Journal of Hazardous Materials 184 (2010) 448–456

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Sorption of quaternary ammonium compounds in soils: Implications to the soil microbial activities

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ARTICLE INFO

*Article history:*
Received 5 May 2010
Received in revised form 11 August 2010
Accepted 13 August 2010
Available online 21 August 2010

*Key words:*
Quaternary ammonium compounds (QACs)
Release of sorbed QACs
Soil microbial activity
Toxicity
Dehydrogenase activity
Potential nitrification

ABSTRACT

Despite their widespread use in household activities and various industries, information on the toxicity of quaternary ammonium compounds (QACs) to microbial activities in soil is scant. This study investigated the effect of three commonly used QACs namely hexadecyltrimethyl ammonium bromide (HDTMA), octadecyltrimethyl ammonium bromide (ODTMA) and Arquad on dehydrogenase and potential nitrification activities in three different soils. The toxicity of QACs on the dehydrogenase activity and potential nitrification in these soils followed the order: HDTMA > ODTMA > Arquad and Arquad > HDTMA > ODTMA, respectively. HDTMA, ODTMA and Arquad exhibited toxicity to dehydrogenase activity at concentration of 50, 100 and 750 mg $kg^{-1}$ soil, respectively, whereas potential nitrification was inhibited by HDTMA and ODTMA even at 50 mg $kg^{-1}$ soil. Arquad exhibited toxicity to potential nitrification at comparatively higher concentration of 250 mg $kg^{-1}$ soil, with the severity of toxicity very intense at higher concentrations. The nature of QACs and soil properties influenced the toxicity. The toxic effect of QACs on soil microbial activities was more influenced by the relative release of sorbed QACs in soils. This study provides valuable information on the toxicological properties of some widely used QACs on important soil microbial activity parameters. To our knowledge, this is the first report.



## 1. Introduction

Living organisms are exposed to numerous organic and inorganic toxic chemicals in the environment (soil and water) as a result of industrial, agricultural and daily household activities. This is a serious environmental problem, sometimes worsened by accidental release or uncontrolled use of certain chemical agents. For example, quaternary ammonium compounds (QACs), commonly known as cationic surfactants (CSs), have found widespread use in industries and household activities during recent years. These compounds have unique properties in terms of surface activity, interaction with negatively charged solids, participation in ion exchange phenomena, and biocidal activity. As a result, they are widely used as detergents, cleansers, deodorisers, wetting and softening agents, hydrophobic agents, emulsifiers, biocides and germicides. It is estimated that consumption of these compounds in Europe and USA individually may exceed 32,000 tonnes [1]. They are mostly used as fabric softeners (66%), coated clays (16%) and biocides (8%) [2]. Most of the uses of these chemicals lead to their release into soil and water systems. In under developed and developing countries, where sewage system is poor, the household waste water is released directly into soils or water stream without adequate treatment. As a result of this uncontrolled discharge, localised high concentrations of QACs may be found in soils. Also, the use of QACs has increased vastly in the recent years in the environmental industry necessitating investigation into new surfactants, especially those which are used in QAC-assisted remediation of contaminants in soil [3–6]. QACs are also largely used in the preparation of coated clays and organoclays [7–10].

QACs are usually toxic to microorganisms. For example, aqueous phase hexadecyltrimethylammonium (HDTMA) is toxic to bacteria at concentrations as low as 10 μM (~2.85 mg $L^{-1}$) [11]. QAC molecules are generally more toxic to Gram-negative than to Gram-positive soil microorganisms and spore formation is one of the survival mechanisms for microorganisms to overcome aqueous QAC toxicity [11]. The toxicity of HDTMA is apparent even at 2.85 mg $L^{-1}$ concentration, with significant inhibition of growth of soil microbes at higher concentrations [12]. Although researchers have attempted to unfold the impact of HDTMA, which is one of the most commonly used QACs, on microbial toxicity, comprehensive study about other frequently used surfactant compounds remains largely unreported.

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doi:10.1016/j.jhazmat.2010.08.055

Stress caused by changes in the soil environment due to presence of foreign chemicals can be judged in advance through sensitive soil quality parameters [13]. Microbial activities are considered very sensitive indicators to environmental disturbances in soils caused by the presence of foreign chemicals such as QACs. Information on the influence of QACs on the microbial activities in soil is rarely available in the literature. Earlier reports mostly dealt with the influence of QACs on soil microorganisms in isolated pure culture and those effects were expressed directly in terms of microbial growth or viable counts [11,12]. Moreover, the focus of those studies was on the toxicity of HDTMA as it had been the most extensively used QAC for environmental application [14–17]. Many other QACs are frequently released into the environment with the household discharge because majority of the cleaning agents, shampoo, etc., contains these compounds. The present study attempts to investigate the impact of some frequently used QACs on two different soil microbial processes, namely dehydrogenase activity and nitrification. Dehydrogenase activity reflects the oxidative activity or intensity of metabolism of the total microflora present in the soil, whereas nitrification is a soil function carried out by a specific group of microorganisms called nitrifiers. We hypothesise that QACs may affect both the microbial parameters differently in soils having dissimilar physicochemical properties. The sorption–desorption behaviour of these compounds in soils may influence their effects on the soil microbial activities. To the best of our knowledge, there has not been any report on the effect of QACs on the microbially mediated processes and functions such as dehydrogenase activity and potential nitrification in soils till date.

## 2. Materials and methods

### 2.1. Soils and chemicals used in the study

Three soils having different physico-chemical properties and land uses were included in this study. Soils (0–10 cm depth) were collected from three different locations, namely Adelaide Hills, Mawson Lakes and Gawler in South Australia. Adelaide Hills (AH) soil was acidic in reaction, whereas Mawson Lakes (ML) and Gawler (GLR) soils were neutral and slightly alkaline in nature, respectively. After collection, the soils were mildly ground to pass through 2 mm sieve and stored at 4 °C temperature for further use. The physico-chemical properties of the experimental soils were determined by standard procedures [18]. Determination of CEC in acidic and alkaline soils was carried out using appropriate methods [18].

All three QACs were purchased from Sigma–Aldrich and used without further purification. Two of the QACs are hexadecyltrimethyl ammonium bromide (HDTMA) and octadecyltrimethyl ammonium bromide (ODTMA), whereas the third is a commercially available relatively inexpensive surfactant, Arquad 2HT. Chemically, Arquad is di(hydrogenated tallow) dimethylammonium chloride having propylene glycol (11%) and water (14%) as impurities. Reagent grade chloroform and Orange II were also purchased from Sigma–Aldrich.

### 2.2. Adsorption–desorption study

Adsorption of QACs to soils was measured in batch experiments. A portion of 0.2 g sieved air dried soil, in triplicate, was equilibrated with 10 mL of QACs solution ranging in concentrations from 72.9 to 883.3 mg $L^{-1}$. The mixture was taken in 50 mL centrifuge tube and agitated on an end-over-end shaker for 3 h at 23 °C, followed by centrifugation at 4000 rpm for 30 min. The clear supernatant was collected for QACs analysis as described in the following section.

The soil sample loaded with QACs (equivalent to 1 mM initial concentration of the QACs) during the sorption experiment was subjected to desorption in 10 mL of deionised water on an end-over-end shaker for 3 h at 23 °C. Following centrifugation at 4000 rpm for 30 min, the desorbed QAC concentration was measured in the clear supernatant. The volume of liquid entrapped by the soils after completion of the adsorption experiment and the amount of QACs held therein was taken into consideration during the calculation of QACs desorption. The amount of QACs desorbed is expressed as the percentage of amount adsorbed.

### 2.3. Analysis of QACs

QAC concentration in the aliquots was analysed by modifying the Orange II method originally described by Scott [19]. In short, 2 mL buffer solution (0.2 M $NaHCO_3$ at pH 9.2) was added to 1 mL of the sample aliquot in 40 mL clear glass vial. The mixture was reacted with 1 mL Orange II solution (2000 mg $L^{-1}$) by intermittent vigorous shaking, followed by extraction with 5 mL chloroform. QAC concentration in the chloroform extract was measured at 485 nm wavelength against chloroform blank on a Synergy HT micro plate reader (BIO-TEK® Instruments Inc., USA) using 96-wells plate.

### 2.4. Microcosm experiment

Microcosm experiments were conducted with 5 g field moist soils, in triplicate, placed in 50 mL polypropylene centrifuge tubes. The soils were spiked with different amounts of QACs (concentration ranging from 0 to 3000 mg $kg^{-1}$ soil). After spiking, the tightly capped centrifuge tubes were agitated on an end-over-end shaker for 24 h to ascertain uniform mixing of the QACs in the soils. Then the microcosms were incubated at 23 °C for 14 days. Untreated soils incubated likewise served as controls. All the soils were maintained at 70% of the total moisture holding capacity throughout the experiment to facilitate optimum growth and proliferation of the soil microorganisms. At the end of incubation, samples were analysed for dehydrogenase activity and potential nitrification. The values of soil microbial activities were expressed against the initial spiked concentration of QACs in the present study. The concentration of the QACs in microcosm soils after incubation was not analysed, rather a separate set of experiment was conducted to examine the sorption–desorption of QACs in the soils as described in the previous sections.

### 2.5. Determination of dehydrogenase activity

Dehydrogenase activity was determined by monitoring the rate of triphenylformazan (TPF) production from triphenyltetrazolium chloride (TTC) [20]. A 1.0 mL of TTC solution (3%) was added to the soil microcosm, in triplicate, followed by gentle tapping to remove the entrapped air to result in a thin layer of water on the soil surface to make the system free from gaseous oxygen. After incubating for 24 h at 37 °C, TPF was extracted with methanol by vigorous shaking and its concentration determined at 485 nm wavelength using Agilent 8453 UV–VIS spectrophotometer [20].

### 2.6. Determination of potential nitrification

Potential nitrification was assayed based on the determination of nitrite ($NO_2^-$) produced by soil incubated aerobically with ammonium sulphate [$(NH_4)_2SO_4$] as substrate. Sodium chlorate ($NaClO_3$) was used to inhibit the formation of nitrate ($NO_3^-$) from nitrite ($NO_2^-$) [21]. To a 5 g soil taken in a 50 mL centrifuge tube, 0.10 mL $NaClO_3$ (1.50 M) and 20 mL 1 mM $(NH_4)_2SO_4$ solution were added and incubated overnight at 25 °C temperature. After incubation, $NO_2^-$ was extracted into the supernatant by shaking the mixture with 5 mL of 2 M potassium chloride (KCl) followed by centrifugation at 4000 rpm for 20 min. Nitrite in the supernatant was