

Assessment of the pro-inflammatory activity of water sampled from major water treatment facilities in the greater Pretoria region

SA Adebayo¹, LJ Shai^{1*}, MC Cholo², R Anderson² and D du Toit¹

¹Dept of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

²Medical Research Council Unit for Inflammation and Immunity, Dept of Immunology, Faculty of Health Sciences, University of Pretoria, and Tshwane Academic Division of the National Health Laboratory Service, Pretoria, 0001, South Africa

ABSTRACT

Notwithstanding direct detection of microbial/viral pathogens or their associated toxins, the quality of drinking water may also be evaluated according to its pro-inflammatory potential. In this latter setting, contamination with pathogens or their products is determined according to the magnitude of activation of blood-derived immune/inflammatory cells following exposure to test water samples *in vitro*, usually by monitoring the synthesis of pro-inflammatory cytokines. The primary objective of the current study was to apply this procedure to evaluate the pro-inflammatory potential of water sampled at entry, as well as at various stages of treatment, from 3 major water treatment facilities in the greater Pretoria region, viz., the Daspoort, Hartbeespoort, and Rietvlei Water Treatment Facilities. Control water samples included domestic tap water, bottled water from a commercial source, and distilled water. Peripheral blood mononuclear leukocytes (MNL) were isolated from the blood of healthy, adult, human volunteers ($n=3$), enumerated, suspended in tissue culture medium RPMI 1640 containing antibiotics at a concentration of $1 \times 10^6/\text{mL}$, and exposed to the various water samples (10%) for 18 h at 37°C. Following incubation, the cell-free supernatants were assayed for the cytokine, interleukin-6 (IL-6), using a quantitative, sandwich, enzyme immunoassay procedure. The mean values for the untreated control system and for a positive control system exposed to bacterial endotoxin (120 ng/mL) were 153.5 ± 17 and $1\,561 \pm 30$ pg/mL, respectively ($p=0.03$). The production of IL-6 was unaffected following exposure of MNL to the control water samples. However, inlet water sampled from all three facilities, especially Hartbeespoort, resulted in significant activation of production of IL-6 by MNL, which declined with progressive treatment, consistent with removal of pro-inflammatory contaminants. Surprisingly, however, a rebound in pro-inflammatory activity was evident in outlet water sampled from Hartbeespoort. In conclusion, the results of the current study appear to support the efficiency of water treatment procedures at the Daspoort and Rietvlei Treatment Facilities, while confirming the usefulness of IL-6-based assays as adjuncts to conventional water quality testing procedures.

Keywords: Endotoxin, inflammatory activity, interleukin-6, mononuclear leukocytes, water quality

INTRODUCTION

Traditional water quality testing techniques test for the presence of indicator organisms (Hunter, 2003). However, certain substances such as endotoxin may still be present in the water, and are rarely detected by traditional methods (Wichmann et al., 2004; Gorbet and Sefton, 2005; Slabbert et al., 2008; Pool, 2008). The inflammatory activity in environmental water samples has been investigated using *in vitro* human immune response tests, specifically the production of pro-inflammatory cytokines by whole blood or isolated leukocytes (Pool et al., 2003; Wichmann et al., 2004; Pool and Magcwebeba, 2009). In the study reported by Wichmann et al. (2004), exposure to treated drinking water samples did not induce cytokine secretion by human leukocytes *in vitro*, while contrasting effects were observed following exposure to all the river water samples tested, confirming the findings of an earlier study (Pool et al., 2003). These latter authors reported that induction of secretion of the cytokine, interleukin (IL)-6, was dependent on the concentration of the contaminant(s) and origin of the

water samples. More recently, exposure of whole blood from healthy adult human volunteers to hydrophobic extracts of contaminated surface water samples resulted in the synthesis of pro-inflammatory IL-6, while suppressing biomarkers of both cell-mediated and humoral immunity (Pool and Magcwebeba, 2009). In addition, several reports have documented that treated drinking water samples, sewage effluents entering river water consumed by humans, and river water treated with insecticides, possess pro-inflammatory activities (Pool, 2008; Slabbert et al., 2008; Mompelat et al., 2009). Clearly, difficult-to-detect pro-inflammatory substances in water may pose a threat to public health.

With increasing awareness of this threat, the measurement of the pro-inflammatory activity of drinking water from various sources is clearly a useful adjunctive strategy to complement traditional testing procedures, as described in several previous reports alluded to above (Pool et al., 2003; Poole et al., 2003; Wichmann et al., 2004; Pool and Magcwebeba, 2009). Although these procedures have been used to assess the human health-related quality of water from various sources in several provinces in South Africa, they have not, to our knowledge, been used in the comparative analysis of water from major treatment facilities in Gauteng Province. Accordingly, the primary objective of the current study was to assess the pro-inflammatory activity of water samples from the Daspoort, Hartbeespoort, and Rietvlei treatment facilities, using

* To whom all correspondence should be addressed.

+27 12 382 6342, fax: +27 12 382 6262;

e-mail: ShaiLJ@tut.ac.za

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