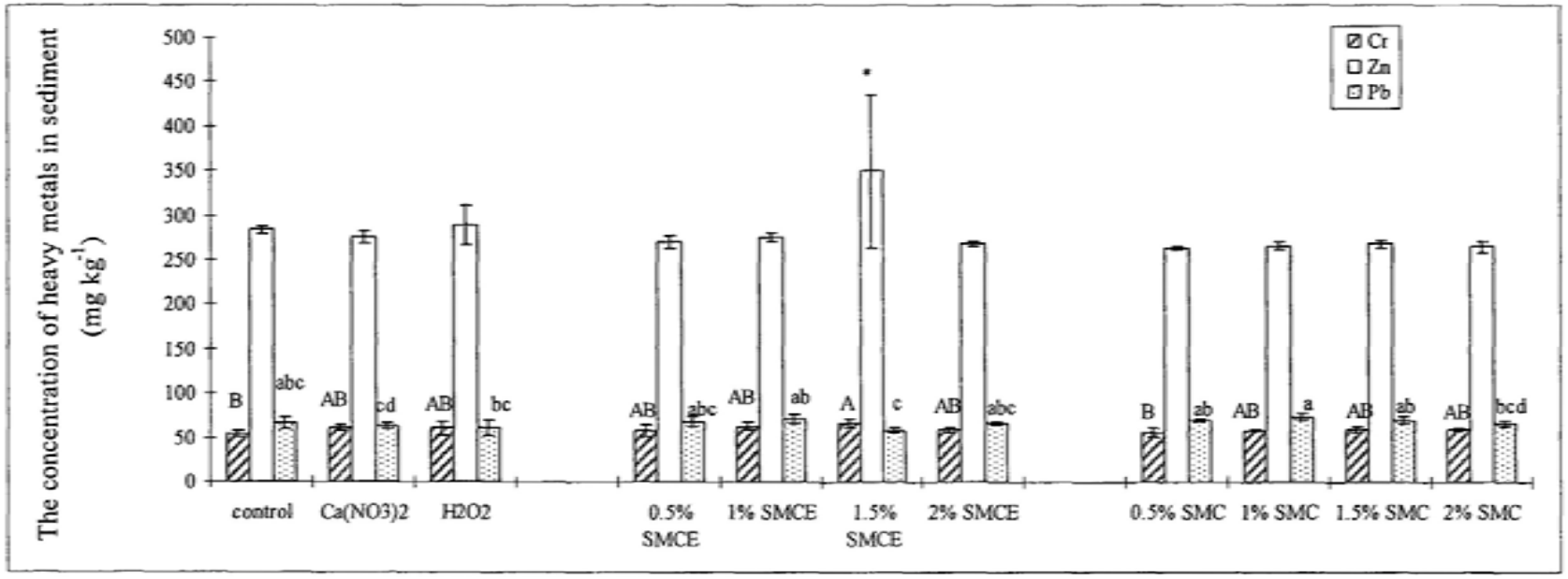


Figure 3.4 Chromium, zinc and lead contents in sediment after 7-day treatment.

Data are presented in mean ± SD of 5 replicates. Means indicated with different small and capitals letter superscripts shows significant difference at $P < 0.05$ within different treatments on metals' content. (One way ANOVA with Tukey test, $p < 0.05$)

3.3.2.3 The nutrients effect

All SMC treatments raised the N contents in the sediment, especially 1% and 1.5% SMC, but had no effect on C content except 2% SMC (Table 3.5). SMCE and nitrate did not cause any significant change in N and H contents. Hydrogen peroxide showed a neutral effect on C as expected while it decreased N and H contents slightly (Table 3.5). In this treatment, it was expected for hydrogen peroxide to generate water and release oxygen to the aquatic body beside the ·OH radical attack on the organopollutants. This strong oxidizing agent, calcium nitrate, SMC and SMCE could decrease S contents of sediments significantly, and nuisance odor did not appear after nitrate treatment. Yet hydrogen peroxide showed a transitional effect, decolorization of sediment did not last for 7 days after application whereas nuisance odor also emerged again at day 7 afterwards.

3.3.2.4 Change of physical parameters

When SMCE or SMC applied to the system, the electrical conductivity and salinity were not increased significantly (Table 3.5). In contrast, calcium nitrate application significantly increased salinity (Table 3.5). For pH, only calcium nitrate showed a slight drop in pH value but all the treatments and control systems were still in alkaline pH (Table 3.5). The addition of hydrogen peroxide increased dissolved oxygen (DO) to 0.21 mg/l in the system. The increase of dissolved oxygen can stimulate the oxidization of organic pollutants. Therefore, in general, the application of SMC or SMCE did not change the natural chemical characteristics of sediment.

Table 3.5 The physical-chemical analysis result of sediment and water samples for the experiment to test the removal capacity of SMC/SMCE compared with nitrate and H_2O_2. Data are presented in mean ± SD of 5 replicates. Data indicated with different small letter superscripts show significant difference at $P < 0.05$ within different treatments. (One way ANOVA with Tukey test, $p < 0.05$)

Parameters	control	Nitrate	H_2O_2	0.5%SMC	1%SMC	1.5%SMC	2%SMC	0.5%SMCE	1%SMCE	1.5%SMCE	2%SMCE
DEHP (mg/kg)	99.0±21.7	90.2±24.8	84.5±18.1	93.3±19.6	75.8±17.7	67.2±31.6	74.7±25.5	90.4±19.1	84.5±17.9	84.4±10.0	73.7±25.8
Cu (mg/kg)	218.9±17.4 ^{ab}	231.7±39.7 ^{ab}	241.9±31.9 ^{a}	173.8±17.2 ^{abc}	124.7±24.4 ^{abc}	147.4±9.0 ^{abc}	156.5±11.8 ^{bcd}	206.9±47.9 ^{bcd}	202.9±21.2 ^{d}	192.2±49.3 ^{bc}	170.9±5.9 ^{bc}
Zn (mg/kg)	283.4±4.1 ^{b}	275.5±7.3 ^{b}	288.9±21.5 ^{b}	263.2±2.1 ^{b}	266.1±5.1 ^{b}	268.0±4.0 ^{a}	264.7±5.5 ^{b}	270.5±7.4 ^{b}	275.3±4.9 ^{b}	349.6±86.3 ^{b}	268.2±2.9 ^{b}
Pb (mg/kg)	66.7±6.8 ^{abc}	62.8±4.1 ^{cd}	61.3±8.5 ^{bc}	69.3±1.7 ^{abc}	73.4±4.2 ^{ab}	70.5±3.7 ^{c}	66.7±3.7 ^{abc}	68.7±5.5 ^{ab}	71.1±5.4 ^{a}	58.6±3.0 ^{ab}	66.7±1.7 ^{bcd}
Ni (mg/kg)	44.9±4.3 ^{abc}	56.1±11.7 ^{a}	41.5±21.7 ^{abc}	44.9±0.7 ^{abc}	46.6±6.6 ^{c}	51.2±7.6 ^{bc}	45.3±17.2 ^{c}	32.5±5.3 ^{abc}	25.8±7.0 ^{abc}	31.4±12.9 ^{ab}	26.5±8.3 ^{abc}
Cr (mg/kg)	54.6±3.8 ^{b}	61.6±3.9 ^{ab}	60.6±7.4 ^{ab}	56.1±4.3 ^{ab}	58.2±1.0 ^{ab}	59.5±2.8 ^{a}	59.4±59.4 ^{ab}	57.8±7.5 ^{b}	63.2±4.3 ^{ab}	66.9±5.3 ^{ab}	59.1±2.2 ^{ab}
C(%)	7.37±0.62 ^{ab}	7.08±0.53^{ab}	7.20±1.09^{ab}	7.49±0.16^{ab}	7.07±0.34^{ab}	7.49±0.45^{ab}	8.05±0.35^{a}	7.21±0.29^{ab}	7.18±0.20^{ab}	6.77±0.15^{b}	7.28±0.16^{ab}
H(%)	1.13±0.38 ^{ab}	0.77±0.14^{b}	0.83±0.09^{b}	0.90±0.06^{b}	1.02±0.09^{ab}	0.97±0.15^{ab}	1.31±0.23^{a}	0.86±0.05^{b}	1.04±0.17^{ab}	0.90±0.16^{b}	1.06±0.13^{ab}
N(%)	0.91±0.05 ^{bc}	0.94±0.03^{ab} ^{c}	0.88±0.06^{c}	1.04±0.09^{ab}	1.08±0.12^{a}	1.07±0.09^{a}	1.02±0.05^{abc}	0.87±0.09^{c}	0.95±0.04^{abc}	0.98±0.08^{abc}	1.00±0.06^{abc}
S(%)	1.26±0.54 ^{a}	0.66±0.14^{b}	0.73±0.18^{b}	0.51±0.13^{b}	0.51±0.18^{b}	0.46±0.18^{b}	0.65±0.07^{b}	0.65±0.06^{b}	0.63±0.08^{b}	0.61±0.17^{b}	0.67±0.09^{b}
Electrical conductivity (μS/cm)	41784± 1823	44368± 1564	39728±3317	45472±11512	42144± 1247	40600± 1060	41680±438	41784±948	41136± 1090	40416± 1003	39016± 2637
Salinity (‰)	24.4±0.6^{bc}	26.2±0.8^{a}	25.4±0.6^{abc}	25.4±0.6^{abc}	24.8±0.5^{bc}	25.6±0.6^{ab}	25.6±0.9^{ab}	24.6±0.6^{bc}	24.6±0.6^{bc}	24.6±0.6^{bc}	24.2±0.8^{c}
pH	8.25±0.34	7.80±0.19	8.06±0.34	8.25±0.28	8.21±0.16	8.15±0.35	7.96±0.62	8.27±0.46	7.99±0.21	7.99±0.24	8.29±0.24

3.3.3 Dose response of SMC/SMCE+Nitrate to sediment on the removal of pollutants

3.3.3.1 Removal of DEHP

There was no significant difference on the contents of DEHP between control and all treatments after 7 days incubation (Table 3.6). In addition, the application of the combination of calcium nitrate+SMC/SMCE removed nuisance odor in sediment and the odor did not appear again.

3.3.3.2 Removal of heavy metals

SMC/SMCE+Nitrate showed the effect of mobilizing heavy metals, especially copper, lead and chromium from sediment. After one-way ANOVA analysis, 2.25%SMC+0.25%Nitrate showed the significant decrease effect on the mobilization of copper and lead, and 3% SMCE had the significant effect on chromium. However, SMCE or SMC had no effect on the removal of zinc from sediment as similar with experiment mentioned in Section 3.3.2.2.

3.3.3.3 The nutrients effect in seawater

Concentration of NH_4^+ had a sharp increase with the addition of nitrate in pore water, while NO_x content showed no significant difference between control and

treatments (Table 3.6). Also, the addition of SMC/SMCE improved the content of PO_4^{3-} slightly (Table 3.6).

3.3.3.4 Change of physical parameters

When the combinations of nitrate and SMC/SMCE were applied into sediment, pH was decreased with the increase of nitrate content significantly, and the neutralization of pH was attributed to the addition of nitrate (Table 3.6). Also, calcium nitrate significantly stimulated the increase of salinity. However, electrical conductivity and salinity did not show a regular pattern along incubation (Table 3.6).

Table 3.6 The physical-chemical analysis result of sediment and water samples for the dose response experiment.

Parameters	control	1%Nitrate	0.75%SMC+0.75%Nitrate	1.5%SMC+0.5%Nitrate	2.25%SMC+0.25Nitrate	3%SMC	0.75%SMCE+0.75Nitrate	1.5%SMCE+0.5Nitrate	2.25%SMCE+0.25Nitrate	3%SMCE
DEHP (mg/kg)	84.9±14.4	69.7±2.9	57.5±3.3	61.7±10.1	57.5±8.0	63.3±19.3	69.8±16.1	79.1±31.9	54.5±5.3	60.7±11.5
Cu (mg/kg)	218.1±30.0^{a}	218.2±14.2^{a}	181.3±26.2^{abc}	154.7±17.9^{c}	156.0±30.1^{c}	191.5±4.6^{abc}	214.7±2.9^{ab}	220.1±18.0^{a}	186.1±9.5^{abc}	158.8±16.7^{bc}
Zn (mg/kg)	154.9±1.3^{ab}	149.9±4.2^{ab}	144.0±2.7^{b}	149.1±4.8^{ab}	149.8±5.5^{ab}	151.0±4.7^{ab}	155.0±0.6^{ab}	153.3±5.4^{ab}	156.5±4.1^{a}	149.3±0.3^{ab}
Pb (mg/kg)	73.7±1.7^{a}	73.8±6.6^{a}	68.2±13.3^{ab}	59.4±4.7^{ab}	51.7±8.7^{b}	58.3±5.0^{ab}	51.3±6.3^{b}	57.7±6.2^{ab}	51.9±4.6^{b}	65.4±6.9^{ab}
Ni (mg/kg)	48.0±6.5	41.9±4.2	40.6±6.1	36.0±3.3	35.9±8.7	42.5±2.2	40.0±2.2	44.3±4.7	43.6±10.5	36.3±2.0
Cr (mg/kg)	69.8±2.8^{b}	86.5±10.6^{a}	65.2±2.6^{bc}	76.1±9.8^{ab}	60.4±6.5^{bc}	60.0±2.2^{bc}	59.9±5.0^{bc}	61.4±3.3^{bc}	51.4±3.2^{c}	49.6±2.5^{c}
NO_X $^{water-soluble}$ (mg/l)	9.0±9.7	15.7±2.8	15.9±7.1	10.1±3.9	9.3±3.1	8.3±3.6	17.9±11.1	15.3±7.4	11.3±7.2	5.8±2.8
NH_4^+ $^{water-soluble}$ (mg/l)	10.7±11.7^{b}	27.8±5.4^{a}	28.4±5.2^{a}	30.1±1.0^{a}	28.4±3.7^{a}	27.7±3.7^{a}	27.0±3.4^{a}	25.4±3.2^{ab}	24.9±2.7^{ab}	23.2±4.4^{ab}
P $^{water-soluble}$ (mg/l)	12.2±6.0	13.5±5.0	12.7±1.7	30.0±12.1	20.8±3.2	26.4±5.9	14.7±4.9	10.3±3.6	15.2±15.6	15.8±11.9
Electrical conductivity (μS/cm)	34560±277^{ab}	35600±348^{a}	35146±790^{ab}	34320±320^{ab}	33907±300^{ab}	33947±477^{ab}	34000±317^{ab}	34800±577^{ab}	33547±439^{b}	34253±1449^{ab}
Salinity (‰)	23.3±0.6^{bc}	25.0±0.0^{a}	23.0±0.0^{bc}	22.5±0.7^{c}	22.7±0.6^{c}	24.3±0.6^{ab}	23.0±0.0^{bc}	24.3±0.6^{ab}	23.7±0.6^{abc}	25.0±0.0^{a}
pH	8.51±0.08^{a}	7.63±0.12^{b}	7.64±0.31^{b}	7.66±0.12^{b}	7.79±0.39^{ab}	8.16±0.20^{ab}	7.59±0.46^{b}	7.77±0.40^{ab}	8.02±0.25^{ab}	8.20±0.15^{ab}

Data are presented in mean ± SD of 3 replicates. Data indicated with different small letter superscripts show significant difference at $P < 0.05$ within different treatments. (One way ANOVA with Tukey test, $p < 0.05$)

3.3.4 Time effect of 2.25% SMCE+0.25% Nitrate on the removal of pollutants in sediment

3.2.4.1 Removal of DEHP and odor

Three percent of SMCE and 2.25% SMCE+0.25% nitrate showed the similar effect on the removal of DEHP from sediment. At the first week, DEHP had a sharp decrease after the addition of SMCE or the mixture. Continually, in the second week, there was a slight drop on the content of DEHP in sediment (Figure 3.5; Table 3.7). Compared with two treatments, control injected with the same volume of distilled water had no effect on the content of DEHP versus the time. As two major malodors in anaerobic environment, NH_3 and H_2S, could be removed significantly after 7-day treatment with 2.25% SMCE+0.25% nitrate which suppressed the odor generation for over one month. The treatment by SMCE decreased the malodors, but could not completely remove the malodors (Figure 3.6; Table 3.8 and 3.9).

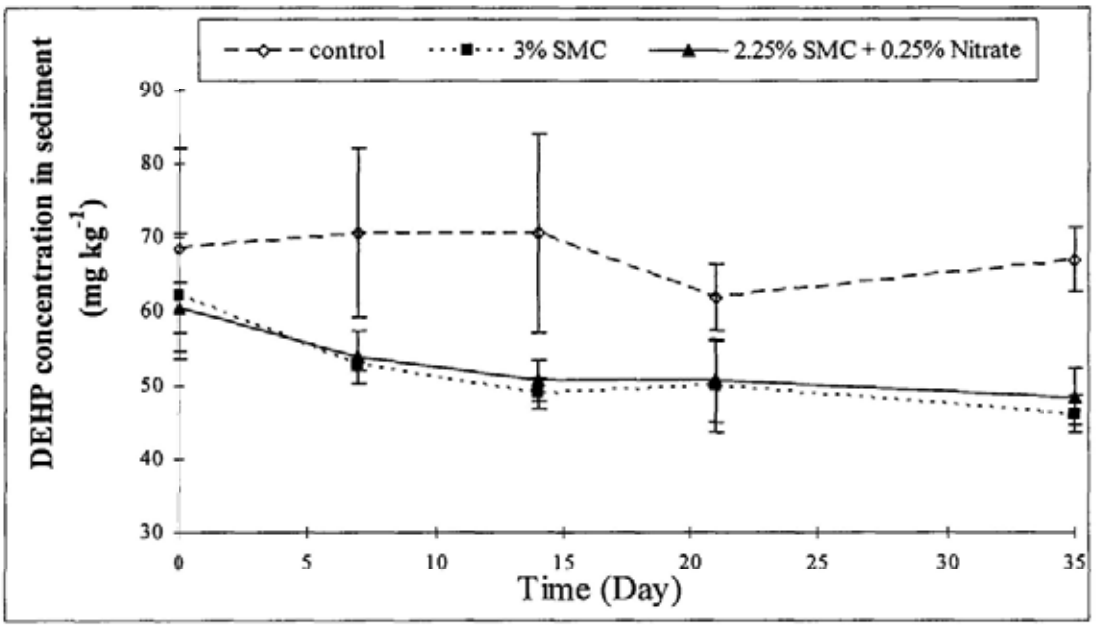


Figure 3.5 The change of DEHP content in sediment in time effect experiment. Data are presented in mean ± SD of 3 replicates (Two-way ANOVA with Tukey test, $p < 0.05$).

Table 3.7 The results of the two-way ANOVA analyses on the study of DEHP content.

(a) The results of the two-way ANOVA analyses on the study of DEHP content between control and 3% SMCE treatment. A probability value of $p<0.05$ is considered as significant.

	df	F	*p* value
Treatment	1	28.088	0.000
Time	4	1.372	0.279
Treatment x Time	4	0.938	0.462

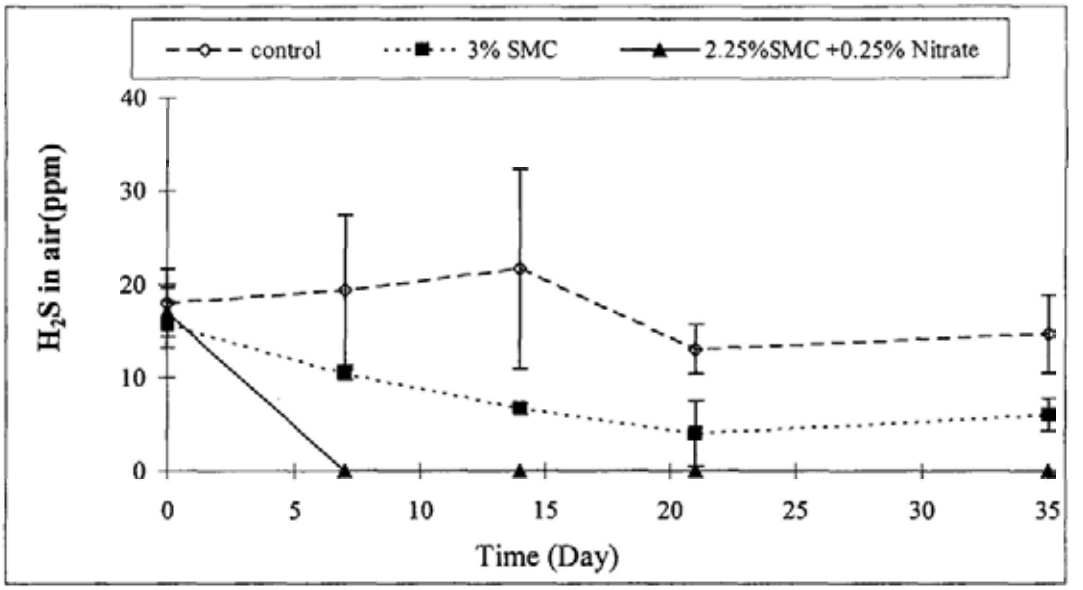
(b) The results of the two-way ANOVA analyses on the study of DEHP content between control and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	26.662	0.000
Time	4	1.087	0.390
Treatment x Time	4	0.062	0.650

(c) The results of the two-way ANOVA analyses on the study of DEHP content between 3% SMCE and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	0.302	0.589
Time	4	8.916	0.000
Treatment x Time	4	0.164	0.954

(a)



(b)

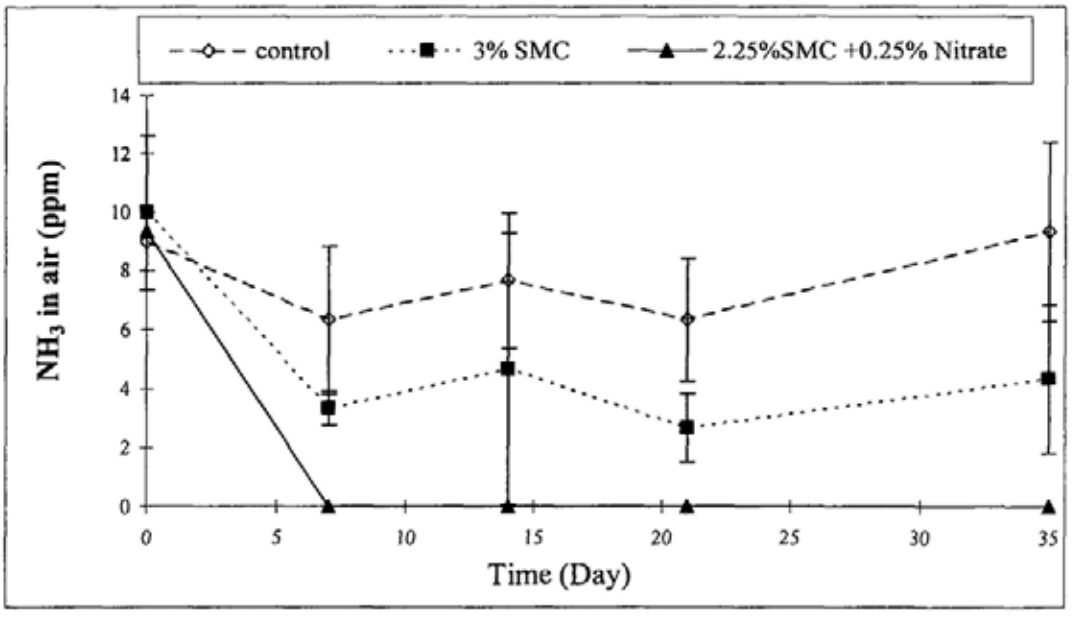


Figure 3.6 The change of (a) H_2S and (b) NH_3 contents in the air with time. Data are presented in mean $\pm$ SD of 3 replicates. (Two-way ANOVA with Tukey test, $p < 0.05$).

Table 3.8 The results of the two-way ANOVA analyses on the study of H_2S content.

(a) The results of the two-way ANOVA analyses on the study of H_2S content between control and 3% SMCE treatment. A probability value of $p<0.05$ is considered as significant.

	df	F	*p* value
Treatment	1	8.941	0.007
Time	4	3.809	0.018
Treatment x Time	4	1.202	0.341

(b) The results of the two-way ANOVA analyses on the study of H_2S content between control and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	96.800	0.000
Time	4	14.263	0.000
Treatment x Time	4	7.613	0.001

(c) The results of the two-way ANOVA analyses on the study of H_2S content between 3% SMCE and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	20.082	0.000
Time	4	20.127	0.000
Treatment x Time	4	1.036	0.414

Table 3.9 The results of the two-way ANOVA analyses on the study of NH_3 content.

(a) The results of the two-way ANOVA analyses on the study of NH_3 content between control and 3% SMCE treatment. A probability value of $p<0.05$ is considered as significant.

	df	F	*p* value
Treatment	1	24.301	0.000
Time	4	2.934	0.046
Treatment x Time	4	1.261	0.318

(b) The results of the two-way ANOVA analyses on the study of NH_3 content between control and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	65.001	0.000
Time	4	5.072	0.005
Treatment x Time	4	4.313	0.011

(c) The results of the two-way ANOVA analyses on the study of NH_3 content between 3% SMCE and 2.25% SMCE+0.25% nitrate treatment. A probability value of $p<0.05$ is considered as significant

	df	F	*p* value
Treatment	1	68.149	0.000
Time	4	72.195	0.000
Treatment x Time	4	9.471	0.000

3.3.4.2 Removal of heavy metals

The results were similar to the two experiments above mentioned, and copper in sediment had a decrease tendency after addition of 2.25% SMCE+0.25% nitrate (Figure 3.7). Owing to the heterogeneity of sediment resulting in the large SD, there was no significance between the control and treatments on copper contents. There were still no significant effect on other metals such as zinc and chromium (Figures 3.9 and 3.10).

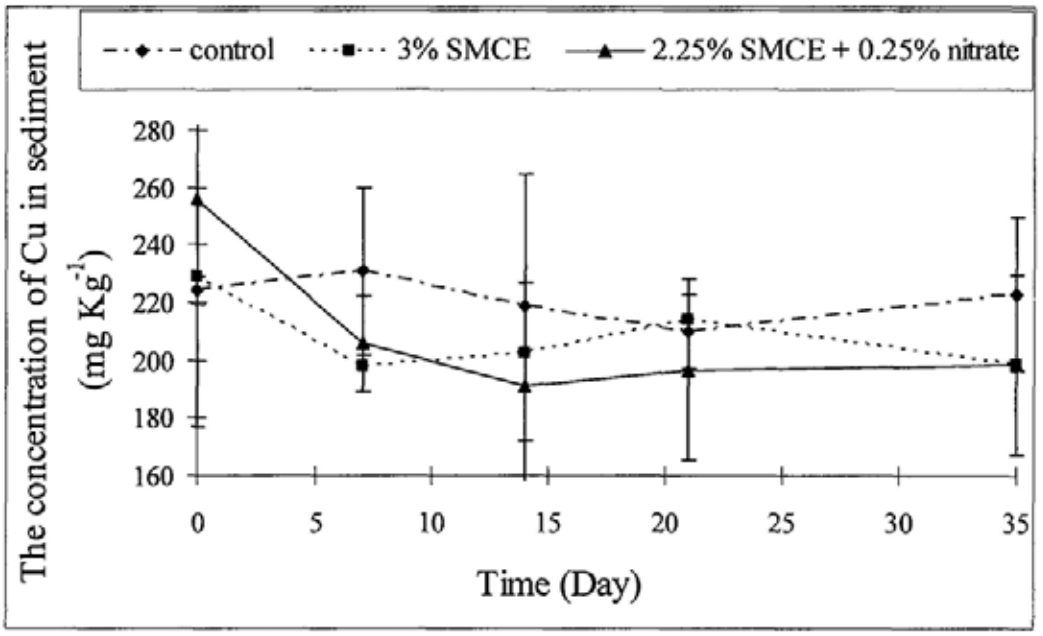


Figure 3.7 The change of copper content in sediment in time effect experiment. Data are presented in mean ± SD of 3 replicates. There is no significant difference after two-way ANOVA with Tukey test ($p < 0.05$).

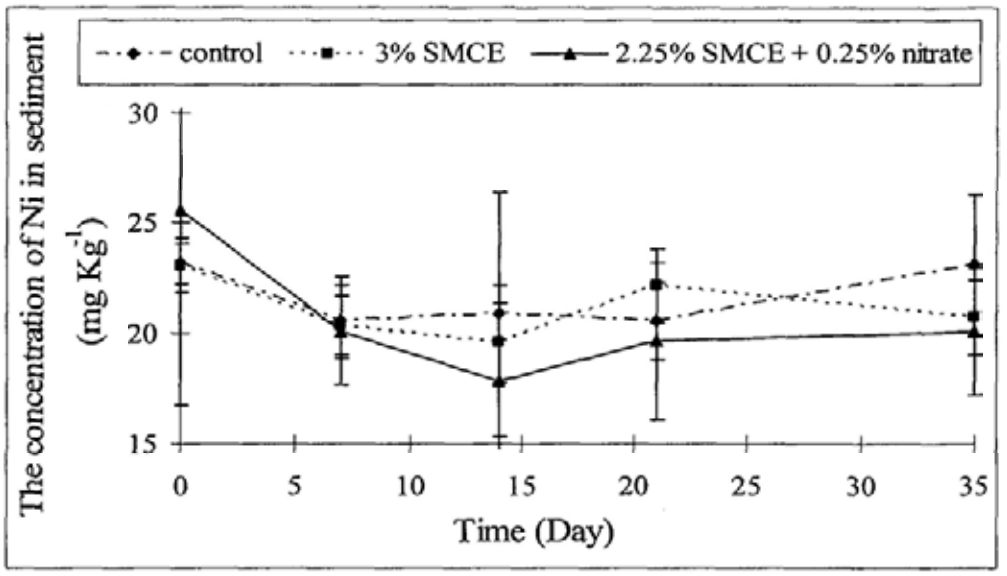


Figure 3.8 The change of nickel content in sediment in time effect experiment. Data are presented in mean ± SD of 3 replicates. There is no significant difference after two-way ANOVA with Tukey test ($p < 0.05$).

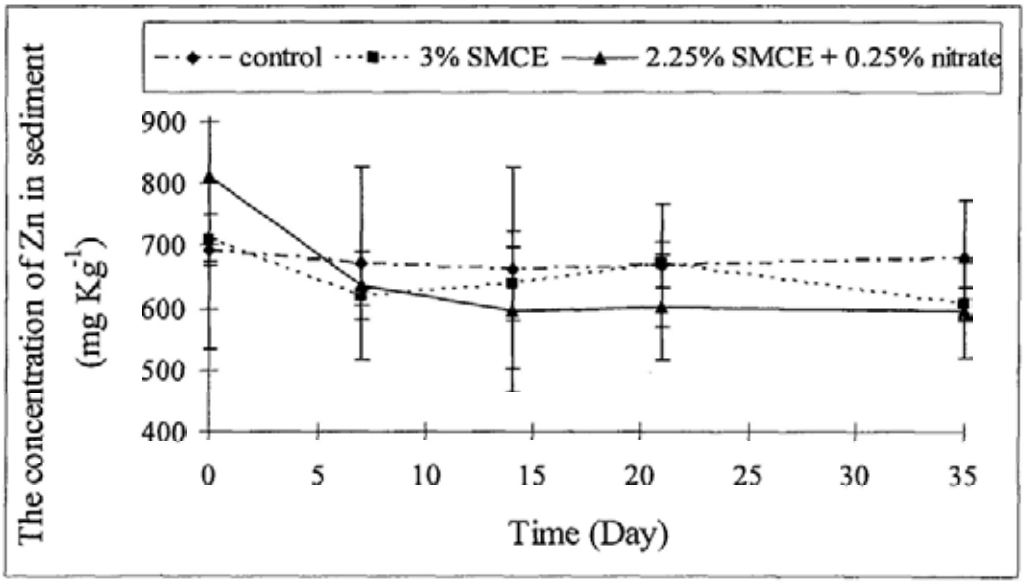


Figure 3.9 The change of zinc content in sediment in time effect experiment. Data are presented in mean ± SD of 3 replicates. There is no significant difference after two-way ANOVA with Tukey test ($p < 0.05$).

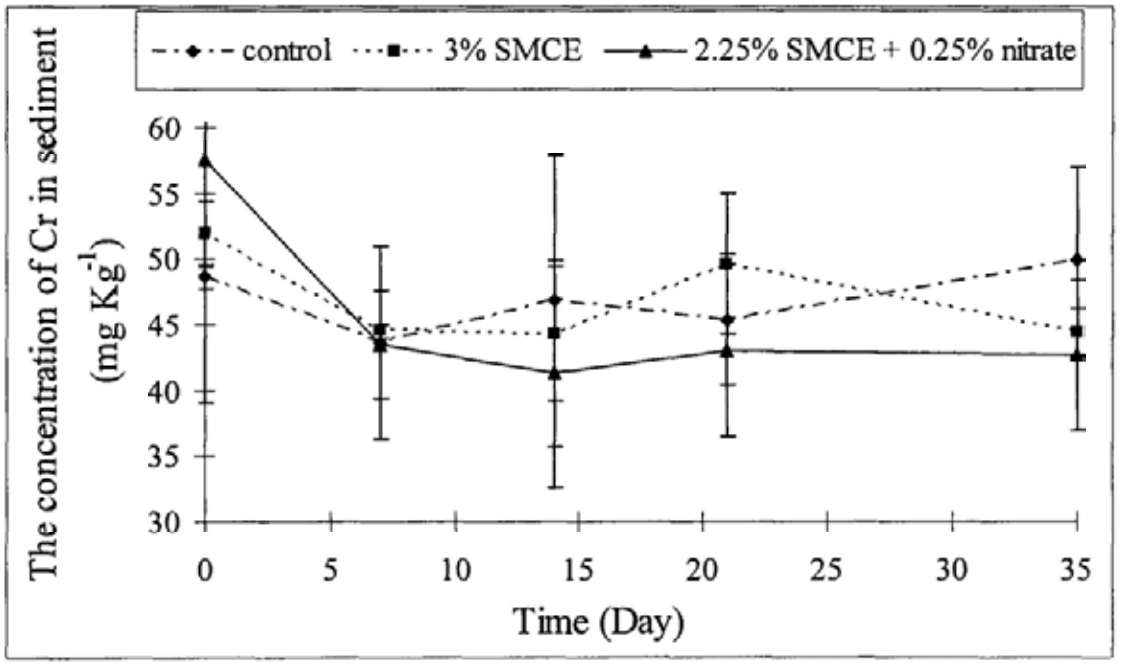


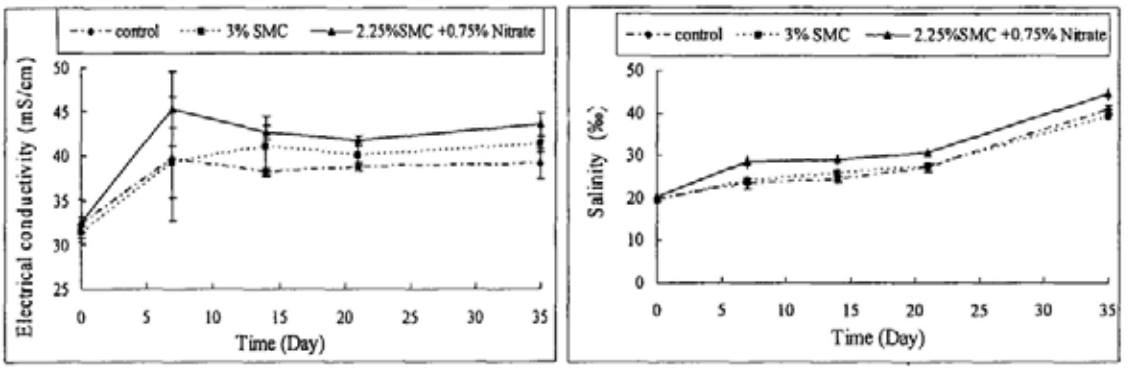
Figure 3.10 The change of chromium content in sediment in time effect experiment. Data are presented in mean ± SD of 3 replicates. There is no significant difference after two-way ANOVA with Tukey test ($p < 0.05$).

3.3.4.3 Change of physical parameters

Compared with the application of SMCE, the addition of the combination of SMCE and nitrate increased the electrical conductivity and salinity slightly in pore water after 7-day treatment (Figure 3.11). In addition, the neutralization of pH by nitrate could be observed again in this experiment. In pore water, NO_x was increased significantly and maintained at a higher level in the treatment of nitrate+SMCE compared with control and the treatment with SMCE, while NH_3 content dropped with time. PO_4^{3-} as another nutrient in water was increased in the two treatments due to the addition of SMCE, and

then continued to decrease with time. During the treatment period, NH_4^+, NO_x and PO_4^{3-} presented decreasing trend (Figure 3.12).

(a) (b)



(c)

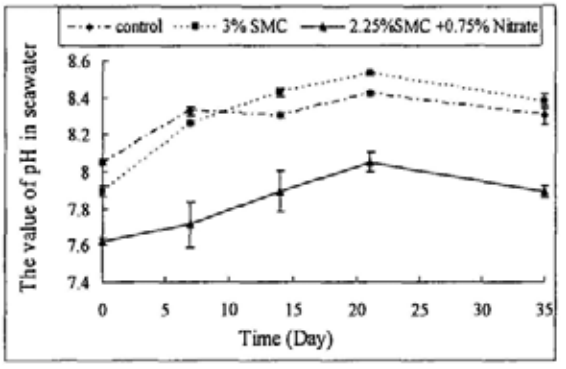
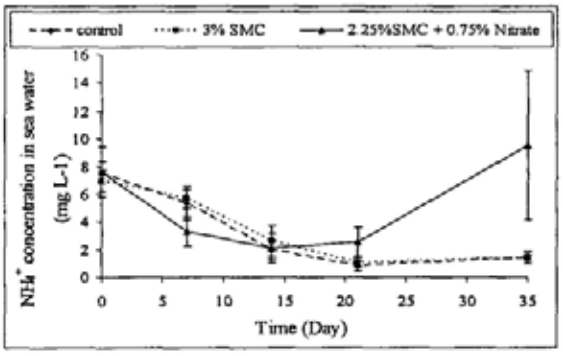
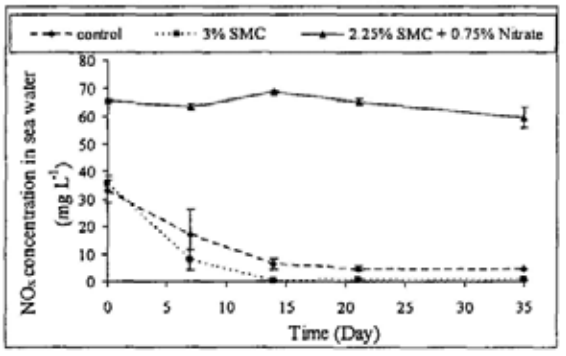


Figure 3.11 The change of physical parameter (a) electrical conductivity, (b) salinity and (c) pH value in pore water in time effect experiment. Data are presented in mean ± SD of 3 replicates.

(a)



(b)



(c)

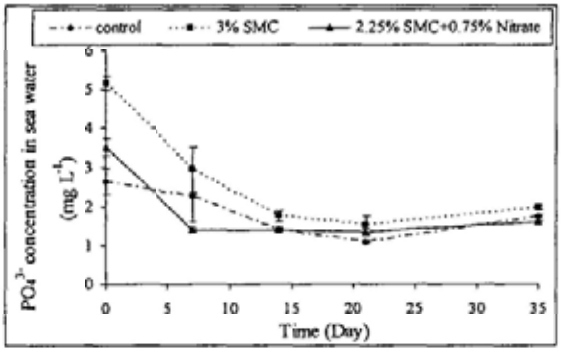


Figure 3.12 The change of nutrients in pore water in time effect experiment: (a) NH_4^+ content in pore water with time. (b) NO_x content in pore water with time. (c) PO_4^{3-} content in pore water with time. Data are presented in mean $\pm$ SD of 3 replicates.

3.3.4.4 Microbial population in sediment and seawater

For biological parameter, aerobic and anaerobic bacteria in sediment had a similar tendency. At the beginning bacteria had a rapid growth stimulated by treatments due to the addition of nutrients, and the treatment with SMCE had the largest population. After 7 days bacterial population began to decrease, but the number of anaerobic bacteria showed a slower decrease than aerobic bacteria. After three weeks, the population of bacteria in

treatments reached the same level as control. For the bacterial population in pore water, they showed a similar phenomenon, increase at the first week and then decrease to the same level as control after two weeks. At the initial, the treatment with 3% SMCE could increase the number of aerobic bacteria in pore water, while the treatment with SMCE+nitrate did not change the aerobic bacteria population significantly (Figure 3.13).

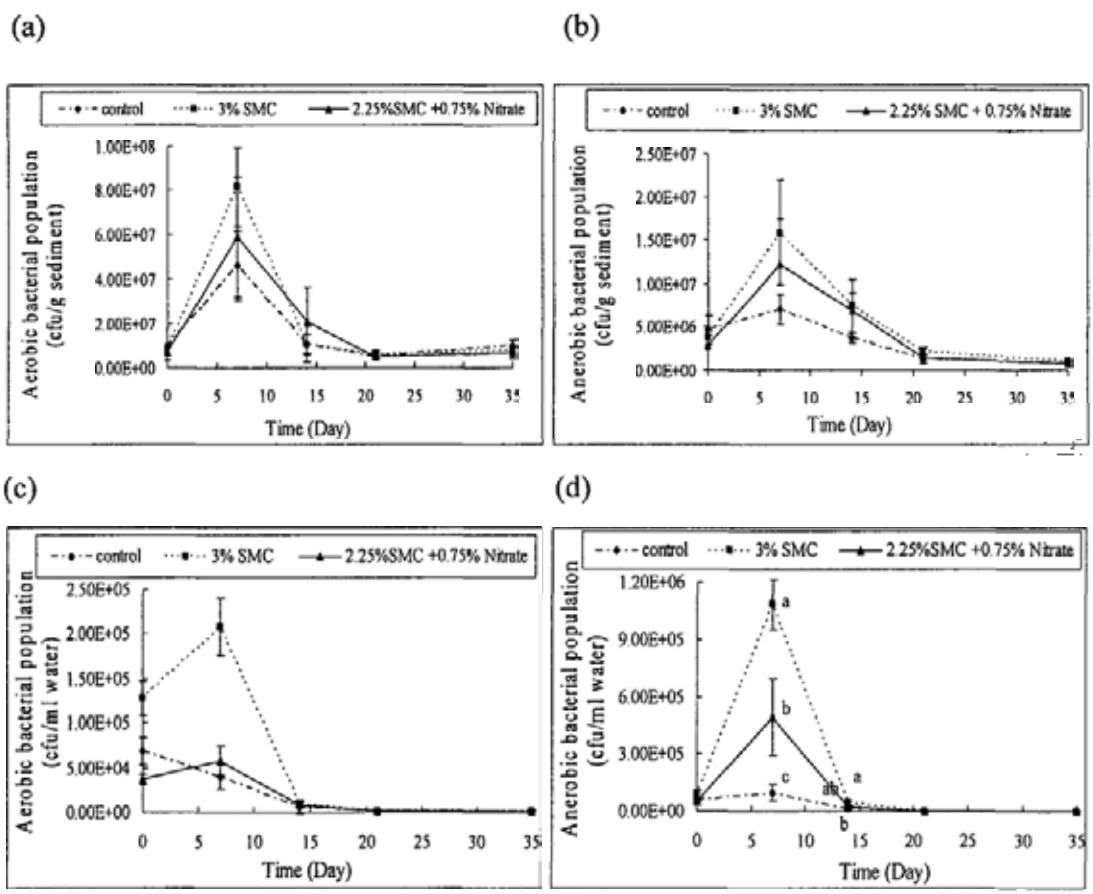


Figure 3.13 The change of (a, c) aerobic and (b, d) anaerobic bacterial population in sediment (a, b) and water (c, d) in time effect experiment. Data are presented in mean ± SD of 3 replicates. Means indicated with different small letter superscripts show significant difference at $P<0.05$ within different treatments at the same time point. (One way ANOVA with Tukey test, $p<0.05$)

3.3.5 A comparison of the effects of different amounts of SMCE on raw sediment and nitrate-treated sediment

3.3.5.1 Removal of DEHP

Comparing GC chromatograms of original sediment treated by 5% SMCE at first day, after one week and two weeks treatment, organic pollutant extracted showed decrease with time (Figure 3.14). As shown in the Figure 3.15, there was no significant difference on the contents of DEHP between different treatments.

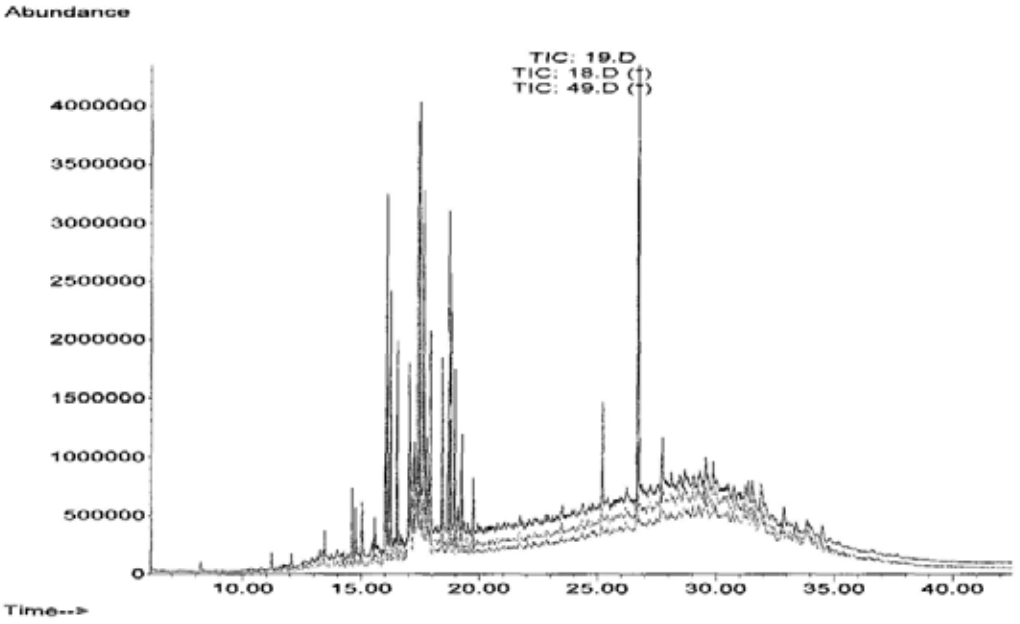


Figure 3.14 GC profiles of sediments (a) before (black curve) and after 5% SMCE treatment for (b) 7 days (red curve) and (c) 14 days (green curve).

(a) (b)

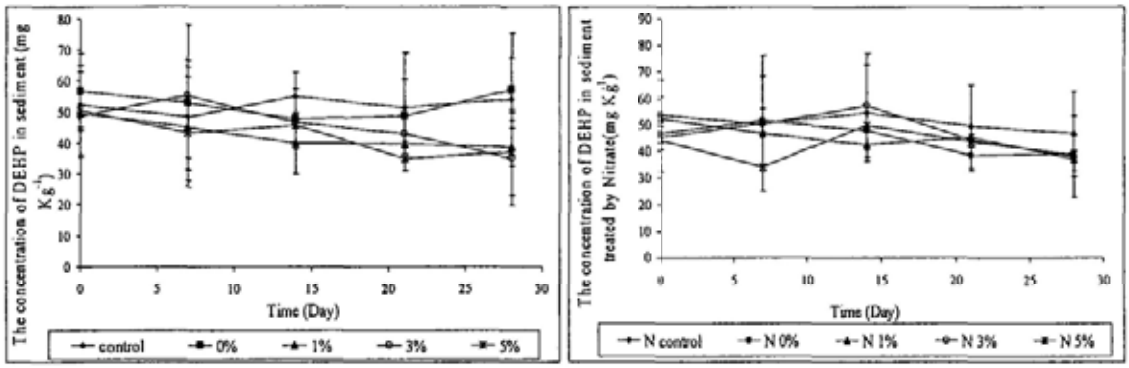


Figure 3.15 The change of DEHP contents in (a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

3.3.5.2 Removal of heave metals

For heavy metals, only copper had a decreasing trend and after one month, 3% SMCE mobilized 23% copper from raw sediment after one month (Figure 3.16), while there was no pattern in nitrate-treated sediment. Other heavy metals, such as zinc (Figure 3.17), nickel (Figure 3.18), and chromium (Figure 3.19) did not show the mobilization from sediment. Compared with raw sediment, heavy metals contents in nitrate-treated sediment had a larger fluctuation.

3.3.5.3 The changes of physical parameters

As shown in Figures 3.20 and 3.21, pH values also showed a slight increase and electrical conductivity had no significant change in both raw sediment and nitrate-treated sediment after treatment of SMCE as in the previous experiments. Similarly, pH and

electrical conductivity showed larger fluctuation in nitrate-treated sediment than those in raw sediment.

(a) (b)

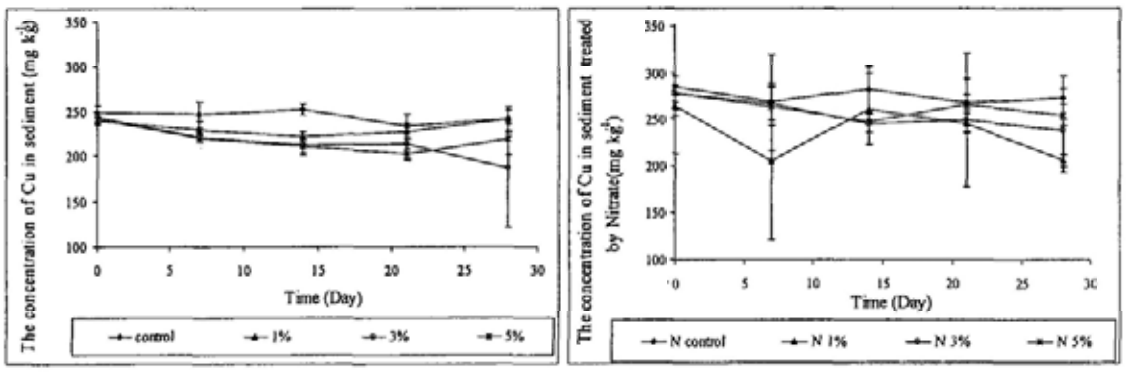


Figure 3.16 The change of Cu contents in (a) original sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

(a) (b)

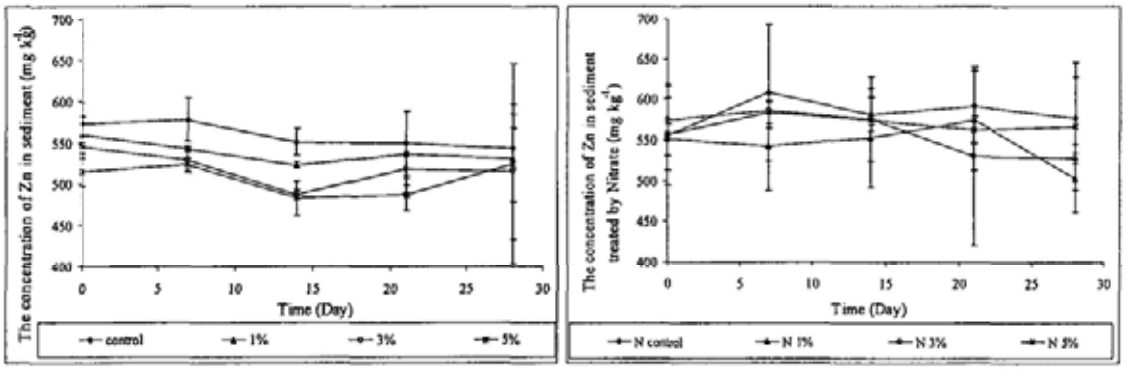


Figure 3.17 The change of Zn contents in (a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

(a) (b)

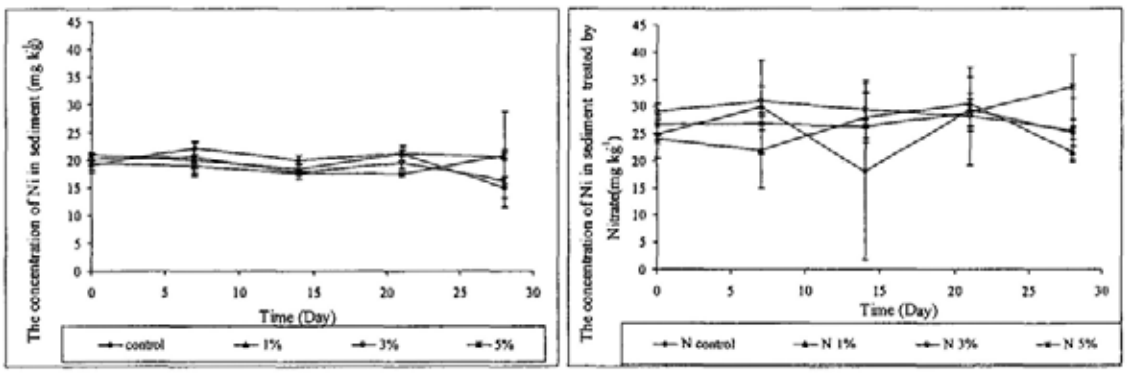


Figure 3.18 The change of Ni contents in (a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

(a) (b)

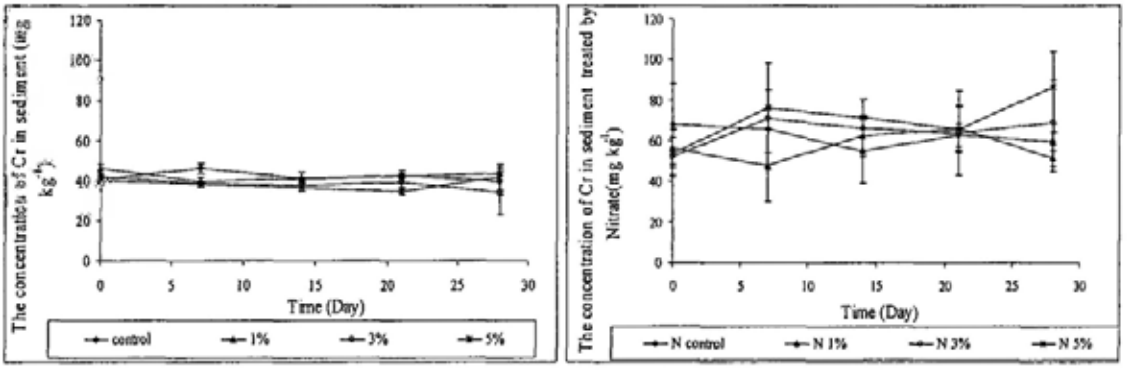


Figure 3.19 The changes of Cr contents in (a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

(a) (b)

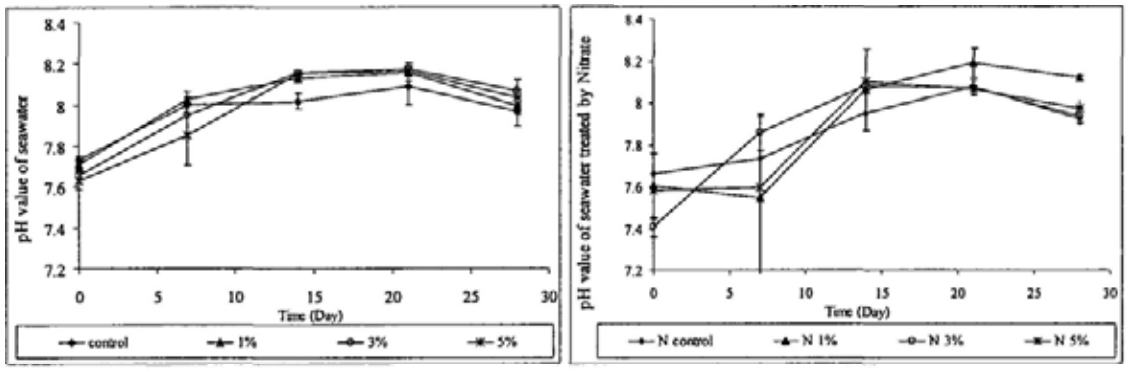


Figure 3.20 The change of pH values in seawater above (a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

(a) (b)

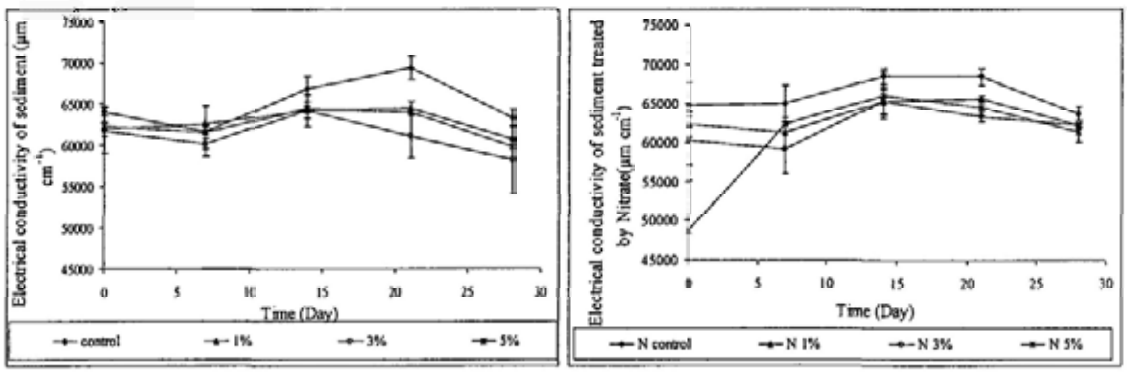


Figure 3.21 The change of electrical conductivity in seawater above ((a) raw sediment and (b) nitrate-treated sediment. Data are presented in mean ± SD of 3 replicates.

3.4 Discussions

3.4.1 The characteristics of sediment and SMC

In this study, sediment samples were collected from KTAC, and were heavily contaminated with heavy metals and organic pollutants. Cu, Pb, Cr, Ni, Zn and DEHP detected were higher than the safety guideline. However, sediment contained abundant nutrients. Thus there were nutrients in the sediment to support microbial biodegradation of organopollutants.

The white-rot fungi are considered the effective organic pollutant degraders, being able to use their non-specific lignolytic degradation system as well as intracellular enzymatic mechanisms. As waste of mushroom cultivation, the *Pleurotus pulmonarius* spent mushroom compost also immobilizes various extracellular enzymes such as laccase and manganese peroxidase. SMC was a good nutrient source. Addition of SMC to the sediment would introduce nutrients to sediment. When the nutrient condition in sediment was improved, the microbial population would be increased leading to improvement in biodegradation.

3.4.2 Pollutant removal capacity of SMC/SMCE compared with nitrate and H_2O_2

3.4.2.1 Removal of DEHP

SMC/SMCE, nitrate and H_2O_2 were tested their removal capacities of pollutants from sediment in this experiment. In previous reports, it has been indicated that calcium nitrate and SMC are effective in removal of organopollutants (Chiu et al., 1998; Lau et al., 2003; Law et al., 2003; Tsang, 2004; Gong et al., 2006). In addition, SMCE extract contains the water-soluble lignocellulolytic enzymes which belong to oxidoreductases and generate highly reactive hydroxyl (·OH) radicals and aromatic radicals to degrade organopollutants non-specifically. In this study, the enzyme activities of laccase (0.63 mmoles/min/g) and manganese peroxidase (0.48 mmoles/min/g) were detected in SMC used. This is the first time to report the degradation of organic pollutants and DEHP in sediment by spent mushroom compost. Moreover, not only microorganisms contained in SMC or SMCE could stimulate the degradation of organic matter, but also the addition of SMC or SMCE benefits the activities of original microorganisms in sediment to degrade organic pollutants. Yet, there was no significant difference between control and treatments in DEHP removal. Presumably the aerobic setup used in the experiments has stimulated the natural biodegradation.

3.4.2.2 Removal of heavy metals

Marine sediments are in fact constituted by a complex heterogeneous mix of geochemical phases, which are able to bind metals by means of precipitation, ionic exchange and adsorption reactions (Di Palma & Mecozzi, 2007). As a result, heavy metals can be found in sediments in several forms, including exchangeable forms such as carbonates, iron or manganese hydroxides, organic matter and sulfides (Di Palma &

Mecozzi, 2007). As the result shown, SMC and SMCE affected the nutrient contents of sediments and enhance the indigenous microbial activities. In addition, enzymes and microorganisms contained in SMC or SMCE also could stimulate the degradation of organic matter, which also benefits to mobilize heavy metals from sediment. However, different metals showed different removal efficiencies, which may be due to the combined forms and species of heavy metals with sediments.

3.4.2.3 The nutrient effects and the change of physical parameters

As the results shown (Table 3.5), calcium nitrate and SMC and SMCE could decrease S contents of sediment significantly. This benefits the removal of hydrogen sulfide, the potential source of malodors. SMC and SMCE showed greater S removal and the odor removal was the greatest after nitrate treatment. The results may be due to the different principles of them. For the removal of odor, the principle of the application of nitrate mainly is due to the oxidation of nitrate. Sulfides could be oxidized as in the following: $S^{2-} + 2NO_3^- + 4H^+ \rightarrow SO_4^{2-} + N_2 + 2H_2O$, and the content of S was declined after treatment by nitrate. However, the declining of S content by SMC has not been reported. The action mechanism is not known.

Alkaline pH in sediment system is a limitation for the mobilization of heavy metal from sediment. The application of nitrate decreased pH value, favouring the mobilization of heavy metals. However, nitrate increased the salinity and electrical conductivity of

sediment. In comparison, the application of SMC and SMCE did not affect the chemical properties of sediment.

3.4.3 Dose response of SMC/SMCE+Nitrate to sediment on the removal of pollutants

3.4.3.1 Removal of DEHP and odor

Considering the merit of nitrate to remove odor, different proportions of SMC/SMCE with nitrate were applied into sediment. Finally the odor was completely removed using a combination 2.25% SMCE and 0.25% nitrate.

3.4.3.2 Removal of heavy metals

Similarly to the first experiment, copper was the metal that was easily mobilized from sediment. According to most researchers, copper occurs in the bottom sediments mainly in the sulfide-organic fraction (60%) (Kersten, et al., 1987; Rauret, et al., 1991; Kwapulinski and Wiechula, 1993; Rule et al., 1996; Loska and Wiechuła, 2000) and in residual form: organically bound. Copper compounds were of the following abundance: carbonates>residual>adsorbed>exchangeable (Zwebe, et al., 1999; Loska and Wiechuła, 2000). As the results shown in first experiment, the treatment could decrease S content significantly. Therefore, copper could migrate from the sulfide-organic fraction easily. Zinc occurs as a zinc iron sulfide with metal-to-sulfur ratios in the range 0.59 to 0.87 in

sediment. Copper sulfides tend to nucleate on surfaces whereas the zinc sulfides occur both on surfaces and as floccular precipitates in open pore space (Large, et al., 2001).

3.4.3.3 Change of physical parameters

In the sediment, adverse environmental conditions such as higher pH did not benefit degradation of DEHP and mobilization of heavy metals. In addition, leaching of copper was the highest from the bottom sediment in the non-aerated systems when pH reaching 2.0-3.5, while the pH range was from 2.0 to 4.5 in the aerated systems (Loska and Wiechuła, 2000). Therefore, the mixture of a small quantity of nitrate and SMCE may be better than SMCE because nitrate could neutralize pH in this study.

3.4.4 Time effect of 2.25% SMCE+0.25% Nitrate on the removal of pollutants in sediment

3.4.4.1 Removal of DEHP and odor

From the results of dose response experiment, the combination of 2.25% SMCE+0.25% nitrate was selected for the time effect experiment. In time-response experiment, the fast decrease of DEHP occurred in the first week. This might be due to the injection of enzymes of SMC. At later time, the drop in activities of SMC enzymes resulted in the slowdown of degradation of DEHP. Moreover, heavy metals in sediment

inhibited the degradation of DEHP, because the toxicity of heavy metals is a known factor in the suppression of microbial activity (Giller et al., 1998; Chang et al., 2005).

The combination of SMCE and nitrate could remove the malodors H_2S and NH_3 gas contents. Previous studies had reported the principle of malodors removal by the oxidation of nitrate. However, there are no reports about the removal of NH_3 by nitrate. For another odor resource NH_3, some information could be obtained from the change of nutrients in dose response and time effect experiment. The content of NH_4^+ declined not only in water but also in air after treatment, and the PO_4^{3-} also decreased with time at the same time, which was likely due to their depletion during the period of microbial action (Xu et al., 2005). This suggests that the removal of NH_3 may be due to other reasons, e.g. microbial action. SMC may act in enhancing microbial action to remove the malodor compounds.

3.4.4.2 Removal of heavy metals

Due to the large SD of heavy metal contents at initial, it could not be judged whether there was any effect by treatment on the removal of heavy metals. However, SMCE showed different effects compared with the results from dose response experiment. It was possible that SMCE had the capacity to mobilize copper, but the principles are still not known.

3.4.4.3 Change of microbial population

Microbial action also plays an important role in bioremediation. The studies of Chang et al. (2005) reported DEHP degradation in river sediment was the result of microbial action and the respective half-lives of DEHP was 25.7 days under optimal conditions of 30°C and pH 7.0. In addition, 24 pure microbial strains were isolated by Chang et al. from sediment and were found that they had the capacity of anaerobically degrading DEHP by using DEHP as a carbon source (2005). Spent mushroom of *P. pulmonarius* contains a lot of nutrients and microorganisms (Law et al., 2003; Gong, 2006). The stimulation of SMCE and its combination with low concentration of nitrate on the native microflora in sediment and pore water could be observed from the increase of the microbial population in the first two weeks from the time effect experiment (Figure 3.13). A higher increase in microbial population, corresponding to the higher DEHP removal was recorded in the first week. This suggests that microbial action may be another main reason in bioremediation of sediment. Compared to control, the content of DEHP dropped in treatments below the standard level; however, the effect of anaerobic degradation was still unsatisfactory and only 20% DEHP were degraded after one month. In addition, anaerobic microorganisms preferred an environment with a pH of approximately 7.0 and are usually inhibited at pH values lower than 6 or higher than 9 (Widdel, 1988).

3.4.5 A comparison of the effects of different amounts of SMCE on raw sediment and nitrate-treated sediment

In this experiment, sediment was collected from KTCA, and some areas of that site had been treated by calcium nitrate for several months. In general, nitrate-treated sediment had a larger fluctuation than raw sediment using SMCE to treat them. However, in the dose-response and time effect experiments, nitrate did not present the impact on treatment of SMCE. Therefore, the application of nitrate may change some physical characteristics of sediment, such as expansion of sediment volume, the change of combination form of chemicals and so on, which resulted in the larger heterogeneity of sediment. So when SMCE was applied into nitrate-treated sediment, large fluctuation occurred. Because many mechanisms of SMCE on the metal mobilization are unknown, further studies should be designed and carried out.

Chapter 4 Using SMC to remove DEHP and DBP in water system

4.1 Introduction

4.1.1 Contaminated water

Contaminated water was provided from China Light & Power Company Limited (CLP), Tuen Mun, New Territories, Hong Kong. The water was contaminated with dibutyl phthalate (127.5±20.7 μg/l) and DEHP (67.0±7.7 μg/l), exceeding the standard level of total phthalates in water (6 μg/l). Two important sources for contaminated water with phthalates are the drainage of industrial wastewater and the slow-release of plastic PVC from solid wastes. In industrial companies, PVC coating is commonly used in cables and lines for the transfer of electricity and information, and soft PVC is found in electrical products, as well as in water supply and drainage pipe (www.greenpeace.org). Therefore, the contaminated wastewater should be treated for drainage.

4.1.2 DEHP in contaminated water

Due to the widespread usages of plastic products in large quantities, DEHP was found in various environmental compartments including groundwater, surface water, coastal seawater, sediment as well as organisms (Wang et al., 1995; Mackintosh et al., 2004). The reported concentrations of DEHP in samples taken from various places worldwide range from 0.09–93.8 μg/l in surface water, 1.74–182 μg/l in municipal

wastewater, and 0.21–8.44 mg/kg in sediment (Staples et al. 1997; Boonyatumanond et al., 2002; Birkett and Lester, 2003). It has been reported that the half-life of dissolved DEHP in surface water ranges from 2–15 days (O'Grady et al., 1985; Staples et al., 1997).

4.1.3 Dibutyl phthalate (DBP) in contaminated water

Worldwide production of phthalates increased from 1.8 million tons in 1975 to 3 million tons in 2001 (Teil et al., 2005). In 1997, consumption of dibutylphthalate (DBP; Figure 4.1) was 20 to 50 thousand tons per year in Europe (Teil et al., 2005). More than one million pounds of DBP are produced each year in the United States (www.weitzlux.com, 2006). Consequently, the environmental contamination level is increased due to the increasing application of phthalates. It has been reported that the acute toxicity of DBP is low (Ema et al., 1998; Fatoki and Noma, 2002; Zhang et al., 2004). A worker who swallowed 10 g of DBP became nauseated, dizzy, and developed photophobia, tearing, conjunctivitis, keratitis with loss of corneal epithelium (www.osha.gov). However, DBP in diverse environmental samples has xenoestrogenic and endocrine-disrupting effects. The animal research and one recent human study show that prenatal DBP exposure disrupts development of the male reproductive system in ways that may increase the risk of testicular cancer (Gray et al., 1983; NTP, 1991; ATSDR, 2002; Zhang et al., 2004). Cellular studies also suggest for concern among females. DBP increases proliferation of MCF-7 breast cancer cells in culture and promotes drug resistance against the action of tamoxifen in these cells (Olsen et al.,

2001; Okubo et al., 2003). Because DBP is used in some cosmetics products, especially nail polishes and perfumes, its ability to inhibit tamoxifen-induced apoptosis (cell death) could affect breast cancer women who take the drug to prevent recurrence. DBP passes through the body relatively quickly after exposure, in contrast to biopersistent chemicals such as DDT and PCBs, which remain in the body for years or even decades (Olsen et al., 2001; Okubo et al., 2003). Hence, DBP is listed as a priority pollutant by the United States Environmental Protection Agency.

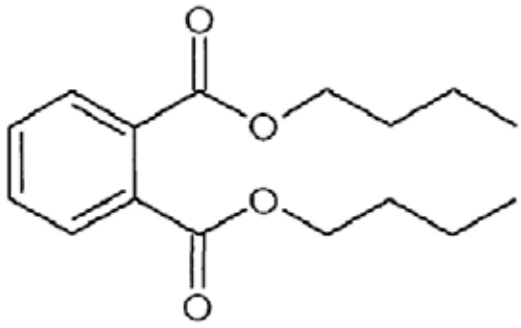


Figure 4.1 Structure of dibutyl phthalate.

Moreover, DBP has been considered as a teratogenic compound to aquatic organisms (GESAMP, 1989). The toxic properties of DBP are even more important considering its high bioaccumulation rate (range from 100 to 3000) in different organisms (Staples et al., 1997). DBP is a rather stable compound in the natural environment. The hydrolysis half-life was estimated to be about 20 years (Gray et al., 1982 & 1983; Staples et al., 1997 a,b). Studies of its biodegradation in fresh waters, marine waters, sediments, wastewaters and sludge revealed rather low degradation rate in the range of several days up to a few months (Staples et al., 1997 a,b).

Taking into account the above-mentioned facts DBP as a pollutant is quite important. Thus, an efficient method for its removal from wastewater would be very important.

Table 4.1 Physical properties of DBP (www.osha.gov).

Molecular weight	278.35 g/mol
Melting point	-35°C
Boiling point	2340°C
Color/form	Colorless, oily liquid
Density	1.05 g/cm^3 at 20°C
Octanol/water partition coefficient	Log K_{ow}=4.72
Aqueous solubility	0.013 g/l

4.1.4 The treatment of contaminated water

There are different methods for pollutants removal from contaminated water, such as physical, chemical and biological treatment. These methods can be further divided into *ex-situ* and *in-situ* remediation technologies. A summary of several remediation technologies for organic compounds removal is listed in Table 4.2. *Ex-situ* remediation is also referred to as the conventional method ''pump and treat''. In this technique, the underground contaminated water is pumped to the surface using a series of extraction wells, treated at the surface to remove the contaminants and then reused or re-injected underground or disposing of it off site. *In-situ* remediation is the treatment of the pollutant at the original place. Physical methods such as adsorption by granular activated carbon (GAC) is technically feasible for water purification, but replacement

and disposal of activated carbon as hazardous waste is a major expense in GAC adsorption water treatment systems (Daifullah and Girgis, 2003; Shih et al., 2003; Ayotamuno et al., 2006; Yin et al., 2007). Chemical methods include coagulation, flocculation, precipitation and oxidation (either chemical or photochemical) (Tiburtius et al., 2005). However, most of these processes are poorly efficient for aromatic compounds, while chemical oxidation alone or photo-oxidation is not cost-effective and may even give undesirable residuals. Bioremediation is an alternative method that has emerged in recent years to treat the wastewater instead of the traditional processes (Vidali, 2001; Shim et al., 2002).

Table 4.2 Summary of remediation methods available for waters contaminated with organic pollutants (Krishna, 2008).

Category	*In-situ* technologies	*Ex-situ* technologies
Physical	Electro remediation	Electro remediation (electrodialysis)
	Capping	Air stripping
	Barrier	Surfactant-modified zeolite
	Hydraulic containment	Carbon adsorption
	Air sparging	Filtration (membrane)
Chemical	Injection of chemical oxidation compounds (O_3, O_2, H_2O_2, Cl_2)	Photocatalysis remediation (nanoparticle TiO_2/UV)
	Phytoremediation	Coagulation, flocculation and precipitation
		Application of chemical oxidation compounds (O_3, O_2, H_2O_2, Cl_2)
Biological	*In-situ* bioremediation (natural and engineered bioremediation)	*Ex-situ* bioremediation (aerobic and anaerobic bioreactors)

Bioremediation is a process to remove organic compounds or to transform them to less harmful substances by utilizing the micro-organism's catabolic (energy producing) and anabolic (cell synthesizing) activities (Yoon et al., 1999). This process is enhanced by the injection of an electron acceptor (e.g. oxygen) or nutrients (e.g. phosphorus and nitrogen) to promote microbial growth. These technologies can be performed *in-situ* or *ex-situ* under aerobic or anaerobic conditions. Classification of bioremediation options and important factors affecting the water bioremediation processes are shown in Figures 4.2 and 4.3 (Boopathy, 2000; Schreiber and Bahr, 2002; Bittkau et al., 2004; Farhadian et al., 2006; Atteia and Guillot, 2007). *In-situ* groundwater bioremediation is a technology that encourages growth and reproduction of indigenous micro-organisms to enhance biodegradation of organic constituents in the saturated zone (www.epa.gov). *In-situ* groundwater bioremediation can effectively degrade organic constituents which are dissolved in groundwater and adsorbed onto the aquifer matrix (Dott et al., 1995). *Ex-situ* bioremediation through biological reactors, both under aerobic and/or anaerobic conditions, has been successfully used in the treatment of water contaminated with chemical pollutants including perchlorate (Min et al., 2004), bromate (Butler et al., 2006), chlorinated hydrocarbons such as trichloroethylene (TCE), phenol, methyl tert-butyl ether (MTBE) and other oxygenated compounds (Kharoune et al., 2001; Vainberg et al., 2002; Zein et al., 2006), alkylate (Cho et al., 2007), and polycyclic aromatic hydrocarbon (PAH) (Guieysse et al., 2000).

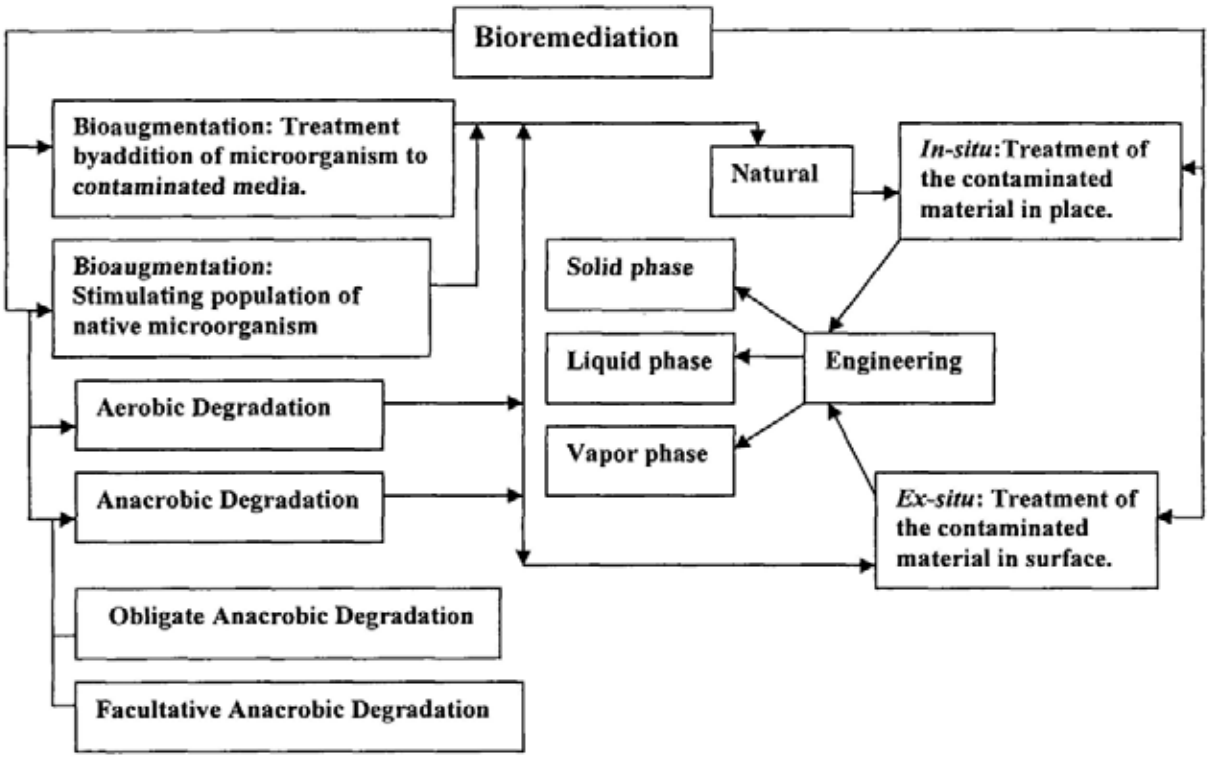


Figure 4.2 Classification of bioremediation options. (Krishna, 2008)

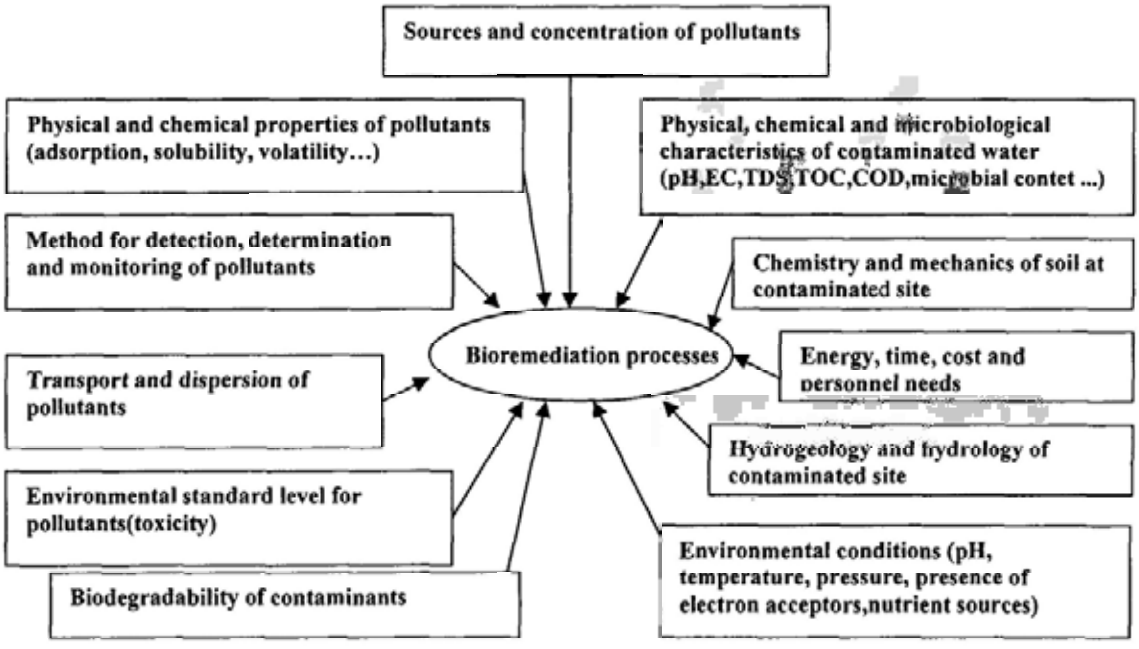


Figure 4.3 Important factors that have to be taken into account to establish an *in-situ* water bioremediation process. (Krishna, 2008)

The term bioreactor refers to a vessel where the biological degradation of contaminants is performed in fully controlled conditions, i.e. parameters such as temperature, pH, aeration and stirring rates are known and controlled. Bioreactors have also been widely applied for treatment of VOCs (volatile organic compounds; such as monoaromatic hydrocarbons) and contaminated gases (Pedersen and Arvin, 1995; Bielefeldt and Stensel, 1999; Lu et al., 2002). Research works have already reported that *ex-situ* bioremediation can be successfully applied for organic compounds removal from water. They can be considered as the best technology in this area, even if drawbacks such as the need for water pumping, power supply, energy consumption, sludge production, VOC stripping and BTEX adsorption on solids are reported. Biological processes such as biosorption (Ramakrishna et al., 1997), bioaccumulation (Gupta et al., 1992; Aksu et al., 2003) and biodegradation (Chao et al., 1994; Banat et al., 1996) have been proposed as potential methods for the removal of pollutants from wastewater. Among these, biosorption is more advantageous for water treatment in which dead organisms are not affected by toxic wastes as they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles (Vieira and Vieira, 2000). Several investigators have reported the potential of different biomaterials to biosorb pollutants from aqueous solutions, including bacteria (Chang et al., 2001), fungi (Fu et al., 2000) and microalgae (Wafaa et al., 2003).

4.1.5 The aim of this study

In this experiment SMC as a biosorption and biodegradation material was used

in bioremediation of contaminated water with DEHP and DBP. The purpose of this experiment was to test the potential capacity of SMC to remove plasticizers in water system.

4.2 Material and methods

4.2.1 Water collection

Contaminated water was collected from the China Light & Power Company Limited (CLP) and was kept in black glass bottles. The dark bottles were transferred to CUHK in ice box and kept in cool room before further study. In the water sample, DBP and DEHP were found exceeding the environmental standard level (6 μl/l).

4.2.2 The characteristics of water

The physical and chemical parameters of water sample were measured as with soil samples mentioned in Chapter 2.

4.2.3 Optimization of the SMC to water ratio

Water samples (10 ml/sample) in glass bottle were mixed with different amounts of SMC (0.0%, 0.2%, 0.4%, 0.6%, and 1.0%; g/ml, SMC/water volume). The mixture was incubated and agitated at 150 rpm at room temperature. At 0, 15, 30 min, 1, 2, 4, 8 and 24 h water samples were taken for the measurement of pollutants by HPLC

(Shimadzu, Japan). A gradient elution was used as the mobile phase on Waters C_{18} Spherisorb ODS column with the program of 60% acetonitrile and 40% water at the initial 5 min, and 100% acetonitrile from 6-20 min (Wang et al., 2005). The flow rate was 1 ml/min. The detection wavelength was 228 nm with the UV- VIS detector. The linear range of the method was determined to be from 2.0 to 500.0 μg/l. Retention time of DBP and DEHP were 8.03 and 14.6 min, respectively. The results reflected the removal of pollutants in water system. Figure 4.4 shows the HPLC chromatogram of 6 μl/l DEHP and 6 μl/l DBP in water.

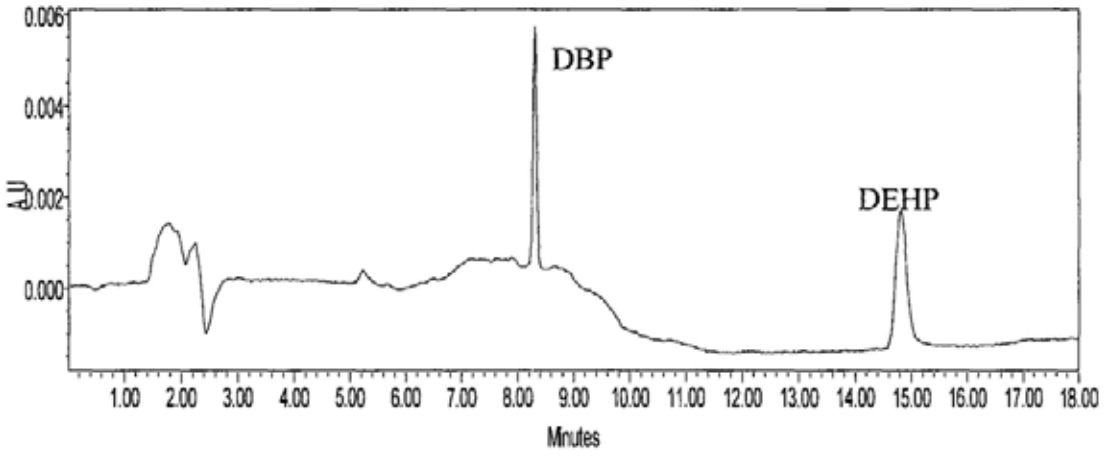


Figure 4.4 The HPLC chromatogram of 6 μl/l DEHP and 6 μl/l DBP in water.

In order to measure biosorption efficiencies of DBP and DEHP, the same setup with different treatment time (1, 2, and 24 h) was designed. After treatment, the whole setups were centrifuged and water was removed. The freeze-dried SMC was extracted with 10 ml DCM twice and concentrated at 60°C by a rotary evaporator with a vacuum pump. One ml HPLC grade methanol was used to redissolve the sample after concentration. The sample was filtered by a 0.45 μm filter (Acrodisc syringe filters 4CR

PTFE) for gas chromatography-mass spectrometry (GC-MSD) measurement. The temperature profile and condition were the same as in Section 2.2. Thus biosorption removal could be obtained.

Biodegradation removal was calculated by subtracting the total residual phthalates measured (residue level in supernatant + in SMC) from the initial amount added.

4.2.4 Optimization of the SMCE to water ratio

According to the ratio of SMC to water (0.0%, 0.2%, 0.4%, 0.6%, and 1.0%), the corresponding volumes of SMCE added into 10 ml water system were 0, 0.05, 0.1, 0.15, 0.25 ml. SMCE was mixed with water sample. The mixtures were incubated at 150 rpm at room temperature. At 0, 15, 30 min, 1, 2, 4, 8, 24 h water samples were taken and measured by HPLC.

4.2.5 The effect of shaking speed on the removal of pollutants by SMC

One percent of SMC was used to test the effect of shaking speed on the removal efficiency. The parameters selected in this experiment were 0, 50, 100, 150, 200 rpm. After incubation for one hour, the contents of DBP and DEHP were measured by HPLC. The optimal shaking speed was obtained.

4.2.6 Effect of sorption-desorption cycles on the removal capacity of SMC

One percent of SMC was added into water system and incubated at room temperature with shaking speed at 150 rpm for one hour. After treatment, the contents of DBP and DEHP in water samples were measured by HPLC. After centrifugation, SMC was collected for sorption removal. The same experiment was repeated 8 times and then the final experiment was incubated for 24 h. Finally, SMC was centrifuged and collected. After freeze-dried, SMC was extracted and the contents of DBP and DEHP were measured by GC-MS.

4.2.7 Isotherm plots and fitting into monolayer model

Langmuir and Freundlich adsorption isotherms were used to characterize the adsorption processes of the DEHP and DBP by SMC. Linear regression analysis with the results at 1 h treatment was employed to assess which model the sorption of SMC fits most. The parameters of the isotherms were determined by the following equations:

$$C_f/q = C_f/q_{max} + 1/(q_{max}b) \quad \text{(Langmuir adsorption isotherm)}$$

$$\text{Log } q = \log k + (1/n) \log C_f \quad \text{(Freundlich adsorption isotherm)}$$

Where q is the removal capacity by sorption (μg/g), C_f is the final concentration of DEHP or DBP (μg/l), q_{max} the maximum adsorption capacity (μg/g), b the affinity constant (l/μg), k the adsorbent capacity, (1/n) the adsorption intensity.

4.3 Results

4.3.1 The characteristics of the contaminated water samples

Table 4.3 shows the characteristics of water sample and spent mushroom compost in this experiment. The water had a high electrical conductivity and salinity. There were small amounts of nutrients in water. However, DBP and DEHP were found exceeding the environmental standard (6 μl/l). In addition, SMC contained many nutrients (N, P and K) and enzymes (specific laccase activity: 1.28±0.92 m mol/min/ g protein; specific manganese peroxidase activity: 0.75±0.52 m mol/min/ g protein).

Table 4.3 The characteristics of water sample and SMC used in this experiment. Data are presented in mean ± SD of 5 replicates.

Parameters	SMC	water
$NO_{X\ water\text{-}soluble}$ (mg kg^{-1}/mg l^{-1})	280±33	1.18±0.05
$NH_{3water\text{-}soluble}$ (mg kg^{-1}/mg l^{-1})	237±59	< 0.5
$P_{water\text{-}soluble}$ (mg kg^{-1}/mg l^{-1})	220±32	< 0.5
K^+ (mg kg^{-1}/mg l^{-1})	3680±384	188±25
pH	7.12±0.06	7.34±0.10
Electrical conductivity (μS cm^{-1})	3016±150	1579±28
Salinity (‰)	33.0 ± 0.7	15±0.3
DBP (μg kg^{-1}/μg l^{-1})	—	127.5±20.7
DEHP (μg kg^{-1}/μg l^{-1})	—	67.0±7.7

— not detectable

4.3.2 Optimization of the SMC to water ratio

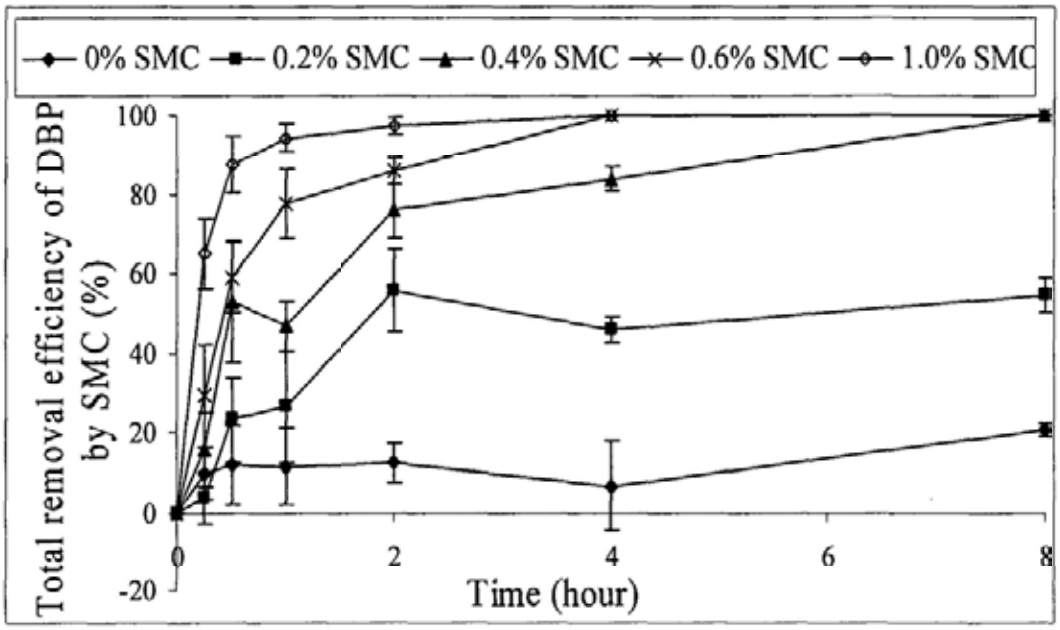
Figure 4.5 shows the effect of the amounts of SMC on the total removal efficiencies of DBP and DEHP. One percentage of SMC had the fastest removal rate 94.2±3.6% and 100% for DBP and DEHP respectively after one hour. After 4 h, the minimum amount of SMC (0.2%) could remove all DEHP and 45.9±3.1% DBP from water. After 8 h, all DBP and DEHP were removed by SMC except 0.2% SMC for DBP.

However, the removal by biodegradation was increased by higher amount of SMC used (Figure 4.6). In addition, removal of DBP by biosorption was increased with the increase in SMC amount, while removal of DEHP was stabilized with 0.6 and 1.0% SMC at 1 and 2 h.

4.3.3 Optimization of the SMCE to water ratio

SMCE showed a similar tend with SMC on the removal of DBP and DEHP from water. However, SMCE needed more time to remove pollutants in water (Figures 4.5 and 4.7). After 24 h, DBP and DEHP could be degraded completely by SMCE except 0.2% SMCE for DBP (Figure 4.7). Similarly, higher amount of SMCE showed faster removal rate. After 4-hour treatment, the maximum amount of SMCE (1.0%) removed all DEHP and 65.3±4.6% DBP from water. Within 8 hours, 1.0% SMCE degraded all DEHP and DBP.

(a)



(b)

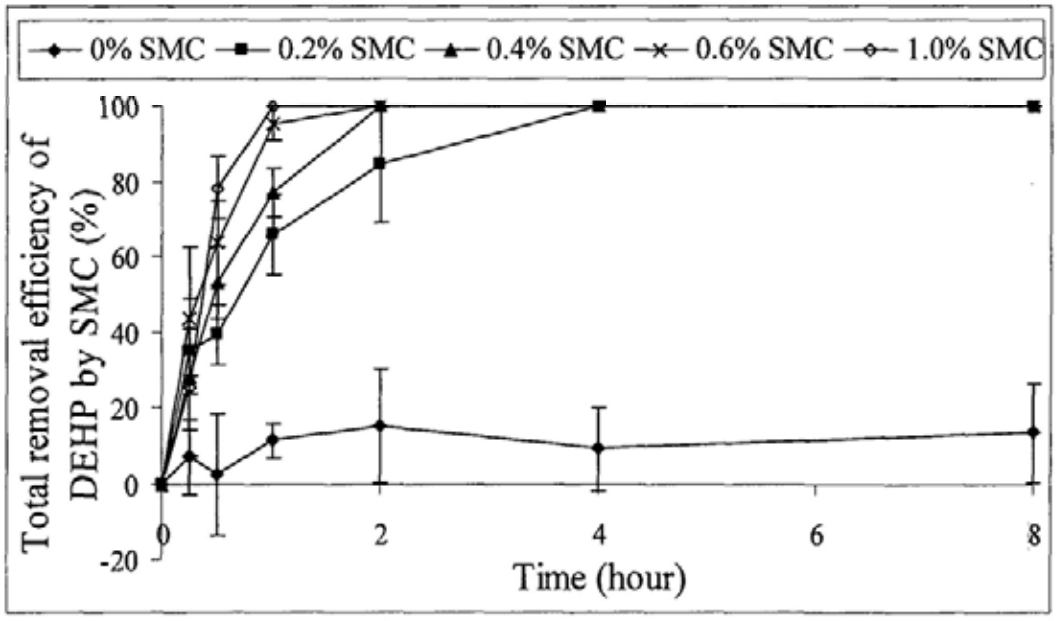


Figure 4.5 The effects of different amounts of SMC on the total removal efficiencies of (a) DBP and (b) DEHP. Data are presented in mean ± SD of 5 replicates.

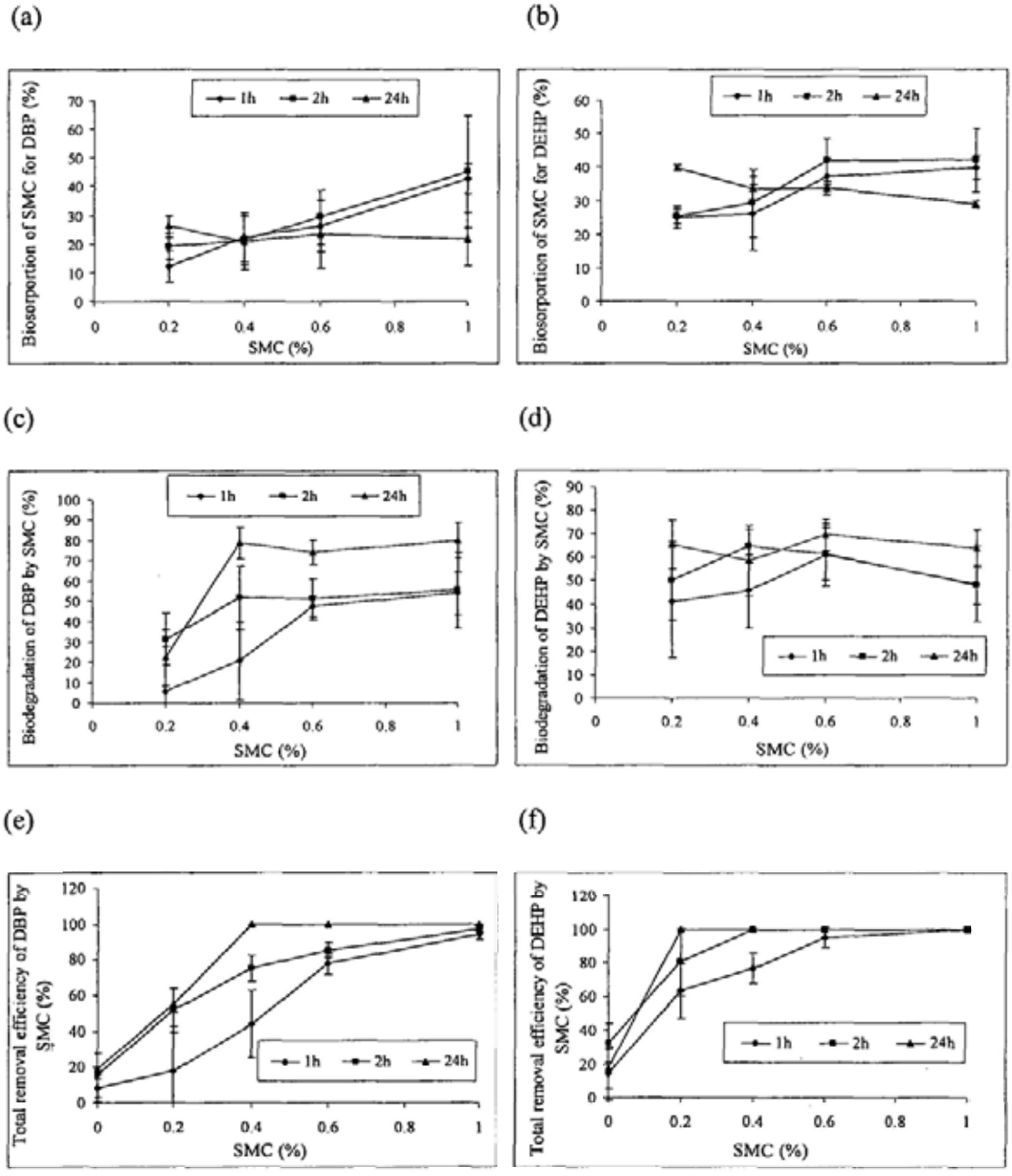
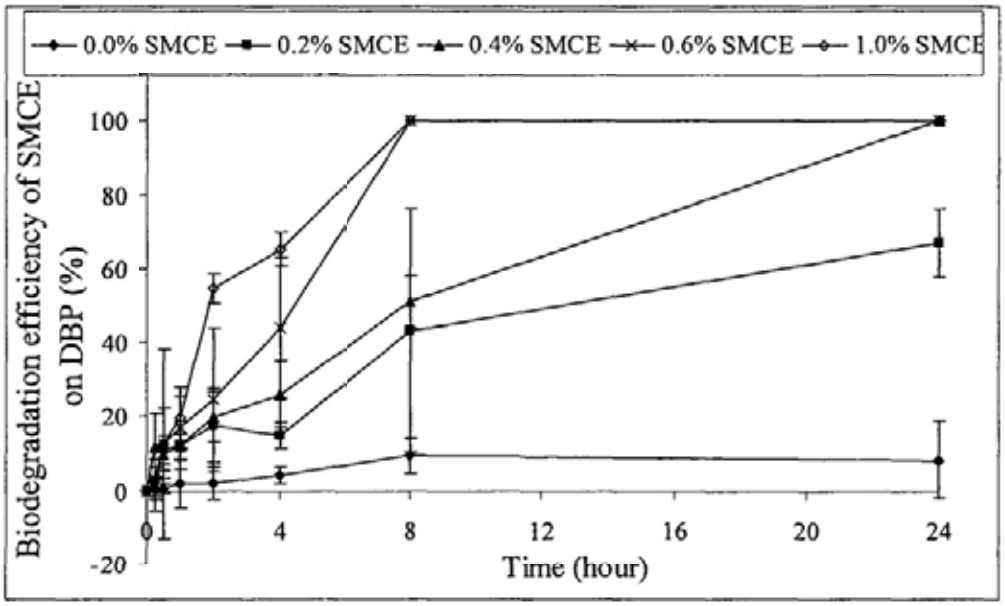


Figure 4.6 The effects of different amounts of SMC on the removal of DBP and DEHP at 1, 2 and 24 h. (a) and (b) Biosorption of DBP and DEHP by SMC; (c) and (d) Biodegradation of DBP and DEHP by SMC; (e) and (f) Total removal efficiencies of DBP and DEHP by SMC. Data are presented in mean ± SD of 5 replicates.

(a)



(b)

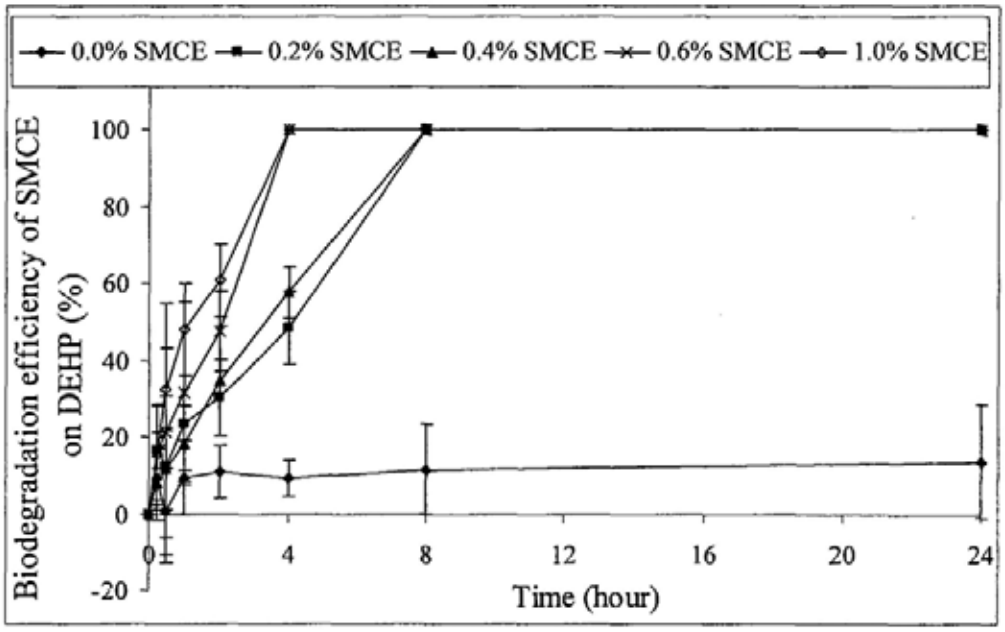


Figure 4.7 The effects of different amounts of SMCE on the total removal efficiencies of (a) DBP and (b) DEHP. Data are presented in mean ± SD of 5 replicates.

4.3.4 The effect of shaking speed on the removal of pollutants by SMC

Shaking speed also showed a significant impact on the removal of pollutants by SMC. After 1-h treatment, only 12.5±6.2% DBP and 19.8±9.7% DEHP were decreased by 1.0% SMC without shaking, while SMC could remove 85.1±3.5% DBP and 92.0±8.5% DEHP when shaking at 50 rpm, and DBP and DEHP were removed completely at 150 or 200 rpm (Figure 4.8).

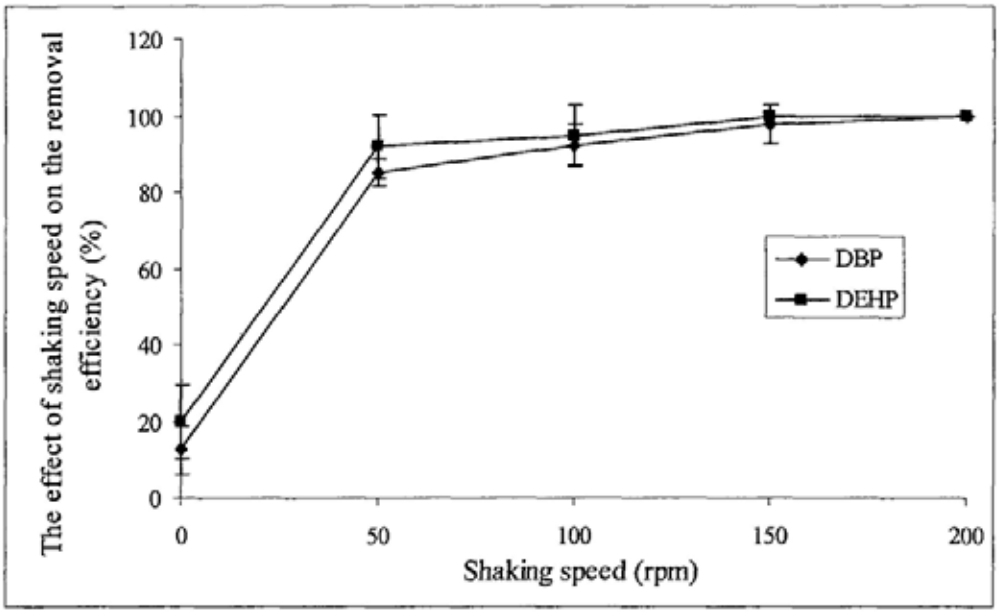


Figure 4.8 The effect of shaking speed on the removal of pollutants by SMC. Data are presented in mean ± SD of 5 replicates.

4.3.5 Effect of sorption-desorption cycles on the maximum removal capacity of SMC

After treatment, DBP and DEHP could not be detected in all water samples. However, 17.3±2.1 μg DBP and 7.7±1.8 μg DEHP were biosorbed per g SMC. Figure 4.9 shows the GC-MS chromatogram of the DBP and DEHP biosorbed in SMC.

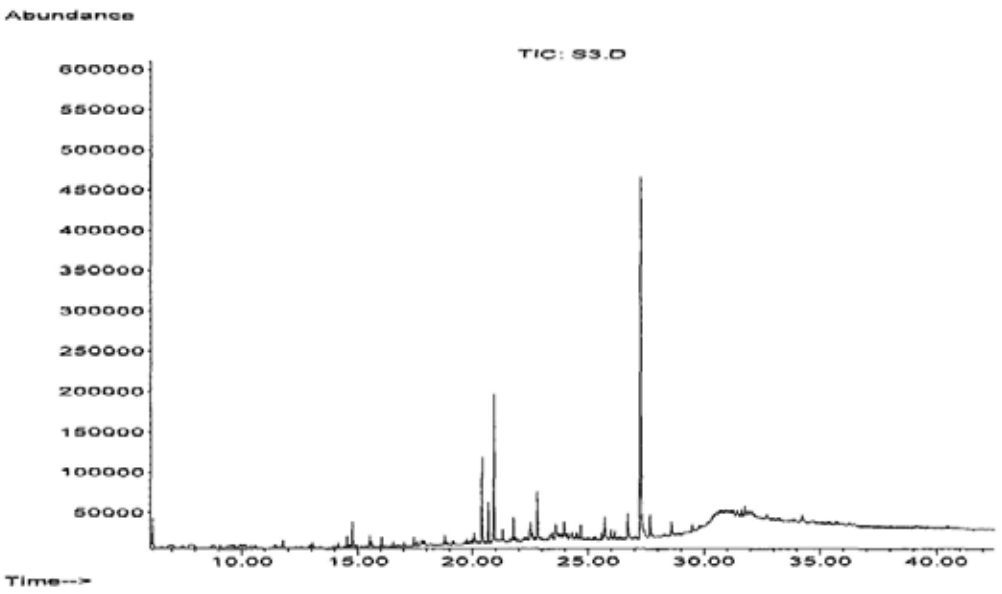


Figure 4.9 The GC-MS chromatogram of SMC after treatment. Peaks at 20.37 and 27.06 min show the DBP and DEHP, respectively.

4.3.6 Sorption kinetics of DBP and DEHP by SMC

The mechanism of adsorption of DBP and DEHP by SMC could be inferred from the adsorption isotherm study. Langmuir isotherm was determined by plotting C_f/q versus C_f while Freundlich isotherm was determined by plotting Log q against Log C_f. Table 4.4 shows constants of Langmuir and Freundlich isotherms for adsorption of DBP and DEHP by SMC. The r^2 of Freundlich isotherm of DBP and DEHP were larger than 0.9, which were greater than that of Langmuir isotherm of DBP and DEHP, respectively.

The value of 1/n for DBP was less than that of DEHP. In addition, the adsorbent capacity (k) of DBP by SMC was higher than that of DEHP.

Table 4.4 Summary of (A) Langmuir and (B) Freundlich isotherms for adsorption of DBP and DEHP by SMC.

Pollutants	(A) Langmuir isotherm		
	q_{max} (μg/g)	b (l/μg)	r^2
DBP	55.56	0.36	0.8048
DEHP	90.09	0.12	0.8714
	(B) Freundlich isotherm		
	K	1/n	r^2
DBP	27.31	0.20	0.9593
DEHP	14.60	0.50	0.9222

4.4 Discussions

4.4.1 The biodegradation of DBP and DEHP

The results shown in both Figures 4.6 and 4.7 demonstrate the capacities of SMC to degrade DBP and DEHP. Previous studies reported the bioremediation capacity of SMC on PCP, PAH and so on (Chiu et al., 1998; Eggen, 1999; Lau et al., 2003; Law et al., 2003; Tsang, 2004; Gong et al., 2006). The proposed pathway of SMC biodegradation was similar to that of photocatalytic oxidation (Law et al., 2003). This is because both systems are oxidative and utilize hydroxyl radicals to cleave organopollutants (Jardim et al., 1996; Guillén et al., 2000; Puplampu and Dodoo, 2000).

As shown in Figure 4.6, with the increase in the amount of SMC, the biosorption capacity of SMC increased at 1 and 2 h. However, there was the same biosorption concentration by different amounts of SMC at 24 h for DBP, in contrast to decreasing tendency for DEHP with the increasing amount of SMC at 24 h. For degradation efficiencies, there was an increasing tendency with time. Both time and the amount of SMC showed the impact on the total removal efficiency. Therefore, biosorption and biodegradation of SMC occur simultaneously and interact between each other. When using SMC to treat water pollutants, soluble enzymes of SMC are distributed into water, while some pollutants are adsorbed on SMC, and maybe the adsorption form impacts the contact of enzymes with pollutants freely, while with the increase in time, it enables enzymes to degrade pollutants with the increase in contact.

4.4.2 The biosorption of DBP and DEHP

Lau et al. (2003) reported that there were various groups (–OH, –NH, etc.) in the SMC for sorption and rich chitin (27.17±1.37 %) were contained in SMC (Law et al., 2003). Thus there were heterogeneous sites for sorption.

Figures 4.6 and 4.9 show the biosorption capacity of SMC. In this study, results from Figure 4.6 show that SMC had a rapid adsorption at the first 2 h, and 0.6 and 1.0% SMC showed higher adsorption contents at 2 h than those at 24 h. Then some pollutant

molecules began to be degraded with the time. Therefore, adsorption of SMC was faster than the degradation of SMC.

In Law et al. study (2003), SMC was compared with activated carbon, chitin and chitosan. The results showed SMC's performance was comparable to that of chitin and chitosan which have been applied for environmental cleanup of PCP from water (Tanjore and Viraraghavan, 1996), but it was not as good as activated carbon (Brandt et al., 1997; Wang et al., 2000). However, SMC was cheaper than activated carbon.

4.4.3 The effect of shaking speed on the removal of DBP and DEHP

For bioremediation in soil and sediment systems, bioavailability is one of the limiting factors. Bioavailability is related to a compound's intrinsic physico-chemical properties with aqueous solubility (Alexander, 1994; Linz and Nakles, 1997; Adriaens *et al.*, 1999). However, in water system, shaking speed was an important factor in affecting the adsorption of DBP and DEHP by the biosorbents. Higher shaking speed provides better mixing between the biosorbents and the sorbates. This allowed SMC to have sufficient contact with DBP and DEHP molecules (Leusch and Volesky, 1995; Chan, 2004)

4.4.4 The removal capacity of SMC

SMC has an integrated system of biosorption and biodegradation to remove DBP and DEHP. SMC could provide adsorption sites for sorption and the enzymes in SMC could act as Fenton reagents to produce reactive radicals for non-specific cleavage of a wide range of highly recalcitrant organopollutants (Cajthaml et al., 2002; Tsang, 2004; Gong et al., 2006). In addition, SMC offers a nutrient source and an attachment support for a consortium of microorganisms. In Law et al.'s study, PCP-tolerant and degradative bacteria were isolated from SMC. However, the degradation was predominately contributed by the immobilized enzymes in SMC (Law et al., 2003).

4.4.5 Sorption kinetics of DBP and DEHP by SMC

The results from Table 4.4 show r^2 of Freundlich isotherm of DBP and DEHP were greater than that of Langmuir isotherm. This means that the adsorption of DBP and DEHP by SMC fitted the Freundlich isotherm much better than the Langmuir isotherm, and the mechanism of biosorption of SMC is better described by Freundlich isotherm, which suggests that binding sites of SMC are not equivalent (Noll et al., 1991; Masel, 1996; Law et al., 2003) and DBP and DEHP are being adsorbed by SMC as a heterogeneous monolayer. For the Freundlich isotherm, Sag et al. reported that when adsorption intensity (1/n) is below 1, the adsorption of the adsorbate by the adsorbent is favored (Sag et al., 2000). The values of 1/n were 0.20 and 0.50 for DBP and DEHP respectively in this study. Therefore, SMC is considered as favorable for the adsorption of DBP and DEHP. The adsorbent capacity (k) of DBP (27.31) by SMC was higher than

that of DEHP (14.60), which indicates SMC should adsorb relatively more DBP than DEHP.

Chapter 5 Contribution of SMC components on removal of mixed pollutants including DEHP

5.1 Introduction

SMC with lignocellulosic materials as the major component contains significant amounts of essential macro- and micro-nutrients, e.g. calcium, ferric and nitrate, etc. (Ching, 1997; Chiu et al., 1998; Lau et al., 2003; Law et al., 2003). Moreover, a consortium of micro-organisms is inhabited in the SMC with some of them degrading persistent organopollutants (Law et al., 2003). In addition, various extracellular enzymes are secreted during the growth of the mushroom, and these residual enzymes in SMC can oxidize and degrade a variety of highly recalcitrant organopollutants, e.g. PAHs, PCP and DDT, by Fenton reactions in soil and water systems (Chiu et al., 1998; Eggen, 1999; Lau et al., 2003; Law et al., 2003; Tsang, 2004; Gong et al., 2006). The SMC enzymes of mushroom *Pleurotus pulmonarius* include cellulases, hemicellulase, manganese peroxidase and laccase (Eggen, 1999; Tsang, 2004). Moreover, Law et al. (2003) reported that there were various groups (–OH, –NH, etc.) in the SMC for sorption and rich chitin were contained in SMC. Thus there were heterogeneous sites for sorption in SMC. Therefore, in this study artificially contaminated soil with mixed pollutants diesel and DEHP was used to test the mechanisms of SMC. From this experiment, the optimal amount of SMC was observed for the removal of single pollutant and mixed-pollutants in soil. The key component contributing to the pollutant removal was traced.

5.2 Material and methods

5.2.1 Treatments of petroleum and DEHP in soil system by spent compost of *P. pulmonarius*

5.2.1.1 Optimization of removal of diesel and DEHP in artificial soil system by SMC

5.2.1.1.1 Soil preparation and the preparation of diesel and DEHP stock solution

Agricultural soil from the Gene Garden, CUHK, was used in this study. Soil was first oven-dried and sieved through 2 mm sieve.

Diesel stock solution (100,000 mg/l) was prepared by dissolving 10 g diesel (diesel Caltex Eavironmental Diesel (Euro V)) into 100 ml hexane (HPLC grade, Labscan). A stock solution of 1,000 mg/l DEHP was prepared by dissolving 0.1000 g DEHP (Analytical standard, cat no.: 36735, Riedel-de Haën®, Seelze, Germany) into 100 ml methanol (HPLC grade, Fisher Scientific). The stock solution was then kept at –18°C for later experiments.

5.2.1.1.2 Optimization of diesel removal by SMC in soil system

In this experiment 10.0±0.1 g soil with 50% moisture content were put into a glass flask. The whole setup was autoclaved at 121°C for 20 min (HA-300P, Hirayahan, Japan). Sterilized soil was spiked with diesel stock solution. The hexane used to dissolve diesel was let evaporate overnight. The concentrations of diesel in soil tested were 0, 1000, 5000, 10000, 15000 and 20000 mg/kg (Dutch B = 1000 mg/kg, Dutch C = 5000 mg/kg). Different amounts of SMC (0%, 1%, 2%, 3% and 5%) were added into soil contaminated with diesel. Flasks were incubated at room temperature (25 – 28°C) and shaken at 150 rpm for one week and every treatment/control had 5 replicates.

The final soil samples were collected and freeze-dried for the analysis of TPH and oil and grease. Removal efficiency (RE) and removal capacity (RC) of SMC were calculated. The optimal amount of SMC was obtained and used for the bioremediation of soil with different concentrations of diesel according to degradative removal efficiencies of TPH and oil and grease.

RE was defined as the ratio of the amount of pollutant removed to the corrected initial pollutant amount, whereas RC is the amount of pollutants removed by per unit mass of spent mushroom compost.

RE (%) = (amount of pollutants removed/corrected initial amount of pollutants added) x 100%

RC (mg pollutants mg^{-1} biomass) = amount of pollutants removed / fungal biomass

The corrected values refer to the measured content extracted and quantified by the instrument (GC-MS) with accounts for the extraction efficiency and the stability of pollutants.

5.2.1.1.3 Optimization of DEHP removal by SMC in soil system

For DEHP-contaminated soil, the same experimental design was used. Sterilized soil was spiked with DEHP stock solution. The methanol used to dissolve diesel was let evaporate overnight. The concentrations of DEHP in soil tested were 0, 30, 60, 90, 120 and 150 mg/kg (series risk concentration = 60 mg/kg). Different amounts of SMC (0%, 1%, 2%, 3% and 5%) were added into soils artificially spiked with DEHP. Flasks were incubated at room temperature (25 – 28°C) and shaken at 150 rpm for one week and every treatment had 5 replicates. After incubation, soil samples were freeze-dried and extracted by organic solvent for the measurement of DEHP. The concentration of DEHP in final soil samples was detected by GC-MS, and removal efficiency and removal capacity were calculated.

5.2.1.1.4 Optimization of removal of the mixed pollutants (diesel and DEHP) by SMC in soil system

The highest concentrations of diesel (20,000 mg/kg) and DEHP (150 mg/kg) were selected for further study. Sterilized soil was spiked with DEHP and diesel stock

solutions. The organic solvent used to dissolve DEHP and diesel was let evaporate overnight. In this experiment 20.0±0.1 g soil were used for each glass flask. For mixed pollutants-spiked soil, different amounts of SMC (0%, 1%, 2%, 3% and 5%) were added into soil. After one week's incubation, the final samples were collected and freeze-dried. TPH, oil & grease and DEHP contents were detected and the optimal amount of SMC was obtained for the removal of pollutants in the contaminated soil. Removal efficiency and removal capacity were calculated.

5.2.1.1.5 Time effect of SMC on the removal pollutants in soil

In this experiment, 5% SMC were used for monitoring the time effect of SMC on the removal of DEHP and diesel. Also the highest concentrations of diesel (20,000 mg/kg) and DEHP (150 mg/kg) were selected for this study. The samples were collected and freeze-dried at day 2, 4, 7, 14, 21 and 28. TPH, oil and grease and DEHP contents were measured. Removal efficiency and removal capacity were calculated.

5.2.1.1.6 Ecotoxicity of soil contaminated with diesel and DEHP

Soil samples contaminated with diesel and DEHP were tested whether the toxicity of the soil was increased with the increasing pollutant concentration by microbial ecotoxicity tests. The microorganisms (bacteria and fungi) used in the indigenous ecotoxicity test were described in Section 2.2.3.4. Similarly, the difference between the changes in the number of bacterial colonies of control and treatment were used as an

indicator of soil toxicity. For fungal ecotoxicity test, the ergosterol content of one-ml inoculum and the changes in the ergosterol content of control and treatment soil were used as a measure of soil toxicity.

5.2.2 Contribution of SMC components on removal of mixed pollutants including DEHP

Artificially contaminated soil with 20,000 mg/kg diesel and 150 mg/kg DEHP was prepared according to the description at Section 5.2.1.1.1.

5.2.2.1 Comparison of SMC with different solid materials: sawdust, fermented compost, autoclaved SMC

In order to test the mechanism(s) of SMC to removal pollutants, four types of solid materials were compared for their capacities of removing pollutants besides contaminated soil: sawdust, fermented sawdust compost, SMC, autoclaved SMC. Autoclaved materials without life only provide the physical properties and nutrients.

In this experiment 20.0±0.1 g soil with 50% moisture content were put into a glass flask. The whole setup was autoclaved at 121°C for 20 min before spiking with the pollutants. Five percent of different solid materials were added into contaminated soil. The flasks were incubated at ambient temperature (25 – 28°C) and shaken at 150 rpm for one week and every treatment had 5 replicates. After incubation, soil samples were

freeze-dried for the measurement of pollutants, TPH, oil and grease and DEHP.

5.2.2.2 Comparison of the removal ability with different liquid materials: SMCE, autoclaved SMCE, membrane filtrate, micro-organisms in SMCE

For liquid materials, SMCE contains the soluble matters (water-soluble nutrients and enzymes) and the microorganisms. Specific laccase and manganese peroxidase activities in SMC used in this study were 0.96±0.62 and 0.70±0.37 m mol/min/ g protein respectively. Autoclaved SMCE provide nutrients and devoid of viable micro-organisms and inactivated enzymes. Membrane (0.45 μm) filtration yielded 2 fractions: the residue on membrane (the microorganisms pool) and the filtrate (the enzymes and nutrients). The residue (microbial biomass) in the filter was enriched by incubating in nutrient broth at 28°C for 5 days to cell density 2.0×10^{6}. The enriched microbial cultures were concentrated by centrifugation and used as an inoculum to the contaminated soil for treatment. In parallel, the filtrates consisted of enzymes and water-soluble nutrients from SMC were also studied.

After preparation, SMCE and other derivatives were added into soil, and the moisture was kept at 50% through the addition of autoclaved water. Then flasks were incubated at ambient temperature (25 – 28°C) and shaken at 150 rpm for one week and every treatment had 5 replicates. After incubation, soil samples were freeze-dried for the measurement of residual pollutants.

5.3 Results

5.3.1 Treatment of diesel and DEHP in soil system by spent compost of *P. pulmonarius*

5.3.1.1 Soil characterization

Table 5.1 shows the physical and chemical properties of the soil and SMC used in this experiment. The soil consisted of 69.5±1.2% sand, 7.5±1.8% silt and 23.0±2.9% clay. Thus the soil was categorized as a sandy-clay-loam soil. In soil and SMC, no pollutants were detected. SMC contained high concentrations of N, P and K, which are essential nutrients for organism's growth.

5.3.1.2 Optimization of diesel removal by SMC in soil system

Figure 5.1 shows the effects of initial diesel concentration and the amount of the SMC on the total removal efficiencies. Five percent SMC had the maximum removal of 69.5±10.0% total petroleum hydrocarbon and 56.8±8.4% oil & grease at a low initial concentration of diesel at 1,000 mg/kg. For other initial concentrations ranging from 1,000 to 20,000 mg diesel per kg soil, the trends of removal were similar, and degradation efficiencies were not significantly different when treated by 3% and 5% SMC.

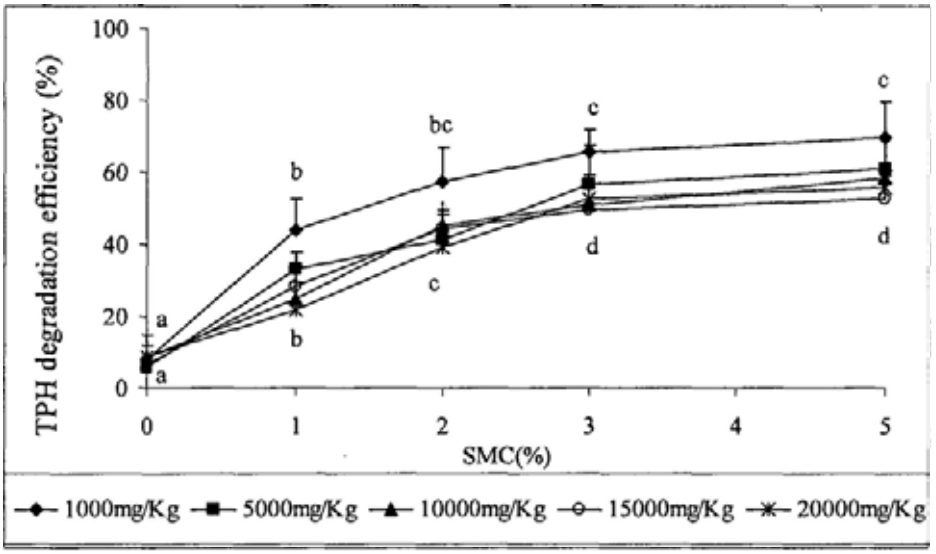
Figure 5.2 shows that total removal capacities increased with the increasing initial diesel concentrations. The maximum total removal capacity occurred at 20,000 mg diesel per kg soil by 1% SMC was 439.1±156.3 mg TPH and 464.9±132.4 mg oil and grease per g SMC.

Table 5.1 Physical and chemical properties of the soil and SMC used in the study. Data are presented in mean ± SD of 5 replicates.

Parameters	SMC	Garden Soil
Total organic carbon (%)	22.0±3.8	0.8±0.2
$N_{Kjeldahl}$ (mg/kg)	1632±218	130±7
$NO_{X\ water\text{-}soluble}$ (mg/kg)	198±26	74±12
$NH_4^{+}{}_{water\text{-}soluble}$ (mg/kg)	267±27	9.4±3.3
P_{total} (mg/kg)	612±2	135±21
$P_{water\text{-}soluble}$ (mg/kg)	66.6±3.4	117±18
K^+ (mg/kg)	2483±212	720±14
Electrical conductivity (μS/cm)	2241±29	320±27
Salinity (‰)	32±1.0	nd
pH	7.2±0.4	6.9±0.1
Stone (%)	0	25±4
Moisture (%)	77.8±1.4	—
TPH (mg/kg)	—	—
Oil & grease (mg/kg)	—	—
DEHP (mg/kg)	nd	nd

—, not determined; nd, not-detectable

(a)



(b)

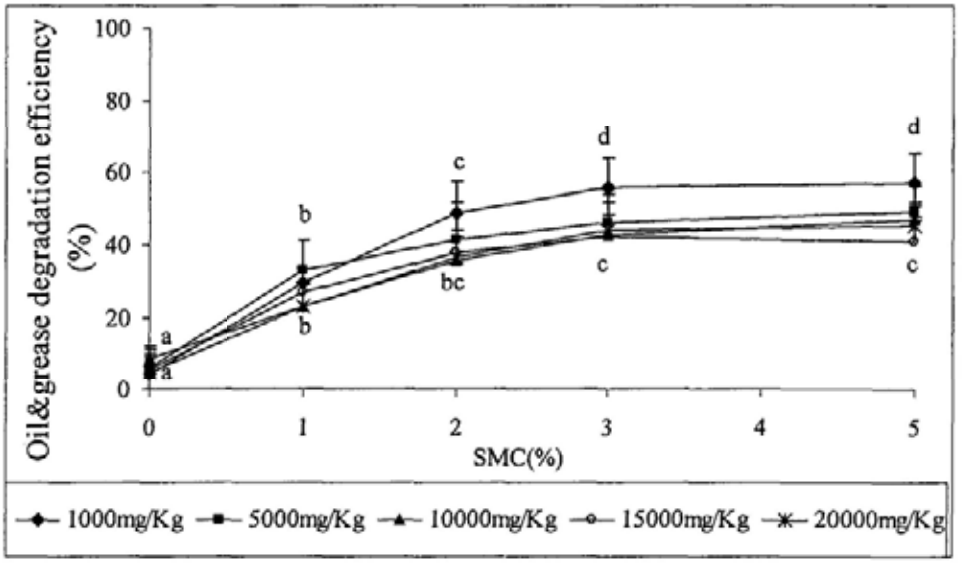
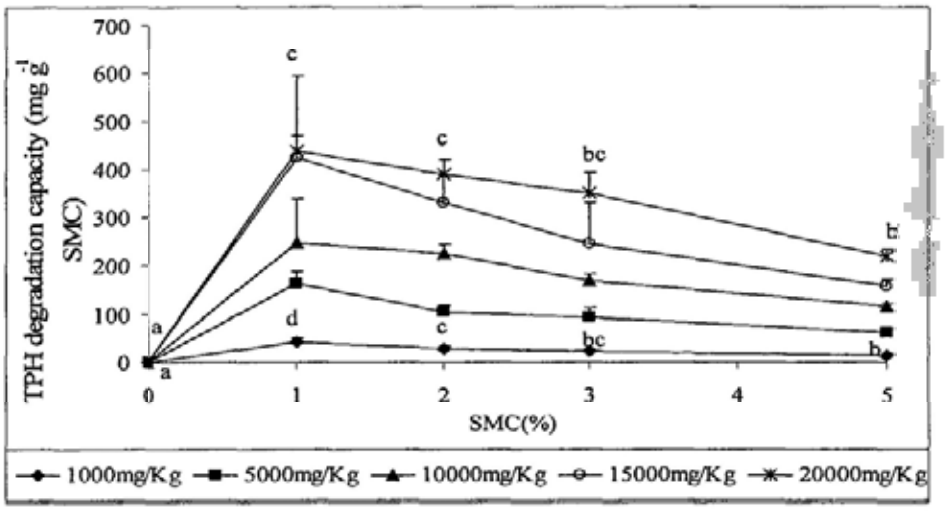


Figure 5.1 The effects of initial diesel concentrations and amounts of spent mushroom compost of *P. pulmonarius* used on the total removal efficiency of diesel in soil system. (a) TPH degradation efficiency; (b) Oil & grease degradation efficiency. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed for the same diesel concentration and followed by ranking using Tukey test at 5% probability.

(a)



(b)

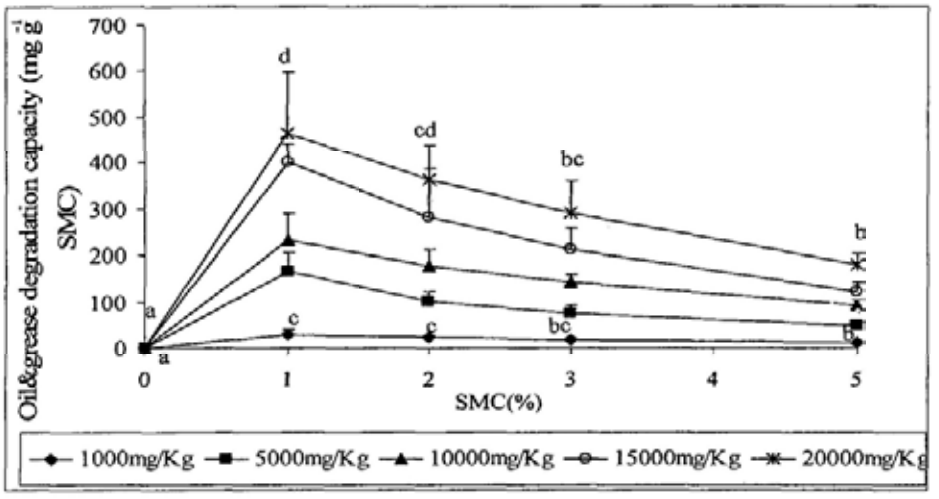


Figure 5.2 The effects of initial diesel concentrations and amounts of spent *P. pulmonarius* compost used on the total removal capacity of diesel in soil system. (a) The capacity of SMC on the degradation of TPH; (b) The capacity of SMC on the degradation of oil & grease. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed for the same diesel concentration and followed by ranking using Tukey test at 5% probability.

5.3.1.3 Optimization of DEHP removal by SMC in soil system

The effects of SMC on the removal of DEHP in soil are shown in Figure 5.3. From the results of one way ANOVA, further increase of SMC from 2% to 5% did not show significant difference on the removal efficiency of DEHP. Five percent of SMC gave degradation efficiency of 31.8±13.7% for 30 mg DEHP per kg soil.

The degradation capacity of SMC on the removal of DEHP shows similar trend to the removal of diesel (Figure 5.4). One percent of SMC showed the maximum removal capacity on different initial concentrations of DEHP. With the increase of DEHP concentration, SMC showed larger removal capacity.

5.3.1.4 Optimization of mixed pollutants (diesel and DEHP) removal by SMC in soil system

After studying the degradation of single pollutant, the degradation capacity of SMC (0-10%) on mixed pollutants was tested. The highest concentrations of diesel (20,000 mg/kg) and DEHP (150 mg/kg) were selected. Seven point five percent of SMC gave the highest degradation efficiencies: 74.1±1.1% for TPH and 55.0±3.5% for oil & grease (Figure 5.5 (a)). However, there was no significant differences on the removal efficiency of DEHP from 1 to 10% SMC (Figure 5.5 (b)). Seven point five percent SMC gave 50% removal efficiency for DEHP. In terms of removal capacity, 1% SMC showed the best removal for both diesel and DEHP (Figure 5.6 (a) and (b)).

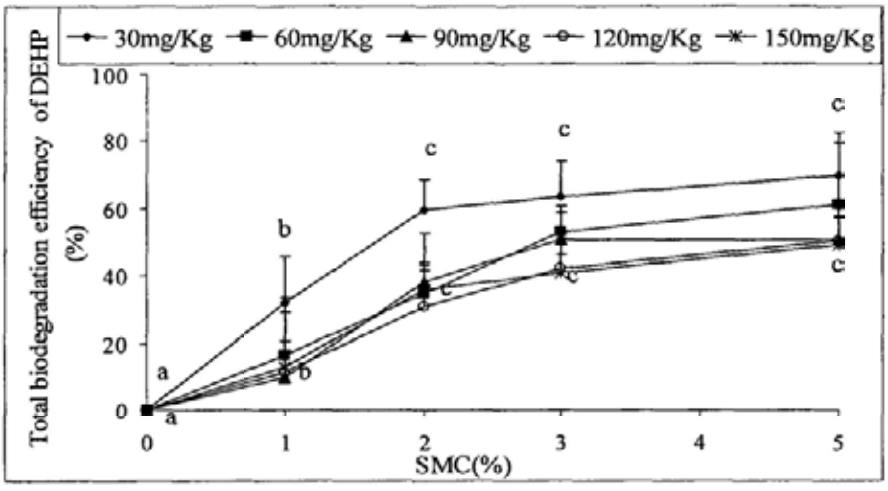


Figure 5.3 The effects of initial DEHP concentrations and amounts of spent mushroom compost of *P. pulmonarius* used on the total removal efficiency of DEHP in soil system. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed for the same diesel concentration and followed by ranking using Tukey test at 5% probability.

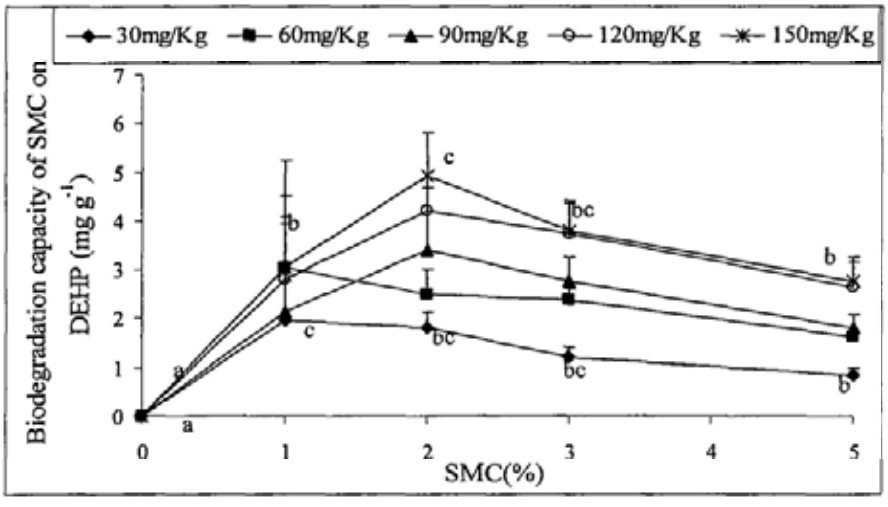
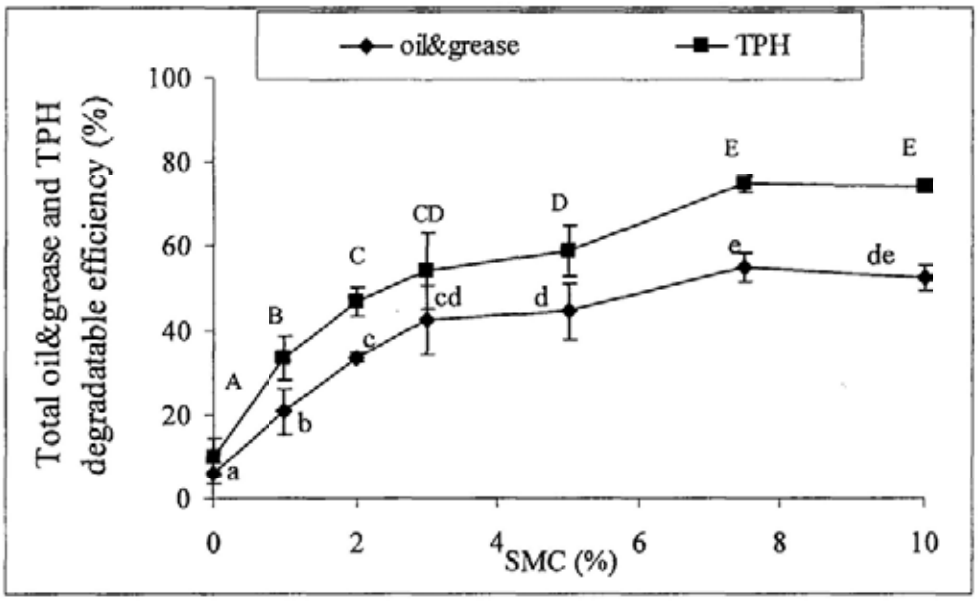


Figure 5.4 The effects of initial DEHP concentrations and amounts of spent *P. pulmonarius* compost used on the total removal capacity of DEHP in soil system. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed for the same diesel concentration and followed by ranking using Tukey test at 5% probability.

(a)



(b)

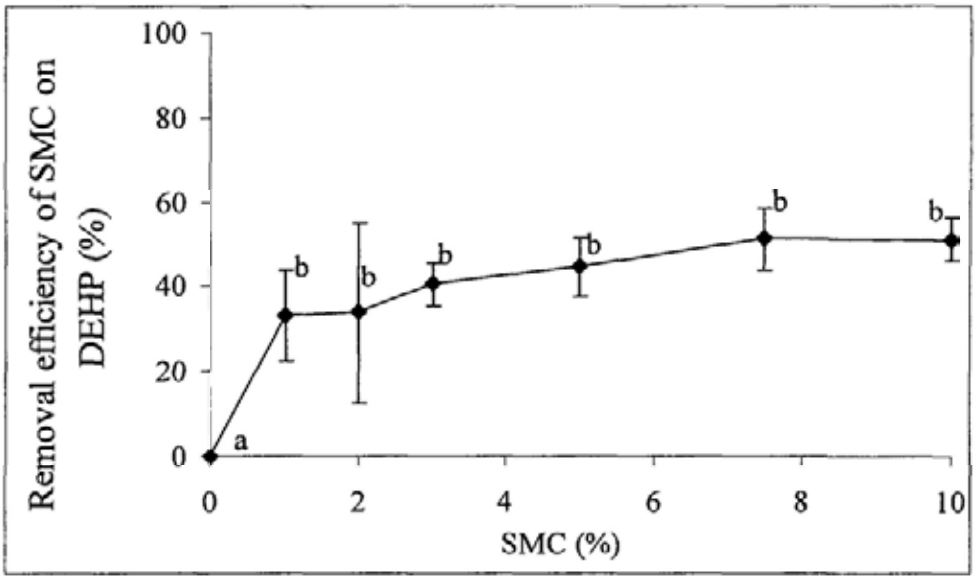
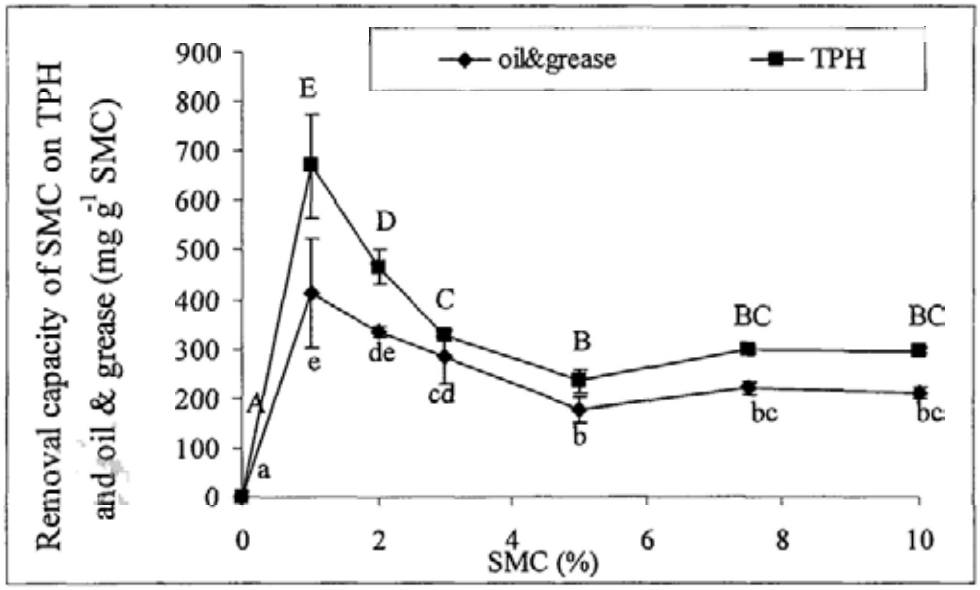


Figure 5.5 The removal effects of spent *P. pulmonarius* compost on the pollutants in soil system. (a) Removal efficiency of SMC on diesel; (b) Removal efficiency of SMC on DEHP. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed and followed by ranking using Tukey test at 5% probability.

(a)



(b)

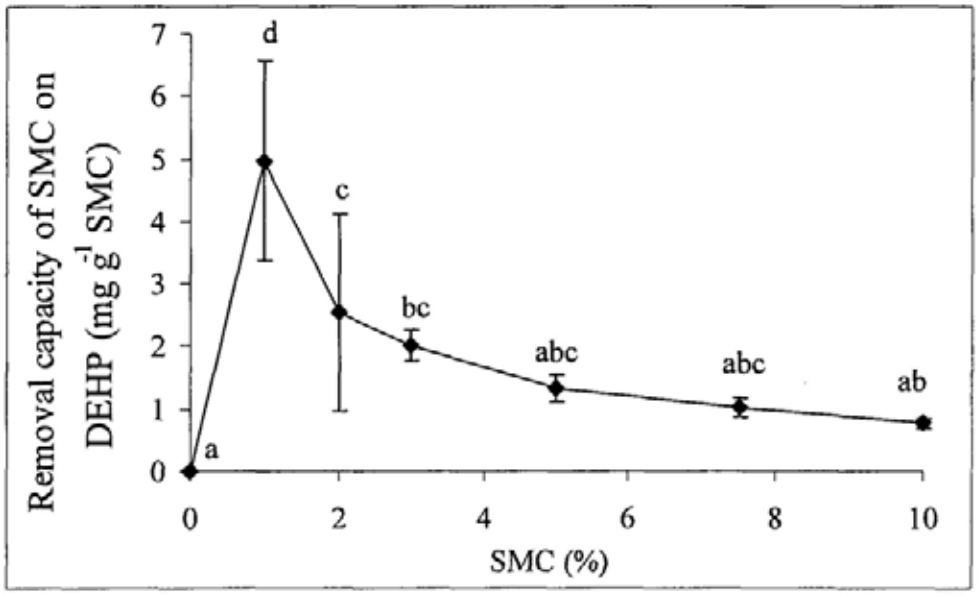


Figure 5.6 The effects of spent *P. pulmonarius* compost on the total removal capacity of diesel and DEHP in soil system. (a) Removal capacity of SMC on diesel; (b) Removal capacity of SMC on DEHP. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed and followed by ranking using Tukey test at 5% probability.

5.3.1.5 Time effect on the pollutants removal in soil

In Figure 5.7, degradation occurred even after 2-day incubation with the removal efficiencies of 12.2±1.9% DEHP, 12.5±6.1% total petroleum hydrocarbon and 9.9±2.5% oil & grease. DEHP removal continued and increased steadily along incubation with a final removal efficiency of 43.6±6.1% at the end of incubation. Similar pattern was also obtained for the removal efficiency of diesel along the 28-day incubation with the removal efficiencies of 59.3±4.1% TPH and 44.2±3.1% oil and grease at day 28. In fact, the maximum removal efficiencies had been obtained at day 21, and the results did not show the significant difference on the removal efficiencies at day 21 and 28 either for diesel or DEHP.

Figure 5.8 shows the overlaid GC/MS chromatograms and the possible breakdown products of SMC-soil mixture before and after 28-day incubation. The abundance of DEHP (retention time 26.71 min) decreased by around 45% after treatment. No novel peaks or prominent increase in a particular peak as possible breakdown products were observed. Thus, it is likely that DEHP was mineralised, and/or non-volatile breakdown products which were not detectable by GC were produced.

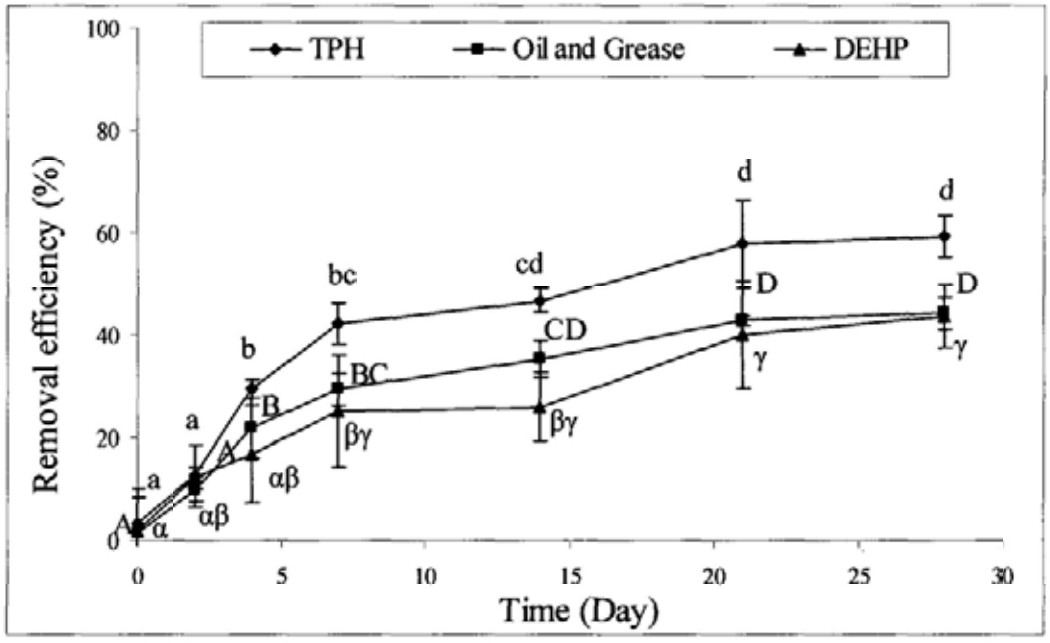


Figure 5.7 Time effect of SMC on the removal of diesel and DEHP in soil system. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed and followed by ranking using Tukey test at 5% probability.

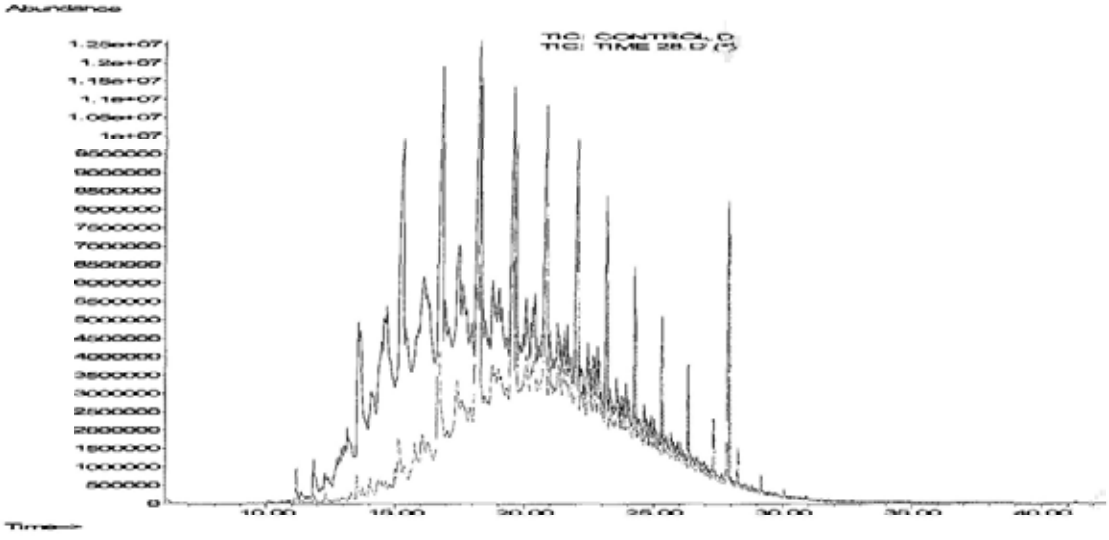


Figure 5.8 The overlaid GC/MS chromatograms and the possible breakdown products of SMC-soil mixture before and after 28-day incubation.

5.3.1.6 Ecotoxicity of soil contaminated with diesel and DEHP

5.3.1.6.1 Ecotoxicity of soil contaminated with diesel

The toxicities of soil contaminated with diesel on bacterial growth are shown in Figure 5.9. With the increase of diesel concentrations, the growth of bacterial population decreased, and the trend showed a linear tendency. Except *Flavobacterium* sp., the other 3 bacteria showed higher sensitivity towards diesel (the larger slope values in the linear regression curves in Figure 5.9).

Results of diesel toxicity in soil system on fungal growth are shown in Figure 5.10. The decrease of fungal population with the increase of diesel concentration indicated the growths of these fungi were inhibited. *Fusarium solani*, *Trichoderma harziauum* and *Trichoderma asperellum* isolated from Tsing Yi site showed higher sensitivities than those from Lau Fau Shan site to diesel. When diesel concentration was below 5,000 mg per kg soil, fungal population showed a relativity linear relationship with the diesel concentration.

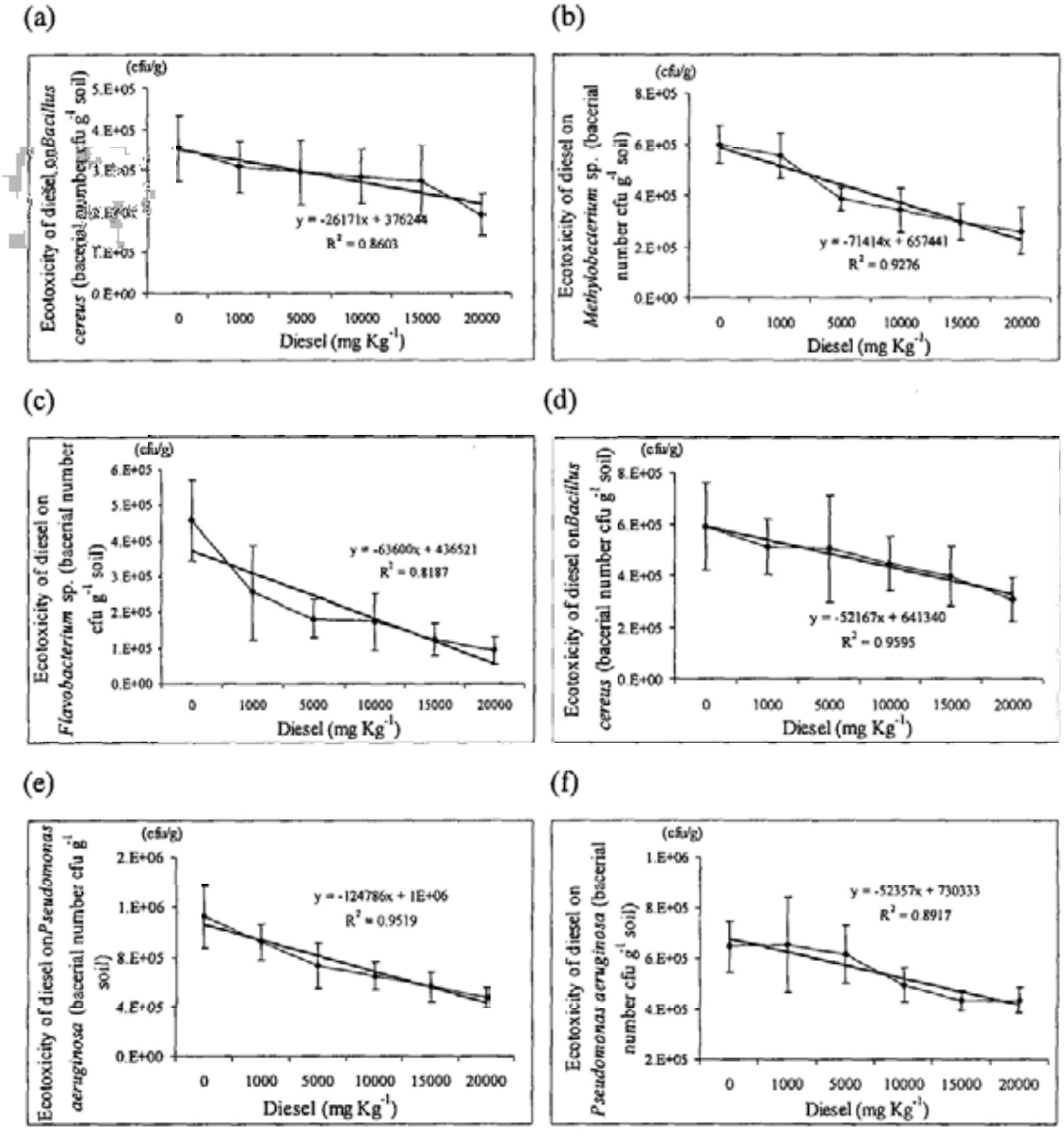


Figure 5.9 Ecotoxicities of diesel-spiked soils towards bacteria. (a) (b) and (c): Ecotoxicities of diesel-spiked soil towards bacteria isolated from Tsing Yi site: *Bacillus cereus*, *Methylobacterium* sp., *Pseudomonas aeruginosa*. (d) (e) and (f): Ecotoxicities of diesel-spiked soil towards bacteria isolated from Lau Fau Shan site: *Pseudomonas aeruginosa*, *Flavobacterium* sp., *Bacillus cereus*. Data are presented in mean $\pm$ SD of 5 replicates

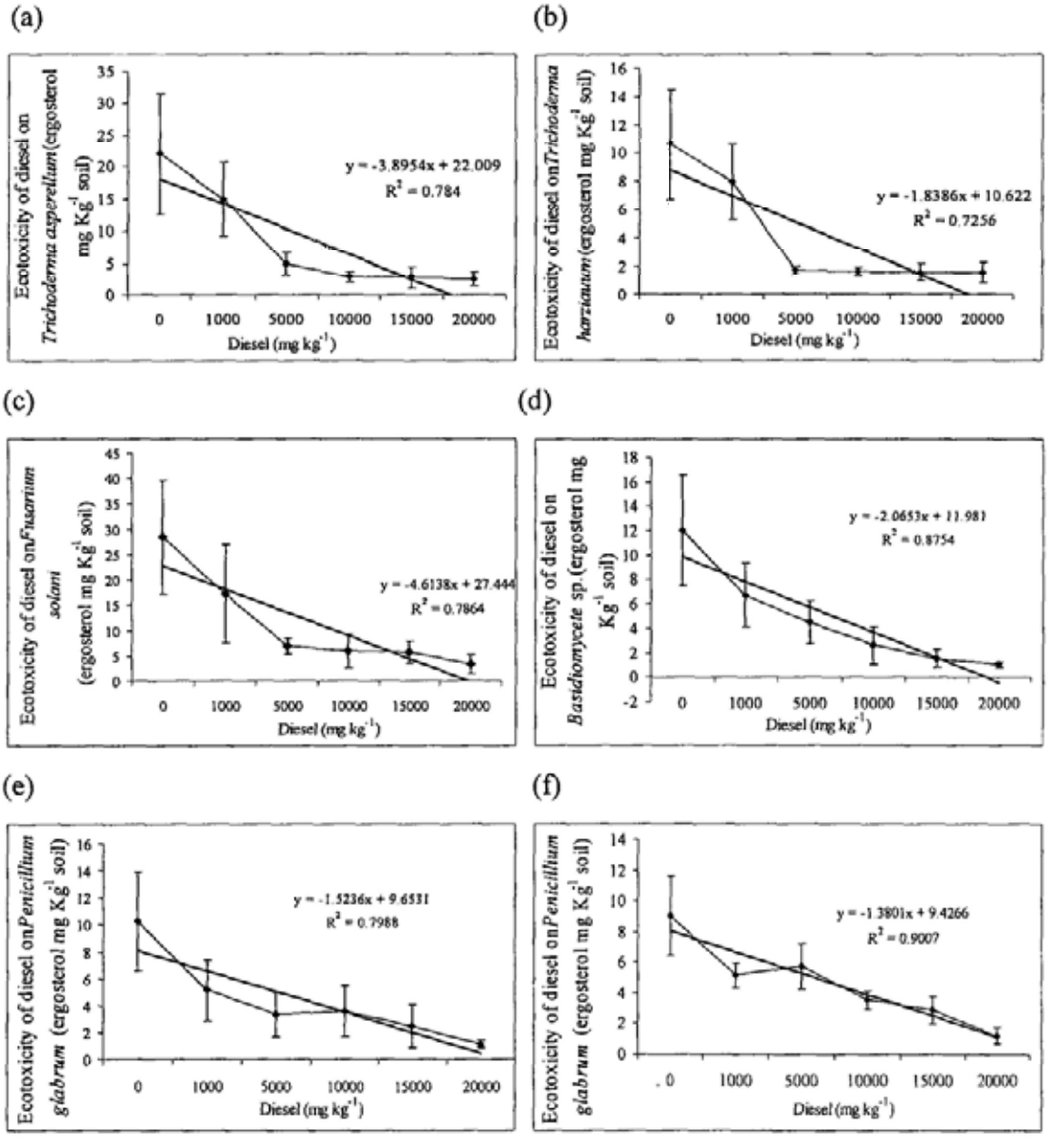


Figure 5.10 Ecotoxicities of diesel-spiked soils towards Fungi. (a) (b) and (c): Ecotoxicities of diesel-spiked soil towards fungi isolated from Tsing Yi site: *Fusarium solani*, *Trichoderma harziauum*, *Trichoderma asperellum*. (d) (e) and (f): Ecotoxicities of diesel-spiked soil towards fungi isolated from Lau Fau Shan site: Basidiomycete sp., *Penicillium glabrum*, *Penicillium glabrum*. Data are presented in mean ± SD of 5 replicates.

5.3.1.6.2 Ecotoxicity of soil contaminated with DEHP

Figure 5.11 shows the ecotoxicity of DEHP on the growth of bacterial population. The more DEHP in soil, the lower population sizes of bacteria in the soil system contaminated with DEHP.

The ecotoxicity of DEHP on fungi in soil system is indicated by the results in Figure 5.12. All the tested fungi showed population drop with the increase of DEHP, especially *Penicillium glabrum*.

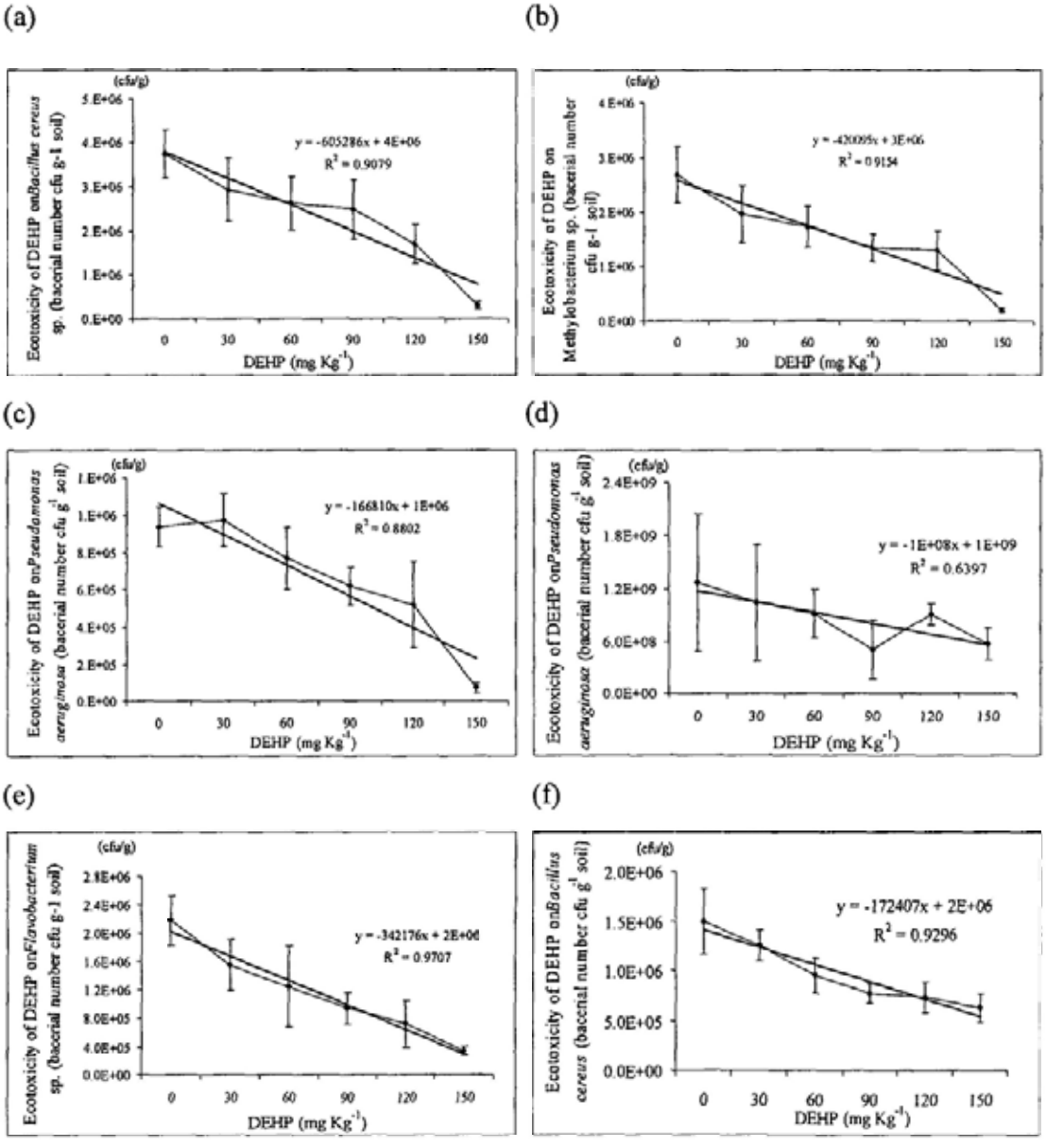


Figure 5.11 Ecotoxicities of DEHP-spiked soils towards bacteria. (a) (b) and (c): Ecotoxicities of DEHP-spiked soil towards bacteria isolated from Tsing Yi site: *Bacillus cereus*, *Methylobacterium* sp., *Pseudomonas aeruginosa*. (d) (e) and (f): Ecotoxicities of DEHP-spiked soil towards bacteria isolated from Lau Fau Shan site: *Pseudomonas aeruginosa*, *Flavobacterium* sp., *Bacillus cereus*. Data are presented in mean ± SD of 5 replicates.

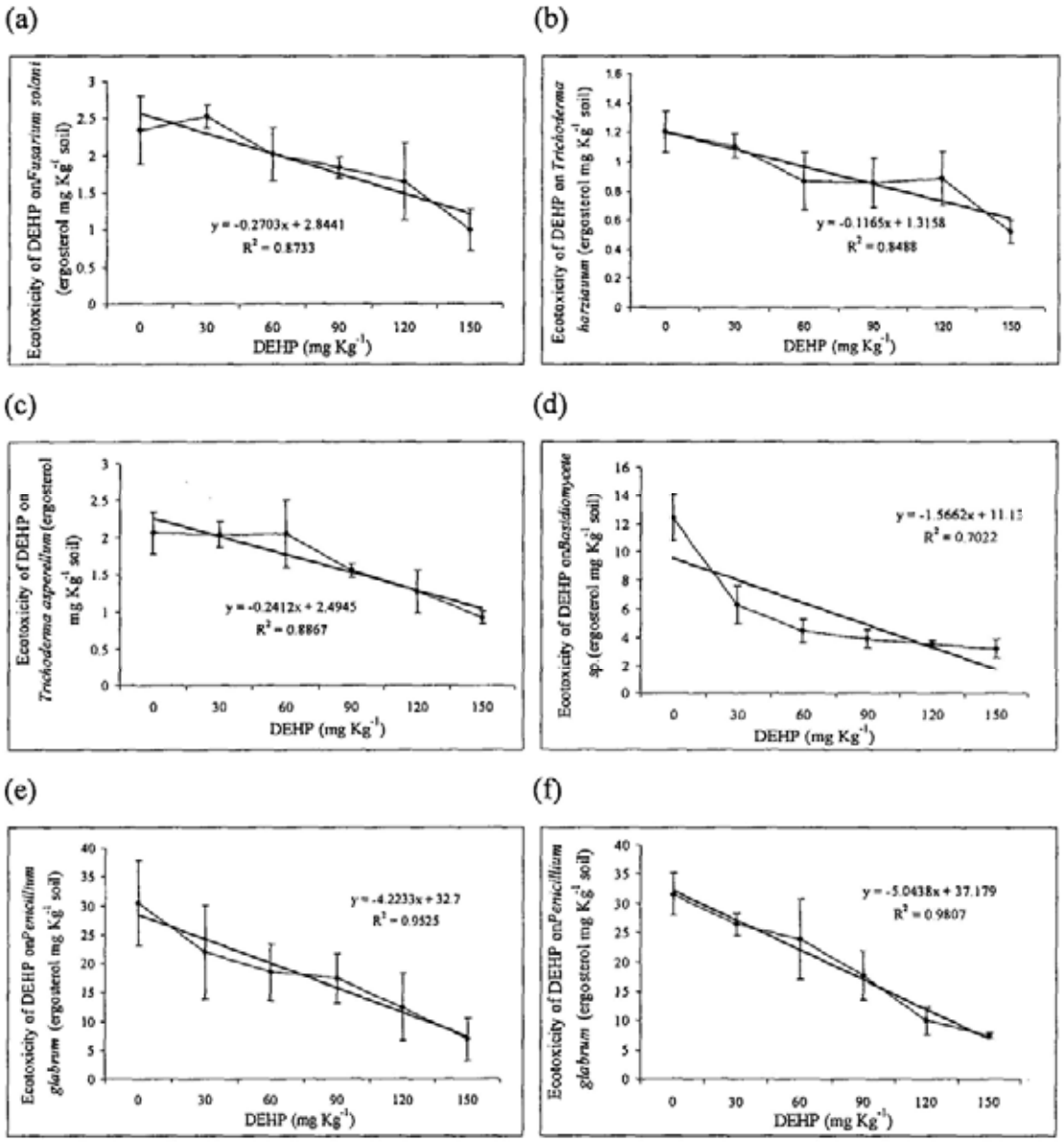


Figure 5.12 Ecotoxicities of DEHP-spiked soils towards fungi. (a) (b) and (c): Ecotoxicities of DEHP-spiked soil towards fungi isolated from Tsing Yi site: *Fusarium solani*, *Trichoderma harziauum*, *Trichoderma asperellum*. (d) (e) and (f): Ecotoxicities of DEHP-spiked soil towards fungi isolated from Lau Fau Shan site: Basidiomycete sp., *Penicillium glabrum*, *Penicillium glabrum*. Data are presented in mean $\pm$ SD of 5 replicates.

5.3.2 Contribution of SMC components on removal of mixed pollutants including DEHP

In order to identify the contributing components of SMC in the removal of pollutants, four types of solid materials and four types of liquid materials were compared for the pollutant removal efficiencies. Sawdust, fermented compost, autoclaved SMC and autoclaved SMCE showed nil removal of TPH and DEHP when compared with original contamination levels in soil, while both SMC and SMCE had significant differences with original contaminated soil on the bioremediation of diesel and DEHP (Figures 5.13 and 5.14). The removal efficiencies of SMC were 41.7±9.8% and 36.1±8.4% for TPH and DEHP, respectively. SMCE had about half the removal efficiency of SMC on the pollutant removal. In addition, filtrate containing enzymes and SMC microbes in SMCE had the removal efficiencies of 6.0±11.9% and 11.3±5.8% for TPH, and 15.7±8.1% and 19.0±6.4% for DEHP, respectively.

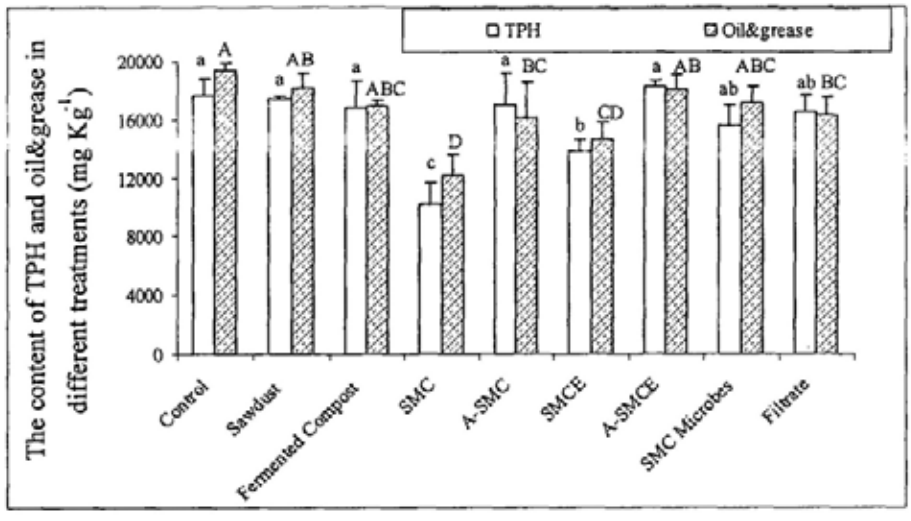


Figure 5.13 The contents of total petroleum hydrocarbon and oil & grease in soil contaminated with 20,000 mg diesel per kg soil after different materials' treatments for 7 days. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed and followed by ranking using Tukey test at 5% probability.

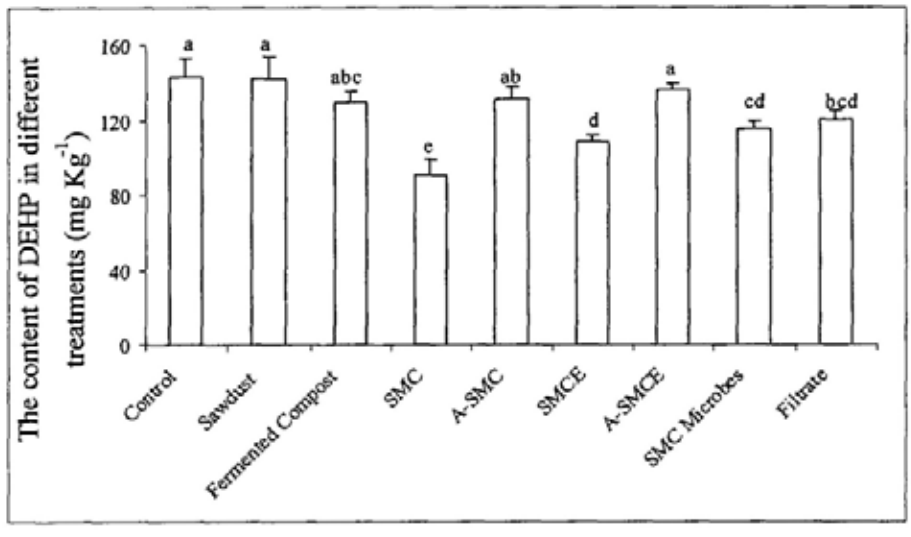


Figure 5.14 The contents of DEHP in soil contaminated with 150 mg DEHP per kg soil after different materials' treatments. Data are presented in mean ± SD of 5 replicates. One-way ANOVA was performed and followed by ranking using Tukey test at 5% probability.

5.4 Discussions

5.4.1 Optimization of diesel removal by SMC in soil system

Without addition of SMC, there was about 5% background removal of TPH and oil and grease in the soil. These may be because the extraction efficiencies of them were about 94% to 96%.

The addition of SMC can significantly enhance the removal of diesel in the artificial soil system. The RE increased from 5% to about 40% at diesel concentration of 1,000 mg/kg with the addition of 1% SMC. SMC may remove pollutants by several processes.

SMC contains various extracellular lignolytic enzymes, which were secreted during cultivation of mushrooms. The enzymes of spent compost of mushroom *Pleurotus pulmonarius* that degrade TPH and oil and grease include laccase and mangnese peroxidase, which can degrade a wide variety of pollutants through the production of hydroxyl radical.

Another possible degradation process is due to the microorganisms induced by SMC. SMC contains plenty of microbes with some of them possessing degradation capacity (Law et al., 2003). The microorganisms can utilize diesel as carbon and energy

source to support their growth, or they can metabolize the diesel along with the utilization of other organic carbon present in the soil-SMC system.

The third is the adsorption by SMC, which could promote more contact opportunities to enzymes from *Pleurotus pulmonarius* with pollutants during incubation. Thus, the degradation rate could be speeded up.

When the SMC amount was increased, RE increased and then leveled off. The enhancement in removal is due to the increase in adsorption sites, microorganism populations and enzymes, and the plateau may be due to the limitation by mass transport of pollutants to the sites of degradation (Facundo et al., 1999). On the other hand, with the increase of pollutant concentrations, RE generally decreased. It was expected as increasing pollutants saturated the treatment ability of SMC.

The removal capacity usually shows a decreasing trend with increasing adsorbent or reactant concentration. This is due to the excess of adsorbent in the system and so the amount of pollutant removed per adsorbent will decrease. This happened as the concentration of SMC increased from 1 to 5%. On the other hand, RC increased with the increase of concentrations of pollutants when using the same amount of SMC. Although the percentage of pollutants removed (RE) decreased, the absolute amount of pollutant removed may actually increase with the increase of pollutant concentrations. The increase of RC may due to the fact that the increase in absolute amount removed is smaller than the increase of concentrations.

5.4.2 Optimization of DEHP removal by SMC in soil system

Similar to diesel, the RE of DEHP also increased with increasing SMC concentration. Although the RE of DEHP was similar to that of diesel, the initial concentration of DEHP was much lower. This may indicate that DEHP is more resistant to the treatment by SMC.

The RC of DEHP also shows similar trend to diesel. When increasing the concentration of SMC, RC first increased then decreased. The increase in RC from 1% SMC to 2% SMC indicated that the removal capacity of SMC is fully utilized in 1% SMC concentration, so increasing SMC content can increase RC. Nevertheless further addition might lower contact efficiency and leading to lower removal capacity. Moreover, from Figure 5.4 we can see that at low DEHP concentrations (30 and 60 mg/kg), 1% SMC was the optimum concentration and RC decreased when more SMC was added; in higher DEHP concentrations, the optimum SMC concentration increased to 2%. This may further support that the treatment ability of SMC was fully utilized in high DEHP concentrations so that adding more SMC can improve the treatment capacity.

5.4.3 Optimization of mixed pollutants (diesel and DEHP) removal by SMC in soil system

In real situations, the pollutants usually present as complex mixture consisting of different types of organic and inorganic compounds. The presence of a mixture of pollutants will affect the removal efficiency in the system (Zytner et al., 2006). Theoretically, the removal may be enhanced, reduced, or no change when more than one type of pollutants are present.

In our system, the presence of pollutant mixture has a positive effect on the removal of the single component in the soil. When comparing the RE at 1 or 2% SMC, the mixture showed a higher RE than that in single component. As seem from Section 5.3.2, the autoclaved samples did not show significant removal of diesel as well as DEHP, this may indicate that the removal was mainly due to biological activity and the presence of pollutants may induce the expression of degrading enzymes which enhance the overall removal of pollutants.

5.4.4 Time effect on the pollutants removal in soil

The removal of pollutants can be roughly divided into two phases. In the first seven days, the removal was a relatively rapid process; after the first week of incubation the removal began to slow down and finally plateau after 28-day treatment. During the first phase, the available reaction sites (adsorption sites and enzymes) were enough to remove the pollutants present. After the exhaustion of these reaction sites, the removal of pollutants started to slow down and the process entered the second slow phase.

Moreover, in the case of degradation, the production of intermediates may have a negative effect on the degradation and further inhibits the degradation.

5.4.5 Ecotoxicity of soil contaminated with diesel and DEHP

The microorganisms isolated from soil samples showed different sensitivities towards the pollutants. The toxicity of diesel and DEHP showed a dose-response relationship with the concentration of pollutants.

5.4.6 Contribution of SMC components on removal of mixed pollutants including DEHP

The test identifies the contribution of the SMC components on the removal of pollutants. The autoclaved samples contain no living organisms and denatured enzymes. The results showed that autoclaving can greatly reduce the remediation of pollutants by SMC and abolished the removal by SMCE, indicating that biodegradation was one of the main processes that remove the pollutants spiked into the soil. On the other hand, the water extract of SMC contained some of the degrading abilities of SMC, but there was still deviation between the SMC and SMCE, which suggested that biosorption also contributes to removal of pollutants.

Both SMC microbes and filtrate showed the degradation of pollutants in soil, suggesting microbes and enzymes in filtrate may be two main components to degrade

pollutants. The stability of enzymes from *Pleurotus* fungi has been reported in previous studies. Hublik & Schinner (2000) reported the laccase activity of *Pleurotus ostreatus* only lost 2% during 10 days at 25°C storage on average. Yuen et al. (2004) found the loss of laccase and manganese peroxidase activities of *Pleurotus Pulmonarius* were about 30% and 55% in crude enzyme preparation under liquid storage at room temperature after two weeks, respectively. However, the filtrate containing enzymes and nutrients of SMC did not degrade the pollutants as good as SMCE, so did SMC microbes that retained on the filter membrane. This implies that these two components could enhance the total degradation ability of the SMCE when they are present together, and the nutrients in the filtrate may also stimulate the growth of microbes that provide more microorganisms for the degradation of pollutants (Adoki and Orugbani, 2007; Rosa et al., 2007).

Chapter 6 Overall discussions

6.1 Overall discussions

In this project, the application of SMC was tested in various environmental samples containing DEHP and other pollutants. The removal of pollutants was found to be effective in soil, sediment and water systems, indicating that this technique is applicable to many environmental systems to remediate the pollutants present.

In soil experiment, the environmental soil samples were collected from Lau Fou Shan, which were contaminated with DEHP and diesel heavily. Compared with other studies (Murai et al., 1998; Azarova et al., 2003; Chang et al., 2004; Nalli et al, 2006), less persistent and toxic metabolites, and the fast degradation rate are the advantages for the application of SMC. The main pollutant DEHP was removed about 54% within one month after adding SMC. In addition, except to DEHP, another main pollutant (spilled oil) also was treated successfully. The degradation of TPH with SMC showed faster degradation rate than that in land farming experiment described in the study of Al-Awadhi et al. (1996). Enzymes are one of the main machineries for the degradation of pollutants, and microorganisms also showed

positive effect on the degradation of pollutants from the results in Section 2.3. The co-existence of DEHP and diesel may enhance, reduce, or not change the removal efficiency. Results from chapter 5 have demonstrated the presence of DEHP and diesel mixture had a positive effect on the removal of the single component in the soil. In addition, in soil system, SMC could be added to improve the quality of soil and increase nutrient contents as additional value for the application of SMC in soil system.

Compared with soil system, sediment is under water. Therefore, there are many limitations for the removal of pollutants in the sediment, such as the anaerobic situation, the toxicities of the sediment and the difficulty of operation. In sediment experiment, DEHP degradation showed lower degradation percentage compared with soil system and only 20% DEHP were degraded after one month. This may be due to anaerobic situation in sediment, and toxicities of sediment to fungi and enzymes in SMC. In the sediment of this study, the toxicity of heavy metals is a known factor in the inhibition of microorganism activity (Giller et al., 1998; Chang et al., 2005), not benefit to the degradation of DEHP. The increase of the microbial population by the stimulation of the combination of SMC/SMCE and nitrate decreased after the first week, corresponding to the DEHP removal. In addition, in face of the difficulty in

adding SMC into the sediment, SMCE was more convenient for application with an injector by gravity. As water is diffusible, the SMCE spreads into the sediment and acts on greater area. i.e. The effect is not at a point only. However, SMCE application cannot stay in sediment for a longer time due to the diffusion of SMCE and the flowage of seawater. The nutrients and enzymes in SMCE (extracted with same percentage of SMC) were less than those in SMC (Table 3.4). Therefore the action time of SMCE cannot last as long as SMC. It is positive for SMC to degrade organic pollutants, but the effect of SMC on the mobilization of heavy metals is not sure. For the removal of odor, the principle of the application of nitrate is mainly because sulphides could be oxidized to sulphate by nitrate, while the removal of NH_3 by nitrate has not been reported. Moreover, the application of SMC/SMCE could decrease the content of NH_3 in air, which may be due to the microbial action, the stimulation of SMC and so on. The principle is still not clear, but the effect of SMC is positive on the removal of odor from sediment. Nitrate showed better effect than SMC/SMCE and H_2O_2. However, the massive application of nitrate increased the content of nitrogen in seawater, while the increase of nitrogen may cause eutrophication of aquatic systems. Therefore, their application has to be assessed in terms of ecological impacts.

By comparing the results of DEHP degradation, it was found that DEHP in water system degraded much faster than those in soil and sediment. Although the initial conditions was not exactly the same in these systems, less SMC extract (1%) used in water sample can completely degrade DEHP in much shorter period of time (24 h) as compared with sediment (3%, 14 days, 20% RE) and soil (5%, 20 days, 50% RE). This implies that DEHP in aqueous phase is easier to be degraded than adsorbed on solid phase. As can be seen in the results of Section 5.3.2, the main components of SMC responsible for the removal of pollutants are the enzymes and microorganisms. In aqueous phase, the pollutants have better contact with the enzymes and microorganisms in SMC due to shaking. In contrast the adsorbed pollutants were immobilized and so the degradation rate was limited by desorption of molecules from the solid surface and then diffusion to the enzymes and microorganisms in soil and sediment systems. Thus the degradation rate was much lower in solid samples. Shaking speed study had demonstrated that higher shaking speed provides better mixing between the biosorbents and the sorbates. SMC with the capacity of biosorption and biodegradation showed fast removal efficiencies for DEHP and DBP, and certain amounts of DBP and DEHP still could be detectable in SMC after 24-h treatment, while SMCE has a better effect on the environment due to the complete degradation of pollutants. Moreover, the application of SMCE is more

convenient as SMCE does not need recovery, and does not need the immobilization materials. SMCE also showed a good bioremediation capacity on the removal of DBP and DEHP.

In general, SMC/SMCE has the capacity to remove pollutants from the three systems. The fast decrease period is shown in the first one to two weeks in solid systems, and fourteen days was needed to reach the plateau when treating DEHP in soil and sediment. Similarly, there are also fast degradation and plateau periods in the aqueous system. The main degradation machinery is attributed to the application of SMC/SMCE, either enzymes or the microbes in SMC. Therefore, favorable conditions should be provided for SMC to improve enzyme and microbe activities such as moisture, when SMC is applied into environment for the treatment of DEHP.

Consequently, DEHP was optimally removed about 54% within one month after adding SMC into soil. SMC treatment not only degrades the persistent organopollutants but also reduces toxicity of the treated soil. However, sediment DEHP degradation showed lower degradation efficiency compared with soil system and only 20% DEHP were removed in sediment. In the application of SMC into sediment, SMCE was more convenient for application with an injector compared

with SMC treatment into sediment. In addition, SMCE when mixed with nitrate could remove odor gases H_2S and NH_3 from sediment. Moreover, SMC has better removal efficiency than SMCE for the treatment of DEHP and DBP in water as an integrated system of biosorption and biodegradation.

6.2 Limitation of Study

In our study, environmental samples were used to test the potential capacity of SMC on the removal pollutants from solid and aqueous systems, but there are still some limitations in the application of SMC and the detection methods.

SMC as an organic fertilizer has been used in horticulture (Williams, et al., 2001). However, SMC, especially made by sawdust, has the high carbon (C) to nitrogen (N) ratio (C/N) which is in favor of microorganism growth. So the application of SMC in the farmland is not always beneficial to utilization of plants after treatment. In our study, wheat was sown in treated soil compared with the wheat in agriculture soil. The results showed there are no significant differences on the growth of wheat between in treatment soil and in agriculture soil. In addition, some heavy metal contents in wheat were higher in treatment soil than in agriculture

soil. Although the treated industrial soil could not be used for growing of crops for human use, this SMC bioremediation followed by greening to reduce erosion of the soil with residuals heavy metals is possible to create an environmentally friendly site. Phytoremediation may be a proper strategy for removal of the heavy metals from the site.

When the results in bench-scale experiment are applied into field application, the efficiency often cannot meet the expectation. The performance of the SMC/SMCE on the sediment pollutants is low, and thus further investigation is needed for optimization. Further, the fluctuating environmental conditions including the rise and fall of the tides, possible ecological disturbance, eutrophication and ecological impacts may need to be concerned.

6.3 Further investigation

The removal of DEHP in environmental samples by spent mushroom compost was performed. The results showed that SMC enhanced biodegradation of organopollutants and lowered DEHP content below the environmental standard levels after treatment. This method is an environmental friendly and sustainable

technology. However, the results have not been applied into field application. Field trial to DEHP-contaminated sites is the ultimate goal to confirm the performance and put laboratory theory into real application.

Environmental conditions like temperature, moisture and oxygen supply should be controlled and monitored well. In soil system, environmental samples and landfarming experiment were used, and 3% SMC were applied into contaminated soil. The results showed the moisture is the key factor for the experiment, while tilling illustrated the oxygen is less important than moisture. In addition, biodegradation is very site specific and no two contaminated soil environment are exactly alike. Therefore, for the field application, a pilot study should be carried out to select the suitable ratio of SMC to soil, and the moisture.

It is anaerobic situation in sediment, so many physical conditions impact the application of SMCE and nitrate. Although the injection method was used in bench scale experiment, it should be assessed during the real application.

In water system, SMC and SMCE had been tested on the removal of pollutants. Multiple cycles experiment also demonstrated the capacity of SMC on

biosorption. Continuous system could be designed for the application of SMC to remove pollutant in water. In industry, the fixed biosorbents were used to remove the pollutants in water through the flow of contaminated water. Therefore, the flow rate and the amount of SMC are two key factors for the continuous system.

Enzymes are one of the main components for the degradation of organic pollutants. However, the principle of laccase is still not clear. This is a direction for the theory research.

6.4 Conclusion

Spent mushroom compost (SMC) is a bulky waste by-product of mushroom industry and produced abundantly. In this study, SMC was applied to contaminated soil, sediment, and water to test the potential capacity of SMC as a bioremediation agent. Various residual extracellular enzymes in SMC can oxidize and degrade a variety of highly recalcitrant organopollutants (Chiu et al., 1998; Eggen, 1999; Lau et al., 2003; Law et al., 2003; Tsang, 2004; Gong et al., 2006). In addition, SMC contains a plentiful of microorganisms with some of them possessing degrading capacities (Law et al., 2003). Moreover, various groups (–OH, –NH, etc.) and rich

chitin endow SMC with the sorption capacity.

In soil experiment, the environmental soil samples were collected from Lau Fou Shan, and were contaminated with DEHP and spilled-oil heavily. The application of SMC could decrease the content of TPH within one month from 20 g/kg to 1 g/kg, and remove about 54% DEHP from soil, as well as reduce the toxicity of soil. The removal of pollutants occurred mainly in the first week after adding SMC, which was due to the enzyme activities in SMC. Moreover, higher microbial population present with addition of SMC and higher moisture contributed to the enhanced degradation of pollutants.

In sediment experiment, both SMC and SMCE, the water extract of SMC, could lower the contents of organopollutants including DEHP in KTAC sediment. This might be due to the presence of microorganisms and lignolytic enzymes which could generate highly reactive hydroxyl radicals (·OH) to degrade organopollutants non-specifically (Chiu et al., 1998; Lau et al., 2003; Law et al., 2003; Tsang, 2004; Gong et al., 2006). Further the enhanced microbial activities might promote the degradation of sediment organic pollutants. Based on the capacity of SMC to decrease organic pollutants, nitrate was applied with SMCE into sediment to remove

odor. However, SMC did not present better effect on the mobilization of heavy metals from sediment. In general, to circumvent the treatment problems underwater, the application of SMCE was more convenient with an injection method compared with SMC. However, further optimization is still needed to improve the removal efficiency with sediment samples.

In water system, SMC had an integrated system of biosorption and biodegradation to remove DBP and DEHP. SMC could provide adsorption sites for sorption and the Freundlich isotherm could describe the sorption kinetics of SMC. The enzymes in SMC could act as Fenton reagents to produce reactive radicals for non-specific cleavage of a wide range of highly recalcitrant organopollutants (Cajthaml et al., 2002; Tsang, 2004; Gong et al., 2006). In addition, the nutrient source and consortium of microorganisms in SMC could stimulate the degradation of pollutants (Law et al., 2003). In addition, it is economically feasible to use SMC several times. In this study, SMCE also showed a good bioremediation capacity on the removal of DBP and DEHP. Though it takes longer time to remove pollutants for SMCE than SMC, SMCE does not need the immobilization materials and recovery.

Mechanism study demonstrated the biodegradation was one of the main

processes to remove pollutants by SMC. Immobilized enzymes and microorganisms were the main components that contributed to the remediation of pollutants.

In conclusion, this study demonstrates that SMC or SMCE have the potential in the bioremediation of contaminated soil, sediment and water. DEHP, as a main persistent plasticizer, could be removed by SMC/SMCE in these systems. Beside DEHP, the removal of other pollutants, such as spilled-oil, other plasticizers, was also observed.

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