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Clayey materials in river basin enhancing microbial contamination of river water

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ABSTRACT

Mineral constituents of clay materials may promote interaction, adsorption and attachment of microorganisms, often resulting in biofilms' formation. In this study investigation is made to determine how littoral clayey materials on the shores of a river promote accumulation of bacteria and increase contamination of river water. Clayey samples were collected at various points along the shore of a river around Mondeor in Johannesburg and the mineralogical composition was determined using XRD and XRF. Microorganisms in clay-biofilm and river water were identified by DNA sequencing and plate count. Results showed that total coliforms, *Escherichia coli*, *Pseudomonas sp.* and presumptive indigenous microorganisms attached to littoral clayey materials containing the mineral muscovite (characterising argillaceous soils). Bacteria number on clayey materials was significantly higher than on overlying water especially before rainy season. However a decrease of the number of bacteria in clayey materials concurrent with an increase in the number of suspended bacteria after rain events, was the result of the action of high and fast flows in the basin, eroding the biofilms. Attachment of microorganisms in clayey material as observed in this study could be ascribed to the glue-like aspect of soil (due to muscovite) that facilitates adhesion. It therefore demonstrates the potential of clayey materials to encourage biofilm formation and enhance microbial contamination of river water as shown here.

1. Introduction

Biofilm structures often develop on clayey material along the river basin. This development can result from adherence of microorganisms directly to the surface via appendages that extend from the cell membrane, while other bacteria may form a capsular material of extracellular polysaccharides (EPS), sometimes called a glycocalyx that anchors the bacteria to the surface (Geldreich, 1988). Biofilm is initiated by seed microorganisms, and rapidly becomes a complex microenvironment, that encompasses processes such as metabolism, growth and product formation, and finally detachment, erosion, or "sloughing" of the biofilm from the surface. Most bacteria exist in natural habitats in the form of biofilm. They attach to solid surfaces in order to have access to nutrients in the overlying liquid or from the solid matrix. Adhesion of bacteria to surface is facilitated by exopolymeric substances, but hydrodynamic conditions and the availability of substrate and nutrients can shape biofilm architecture (Battin et al., 2007; Perdrial et al., 2009). Physical and chemical interactions at bacteria-mineral interfaces often determine the force of bacterial adhesion, and are due to several factors. These factors include the mosaic of spatially discrete macromolecular cell envelope structures on bacteria, the dynamic nature of these structures imposed by various environmental conditions and the diversity of mineral surface functionality and crystallography. When bacteria and minerals are separated by some finite distance and by adhesion forces, the cumulative effects of interfacial forces determine the interactions (Israelachvili and McGuiggan, 1988; Israelachvili, 1992; Kendall, 1994; Butt et al., 1995; Fletcher, 1996; Gay and Leibler, 1999; Lower et al., 2000).

Clayey materials could provide suitable surface for bacteria adhesion, as they have high specific surface area which allows physical and chemical interactions, but the adsorption capabilities also result from the net negative charge on the structure of minerals and their porosity (Nayak and Singh, 2007). In some instances clayey materials can be the source of carbon and cations require by microorganisms. There are several groups of clays, including kaolin, smectite, clay-mica and chlorite groups. Attachment of microbial cells on clay surface has been investigated by some

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researchers in order to determine the adsorption capabilities of some minerals as well as the preferential bindings (Perdrial et al., 2009; Cao et al., 2011).

Biofilms can be composed of many different organisms, but usually bacteria comprise the largest portion of the biofilm population. Some heterotrophic bacteria that live in biofilms may cause exthetic problems with water quality, including off-tastes, odours, and coloured water problems; Geldreich (1990) identified some of these bacteria as Actinomyces, Streptomyces, Nocardia and Arthrobacter. Few of the microorganisms present in biofilm may pose a serious threat to human health as they may be 1000-fold more resistant to antibiotic treatment than the same organism grown planktonically (Gilbert et al., 1997; Davey and O'Toole, 2000). Bacteria, viruses, fungi, protozoa, and other invertebrates have been isolated from drinking water biofilms (USEPA, 1992).

Biofilms occurring on clayey material in river basin can be washed away or eroded during heavy rains and normal river flow contributing to further degradation of the quality of river waters through contamination with bacteria. These river waters sometimes happen to be the only source of water available and used by communities in poor urban areas of South Africa (Fosso-Kankeu et al., 2008).

The focus of this study was to characterise and analyse clayey materials encountered along the shore and in the basin of Mondeor River and correlate their mineralogical and physico-chemical properties with the occurrence and abundance of microorganisms in biofilms on the shore of the river.

2. Methodology

2.1. Sampling of water and clay

Both water and clay samples were collected at the beginning and towards the end of the rainy period of summer's season in South Africa. This was done to determine the possible contribution of clay in microbial contamination of river water following rain events.

Water samples were collected manually in 500 mL sterile plastic bottle at eight selected sampling points along the length profile of the river in Mondeor-Johannesburg. Samples were kept cold during transportation and immediately analysed upon arrival in the laboratory to minimise the possible changes in the samples and contamination.

At each water sampling point, clayey materials (about 500 g) were collected from the adjacent shore. A metallic forceps was used to dislodge the clayey material which were collected in sterile plastic bags then stored in a cooler bag during transportation.

2.2. Mineralogical characterisation of clayey materials

2.2.1. Samples preparation

Clay samples were dried in an oven (Digital Oven 700 L, model code 385) at 100 °C for five hours and pulverised using a Sibstechnik pulveriser (GMBH-Melheim-RUHR, Labor-Schobenschwingmuhle, type 250) to obtain finer powders.

2.2.2. XRD analysis

Powdered clayey materials were screened in a sieve and the particles <75 μm were recovered, then loaded into the XRD sample holder and the XRD carried out. The diffractometer used was the Philips model X'Pert pro MPD, equipped with a 15 place automatic sample changer. The utilisation parameters were copper anode tube with a power of 1.6 kW used at 40 kV, 40 mA; Programmable divergence and anti-scatter slits; primary Soller slits: 0.04 Rad; 2θ range: 4–79.98; step size: 0.017°; X'celerator detector with a nickel filter and sample spinner.

2.2.3. XRF analysis

Powdered clayey materials were mixed with binder, grinned and pelletized in an aluminium cuvetted for uniform grain size suitable for XRF analysis. The XRF was performed on the MagiX PRO & SuperQ Version 4 (Panalytical, Netherland); the sample was suspended above the X-ray tube in a two position carousel in a holder. A rhodium (Rh) anode was used in the X-ray tube and operated at 50 kV and current 125 mA; at power level of 4 kW. The X-ray spectra were evaluated with the IQ + program which is part of SuperQ.

2.3. Physico-chemical and microbiological analyses

2.3.1. Physico-chemical analysis

The temperature and pH of the water samples were measured in situ using the portable Eutech Instruments Cyberscan pH11 Waterproof pH meter (made in Singapore). Turbidity informs about the clarity of the water related to the presence or not of soils, organic and inorganic matters as well as microorganisms in water; this parameter was measured in the laboratory according to standard methods (APHA, AWWA, WEF, 2005) using a Nephla turbidity meter (Dr. Lange GmbH-Berlin).

2.3.2. Heterotrophic microorganisms

To determine the presence of microorganisms in water samples, one millilitre of the solutions was serially diluted in phosphate buffered saline (0.01 M, pH 7.4 at 25 °C), the spread plate method was then used to inoculate 0.1 L of the enriched solution onto Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 37 °C overnight. For clayey samples, one gram was suspended in ten millilitres of phosphate buffered saline (0.01 M, pH 7.4 at 25 °C) and one millilitre of the resulting solution serially diluted in phosphate buffered saline (0.01 M, pH 7.4 at 25 °C) and then plated as above. Colonies were identified based on their respective colour on the plate as specified by the manufacturer and counted manually according to standard methods (APHA, AWWA, WEF, 2005). Unknown colonies were subcultured on supplemented *Pseudomonas* agar base or identified by DNA sequencing technique at Inqaba Biotech, SA.

2.3.3. Phytoplankton

The surface of clayey materials at the shore of Mondeor River was collected and suspended in sterile distilled water and later fixed with formaldehyde (2% v/v). Aliquot (1 mL) of each sample was then poured into the sedimentation chamber and centrifuged, to allow algal cells to settle to the bottom. Thereafter, the cells were then identified and enumerated using an inverted light microscope according to the procedure described by Swanepoel et al. (2008).

2.4. Statistical analysis

The experiments were conducted in triplicate and data recorded on a Microsoft Excel® spreadsheet. Difference between parameters influenced by rain events was determined by statistical method of Mann Whitney test (Helsel and Hirsch, 2002; Hodges and Lehmann, 1963). The difference was deemed significant when p value was <0.05.

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