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Toxicity of organoclays to microbial processes and earthworm survival in soils

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HIGHLIGHTS

- Organoclays are toxic to soil dehydrogenase and nitrification activities.
- They are also toxic to soil inhabiting earthworms.
- Extent of toxicity depends on the type, structure and chain length of the surfactants.
- Application rates of organoclays to soils also affect the toxicity levels.

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ABSTRACT

Organoclays have wide spread application in environmental remediation and nanocomposites synthesis. Some of the quaternary ammonium compounds (QACs) commonly used to prepare organoclays are toxic to biota. However, information on the toxicity of organoclays is rarely available in the literature. This study assessed the toxicity of three laboratory prepared bentonite organoclays on the soil microbially mediated processes (such as dehydrogenase activity and potential nitrification) and soil inhabiting animals, such as earthworms. Toxicity to both microbial processes and earthworm followed the order: hexadecyltrimethyl ammonium modified bentonite > octadecyltrimethyl ammonium modified bentonite > arquad modified bentonite > unmodified bentonite. The organoclays were able to cause slight improvement (up to 25%) in the potential nitrification in some soils when they were added at low application rates up to 5%, but caused reduction (3–86%) in the dehydrogenase activity in all the soils irrespective of loading rates. The organoclays were extremely toxic to the survival and vigour of the earthworms. The average body weight loss of the worms reached as high as 62% in hexadecyltrimethyl ammonium modified bentonite treated soil even at 1% loading. This study holds utmost importance in assessing the toxicity of organoclays to soil microbially mediated processes and earthworms.

1. Introduction

The use of organoclay minerals for remediating contaminants in soil and water are widely reported during the last two decades. These modified clay minerals are prepared by introducing organic molecules into the clay mineral structure. The group of organic molecules most commonly used to prepare organoclays is quaternary ammonium compounds (QACs) which are also known as surfactants. Chemically, they are organically substituted ammonium compounds, in which the nitrogen atom has a valence of 5, four of the substituent radicals (R1–R4) are alkyl or heterocyclic radicals of a given size or chain length, and the fifth (X) is a halide, sulphate or similar radical. The QAC molecules contain at least one hydrophobic long alkyl chain attached to the positively charged nitrogen atom and other alkyl groups are mainly short chain substituents. Due to modification of natural clay minerals with QACs, the resultant organoclay minerals become hydrophobic in nature. When prepared with QAC concentrations greater than the cation exchange capacity (CEC) of the clay minerals, the organoclay minerals can also exhibit positive charges on their surface [1,2]. The organoclay minerals are efficient adsorbents of many organic and inorganic pollutants [1–7]. They are also used for the synthesis of various polymer nanocomposites [8].

Available literature indicates that the QACs can exhibit significant harmful effects to the living organisms including bacteria, protists and animals [9–16]. For example, QACs exhibited inhibitory effects on anaerobic biogas producing bacteria and the toxic effects increased with decreasing alkyl chain length of the QACs [9,14]. However, acute toxicity on a fresh water amphipod *Echinogammarus tibaldii* increased as a result of an increase in alkyl chain length and head group hydrophobicity of a single chain QAC [12].

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The toxicity of QAC molecules involves both acute effects and chronic effects like DNA damage and mutagenicity [13,15,16]. The QACs commonly used to prepare organoclay minerals can also cause toxic effects to the soil inhabiting microorganisms [17–21]. For example, the LC_{50} values of heterotrophic soil bacteria to aqueous hexadecyl trimethylammonium (HDTMA) cation ranged between 1.14 and 146.26 mg L^{-1} and Gram-negative bacteria were more sensitive to the QAC compared to Gram-positive bacteria [19]. The presence of clay mineral could partially reduce the toxicity by binding the HDTMA molecules on its surface [19]. Free QACs in soil could also impact the microbial community responsible for specific soil function such as nitrification [17]. However, very limited information is available on this. The extent to which the interaction of QACs with clay minerals can reduce the toxicity to soil microorganisms is also unknown.

One important issue concerning the use of organoclay minerals for environmental remediation is the potential toxicity of these modified materials to the native microorganisms in the environment including the beneficial microbes that are involved in biodegradation of contaminants [6,17,21]. Thus, organoclays may potentially hinder the natural attenuation process. Some organoclay minerals specially the quaternary phosphonium and cetylpyridinium derivatives of clay minerals are reported to have antimicrobial activities [22–24]. However, the antimicrobial activities of quaternary ammonium organoclay minerals, which are most commonly used for environmental remediation purposes, were not studied in detail [24–26]. Although the release of clay-intercalated surfactant molecules into the soil is unlikely, still it is uncertain that these modified clay minerals are harmless to soil microorganisms. So far only one study attempted to evaluate the toxicity of organoclays on the soil eubacterial community, but the interpretation was not decisive because some organoclays showed toxic effects whereas others stimulated bacterial growth [21]. In addition, organoclay minerals are prepared from numerous QACs which differ in their individual chemical structure and properties. The fate, biodegradability and toxicity of QACs vastly depend on their alkyl chain length, chemical structure and properties [17,27]. Therefore, it is necessary to investigate the toxicity of organoclays prepared with those QACs in order to ensure the environmental friendliness of the modified clay products. The toxicity of the clay minerals modified with QACs towards soil microbially mediated processes and functions and also on the soil inhabiting animals is totally unknown. Hence, this study investigated the influence of three laboratory prepared organoclays on the dehydrogenase activity and potential nitrification in soils and also soil inhabiting earthworms.

2. Materials and methods

2.1. Reagents and organoclays

Organoclays were prepared using bentonite clay (QB) obtained from Queensland, Australia. The cation exchange capacity (CEC) of the bentonite was 66.7 cmol (p^+) kg^{-1}. Three QACs, supplied by Sigma–Aldrich, were used to prepare the organoclays, namely, hexadecyl trimethylammonium bromide (HD), octadecyl trimethylammonium bromide (OD) and Arquad® 2HT-75 (di(hydrogenated tallow) dimethylammonium chloride (~75%) with 2-propanol (~14%) and water (~11%) impurity; denoted as Aq). Arquad is a commercially available relatively cheap surfactant, and less detrimental to soil microorganisms than HD and OD [17]. The organoclays were prepared at surfactant loadings equivalent to the CEC of the clay as described earlier [4]. On molar basis the surfactant loadings corresponded to about 0.67 mM g^{-1}. The three organoclays thus obtained were denoted as QB–HD, QB–OD and QB–Aq.

2.2. Characterisation of organoclays

The prepared organoclays were characterised by X-ray diffraction (XRD) using CuKα radiation (λ = 1.5418 Å) on a PANalytical, Empyrean X-ray diffractometer (PANalytical, Australia) operating at 40 kV and 40 mA between 2.0° and 80° (2θ) at a step size of 0.016°. The organoclays were also characterised by Fourier transformed infrared spectroscopy (FTIR). Infrared (IR) spectra were obtained using an Agilent Cary 600 Series FTIR Spectrometer (Agilent Technologies, USA) equipped with DRIFT (Diffuse Reflectance Infra-red Fourier Transform) accessories. Spectra over the 4000–400 cm^{-1} range were obtained by the co-addition of 64 scans with a resolution of 4 cm^{-1}. The micro-morphology of the bentonite and the organoclays were examined under a scanning electron microscope (SEM) (FEI Quanta 450 FEG ESEM). Images were taken in high vacuum mode and with a 20 kV accelerating voltage using an Everhart–Thornley detector (ETD).

2.3. Soils

Three surface soils having different physico-chemical properties were collected from the 0–10 cm depth at three locations, namely Adelaide Hills, Mawson Lakes and Gawler in South Australia. Selected physico-chemical properties of the experimental soils are listed in Table 1 [17]. After collection, the soils were mildly ground to pass through 2 mm sieve and stored at 4 °C temperature for further use.

2.4. Organoclay treatments and incubation

Microcosms were set up in triplicate with 5 g field moist soil placed in 50 mL polypropylene centrifuge tubes. Organoclays were added to the soils at 1, 5 and 10% (w/w) loading rates. The loading concentrations were selected to simulate conditions usually used to stabilise contaminants in soils by applying organoclays (as high as 20%) [7,28]. In another set of microcosms, soils were treated with unmodified bentonite at similar loadings. A control microcosm having only soils without any clay mineral treatment was also maintained. The organoclays were uniformly mixed with the soils by agitating the centrifuge tubes in an end-over-end shaker for 24 h. The microcosms were then incubated at 23 °C for 30 days with the caps loosely closed. To facilitate optimum growth and proliferation of the microorganisms throughout the experiment, all the treatments and controls were maintained at 70% of the total moisture holding capacity of the soils.

2.5. Soil analysis

After incubation, soil microcosms were analysed for dehydrogenase activity, potential nitrification, pH and electrical conductivity (EC). The dehydrogenase activity was measured by determining the rate of triphenylformazan (TPF) production by the soils treated with triphenyltetrazolium chloride (TTC) [17,29]. The assay of potential nitrification was based on the production of nitrite (NO_2^-) by the soils treated with ammonium sulphate [$(NH_4)_2SO_4$] [17,30]. The pH and EC of the treated soils were analysed in 1:2.5 (w/v) soil–water suspension and are shown in Table 2.

2.6. Earthworms experiment

The soil with nearly neutral pH (pH = 6.65) collected from Mawson Lakes, South Australia was used in the earthworm experiment. Triplicate microcosms were set up by placing 150 g soil in 500 mL clear glass jars. The organoclays were applied to the soil at 1, 5 and 10% (w/w) loading rates and uniformly mixed in an end-over-end shaker. The moisture content of the treated soils were maintained