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Occurrence of *Escherichia coli* in *Brassica rapa* L. *chinensis* irrigated with low quality water in urban areas of Morogoro, Tanzania

Ofred J. Mhongole[1]*, Robinson H. Mdegela[1], Lughano J. M. Kusiluka[1] and Anders Dalsgaard[2]

[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, Tanzania.
[2]Department of Veterinary Disease Biology, Copenhagen University, Denmark.

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Low quality water has become valuable resource with restricted or unrestricted use in food production depending on its quality. This study has quantified the occurrence of *Escherichia coli* in *Brassica rapa* L. *chinensis* (Chinese cabbage) vegetables and low quality irrigation water. A total of 106 samples including Chinese cabbage (69) and water (37) were collected. The *E. coli* were cultured in petri film selective *E. coli* plates at 44°C. The Chinese cabbage irrigated with river water at Fungafunga area indicated significantly ($P<0.001$) high prevalence 86% (n=21, 0.00-4.10 log cfu/g) of *E. coli* than those irrigated with treated wastewater at Mazimbu 10% (n=48, 0.00-1.36 log cfu/g). The mean counts of *E. coli* in untreated wastewater ranged from 4.59 to 5.56 log cfu/mL, while in treated wastewater was from 0.54 to 1.05 log cfu/mL and in river water it was 2.40 log cfu/mL. Treated wastewater of the quality found in this study could be used for food production.

Key words: Agricultural irrigation, Chinese cabbage, incidental inputs, river water, wastewater.

INTRODUCTION

The term low quality irrigation water (LQIW) used in this study covers different types of water used for irrigation of crops in urban and peri urban areas of Morogoro municipality. The LQIW include domestic wastewater and polluted downstream rivers. The use of LQIW in food production is considered as an alternative source of water due to physical and economical water scarcity (Mateo-Sagasta et al., 2015). Low quality irrigation water has become valuable resource with restricted or unrestricted use in food production depending on its quality (World Health Organization, 2006a, b, c). It is a valuable and reliable resource for irrigation and fertilizing soils (Babayan et al., 2012) particularly in the Middle East (Ensink et al., 2007) as well as in African countries (Alemayehu et al., 2015). Agricultural irrigation by utilizing wastewater plays a significant role in food security as it improves crop yields and allows year round production (Jiménez, 2006). In Tanzania, for instance in urban and

*Corresponding author. E-mail: ofredjonas@gmail.com, ojmmhongole@yahoo.co.uk. Tel: +255 717 041676, +255 23 2 604542. Fax: + 255 23 2 604647.

peri-urban areas of Morogoro, small-scale farmers around wastewater treatment systems use LQIW (Mayilla et al., 2015) with partially or without any treatment. The growing water scarcity in urban and peri-urban areas in developing countries is among the drivers for farmers to use readily available LQIW (Qadir et al., 2010) which is often cheap or free. The sustainable use and management of LQIW for food production systems may certainly increase crop yields (Valipour, 2013).

Low quality irrigation water is generally contaminated with humans or animals faecal pathogenic microorganisms. The faecal pathogens may cause diseases to farmers, consumers and communities (Abakpa et al., 2013; Cobbina et al., 2013). These studies reported faecal bacterial contamination in vegetables including lettuce and Swiss chards irrigated with LQIW. These vegetables in particular, lettuce are often consumed raw and so they may pose health risks to consumers from faecal bacteria contamination. The green leafy vegetables have been associated with food-borne outbreaks caused by pathogenic bacteria such as *E. coli, Salmonella* spp., *L. monocytogenes* and *Shigella* spp. (EFSA Panel on Biological Hazards (BIOHAZ), 2011). Although Chinese cabbage is heated prior to consumption, inadequate preparation could expose consumers to potential health risks. Although in developing countries, untreated wastewater is often used for irrigation of crops (Jung et al., 2014), data on occurrence of pathogenic bacteria on green leafy vegetables including Chinese cabbage are limited (Erickson, 2010). The aim of this study was, therefore, to determine the extent of contamination of E. coli in Chinese cabbage and low quality irrigation water.

MATERIALS AND METHODS

This study was carried out in November, 2012 at Mazimbu, Mafisa and Mzumbe wastewater treatment systems (Figure 1) as well as at Fungafunga area (Morogoro River). Field visits were conducted to the farms to identify types of vegetables and farming practices. The observation was guided by prepared checklist which was administered to individuals or groups of farmers participated in this study. The Chinese cabbage was chosen from among the identified vegetables in the fields at Mazimbu and Fungafunga area, study sites. The reason for choosing Chinese cabbage was that it takes 3 to 4 months in the field compared to shortlived or once harvested vegetables such as amaranthus and pumkin leaves.

A total of 106 samples including Chinese cabbage (69) and water samples (37) were collected from four study sites; Mazimbu, Mafisa, Mzumbe and Fungafunga area. Out of 69 samples of Chinese cabbage, 48 were collected at Mazimbu and 21 at Fungafunga. Mzumbe and Mafisa study sites were not included because during the period of this study there were no Chinese cabbage being grown. A bundle of three to five leaves from different Chinese cabbage plants were harvested and placed in the sterile polythene bags. A total of 37 samples of water were collected from Mafisa (11), Mzumbe (13), Mazimbu (9) and Fungafunga area (4). Using sterile falcon tubes, about 50 mL of water samples were collected from untreated wastewater inlets and treated wastewater outlets, while at irrigation fields water samples were collected from a hose pipe, canal or intake points and further from treated wastewater downstream. All samples of Chinese cabbage and water were put into a cool box with ice cubes and immediately transported to the Pest Management Centre Laboratory, Sokoine University of Agriculture and analysed in the same day.

The Chinese cabbage vegetables were chopped using a sterile blade to make a composite sample of 50 g into sterile plastic bags. A total of 100 mL of 0.025% sodium dedocyl sulfate solution (SDS) was added into the bag containing 50 g of vegetables, then swirled ten times to recover bacteria from the sample. A small hole was cut at a corner of the plastic bag by a sterile scissor and about 50 mL of homogenate was transfered into sterile falcon tubes and stored at 4 to 8°C until analysis.

Enumeration of *E. coli* was done on 3 M petri film Select *E. coli* (SEC) plates as per 3 M Microbiology Products protocol (St. Paul, USA). The undiluted Chinese cabbage homogenate and water were serially diluted from 1/10; 1/100; 1/1000, continued as required. About 1 mL of the sample from selected dilutions was inoculated on SEC plates and incubated at 44°C for 24 h. Following incubation, all typical blue colonies of *E. coli* regardless of their size and colour were counted and calculated against the dilution factor and reported in cfu/g or mL for Chinese cabbage and water respectively.

The mean counts of *E. coli* in Chinese cabbage and water samples were compared between study sites by Student's *t*-test using SPSS statistics 20.0 of 2011 (IBM, California, USA). The differences of counts of *E. coli* in Chinese cabbage and water between the study sites were reported at P<0.05 (Kamoutsis et al., 2012).

RESULTS AND DISCUSSION

Different types of vegetables are grown in the study sites including Sweet potato, Chinese cabbage (*Brassica rapa* L. c*hinensis*), pumpkin leaves, Swiss chard, *Brassica carinata*, amaranthus and cowpeas. Others were okra, tomatoes, eggplant, African eggplant and paddy. The Chinese cabbage was selected for this study. Table 1 shows the irrigation methods, farm pre-harvest practices and possible sources of contamination on Chinese cabbage in different steps. Main types of irrigation methods observed were surface flooding and furrow aided by pumping and or conveyed by gravity. Various types of composite manures, and fertilizers used include poultry manure, tobacco leafy stalks and dust as well as industrial fertilizers such as Calcium, Ammonia and Nitrogen (CAN), UREA and Sulfate of Ammonia (SA), respectively.

This study found high concentration of *E. coli* (4.00 log cfu/g) in Chinese cabbage at the farm level. The potential sources of pathogen contamination during pre-harvest practices may include dust, soil, polluted irrigation water and rodents (Jung et al., 2014). Previous studies conducted on leafy vegetables in Ghana (Keraita et al., 2007) and in Pakistan (Ensink et al., 2007) during pre- and post-harvest handling practices reported faecal pathogens contamination. This may be caused by unhygienic practices during applying the incidental inputs and handling of harvested vegetables at the farms as habitually are placed in contact with soil and often washed with LQIW. There is also a possibility of growth of faecal pathogens during post-harvest handling, transportation and overnight storage at home, and

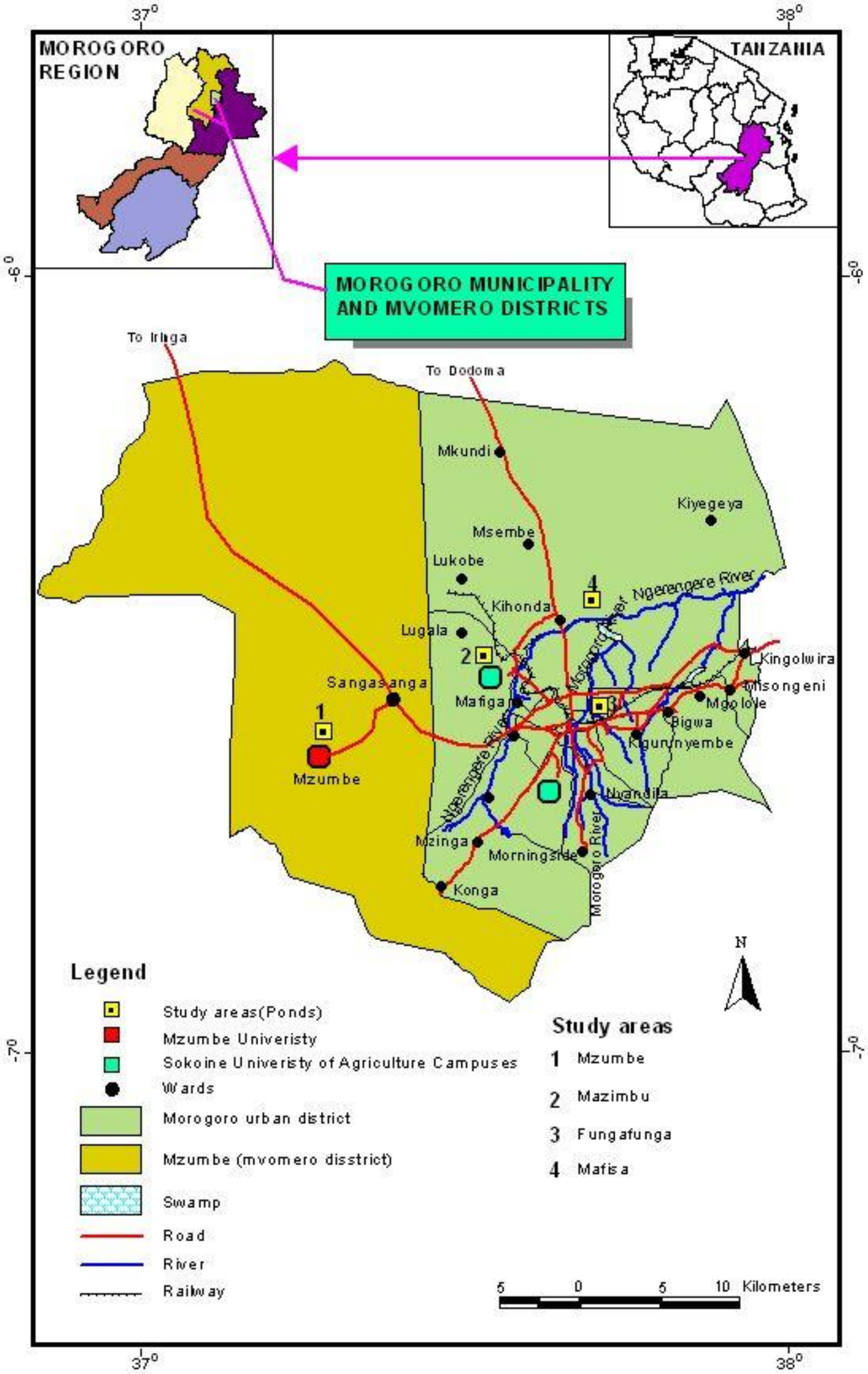

Figure 1. Map of Morogoro urban and peri-urban areas showing study sites (Source: Field Data and Tanzania Administrative Boundaries Map (2002))

display at markets.

The Chinese cabbage irrigated with river water at Fungafunga area indicated significantly (P<0.001) high prevalence 86% (n=21, 0.00-4.1 log cfu/g) of *E. coli* than those irrigated with treated wastewater at Mazimbu 10% (n=48, 0.00-1.36 log cfu/g) (Figure 2). Although there is no established criteria for concentration of *E. coli* in the raw leafy vegetables (ICMSF, 1986), concentration of *E. coli* >3 log cfu/g observed in this study may pose health risks to consumers. This was expected, because

Table 1. Farm practices and possible faecal contamination in Chinese cabbage in urban areas of Morogoro, Tanzania.

Irrigation methods	Steps	Farm practices	Possible contamination/growth of bacteria
Chinese cabbage farms irrigated by flooding using hose pipe, in interval of 2 to 5 days depending on the weather. And or two days prior each harvest.	Field preparation	Fields prepared with or without applying cow or poultry manure, or tobacco stalks	Soil contamination by faecal pathogens from manure, water and incidental inputs
	Seedling preparation and transplanting	Seedlings were planted and left to grow up to the maximum of 1 month and transplanted into the main field	Seedling cross contamination from farmers, water and incidental inputs
	Direct seeding	Some farmers saw seed direct into field by spreading them randomly.	Soil contamination by water and incidental inputs
	Weeding	Weeding depend on growth of weeds.	Contamination with pathogens from soil and incidental inputs
	Fertilizing	Fertilizers were applied during the 3rd week. Most of farmers use poultry manure and sometimes industrial fertilizers (Calcium, Ammonia and Nitrogen – (CAN), UREA and Sulfate of Ammonia (SA)	Possible contamination from poultry manure
	First leafy harvest	First leafy harvest was after 4-6 weeks depending on the early or late maturity	Cross contamination from farmers, handlers and incidental inputs
	Harvesting	Only the mature leaves are harvested every week up to the period of 12 to 16 weeks	Contamination from farmers, handlers and incidental inputs
	Preparation/ consumption	Proper cooking	Reduce or eliminate faecal pathogens load to an acceptable level

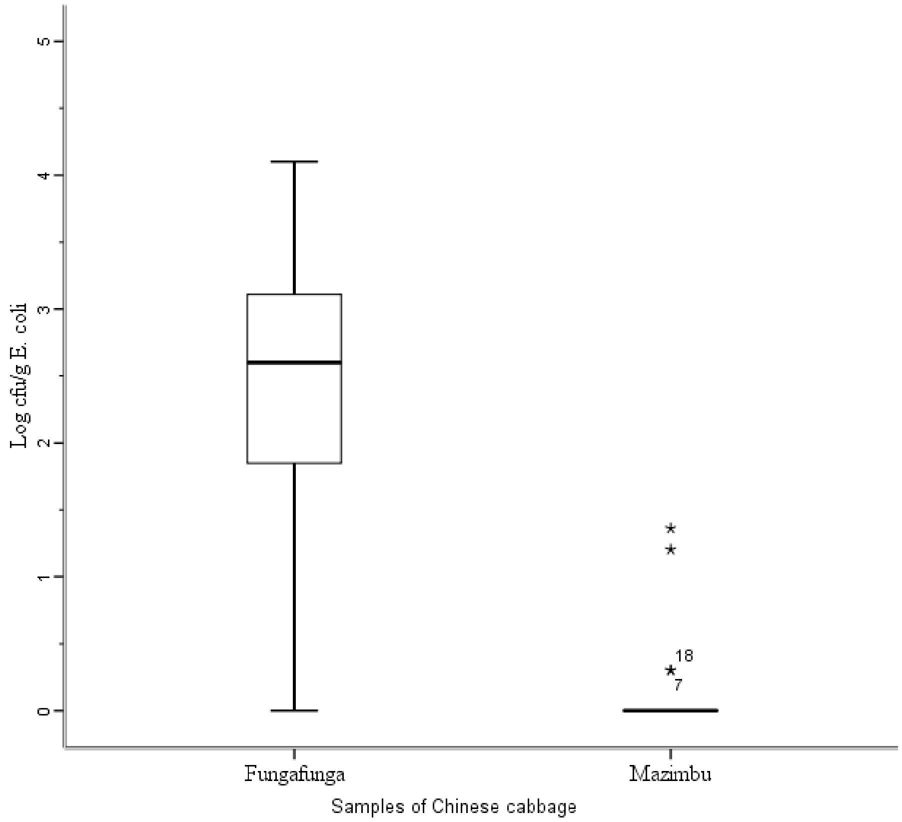

Figure 2. Concentration of *E. coli* in Chinese cabbage vegetables from Mazimbu and Fungafunga. *Concentration of *E. coli* >0 log cfu/g.

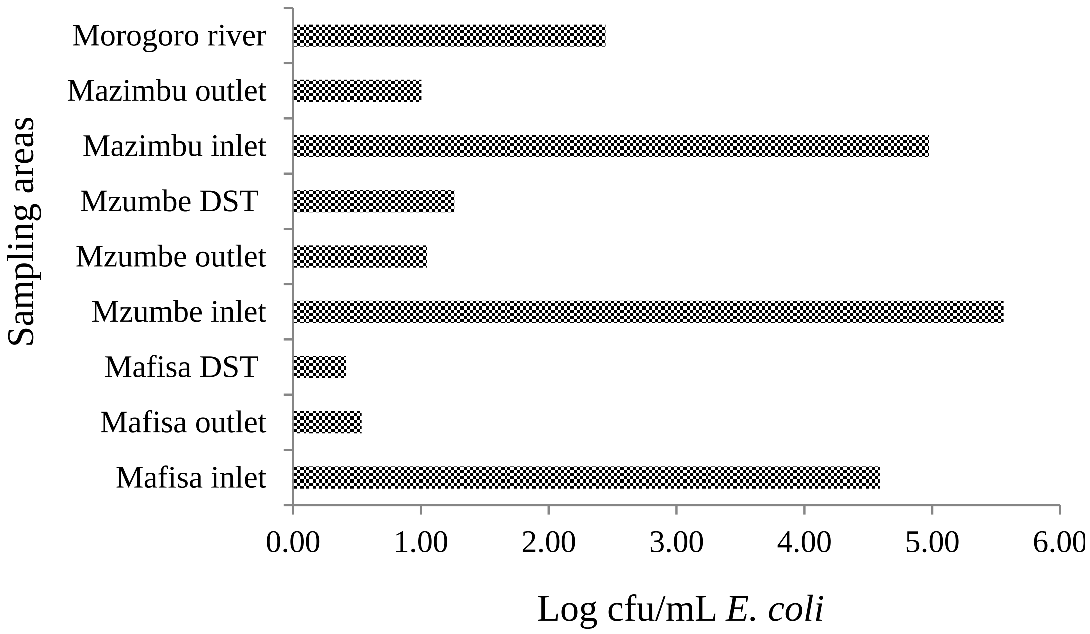

Figure 3. Concentration of *E. coli* in different sources of low quality irrigation water in urban and peri-urban areas of Morogoro (DST, downstream treated wastewater).

Fungafunga area is a home of the old people, located in urban Morogoro. It is, therefore, characterized with high human traffic and activities. Since Morogoro river is not stationary, and if the up-stream experience high domestic activities of effluent of wastewater high contamination levels are expected. The other sources of contamination could be incidental agricultural inputs including animal manure or non-incidental inputs from animals, humans and farmers practices than the irrigation water (FAO/WHO [Food and Agriculture Organization of the United Nations/World Health Organization], 2008; Jung et al., 2014). However, Chinese cabbage which has a natural epiphytic flora, it may acquire contamination from various incidental and accidental inputs.

Figure 3 illustrates the level of *E. coli* contamination of different types of LQIW. The *E. coli* contamination level of untreated wastewater was significantly ($P<0.05$) higher (4.59-5.56 log cfu/mL) than treated wastewater effluent (0.54-1.05 Log cfu/mL) and river water (2.4 log cfu/mL). The untreated wastewater from Mzumbe had the highest level of *E. coli* contamination (5.56 log cfu/mL), while sample from Mafisa was the least (4.59 log cfu/mL) (Figure 2). Likewise, treated wastewater downstream from Mzumbe had highest level of *E. coli* contamination (1.26 log cfu/mL) than the one from Mafisa (0.41 log cfu/mL). If wastewater is not collected from the source and discharged into the wastewater treatment units, it is likely to be disposed indirectly and or directly to the surface water bodies or soil (SAI Platform, 2010). The increase in concentration of *E. coli* in treated wastewater downstream could be due to cross contamination from incidental inputs including environment, human activities, droppings of birds and animal faeces. This may be attributed to the limited or inadequate plans and wastewater treatment facilities, and it thus, may lead to environmental contamination (Sato et al., 2013).

Risk of leafy vegetables contamination with LQIW has been reported to increase in the order of potable/rain water, deep wells and shallow wells. Followed by the surface water in proximity to animals, human habitation and associated wastes; and untreated or partially treated wastewater (Mateo-Sagasta et al., 2013). Low quality water close to human and animal habitation waste or activities may present potential risk when used for food production. The quality of treated wastewater found in the study sites may be used for crop irrigation as recommended by the WHO guidelines (World Health Organization, 2006b). Safe use of treated wastewater for irrigation depends on awareness, knowledge and hygiene practices by the farmers (Mateo-Sagasta et al., 2013). The use of LQIW with *E. coli* >1.00 log cfu/mL for irrigation of vegetables may pose potential health risks to the public and environment. Training of farmers on safe use of wastewater and good agricultural practices will, therefore, help to reduce the potential public health risks (Keraita and Akatse, 2012). Implementation of good agricultural and hygiene practices, good pre- and post-harvest handling practices, may reduce faecal bacterial contamination in irrigated foodstuffs. To our knowledge this is a first study on occurrence of *E. coli* in Chinese cabbage irrigated with treated wastewater in Tanzania. Generally water from rivers is regarded as of good microbiological quality than treated wastewater, and vegetables irrigated by treated wastewater are perceived of poor quality. Well treated wastewater could be used for crop irrigation with minimal health risks. However, further studies on contamination of pathogenic bacteria in other green leafy vegetables, during pre-and post-harvest handling practices is recommended. Use of tobacco stalks and dust as organic fertilizers in vegetables need to be investigated for their antimicrobial effects.

Conflicts of Interests

The authors have not declared any conflict of interests.

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