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Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa

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ABSTRACT

The use of wastewater for irrigation is widely practiced in developing countries such as South Africa due to local fresh water scarcity, but little is understood in these developing nations about the potential risks associated with its use. This study was conducted to establish potential links between river water quality and the microbiological quality of fresh produce irrigated with this water. Selected physico-chemical (pH, temperature and chemical oxygen demand) and microbiological parameters (aerobic plate count, total coliforms, fecal coliforms and *Escherichia coli*) of irrigation water and fresh produce were analyzed. The samples were collected from the Baynespruit River in Sobantu, a sub-urban area in Pietermaritzburg consisting of low cost housing. Fecal coliform counts frequently exceeded the recommended maximum values suggested by the World Health Organization and the South African Department of Water Affairs for safe irrigation, as well as the recommended maximum values set by the Department of Health (South Africa) for consumption of raw produce. Fecal coliform counts of up to 1.6×10^6 ($\log=6.20$) per 100 ml of irrigation water and 1.6×10^5 ($\log=5.20$) per gram of produce were observed. This indicates that fecal matter might have entered the Baynespruit River and that microbes present therein can be transferred via irrigation to fresh produce.

1. Introduction

Africa is experiencing large scale urban growth (De Bon, Parrot, & Moustier, 2010). As a result, the agricultural sector is facing increased pressure to produce sufficient quantities of food to meet the growing demand (Addo, 2010; De Bon et al., 2010). Coupled with climate change scenarios causing reduced rainfall, this may lead to diminishing availability and quality of water sources (Jorgensen, Graymore, & O'Toole, 2009). African countries are therefore facing food and water security problems as well as water safety issues (Costello et al., 2009) as poor water quality is estimated to cause about 80% of illness and death in the developing world (Schaefer, 2008). In South Africa, the increasing population, urbanization and industrialization has resulted in a large proportion of mostly rural communities lacking adequate sanitation, waste disposal and access to potable water (Drechsel, Graefe, Sonou, & Cofie, 2006; Nevondo & Cloete, 1999; Obi, Potgieter, Bessong, & Matsaung, 2002). They therefore use available water sources such as rivers for their daily water needs (Nevondo & Cloete, 1999; Obi et al., 2002). The lack of adequate sanitation results in fecal contamination of these water sources leading to serious consequences for human and environmental health (Drechsel et al., 2006; Germer, Boh, Schoeffler, & Amoah, 2010; Ibenyassine, Aitmhand, Karamoko, Cohen, & Ennaji, 2006; Schaefer, 2008; Scott, Faruqui, & Raschid-Sally, 2004). Rural community members in South Africa are exposed to water-borne diseases and by using fecally contaminated water for irrigation, both subsistence and commercial farmers are potentially reducing the microbiological quality and safety of fresh produce (Drechsel, Graefe, Sonou & Cofie, 2006; Keraita, Drechsel, & Konradsen, 2008; Mara, Sleigh, Blumenthal, & Carr, 2007; Obi et al., 2002; Shuval, Adin, Fattal, Rawitz, & Yekutiel, 1986). Depending on climatic conditions, up to 100 l of water are required to produce 1 kg of tomatoes. Given diminishing freshwater resources the use of potable water for irrigation is not a feasible option (De Bon et al., 2010). Consequently, developing countries are becoming increasingly reliant on the use of wastewater for irrigation (Amoah, Drechsel, & Abaidoo, 2005; De Bon et al., 2010; Huibers & van Lier, 2005; Seidu et al., 2008). Although the use of wastewater can be beneficial by providing nutrients for crops thereby saving fertilizer costs (De Bon et al., 2010; Raschid-Sally, Carr, & Buechler, 2005; Van der Hoek et al., 2002), the potential health risks associated with its use need to be

Abbreviations: BGLB, Brilliant-green lactose bile broth; COD, Chemical oxygen demand; DOH, Department of Health (South Africa); DWAF, Department of Water Affairs and Forestry; EC Broth, *Escherichia coli* broth; L-EMB, Levine Eosine Methylene Blue; *Gada*, Glutamate decarboxylase A; LST, Lauryl Sulphate Tryptose broth; MPN, Most probable number; MUG, 4-methylumbelliferyl-β-D-glucuronide; PCR, Polymerase chain reaction; SANS, South African National Standards; UV, Ultra violet; WHO, World Health Organization.

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considered (Hamilton et al., 2007; Huibers & van Lier, 2005; Mara et al., 2007; Seidu et al., 2008; Shuval et al., 1986; Toze, 2006).

There is sufficient evidence for the presence of excreted pathogens on the surface of vegetables irrigated or fertilized with products related to fecal matter (Beuchat, 2002). Shuval et al. (1986) established that bacteria, viruses, protozoa and helminths can survive for days, weeks or even months on crops irrigated with microbially contaminated wastewater. The presence of pathogens in wastewater or contaminated sources such as rivers is therefore a serious concern (Mrayyan, 2005) in view of irrigating minimally processed crops such as lettuce, spinach and parsley (Blumenthal & Peasey, 2002; Keraita, Konradsen, Drechsel, & Abaidoo, 2007).

As it is virtually impossible to identify all potential pathogens present in water sources and on produce samples, selected indicator organisms are often used in risk assessments (Maimon, Tal, Friedler, & Gross, 2010) as an indicator for the presence of fecal contamination. The presence of fecal coliforms is usually assumed to indicate the potential presence of other fecal pathogens such as *Salmonella* spp., *Shigella* spp. or pathogenic strains of *Escherichia coli* (DWAF, 1996b; Maimon et al., 2010; Mara et al., 2007; WHO, 1989, 2006). These organisms can cause gastroenteric illnesses via the fecal/oral route through the consumption of raw produce irrigated with contaminated water (DWAF, 1996b). According to South Africa's department of Water Affairs (DWAF, 1996a) there is a correlation between the risk of being infected with the degree of produce contamination and the quantity of contaminated produce consumed. Therefore, higher counts of fecal coliforms in irrigation water can indicate an increased risk in contracting a waterborne disease, even if small quantities of this produce are consumed raw.

In this study, indicators of microbiological pollution (aerobic plate counts, total coliforms, fecal coliforms and *E. coli*) were used to assess the microbial quality of water sources used by rural communities in Pietermaritzburg (KwaZulu-Natal) in comparison with recommended guideline values (DOH, 2001; DWAF, 1996b; WHO, 1989, 2006). In addition, the microbiological quality of fresh produce irrigated with this water source was assessed to establish whether a transfer of potential pathogens from irrigation water onto fresh produce might take place.

2. Materials and methods

2.1. Study site

The study site (29°35′33.86″S, 30°25′12.73″E) is a sub-urban community (Sobantu) in the Pietermaritzburg region of KwaZulu-Natal, a province with a high number of informal settlements living adjacent to surface water sources such as rivers. The Baynespruit River is commonly used by communities along its course to irrigate small-scale market gardens (the produce is typically sold in the city center) and subsistence gardens. Irrigation is conducted manually with watering cans or buckets directly onto the produce. Crops studied because of their ease to cultivate comprised spinach, cauliflower and parsley which are known to be frequently consumed raw. Analyses were carried out for 13 months in both rainy (January to March 2010 and October 2010 to January 2011) and dry seasons (April to September 2010). Irrigation of the produce with the Baynespruit River water continued every morning until harvest day, thereby mimicking the procedure employed by local community members.

2.2. Sample collection

2.2.1. Water sampling

River water samples were collected once a month at the same location between 8 am and 11 am in a sterile 1 l Schott glass bottle from the river source at the same point that irrigation water for the produce is extracted. Water was obtained from areas of fast flow at a depth half that of the total in order to avoid debris and collecting exclusively surface water. Samples were transported on ice and analyzed within 2 h.

2.2.2. Produce sampling

Once a month, produce samples (the choice of which was dependent on their respective availability i.e. the growing season) were collected at the study site aseptically into a sterile Erlenmeyer flask by randomly removing not less than 20 g of produce material (leaves in the case of spinach and parsley and florets in the case of cauliflower) from at least three different plants. To avoid soil based contamination, material furthest away from the soil surface was harvested. Samples were stored on ice before 10 g of produce material was analyzed within 2 h.

2.2.3. Microbiological analysis

River water samples (10 ml) were aseptically pipetted into a sterile Erlenmeyer flask and diluted tenfold by adding 90 ml of quarter-strength Ringers solution followed by subsequent decimal dilution (up to 10^{-5}) using the same diluent. Produce samples were prepared for subsequent analyses by adding 90 ml quarter-strength Ringers solution to 10 g of uncut leaf material (or florets in the case of cauliflower) in a sterile Erlenmeyer flask followed by a 10 min treatment on an orbital shaker at 200 rpm at ambient temperature prior to decimal dilution (up to 10^{-5}) using the same diluent as above. Aerobic plate counts for water and produce were conducted in triplicate according to the South African National Standards (SANS, 2007) procedure 4833 using plate count agar as specified therein. Results are expressed as the weighted mean ($\log_{10}$) with the calculated standard error indicated. Total and fecal coliforms were enumerated by using a MPN (most probable number) method according to the method MFHPB-19 suggested by Health Canada (2002). This involved an initial presumptive test in Lauryl Sulphate Tryptose broth (LST) (Oxoid) and a confirmation test by inoculating Brilliant-green lactose bile broth (BGLB) (Merck) with one loopful from the gas-positive LST tubes. *Escherichia coli* was quantified by inoculating gas-positive LST tubes into *E. coli* (EC) broth (Merck) containing 4-methylumbelliferyl-β-D-glucuronide (MUG). Fluorescence under ultra violet (UV) light, visible growth and the production of gas indicated a positive test, and *E. coli* presence was confirmed using Levine-Eosin Methylene Blue (L-EMB) agar (Conda) and the prescribed biochemical tests (i.e. gas production at 45 °C (G), indole formation from tryptophane (I), Methyl-Red (M), Voges-Proskauer (Vi) and Simmon's citrate assimilation (C) = GIMViC). The results are expressed as $\log_{10}$ MPN counts per 100 ml of river water or gram of produce sample with a 95% confidence interval established according to Garthright and Blodgett (2003).

In addition to the above procedure, the presence of *E. coli* was confirmed using a PCR based amplification of the *gadA* gene (glutamate decarboxylase A) according to Kim, Demeke, Clear, and Patrick (2006). Aliquots of EC broth showing gas formation and/or UV fluorescence (phenotypic features such as the β-glucuronidase activity are not always expressed and sometimes repressed on carbohydrate rich media (Grant, Weagant, & Feng, 2001; Fricker, DeSarno, Warden, & Eldred, 2008)) after 48 h of incubation at 45 °C, as well as typical *E. coli* colonies obtained from L-EMB agar were analyzed. DNA was extracted by a simple freeze–thaw procedure involving boiling 100 µl of the above mentioned EC-broth or a typical *E. coli* colony suspended in 100 µl of sterile distilled water for 10 min followed by 5 min freezing in liquid nitrogen. The samples were thawed and centrifuged at 13 000 ×*g* for 3 min and 1 µl of the supernatant was used for PCR analysis. The PCR protocol, the specific primers and subsequent gel electrophoresis procedure to detect the amplification product were as reported by Kim et al. (2006).