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SEPARATE AND COMBINED ENVIRONMENTAL BEHAVIOUR OF SURFACTANTS AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

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For my dear mother, my dear wife Xiaoye and son Yingnan

Abstracts

Surfactants and their effects on the behaviour and toxicity of polycyclic aromatic hydrocarbons (PAHs) were studied. Six surfactants covering a wide range of different types and characteristics were examined: anionic LAS (linear alkylbenzene sulfonate), cationic TDTMA (tetradecyltrimethyl-ammonium bromide), nonionic Tween-80 (polyoxyethylene sorbitanmonooleate), Brij30 (polyoxyethylene-4-lauryl ether), 10LE (polyoxyethylene -10-lauryl ether), and Brij35 (polyoxyethylene-23-lauryl ether). With emphasis on phenanthrene, PAHs studied also include pyrene and benzo[a]pyrene representing PAHs of different benzo-ring numbers.

Surfactants were found to have significant influences on the environmental behaviour and toxicity of PAHs. Their influences depended on the characteristics of both surfactants and PAHs, type of organisms and environmental matrices. They could vary from synergism to antagonism among surfactants and organisms. In addition, the effect of surfactants was also a function of their concentration and could shift from positive to negative and *vice versa* as their concentration changed.

Adsorption of LAS on soils underwent two stages: linear and exponential adsorption. Its mechanism was mainly specific site surface interactions and adsorption capacity depended on soil physical clay content. LAS significantly influenced the adsorption of phenanthrene on soil. LAS at high concentrations decreased the adsorption. At low concentrations, it could either increase or decrease phenanthrene adsorption and the key factor was their contacting sequence with soil. The velocity of LAS migration in soil was dependent on water flow flux and independent of its concentration. The migration of selected PAHs in soil was slow and followed the order of phenanthrene > pyrene > benzo[a]pyrene. The presence of LAS or Tween-80 could enhance their migration. Preferential migration of all test chemicals was observed in natural soil.

Phenanthrene and all test surfactants were toxic to *Vibrio fischeri* in a descendent order of TDTMA > phenanthrene > Brij30 > 10LE > LAS > Brij35 > Tween-80. The impact of nonionic surfactants and phenanthrene was reversible. The combination of phenanthrene and surfactants was more toxic to the bacteria in an order of TDTMA > Brij30 > LAS > Brij35 > 10LE > Tween-80. The combined effects of phenanthrene with the later four surfactants were underadditive or antagonistic while overadditive

or synergistic with TDTMA and Brij30.

Mycobacterium sp. could grow on Tween-80 and Brij surfactants as sole carbon and energy source but not on LAS and TDTMA. The toxicity of Brij surfactants increased with the increase of ethoxylate group in their molecules, which was contrary to the responses of *Vibrio fischeri*. TDTMA was the most toxic, followed by LAS and Brij35. The combined effects of phenanthrene with Tween-80 or Brij surfactants were positive and additive while negative with LAS or TDTMA. LAS and Tween-80 had similar effects on soil microorganisms as on *Mycobacterium* sp. TDTMA was less toxic to soil microorganisms than LAS though the former was much more toxic to *Mycobacterium* sp. and *Vibrio fischeri* than the later. Phenanthrene exhibited completely contrary effects on soil microbial activities in different soils. The coexistence of any test surfactants could generally increase soil microbial activities. The combined effects of PAHs with TDTMA were overadditive while with Tween-80 was slightly overadditive at low respiration levels and underadditive at high levels.

The presence of surfactants inhibited the biodegradation of phenanthrene by *Mycobacterium* sp. in a descent order of TDTMA > LAS > Tween-80. It was either because of their (LAS and TDTMA) toxic to the bacteria or (Tween-80) utilization by bacteria as preferential substrates. The biodegradation of pyrene in soil was much lower than that of phenanthrene. LAS and TDTMA at low concentrations and Tween-80 enhanced the biodegradation of both PAHs. Surfactant concentrations, at which the biodegradation of PAHs was enhanced or inhibited, varied significantly among soils. The relationship between enhancement of phenanthrene biodegradation and microbial activities was also remarkably different among soils and among surfactants. The efficiency of surfactant's enhancement of PAH biodegradation depended on the intrinsic PAHs-degrading capacities and the inherent microbial activities of the soils.

Mycobacterium sp. strain KR2 could grow on solid phenanthrene through direct contact. It was inhibited by LAS, TDTMA, Brij30 and 10LE, while enhanced by Tween-80 at <20 mg l^{1} and Brij35 at 5 to 80 mg l^{-1}. The mineralization and metabolism of phenanthrene were quick in wheat-solution-lava microcosm. At least 90% of the applied phenanthrene were transformed within 24 days. 70% of the applied ^{14}C-activity was transferred to wheat and 53% constructed into wheat tissues. The presence of Tween-80 significantly increased the metabolism of phenanthrene.

Zusammenfassung

Die vorliegende Arbeit beschäftigt sich über die Wechselwirkungen von sechs Tensiden (anionische, kationische und nichtionogene), die als Vertreter der im kommerziellen Einsatz befindlichen ausgesucht wurden, mit Phenanthren, Pyren, Benz[a]pyren als Vertreter der PAKs (Polyzyklischer Aromatischer Kohlenwasserstoffe) in unterschiedlichen Bodentypen. Neben den Aspekten wie das Adsorptions- und Migrations-Verhalten der Testchemikalien, sowie deren Bioabbaubarkeit und toxische Wirkung auf Bakterien in unterschiedlichen Böden, steht auch die mikrobiologische Abbaubarkeit von Phenanthren und sowie die Einwirkung von Detergentien auf diesen Abbauvorgang in unterschiedlichen Matrizes im Mittelpunkt der Untersuchungen.

Die Adsorption von LAS (Lineares Alkylbenzolsulfonat) an untersuchten Böden verlief je nach der physikalischen Beschaffung der Böden linear oder exponential. LAS beeinflusste sehr stark das Adsorptionsverhalten des Pheanthrens. Hohe LAS Konzentrationen im Boden reduzierten die Adsorption. Die Perkolationsgeschwindigkeit von LAS war konzentrationsabhängig. Die Mobilität der drei PAKs im Boden war gering und folgte der Rangordnung Phenanthren> Pyren> Benz[a]pyren. Bei Anwesenheit von LAS und Tween-80 war die Bodenmigration erhöht.

Phenanthren und alle sechs getesteten Tenside zeigten eine starke Hemmung im Leuchtbakterienhemmtest mit *Vibrio fischeri.* Die Toxizität war höher wenn die beiden Chemikalien zusammen appliziert wurden.

Mycobacterium sp. akzeptierte Tween-80 und Brij 30/35 als alleinige Kohlenstoff- und Energie-Quelle und wuchs in deren Anwesenheit, während bei Zugabe von LAS und TDTMA keine wachstumsfördernde Wirkung zu sehen war. Unter den eingesetzten Tensiden hatte TDTMA das höchste toxische Potential, danach folgten LAS und Brij35. Die kombinierte Wirkung von Phenanthren mit Tween-80 und Brij 30/35 verursachte eine positive und additive Toxizität, während LAS zusammen mit TDTMA keine toxische Wirkung erzeugten. LAS und Tween-80 hatten eine ähnliche Wirkung auf die Aktivität der Mikroorganismen im Boden wie bei dem *Mycobacterium sp.* Im allgemeinen erhöhte die Koexistenz von eingesetzten Tensiden

im Boden die bakterielle Aktivität. PAKs mit TDTMA zeigten einen gesteigerten „additiven“ Effekt, während Tween-80 eine von der Atmungsaktivität der Bakterien abhängige Wirkung zeigte.

Die biologische Abbaubarkeit von Phenanthren und Pyren nahm in der Reihenfolge von den Tensiden wie folgt ab TDTMA> LAS> Tween-80. Es kann vermutet werden, dass es entweder auf die toxische Wirkung der LAS und TDTMA zurückzuführen ist oder aber die Bakterien bevorzugen die Tween-80 als Kohlenstoffquelle solange es vorrätig ist. Die mikrobiologische Abbaubarkeit von Pyren war schlechter als für Phenanthren. Niedrige Konzentrationen von LAS und TDTMA und Tween-80 erhöhten die biotische Aktivität, bzw die Abbaubarkeit der Testsubstanzen. Die Fähigkeit der Tenside die Abbaubarkeit der PAKs zu erhöhen war von der Abbaukapazität und von der inhärenten, mikrobiellen Aktivität des Bodens abhängig.

Mycobacterium sp. KR2 akzeptierte die festen Phenanthren-Kristalle als Energiequelle und wuchs auch auf diesem Substrat. Das Wachstum wurde von LAS, TDTMA, Brij30 und 10LE negativ beeinflusst, dagegen zeigten Tween-80 (<20 mg l^{-1}) und Brij35 (5-10 mg l^{1}) eine wachstumsfördernde Wirkung. Die Mineralisierung und Umwandlung von Phenanthren im Mikro-Ökosystem „Weizen-Nährlösung-Lava“ verlief rasch wobei 90% der applizierten Phenanthrens bereits nach 24 Tagen umgewandelt war. 70% der eingesetzten ^{14}C-aktiven Phenanthrens wurde von der Pflanze aufgenommen und 53% in die diversen Weizengewebe transportiert. Eine signifikante Erhöhung der Metabolisierungsrate von Phenathren wurde bei Zugabe von Tween-80 festgestellt.

LAS (linear alkylbenzene sulfonate), cationic TDTMA (tetradecyltrimethyl-ammonium bromide), nonionic Tween-80 (polyoxyethylene sorbitanmonooleate), Brij30 (polyoxyethylene-4-lauryl ether), 10LE (polyoxyethylene -10-lauryl ether), and Brij35 (polyoxyethylene-23-lauryl ether).

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List of abbreviation

10LE - polyoxyethylene 10 lauryl ether
ABS - absorbance
ACN - acetonitrile
BaP - benzo[a]pyrene
Brij30 - trade name of polyoxyethylene 4 lauryl ether
Brij35 - trade name of polyoxyethylene 23 lauryl ether
CK - control (experiments without test chemicals)
CMC - critical micelle concentration
CSA - cross section area
DAD - diode-array detector
DCM - dichloromethane
EC - effective concentration
FLD - fluorescent detector
EMME - ethylene glycol monomethyl ether
HLB - hydrophile-lipophile balance
HASs - hydrophobic adsorption sites
HOCs - hydrophobic organic compounds
HPLC - High performance liquid chromatography
KDE - Kuderna-Danish-Evaporator
K_{ow} - Octanol-water partition coefficient
LAS - linear alkylbenzene sulfonate
LSC - liquid scintillation counter
MSM - Mineral salt medium
MSM-Ca - Mineral salt medium without calcium
MW - molecular weight
MPW - Millipore water (water purified by Millipore purification system)
NAPL - non-aqueous-phase liquid
PAHs - polycyclic aromatic hydrocarbons
RSD - relative standard deviation
SES - Shenyang Ecological Station, Shenyang, China
SMPW - Sterile Millipore water (MPW)
SPE - solid phase extraction
TDTMA - tetradecyltrimethylammonium bromide
THF - tetrahydrofuran
ThOD - theoretical oxygen demand
TLC - thin layer chromatography
Tween-80 - Trade name of polyoxyethylene sorbitanmonooleate
VOCs - volatile organic chemicals

CONTENTS

1 GENERAL INTRODUCTION

1.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are the most commonly found environmental pollutants that belong to hydrophobic organic compounds (HOCs) based on their properties. The fate of PAHs in nature is of great environmental and human health concerns due to their carcinogenic, mutagenic and teratogenic properties (Pahlmann and Pelkonen, 1987; Wilson and Jones, 1993; Shaw and Connell, 1994) as well as their high concentration and frequency found in the environment (LaFlamme and Hites, 1978; Sims and Overcash, 1983; Jones *et al*., 1989a). These facts make PAHs priority pollutants needed to be controlled (Keith and Telliard, 1979; Volkering *et al*., 1995).

PAHs are compounds containing carbon and hydrogen with fused benzene rings in linear, angular or cluster arrangements. PAHs may also contain alkyl and nitro substituents and can be considered heterocyclic molecules with the substitution of an aromatic ring carbon with nitrogen, oxygen or sulphur. During the last three centuries, a relationship between the higher incidence of cancer in urban and industrial areas than in rural areas, and the exposure of humans to PAHs, have prompted considerable research on the sources, occurrence, bioaccumulation, metabolism, and disposition of these pollutants in aquatic and terrestrial ecosystems (Cerniglia, 1985 and 1992; Santodonato, 1997). Generally, PAHs of higher molecular weight, containing four or more fused rings, pose the greatest hazard to both environment and human health (US EPA, 1982).

Occurrence of PAHs in the environment is due to both natural and anthropogenic processes (Blumer, 1976). Natural PAHs come from petrogenic and phytogenic sources (NAS, 1983; Mueller *et al*., 1989). However, most PAHs in the environment possessing potential hazard to human health are anthropogenically produced. Environment contaminated by large amounts of these chemicals is considered hazardous owing to potential harmful effects of specific PAHs to human health

(Moore *et al*., 1989). PAHs are universal products of the combustion of organic matter. They are formed during the combustion of a wide variety of materials such as fossil fuels, saturated and unsaturated hydrocarbons, peptides, and carbohydrates. Several theories on PAH pyrosynthesis have been proposed and extensive reviews on this subject are available (Badger *et al*., 1958 and 1964; Blumer, 1976). Some major sources of PAHs are forest and prairie grass fires, volcanic ash, heat and power generation, refuse burning, motor vehicle emissions, industrial processes, petroleum leakage and spills, fallout from urban air pollution, coal liquefaction and gasification processes, and cigarette smoke.

PAHs enter the environment from a multiplicity of sources which include: wet and dry deposition, chronic leakage of industrial or sewage effluents, accidental discharges during the transport, use and disposal of petroleum products, or from natural sources such as oil seeps and surface water run-off from forest and prairie fire sites (Giger and Blumer, 1974; LaFlamme and Hites, 1978; Niessner, 1994; Ou *et al*., 1995a; Grimmer et al., 1997; Jacob *et al*., 1997; Koeber *et al*., 1999). More specifically, industrial effluents from coal gasification and liquefaction, and other petroleum-derived products release high quantities of PAHs into the environment. Anthropogenic combustion activities are principal sources of PAHs to plant-soil systems in industrialized countries via atmospheric deposition and in developing countries via irrigation with sewage or wastewater (Jones, 1991; Ou *et al*., 1992b). As a result, soil concentrations of PAHs have tended to increase over the last 100 to 150 years, particularly in urban areas (Jones *et al*., 1989a and 1989b).

The possible fates of PAHs in the environment include volatilization, photooxidation, chemical oxidation, bioaccumulation, adsorption to soil particles or sediments, and leaching and microbial degradation. PAHs are highly persistent in the environment. This is a consequence of the resistance of PAHs to decomposition processes (Zaidi and Imam, 1999), their high affinity for organic matter (Kögel-Knabner and Totsche, 1998) and their low water solubility (Rippen, 1998). PAHs are hydrophobic compounds and rapidly become associated with soil particles or sediments, where

they may become buried and persist for long periods. Their lipophilicity, environmental persistence, and genotoxicity increase as the molecular size of PAHs increases up to 4 or 5 fused benzene rings, and their toxicological concern shifts towards chronic toxicity, primarily carcinogenesis (Miller and Miller, 1981; Jacob *et al*., 1986). The bioaccumulation of PAHs along food chains can lead to a great risk to human being. Understanding the interactions of PAHs with soil and organisms and the behaviour of PAHs in the environment are essential to minimize or eliminate their harmful effects to human health. Therefore, the fate and behaviour of PAHs in the environment are the subject receiving great attention for decades.

1.2 Surfactants

Surfactants have received attention as environmental and human health concerns not only due to their direct toxicity (Cserháti *et al*., 1991; Kusk and Petersen, 1997; Shcherbakova, *et al*., 1999) but also to their impacts on environmental processes and on the fate and behaviour of other contaminants to a great extent (Kuhnt and Knief, 1991; Haigh, 1996). Although little is known about the modes of interaction of surfactants and soil components (Haigh, 1996; Jensen, 1999), it is found that nearly all of the natural soil functions can be altered by surfactants and the possibility of negative effects should never be excluded (Kuhnt and Knief, 1991). In particular, surfactants are believed to be able to enhance the solubility and bioavailability of hydrophobic organic compounds (HOCs). Intensive attempts have been made in recent years to increase the biodegradation of HOCs in remediation of HOCs-contaminated soils by addition of surfactants. Therefore, there is currently a growing interest on the behaviour of surfactants in the environment and their influences on solubility, adsorption/desorption, migration, bioavailability, degradation of HOCs (West and Harwell, 1992; Rouse *et al*., 1994; Sánchez-Camazano *et al*., 1995; Haigh, 1996; Iglesias-Jiménez *et al*., 1996; Volkering *et al*., 1998).

Surfactants are a group of surface active agents. They are amphipathic molecules consisting of a hydrophilic polar head moiety and a hydrophobic nonpolar tail moiety. Surfactants are characterized by their ability to concentrate at surfaces and reduce the

surface tension by forming aggregates. Surfactants can be produced chemically (synthetic surfactants) and biologically (biosurfactants). The hydrophobic moiety of a surfactant is a relatively long aliphatic hydrocarbon chain (10 to 20 carbon atoms). The most common hydrophobic parts of synthetic surfactants are the alkyl chain of fatty acids, paraffins, olefins, alkylbenzenes, alcohols, alkylphenols, or polyoxypropylene; the hydrophilic moiety can be a sulphonate, sulphate, carboxylate (anionic), quaternary ammonium (cationic), sucrose, polypeptide, or polyoxyethylene (nonionic). Therefore, surfactants can be categorized by the ionic charge associated with their polar moiety: nonionic, anionic, cationic, and zwitterionic (amphoteric). The major classes of biosurfactants include glycolipids, phospholipids, fatty acids, lipopeptides/lipoproteins, and biopolymeric surfactants.

A primary characteristic of surfactants is their ability to form aggregates called micelles in solution. The formation of these micelles occurs when the critical micelle concentration (CMC) is exceeded. Micelles form through self-association of surfactant molecules, in which hydrophobic tail groups come together to create a thermodynamically favourable hydrocarbon pseudophase (hydrophobic core), an outer ionic layer and an intermediate region termed the palisade layer. Below CMC, surfactants in solution exist solely as individual molecules or monomers. Above CMC, a constant monomer concentration is maintained in equilibrium with the micelles. The possible forms of surfactants in soil-water systems are schematically illustrated in Fig. 1. When small quantities of surfactant are added to soil-water systems, parts of them are dissolved or adsorbed on soil particles as monomers and part forms a monolayer at the air/water interface leading to a reduction of interfacial tension. In high concentrations, surfactant adsorption onto soil surfaces leads to the formation of single (hemimicelles) layers of surfactant molecules at the interface. When concentration is above CMC, surfactant monomers aggregate to form micelles in solution and double (admicelles) layer of surfactant molecules on soil surface. The concentration of surfactant molecules at soil surfaces also leads to a reduction of the interfacial tension, which may improve the contact between the aqueous and solid phases. The molecules in the monolayer, micelles, hemimicelles and admicelles are in equilibrium with monomers in the bulk solution. In the presence of a non-aqueous

phase liquid (NAPL), surfactants concentrate at the liquid-liquid interface, reducing the interfacial tension. This may cause dispersion of NAPL droplets and stabilization of emulsions (Helenius and Simons, 1975; Ou *et al*., 1995b; Volkering *et al*., 1998).

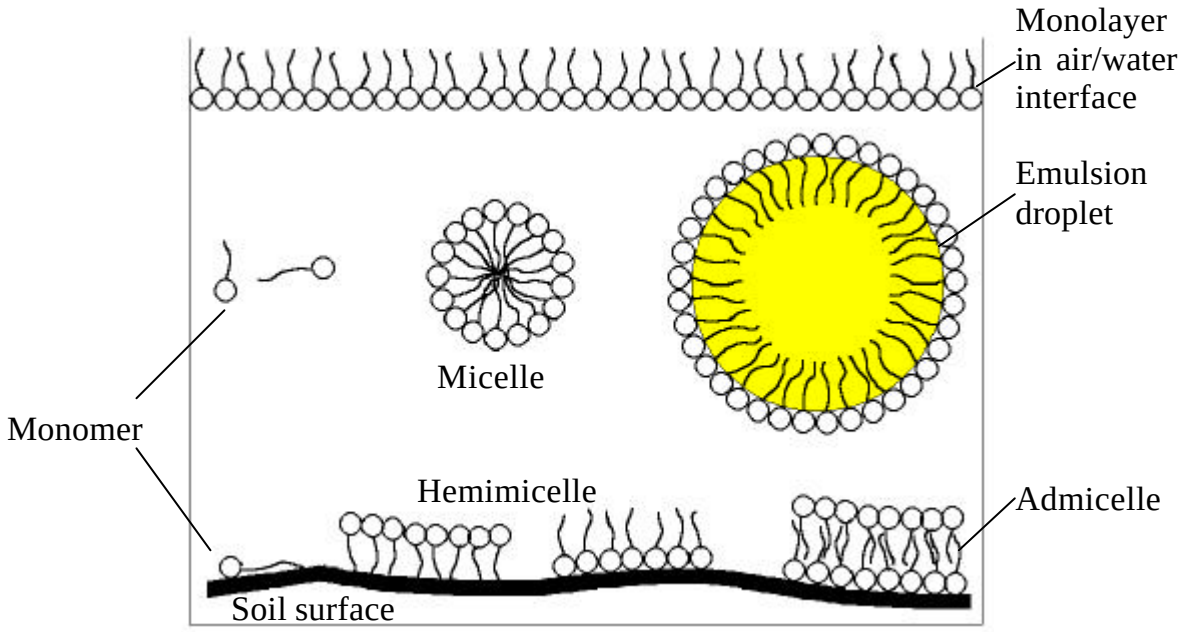


Fig. 1. Schematic drawing of different surfactant forms in soil-water system

Surfactants can also be of natural and anthropogenic occurrence. Many microorganisms can produce their own surfactants i.e. biosurfactants. However, only anthropogenic or synthetic surfactants are of environmental and health concern since they are widely used in daily life and in almost all industrial sectors. Fig. 2 shows the use of surfactants in various purposes. Of the various types, the anionic surfactants are commercially and quantitatively the most important (Marchesi et al., 1991). Among them, anionic linear alkylbenzene sulfonate (LAS) is most widely used in both percentage and absolute amount (Inoue *et al*., 1978; He *et al*., 1991, Jensen, 1999). The worldwide use of LAS was approximately 2.5×10^6 tons in 1995 (de Ferrer *et al*., 1996).

The extensive use of detergents and existing methods of disposal mean that

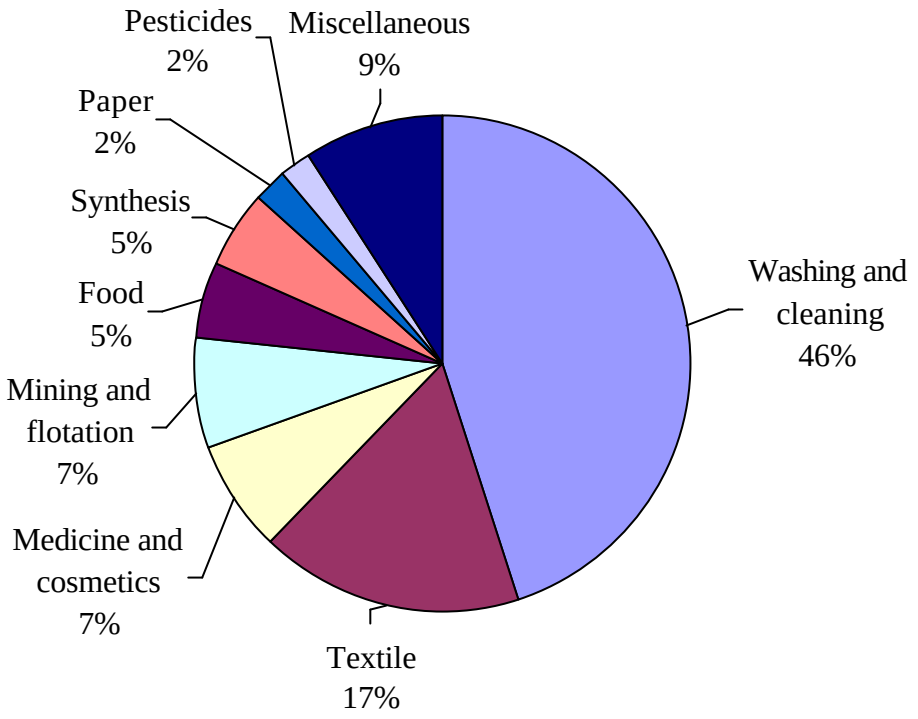


Fig. 2. Utilization of surfactants (Fabry, 1991)

surfactants are widely dispersed in the environment. Surfactants enter the aquatic environment from wastewater treatment plants and in effluent discharge from industry and households (Marchesi *et al*., 1991; Ahel *et al*., 1994). Surfactants reach plant-soil systems by various ways including irrigation with water contaminated by surfactants and agricultural or municipal wastewater, application of manure or sludge on land, and the use of detergent pesticides, etc. (Cserháti *et al*., 1991; Jones, 1991; Ou *et al*., 1992b). As most widely used, LAS at the same time is also the most common pollutant found in almost all environmental compartments (Gullivan and Swisher, 1969; McEvoy and Giger, 1986; Takada and Ishiwatari, 1987; Yediler *et al*., 1989; Papaport and Eckhoff, 1990; McAvoy *et al*., 1993; Kusk and Petersen, 1997; Perales *et al*., 1999). Of the annual production of 317,000 tons in the United States only, approximately 16,000 tons per year is discharged to surface waters in municipal sewage effluent. The concentration of LAS in treated sewage effluent ranges 0.02 to 0.8 mg l^{-1} (Painter and Zabel, 1989). But the concentration of LAS in the environment varies broadly. Typical surface water concentrations immediately below wastewater

treatment plant outfalls are less than 50 µg l^{1} (McAvoy *et al*., 1993), although in some countries levels may be as high as 1 to 2 mg l^{-1}. Concentrations of LAS in soil range from <3 to 47 mg kg^{-1} shortly after sludge amendment (Papaport and Eckhoff, 1990), and <1 to 5 mg kg^{-1} in soil not received sewage sledge recently (Jensen, 1999).

1.3 Combined behaviour of surfactants and PAHs

The coexistence of different kinds of contaminants including surfactants and PAHs in environmental matrices is a common fact. As a consequence, environmental contamination is usually a result of many pollutants of different sources, types and characteristics. The exposure of organisms in the environment to toxic chemicals in fact occurs as exposure to the mixtures of coexistent chemicals (Tøraløv and Lindgaard-Jørgensen, 1994). The idea that the effects of an agent may be altered - enhanced or diminished - by a number of other compounds is a well-known concept in pharmacology and toxicology (Kortenkamp and Altenburger, 1999). It is also true in the environment that toxicity and physicochemical behaviour of one chemical can be altered by another. For examples, Liu (1989) observed synergistic toxicity of pentachlorophenol and 2,3-dichlorophenol to bacterial dehydrogenase activity. Tøraløv and Lindgaard-Jørgensen (1994) reported antagonistic effects of nonylphenolethoxylate and tetrapropylenbenzenesulfonate on *Pseudomonas fluorescens*. The existence of such combination effects implies that the effect of the mixture somehow deviates from what is expected on the basis of the single pollutant's effects. Therefore, it is important to have the knowledge of the interaction of coexistent chemicals, their combined behaviour in the environment and combined effects on organisms in order to properly predict and control their fate in the environment and assess their ecotoxicity.

The fate and behaviour (adsorption/desorption and mobilization, biotic and abiotic degradation, volatilization, uptake and utilization by organism, effects on organism, or accumulation in soil and organism) of PAHs in plant-soil systems is affected by the coexistent surfactant in many ways, for example:

- solubilization and mobilization (Edwards *et al*., 1991; Yediler *et al*., 1991; Laha

and Luthy, 1992; West and Harwell, 1992),
- bioavailability (Volkering *et al*., 1995; Guha and Jaffé, 1996b),
- accessibility of micellar hydrocarbons into the cells (Miller and Bartha, 1989; Bury and Miller, 1993), and
- adhesion of bacteria to NAPL hydrocarbons or sorbed HOCs (Rosenberg *et al*., 1983; Efroymson and Alexander, 1991; Ortega-Calvo and Alexander, 1994).

These effects are sometimes decisive for the fate and behaviour of PAHs. It is believed that the interaction among surfactant and HOCs, surfactant and soil components, and between surfactant and organisms plays an important role in the effects of surfactants on behaviour and fate of HOCs in the environment. However, these interactions in soil or sediment systems and their mechanisms behind these interactions are not well understood (Edwards *et al*., 1991; Rouse *et al*., 1994; Haigh, 1996; Volkering *et al*., 1998).

Reports about the effects of surfactants on the overall biodegradation of HOCs are also controversial (Haigh, 1996). While some researches have found that the presence of surfactants enhances biodegradation of hydrocarbons (Efroymson and Alexander, 1991; Aronstein and Alexaner, 1992 and 1993; Bury and Miller, 1993; Tiehm, 1994; Churchill *et al*., 1995; Lantz *et al*., 1995; Liu et al., 1995; Volkering *et al*., 1995; Jahan *at al*., 1999), others have reported that the presence of surfactants inhibits biodegradation (Aronstein *et al*., 1991; Efroymson and Alexander, 1991; Laha and Luthy 1991 and 1992; Putcha and Domach, 1993; Tiehm, 1994; Churchill *et al*., 1995; Deschênes *et al*., 1995; Grimberg and Aitken, 1995; Wilson *et al*., 1995). Explanations for negative effects vary from the surfactant being used as preferential substrates (Tiehm, 1994), micelles shielding HOCs from microbial attack (Putcha and Domach, 1993), decrease of bioavailable equilibrium free HOCs as a result of micellization (Laha and Luthy 1991), to surfactant toxicity (Aronstein *et al*., 1991; Tiehm, 1994). These discrepancies within the literature indicate that there is a need to understand the mechanism of biodegradation in the presence of surfactants.

There is little information and therefore understanding about the combined toxicity of PAH and surfactant mixtures on organisms though they occur in all environmental

compartments (Volkering et al., 1998; Schramm et al., 1999). Tøraløv and Lindgaard-Jørgensen (1994) studied the combined toxicity of two surfactants nonylphenolethoxylate and tetrapropylenebenzenesulfonate on bacteria *Pseudomonas fluorescens* and found underadditive effects of the mixture. Grimme *et al*. (1996a and 1996b) investigated the combined effects of surfactants and pesticides on green algae and found additive effects of the mixtures in range of EC_{20} to EC_{80}. Schramm *et al*. (1999) examined the combined toxicity of terbuthylazine and LAS on green algae *Scenedesmus subspicatus* and also found the increased toxicity of the mixture could be described by additive model.

Research on the combined behaviour of surfactants and HOCs has a twofold purpose, namely (1) to determine how the behaviour and fate of HOCs is altered by the presence of surfactants when both coexist in soil as a result of human activities, and (2) to investigate the potential use of surfactants in the remediation of HOCs-contaminated soil (Abdul *et al*., 1990; Fountain, *et al*., 1991; West and Harwell, 1992; Sánchez-Camazano *et al*., 1995; Schramm *et al*., 1997). The first is important for the assessment of ecological risk of HOCs in the environment.

1.4 Surfactant's enhancement of bioremediation of HOCs-contaminated soils

Sites of closed-down coking plants and gas works are frequently contaminated with HOCs. Waste sites are currently a problem in every country. It was estimated that there were 240,000 contaminated sites in Germany, of which the main contaminants were PAHs (Hagedorn, 1995). Soil contamination is a serious environmental problem in the Netherlands, where the number of more or less strongly contaminated sites is about 100,000 involving a total of 200 million tons of contaminated soil. It has been estimated that remediation of these sites will cost more than 30 billion US$ (Rulkens and Honders, 1996). In the United Kingdom, there are approximately 50,000 to 100,000 contaminated sites of 100,000 ha (Overcash, 1996). As in most of Europe, PAHs are the focus of decontamination researches of contaminated sites (Overcash, 1996). In the USA, the Environmental Protection Agency (EPA) has estimated that of the more than 3 million potential contaminated sites, over 400,000 sites are likely to require some form of remediation (US EPA, 1993).

The remediation and reclamation of land contaminated with hazardous materials has received increasing attention internationally in recent years, with enhanced awareness of the potential adverse effects on human health and the environment (Wilson and Jones, 1993). Biological remediation techniques are in particular the focus in this field. Biodegradation is an attractive method for remediation of contaminated soil because of its economic viability and environmental soundness. Bioremediation is the use of microorganisms to break down hazardous organic materials to harmless compounds. It has advantages over thermal and some physicochemical techniques in terms of low costs and because the soil as a living system for plant growth is not destroyed.

It is generally accepted that a low level of bioavailability (i.e. low water solubility, high partitioning onto the soil matrix, and slow release of the adsorbed pollutant from solid phases to the aqueous phase) is one of the most important factors limiting the biodegradation of HOCs in soil (Volkering *et al*., 1995), which is a potential problem for bioremediation of PAHs-contaminated soil (Grimberg *et al*., 1996). A possible way of enhancing the bioavailability of HOCs is the application of surfactants. The use of surfactants in biological soil remediation is somewhat controversial, however, as the mechanism of their function and interactions with HOCs and soil components is not well understood (Edwards *et al*., 1991; Rouse *et al*., 1994; Haigh, 1996; Volkering *et al*., 1998). As stated previously, there are some reports that surfactants can enhance the biodegradation of HOCs in bioremediation processes while others report that surfactants inhibit their biodegradation.

Surfactants can have positive and negative effects on biodegradation of HOCs. Positive effects, i.e. the enhancement of a surfactant on the biodegradation of HOCs, can be expected though five main mechanisms:

- dispersion of solid or NAPL hydrocarbons, leading to an increase in contact area, which is caused by a reduction in the interfacial tension between the aqueous phase and the nonaqueous phase (Zhang and Miller, 1992);
- increase of HOCs solubility by surfactants above CMC, micelles of which may contain high concentrations of HOCs (Edwards *et al*., 1991; Laha and Luthy, 1992);

- desorption of sorbed HOCs from soil, caused by solubilization or competition of surfactants for adsorption sites on soil (Ou *et al*., 1995b);
- facilitated transport of HOCs from the solid phase to the aqueous phase, which can be caused by a number of phenomena, such as lowering of the surface tension of the pore water in soil particles, interaction of the surfactant with solid interfaces, and interaction of HOCs with single surfactant molecules (Volkering *et al*., 1995);
- enhancement of the uptake rate of HOCs by facilitating the transport of the hydrocarbon over the bacterial membrane into the cells (Miller and Bartha, 1989; Bury and Miller, 1993), caused by the transport of micellar HOCs or by incorporation of surfactant into cell membranes and change their properties (Breuil and Kushner, 1980).

The first mechanism is involved only when liquid-phase hydrocarbon is present. It should be noted that, however, increased dispersion does not always lead to increase biodegradation (Zhang and Miller, 1995). The second mechanism influences the amount of HOCs released from solid phase. The third and fourth mechanisms can cause an increase in the rate of mass transfer to the aqueous phase and therefore bioavailability. The fifth mechanism can increase the rates of HOC degradation. However, it is controversial over the bioavailability of micellar HOCs. Some authors report that micellar HOCs are accessible by organisms (Guha and Jaffé, 1996b) while others found micellar HOCs are essentially unavailable to the microorganism (Grimberg and Aitken, 1995), indicating that this enhancement is different among organisms as well as among surfactants. The most recognized positive effects are:

- increase of HOCs solubility by surfactants above their CMC,
- desorption of sorbed HOCs from soil, and
- facilitated diffusion of HOCs from the solid phase to the aqueous phase, which will potentially increase the bioavailability of HOCs.

In addition, surfactants may also act as inducers of enzymes necessary for the degradation of HOCs (Breuil and Kushner, 1980).

Negative effects can be caused by:

- depletion of minerals or oxygen;

- toxicity of some surfactants and their intermediates, which are often more toxic than the parent compounds (Holt *et al*., 1992);
- preferential degradation of the surfactant, slowing the pollutant degradation (Tiehm, 1994; Deschênes *et al*., 1995);
- possible reduction of bioavailability of the pollutant due to the partition of HOCs into surfactant hemimicelles/admicelles formed on the soil surface (Ou *et al*., 1995b) or less accessibility of micellar HOCs by microorganisms (Grimberg and Aitken, 1995).

The lack of understanding of mechanisms of surfactant interaction with HOCs, organisms and soil components leads to so many controversies in the application of surfactants in bioremediation of HOCs-contaminated soils. This also indicates that further researches on the combined behaviour of surfactants and HOCs as well as their separate behaviour are crucially needed before surfactants can be successfully applied to enhance bioremediation of contaminated soil in practice.

2 OBJECTIVES

Knowledge of the separate behaviour of individual chemicals in the environment is essential for understanding and predicting their combined environmental behaviour and ecological effects. Therefore, the present work focuses not only on the combined behaviour and effects of surfactants and PAHs but also on their separate behaviour in the environment. The overall aims of this work are for a better understanding of the separate and combined behaviour and fate of surfactants and PAHs in the environment and mechanisms governing the influence of surfactants on PAH's behaviour, and for the need of practical application of surfactants in the enhancement of bioremediation of PAHs-contaminated soil. Therefore, the specific objectives of this work are multifold and summarized under four readings:

(1) Studies on adsorption and migration of surfactants in soil and their influence on the corresponding behaviour of PAHs: These investigations are to provide necessary information for proper assessment of their transport in soil and possibility of contamination of ground water, and for better understanding of the mechanism altering the bioavailability of PAHs by surfactants. These information and mechanism are important for ground water pollution control as well as bioremediation of PAHs-contaminated soil.

(2) Studies on the separate and combined effects of surfactants and PAHs on microorganisms: These studies would lead to a correct assessment of PAH's risk in real environment where many pollutants coexist. Furthermore, they should promote the understanding of mechanisms how surfactants influence PAH's toxicity on organisms, and give information on optimal surfactant concentrations for bioremediation.

(3) Studies on the degradation of surfactants and PAHs in the presence and absence of surfactant in mono bacterium culture, in soils and in wheat-nutrient-lava microcosm: These investigations should aim more directly to the bioremediation of PAHs-contaminated soil. The controversy in literature concerning the effects of

surfactants on the biodegradation of HOCs during bioremediation is to be solved only when their mechanisms are understood. The present study will also provide necessary parameters, such as type of surfactants and their optimal concentrations, for the application of surfactants in bioremediation process. Influence of Tween-80 on the mineralization and metabolism of phenanthrene in wheat-nutrient-lava microcosm will provide necessary information for better understanding how surfactants affects the behaviour and fate of PAHs in plant-soil systems.

(4) Studies on microbial utilization of solid phenanthrene in the presence and absence of surfactants: Considerations underlined these studies are how surfactant affects the adherence and growth of microorganisms on HOCs existing as solid particles or NAPLs.

With emphasis on phenanthrene, PAHs studied in the present work also include pyrene and benzo[a]pyrene. They are selected as model compounds representing PAHs of different benzo-ring numbers i.e. 3 to 5 benzo-rings, respectively. Phenanthrene, although not particularly hazardous itself (Phillips, 1983), is a good model compound for studying PAH biogeochemistry because it is intermediate in solubility and physical behaviour (May *et al*., 1978; Readman *et al*., 1982).

Six surfactants commonly used in surfactant-enhanced bioavailability experiments or found in the environment are examined: LAS (linear alkylbenzene sulfonate), TDTMA (tetradecyltrimethyl-ammonium bromide), Tween-80 (polyoxyethylene sorbitanmonooleate), Brij30 (polyoxyethylene-4-lauryl ether), 10LE (polyoxyethylene -10-lauryl ether), and Brij35 (polyoxyethylene-23-lauryl ether). They are selected to cover a range of different types and characteristics of surfactants and as representatives of anionic, cationic and nonionic surfactants, respectively. Since LAS is used in larger amount and more frequently found in the environment, its behaviour is studied with emphasis. Nonionic Brij series surfactants (Brij30, 10LE and Brij35) are chosen based on the length of their oxyethylene chain for comparative studies.

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

3.1.1.1 Water and solvents

Unless specified, water used in all experiments was generated by a Millipore Water Purifying System (Milli-RO plus + Mill-Q plus) and is abbreviated as MPW (Millipore water) in all following text. Sterile MPW (SMPW) was obtained by autoclaving MPW at 121°C for 30 min.

All solvents were GC or HPLC grade and used as received. Methanol, n-hexane, cyclohexane, toluene, chloroform, and ethylene glycol monomethyl ether (EMME) were obtained from MERCK (Darmstadt, Germany). Acetonitrile and dichloromethane came from Riedel-deHaën (Seelze, Germany).

3.1.1.2 PAHs

With emphasis on phenanthrene, PAHs (polycyclic aromatic hydrocarbons) studied also include pyrene and BaP (benzo[a]pyrene). They were selected as model compounds representing PAHs of different benzo-ring numbers i.e. 3 to 5 benzo-rings, respectively. Commercial PAHs were used as supplied.

Phenanthrene with a purity of 99.5% (HPLC) from Aldrich-Chemie (Steinheim, Germany) and >96% (HPLC) from Sigma-Aldrich Chemie (Steinheim, Germany) was used as standards for HPLC analysis and as chemicals for experimental studies, respectively. ^{14}C-phenanthrene ([9-^{14}C]phenanthrene) with a purity of >97% and specific activity of 55 mCi mmol^{-1} was purchased from Biotrend Chemical Co. (Köln, Germany). The purity of the ^{14}C-phenanthrene was checked by TLC (thin layer chromatography) prior to use.

Pyrene with a purity of 99% from Sigma-Aldrich (Steinheim, Germany) was used for

both HPLC analysis and experimental studies. ^{14}C-pyrene ([4,5,9,10-^{14}C] pyrene) obtained from Co. Sigma Chemical (St Louis, MO, USA) had a radiochemical purity of =95% and specific activity of 56 mCi mmol^{-1}.

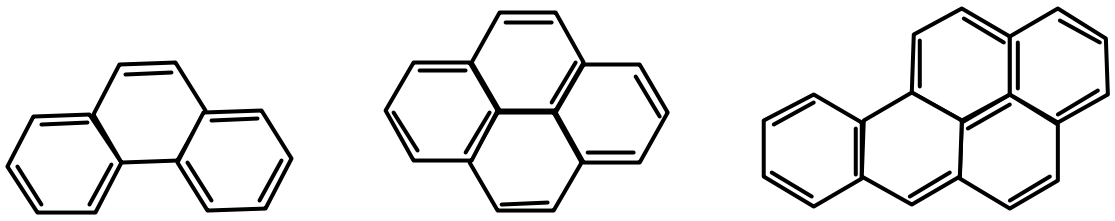
BaP with a purity of >97% was a product of Fluka (Buchs, Switzerland).

The related properties of the PAHs selected for this study are listed in Table 1 and their structure illustrated in Fig. 3.

Table 1. Formulas and properties of PAHs for this study

Compounds	mol formula	MW	Solubility [a], mol l^{-1} (mg l^{-1})	Log K_{ow}
Phenanthrene	$C_{14}H_{10}$	178.2	7.2x10^{-6} (1.28)	4.57 [b]
Pyrene	$C_{16}H_{10}$	202.2	6.8 x 10^{-7}(0.14)	5.18 [b]
Benzo[a]pyrene (BaP)	$C_{20}H_{12}$	252.3	1.6 x 10^{-8} (0.004)	6.34 [c]

[a] Mackay and Shiu, 1981.
[b] Karickhoff *et al*., 1979.
[c] Steen and Karickhoff, 1981.



Phenanthrene Pyrene Benzo[a]pyrene

Fig. 3. Molecular structure of PAHs for this study

3.1.1.3 Surfactants

With emphasis on linear alkylbenzene sulfonate (LAS) which is used in larger amount and more frequently found in the environment, six surfactants were selected for study to cover a range of different types and characteristics of surfactants. LAS, Tween-80 (polyoxyethylene sorbitanmonooleate) and TDTMA (tetradecyltrimethyl-ammonium bromide) were selected as representatives of anionic, nonionic and cationic surfactants, respectively. Nonionic Brij series surfactants of different length of oxyethylene chain were selected for comparative studies, i.e. Brij30 (polyoxyethylene 4 lauryl ether), 10LE (polyoxyethylene 10 lauryl ether) and Brij35 (polyoxyethylene 23 lauryl ether). All surfactants were used as received.

Except for Tween-80 which was obtained from MERCK (Hohengrunn, Germany), LAS (sodium salt of dodecylbenzene sulfonate with a purity of *ca* 80%), TDTMA, Brij30, 10LE and Brij35 were all purchased from Sigma-Aldrich Chemie (Steiheim, Germany).

Commercial LAS is a complex mixture of various homologues and phenyl positional isomers. Alkyl chain lengths typically range from C_{10} to C_{14} with an average carbon chain length of *ca* 12. The phenyl attaches to the alkyl chain at all points with the exception of the primary carbon in the alkyl chain (Holt *et al*., 1989; Trehy *et al*., 1996). The LAS used in this work was reported as well as revealed by our analysis (Fig. 4) to be a mixture of homologues dominated by the dodecylbenzene sulfonate although analysis by House and Farr (1989) indicated that C_{11} homologue was the major component. Our analysis (Fig. 4) showed that the LAS composed of 1.9, 21.1, 37.0, 22.9 and 17.1% of C_{10} to C_{14} homologues, respectively (Ou *et al*., 1996a). The mixture was also reported to contain 15% by weight of sodium sulphate and ~5% organic impurities. The impurities are probably more polar compounds formed during sulfonation process, e.g. disulfonated material (House and Farr, 1989).

The basic properties and structures of the selected surfactants are presented in Table 2 and Fig. 5, respectively. As it can be seen from Table 2, critical micelle concentrations

Table 2. Basic properties of surfactants studied

Trade name/ abbreviation	Chemical name	Symbol [a]	Average mol formula	Type	Average MW [b]	HLB [c]	CMC [d] ($mg\ l^{-1}$)	ThOD [e] ($g\ O_2\ g^{-1}$)
LAS	linear alkylbenzene sulfonate (dodecylbenzene sulfonate)	$C_{12}BSoNa$	$C_{21}H_{35}SO_3Na$	Anionic	390		433.5	
Tween-80	Polyoxyethylene sorbitanmonooleate	$C_{18}S_6E_{20}$	$C_{64}H_{125}O_{26}$	Nonionic	1309	15.0 [f]	13 [g],40 [h]	2.01
TDTMA	tetradecyltrimethylammonium bromide	$NC_{14}Br$	$C_{17}H_{38}NBr$	Cationic	336.4		100 [h]	
Brij30	polyoxyethylene 4 lauryl ether	$C_{12}E_4$	$C_{20}H_{42}O_5$	Nonionic	362	9.7 [f]	10 [i], 20 [f]	2.48
10LE	polyoxyethylene 10 lauryl ether	$C_{12}E_{10}$	$C_{32}H_{66}O_{11}$	Nonionic	626	14.1 [f]	48 [i], 63 [f]	
Brij35	polyoxyethylene 23 lauryl ether	$C_{12}E_{23}$	$C_{58}H_{118}O_{24}$	Nonionic	1198	16.9 [f]	40 [i], 110 [f]	2.02

[a] C represents alkyl chain ($-CH_2-$), B represents a benzene ring, So represents a sulfonic group, S_6 represents a sorbitan ring, N represents a trimethylamino group, and E represents an ethoxylate group ($-C_2H_4O-$). [b] MW, molecular weight. [c] HLB, hydrophile-lipophile balance. [d] CMC, critical micelle concentration. [e] ThOD, theoretical oxygen demand. [f] Yeom *et al*., 1996. [g] Yeh *at al*., 1998. [h] Sánchez-Camazano *et al*., 1995. [i] Guha and Jaffé, 1996a.

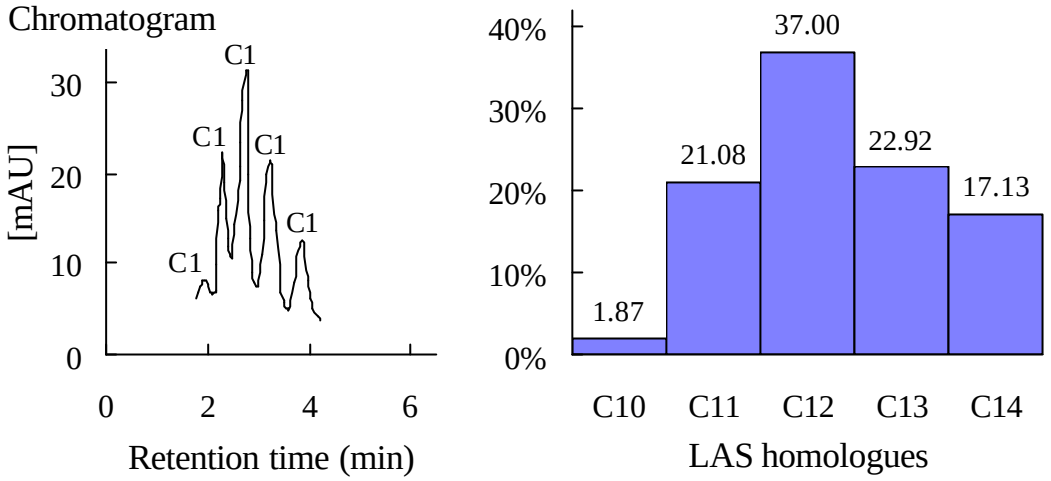
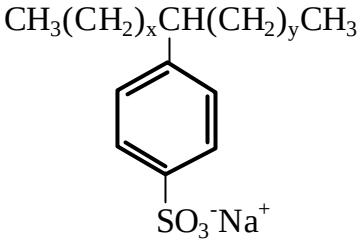


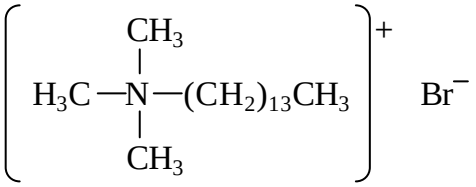
Fig. 4. Composition of homologues of LAS used in this work

LAS:

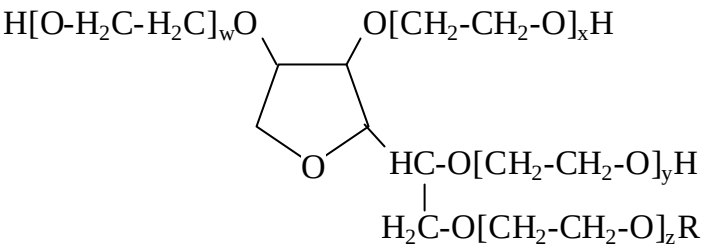


where x + y = n and n = 7-15 carbon units

TDTMA:



Tween-80:



where w + x + y + z = 20 and R = $C_{18}H_{34}O_2$

Brij30 (n = 4), 10LE (n = 10) and Brij35 (n = 23):

$C_{12}H_{25}$ $(CH_2CH_2O)_nOH$

Fig. 5. Molecular structure of surfactants used in this study

(CMCs) of the same surfactant cited from literature varies in a rather wide range. In this study, CMC of LAS in clean-water (SMPW) was determined by surface tension measurement using ring method to be 433.5 mg l^{-1} (Fig. 6) (Ou *et al*., 1996b).

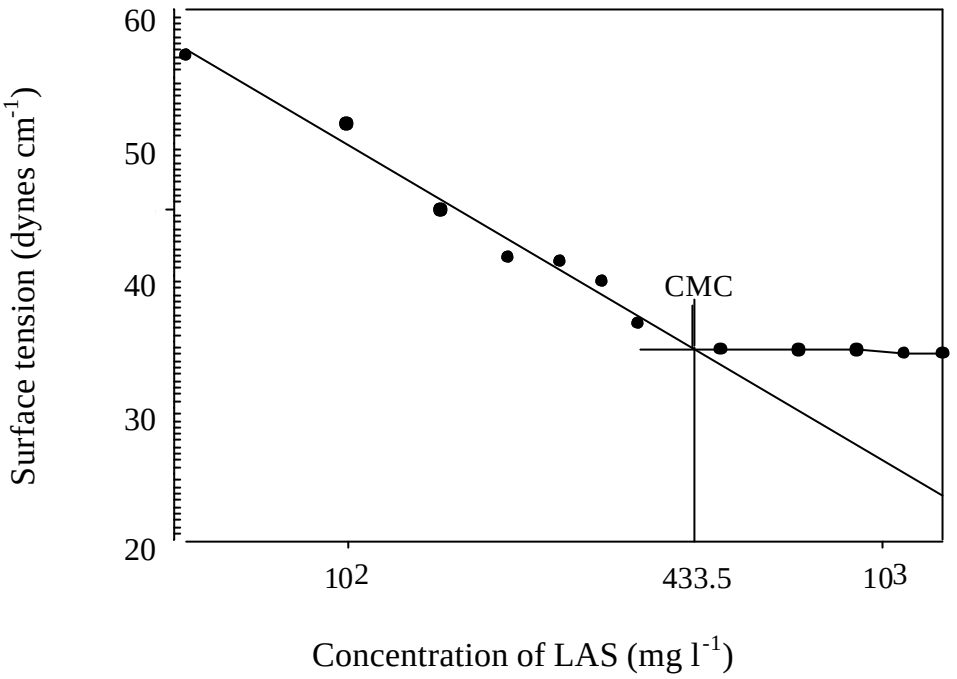


Fig. 6. Determination of CMC by surface tension measurement for LAS in clean-water (Ou *et al*., 1996b)

3.1.1.4 Other chemicals, reagents and consumables

Reagents and consumables used for microbial cultural medium, protein assay, luminescent test and measurement of ^{14}C-activity and experiments are listed in Table 3 with information on quality and manufacturers.

Cocktail-I for measuring ^{14}C-volatile organic chemicals (VOCs) consisted of 11 g of PERMABLEND® III dissolved in 1 liter toluene.

Cocktail-II for absorption and measurement of $^{14}CO_2$ was a mixture of CARBO-SORB® E and PERMAFLUOR® E^+ in a ratio of 2:3 (v/v).

Table 3. Other chemicals, reagents and consumables

Chemicals, reagents and consumables	Quality	Manufacturers
For microbial cultural medium		
Bacto® yeast extract		BIFCO Laboratories, Detroit, USA
Proteose peptone		
Bacto® agar		
Glucose (D(+)-glucose anhydrous)	GR	MERCK, Darmstadt, Germany
Casein hydrolysate	GR	
Starch soluble	GR	
Pyruvic acid sodium salt	GR	
Potassium dihydrogen phosphate (KH_2PO_4)	GR	
Dibasic potassium phosphate (K_2HPO_4)	GR	
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	GR	
Sodium chloride (NaCl)	GR	
Potassium nitrate (KNO_3)	GR	
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	GR	
Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)	GR	
Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)	GR	
Cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)	GR	
Copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$)	GR	
Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	GR	
Manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	GR	
Sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)	GR	
Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$)	GR	
Ethylenedinitrillo tetraacetic acid disodium salt dihydrate (EDTA-Na $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$)	GR	
Boric acid (H_3BO_3)	GR	
Reagents for luminescent test		
Reactivation solution (LCK482)		Dr. Bruno Lange, Düsseldorf, Germany
7.5% NaCl standard solution (LCK480/LCK482)		

Table 3. Other chemicals, reagents and consumables (Continued)

Chemicals, reagents and consumables	Quality	Manufacturers
For protein assay		
Lyophilized bovine plasma gamma globulin (Bio-Rad protein assay standard I)		Bio-Rad Laboratories, München, Germany
Dye Reagent Concentrate		Bio-Rad Laboratories, München, Germany
Sodium hydroxide (NaOH)		MERCK, Darmstadt, Germany
For measurement of ^{14}C activity		
PERMABLEND® III (PPO 91% + bis-MSB 9%)		PACKARD Instrument, Meriden, USA
PERMAFLUOR® E+ (a)		PACKARD BioScience B.V., Groningen, The Netherlands
CARBO-SORB® E (3-methoxypropylamine)		PACKARD BioScience B.V., Groningen, The Netherlands
ULTIMA GOLD™ (XR) (b)		PACKARD BioScience B.V., Groningen, The Netherlands
For other tests		
Coomassie Brilliant Blue (G 250)	GR	MERCK, Darmstadt, Germany
Soda lime pellets with indicator	GR	MERCK, Darmstadt, Germany
Silica gel LiChroprep® Si 60 (particle size 25-40 µm)	GR	MERCK, Darmstadt, Germany
Neutral aluminum oxide (100-200 mesh)	GR	MERCK, Darmstadt, Germany
C_{18} columns (c)		J. T. Baker, USA
GF-5 glass fiber membrane filter (*ca* 0.4 µm)		Macherey-Nagel, Germany
Filter paper (S&S 595)		Schleicher&Schuell, Dassel, Germany
Polyethylene vials (20 ml) for LSC		PACKARD Instrument, Meriden, USA
Glass beads (5 mm and *ca* 1 mm d.)		Scherf Praezision, Germany
PVC tubing (2.05 mm i.d.)/peristaltic pump		Abimed, Germany
Teflon tubing (2 mm i.d. and 0.5 mm thickness)		MERCK, Darmstadt, Germany
Disposable cuvette of 1-cm light path		BRAND, Wertheim, Germany

(a) a blend of 1,2,4-trimethylbenzene (Pseudocumene) and propyleneglycol[mono] methylether with 2,5-diphenyloxazole[PPO] and 1,4-bis[2-methylstyryl]benzene [bis-MSB]),

(b) a blend of alkylnaphtalenes with scintillators PPO and bis-MSB and emulsifiers

(c) Three-ml bonded silica C_{18} columns (Octadecyl disposal extraction column, glass with PTFE-frits and filled with 500 mg octadecylsilane)

3.1.2 Bacterial strains

Luminescent bacteria *Vibrio fischeri* (NRLL-B-11177) in liquid-dried formulation was purchased from Dr. Bruno Lange (Düsseldorf, Germany).

Mycobacterium sp. strain KR2 was a kind gift from Dr. K. Rehmann, GSF-IÖC. It was isolated from a PAHs-contaminated soil originating from a former gas work plant and capable of utilization of phenanthrene and pyrene as sole carbon and energy source (Rehmann *et al*., 1996 and 1998). *Mycobacterium* sp. has been reviewed to be able to oxidize a number of PAHs, e.g. naphthalene, anthracene, phenanthrene, fluoranthene, pyrene and benzo[a]pyrene (Cerniglia, 1992).

3.1.3 Equipment and instrument

High performance liquid chromatography (HPLC) and columns - HP1090 HPLC instrument (Hewlett-Packard, CA, USA) was equipped with a diode-array detector (DAD), a fluorescent detector (FLD, HP1064A) and an autosampler. The system was controlled by a HP Chemstation which also performed data acquisition and analysis. Analysis of 16 priority PAHs listed by US EPA was conducted on a RP-C18 column (VYDAC^{TM}, USA) using FLD. Separation and measurement of LAS were performed on a 250 mm $\times$ 4.0 mm i.d. stainless-steel analytical C_{18} column packed with 5µm LiChrospher 100 RP-18 (Hewlett-Packard, USA) using DAD. All other samples were analyzed using an ODS-HYPERSIL RP-C18 column (particle size of 5 µm, 250 mm $\times$ 4.6 mm i.d. from BISCHOFF, Germany) and measured with DAD.

Another HPLC (655A-40[auto sampler]/L-6200A[intelligent pump]/655A-22[variable wavelength UV monitor], MERCK-HITACHI, Darmstadt, Germany) equipped with an isotope detector (Ramona 2000, Raytest, Sprockhövel, Germany) was used for analysis of ^{14}C-samples. An ODS-HYPERSIL RP-C18 column (5 µm, 25 cm $\times$ 4.6 mm i.d., BISCHOFF, Leonberg, Germany) was employed for separation at conditions of 35 ^{o}C oven temperature and mobile phase of methanol/water (85:15/v:v, 0.8 ml min^{-1}, isocratic). Phenanthrene was measured with detection wavelength of 254 nm.

Respirometer - Sensitive respirometric equipment (Sapromat® C, VOITH, Heidenheim, Germany) was used to measure microbial oxygen consumption. Each one consisted of twelve 500-ml conical flasks placed in a water bath thermostated at desired temperature. Fig. 7 is the schematic drawing of the system. In the Sapromat® culture flasks (6), oxygen was kept at a constant level by electrolytic production and its consumption was continuously recorded by a computer (11) in pre-set time intervals. When CO_2 produced due to the microbial activity was removed by soda lime pellets (8) in a glass cup (9) in the headspace of the culture flask (6), air pressure in the culture flask decreased, which was sensed by the pressure meter (3). It then triggered off the control unit (13) to supply power to the electrolytic cell (5) to produce oxygen. After the air pressure in the culture flask returned to normal, oxygen production stopped. The power consumption (coulomb) which was proportional to the oxygen produced was recorded by the computer (11) and converted to oxygen consumption in mg O_2 l^{-1} of culture medium (based on 250 ml liquid medium). The

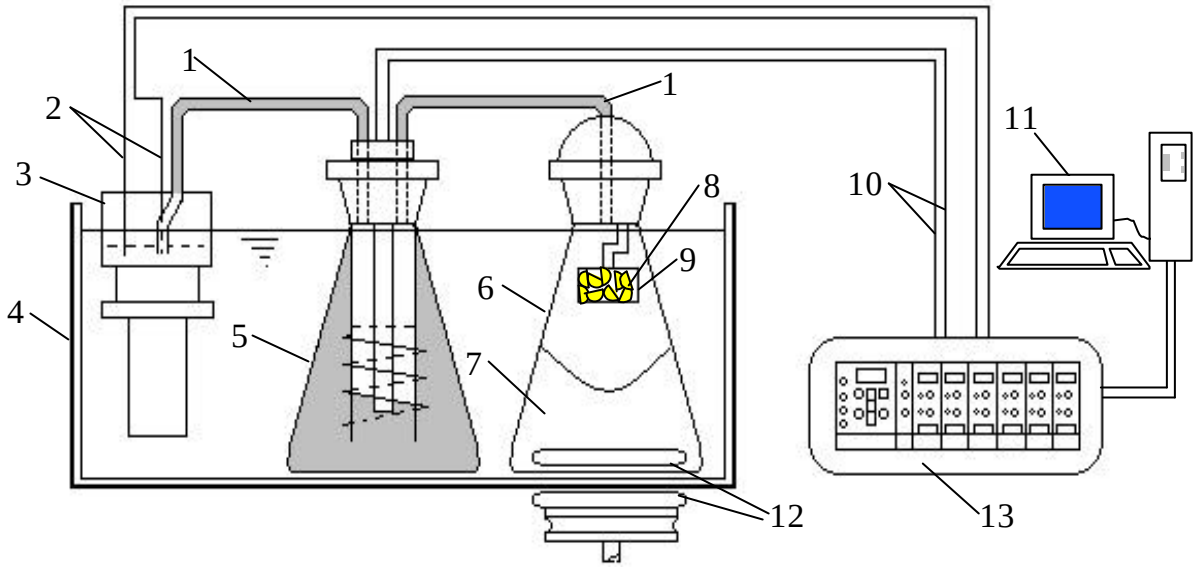


Fig. 7. Schematic drawing of respirometer (Sapromat® C): 1- air connecting tubing, 2- pressure-sensor electrodes, 3- pressure meter, 4- thermostated water bath, 5- electrolytic cell, 6- culture flask, 7- culture medium (liquid or solid such as soil), 8- soda lime pellets, 9- glass cup, 10- wire for power supply to electrolysis, 11- computer (data recording and processing), 12- magnetic stirrer and stirring rod (no stirring when solid medium was cultured), 13- controlling and data recording unit.

sensitivity of the Sapromat® equipment was 0.25 mg of oxygen produced per impulse. Time intervals of data output could be selected for 15 min, 30 min, 1 hour, 4 hour or 1 day as required.

Other equipment and instrument - They are listed in Table 4.

Table 4. Equipment and instrument

Equipment and instrument	Manufacturers
LC column (100 mm × 25 mm i.d.)	Sigma-Aldrich Chemie, Steinheim, Germany
Multiple stirring unit with 15 stirrers (Variomag® Electronic MULTIPOINT HP15)	H+P Labortechnik, Oberschleißheim, Germany
Tube shaker (REAX2000)	Heidolph, Germany
Tissue homogenizer(Ultra Turrax T25)	Janke & Kunkel, Germany
Soil mixer mill (MM 2000)	Retsch, Germany
Freeze-drier (VIRTIS SENTRY™ 5L)	VIRTIS, New York, USA
Peristaltic pumps (Minipuls 3)	Abimed, Villiers-le-Bel, France
Ultrasonic water baths (SCQ-5)	Shanghai, China
Ultrasonic water baths(SONOREX RK510S)	BANDELIN electronic, Berlin, Germany
Centrifuge (Biofuge 22R)	Heraeus Sepatech, Germany
Rotary evaporator (LABO ROTA S300)[(a)]	Resona Technics, Switzerland
Vacuum controller (vacu-Box PVK600)	Labor-Technik,Wagen,Switzerland)
Autoclave (Model C)	Webco, München, Germany
Visible spectrometer (UNICAM UV5675)	ATI UNICAM,Cambridge, England
UV-Visible spectrophotometer (CARY 1)	Varian, Darmstadt, Germany
Luminescent analyzer (LUMIStox 300)[(b)]	Dr. Bruno Lange, Berlin, Germany
Thermostated (15 °C) unit LUMIStherm	
Liquid scintillation counter (TRI-CARB 1600TR)	PACKARD Instrument, Meriden, USA
Tri-Carb Sample Oxidizer (Model 307)	
Automatic TLC-Linear Analyzer (TRACEMASTER 20)	Laboratorium BERTHOLD, Wildbad, Germany
Pre-coated TLC plates (Silica gel 60 F_{254}, 0.25mm)	MERCK, Darmstadt, Germany

[(a)] equipped with a vacuum controller (vacu-Box PVK600),

[(b)] controlled by a micro-processor and equipped with a thermostated (15 °C) unit LUMIStherm.

3.1.4 Crops

Paddy rice (Shennong No.254) and soybean (Tiefeng No.27) were obtained from Shenyang Agricultural University, China. Wheat (Tiechun No.1) was obtained from Research Institute of Paddy Rice of Liaoning Province, Sujiatun, Liaoning, China.

3.1.5 Soils

Seven soils were selected in this work for comparative purposes though with different emphasis on each soil. Three of them (Soil $13^{\#}$: 0-20 cm topsoil, Soil $14^{\#}$: 20-60cm subsoil, Soil $15^{\#}$: 60-100cm subsoil) were meadow burozem and collected from Shenyang Ecological Station (SES), Chinese Academy of Sciences, located in *ca* 40 Km southwest to Shenyang City, Liaoning Province in Northeastern China. Soil samples were air-dried and sieved through a 1 mm sieve before use for laboratory experiments, through a 2 mm sieve prior to use for column experiments, or through a 5 mm sieve for use for pot culture. Two reference soils (Soil 2.2 and Soil 2.3) were obtained from LUFA, Speyer, Germany and freeze-dried prior to use. The basic characteristics of the soils are given in Table 5. Their background levels of LAS, phenanthrene and pyrene were measured using methods described in Section *3.2.2.1.2* and *3.2.1.1.5*, respectively.

Another soil was H_2O_2-treated Soil $13^{\#}$. The purpose of H_2O_2 treatment was to remove the organic matter in soil using the same method as described by House and Farr (1989).

To study the effects of surfactants on microbial activity in contaminated soil, a soil, collected from a former petroleum tank farm in Baden Württemberg, Germany, was also studied. The site had a history of contamination about 30 years.

Table 5. Physical and chemical characteristics of experimental soils

Soils [a]	pH	Organic Carbon (%)	CEC [C mol (+) kg^{-1}]	Volume weight (g cm^{-3})	Total Nitrogen (%)	Composition of soil particles (%)			
						Clay	Silt	Fine sand	Coarse sand
						(<0.002mm)	(0.002-0.02mm)	(0.02-0.2mm)	(>0.2mm)
SP2.2	5.6	2.24	10	NM	0.17	6.1	4.9	28.3	60.7
SP2.3	6.4	1.08	8	NM	0.10	8.3	11.2	43.3	37.2
						(<0.001mm)	(0.001-0.01mm)	(0.01-0.25mm)	(>0.25mm)
Soil 13#	6.7	0.93	19.4	1.30	0.14	22.0	24.0	51.5	2.5
Soil 14#	6.8	0.74	23.2	1.31	0.13	22.0	26.0	49.8	2.2
Soil 15#	6.8	0.46	26.0	1,37	0.12	32.0	28.0	38.5	1.5

Soils [a]	Phenanthrene (µg kg^{-1})	Pyrene (µg kg^{-1})	LAS (mg kg^{-1})	Mineral composition (%)					
				Quartz	Albilte	Microline	Illite	Kaolinite	Montmoril
SP2.2	NM	NM	NM	NM	NM	NM	NM	NM	NM
SP2.3	35.4	84.8	ND	96.6	0	2.4	1.0	0	0
Soil 13#	2291.4	ND	ND	82.0	12.8	0	4.8	0.1	0.3
Soil 14#	NM	NM	NM	78.9	12.0	0.3	8.3	0	0.5
Soil 15#	NM	NM	NM	77.1	11.2	4.0	7.3	0.2	0.2

[a] Data for reference Soil 2.2 and Soil 2.3 were provided by LUFA, Speyer, Germany, from whom the soils were obtained. Soil 13# (top soil 0-20 cm), Soil 14# (subsurface soil 20-60 cm) and Soil 15# (60-100 cm) were collected from Shenyang Ecological Station (SES), Institute of Applied Ecology, Academia Sinica, China.

ND - not detectable. NM - not measured.

3.1.6 Field experiments - lysimeters and site description

Field lysimeters are situated in Shenyang Ecological Station (SES). SES is a complex experimental station for agriculture, biology, ecology, forestry etc. The main meteorological conditions of the area are presented in Table 6. Lysimeters are reinforced concrete of 1 m i.d. with a sloping base and contain a soil monolith of 1.5 m depth. They were repacked in 1991 with the same soils (i.e. Soil $13^{\#}$, Soil $14^{\#}$ and Soil $15^{\#}$) as those used for laboratory study (Table 5) and had been cultivated under normal agricultural practice and management for 3 years before launching this experiment in 1995. Soils in the lysimeters, therefore, had typical structure and morphology as normal cultivated soil.

Table 6. Monthly meteorological conditions of field experimental site (monthly mean values of 10-year average) (Ou *et al*., 1992a)

Month	Temperature (^{o}C)	Precipitation (mm)	Evaporation (mm)	Wind speed (m s^{-1})
January	-12.2	4.9	21.8	2.8
February	-8.6	6.0	35.8	2.9
March	0	18.6	89.5	3.9
April	9.5	41.5	176.4	4.7
May	16.8	42.5	242.2	4.2
June	21.5	84.6	202.0	3.4
July	23.8	153.4	159.1	2.6
August	23.1	178.0	147.2	2.5
September	16.8	83.7	121.7	2.5
October	9.2	31.8	98.1	3.3
November	-0.8	13.3	48.5	3.4
December	-8.0	3.1	26.5	2.9
Yearly	7.6	661.4	1368.8	3.3

3.2 Methods

3.2.1 Experimental methods

3.2.1.1 Adsorption and migration

All adsorption measurements were performed in the apparatus shown in Fig. 8 at 25 ± 0.1°C using a soil suspension of 20 g l^{-1}.

3.2.1.1.1 Adsorption of LAS on soils

The adsorption of LAS on soils was measured using the adsorption cell and apparatus shown in Fig. 8. Basic parts of the apparatus were similar to those used by House and Farr (1989) for studies of adsorption of LAS on kaolinite. The equipment overcomes many of the problems associated with the traditional batch method, e.g. temperature changes during the centrifugation or filtration steps prior to the supernatant analysis, and variation of the adsorbent in particularly for soil and sediment. Other advantages of the systems are that adsorption and desorption can be conducted on the same adsorbent without any separation procedure (e.g. centrifugation) introduced, and continuous and automatic online measurements are possible. This is particularly important for measurement of adsorption kinetics. Adsorption reaction took place inside an adsorption cell (4). Supernatant was collected in specified time intervals by filtration of the soil suspension through a glass fiber membrane filter (5). The solution in contact with the suspended soil was circulated using a syringe pump (8) through the glass fiber membrane filter (5) via valve (7). This enabled the PTFE tubing and any "dead volume" to be flushed thoroughly before a supernatant was directed to the supernatant collector (9). The tube leading to the collector was flushed with air after each sampling.

Preparation of aqueous LAS solution

A 500 mg l^{-1} aqueous LAS solution was prepared in sterilized Millipore water (SMPW). 0.1562 g commercial LAS (equivalent to 0.125 g pure LAS) was weighed into a 250-ml volumetric flask and SMPW was added to the mark.

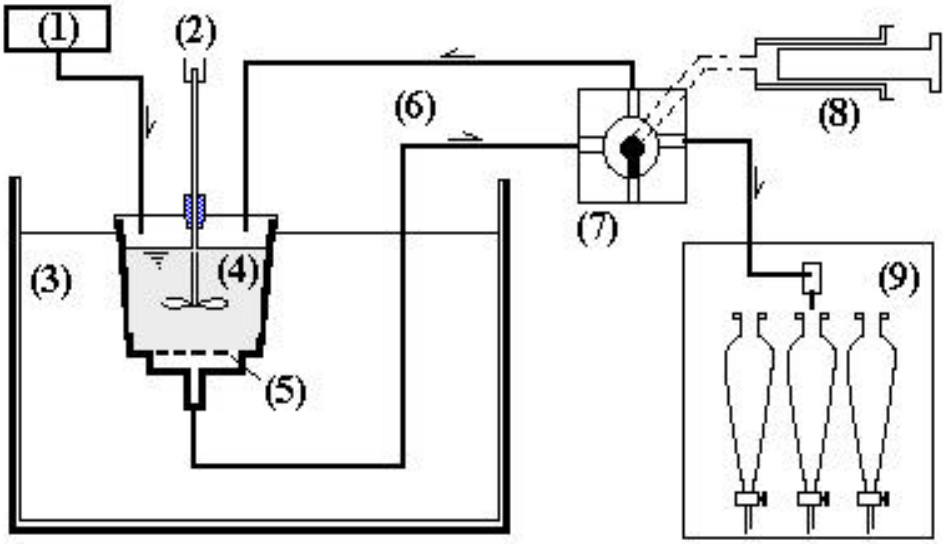


Fig. 8. Adsorption apparatus: (1) stock solution, (2) stirring rod, (3) thermostated water-bath, (4) adsorption cell, (5) glass fiber membrane filter, (6) PTFE tubing, (7) valve, (8) syringe pump, and (9) supernatant collector.

Adsorption experiment

250 g SMPW was weighed into the adsorption cell and allowed to come to thermal equilibrium. Five g soil (air- or freeze-dry) was added to the adsorption cell ((4) in Fig. 8) and left for 0.5 - 1 h before 2.5 ml supernatant was removed for the analysis of LAS. 2.5 ml of 500 μg ml^{-1} stock LAS solution was added immediately after removal of supernatant. After another 1 hr, a second sample (5 ml supernatant) was removed and stock LAS solution (5 ml) was added successively. The procedure was then repeated with successive removal of 10 ml supernatant and addition of 10 ml stock LAS solution in 1 hr intervals until the desired concentration was reached.

Calibration of adsorption apparatus

Prior to all adsorption measurements, the adsorption apparatus was calibrated with LAS using the above procedure with and without the membrane filter installed in the adsorption cell and in the absence of soil.

Kinetics of adsorption

In order to obtain the adsorption equilibrium time, before the adsorption measurements, the kinetics of LAS adsorption was also evaluated. This was done by adding a single dose of LAS solution into the cell with soil suspension and then measuring the adsorption change with time.

3.2.1.1.2 Influence of LAS on the adsorption of phenanthrene on soils

Preparation of aqueous phenanthrene solution

Aqueous phenanthrene solution for adsorption experiments was prepared in SMPW using a column method in order to obtain a true aqueous solution of phenanthrene of high concentration. A glass tube (1.5 cm i.d. x 30 cm length) with stopper and a small piece of glass wool was packed with glass beads (*ca* 1 mm) to a length of *ca* 25 cm and rinsed with acetone (50 ml), dichloromethane (50 ml) and cyclohexane (50 ml) successively. Ten ml of 500 mg l^{-1} phenanthrene in dichloromethane was slowly applied to the column while nitrogen gas was blowing upwards through the column to evaporate the solvent. After the solvent had completely evaporated, phenanthrene in the column was eluted by circulating SMPW (2 liters) through it for more than 24 h. The resulted aqueous phenanthrene solution was filtrated through a glass fiber membrane filter and kept in 4 ^{o}C in dark prior to use. Phenanthrene concentration of the solution was measured using HPLC before use.

Adsorption experiment

Basic steps of the adsorption experiment were the same as those described in Section *3.2.1.1.1*. In order to simulate different environmental conditions and to compare the adsorption mechanism, the measurement of LAS effects on the adsorption of phenanthrene on soils was conducted using two methods to represent two different exposure modes of soil to LAS and phenanthrene:

Method-1 (soil+phenanthrene+LAS):

This method was designed to simulate irrigation of phenanthrene-contaminated fields with surfactant-containing wastewater and remediation processes of

phenanthrene-contaminated soils with surfactants. In this mode, soil was exposed first to phenanthrene and then to LAS. Soil was allowed to contact with phenanthrene until equilibrium before addition of LAS. 250 g of aqueous phenanthrene solution of predetermined concentration was weighed into the reaction cell and after the thermal equilibrium (15 min), 5 g soil was added. In 1 h when adsorption reached equilibrium, 5 ml supernatant was removed for analysis and solid LAS was added to obtain the desired LAS concentration. A predetermined volume of aqueous phenanthrene solution was added as well to keep the phenanthrene concentration and the volume of solution in the cell unchanged.

Method-2 (soil+LAS+phenanthrene):

It was intended to imitate conditions of LAS-contaminated land further irrigated with phenanthrene-containing wastewater. In this mode, soil was exposed to LAS before to phenanthrene. Soil was allowed to equilibrate with LAS prior to addition of phenanthrene. 250 g SMPW was weighed into the adsorption cell and after the thermal equilibrium (15 min), 5 g soil and a designed amount of solid LAS were added successively. After 1.5 h, when adsorption of LAS reached equilibrium, 20 ml supernatant was removed for analysis and 20 ml aqueous phenanthrene solution containing a predetermined amount of LAS was added to obtain the desired phenanthrene concentration and to keep the LAS concentration constant.

The procedure for both methods was thus repeated with successive removals of supernatant and additions of aqueous solution of phenanthrene and /or LAS in 1 h intervals.

Calibration of adsorption apparatus

Prior to all adsorption measurements, the adsorption apparatus was calibrated with phenanthrene using the above procedure with and without the membrane filter installed in the adsorption cell and in the absence of soil.

Kinetics of adsorption

In order to obtain the adsorption equilibrium time, before the adsorption measurements, the kinetics of phenanthrene adsorption was also evaluated. This was conducted by adding a single dose of phenanthrene solution into the cell with soil suspension and then measuring the adsorption change with time.

Extraction of phenanthrene in supernatant

Phenanthrene in supernatant removed from the adsorption cell was extracted using a liquid-liquid method. Twenty ml supernatant in a 100-ml separation funnel was successively extracted with 20 ml and two aliquots of 5 ml cyclohexane. The extract was evaporated to the dryness at 40 ^{o}C and 150 mbar in a rotary evaporator and reconditioned with 1 ml methanol. After spiking it with pyrene as internal standard for both qualification and quantitation of phenanthrene, the extract was analyzed on HPLC.

3.2.1.1.3 Migration of LAS applied directly to topsoil in repacked soil columns

Preparation of soil columns

Glass columns of 4.5 cm i.d. x 1 m length and with an outlet in one end were used as containers for soil columns. A small piece of glass wool was placed in the outlet to prevent soil falling off the column. Air-dried and sieved (2 mm) Soil 15$^{\#}$ (60 - 100 cm subsoil), 14$^{\#}$ (20 - 60 cm subsoil) and 13$^{\#}$ (0 - 20 cm topsoil fortified with LAS) were packed to the glass column successively according to their volume weights (Table 5).

Application of LAS

LAS dissolved in methanol was applied to the topsoil and mixed completely. The resulted LAS concentrations in topsoil were 100, 500 and 1000 mg kg^{-1}, respectively. The LAS-fortified topsoil was then packed to the soil column. Triplicate columns were prepared for 100 and 500 mg kg^{-1} and duplicate columns for 1000 mg kg^{-1}.

Wetting of soil columns

The bottom end of the repacked soil columns were soaked vertically in distilled water to a depth of *ca* 20 cm until the entire column was wetted by the capillary-rising

water. Wetting soil columns in this way could prevent the formation of soil cracks.

Leaching experiment

Above soil columns were eluted with distilled water. For LAS concentrations of 100 and 500 mg kg^{-1}, three levels of leaching intensity, i.e. low (8 cm^3 d^{-1}), medium (16 cm^3 d^{-1}) and high (32 cm^3 d^{-1}), were applied. They were corresponding to column CSA(cross section area)-normalized leaching intensity of 5 mm^3 mm^{-2} d^{-1} (or 5 mm d^{-1}), 10 mm^3 mm^{-2} d^{-1} (10 mm d^{-1}) and 20 mm^3 mm^{-2} d^{-1} (20 mm d^{-1}), respectively. Two levels (medium and high) of leaching intensity were applied to columns with 1000 mg kg^{-1} LAS. Water was added once per day according to the designed leaching intensity. Leachate was collected continuously and one mixed sample was removed for analysis daily. After the termination of the experiment, soil samples were taken from all columns in 5-cm increments for extraction and analysis of residual LAS.

3.2.1.1.4 Migration of LAS applied directly to topsoil in field lysimeters cropped with rice and soybean

Application of LAS and growth of crops in lysimeters

LAS was applied in early May 1997 before crops were planted. Three concentrations of LAS, i.e. 0 (CK), 80 and 400 mg kg^{-1} in 0 - 20 cm topsoil, were investigated. Appropriate amount of topsoil in lysimeter was removed into a metal basin and designed amount of LAS dissolved in methanol was added to the soil while mixed. After completely mixed, the LAS-fortified soil was spread evenly over the surface of lysimeter. The 0 - 20 cm topsoil was then mixed forward and backward three times with a spade. Paddy rice and soybean were grown and managed following normal agricultural practice, respectively. The growth of crops was monitored and recorded in each growth stages.

Collection of leachate and analysis of LAS

Leachate from lysimeters was collected in glass vessels once a week. Sampling ended when no more leachate drained out of the lysimeter. After the volume of the leachate recorded, partial leachate was removed for extraction and analysis for LAS using method employed in Section *3.2.1.1.1*.

3.2.1.1.5 Migration of LAS and Tween-80 applied via irrigation water and their influence on the migration of PAHs in repacked soil columns not subjected to freeze-thaw treatment

Preparation of soil columns

The same methods described in Section *3.2.1.1.3*. were used to repack and wet the soil columns except the top 0 - 20 cm was filled with PAHs-spiked soil.

Application of PAHs

A mixture of PAHs (phenanthrene, pyrene and BaP) dissolved in dichloromethane (DCM) was applied to the topsoil (0 - 20 cm) to reach a concentration of 5 mg kg^{-1} for each PAH compound. After mixed and DCM evaporated completely, the PAHs-fortified topsoil was then packed to the soil column.

Leaching experiment

Duplicate soil columns were eluted with LAS solution at concentrations of 56.25 (1/8CMC), 225 (1/2CMC), 450 (CMC) and 900 (2CMC) mg l^{-1}, and with Tween-80 solution of 5 (1/8CMC), 20 (1/2CMC), 40 (CMC) and 80 (2CMC) mg l^{-1}, respectively, for 30days. For comparison of the influence between the two surfactants of different concentrations on the migration of PAHs, a single leaching intensity of 56 cm^3 d^{-1} (or 35 mm d^{-1} expressed as normalized to column CSA) was applied. Leachate was collected continuously and a 24-hours composite sample was removed for analysis of LAS and Tween-80 before their breakthrough the column. Analysis of LAS and Tween-80 was conducted on 3-days composite leachate samples after their breakthrough was observed. PAHs were measured on three-day mixed leachate samples from the beginning due to more leachate was needed for their analysis.

Analysis of Tween-80 in leachate

Tween-80 in leachate was analyzed using Coomassie Brilliant Blue (G 250) methods. It was dissolved in 3% $HClO_4$ solution until absorption (ABS) at 465 nm reached 0.68 to 1.6. Filtration or centrifugation was imposed to remove solid particles of Coomassie Brilliant Blue if necessary. About 1 ml of leachate (depending on the

concentration of Tween-80) was mixed with 1 ml of above Coomassie Brilliant Blue solution. ABS of the mixture was measured at 600 nm against blank (1 ml of distilled water + 1 ml of Coomassie Brilliant Blue solution) within 2 h. For the purpose of quantitation, a standard curve (Fig. 9) was established using a series concentration of Tween-80. The detection limit of this method was *ca* 5 mg l^{-1}.

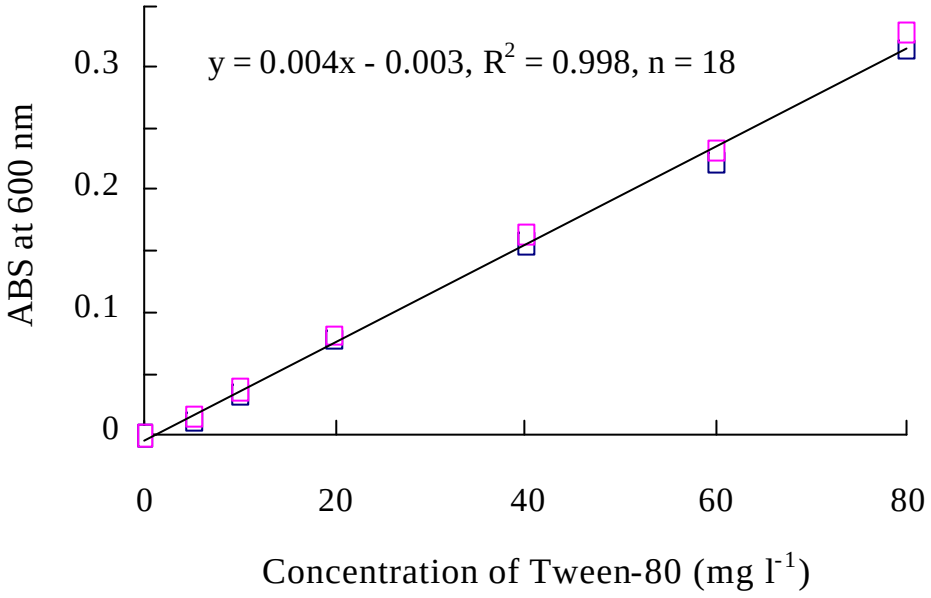


Fig. 9. Standard curve of Tween-80 measured using Coomassie Brilliant Blue

Extraction and analysis of PAHs in soil

After the termination of leaching experiment at day 30, soil samples were taken from all columns in 5-cm increments for extraction and analysis of residual PAHs. Freeze-dried soil samples were extracted and analyzed using the following methods. Before the extraction began, clean-up columns were prepared. One g silica gel (0.063-0.200 mm, 70-230 mesh) was weighed into a 50-ml glass beaker and *ca* 10 ml n-hexane was added to soak the silica gel for at least 10 min. The wet silica gel was transferred with n-hexane to a Pasteur pipette plugged with a small piece of glass wool and pre-washed with acetone and n-hexane for 2 to 3 times. It was kept n-hexane wet all the time before use.

Five g freeze-dried soil sample was weighed into a 25-ml centrifuge tube with screw cap and 20 ml tetrahydrofuran (THF) was added. The soil in the tube underwent ultrasonic extraction in an ultrasonic water bath for 2.5 hr under conditions of maximum ultrasonic and water temperature <40 ^{o}C. Water temperature was controlled by addition of ice to water bath when necessary. The extract was centrifuged at 4000 rpm for 5 min and 10 ml supernatant was transferred to a 25-ml conical-bottom flask. The extracts were evaporated to the dryness on a rotary evaporator under conditions of 40 ^{o}C (for heating)/4 ^{o}C (for cooling) and 150 mbar. The flask was washed with three aliquots of 0.5 ml n-hexane, which were transferred to a 2-ml volumetric flask and additional n-hexane was added to the mark. 1.0 ml extract was transferred onto above clean-up column when the meniscus of n-hexane just reached the silica gel. Collection of eluate started immediately after the addition of extract onto the column. The first 1 ml eluate was discarded. Immediately after the meniscus of the extract reached the silica gel, n-hexane/dichloromethane (1:1, v/v) was added to elute the column until about 2.5 ml eluate is collected in a 2-ml volumetric flask. The eluate was evaporated to the dryness with gentle nitrogen flow and reconditioned with methanol to the 2-ml mark. This extract was analyzed for PAHs on HPLC as described in Section *3.2.2.2.3*.

3.2.1.1.6 Migration of LAS applied via irrigation water and its influence on the migration of PAHs in repacked soil columns subjected to freeze-thaw treatment

Preparation of soil columns

PVC columns of 10 cm i.d. x 1 m length and with an outlet in one end were used as containers for soil columns. A small amount of sand was place in the bottom of columns to serve as a filter. Air-dried and sieved (2 mm) Soil 15$^{\#}$ (60 - 100 cm subsoil), 14$^{\#}$ (20 - 60 cm subsoil) and 13$^{\#}$ (0 - 20 cm topsoil fortified with PAHs) were packed to the column successively according to their volume weights (Table 5).

Application of PAHs

The same PAHs and methods described in Section *3.2.1.1.5* were used.

Freeze-thaw treatment of soil columns

The soil columns were wetted using the same methods employed in Section *3.2.1.1.3*. After no more water flew out of the column by gravity, soil columns covered with plastic sheet on top were buried under outdoor ground vertically. The surface of the soil in soil column was in the same level as that of ground surface. Placed outdoor underground in October 1997 and dug out for leaching experiments in May 1998, soil columns underwent freeze (mean minimum temperature of -12.2 ^{o}C) and thaw (mean maximum temperature of 16.8 ^{o}C) processes from early autumn to spring (Table 6). The minimum air temperature in the area can reach -25 ^{o}C. The maximal depth of frozen soil in the area is 1.5 m in normal year.

Leaching experiment

The freeze-thaw treated columns were leached for 37 days with LAS and Tween-80 solution of the same concentrations and at the same hydraulic loading as those for columns not subjected to freeze-thaw treatment (Section *3.2.1.1.5*). Collection and monitoring of leachate also followed the same frequency and methods.

3.2.1.2 Effects of surfactants and PAHs on microbial activities

3.2.1.2.1 Acute toxicity of phenanthrene on luminescent bacteria Vibrio fischeri

Preparation of aqueous phenanthrene solution of 2% NaCl

Appropriate volume of 50 mg l^{1} phenanthrene DCM solution was added to a 20-ml glass vial with screw cap lined with aluminium foil. After DCM was evaporated to the dryness, 10 ml MPW was added to the vial. The final concentrations of phenanthrene were 0.125, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 1.80, 1.85, 1.90, 1.95, 2.00, 2.10, 2.25 and 2.50 mg l^{-1}. After addition of this aqueous phenanthrene solution to equal volume of luminescent bacteria solution, the phenanthrene concentrations which the *Vibrio fischeri* actually exposed to were half of those of the prepared solutions, i.e. the exposure concentrations tested were 0.0625, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875, 0.900, 0.925, 0.950, 0.975, 1.000, 1.050, 1.125 and 1.250 mg l^{-1}. Duplicate vials were prepared for each concentration. After capped, the

vials were shaken on a rotary shaker at 140rpm/20 ^{o}C over night and then treated with ultrasonic for 1 h to ensure a true aqueous phenanthrene solution was obtained. 0.2 g NaCl was added to each vial and shaken until NaCl dissolved.

Activation of Vibrio fischeri

Twelve ml reactivation solution (LCK482) was added to a reaction test tube which was placed in LUMIStherm and stood for at least 15 min to reach thermal equilibrium at 15 ^{o}C. One ml of the reaction solution was transferred to one tube containing *Vibrio fischeri* which had been warmed up at room temperature for 2 min. The tube was shaken until all bacteria dissolved. The bacteria were then all transferred to the reaction test tube and shaken so that they were completely mixed. 0.5 ml of the reactivated bacteria was removed to each of 20 cuvettes placed in LUMIStherm.

Measurement of inhibition rate

1.5 ml of each above phenanthrene solution was added to a glass cuvette placed in LUMIStherm. Another two cuvettes had 1.5 ml of control solution (2% NaCl) and 7.5% NaCl standard solution (LCK480/LCK482), respectively. The former served as blank and the later as a check for the activity of the luminescent bacteria which should produce *ca* 50% inhibition. After thermal equilibrium was reached, 0.5 ml of each solution was transferred to a cuvette containing 0.5 ml activated bacteria solution. Duplicate cuvettes were prepared for each test solution. Inhibition rates were measured on LUMIStox against control in incubation time intervals of 5, 15 and 30 min, respectively.

3.2.1.2.2 Acute toxicity of surfactants on luminescent bacteria Vibrio fischeri

Preparation of aqueous surfactant solution of 2% NaCl

20 g l^{-1} stock solution of six surfactants (LAS, Tween-80, TDTMA, Brij30, 10LE and Brij35) with 2% NaCl was prepared by weighing appropriate amount of surfactants into a volumetric flask and adding 2% NaCl solution to the mark. Further dilution of the stock surfactant solution with 2% NaCl solution produced a series surfactant solution for toxicity test. The final exposure concentrations of surfactants

are presented in Table 7.

Measurement of inhibition rate

Toxicity of above surfactant was measured using the same methods as described in Section *3.2.1.2.1.*

Table 7. Exposure surfactant concentrations of *Vibrio fischeri* in acute toxicity test

Surfactants	Exposure concentrations (mg l^{-1})
LAS	5, 7.5, 10, 12.5, 15, 17.5, 20, 40, 50, 80, 100, 225, 450
Tween-80	5, 10, 20, 40, 50, 80, 100, 225, 325, 450, 750, 1000, 1250, 1500, 2000
TDTMA	0.05, 0.1, 0.2, 0.3, 0.35, 0.4, 0.45, 0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 10
Brij30	0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 10, 20, 40, 50, 80, 100, 225
10LE	1.25, 2.5, 5, 7.5, 10, 20, 40, 50, 80, 100, 225
Brij35	5, 10, 20, 40, 50, 80, 100, 225

3.2.1.2.3 Combined acute toxicity of phenanthrene and surfactants on luminescent bacteria Vibrio fischeri

Combined acute toxicity of phenanthrene and surfactants was tested on six phenanthrene concentrations (0.05, 0.25, 0.5, 0.75, 1.0 and 1.25 mg l^{-1}) fortified with a series of surfactant doses (Table 8). Concentrations of surfactants covered a wide range depending on the toxicity of each surfactant to the bacteria.

Preparation of aqueous phenanthrene + surfactant solution of 2% NaCl

Appropriate volume of 50 mg l^{1} phenanthrene DCM solution was added to a 20-ml glass vial with screw cap lined with aluminium foil. After DCM evaporated to the dryness, 10 ml of 2% NaCl solution of surfactants of different concentrations was added to the vial. The resulted concentrations of both phenanthrene and surfactant were double of the exposure concentrations (Table 8). After addition to equal volume of activated luminescent bacteria solution during bioassay, their concentrations were

diluted one time and equalled to exposure concentrations shown in Table 8. Duplicate vials were prepared for each concentration. After capped, the vials were shaken on a rotary shaker at 140rpm/20^{o}C over night and then treated with ultrasonic for 1 h.

Measurement of inhibition rate

Toxicity of above surfactant/phenanthrene combination was measured using the same methods as described in Section *3.2.1.2.1*.

Table 8. Combination of phenanthrene and surfactants for jointed toxicity test of *Vibrio fischeri* (exposure concentrations)

Concentrations of phenanthrene (mg l^{-1})	Surfactant	Concentrations (mg l^{-1})							
0.05, 0.25, 0.5, 0.75, 1.0, 1.25	LAS	0	2.5	5	10	15	20	25	30
	TDTMA	0	0.025	0.05	0.2	0.3	0.4	0.5	0.7
	Tween-80	0	12.5	25	50	125	250	500	750
	Brij35	0	5	10	25	50	75	100	125
	10LE	0	0.5	2.5	5.5	7.5	10	12.5	15.0
	Brij30	0	0.25	0.5	1.0	1.5	2.0	2.5	3.0

3.2.1.2.4 Effects of surfactants on the respiration of microorganism (Mycobacterium sp.) in liquid culture

Effects of surfactants on the activities of *Mycobacterium* sp. strain KR2 were measured by oxygen consumption on respirometers (Fig. 7) in the presence and absence of phenanthrene. These experiments were conducted also to investigate if the bacteria could utilize the test surfactants as sole carbon and energy sources. The measurement was conducted in 250 ml culture and at 20±0.1 ^{o}C in dark..

Preparation of culture medium

Mineral salts medium (MSM) (Lockhead and Chase, 1973) used as cultural medium for respiration measurement contained no carbon source. Test chemicals

added to the medium were the sole carbon sources. The composition of the MSM and their concentrations are presented in Table 9. Stock solution of each component was prepared in MPW according to Table 9. Appropriate volume of each stock solution was added to MPW to make up MSM, pH of which was then adjusted to 7.1 using K_2HPO_4 or KH_2PO_4 buffer solution. The MSM was then autoclaved at 121°C for 30 min.

Table 9. Composition of mineral salt medium (MSM)

Salts	Final concentrations	Volume of stock solution taken for 1 liter deionized water
K_2HPO_4	0.08%	4 ml 20%(w/v) K_2HPO_4
KH_2PO_4	0.02%	1 ml 20%(w/v) KH_2PO_4
KNO_3	0.1%	10 ml 10%(w/v) KNO_3
$MgSO_4 \cdot 7H_2O$	0.02%	1 ml 20%(w/v) $MgSO_4 \cdot 7H_2O$
$CaCl_2 \cdot 2H_2O$	0.01%	0.5 ml 20%(w/v) $CaCl_2 \cdot 2H_2O$
$FeCl_3 \cdot 6H_2O$	0.001%	100 µl 10%(w/v) $FeCl_3 \cdot 6H_2O$
NaCl	0.01%	500 µl 20%(w/v) NaCl
Trace elements*	*	1 ml of stock solution*
pH	7.10	Adjusted using K_2HPO_4/ KH_2PO_4

* 1 liter of stock solution contained the following amount of trace elements: 500 mg EDTA-Sodium salt, 200 mg $FeSO_4 \cdot 7H_2O$, 30 mg H_3BO_3, 20 mg $CoCl_2 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 10 mg $ZnSO_4 \cdot 7H_2O$, 6 mg $NiCl_2 \cdot 6H_2O$, 3 mg $MnCl_2 \cdot 4H_2O$, and 3 mg $Na_2MoO_4 \cdot 2H_2O$. The stock trace element solution was stored in about 2 ml aliquot in small vials in -30°C and warmed up at room temperature before use.

Stock culture of bacteria

The test bacteria *Mycobacterium* sp. strain KR2 were cultured in MSM containing phenanthrene as the sole carbon and energy source to keep their ability to utilize phenanthrene. The stock culture was grown in 10 ml MSM containing 500 mg l^{-1} phenanthrene in 100-ml Erlenmeyer flasks with rid (EHS, Germany). 0.25 ml of 20

g l^{-1} phenanthrene in DCM was added to a flask. After the solvent was evaporated to the dryness, 10 ml sterile MSM was added and inoculated with 1 ml bacteria solution of previous culture. The vessels were kept tightly closed using teflon lined screw cap. All procedures were carried out aseptically. The bacteria were then incubated on a rotary shaker at 100 rpm and 20 ^{o}C. The stock culture was renewed every two weeks.

Pre-culture of bacteria

To obtain enough bacteria for respiration test, the test bacteria were cultured at 100 rpm and 28^{o}C on a rotary shaker for 5 days using the test chemicals (phenanthrene in this study) as a sole carbon and energy source. 2.5 ml of 20 g l^{-1} phenanthrene in DCM was added to a 500-ml triangle flask with rid (Schott, Duran, Germany) and the DCM was allowed to evaporate to the dryness inside aseptic fume cupboard. 100 ml MSM was added and inoculated with 1 ml stock bacteria solution. The final concentration of phenanthrene was 500 mg l^{-1}. The bacteria were harvested after 5-days incubation. Culture solution was transferred to sterile 75-ml plastic centrifuge tubes and centrifuged at 6500 rpm/20 ^{o}C for 15 min. The supernatant was discarded. The bacteria were then washed three times with MSM without calcium (MSM-Ca). About 20 ml MSM-Ca was added to the centrifuge tubes and shaken on a tube shaker to re-suspend the bacteria. After centrifugation, supernatant was discarded. The bacteria were finally re-suspended in 75 ml MSM-Ca, which was ready to serve as inoculum for respiration tests. After dilution 10 times with MSM-Ca, the optical density of the inoculum was measured against MSM-Ca at 540 nm, which were in the range of 0.45 to 0.65. Aseptic technique was used for all transfers.

Preparation of test culture

Respiration tests of *Mycobacterium* sp. were conducted at 20 ^{o}C in 250 ml culture medium containing 100 mg l^{-1} phenanthrene in the present and absence of surfactants. Preparation of phenanthrene MSM solution followed the same technique used in pre-culture of the bacteria. 1.25 ml 20 g l^{-1} phenanthrene DCM solution was added to a 500-ml respiration flask (No. 6 in Fig. 7). After the solvent evaporated to the dryness inside aseptic fume cupboard, 250 ml MSM was added, which resulted in phenanthrene concentration of 100 mg l^{-1} in MSM. This culture served as a blank to

check if there was any contamination during the preparation process. All other cultures were inoculated with 2 ml inoculum. For cultures with co-existent surfactants, 20 g l^{-1} surfactant solution was added to obtain designed concentrations ranging from 5 mg l^{-1} to 900 mg l^{-1}, depending on the type of surfactants. Cultures with surfactant (5 to 450 ng l^{-1}) as the sole carbon and energy source were also prepared to study if the bacteria could grow on the surfactant as well as to investigate the effect of surfactant on the bacterial activity without the presence of phenanthrene. Another culture (control) containing 248 ml MSM and 2 ml inoculum was prepared to measure the background respiration level of the test bacteria in the absence of both test chemicals. All processes were conducted aseptically. Triplicate cultures were prepared for each treatment.

Measurement of respiration

Appropriate amount of soda lime was added to the glass cup (No. 9 in Fig. 7) and the flask was capped and assembled. The apparatus was set up as shown in Fig. 7. The cultures were incubated at 20±0.1 ^{o}C in dark. After 2 h when thermal equilibrium between culture solution inside the flask and water in the thermal water bath was reached, record of oxygen consumption began by a computer in 1 min intervals. Triplicate cultures were measured for each treatment. After the termination of the measurement, data were transferred to Excel for further process in selectable time intervals of 15 min, 30 min, 1 h, 4 h or 1 day.

3.2.1.2.5 Effects of surfactants on the growth of Mycobacterium sp. on non-toxic soluble substrate

Effects on surfactants on the growth of *Mycobacterium* sp. strain KR2 in normal cultural substrate R2A (Reasoner and Geldreich, 1985) were investigated for comparison with the bacterial activity when growing on scarcely soluble phenanthrene. The purpose of this part of study was to find out the effect of surfactants on the microbial activities in different substrates. Besides measurement of bacterial density (optical density) in culture medium, observation using electron microscope was also conducted to investigate effects of substrates on morphology of the bacteria.

Preparation of R2A culture medium

0.5 g yeast extract, 0.5 g proteose peptone, 0.5 g casein hydrolysate, 0.5 g soluble starch, 0.5 g dextrose, 0.3 g sodium pyruvate, 0.3 g dibasic potassium phosphate (K_2HPO_4), and 0.05 g magnesium sulphate ($MgSO_4 \cdot 7H_2O$) were added to 1 liter MPW. After pH was adjusted to 7.2 using K_2HPO_4 or KH_2PO_4 buffers, the medium was autoclaved at 121 ^oC for 30 min.

Preparation of R2A test culture

The R2A test culture was prepared basically as described in previous sections except R2A culture medium instead of MSM was used and without phenanthrene. Appropriate volumes of sterile R2A medium and sterile 20 g l^{-1} surfactant solution were added to a 500-ml flask to obtain designed surfactant concentrations ranging from 5 mg l^{-1} to 900 mg l^{-1}. They were then inoculated with 1.6 ml inoculum. All transfers were conducted under aseptic conditions.

Incubation and measurement

Cultures were incubated on a rotary shaker at 100 rpm and 20±0.2 ^oC. Samples were removed under aseptic conditions to 1-cm/4-ml disposable cuvettes in specific time intervals for measurement of optical density at 540 nm. Measurement was ended when growth plateau was observed in R2A culture or 24 days in phenanthrene-MSM culture. In case of R2A culture, optical density was measured in 2 h intervals in the first 2 to 3 days and in 4 h intervals afterward. For phenanthrene-MSM culture, optical density was measured in 2 h intervals in the first two days, in 24 h intervals up to 10 days and in 48 h intervals later on.

3.2.1.2.6 Effects of PAHs and surfactants on the respiration of inherent microorganisms in uncontaminated soils

Effects of surfactants on the inherent microbial activities were measured as oxygen consumption on respirometer in the presence and absence of phenanthrene or pyrene in two reference soils (Soil 2.2 and Soil 2.3) and in an agricultural soil (Soil $13^{\#}$). The measurement was conducted with 100 g soil and at 20±0.1 ^oC in dark and 20% soil

moisture. The thickness of 100 g soil layer in the culture flask (No.6 in Fig. 7) was about 1 cm, which could insure the penetration of enough oxygen to the bottom of the soil.

Preparation of phenanthrene and pyrene solution

60 ml of 25.0 mg l^{-1} phenanthrene or pyrene dissolved in methanol was added to 400 ml MPW in a 600-ml beaker and mixed with Pasteur pipette. This solution was ready to be applied to 3000 g soil to produce a test soil containing 0.5 mg kg^{-1} phenanthrene or pyrene.

Preparation of phenanthrene- or pyrene-test soil

3000 g soil was weighed into a 5000-ml beaker. Phenanthrene or pyrene solution prepared above was added to the soil while being mixed. The solution beaker was rinsed with two aliquots of 9 ml MPW, which were also added to the soil. Three aliquots of 113.4 g of the PAH-fortified soil was weighed into triplicate incubation flasks (No. 6 in Fig. 7) and 6.6 ml MPW was added to obtain a soil moisture of 20%. These soils were used for investigation of microbial activity without the presence of surfactants.

Preparation of PAH- and surfactant-test soil

The rest of the soil was distributed evenly to nine 600-ml beakers (340.2 g each). Twenty ml surfactant solution of designed concentration was added to each beaker while being mixed to obtain desired surfactant concentration. The PAH- and surfactant-spiked soil was then distributed into 3 culture flasks, which served as triplicate measurements. Each culture flask contained 100 g freeze-dried soil with a moisture content of 20%.

Preparation of surfactant-test soil

100 g soil was weighed into an incubation flask. In order to be comparable with PAH- and surfactant-test soils, 15.9 ml MPW of 14% methanol was added to the soil while being mixed. 6.7 ml of aqueous surfactant solution of designed concentration

was then added to obtain desired concentration in soil and 20% soil moisture. Triplicates were prepared for each concentration.

Preparation of control test soil

In order to investigate the background respiration level of the soil without the presence of test chemicals, control test soil was prepared and measured. 100 g soil was weighed into an incubation beaker and 20 ml MPW of 14% methanol was added while being mixed. Triplicate control test soils were prepared.

Measurement of respiration

Assemble of the culture flasks and respirometer and measurement of oxygen consumption were the same as described in Section *3.2.1.2.4*.

3.2.1.2.7 Effects of surfactants on the respiration of inherent microorganisms in PAHs-contaminated soil

The effects of surfactants on inherent microbial activities in a PAHs-contaminated soil were studied with consideration that surfactants would increase the bioavailability of aged PAHs. This is the main principle based on which surfactants are used in order to enhance the degradation of chemicals in bioremediation of contaminated soils. The measurement of microbial activities expressed as oxygen consumption was made on the same respirometers used for uncontaminated soils.

Preparation of test soil

100 g contaminated soil was weighed into the culture flask of respirometer. 20 ml MPW or aqueous surfactant solution of different concentrations was added while mixed to obtain desired surfactant concentrations (20 and 40 mg kg^{-1}) and a soil moisture of 20%. The test soil without surfactants served as a control to measure the background activities of the inherent soil microorganisms.

Measurement of respiration

The same method described in Section *3.2.1.2.4* was used.

3.2.1.3 Influence of surfactants on the biodegradation of PAHs

3.2.1.3.1 Influence of surfactants on the biodegradation of phenanthrene by Mycobacterium sp.

Biodegradation of phenanthrene by PAH-degrading *Mycobacterium* sp. strain KR2 in the presence and absence of surfactants was conducted using a trap system as shown in Fig. 10. The system consisted of an air inlet filter (1), a 250-ml narrow-mouthed glass culture bottle (3), 3 glass absorption traps linked in series, a charcoal tube (7) to protect the pump, an air-flow control valve (8), a vacuum pump and a timer. The culture bottle was connected to the traps by a syringe needle going through the rubber cap of the culture bottle and connecting to the trap. Another syringe needle in the cap served as air inlet to the culture bottle. A sterile 0.2 µm membrane filter was installed on the air inlet needle to prevent bacteria from the air entering the culture bottle. The pump controlled by a timer ran 15-min every 2.67 h (i.e. 9 15-min run a day) during the exposure period to aerate the culture medium to provide enough dissolved oxygen.

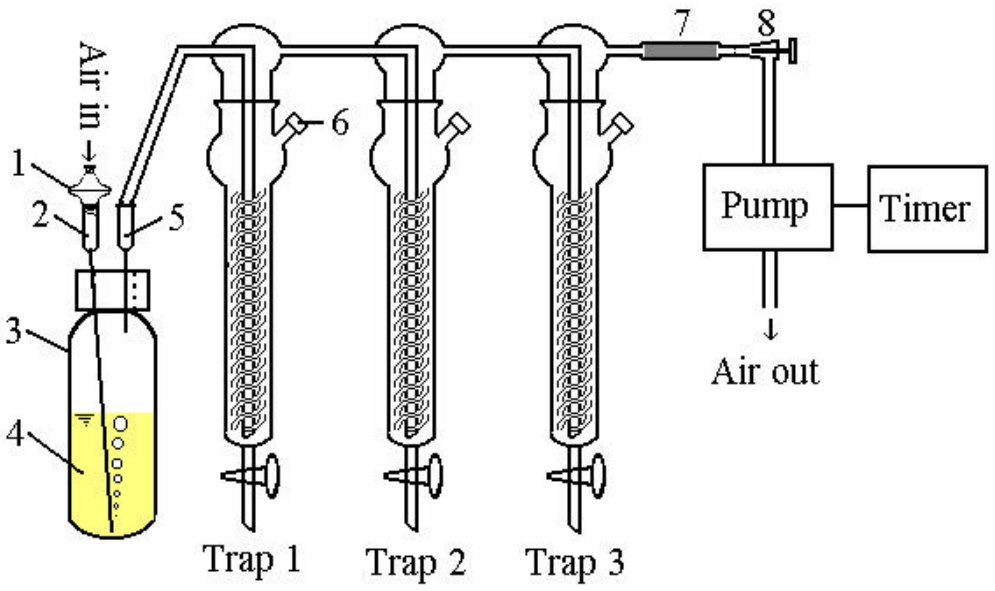


Fig. 10. Experimental apparatus for measuring ^{14}C-volatile organic chemicals (VOCs) and ^{14}C-CO_2 as results of the biodegradation of ^{14}C-phenanthrene in liquid culture: 1- 0.2 µm sterile membrane filter, 2- air inlet (syringe needle), 3- incubation bottle, 4- culture, 5- air outlet, 6- glass stopper with teflon liner, 7- charcoal, 8- control valve

When the vacuum pump was running, air was drawn through the air filter (1), entered culture medium (4), left the culture bottle through air outlet (5), and then went through the three traps in series, charcoal, valve and pump to the air outlet of the system. When air going through the culture medium containing ^{14}C-phenanthrene, mineral salts and inoculum in culture bottle (3), volatile organic compounds (VOC, parent compound and metabolites of ^{14}C-phenanthrene) and $^{14}CO_2$ were carried by the air flow through the traps. ^{14}C-VOCs other than $^{14}CO_2$ were trapped in Trap 1 containing 10 ml EMME (ethylene glycol monomethyl ether). $^{14}CO_2$ was then trapped in Trap 2 and Trap 3 containing 15 ml Cocktail-II (CARBO-SORB® E + PERMAFLUOR® E$^+$ in a ratio of 2:3 (v/v)) each. The $^{14}CO_2$-absorbed Cocktail-II in Trap 2 or Trap 3 was collected into a 20-ml LSC vial. Each trap was rinsed with 5 ml Cocktail-II which was combined to the same vial. The vial was then counted for $^{14}CO_2$ on LSC (liquid scintillation counter). Trap 1 solution (^{14}C-VOC-absorbed EMME) was also collected into a 20-ml LSC vial and the trap was washed with 10 ml Cocktail-I. The vial was then counted for ^{14}C-VOC on LSC.

Preparation of culture medium and inoculum

Mineral salts medium (MSM) used as culture medium consisted of components listed in Table 9. They are prepared using the same method as described in Section *3.2.1.2.4*.

Preparation of test culture

The basic steps for preparation of test culture were the same as described in Section *3.2.1.2.4*. 0.5 ml 20 g l^{-1} phenanthrene DCM solution and 0.5 ml ^{14}C-phenanthrene solution (25 mg l^{1}, 2.7x10^6 dpm ml^{-1}) were added to a 250-ml culture bottle (No. 3 in Fig. 10). After the solvent evaporated to the dryness, appropriate volume (100 ml minus the volume of surfactant solution and inoculum added) of MSM was added, resulting in phenanthrene concentration of 100 mg l^{-1} in MSM. 20 g l^{-1} aqueous surfactant solution was added to obtain designed surfactant concentrations ranging from 10 mg l^{1} to 80 mg l^{-1}. The culture solution was then inoculated with 1.2 ml inoculum, which produced the same bacteria densities in the

culture solution as those used in respiration tests (Section *3.2.1.2.4*). One culture was prepared to measure the degradation of phenanthrene without the presence of surfactants, in which phenanthrene was the sole carbon and energy source. Duplicates were prepared for each treatment. All processes were conducted aseptically.

Measurement of degradation

The culture bottles and the system were assembled as shown in Fig. 10. The culture was incubated at room temperature (20±3 ^{o}C) and in dark (wrapped with aluminium foil) for more than 20 days. Trap solutions were collected to measure ^{14}C-VOCs and ^{14}C-CO_2 in 24-h intervals in the first week of incubation and in 48-h intervals afterward. After removal of trap solution, Trap 1 was refilled with 10 ml EMME and Trap 2 and Trap 3 with 15 ml Cocktail-II each. At the end of the test, residual ^{14}C-activity in the culture medium was measured. 5 ml of the culture medium was removed to a LSC vial containing 10 ml cocktail (ULTIMA GOLD^{TM}) and counted on LSC.

3.2.1.3.2 Influence of surfactants on the biodegradation of PAHs by inherent microorganisms in soils

Biodegradation tests of phenanthrene and pyrene by inherent soil microorganisms were conducted in two reference soils (Soil 2.3 and Soil 2.2) and three agriculture soils (Soil 13$^{\#}$, 14$^{\#}$ and 15$^{\#}$) in the presence and absence of surfactants (LAS, Tween-80 or TDTMA). The soil was fortified with ^{14}C-phenanthrene or ^{14}C-pyrene to a concentration of 0.5 mg kg^{-1} and with one of the surfactants to a series of concentrations. The fortified soils were incubated at room temperature (20±3 ^{o}C) in dark (wrapped with aluminium foil) for more than 20 days. Biodegradation of ^{14}C-PAHs was monitored by measuring liberated ^{14}C-VOCs and ^{14}C-CO_2 using a trap system illustrated in Fig. 11. It was modified from the system (Fig. 10) used for the examination of phenanthrene degradation in liquid culture as described in Section *3.2.1.3.1*. Except the parts of culture tube (3) and air moisture bottle (1), both systems were basically the same and operated based on the same principle. Air moisture bottle was introduced to minimize the water loss due to evaporation during aeration.

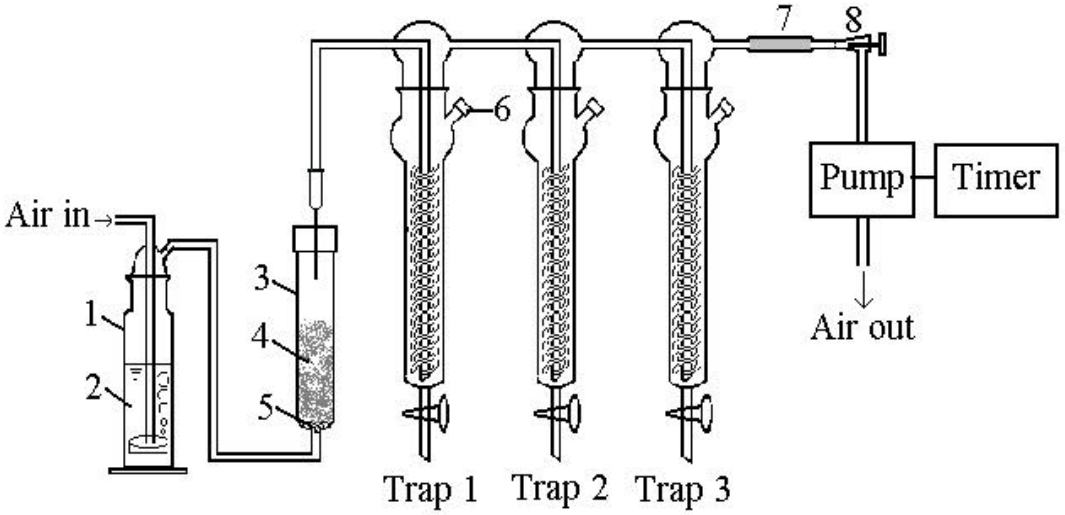


Fig. 11. Experimental apparatus for measuring ^{14}C-volatile organic chemicals (VOCs) and ^{14}C-CO_2 as results of the biodegradation of ^{14}C-phenanthrene in soil: 1- air moisture bottle, 2- water, 3- culture tube, 4- soil, 5- glass wool, 6- glass stopper with teflon liner, 7- charcoal, 8- control valve

Preparation of aqueous ^{14}C-phenanthrene and ^{14}C -pyrene solution

Five ml of 25.0 mg l^{-1} ($2.7x10^6$ dpm ml^{-1}) ^{14}C-phenanthrene or ^{14}C-pyrene dissolved in methanol was added to 30 ml MPW in a 50-ml beaker and mixed using a Pasteur pipette. This aqueous solution was ready to be applied to 250 g soil to produce a phenanthrene or pyrene concentration of 0.5 mg kg^{-1}.

Preparation of test soil

After the above aqueous ^{14}C-phenanthrene or ^{14}C-pyrene solution was applied to 250 g soil, the beaker was rinsed twice with two aliquots of 2.5 ml MPW which was also added to the soil. The soil was divided into 4 sub-soils of 68.59 g each. The rest of ^{14}C-spiked soil was reserved for measurement of ^{14}C-activity after combusted on an oxidizer, which was used for calculation of total ^{14}C-activity applied to the soil. One of the sub-soils was added with 4 ml MPW and served as a control (CK) to investigate the biodegradation of test PAH by inherent microorganisms in the absence of surfactants. To each of the rest sub-soils, 4 ml surfactant solution of appropriate concentrations was added to reach the required surfactant concentration in soil. As a result, the final soil moisture content was 20% which was about 60 to 70 % of its field

capacity. Each of the test sub-soils was further divided and transferred to three special incubation tubes (3) as shown in Fig. 11 serving as triplicates.

Apparatus and experimental set-up

The incubation tube (3) (25 mm i.d. x 150 mm with screw cap) had an air inlet in the bottom in which a small piece of glass wool was placed. The upper end of the tube was closed by a screw cap, on which there was an open sealed by a teflon-lined rubber septum. A syringe needle piercing through the septum served as an air outlet. The culture bottles and the system were assembled as shown in Fig. 11.

Measurement of degradation

The culture was incubated at room temperature (20±3 ^{o}C) and in dark (wrapped with aluminium foil) for more than 20 days. The methods and frequency for measuring ^{14}C-VOCs and ^{14}C-CO_2 were the same as those described Section *3.2.1.3.1*. At the end of the test, residual ^{14}C-activity in the soil was measured. One g freeze-dried soil was combusted on an oxidizer and counted on LSC.

3.2.1.3.3 Influence of Tween-80 on the mineralization and metabolism of phenanthrene in wheat-nutrient-lava microcosm

In order to quantify the effects of Tween-80 on the behaviour of phenanthrene, mineralization and metabolism of phenanthrene and its uptake by wheat in the presence and absence of Tween-80 were studied using a controlled wheat-nutrient-lava microcosm as shown in Fig. 12. The basic parts of the system were the same as those shown in Fig. 10 and Fig. 11 used for the measurement of phenanthrene biodegradation in liquid culture and in soil. Wheat (3) was grown in a 1-liter narrow- mouthed glass culture bottle i.e. the microcosm (2) containing nutrient solution (4) and lava (5) under continuous air supply via air inlet (1) filled with glass wool. ^{14}C-VOCs (both parent compound and metabolites of ^{14}C-phenanthrene) and $^{14}CO_2$ as a result of ^{14}C-phenanthrene degradation in the system were absorbed by solvents in three traps for measurement, respectively. Operation and monitoring of ^{14}C-VOCs and ^{14}C-CO_2 were the same as described in Section *3.2.1.3.1* and *3.2.1.3.2*.

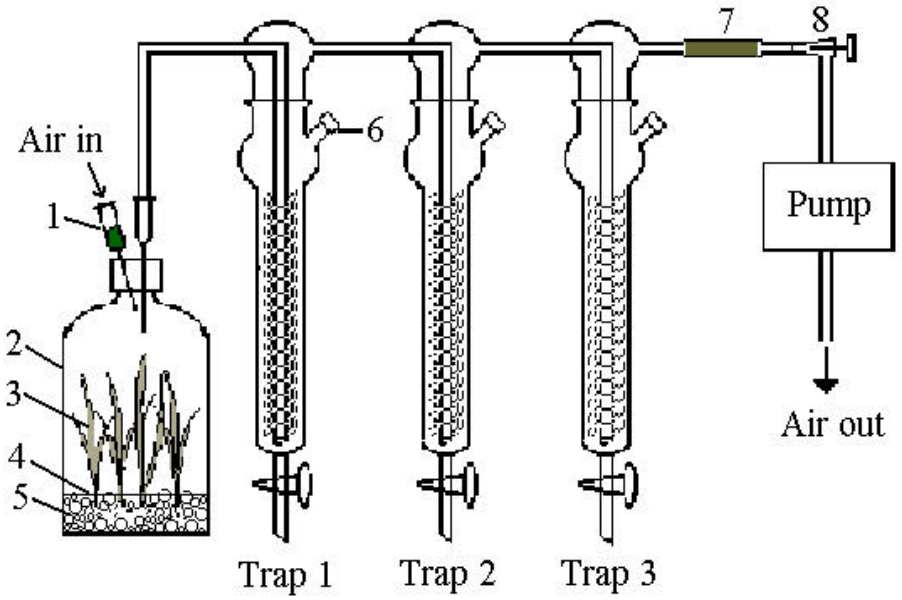


Fig. 12. Experimental apparatus for measuring ^{14}C-volatile organic chemicals (VOCs) and ^{14}C-CO_2 as results of the degradation of ^{14}C-phenanthrene in wheat-nutrient-lava microcosm: 1- air inlet filter (syringe tube filled with glass wool), 2- culture bottle (microcosm), 3- wheat, 4- nutrient solution, 5- lava, 6- glass stopper with teflon liner, 7- charcoal, 8- control valve

Table 10. Compositions of nutrient solutions for liquid culture of crops

Nutrient solution	Salts	Concentration
Normal elements	KNO_3	0.006 mol
	$Ca(NO_3)_2 \cdot 4H_2O$	0.004 mol
	$MgSO_4 \cdot 7H_2O$	0.002 mol
	KH_2PO_4	0.001 mol
Trace elements No. 1	H_3BO_3	1.34 mg l^{-1}
	$ZnSO_4 \cdot 7H_2O$	0.11 mg l^{-1}
	$MnSO_4 \cdot 4H_2O$	1.05 mg l^{-1}
	$CuSO_4 \cdot 5H_2O$	0.04 mg l^{-1}
	$H_2MoO_4 \cdot H_2O$	0.01 mg l^{-1}
Trace element No. 2*	Iron citrate	0.5%

* Applied twice a week and 4 drops once.

Preparation of aqueous phenanthrene- and/or Tween-80-fortified nutrient solution

Designed amount of stock ^{14}C-phenanthrene solution in methanol was added to nutrient solution (Table 10) to obtain phenanthrene concentration of 0.5 mg l^{-1} and ^{14}C-activity of 330000 dpm per culture bottle in the nutrient solution. The solution was then treated with ultrasonic for 1 hour. When Tween-80 was used, it was added before ultrasonic treatment to reach a concentration of 13.2 mg l^{-1} in nutrient solution. Before the test started, the exact concentration of phenanthrene and ^{14}C-radioactivity were measured on HPLC and LSC, respectively.

Preparation of lava

Lava was used as a supporting culture media for plant growth. It contained low organic matter (<0.2%) and therefore showed rather weak adsorption of PAHs (He *et al*., 1995). It was used after oven-dried (105 ^{o}C) to stable weight and passed through a 2.0-mm sieve.

Apparatus and experimental set-up

When wheat seedlings reached 0.5 cm height (3 days old), they were transplanted to the microcosm (2) containing 100 g lava and 60 ml nutrient solution. They were allowed to grow 3 days (2nd leaf stage) before addition of test chemicals. Then all nutrient solution was removed and 45 ml chemicals-spiked nutrient solution was added to the microcosm.

Culture and measurement of degradation

After the system was assembled as illustrated in Fig. 12, pump ran continuously till the end of culture except during sample collection. The culture was conducted at room temperature (20±3 ^{o}C). During culture period, nutrient solution was added to the microcosm to compensate the water losses due to evapotranspiration. Evapotranspiration rate was 2.5 ml day^{-1} during the first 6 days and decreased to zero at the end of the experiment at day 24. Trap solution and nutrient solution were collected for measurement of ^{14}C-activity. The methods for measuring ^{14}C-VOCs and ^{14}C-CO_2 were the same as those used Section *3.2.1.3.1*. After the termination of the

test, nutrient solution, wheat and lava were extracted and analyzed for residual ^{14}C-activity and metabolites.

Collection and measurement of trap solution during culture

Trap solutions were collected once a day for measuring ^{14}C-VOCs and ^{14}C-CO_2. The methods and cocktails used are described in Section *3.2.1.3.1*.

Collection and measurement of nutrient solution during culture

Nutrient solution in microcosm was collected for measurement of ^{14}C-activity at 10 min, 30 min, 2 h, 6 h, 20 h, 28 h, 2 d, 3 d, 4 d, 5 d, 10 d, 20 d, 23 d, and 24 d after addition of test chemicals. 0.5 ml nutrient solution was removed from the microcosm, added to 15 ml ULTIMA GOLD^{TM} in a 20-ml LSC vial and counted on LSC for ^{14}C-activity.

Extraction and analysis of nutrient solution after termination of culture

The procedure for extraction and analysis of nutrient solution is shown in Fig. 13. To reduce the evaporative losses of ^{14}C-activity and make up enough concentration of test chemical so that it could be identified under UV light after applied to TLC plate, 1 µg unlabelled phenanthrene was added to the extract of nutrient solution (as well as to the following extracts of lava and plant samples) before evaporation process.

Forty ml of nutrient solution was added to a 250-ml separation funnel and extracted with 40 ml cyclohexane. After separation, aqueous phase was extracted again twice successively with 30 and 20 ml cyclohexane, and organic phase was combined. The extracted aqueous phase was measured for ^{14}C-activity using cocktail ULTIMA GOLD^{TM}, which represented polar metabolites derived from ^{14}C-phenanthrene.

After taken for measurement of ^{14}C-activity representing the total non-polar compounds derived from ^{14}C-phenanthrene, the extract was dehydrated by filtrating through 30 g anhydrous Na_2SO_4 held in a funnel plugged with glass wool. It was then

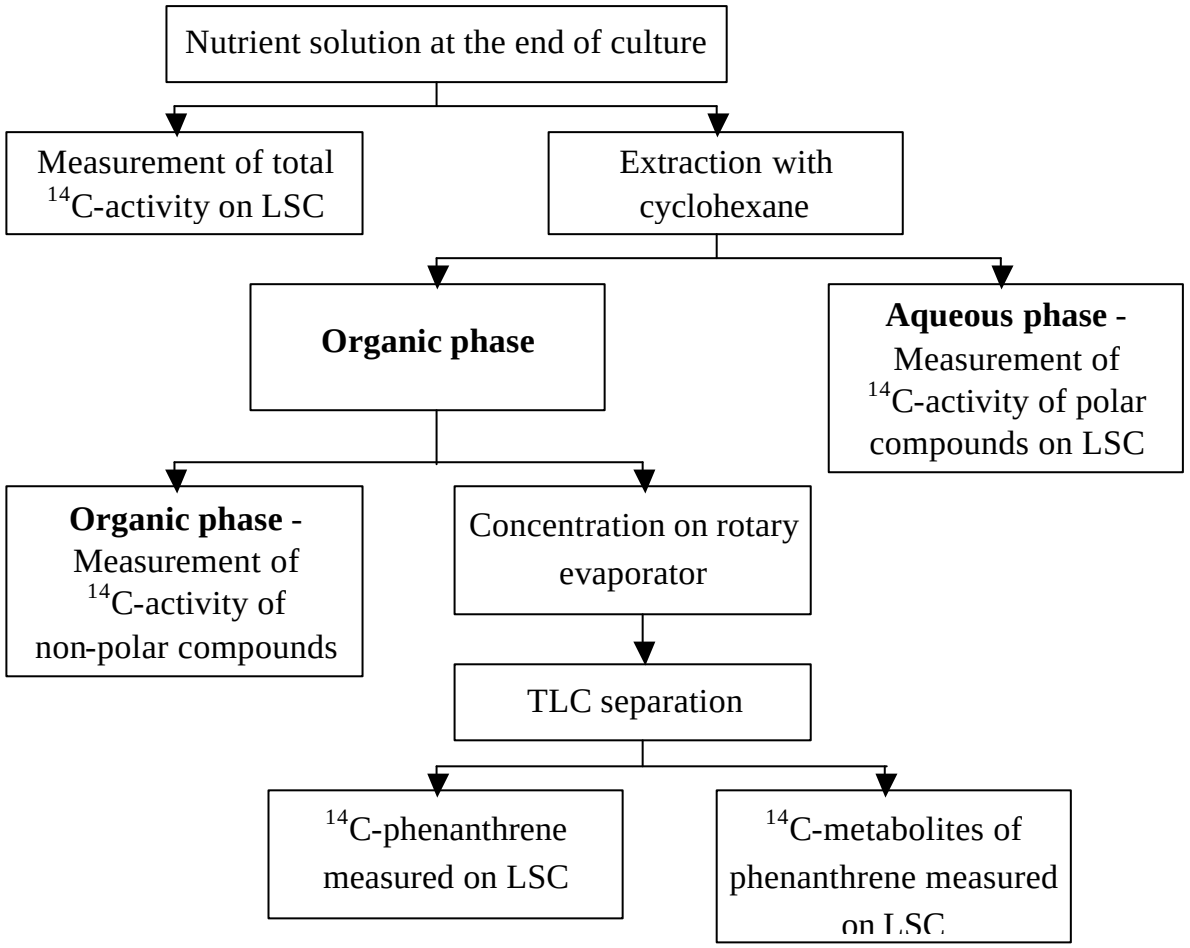


Fig. 13. Diagram for extraction and analysis of nutrient solution for ^{14}C-phenanthrene and its metabolites

evaporated to dryness on a rotary evaporator at a water bath temperature of 35 ^{o}C, cooling temperature of 4 ^{o}C and vacuum of 150 mbar. It was reconditioned with 1 ml methanol. Separation of parent ^{14}C-phenanthrene and its metabolites was achieved using TLC. 400 µl of the methanol extract was applied on a TLC plate. Non-labelled phenanthrene standard solution was also applied on the same plate for identification purpose. The plate was then developed using a mixture of chloroform/acetic acid/methanol (90:5:5, v/v/v). After air-dried, the plate was placed under UV light and ^{14}C-phenanthrene was identified by comparison with the standard phenanthrene. The R_f value for phenanthrene was about 0.9. Under dim light, spots of phenanthrene and its metabolites were scraped into LSC vials, respectively. A known amount of cocktail ULTIMA GOLD^{TM} was added and shaken until they were completely mixed. After

the suspended solids precipitated, ^{14}C-activity was counted on LSC.

Extraction and analysis of lava after termination of culture

The procedure for extraction and analysis of lava is illustrated in Fig. 14. Parts of lava samples were air-dried and milled to fine powder. One g aliquots of the powder were combusted on a sample oxidizer for measurement of ^{14}C-activity on LSC, representing the total ^{14}C-compounds sorbed on lava.

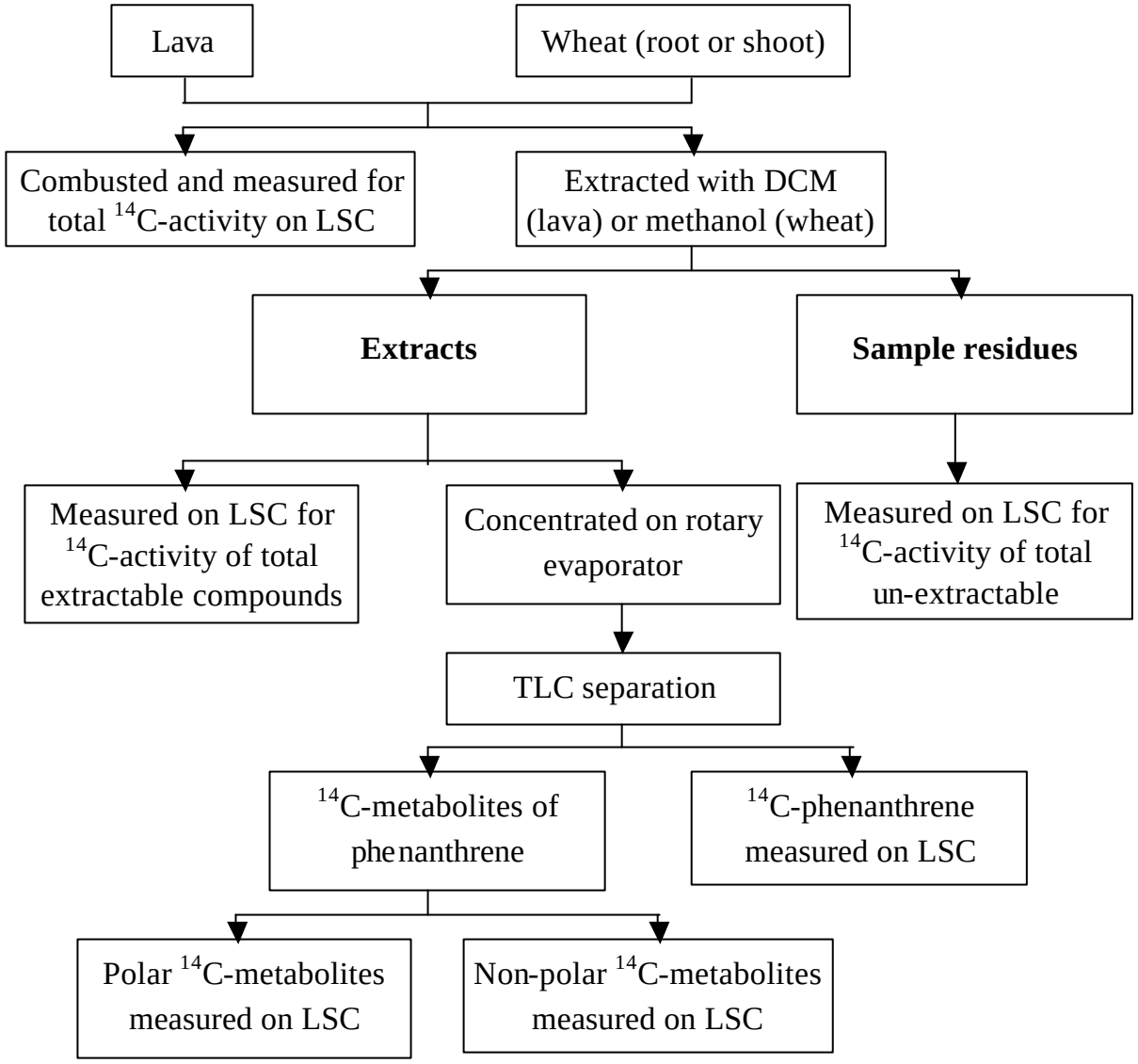


Fig. 14. Diagram for extraction and analysis of lava and wheat for ^{14}C-phenanthrene and its metabolites

Twenty g wet lava was extracted with 50 ml dichloromethane (DCM) using ultrasonic for 1 hour, and the extract was allowed to settle down overnight. One part of the supernatant was counted for ^{14}C-activity on LSC, which depicted the total extractable ^{14}C-compounds associated with lava. The remaining extract of known volume was dried on a rotary evaporator and reconditioned with 1 ml methanol. To separate phenanthrene and its metabolites, 400 µl of the methanol extract was applied to a TLC plate and developed with cyclohexane. After identified under UV-light, spots of phenanthrene (with an R_f value of about 0.5 to 0.6) and its metabolites were scraped into separate LSC vials under dim light. ^{14}C-activity was measured on LSC using the same cocktail as that used for nutrient solution. Compounds having R_f values higher and lower than that of phenanthrene were defined as non-polar and polar metabolites, respectively.

After extracted with DCM, remained lava was combusted and counted for ^{14}C-activity representing un-extractable ^{14}C-compounds in lava.

Extraction and analysis of wheat after termination of culture

The procedure for extraction and analysis of wheat was the same as that for lava (Fig. 14). After harvested, wheat was separated into two parts, i.e. roots and shoots, and washed with acetone/water (1:1, v/v) to remove ^{14}C-compounds adsorbed on their surface. The ^{14}C-activity of the washing waste was measured on LSC. Then they were stored at -80 ^{o}C before analysis. After dried with paper, 0.05 g of the wheat tissue was combusted on oxidizer and measured for ^{14}C-activity on LSC. The remaining tissues (0.3 to 0.8 g fresh weight) were cut into small pieces (ca. 3 mm) and put into a 100-ml glass extraction bottle containing 20 ml methanol. The sample was homogenized for 5 min using a homogenizer and then extracted using ultrasonic for 1 hour. In order to prevent loss due to evaporation, the extraction bottle was placed in ice-water to keep the solution cool during the process of homogenization and ultrasonic extraction. The homogenate was filtered through pre-weighed glass filter paper with the aid of vacuum. Residues on the filter paper were washed with two aliquots of 5 ml methanol which was also collected and combined into the filtrate. After dehydrated with ca. 5 g

Na_2SO_4, ^{14}C-activity and volume of the extracts were measured. The extract was then evaporated to small volume, dried to dryness with nitrogen flow and reconditioned in 2 ml methanol. After separated on TLC plate and collected into LSC vials containing cocktail, ^{14}C-activity of the phenanthrene and its metabolites was determined in the same way as lava.

The methanol-washed residues on the filter was combusted on oxidizer after air-dried and counted on LSC for ^{14}C-activity, which represented the un-extractable compounds in wheat tissues.

3.2.1.3.4 Influence of surfactants on the utilization and growth of Mycobacterium sp. on solid phenanthrene in the interface of phenanthrene and water

Utilization and growth of *Mycobacterium* sp. strain KR2 on solid phenanthrene in the interface of phenanthrene and water were investigated in the presence and absence of surfactants. Studies on the effects of surfactants on bacteria's utilization and growth on solid organic chemicals are of environmental interests in recent years (Stelmack *et al*., 1999). Measurement of bacteria growth on solid chemicals requires the separation of bacteria between growing on solid phase and in liquid phase. Methods used for such study so far, however, could not properly separate the bacteria between the two phases. In order to obtain the biomass truly growing on the solid phenanthrene, a column method was developed and employed for this study. Fig. 15 is the illustration of the apparatus. Phenanthrene was coated on glass beads contained in a column. Inoculated nutrient solution was circulated through the column by a peristaltic pump. Bacteria growing on the surface of phenanthrene-coated glass beads were extracted for measurement of biomass after nutrient solution drained out of the column.

Preparation of phenanthrene-coated glass beads and culture columns

Appropriate amount of phenanthrene was put into an earthen bowl and heated on an electric stove until it melted. Sterile glass beads were added to the melted phenanthrene. The phenanthrene-coated glass beads were removed to a beaker. The beaker was shaken while the coated phenanthrene was cooling down. The

phenanthrene-coated glass beads were packed into a glass column while being tapped. All processes were conducted under aseptic conditions. The total effective column was 62.3 cm^3. After a total volume of 34.2 cm^3 of glass beads was packed, there was a total pore volume of 28.1 cm^3. The total surface area of phenanthrene coated on glass beads was 19.6 cm^2.

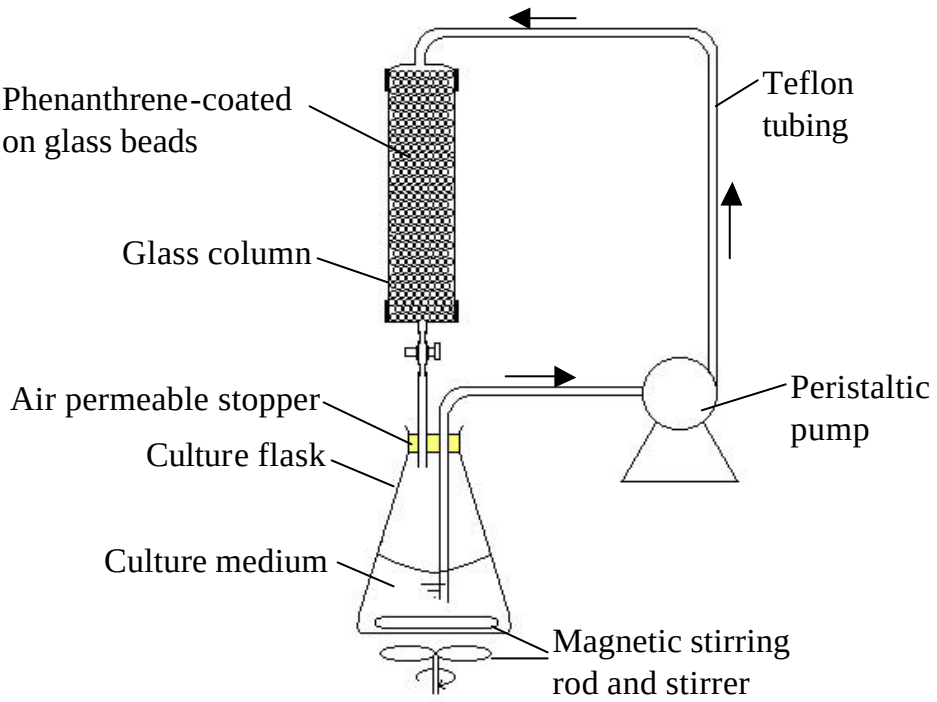


Fig. 15. Apparatus for measuring bacteria growing on solid phenanthrene

Preparation of culture medium

MSM containing no carbon and energy sources was used to prepare culture medium. Components and preparation of MSM were described in Section *3.2.1.2.4*. MSM and stock surfactant solution (20 g l^{-1}) were added to a 100-ml flask to obtain surfactant concentrations ranging from 0 to 200 mg l^{-1}. Then 1.2 ml inoculum was added. Duplicates were prepared for each treatment except for that of surfactant concentration of 200 mg l^{-1} due to the limit of stirring port. All transfers were made aseptically.

Assemble of apparatus and running of experiment

Column and tubing were connected and assembled as illustrated in Fig. 15. All tubing was autoclaved at 121 ^{o}C for 30 min before used. A total of 15 culture flasks were placed on a 15-ports magnetic stirrer. The stirrer was set at a speed of 600 rpm to ensure enough dissolved oxygen in the culture medium. The peristaltic pump was adjusted to obtain a flow of 4.6 ml min^{-1} or to replace one column volume of culture medium in 6 min. The culture was incubated in room temperature (20±3 ^{o}C).

Extraction of protein on solid phenanthrene coated on glass beads

In the end of test, the stirrer was stopped to let bacteria in the flask to settle down. The peristaltic pump was turned to its highest speed to flush bacteria not adhering to solid phenanthrene out of the column while it was turned left and right to shake the bacteria. After the culture solution completely drained off the column, glass beads were transferred to a 250-ml flask. Six ml MPW and 6 ml 0.075 M NaOH were added to extract the protein of bacteria growing on the solid phenanthrene. The flask was shaken from time to time for 30 min. The extract was then filtrated through a filter paper, which was ready for protein assay.

Extraction of protein in culture solution

5 ml of culture solution was removed to a test tube and 5 ml 0.075 M NaOH was added. After mixed completely, it was let to stand still for 30 min. The solution was then filtrated through a filter paper. The extract was ready for protein assay.

Protein assay

Appropriate volume of extracts was transferred to a test tube (150 mm x 13 mm i.d.). The volume removed for protein assay depended on protein concentration of the extract. If less than 4 ml extract was taken, MPW was added to make up to 4 ml. One ml Dye Agent Concentrate was added and shaken on tube shaker till completely mixed. After standing still for 30 min, absorbance (ABS) at 595 nm was measured in 1-cm cuvette against blank (2 ml MPW and 2 ml 0.075 M NaOH plus 1 ml Dye Agent Concentrate). The quantitation of protein was made by comparison of sample's ABS

with that of standard protein curve (see below).

Preparation of standard curve for protein assay

Bio-Rad protein assay standard I was diluted with MPW to obtain a series standard solutions of 0.014, 0.070, 0.139, 0.174, 0.222, 0.348, 0.695, 0.834, 1.112, and 1.390 mg ml^{-1}. Two ml of standard protein solution was transferred to a test tube containing 2 ml 0.075 M NaOH and shaken. After 30 min, 1 ml Dye Agent Concentrate was added and shaken till completely mixed. The solution was incubated at room temperature for 30 min before measured for ABS at 1-cm cuvette and at 595 nm. ABS should be measured within one hour. A standard curve was plotted with ABS against protein concentrations (Fig. 16).

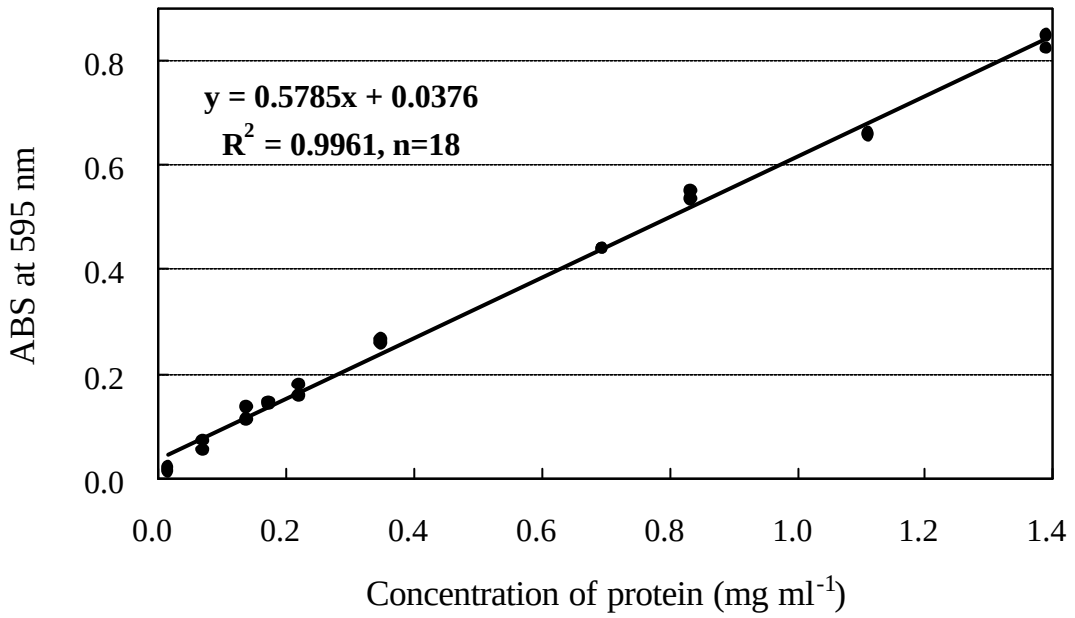


Fig. 16. Standard curve of protein assay

3.2.2 Analytical methods

3.2.2.1 Extraction and clean-up

3.2.2.1.1 Analysis of LAS in water

Method 1 - for high concentrations of LAS

Filtration: Glass fiber membrane filters (MN GF-5, pore size of *ca* 0.4 µm)

Analysis: Direct injection to HPLC

Method 2 - for low concentrations of LAS

Details of the method have been described by Ou *et al*. (1996a).

Extraction: Three-ml (500 mg) bonded silica C_{18} column

Eluate: Methanol

Final solvent: Methanol

Analysis: HPLC

3.2.2.1.2 Analysis of LAS in soil

Details of the method have been described by Ou *et al*. (1996a).

Sample preparation: Freeze-dried and sieved (1 mm)

Extraction: Methanol and ultrasonic for 1 h

Separation: Centrifugation (4000 rpm for 5 min)

Analysis: Supernatant injected to HPLC.

3.2.2.1.3 Analysis of LAS in rice

Details of the method have been described by Ou *et al*. (1997).

Sample preparation: Freeze-dried and *ca* 1 cm in length

Extraction: Methanol and ultrasonic for 1 h

Separation: Centrifugation (4000 rpm for 5 min)

Condensation: Evaporation in KDE

Dried to dryness with N2

Reconditioned with water

Clean-up: Neutral aluminium oxide

Eluted with water

Enrichment: Three-ml (500 mg) bonded silica C_{18} SPE column

Eluted with methanol

Analysis: Eluate injected to HPLC.

3.2.2.1.4 Analysis of phenanthrene in water

Extraction: Liquid-liquid method with cyclohexane

Condensation: Evaporation in rotary evaporator to dryness

Solvent exchange: Reconditioned with methanol

Analysis: Extract injected to HPLC.

3.2.2.1.5 Analysis of PAHs in soil

Sample preparation: Freeze-dried

Extraction: Tetrahydrofuran (THF) and ultrasonic for 2.5 h

Separation: Centrifugation (6000 rpm for 5 min)

Condensation: Evaporation to the dryness on rotary evaporator

Reconditioned with n-hexane

Clean-up: Silica gel

Eluted with n-hexane/dichloromethane (1:1, v/v)

Solvent exchange: Evaporation of eluate to dryness with N_2

Reconditioned with methanol

Analysis: Extracts injected to HPLC.

3.2.2.2 HPLC conditions

3.2.2.2.1 HPLC conditions for analysis of total LAS

Detector: DAD 225 nm

Column: LiChrospher 100 RP-18 for method development etc. or

ODS-HYPERSIL C18 column

Oven temperature: 38 ^{o}C

Mobile phase: methanol/water (4:1, v/v) 0.8 ml min^{-1}, isocratic.

3.2.2.2.2 HPLC conditions for analysis of LAS homologues

Detector: DAD 225 nm

Column: LiChrospher 100 RP-18

Oven temperature: 38 ^{o}C

Mobile phase: 0.02 mol l^{-1} $NaClO_4$ methanol/water (4:1, v/v) 0.8 ml min^{-1},

isocratic.

3.2.2.2.3 HPLC conditions for analysis of PAHs

Detector: DAD 255 nm

Column: ODS-HYPERSIL C18

Oven temperature: 38 ^{o}C

Mobile phase: methanol/water (4:1, v/v) 0.8 ml min^{-1}, isocratic.

3.2.2.2.4 HPLC conditions for analysis of ^{14}C-phenanthrene and its metabolites

Detector: UV detector 254 nm and isotope detector

Column: ODS-HYPERSIL C18

Oven temperature: 35 ^{o}C

Mobile phase: methanol/water (85:15, v/v) 0.8 ml min^{-1}, isocratic.

3.2.2.3 Chemical analysis

3.2.2.3.1 Analysis of Tween-80 in water

Reagents: Coomassie Brilliant Blue dissolved in 3% $HClO_4$ solution

Reaction: Sample + reagent within 2 h

Measurement: ABS at 600 nm against blank on spectrophotometer.

3.2.2.3.2 Protein assay

Reagents: Bio-Rad Dye Agent Concentrate
0.075 M NaOH

Reaction: 2 ml sample + 2 ml 0.075 M NaOH + 1 ml Dye Agent Concentrate within one hour

Measurement: ABS at 595 nm against blank on spectrophotometer.

4 RESULTS AND DISCUSSIONS

4.1 Adsorption and migration

The behaviour and fate of surfactants and PAHs in plant-soil systems (leaching to groundwater, biotic and abiotic degradation, bioavailability, uptake and accumulation by plant, persistence in soil, separate and combined ecological effects and so on) are governed, to a great extent, by their interaction between interface of soil particle and solution i.e. the adsorption/desorption process (Karickhoff *et al*., 1979; Schwarzenbach and Westall, 1981; Sims and Overcash, 1983; Litz *et al*., 1987; Weber and Miller, 1989; Larsen *et al*., 1992; Beck *et al*., 1993; Kowalska, *et al*., 1994; Jesen, 1999). The success of physically based management and research models in describing the movement and fate of organic chemicals in the soil system is largely dependent on our understanding of their adsorption/desorption mechanisms on soil and migration processes in soil. Regardless of the physicochemical properties of most organic contaminants, their behaviour in soils in most likely to be determined by their relative partitioning between the solid and solution phase (Beck *et al*., 1993). The adsorption/distribution coefficient of organic compounds between the solid and aqueous phase of soil is the indispensable parameter of mathematical models describing their leaching in soil (House and Farr, 1989; Beck *et al*., 1993; Edwards *et al*., 1994). Thus, it is important to study the adsorption/desorption mechanism and migration processes of organic compounds in soil.

4.1.1 Adsorption of LAS and phenanthrene on soils and influence of LAS on the adsorption of phenanthrene on soils

4.1.1.1 Adsorption of LAS on soils

Few studies have been conducted on the adsorption/desorption of LAS on soils and sediments. The little understanding of its adsorption/desorption behaviour evokes much controversy on its adsorption/desorption mechanisms. It is still difficult to make any general conclusion. Some authors reported a correlation between sorption and organic carbon content of sediments and soils (Urano *et al*., 1984; Matthijs and de

Henau, 1985; Fytianos *et al*., 1998) and others give a poor correlation with organic carbon content but positive correlation with silt (Hand and Williams, 1987). Cooperative adsorption has been observed on kaolinite (House and Farr, 1989) and soils (Fytianos *et al*., 1998) but not on sediments (Marchesi *et al*., 1991; Rubio et al., 1996). The degree of reversibility of sorption is also uncertain, with reports of almost complete reversibility (Hand and Williams, 1987; Siracusa and Somasundaran, 1987; House and Farr 1989) to those of considerable irreversibility (Hanna and Somasundaran, 1979; Matthijs and de Henau, 1985). In spite of these difference, there are come reports that the mechanisms for the sorption is dominated by hydrophobic interaction (Hand and Williams, 1987; House and Farr, 1989; Marchesi *et al*., 1991).

The purpose of this part of our work was to study the behaviour and mechanism of LAS adsorption on soils through experiments to evaluate the distribution coefficient of LAS between solid/aqueous phases of soils, to provide basic theory and key parameters for mathematical models that simulate the migration of LAS in plant-soil systems, and to serve as a base for understanding surfactant's effects on the adsorption/desorption of HOCs on soil.

4.1.1.1.1 Kinetics of LAS adsorption on soils

Results shown in Fig. 17 indicate that adsorption of LAS on soils occurred rapidly and equilibrium established in 40 min under experimental conditions. The fast establishment of equilibrium may be the result of strong mechanical stirring employed. Results also show that there were no observable effects of LAS concentration on equilibrium within the LAS concentrations measured, which suggest that diffusion of LAS molecules from aqueous phase to solid phase was not the process restricting the kinetics of LAS adsorption. Thus, 1 h of equilibrium was allowed for all adsorption measurements.

The equilibrium time observed in our experiments was longer than that reported (20 min) for LAS adsorption on kaolinite (House and Farr, 1989) and on sediments (Marchesi *et al*., 1991) using similar equipment. The adsorption amounts were

constant, within experimental error, in the time period measured up to 14 h, which suggested that no measurable degradation of LAS occurred in the time period for adsorption measurement which was usually less than 14 h.

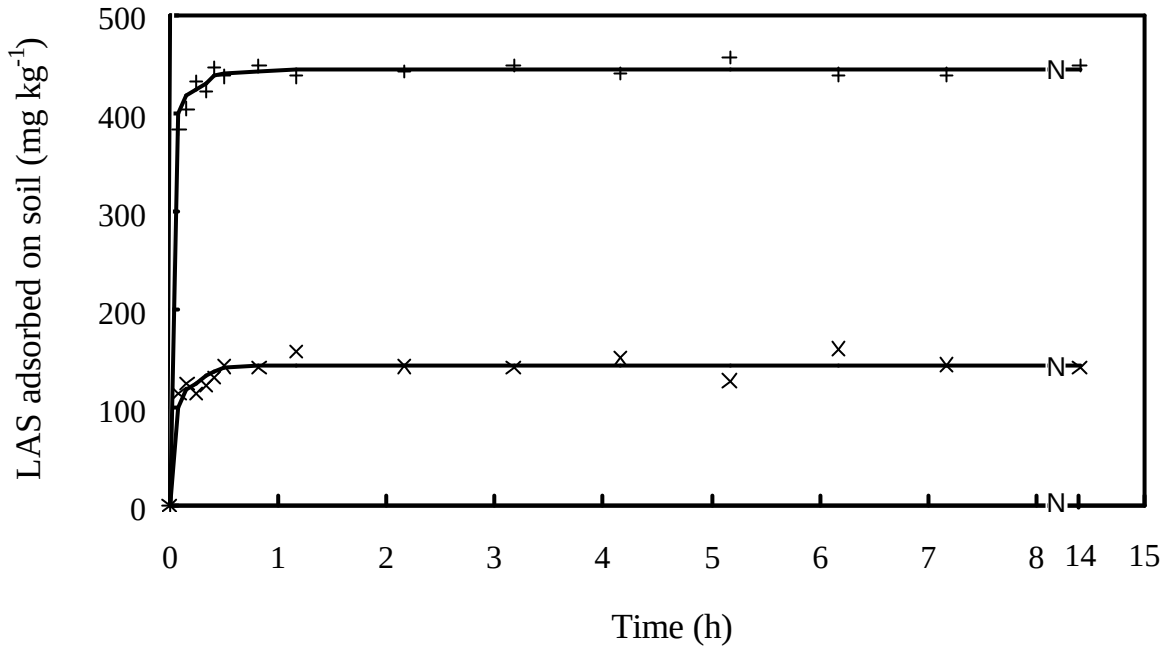


Fig. 17. Kinetics of LAS adsorption on Soil 13# (+) and 15# (×) collected from SES, Shenyang, China

4.1.1.1.2 LAS adsorption isotherms and mechanisms

Adsorption isotherms of LAS on natural soils are shown in Fig. 18. It is obvious that the adsorption of LAS on soils could be divided into two stages. First stage is linear adsorption, where the amount of LAS adsorbed on soil increase proportionally with the increasing of equilibrium LAS concentrations in solution. It can be described by Freundlich equation $S = KC^n$, where S is the amount of LAS adsorbed on soil (mg kg^{-1}), C is the equilibrium concentration of LAS in solution (mg l^{-1}), K and n are constants. As n = 1, Freundlich equation could be written as $S = K_dC$, where K_d is distribution coefficient. The K_d values of LAS on four natural soils were 1.23, 1.30, 2.02 and 1.77 for Soil 13#, 14#, 15# and Soil 2.3, respectively. Adsorption of LAS on the four soils was all very weak. Except for Soil 2.3 having a linear adsorption range of 0 to 60 mg l^{-1} of equilibrium LAS concentrations in solution, the linear range for all

other three soils was in 0 to 90 mg l^{-1}, which implied that the soil properties in charge of LAS adsorption of the three soils from SES ($13^{\#}$, $14^{\#}$, $15^{\#}$) were essentially similar to each other and different to those of Soil 2.3. In order to approach the adsorption mechanism, correlation between soil properties and K_d are analyzed only for the "essentially similar" soils from SES. Results (Table 11) show that the adsorption intensity of LAS (K_d) correlated significantly or most significantly and positively with clay ($P < 0.05$) and microline ($P < 0.01$) contents of soils, and most significantly and negatively with fine sand ($P < 0.01$) and coarse sand ($P < 0.01$) contents of soils, respectively. Further analysis indicates that K_d had a most significantly positive and

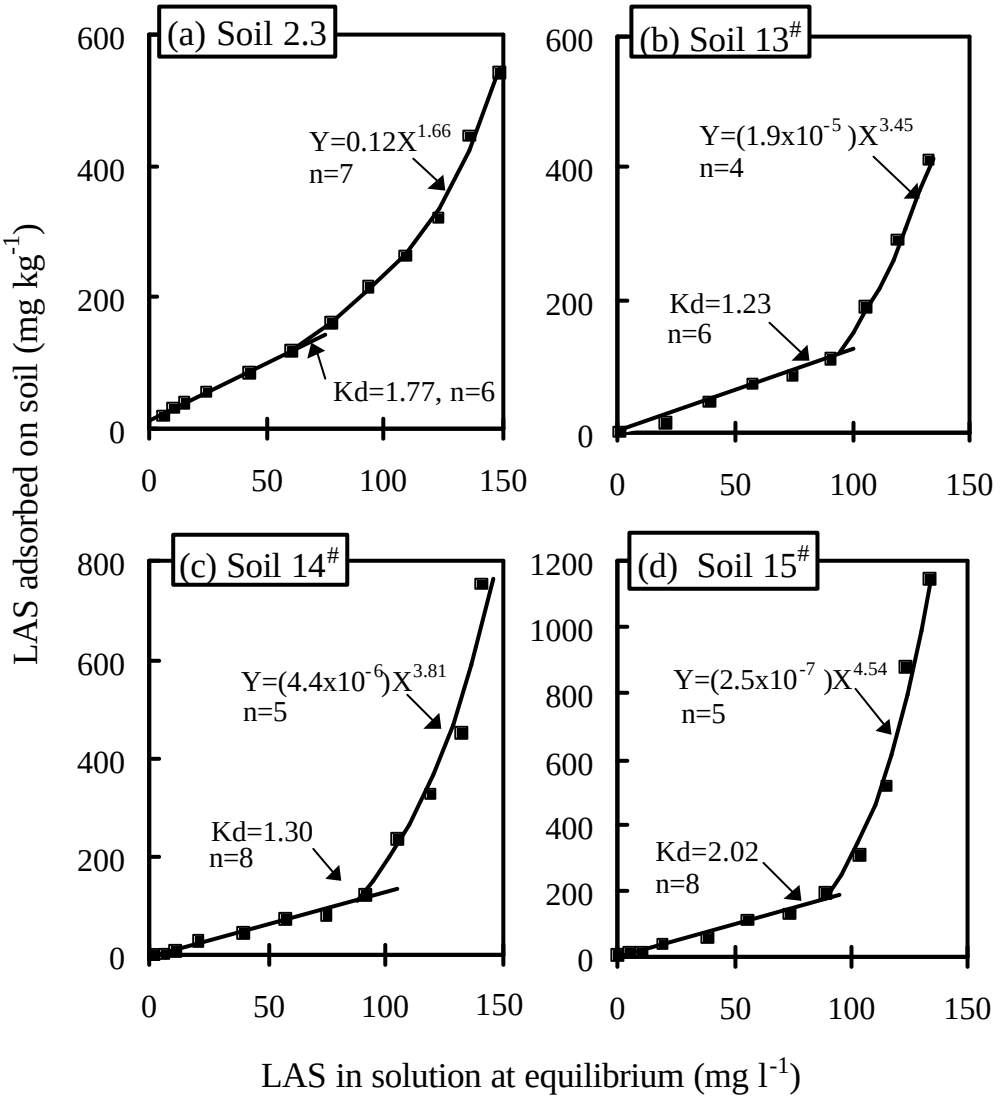


Fig. 18. Isotherms of LAS adsorption on soils from (a) LUFA, Speyer, Germany and (b)(c)(d) SES, Shenyang, China

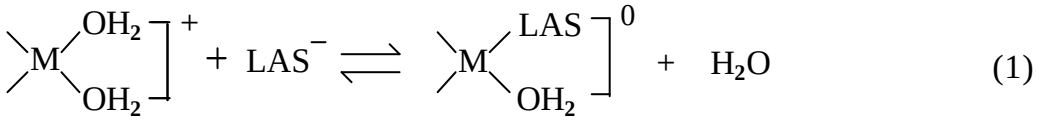
Table 11. Correlation between soil properties and K_d for LAS adsorption on soils from SES, Shenyang, China (n=3)

Soil property	Equation	Coefficient
Organic carbon	y = -1.75x + 2.76	-0.945
CEC	y = 0.11x - 1.09	0.862
Clay	y = 0.08x - 0.40	0.997*
Silt	y = 0.20x - 3.62	0.903
Fine sand	y = -0.06x + 4.40	-0.999**
Coarse sand	y = -0.83x + 3.24	-0.977
Physical clay (<0.01mm)	y = 0.06x - 1.44	0.999**
Physical sand (>0.01mm)	y = -0.06x + 4.32	-0.999**
Quartz	y = -0.15x + 13.10	-0.828
Microline	y = 0.20x + 1.24	1.000**
Albite	y = 0.04x + 12.1	0.511

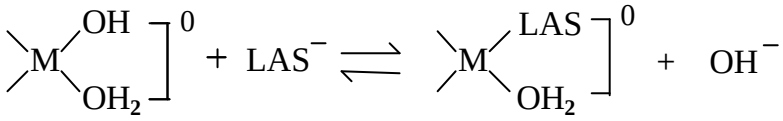
* P< 0.05, ** P< 0.01

negative correlation with physical clay (<0.01 mm) and physical sand (>0.01 mm) contents, respectively, which suggested that the adsorption of LAS on soils mainly depended on the content of clay minerals with larger specific surface areas. Effect of soil sand content on K_d was a result of its growth and decline correlation with clay content. The excellent correlation between microline content and K_d may be due to its relation to physical clay content. In addition, experimental results also suggested that mechanisms of LAS adsorption on soils of low organic carbon content (<1%) were not distribution or hydrophobic interactions. It is likely that LAS adsorbs on soil by hydrophilic head (negatively charged sulfonate group) through specific site surface interactions (Mingelgrin and Gerstl, 1983; Litz *et al*., 1987) or hydrogen bonding, e.g. those formed by sulfonate group of LAS molecules with polar groups (e.g. hydroxyl and phenol groups) of soil humus or -OH groups on the surface of minerals and oxides (Laha and Luthy, 1992; West and Harwell, 1992; Schwarzenbach *et al*., 1993; Kowalska *et al*., 1994). Specific site surface interactions include electrostatic bonding

of negatively charged sulfonate groups of LAS with positively charged sites (e.g. $-NH_3^+$ on organic matter and oxides on mineral surface) on soil surface (Schwarzenbach *et al.*, 1993), exchange of anions (Xiong *et al.*, 1990) and exchange of ligands (Inoue *et al.*, 1978; Xiong *et al.*, 1990). The following adsorption of LAS on soil colloids through ligand exchange could occur:



(1)



(2)

where M stand for Fe or Al. Sites where ligand exchange can happen include bare M-$(OH)H_2O$ groups on the surface or edge of ferric and aluminium oxides or crystals of layer silicate minerals. If the adsorption of LAS occurs by means of ligand exchange with hydrate groups as reaction (1), the amount of positive charges on colloid surface reduces while the pH of the system does not change. If the adsorption of LAS happens by ligand exchange with hydroxyl as reaction (2), the result is just opposite, i.e. the pH value of system increases (Inoue *et al.*, 1978). The pH value of our experimental systems did not change after adsorption, which may imply that ligand exchange adsorption of LAS on the experimental soils mainly followed reaction (1).

In the linear adsorption stage, mechanisms of LAS adsorption on soils are schematically illustrated in Fig. 19(a). On soil surface, thus, the LAS monomers adsorbed form a hydrophobic (nonpolar) monolayer (hemimicelle) with their hydrophobic tails (alkyl chains) sticking out into aqueous phase (West and Harwell, 1992; Park and Jaffé, 1993). The tendency of the surfactant to form hemimicelles is dependent on the interactions between hydrophilic moiety of the surfactant and the soil surface (West and Harwell, 1992). Because of the noncontinuance of active adsorption sites on soil surface, the hemimicelles formation were not continuous but

in patches.

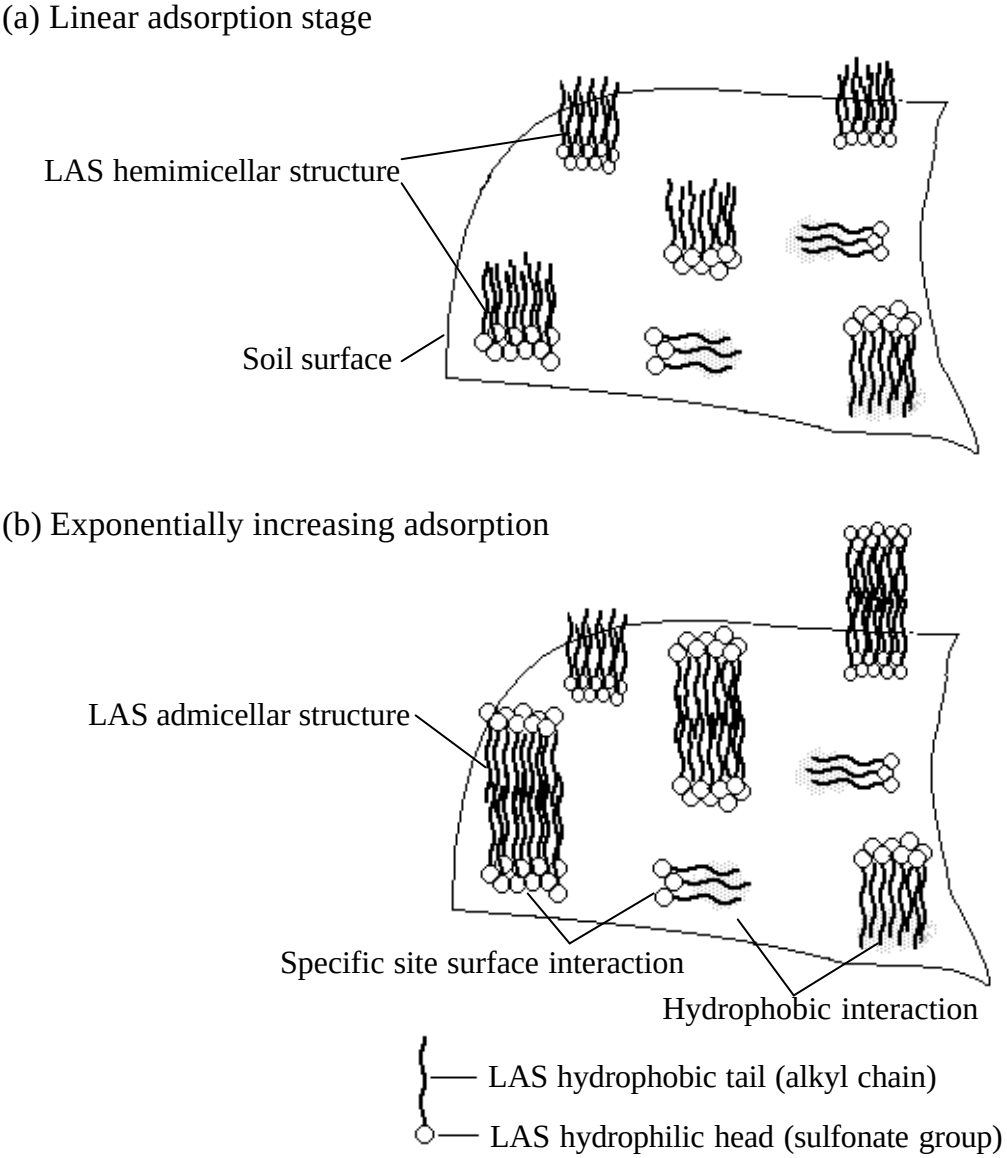


Fig. 19. Schematic sketch of LAS adsorption on soil surface: (a) linear adsorption stage, and (b) exponentially increasing adsorption stage

The second stage of LAS adsorption on soils was the exponentially increasing stage of the adsorption isotherm. The amount of LAS adsorbed increased exponentially with the increase of LAS concentration in solution, which indicated a cooperative adsorption mechanism (House and Farr, 1989). As the increase of LAS concentration in solution, active adsorption sites on soil surface become less and less available, and

on the other hand, more and more hemimicelles form, which changes the properties of the soil surface to become hydrophobic (Schwarzenbach *et al.*, 1993). The increase of LAS monomers in the solution and hydrophobity on soil surface will increase the energy of the system and make it unstable. To make the system stable, hydrophobic tails of LAS monomers adsorb or aggregate on the hemimicelles on soil surface through van der Walls force, hydrophobic binding or thermodynamics ways to form a bilayer (admicelles) (Inoue *et al.*, 1978; House and Farr, 1989) (Fig. 19(b)). Hydrophilic heads of LAS in admicelles extend into solution with partial removal of hydrophobic alkyl chains from solution by rapping into micelles, which reduces the energy of the system and keep it stable (House and Farr, 1989). As the continuous increasing of LAS concentration in solution, coverage of LAS on soil surface become larger and larger, affinity of LAS to soil surface enhance, and the amount of LAS adsorbed increases exponentially. Once these structures form on soil surface, adsorption of additional surfactant may rapidly increase until a complete bilayer of surfactant covers the soil surface (West and Harwell, 1992). House and Farr (1989) also observed the similar shape LAS adsorption isotherms on kaolinite.

The adsorption intensity of LAS on H_2O_2–treated Soil $13^{\#}$, whose organic matter was removed, increased significantly. The value of K_d increased more than 4 times from 1.23 to 5.64. Moreover, within the range of concentrations measured (0 - 150 mg l^{-1}), adsorption isotherm was linear, i.e. the adsorption amount increased linearly with the increasing of LAS concentration in solution (Fig. 20). There are two possible mechanisms for the increase of adsorption ability of LAS on soil after being treated by H_2O_2. One is the removal of organic matter, which results in the decrease of negative charges on soil surface and favourable to the access and contact of LAS with soil surface, and on the other hand, the release of active ferric and aluminium oxides from organic chelators (Inoue *et al.*, 1978) proving more active adsorption sites. The other one is the protonization of soil surface, which results in the soil surface positively charged and the increase of its electrostatic bonding capacity (Urano *et al.*, 1984). All these results further indicate that the mechanism of LAS adsorption on soil is not partitioning or hydrophobic interaction but specific site adsorption.

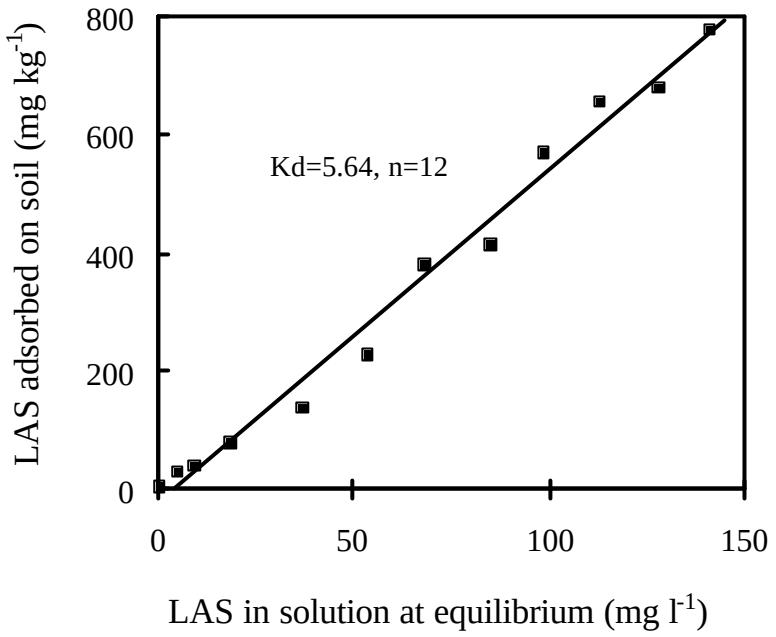


Fig. 20. Isotherm of LAS adsorption on H_2O_2-treated Soil 13#

4.1.1.2 Adsorption of phenanthrene on soils

4.1.1.2.1 Kinetics of phenanthrene adsorption on soils

Results (Fig. 21) indicate that adsorption of phenanthrene on soils occurred rapidly and equilibrium established in 1 h under experimental conditions. The fast establishment of equilibrium may be the result of strong mechanical stirring employed. Compared with the adsorption kinetics of LAS under the same conditions (Fig. 17), phenanthrene required longer time than LAS did (40 min) to reach equilibrium. Results also show that there were no observable effects of phenanthrene concentration on adsorption kinetics within the phenanthrene concentrations measured, which suggest that diffusion of phenanthrene molecules between the aqueous and solid phases was not the process restricting the equilibrium of phenanthrene adsorption. Thus, 1 h of equilibrium was allowed for all adsorption measurements. Moreover, the adsorption amounts were constant, within experimental error, in the time period measured up to 14 h. It suggested that no measurable degradation of phenanthrene occurred in the time period for adsorption measurement which was usually less than 14 h.

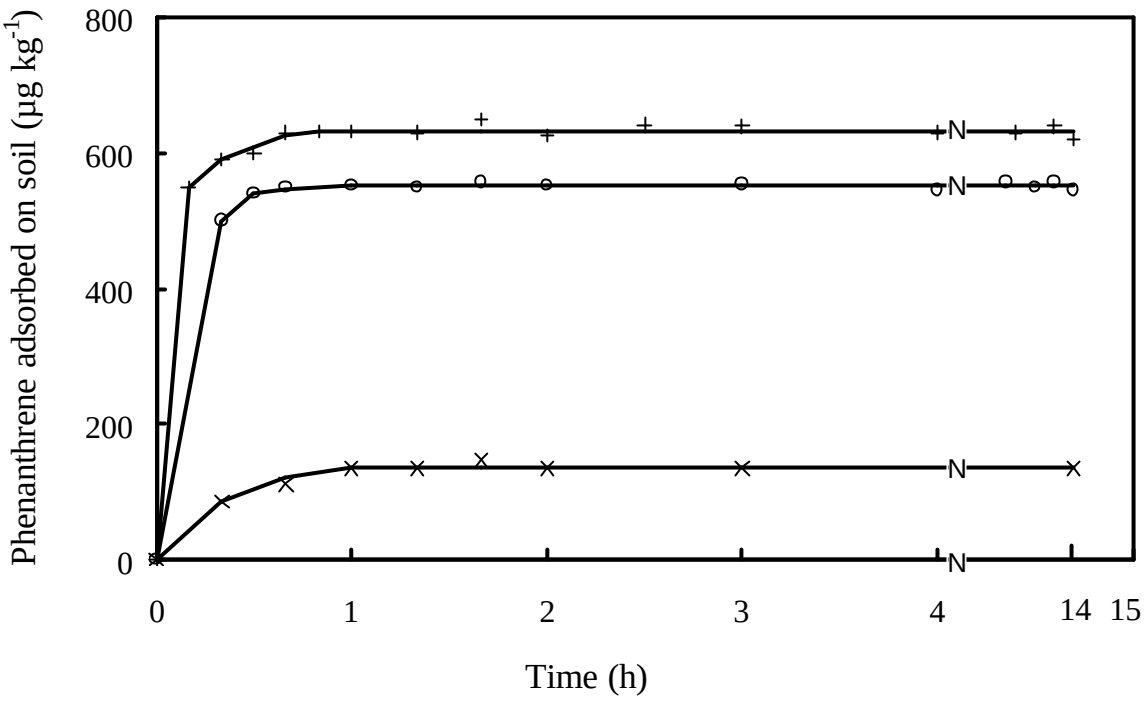


Fig. 21. Kinetics of phenanthrene adsorption on soils from LUFA, Speyer (Soil 2.3), Germany (+) (o) and from SES (Soil 13#), Shenyang, China (×): (+) and (×) without LAS, and (o) with the presence of 500 mg l^{-1} LAS in solution.

4.1.1.2.2 Isotherms of phenanthrene adsorption on soils

Fig. 22 shows the isotherms of phenanthrene adsorption on two soils. The adsorption isotherms were linear over the concentrations examined up to *ca* 60 µg l^{-1} and can be described by the special form of Freundlich equation, i.e. $S = K_dC$ (see Section *4.1.1.1.2*). The distribution coefficients (K_d) were 250.7 and 206.3 for Soil 2.3 and Soil 13#, respectively. When K_d normalized to organic carbon content of the test soil, the values of log K_{oc} of the two soils were almost exactly the same (4.37 and 4.34 for Soil 2.3 and Soil 13#, respectively). Results suggested that the adsorption of phenanthrene was hydrophobic interaction in mechanism (Chiou *at al*., 1979; Karickhoff et al., 1979).

4.1.1.3 Influence of LAS on phenanthrene adsorption on soils

The enhancement of solubility and therefore mobility of HOCs by surfactants has

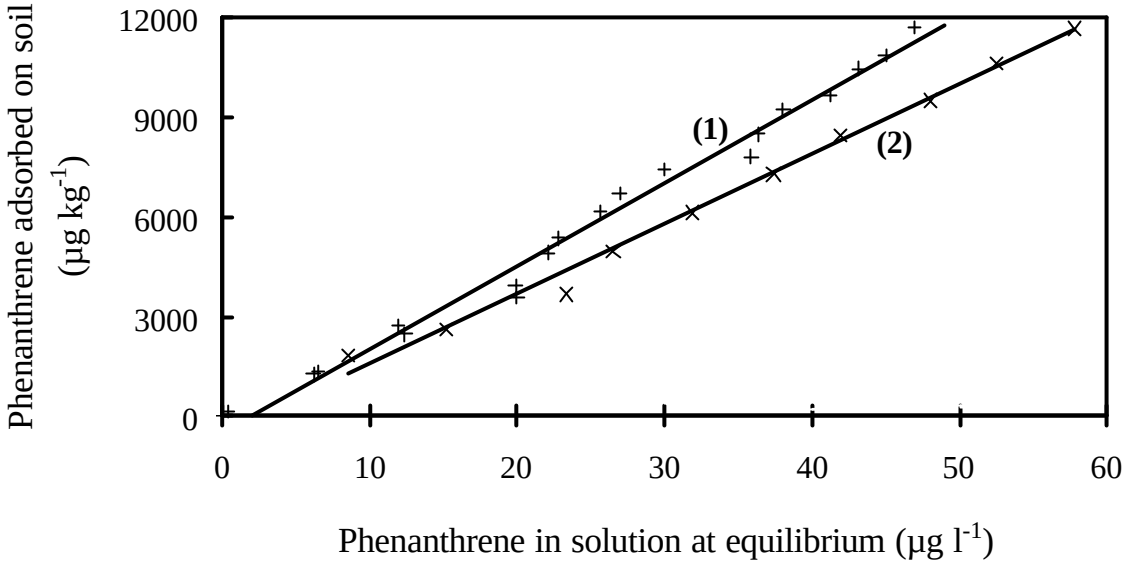


Fig. 22. Adsorption isotherms of phenanthrene on (+) Soil 2.3 from LUFA, Speyer, Germany, and on (×) Soil 13# from SES, Shenyang, China

been observed by many researchers and this theory has been applied to the remediation of contaminated soils and restoration of aquifers (West and Harwell, 1992). From results obtained so far (e.g. by Kile and Chiou, 1989; Edwards *et al*., 1991; Liu *et al*., 1991; Laha and Luthy, 1992), it can be generalized that surfactant solubilization is a micellar phenomenon. Surfactant solubilization commences at aqueous-phase surfactant CMC in which pseudophase micelles form through self-association of surfactant molecules. Micelles formed by ionic surfactants like LAS have an inner hydrocarbon layer, an outer ionic layer and an intermediate region termed the palisade layer. Hydrophobic solutes can remain dissolved in the hydrophobic core, adsorbed on the ionic layer or sandwiched in the palisade layer. HOCs are able to escape the unfavourable aqueous environment by partitioning either partially or entirely into the hydrophobic core of the micelles (Kibbey and Hayes, 1993). For most non-polar HOCs, therefore, the locus of solubilization is the hydrocarbon core of the micelles (Atwood and Florence, 1983). The solubility of nonionic organic chemicals (including PAHs) increases approximately linearly with nonionic surfactant concentration above CMC in the presence of excess organic chemicals, while very slight solubility enhancement is observed in surfactant concentration below CMC (Edwards *et al*., 1991; Liu *et al*., 1991; Laha and Luthy,

1992). Only the organic compounds having very sparing solubility (e.g. DDT) exhibit significant solubility enhancement below CMC (Kile and Chiou, 1989).

Presently, there is little understanding of the influence of surfactants on solubilization and adsorption behaviour of HOCs in soils and sediments. Most studies indicate that the amount of surfactant required to solubilize HOCs in soil- and sediment-water systems is considerably greater than the corresponding clean-water CMC, i.e. the effective CMC (CMC_{eff}) in the presence of soil or sediment is greater than clean-water CMC (CMC_w). The difference between CMC_{eff} and CMC_w depends on the kind of surfactants as well as on solution conditions (Liu *et al*., 1991; Laha and Luthy, 1992). Yediler *et al*. (1991) reported that linear alkylbenzene sulfonate (LAS) increases the mobility of o-cresol in soil. Much controversy, however, appears over the effects of surfactant in concentrations much lower than CMC on the solubilization, adsorption and consequent mobilization of HOCs. Results obtained by Jafvert (1991) show that solubility of phenanthrene decreases slightly in the presence of low concentrations of dodecylsulfate (DS) and then steadily increased above DS CMC_{eff}. Similar effects reported by Yediler *et al*. (1991) indicate that adsorption of o-cresol of high concentrations on soil increases in the presence of 500 mg l^{-1} LAS but decreases in LAS concentrations of 1000 mg l^{1} and 2000 mg l^{1}. Other investigators, however, report that the solubility of HOCs is approximately constant or increases slightly in the presence of surfactant below CMC_{eff} (Edwards *et al*., 1991; Liu *et al*., 1991; Laha and Luthy, 1992).

The purpose of this part of the study was to evaluate the effects of LAS on the adsorption behaviour of phenanthrene on soils. The adsorption of phenanthrene on two soils was studied in the presence of LAS in concentration around CMC with emphasis on low LAS levels which are relevant to the environment. In order to simulate different environmental conditions and for better understanding of the mechanism, two contacting sequences of soils with phenanthrene and LAS were examined: (1) "soil+phenanthrene+LAS (Method-1)" and (2) "soil+LAS+ phenanthrene (method-2)".

4.1.1.3.1 Influence of LAS on kinetics of phenanthrene adsorption on soils

No obvious effects of LAS on kinetics of phenanthrene adsorption were observed under the experimental conditions used (Fig. 21). In both situations with the presence and absence of LAS, adsorption of phenanthrene occurred rapidly and equilibrium established in 1 h. There was no significant difference in the kinetics of phenanthrene adsorption between those with and without the presence of LAS within experimental error. Thus, 1 h of equilibrium was allowed for all adsorption measurements.

4.1.1.3.2 Influence of LAS on isotherms of phenanthrene adsorption on soils

Results obtained using Method-1 (soil+phenanthrene+LAS) are illustrated in Fig. 23 and Fig. 24 and those obtained with Method-2 (soil+LAS+phenanthrene) are shown in Fig. 25 and Table 12. For high aqueous LAS concentrations (above 250 mg l^{-1}), both methods gave similar results, i.e. the amount of phenanthrene adsorbed on soils decreased with increases of aqueous LAS concentrations (up to 2000 mg l^{-1}). The distribution coefficient (K_d) dropped from 250.7 in the absence of LAS to 112.3 and 31.9 in the presence of 500 and 1000 mg l^{-1} of LAS, respectively (Table 12). The

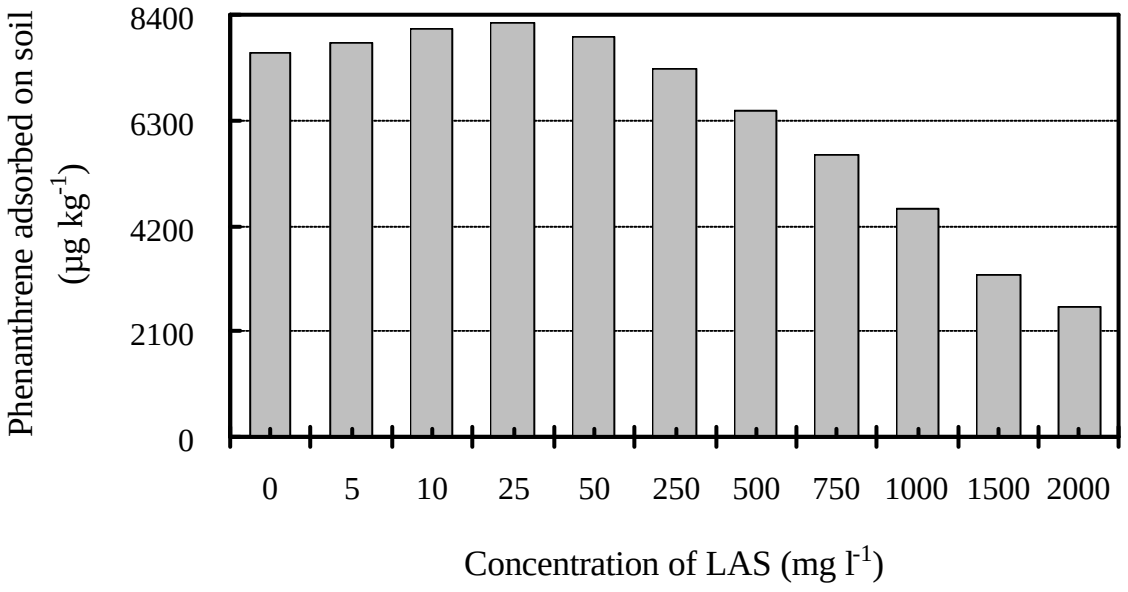


Fig. 23. Phenanthrene adsorption on soil in the presence of LAS obtained with Method-1 (soil+phenanthrene+LAS): Soil 2.3 from LUFA, Speyer, Germany; means of duplicates.

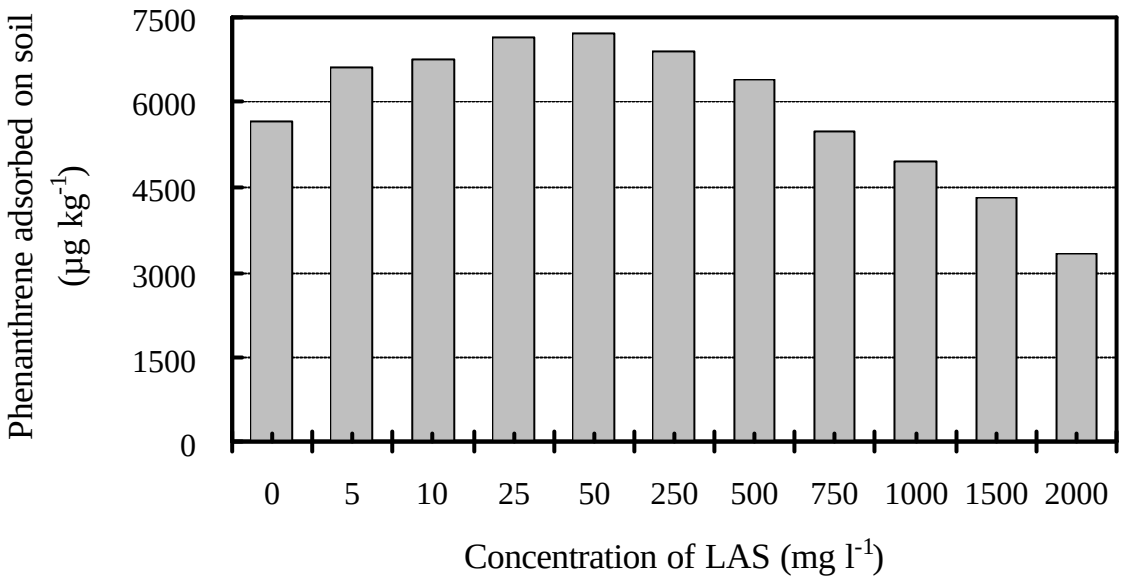


Fig. 24. Phenanthrene adsorption on soil in the presence of LAS obtained with Method-1 (soil+phenanthrene+LAS): Soil 13# from SES, Shenyang, China; means of duplicates.

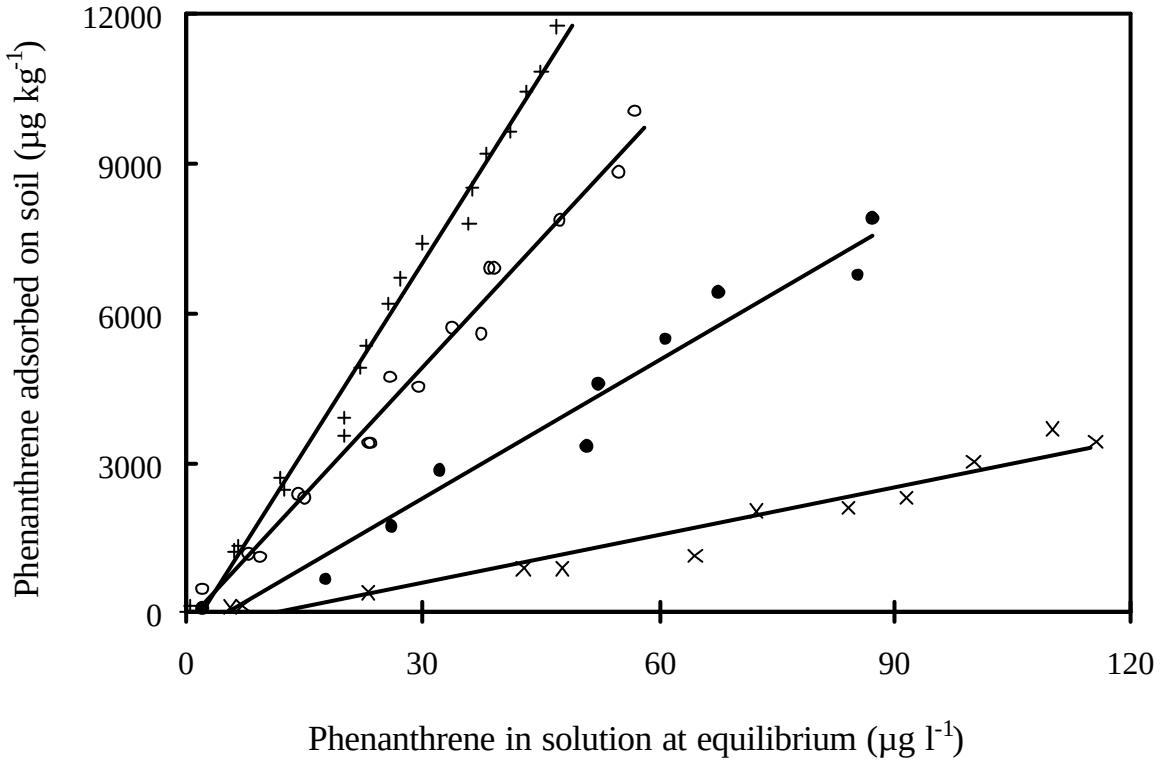


Fig. 25. Adsorption isotherms of phenanthrene on Soil 2.3 from LUFA, Speyer, Germany, obtained with Method-2 (soil+LAS+phenanthrene), in the presence and absence of LAS: (+) no LAS; (o) LAS = 25 mg l^{-1}; (•) LAS = 500 mg l-1; (×) LAS = 1000 mg l^{-1}.

Table 12. Distribution coefficients (K_d) and K_{oc} values of phenanthrene adsorption onto soil in the presence of different concentrations of LAS in solution [a]

LAS concentration (mg l^{-1})	0	25	500	1000
Kd	250.7	210.3	112.3	31.9
Log Koc	4.37	4.29	4.02	3.47

[a] Soil 2.3 from LUFA, Speyer, Germany. Results obtained with Method-2 (soil+LAS+phenanthrene).

rapid decrease of the adsorption of phenanthrene due to the significant solubilization of HOCs when CMC was approached as reported (Kile and Chiou, 1989; Edwards *et al*., 1991; Liu *et al*., 1991; Laha and Luthy, 1992), however, was not obvious around LAS CMC (in clean water 433.5 mg l^{-1} measured in this study and shown in Fig. 6 or *ca* 520 mg l^{-1} reported by Mukerjee and Mysels, 1971) under the described experimental conditions (both Method-1 and Method-2). In fact, the decrease of adsorption occurred well below LAS CMC e.g. 50 mg l^{-1} and 25 mg l^{-1} for Soil 2.3 obtained in Method-1 and Method-2, respectively. When adsorption of LAS on soil considered, those concentrations were even much lower than that (CMC_{eff}) required for LAS to form micelles. This suggested that there were other mechanisms causing the desorption of phenanthrene from soil beside solubilization.

At low aqueous LAS concentrations, the two methods delivered completely different results. When soil equilibrated with LAS prior to the addition of phenanthrene (Method-2), the adsorption of phenanthrene decreased in the presence of 25 mg l^{-1} LAS (K_d = 210.3) compared with results (K_d = 250.7) obtained in the absence of LAS (Fig. 25 and Table 12). This was consistent with the results obtained in high aqueous LAS concentrations. When the soil was exposed to phenanthrene prior to LAS (Method-1), however, the adsorption of phenanthrene increased with the increasing aqueous LAS concentrations from 0 to 25 mg l^{-1} for Soil 2.3 (Fig. 23) or from 0 to 50 mg l^{-1} for Soil 13$^{\#}$ (Fig. 24). Data obtained by Jafvert (1991) also indicate that aqueous concentrations of phenanthrene decrease slightly in the presence of dodecylsulfate of low concentrations in a soil-water system, which implies that the

adsorption of phenanthrene onto soil increases. Our results indicate that at low LAS concentration, the exposed sequence of soil to phenanthrene and LAS significantly affects their competition for active adsorption sites and consequently the adsorption amount on soils. A similar effect has been also observed by Yediler *et al*. (1991) that an increase of adsorption of o-cresol onto soils was caused by LAS in low concentrations.

In a system with the coexistence of phenanthrene, LAS, soil and water, the following interactions may occur simultaneously:

- competition for active hydrophobic adsorption sites (HASs) on soil surface between phenanthrene and LAS,
- equilibria of LAS among monomers, micelles and hemimicelles/admicelles (monomers adsorbed on soil surface),
- partition of phenanthrene among soil HASs, LAS micelles (i.e. solubilization) and organo minerals (hemimicelles/admicelles formed on soil surface), and
- interaction of phenanthrene with LAS monomers (Valsaraj and Thibodeaux, 1989; Jafvert, 1991; Liu *et al*., 1991; West and Harwell, 1992; Park and Jaffé, 1993).

Fig. 26 demonstrates the possible interactions among LAS and phenanthrene and soil surface. The apparent adsorption of phenanthrene depends on the extent and competition of each interaction. The interaction between PAHs and surfactant monomers is usually very weak and negligible (Edwards, *et al*., 1991; Liu *et al*., 1991; Park and Jaffé, 1993). When LAS concentration is low, e.g. less than 50 mg l^{-1}, which is much lower than CMC, there is no obvious solubilization of phenanthrene (Edwards *et al*., 1991; Liu *et al*., 1991; Laha and Luthy, 1992). Therefore, in low LAS concentrations, the apparent adsorption of phenanthrene mainly depends on the results of competition for active HASs on soil surface between phenanthrene and LAS, the formation of LAS hemimicelles/admicelles on soil surface, and the partition of phenanthrene to LAS hemimicelles/admicelles. The one adsorbed on active HASs on the soil surface is not so readily replaced by the other, which explains the decrease of phenanthrene adsorption at low LAS concentration when soil equilibrated with LAS prior to phenanthrene, as shown in the results of Method-2. At an equilibrium LAS

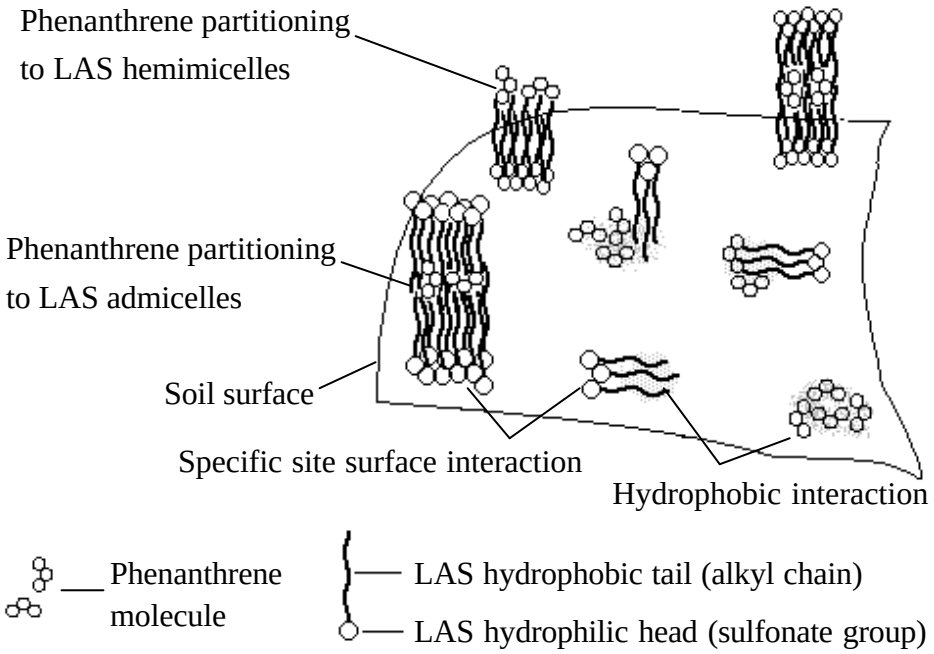


Fig. 26. Schematic sketch of phenanthrene adsorption on soil surface and partitioning to hemimicelles/admicelles of LAS formed on soil surface

concentration of 25 mg l^{1}, the amount of LAS adsorbed on soil by hydrophobic binding measured 44.3 mg kg^{-1} for Soil 2.3 or 30.8 mg kg^{-1} for Soil 13# (Fig. 18). This amount was much higher than that of adsorption of the coexistent phenanthrene (8.2 mg kg^{-1} and 6.8 mg kg^{-1} for Soil 2.3 and 13#, respectively, Fig. 23 and Fig. 24). The LAS so adsorbed probably occupied, to some extent, the active HASs on the soil surface and it might be that only a few of them could be replaced by phenanthrene added afterward. This caused the decrease of phenanthrene adsorption. It can be assumed that the rationale also applies to the results obtained with Method-1 in low LAS concentrations. When soil contacts with phenanthrene before LAS, phenanthrene adsorbed on soil is scarcely removed by LAS. On the other hand, LAS added after phenanthrene can adsorb on soil by their hydrophilic heads through specific site interactions or hydrogen bonding forming hemimicelles or admicelles, which creates more HASs to which additional phenanthrene partitioned (Fig. 26) (West and Harwell, 1992; Park and Jaffé, 1993). This resulted in an increase of apparent adsorption of phenanthrene (Fig. 23 and Fig. 24). The adsorption of phenanthrene on LAS

hemimicelles or admicelles occurred as well in the case when soil contacted with LAS before phenanthrene. But the amount of phenanthrene adsorption increased via this mechanism was much less than that decreased due to LAS occupation of active HASs, resulting in a decrease in apparent adsorption of phenanthrene (Fig. 25).

For LAS concentrations higher than 50 mg l^{-1}, competition of LAS for active HASs and its solubilization of phenanthrene both increased. When LAS concentration exceeded 60 mg l^{-1}, rapid and significant increase of LAS adsorption on soil due to cooperative adsorption was measured, which made active HASs further less available to phenanthrene. The results of these interactions were that less phenanthrene adsorbs on soils, regardless of the LAS- and phenanthrene-contacting sequence with soils. The adsorption of phenanthrene decreased almost linearly with the increase of LAS concentration above 50 mg l^{-1}. Only slightly rapid decline of adsorption of phenanthrene was observed at LAS concentrations from 1000-1500 mg l^{-1} and 500-750 mg l^{-1} for Soil 2.3 and Soil 13$^{\#}$, respectively (Fig. 23 and Fig. 24). It was likely that LAS micelles formed at these concentrations and that solubilization of phenanthrene considerably increased due to its partitioning into LAS micelles (Mori *et al*., 1982; Jafvert, 1991; Liu *et al*., 1991; Laha and Luthy, 1992). According to Valsaraj and Thibodeaux (1989), LAS-micelle-water partition coefficient (K_m) of HOCs can be estimated from their octanol-water partition coefficient (K_{ow}), i. e.

$$\log K_m = 0.865 \log K_{ow} + 0.92 \qquad (3)$$
$$= 0.865 \times 4.57 + 0.92 = 4.87$$

where $\log K_{ow} = 4.57$ for phenanthrene (Karickhoff *et al*., 1979). The value of $\log K_m$ is higher than of $\log K_{oc}$ (4.37 in the absence of LAS and 4.02 and 3.47 in the presence of 500 mg l^{1} and 1000 mg l^{1} of LAS, respectively (Table 12)), which suggests that LAS did enhance the solubility of phenanthrene in the studied suspension concentration and affinity of phenanthrene to LAS micelles was higher than that to the soils studied.

The solubilization of phenanthrene by LAS is demonstrated is Fig. 27. For both soils,

the aqueous concentrations of phenanthrene slightly decreased at low LAS concentrations and then steadily increased when LAS concentrations were above 50 mg l^{-1}. A similar solubilization pattern of phenanthrene by dodecylsulfate was also observed by Jafvert (1991). The increase of solubilization of phenanthrene by LAS was slightly higher at LAS concentrations in the range of 500 -1000 mg l^{-1}.

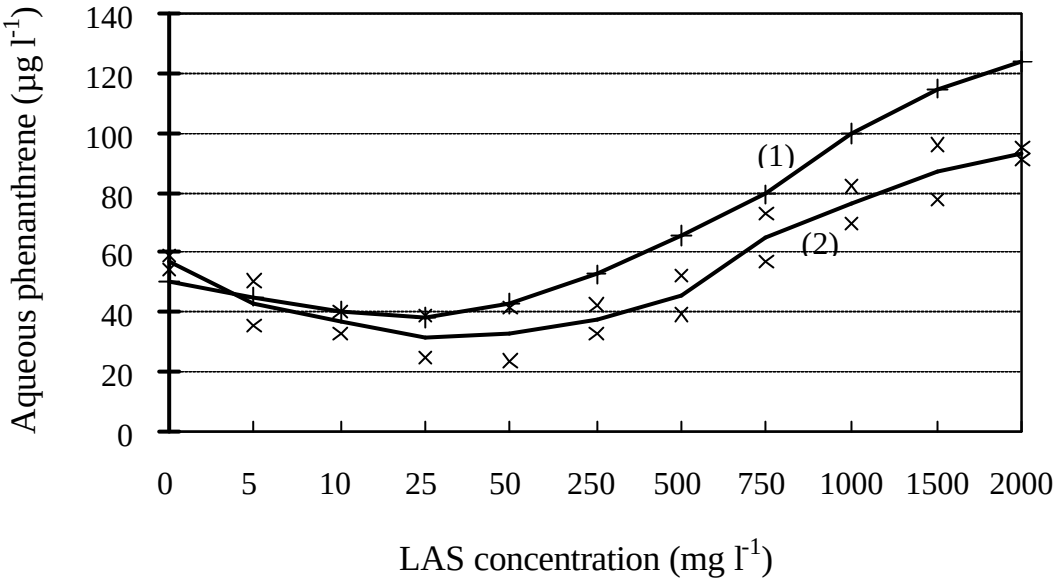


Fig. 27. Solubilization of phenanthrene by LAS in soil suspension: (1) Soil 2.3 from LUFA, Speyer, Germany (+); (2) Soil 13# from SES, Shenyang, China (×).

4.1.2 Migration of LAS in soils

4.1.2.1 LAS applied directly to top layer of soil

4.1.2.1.1 Migration of LAS in repacked soil columns

Results of LAS migration via water in repacked soil column with three different initial LAS concentrations applied in top 20-cm soil (100, 500 and 1000 mg kg^{-1}) and under three leaching intensities (8, 16 and 32 cm^3 d^{-1} or CSA-normalized 5, 10 and 20 mm d^{-1}, respectively) are shown in Fig. 28 and Fig. 29.

At high leaching intensity (32 cm^3 d^{-1} or 20 mm d^{-1}), LAS broke through the 1-m soil monolith in 13 to 16 days, which corresponded to leaching volumes of 445-493 cm^3

(or 280-310 mm after normalized to column CSA), regardless initial LAS concentrations. At medium leaching intensity (16 cm^3 d^{-1} or CSA-normalized 10 mm d^{-1}), breakthrough of LAS was detected at day 28 to 30. Breakthrough of LAS could

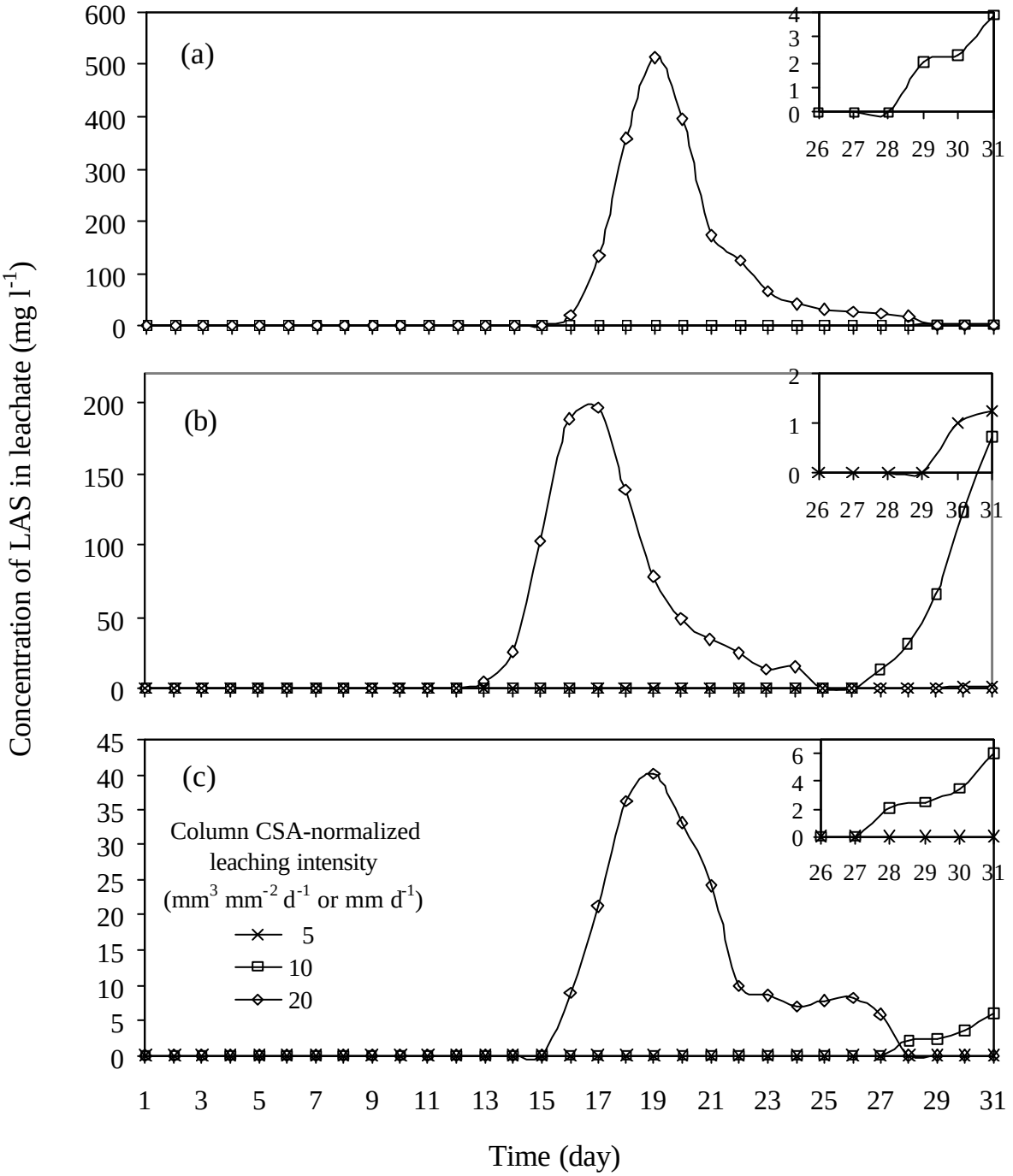


Fig. 28. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China), expressed as LAS concentrations in leachate as function of time. Initial LAS concentration in top 20-cm soil: (a) 1000 mg kg^{-1}, (b) 500 mg kg^{-1}, and (c) 100 mg kg^{-1}.

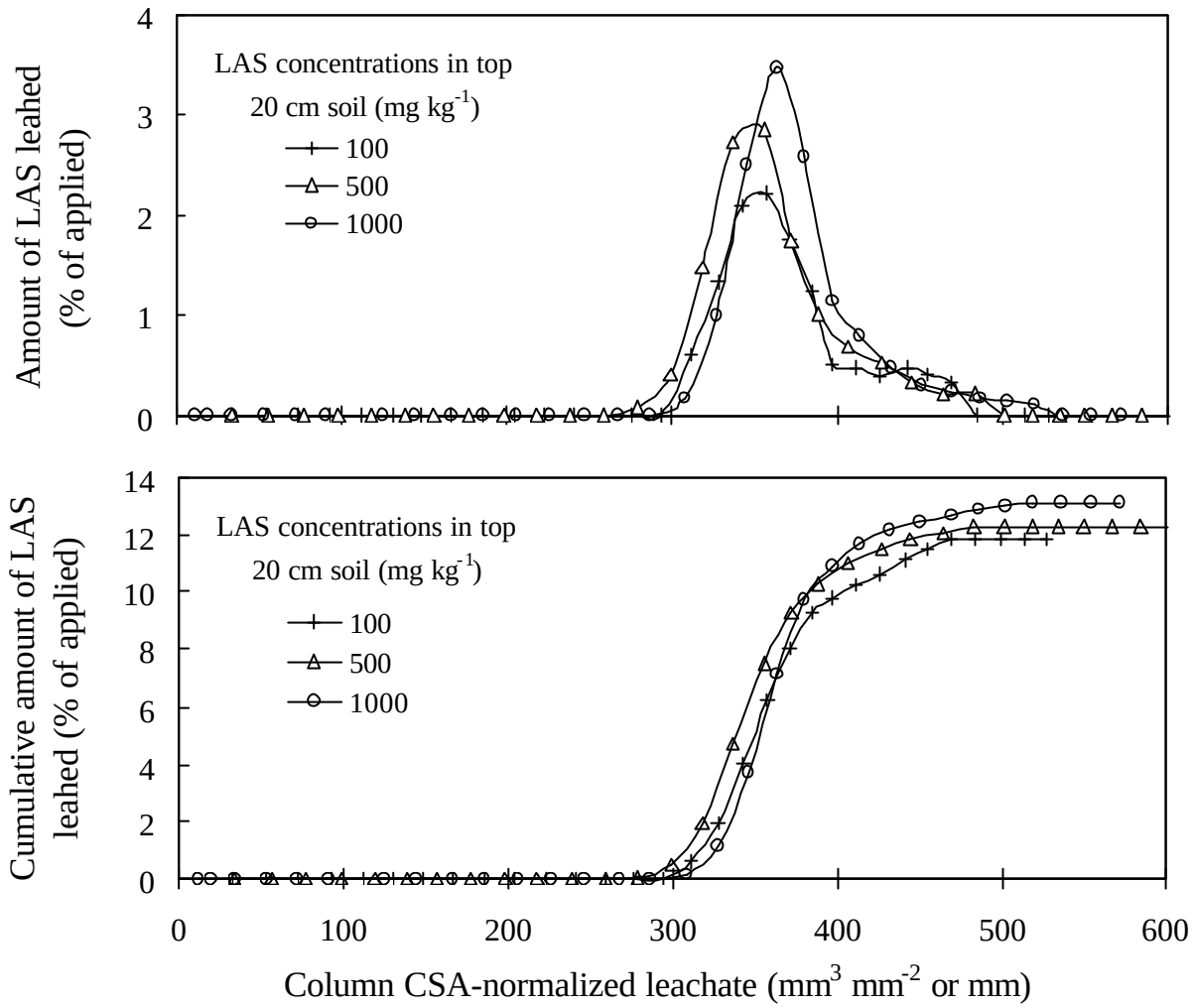


Fig. 29. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China) at high leaching intensity (32 cm^3 d^{-1} or 20 mm d^{-1}), expressed as amount of LAS in leachate as function of leachate collected. (CSA = cross section area)

not be observed basically for low leaching intensity (8 cm^3 d^{-1} or 5 mm d^{-1}). There was no correlation between breakthrough time and initial LAS concentrations. These results suggest that velocity of LAS migration in soil was merely function of water flow and adsorption/desorption equilibrium. This was proved by the fact that LAS breakthrough time was significantly and linearly correlated to the leaching intensity negatively as shown in Fig. 30 even though the data were obtained at different LAS concentrations. The breakthrough time declined form 30 days at leaching intensity of 5 cm^3 d^{-1} (or 3 mm d^{-1}) to 13 days at leaching intensity of 35 cm^3 d^{-1} (or 22 mm d^{-1}).

The initial LAS concentrations, however, did govern the effluent concentration and

amount (both peak and cumulative values) of LAS leached, i.e. the higher initial concentration, the higher effluent concentration and absolute amount leached. A significant correlation existed between initial concentration and effluent concentration (data not shown) or amount of LAS leached (Fig. 31). The total amount leached after 31 days increased from 5 mg (11.9% of the applied LAS) to 54 mg (13.1% of the applied LAS) with the increase of soil initial LAS concentration from 100 to 1000 mg kg^{-1}, respectively.

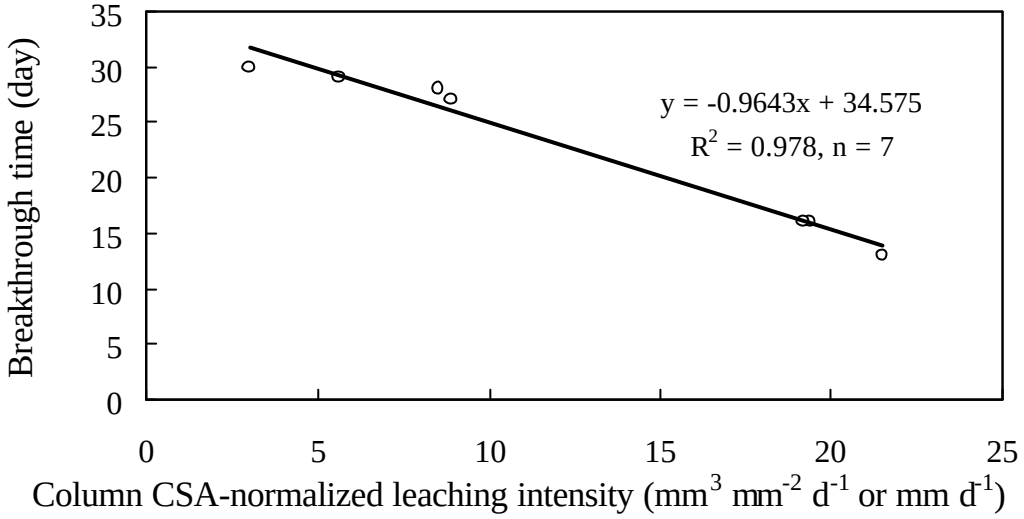


Fig. 30. Relationship between LAS breakthrough time and leaching intensity in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China)

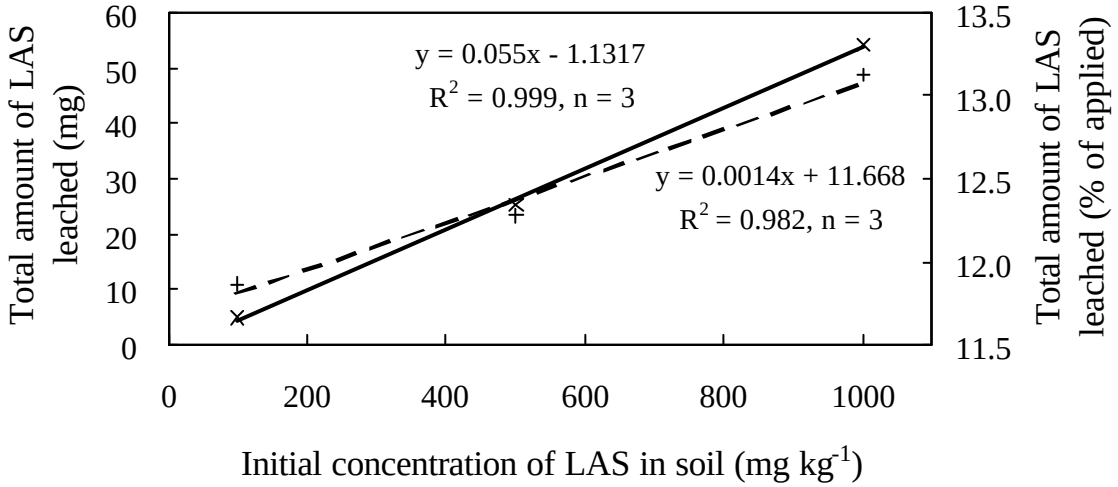


Fig. 31. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China): relationship between amount of LAS leached and initial LAS concentration in top 20-cm soil at leaching intensity of 32 cm^3 d^{-1} (or CSA-normalized 20 mm d^{-1}), (×) absolute amount, and (+) percentage of applied.

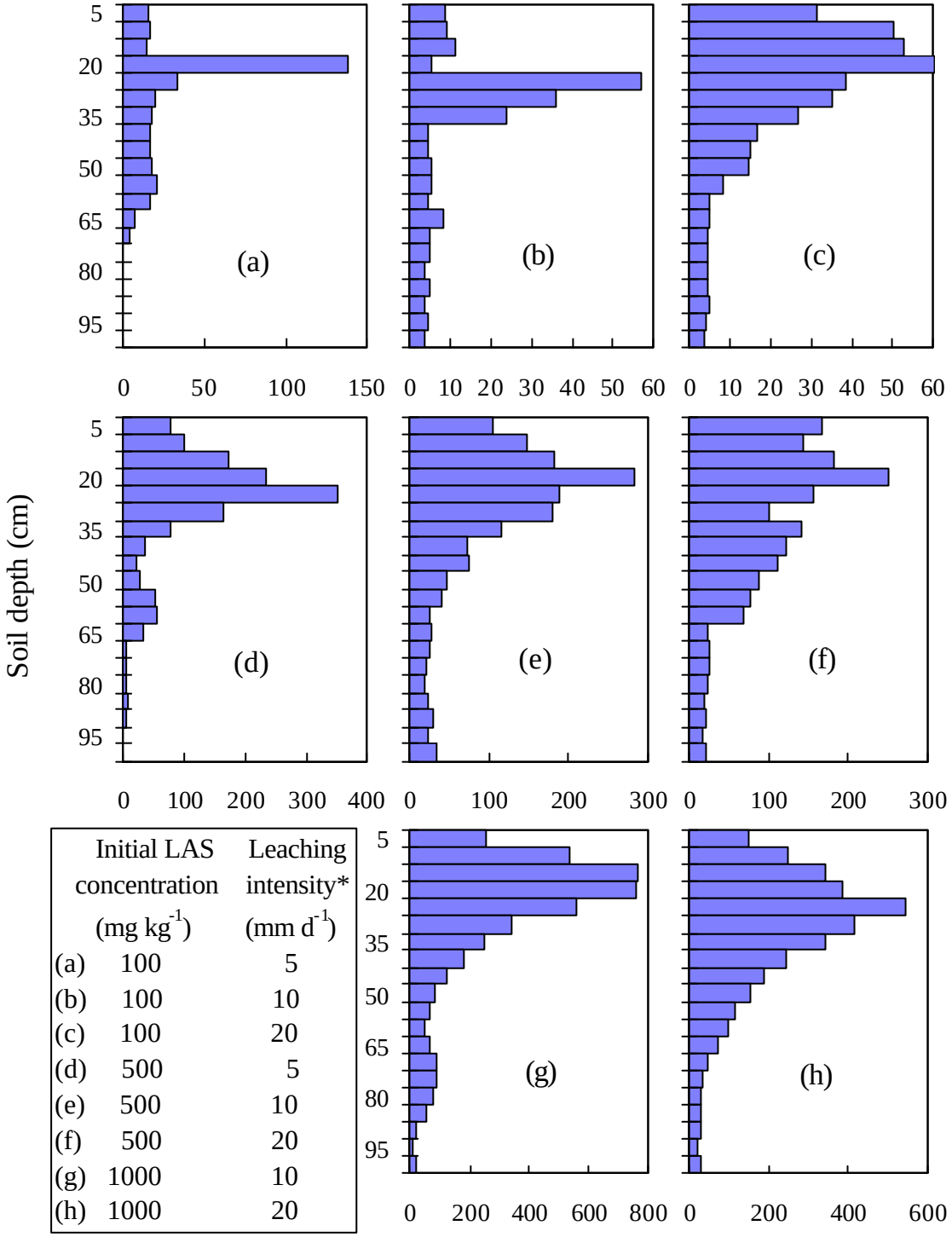
Data of residual LAS in soil (Fig. 32) also show the dominant effect of leaching intensity and volume on the migration of LAS in soil. At low leaching intensity (8 cm^3 d^{-1} or CSA-normalized 5 mm d^{-1}) and after leached for 31days, LAS migrated to 70 cm and 90 cm depth for initial LAS concentrations of 100 and 500 mg kg^{-1}, respectively. At higher leaching intensity (16 and 32 cm^3 d^{-1} or CSA-normalized 10 and 20 mm d^{-1}), LAS moved deeper to the bottom 5-cm soil (a depth of 100 cm) for the same as well as for the 1000 mg kg^{-1} initial LAS concentration.

Although LAS broke through the soil columns in relatively short time at high leaching intensity (13 to 16 days) and LAS was detected in almost all layer of the soil profile (except the two columns leached under low intensity), the highest LAS concentrations occurred at 20 to 25 cm soil layer and 64 to 81% (74±4% average) of LAS remained in the top 35-cm soil layer. Further analysis shows that 41(±12)%, 36(±11)%, 13(±5)%, 6(±2)% and 4(±3)% of the average residual LAS for all columns were found in soil layers of 0-20, 20-40, 40-60, 60-80 and 80-100 cm, respectively. Accounting for the amount of LAS leached with water, less than 40% of the applied LAS remained in the top layer of 20-cm soil where it was applied. A total of about 20% of the applied LAS moved beyond 40 cm. These results suggest that the migration of LAS in soil was significant.

4.1.2.1.2 Migration of LAS in soils of field lysimeters cropped with rice and soybean

Fig. 33 is the effluent from lysimeters cropped to paddy rice and soybean in the whole crop growth season with comparison of rainfall of the same period. Effluent from lysimeters of the same crop varied in almost the same pattern and magnitude suggesting that those lysimeters had similar hydraulic characteristics and providing the basis for comparison among lysimeters. The variation of effluent was consistent with the change of rainfall especially for soybean which received no irrigation and water mainly came from rainfall. Since irrigated whenever rice needed, lysimeters cropped with paddy rice had a higher and steadier effluent over the whole growth period.

Fig. 34 and Fig. 35 show the monitoring results of LAS in effluent of field lysimeters.



	Initial LAS concentration (mg kg^{-1})	Leaching intensity* (mm d^{-1})
(a)	100	5
(b)	100	10
(c)	100	20
(d)	500	5
(e)	500	10
(f)	500	20
(g)	1000	10
(h)	1000	20

Fig. 32. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China): Distribution of residual LAS in soil.

* Leaching intensities (mm^3 mm^{-2} d^{-1} or mm d^{-1}) after normalized to column cross section area.

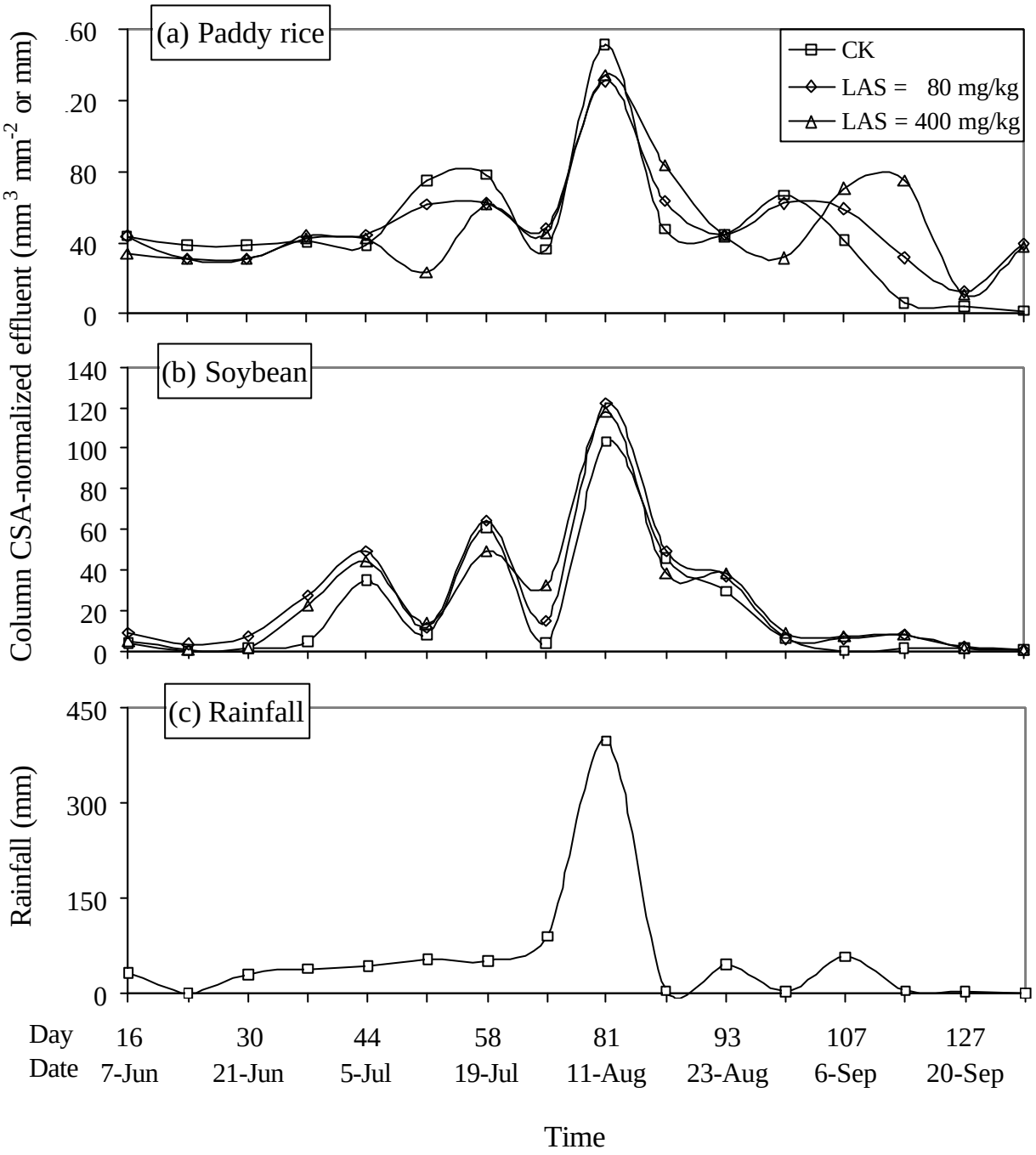


Fig. 33. Effluent from field lysimeters cropped with (a) paddy rice and (b) soybean *vs* time in comparison with (c) rainfall, located at SES, Shenyang, China. (CSA = cross section area)

The following phenomena can be obviously seen from these curves showing the changing of LAS concentration in leachate. For soybean lysimeters, in 30 days after the beginning of experiment, LAS concentrations in leachate reached their first peaks for both LAS concentrations studied (Fig. 34). Then LAS concentrations gradually

declined. LAS concentrations increased again at day 58 and reached their second peaks at day 100 and day 107 for lysimeters with initial soil LAS concentrations of 80 and 400 mg kg^{-1}, respectively. LAS concentrations decreased afterward till the end of experiments. The peak leachate LAS concentrations were proportional to the amount of LAS applied. The migration of LAS in paddy soil was similar. First peak leachate LAS concentrations appeared at day 30 and 37 for lysimeters applied with 400 and 80 mg kg^{-1} LAS, respectively. Then, LAS concentrations declined gradually. The second corresponding peak occurred at day 81 and 86, respectively.

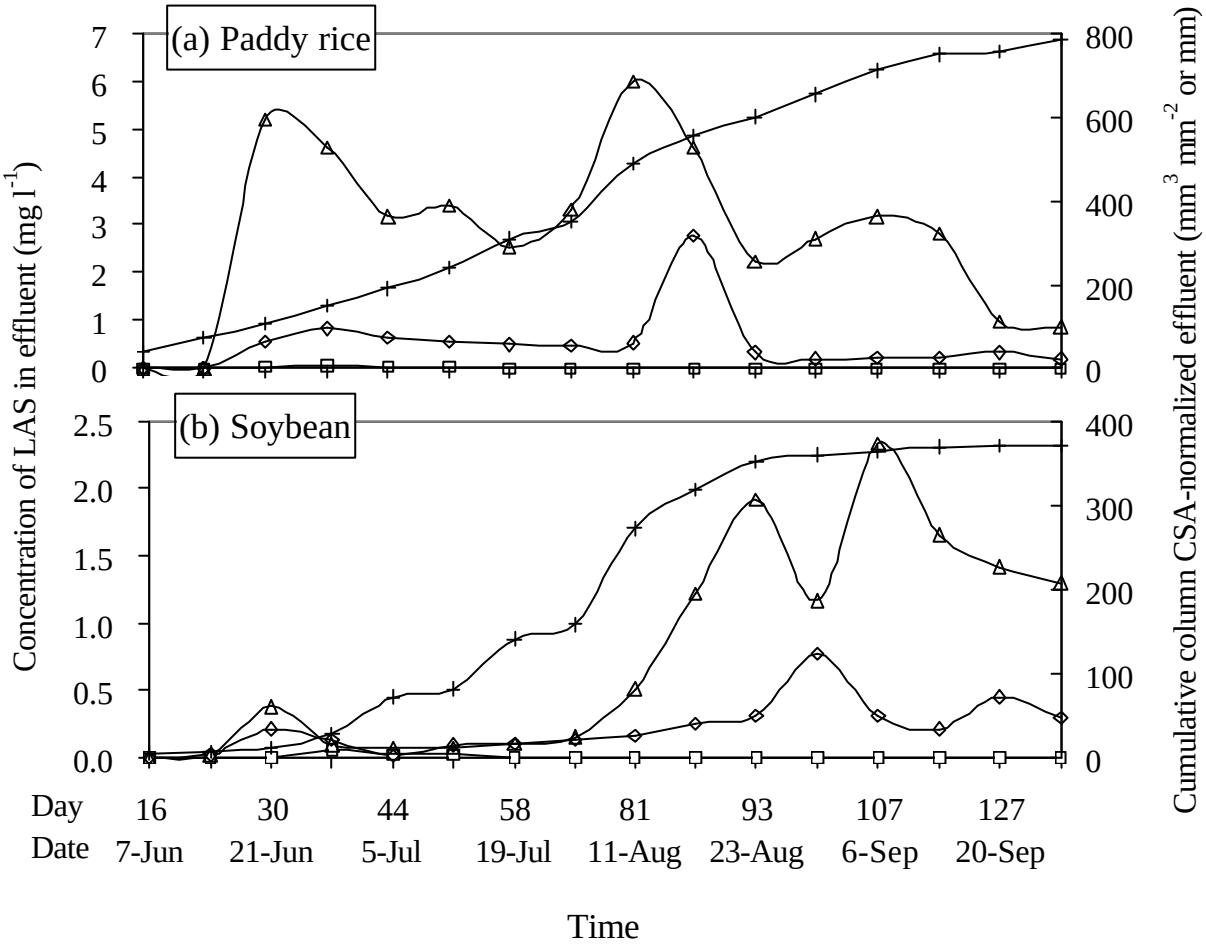


Fig. 34. Migration of LAS in 1.5-m soil monolith of field lysimeters located at SES, Shenyang, China, expressed as LAS concentration in effluent *vs* time in comparison with (+) average cumulative volume of effluent. Initial LAS concentrations in top 20-cm soil: (□) 0 (CK), (◇) 80 mg kg^{-1}, and (△) 400 mg kg^{-1}.

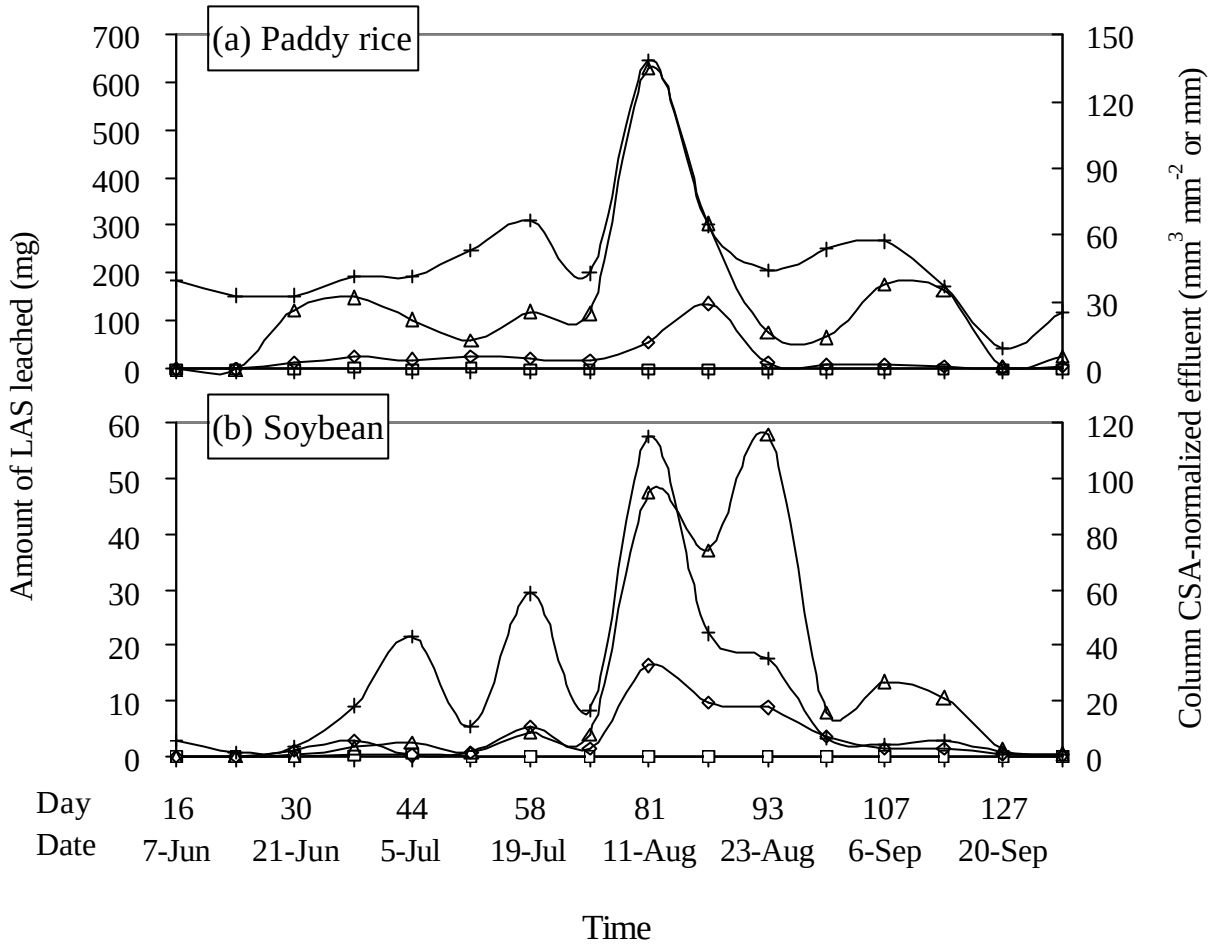


Fig. 35. Migration of LAS in 1.5-m soil monolith of field lysimeters located at SES, Shenyang, China, expressed as the amount of LAS in effluent *vs* time in comparison with (+) average volume of effluent. Initial LAS concentrations in top 20-cm soil: (□) 0 (CK), (◇) 80 mg kg^{-1}, and (△) 400 mg kg^{-1}. (CSA = cross section area)

If the breakthrough of LAS in lysimeters is defined as when LAS concentrations in the leachate of lysimeters applied with LAS (C_{out}) are two times higher than the standard deviation of LAS concentrations in leachate from control lysimeters (CKC_{out}), i.e. $C_{out} > 2Sx,CKC_{out}$, the breakthrough of LAS happened at day 23 for both paddy soil and soybean soil. After the first LAS concentration peaks, however, the times at which LAS concentrations started to increase again (second breakthrough times) were much different for the two soils, i.e. 44 days for paddy soil and 58 days for soybean soil (Fig. 34).

Although the first breakthrough times of LAS were the same for both paddy and soybean soils, their first breakthrough leachate volumes were much different, i.e.

54165 cm^3 (or 69 mm after normalized to column CSA) for paddy soil and only 7065 cm^3 (or CSA-normalized 9 mm) for soybean soil (Fig. 34). In other words, the first breakthrough of LAS in both soils was time constant and independent of leachate volume in the range of 7065 to 54165 cm^3 (or 9 to 69 mm) monitored. This suggests that at this stage, water and LAS leached through soil monolith at a constant speed as long as the leachate volume met certain amount, and the adsorption/desorption of LAS on soil was far from equilibrium. The difference of breakthrough volumes of the two soils implies that the process of first LAS breakthrough was not controlled by soil adsorption/desorption mechanism. Reasonable explanation for these phenomena is that there were macropores and preferential flow in the test soil. LAS broke through soil monolith rapidly via preferential flow before it interacted with soil. Based on breakthrough time, peak concentrations and the changing of LAS concentrations over the whole period, it can be also concluded that the first breakthrough was a result of LAS preferential migration caused by preferential water flow. The phenomenon of preferential breakthrough of LAS could not be observed in soil monolith without macropores (see Section *4.1.2.1.1.* and Fig. 28).

Freeze-thaw cycle can cause the formation of soil macropores and associated preferential flow (Ou *et al*., 1999). In Shenyang area where winter is extremely cold (lowest mean monthly temperature of -12.2 ^oC in January (Table 4)) and soil freeze-thaw effects are very strong, macropores and preferential flow developed to a certain extent in these two soils. Water conditions of paddy and soybean soils are different in growing seasons, but soil moisture in winter period is similar and the effects of freeze-thaw cycle on both soils can be expected to be the same. Therefore, both soils should have similar macropores in magnitude and morphology. This explains why the preferential breakthrough time of LAS in both soils was the same.

The second breakthrough volumes for soils cropped to paddy rice and soybean were very close, i.e. 145225 cm^3 (or 185 mm) and 139730 cm^3 (or 178 mm), respectively, though their breakthrough times were much different (44 days for paddy soil and 58 days for soybean soil). This suggested that equilibrium between adsorption and desorption of LAS on soil had established. The second breakthrough was a “real”

breakthrough controlled by soil adsorption/desorption processes, which depends on soil characteristic and flux of water flow. Although cropped to different crops for 3 years, the differentiation of the two soils, which originally was the same soil when packed to lysimeters, in characteristic was still not significant to affect their adsorption/desorption process.

The preferential breakthrough of LAS was 21 and 35 days ahead of “normal” breakthrough governed by soil adsorption/desorption processes for paddy and soybean soils, respectively. These results indicate that preferential flow is of significant effects on migration velocity and transport distance of solute in soil. In soybean soil, the peak leachate LAS concentrations resulted from preferential breakthrough accounted for 20-30% (depending on initial LAS concentrations) of those measured at equilibrium breakthrough. These ratios were as high as 30-86% for paddy soil. These data show that preferential flow also has significant effects on the amount of solute migration in soil. Significantly greater preferential migration of LAS in soils cropped to paddy rice than in those cropped to soybean was a result of much greater preferential flow flux in the former than the latter.

Data obtained in field lysimeters confirmed some of the results obtained with repacked columns in laboratory studies. As shown before, the migration velocity of LAS in soil was a function of water flow flux in the soil and independent of LAS concentration in the soil. Good correlation could be expected between breakthrough time and leaching intensity (Fig. 30). Concentration of LAS in effluent and amount of LAS leached depended on its concentration in the soil (Fig. 31). Excellent correlation was also found in field lysimeters between the effluent concentration and amount of LAS leached and the initial LAS concentration in top 20-cm soil for both lysimeters cropped to rice and soybean (Fig. 36). The total amount of LAS leached was also a function of the volume of leachate. This could be observed in field lysimeters as well that total amount of LAS leached varied along with the volume of effluent (Fig. 35).

Migration studies using repacked soil columns under more controlled conditions are necessary to obtain some parameters that are difficult or impossible to gain in fields. It

should be pointed out, however, that caution should be taken when predicting LAS migration behaviour in field from laboratory data. In addition to possible preferential migration in the field, the equilibrium migration of LAS also differed between repacked soil and natural soil. For example, LAS breakthrough time and effluent in laboratory studies with repacked columns were 13 to 16 days and 445 to 493 cm^3 (or 280 to 310 mm after normalized to 5cm-column CSA), respectively. The corresponding breakthrough time and effluent in field lysimeters, however, were 44 (for rice) to 58 days (for soybean) and 139730 to 145225 cm^3 (or 178 to 185 mm after normalized to 100cm-column CSA).

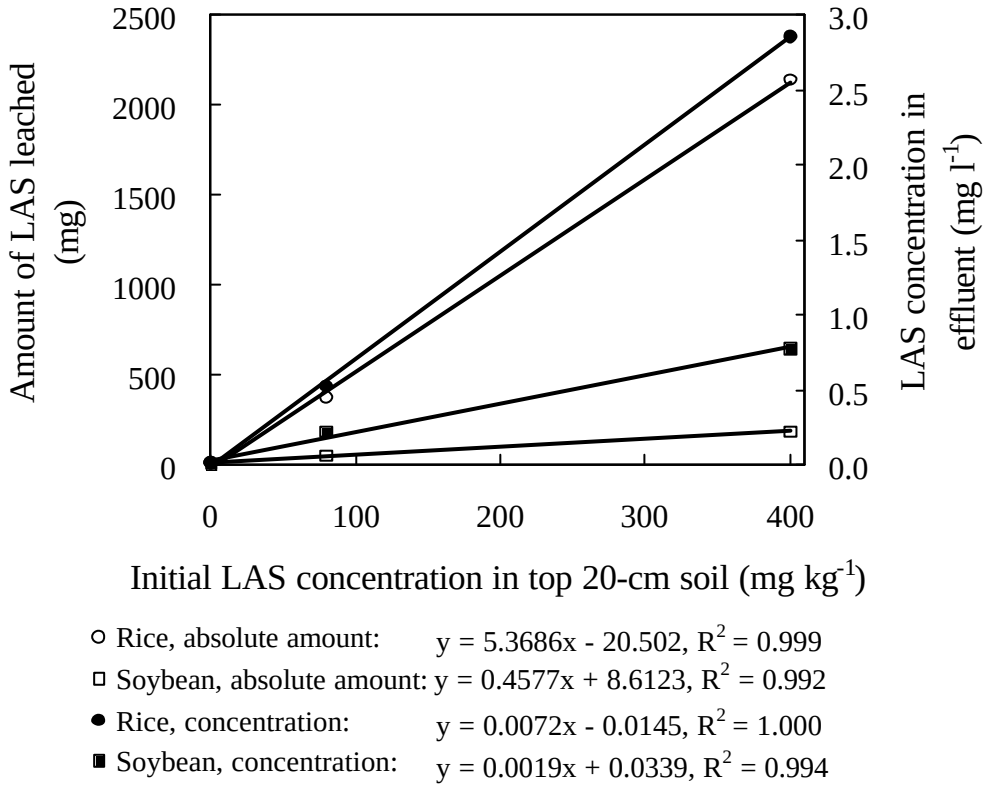


Fig. 36. Migration of LAS in 1.5-m soil monolith of field lysimeters located at SES, Shenyang, China: relationship between amount and concentration of LAS in effluent and initial LAS concentration in top 20-cm soil.

4.1.2.2 LAS applied to soil via irrigation water

4.1.2.2.1 Migration of LAS in repacked soil columns not subjected to freeze-thaw treatment

The size of column and soil used for this study were the same for migration of LAS in

repacked soil columns with application of LAS directly to top soil. The breakthrough of LAS applied via irrigation water was at day 8 to 9 (data not shown) regardless of LAS concentrations in the leaching solution. The corresponding effluent volumes were 382 to 461 cm^3 (or 240 to 290 mm) (Fig. 37). Although these LAS breakthrough times were shorter than those with LAS applied directly to the top soil (13 to 16 days), their breakthrough volumes of effluent were very close, i.e. 445 to 493 cm^3 (or 280 to 310 mm) for the later (see Section *4.1.2.1.1*.). Since the two experiments used different leaching intensities, it is not reasonable to directly compare their breakthrough time. 8 to 9 days breakthrough time was obtained under a leaching intensity of 56 cm^3 d^{-1} (or 35 mm d^{-1}) while 13 to 16 days breakthrough time was a result under leaching intensity of 32 cm^3 d^{-1} (or 20 mm d^{-1}). The difference of their leaching intensities was a factor of 1.75, which was very closed to that of breakthrough time (1.71). The migration distance of LAS was almost the same (290 to 298 mm) in the two experiments. This indicates that LAS had exactly the same migration velocity in the two leaching regimes when leaching intensity taken into account. This also implies the importance to mention leaching intensity whenever breakthrough time is referred.

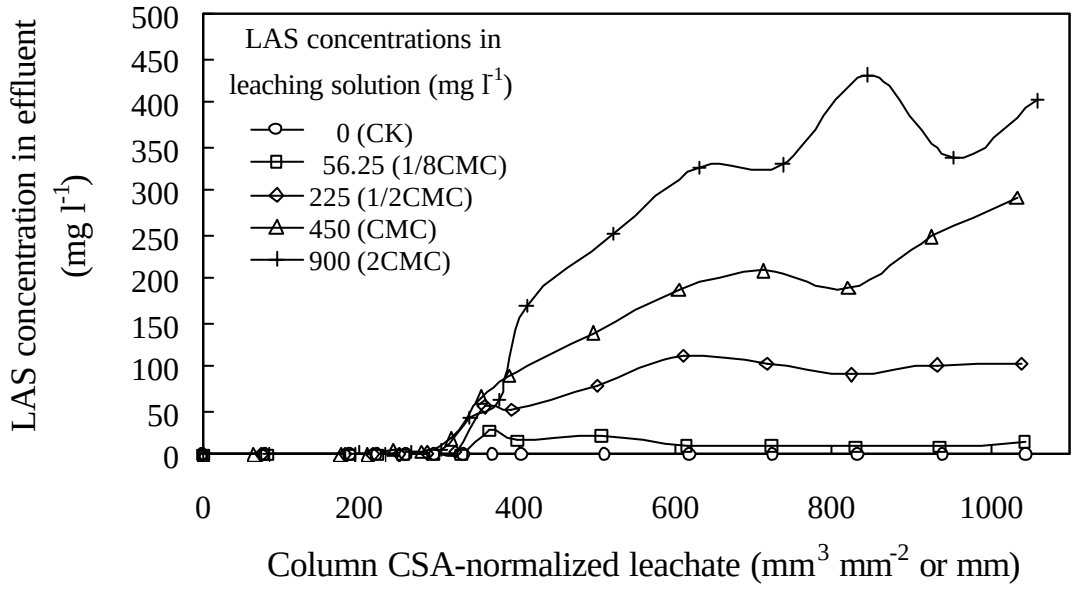


Fig. 37. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China) at leaching intensity of 56 cm^3 d^{-1} or 35 mm d^{-1}, expressed as LAS concentrations in leachate as function of effluent volume. (CSA = cross section area)

Above results proved again that the velocity of LAS migration in soil was governed by the water flow flux in the soil and independent of the amount as well as the methods of LAS application. When referred to the breakthrough effluent volume, there was no significant difference in migration velocity between LAS applied directly to soil and applied via irrigation water. That is LAS breakthrough of 1-m soil column at 445 to 493 cm^3 (or 280 to 310 mm) leachate whether it was applied directly to the soil or via solution.

At low LAS concentrations of leaching solution, excellent linear relationship was also found between the amount of LAS leached and the volume of effluent in this study in which LAS was continuously applied via leaching solution (Fig. 38). But when LAS concentrations were equal to or higher than CMC, the amount of LAS leached increased polynomially. After LAS breakthrough the columns, the amount of LAS leached (M_L) increased with the increase of leaching volume (L). At LAS concentration lower than CMC, the relationship between M_L and L can be described using a linear equation:

$$M_L = K_{c1}L - K_{c2} \quad (4)$$

where M_L is in unit of mg and L is expressed as column CSA-normalized leaching volume in $mm^3\ mm^{-2}$ or mm. K_{c1} (mg $mm^2\ mm^{-3}$) and K_{c2} (mg) assigned as concentration factor 1 and 2, respectively, were function of LAS concentration in leaching solution (Fig. 39), i.e.

$$K_{c1} = 6\times10^{-4}C + 7\times10^{-7}\,C^2 - 7\times10^{-10}\,C^3 - 0.006 \quad (5)$$

and

$$K_{c2} = 0.18\,C + 4\times10^{-4}\,C^2 - 4\times10^{-7}\,C^3 - 2.7973 \quad (6)$$

Equation (4) to (6) can be also used to estimate the amount of LAS leached at LAS concentrations ≥ CMC.

The physical meanings of the model can be explained as following. In the beginning of application of LAS via leaching solution, LAS was completely retained by the soil

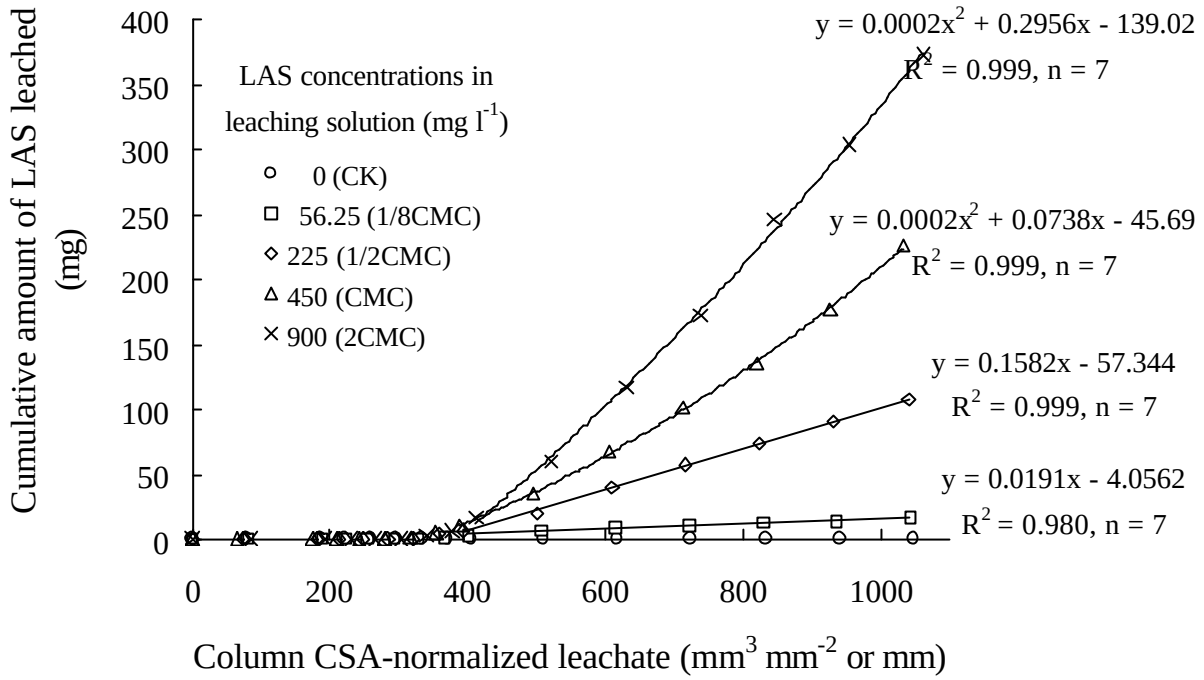


Fig. 38. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China) at leaching intensity of 56 cm^3 d^{-1} or 35 mm d^{-1}, expressed as amount of LAS leached *vs* the volume of effluent. (CSA = cross section area)

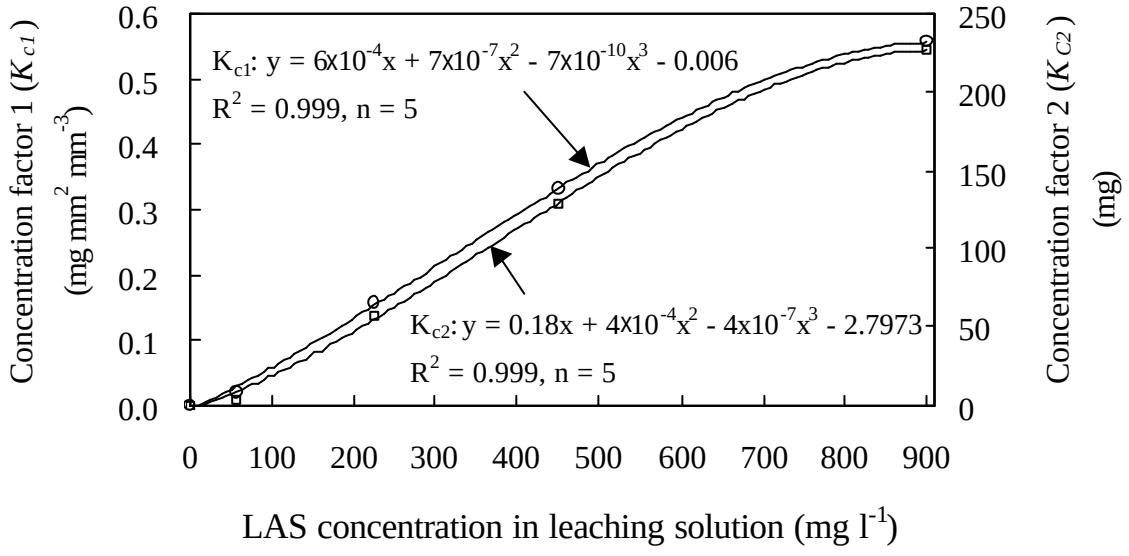


Fig. 39. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China) at leaching intensity of 56 cm^3 d^{-1} or 35 mm d^{-1}: influence of LAS concentration in leaching solution on the concentration factor 1 (K_{c1}) and 2 (K_{c2}) defined as the slope and intercept of linear regression equation of 'LAS leached'-'leaching volume', respectively.

column until breakthrough-leaching volume was reached. After LAS broke through the column, the amount of LAS leached increased linearly as the leaching volume increased. The extent to which leached-LAS increase depended on LAS concentration in the leaching solution (Fig. 40). The more LAS was applied, the more LAS leached out off the column.

Fig. 41 shows the relative LAS concentration (ratio of LAS concentration in the effluent (C_i) over that in leaching solution (C_0)) changing with leaching volume expressed as pore volume. LAS broke through soil columns at 1.3 to 1.4 pore volumes for all test concentrations. At LAS concentration of 1/8 CMC, a peak appeared at 1.7 pore volume and then declined till 4.3 pore volume. Relative concentration peak was not obvious for the other concentrations. However, their relative concentrations increased till almost 5 pore volume as the column was leached with more LAS solution. At pore volume of 4.8 in the end of the test after leaching for a total of 30 days, LAS concentrations in the effluent were lower than half (0.45 to 0.46) of those in leaching solution except for LAS concentration at CMC. The highest relative concentration (0.65) was at CMC and the lowest (0.27) was at 1/8 CMC. Till the end of the test, 74 to 85% of the LAS applied were retained in the soil column and only 15 to 26% of the LAS applied were leached out off the column. Fig. 42 shows that the

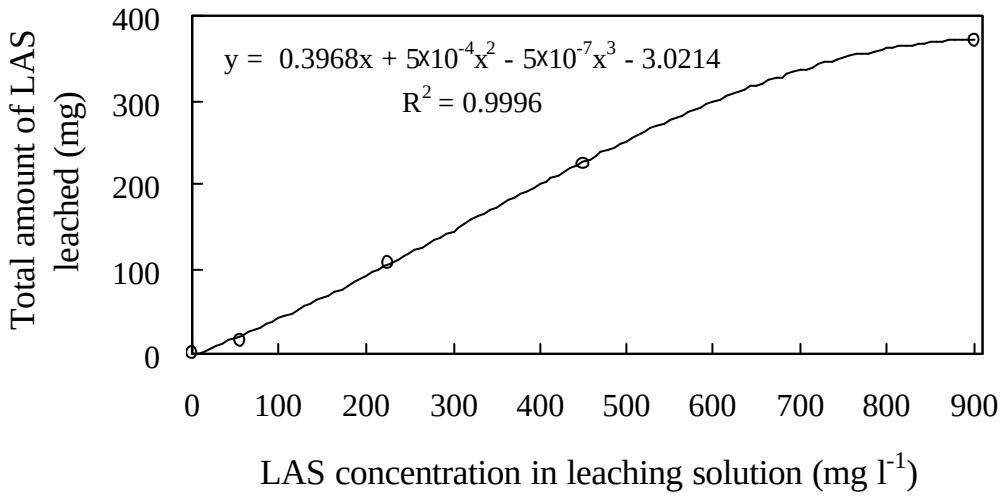


Fig. 40. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China): relationship between the amount of LAS leached and the concentration of LAS in leaching solution.

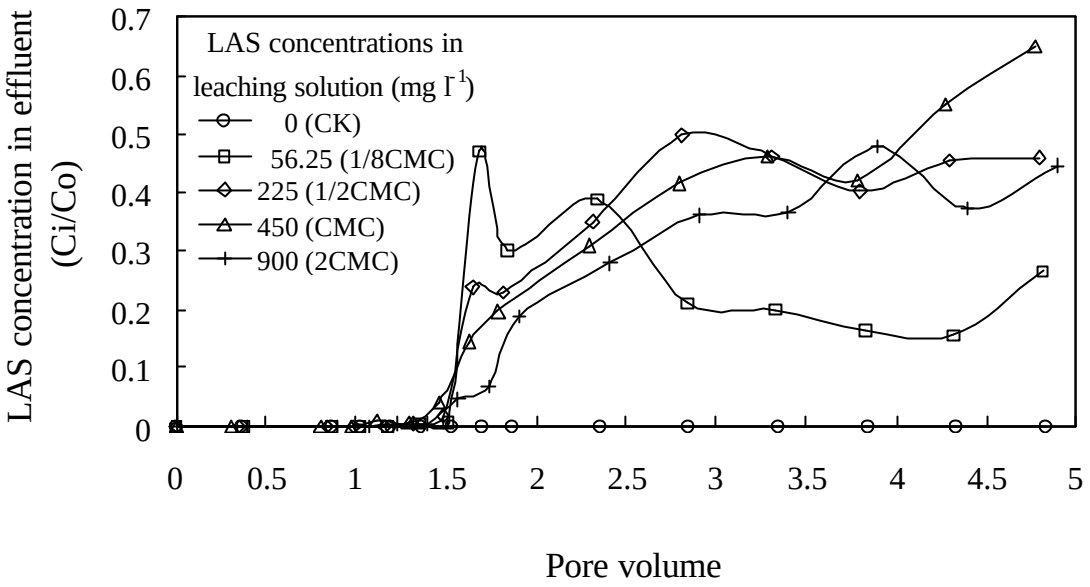


Fig. 41. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China): breakthrough curve.

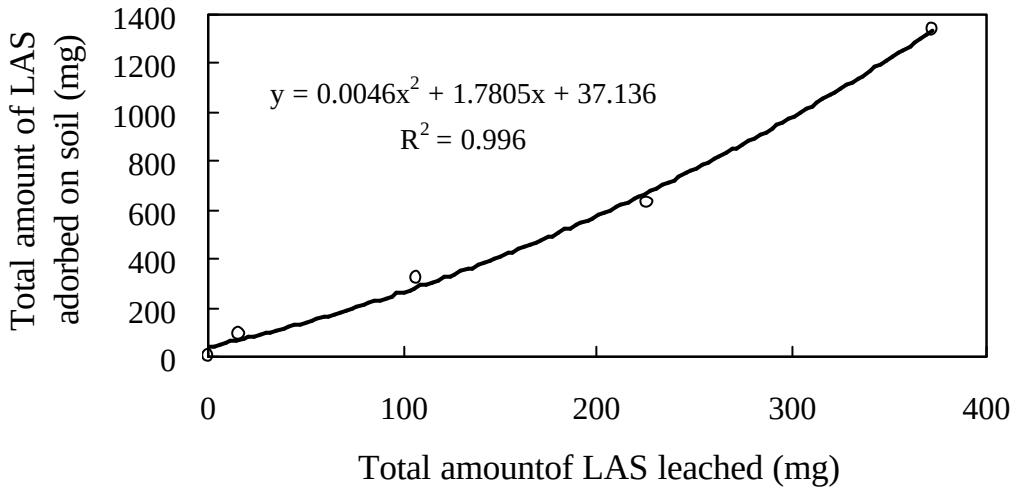


Fig. 42. Migration of LAS in 1-m repacked soil column (Soil 13#, 14# and 15# from SES, Shenyang, China): relationship between total amount of LAS retained in the column and that leached out off the column

amount of LAS adsorbed on soil increased faster than that leached via effluent, indicating more LAS was retained by soil than migrated via leachate and the soil was far from saturated with LAS under experimental conditions employed.

4.1.2.2.2 Migration of LAS in repacked soil columns subjected to outdoor freeze-thaw treatment

Although the columns were subjected to outdoor freeze-thaw treatment from early autumn to late spring, no preferential migration of LAS was observed as expected (Fig. 43 and Fig. 44). Similar to soil columns not subjected to freeze-thaw treatment, there was a hysteresis period before the breakthrough of LAS. The breakthrough of LAS was at day 12 to 13 and 2983 to 3376 cm^3 (or 380 to 430 mm) leachate. It was higher than that without freeze-thaw treatment (240 to 290 mm) in terms of leaching volume. However, other trends were the same, i.e. LAS concentrations in effluent increased with the increase of leaching volume, and the higher the concentration of LAS in the leaching solution, the higher the increase of LAS concentration in effluent (Fig. 43).

When the relative concentration of LAS in effluent was examined (Fig. 44), a peak could also been observed for freeze-thaw-treated soil columns leached with 1/8 CMC and 1/2 CMC LAS solutions, which was consistent with results obtained for soil columns without treated by freeze-thaw processes (Fig. 41). However, the relative LAS concentrations at 1/8 CMC in the former was the highest at pore volumes from 2 to 4.8 compared with other leaching solution, while in the later they were the lowest.

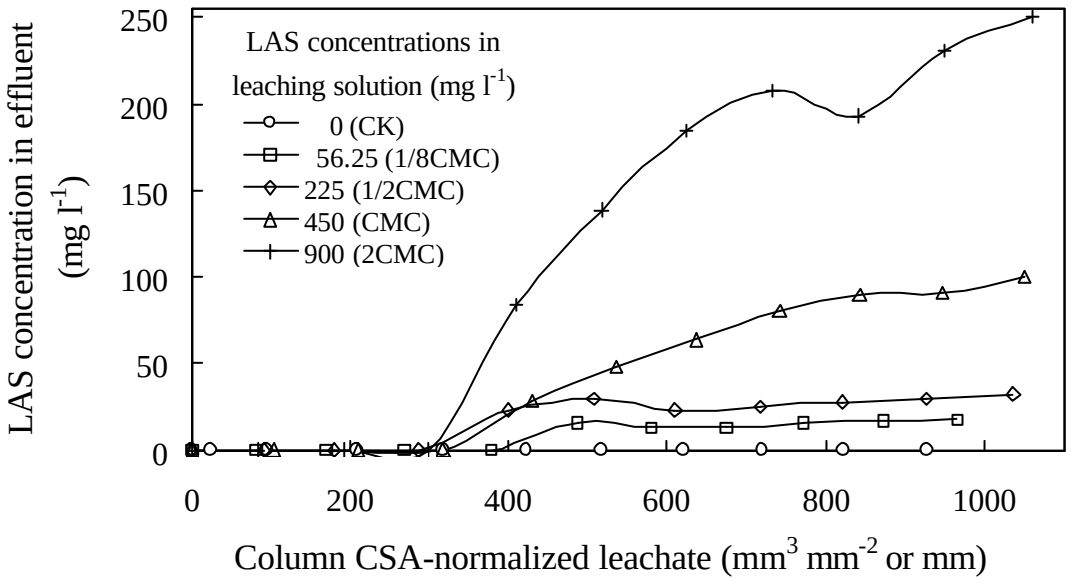


Fig. 43. Migration of LAS in 1-m repacked soil column subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China), expressed as LAS concentrations in leachate as function of effluent volume. (CSA = cross section area)

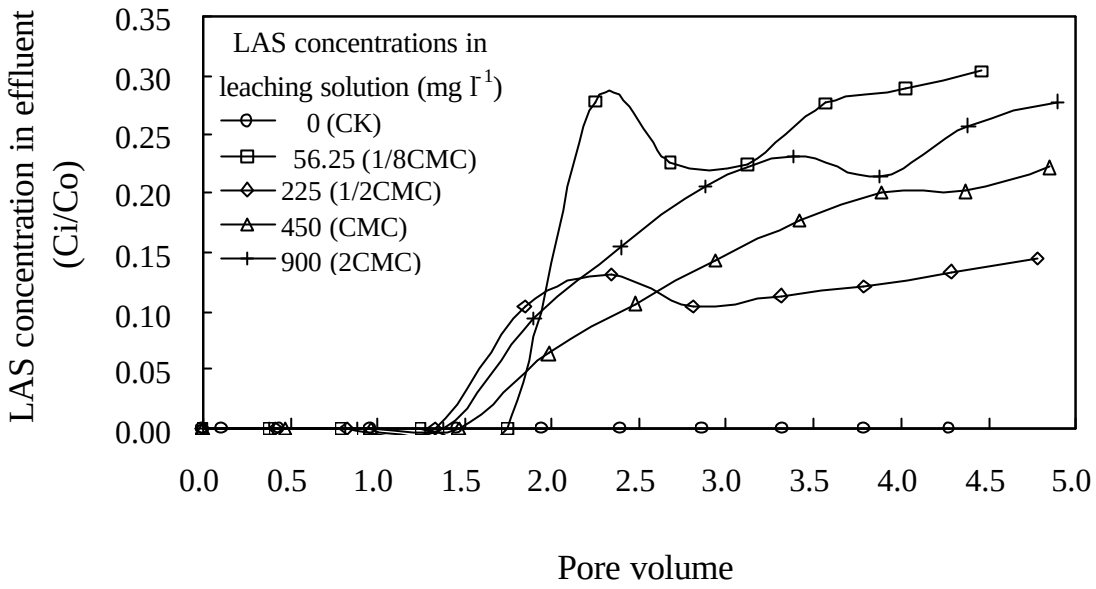


Fig. 44. Migration of LAS in 1-m repacked soil column subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China): breakthrough curve.

Under the same leaching conditions, LAS concentrations in effluent of soil columns not subjected to freeze-thaw treatment were higher than those of freeze-thaw treated soil columns (Fig. 37 and Fig. 43). Effluent LAS concentrations in the former were about half of those of leaching solution (Fig. 41), while they were only 20 to 30% in the later (Fig. 44). These results imply that more LAS was retained by freeze-thaw treated soil than freshly repacked soil. When compared based on section area of the column, the amount of LAS leached from untreated soil columns was about 4 times higher than that from freeze-thaw treated soil (Fig. 45).

The amount of LAS leached from freeze-thaw treated soil columns can be also calculated from leaching volume using equation (4) at LAS concentrations ≤ 1/2 CMC or estimated when LAS concentrations ≥ CMC (Fig. 46). Values of K_{c1} and K_{c2} can be obtained from Fig. 47. The amount of LAS leached was increased with the increase of LAS concentrations in leaching solution (Fig. 48).

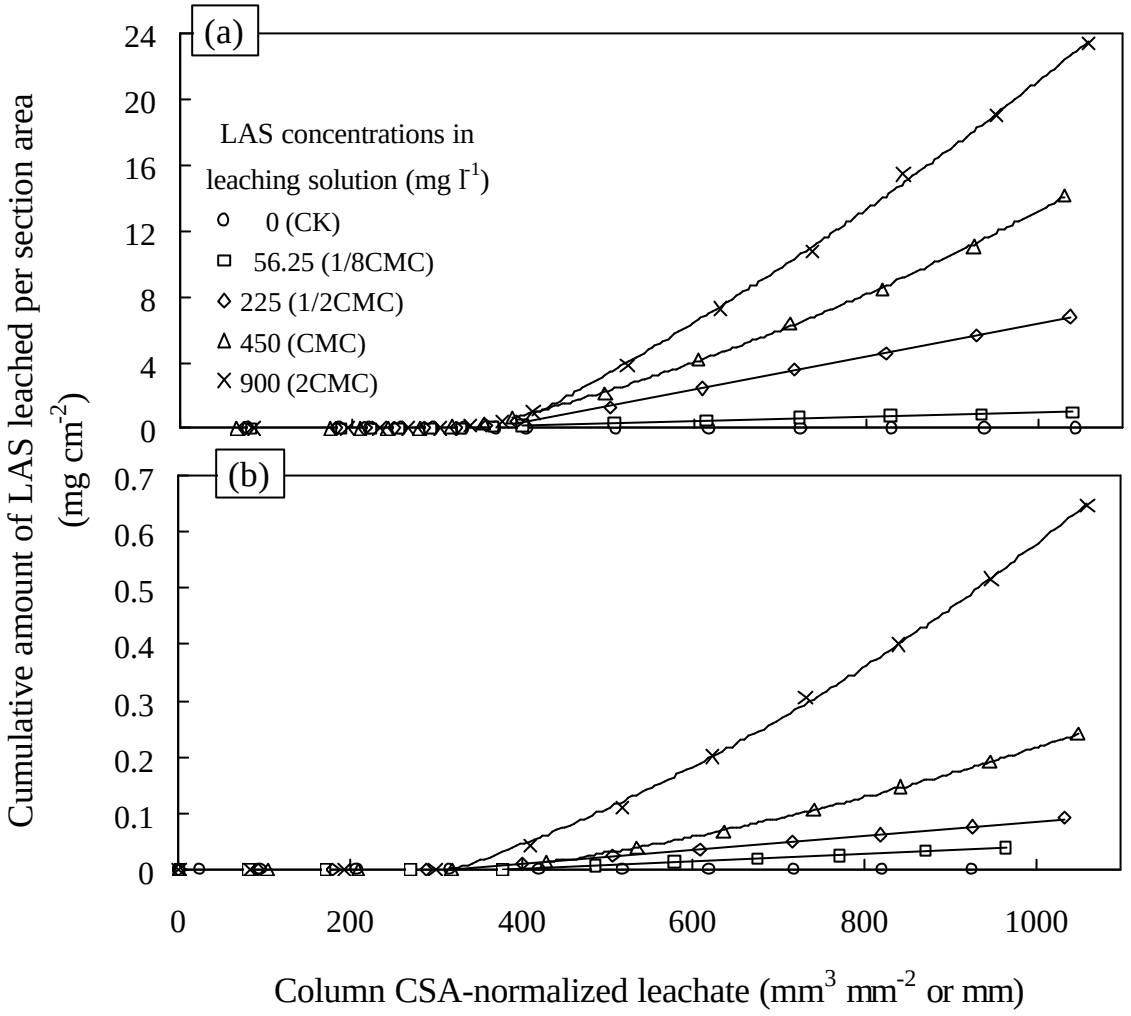
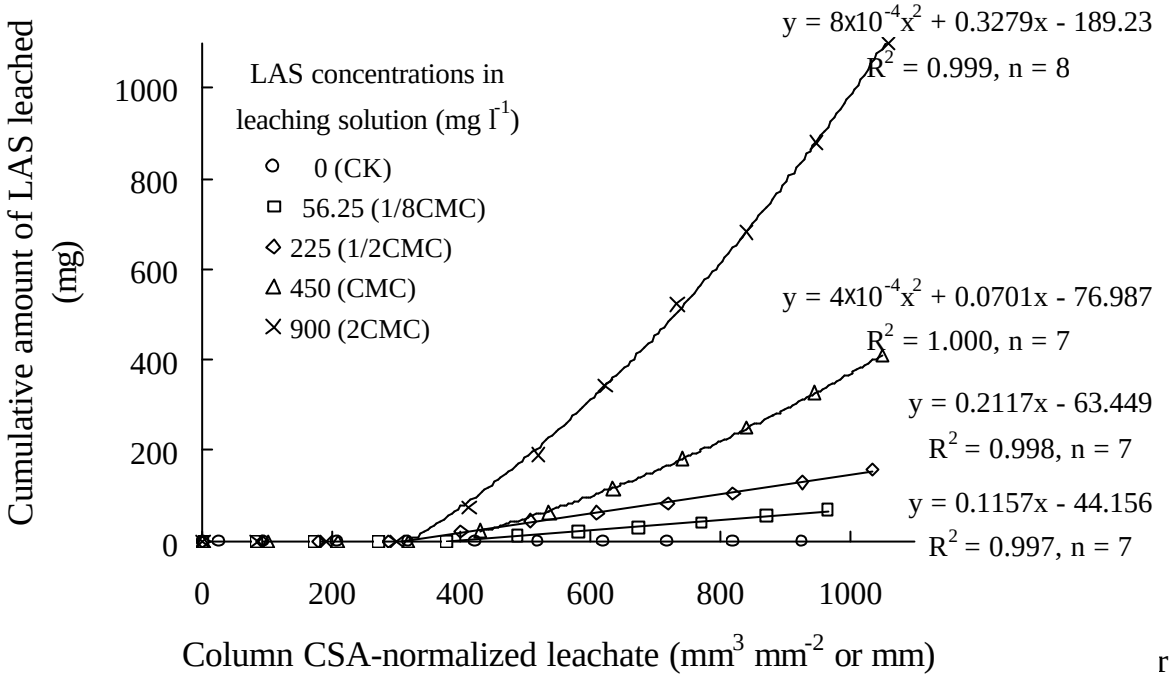


Fig. 45. Comparison of LAS leaching between soils (a) un-subjected and (b) subjected to freeze-thaw treatment (CSA = cross section area)

4.1.3 Influence of surfactants on the migration of PAHs in soils

4.1.3.1 Influence of LAS on the migration of PAHs in repacked soil columns not subjected to freeze-thaw treatment

One meter long repacked soil columns containing a mixture of phenanthrene, pyrene and benzo[a]pyrene at 5 mg kg^{-1} each in top 20-cm soil were leached with LAS solution of different concentrations covering 1/8 CMC to 2 time CMC (2CMC). Phenanthrene and pyrene broke through the columns at day 6 and day 15, corresponding to a total of leaching volume of 277-308 cm^3 (or 174-194 mm after normalized to column CSA) and 789-828 cm^3 (or 496-521 mm), respectively (Fig.



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freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China), expressed as amount of LAS leached *vs* the volume of effluent. (CSA = cross section area)

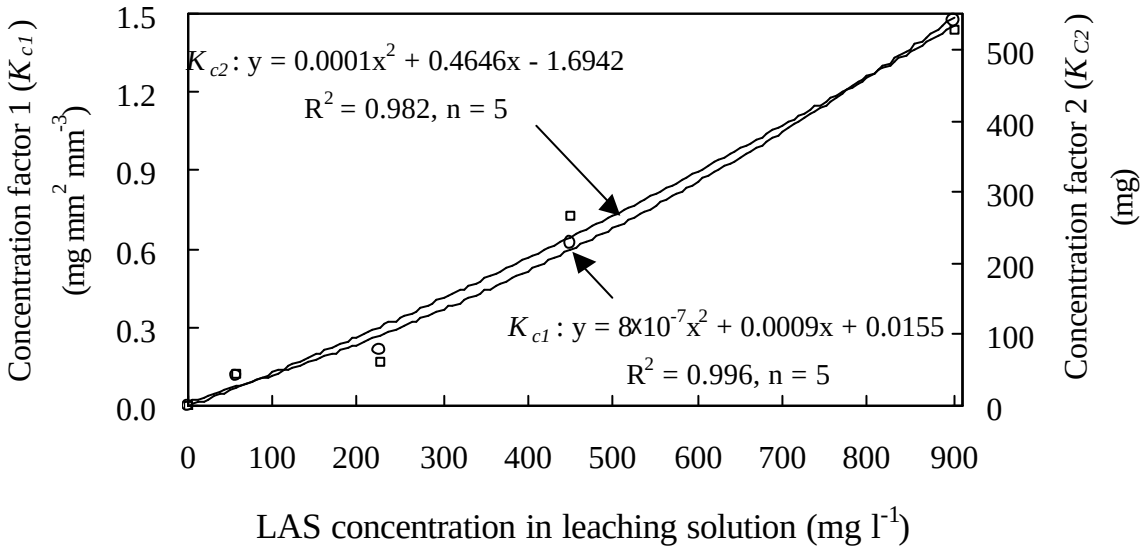


Fig. 47. Migration of LAS in 1-m repacked soil column subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China): influence of LAS concentration in leaching solution on the concentration factor 1 (K_{c1}) and 2 (K_{c2}) defined as the slope and intercept of linear regression equation of 'LAS leached'-'leaching volume', respectively.

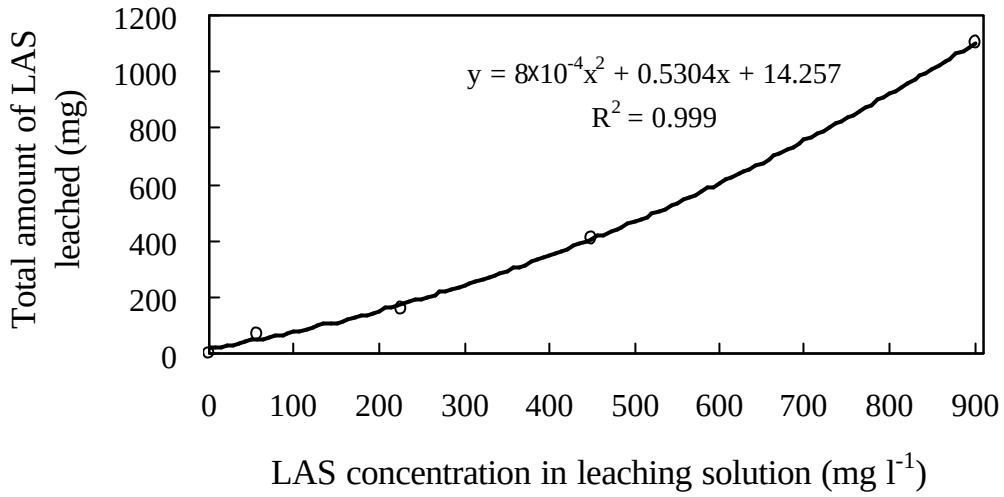


Fig. 48. Migration of LAS in 1-m repacked soil column subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China): relationship between the amount of LAS leached and the concentration of LAS in leaching solution.

49). LAS at all concentrations studied could not alter the breakthrough time or migration velocity of both phenanthrene and pyrene. At the end of the experiment with a total leaching volume of 1670 cm^3 (or 1050 mm) solution with and without LAS, still no benzo[a]pyrene was detected in the leachate. The migration of phenanthrene in soil was faster than pyrene followed by benzo[a]pyrene, which was consistent to the order of their water solubility or octanol-water partition coefficients (K_{ow}) (Table 1).

No significant difference in effluent concentrations of phenanthrene and pyrene could be observed among columns leached with LAS solution of different concentrations. But when cumulative leached amounts of phenanthrene or pyrene were examined, the presence of LAS did enhance the extent of their migration (Fig. 50). Generally, the amounts of phenanthrene and pyrene leaching out off the column increased with the increase of LAS concentration. However, some exceptions existed. The order of LAS-enhanced migration of phenanthrene was 2CMC > CMC > 1/8CMC > 1/2CMC > CK (control, no LAS), while for pyrene CMC > 1/8CMC > 1/2CMC > 2CMC = CK.

Less amount of phenanthrene or pyrene migrating through the soil column at some

LAS concentrations might be the result of the formation of LAS hemimicelles or admicelles on soil surface and the partitioning of phenanthrene and pyrene to them (see Section *4.1.1.3.2*.). Kögel-Knabner and Totsche (1998) have reported that dissolved organic matter (DOM) reduce the mobility of PAHs in soil due to co-sorption or cumulative sorption of DOM and PAHs. The reduced migration of PAHs caused by LAS in this study was more likely because of cumulative sorption resulted from increased sorptive capacity of the bulk phase due to sorption of LAS and thus increased organic carbon content as well as hydrophobicity.

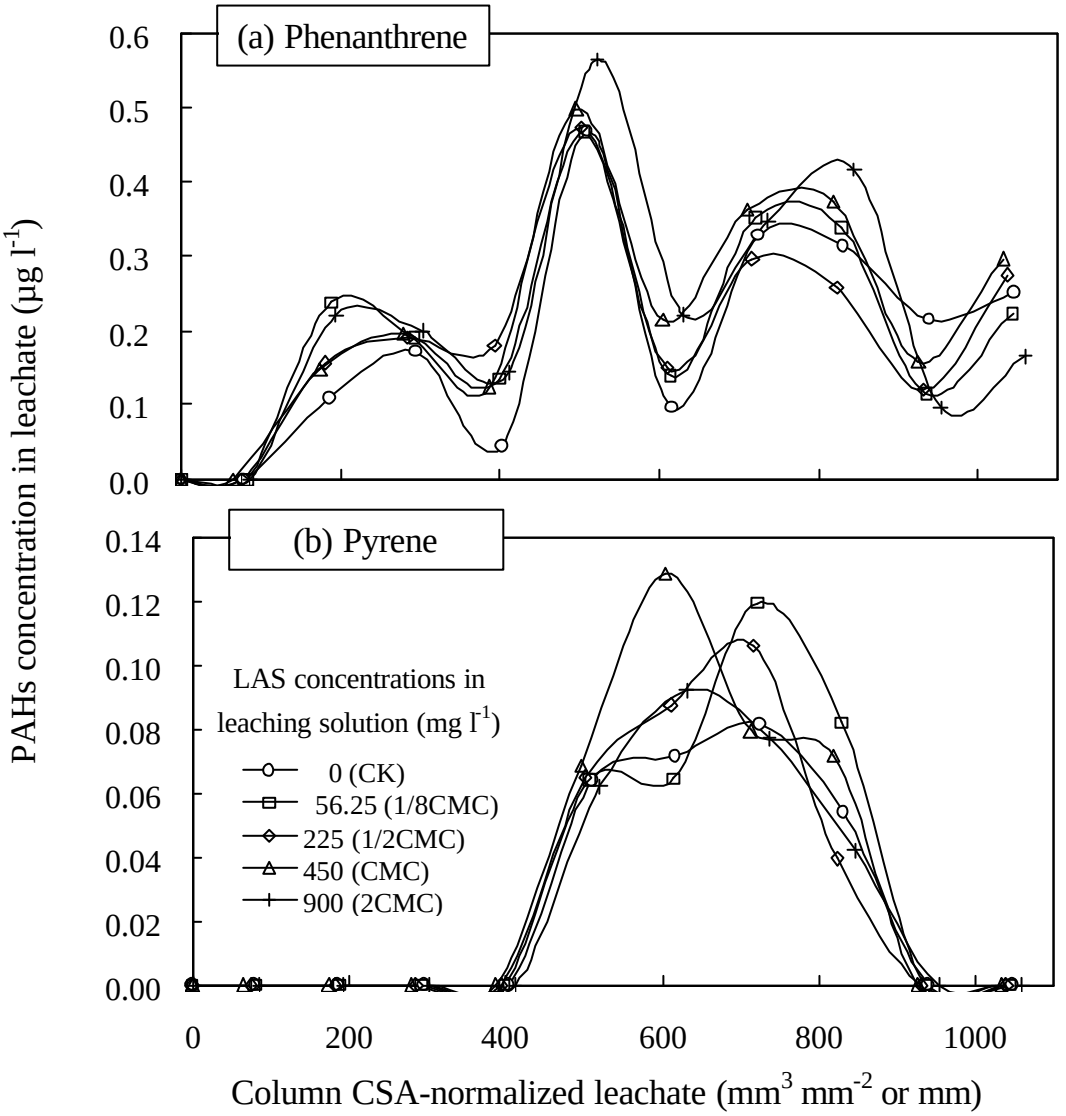


Fig. 49. Migration of PAHs in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China) leached by LAS solution: concentration of PAHs in leachate. (CSA = cross section area)

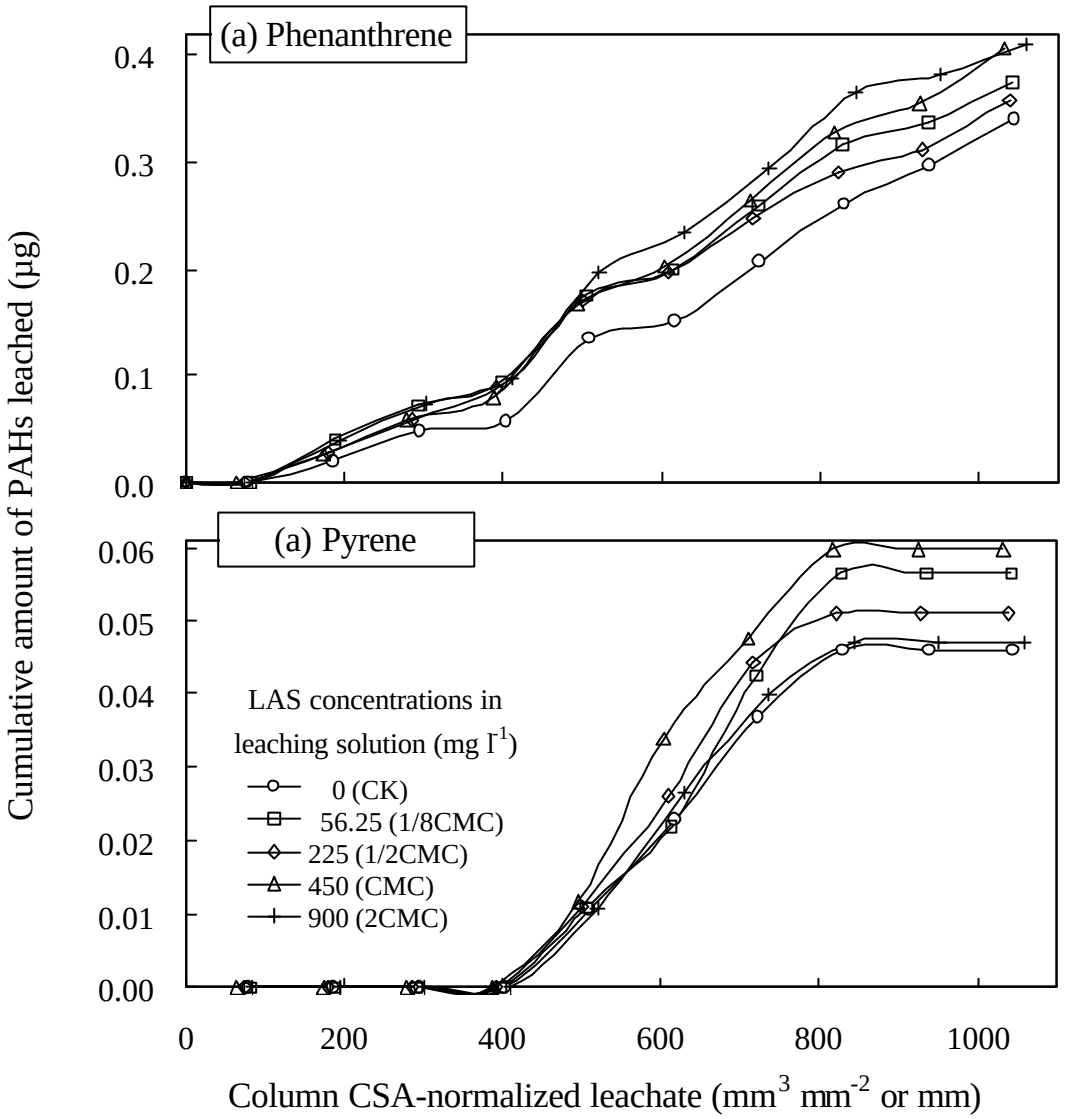


Fig. 50. Migration of PAHs in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China) leached by LAS solution: cumulative amount of PAHs leached. (CSA = cross section area)

When leaching was terminated, the concentrations of LAS in the leachate were 15.0, 103.6, 291.5 and 402.2 mg l^{-1} for 1/8CMC, 1/2CMC, CMC and 2CMC LAS leaching solutions, respectively (Fig. 37). They were obviously lower than the clean water CMC (CMC_w) of LAS ($CMC_{w\text{-}LAS}$ = 433.5 mg l^{-1} (Fig. 6)), needless to say effective CMC (CMC_{eff}) which is usually higher than CMC_w in soil- or sediment-systems. Surfactant solubilization of HOCs was limited at concentrations below CMC (Edwards *et al.*, 1991; Liu *et al.*, 1991; Laha and Luthy, 1992). Therefore, the

reasonable explanation for the enhancement of phenanthrene and pyrene migration in this work was the competition of active hydrophobic adsorption sites on soil surface between LAS and PAHs (see Section *4.1.1.3.2*.) (Ou *et al*., 1995b). It has been shown in Section *4.1.1.3.2* that the presence of LAS could decrease the adsorption of phenanthrene on soil.

Fig. 51 shows the distribution of residual phenanthrene, pyrene and benzo[a]pyrene in soil profile after the termination of leaching experiment. The migration ability of all three PAHs was much lower than that of LAS (Fig. 32). Most amounts of the applied PAHs remained in the top 20 to 25-cm soil. In most cases, PAHs could not be found in soil below 65 cm. Except the soil columns leached with LAS solution at CMC, concentrations of the three PAHs in the very top 5-cm soil decreased as LAS concentrations increased. The presence of high concentration LAS also caused the PAHs migrating to deeper layer. From these points of views, LAS could enhance the migration of PAHs in soil.

4.1.3.2 Influence of LAS on the migration of PAHs in repacked soil columns subjected to freeze-thaw treatment

One meter long repacked soil columns containing a mixture of phenanthrene, pyrene and benzo[a]pyrene at 5 mg kg^{-1} each in top 20-cm soil were first subjected to outdoor freeze-thaw treatment from early autumn to late spring and then leached with LAS solution of different concentrations covering 1/8 CMC to 2 time CMC. Results (Fig. 52 and Fig. 53) show that the migration of selected PAHs was much different from that in columns not subjected to freeze-thaw treatment. Phenanthrene broke through the 1-m soil monolith immediately in the first collection of 393-628 cm^3 (or 50-80 mm after normalized to column CSA) leachate at day 3 (Fig. 52(a)), while the corresponding breakthrough was at 170-190 mm (CSA-normalized) or day 6 for columns without freeze-thaw treatment (Fig. 49). Similar phenomenon was also observed for pyrene (Fig. 52(b)). The breakthrough of pyrene was at 1021-1570 cm^3 (or 130-200 mm) (day 6) variant among LAS concentrations, which was 320-370 mm (CSA-normalized) or 9 days less than that for non-freeze-thaw-treated soil columns. Furthermore, the breakthrough of benzo[a]pyrene was observed in 240-270 mm (for

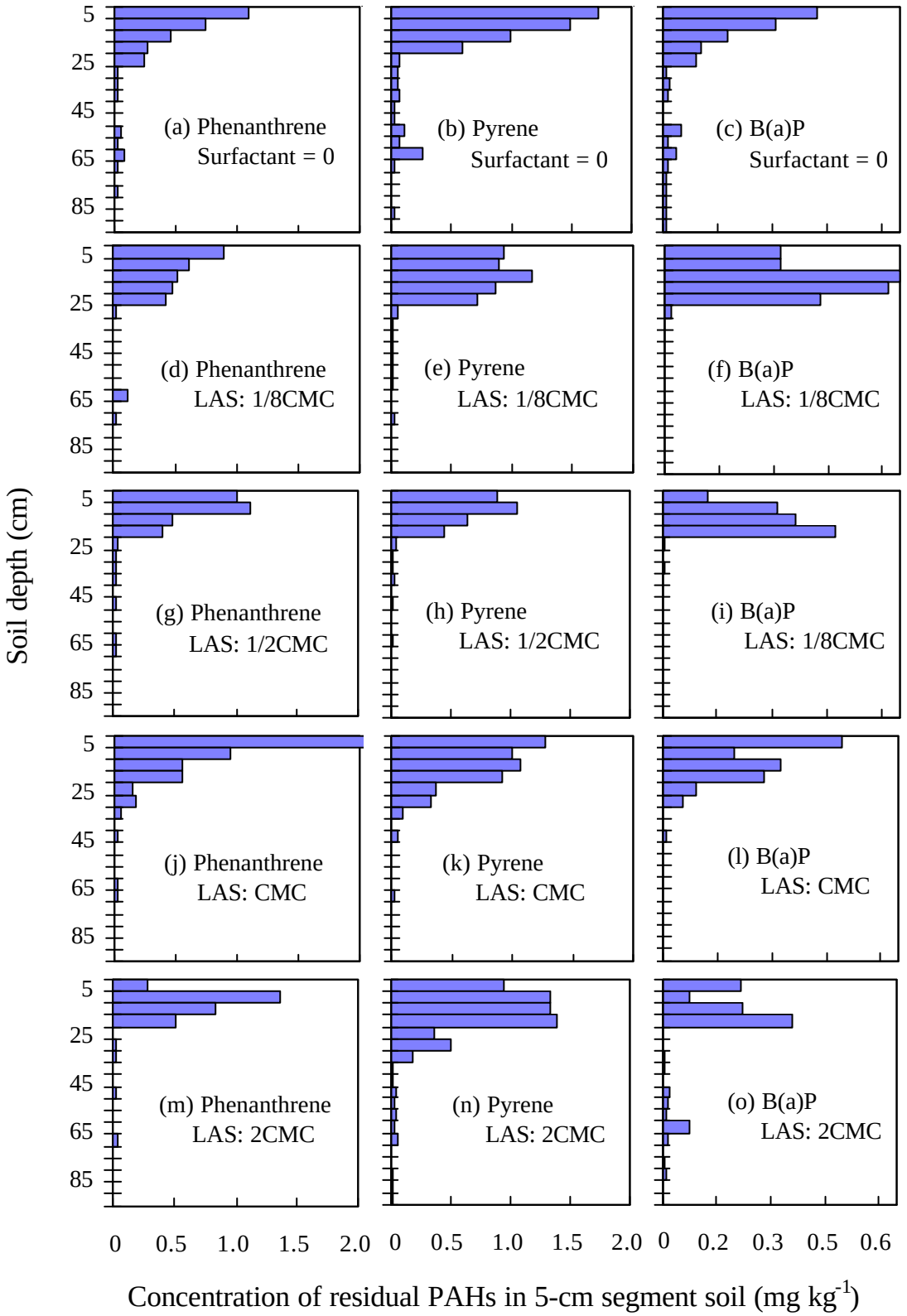


Fig. 51. Migration of PAHs in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China) leached by LAS solution: distribution of residual PAHs.

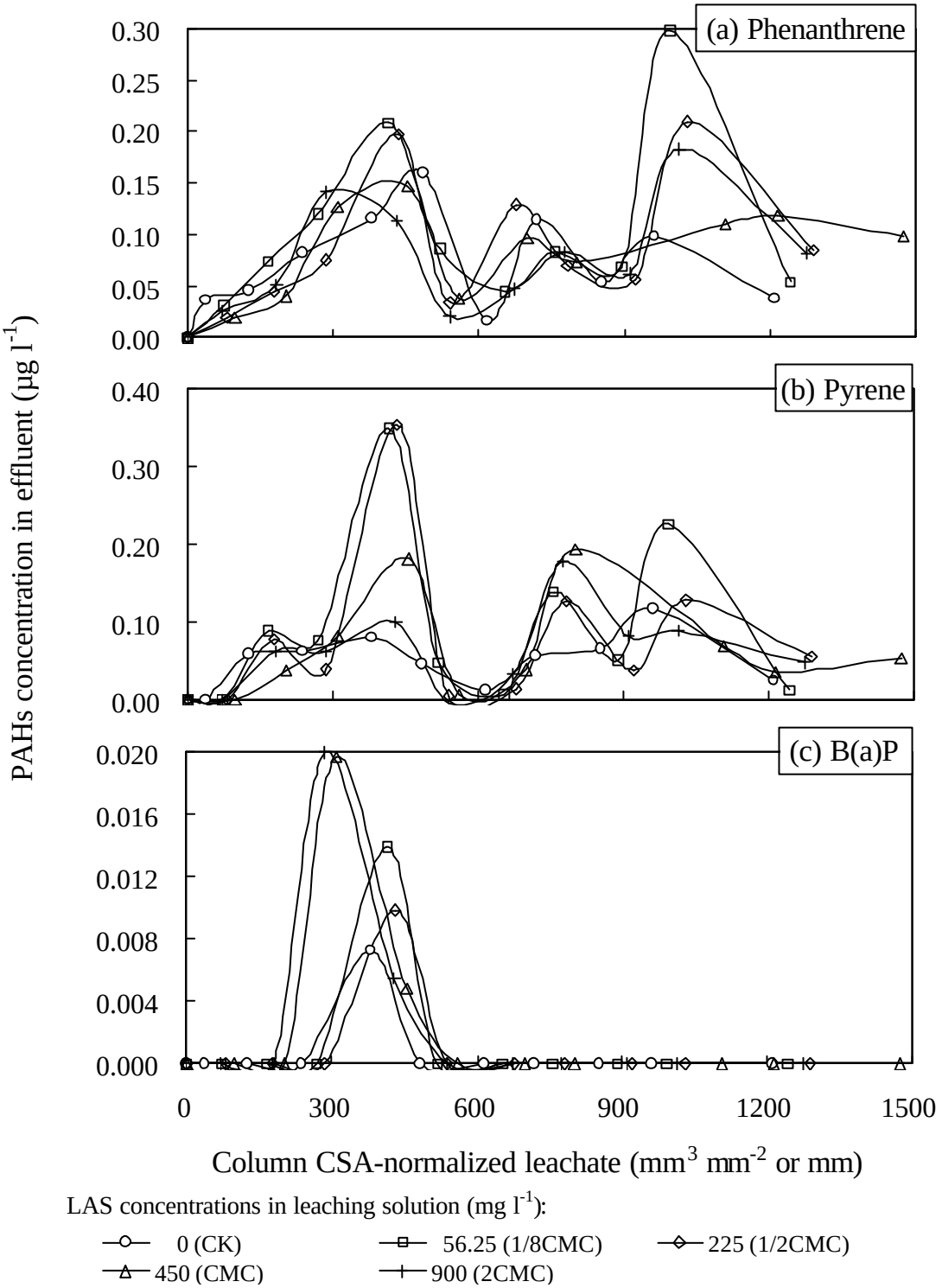


Fig. 52. Migration of PAHs in 1-m repacked soil columns subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China) and leached by LAS solution: concentration of PAHs in leachate. (CSA = cross section area)

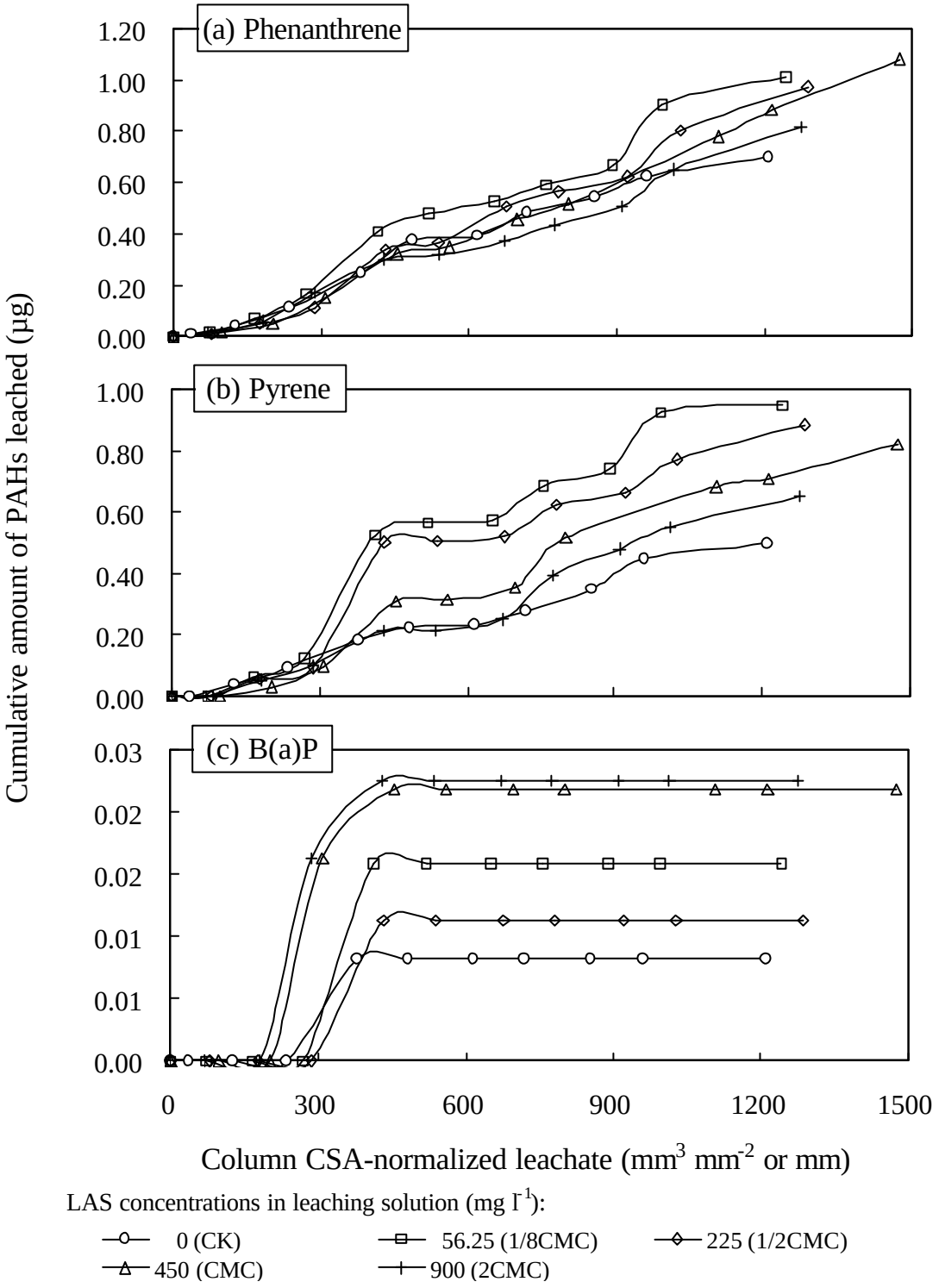


Fig. 53. Migration of PAHs in 1-m repacked soil columns subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China) and leached by LAS solution: cumulative amount of PAHs leached. (CSA = cross section area)

LAS at CMC and 2CMC) to 380-450 mm (for all other LAS concentrations) leachate or at day 9 to 13, while no breakthrough of benzo[a]pyrene was found in non-freeze-thaw-treated soil columns (see Section *4.1.3.1*).

Generally, two concentration peaks existed in the leaching curves of phenanthrene and pyrene (Fig. 52(a) and (b)), which was very similar to the preferential migration patterns of LAS in field lysimeters (Fig. 34). These phenomena could not be observed in soil columns not subjected to freeze-thaw treatment (Fig. 49). After reaching peak values at 2277-3768 cm^3 (or 290-480 mm) and 2983-3522 cm^3 (or 380-450 mm) leachate for phenanthrene and pyrene, respectively, concentrations of both PAHs in the effluent decreased to the same values as those of first breakthrough for phenanthrene or to almost non-detectable levels for pyrene. Although the leachate volumes for phenanthrene and pyrene were different, their breakthrough times were the same (13 days). As explained in Section *4.1.2.1.2*, this is a typical phenomenon of preferential breakthrough and migration of solute in soil. Then they increased again as leaching continued and reached their second peaks at 7536-9420 cm^3 (or 960-1200 mm) leachate or day 30 for both phenanthrene and pyrene. The second peaks were obviously the result of equilibrium migration of phenanthrene and pyrene. The breakthrough and concentration peaks of benzo[a]pyrene were also resulted from its preferential migration along with preferential water flow. As the same as no breakthrough could be observed in soil without preferential water flow in non-freeze-thaw-treated columns, the adsorption/desorption of benzo[a]pyrene in freeze-thaw-treated soil also did not reach equilibrium. Therefore, after preferential breakthrough, no second breakthrough or equilibrium breakthrough could be observed for freeze-thaw-treated soil. Results approve that soil undergone natural freeze-thaw processes can result in the preferential migration of HOCs. The preferential migration can significantly change the fate of HOCs in soil. Preferential migration caused phenanthrene and pyrene braking through 1-m soil monolith 3 days earlier than equilibrium breakthrough, and altered the migration of benzo[a]pyrene from non-breakthrough in non-freeze-thaw-treated columns to preferential breakthrough in 9-13 days in freeze-thaw-treated columns. Not only the migration velocity but also the migration intensity was affected by preferential migration. The amount of

phenanthrene and pyrene leaching out off the soil columns via preferential migration accounted for 37% and 47% of the total amount leached in average, respectively.

Similar to the results obtained from non-freeze-thaw-treated soil columns, there was no significant difference in effluent concentrations of phenanthrene and pyrene among freeze-thaw-treated columns leached with LAS solution of different concentrations. However, peak levels and cumulative amount of benzo[a]pyrene leached obviously increased with the increase of LAS concentrations in leaching solution (Fig. 52(c) and Fig.53(c)). The total amounts of phenanthrene and pyrene leached were highest when leached with 1/8CMC LAS solution followed by 1/2CMC, CMC, 2CMC and CK. This might be explained using above hypothesis that phenanthrene and pyrene adsorbed on LAS hemimicelles and admicelles formed on soil surface. As can been seen from Fig. 43 that LAS concentrations in effluent were much less than CMC_w, e.g. only about half of CMC in effluent from columns leached with 2CMC LAS solution, LAS solubilization of the PAHs would be very limited and therefore the LAS enhancement of migration would not be significant.

4.1.3.3 Influence of Tween-80 on the migration of PAHs in repacked soil columns not subjected to freeze-thaw treatment

Soil columns, PAHs concentrations and other experimental conditions were the same as those used in LAS study. Tween-80 concentrations in leaching solution also covered a range from 1/8CMC to 2CMC as LAS did. Similar to LAS, no breakthrough of benzo[a]pyrene could be observed for all columns leached with solutions containing Tween-80 or not. The breakthrough for phenanthrene and pyrene were at 350-334 cm^3 (or CSA-normalized 220-210 mm) (day 6) and 652-684 cm^3 (or 410-430 mm) (day 12) leachate (Fig. 54), respectively, which was also very similar to those obtained for LAS. Tween-80 at all concentrations studied could not alter the breakthrough or migration velocity of both phenanthrene and pyrene, which was consistent with results for LAS. The patterns of the breakthrough curves of phenanthrene and pyrene were similar between leaching with LAS (Fig. 49) and Tween-80 (Fig. 54). No significant difference in effluent concentrations of both PAHs could be found among columns leached with Tween-80 solution of different

concentrations. But their concentrations in effluent were generally higher when leached with LAS than those leached with Tween-80 in a factor of 4 to 5. This was because LAS concentrations in leaching solution were much higher than those of Tween-80 though they covered the same CMC range. The CMC of LAS is one magnitude higher than that of Tween-80. Results suggest that absolute concentration of surfactant is important in affecting the migration of HOCs particularly when competition of hydrophobic adsorption sites between surfactant and HOCs is the main mechanism for surfactant enhancing desorption of HOCs from soil.

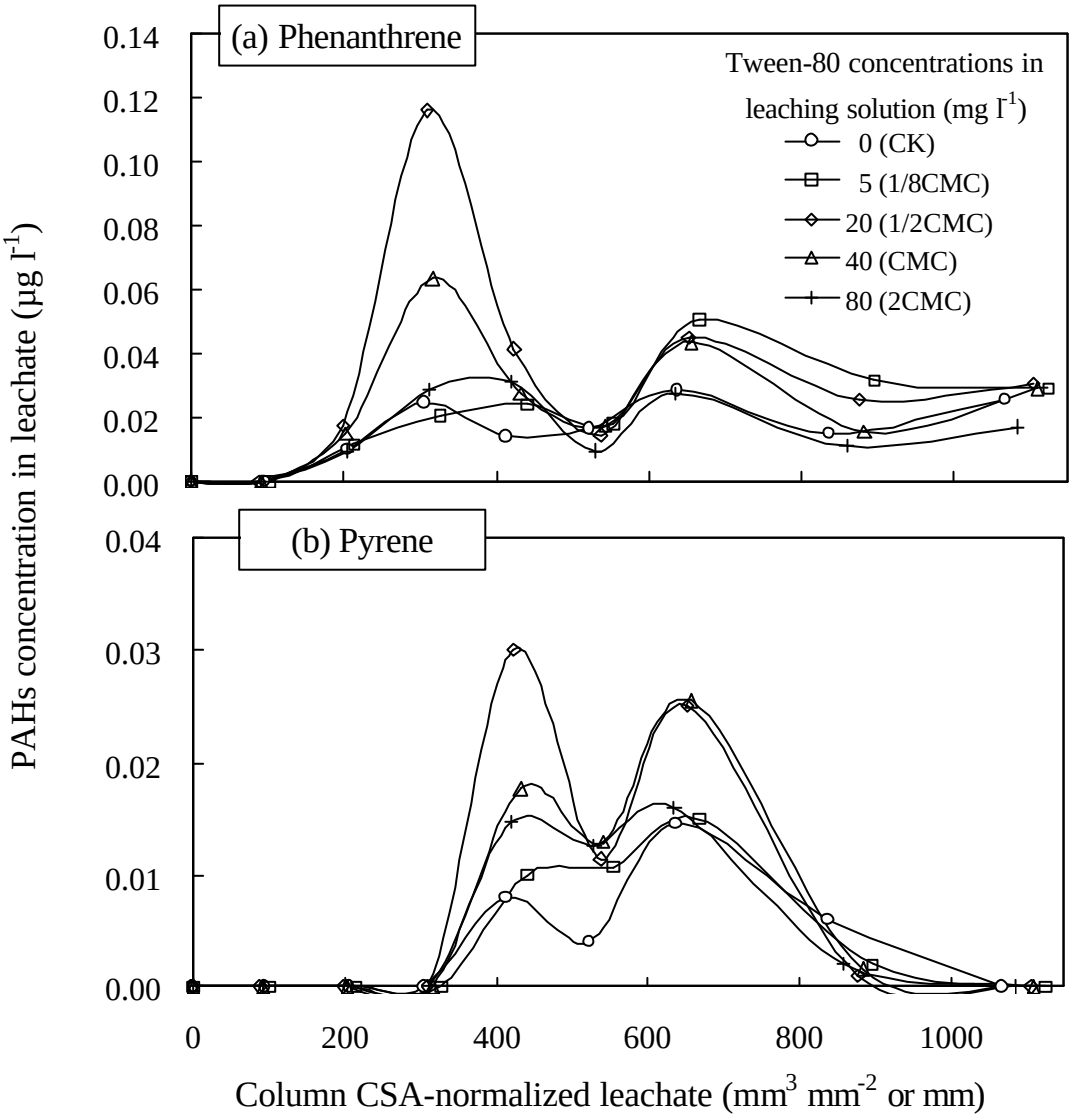


Fig. 54. Migration of PAHs in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China) leached by Tween-80 solution: concentration of PAHs in leachate. (CSA = cross section area)

The order of Tween-80-enhanced migration of phenanthrene was 1/2CMC > CMC = 1/8CMC > 2CMC = CK (control, no Tween-80), while for pyrene 1/2CMC > CMC > 2CMC > 1/8CMC = CK (Fig. 55). 1/2CMC Tween-80 solution enhanced the leaching of both PAHs to the greatest extend followed by CMC. However, it should be pointed out that their differences were not significant either in terms of effluent concentration or total amount leached. It was probably because of the low Tween-80 concentrations used (only 80 mg l^{-1} even for 2CMC).

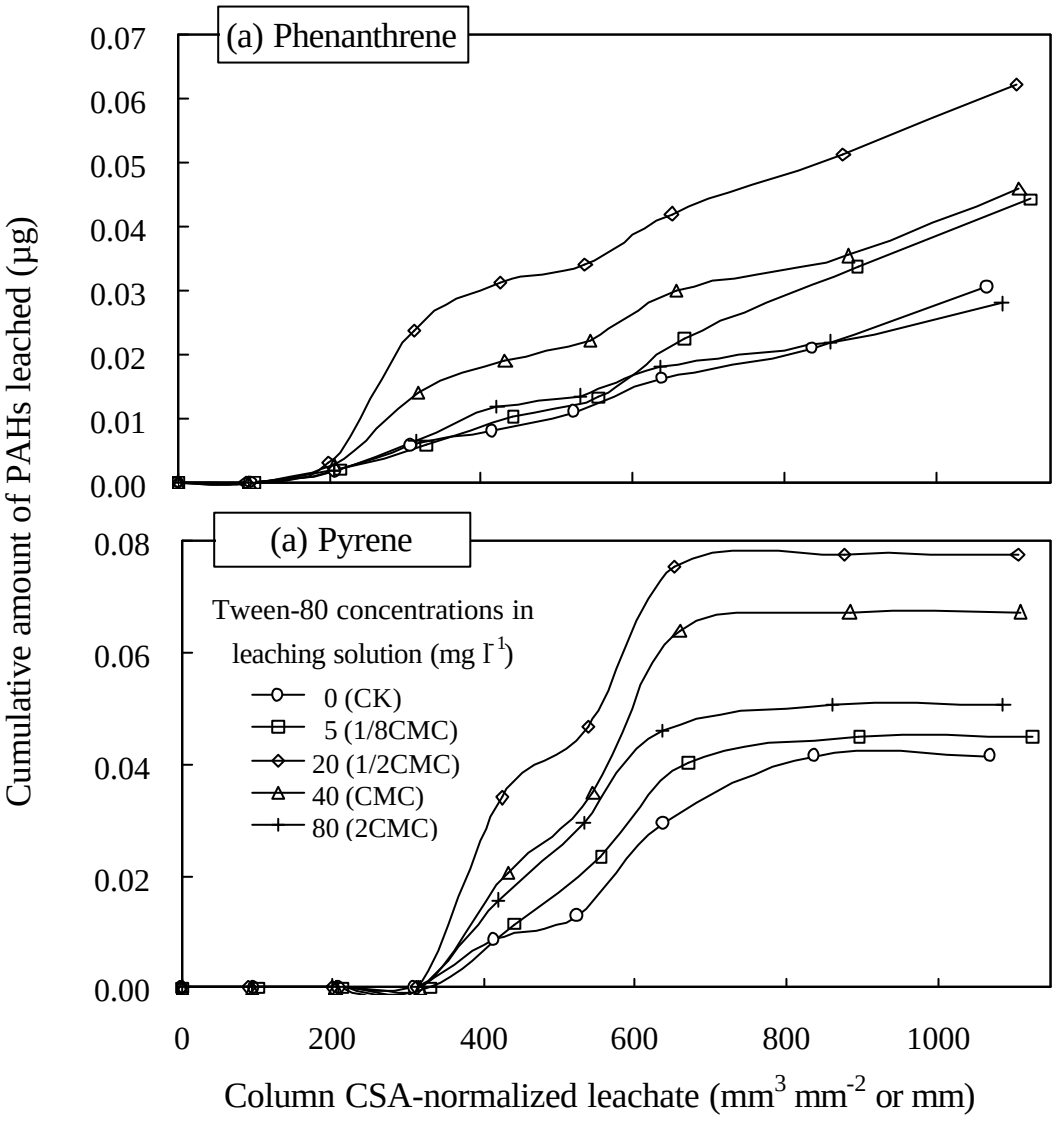


Fig. 55. Migration of PAHs in 1-m repacked soil columns (Soil 13#, 14# and 15# from SES, Shenyang, China) leached by Tween-80 solution: cumulative amount of PAHs leached. (CSA = cross section area)

4.1.3.4 Influence of Tween-80 on the migration of PAHs in repacked soil columns subjected to freeze-thaw treatment

Soil columns, PAHs concentrations, freeze-thaw treatment and other experimental conditions were exactly the same as those used in LAS study. Tween-80 concentrations in leaching solution covered the same range i.e. from 1/8CMC to 2CMC as LAS did. Results (Fig. 56 and Fig. 57) obtained were also very similar to those of LAS. The migration of PAHs was much different from that in soil columns not subjected to freeze-thaw treatment. First, preferential breakthrough of all three PAHs in freeze-thaw-treated soil columns was observed as did for the same treated columns leached by LAS. Preferential breakthrough was at 471-550 cm^3 (or CSA-normalized 60-70 mm) (first leachate collected at day 3), 1256-1413 cm^3 (or 160-180 mm) (day 6) and 2120-2277 cm^3 (or 270-290 mm) (day 9) leachate for phenanthrene, pyrene and benzo[a]pyrene, respectively, which was very close to the corresponding values obtained for LAS. The first breakthrough of phenanthrene and pyrene was 3 and 6 days ahead of that obtained from non-freeze-thaw-treated soil columns, respectively (Fig. 54). Moreover, benzo[a]pyrene did not break through non-freeze-thaw-treated soil columns at all. The amount of phenanthrene and pyrene leaching out off the soil columns via preferential migration accounted for 25 and 22% of the total amount leached in average, respectively (Fig. 57).

Similar to results obtained from non-freeze-thaw-treated soil columns, the differences of either effluent concentrations or total amount of test PAHs leached among Tween-80 concentrations were not significant. The reason for this might be that Tween-80 concentrations were too low to cause desorption or solubilization of PAHs in soil. Even though Tween-80 concentrations covered 1/8CMC to 2CMC, the highest concentration was only 80 mg l^1. It suggests that certain absolute concentration should be reached for surfactant to mobilize adsorbed HOCs from soil.

4.1.4 Sub-summary

The adsorption of LAS on natural soils is very weak and could be divided into two stages: linear and exponentially increasing isotherms. With LAS concentrations (<90 mg l^{-1}) in real environment, the adsorption isotherms were linear. The values of K_d of

four natural soils investigated (25°C) were among 1.2 to 2.0. At higher concentrations, LAS admicelles formed on soil surface, which resulted in cooperative adsorption and the exponential increase of adsorption amount.

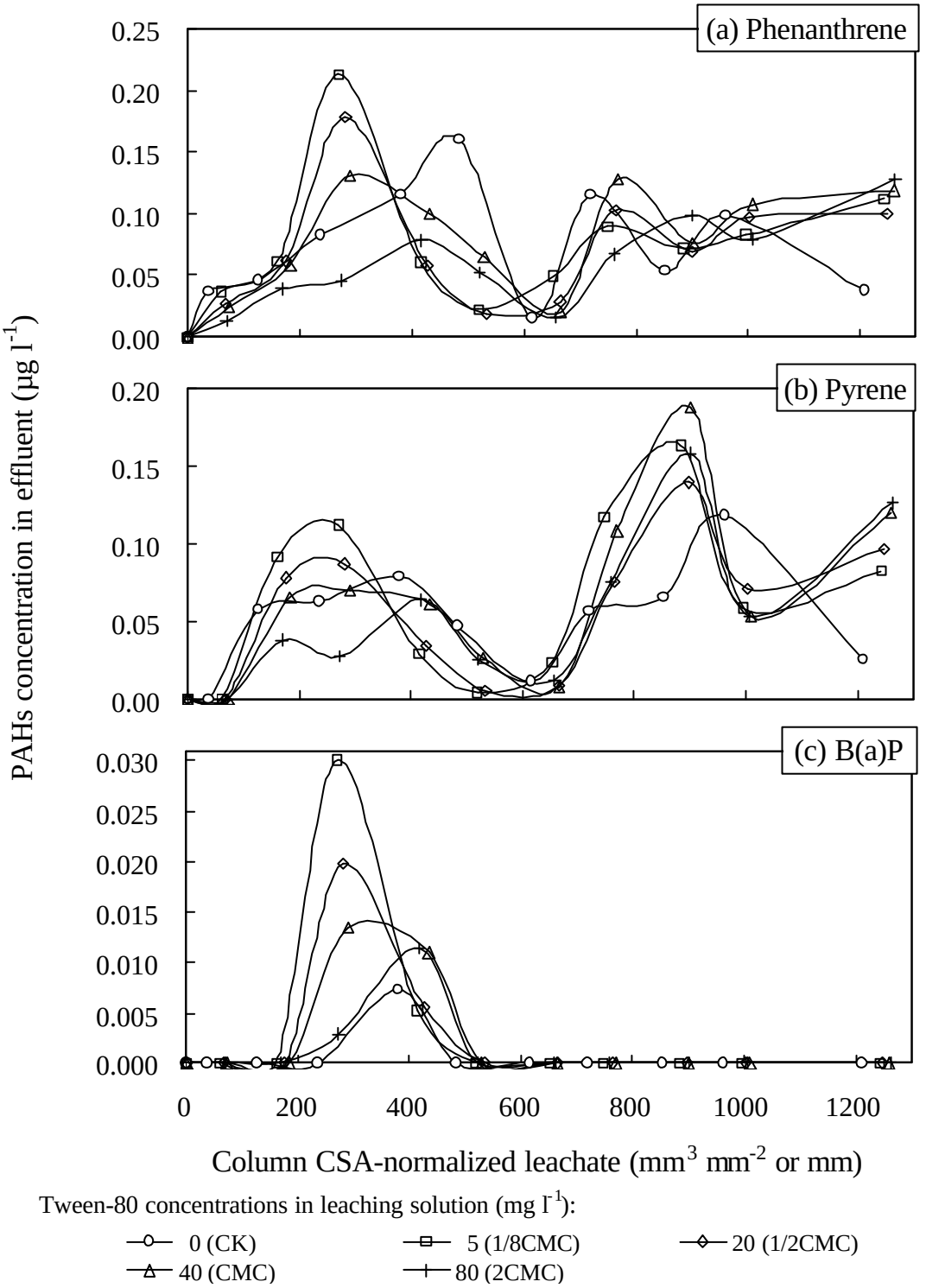


Fig. 56. Migration of PAHs in 1-m repacked soil columns subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China) and leached by Tween-80 solution: concentration of PAHs in leachate. (CSA = cross section area)

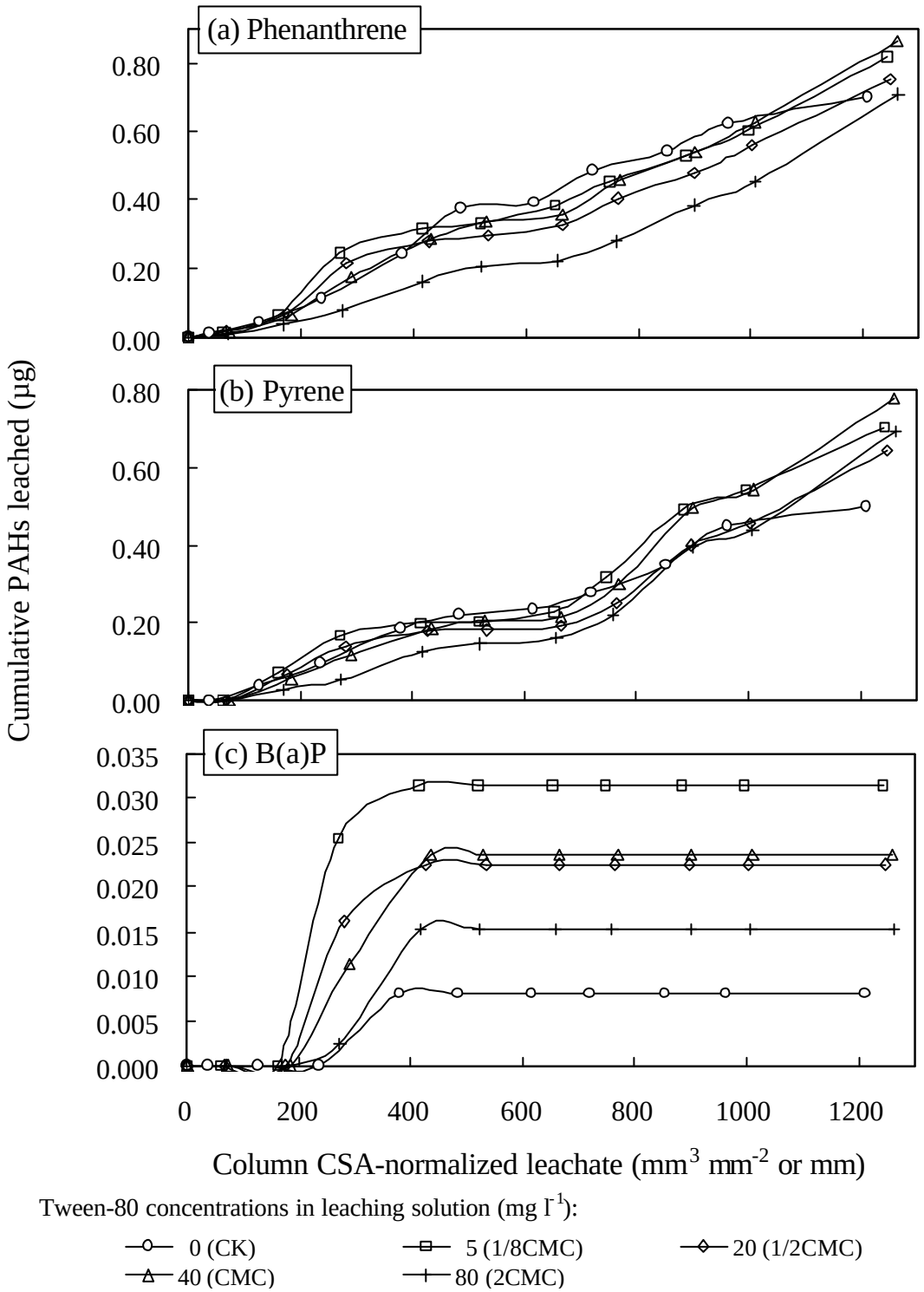


Fig. 57. Migration of PAHs in 1-m repacked soil columns subjected to outdoor freeze-thaw treatment (Soil 13#, 14# and 15# from SES, Shenyang, China) and leached by Tween-80 solution: cumulative amount of PAHs leached. (CSA = cross section area)

The mechanism of LAS adsorption on soils was neither partitioning nor hydrophobic interactions, but mainly specific site surface interactions or hydrogen bonding. Specific site surface interactions can include electrostatic bonding of negatively charged sulfonate groups of LAS with positively charged sites on soil surface, exchange of anions and ligands. The LAS adsorption capacity of a soil mainly depended on its physical clay content. Removal of soil organic matter could increase the adsorption capacity of the soil.

The adsorption behaviour of phenanthrene on soil was partitioning mechanism or hydrophobic interaction and significantly influenced by LAS. LAS at low concentrations (< 50 mg l^{-1}) could result in either an increase or decrease in phenanthrene adsorption onto soil. The key factor was the contacting sequence of LAS and phenanthrene with soil. When soil equilibrated with LAS prior to phenanthrene, the adsorption of phenanthrene decreased due to the occupation of active hydrophobic adsorption sites by LAS. When soil contacted with phenanthrene before LAS, the adsorption of phenanthrene increased due to additional partitioning of the material into LAS hemimicelles/admicelles formed on the soil surface. At higher concentrations of LAS (> 50 mg l^{-1}) the adsorption of phenanthrene always decreased no matter which contacting sequences.

Since its weak adsorption on soil, LAS migration in soil was significant. The velocity of LAS migration depended on water flow flux and adsorption/desorption equilibrium in soil and independent of LAS concentrations either in soil or in leaching solution. The breakthrough time of LAS decreased linearly with the increase of water flow flux expressed as leaching intensity. LAS concentration in soil or leaching solution determined its concentration in leachate. The total amount of LAS leached depended on both concentrations of LAS and volume of water flow and can be predicted using the established model incorporating the two factors. There were no significant differences in term of velocity and amount of migration between LAS applied directly to soil and via water.

The migration of the selected PAHs (phenanthrene, pyrene and benzo[a]pyrene) in soil was slow. Their migration ability followed the order of phenanthrene > pyrene > benzo[a]pyrene, which was consistent to the order of their water solubility or octanol-water partition coefficients (K_{ow}). Generally, the presence of LAS or Tween-80 could enhance the migration of the test PAHs. When both surfactants were in the same level expressed as CMC, LAS showed greater enhancement of PAHs migration than Tween-80 did. The concentration of surfactant is an important factor and should be optimized. In addition, absolute concentration is more meaningful than that expressed as CMC when considering surfactant enhancement of the migration of HOCs.

Great differences existed between migration of test chemicals (both LAS and selected PAHs) in repacked soil columns without macropores and natural soil or repacked soil columns been subjected to freeze-thaw treatment. Preferential migration of all test chemicals was observed in natural soil and freeze-thaw-treated repacked soil columns but not in repacked soil columns not undergone freeze-thaw processes. Preferential migration resulted in the test chemicals moving much faster in soil than that predicted from equilibrium migration. The preferential breakthrough of LAS was 21 and 35 days ahead of equilibrium breakthrough in field lysimeters cropped to paddy rice and soybean, respectively. Preferential migration caused phenanthrene and pyrene breaking through 1-m soil monolith 3 to 6 days earlier than equilibrium breakthrough, and altered the migration of benzo[a]pyrene from non-breakthrough in non-freeze-thaw- treated soil columns to preferential breakthrough in 9-13 days in freeze-thaw-treated soil columns. Not only the migration velocity but also the amount of migration was enhanced by preferential migration. Concentrations of LAS in preferential effluent accounted for up to 20-30% and 30-86% of equilibrium concentrations in field lysimeters cropped to soybean and paddy rice, respectively. The amount of phenanthrene and pyrene via preferential migration could be as high as 20 to 50% of the total amount leached.

Consequently, caution should be taken when predicting migration behaviour of both hydrophilic (e.g. surfactants) and hydrophobic (e.g. PAHs) organic chemicals in field soil from laboratory data. In addition to possible preferential migration in the field,

their equilibrium migration also differed between repacked soil and natural soil.

4.2 Effects of surfactants and PAHs on microorganisms

4.2.1 Acute toxicity of surfactants and phenanthrene on luminescent bacteria *Vibrio fischeri*

4.2.1.1 Acute toxicity of phenanthrene on luminescent bacteria *Vibrio fischeri*

Phenanthrene was toxic to luminescent bacteria *Vibrio fischeri*. As shown in Fig. 58, the inhibition on the emission of luminescence or the toxicity of phenanthrene to the bacteria increased with the increase of its concentration. However, as the concentration of phenanthrene increased towards its solubility, the increase of toxicity was getting slower. For example, the inhibition rates at 30 min were 38.4% at phenanthrene concentration of 0.875 mg l^{-1} while only 39.3% at 1.25 mg l^{-1}. It can be seen from Fig. 58 that the dose-response curves were linear at phenanthrene concentrations lower than 0.5 mg l^{-1} but not at higher concentrations. In addition, the toxicity decreased with the lengthening of exposure time from 5 min to 60 min, implying that the bacterium was able to adapt to phenanthrene and recover from the toxicity of phenanthrene.

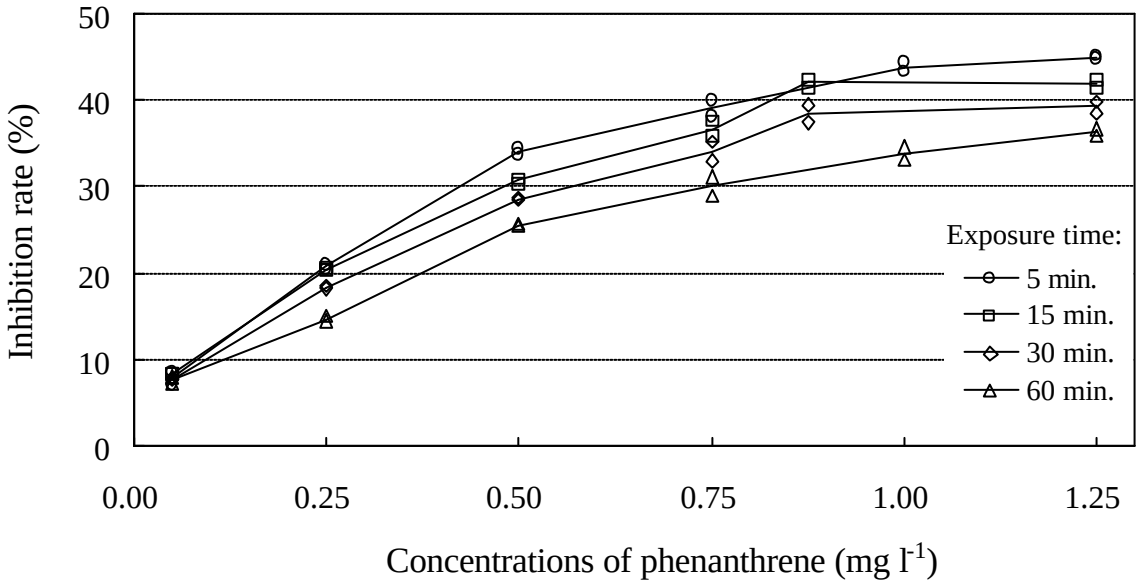


Fig. 58. Acute toxicity of phenanthrene on luminescent bacteria *Vibrio fischeri*

-EC_{10}, -EC_{20} and -EC_{50} are herein defined as effective concentrations causing 10%, 20% and 50% decrease of luminescent emission, respectively. The value of -EC_{50} could not be obtained since the maximal inhibition rate caused by phenanthrene was only 40% at its concentration of solubility. The values of -EC_{10} and -EC_{20} at 30 min exposure time were 0.09 and 0.29 mg l^{-1}, respectively.

4.2.1.2 Acute toxicity of surfactants on luminescent bacteria *Vibrio fischeri*

The toxic effect of surfactants on bacteria can be explained by two main factors: (1) disruption of cellular membranes by interaction with lipid components and (2) reaction of surfactant molecules with proteins essential to the functioning of the cell (Schwuger and Bartnik, 1980; Jensen, 1999). At a pH of 7 and higher, cationic surfactants are the most toxic ones, while anionic surfactants display the most toxic behaviour at lower pH values. Nonionic surfactants are in general less active against bacteria than ionic surfactants (Helenius and Simons, 1975; Volkering *et al*., 1998).

Results obtained in this study confirmed that cationic TDTMA was the most toxic surfactant but not for the nonionic surfactant. At the same pH (6.9), the nonionic Brij30 and 10LE were more toxic to the luminescent bacteria *Vibrio fischeri* than anionic LAS was (Fig. 59 and Fig. 60). At exposure time of 30 min (the following discussion should be referred to an exposure time of 30 min unless specified), emission of luminescent of the bacteria was almost completely inhibited (inhibition rate = 99%) at TDTMA concentration of as low as 3 mg l^{-1}. To reach the same inhibition rate (99%), concentration of Brij30 was 17 times of that of TDTMA, i.e. 50 mg l^{-1}. Such a high inhibition rate, however, could not be observed for the rest of surfactants tested at their concentrations as high as 2000 mg l^{-1} for Tween-80, 450 mg l^{-1} for LAS, or 225 mg l^{-1} for 10LE and Brij35. Table 13 shows the -EC values at 30 min exposure time. No matter -EC_{10}, -EC_{20} or -EC_{50}, TDTMA had the lowest values, followed by Brij30, 10LE, LAS, Brij35 and Tween-80, indicating the order of toxicity was TDTMA > Brij30 > 10LE > LAS > Brij35 > Tween-80. It is interesting to notice that the toxicity of the three Brij surfactants to *Vibrio fischeri* decreased with the

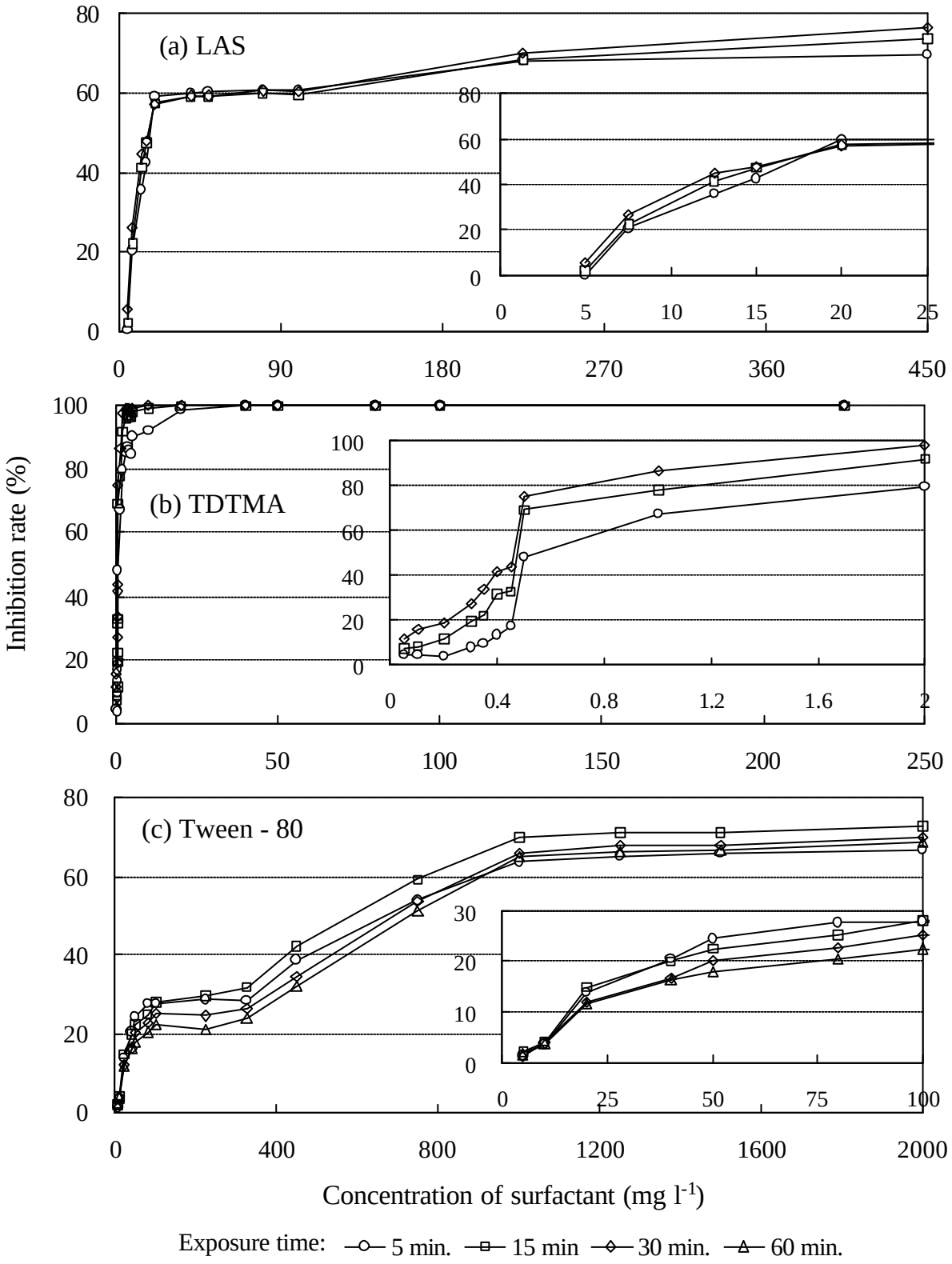


Fig. 59. Acute toxicity of LAS, Tween-80 and TDTMA on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

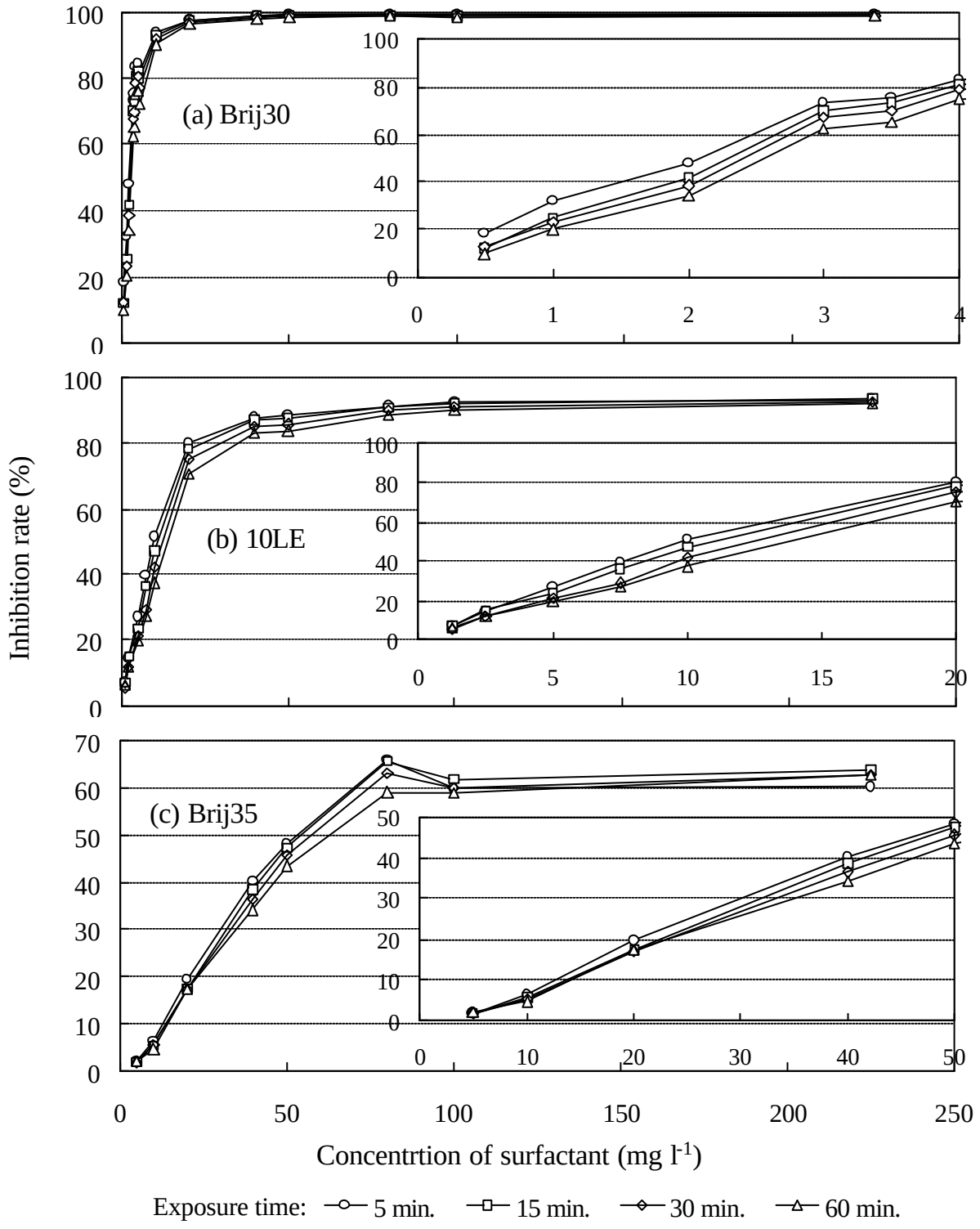


Fig. 60. Acute toxicity of Brij series surfactants on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

Table 13. EC values of acute toxicity of surfactants on luminescent bacteria *Vibrio fischeri*, 30 min exposure. (mg l^{-1})

Surfactants	LAS	Tween-80	TDTMA	Brij30	10LE	Brij35
-EC_{10}	5.5	17.5	0.06	0.4[a]	4.3	13.9
-EC_{20}	6.7	49.9	0.22	0.8	4.6	23.0
-EC_{50}	16.1	695.2	0.46	2.4	12.4	57.5

-EC_{10}, -EC_{20} and -EC_{50} are effective concentrations of surfactant causing 10%, 20% and 50% decrease of luminescent emission, respectively.

[a] extrapolated values.

increase of ethoxylate group in their molecules and that LAS was less toxic than the two Brij surfactants possessing less ethoxylate group, i.e. Brij30 and 10LE.

Unlike phenanthrene, linear dose-response curves were not observed for LAS, Tween-80 and TDTMA even at inhibition rate below 20% and at concentrations of LAS < 12.5 mg l^{-1}, Tween-80 < 40 mg l^{-1} and TDTMA < 0.5 mg l^{-1}. For LAS, the rapid increase of inhibition rates was at concentrations of 5 to 20 mg l^{-1}, at which inhibition rate rose from 5% to 57%. At higher concentrations, the increase of inhibition rates was less significant, indicating a plateau was reached. At LAS concentration of 450 mg l^{-1} (CMC), inhibition rate was 76% (Fig. 59). For Tween-80, there was a rapid rising of inhibition rate (from 1% to 25%) at concentrations of 5 to 100 mg l^{-1}. Then inhibition rate slowly increased to its maximum (66%) at concentration of 1000 mg l^{-1}. At higher concentrations up to 2000 mg l^{-1}, the inhibition rate basically did not change (Fig. 59). TDTMA had similar increasing pattern of toxicity as Tween-80 did. At low concentrations of 0.05 to 0.5 mg l^{-1}, inhibition increased very rapidly from 8% to 72%. From 0.5 mg l^{-1} to 2.0 mg l^{-1}, inhibition rate slowly increased to its maximum (99%). At higher concentrations, emission of luminescence was completely inhibited (Fig. 59).

Different to LAS, Tween-80 and TDTMA, inhibition rates caused by the three Brij series surfactants increased linearly with the increase of their concentrations at 0.5 to

4 mg l^{-1} for Brij30 (inhibition rates from 12% to 79%), 1.25 to 20 mg l^{-1} for 10LE (5% to 75), and 5 to 50 mg l^{-1} for Brij35 (2% to 46%) (Fig. 60). At concentrations of higher than 40 mg l^{-1}, Brij30 almost completely inhibit the bacteria. When concentrations of 10LE and Brij35 were 80 mg l^{-1} and higher, inhibition rates reached their maximum, i.e. 90% for 10LE and 60% for Brij35.

The inhibition eased as time elapsed for all nonionic surfactants, implying that their toxic effects on the bacteria were reversible and the bacteria could adapt to them. In contrast to nonionic surfactants, the toxicity of the ionic surfactants increased with lengthening of exposure, indicating the bacteria could not recover from their effects.

4.2.1.3 Combined acute toxicity of phenanthrene and surfactants on luminescent bacteria *Vibrio fischeri*

The toxicity of a compound may be altered, either enhanced or diminished, by other coexistent compounds. Therefore, the effect of a mixture of chemicals somehow deviates from the effects of the single chemical. If a mixture is more effective than that would be predicted considering the potency of its individual components, the combined effect is synergistic; if it is less effective, there is antagonism. If the combined effect is equal to the summation of single effects, the combined effect is additive (Kortenkamp and Altenburger, 1999).

There are a number of models describing combined effects of chemicals (Pöch *et al*., 1996). The most often and commonly used were additive models, either mechanistic or non-mechanistic. The two popular additive models are concentration-addition model (or called dose-additivity or Loewe additivity) (Loewe and Muischnek, 1926) and response-addition model (or called effect additivity, effect summation or Bliss-additivity) (Bliss, 1939). The concentration-addition model is based on the assumption that chemicals act in a similar way, such that effects can be produced by replacing one compound totally or partially with the other. Each individual component of a multiple mixture is assumed to contribute to the observed overall effect by acting in proportion to its concentration, regardless of any effect thresholds (Kortenkamp and

Altenburger, 1999). This model is described by the equation (Schramm *et al*., 1999):

$$c_{m1}/c_1 + c_{m2}/c_2 = S = 1 \quad (7)$$

where, c_1 and c_2 are effective concentrations of pure substance 1 and 2, respectively, which causes quantitatively the same effect as in the mixture; c_{m1} and c_{m2} are effective concentrations of substance 1 and 2 in the mixture, respectively, which produces quantitatively the same effect as the single substance; *S* is summation of the equation. If *S* equals 1, the combined effect is additivity. If *S* deviates significantly from 1, the combined effect is not additive but rather either synergistic or antagonistic. Values lower or higher than 1 indicates underadditive (antagonistic) or overadditive (synergistic) effects, respectively.

The concept of response-addition was developed on the basis of stochastic considerations and later evolved to assume that compounds act on different sub-systems in organisms, with different sites of action (Pöch et al., 1996; Kortenkamp and Altenburger, 1999). The effect of the mixture is assumed to equal the arithmetic summation of the effects of single agent:

$$E_{c1,2} = E_{c1} + E_{c2} \quad (8)$$

where $E_{c1,2}$ is the effect of the mixture, and E_{c1} and E_{c2} are the effect of single substance 1 and 2, respectively. If the expectation is met, the two chemicals are assumed to act additively. If the mixture produces an effect exceeding that predicted by summation, then there is thought to be synergism (overadditivity); if it is smaller, there is said to be antagonism (underadditivity) (Pöch et al., 1996; Kortenkamp and Altenburger, 1999).

Combined acute toxicity on luminescent bacteria *Vibrio fischeri* was studied with combination of phenanthrene with each of the six surfactants. Results are shown in Fig. 61 to Fig. 66. Concentrations of phenanthrene covered the same range as

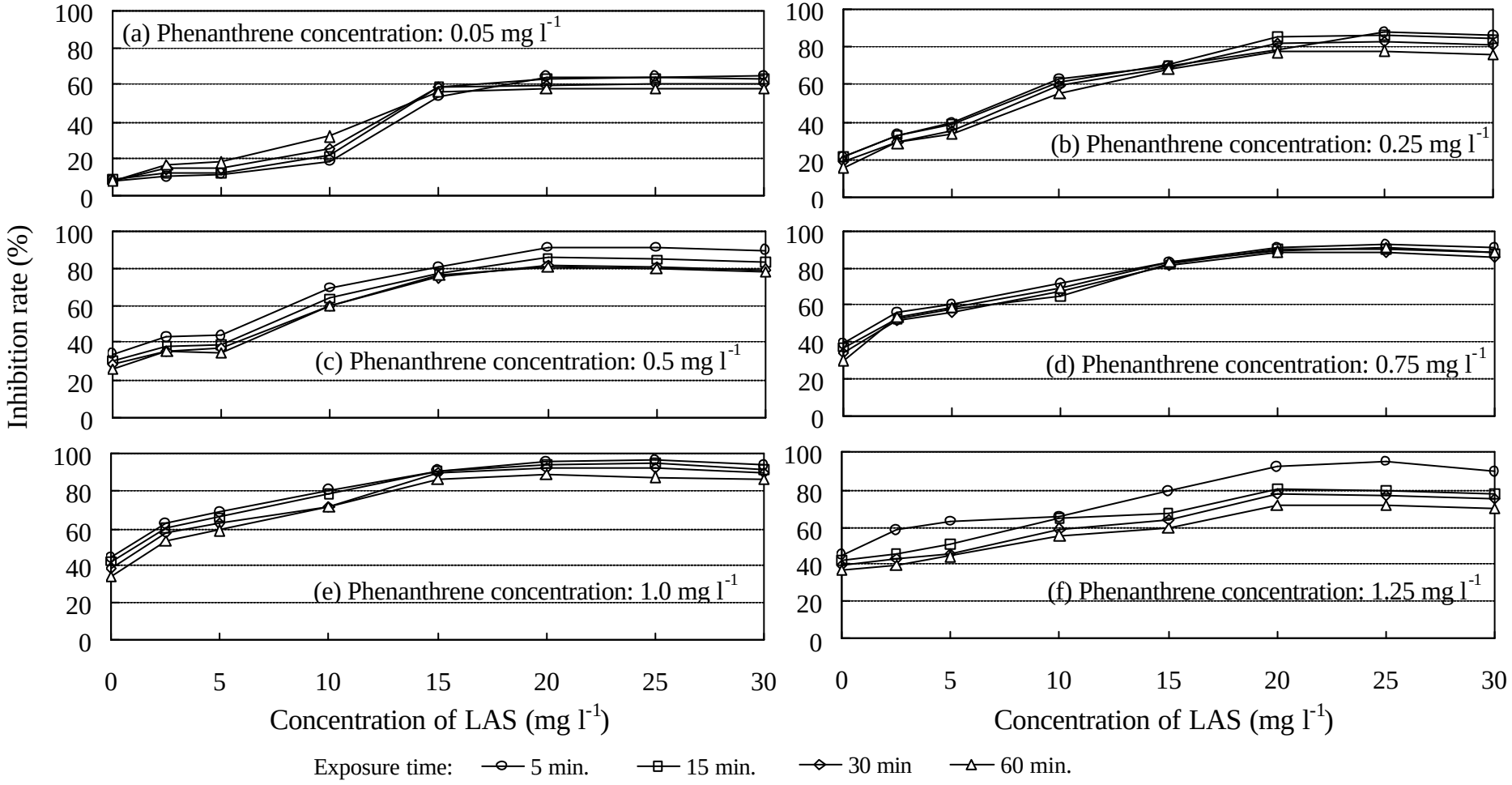


Fig. 61. Combined acute toxicity of phenanthrene and LAS on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

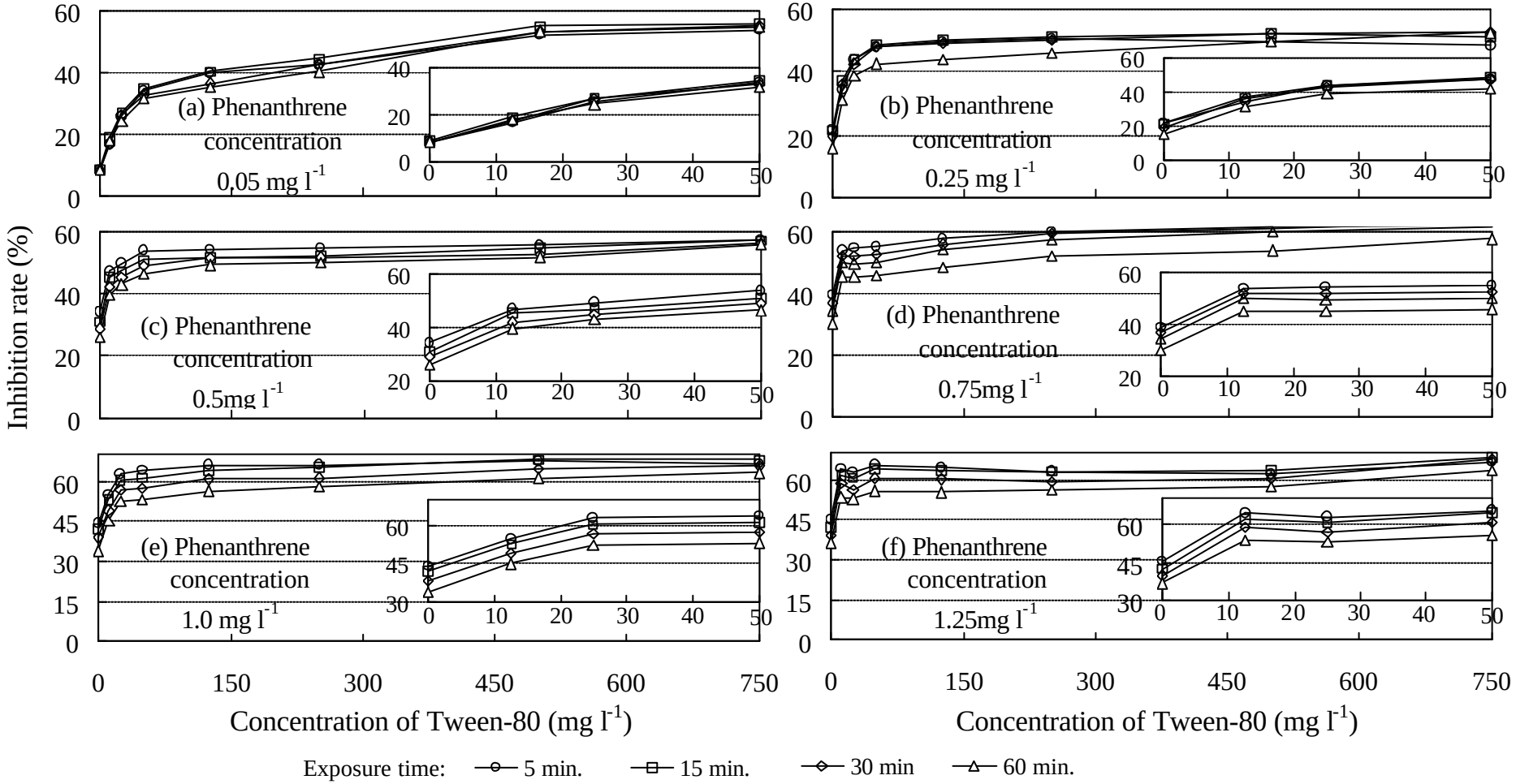


Fig. 62. Combined acute toxicity of phenanthrene and Tween-80 on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

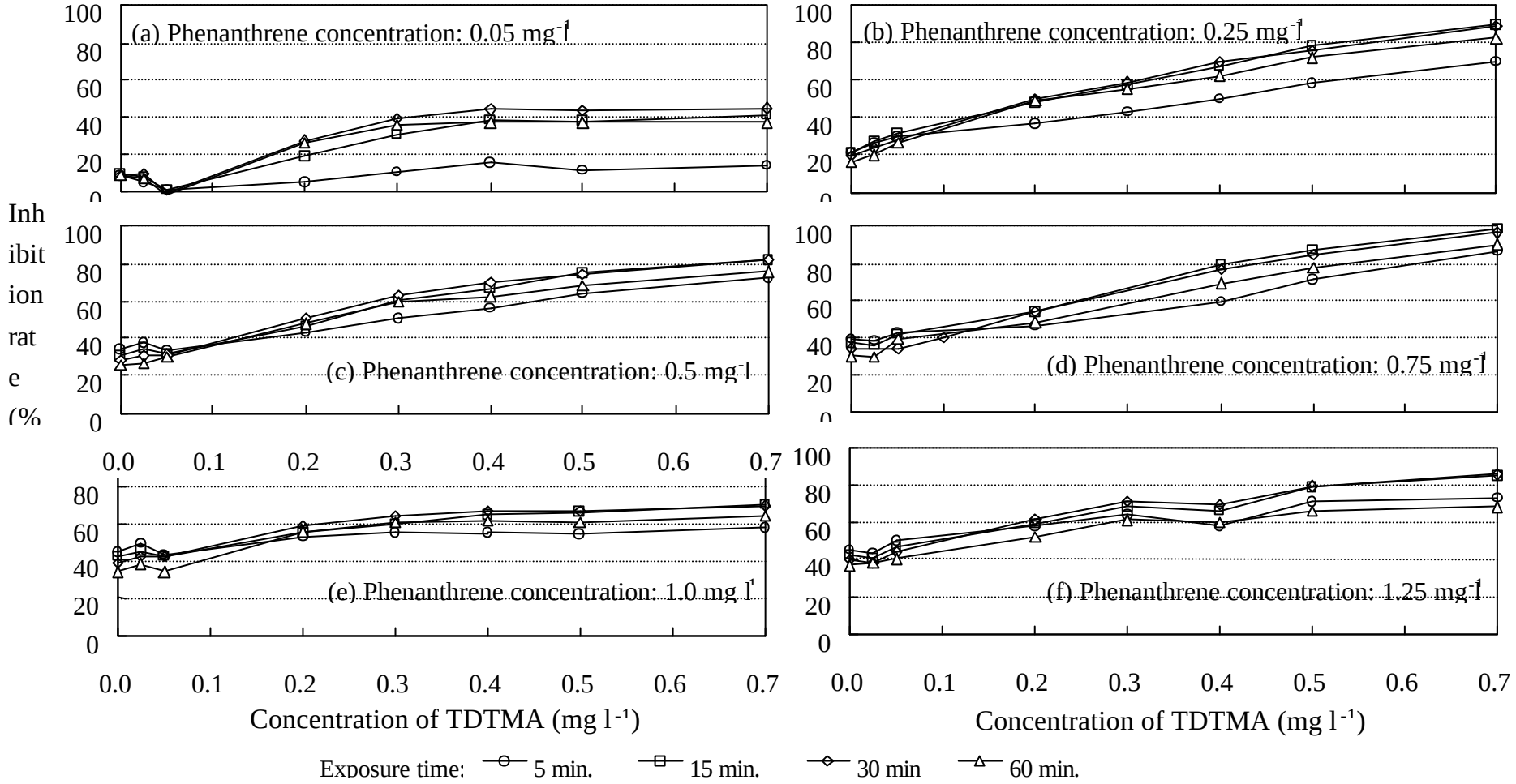


Fig. 63. Combined acute toxicity of phenanthrene and TDTMA on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

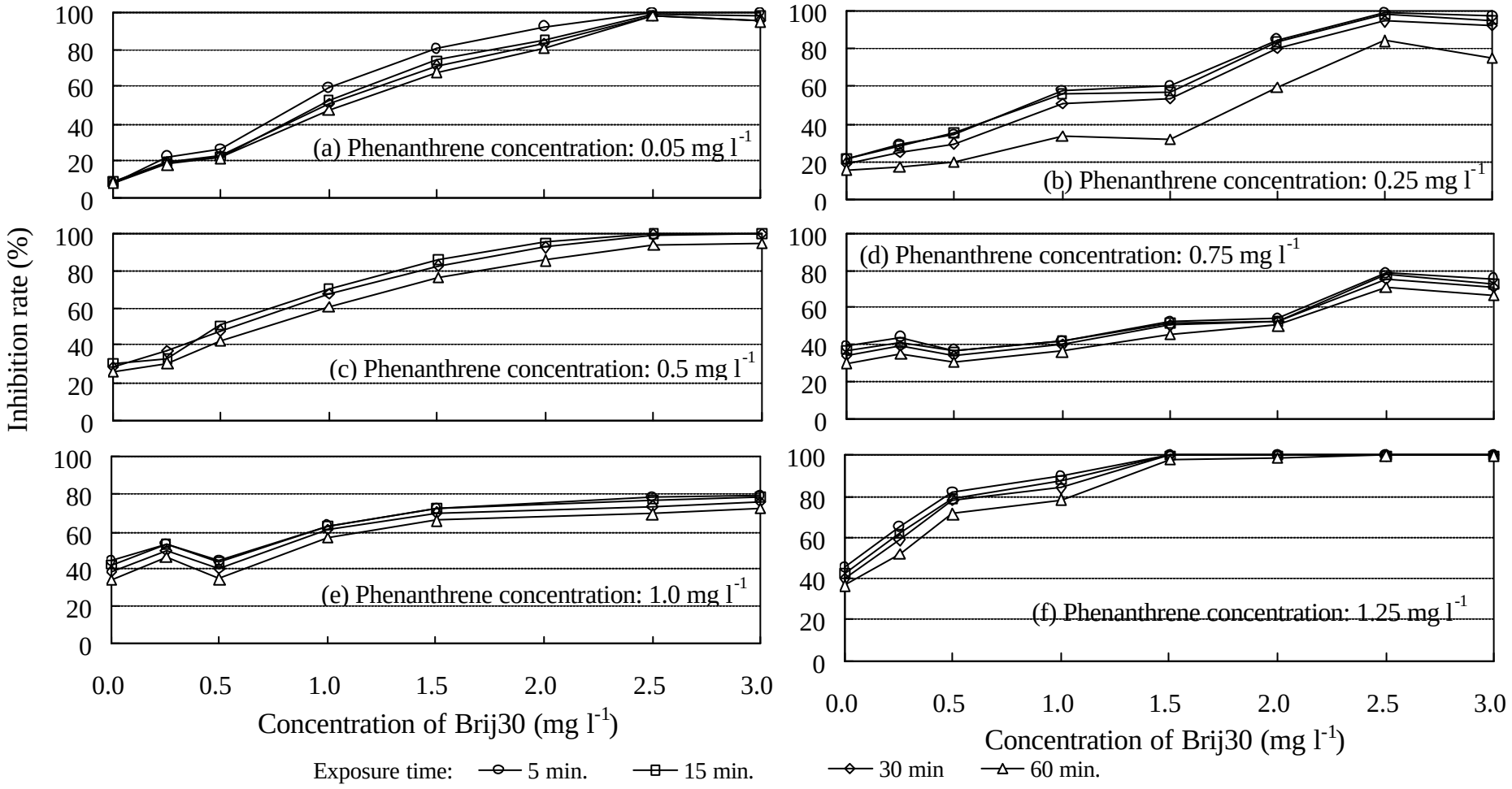


Fig. 64. Combined acute toxicity of phenanthrene and Brij30 on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

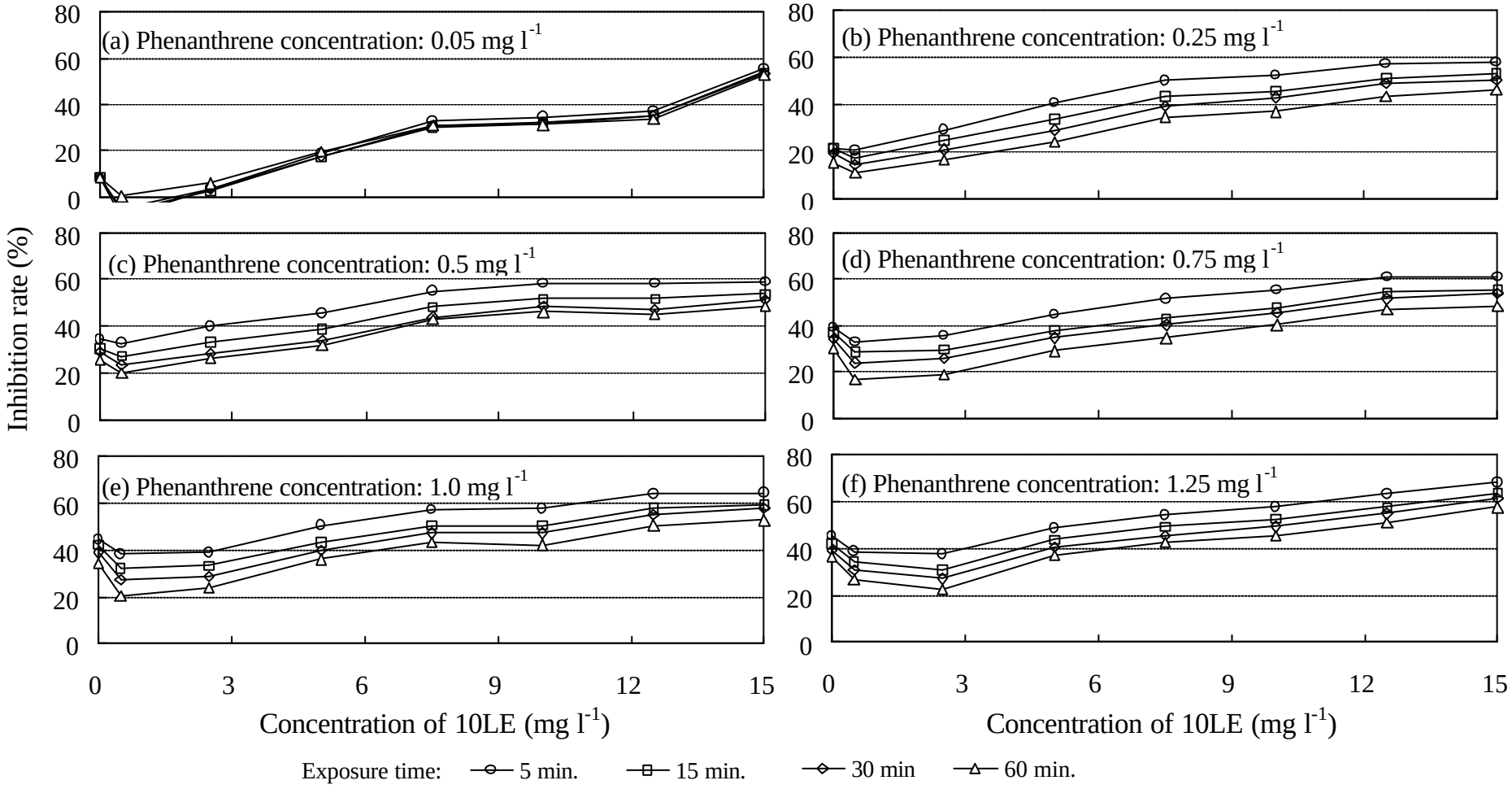


Fig. 65. Combined acute toxicity of phenanthrene and 10LE on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

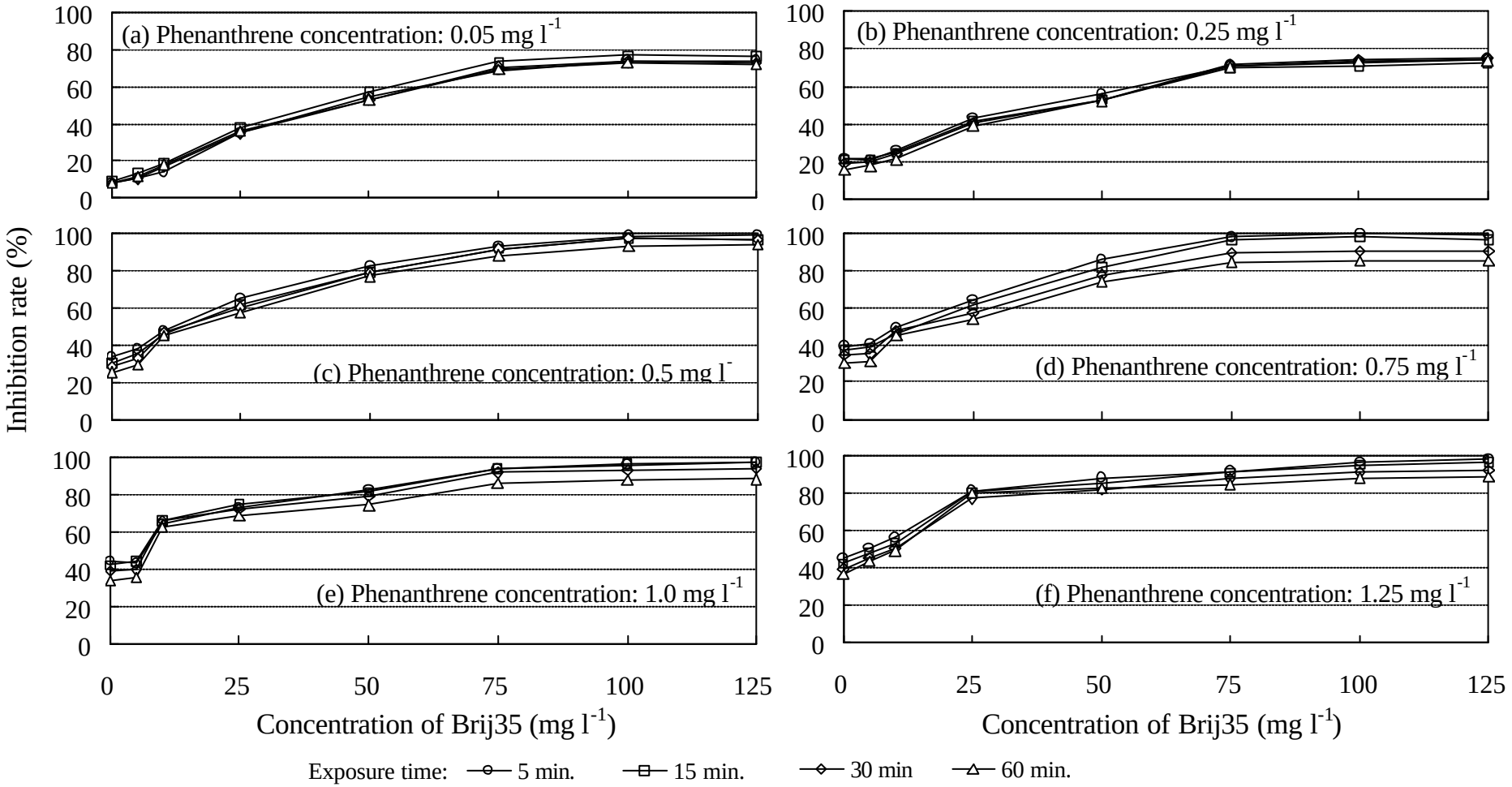


Fig. 66. Combined acute toxicity of phenanthrene and Brij35 on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

measured for its single effects. Concentrations of surfactants were chosen based on results obtained in assay of individual toxicity, which resulted in highest responses of the bacteria (see previous section).

As shown in these figures, combination of phenanthrene and surfactants was more toxic than individual compound (except Brij30, statistically most significant ($P<0.01$) for Tween-80 and 10LE and significant ($P<0.05$) for LAS, TDTMA and Brij35). Generally, the higher doses of the two compounds in combination, the higher toxicity to the bacteria. For example, when exposed separately to 0.25 mg l^{-1} phenanthrene or to 15 mg l^{-1} LAS for 30 min, luminescence emission was inhibited for 18% and 45%, respectively (Fig. 58 and Fig. 59(a)). Combination of the two chemicals of the same doses resulted in 70% inhibition rate (Fig. 61(b)). Combined effects of the two chemicals at higher doses, e.g. 0.5 mg l^{-1} phenanthrene and 20 mg l^{-1} LAS, caused 81% inhibition (Fig. 61(c)). Inhibition rate caused by individual Tween-80 reached first plateau of the dose-response curve at concentration of 100 mg l^{-1} (inhibition rate = 25%) (Fig. 59(c)), while combined with 0.5 mg l^{-1} phenanthrene, the plateau appeared at Tween-80 concentration of 50 mg l^{-1} with an inhibition rate of 47% (Fig. 62(c)). The bacteria were almost completely inhibited by single TDTMA at concentration of 3 mg l^{-1} while the same level of inhibition was obtained by a combination of 0.7 mg l^{-1} TDTMA and 0.75 mg l^{-1} phenanthrene (Fig. 63(d)). To completely inhibit the bacteria, Brij30 concentration was required to reach 40 mg l^{-1} individually (Fig. 60(a)) while only 2.5 mg l^{-1} at the presence of 0.05 mg l^{-1} phenanthrene (Fig. 64(a)). 50 mg l^{-1} Brij35 caused 45% inhibition of luminescent emission (Fig. 60(c)) while at the presence of phenanthrene from 0.05 to 1.25 mg l^{-1}, the same dose of Brij35 produced inhibition rates of 60 to 80% (Fig. 66). Although the combined effects of 10LE and phenanthrene was less significant, the combination increased inhibition rates about 10 to 15% at concentration of 15 mg l^{-1} (Fig. 65) compared with those caused by the same dose of single 10LE (Fig. 60). As clearly shown in Fig. 67, to obtain the same effect e.g. -EC_{50}, concentrations of phenanthrene decreased with the increase of surfactant concentrations and *vice versa*, indicating that combined effects were higher than those of single chemicals.

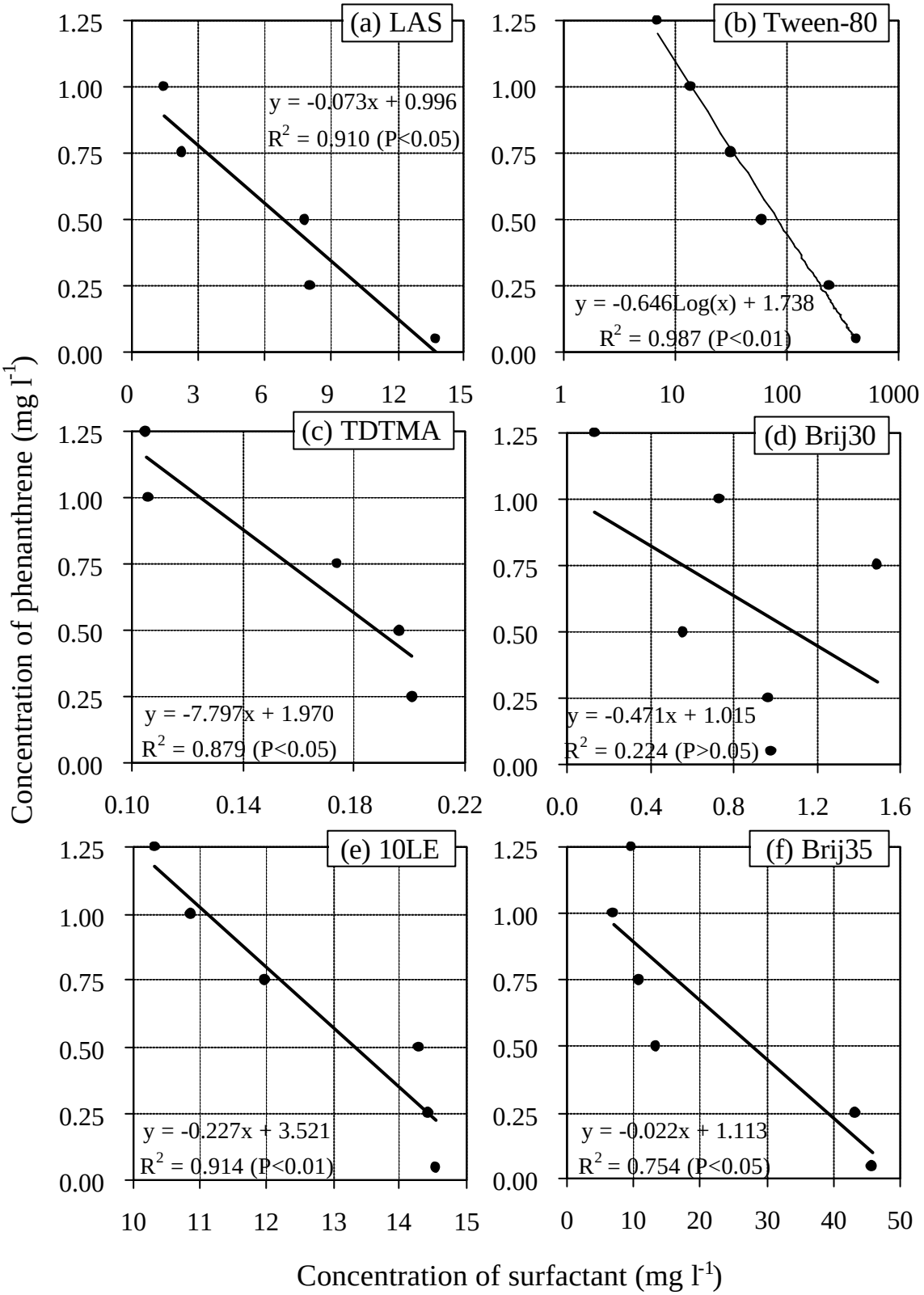


Fig. 67. Combined effects of surfactant and phenanthrene on luminescent bacteria *Vibrio fischeri*: relationship among -EC_{50} and concentrations of phenanthrene and surfactants. Data points are means of duplicates.

Similar to separate effects, responses did not increased linearly with the increase of LAS or Tween-80 doses, even at low concentrations, in the coexistence of phenanthrene (Fig. 61 and Fig. 62). Although the dose-response curves of single TDTMA was not linear, responses to the combined effects of phenanthrene and TDTMA was linear at concentrations investigated (TDTMA < 0.7 mg l^{-1}) (Fig. 63). In contrast to TDTMA, the three Brij surfactants at low concentrations caused linear responses from the bacteria, but their combination with phenanthrene did not produce linear dose-response curves at the same or even lower concentrations. Instead, the responses to the combined effects reached a plateau at lower concentrations than separate effects (Fig. 64 to Fig. 66).

Combined EC values (Table 14) produced by the mixture of phenanthrene and surfactants were generally lower than those caused by separate surfactant or phenanthrene, also indicating that higher toxicity of combination of the chemicals than individual. For example, in combination with 1.0 mg l^{-1} phenanthrene, combined -EC_{50} values for LAS was one magnitude lower than its separate effects, 50 times lower for Tween-80, 4 times lower for TDTMA, 3 times lower for Brij30, and 8 times lower for Brij35 (Table 13 and Table 14). The combined effects of phenanthrene and 10LE were less significant based on -EC values compared with other surfactants. According to -EC values, the combined toxicity of phenanthrene and surfactants was in a descent order of TDTMA > Brij30 > LAS > Brij35 > 10LE > Tween-80, which was somewhat different from the order of their individual effects for the position of 10LE, i.e. TDTMA > Brij30 > 10LE > LAS > Brij35 > Tween-80 in single effects.

The combined effects of phenanthrene and the 6 surfactants were not additive based on the response-addition model (8) (Fig. 68). The combined effects of phenanthrene with LAS, Tween-80, 10LE or Brij35 were underadditive or antagonistic, i.e. they were lower than the sum of their individual effects at the same dose (Fig. 68(a)(b)(e)(f)). The most underadditive was Tween-80, followed by 10LE, LAS and Brij35. The combined effects of phenanthrene and Tween-80 were slightly lower than the half of the sum of their individual effects. The mixtures of phenanthrene and Brij35 caused combined effects slightly lower than the sum of their individual effects

(Fig. 68(f)). Unlike the above 4 surfactants, the combined effects of phenanthrene and TDTMA or Brij30 were overadditive or synergistic at theoretic effects ($E_{c1,2}$ in equation (8)) lower than 70% inhibition rates (i.e. -EC_{70}), corresponding to 70% and 90% measured inhibition rates for TDTMA and Brij30, respectively (Fig. 68(c)(d)). Their combination was more toxic than the sum of the toxicity of the pure chemicals.

Table 14. EC values of combined acute toxicity of phenanthrene and surfactants on luminescent bacteria *Vibrio fischeri*, 30 min exposure.

	Phenanthrene (mg l^{-1})	0.05	0.25	0.50	0.75	1.00	1.25
-EC_{10}							
	LAS (mg l^{-1})	0.6	>	>	>	>	>
	Tween-80 (mg l^{-1})	9.8	>	>	>	>	>
	TDTMA (mg l^{-1})	0.03	>	>	>	>	>
	Brij30 (mg l^{-1})	0.04	>	>	>	>	>
	10LE (mg l^{-1})	3.6	>	>	>	>	>
	Brij35 (mg l^{-1})	3.7	>	>	>	>	>
-EC_{20}							
	LAS (mg l^{-1})	7.6	0.2	>	>	>	>
	Tween-80 (mg l^{-1})	16.5	15.0	>	>	>	>
	TDTMA (mg l^{-1})	0.16	0.01	>	>	>	>
	Brij30 (mg l^{-1})	0.29	0.03	>	>	>	>
	10LE (mg l^{-1})	5.4	2.2	>	>	>	>
	Brij35 (mg l^{-1})	12.7	5.2	>	>	>	>
-EC_{50}							
	LAS (mg l^{-1})	13.7	8.1	7.8	2.3	1.5	6.5
	Tween-80 (mg l^{-1})	427.8	239.6	59.4	31.7	13.7	6.8
	TDTMA (mg l^{-1})	<	0.20	0.20	0.17	0.12	0.11
	Brij30 (mg l^{-1})	0.98	0.97	0.56	1.49	0.73	0.14
	10LE (mg l^{-1})	14.5	14.4	14.3	12.0	10.9	10.3
	Brij35 (mg l^{-1})	45.7	43.3	13.3	10.8	7.0	9.7

Notes: (1) -EC_{10}, -EC_{20} and -EC_{50} are effective concentrations of surfactant causing 10%, 20% and 50% decrease of luminescent emission, respectively.

(2) > and < represent the toxicity was higher or lower than the specified percentage of effects, respectively.

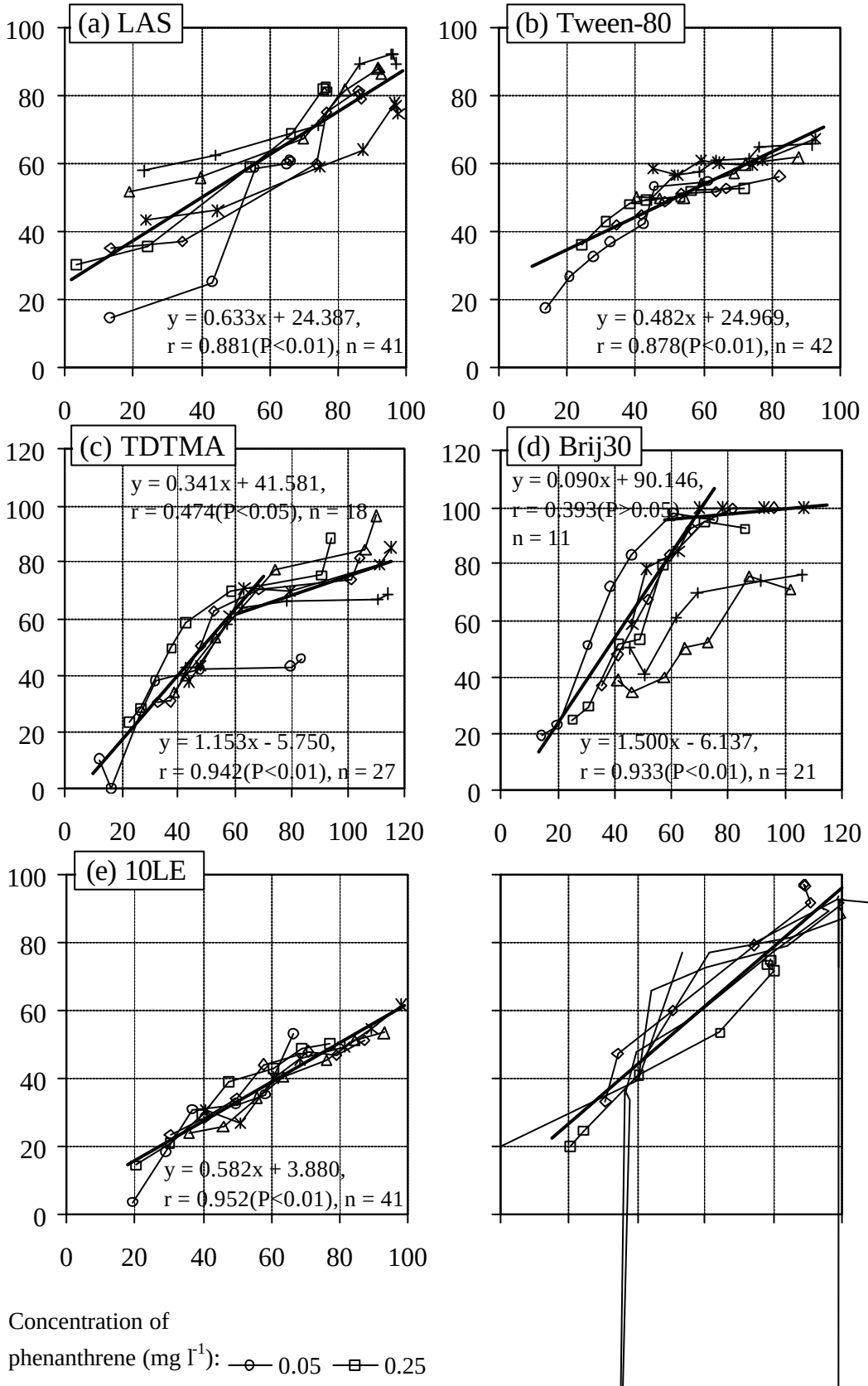


Fig. 68. Mode of combined acute toxicity of surfactant and phenanthrene on luminescent bacteria *Vibrio fischeri*. Data points are means of duplicates.

The most synergistic effects were produced by the mixture of phenanthrene and Brij30, which was 50% higher than the sum of their individual effects. At the theoretic inhibition rate of 70%, almost complete inhibition was measured. The combined effects of phenanthrene and TDTMA were 15% higher than the sum of the effects of single chemical. At higher effect concentration, the combined effects of phenanthrene and TDTMA or Brij30 were underadditive, indicating the effects approaching the maximum or plateau. The order of combined effects of phenanthrene and the 6 surfactants from synergism to antagonism was Brij30 > TDTMA > Brij35 > LAS > 10LE > Tween-80.

4.2.2 Effects of surfactants on the respiration of microorganism (*Mycobacterium* sp.) in liquid culture

Fig. 69 shows the effects of LAS on the respiration of *Mycobacterium* sp. (strain KR2) in the presence and absence of phenanthrene. *Mycobacterium* sp. has been reported to be able to utilize a number of PAHs as sole carbon and sole energy source, e.g. naphthalene, anthracene, phenanthrene, fluoranthene, pyrene and benzo[a]pyrene (Cerniglia, 1992). Strain KR2 has demonstrated the capacity to oxidize both phenanthrene and pyrene (Rehmann *et al*., 1996 and 1998).

When LAS was present as the sole carbon and energy source, the activity of *Mycobacterium* sp., expressed as oxygen consumption, was inhibited at all LAS concentrations studied from 5 to 450 mg l^{-1} and its respiration levels were all lower than the background level (i.e. only the bacteria existed). When LAS concentration reached 200 mg l^{-1}, the respiration was completely inhibited. Further more, the oxygen consumption levels declined with the increase of LAS concentration (Fig. 69(a)). The results clearly indicated that (1) *Mycobacterium* sp. could not utilize LAS as sole carbon and energy source and (2) LAS was toxic to the bacteria. If -EC_{20} and -EC_{50} are assigned for effective concentrations which cause 20% and 50% reduction of microbial activity (e.g. expressed as oxygen consumption), the values of -EC_{20} and -EC_{50} for LAS effecting on *Mycobacterium* sp. were 21.5 and 63.0 mg l^{-1}, respectively (Table 15).

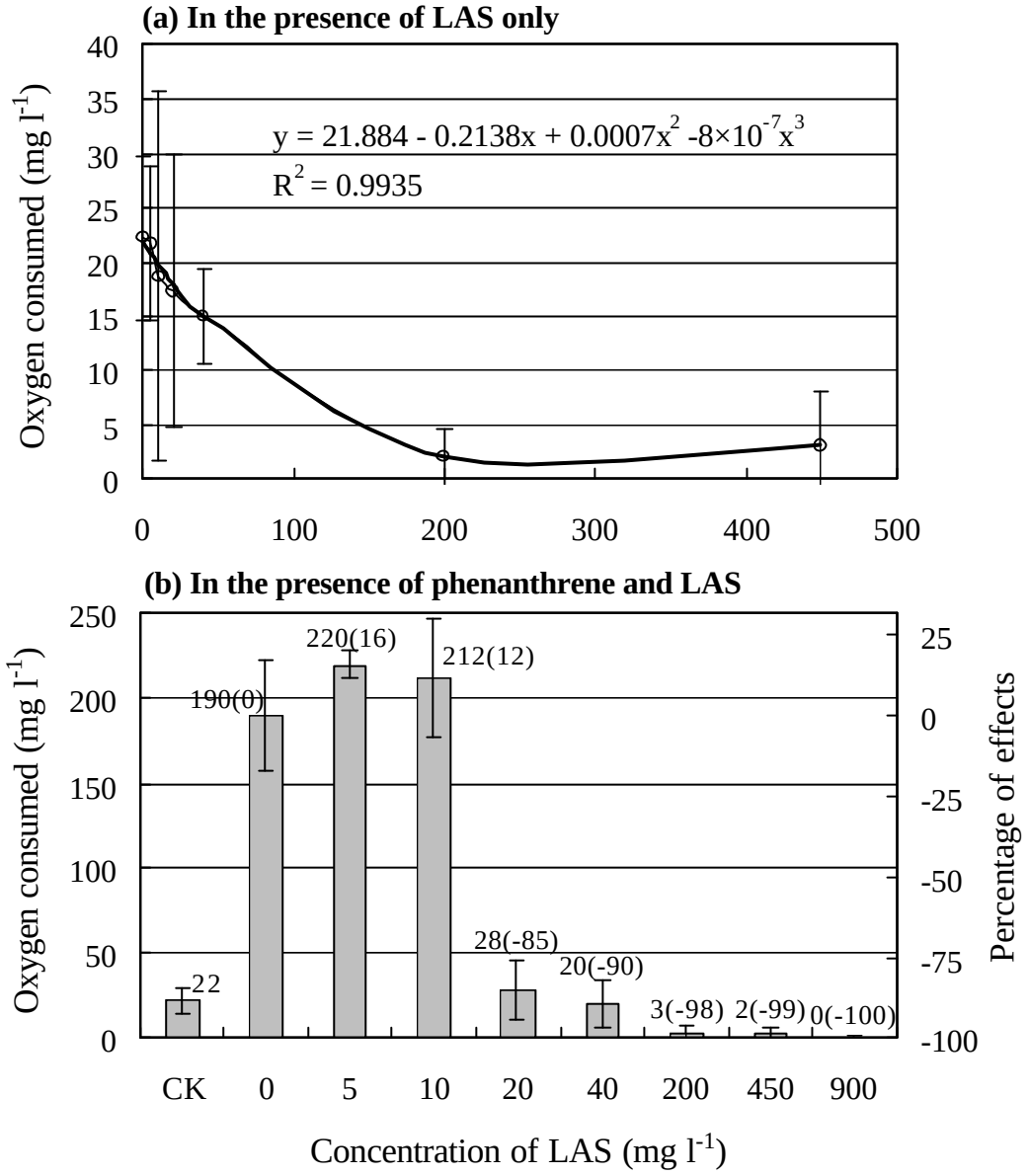


Fig. 69. Effects of LAS on the respiration of *Mycobacterium* sp. in the (a) absence and (b) presence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for 20 days. CK represents treatment with neither phenanthrene nor LAS. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols. Data in parenthesis are percentage of increase or decrease compared with that without surfactants.

However, when phenanthrene coexisted as substrate, LAS at low concentrations of 5 and 10 mg l^{-1} increased the respiration levels of *Mycobacterium* sp. for 16 and 12%, respectively, compared with that with the presence of phenanthrene alone (Fig. 69(b)).

The increase of microbial activity at low levels of LAS suggested that (1) the toxicity of LAS on the bacteria eased at the presence of phenanthrene, (2) LAS probably increased the bioavailability of phenanthrene, and (3) *Mycobacterium* sp. could probably utilize LAS as co-substrate. Further experiments using ^{14}C-labeled phenanthrene have been conducted to verify if LAS could increase the bioavailability of phenanthrene (see Section 4.3.1).

Table 15. EC values of surfactant's effects on *Mycobacterium* sp. in the presence (100 mg l^{-1}) and absence of phenanthrene (mg l^{-1})

	Surfactants	$+EC_{20}$	$+EC_{50}$	$-EC_{20}$	$-EC_{50}$
In the absence of phenanthrene					
	LAS	NE	NE	21.5	63.0
	Tween-80	12.2	30.6	NE	NE
	TDTMA	NE	NE	1.8	4.5
	Brij30	6.8	16.9	NE	NE
	10LE	0.8	1.9	NE	NE
	Brij35	5.8	7.1	ND	ND
In the presence of phenanthrene					
	LAS	ND	ND	13.3	16.4
	Tween-80	77.6	193.9[a]	NE	NE
	TDTMA	NE	NE	1.0	2.6
	Brij30	24.6	61.4	NE	NE
	10LE (<20mg l^{1})	3.2	8.8	NE	NE
	10LE (>20mg l^{1})	20.0	164	NE	NE
	Brij35	3.8	ND	ND	ND

Notes: $+EC_{20}$ and $+EC_{50}$ are assigned for effective concentrations of surfactants which cause 20% and 50% increase of microbial activity (oxygen consumption), respectively; whereas, $-EC_{20}$ and $-EC_{50}$ for those causing 20% and 50% decrease of microbial activity, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

[a] extrapolated values.

At higher concentrations, LAS inhibited the activity of the bacteria as well as the utilization of phenanthrene by the bacteria (Fig. 69(b)). Similar to the case where there was only LAS present, the respiration completely stopped at LAS concentration above 200 mg l^{-1}, indicating the toxicity of LAS to the bacteria. The values of -EC_{20} and -EC_{50} were 13.3 and 16.4 mg l^{1}, respectively, which were lower than those obtained with the presence of LAS alone (Table 15).

The cationic surfactant TDTMA inhibited the respiration of *Mycobacterium* sp. no matter if phenanthrene coexisted or not (Fig. 70). At concentration as low as 10 mg l^{-1}, it completely inhibited the respiration. The -EC_{20} and -EC_{50} were 1.8 and 4.5 mg l^{-1} with the absence of phenanthrene or 1.0 and 2.6 mg l^{1} with the co-presence of phenanthrene, respectively (Table 15). They were one order of magnitude lower than those of LAS. Results indicated that TDTMA was much more toxic to *Mycobacterium* sp. than LAS did.

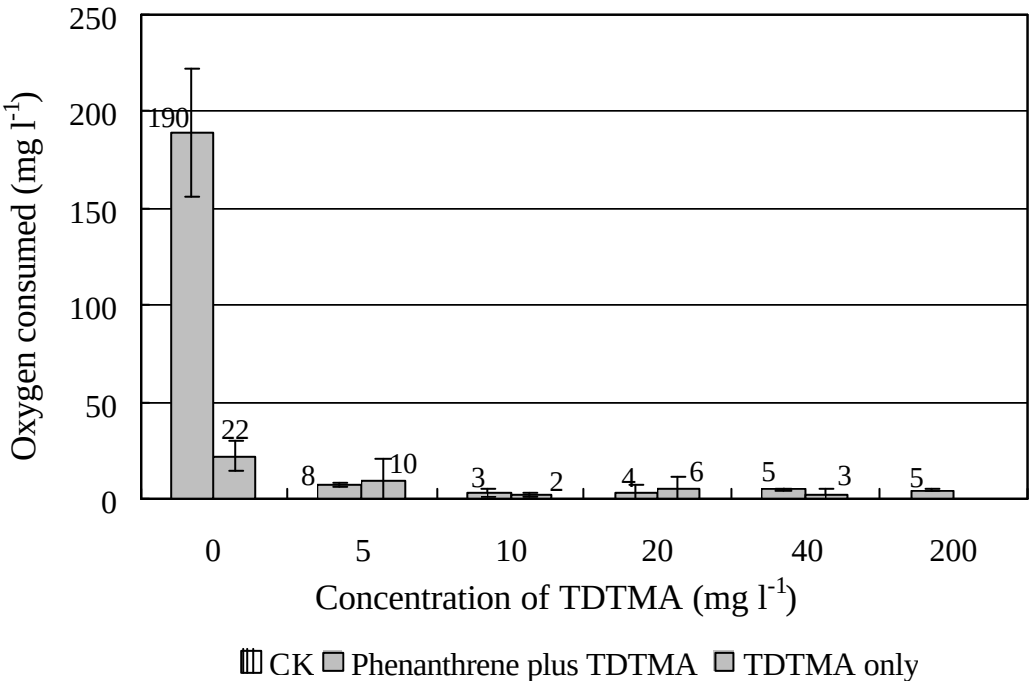


Fig. 70. Effects of TDTMA on the respiration of *Mycobacterium* sp. in the presence and absence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for 20 days. CK represents treatment with neither phenanthrene nor TDTMA. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

Unlike LAS and TDTMA, nonionic surfactant Tween-80 increased the activity (oxygen consumption) of *Mycobacterium* sp. at all concentrations studied from 5 to 80 mg l^{-1} or from 1/8CMC to 2CMC, no matter if phenanthrene was present or not (Fig. 71). The oxygen consumption levels were increased 20% and 120% by Tween-80 at 80 mg l^{-1} in the presence and absence of phenanthrene, respectively. The values of $+EC_{20}$ and $+EC_{50}$, defined as effective concentrations causing 20 or 50% increase of microbial activities, were 12.2 and 30.6 mg l^{-1} in the absence of phenanthrene and 77.6 and 194 mg l^{-1} in the presence of the material, respectively (Table 15). The respiration levels increased linearly with the increasing of Tween-80 concentrations in both cases with the presence and absence of 100 mg l^{-1} phenanthrene. The linear increasing of the respiration lasted up to 30 days and maybe even longer. *Mycobacterium* sp. could obviously utilize Tween-80 as sole carbon and energy source. The oxygen consumption increased to a greater extent in the presence of phenanthrene than that in the absence of the material as shown by that the slopes of the curves were 0.5-0.6 for the former and 0.4 for the later. It implied that Tween-80 could probably increase the bioavailability of phenanthrene. Further experiments using ^{14}C-labeled phenanthrene have been conducted to verify the possibility (see Section 4.3.1). The slope of the curve also represents the specific oxygen consumption of Tween-80, i.e. 0.5-0.6 mg O_2 was consumed per mg Tween-80 in the presence of phenanthrene or 0.4 mg O_2 per mg Tween-80 in the absence of phenanthrene.

Effects of the three Brij series nonionic surfactants, i.e. Brij30, 10LE and Brij35, were very differences from each other (Fig. 72 to Fig. 74). Brij30 had similar behaviour as Tween-80 did. It increased linearly the microbial activities at all concentrations covering from 5 mg l^{-1} to 80 mg l^{-1}, which was the same as for Tween-80 (Fig. 72). Results clearly indicated that *Mycobacterium* sp. could grow on Brij30 as sole carbon and energy source. There was also the possibility that Brij30 could increase the bioavailability of phenanthrene, which have been further investigated using ^{14}C-labeled phenanthrene (see Section 4.3.1). The values of $+EC_{20}$ and $+EC_{50}$ were 6.8 and 16.9 mg l^{-1} in the absence of phenanthrene or 24.6 and 61.4 mg l^{-1} in the presence of the material (Table 15). Brij30 had a higher oxygen demand than

Tween-80, i.e. 1.6 and 1.2 mg O_2 mg^{-1} Brij30 compared with 0.5 and 0.4 mg O_2 mg^{-1} Tween-80 in the presence and absence of phenanthrene, respectively. These results indicated that Brij30 had stronger positive effects on the activity of *Mycobacterium* sp. than Tween-80 did. At the same concentration of 80 mg l^{-1}, Brij30 increased 80% and 500% of the oxygen consumption in the presence and absence of phenanthrene, respectively, while only 20% and 120% for Tween-80.

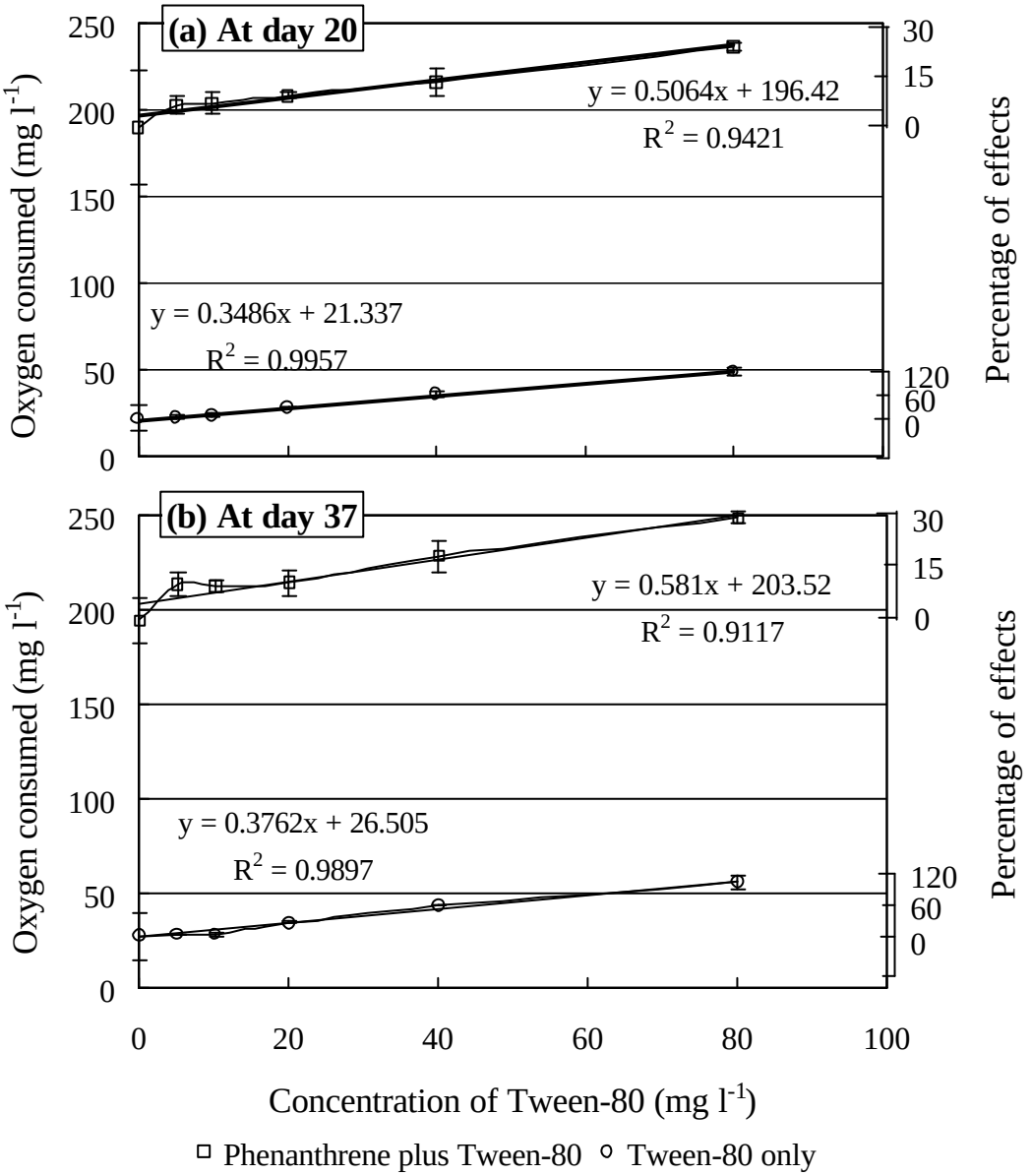


Fig. 71. Effects of Tween-80 on the respiration of *Mycobacterium* sp. in the presence and absence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for (a) 20 days and (b) 37 days, respectively. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

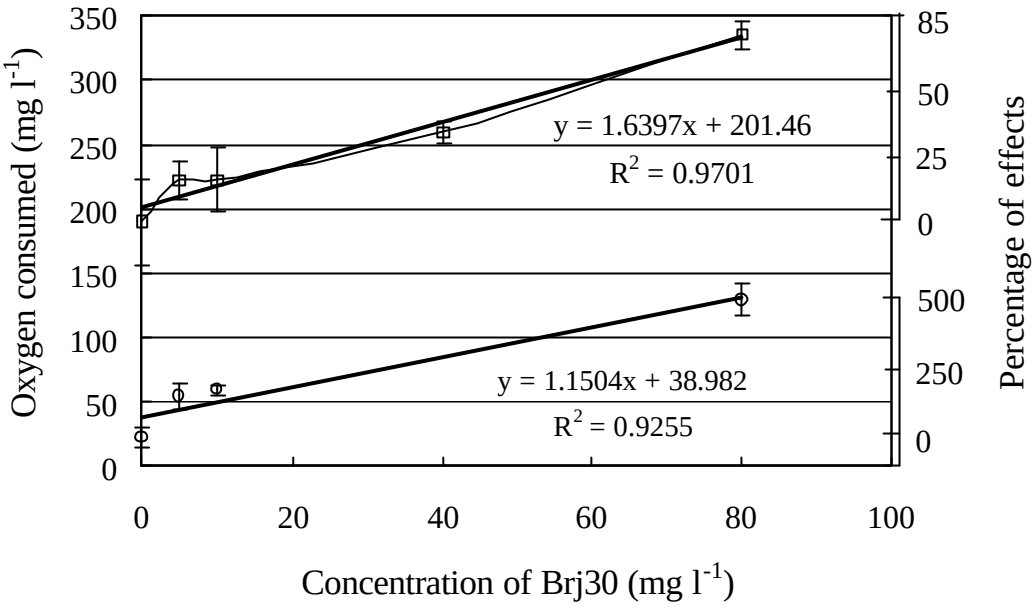


Fig. 72. Effects of Brij30 on the respiration of *Mycobacterium* sp. in the presence and absence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for 20 days. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

10LE generally increased the respiration of *Mycobacterium* sp. both in the presence and absence of phenanthrene (Fig. 73). However, the increase was not linear against the concentration of 10LE as Brij30 did. The respiration levels increased with the increase of 10LE concentrations from 5 to 10 mg l^{1} but decreased when 10LE concentrations further increased. Then the respiration increased again gradually till the highest concentration (200 mg l^{-1}) studied. But the oxygen consumption level at 10LE concentration of 200 mg l^{-1} was the same as that at 10 mg l^{-1}, which caused 56% increase of respiration in the presence of phenanthrene. The values of $+EC_{20}$ and $+EC_{50}$ were 0.8 and 1.9 mg l^{-1} in the absence of phenanthrene, respectively (Table 15). In the presence of phenanthrene, when concentrations of 10LE < 20 mg l^{-1}, $+EC_{20}$ and $+EC_{50}$ were 3.2 and 8.8 mg l^{-1}; at concentrations of 10LE > 20 mg l^{-1}, corresponding values were 20 and 164 mg l^{-1}, respectively.

The effects of Brij35 were similar to those of LAS, i.e. increasing the microbial

activity at low concentrations while inhibiting at high concentrations (Fig. 74). The respiration levels of *Mycobacterium* sp. were the same at Brij35 concentrations of 5 and 10 mg l^{-1}, which was 25% higher than that with the presence of phenanthrene alone. Although the oxygen consumption at Brij35 concentrations of 40 and 80 mg l^{-1} was still higher than that with phenanthrene only, it was lower than that at lower Brij35 concentrations. Similar results were obtained in test without the presence of phenanthrene (Fig. 74). These results indicated that (1) Brij35 was toxic to the bacteria at high concentrations, (2) Brij35 had the potential ability increasing the bioavailability of phenanthrene, and (3) *Mycobacterium* sp. could probably utilize Brij35 as co-substrate. Further experiments using ^{14}C-labeled phenanthrene have been conducted to verify if Brij35 could increase its bioavailability (see Section 4.3.1). The values of $+EC_{20}$ and $+EC_{50}$ were 5.8 and 7.1 mg l^{-1} in the absence of phenanthrene, respectively. In the presence of phenanthrene, $+EC_{20}$ was 3.8 mg l^{-1} and $+EC_{50}$ could not been observed at the concentrations studied.

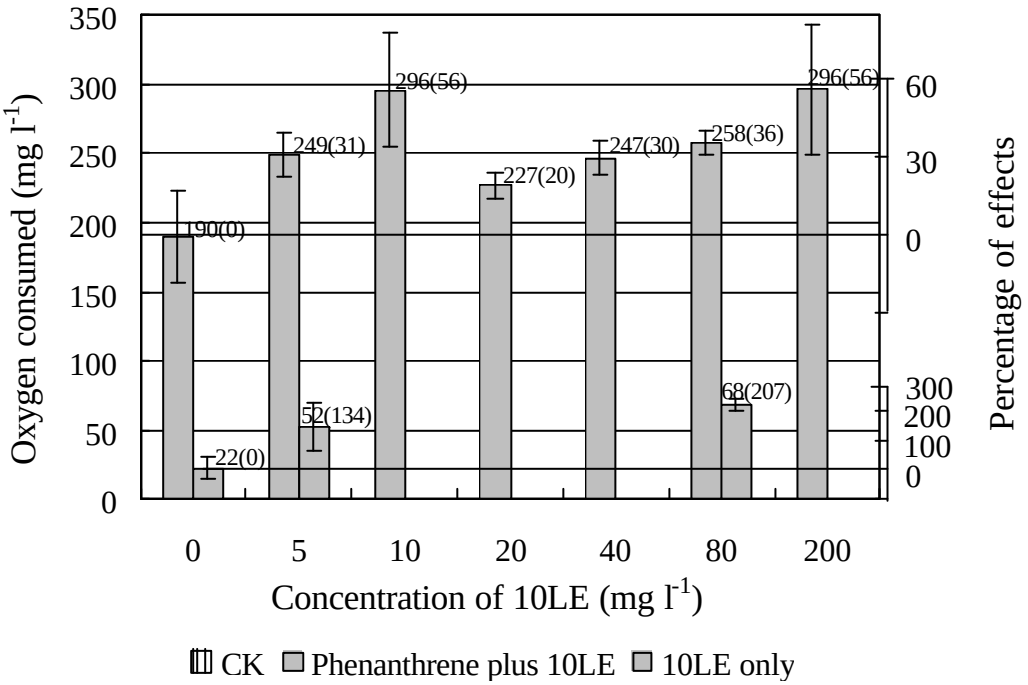


Fig. 73. Effects of 10LE on the respiration of *Mycobacterium* sp. in the presence and absence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for 20 days. CK represents treatment with neither phenanthrene nor 10LE. The error bars indicate standard deviations based on triplicate measurements. Data in parenthesis are percentage of increase or decrease compared with those without surfactants.

The effects of Brij series surfactants on the microbial activity were closely related to their molecular structure. The three Brij series surfactants have the same molecular structure except the difference in the length of ethoxylate group (Fig. 5). The toxicity of the surfactants increased with the increase of ethoxylate groups in the molecule. Brij30 containing 4 ethoxylate groups showed no toxic effects to *Mycobacterium* sp., while 10LE of 10 ethoxylate groups exhibited a bit toxicity followed by Brij35 containing 23 ethoxylate groups. The extent to which they increased the respiration levels of the bacteria also decreased with the increase of ethoxylate groups from Brir30 to Brij35 both in the presence and absence of phenanthrene (Fig.72 to Fig. 74).

Comparison among the six surfactants studied shows that the cationic TDTMA was the most toxic to *Mycobacterium* sp., followed by anionic LAS and nonionic Brj35. Nonionic Tween-80, Brij30 and 10LE were not toxic to the bacteria at all at

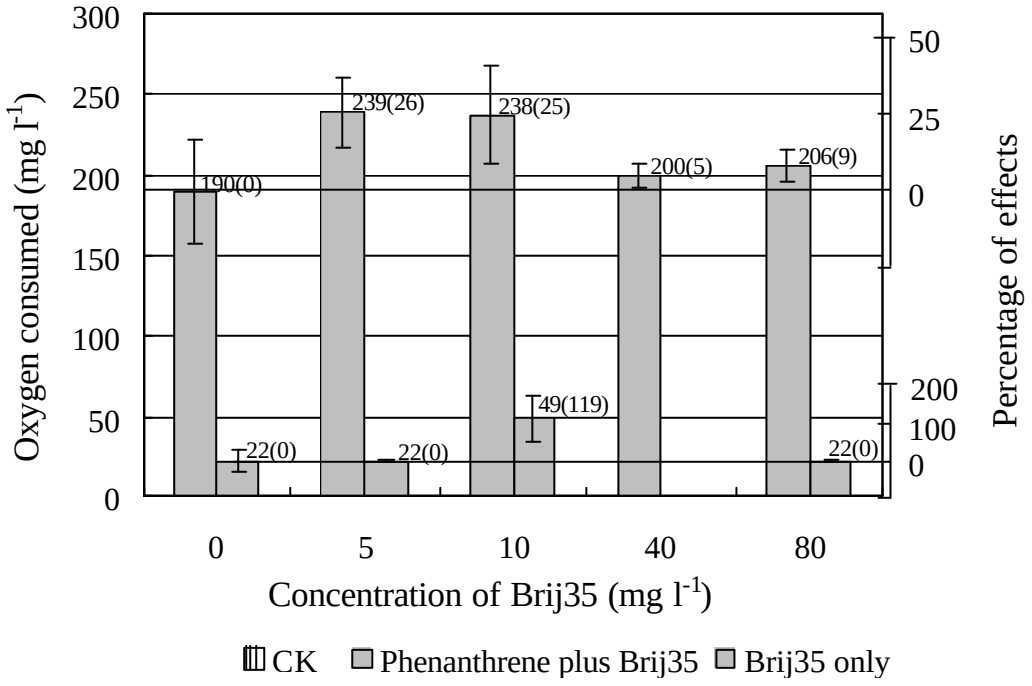


Fig. 74. Effects of Brij35 on the respiration of *Mycobacterium* sp. in the presence and absence of phenanthrene (100 mg l^{-1}) in liquid culture at 20°C for 20 days. CK represents treatment with neither phenanthrene nor Brij35. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols. Data in parenthesis are percentage of increase or decrease.

concentrations studied. This toxic order was also reflected by their values of -EC_{20} and -EC_{50}. TDTMA had the lowest values, followed by LAS. Brij35 showed sign of toxicity though -EC_{20} and -EC_{50} could not be measured at the concentrations examined. Fig. 75 shows the mode of combined effects of surfactants and phenanthrene on the respiration of the bacteria. Two different modes could be obviously seen. The combined effects of phenanthrene and Tween-80 or the three Brij series surfactants were positive and close to additivity, which can be described the response-addition model (Equation (8)). It suggested that the coexistence of phenanthrene and these surfactants increase the activity of the bacteria. The combined effects equalled to the sum of their individual effects, which might imply that the combined effects were results of substrate summation of the individual chemicals. The second type of combined effect mode was negative for the mixture of phenanthrene and LAS or TDTMA, indicating the toxicity of the surfactants to the bacteria.

Fig. 76 is the respiration kinetics of *Mycobacterium* sp. in the presence and absence of test surfactants and phenanthrene. The background respiration level of the bacteria without the presence of substrate was low (oxygen consumption of 22 mg l^{-1} at day 20). It reached the 50% of the final 20-days' respiration levels at day 3. Addition of

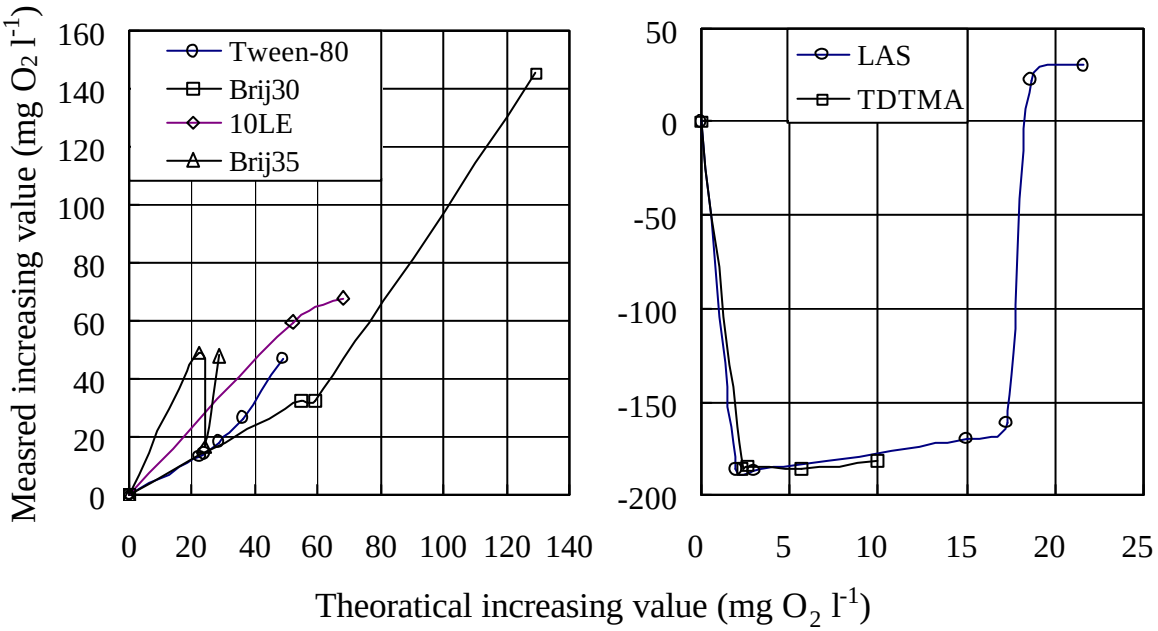


Fig. 75. Mode of combined effects of surfactant and phenanthrene on the respiration of *Mycobacterium* sp. in liquid culture at 20°C for 20 days

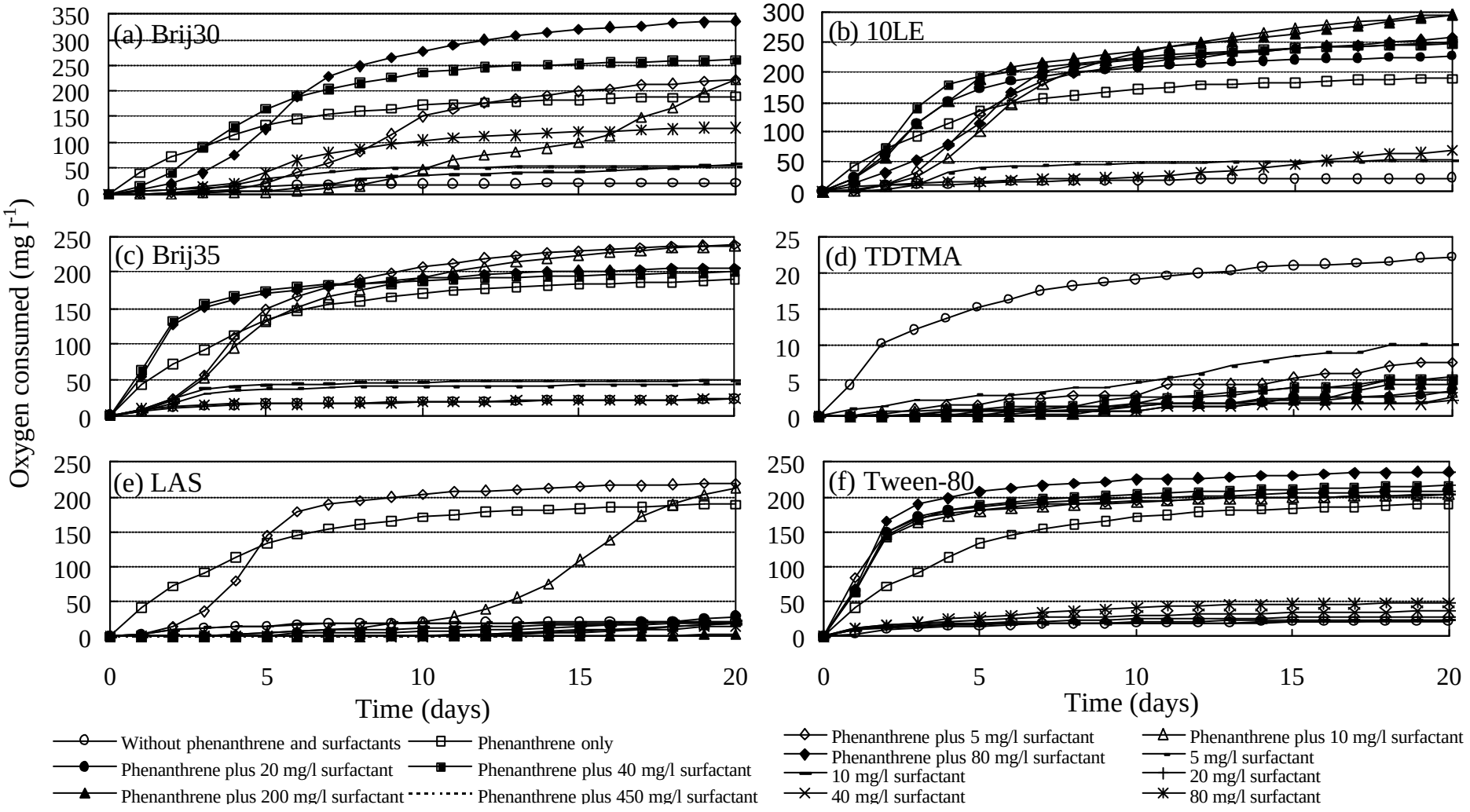


Fig. 76. Respiration kinetics of *Mycobacterium* sp. at 20°C in the presence and absence of phenanthrene (100 mg l^{-1}) and surfactants. Data points are means of triplicates.

phenanthrene could dramatically increase the respiration of the bacteria to 190 mg l^{-1} oxygen consumption at day 20. Although the coexistence of LAS at concentrations of 5 and 10 mg l^{-1} increased the final respiration levels, the activity of the bacteria was inhibited or lower than that without the presence of LAS before day 5 and day 28 for LAS concentrations of 5 and 10 mg l^{-1}, respectively. It indicated that there was a lag phase before the bacteria adapted to the surfactant. The length of the lag phase was proportional to the concentration of the surfactant. The three Brij series surfactants at some concentrations also caused hysteresis but acted in different mode compared with that of LAS. For example, the lag time for Brij30 was shorter at high concentrations of 40 and 80 mg l^{1} than at low concentrations of 5 and 10 mg l^{1}. There was no hysteresis at Birj35 concentrations of 40 and 80 mg l^{-1} at all while 5 day lag time was observed at 5 and 10 mg l^{-1}. The hysteresis caused by the Brij series surfactants was not because of their toxic effects as LAS did. It was probably due to the low bioavailability to the bacteria at low concentrations. Tween-80 did not cause any hysteresis at all concentrations indicating that it was more easily assessable to the bacteria. Because of its high toxicity to the bacteria, respiration levels at the presence of TDTMA were all lower than the background level during the whole time window monitored.

4.2.3 Effects of surfactants on the growth of *Mycobacterium* sp. on non-toxic soluble substrate

To investigate the influence of substrate on surfactant's effects on *Mycobacterium* sp., growth rates of the bacteria were measured on non-toxic soluble nutrient medium (R2A) at the presence of surfactants. Results are shown in Fig. 77 and Fig. 78. Effects of LAS on the growth of the bacteria on phenanthrene were also measured in order to verify the comparability between measurements of growth rates and oxygen consumption (Fig. 79). Results indicate that the two measurements are comparable or consistent. LAS enhanced both growth rates and oxygen consumption at low concentrations ($\leq$ 10 mg l^{-1}) and inhibited at high concentrations (Fig. 69(b) and Fig. 79) though there were differences in kinetic data at low concentrations (Fig. 76(e)). No hysteresis was observed at optical density measurements while lag phases of 5 and

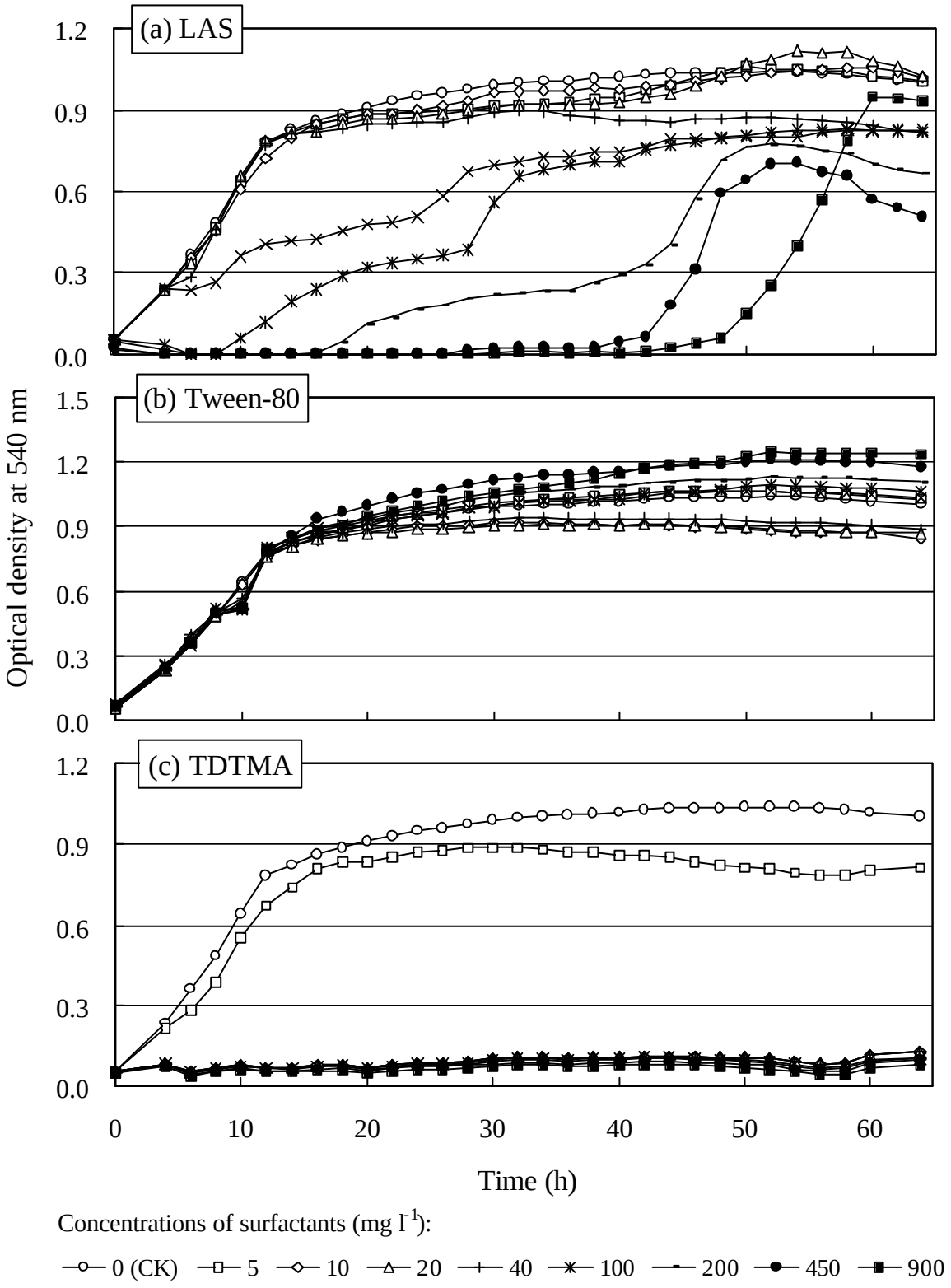


Fig. 77. Effects of LAS, Tween-80 and TDTMA on the growth of *Mycobacterium* sp. on non-toxic soluble substrate (R2A). Data points are means of triplicates.

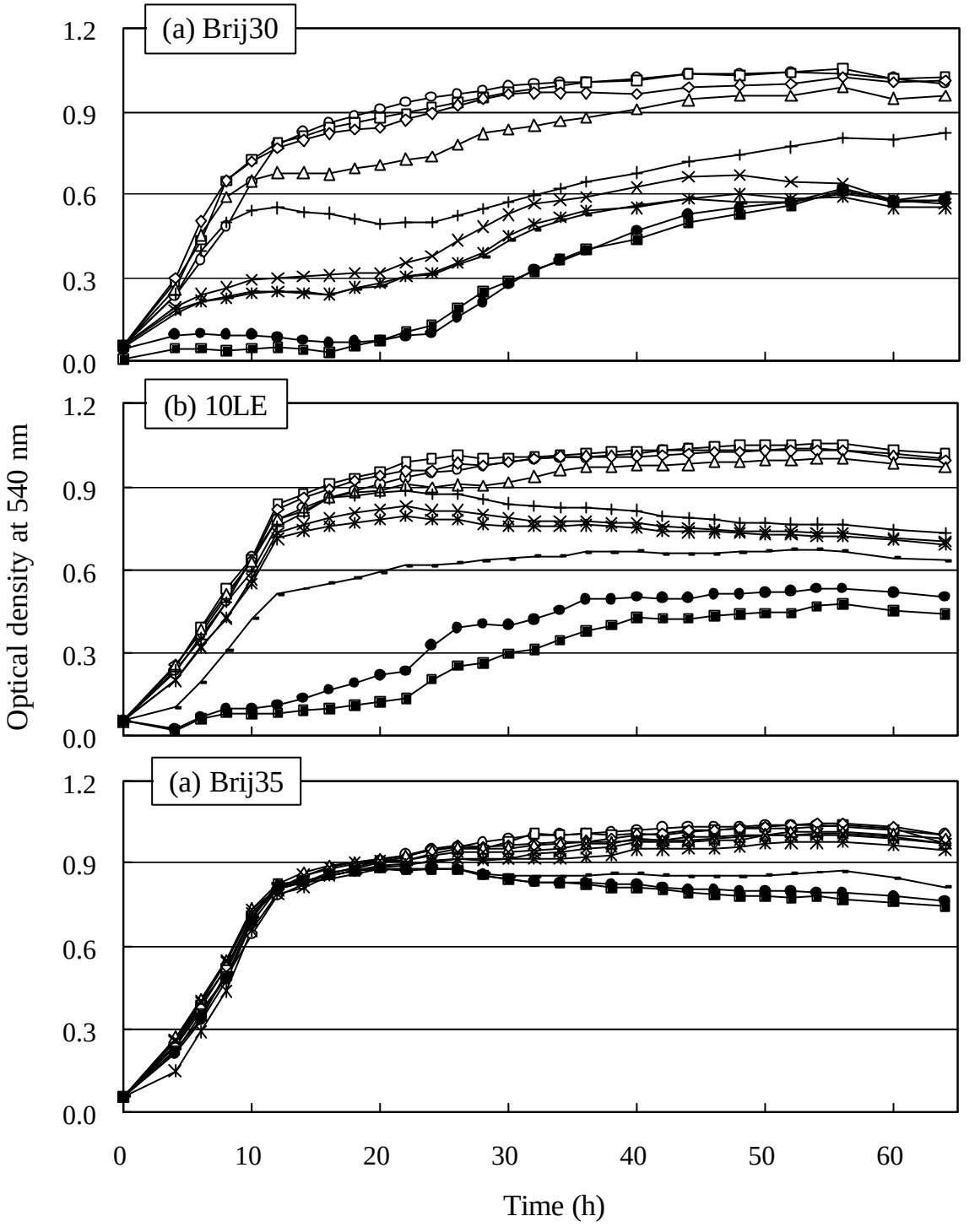


Fig. 78. Effects of Brij surfactants on the growth of *Mycobacterium* sp. on non-toxic soluble substrate (R2A). Data points are means of triplicates.

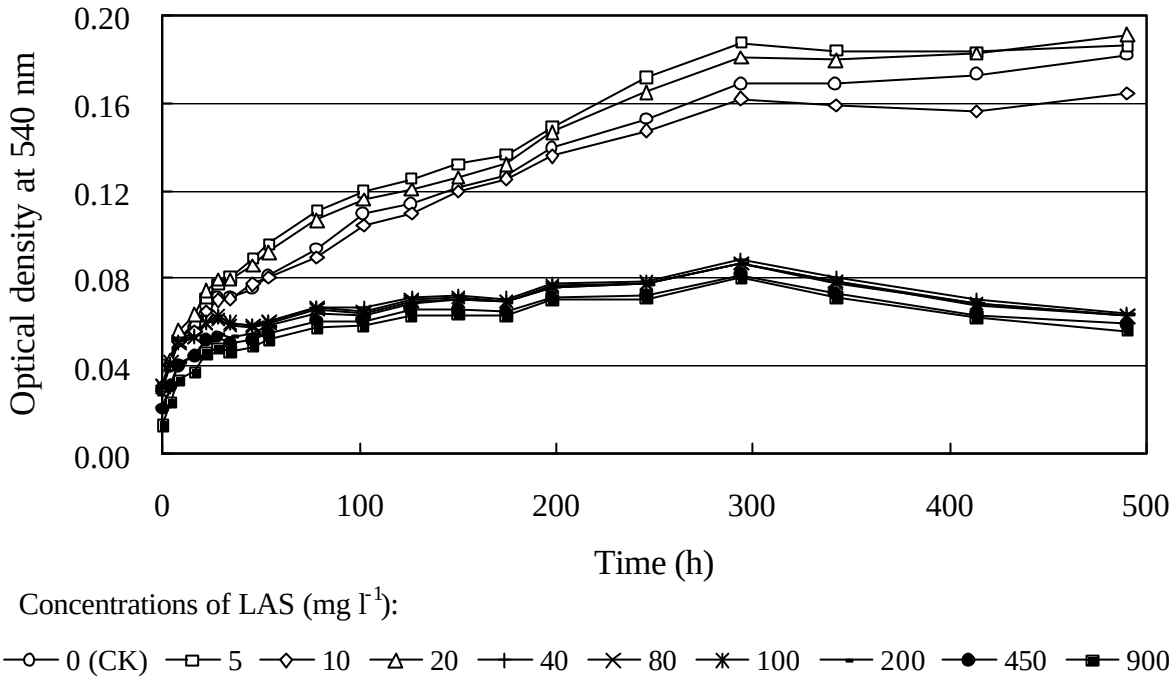


Fig. 79. Effects of LAS on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of triplicates.

18 days were found at oxygen measurements for LAS concentrations of 5 and 10 mg l^{-1}, respectively.

LAS at low concentrations (≤ 10 mg l^{-1}) increased the activities (both growth rates and respiration levels) of *Mycobacterium* sp. growing on phenanthrene (Fig. 69(b) and Fig. 79) but not on soluble substrate at all concentrations investigated from 5 to 900 mg l^{-1} (Fig. 77(a)). This suggested that surfactants could only increase the activity of the microorganism when growing on scarcely soluble HOCs such as phenanthrene. It was probably due to the increase of the bioavailability of phenanthrene or the surfactant was co-metabolized by the bacteria. When there was abundant soluble substrate available, the bacteria would utilize the soluble substrate instead of the surfactant. This conclusion was also supported by the results obtained for Tween-80. When growing on phenanthrene, the activity of the bacteria increased proportionally to the concentrations of Tween-80 from as low as 5 mg l^{-1} (Fig. 71). When growing on soluble substrate, however, the growth of the bacteria only slightly increased at

Tween-80 concentrations as high as 200 mg l^{-1} (Fig. 77(b)).

On the other hand, LAS at concentrations above 20 mg l^{-1} inhibited the activity and growth of the bacteria growing on phenanthrene from the beginning of incubation till ended at 20 days, showing its high toxicity to the microorganism. However, when growing on soluble substrate, the growth of the bacteria was almost normal at LAS concentration of 40 mg l^{-1}. Although inhibited in early stage of incubation at higher LAS concentrations, the growth partially recovered after 48 h. At LAS concentrations as high as 900 mg l^{-1}, the growth of the bacteria almost reached the control level after 60 h. These effects were also observed on TDTMA. The microbial activity was almost completed inhibited at the presence of 5 mg l^{-1} TDTMA when the bacteria growing on phenanthrene (Fig. 70), while it was only slightly inhibited when growing on soluble substrate (Fig. 77(c)). These data indicate that surfactants were less toxic to *Mycobacterium* sp. at the presence of abundant soluble substrate.

The morphology of *Mycobacterium* sp. was affected by the type of substrate. When growing on soluble substrate, the bacteria were in a fat and round shape and the outer wall of the cell was fuzzy (Fig. 80(a)). When phenanthrene was the sole carbon and energy source, the bacteria grew in a narrow and long shape and the outer wall of the cell was clear (Fig. 80(b))

(a) Normal substrate (R2A) (b) Phenanthrene (5 mg l^{-1}) as substrate

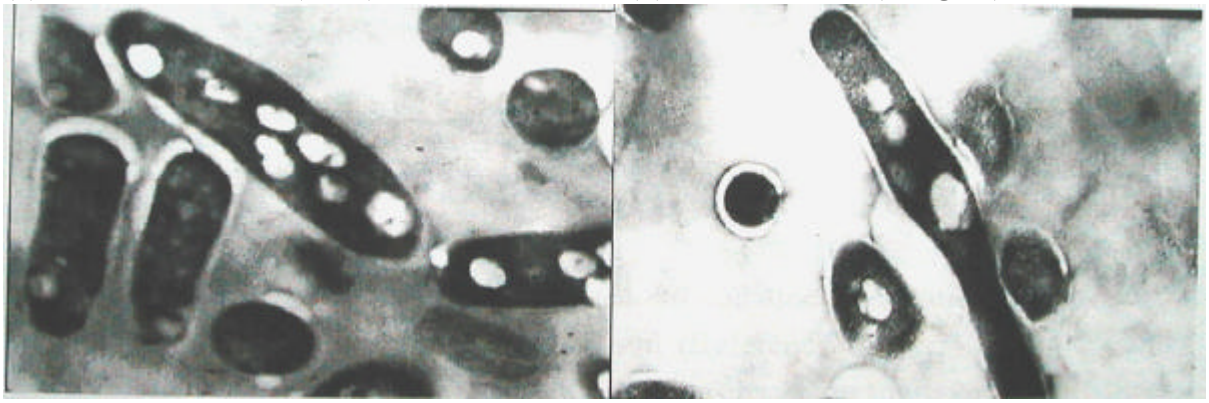


Fig. 80. Morphology of *Mycobacterium* sp. grew on different substrates (x 20,000)

4.2.4 Effects of surfactants and PAHs on the respiration of inherent microorganisms in soils

Separate and combined effects of surfactants (non-ionic Tween-80, Brij30, 10LE, Brij35, anionic LAS and cationic TDTMA) and PAHs (phenanthrene and pyrene) on the microbial activities (respiration) were studied on two uncontaminated soils (Reference soil 2.2 and 2.3) and a PAHs-contaminated soil.

4.2.4.1 Effects of surfactants on uncontaminated soils

Fig. 81 to Fig. 83 shows the effects of LAS, Tween-80 and TDTMA on the microbial activity, measured by oxygen consumption at 20 ^{o}C, of an uncontaminated soil (Reference soil 2.3). The effects of LAS and Tween-80 were somewhat similar to their effects on *Mycobacterium* sp. LAS decreased linearly the respiration as its concentrations increased from 45 mg l^{-1} to 900 mg l^{-1}, indicating that not only the soil

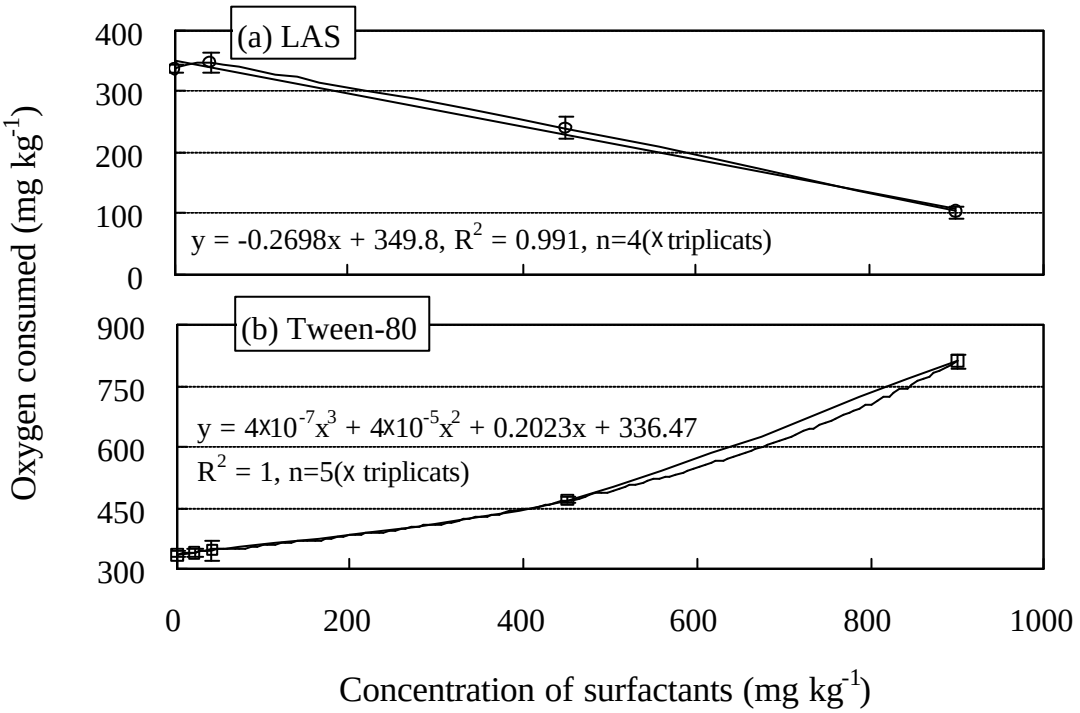


Fig. 81. Effects of LAS and Tween-80 on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) at day 20 and 20^{o}C. The error bars indicate standard deviations based on triplicates; where there are no error bars, the standard deviations were smaller than the symbols.

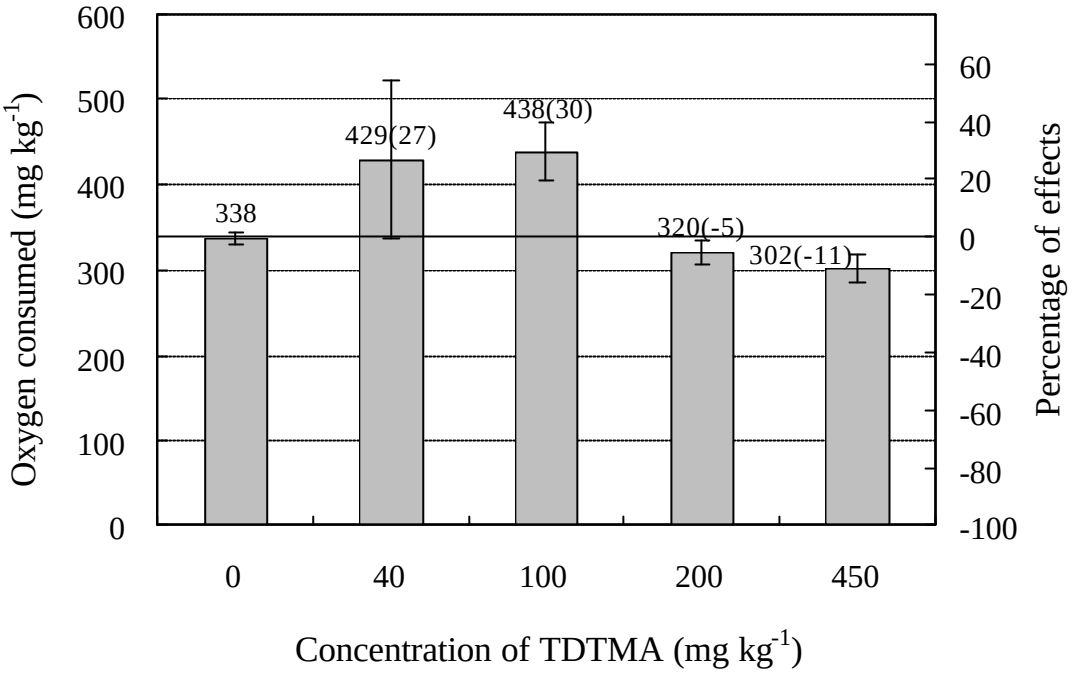


Fig. 82. Effects of TDTMA on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) at day 20 and 20°C. Data in parenthesis are percentage of effects. The error bars indicate standard deviations based on triplicate measurements.

microorganisms could not utilize LAS but also LAS was highly toxic to the soil microorganisms (Fig. 81(a)). The values of $-EC_{20}$ and $-EC_{50}$ were 259 and 648 mg kg^{-1}, respectively (Table 16). In contrast to LAS, Tween-80 increased the oxygen consumption of the soil as it did on *Mycobacterium* sp. but not linear rather to a higher extent (Fig. 81(b)). The values of $+EC_{20}$ and $+EC_{50}$ were 276 and 513 mg kg^{-1}, respectively (Table 16).

The effects of TDTMA on the activity of the soil microorganisms were completely different from those of LAS and Tween-80 as well as the effects of itself on *Mycobacterium* sp. (Fig. 82). At concentrations of 40 and 100 mg kg^{-1}, TDTMA increased the respiration levels of the soil up to 30%, probably due to oxidation of TDTMA by soil microorganisms. The value of $+EC_{20}$ was 29 mg kg^{-1} though $+EC_{50}$ could not calculated based on concentrations studied. At higher concentrations,

TDTMA inhibited the activity of the soil microorganisms probably due to its toxicity. At TDTMA concentration of 450 mg kg^{-1}, oxygen consumption was reduced 11% while the respiration of *Mycobacterium* sp. was completely inhibited at concentration as low as 10 mg l^{-1} (Fig. 70). It suggests that mixed communities of microorganisms can tolerate higher toxicity than mono microbial and can degrade more toxic substance than mono bacteria.

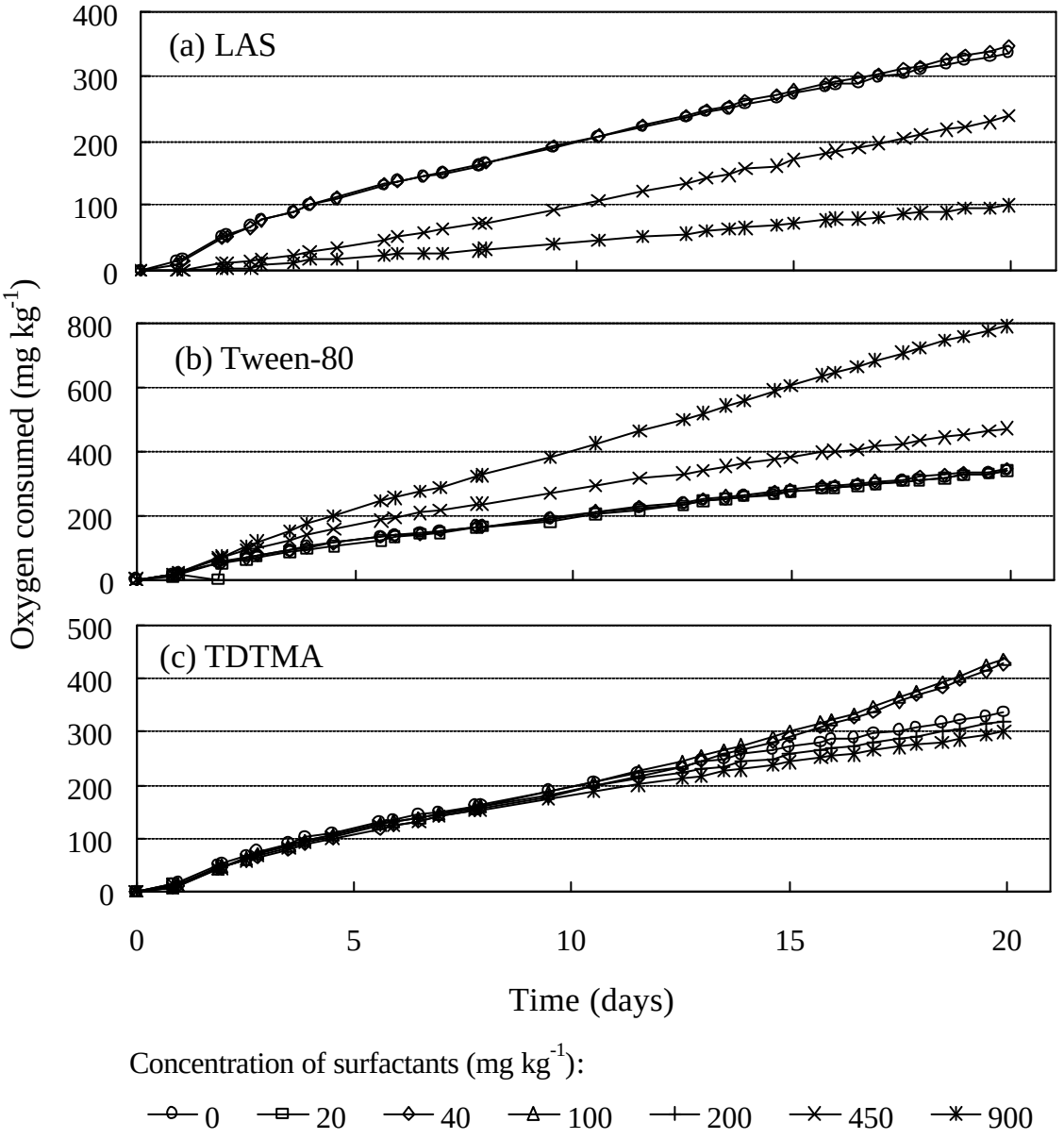


Fig. 83. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference Soil 2.3) in the presence of surfactants at 20°C. Data points are means of triplicates.

Table 16. EC values of surfactant's effects on inherent microorganisms of an uncontaminated soil (Reference soil 2.3) in the presence (0.5 mg kg^{-1}) and absence of phenanthrene (20 days) (mg kg^{-1})

	Surfactants	$+EC_{20}$	$+EC_{50}$	$-EC_{20}$	$-EC_{50}$
In the absence of phenanthrene					
	LAS	NE	NE	259.3	648.2
	Tween-80	276.0	512.9	NE	NE
	TDTMA	29.4	ND	ND	ND
In the presence of phenanthrene					
	LAS	11.4	14.2	429.6	869.6
	Tween-80	8.4	294.8	NE	NE
	TDTMA	4.3	10.8	884.0	1159.9[a]
	Brij30	ND	ND	ND	ND
	10LE	ND	ND	ND	ND
	Brij35	23.7	31.3	ND	ND

Notes: $+EC_{20}$ and $+EC_{50}$ are assigned for effective concentrations of surfactants which cause 20% and 50% increase of microbial activity (oxygen consumption), respectively; whereas, $-EC_{20}$ and $-EC_{50}$ for those causing 20% and 50% decrease of microbial activity, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

[a] extrapolated values.

Fig. 83 shows the respiration kinetics of inherent soil microorganisms in the presence of different surfactants, i.e. LAS, Tween-80 and TDTMA. The respiration levels in the presence of LAS were always lower than that of control (no surfactants) (Fig. 83(a)). There was no lag phase for respiration at the presence of Tween-80. Except at concentrations lower than 40 mg kg^{-1}, Tween-80 significantly increased the oxygen consumption of the soil during the whole incubation period. The respiration levels at the presence of TDTMA at concentrations of 20 and 40 mg kg^{-1} was not higher than the control until 14 days. Before that time, there was no significant difference among the microbial activities at control and at the presence of TDTMA of all concentrations.

4.2.4.2 Combined effects of surfactants and phenanthrene on uncontaminated soils

Effects of LAS, Tween-80, TDTMA and three Brij series surfactants on the activity of soil inherent microorganisms in the presence of 0.5 mg kg^{-1} phenanthrene were measured by oxygen consumption at 20 ^{o}C on two Reference Soils 2.3 and 2.2. Addition of phenanthrene to the two soils to a concentration of 0.5 mg kg^{-1} resulted in completely different responds from the two soils. It increased slightly (*ca* 3%) the respiration level of Soil 2.3 (Fig. 84 to Fig. 92) but decreased (20%) that of Soil 2.2 (Fig. 93 to Fig. 97), indicating that the toxicity of the same chemical was different from soil to soil. The microorganisms in Soil 2.3 had the capacity to utilize phenanthrene even the soil had had no contamination history while Soil 2.2 did not.

The coexistence of any of above surfactants could generally increase the respiration of Soil 2.3 except for LAS and TDTMA at very high concentrations. In Soil 2.2, Tween-80 and TDTMA had similar effects as those in Soil 2.3. But the effects of LAS on Soil

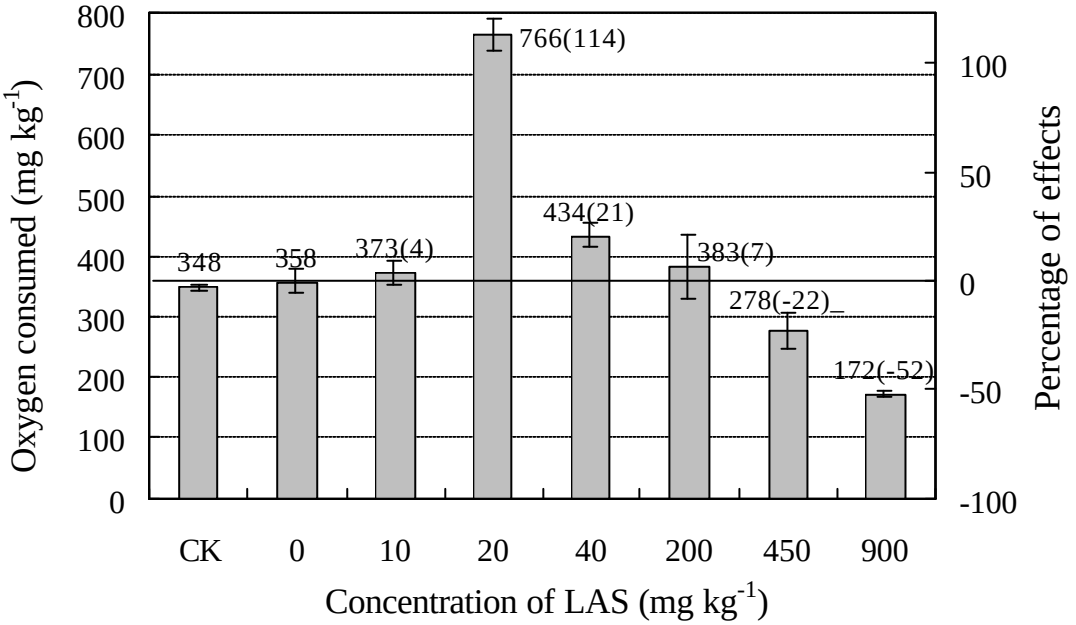


Fig. 84. Effects of LAS on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20^{o}C. CK represents treatment with neither phenanthrene nor LAS. The error bars indicate standard deviations based on triplicates. Data in parenthesis are percentage of increase or decrease compared with that without LAS.

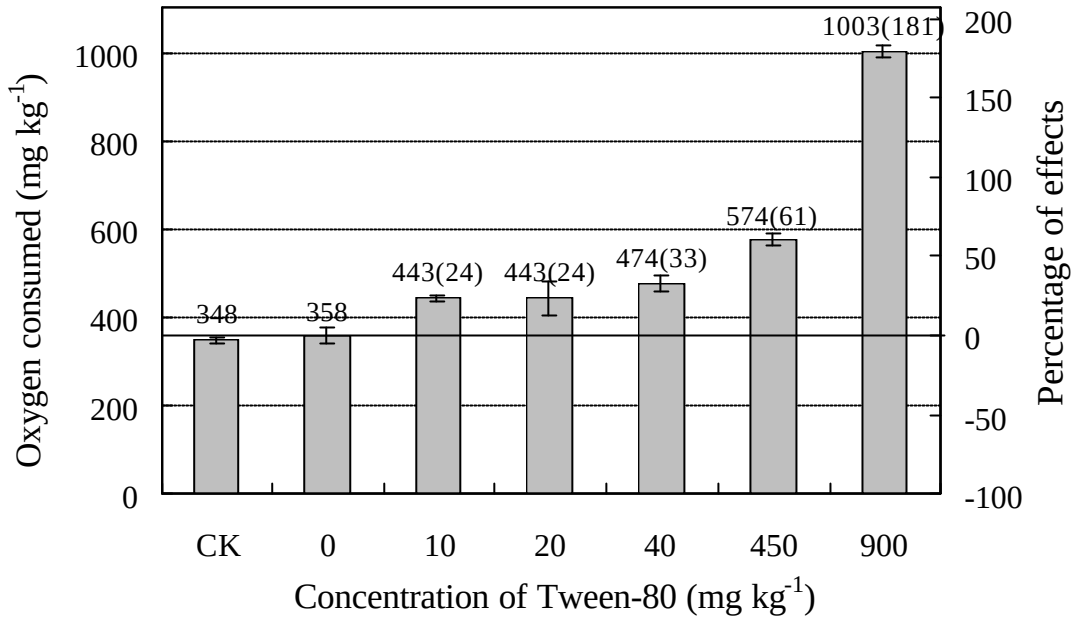


Fig. 85. Effects of Tween-80 on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20°C. CK represents treatment with neither phenanthrene nor Tween-80. The error bars indicate standard deviations based on triplicates. Data in parenthesis are percentage of increase or decrease compared with that without Tween-80.

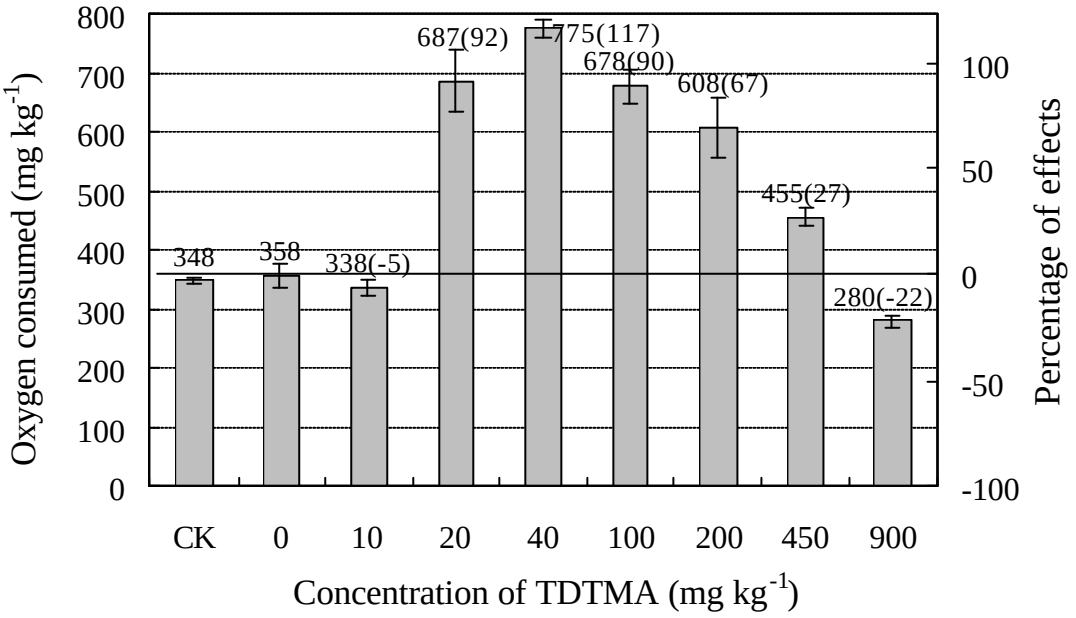


Fig. 86. Effects of TDTMA on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of phenanthrene (0.5 mg kg^{-1}) at day 20 and 20°C. CK represents treatment with neither phenanthrene nor TDTMA. The error bars indicate standard deviations based on triplicates. Data in parenthesis are percentage of increase or decrease compared with that without TDTMA.

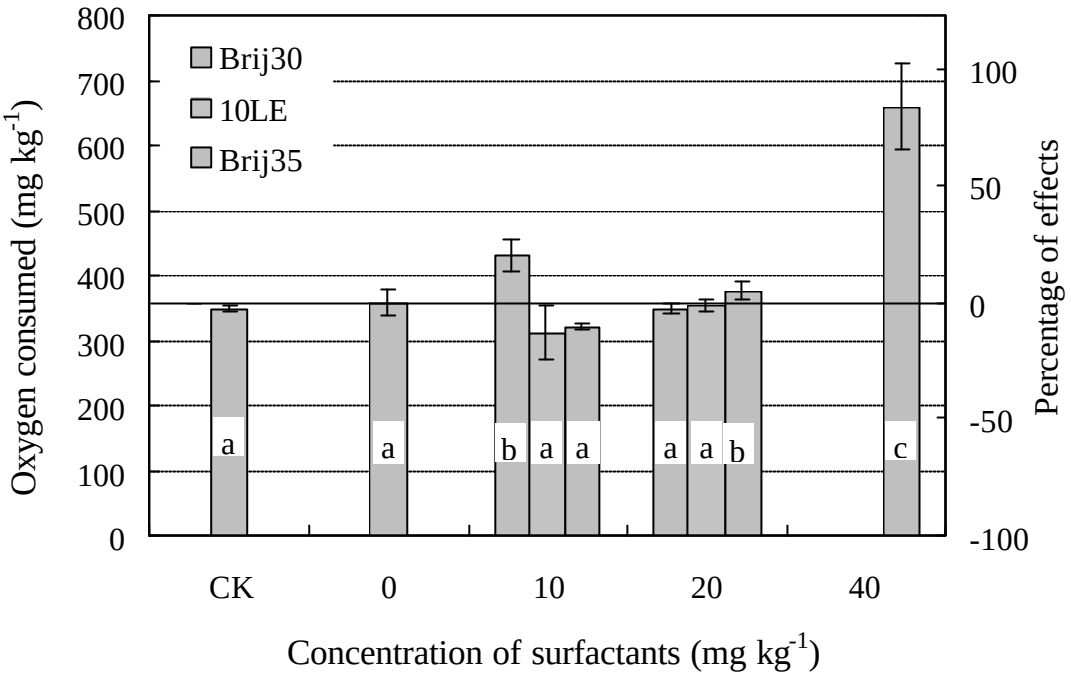


Fig. 87. Effects of Brij series surfactants on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20°C. CK represents treatment with neither phenanthrene nor surfactants. The error bars indicate standard deviations based on triplicate measurements. Columns with the same letter are in the same statistic significant level (>0.05) and different letters represent significant difference (<0.05) among the columns.

2.2 were different from those on Soil 2.3. It did not increase the respiration of Soil 2.2 at low concentrations as it did in Soil 2.3.

LAS at concentrations of 10 to 200 mg kg^{-1} increased the oxygen consumption levels of Soil 2.3 compared with that in the presence of phenanthrene alone (Fig. 84). $+EC_{20}$ and $+EC_{50}$ accounted for 11 and 14 mg kg^{-1}, respectively. The highest respiration level was at LAS concentration of 20 mg kg^{-1}, in which respiration increased 114%. The microbial activities started to decline when LAS concentrations getting higher. At LAS concentrations between 200 and 450 mg kg^{-1}, the respiration level became lower than that with phenanthrene only. The values of $-EC_{20}$ and $-EC_{50}$ were 430 and 870 mg kg^{-1}, respectively. The presence of LAS at low concentrations increased the soil respiration indicated that LAS either increased the bioavailability of phenanthrene or

was utilized as co-substrate by soil microorganisms. Because the inherent soil microorganisms could not oxidize LAS without the presence of phenanthrene (Fig. 81), it was highly likely that LAS increased the bioavailability of phenanthrene. This was proved by experiments using ^{14}C-labeled phenanthrene (see Section 4.3.2).

In the co-presence of phenanthrene at 0.5 mg kg^{-1}, Tween-80 increased the respiration of Soil 2.3 at its concentrations from 20 mg kg^{-1} up to 900 mg kg^{-1} (Fig. 85), which was consistent to that without the presence of phenanthrene (Fig. 81(b)). The values of $+EC_{20}$ and $+EC_{50}$ were 8 and 295 mg kg^{-1}, respectively (Table 16). The combined effects of phenanthrene and Tween-80 on the respiration were slightly overadditive (Fig. 88). This implied that Tween-80 increased the respiration by acting as substrate as well as by increasing the bioavailability of phenanthrene. The later was approved by measuring ^{14}C-CO_2 derived from the degradation of ^{14}C-phenanthrene in the presence of Tween-80 (see Section 4.3.2).

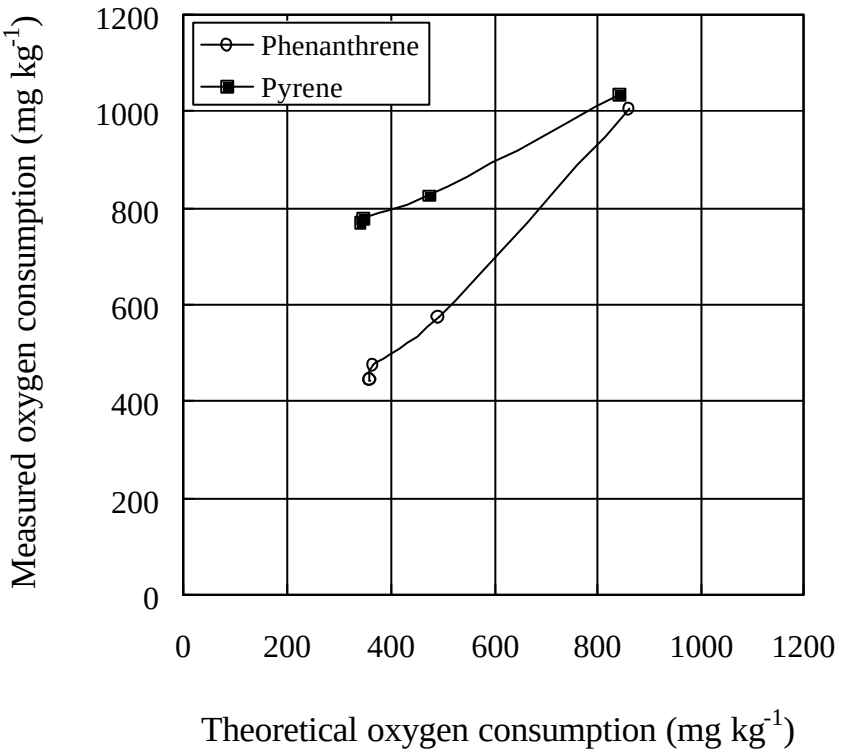


Fig. 88. Mode of combined effects of Tween-80 and phenanthrene or pyrene on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) at 20^{o}C

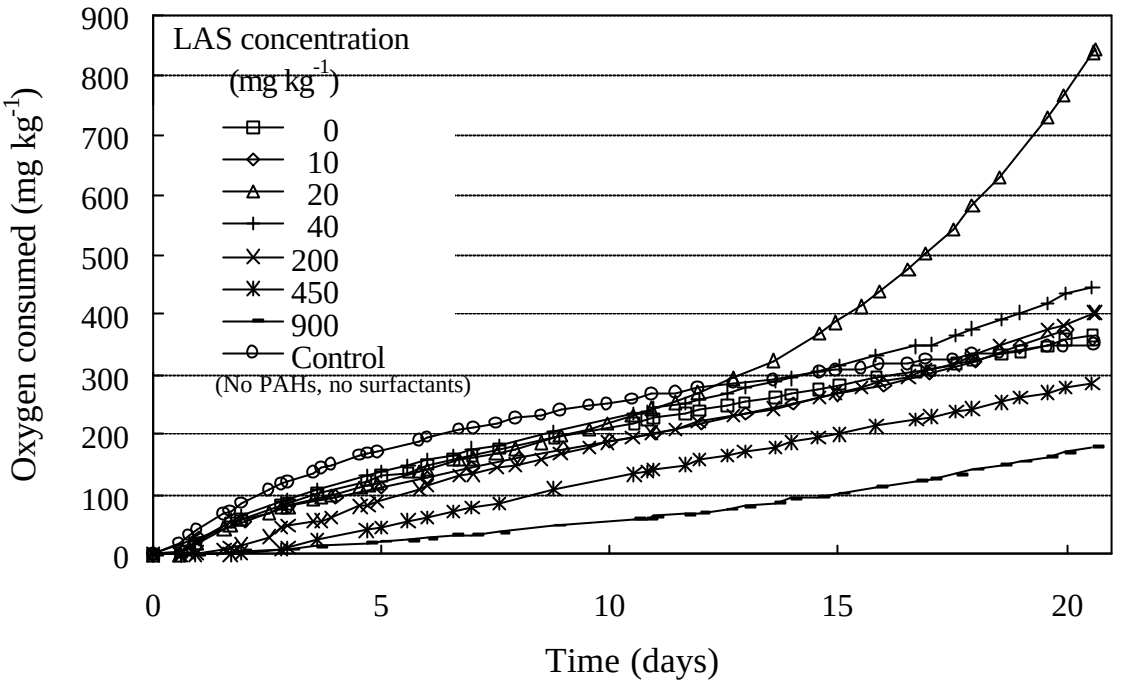


Fig. 89. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) incubated at 20°C in the presence of phenanthrene (0.5 mg kg^{-1}) and LAS. Data points are means of triplicates.

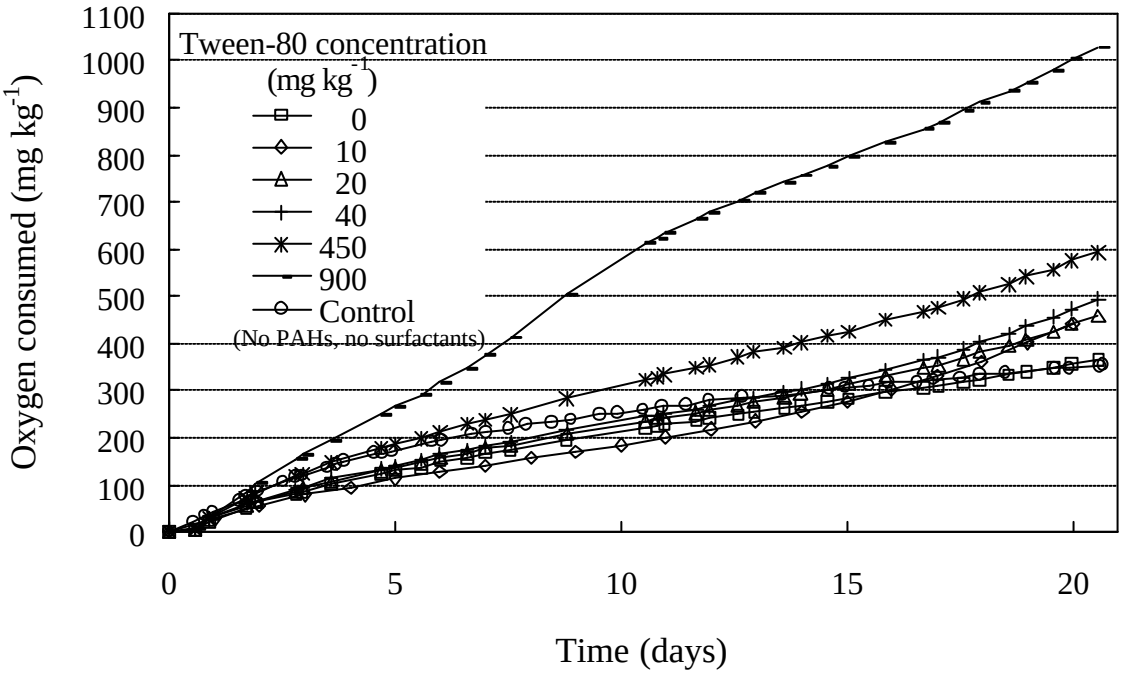


Fig. 90. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) incubated at 20°C in the presence of phenanthrene (0.5 mg kg^{-1}) and Tween-80. Data points are means of triplicates.

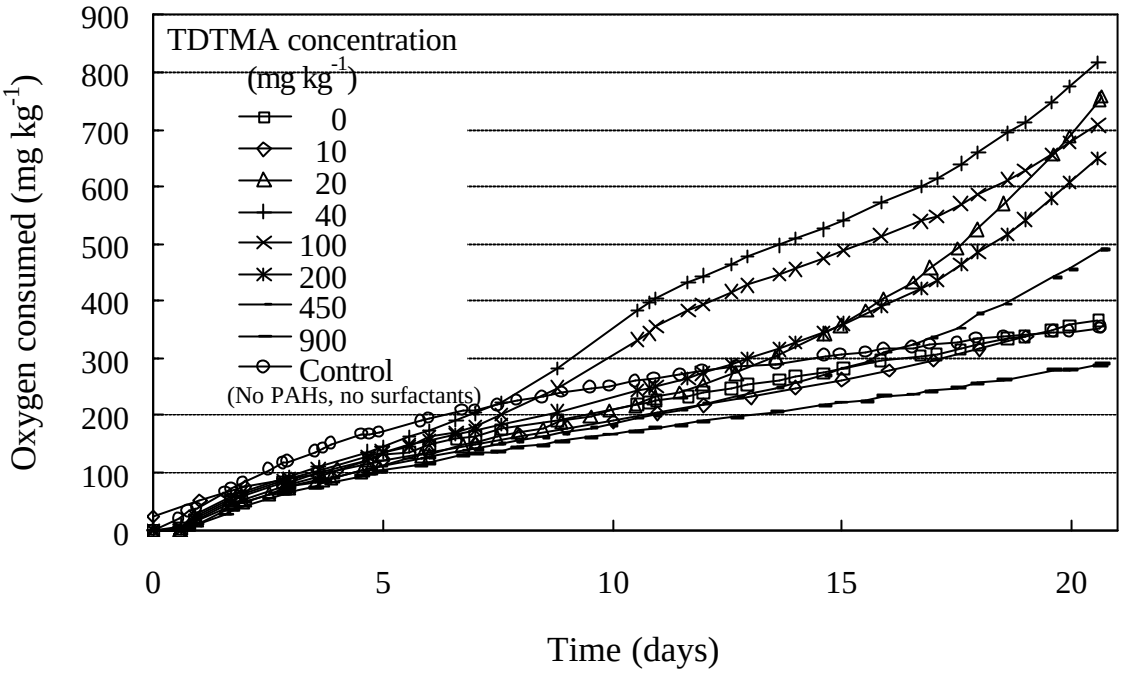


Fig. 91. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) incubated at 20°C in the presence of phenanthrene (0.5 mg kg^{-1}) and TDTMA. Data points are means of triplicates.

TDTMA at concentrations from 20 mg kg^{-1} to 450 mg kg^{-1} increased the respiration levels of Soil 2.3 in the co-existence of 0.5 mg kg^{-1} phenanthrene (Fig. 86). The highest respiration level was at TDTMA concentration of 40 mg kg^{-1}, which was 117% higher than that in the absence of TDTMA. The values of $+EC_{20}$ and $+EC_{50}$ were 4 and 11 mg kg^{-1}, respectively (Table 16). Compared with the case that respiration started to decline below background level in the presence TDTMA alone at 200 mg kg^{-1} (Fig. 82), oxygen consumption levels in the co-presence of phenanthrene were still 27% higher than the background value at TDTMA concentrations up to 450 mg kg^{-1}. It suggested that the coexistence of phenanthrene might reduce the toxicity of TDTMA by providing available substrate. The combined effects of phenanthrene and TDTMA on microbial respiration were overadditive, implying not only the oxidation of both chemicals by soil microorganisms but also additional substrate available probably due to the increase of bioavailability of phenanthrene by TDTMA. This was also verified by measuring the release of $^{14}CO2$ from ^{14}C-phenanthrene in the presence of TDTMA (see Section 4.3.2).

For the three Brij surfactants, no significant differences were observed among the background respiration level, that in the presence of phenanthrene alone and those in the coexistence of the surfactants at concentrations below 20 mg kg^{-1} (Fig. 87). Increase of soil respiration was measured only at the presence of Brij30 at 10 mg kg^{-1} and Brij35 at 40 mg kg^{-1}. Values of $+EC_{20}$ and $+EC_{50}$ could be calculated for Brij35 to be 24 and 31 mg kg-1, respectively (Table 16).

Fig. 89 to Fig. 92 shows the respiration kinetics of inherent microorganisms in Soil 2.3 at the presence of phenanthrene (0.5 mg kg^{-1}) and selected surfactants. As can be seen from these figures, soil microorganisms took almost 20 days to adapt to phenanthrene before they could utilize it. Addition of LAS ($\leq$ 200 mg kg^{-1}), Tween-80 (up to 900 mg kg^{-1}), TDTMA (20 to 450 mg kg^{-1}), Brij30 (10 mg kg^{-1}) or Brij35 (40 mg kg^{-1}) could reduce the lag phase. This indicated that either the surfactant was utilized by soil microorganisms as preferential substrate or the bioavailability of phenanthrene was increased by the surfactant. However, when compared with results obtained from the same soil in the presence of surfactants alone, it was more likely that either the bioavailability of phenanthrene was increased or phenanthrene and surfactants were co-metabolized by soil microorganisms.

In the co-presence of LAS at 20 mg kg^{-1}, the lag phase was reduced to 12 days, followed by 40 mg kg^{-1} (15 days), 200 mg kg^{-1} (18 days) and 450 mg kg^{-1} (19 days) (Fig. 89). The respiration levels at LAS concentration $\geq$ 450 mg kg^{-1} were all lower than the background level in the whole incubation period no mater whether there was the presence of phenanthrene or not (Fig. 83). The presence of TDTMA had similar effects, in which the lag phase was reduced to 7days at concentration of 40 mg kg^{-1}, to 8 days at 100 mg kg^{-1}, to 12 days at 200 mg kg^{-1}, and to 17 days at 450 mg kg^{-1} (Fig. 91). The respiration levels at TDTMA of 900 mg kg^{-1} were lower than the background level all the time during incubation. These results indicate that the two surfactants inhibited the microbial activities as their concentrations increased due to their toxicity to soil microorganisms. Contrasted to LAS and TDTMA, the respiration levels in the presence of Tween-80 at concentration above 450 mg kg^{-1} were all higher than the

background level during the whole incubation period in both cases with the presence and absence of phenanthrene (Fig. 83 and Fig. 90). In addition, the lag phase was shorter as the increase of Tween-80 concentrations, suggesting that Tween-80 was not toxic to soil microorganisms at all and was ready to be utilized by soil microbials.

The presence of Brij surfactants reduced the respiration levels of the soil. Except at 10 mg kg^{-1} Brij30 and at 40 mg kg^{-1} Brij35, the oxygen consumption levels at other

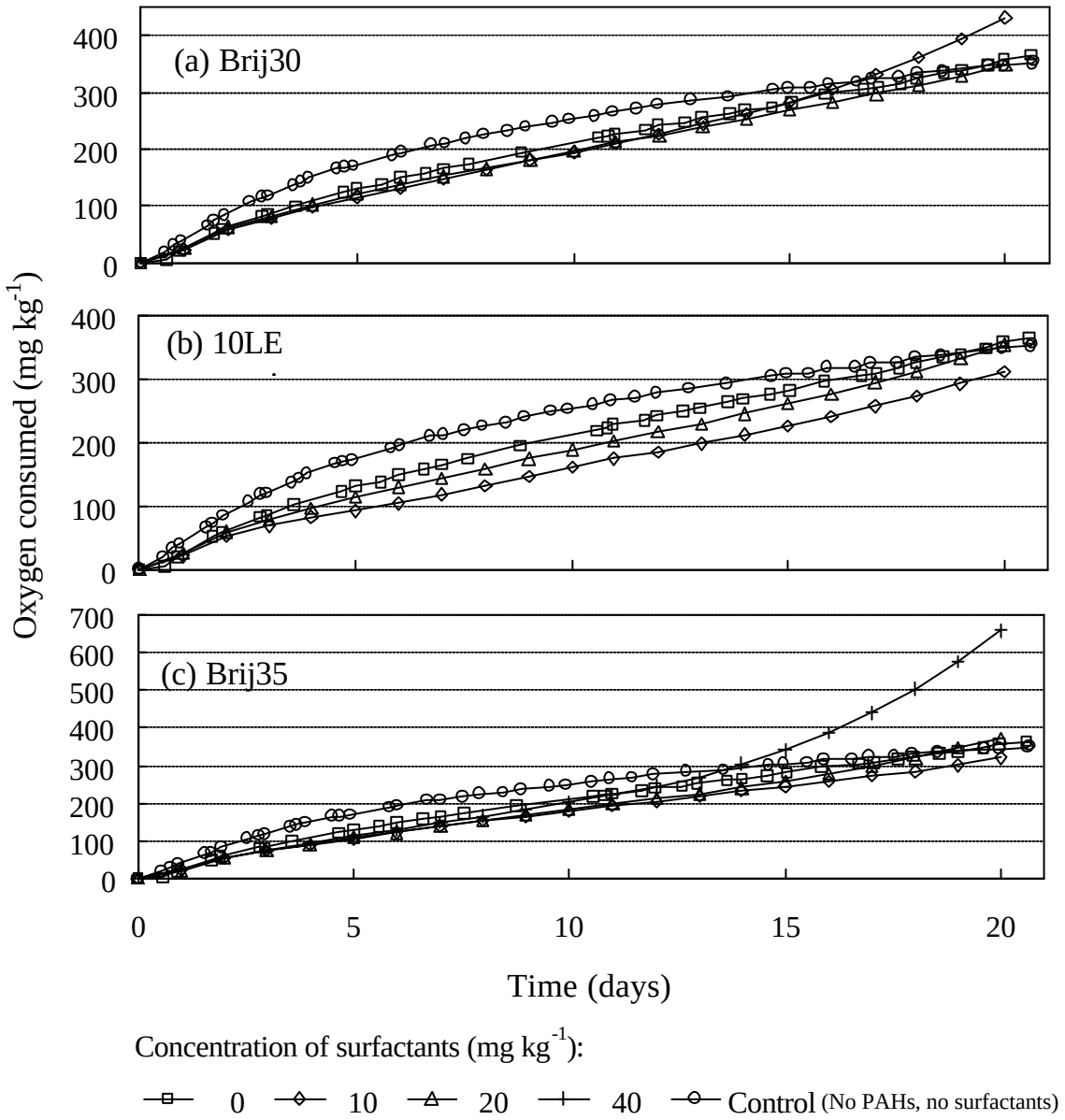


Fig. 92. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) incubated at 20°C in the presence of phenanthrene (0.5 mg kg^{-1}) and Brij series surfactants. Data points are means of triplicates.

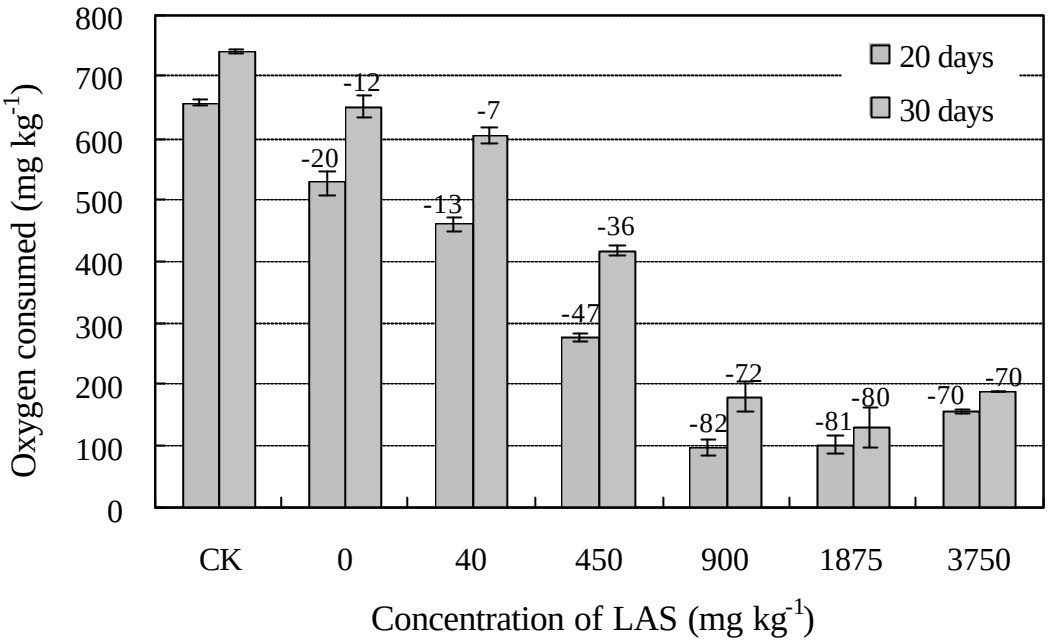


Fig. 93. Effects of LAS on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20°C for different time. CK represents treatment with neither phenanthrene nor LAS. Data labels are percentage of decrease caused by phenanthrene or LAS. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

concentrations were all lower than the background level during the whole incubation period (Fig. 92). It indicated the toxicity of the surfactant to soil microorganisms.

For comparison of effects on different soil, the effects of LAS, Tween-80, and TDTMA on the activity of soil inherent microorganisms were measured on another uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene at the same concentration (0.5 mg kg^{-1}). In order to investigate the influence of temperature, the effects of TDTMA were monitored at 20 and 30 °C. Prolong measurement up to 32 days was also conducted for LAS and Tween-80. Fig. 93 to Fig. 97 shows the results obtained.

In contrasted to Soil 2.3, addition of phenanthrene to Soil 2.2 caused significant

decrease (20% at 20 ^{o}C and 12% at 30 ^{o}C) of the respiration level of the soil (Fig. 93 to Fig. 95). Kinetics data (Fig. 96 and Fig. 97) showed that the respiration declined from the beginning of incubation and lasted until the termination of the measurement in 32 days. This implied that microorganisms in the soil did not have the capacity to utilize phenanthrene that was toxic to them.

At the co-presence of phenanthrene, addition of LAS to Soil 2.2 could not ease the toxicity of phenanthrene as it did on Soil 2.3 at low concentration, but rather it further reduced the microbial activities (Fig. 93). The respiration decreased with the increase of LAS concentration from 40 mg kg^{-1} to 3750 mg kg^{-1}, indicating the higher the LAS concentration, the hither its toxicity to the soil microorganisms. None of the respiration levels at the presence of LAS was higher than the background level.

Prolong incubation up to 32 days could increase the oxygen consumption levels indicating the adaptation of microorganisms to the chemicals but could not changed

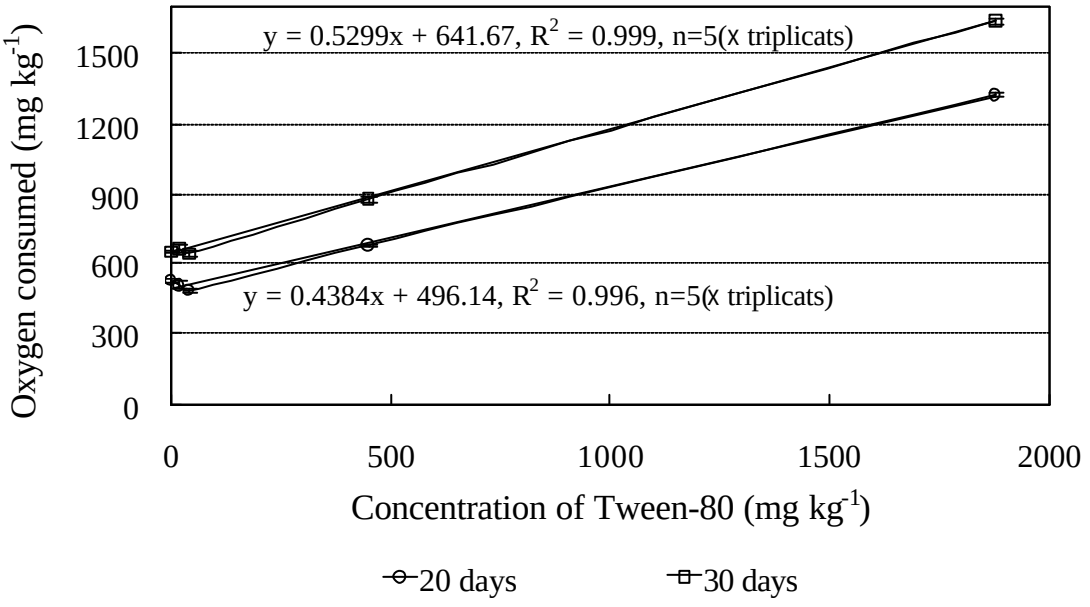


Fig. 94. Effects of Tween-80 on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20^{o}C for different time. CK represents treatment with neither phenanthrene nor Tween-80. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

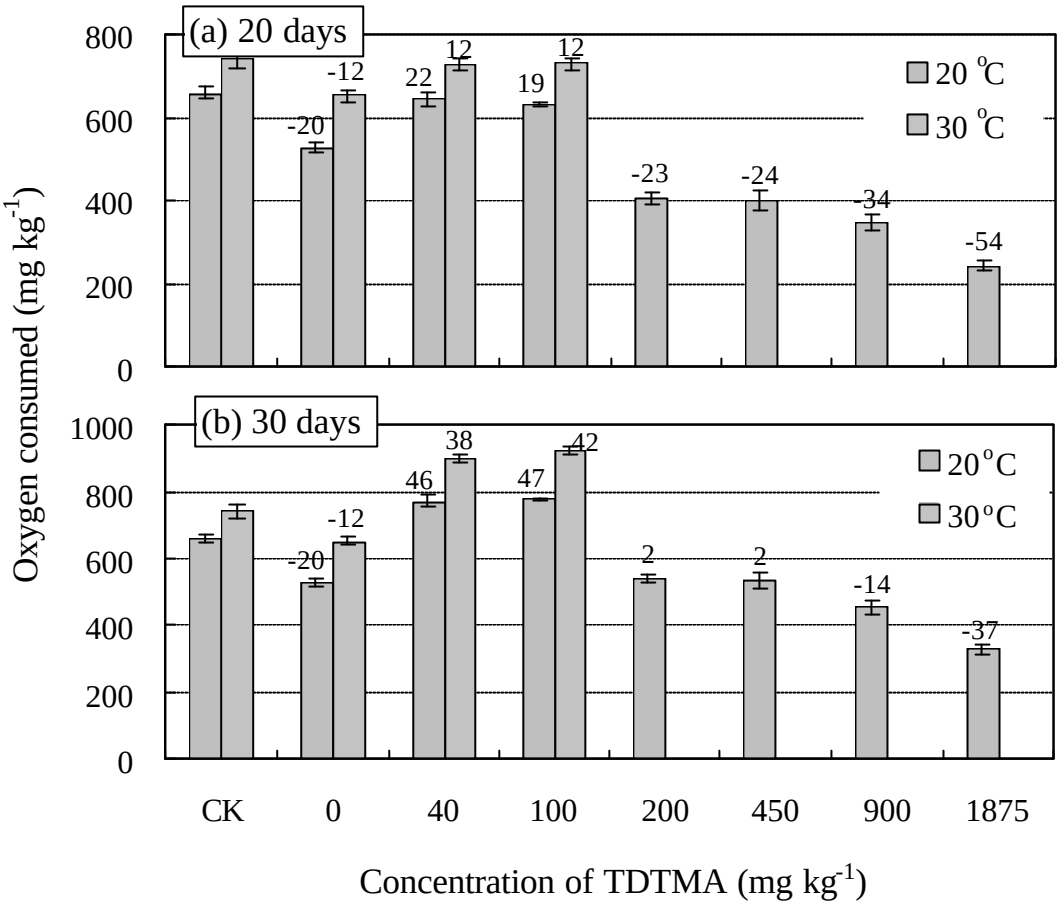


Fig. 95. Effects of TDTMA on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene (0.5 mg kg^{-1}) at 20°C and 30°C for different time. CK represents treatment with neither phenanthrene nor TDTMA. Data labels are percentage of effects caused by phenanthrene or TDTMA. The error bars indicate standard deviations based on triplicate measurements.

the trend. The values of $-EC_{20}$ and $-EC_{50}$ were 124 and 484 mg kg^{-1} for 20 days and 222 and 624 mg kg^{-1} for 32 days, respectively (Table 17).

In contrasted to LAS, the addition of Tween-80 to Soil 2.2 at concentrations above 450 mg kg^{-1} could increase the respiration without lag phase, showing the ease of phenanthrene toxicity by Tween-80 (Fig. 94 and Fig. 96). At concentration of 450 mg kg^{-1}, there was a lag phase of 16 days before the respiration reached the background level though it was higher than that in the presence of phenanthrene alone from the

beginning of incubation. However, at lower concentrations, Tween-80 could not increase the microbial activities. Results suggested that abundance of Tween-80 acting as substrate was necessary to ease the toxicity of phenanthrene.

The effects of TDTMA on the activity of inherent microorganisms in Soil 2.2 were different from those of LAS and Tween-80 (Fig. 95 and Fig. 97). It increased the soil respiration at concentrations ≤ 100 mg kg^{-1} while decreased the activity at higher concentrations, which was similar to its effects on Soil 2.3 (Fig. 86). The values of $+EC_{20}$, $-EC_{20}$ and $-EC_{50}$ were 36, 193 and 1700 mg kg^{-1} at 20 °C for incubation of 20

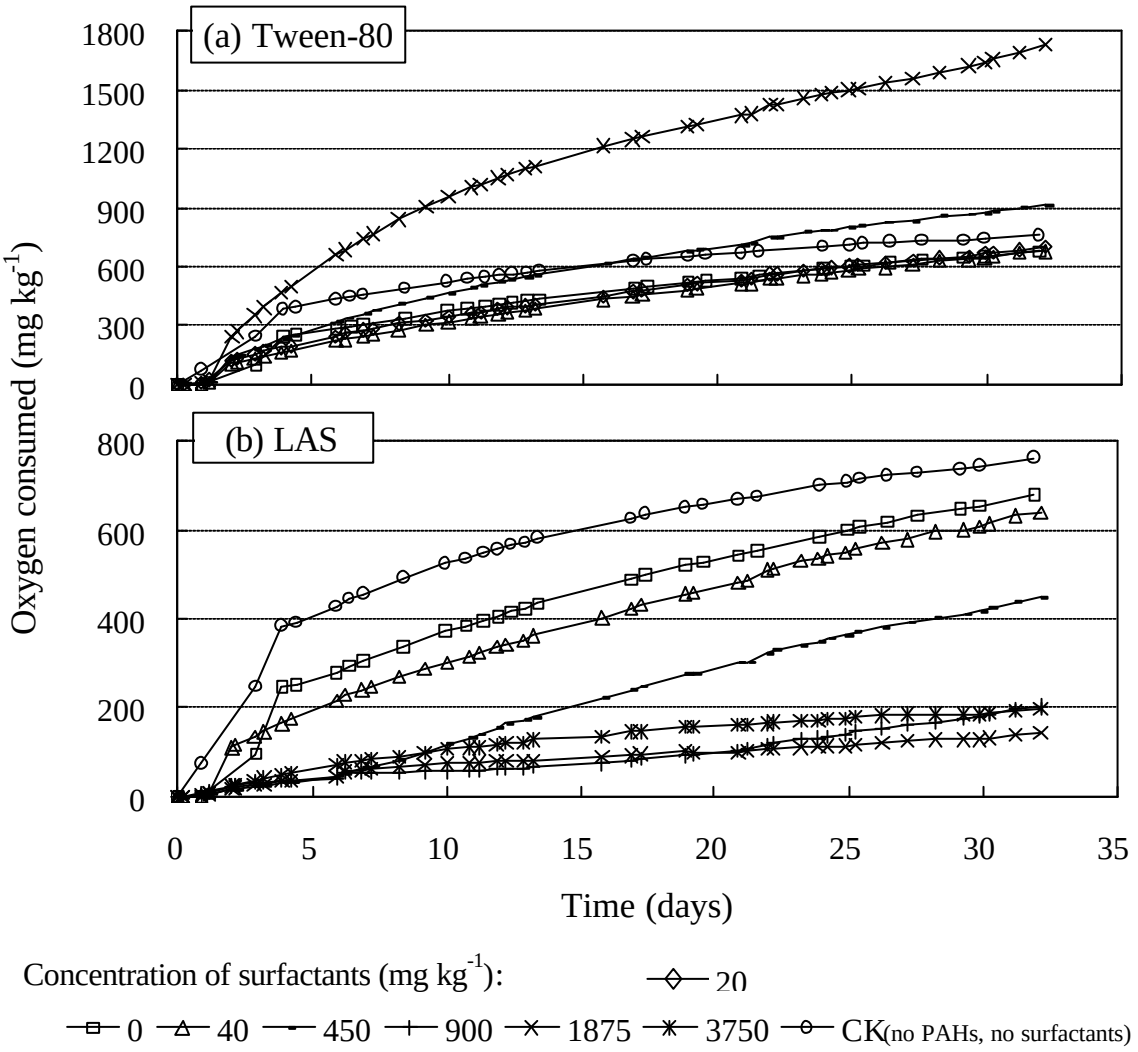


Fig. 96. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene (0.5 mg kg^{-1}) and surfactants at 20°C. Data points are means of triplicates.

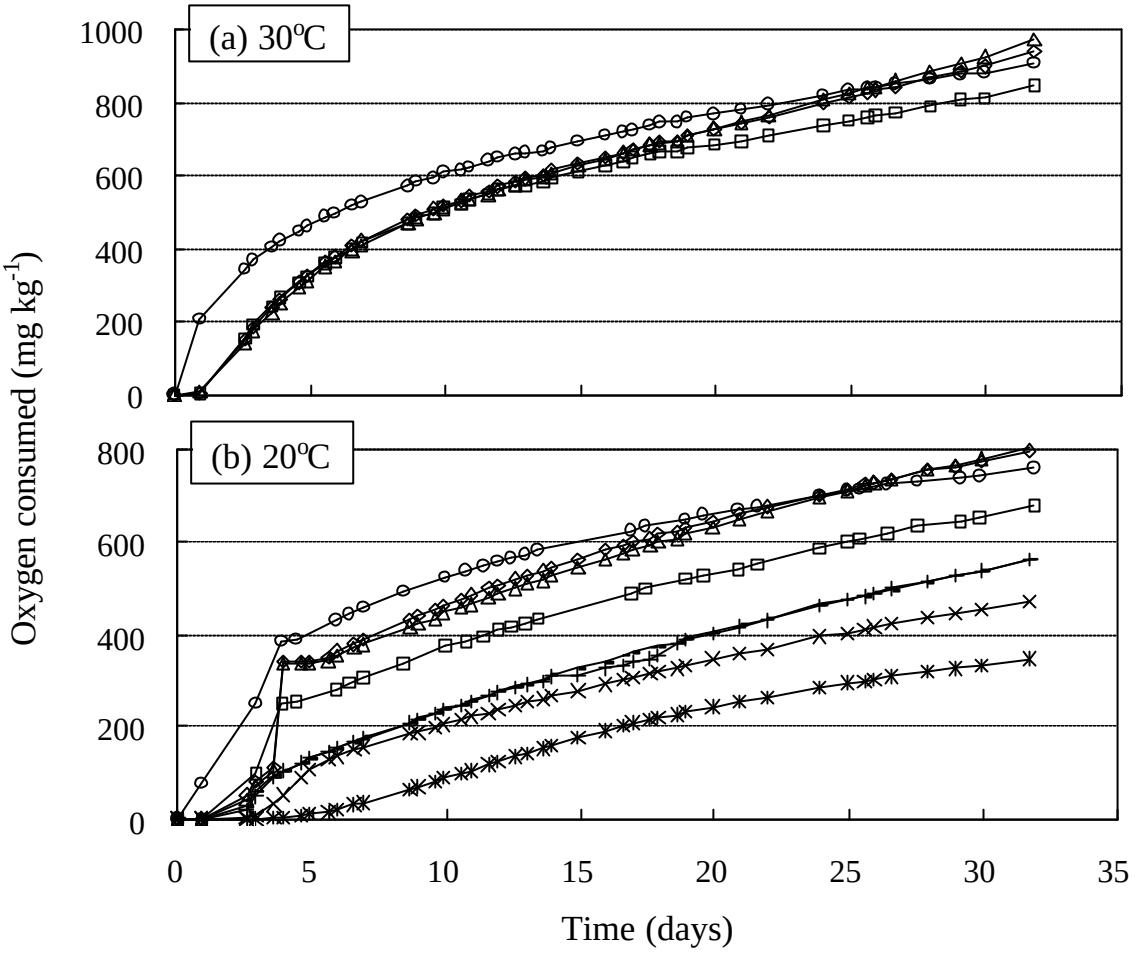


Fig. 97. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.2) in the presence of phenanthrene (0.5 mg kg^{-1}) and TDTMA at different temperature. Data points are means of triplicates.

days, respectively (Table 17). $+EC_{50}$ could not obtained under the TDTMA concentrations investigated. A lag phase of 24 days existed at TDTMA concentrations of 40 and 100 mg kg^{-1} compared with the background respiration of the soil but no lag phase when compared with that at the presence of phenanthrene alone (Fig. 97). It indicated that TDTMA could ease the toxicity caused by phenanthrene by probably acting as substrate. At higher concentrations, the respiration was below the level at the presence of phenanthrene alone all though the incubation.

Temperature could increase the overall activity of the soil microorganisms no matter there were test chemicals or not (Fig. 95 and Fig. 97). But temperature could not alter the relative respiration levels among treatments, i.e. the trend was the same at 20 ^{o}C and 30 ^{o}C. Prolonging incubation could lead to the adaptation of soil microorganisms to the chemicals and increase their activities. For instance, the oxygen consumption levels at 200 and 450 mg kg^{-1} TDTMA at 20 ^{o}C increased from 24% below that without TDTMA in 20 days to 2% above that of the same treatment in 30 days. But the overall trend remained the same.

Fig. 98 compares the effects of surfactants and phenanthrene on the activities of inherent soil microorganisms between Soil 2.2 and Soil 2.3. Soil 2.2 had a higher background respiration or microbial activities than Soil 2.3. Addition of phenanthrene to the soils increased the respiration of Soil 2.3 slightly (3%) but decreased that of

Table 17. EC values of surfactant's effects on inherent microorganisms of an uncontaminated soil (Reference soil 2.2) in the presence (0.5 mg kg^{-1}) of phenanthrene (mg kg^{-1})

	Surfactants	Temperature	+EC_{20}	+EC_{50}	-EC_{20}	-EC_{50}
20 days						
	LAS	20^{o}C	NE	NE	123.6	484.1
	Tween-80	20^{o}C	1358.0	1697.6	NE	NE
	TDTMA	20^{o}C	36.1	ND	192.7	1699.6
	TDTMA	30^{o}C	ND	ND	ND	ND
30 days						
	LAS	20^{o}C	NE	NE	222.2	623.6
	Tween-80	20^{o}C	1453.1	1816.4	NE	NE
	TDTMA	20^{o}C	17.3	ND	1149.0	ND
	TDTMA	30^{o}C	21.0	ND	ND	ND

Notes: +EC_{20} and +EC_{50} are assigned for effective concentrations of surfactants which cause 20% and 50% increase of microbial activity (oxygen consumption), respectively; whereas, -EC_{20} and -EC_{50} for those causing 20% and 50% decrease of microbial activity, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

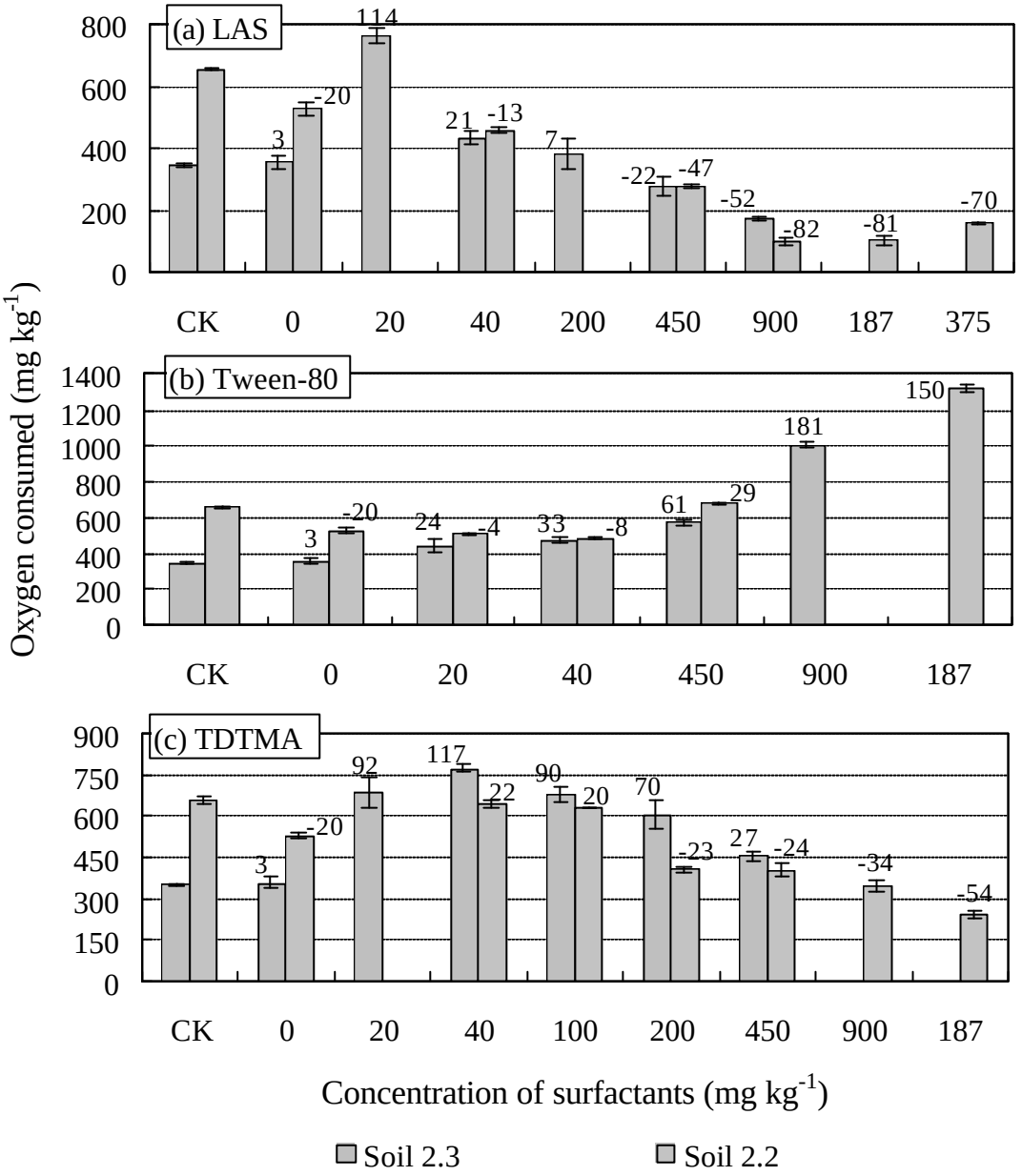


Fig. 98. Comparison of combined effects of surfactants and phenanthrene (0.5 mg kg^{-1}) on the respiration of inherent microorganisms between two uncontaminated soils (Reference soil 2.2 and 2.3) at day 20 and 20°C. CK represents treatment with neither phenanthrene nor surfactants. Data labels are percentage of effects. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

Soil 2.2 significantly (20%). It implied that the microorganisms in Soil 2.3 had the capacity to adapt to and degrade the material while Soil 2.2 did not though it had higher microbial activities. The coexistence of LAS at low concentrations (≤ 40 mg

kg^{-1}) further increased the respiration level of Soil 2.3 though inhibited at higher concentrations. In contrast to Soil 2.3, the coexistence of LAS at all concentrations investigated further decreased the microbial activities of Soil 2.2. The respond of Soil 2.2 and Soil 2.3 to the combined effects of phenanthrene and Tween-80 or TDTMA was similar. Tween-80 increased the microbial activities of both soils with the increase of its concentrations. The microbial activities of Soil 2.2 did not recover from the depression of phenanthrene until Tween-80 concentration above 40 mg kg^{-1}. At low concentrations, the respiration levels were higher than that at the presence of phenanthrene alone but lower than the background respiration. TDTMA at low concentrations increased the respiration of both soils while inhibited at high concentrations. The maximal respiration was at TDTMA concentration of 40 mg kg^{-1} for both soils, at which respiration increased 117% for Soil 2.3 while only 22% for Soil 2.2.

4.2.4.3 Combined effects of surfactants and pyrene on uncontaminated soils

Combined effects of pyrene and surfactants (LAS, Tween-80 or TDTMA) on the respiration activity of inherent microorganisms in Soil 2.3 in the presence of 0.5 mg kg^{-1} pyrene were measured at 20 ^{o}C. Addition of pyrene to the soil did not changed its respiration level at 20 days (Fig. 99 to Fig. 102), implying that pyrene did not possess toxicity to the soil microorganisms which in turn could not utilize pyrene. The coexistence of any of the three surfactants could generally increase the respiration of Soil 2.3 except for LAS and TDTMA at very high concentrations. This was similar to the combined effects of phenanthrene and the surfactants on the same soil (Fig. 103).

LAS at concentrations of 20 to 40 mg kg^{-1} increased the oxygen consumption levels of the soil compared with that in the presence of pyrene alone (Fig. 99). $+EC_{20}$ and $+EC_{50}$ accounted for 8 and 20 mg kg^{-1}, respectively (Table 18). The highest respiration level was at LAS concentration of 40 mg kg^{-1}, in which respiration increased 134%. The microbial activities started to decline when LAS concentrations were getting higher. At LAS concentrations of 200 mg kg^{-1}, the respiration became lower than that with pyrene only and background level. The value of $-EC_{20}$ was 708

mg kg^{-1}. -EC_{50} could not be observed at LAS concentration up to 900 mg kg^{-1}. Similar to the combined effects with phenanthrene, the increase of soil respiration by LAS at low concentrations indicated that LAS either increased the bioavailability of pyrene or was utilized as co-substrate by soil microorganisms. Because the inherent soil microorganisms could not oxidize LAS without the presence of PAHs (Fig. 81(a)), it was highly likely that LAS increased the bioavailability of pyrene.

In the co-presence of pyrene, Tween-80 increased the respiration of the soil at its concentrations from 20 mg kg^{-1} up to 900 mg kg^{-1} (Fig. 100), which was consistent to those in the presence of Tween-80 alone (Fig. 81(b)) or to those in the coexistence of phenanthrene and Tween-80 (Fig. 84). The values of +EC_{20} and +EC_{50} were 3 and 9 mg kg^{-1}, respectively (Table 18). The combined effects of pyrene and Tween-80 on the respiration were overadditive at low oxygen consumption levels but underadditive

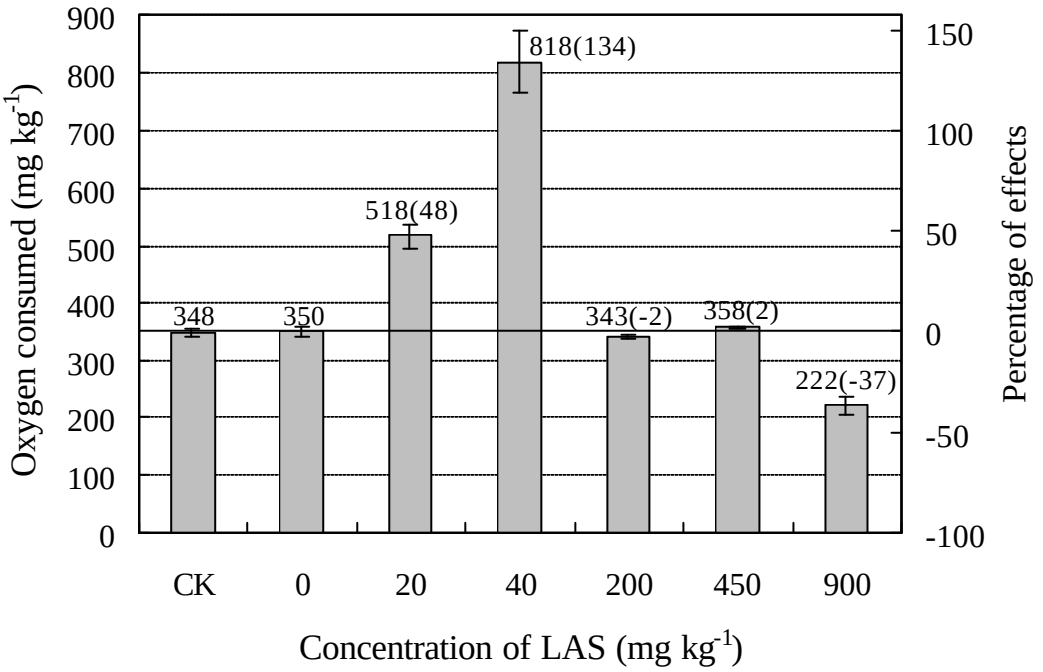


Fig. 99. Effects of LAS on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of pyrene (0.5 mg kg^{-1}) at day 20 and 20^{o}C. CK represents treatment with neither pyrene nor LAS. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols. Data in parenthesis are percentage of effects.

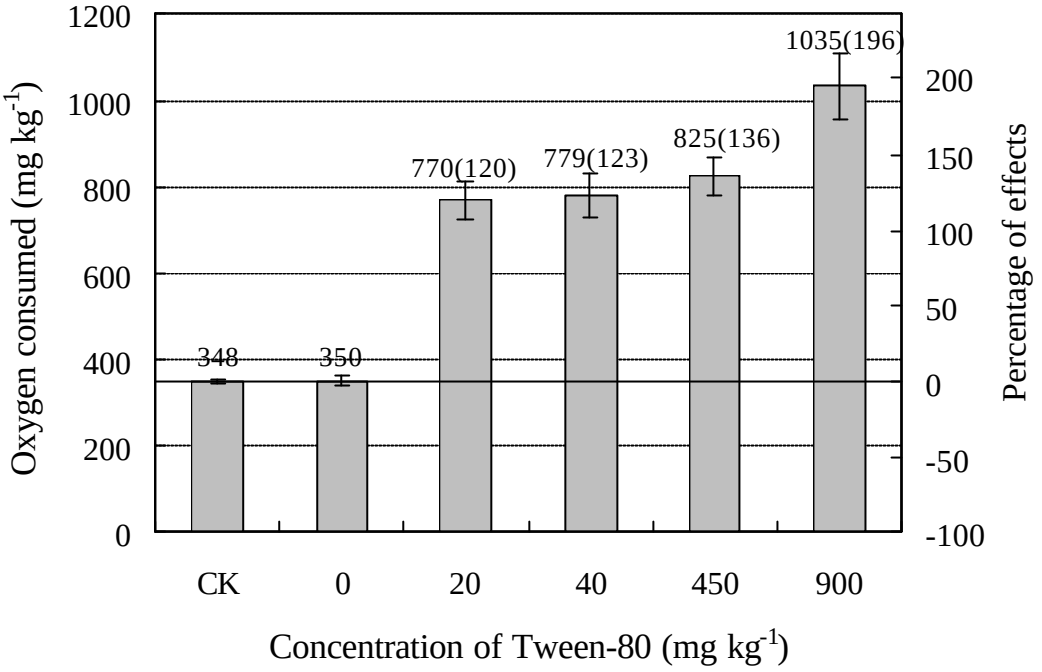


Fig. 100. Effects of Tween-80 on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of pyrene (0.5 mg kg^{-1}) at day 20 and 20°C. CK represents treatment with neither pyrene nor Tween-80. The error bars indicate standard deviations based on triplicate measurements. Data in parenthesis are percentage of effects.

Table 18. EC values of combined effects of surfactants and pyrene (0.5 mg kg^{-1}) on inherent microorganisms of an uncontaminated soil (Reference soil 2.3) (20 days)

(mg kg^{-1})

Surfactants	$+EC_{20}$	$+EC_{50}$	$-EC_{20}$	$-EC_{50}$
LAS	8.4	20.5	707.8	ND
Tween-80	3.3	8.8	NE	NE
TDTMA	3.3	24.3	ND	ND

Notes: $+EC_{20}$ and $+EC_{50}$ are assigned for effective concentrations of surfactants which cause 20% and 50% increase of microbial activity (oxygen consumption), respectively; whereas, $-EC_{20}$ and $-EC_{50}$ for those causing 20% and 50% decrease of microbial activity, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

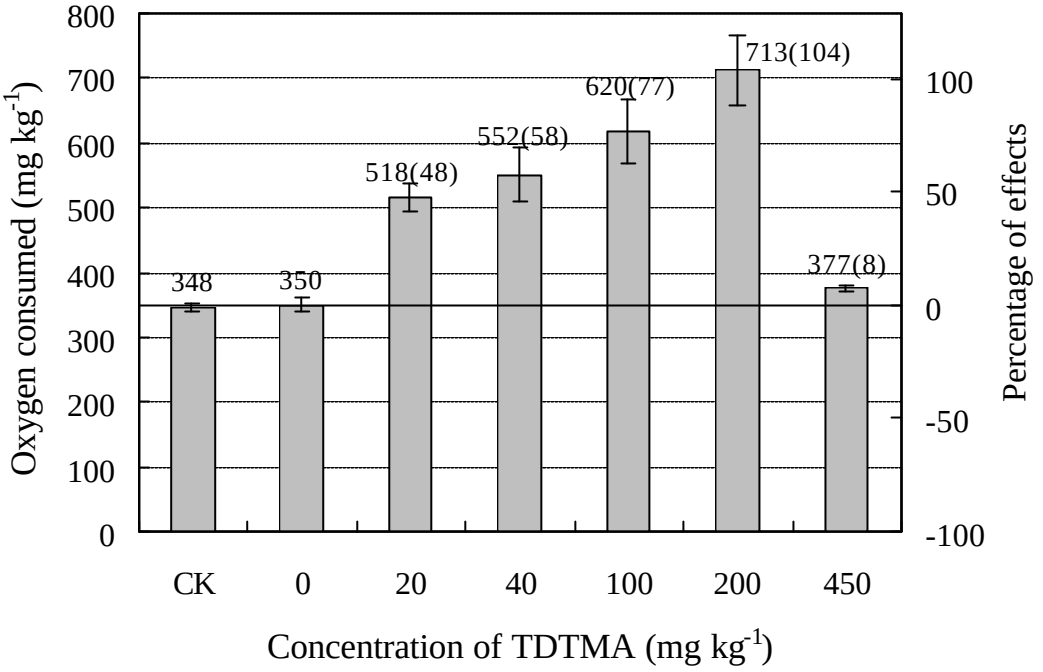


Fig. 101. Effects of TDTMA on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of pyrene (0.5 mg kg^{-1}) at day 20 and 20°C. CK represents treatment with neither pyrene nor TDTMA. The error bars indicate standard deviations based on triplicate measurements. Data in parenthesis are percentage of effects.

at high levels (Fig. 88). This implied that Tween-80's increase of respiration and bioavailability of pyrene was more efficient at low concentrations.

TDTMA at concentrations from 20 mg kg^{-1} to 450 mg kg^{-1} increased the respiration of the soil in the co-existence of 0.5 mg kg^{-1} pyrene (Fig. 101). The highest respiration level was at TDTMA concentration of 200 mg kg^{-1}, which was 104% higher than that in the absence of TDTMA. The values of $+EC_{20}$ and $+EC_{50}$ were 3 and 24 mg kg^{-1}, respectively (Table 18). Compared with the case that respiration started to decline below background level in the presence TDTMA alone at 200 mg kg^{-1} (Fig. 82), oxygen consumption levels in the co-presence of pyrene were still 8% higher than the background value at TDTMA concentrations up to 450 mg kg^{-1}. It suggested that the coexistence of pyrene reduced the toxicity of TDTMA probably by providing

available substrate. The combined effects of pyrene and TDTMA on microbial respiration were overadditive, implying not only the oxidation of both chemicals by soil microorganisms but also additional substrate available probably due to the increase of bioavailability of pyrene by TDTMA.

Fig. 102 shows the combined effects of surfactants and pyrene on the respiration kinetics of inherent microorganisms of the soil. Addition of pyrene inhibited the

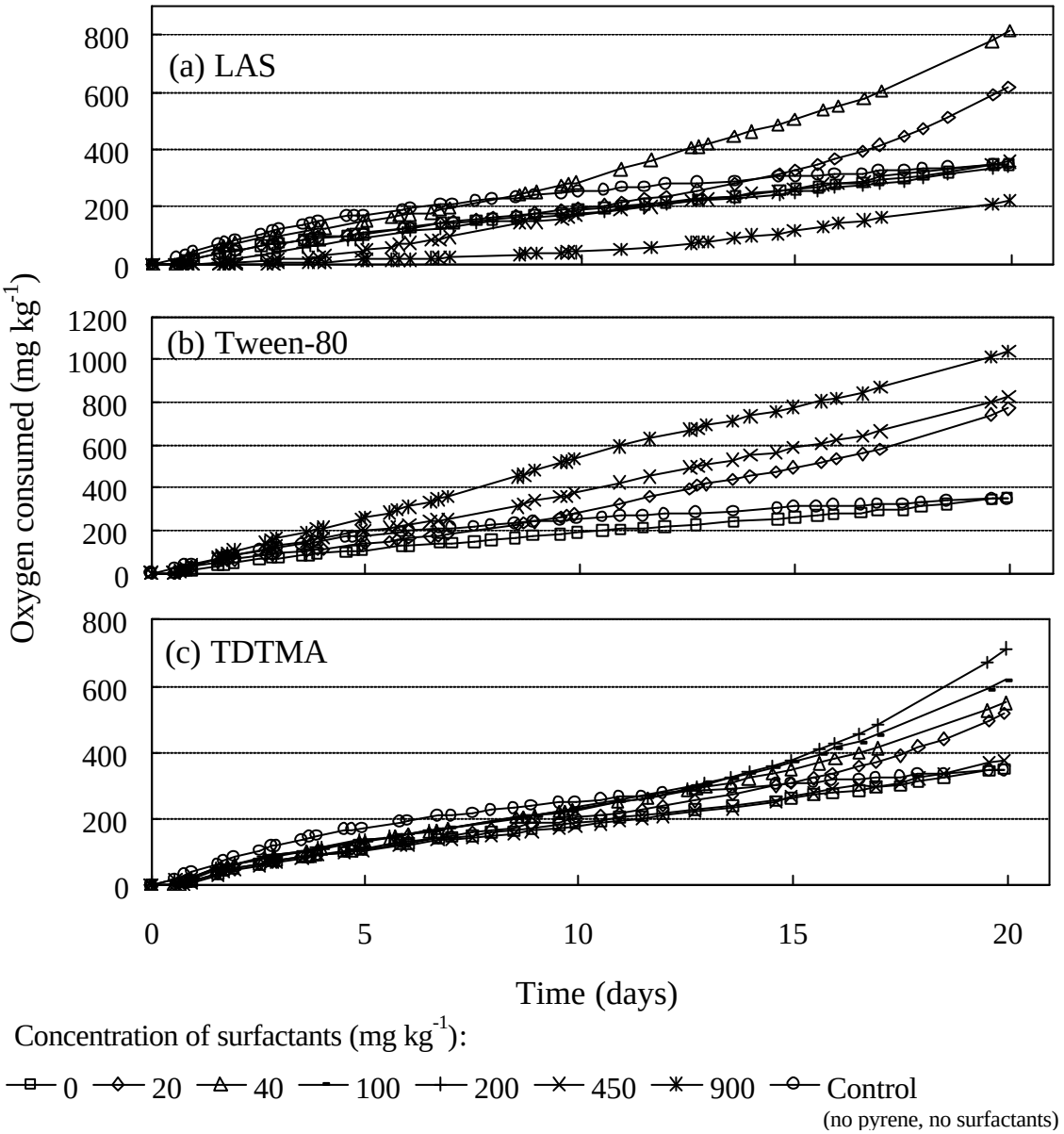


Fig. 102. Respiration kinetics of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of pyrene (0.5 mg kg^{-1}) and surfactants at 20°C. Data points are means of triplicates.

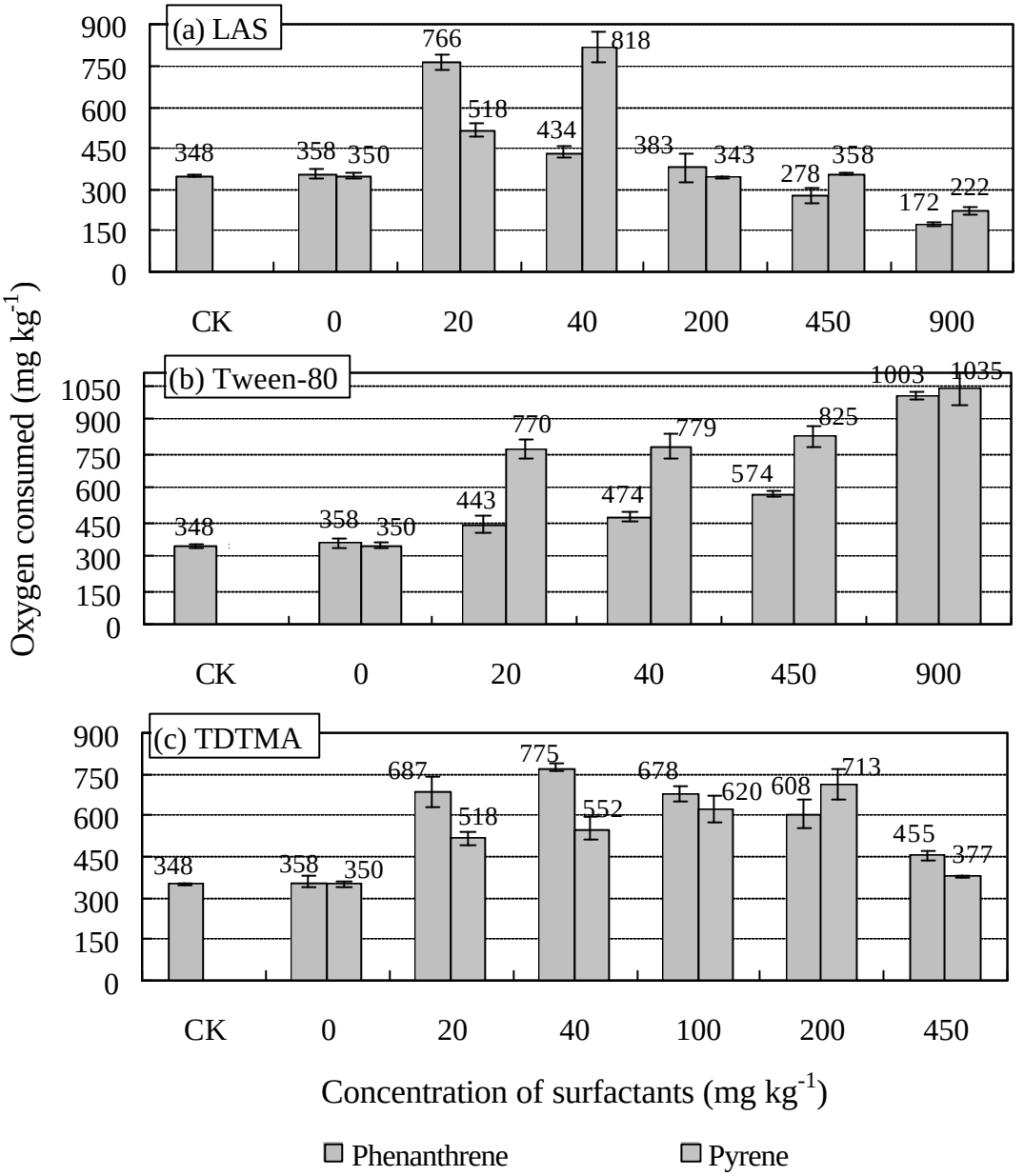


Fig. 103. Comparison of effects of surfactants on the respiration of inherent microorganisms in an uncontaminated soil (Reference soil 2.3) in the presence of phenanthrene (0.5 mg kg^{-1}) and pyrene (0.5 mg kg^{-1}) at day 20 and 20°C. CK represents treatment with neither phenanthrene nor surfactants. The error bars indicate standard deviations based on triplicate measurements; where there are no error bars, the standard deviations were smaller than the symbols.

microbial respiration in almost the whole incubation period though the respiration level reached to the background level after 20 days. The presence of LAS at 20 mg l^{-1}

shortened the lag phase to 15 days from 20 days without LAS (Fig 102(a)). As the LAS concentration increased to 40 mg l^{-1}, the lag phase further reduced to 9 days. It indicated that the presence of LAS increased the bioavailability or LAS was utilized as co-substrate since the soil microorganisms could not oxidize LAS alone (Fig. 81(a)). But at higher concentrations, LAS inhibited the respiration from the beginning to the end of incubation. The presence of Tween-80 at all concentrations studied from 20 to 900mg l^{1} caused the microbial respiration recovering from the toxicity of pyrene almost immediately in the beginning of incubation (Fig. 102(b)). The higher the concentrations, the higher the respiration increased. At concentrations below 200 mg l^{-1}, TDTMA reduced the lag phases to 12 to 15 days (Fig. 102(c)). But at higher concentrations, the lag phase lengthened again.

The combined effects of pyrene and these three surfactants were similar to those of phenanthrene and the same surfactants (Fig. 89 to Fig. 91). It indicated that surfactants rather than PAHs were the main factors affecting the microbial activities in the same soil. Therefore, similar reactions should be expected. However, differences still existed in the aspect such as surfactant concentrations which evoked the maximal respiration. At the presence of phenanthrene, the maximal respiration was at 20 mg l^{-1} LAS or 40 mg l^{1} TDTMA (Fig. 103(a)(c)). While at the presence of pyrene, they were 40 mg l^{-1} and 200 mg l^{-1}, respectively. It suggested that higher concentrations of surfactants were required to increase the bioavailability of pyrene than phenanthrene.

4.2.4.4 Effects of surfactants on PAHs-contaminated soil

Effects of the six surfactants of different doses on the respiration of inherent microorganisms of a PAHs-contaminated soil were studied considering their potential application in enhancing bioremediation of contaminated soils. LAS and the Brij surfactants at concentrations as low as 20 mg kg^{-1} could significantly increase the respiration levels to 13-37% (Fig. 104). It is interesting to find that Tween-80 could not increase the respiration at this concentration as it always did in uncontaminated soils after incubation for such a long time. Ten percents of increase of respiration level were measured at 40 mg kg^{-1} Tween-80. TDTMA did not change the respiration

levels at two concentrations (20 and 40 mg kg^{-1}) investigated, indicating TDTMA could neither be utilized by the soil microorganisms nor possessed toxicity to them. At concentration of 40 mg kg^{-1}, LAS caused the highest increase of respiration level (37%). The respiration at this concentration of Brij30 was not obviously different from that at 20 mgkg^{-1}. Significant decrease of respiration was found for Brij35 as its concentration increased from 20 mg kg^{-1} to 40 mg kg^{-1}, indicating its toxicity at high concentration. 10LE resulted in 14% increase of respiration at concentration of 40 mg kg^{-1}.

Fig. 105 presents the respiration kinetics of the PAHs-contaminated soil in the presence and absence of surfactants. Unlike acting on uncontaminated soils, LAS did not caused hysteresis of respiration on contaminated soil, indicating the soil contained those microorganisms ready to utilized LAS. Tween-80 showed a lag phase of 13 days at 40 mg kg^{-1}, which was similar to the effects on uncontaminated soils, i.e. shortening of lag phase as its concentration increased. Weaker hysteresis was observed for Brij surfactants on contaminated soil compared with uncontaminated soils.

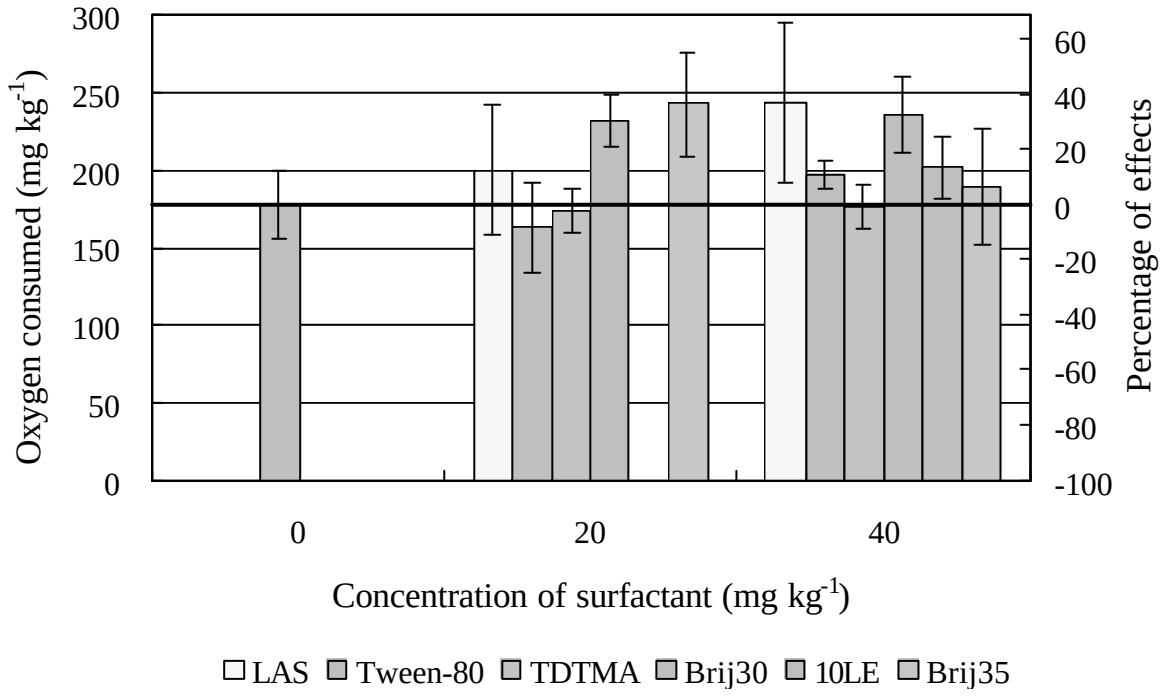


Fig. 104. Effects of surfactants on the respiration of inherent microorganisms in a PAHs-contaminated soil incubated at 20°C for 20 days. The error bars indicate standard deviations based on triplicate measurements.

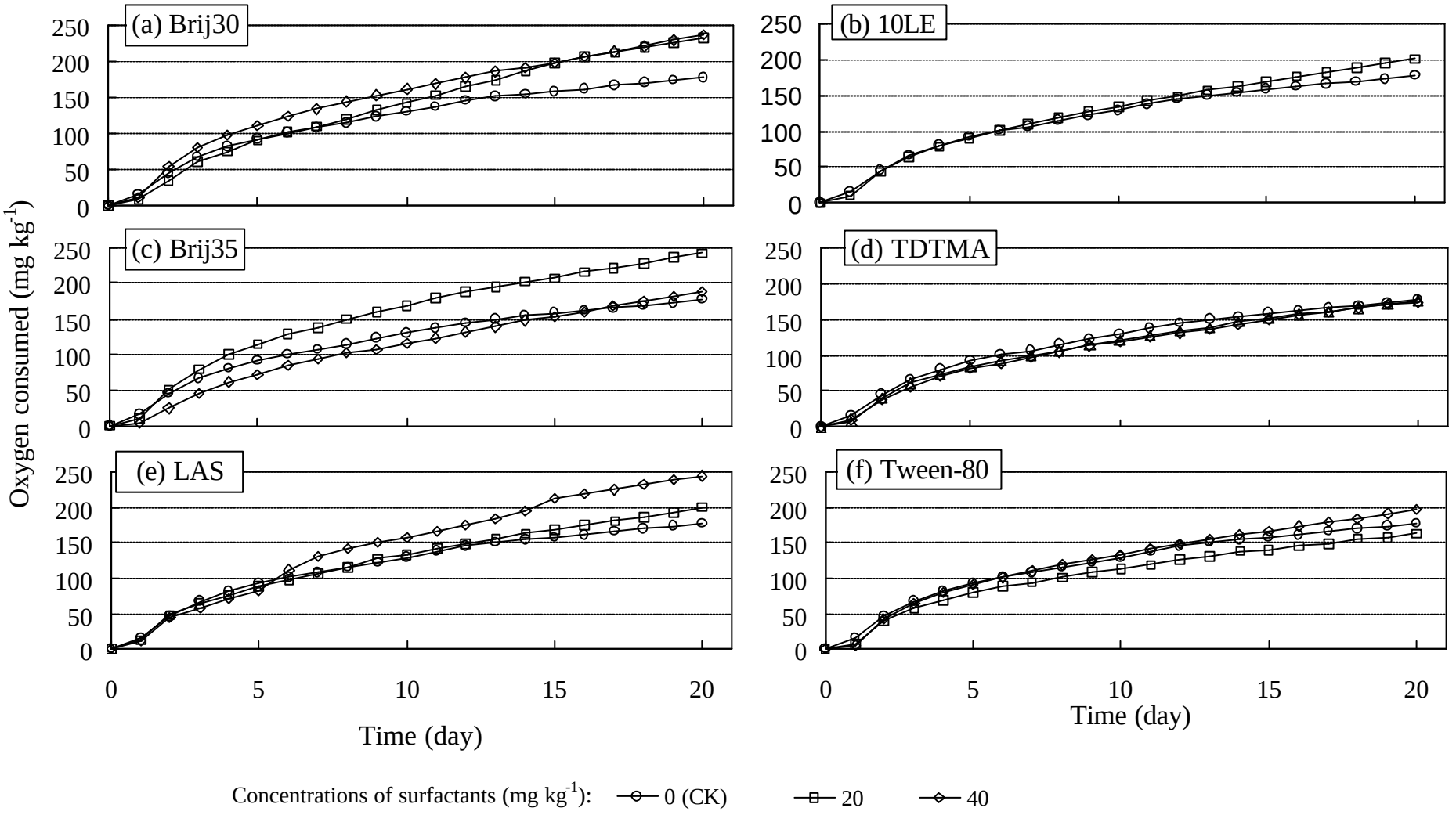


Fig. 105. Respiration kinetics of inherent microorganisms in a PAHs-contaminated soil incubated at 20°C in the presence and absence of surfactants. Data points are means of triplicates.

4.2.5 Sub-summary

Phenanthrene and all 6 surfactants tested were toxic to luminescent bacteria *Vibrio fischeri*. The dose-response curves of the bacteria to phenanthrene and Brij surfactants were linear at low concentrations but not to LAS, Tween-80 or TDTMA. Cationic surfactant TDTMA was the most toxic one. Its toxicity was even higher than phenanthrene. It is interesting to find that anionic LAS was less toxic than nonionic Brij30 and 10LE and that the toxicity of the Brij surfactant decreased with the increase of ethoxylate group in their molecules. The toxicity of all chemicals tested was in a descendent order of TDTMA > phenanthrene > Brij30 > 10LE > LAS > Brij35 > Tween-80. The toxic impact of the nonionic surfactants and phenanthrene on the bacteria was reversible or the bacteria were able to adapt to these chemicals but not to the ionic surfactants TDTMA and LAS.

The combination of phenanthrene and surfactants was more toxic to luminescent bacteria *Vibrio fischeri* than individual compounds. According to -EC values, the combined toxicity of phenanthrene and surfactants was in a descent order of TDTMA > Brij30 > LAS > Brij35 > 10LE > Tween-80. Generally, the higher dose of the two compounds in combination, the higher toxicity to the bacteria. Similar to separate effects, however, responses did not increased linearly with the increase of LAS and Tween-80 doses. But unlike separate effects, the dose-response curves of combined effects of phenanthrene and TDTMA were linear. In contrast to TDTMA, the three Brij surfactants alone at low concentrations caused linear responses but not in combinations with phenanthrene. The combined effects of phenanthrene with all 6 surfactants were not additive. The combined effects of phenanthrene with LAS, Tween-80, 10LE or Brij35 were underadditive or antagonistic, i.e. they were lower than the sum of their individual effects at the same dose. The most underadditive was Tween-80, followed by 10LE, LAS and Brij35. In contrast, the combined effects of phenanthrene and TDTMA or Brij30 were overadditive or synergistic at concentrations below -EC_{70}. The most synergistic effects were produced by the mixture of phenanthrene and Brij30. At higher effect concentrations, the combined effects of phenanthrene and TDTMA or Brij30 were underadditive. The order of combined effects of phenanthrene and the 6 surfactants from synergism to antagonism was Brij30 > TDTMA > Brij35 > LAS > 10LE > Tween-80.

Mycobacterium sp. could utilize Tween-80, Brij30, 10LE and Brij35 as sole carbon and energy source but not LAS and TDTMA. Tween-80, Brij30 and 10LE did not show any toxicity to the bacteria. Brij35 was toxic at high concentrations. TDTMA was the most toxic one, followed by LAS and Brij35 at high concentrations. The toxicity of the three Brij surfactants increase with the increase of ethoxylate group in their molecules, i.e. Brij35 > 10LE > Brij30. It was completely contrast to the responses of luminescent bacteria *Vibrio fischeri*, indicating that the toxicity of the same chemical varied significantly to different microorganisms.

The toxicity of LAS on *Mycobacterium* sp. eased in the coexistence of phenanthrene. LAS and Brij35 at low concentrations increased the activity of the bacteria. Probably *Mycobacterium* sp. could utilize them as co-substrate at low concentrations. Or LAS and Brij35 at low concentrations as well as Tween-80 and Brij30 probably increased the bioavailability of phenanthrene. But at higher concentrations, LAS and Brij35 were toxic to the bacteria. TDTMA was the most toxic surfactant. Its toxicity to the bacteria at the presence of phenanthrene was the same as at the absence of the material. Like in separate effects, Tween-80, Brij30 and 10LE in combination with phenanthrene were also not toxic to the bacteria. The combined effects of phenanthrene and Tween-80 or the three Brij surfactants were positive and additive. They were close to the sum of their individual effects, which might imply that the combined effects were results of substrate summation of the individual chemicals. The combined effect mode was negative for the mixture of phenanthrene and LAS or TDTMA, indicating the toxicity of the surfactants to the bacteria.

At the presence of abundant soluble substrate, either increase or decrease of growth of *Mycobacterium* sp. caused by surfactants was much less significant than that at the presence of scarcely soluble phenanthrene as sole carbon and energy source. It indicated that the bacteria would utilize the soluble substrate instead of surfactants. On the other hand, surfactants were less toxic to the bacteria at the presence of abundant soluble substrate. Results suggested that either positive effects or toxicity of surfactants depended on the type of substrates. The decedent order of toxicity of the

surfactants was TDTMA > LAS > Tween-80 (non-toxic), which was consistent to that of phenanthrene as substrate.

LAS and Tween-80 had similar effects on inherent microorganisms in uncontaminated soils as those on mono bacteria culture *Mycobacterium* sp. The soil microorganisms could not utilize LAS which was toxic to them. Tween-80 increased the microbial activities more significantly than did on *Mycobacterium* sp. The effects of TDTMA, however, were much different. At low concentration (≤ 100 mg kg^{-1}), TDTMA increase the activity of inherent microorganisms in soil which could oxidize TDTMA. It suggested that mixed communities of microorganisms could tolerate higher toxicity than mono microbial and degrade more toxic substance than mono bacteria. In addition, TDTMA was less toxic to inherent soil microorganisms than LAS though the former was much more toxic to *Mycobacterium* sp. and to luminescent bacteria *Vibrio fischeri* than the later. Therefore, it should be caution when conducting ecotoxicological assessment of chemicals in environment based on results obtained from mono bacteria.

It is also true for phenanthrene which had completely contrary effects on microorganisms in different soils. The microorganisms in Soil 2.3 had the capacity to utilize phenanthrene even the soil had had no contamination history while Soil 2.2 did not. The coexistence of any of above surfactants could generally increase the respiration of the soils except for LAS and TDTMA at very high concentrations and for LAS on Soil 2.2. These results indicate that both separate and combined effects of chemicals are different from soil to soil. LAS at low concentrations increased the activity of inherent microorganisms of Soil 2.3 in the presence of phenanthrene but did not in the absence of the material and not for Soil 2.2 either. Effects of Tween-80 were similar in the presence and absence of phenanthrene as well as in Soil 2.3 and Soil 2.2. It always increased the activity of inherent soil microorganisms as it did on *Mycobacterium* sp. TDTMA produced similar effects on both soils and in both presence and absence of phenanthrene, i.e. it increased the soil activity at low concentrations but inhibited at high concentrations. Temperature and prolonging

incubation could increase the overall microbial activity no matter there were test chemicals or not, but could not alter the relative respiration levels among treatments.

Pyrene could not increase the microbial activities in Soil 2.3 as phenanthrene did. It also did not exhibit toxicity to the microorganisms. The combined effects of pyrene and surfactants on the microbial activities were similar to those of phenanthrene and the same surfactants, indicating surfactants were the main factors influencing the microorganisms. The maximal respiration occurred at higher LAS and TDTMA concentrations at the presence of pyrene than phenanthrene, suggesting that higher doses of surfactants were required to increase the bioavailability of less soluble pyrene than phenanthrene.

The presence of LAS or TDTMA at low concentrations increased the soil respiration, indicating that they either increased the bioavailability of PAHs (phenanthrene and pyrene) or were utilized as co-substrates by soil microorganisms. Because the inherent soil microorganisms could not oxidize LAS without the presence of PAHs, it was highly likely that LAS increased the bioavailability of both PAHs. The combined effects of PAHs and TDTMA on microbial respiration were overadditive, implying not only the oxidation of both chemicals by soil microorganisms but also additional substrate available probably due to the increase of bioavailability of PAHs by TDTMA. It also suggested that the coexistence of PAHs reduced the toxicity of TDTMA probably by providing available substrate. In contrast to LAS and TDTMA, Tween-80 increased the respiration by acting as substrate as well as by increasing the bioavailability of PAHs. The combined effects of both PAHs and Tween-80 on the respiration were slightly overadditive at low respiration levels while underadditive at high levels.

TDTMA, which increased microbial activities of both uncontaminated soils either at the presence of PAHs or not, could not enhance the respiration of contaminated soil. The other surfactants could increase the activities of the inherent microorganisms of the contaminated soil. LAS did not cause hysteresis of respiration on the contaminated

soil as it did on uncontaminated soils. The hysteresis effects of Brij surfactants were also weaker on the contaminated soil than uncontaminated soils. Tween-80 had similar effects on both contaminated and uncontaminated soils. The positive effects of the six surfactants were generally lower on inherent microbial activities in PAHs-contaminated soil than in uncontaminated soils. It was probably because there were less variety of microorganisms in contaminated soil than in uncontaminated soils due to the toxicity of PAHs.

4.3. Influence of surfactants on the biodegradation of PAHs

The persistence of PAHs in soil depends mainly on the sorption, volatilization, leaching and biological or chemical degradation processes, which may be influenced by a variety of environmental factors (Sims and Overcash, 1983; Bulman *et al*., 1985). Microbiological degradation of PAHs is the major process that results in the decontamination of sediment and soil (Sims *et al*., 1990; Cerniglia, 1992; Wilson and Jones, 1993). The biodegradability of PAHs, in one hand, depends on the complexity of their chemical structure and corresponding physicochemical properties. On the other hand, it is also governed to a great extent by interactions of PAHs with soil particles, which influence their bioavailability.

Surfactants have the potential to increase the bioavailability of HOCs. However, the discrepancy within the literature about the effects of surfactants on the biodegradation of HOCs (see Section 1.3 and 1.4) indicates that there is a need to understand the mechanism of biodegradation in the presence of surfactants. The overall impact of a surfactant on biodegradation depends on how the bioavailability of HOCs is altered and whether the surfactant itself affects the cells. More specifically, the processes via which surfactants alter bioavailability of HOCs include dissolution of HOCs in micelles from non-aqueous-phase liquid (NAPL), desorption from soil particles into the aqueous phase, dissolution from the micelles into aqueous solution, access of micellar hydrocarbons into the cells, adhesion of bacteria to NAPL HOCs. The most important effect of surfactants on bacteria is toxicity. Alternation of characteristics of cell surface and membrane, and bacteria utilization of the surfactant as a substrate can

also affects the overall biodegradation of HOCs. If the surfactant is neither toxic nor a growth substrate, it can either increase the rate of biodegradation by carrying HOCs in relatively accessible micelles or it can decrease the rate by inhibiting the adhesion of cells to the NAPL-water interface. The overall impact depends on the importance of each process. If the surfactant is not toxic but a growth substrate, it can also either increase the rate of biodegradation by increasing microbial activity or it can decrease the rate by acting as a preferential substrate that the bacteria utilize the surfactant rather than the hydrocarbon. The overall impact depends on the concentration of surfactant besides the solubilization effects. Although it is still difficult to generalize the effects of surfactants on the biodegradation of HOC, it is commonly recognized that their effects are functions of the property, structure and concentration of surfactants.

4.3.1 Influence of surfactants on the biodegradation of phenanthrene by *Mycobacterium* sp.

The influence of surfactants on the biodegradation of phenanthrene by *Mycobacterium* sp. in liquid culture was measured by the release of $^{14}CO_2$ from ^{14}C-phenanthrene (100 mg l^{-1}) at the presence of different surfactants at various concentrations. The concentrations of surfactants were chosen based on their effects on the respiration of the bacteria (Section 4.2.2) to verify if they increased the bioavailability of phenanthrene at their concentrations which increased the respiration. The four nonionic surfactants at all concentrations (up to 80 mg l^{-1}) investigated increased the activities of the bacteria no mater whether phenanthrene was present or not, indicating that they can be utilized by the bacteria as sole- or co-substrate (Fig. 71 to Fig. 74). LAS increased the bacterial respiration at concentrations below 10 mg l^{1} at the presence of phenanthrene (Fig. 69). These phenomena further implied that those surfactants had the potential to increase the bioavailability of phenanthrene.

However, the biodegradation of phenanthrene was inhibited by LAS, Tween-80 and TDTMA at all concentrations (<40 mg l^{-1}) investigated (Fig. 106(a)). TDTMA caused the decrease of phenanthrene biodegradation to the greatest extent, followed by LAS

and Tween-80. The three Brij surfactants did not significantly alter the degradation (Fig. 106(b)) though trend can been seen that the biodegradation of phenanthrene was lower at the presence of Brij30 and 10LE than control and Brij35.

The influence of TDTMA on the biodegradation of phenanthrene was consistent to its effects on the respiration of the bacteria (Fig. 70 and Fig. 106(a)). It suggested that the inhibition of phenanthrene biodegradation was due to the toxicity of TDTMA. At TDTMA concentration of 5 mg l^{-1}, the bacterial respiration was almost completely (96%) inhibited, however, the phenanthrene degradation was only decreased 42%, indicating that the bacteria could still metabolize the chemical even at very low activity (8 mg O_2 l^{-1}). Degradation kinetics showed that there was a lag phase of about 3 days before the bacteria could significantly utilize the chemical (Fig. 107). As TDTMA concentrations increased above 10 mg l^{-1}, both respiration and

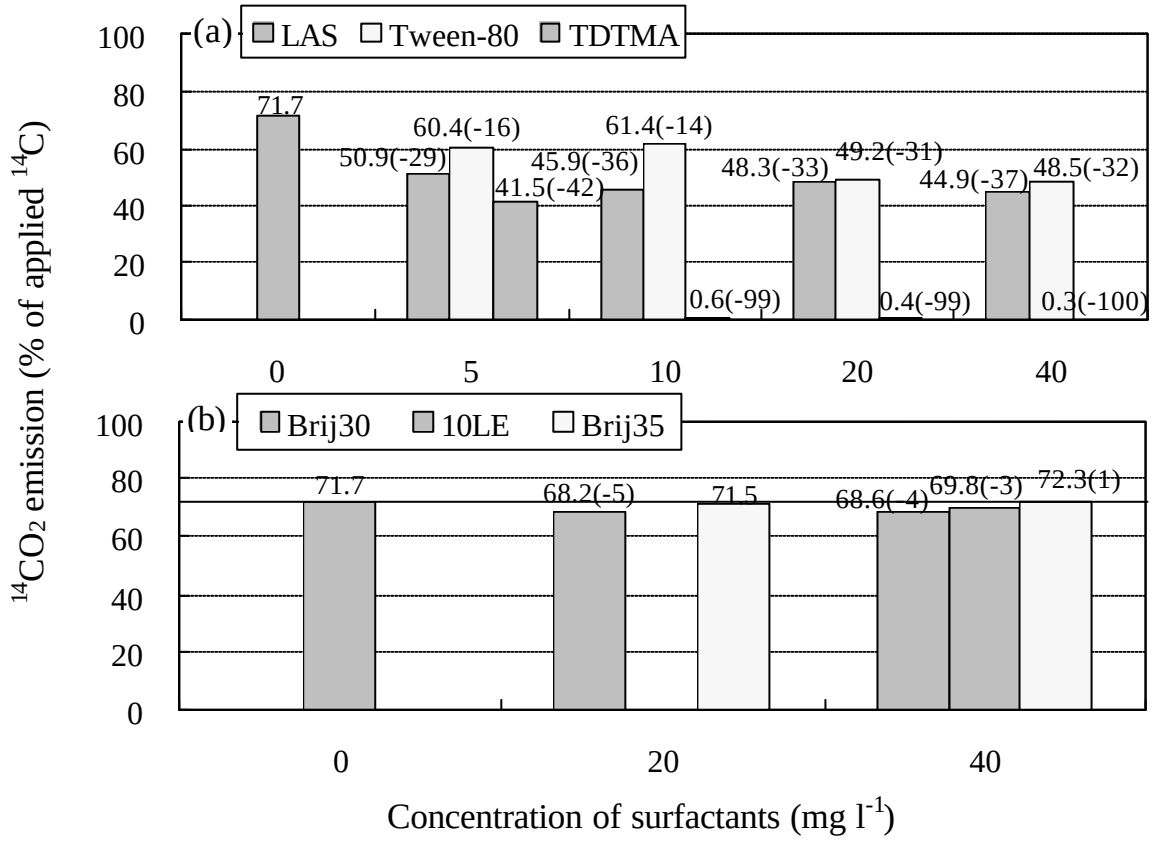


Fig. 106. Influence of surfactants on the biodegradation of ^{14}C-phenanthrene (100 mg l^{-1}) by *Mycobacterium* sp. in liquid culture at 20°C for 20 days. Results are means of duplicates. Data in parenthesis are percentage of increase or decrease compared with that without surfactants.

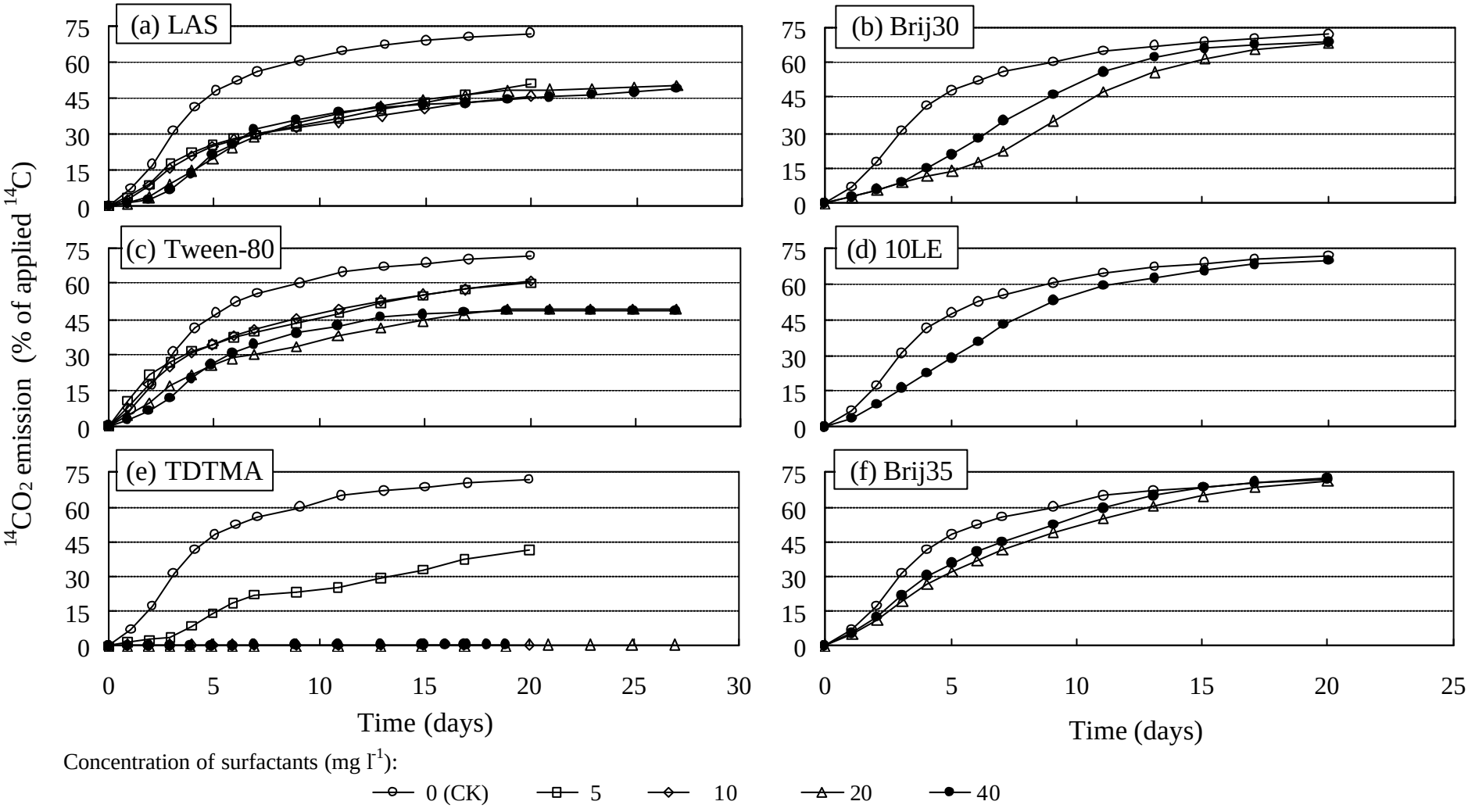


Fig. 107. Influence of surfactants on the biodegradation kinetics of ^{14}C-phenanthrene (100 mg l^{-1}) by *Mycobacterium* sp. in liquid culture at 20 ^{o}C. Data points are means of duplicates.

biodegradation processes were completely inhibited. Values of -EC_{20} and -EC_{50} were 2.4 and 5.7 mg l^{-1}, respectively.

The biodegradation of phenanthrene decreased as the concentrations of LAS and Tween-80 increased from 5 to 40 mg l^{-1}, resulting in -EC_{20} values of 3.4 and 13.5 mg l^{-1}, respectively (Fig. 106(a)). At concentration of 5 mg l^{-1}, LAS and Tween-80 caused 29% and 16% decrease of the degradation compared with control, respectively. At concentration of 40 mg l^{-1}, the degradation was inhibited 37% and 32% for LAS and Tween-80, respectively. Since respiration data showed that Tween-80 was not toxic to the bacteria and could be utilized by the bacteria as growth substrate (Fig. 71), the decrease of phenanthrene biodegradation in the presence of Tween-80 can only be explained by that the bacteria used it as a preferential substrate, resulting in mineralization of less phenanthrene. The decrease of phenanthrene biodegradation at the presence of LAS at concentrations ≤10 mg l^{-1} was probably also due to the same mechanism as the bacteria were more active at the presence of LAS than control (Fig. 69(b). It indicated that the utilization of LAS as co-substrate by the bacteria reduced the utilization of phenanthrene by the bacteria. At higher concentrations, the inhibition of phenanthrene degradation was a result of LAS toxicity to the bacteria.

However, the decrease of phenanthrene biodegradation as a result of preferential utilization of surfactants was not proportional to their increase of the bacterial respiration levels. The Brij surfactants significantly increased the bacterial activities (Fig. 72 to Fig. 74) but did not alter the biodegradation of phenanthrene within experimental deviation (Fig. 106(b)). It suggests that besides preferential metabolism of the co-existent surfactants, other mechanisms may exist such as effects of surfactants on the function of cell membrane, and the uptake and metabolic path of the chemical by the bacteria. As shown in Fig. 108 of some culture solution as examples, colour of the culture with the absence and presence of surfactants was different, implying that the metabolic products might be different (Guerin and Jones, 1988).

Kinetics data showed that the biodegradation of phenanthrene was low at the

beginning of incubation in the presence of all surfactants except for Tween-80 at 5 and 10 mg l^{-1} (Fig. 107). The biodegradation levels at the presence of Brij surfactants increased close to the control levels at the end of incubation at day 20 but not for the other surfactants.

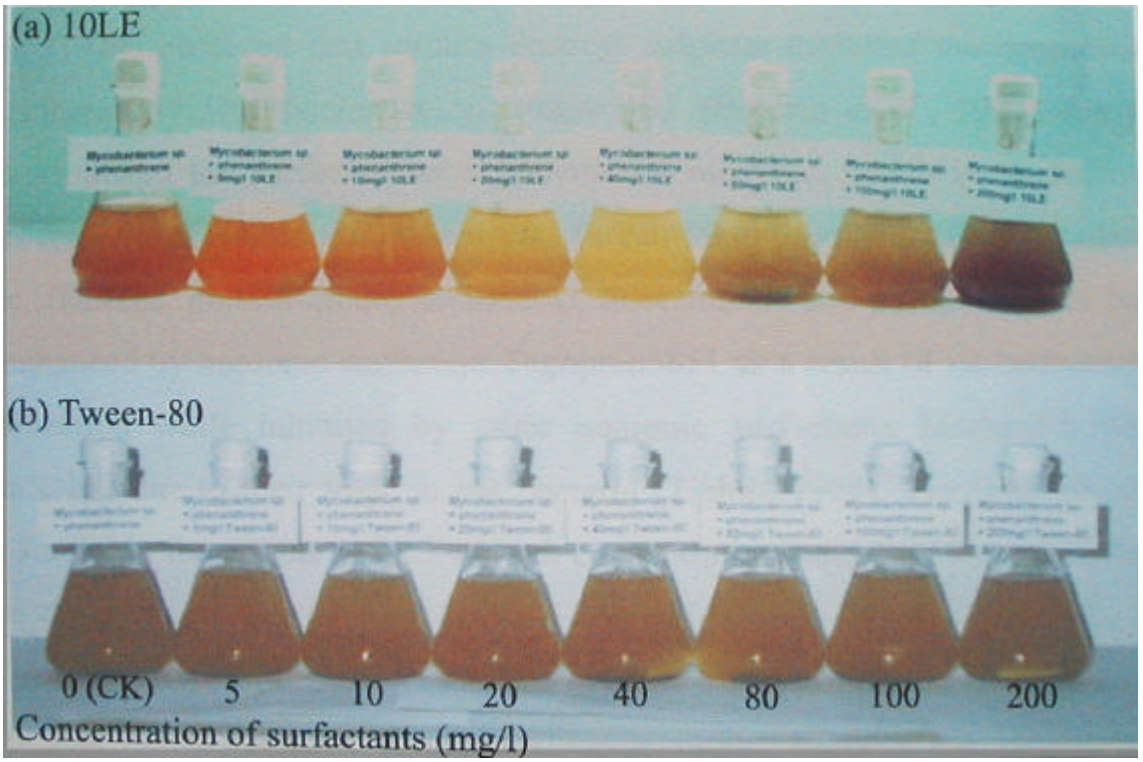


Fig. 108. Colour of *Mycobacterium* sp. culture on phenanthrene (100 mg l^{-1}) in the presence and absence of surfactants

It can be concluded from this study that the surfactants did not increase the bioavailability and the biodegradation of phenanthrene in liquid culture where both chemicals were in dissolved state ready to be utilized by bacteria.

However, Guerin and Jones (1988) found that Tween-80 (100 mg l^{-1}) could slightly increase the degradation of phenanthrene (100 mg l^{-1}) by *Mycobacterium* sp. strain BG1. They attributed the enhancement of degradation to the increased solubilization of phenanthrene by the surfactant while suggested other physiological as well as physicochemical effects of the surfactant might be involved. In addition, they compared the effect of various Tween surfactants and found that Tween-80 was not

the most conducive to phenanthrene utilization by the bacteria. Further more, their results indicated that the bacteria strain did not grow on any the tested Tween surfactants as sole carbon source. Respiration data in this study, however, revealed that the *Mycobacterium* sp. strain KR2 used in this measurement could grow on Tween-80 as the sole carbon and energy source (see Section 4.2.2).

Tiehm (1994) observed that sodium dodecyl sulphate inhibited the degradation of phenanthrene by *Mycobacterium* sp. strain BB1 (Boldrin *et al*., 1993) due to the bacteria utilized the surfactant as a preferential substrate, which is consistent with results for LAS at low concentrations and Tween-80 in this study. The degradation of PAHs (fluorene, phenanthrene, anthracene, fluoranthene and pyrene) by the bacteria was enhanced by nonionic surfactant Tegopren5851 as a result of its increasing their solubilization while inhibited by other nonionic surfactants Marlipal013/90 and TritonX-102 due to their toxic to the bacteria (Tiehm, 1994). The later phenomena were similar to the effects of LAS at high concentrations and TDTMA on strain KR2 in this study.

Brij30 was found not toxic to naphthalene-degrading bacteria nor inhibiting their mineralization of naphthalene in a mixed microbial culture (Liu *et al*., 1995). However, Laha and Luthy (1991) reported that Brij30 at concentration of above CMC inhibited the mineralization of phenanthrene in water-soil systems and the inhibition was reversible on diluting the surfactant to a concentration below CMC. Although Brij35 was found to be able to increase the apparent solubility of phenanthrene and naphthalene and not toxic to bacteria strain 8909N and strain 8803F, the PAHs present in its micellar phase was not readily available for degradation by the microorganisms (Volkering *et al*., 1995). These phenomena may explain why Brij surfactants did not influence the degradation of phenanthrene in this study.

4.3.2 Influence of surfactants on the biodegradation of PAHs by inherent soil microorganisms

Influences of selected surfactants (LAS, Tween-80 and TDTMA) on the

biodegradation of phenanthrene and pyrene (0.5 mg kg^{-1}) were studied in uncontaminated soils - Reference soils 2.2 and 2.3, and agricultural soils $13^{\#}$, $14^{\#}$ and $15^{\#}$. Effects of the three surfactants on the microbial activities of the same soils are presented in Section 4.2.4.

4.3.2.1 Biodegradation of phenanthrene in soils

4.3.2.1.1. Biodegradation of phenanthrene in Reference soil 2.3

The influence of LAS, Tween-80 and TDTMA on the biodegradation of phenanthrene by inherent soil microorganisms (Fig. 109) was consistent to their effects on the respiration of the same soil (Soil 2.3). At low concentrations, LAS (10 to 167 mg kg^{-1}) and TDTMA (10 to 200 mg kg^{-1}) increased the biodegradation of phenanthrene compared with control, giving $+EC_{20}$ and $+EC_{50}$ of 3.9 and 9.8 mg kg^{-1} for LAS and $+EC_{20}$ of 153.4 mg kg^{-1} for TDTMA, respectively (Table 19). At higher concentrations (LAS $\geq$ 200 mg kg^{-1} and TDTMA $\geq$ 450 mg kg^{-1}), both surfactants significantly inhibited the biodegradation of phenanthrene, resulting in $-EC_{20}$ and $-EC_{50}$ values of 178.8 and 189.2 mg kg^{-1} for LAS and 397.6 and 670.6 mg kg^{-1} for TDTMA, respectively (Fig. 109(a)(c)). Tween-80 at all concentrations (10 to 1875 mg kg^{-1}) enhanced the biodegradation of phenanthrene (Fig. 109(b)).

The enhancement of biodegradation of phenanthrene by LAS was mainly due to the increase of bioavailability of phenanthrene by the surfactant, as it could not be utilized by the inherent soil microorganisms without the presence of phenanthrene (Fig. 81(a)). This result proved that LAS's increase of the soil respiration was resulted from its increase of the bioavailability of phenanthrene. Since both Tween-80 and TDTMA could be utilized by the soil microorganisms, the enhancement of phenanthrene biodegradation by the two surfactants was results of their increases of bioavailability of phenanthrene and of microbial activities (Fig. 81(b) and Fig. 82). The possible mechanisms for the increased bioavailability of phenanthrene by the surfactants may include increase of phenanthrene desorption from soil particles due to competition of adsorption site on soil surface (Ou *et al.*, 1995b), overcoming transport limitation from soil particles to aqueous phase (Volkering *et al.*, 1995), and increase of

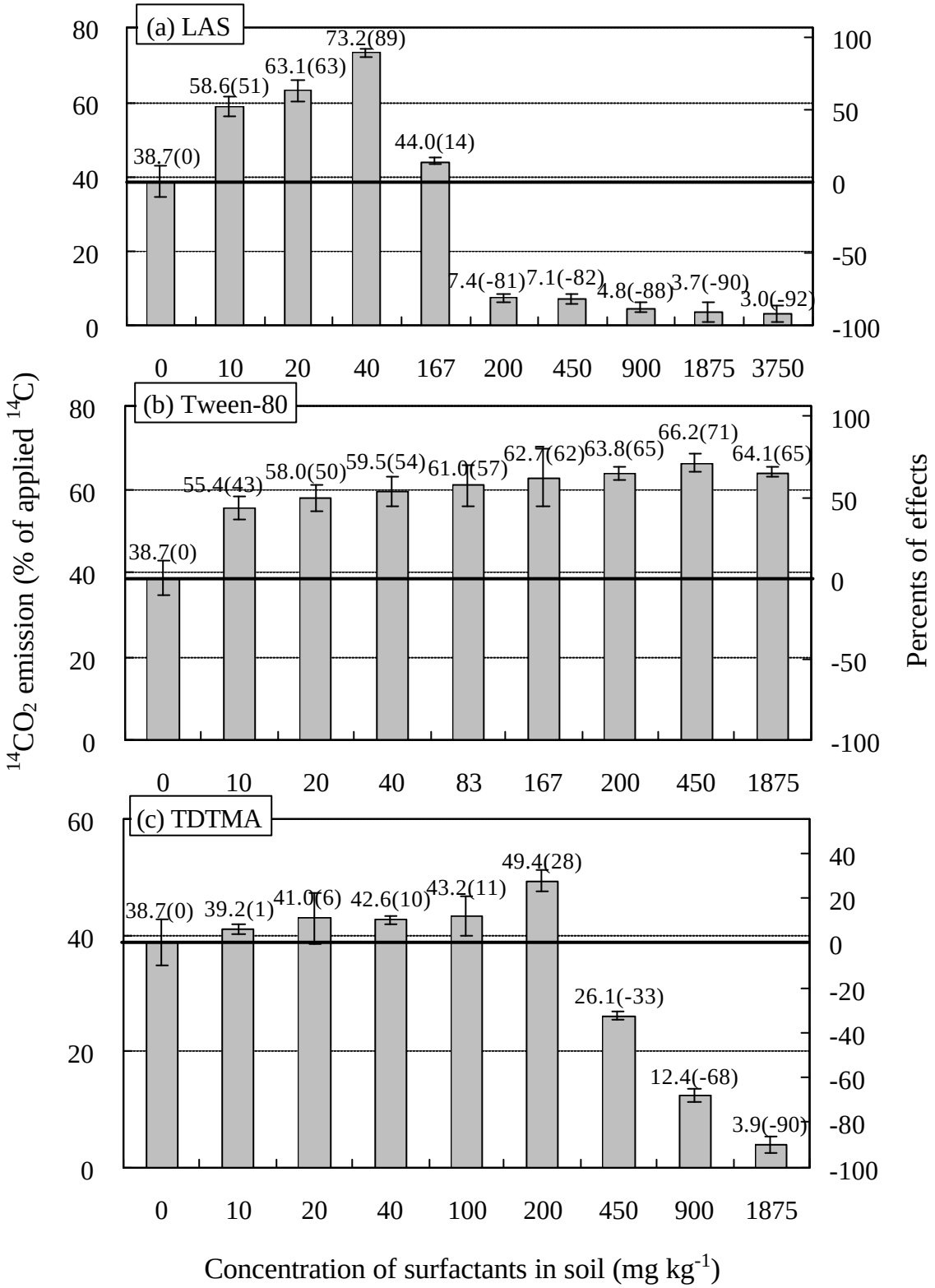


Fig. 109. Influence of surfactants on the biodegradation of phenanthrene in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} ^{14}C-phenanthrene at 20^{o}C for 20 days. The error bars indicate standard deviations based on triplicates. Data in parenthesis are percentage of increase or decrease compared with that without the presence of surfactants.

Table 19. EC values of surfactant's influence on the biodegradation of phenanthrene in different soils amended with 0.5 mg kg^{-1} phenanthrene at 20°C (mg kg^{-1})

Soil	Surfactants	$+EC_{20}$	$+EC_{50}$	$-EC_{20}$	$-EC_{50}$
Reference soil 2.3					
	LAS	3.9 [a]	9.8 [a]	178.8 [a]	189.2 [a]
	Tween-80	4.6 [a]	21.1 [a]	ND [a]	ND [a]
	TDTMA	153.4 [a]	ND [a]	397.6 [a]	670.6 [a]
Reference soil 2.2					
	LAS	1.9 [b]	4.8 [b]	3702.2 [b]	3720.1 [b]
	Tween-80	0.9 [b]	2.3 [b]	ND [b]	ND [b]
	TDTMA	3.9 [b]	9.8 [b]	397.6 [b]	670.6 [b]
	TDTMA	17.3 [bc]	ND [bc]	ND [bc]	ND [bc]
SES Soil 13#	LAS	ND [d]	ND [d]	100.2 [d]	174.7 [d]
SES Soil 14#	LAS	ND [d]	ND [d]	63.3 [d]	108.2 [d]
SES Soil 15#	LAS	ND [d]	ND [d]	60.0 [d]	100.7 [d]

Notes: $+EC_{20}$ and $+EC_{50}$ are assigned for effective concentrations of surfactants which cause 20% and 50% increase of phenanthrene biodegradation, respectively; whereas, $-EC_{20}$ and $-EC_{50}$ for those causing 20% and 50% decrease of biodegradation, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

a, b, d - exposure time: 20, 32 and 18 days, respectively.

c - incubation temperature: 30°C.

phenanthrene solubility (Edwards *et al.*, 1991; Laha and Luthy, 1992) due to hydrophobic interactions between surfactant monomers and phenanthrene when surfactant below CMC and due to surfactant encapsulation of phenanthrene into micelles when surfactant above CMC (Zhang and Miller, 1992). The inhibition of phenanthrene biodegradation by LAS and TDTMA at high concentrations was caused by their toxicity to the soil microorganisms (Fig. 84 and Fig. 86).

Fig. 110 compares the influence of the three surfactants on the biodegradation of

phenanthrene. LAS was the most significant and efficient in increase of phenanthrene biodegradation among the three surfactants. The highest increase of phenanthrene biodegradation caused by each surfactant occurred at 40 mg kg^{-1} LAS (89% increase), 450 mg kg^{-1} Tween-80 (71%) and 200 mg kg^{-1} TDTMA (28%). The lower efficiency of Tween-80 and TDTMA in enhancement of phenanthrene biodegradation than LAS was partially because they were utilized by the soil microorganisms as preferential substrates, resulting in less phenanthrene was mineralized by the microorganisms. It had been proved that the two surfactants could be oxidized by the soil microorganism (Fig. 81(b) and Fig. 82).

Fig. 111 shows the influence of surfactants on the kinetics of phenanthrene biodegradation. There was a lag phase of 3 to 4 days after addition of phenanthrene to the soil before it started to be degraded. LAS at concentrations ≤40 mg kg^{-1}, Tween-80 at concentrations ≤1875 mg kg^{-1} and TDTMA at concentrations ≤200 mg kg^{-1} did not alter the length of the lag phase. The surfactant's enhancement of phenanthrene

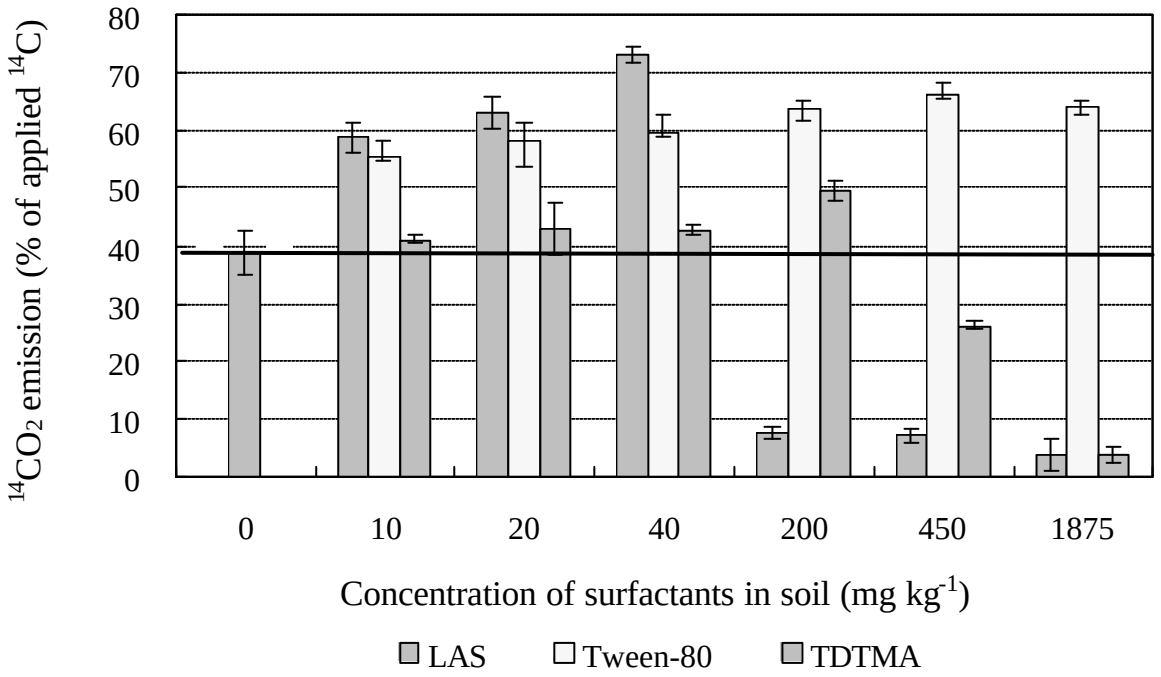


Fig. 110. Comparison among surfactants' influences on the biodegradation of phenanthrene in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} ^{14}C-phenanthrene at 20°C for 20 days. The error bars indicate standard

deviations based on triplicates.

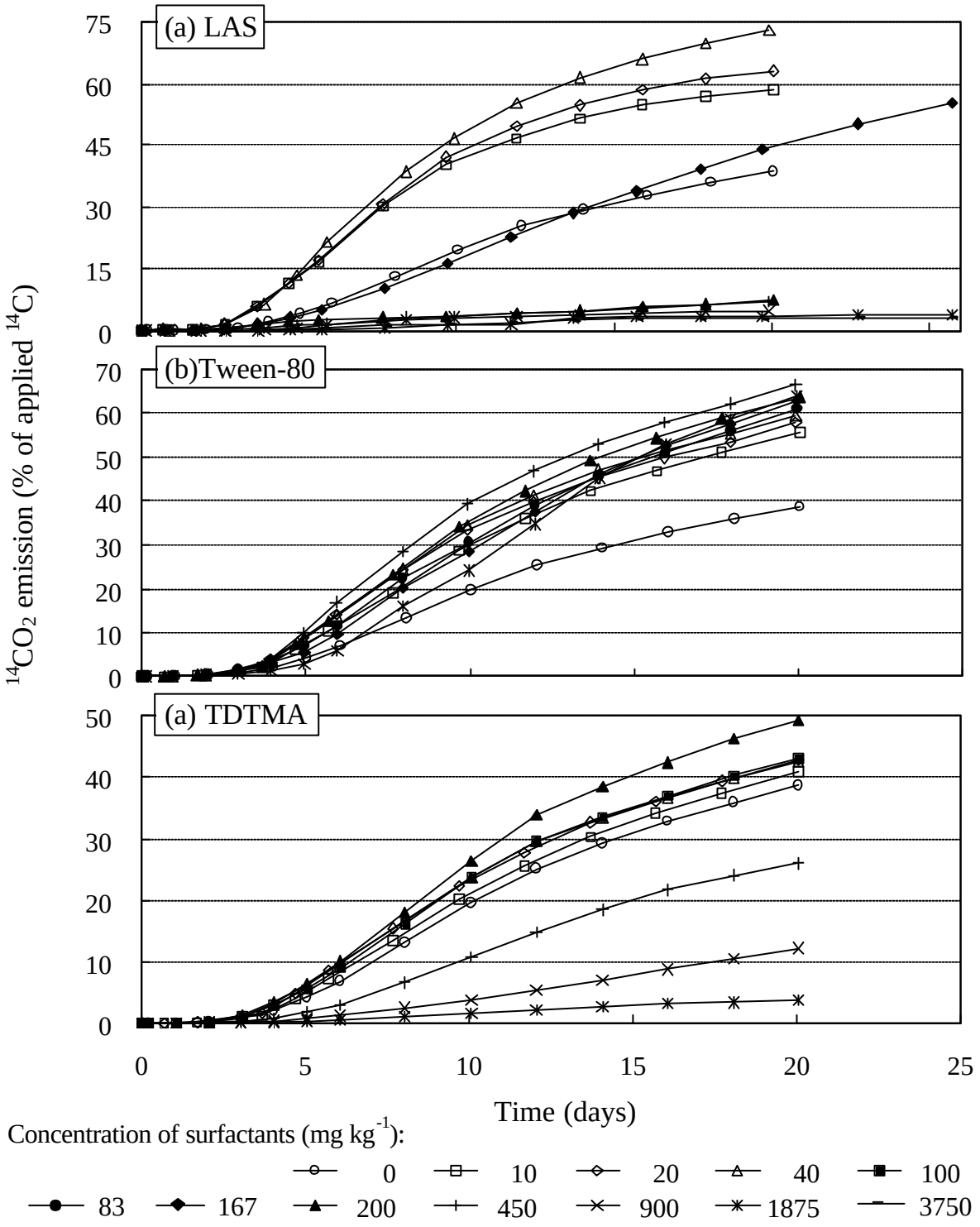


Fig. 111. Influence of surfactants on the kinetics of phenanthrene biodegradation in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} ^{14}C-phenanthrene at 20^{o}C. Data points are means of triplicates.

biodegradation occurred as that of control. Measured in the same soil and under the same conditions, the respiration kinetics did not show any lag phase (Fig. 96 and Fig. 97). Therefore, the lag phase monitored in degradation kinetics indicated that the soil microorganisms utilized other materials (e.g. surfactants applied) as substrates rather than phenanthrene at the early stage of incubation.

4.3.2.1.2. Biodegradation of phenanthrene in Reference soil 2.2

Like influenced on Soil 2.3, LAS also enhanced the biodegradation of phenanthrene in Soil 2.2 at low concentrations and inhibited at high concentrations (Fig. 112). However, LAS concentrations at which the biodegradation of phenanthrene was increased or decreased were quite different between the two soils. In Soil 2.2, the biodegradation of phenanthrene increased with LAS concentrations increased from 40 mg kg^{-1} up to as high as 1875 mg kg^{-1}, while in Soil 2.3 the biodegradation started to decline when LAS concentrations above 200 mg kg^{-1}. As a result, LAS produced low +EC (+EC_{20} = 1.9 and +EC_{50} = 4.8 mg kg^{-1}) and very high -EC values (-EC_{20} = 3702 and -EC_{50} = 3720 mg kg^{-1}) in Soil 2.2 compared with Soil 2.3 (Table 19).

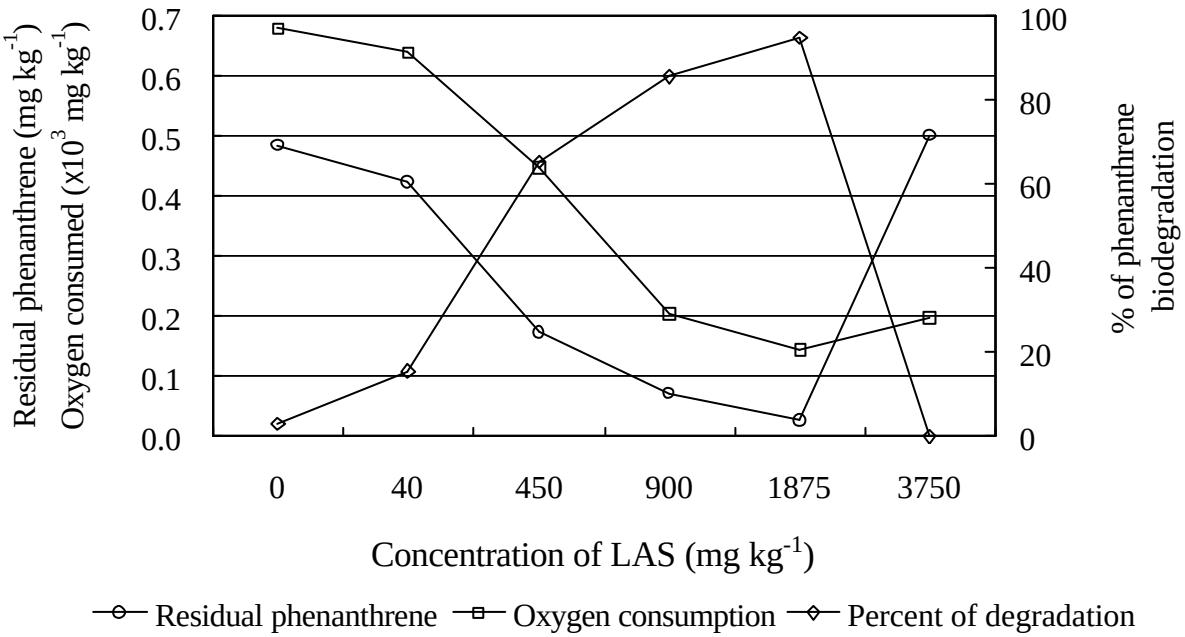


Fig. 112. Influence of LAS on the biodegradation of phenanthrene in an uncontaminated soil (Reference soil 2.2) amended with 0.5 mg kg^{-1} phenanthrene at

20°C for 32 days, in comparison with oxygen consumption of the same soil samples. Another interesting difference between the influence of LAS on the two soils was the relationships between biodegradation of phenanthrene and microbial activities. LAS enhanced the biodegradation of phenanthrene in both soils, but the respiration of Soil 2.2 decreased with the increase of LAS concentrations while that of Soil 2.3 increased. It indicated that the enhancement of biodegradation of phenanthrene by LAS in Soil 2.2 was mainly due to its increase of bioavailability of phenanthrene. At LAS concentration of 1875 mg kg^{-1}, 96% of the applied phenanthrene was degraded even though the oxygen consumption was the lowest (150 mg kg^{-1}) among all concentrations. The differences between microbial communities of the two soils may be the main reason causing different responses as it can be seen that the background respiration level of Soil 2.2 was almost double that of Soil 2.3 (Fig. 84 and Fig. 93).

In contrast to LAS, Tween-80's enhancement of the biodegradation of phenanthrene in Soil 2.2 was accompanied by the increase of microbial activities (Fig. 113), which was consistent to its effects on Soil 2.3. At Tween-80 concentrations ranging from 20 to 900 mg kg^{-1}, the biodegradation of phenanthrene increased from 16% to 99% of

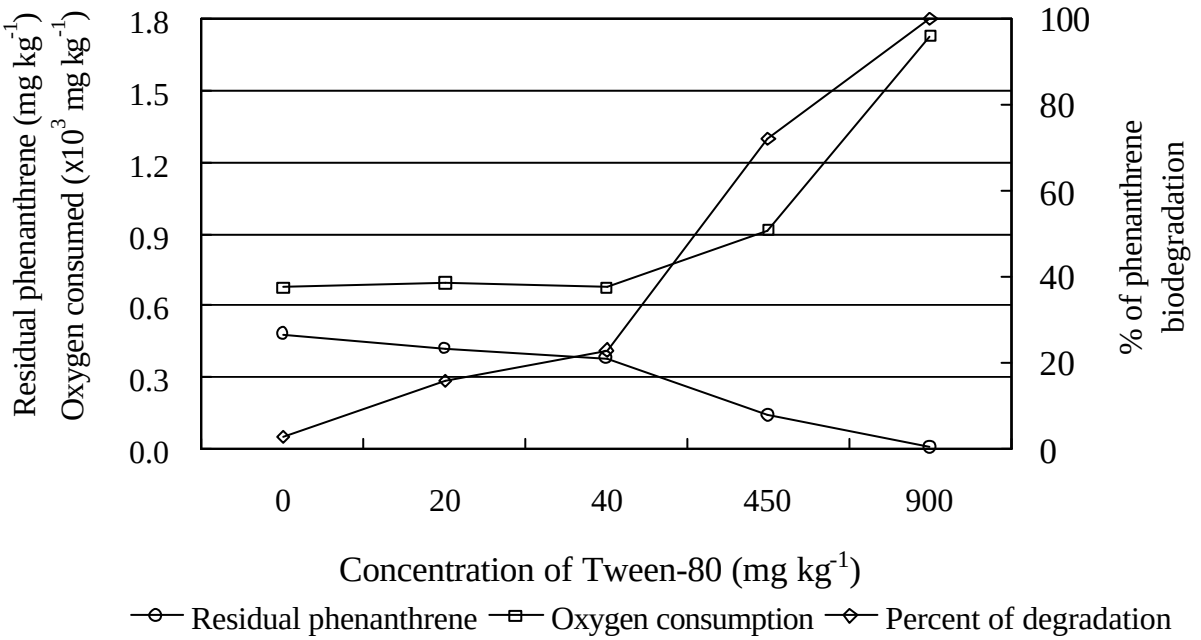


Fig. 113. Influence of Tween-80 on the biodegradation of phenanthrene in an uncontaminated soil (Reference soil 2.2) amended with 0.5 mg kg^{-1} phenanthrene at

20°C for 32 days, in comparison with oxygen consumption of the same soil samples. applied phenanthrene while oxygen consumption increased from 700 to 1700 mg kg^{-1}. Values of $+EC_{20}$ and $+EC_{50}$ were 0.9 and 2.3 mg kg^{-1}, respectively.

TDTMA had similar influence on the biodegradation of phenanthrene in Soil 2.2 and Soil 2.3, i.e. increased the degradation at low concentrations while inhibited at high concentrations (Fig. 114). Like Tween-80, the enhancement of biodegradation of phenanthrene by TDTMA was also accompanied by the increase of microbial oxygen consumption levels. At high concentrations, TDTMA decreased both microbial respiration and biodegradation of phenanthrene due to its toxicity to the microorganisms. High temperature increased the overall biodegradation of phenanthrene and microbial respiration but did not change the relative trend of TDTMA's influence of both parameters.

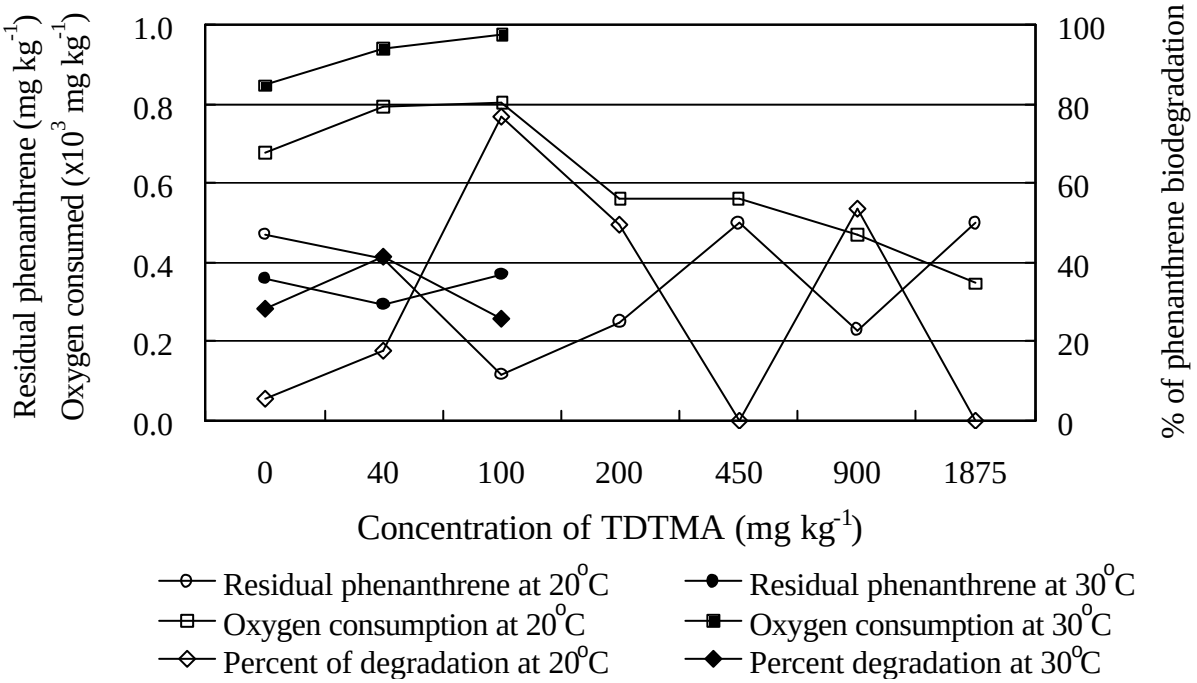


Fig. 114. Influence of TDTMA on the biodegradation of phenanthrene in an uncontaminated soil (Reference soil 2.2) amended with 0.5 mg kg^{-1} phenanthrene for 32 days, in comparison with oxygen consumption of the same soil samples.

4.3.2.1.3. Biodegradation of phenanthrene in agricultural soils

The influence of LAS on the biodegradation of phenanthrene was examined in three different layers of soil collected from farmland in SES. Unlike in Soil 2.2 and Soil 2.3, LAS only slightly (6%) increased the biodegradation of phenanthrene in top layer soil (Soil 13#) at concentration of 40 mg kg^{-1}, and decreased the degradation at higher concentrations (Fig. 115). In the two subsurface soils (Soil 14# and 15#), the degradation was inhibited at all concentrations of LAS tested. The inhibition of LAS on the degradation was more significant in subsurface soils than in topsoil due to the less amount and diversity of microorganisms in the former than in the later (Ou *et al*., 1996c). At LAS concentration of 167 mg kg^{-1}, the degradation was almost inhibited in Soil 15# (60-100 cm) while the same level of inhibition in Soil 14# (20-60 cm) was observed only when LAS concentrations increased to 450 mg kg^{-1}. At this LAS concentration, there were still about 3% of applied phenanthrene was degraded. This is also reflected in their -EC values which were lower in subsurface soils than topsoil (Table 19). The inhibition of LAS was due to its toxicity to the soil microorganisms as it decreased the microbial respiration of these soils (data not shown).

However, it should be noticed that the three soils had much higher degrading capacities for phenanthrene than Soil 2.2 and Soil 2.3. In the absence of surfactants,

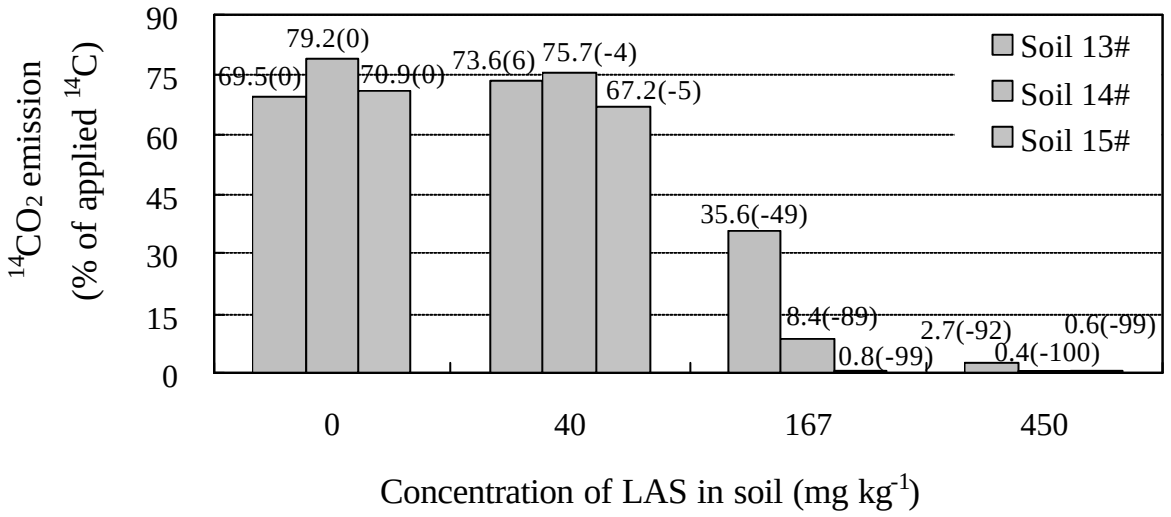


Fig. 115. Influence of LAS on the biodegradation of phenanthrene in an agricultural soil of different layers (Soil 13#: 0-20cm, Soil 14#: 20-60 cm, and Soil 15#: 60-100cm) amended with 0.5 mg kg^{-1} ^{14}C-phenanthrene at 20^{o}C for 18 days. Data points are means of duplicates.

the amount of phenanthrene degraded in Soil 13#, 14# and 15# was almost double that in Soil 2.3 and 17 times of that in Soil 2.2. At LAS concentration of 40 mg kg^{-1}, although the biodegradation of phenanthrene was increased in both Soil 2.2 and 2.3 and deceased in Soil 14# and 15#, the amounts of phenanthrene degraded in the later two soils were still almost as the same as that in Soil 2.3 and higher than that in Soil 2.2. It suggested that the surfactant's enhancement efficiency would be low or even there would be no enhancement when the soil already had high degrading capacities for phenanthrene.

Fig. 116 shows the influence of LAS on the kinetics of phenanthrene biodegradation in Soil 13#, Soil 14# and Soil 15#. Similar to Soil 2.3, there was 3 to 4 days lag time in these soil. At LAS concentration of 40 mg kg^{-1}, no significant increase of hysteresis was observed compared with control. At higher concentrations, the lag phase increased with the increase of LAS concentrations. At LAS concentration of 450 mg kg^{-1}, no biodegradation of phenanthrene was observed at all soils till the end of measurement in 18 days.

4.3.2.2 Biodegradation of pyrene in soils

The biodegradation of pyrene (Fig. 117) was much lower than that of phenanthrene (Fig. 110) in the same soil (Soil 2.3), indicating that pyrene was more persistent in soil due to its lower water solubility and higher affinity to soil particles than phenanthrene. In the absence of surfactants, only 4.8% of pyrene were degraded by inherent soil microorganisms while the degradation of phenanthrene was as high as 38.7% under the same conditions. The bioavailability of pyrene was 8 times lower than that of phenanthrene.

LAS at low concentration (40 mg kg^{-1}) and Tween-80 at all concentrations (20 to 450 mg kg^{-1}) investigated increased the biodegradation of pyrene (Fig. 117). LAS at high concentrations (≥450 mg kg^{-1}) and TDTMA at all concentrations (40 to 200 mg kg^{-1}) tested decreased the biodegradation of the chemical. These phenomena were similar to their influences on the biodegradation of phenanthrene except for TDTMA. But the

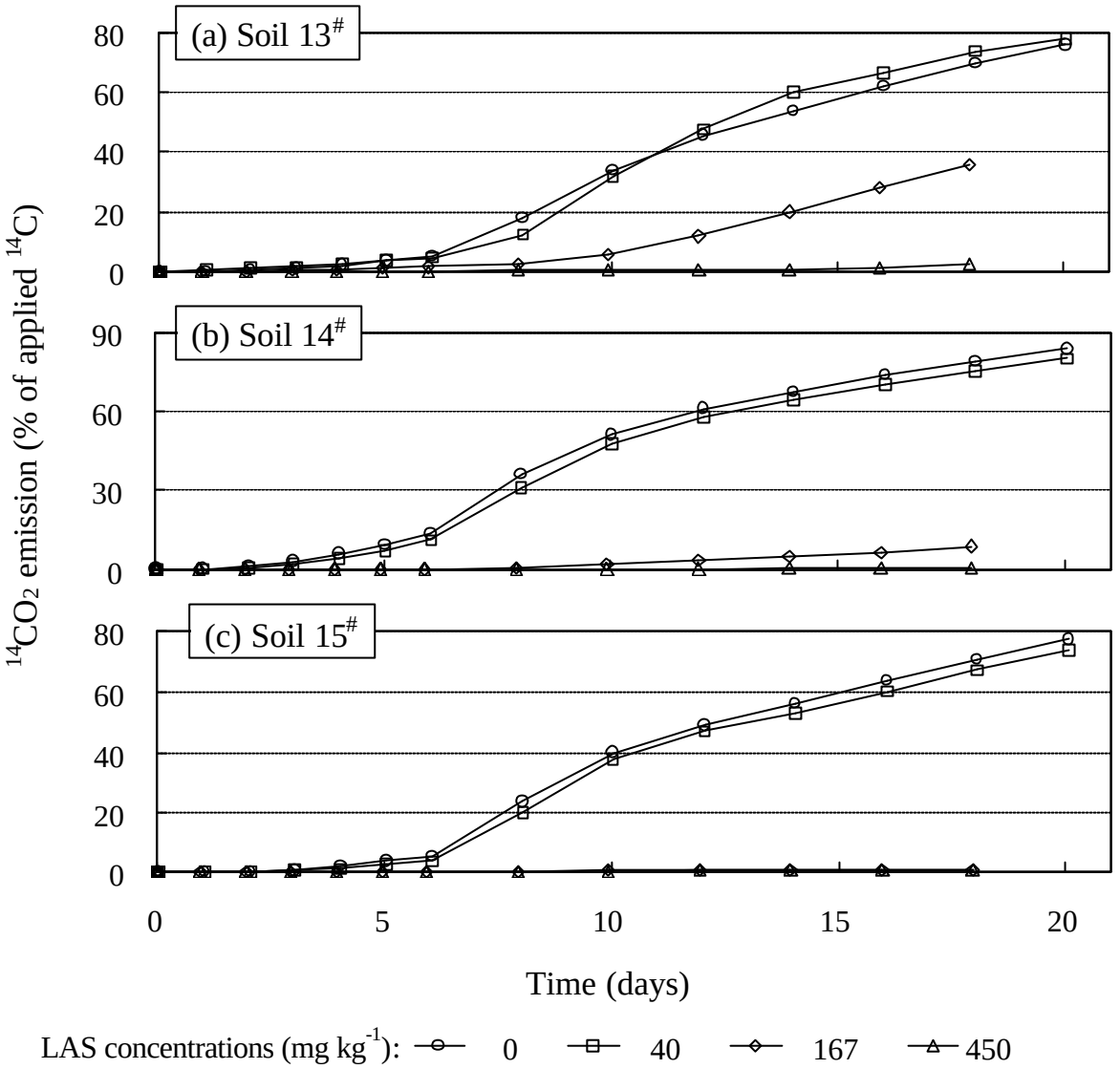


Fig. 116. Influence of LAS on the kinetics of phenanthrene biodegradation in an agricultural soil of different layers ((a) Soil 13#: 0-20cm, (b) Soil 14#: 20-60 cm, and (c) Soil 15#: 60-100cm) amended with 0.5 mg kg^{-1} ^{14}C-phenanthrene in the presence and absence of LAS at 20°C. Data points are means of duplicates.

efficiency of surfactant's enhancement of biodegradation of pyrene was much lower than that of phenanthrene. At concentrations of 40 mg kg^{-1}, LAS and Tween-80 increased the biodegradation of phenanthrene by 89% and 54% while only caused 17% and 23% increase of the biodegradation of pyrene, respectively. This was also reflected in +EC values (Table 20). For example, to achieve 50% increase of pyrene biodegradation, Tween-80 concentration of more than 10 times higher than that for phenanthrene was required. It suggested that higher doses were required to increase

the bioavailability of pyrene than phenanthrene. However, at the presence of Tween-80 at high concentration (450 mg kg^{-1}), the biodegradation of pyrene and phenanthrene increased to similar extent, i.e. 67% for the former and 71% for the later, though the absolute degradation amounts of the two chemicals were much different, 8% and 66% of applied pyrene and phenanthrene, respectively.

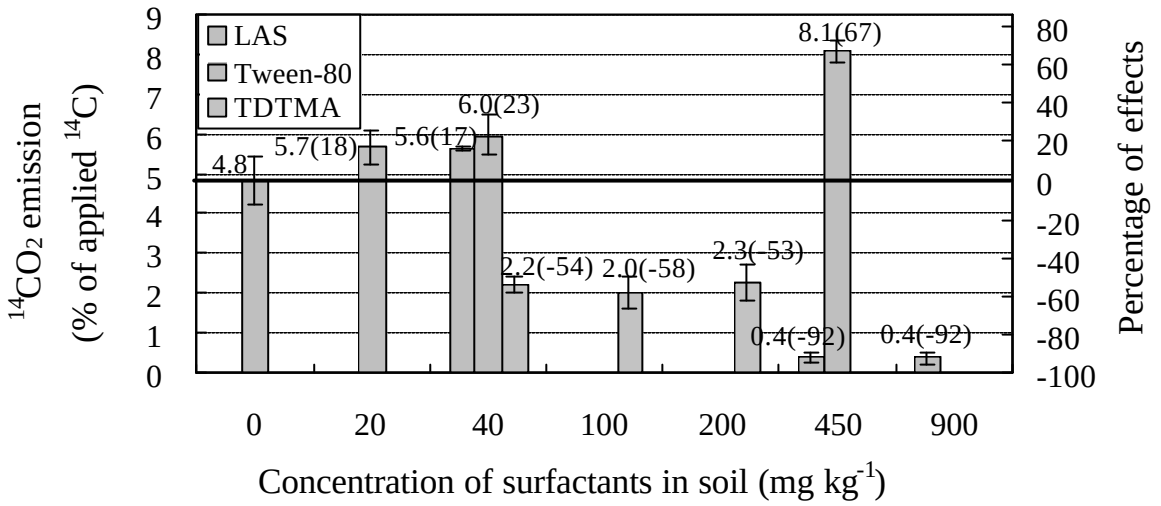


Fig. 117. Influence of surfactants on the biodegradation of pyrene in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} ^{14}C-pyrene at 20^{o}C for 20 days. The error bars indicate standard deviations based on triplicates. Data in parenthesis are percentage of increase or decrease compared with that without the presence of surfactants.

Table 20. EC values of surfactant's influence on the biodegradation of pyrene in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} pyrene at 20^{o}C for 20 days (mg kg^{-1})

Surfactants	$+EC_{20}$	$+EC_{50}$	$-EC_{20}$	$-EC_{50}$
LAS	ND	ND	148.1	274.8
Tween-80	28.2	289.4	ND	ND
TDTMA	ND [a]	ND [a]	14.8	37.0

Notes: $+EC_{20}$ and $+EC_{50}$ are assigned for effective concentrations of surfactants which cause 20% and 50% increase of phenanthrene biodegradation, respectively; whereas, $-EC_{20}$ and $-EC_{50}$ for those causing 20% and 50% decrease of biodegradation, respectively. NE denotes that this effect was not observed. ND denotes that this effect could not be obtained under surfactant concentrations studied.

a - exposure time: 18 days.

Tween-80 increased the microbial respiration levels more significantly in the presence of pyrene than phenanthrene in the same soil. LAS or TDTMA had similar effects on the soil microbial activities in the presence of pyrene and phenanthrene though the maximal respiration occurred at different surfactant doses (Fig. 103). The increase of biodegradation of pyrene by LAS, however, was much lower than that of phenanthrene. Moreover, although TDTMA increased the microbial activity at concentrations from 20 mg kg^{-1} to as high as 200 mg kg^{-1} (Fig. 101), it inhibited the biodegradation of pyrene. It suggests that increase of microbial activities dose not necessarily enhance the biodegradation of HOCs. The increase of bioavailability of HOCs is more directly relevant to the enhancement of biodegradation of the chemicals.

In the same soil and under the same conditions, TDTMA increased the biodegradation of phenanthrene (Fig. 109(c)) while inhibited the biodegradation of pyrene. The inhibition of pyrene degradation can not be explained either by toxic effects of the surfactant as it increased the microbial activities (Fig. 101) or by preferential utilization of the surfactant by microorganisms as it enhanced the degradation of phenanthrene. Reasonable explanation was that TDTMA decreased the bioavailability of pyrene probably due to its increase of the adsorption and bonding of pyrene to TDTMA-soil complexes (Smith *et al*., 1990). As its lower solubility and higher affinity to organic matter than phenanthrene, pyrene strongly adsorbed to the TDTMA complexes, resulting in unavailable to microorganisms.

Fig. 118 shows the influence of surfactants on the kinetics of biodegradation of pyrene in the same soil. Similar to phenanthrene, a lag phase of 3 to 4 days was also observed. But the addition of surfactants did not shorten the lag phase in the presence of pyrene as they did on phenanthrene (Fig. 111). In the same soil and under the same conditions, no lag phase existed in the respiration kinetics data (Fig. 102), confirming the same fact found for phenanthrene that the increase of microbial activities dose not necessarily result the increase of biodegradation of HOCs.

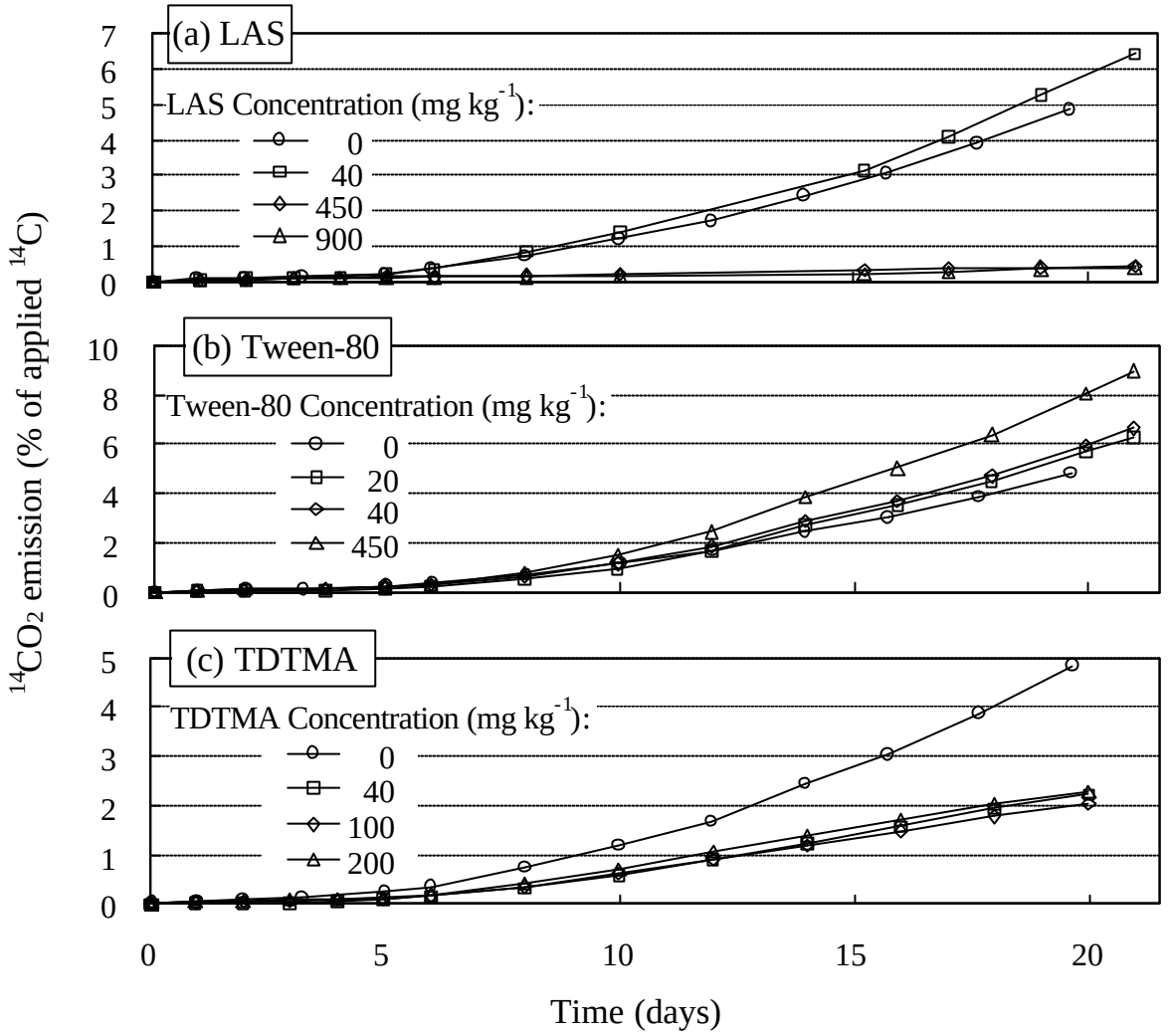


Fig. 118. Influence of surfactants on the kinetics of pyrene biodegradation in an uncontaminated soil (Reference soil 2.3) amended with 0.5 mg kg^{-1} ^{14}C-pyrene at 20^{o}C. Data points are means of triplicates.

4.3.3 Influence of Tween-80 on the mineralization and metabolism of phenanthrene in wheat-nutrient-lava microcosm

4.3.3.1 Mass balance of ^{14}C-activity in test system

Mass balance of ^{14}C-activity within the test system (Fig. 12) was examined by measuring ^{14}C-activity in all components in the system. Results, expressed as percentage of ^{14}C-activity recoveries, are presented in Fig. 119. Satisfied mass recoveries were obtained, proving that the system was suitable for the present study. The total recoveries of ^{14}C-activity were 93% and 97% of the ^{14}C-activity applied in

the absence and presence of Tween-80, respectively. At the end of experiment, 18 to 21% of the total ^{14}C-activity applied was found in the wheat roots, about 50% in the wheat shoots (stems and leaves), 2 to 3% in culture solution and 3 to 5% in lava. 4 to 5% and 12 to 16% of the total applied ^{14}C-activity volatized in forms of volatile organic chemicals (VOCs) and CO_2, respectively. Only small amount of ^{14}C-activity (less than 0.3% of the total ^{14}C-activity) was adsorbed on the surface of the apparatus. The presence of Tween-80 did not significantly alter the total recovery within experimental deviations.

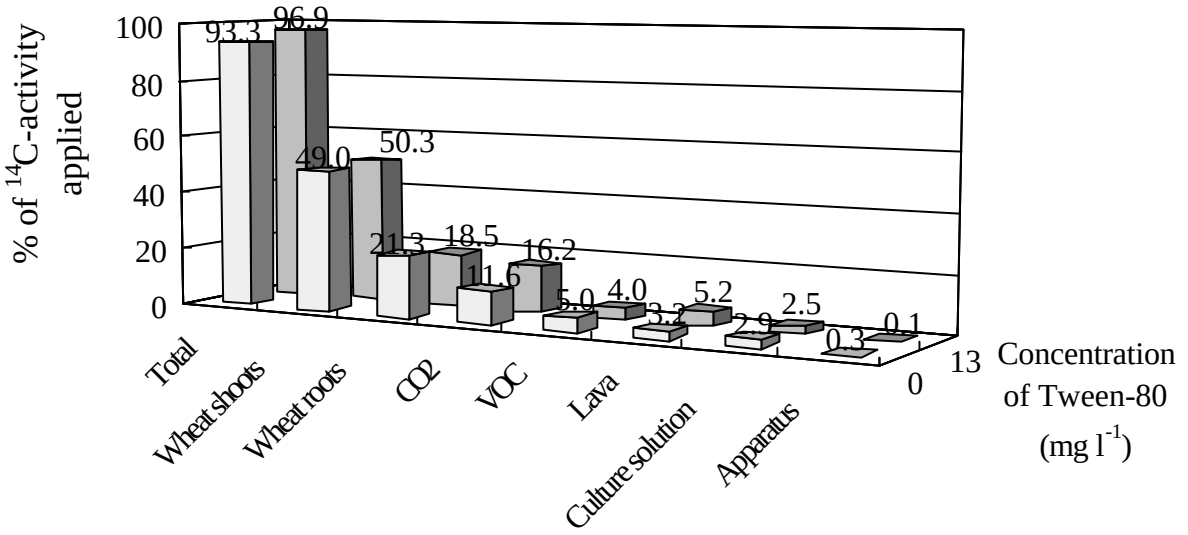


Fig. 119. Mass balance and influence of Tween-80 on the distribution of ^{14}C-activity in the test system at the end of experiment. Data points are means of 5 replicates.

4.3.3.2 Influence of Tween-80 on the kinetics of volatilization and degradation of ^{14}C-phenanthrene

Fig. 120 presents the kinetics of the emission of VOCs and CO_2 and the decrease of ^{14}C-activity in culture solution during the exposure period in wheat-solution-lava microcosm. The production of VOCs increased rapidly in 3 days and reached its highest in day 6. Then, the volatilization of VOCs decreased dramatically and came to a stable level of about 0.05 to 0.1% of the applied ^{14}C-activity after 10 days. The emission of CO_2 showed similar trend though its highest emission occurred in day 8

and remained till day 12. The cumulative production of CO_2-associated ^{14}C-activity was lower than that of VOCs in the first 9 days but higher afterwards. The emission of CO_2 emerged in day 5 and then rapidly increased to its highest in day 8. There was a plateau in the $^{14}CO_2$-release curve, indicating the highest degradation of ^{14}C-phenanthrene lasted for about 5 days. The production of $^{14}CO_2$ declined afterwards and came to a stable level of 0.4 to 0.5% of the applied ^{14}C-activity in day 17 till the end of measurement in 23 days.

^{14}C-activity in culture solution decreased dramatically at an exponential rate to about 20% of the applied ^{14}C-activity within the first 2 hours. During that period, the

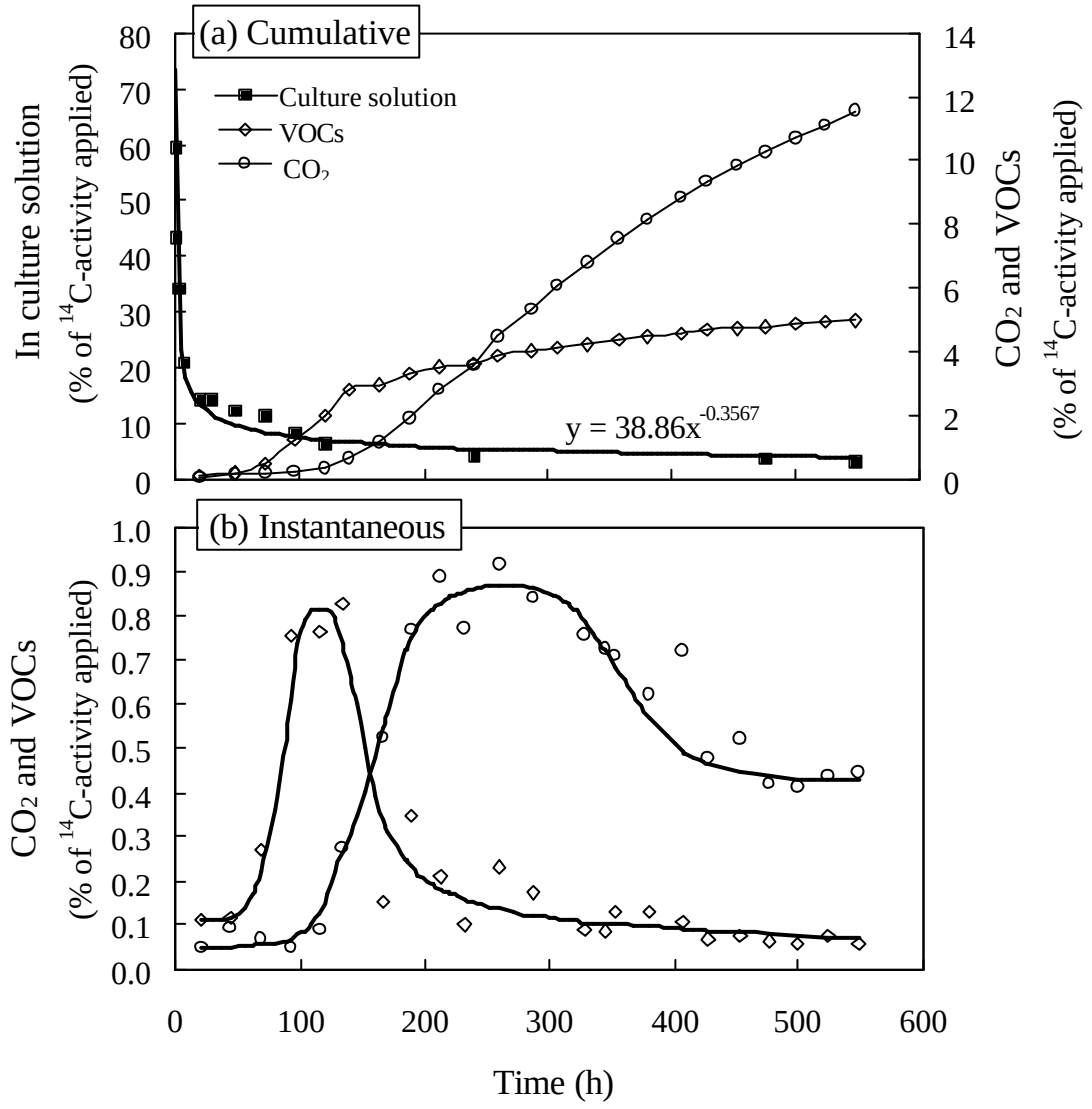


Fig. 120. Kinetics of volatilization and degradation of ^{14}C-phenanthrene in wheat-solution-lava microcosm. Data points are means of 5 replicates.

production of both VOCs and CO_2 was very low, which suggested that the decline of ^{14}C-activity from culture solution was mainly due to the adsorption of phenanthrene onto wheat roots, culture bottle and lava. The ^{14}C-activity level in the culture solution remained relative constant after 4 days, indicating a dynamic equilibrium was established among the adsorption components. As the increase of ^{14}C-activity loses due to the emission of VOCs and CO_2 and the uptake by wheat in the following days, ^{14}C-activity adsorbed was constantly released to the culture solution. This was also supported by the fact that only 3 to 5% of ^{14}C-activity applied were found in lava at the end of the experiment (Fig. 119). The kinetics of ^{14}C-activity decrease in the culture solution can be described by an equation of $y = 38.86x^{-0.3576}$ with a "half life" ($t_{1/2}$) of 0.49 h.

There was no observable difference in the kinetics of the production of VOCs and CO_2 between the presence and absence of Tween-80. However, the presence of Tween-80 did significantly increased the production of CO_2, i.e. enhanced the degradation of phenanthrene in a wheat-solution-lava microcosm (Fig. 121). As presented by the curves, in the presence of Tween-80, the amount of $^{14}CO_2$ production was significantly higher than that without the surfactant after 5 days of culture, and the difference became biggest at the maximal $^{14}CO_2$ production. The accumulated amount of $^{14}CO_2$ release in the presence of Tween-80 was 40% higher than that in absence of the surfactant. The enhancement of phenanthrene degradation by Tween-80 could be attributed to its increase of microbial activities in the system as Tween-80 had been found to be able to increase microbial activities in many systems, e.g. mono-culture of bacteria (Section 4.2.2) and inherent soil microorganisms in uncontaminated soils (Section 4.2.4.1) and in contaminated soil (Section 4.2.4.4).

4.3.3.3 Distribution of ^{14}C-phenanthrene metabolites among wheat tissues and culture media

The distribution of ^{14}C-activity among system components is illustrated in Fig. 119. The highest ^{14}C-activity was found in wheat shoots (stems and leaves) (half of the applied ^{14}C-activity), followed by wheat roots (*ca* 20%), CO_2 (*ca* 14%), VOCs (*ca*

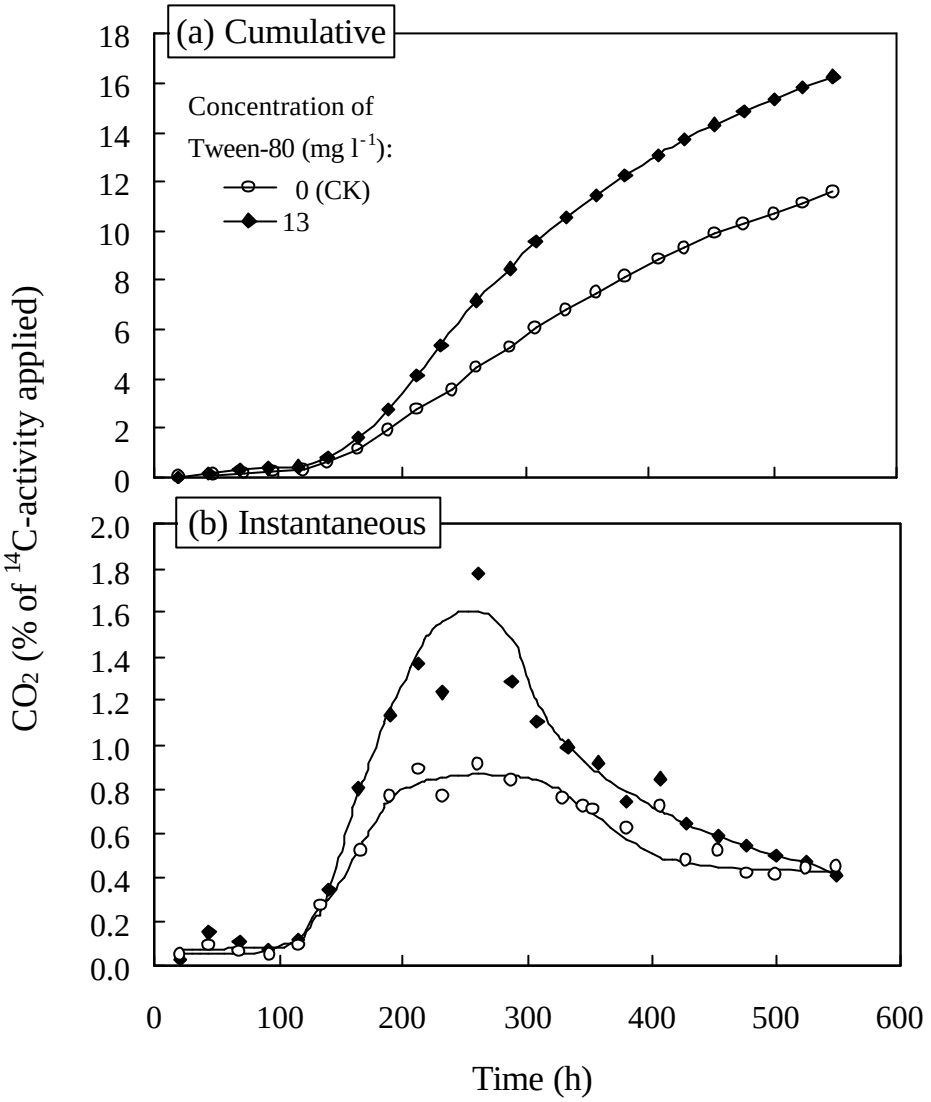


Fig. 121. Influence of Tween-80 on the degradation kinetics of ^{14}C-phenanthrene in wheat-solution-lava microcosm. Data points are means of 5 replicates.

5%), lava (*ca* 4%), culture solution (*ca* 3%), and apparatus (<0.3%). A total of 70% of the applied ^{14}C-activity was absorbed by wheat.

Most of applied ^{14}C-activity (56%) had been constructed into plant tissues or bonded to organic or mineral components of lava, which could not be extracted with organic solvents i.e. methanol and dichloromethane, respectively (Fig. 122). In the un-extractable part, 60% (34% of the applied ^{14}C-activity) and 35% (20% of the applied ^{14}C-activity) were associated with wheat shoots and roots, respectively; only 5% (3% of the applied ^{14}C-activity) were found in lava. Polar metabolites of ^{14}C-phenanthrene

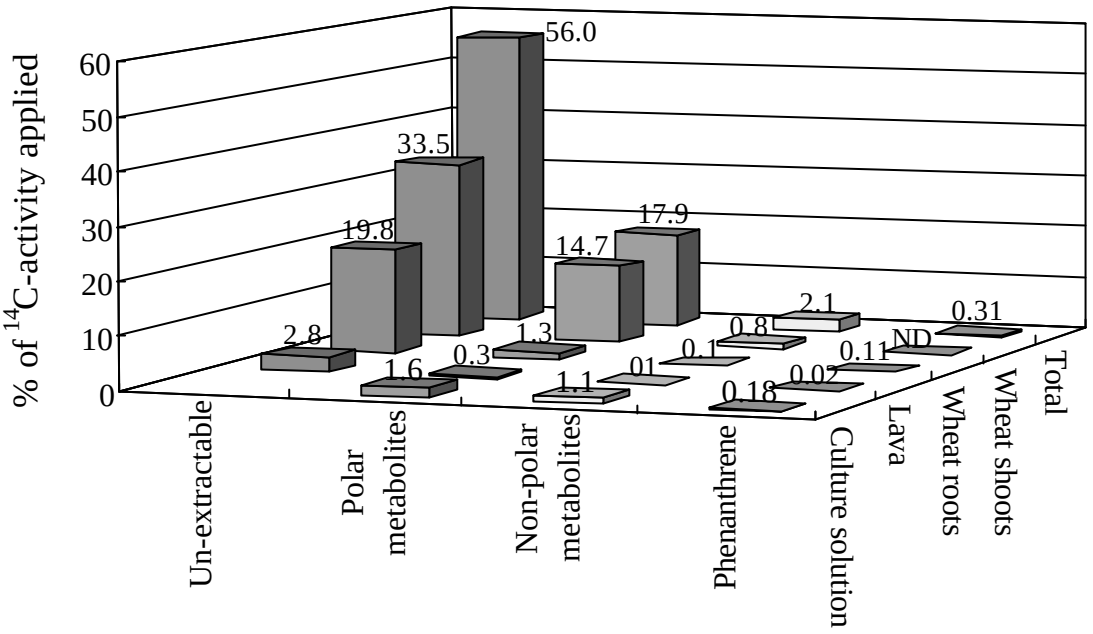


Fig. 122. Distribution of ^{14}C-activity in wheat tissues and culture media expressed as % of the applied ^{14}C-activity. Data points are means of 5 replicates. ND = not detectable.

accounted for 18% of the applied ^{14}C-activity, in which 82% (15% of the applied ^{14}C-activity) were found in wheat shoots, 9% (1.6% of the applied ^{14}C-activity) in culture solution, 7% (1.3% of the applied ^{14}C-activity) in wheat roots and 2% (0.3% of the applied ^{14}C-activity) in lava. Very small portions were identified as non-polar metabolites (2.1% of the applied ^{14}C-activity) and parent phenanthrene (0.3% of the applied ^{14}C-activity).

Further analysis shows that the un-extractable ^{14}C-activity accounted for 68%, 93% and 88% of ^{14}C-activity in corresponding components for wheat shoots, wheat roots and lava, respectively (Fig. 123(a)). Higher proportion of the absorbed ^{14}C-activity was constructed into plant cells in wheat roots than in shoots. The second abundant ^{14}C-activity was found to be associated with polar metabolites in both wheat tissues and culture media. 95%, 85% and 76% of the extractable ^{14}C-activity in wheat shoots, wheat roots and lava (Fig. 123(b)), and 56% of ^{14}C-activity in culture solution (Fig. 123(a)) were polar metabolites derived from ^{14}C-phenanthrene, respectively. It has

been reported that the metabolic products of phenanthrene are mainly polar compounds such as naphthoic acid, phthalic acid, protocatechuic acid, dihydrodial, carboxybenzaldehyde and hydroxyphenanthrene (Cerniglia, 1985; Rehmann *et al*., 1996; Papaderos *et al*. 1998). The rest of ^{14}C-activity was found in associated with non-polar metabolites and parent phenanthrene. 5%, 8% and 19% of the extractable ^{14}C-activity in wheat shoots, wheat roots and lava, and 38% ^{14}C-activity in culture solution were identified as non-polar metabolites derived from ^{14}C-phenanthrene, respectively. 7% and 6% of the extractable ^{14}C-activity in wheat roots and lava, and 6% of ^{14}C-activity in culture solution were parent phenanthrene. No phenanthrene was detected in the extracts of wheat shoots, indicating that only metabolites of phenanthrene were transported to the wheat stems and leaves.

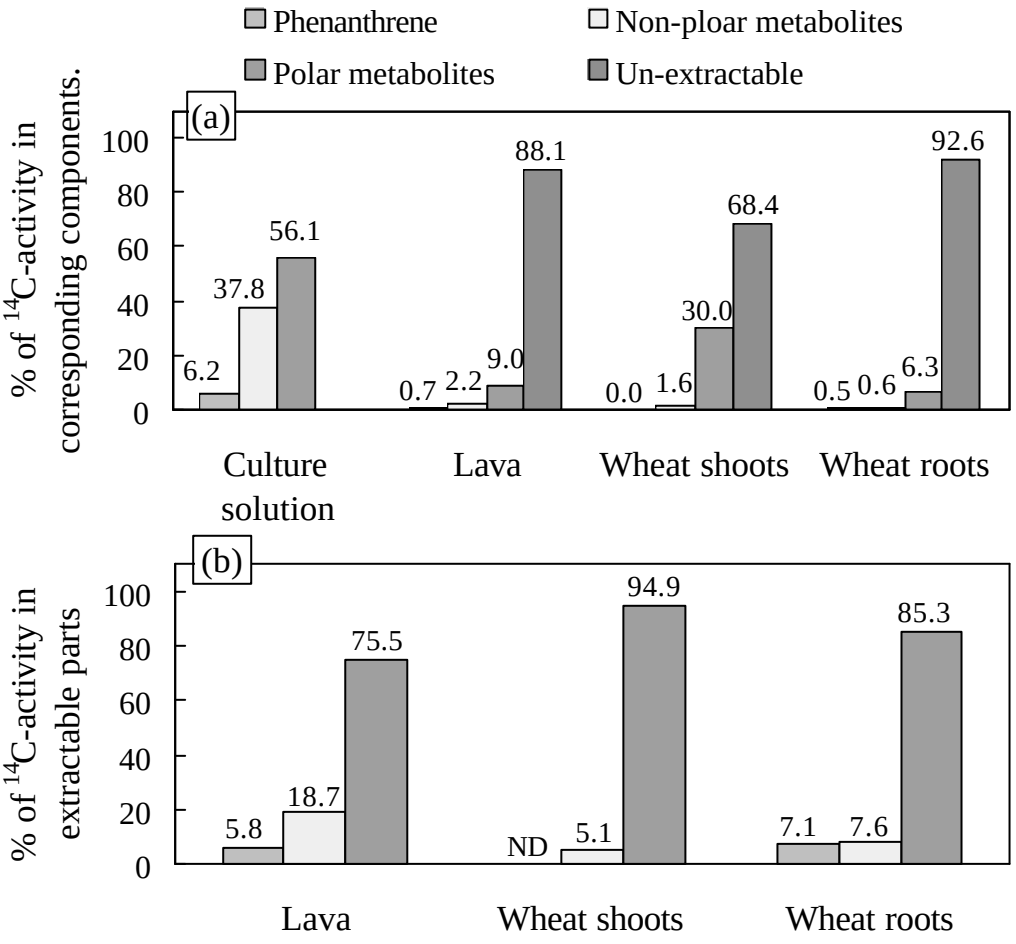


Fig. 123. Distribution of ^{14}C-activity in wheat tissues and culture media expressed as % of ^{14}C-activity in corresponding components. Data points are means of 5 replicates. ND = not detectable.

Fig. 124 shows the concentrations of ^{14}C-activity of each component in wheat tissues and culture media. Except phenanthrene, concentrations of other components (bonded ^{14}C, polar and non-polar metabolites, and total ^{14}C) were all in a descent order of wheat shoots > wheat root > culture solution > lava, indicating more ^{14}C-activity was accumulated in wheat than in culture media. This was proved by their extremely high bioaccumulation factors of ^{14}C-activity (597 and 396 for wheat shoots and roots, respectively), i.e. ratios of ^{14}C-activity concentration in plant tissue over that in culture solution. The higher bioaccumulation of ^{14}C-activity in wheat shoots than in wheat roots indicated that foliar uptake of ^{14}C-activity via air in form of $^{14}CO_2$ occurred, which came from the degradation of ^{14}C-phenanthrene in culture media. Higher concentration of ^{14}C-activity in roots than in shoots of bush bean has been reported in a close root chamber experiment with ^{14}C-anthrathene in which foliar uptake of $^{14}CO_2$ was avoided (Edwards, 1986).

4.3.3.4 Influence of Tween-80 on the distribution of ^{14}C-activity among wheat tissues and culture media

Except higher emission of $^{14}CO_2$ in the presence of Tween-80, no significant

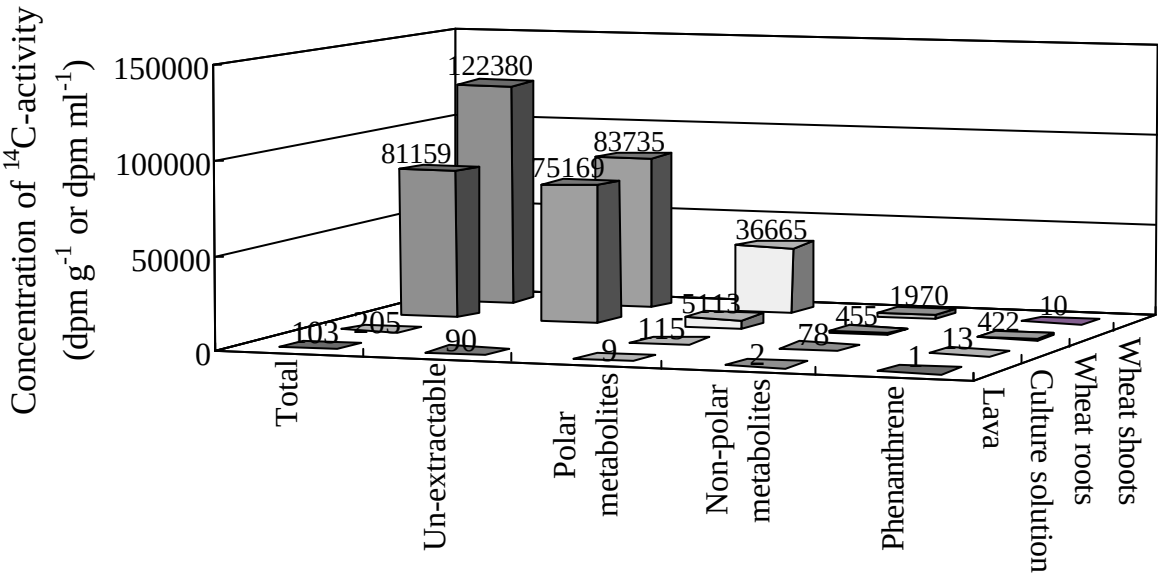


Fig. 124. Concentration of ^{14}C-activity in wheat tissues and culture media. Data points are means of 5 replicates.

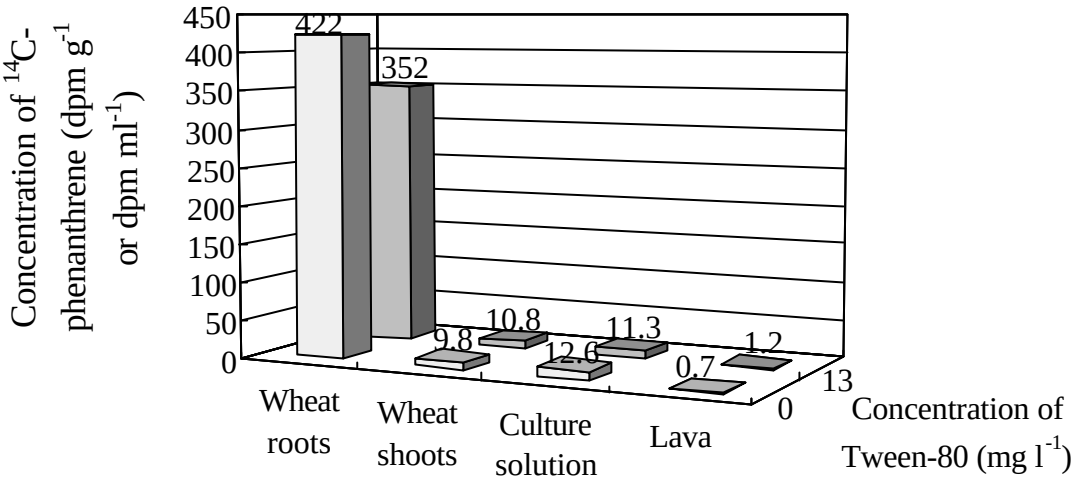


Fig. 125. Influence of Tween-80 on the uptake of ^{14}C-phenanthrene by wheat and the distribution of ^{14}C-phenanthrene in different components. Data are means of 5 replicates.

differences could be observed in the distribution of ^{14}C-activity in all other components in the system (Fig. 119). However, when ^{14}C-phenanthrene concentrations in wheat tissues and culture media were examined, it can be obviously seen that the surfactant significantly ($P < 0.05$) reduced the phenanthrene level in wheat roots compared with that in the absence of Tween-80 (Fig. 125). The reduction might result from desorption of phenanthrene from root surface into the culture solution caused by the surfactant, which accordingly led to less uptake of phenanthrene by wheat roots. This might also imply that the micellar phenanthrene was less available to the plant or the surfactant inhibited the absorption of phenanthrene by the plant. As mainly metabolites of phenanthrene was absorbed and transported to the upper parts of the plant and foliar uptake of $^{14}CO_2$ occurred, Tween-80 did not cause observable differences in both total amount of ^{14}C-activity and ^{14}C-phenanthrene concentrations in wheat shoots compared with that without the surfactant.

4.3.4 Influence of surfactants on the utilization and growth of *Mycobacterium* sp. on solid phenanthrene in the interface of phenanthrene and water

The importance of adherence in growth physiology of many bacterial species is well

documented. The influence of bacterial adhesion to surface of water-insoluble HOCs, existing either as solid or NAPL, on their utilization and degradation of the chemicals has been the subject of recent concerns (Tongpim and Pickard, 1996; Stelmack *et al*., 1999). In cases where the carbon and energy source is a water-insoluble material such as cellulose or chitin, cell adhesion can facilitate growth though cell contact is not an absolute requirement because extracellular enzymes can degrade these polymers into water-soluble substrates. However, the growth of microorganisms on hydrocarbons may be a problem, since not only are hydrocarbons poorly soluble in water, but also their breakdown cannot occur extracellularly. The first step in aromatic or aliphatic hydrocarbon degradation is the introduction of molecular oxygen into the molecules by cell-associated enzymes (Foster, 1962).

There are three possible modes of uptake of poorly water-soluble hydrocarbons by microbial cells:

- Homogenous mode that uptake of hydrocarbons by microorganisms from aqueous phase as dissolved state. There is no doubt that microorganisms can grow on hydrocarbons via this mode as revealed by all related researches (e.g. Chakravarty *et al*, 1972; Wodzinski and Bertolini, 1972; Reddy *et al*., 1982). The degradation rates of hydrocarbons utilized in this mode are restricted due to the extremely low rates of solubilization of these substrates.
- Pseudosolubilization mode that the uptake of hydrocarbons by microorganisms from aqueous phase as emulsified state. Microorganisms may produce metabolites such as biosurfactants which help to dissolve liquid or solid hydrocarbons by emulsion.
- Interfacial adsorption mode that the uptake of hydrocarbons by microorganisms by direct contact and transfer. For examples, the uptake through direct contact of cells with hydrocarbon drops larger than the cells have been reported (Velankar *et al*., 1975; Wodzinski and Larocca, 1977; Rosenberg and Rosenberg, 1981). The adherence of microorganisms to hydrocarbons may vary widely among organisms, culture age and growth conditions (Rosenberg and Rosenberg, 1985). The possibility of growth of microorganisms through direct contact with solid

hydrocarbon is remote because of inability of the solid substrates to penetrate the cell membrane. But some studies have found that microorganisms can grow on solid hydrocarbons by direct contact (e.g. Goswami *et al*., 1983; Miller and Bartha, 1989; Tongpim and Pickard, 1996) though others reported not (e.g. Chakravarty *et al*, 1972; Wodzinski and Coyle, 1974).

The presence of surfactants can alter the characteristics of both the surface of solid hydrocarbons and the microbial cell wall and membrane and thus their interaction. On the other hand, surfactants can also increase the dispersion (Zhang and Miller, 1992) and the solubilization (Helenius and Simons, 1975; Ou *et al*., 1995b; Volkering *et al*., 1998) of hydrocarbons by emulsion. Therefore, it can be expected that surfactants will influence the adherence of bacteria to hydrocarbons, thus the degradation of hydrocarbons and the growth of microorganisms. These influences of surfactants are not yet clear. There are reports that surfactants inhibit the adherence of bacteria to hydrocarbons (Rosenberg *et al*., 1983; Zhang and Miller, 1994; Stelmack *et al*., 1999).

The influence of surfactants on the adhesion and growth of *Mycobacterium* sp. on solid phenanthrene was measured by protein assay for protein contents on the surface of solid phenanthrene and in culture solution. The amount of protein represents the amount of bacterial biomass, as the former is proportion to the later. The amounts of bacteria growing on the surface of solid phenanthrene generally decreased with the increasing concentrations of LAS, TDTMA, Brij30 and 10LE (Fig. 126 to Fig. 129). It indicated that these surfactants inhibited the adherence and growth of the bacteria on solid phenanthrene. Stelmack *et al*. (1999) have observed the same effects that surfactants (Triton-X-100 and Dowfac8390) inhibit the adhesion of a *Mycobacterium* strain to NAPLs and the growth of the bacteria on solid anthracene. At the presence of LAS, TDTMA or Brij30, the reduction of the bacteria growth on solid phenanthrene was accompanied by the decrease of the bacteria growth in culture solution, indicating they inhibited the growth of the bacteria in the whole culture. The inhibition caused by LAS and TDTMA may be explained by their toxicity to the bacteria as shown by respiration data (Fig. 69(a) and Fig. 70). At the presence of phenanthrene, however,

LAS at low concentrations (<10 mg l^{1}) did not show toxic to the bacteria as it increased their respiration (Fig. 69(b)). LAS exhibited toxicity in this experiment may be due to the low concentration of phenanthrene as a result of its application in solid. In respiration experiments, phenanthrene was applied using a re-crystallization method which has been reported to be able to result in higher bioavailability of the chemical (To ngpim and Pickard, 1996; Bouchez *et al*., 1997). As Brij30 was not toxic

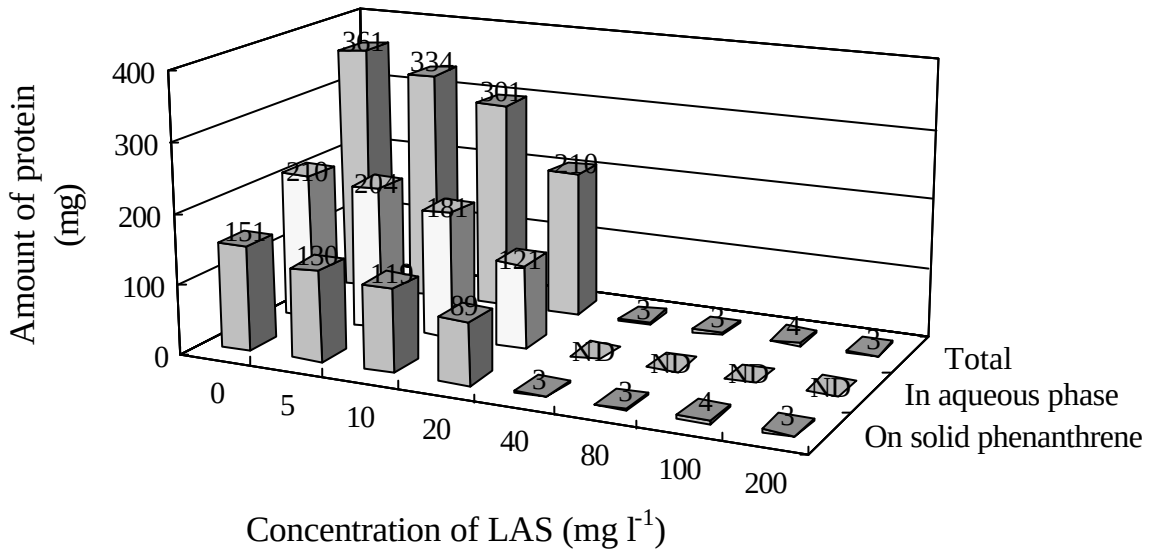


Fig. 126. Influence of LAS on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

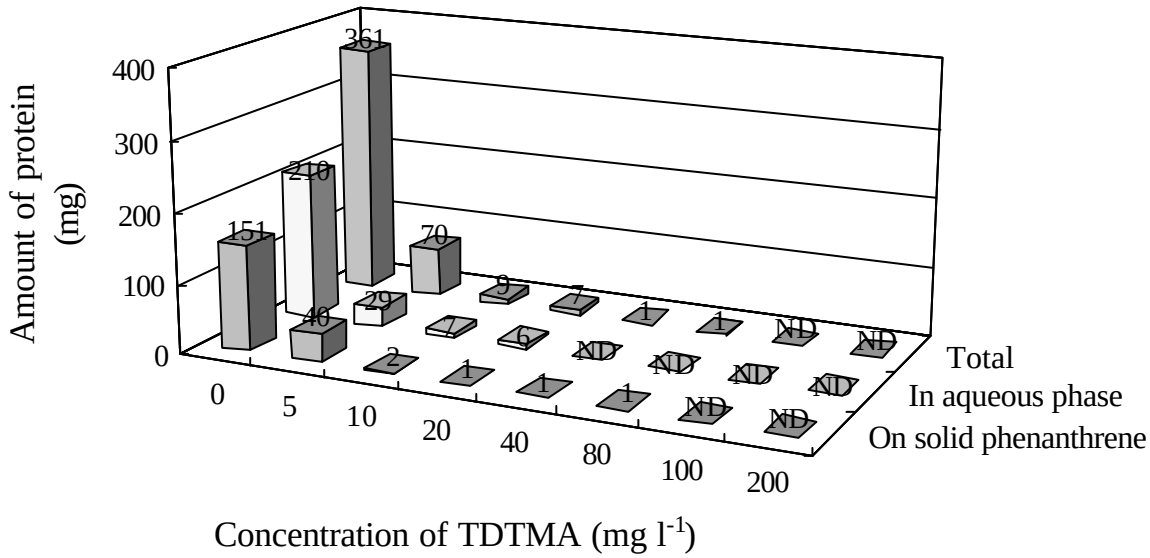


Fig. 127. Influence of TDTMA on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

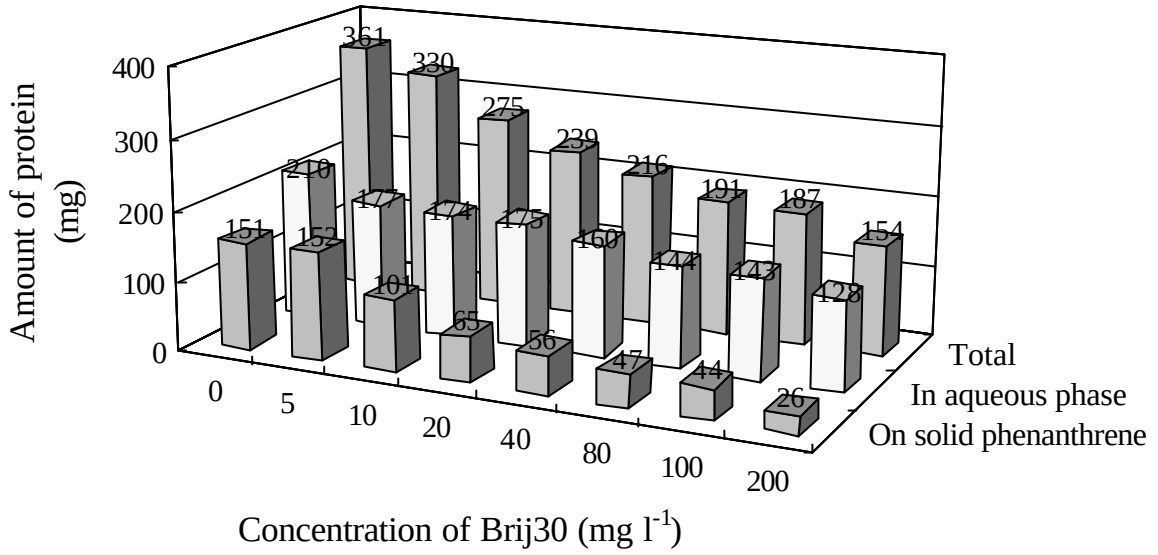


Fig. 128. Influence of Brij30 on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

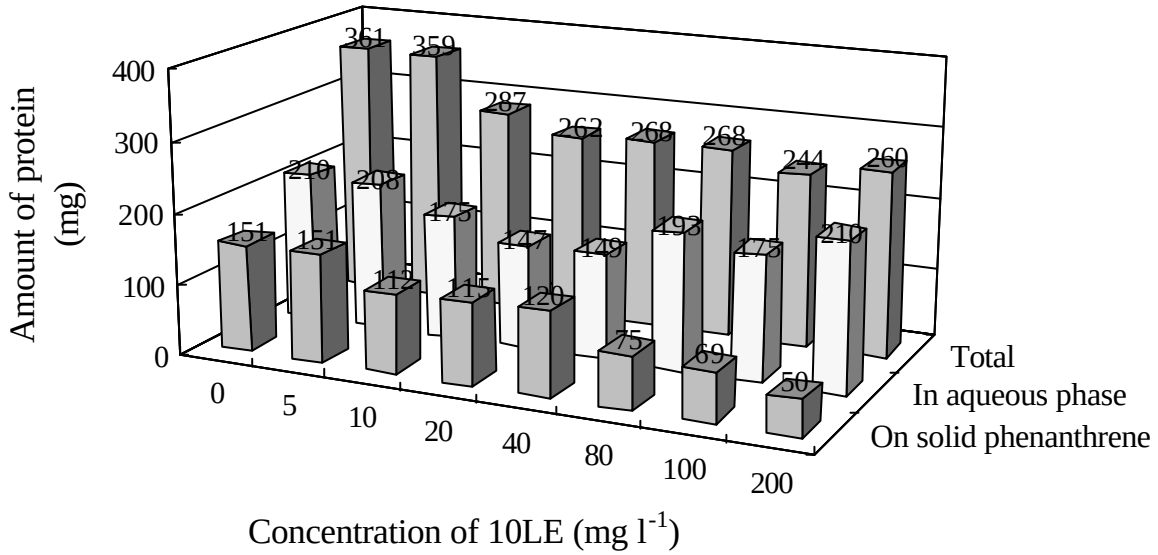


Fig. 129. Influence of 10LE on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

to the bacteria both in the presence and absence of phenanthrene (Fig. 72), its inhibition on the growth of the bacteria in the presence of solid phenanthrene might be due to a reduction in the uptake of the solid carbon source (Stelmack *et al*., 1999).

Tween-80 at low concentrations (<20 mg l^{-1}) enhanced the adhesion and growth of the

bacteria on solid phenanthrene and the maximal growth of the bacteria was at Tween-80 concentration of 1/2CMC (20 mg l^{-1}) (Fig. 130). The amount of the bacteria growing on solid phenanthrene increased as the concentration of Brij35 increased from 5 to 80 mg l^{-1} with a maximal growth also at Brij35 concentration of 1/2CMC (20 mg l^{-1}) (Fig. 131). At higher concentrations, both surfactants inhibited the adhesion and growth of the bacteria on solid phenanthrene. The enhancement of the

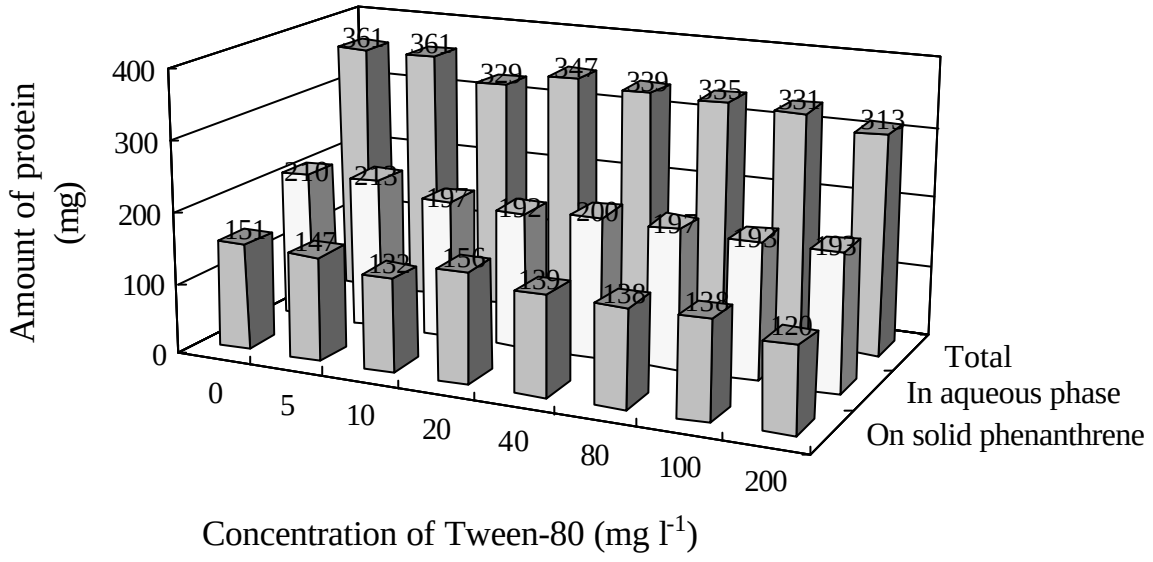


Fig. 130. Influence of Tween-80 on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

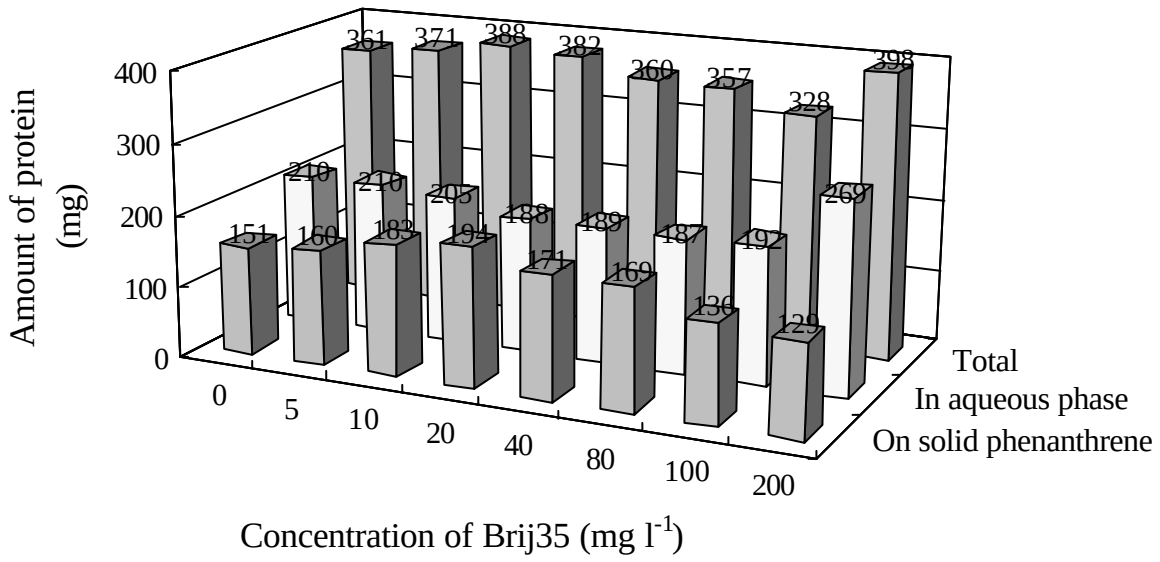


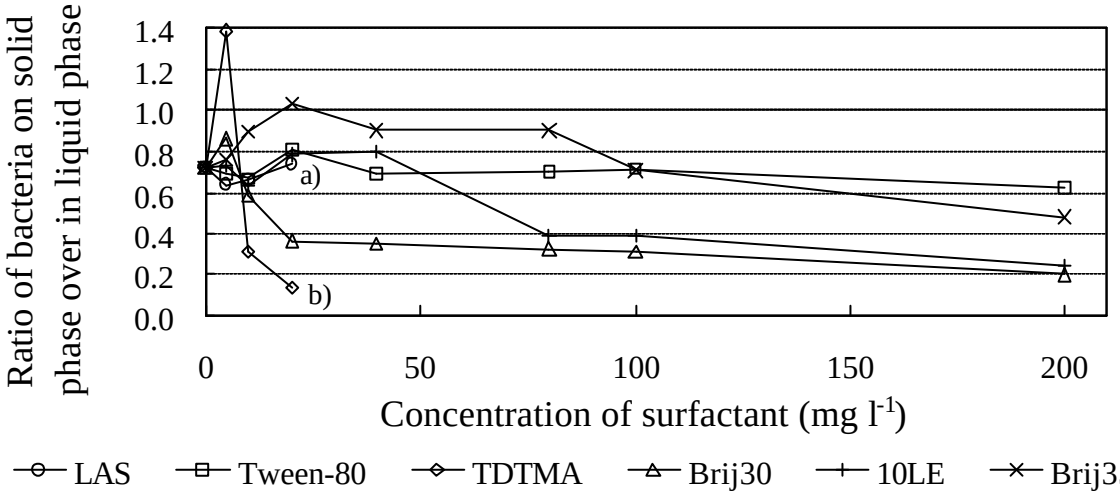
Fig. 131. Influence of Brij35 on the growth of *Mycobacterium* sp. on phenanthrene. Data points are means of duplicates.

two surfactants on the adhesion and growth of the bacteria on solid phenanthrene was probably a result of their modification of the cell surface to increase its affinity to the hydrophobic phenanthrene (Stucki and Alexander, 1987) and thus increased the direct interfacial transfer of substrate to microorganisms (Bouchez *et al*., 1997).

Another possible mechanism reducing the growth of bacteria on the surface of solid phenanthrene was the interference of surfactants on the interaction between the microbial cells and phenanthrene, which prevented their contact (Zhang and Miller, 1994). Surfactants can alter adhesion by adsorbing to the cell surface, to the hydrocarbon surface, or to both (Neu, 1996). The hydrophobic tails of the surfactant molecules can adsorb to hydrophobic surfaces of solid phenanthrene while the hydrophilic heads remain in the aqueous phase, i.e. formation of hemimicelles (or surfactant-hydrocarbon complex). This should result in the surface of solid phenanthrene more hydrophilic. Adsorption of surfactant to hydrophobic domains on the cell surface should give the same results, i.e. make the cell surface more hydrophilic (Zhang and Miller, 1994). As the contact of bacterial cells and hydrocarbons is regulated by hydrophobic interaction (Dahlbäck *et al*., 1981; Rosenberg *et al*., 1983), either surface modification should decrease the hydrophobic interactions between the cells and the solid phenanthrene and reduce adhesion (Stelmack *et al*., 1999). Zhang and Miller (1994) have proposed the inhibition of surfactant on the adherence of bacterial cells to hydrocarbons is due to the electrostatic repulsion between the negatively changed cells and the similarly changed surfactant-hydrocarbon complexes. But this hypothesis is not applicable to the cases in the presence of non-ionic surfactants in this study. Dispersion of surfactant-modified cells is probably the main reasons causing the reduction of bacterial adhesion to hydrocarbons.

Detection of bacterial protein on the surface of solid phenanthrene and in culture solution indicated that *Mycobacterium* sp. strain KR2 could utilize the hydrophobic compound through direct contact of microbial cells and solid hydrocarbon and through uptake of solubilized hydrocarbon. Tongpim and Pickard (1996) have also

observed the growth of a *Mycobacterium* sp. (previously described as a *Rhodococcus* sp. (Stelmack *et al*., 1999)) as a biofilm on solid anthracene. As shown in Fig. 132, however, the amount of bacteria growing on solid phenanthrene was less than that in culture solution in the presence and absence of all surfactant at all concentrations except at 5 mg l^{-1} TDTMA and 20 mg l^{-1} Brij35. It indicated that the bacteria grew preferentially on dissolved phenanthrene to solid phenanthrene. As the increase of surfactant concentration, besides inhibition of bacteria adherence to solid phenanthrene by surfactants, more phenanthrene was dissolved by emulsification and relatively more microbial growth in culture solution compared with that on solid surface. This resulted in the decrease of the ratios of bacteria growth on solid phase over those in liquid phase with the increase of surfactant concentrations.



a) Bacteria in liquid phase were not detectable by protein assay beyond this LAS concentration.
b) Bacteria in both phases were not detectable by protein assay beyond this TDTMA concentration.

Fig. 132. Influence of surfactants on the ratios of growth of *Mycobacterium* sp. on solid phenanthrene phase and in liquid phase of culture solution

4.3.5 Sub-summary

Surfactants did not increase the bioavailability and the biodegradation of phenanthrene in liquid mono-culture of *Mycobacterium* sp. where both chemicals were in dissolved state ready to be utilized by the bacteria. In such a system, there was

enough solubilized phenanthrene and surfactants available to the bacteria. Therefore, the role of surfactant in solubilization of HOCs and thus increasing their bioavailability was not significant. On the contrary, the presence of surfactants inhibited the biodegradation of phenanthrene. It was either because the surfactants (e.g. LAS at high concentrations and TDTMA) were toxic to the bacteria or due to they (e.g. LAS at low concentration and Tween-80) were utilized by the bacteria as preferential substrates if the surfactants were not toxic to the bacteria and could be utilized as sole- or co-substrates. In addition, other mechanisms may be also involved such as effects of surfactants on the function of cell membrane, and the uptake and metabolic path of the chemical by the bacteria. Results in this study indicated that the inhibition of biodegradation of phenanthrene by the surfactants was in a descent order of TDTMA > LAS > Tween-80. The influence of Brij surfactants on the biodegradation was not significant under the same conditions.

In contrast to their influences on liquid mono-culture of *Mycobacterium* sp., LAS and TDTMA at low concentrations and Tween-80 at all concentrations enhanced the biodegradation of phenanthrene in soils. At higher concentrations, both LAS and TDTMA inhibited the biodegradation of phenanthrene. But their influences, i.e. positive or negative effects on the biodegradation, also depended on soils. A surfactant enhancing the biodegradation in one soil may exhibit inhibition in other soils. For example, LAS at the same concentrations inhibited the biodegradation of phenanthrene in Soil $14^{\#}$ and Soil $15^{\#}$ while enhanced in Soil 2.3. In addition, their concentrations at which the biodegradation of phenanthrene was enhanced or inhibited, i.e. the critical concentrations, were soil specific and varied significantly among soils. This was reflected in the variation of their EC values in different soils. The relationship between enhancement of phenanthrene biodegradation and increase of microbial activities was also remarkably different among soils and among surfactants in the same soil. In some soils (e.g. Reference soil 2.3), LAS's enhancement of degradation was accompanied by the increase of soil microbial activities, while in other soils (e.g. Reference soil 2.2), the opposite was true. In the same soil (e.g. Reference soil 2.2), LAS enhanced the biodegradation of phenanthrene while

decreasing the soil microbial activities. On the contrary, Tween-80 and TDTMA increased both biodegradation of the chemical and microbial activities of the soil simultaneously. Therefore, the increase of microbial activities dose not necessarily mean enhancing the biodegradation of HOCs. The increase of bioavailability of HOCs is more directly relevant to the enhancement of biodegradation of the chemicals.

The enhancement of biodegradation of phenanthrene by LAS was mainly due to the increase of bioavailability of phenanthrene by the surfactant. This result proved that LAS's increase of the soil respiration was resulted from its increase of the bioavailability of phenanthrene. Tween-80 and TDTMA enhanced the biodegradation of phenanthrene by increasing both bioavailability of the chemical and microbial activities of the soil. The possible mechanisms for the increased bioavailability of phenanthrene by the surfactants may include increase of phenanthrene desorption from soil particles due to competition of adsorption site on soil surface, overcoming transport limitation from soil particles to aqueous phase, and increase of phenanthrene solubility due to hydrophobic interactions between surfactant monomers and phenanthrene when surfactant below CMC and due to surfactant encapsulation of phenanthrene into micelles when surfactant above CMC. The inhibition of phenanthrene biodegradation by LAS and TDTMA at high concentrations was caused by their toxicity to the soil microorganisms.

Results showed that the efficiency of surfactant's enhancement on biodegradation of phenanthrene depended on the intrinsic phenanthrene-degrading capacities of the soils as well as the inherent microbial activities of the soils. The surfactant's enhancement efficiency would be low or even there would be no enhancement when the soil already had high degrading capacities. This resulted in quite different EC values of the same surfactant in different soils.

The biodegradation of pyrene was much (8 times) lower than that of phenanthrene in the same soil (Soil 2.3), indicating that pyrene was more persistent in soil due to its lower water solubility and higher affinity to soil particles than phenanthrene. In

Reference soil 2.3, the influences of LAS and Tween-80 on the biodegradation of pyrene were similar to their effects on phenanthrene. LAS at low concentration and Tween-80 increased the biodegradation of pyrene. LAS at high concentrations and TDTMA decreased the biodegradation of the chemical. The efficiency of surfactant's enhancement of pyrene biodegradation was much lower than that of phenanthrene, indicating that the surfactant's efficiency was also dependent on the characteristics of the chemicals.

Mycobacterium sp. strain KR2 could grow on solid phenanthrene through direct contact with the solid hydrocarbon though preferably to utilize solubilized phenanthrene. The influence of surfactants on the adherence and growth of the bacteria on solid phenanthrene depended on the characteristics of surfactants and their concentrations. The adhesion and growth of the bacteria on solid phenanthrene were inhibited by LAS, TDTMA, Brij30 and 10LE, while enhanced by Tween-80 (<20 mg l^{-1}) and Brij35 (5 to 80 mg l^{-1}). However, at higher concentrations, both Tween-80 and Brij35 exhibited inhibition effects. Both surfactants had a maximal enhancement at concentration of 1/2CMC (20 mg l^{1}). The inhibition caused by LAS and TDTMA may be explained by their toxicity to the bacteria. For Brij30 which was not toxic to the bacteria, its inhibition might be due to a reduction in the uptake of the solid carbon source. Another possible mechanism for the inhibition was the interference of surfactants on the interaction between the microbial cells and phenanthrene, which prevented their contact. Surfactants can alter adhesion by adsorbing to the cell surface, to the hydrocarbon surface, or to both. Either surface modification would decrease the hydrophobic interactions between the cells and the solid phenanthrene and reduce adhesion. Dispersion of surfactant-modified cells was probably another mechanism causing the reduction of bacterial adhesion to solid phenanthrene. The enhancement of the two surfactants on the adhesion and growth of the bacteria on solid phenanthrene was probably a result of their modification of the cell surface to increase its affinity to the hydrophobic phenanthrene and thus increased the direct interfacial transfer of substrate to microorganisms.

The mineralization and metabolism of phenanthrene were quick in wheat-solution-lava microcosm. At least 90% of the applied phenanthrene were transformed within 24 days to CO_2, VOCs, plant-building materials, and polar and non-polar metabolites in wheat and culture media. Only 0.3% of the applied ^{14}C-activity were identified to be the parent phenanthrene and no phenanthrene was found in wheat shoots. Most of the applied ^{14}C-activity (70%) was recovered from wheat, in which *ca* 70% were associated with wheat shoots (stems and leaves) and *ca* 30% in wheat root. The ^{14}C-activity recovered in forms of CO_2 and VOCs and in lava and culture solution was 12 to 16%, 4 to 5%, 3 to 5%, and 2%, respectively. Most of applied ^{14}C-activity (56%) had been constructed into wheat shoots (33%), wheat roots (20%) or bonded to lava (3%). The major metabolites of phenanthrene were polar compounds (18% of the applied ^{14}C-activity), which accounted for 95% of extractable ^{14}C-activity found in wheat shoots, 85% in wheat roots, 76% in lava, and 56% in culture solution. Very small portions were identified as non-polar metabolites (2.1% of the applied ^{14}C-activity). The higher bioaccumulation of ^{14}C-activity in wheat shoots than in wheat roots indicated that foliar uptake of ^{14}C-activity via air in form of $^{14}CO_2$ occurred.

The presence of Tween-80 significantly increased the production of CO_2, i.e. enhanced the degradation of phenanthrene in wheat-solution-lava microcosm. The enhancement of phenanthrene degradation by Tween-80 could be attributed to its increase of microbial activities in the system. Tween-80 also significantly ($P < 0.05$) reduced the phenanthrene level in wheat roots compared with that in the absence of the surfactant. The reduction might be resulted from desorption of phenanthrene from root surface into the culture solution caused by the surfactant. This might also imply that the micellar phenanthrene was less available to the plant or the surfactant inhibited the uptake of phenanthrene by the plant.

5 SUMMARY

Surfactants were found in this study to have significant influences on the environmental behaviour of PAHs. Their influences depended on the characteristics of both surfactants and PAHs, type of organisms and environmental matrices. They could vary from synergism to antagonism among surfactants and organisms. In addition, the effect of surfactants was also a function of their concentration and could shift from positive to negative and *vice versa* as their concentration changed. Findings of this study are summarized in the following:

Adsorption of LAS on soils could be divided into two stages: linear and exponential adsorption. Its adsorption mechanism was found to be mainly specific site surface interactions rather than hydrophobic interactions as reported in the literature. The adsorption capacity depended on soil physical clay content. Adsorption of phenanthrene on soil was partitioning mechanism and significantly influenced by LAS. LAS at high concentrations decreased the adsorption. At low concentrations, it could result in either an increase or decrease in phenanthrene adsorption and the key factor was the contacting sequence of LAS and phenanthrene with soil.

The velocity of LAS migration in soil was dependent on water flow flux and independent of its concentration in soil or leaching solution. The later determined its content in leachate. The migration of selected PAHs in soil was slow and followed the order of phenanthrene > pyrene > benzo[a]pyrene. The presence of LAS or Tween-80 could enhance their migration. The concentration of surfactant was an important factor. Great differences existed between the migration of LAS and PAHs in repacked soil columns and natural soil or repacked soil columns been subjected to freeze-thaw treatment. Preferential migration of all test chemicals was observed in the later but not in the former. Consequently, caution should be taken when predicting migration of both hydrophilic and hydrophobic chemicals in field soil from laboratory data.

Phenanthrene and all test surfactants were toxic to *Vibrio fischeri* in a descendent order of TDTMA > phenanthrene > Brij30 > 10LE > LAS > Brij35 > Tween-80. The

impact of nonionic surfactants and phenanthrene was reversible. It was interesting to find that the toxicity of Brij surfactants was higher than LAS and decreased with the increase of ethoxylate group in their molecules. The combination of phenanthrene and surfactants was more toxic to the bacteria than individual compounds in an order of TDTMA > Brij30 > LAS > Brij35 > 10LE > Tween-80. The combined effects of phenanthrene with the later four surfactants were underadditive or antagonistic while overadditive or synergistic with TDTMA and Brij30. The significance of their combined effects from synergism to antagonism was Brij30 > TDTMA > Brij35 > LAS > 10LE > Tween-80.

Mycobacterium sp. could grow on Tween-80 and Brij surfactants as sole carbon and energy source but not on LAS and TDTMA. The toxicity of Brij surfactants increased with the increase of ethoxylate group in their molecules, which was contrary to the responses of *Vibrio fischeri*. TDTMA was the most toxic, followed by LAS and Brij35 no matter with the presence of phenanthrene or not. In the coexistence of phenanthrene, the toxicity of LAS eased. The combined effects of phenanthrene with Tween-80 or Brij surfactants were positive and additive while negative with LAS or TDTMA. At the presence of non-toxic soluble substrate, either positive or negative effects of surfactants was much less significant than those at the presence of phenanthrene as sole carbon source, indicating that effects of surfactants were substrate-specific.

LAS and Tween-80 had similar effects on soil microorganisms as on *Mycobacterium* sp. TDTMA was less toxic to soil microorganisms than LAS though the former was much more toxic to *Mycobacterium* sp. and *Vibrio fischeri* than the later. Phenanthrene exhibited completely contrary effects on soil microbial activities in different soils. Pyrene did not cause significant change in soil microbial activities as phenanthrene did. The coexistence of any test surfactants could generally increase soil microbial activities except for LAS and TDTMA at very high concentrations. The combined effects of PAHs with TDTMA were overadditive while with Tween-80 was slightly overadditive at low respiration levels and underadditive at high levels. Results indicate that both separate and combined effects of test chemicals were different among soils.

The presence of surfactants inhibited the biodegradation of phenanthrene by *Mycobacterium* sp. in a descent order of TDTMA > LAS > Tween-80. It was either because the surfactants (e.g. LAS at high concentration and TDTMA) were toxic to the bacteria or due to they (e.g. Tween-80) were utilized by the bacteria as preferential substrates. The influence of Brij surfactants on the biodegradation was not significant.

The biodegradation of pyrene in soil was much lower than that of phenanthrene. LAS and TDTMA at low concentrations and Tween-80 enhanced the biodegradation of both PAHs in soils. But the enhancement of pyrene biodegradation was lower than phenanthrene. At high concentrations, both LAS and TDTMA inhibited their biodegradation. The surfactant concentrations, at which the biodegradation of PAHs was enhanced or inhibited, varied significantly among soils. A surfactant enhancing the biodegradation in one soil may exhibit inhibition in other soils. The relationship between enhancement of phenanthrene biodegradation and microbial activities was also remarkably different among soils and among surfactants. The increase of microbial activities dose not necessarily mean enhancing the biodegradation of HOCs. Results showed that the efficiency of surfactant's enhancement of PAH biodegradation depended on the intrinsic PAHs-degrading capacities and the inherent microbial activities of the soils. The surfactant's enhancement efficiency would be low or even there would be no enhancement when the soil already had high degrading capacities.

Mycobacterium sp. strain KR2 could grow on solid phenanthrene through direct contact though preferably to utilize solubilized phenanthrene. The influence of surfactants on the adherence and growth of the bacteria on solid phenanthrene depended on the characteristics and concentration of surfactants. It was inhibited by LAS, TDTMA, Brij30 and 10LE, while enhanced by Tween-80 at <20 mg l^{-1} and Brij35 at 5 to 80 mg l^{-1}. Both surfactants had a maximal enhancement at 1/2CMC.

The mineralization and metabolism of phenanthrene were quick in wheat-solution-lava microcosm. At least 90% of the applied phenanthrene were transformed within 24 days. Most of the applied ^{14}C-activity was recovered from wheat (70%) and constructed into wheat tissues (53%). The major metabolites of phenanthrene were polar compounds. The presence of Tween-80 significantly increased the metabolism of phenanthrene.

6 OUTLOOK

The common coexistence of HOCs and surfactants in the environment and the significance of effects of the later on the environmental behaviour of the former have evoked the necessity to study their combined behaviours, ecological effects and fates in the environment. It has also been realized that environmental impact assessments carried out nowadays should take these combined interactions into consideration in order to reflect properly the real situation in the environment. The application of surfactants in remediation of contaminated soils and aquifers to overcome the limited bioavailability of contaminants has also attracted much study in the interactions among surfactants, HOCs, organisms and soil components. As can be seen from the results obtained in this study, however, the complexity of their combined interactions makes it very difficult to sum up general rules and to apply this knowledge in environmental assessment. The effect of surfactants on the environmental behaviour of HOCs is surfactant-, HOC-, organism- and environmental matrices-specific as well as vary remarkably from positive to negative and *vice versa* according to the concentration of surfactants. No single phenomenon or factor can realistically be used to assess and predict the effects that surfactants will have on the environmental behaviour of HOCs and the microbial degradation of HOCs. Therefore, more researches both in laboratory and especially in fields are necessary. Insights and mechanisms obtained in laboratory researches using simple model systems are required for batter understanding of the more complicated processes in field situation. Field researches are essential for successful application of the knowledge in environmental assessment and in particular in the surfactant's enhancement of remediation of contaminated soils and aquifers. Criteria of combined effects of contaminants have to be developed before they can be included in environmental assessment. Much more knowledge is required before the application of surfactants can be used as a standard technique in the remediation of contaminated soils and aquifers.

The interactions of surfactants and organisms are the most controversy in this field, which are critical for the understanding of the mechanisms governing surfactant's effects on the biodegradation of HOCs. There is a need to know more of the effect of

surfactants on membrane processes and the physical and biochemical mechanisms by which the surfactants influence the transport of HOCs to and across cell membrane. The steric and ionic compatibilities of surfactants with cell envelope lipids and membrane-bond proteins appear to be important factors. The mechanisms governing the surfactant effects on the uptake of HOCs by plants and their transport in plants are poorly understood. Investigation is also need of the effect of micellar surfactants on the bioavailability of HOCs. Beside effects of surfactants on membrane processes as mentioned above, this should include the bioavailability of micellar HOCs, and the potential of micellar surfactant solubilization to inhibit the accessibility of HOCs to microorganisms due to reduced aqueous HOCs concentrations in the presence of the micellar pseudophase and limited partitioning rates.

The effect of surfactant solubilization on biodegradation of HOCs is needed to be better understood in order to assess the feasibility of application of surfactant for enhancement of bioremediation of contaminated soils and aquifers. Surfactant enhancement of desorption of sorbed HOCs from soil particles to aqueous phase is an important process to increase their bioavailability.

The adherence of microorganisms to contaminants present as NAPLs and solid states is an important step before their microbial mineralization commences. However, little is known about mechanisms underlined the effects of surfactants on the adhesion of microorganisms to solid and NAPL hydrocarbon. Research is needed for better understanding the interaction of cell and solid/NAPL hydrocarbon and the alternation and modification of surfactants on characteristics of cell surface and solid/NAPL hydrocarbons.

The use of surfactants as a tool in bioremediation of contaminated soil has promising prospects though more research is needed. The fate of surfactants used in soil remediation is an additional concern and more study is needed in this aspect. They should not cause secondary pollution as a result of their application in soil remediation.

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ANNEX

Annex-1. List of Figures

Annex-2. List of Tables

Annex-3. *Curriculum vitae*

Name: **Ziqing OU**

Date of birth: March 31, 1956

Place of birth: Guangxi, China

Studying and working experiences:

1996-1999	Ph.D. candidate at Technical University of Munich (Technische Universität München)
1994-present	Professor, Institute of Applied Ecology (IAE), Chinese Academy of Sciences (CAS), Shenyang, China
1994-present	Deputy director, Open Laboratory of Ecological Processes of Trace Substance in Terrestrial Ecosystem, CAS, Shenyang, China
1998	Visiting research scholar, Department of Civil Engineering and Operations Research, Princeton University, USA
1995	Visiting scientist for UNESCO CERP project, IÖC-Institute of Ecological Chemistry, GSF-National Research Center for Environment and Health, Germany
1993-1994	Visiting scientist for UNESCO CERP project, GSF-IÖC, Germany
1993-1994	Associate professor, IAE, CAS, Shenyang, China
1991	Visiting scholar of British Royal Society, Institute of Terrestrial Ecology, Natural Environment Research Council (NERC), UK
1990-1991	Visiting scholar of British Royal Society, Institute of Freshwater Ecology, NERC, UK
1988	Visiting scientist, Department of Toxicology, State University of Ghent, Belgium
1987-1992	Assistant professor, IAE, CAS, Shenyang, China
1985-1986	Research associate, IAE, CAS, Shenyang, China
1982-1985	M.Sc. degree, Institute of Forestry and Soil Sciences, CAS, Shenyang, China
1978-1982	B.Sc. degree, Department of Soil and Agricultural Chemistry, Guangxi Agricultural University, China

Awards:

1999 National Research Funding for Outstanding Young Scientist, awarded by the National Natural Scientific Foundation of China (NSFC)

1999 Second-Grade National Award for Scientific and Technological Progress for project "Renovate/Alternative technologies for the treatment and reutilization of municipal wastewater", awarded by the Ministry of Science and Technology, China

1998 First-Grade Award for Scientific and Technological Progress for project "Renovate/Alternative technologies for the treatment and reutilization of municipal wastewater", awarded by CAS

1997 Third-Grade Award for Scientific and Technological Progress for project "Ecological construction planning and design along Beijing-Shenyang highway in the section of Panshan County", awarded by the Scientific and Technological Committee of Liaoning Province, China

1995 First-Grade Award for Excellent Scientific Thesis, co-awarded by the Scientific and Technological Committee of Liaoning Province and the Ecological Society of Liaoning Province, China

1995 First-Grade Award for Excellent Scientific Thesis, awarded by the 2nd Liaoning Province Annual Academic Conference of Youth under the sponsor of China Scientific and Technological Association

1994 Golden Award for Green Technology, awarded by the China National Committee for Development of Green Technology

1994 Award for Advanced Worker, awarded by IAE, CAS

1993 Special Lifetime Allowance Award for Excellent Scientists, awarded by the State Council of China

1993 Award for Excellent Youth Scientists, awarded by CAS-Shenyang Branch

1993 Award for Advanced Young Worker for Years 1991 and 1992, awarded by IAE, CAS

1992 Award for Advanced Young Scientific and Technological Workers, awarded first time by Provincial Government of Liaoning Province, China and co-awarded by the Scientific and Technological Committee of Liaoning Province, China

1992 First-Grade Awards for Excellent Scientific Thesis, awarded by the 1st Liaoning Province Annual Academic Conference of Youth under the sponsor of the China Scientific Association

1992 Second Award for Scientific and Technological Progress for project "Studies on slow-rate infiltration land treatment of municipal wastewater in west Shenyang", awarded by CAS

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