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

Table 1
Selected physico-chemical properties of the soils included in the study.

Soil	Clay content (%)	Texture	Bulk density ($g\,cm^{-3}$)	pH (1:2.5 soil:water)	Organic carbon (%)	CEC ($cmol\,(p^+)\,kg^{-1}$)	CBD extractable Fe and Al (%)	AO extractable Fe and Al (%)	BET surface area ($m^2\,g^{-1}$)
AH	7.6	Loam	1.51	5.5	4.11	9.05	0.917	0.252	10.5
ML	15.9	Silty loam	1.42	6.7	2.10	17.44	0.260	0.197	18.3
GLR	15.1	Sandy loam	1.48	7.8	1.45	14.36	0.282	0.173	33.0

CEC: cation exchange capacity; CBD: citrate–bicarbonate–dithionate extractable Fe and Al; AO: ammonium oxalate–oxalic acid extractable Fe and Al.

determined spectrophotometrically at 520 nm wavelength by sulphanilamide method [21]. Potential nitrification was expressed as $\mu g\,NO_2^-$ produced g^{-1} soil day^{-1}.

2.7. Statistical analysis

Complete randomized design (CRD) with three replications was followed for the data analysis. Two-way analysis of variance (ANOVA) was performed to determine the effects of treatments (three different surfactants) and their application doses on the dehydrogenase activity and potential nitrification in soil. Duncan's Multiple Range Test (DMRT at $p < 0.01$ or $p < 0.05$) was used to determine whether means differed significantly. For analysis of data, Microsoft Excel (Microsoft Corporation, USA) and/or SPSS window version 17.0 (SPSS Inc., Chicago, USA) packages were used. Probit analysis was done using Minitab 15 software packages at 95% confidence level and corresponding LC_{50} (lethal concentration) values were determined.

3. Results

3.1. Physico-chemical properties of the soils

Selected physico-chemical properties of the soils used in the study are listed in Table 1. Among the three soils studied, AH soil was loamy in texture having a bulk density of $1.51\,g\,cm^{-3}$, whereas ML and GLR soils were silty loam and sandy loam in texture having bulk densities of 1.42 and $1.48\,g\,cm^{-3}$, respectively. The soils varied significantly in pH, organic carbon content and cation exchange capacity (CEC) as shown in Table 1. AH soil was acidic in reaction having pH 5.5, whereas ML soil and GLR soil were near to neutral (pH 6.7) and alkaline (pH 7.8) in nature, respectively. The AH soil was collected from a perennial eucalyptus forest having organic carbon content of 4.1%, while GLR soil belongs to an agricultural field. The organic carbon content in GLR soil was quite low, about 1.45%. The ML soil was collected from an uncultivated paddock which was medium in organic carbon content (2.1%), but slightly higher in CEC ($17.44\,cmol\,(p^+)\,kg^{-1}$) than the other two soils. The CEC of AH and GLR soils were 9.05 and $14.36\,cmol\,(p^+)\,kg^{-1}$, respectively. The three soils also varied in their citrate–bicarbonate–dithionate (CBD) extractable and ammonium oxalate–oxalic acid extractable amorphous iron and aluminium contents and the BET surface area (Table 1).

3.2. Sorption and desorption of QACs in soils

The sorption isotherms of QACs in different soils are shown in Fig. 1. In general, all three soils showed high, but variable sorption capacities ranging from 22.3 to $39.8\,g\,kg^{-1}$. L-type isotherm was observed for QACs adsorption in both ML and GLR soils, whereas the sorption was more similar to S-type isotherm in case of AH soil (Fig. 1a–c). The k_d values (calculated from a general linear isothermal model as the ratio of millimolar adsorbed species per unit mass of solid to the millimolar species in solution per unit volume) for the sorption of QACs in all three soils followed the order: HDTMA > ODTMA > Arquad (Table 2). The k_d values for sorption of

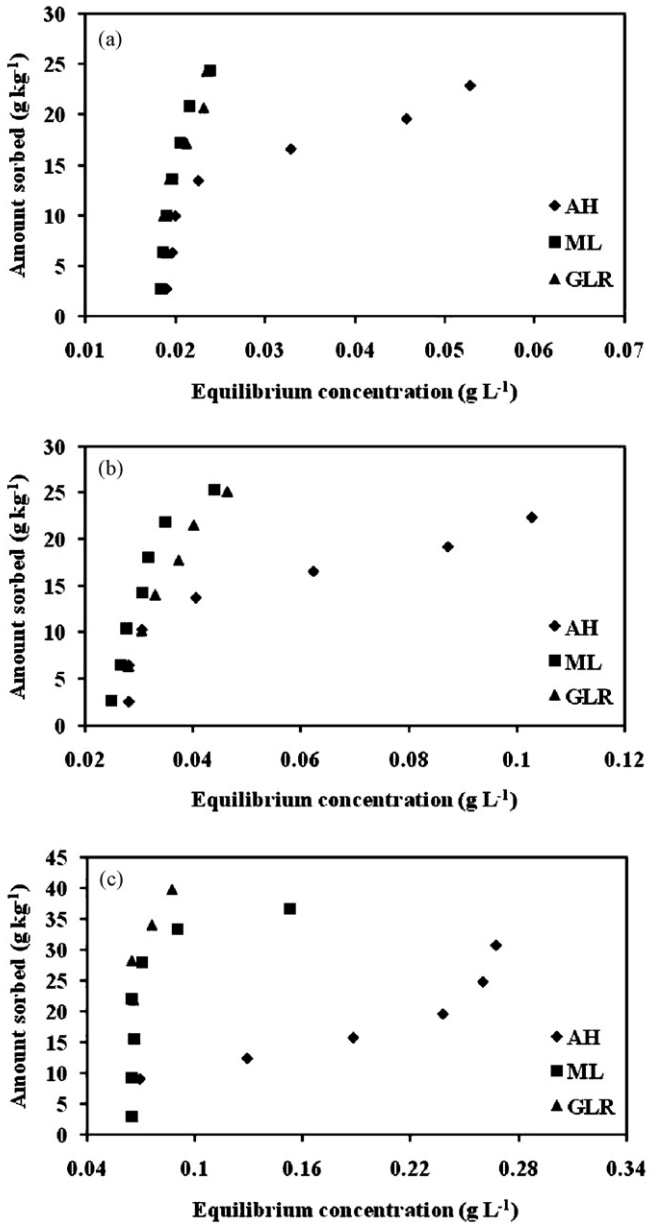

Fig. 1. Sorption of QACs in soils from aqueous solution (a) HDTMA, (b) ODTMA and (c) Arquad.

Table 2
Calculated k_d values ($L\,kg^{-1}$) for the sorption of QACs in three soils.

	HDTMA	ODTMA	Arquad
AH soil	417.7	242.5	92.9
ML soil	646.5	423.4	252.9
GLR soil	637.0	379.2	299.7

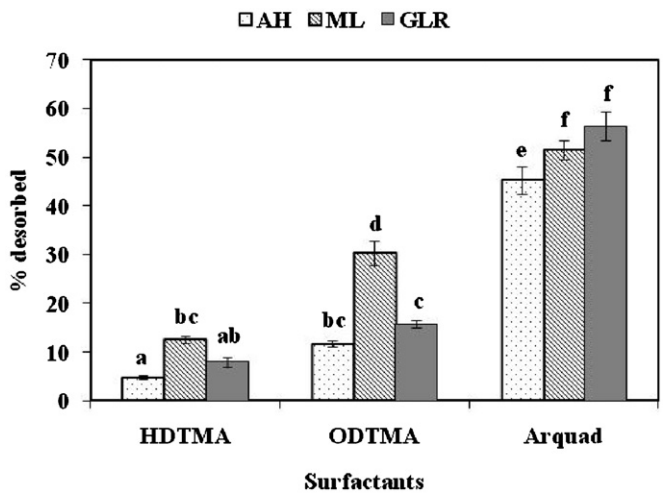

Fig. 2. Percentage desorption of QACs in water from soils sorbed with surfactants.

HDTMA and ODTMA were maximum in ML soil followed by GLR and AH soils. However, Arquad showed maximum k_d value in GLR soil followed by ML and AH soils. The initial and final pH values during the QACs adsorption experiment on AH soil ranged between 5.2 to 5.4 and 5.6 to 5.9, respectively. For ML and GLR soils, the initial and final pH did not vary significantly from the pH of the soils measured in 1:2.5 soil–water suspensions (Table 1).

Fig. 2 shows desorption patterns of QACs in three different soils. Desorption of HDTMA and ODTMA from all three soils followed similar patterns. Similar to the adsorption results, the percentage desorption of these two surfactants followed the order: ML > GLR > AH. However, desorption order for Arquad was GLR > ML > AH. Among the three QACs, Arquad showed the maximum percentage desorption followed by ODTMA and HDTMA (Fig. 2).

3.3. Effect of QACs on dehydrogenase activity in soil

The influence of QACs on dehydrogenase activity in AH soil is shown in Fig. 3a. Up to a concentration of 250 mg kg^{-1} soil, HDTMA did not show any significant ($p < 0.01$) effect on the dehydrogenase activity in AH soil as compared to the control treatment. However, there was a significant gradual decrease in the dehydrogenase activity at HDTMA concentration of 500 mg kg^{-1} soil and higher. It was observed that at 500, 750, 1500 and 3000 mg kg^{-1} HDTMA concentrations, the dehydrogenase activity decreased by 10, 22, 25 and 58%, respectively in comparison to the control. A marginal increase in the dehydrogenase activity (up to 6%) was observed at low HDTMA concentration up to 250 mg kg^{-1} soil. Similar trend was noticed in case of ODTMA in AH soil. Thus, ODTMA toxicity on dehydrogenase activity started to appear at surfactant concentration 250 mg kg^{-1} soil, being the most intense (34% reduction) at 3000 mg kg^{-1} soil. Fig. 3a also shows that among the three QACs studied, Arquad had the minimum toxicity on the dehydrogenase activity in AH soil. At the maximum soil Arquad level applied, there was a reduction of only about 12% dehydrogenase activity, whereas at similar application level HDTMA and ODTMA showed 58 and 35% reduction, respectively. Negative effect of Arquad on soil dehydrogenase activity became apparent at a level of 750 mg kg^{-1} in this soil.

Fig. 3b illustrates the effect of QACs on dehydrogenase activity in ML soil. It is apparent that the trend of soil dehydrogenase activity in ML soil was quite different from that in AH soil. Without any QAC application (control), the dehydrogenase activity in AH soil was found to be about 12 μg TPF g^{-1} soil h^{-1}, whereas in ML soil it was only about 1.8 μg TPF g^{-1} soil h^{-1}. HDTMA, ODTMA and Arquad improved the dehydrogenase activity in ML soil to the extent of 4, 22 and 7%, respectively at 50 mg kg^{-1} QACs application level. Although this improvement with HDTMA was insignificant ($p < 0.01$), it was significant with ODTMA and Arquad at similar statistical level of confidence. Also at 100 mg kg^{-1} soil QACs level, all three surfactants showed significant increase in dehydrogenase activity as compared to the control ML soil. Overall, HDTMA and Arquad did not show any significant effect on dehydrogenase activity even up to 3000 mg kg^{-1}. However, ODTMA did exert significant ($p < 0.01$) toxicity (46%) at 3000 mg kg^{-1} compared to the control treatment.

Fig. 3c depicts the influence of QACs on dehydrogenase activity in GLR soil. Among the three soils investigated, GLR soil represented fertilised agricultural soil in which dehydrogenase activity was about 3.6 μg TPF g^{-1} soil h^{-1}. In this soil, HDTMA showed significant ($p < 0.01$) toxicity (10%) to dehydrogenase activity at soil QAC concentration as low as 50 mg kg^{-1} with a maximum 61% reduction at 3000 mg kg^{-1} soil QAC level. ODTMA also caused significant ($p < 0.01$) negative effect on dehydrogenase activity in GLR soil at surfactant level 100 mg kg^{-1} and higher. The maximum reduction of about 48% was observed at soil ODTMA concentration of 3000 mg kg^{-1}. Interestingly, Arquad showed significantly positive effect ($p < 0.01$) on dehydrogenase activity in GLR soil up to 750 mg kg^{-1} with a maximum increment in dehydrogenase activity (16%) at 250 mg kg^{-1} level. However, significant negative ($p < 0.01$) effect (5–7%) was noticed at 1500 and 3000 mg kg^{-1} soil QAC levels.

3.4. Effect of QACs on potential nitrification in soil

The effect of QACs on potential nitrification in AH soil is shown in Fig. 4a. HDTMA was significantly toxic ($p < 0.01$) to potential nitrification at QAC concentration as low as 50 mg kg^{-1} in AH soil. The toxic effects of HDTMA kept increasing with incremental surfactant concentrations in soil with a maximum 16.3% reduction at 3000 mg kg^{-1} of QAC as compared to the control treatment. Unlike HDTMA, ODTMA showed significant ($p < 0.01$) improvement (13%) in potential nitrification at 50 mg kg^{-1} QAC concentration in soil. However, toxic effects were observed at QAC levels greater than 750 mg kg^{-1} showing maximum toxicity (14%) at ODTMA concentration of 3000 mg kg^{-1}. The influence of Arquad on potential nitrification in AH soil is quite different in comparison to the other two surfactants examined in this study. Arquad showed an increase (up to 29%) in potential nitrification in AH soil at QAC level of 100 mg kg^{-1}. However, toxicity begins to appear at Arquad level of 250 mg kg^{-1} or higher in soil, with 22% reduction at 3000 mg kg^{-1}.

Fig. 4b shows influence of QAC on the potential nitrification in ML soil. HDTMA showed a steady decrease in potential nitrification in this soil with increasing level of applied QACs. The maximum toxicity in the potential nitrification of about 24% reduction, as compared to the control, was observed at 3000 mg kg^{-1} HDTMA level in soil. ODTMA also showed similar trends like HDTMA with the highest toxicity of 22% at 3000 mg kg^{-1} QAC level. The effect of Arquad on the potential nitrification in ML soil was different than the other two QACs. At low concentration, i.e. 50 and 100 mg kg^{-1}, Arquad showed significant positive effect ($p < 0.01$) on the potential nitrification in ML soil. However, at higher level of application, Arquad caused more toxicity compared to the other two surfactants. For example, at 1500 mg kg^{-1} level, HDTMA, ODTMA and Arquad caused about 24, 23 and 58% reduction in the potential nitrification in ML soil, respectively.

The effect of QACs on the potential nitrification in GLR soil is presented in Fig. 4c. Both HDTMA and ODTMA showed a steady decrease in the potential nitrification with an increase in the QACs concentration. Both the surfactants showed maximum toxicity on

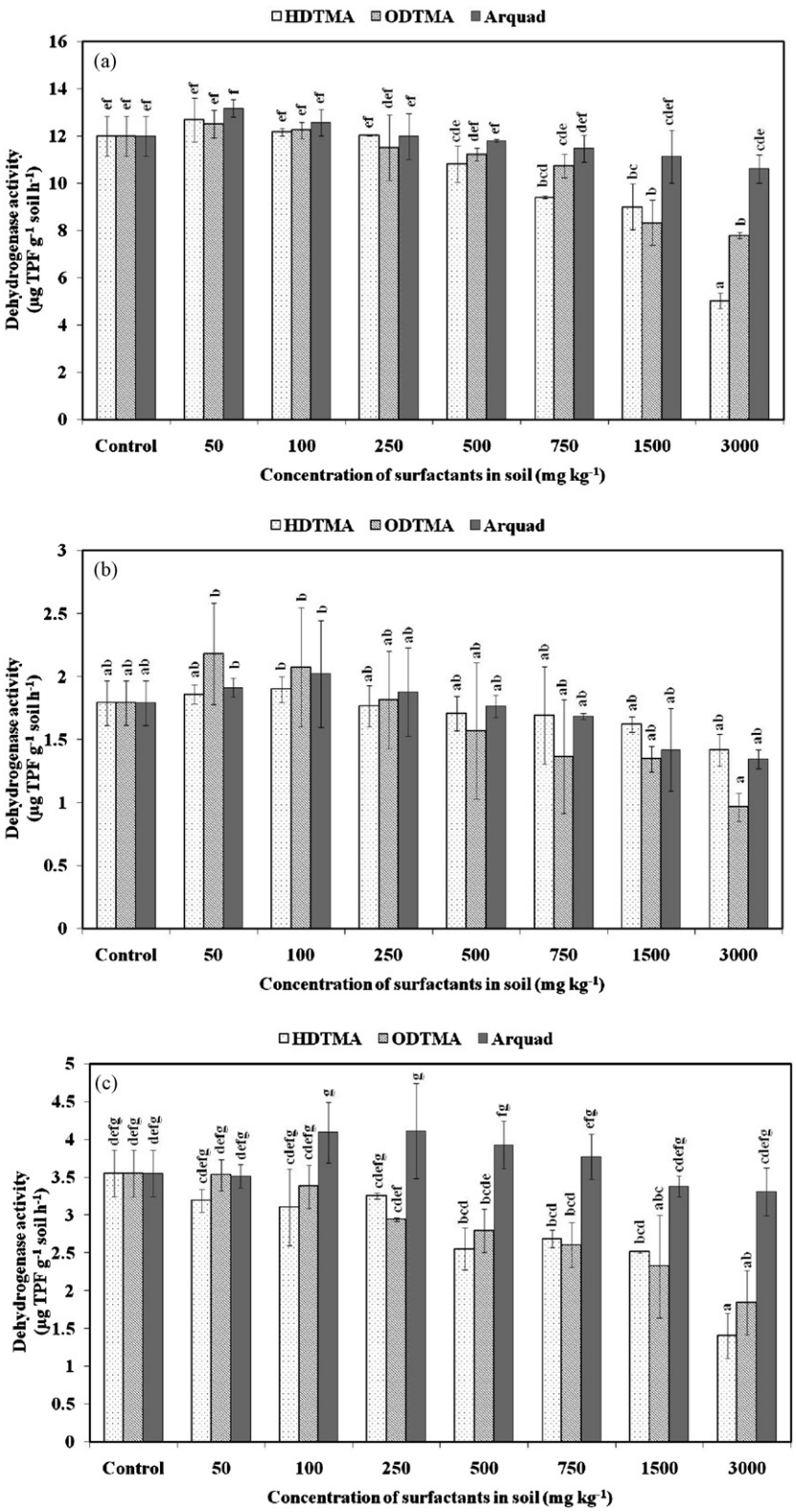

Fig. 3. Effect of QACs on dehydrogenase activity in (a) AH soil, (b) ML soil and (c) GLR soil.

the potential nitrification at 3000 mg kg^{-1} soil level and the reduction corresponds to 45 and 43%, respectively. On the other hand, Arquad showed slight but significant ($p < 0.01$) improvement in the potential nitrification in GLR soil at 50 and 100 mg kg^{-1} QAC level. But at 3000 mg kg^{-1} concentration, Arquad was very toxic (62% reduction) to the potential nitrification in GLR soil.

3.5. LC_{50} values for dehydrogenase activity and potential nitrification

The estimated LC_{50} (g kg^{-1}) values (concentration showing 50% inhibition in the microbial activity) for the dehydrogenase activity and potential nitrification in soils as affected by differ-

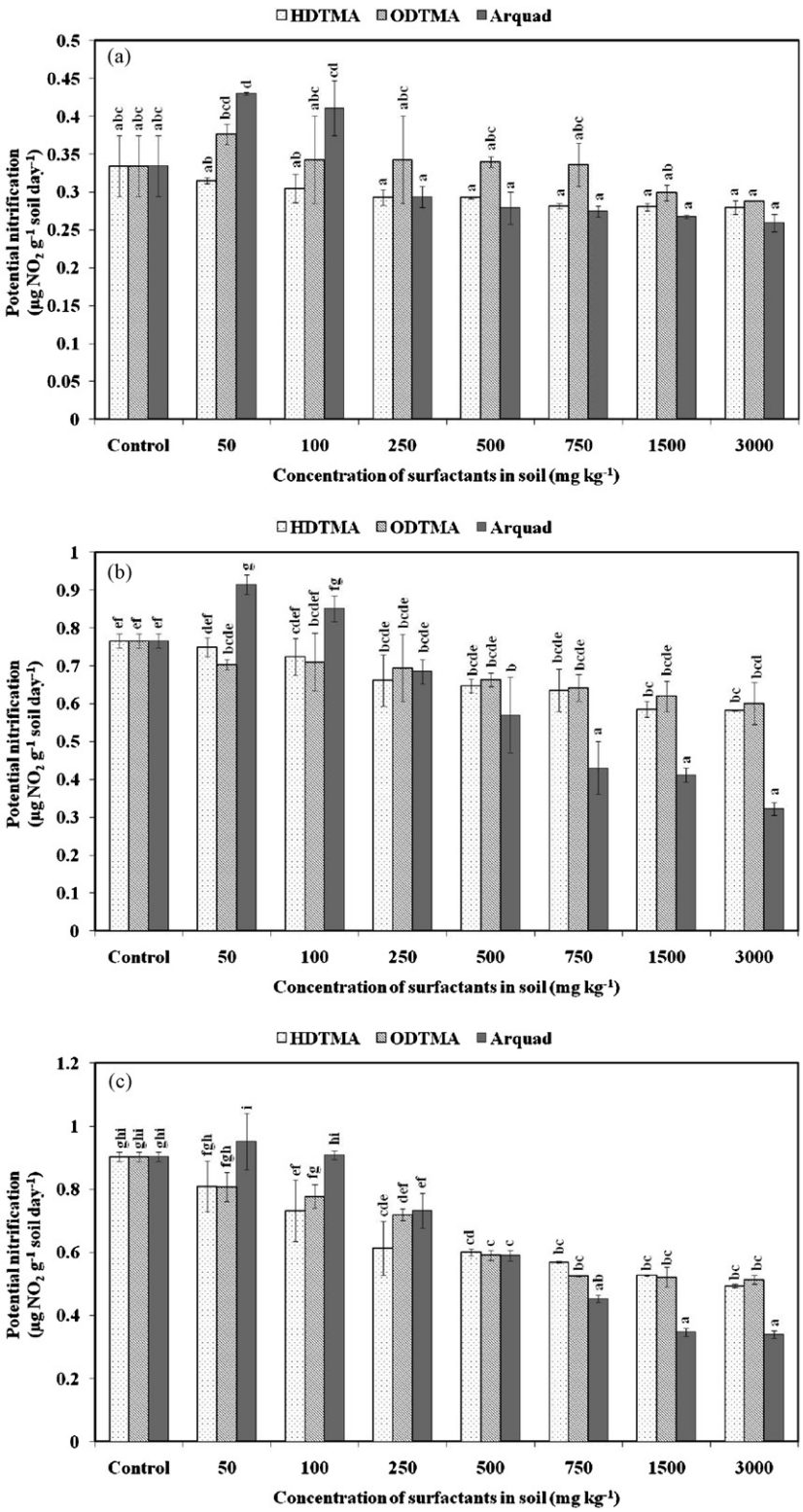

Fig. 4. Effect of QACs on potential nitrification in (a) AH soil, (b) ML soil and (c) GLR soil.

ent QACs applied at various concentrations in the present study are presented in Table 3. It was apparent from the LC_{50} values (Table 3) that the order of toxicity of QACs on dehydrogenase activity in soils was as follows: HDTMA > ODTMA > Arquad. However, the LC_{50} values for potential nitrification followed the order: Arquad > HDTMA > ODTMA. The results indicated that all the three surfactants exhibited different levels of toxicities on both dehydrogenase activity and potential nitrification in the soils studied.

Table 3
LC_{50} values ($g\,kg^{-1}$) at 95% confidence level calculated for the dehydrogenase activity and potential nitrification in soils as affected by QACs.

Soil	Surfactant	Dehydrogenase activity		Potential nitrification	
		LC_{50} ($g\,kg^{-1}$)	Standard error	LC_{50} ($g\,kg^{-1}$)	Standard error
AH	HDTMA	2.43	0.12	7.18	0.33
	ODTMA	3.19	0.21	5.12	0.14
	Arquad	5.73	0.93	3.21	0.07
ML	HDTMA	4.47	0.38	4.56	0.07
	ODTMA	2.33	0.12	5.36	0.11
	Arquad	3.61	0.25	1.68	0.01
GLR	HDTMA	2.40	0.09	2.70	0.03
	ODTMA	2.70	0.10	2.72	0.03
	Arquad	6.15	0.63	1.62	0.01

4. Discussion

4.1. Sorption and desorption of QACs in soils

The adsorption of QACs in ML soil and GLR soil was similar to L-type isotherm (Fig. 1a–c) indicating relatively high affinity between the adsorbent and the adsorbate [22]. The k_d values (calculated from a general linear isothermal model) were also higher in ML and GLR soils as compared to AH soil. Higher adsorption capacity of QACs in ML and GLR soils is in agreement with higher CEC and clay contents of these soils (Table 1). The adsorption in AH soil showed S-type isotherm exhibiting slower adsorption [22–24]. The Fe–Al oxides content in AH soil was higher than two other soils (Table 1). It is well known that the pH-dependent charges predominate on the surfaces of Fe–Al oxides. This results in high zero point charge (ZPC) on Fe–Al oxides. Therefore, positive charges might dominate on Fe–Al oxide surfaces and inhibit QACs sorption particularly in acidic AH soil [25]. However, AH soil had 4.11% organic carbon which is almost 2 times and 2.8 times higher than ML and GLR soils, respectively. As a consequence, the release of QACs from AH soil was minimum among the three soils studied (Fig. 2). High organic content of soils is known to cause irreversible adsorption of surfactant molecules in soils [23,24]. Although ML soil and GLR soil could adsorb significantly higher quantity of QACs due to their high CEC and clay contents, significant amounts of the sorbed surfactant were released subsequently. This could also be attributed to the types of clay minerals in these soils [26]. Although AH soil was lower in total clay content, the quantitative X-ray diffraction data showed relatively higher fraction of 2:1 type clays in AH soil as compared to ML and GLR soils (data not shown). Soils having higher percentage of 2:1 type clay minerals are supposed to adsorb QACs more strongly than soils having 1:1 type clay minerals or quartz as the dominant mineral species [26].

4.2. Effect of QACs on soil microbial activities

We found that all three surfactants studied showed toxicities to various extents towards dehydrogenase activity and potential nitrification in different soils. It was reported that aqueous phase HDTMA inhibited bacterial growth at concentration as low as $2.85\,mg\,L^{-1}$ [11,12]. Nye et al. [11] studied the effect of aqueous HDTMA on 11 pure cultures of bacteria isolated from a HDTMA treated fine loamy soil in addition to 5 pure bacterial cultures obtained from ATCC. The LC_{50} values of these bacteria to aqueous HDTMA ranged between 1.14 and $146.26\,mg\,L^{-1}$. Their study showed Gram-negative bacteria were extremely sensitive to HDTMA compared to Gram-positives. All the Gram-positive bacteria with the exception of *Arthrobacter globiformis* (ATCC 8010) exhibited at least 3-fold higher EC_{50} values than Gram negative-bacteria. However, the addition of smectite clay reduced the toxicity of HDTMA to bacteria suggesting bound HDTMA is unavailable to cause toxicity. In the present study, the LC_{50} values for dehydrogenase activity (ranging between 2.3 and $6.2\,g\,kg^{-1}$) and potential nitrification (ranging between 1.6 and $7.6\,g\,kg^{-1}$) in three different soils were generally high probably because of less availability of the QACs to microorganisms due to their adsorption to soil clay minerals. This phenomenon is supported by the sorption data of the QACs (Fig. 1 and Table 2). However, binding of QACs on acidic AH soil was inhibited to some extent due to the variable charge formation on Fe–Al oxide surfaces in this soil [25]. Nye et al. [11] also reported that the toxicity of HDTMA to soil heterotrophic microorganisms was controlled mainly by (a) group of microorganisms, (b) type of carbon source available and (c) type of soils. For example, aromatic hydrocarbon mineralising microorganisms were most affected by HDTMA, followed by 2,4-dichlorophenoxyacetic acid and salicylate degrading microorganisms, having least effect to glucose mineralising microorganisms [11]. The lag period preceding the mineralisation of these compounds increased accordingly [11]. It was found in the current study that the toxicity of QACs on dehydrogenase activity in soils followed the order: HDTMA > ODTMA > Arquad. However, the extent of toxicity to nitrification followed the order: Arquad > HDTMA > ODTMA. The toxicity patterns on dehydrogenase activity and potential nitrification are different probably because the former represents overall oxidative metabolic activity in soil [27,28], whereas the later is due to activity of a specific microbial community belonging mostly to the genus *Nitrosomonas* and *Nitrobacter* [29]. Also, the propylene glycol present as an impurity in commercial grade Arquad might have affected the soil nitrifiers which require further consideration. Arquad as commercial product is a widely used QAC in both household activities and organoclay manufacturing process. Due to its widespread use and cheap commercial availability, Arquad was included in the present toxicity evaluation study along with HDTMA and ODTMA.

4.3. Effect of soil types on the toxicity of QACs on soil microbial activities

We observed variable effects of the QACs on microbial parameters in three different soils studied. In our experiment, the AH soil showed higher dehydrogenase activity as compared to the other two soils. It could be attributed to the higher organic carbon content (Table 1) in this soil which accelerated soil metabolic activity [30]. Higher organic content of AH soil also caused irreversible binding of QAC molecules to the soil particles and thereby reducing QACs release and bioavailability to soil microorganisms [23,24]. GLR soil showed greater dehydrogenase activity than ML soil probably because the former was an agricultural soil receiving fertilisers which provided sufficient nutrients for the oxidative activity of the soil microorganisms [30,31], whereas the later was a paddock soil receiving no nutrient from external sources. In addition, desorption of HDTMA and ODTMA in ML soil (12.6 and 30.3%, respectively)

Table 4
Pearson correlation matrices (r^2) at 99% level of significance for soil microbial parameters and selected physico-chemical properties after two weeks of incubation of soils spiked with various doses of QACs.

Parameters	Organic carbon	Clay content	pH	CEC	Fe–Al$_{(CBD)}$	Fe–Al$_{(AO)}$	BET surface area	Bulk density
Dehydrogenase activity	0.956	NS	−0.925	−0.782	0.921	0.955	−0.858	NS
Potential nitrification	−0.791	NS	0.717	0.751	−0.810	−0.778	0.628	NS

CEC: cation exchange capacity; CBD: citrate–bicarbonate–dithionate extractable Fe and Al; AO: ammonium oxalate–oxalic acid extractable Fe and Al; NS: not significant.

was also significantly higher ($p < 0.05$) than GLR soil (7.9 and 15.8%, respectively). Desorption of Arquad was little higher in GLR soil than ML soil, but the difference was not significant ($p < 0.05$). However, the potential nitrification value was higher in GLR soil than ML and AH soils which suggested activity of nitrifiers were enhanced by fertiliser application. The 2 M KCl extractable NH_4-N content in GLR soil was 470 mg kg^{-1}, whereas for AH and ML soils were 417 and 427 mg kg^{-1}, respectively. The Pearson correlation matrices (r^2 at $p = 0.01$) for soil microbial parameters and physico-chemical properties after two weeks of incubation of soils spiked with various doses of QACs are shown in Table 4. Higher organic carbon content could significantly ($p < 0.01$) improve dehydrogenase activity in QACs spiked soils because organic carbon causes irreversible sorption of QAC molecules to the soil particles and thereby reducing QACs release [23,24]. Among the three soils studied in this report, AH soil had acidic pH and comparatively higher Fe–Al oxide contents (Table 1). Thus, AH soil could form positive variable charge on soil particles and reduce QACs sorption. However, the Pearson analysis results indicated that Fe–Al oxide content exhibited positive effects on soil dehydrogenase activity. Therefore, the toxic effect of QACs on soil dehydrogenase activity was more influenced by relative release of QACs in soil, not by the total quantity of QACs adsorbed. Higher binding strength of QACs in AH soil, due to its higher organic carbon content, might have reduced the QACs release thereby increasing the dehydrogenase activity in this soil. Moreover, the AH soil with its higher organic carbon content might have higher initial microbial biomass than the other two soils. On the other hand, the presence of Fe–Al oxides also might contribute to the increased sorption of QACs in soils with lower organic carbon content and neutral or alkaline pH. However, further work is required on the role of Fe–Al oxides on sorption of QACs in soils. The Pearson analysis results also showed that higher soil pH negatively affected the dehydrogenase activity which indicated that the added QACs did not have any buffering action on soil pH. If the QACs have some buffering action, they might have improved dehydrogenase activity by neutralising the acidity or alkalinity. Interestingly, the toxicity of QACs on soil dehydrogenase activity was not alleviated by higher CEC and BET surface area of soils probably because the toxicity was more influenced by the desorbed free QAC molecules in soils. The potential nitrification was affected differently than dehydrogenase activity by soil physico-chemical properties (Table 4) because the former is represented by a very sensitive and small group of microorganisms as opposed to total microbial community for the later [27–29]. Contrary to the dehydrogenase activity, higher CEC and BET surface area of soils could alleviate the toxicity of QACs on potential nitrification. Potential nitrification showed a positive correlation with soil pH ($r^2 = 0.72$, $p = 0.01$) (Table 4), which supported earlier evidence that pH in the range of 6–8 could enhance nitrification rate in soils [32]. The pH of the soils in the present study ranged from 5.5 to 7.8. Optimum pH for soil nitrification is 6.6–8 or higher and nitrification activity is reduced at pH below 6 and becomes negligible at pH below 5 [29,32].

This study suggests that dehydrogenase activity and potential nitrification in some soils might be improved by QACs when the chemicals were present at low concentrations (Figs. 3a–c and 4a–c). An organic chemical, generally considered as a toxicant, can sometime enhance soil microbial growth and activity when it is present at very low concentration. In this case, the organic toxicant in question acts as a carbon source to the soil microorganisms. Naturally, this kind of effect, which is also called the priming effect, would be more prominent in an organic carbon deficient soil. At low concentrations, Arquad was most effective to enhance soil metabolic activity probably because Arquad which consisted of di(hydrogenated tallow) dimethylammonium moiety was more easily biodegradable than the other two surfactants [1]. However, the potential nitrification was more enhanced by ODTMA at low concentrations.

4.4. Effect of type of QACs on microbial activities in soils

In the current study, different surfactant molecules showed different levels of toxicities on dehydrogenase activity and potential nitrification in soils due to the variable structure and chain length of the surfactants. The alkyl chain length not only determines the physico-chemical properties (water solubility, octanol/water partition coefficient, adsorption/partition coefficient on sediments, sludges and soils) of a surfactant [33], but also may have a decisive role in the fate and effects of these compounds on microorganisms in the environment. HDTMA has long straight alkyl chain having 16 C atoms, whereas the alkyl chain in ODTMA is longer and constituted of 18 C atoms. In both the QACs, the short alkyl chains associated with the positively charged N atom are represented by three methyl groups. The molecular structure of Arquad, which contains di(hydrogenated tallow) dimethylammonium moiety, is different than the other two surfactants. The tallow is basically an animal fat consisting of glycerol esters of oleic, palmitic and stearic acids (16–18 C atoms). Being a fatty acid ester of animal origin, Arquad would have more biodegradability than the other two QACs examined in this study. For this reason, Arquad was found less toxic to soil dehydrogenase activity although its desorption percentage was higher than the other two surfactants. However, further research is needed to study the relative biodegradability of these QACs by soil microorganisms. The toxicity of QACs on soil dehydrogenase activity in the present study supported previous report by Nye et al. [11] who also observed more toxicity of HDTMA (16 C atoms in single alkyl chain) than other monoalkyl cations such as nonyltrimethyl ammonium (9 C atoms in single alkyl chain), dodecyltrimethyl ammonium (12 C atoms in single alkyl chain) and dioctadecyldimethyl ammonium (18 C atoms each in two alkyl chains). However, higher degree of toxicity imparted by Arquad on potential nitrification in soils was in agreement with higher desorption of this surfactant in all three soils.

5. Conclusion

It could be concluded from the current study that the toxicity of QACs on the dehydrogenase activity and potential nitrification in different soils followed the order: HDTMA > ODTMA > Arquad and Arquad > HDTMA > ODTMA. When present at low concentration, the QACs even could enhance the dehydrogenase activity and potential nitrification in some soils. The nature of QAC molecules and the organic carbon content of soils appear to be the main responsible factors for the observed toxicity of these chemicals. The toxic effect of QACs on soil microbial activities was more

influenced by relative release of QACs in soil, not by the total quantity of QACs adsorbed. This study provides valuable information on the toxicological properties of some widely used QACs on important soil microbial activity parameters and may have implications to QAC-assisted remediation of contaminants in soils. The soil microbial activities studied are very important processes for agriculture and soil health and hence can be used for judging soil quality as affected by QACs which are commonly used in household activities and industries. Further studies should be directed towards the investigation of (a) biodegradability of QACs by soil microorganisms, and (b) long-term effects of QACs on soil microbial diversity and functions under different environmental conditions.

Acknowledgements

Binoy Sarkar is thankful to the University of South Australia for the award of University President Scholarship and Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) for the award of CRC CARE PhD fellowship. The authors would like to acknowledge the financial and infrastructural support from CRC CARE and the Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia. Suggestions from anonymous reviewers to improve the manuscript are also gratefully acknowledged.

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